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Integrated Management of Billbugs (Coleoptera: Dryophthoridae) in Intermountain West Turfgrass

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INTEGRATED MANAGEMENT OF BILLBUGS (COLEOPTERA: DRYOPHTHORIDAE) IN INTERMOUNTAIN WEST TURFGRASS

by

Madeleine M. Dupuy

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Ecology

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UTAH STATE UNIVERSITY
Logan, Utah

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ABSTRACT

Integrated Management of Billbugs (Coleoptera: Dryophthoridae) in Intermountain West Turfgrass

by

Madeleine M. Dupuy, Doctor of Philosophy

Utah State University, 2018

Major Professor: Dr. Ricardo A. Ramirez
Department: Biology

Billbugs (Coleoptera: Dryopthoridae: *Sphenophorus* spp.) are a serious pest of turfgrass in the Intermountain West, where bluegrass (*S. parvulus*), hunting (*S. venatus vestitus*), and Rocky Mountain (*S. cicatristriatus*) billbugs damage turfgrass as a species complex. Billbug larvae severely discolor and eventually kill turfgrass by feeding in stems, on roots, and on crowns of the plant. Given the cryptic nature of the susceptible larval stages in stems and soil, billbugs are typically managed with preventive, calendar-based applications of long-residual, systemic insecticides, including neonicotinoids and anthranilic diamides. Our understanding of billbug biology and management has resulted from research conducted in the eastern U.S., and little is known about billbug biology and best management practices in the Intermountain West. Further, insecticides commonly used against billbugs have been shown to have a negative effect on generalist predatory arthropods that provide natural pest suppression. In Chapter II, I examined the phenology of billbug life stages in Intermountain West turfgrass and developed a predictive degree-day model to better time management strategies against billbugs. I found that an existing degree-day model from the eastern U.S. was not robust enough to predict billbugs in Utah and Idaho. Instead, the model that best predicted adult activity of the billbug complex accumulated degree-days above 3^{o}C after 13 January. In Chapter III, I used the Utah-Idaho degree-day model to test whether eastern U.S. recommendation for preventive management timing at 30% of adult billbug activity and a curative management timing at 50%, or peak, adult billbug activity were effective for billbugs in the Intermountain West. I examined the efficacy of applications of synthetic and biological insecticides at model-predicted management timings. I found that the preventive and curative application timings as predicted by the Utah-Idaho model were effective times to apply systemic, long-residual insecticides such as neonicotinoids and anthranilic diamides. However, newer biological insecticides were highly variable and less effective with a single application. In Chapter IV, I assessed the predatory arthropod community in Intermountain West turf and their impacts on billbug suppression. I found that the predatory arthropod community consisted primarily of carabids and spiders, representing 60% and 28% of all predators, respectively. The greatest consumptive effects of predators were on billbug eggs, with *Anisodactlylus* sp. feeding on 46% of eggs. Predator exposure reduced overall billbug activity by 56%, and for hunting billbugs, specifically, reduced mating activity by 28%. My research not only lays the ground work for development of effective, sustainable integrated management of billbugs in Intermountain West turfgrass, including conservation biocontrol, but also illustrates the necessity of regional predictive models, monitoring, and appropriate timing of management for successful turf pest suppression.

(182 pages)

PUBLIC ABSTRACT

Integrated Management of Billbugs in Intermountain West Turfgrass

Madeleine M. Dupuy

Billbugs are a serious pest of turfgrass in the Intermountain West. Billbug larvae severely discolor and eventually kill turfgrass by feeding in stems, on roots, and on crowns of the plant. Billbugs are typically managed with preventive, calendar-based applications of insecticides. Most of our knowledge on the biology and management of billbugs comes from research in the eastern U.S, and little is known about billbug biology and best management practices in the Intermountain West. First, I examined the seasonal activity of billbug life stages in Intermountain West turfgrass and developed a predictive degree-day model to better time management strategies against billbugs. I found that compared to the eastern U.S., a regional model that starts earlier (January 13) and has a cooler insect development threshold (3^{o}C) was adequately robust to predict billbugs in Utah and Idaho. Next, I used the Utah-Idaho degree-day model to determine whether preventive and curative timings for billbug management developed in the eastern U.S. were effective in the Intermountain West. Testing four insecticides with the Utah-Idaho model and with eastern U.S. management timings I found that there was support to consider adoption of these same recommendations in Utah and Idaho, particularly for current preventive insecticides such as neonicotinoids and anthranilic diamides. Finally, considering that turf insecticides can negatively impact predatory insects, thought to suppress turf pests, I assessed the predatory arthropod community in Intermountain West turf and their impacts on billbugs. I found that the predatory arthropod community consisted primarily of ground beetles and spiders, representing 60% and 28% of all predators, respectively. I found that predators contributed the most by consuming billbug eggs and by changing the behavior of billbug adults with an observed reduction in mating activity. My research not only lays the ground work for development of effective, sustainable integrated management of billbugs in Intermountain West turfgrass, including conservation biocontrol, but also illustrates the necessity of regional predictive models, monitoring, and appropriate timing of management for successful turf pest suppression.

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Madeleine M. Dupuy

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CHAPTER I

BIOLOGY AND MANAGEMENT OF BILLBUGS (COLEOPTERA: CURCULIONIDAE) IN TURFGRASS[1]

Abstract

Billbugs (Coleoptera: Curculionidae: *Sphenophorus* spp.) are a complex of weevil pests affecting turfgrass throughout the United States. Billbug larvae cause damage by feeding in stems, on roots, and on the crowns of turf, causing severe discoloration and eventual plant death. Monitoring efforts have focused on non-destructive pitfall sampling of ground active billbug adults and on destructive sampling using soil cores for larval stages in the soil. Given the cryptic nature of the susceptible larval stages, billbugs are typically managed by preventive applications of long-residual, systemic insecticides, including neonicotinoids and anthranilic diamides. Despite knowledge of effective management practices including pest resistant turf varieties, irrigation management, and microbial controls that contribute to an IPM approach, billbug management continues to rely heavily on prophylactic synthetic insecticides. This review will summarize the identification and biology of billbugs and strategies for their management.

Key words: *Sphenophorus parvulus*, *Sphenophorus venatus vestitus*, *Sphenophorus cicatristriatus*, pitfall trap, Kentucky bluegrass

[1] Dupuy, Madeleine M. and Ricardo A. Ramirez

Turfgrass covers more than 164,000 km^2 (63,321 mi^2) of the United States landscape, over three times the land area of any other irrigated crop (Milesi et al. 2005), and includes golf courses, home lawns, sports fields, and sod farms (Gelernter 2012). In 2005, the revenue generated by the turfgrass industry exceeded $62 billion (Haydu et al. 2008), surpassing the combined value of corn ($21 billion) and soybeans ($17 billion) in the same year (NASS 2006). This revenue depends largely on maintenance of turfgrass quality, aspects of which include density, texture, growth habit, smoothness, and color (Beard 1972). Management practices that enhance turfgrass quality, like regular irrigation, fertilization, and mowing, however, encourage many species of turf-feeding arthropods (Held and Potter 2012).

Turf is grown primarily for its utility and appearance, and discoloration of turfgrass can quickly become unacceptable in settings such as golf courses and sod farms, whose revenues depend largely on turf health and quality. Feeding by billbug (Coleoptera: Curculionidae: *Sphenophorus* spp.) larvae in stems and on roots causes spotty patches of yellow and brown turf, which can expand to large areas of dead grass. Thus, billbugs can be a serious pest of turfgrass, but effective management has been historically difficult due to several aspects of billbug biology, which will be discussed in this review.

Billbugs are a complex of weevils native to and widespread throughout the U.S. (Johnson-Cicalese et al. 1990, Shetlar et al. 2012). The genus *Sphenophorus* contains 71 species, 64 of which occur in North America (Niemczyk and Shetlar 2000). At least ten species are pests of turfgrass in the U.S., including the bluegrass billbug (*Sphenophorus* *parvulus* Gyllenhal) and hunting billbug (*S. venatus vestitus* Chittenden), which are considered most harmful to cool-season grasses and warm-season grasses, respectively (Potter and Braman 1991, Vittum et al. 1999). Though billbugs have been known to infest other agricultural crops such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and range grasses (Satterthwait 1931a, Asay et al. 1983, Kuhn et al. 2013), they were first recognized as a serious pest of turfgrass when bluegrass billbug began to outbreak in several states in the 1960's (Tashiro and Personius 1970). These outbreaks were thought to be caused by resistance of the bluegrass billbug to pesticides that were heavily used at the time and the resulting reduction in natural enemy populations (Tashiro and Personius 1970). Billbugs continue to be problematic for turfgrass managers throughout the country.

Biology

Distribution

Billbugs are found throughout the continental United States and in Hawaii. Their range extends north to southern Canada and south through Mexico (Reynolds 2013), and they are also pests of turfgrass in Japan (Aoyagi et al. 1990, Georgis et al. 2006). Hunting billbug has also been reported in Puerto Rico, the Bahamas, the Dominican Republic, and Martinique (Kuhn et al. 2013). Previously, it was thought that bluegrass billbug and hunting billbug were the only species causing damage to turf in the U.S.; however, Johnson-Cicalese et al. (1990) classified eight species commonly reported in the U.S., including a complex of four species damaging turf in New Jersey—bluegrass billbug, hunting billbug, lesser billbug (*S. minimus* Hart), and unequal billbug (*S. inaequalis* Say). Furthermore, it was determined that different species of billbug dominate different parts of the country: hunting billbug in the southeastern U.S., bluegrass billbug in the northern half of the country, Phoenix billbug (*S. phoeniciensis* Chittenden) in the southwestern U.S., and Rocky Mountain billbug (*S. cicatristriatus* Fabraeus) in the Rocky Mountain region. Three species are prevalent in the wider Intermountain West (in descending order of abundance): bluegrass billbug, hunting billbug, and Rocky Mountain billbug (Fig. 1). This complex is common in the western U.S. with the addition of *S. sayi* (Gyllenhal) in northeast Oregon (Walenta et al. 2004) and Phoenix billbug in California (Flint et al. 2009), Idaho (Fritz and Salaiz 2007), and the southwest (Sutherland 2006). Other species found in U.S. turf include *S. apicalis* (LeConte), *S. coesifrons* (Gyllenhal), the southern corn billbug (*S. callosus* Oliver), and *S. rectus* (Say) (Table 1). Overall, there are at least ten species of billbug causing damage to turf in the U.S. (Held and Potter 2012), though detailed biological observations continue to be limited to bluegrass billbug and hunting billbug.

Host plants

Bluegrass billbug infests mostly cool-season grasses, especially Kentucky bluegrass (*Poa pratensis* L.), but may also inhabit some warm-season grasses and grassy weeds (Vittum et al. 1999) (Table 1). Hunting billbug primarily infests warm-season grasses, especially zoysiagrass (*Zoysia* spp.), and is sometimes called the zoysiagrass billbug. Additional hosts include certain cool-season grasses and a variety of grassy weeds (Table 1). For example, yellow nutsedge (*Cyperus esculentus* L.) was previously determined to be the preferred host of hunting billbug (Satterthwait 1931a). Rocky Mountain billbug is most common in Kentucky bluegrass and perennial ryegrass (Niemczyk and Shetlar 2000). Recently, billbugs—including the bluegrass, hunting, and southern corn billbugs—have been recognized as a serious pest of orchardgrass in Virginia (Kuhn et al. 2013) They can also be pests on other range grasses, corn, and wheat (Satterthwait 1931a, Asay et al. 1983). There is little evidence, however, that billbugs are problematic to adjacent ornamental plants within a turf landscape. Relatively little is known about billbug host ranges for other species of billbug beyond the plants in which they have been observed (Table 1).

Life history

Billbug adults are ground active, and their primary method of locomotion is crawling. Adults have been observed either unsuccessfully attempting flight (Tashiro and Personius 1970, Kindler and Spomer 1986) or flying at very low heights for very short distances when wind conditions increase chances of becoming airborne (Young 2002, Shetlar et al. 2012). Billbug adults are usually found in thicker grasses with a heavy thatch layer that is thought to offer shade and protection (Kindler and Spomer 1986). They prefer grasses with thick, plush stems for oviposition, or simply grasses that are actively growing (Kindler and Spomer 1986, Vittum et al. 1999, Rondon and Walenta 2008). Billbugs overwinter as adults in protected areas, such as thatch, the junction between turf and sidewalk (Niemczyk 1983, Richmond 2015), nearby leaf litter or unmanaged turf areas (Young 2002, Richmond 2015), or buried in the soil head-first at depths of 1 cm or less (Kindler and Spomer 1986). Some species in certain regions may also overwinter as larvae (Doskocil and Brandenburg 2012, Shetlar et al. 2012, Richmond and Duffy 2015). In the southeastern U.S., adults of the hunting billbug are nocturnal (Huang and Buss 2009, Reynolds 2013).

Larvae are legless; therefore, feeding by individuals is restricted to a small area (Kindler and Spomer 1986). It is widely accepted that the larval stage is the damaging one, while adults feed minimally on grass blades and cause only superficial damage. In North Carolina, however, adult hunting billbugs appear to be the damaging life stage on warm-season turf while larvae are rarely found in damaged areas (Doskocil and Brandenburg 2012).

Description of damage

Larval feeding on stems, roots, and crowns causes severe discoloration and can eventually lead to plant death. Feeding damage first appears as yellowing of small patches of turf, which is often mistaken for disease, but quickly expands to larger areas of brown and dying turf under heavy infestation. This more extensive damage is frequently mistaken for drought stress and can be exacerbated under drought conditions (Niemczyk 1983). Heavy larval feeding compromises the root system, and stems of severely damaged turf break and pull away easily from the soil. Often, a sawdust-like frass is present in hollowed-out stems to diagnose billbug feeding (Watschke et al. 2013). Damage by overwintered hunting billbug larvae in spring can appear as delayed green-up in regions where larvae of this species are capable of overwintering (Richmond 2015).

Potential for economic damage

Management decisions in the turf industry are largely driven by aesthetics and consumer culture, and traditional metrics of economics used for field crops do not readily translate to the turfgrass system (e.g., yield loss) (Held and Potter 2012). The level of acceptable damage varies by the intended use of the turf. On golf courses and sports fields, for example, the threshold of allowable damage for any insect is very low. Billbugs can not only damage but also can kill extensive areas of turfgrass in a matter of weeks under heavy infestations (Shetlar et al. 2012). Thus, insecticides with long residual activity are often applied preventively against billbugs on an annual basis regardless of whether or not they will become damaging.

Insecticides for all turf insects account for 31% and 19% of annual chemical expenditures for lawn care companies and golf courses, respectively (Held and Potter 2012). In 2006, lawn and garden products accounted for 16% of all conventional insecticides used in the U.S. (Grube et al. 2011). The cost of insecticides for pest control can account for millions of dollars of the multibillion-dollar turf industry (Haydu et al. 2008). In 2006 for the Georgia turf industry alone, not including golf courses, billbugs contributed to $2,835,000 worth of damage caused by miscellaneous turf pests (including non-fire ants, billbugs, leafhoppers, bermudagrass mites, and stunt mites) (Oetting et al. 2006). The cost of insecticides for preventive billbug management in the Intermountain West can range from $12 per acre to $114 per acre for treatments of imidacloprid and clothianidin, respectively (P. Stokes, personal communication). Unfortunately, there are no published figures on economic losses in turfgrass caused specifically by billbugs nationwide.

Life Stages and Phenology

Description and life cycle

Adult billbugs have hard wing covers and a long beak-like snout with chewing mouthparts at the distal end, typical of weevils (Fig. 2A-D). They have clubbed, elbowed antennae with a long scape inserted at the proximal end of the snout. Depending on the species, adults are black or dull red/brown in color (Reynolds 2013), but when coated in soil can appear lighter in color (Niemczyk and Shetlar 2000, Richmond 2015). Billbug species can be differentiated from one another using pronotal patterns and markings on the elytra, color, and relative size (Shetlar 2011, Shetlar et al. 2012) (Fig. 2A-D). *Sphenophorus* is distinguished from other related genera by the shape of the antennal club, the relative separation of the coxae, the shape of the mesoepimeron, metaepimeron, and intercoxal processes, the claw segment, and the amount and arrangement of hairs on the underside of the third tarsal segment (Vaurie 1951).

Bluegrass and hunting billbugs are univoltine in multiple parts of the country (Johnson-Cicalese et al. 1990, Rondon and Walenta 2008, Kindler and Spomer 1986). Adults emerge from protected overwintering sites with warming temperatures in the spring and mate (April-May). Adult females chew holes in turf stems near the crown and deposit one to three eggs in each opening (Webster 1892, Satterthwait 1931a). Johnson-Cicalese et al. (1990) observed egg laying through August in New Jersey. Billbug eggs are oblong, creamy white, smooth and glossy, and 1-2 mm (0.04-0.08 in) in length (Fig.

3) (Kindler and Spomer 1986). The egg stage generally lasts 6-10 days (Johnson-Cicalese et al. 1990, Rondon and Walenta 2008) before first-instars emerge.

The larval stage has five instars that are cream-colored and robust, with a slightly tapered abdomen and a yellowish-brown to reddish-brown head capsule (Fig. 4). Billbug larvae are legless, which distinguishes them from white grubs (Coleoptera: Scarabaeidae), to which they may otherwise appear similar in initial stages. First instars are typically around 1.3 mm (0.01 in) long and feed in grass stems after egg hatch. They then drop 2-8 cm (0.79-3.15 in) into the soil and continue feeding on the roots and crown of the plant (June-August) (Johnson-Cicalese et al. 1990, Vittum et al. 1999). These later instars range from 6-10 mm (0.24-0.39 in) in length (Shetlar et al. 2012). Currently, no external characters have been identified that can be used to distinguish larval species from one another, but DNA-based larval identification tools have been examined (Richmond et al. 2011). The larval stage generally lasts 35-55 days for bluegrass billbug and 21-35 days for hunting billbug before pupation (Watschke et al. 2013).

Pupae are initially cream colored, then sclerotize and darken to reddish brown. The appendages and wing pads of these exarate pupae are held close to the body, and the characteristic curculionid snout is evident (Fig. 4) (Shetlar et al. 2012). Pupae of different billbug species can be distinguished from one another, using characters such as setae, length of beak, and the width of the pronotum (Satterthwait 1931a). The pupal stage lasts 8-12 days for bluegrass billbug or 3-7 days for hunting billbug before adults emerge in the fall and return to overwintering sites (Johnson-Cicalese et al. 1990, Watschke et al 2013).

Differences in phenology throughout the U.S.

In northeast Oregon, Rocky Mountain billbug is also univoltine, but adults and larvae are present year-round (Rondon and Walenta 2008). Larvae of the hunting and Rocky Mountain billbugs in New Jersey and northeast Oregon, respectively, have also been observed during the winter months, suggesting that a partial second generation occurs for these species in particular regions (Johnson-Cicalese et al. 1990, Rondon and Walenta 2008). In Indiana and North Carolina, the hunting billbug produces two overlapping generations per year and is capable of overwintering both as an adult or larva (Doskocil and Brandenburg 2012, Richmond and Duffy 2015). In Florida, Huang and Buss (2009) observed up to six overlapping generations of hunting billbug per year in greenhouse experiments at 25.8-27^{o}C (78.4-80.6^{o}F), with total development from egg to adult taking only 8-9 weeks on warm-season turfgrasses. Under field conditions, such as those reported in New Jersey where average spring and summer temperatures range from 10^{o}C to 24^{o}C (50-75.2^{o}F) (climate.rutgers.edu), univoltine billbugs develop from egg to adult through the months of April-September. In the Intermountain West, where billbugs also appear to be univoltine, the window of development is extended from March through October (Fig. 1).

Monitoring

Adult activity

Billbug activity can be monitored with pitfall traps because billbug adults are primarily ground active. Pitfall traps can be as simple as a plastic cup placed in the ground so that the lip of the cup is flush with the ground surface, or they can be more complex. Linear pitfall traps use PVC pipe or similar material to capture ground-active insects from a wider area in a single collection cup (Fig. 5A-C). Adults captured in the traps should be counted at least once per week to inform pest management decision-making (Potter 1998). Nocturnal hunting billbug adults may also be monitored easily by searching on greens and fairways at night with a strong light (Reynolds 2013).

An early treatment threshold suggests management is necessary when 15-25 adults can be collected by one person from pavement over a five-minute period (Tashiro and Personius 1970). However, this does not specify the area of pavement to be covered, time of day collection is to be done or other important parameters. Unfortunately, more useful treatment thresholds have not been developed, but information from pitfall traps on first occurrence and increases in activity can be paired with other monitoring techniques to time management strategically.

Larval activity

Billbug larvae are stem- and soil-dwelling, and thus, more difficult to monitor. Stems in areas of suspected billbug feeding can be inspected using the “tug test.” Stems that have been fed on by larvae will break away easily when tugged on, particularly under heavy infestation (Fig. 6 A-B). These stems are often hollow or filled with a sawdust-like frass. Later instars can be sampled by taking a soil core (e.g., using a cup cutter) in areas where larval feeding is suspected and inspecting the crown and root zone. In North Carolina, a standard cup cutter may not be an effective sampling tool because hunting billbug larvae are often found beyond the cup cutter’s sampling range, up to 23 cm (9.05 in) beneath the soil surface (Reynolds and Brandenburg 2015). Larvae can also be found by cutting three sides of a square foot in the turf with a sturdy knife. The turf can then be peeled back to check for the presence of larvae in the root zone and can be easily replaced with minimal damage afterwards (Vittum et al. 1999).

Degree-day model

Predictive degree-day models may be paired with the monitoring tools previously described and have been implemented effectively in many systems, including turfgrass for the annual bluegrass weevil (*Listronotus maculicolis* Dietz) (Syngenta 2015). The degree-day approach assumes that insect development is directly related to ambient temperature and that higher temperatures result in increased growth rate, to a certain threshold (Higley et al. 1986). Heat units based on daily high and low temperatures (degree-days) accumulate from a biofix, or starting date, every day the average temperature is above a pre-determined lower development threshold (a temperature below which the insect does not develop) for a particular species (Higley et al. 1986). A degree-day model for bluegrass billbug was developed in Ohio using the average method of calculation, a March 1 biofix, and a lower development threshold of 10°C (50°F). This model predicts first adult activity at 155-195 DD_{10^oC} (280-352DD_{50^oF}), larval emergence from stems at 513-575 DD_{10^oC} (925-1035 DD_{50^oF}), and apparent visual damage at 739-825 DD_{10^oC} (1330-1485 DD_{50^oF}) (Watschke et al. 2013). However, this model does not appear to be robust, as preliminary calculations do not accurately predict activity in other regions of the country (Fig. 1). Adjustments to the model may need to be considered for different regions, including the western U.S.

Management Options

Billbugs are particularly difficult to manage effectively because of differences in susceptibility of life stages to management methods and the soil- and stem-dwelling nature of larval stages versus the surface-dwelling adult stage. For optimal management, turf managers must first have a sound understanding of billbug seasonal activity and biology.

Cultural control

Resistant turfgrass varieties provide a non-chemical and economic method of long-term billbug management that can be paired with other IPM strategies. Additionally, turfgrass that has already been killed by billbugs can be overseeded with a resistant variety (Shetlar 1991). Many varieties and cultivars of Kentucky bluegrass have been shown to be resistant to feeding by bluegrass billbug, including Park, Arista, NuDwarf, Delta, Kenblue, and South Dakota Certified (Watschke et al. 2013). These varieties have fine stems and leaves and tougher plant tissue, which offer more resistance to feeding and are less preferred for oviposition than non-resistant varieties with thicker stems and leaves (Bruneau et al. 1987, Johnson-Cicalese et al. 1989). Varieties of Kentucky bluegrass with more aggressive growth habits also displayed faster recovery from billbug feeding (Johnson-Cicalese 1989). Several varieties of warm-season grasses resistant to feeding by hunting billbug have also been identified, including the *Zoysia matrello* (L.) cultivars Diamond, Zorro, Cavalier, and Royal (Reinert et al. 2011), and TifEagle bermudagrass (Huang and Buss 2013). *Acremonium* endophytic fungi grow symbiotically with many species of grasses, causing them to produce higher concentrations of plant allelochemicals that deter feeding by many insect herbivores (Breen 1994). Endophyte enhanced ryegrasses and fescues are highly resistant to feeding by billbugs and have been shown to be optimally resistant when they comprise 35-40% of the stand (Johnson-Cicalese and White 1990, Richmond et al. 2000, Watschke et al. 2013).

Billbug damage is most evident in stressed turf (i.e., under drought conditions or inadequate fertility) (Shetlar et al. 2012). Under light to moderate billbug infestation, damage can often be masked with adequate irrigation and fertilization (Watschke et al. 2013). Irrigation should be applied regularly to cool-season grasses when they are preparing for summer dormancy or while billbug larvae are emerging from grass stems to feed at the crown (Shetlar 1991, Shetlar et al. 2012).

Transportation of infested sod is a major cause of the spread of billbugs, especially with hunting billbug on bermudagrass and zoysiagrass sod farms (Watschke et al. 2013). Billbugs from unmanaged sites may also infest nearby managed sites (Watschke et al. 2013).

Biological control

Entomopathogenic nematodes are a potential biological control agent for billbug larvae (Georgis et al. 2006). In the U.S., *Steinernema carpocapsae* ((Weiser) Wouts, Mracek, Gerdin & Bedding), *Steinernema feltiae* ((Filipjev) Wouts, Mracek, Gerdin & Bedding), and *Heterorhabditis bacteriophora* (Poinar) have all been reported to control billbugs at rates comparable to commonly used insecticides in both field and lab trials (Niemczyk 1988, Georgis and Poinar 1994, Niemczyk and Shetlar 2000). The turfgrass system is ideal for use of nematodes because of ease of application and the soil-dwelling nature of many turfgrass pests. Despite promising efficacy results (74-78% mortality of bluegrass billbug; Georgis and Poinar 1994) and availability in commercial preparations, use of entomopathogenic nematodes for billbug management is limited because of the high availability of insecticides that are less expensive, have longer shelf lives, are regarded as more reliable, and require less consideration of application conditions (e.g., UV exposure, pre- and post-application irrigation). In Japan, *Steinernema carpocapsae* was the primary means of control for hunting billbug because of the lack of available effective insecticides and favorable environmental conditions. Since the registration of imidacloprid for use in Japan, however, sales of *Steinernema carpocapsae* have significantly declined (Georgis et al. 2006).

Grandevo® and Venerate™ are two microbial products (active ingredients: *Chromobacterium subtsugae* strain PRAA4-1 and spent fermentation media and heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media, respectively) that have been assessed for use against bluegrass billbug in Kentucky bluegrass. Grandevo® reduced numbers by 79.3% at 25.51g/92.9m^2 (0.90 oz/1000 ft^2), and Venerate™ reduced numbers of larvae and pupae by 93.1% at 177.44mL/92.9m^2 (6 fl oz/1000 ft^2) (Stamm et al. 2014). These rates of control are comparable to many commonly used chemical insecticides, thus these microbial products deserve further consideration.

Billbug adults and larvae are also susceptible to the entomopathogenic fungi *Beauveria* spp. and *Metarhizium* spp. Naturally existing complexes of these fungi rarely kill enough billbugs to have an effect on damage levels, and though commercial preparations of both fungi are available, they are expensive and field trials do not show consistent control (Watschke et al. 2013).

Additionally, there are a few known natural enemies of billbugs. *Zavipio (Vipio) belfragei* (Cresson) is a hymenopteran (Braconidae) parasitoid that has been reared from billbug larvae, however, no studies have been done on percent parasitism or potential impact (Young 2002). *Anaphes (Anaphoidea) calendrae* (Gahan) (Hymenoptera: Myrmaridae) has been reported as a parasitoid of eggs of bluegrass billbug, lesser billbug, and southern corn billbug (Satterthwait 1931b). This parasitoid is distributed throughout the eastern half of the U.S. and reportedly results in relatively high percentages of parasitism, thus, it may deserve further study (Young 2002).

There is a diverse predatory arthropod fauna inhabiting turfgrass, including spiders (Arachnida: Araneae), ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae), and ants (Hymenoptera: Formicidae) (Bixby-Brosi and Potter 2012). Several studies have documented factors impacting predatory arthropod communities and the impact of these predators on certain turf pests (Cockfield and Potter 1984, 1985, Arnold and Potter 1987, Terry et al. 1993, Kunkel et al. 1999, Lopez and Potter 2000, Zenger and Gibb 2003, Peck 2009, Dobbs and Potter 2014). For instance, commonly used turf insecticides have adverse effects on non-target predatory arthropods and their natural pest suppression (Terry et al. 1993, Kunkel et al. 2001), while conservation biocontrol practices (e.g., cultivation of flowering plants and predator refugia) have positive effects (Braman et al. 2002). In a study by Frank and Shrewsbury (2004), not only did “conservation strips” (strips of bunch grass and flowering plants) increase predator abundance, but instances of predation on black cutworm (*Agrostis ipsilon* Hufnagel) were more frequent on golf course fairways adjacent to strips. In our work, we have observed evidence of spider-feeding on billbug adults in pitfall traps. Predators may also have indirect effects on pest populations by changing behavior of pests which can lead to fitness costs. For example, billbugs feign death in response to disturbance, which is thought to be an anti-predator defense (Kindler and Spomer 1986). Further responses to and impacts of predatory arthropods on billbugs have not been documented, but as demand for sustainable turfgrass management increases, conservation biocontrol should be considered.

American toad (*Anaxyrus americanus* Holbrook) and several bird species are also reported billbug predators (Young 2002). Often larger predators like birds become pests themselves as they damage turf while foraging for larvae in the soil. Therefore, predatory arthropods offer better opportunities for pest suppression while maintaining the aesthetics of turf.

Chemical control

Billbugs have historically been managed through use of contact insecticides, such as pyrethroids (e.g., bifenthrin), targeting spring adults emerging from overwintering sites (Watschke et al. 2013). More recently, billbugs have been managed through prophylactic applications of long-residual, systemic insecticides targeting early-instars, against which they are most effective. These preventive insecticides include the neonicotinoids (e.g., clothianidin and imidacloprid) and the anthranilic diamides (e.g., chlorantraniliprole and cyantraniliprole), and are ideally applied approximately a month (neonicotinoids) or more (anthranilic diamides, which are less water soluble) before egg hatch to allow them to be translocated throughout the turf plant before stem-dwelling larvae begin feeding (Potter 1998, Reynolds and Brandenburg 2015). Neonicotinoids also have activity against adults—either by ingestion or contact during foraging and oviposition—and can be applied curatively against adults (Shetlar and Andon 2012).

The existing degree-day model for bluegrass billbug suggests that the latest a contact insecticide against billbug adults is effective is 311-347 DD$_{10^{o}\mathrm{C}}$ (560-624 DD$_{50^{o}\mathrm{F}}$), or at approximately 30% of total adult emergence. Systemic insecticides applied against larvae should be effective from 513-825 DD$_{10^{o}\mathrm{C}}$ (925-1485 DD$_{50^{o}\mathrm{F}}$) (Watschke et al. 2013). Note again that this model may not be applicable to regions beyond the eastern U.S. or to species other than the bluegrass billbug (see billbug captures in the Intermountain West, Fig. 1).

Most work assessing insecticide efficacy against billbugs has been done in cool-season turfgrass with bluegrass billbug and hunting billbug. In field trials, products containing chlorantraniliprole applied preventively against hunting and bluegrass billbugs resulted in 93-100% suppression of larvae and pupae, while bifenthrin provided 82.7% suppression, and imidacloprid provided 62.1-79.4% suppression when compared with controls (Heller et al. 2008a). Furthermore, the preventive application of a combination of bifenthrin+clothianidin against bluegrass and hunting billbugs yielded varied results depending on the rate of application (50.2-83.4% suppression of larvae and pupae when compared with controls) (Heller et al. 2008b). In contrast, Reynolds and Brandenburg (2015) have recently assessed common insecticides against hunting billbug larvae and adults in warm-season turf in greenhouse trials. Bifenthrin, clothianidin, cyantraniliprole, and a combination of bifenthrin+clothianidin all had >80% efficacy against adults while imidacloprid had the greatest efficacy against larvae with just 33.6% mortality. The authors attribute low efficacy of the tested chemicals against larvae to observations that hunting billbug larvae are sometimes found very deep in the soil profile, perhaps beyond the reach of soil insecticides.

Insecticide resistance

The current reliance on prophylactic insecticide applications may be short-lived if insecticide resistance management practices (i.e., IPM and chemical rotations) are not implemented in billbug management plans. Many turfgrass insect pests have evolved resistance to commonly used pyrethroids, including chinch bugs (Hemiptera: Blissidae), fall armyworm (*Spodoptera frugiperda* J.E. Smith), and the annual bluegrass weevil (Silcox and Vittum 2012). Other insect pests have become resistant to the relatively new classes of insecticides that are commonly used against billbugs, including resistance to neonicotinoids in whiteflies (Hemiptera: Alyrodidae), aphids (Hemiptera: Aphididae), houseflies (*Musca domestica* L.), Colorado potato beetle (*Leptinotarsa decemlineata* Say), and codling moth (*Cydia pomonella* L.) (Bass et al. 2015). A substantial portion of resistance issues with neonicotinoids involve imidacloprid (Bass et al. 2015), one of the most widely used active ingredients for billbugs. Additionally, the diamondback moth (*Plutella xylostella* L.) has shown high levels of resistance to chlorantraniliprole, part of the relatively newer class of anthranilic diamides (Teixeira and Andaloro 2013). Although insecticide resistance has not been observed in billbugs since the 70's (bluegrass billbug resistance to the cyclodiene dieldrin; Niemczyk and Frost 1978), it is important to be aware of the potential for resistance because of the limited classes of insecticides available for use in turfgrass and the current reliance on preventive insecticide applications.

In addition to insecticide loss through resistance, neonicotinoids have faced mounting public scrutiny over non-target effects, particularly those on pollinators, and have been recently banned in the European Union (Gross 2013). There have been localized bans elsewhere, including the U.S., where the Environmental Protection Agency is currently assessing the risk of imidacloprid to pollinators to support the review of the registered uses of imidacloprid in the U.S. (Housenger et al. 2016). In turfgrass, flowering weeds can provide a path for neonicotinoid exposure to pollinators (Larson et. al. 2013). Larson et al. (2013) found that mowed clover reduced the effect of neonicotinoids on pollinators compared to unmowed clover, and the authors also found that the anthranilic diamide chlorantraniliprole did not appear to harm pollinators. It is not clear how the availability of neonicotinoids in turfgrass will be affected, but it may become necessary to consider alternative management strategies.

Conclusions

Billbugs remain one of the primary pests of turfgrass in the United States. Chemical control methods for billbugs continue to advance, but as concerns with insecticide resistance and the negative impact of pesticides on the environment, people, and other non-target organisms grow, the demand for alternative management strategies is increasing. Future billbug research should be focused on a path to sustainable management methods, including the development of more robust predictive models, assessment of the effects of existing populations of predatory arthropods, and integration of cultural and biological controls into an IPM approach to billbug management. More broadly, the body of knowledge on billbug biology and management should be expanded from the eastern U.S. to the western U.S., where comparatively little research has been conducted.

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Tables

Table 1. A summary of billbug species found on turf in the U.S., their common host plants, and their geographic distribution, based on literature reports.

Billbug Species	Host Plants	Distribution in the Contiguous U.S.	Sources
Bluegrass billbug (*Sphenophorus parvulus*)	**Cool-season turf** (Kentucky bluegrass, ryegrass, fescues, bentgrass) **Warm-season turf** (Zoysiagrass) **Non-turf** (Orchardgrass, corn, timothy, wheat, quackgrass, barley, rye)	**Northeast** (MA, NJ, NY, OH, PA, VA, WI) **Southeast/Gulf** (FL, NC, SC, TX) **Midwest** (KS, NE, SD) **Intermountain West** (ID, UT) **Northwest** (OR, WA) **Anywhere that Kentucky bluegrass is grown,** most likely throughout the contiguous U.S.	Satterthwait 1931a Tashiro and Personius 1970 Asay et al. 1983 Johnson-Cicalese and Funk 1990 Vittum et al. 1999 Walenta et al. 2004 Huang and Buss 2009 Fry and Cloyd 2011 Kuhn et al. 2013
Hunting billbug (*S. venatus vestitus*)	**Cool-season turf** (Kentucky bluegrass, fescues, perennial ryegrass) **Warm-season turf** (Zoysiagrass, Bermudagrass, St. Augustinegrass, Centipedegrass, Bahiagrass) **Non-turf** (Corn, wheat, sugarcane, yellow nutsedge, orchardgrass, leatherleaf fern, seashore pasalpum)	**Northeast** (NJ, VA) **Southeast/Gulf** (AL, FL, GA, NC, SC, TX) **Midwest** (KS, MO) **Intermountain West** (ID, UT) **Southwest** (CA)	Satterthwait 1931a Johnson-Cicalese and Funk 1990 Vittum et al. 1999 Huang and Buss 2009 Doskocil and Brandenburg 2012 Kuhn et al. 2013 Chong 2015
Rocky Mountain billbug (*S. cicatristriatus*)	**Cool-season turf** (Kentucky bluegrass, perennial ryegrass)	**Midwest** (ND, NE, SD) **Intermountain West** (CO, ID, UT, WY) **Southwest** (NM) **Northwest** (OR)	Vittum et al. 1999 Niemczyk and Shetlar 2000 Walenta et al. 2004
Phoenix billbug (*S. phoeniciencis*)	**Warm-season turf** (Bermudagrass, zoysiagrass, kikuyugrass) **Non-turf** (Johnson grass, oats)	**Intermountain West** (ID, UT) **Southwest** (AZ, CA, NM)	Satterthwait 1931a Vittum et al. 1999 Fritz and Salaiz 2007 Sutherland 2006 Flint et al. 2009
Uneven billbug (*S. inaequalis*)	**Cool-season turf** (Kentucky bluegrass, tall fescue, perennial ryegrass) **Warm-season turf** (Bermudagrass, zoysiagrass)	**Northwest** (NJ) **Southeast** (FL, NC, SC)	Johnson-Cicalese and Funk 1990 Johnson-Cicalese et al. 1990 Vittum et al. 1999 Huang and Buss 2009 Doskocil and Brandenburg 2012 Chong 2015

Lesser billbug (*S. minimus*)	**Cool-season turf** (Kentucky bluegrass, fescues, ryegrass) **Non-turf** (Rice, timothy, wheat, rye)	**Northeast** (NJ, NY, OH, PN) **Southeast** (FL, NC, SC)	Satterthwait 1931a Johnson-Cicalese and Funk 1990 Vittum et al. 1999 Huang and Buss 2009 Chong 2015 Doskocil and Brandenburg 2012
Southern corn billbug (*S. callosus*)	**Warm-season turf** (Bermudagrass) **Non-turf** (Corn, yellow nutsedge, orchardgrass)	**Southeast** (NC, VA)	Doskocil and Brandenburg 2012 Kuhn et al. 2013
S. apicalis	**Warm-season turf** (Bermudagrass, zoysiagrass)	**Northeast** (NJ) **Southeast/Gulf** (FL)	Vaurie 1951 Vittum et al. 1999 Huang and Buss 2009
S. coesifrons	**Warm-season turf** (Bahiagrass) **Non-turf** (Nutsedge)	**Southeast** (FL, GA, SC)	Vaurie 1951 Morrill and Suber 1976 Huang and Buss 2009 Chong 2015
S. rectus	**Cool-season turf** (Kentucky bluegrass)	**Southeast** (NC)	Doskocil and Brandenburg 2012
S. cariosus	**Warm-season turf** (Bahiagrass) **Non-turf** (Nutsedge)	**Southeast** (SC)	Chong 2015

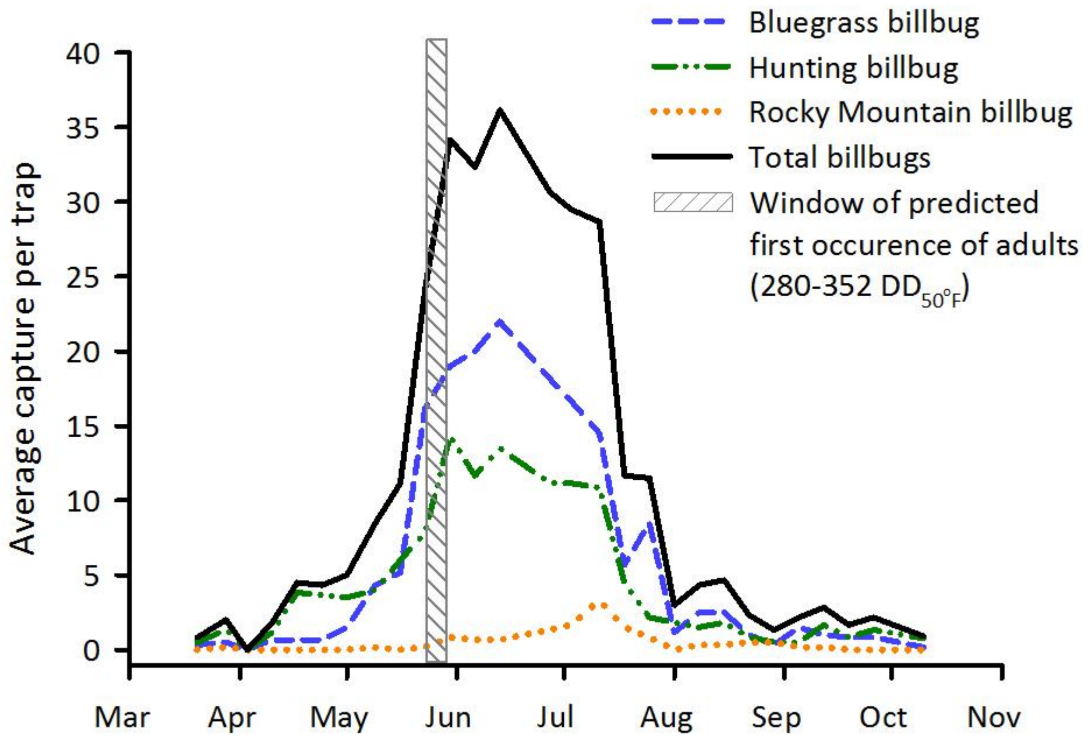

Fig. 1. Adult billbug captures from six linear pitfall traps at an infested golf course in the Intermountain West in 2014. Bluegrass billbug is the dominant species, followed by hunting billbug and Rocky Mountain billbug. Degree-days were calculated using a nearby weather station and the available bluegrass billbug model from the east (Watschke et al. 2013). First adult occurrence is apparently earlier in the Intermountain West than is predicted for the eastern U.S. (280-352 DD_{50}, which was between May 23 and May 28).

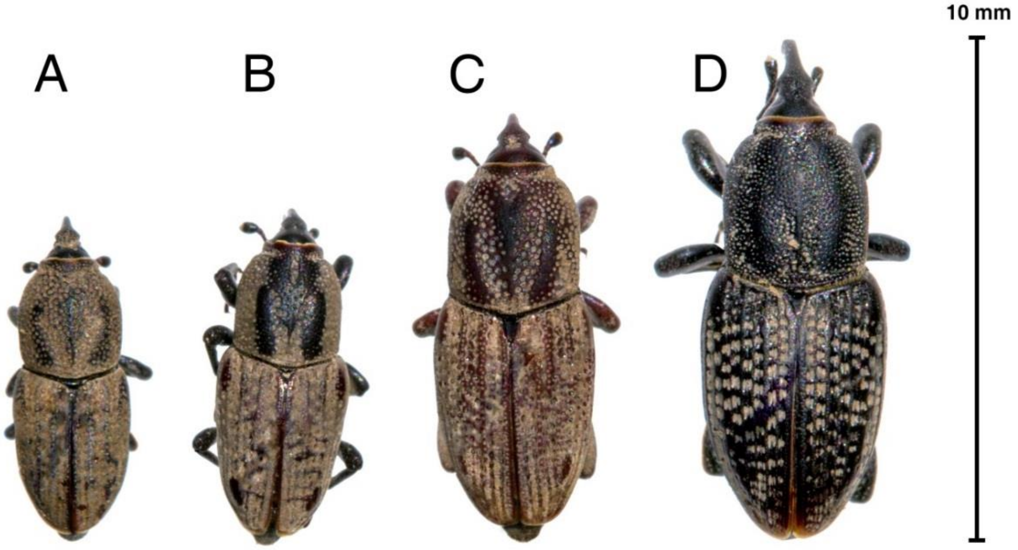

Fig. 2. Commonly occurring species of billbug adults in the western United States can be easily distinguished by markings on the elytra and thorax and relative sizes. (A) Bluegrass billbug (*Sphenophorus parvulus*) has even dimples covering the thorax and is approximately 5-7 mm (0.20-0.28 in) in length; (B) Phoenix billbug (*S. phoeniciensis*) has a raised, smooth M-shape on the thorax and is approximately 6-8 mm (0.24-0.31 in) in length; (C) hunting billbug (*S. venatus vestitus*) has a raised, smooth marking resembling a "Y" in parentheses on the thorax and is approximately 7-9 mm (0.28-0.35 in) in length; (D) Rocky Mountain billbug (*S. cicatristriatus*) has small, even dimples on the thorax and deep, heart-shaped or hoof-shaped punctures on the elytra and is approximately 10-12 mm (0.40-0.47 in) in length. Photo credit: James Bradford.

Fig. 3. Adult female billbugs chew notches in grass stems and lay one to three eggs in the chamber. Photo credit: Madeleine Dupuy, Utah State University.

Fig. 4. Billbugs have an egg stage (left), five larval stages (middle), and a pupal stage (right) before maturing as adults. Photo credit: Madeleine Dupuy, Utah State University.

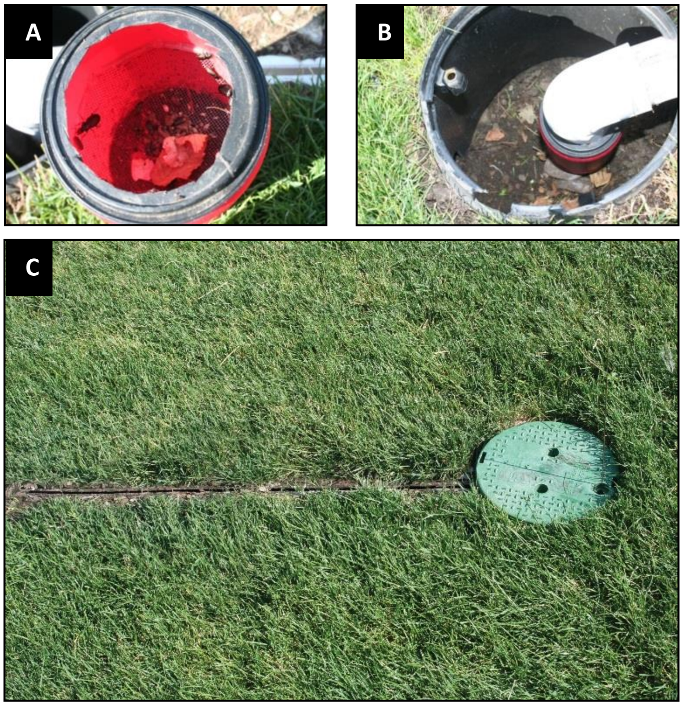

Fig. 5. A linear pitfall trap (similar to Lawrence 1982) is a useful tool for monitoring ground-active adult billbugs. The trap consists of (A) a collection vessel, here made from a recycled coffee container, with a hole cut in the lid for attachment to the end of the PVC pipe. Modifications to the collection vessel can be made including drilling small holes into the bottom for drainage and attaching mesh midway with adhesive to reduce moisture contact with captured insects. (B) The collection vessel attaches to an elbowed end of the pitfall PVC pipe and is housed within an irrigation box. The entire trap (C) consists of a 5.08 cm (2 in) diameter, 1 m (3.28 ft) long PVC pipe with a 1 cm (0.393 in) slit running the length of the pipe. The pipe is dug into the ground with the slit facing upward so that the slit is flush with the surface of the ground. The other end of the PVC pipe is capped. Photo credits: Madeleine Dupuy, Utah State University.

Fig 6. Stems of turfgrass in a heavily billbug-damaged area have broken away easily during a “tug test” (A) to reveal later instars that have dropped into the soil to feed on the roots and crown of the turfgrass (B). Photo credits: Lori Spears, Utah State University.

Research Objectives

Billbugs are a cosmopolitan pest of turfgrass throughout the United States in both cool and warm season turfgrass. However, billbug research has primarily been focused in the eastern United States, and research from the Intermountain West, where billbugs are an equally damaging pest, is lacking. Further, billbug management has primarily relied on preventive applications of synthetic insecticides, and integrated management methods that are effective and sustainable have not been developed. To fill gaps in the knowledge on billbugs in the western United States, I conducted field and laboratory experiments to examine:

1. the seasonal activity of billbug life stages in Intermountain West turfgrass and the development of a predictive degree-day model to assist in management application timing based on billbug seasonal activity (see Chapter II; This is a pre-copyedited, author-produced version of an article accepted for publication in the *Journal of Economic Entomology* following peer review. The version of record Dupuy, M.M., J.A. Powell, and R.A. Ramirez. 2017. Developing a degree-day model to predict billbug (Coleoptera: Curculionidae) seasonal activity in Utah and Idaho turfgrass. Journal of Economic Entomology 110: 2180-2189 is available online at: https://doi.org/10.1093/jee/tox210);
2. the efficacy of conventional and biological insecticide applications timed with degree-day model predictions developed in Chapter II (see Chapter III; formatted according to guidelines for the journal *Crop, Forage, and Turfgrass Management*);

3. and the potential impact of conservation biological control of generalist predatory arthropods on billbug populations (see Chapter IV; formatted according to guidelines for the journal *Biological Control*).

Chapter I was a literature review of billbugs. This is a pre-copyedited, author-produced version of an article accepted for publication in the *Journal of Integrated Pest Management* following peer review. The version of record Dupuy, M.D. and R. A. Ramirez. 2016. Biology and management of billbugs (Coleoptera: Curculionidae) in turfgrass. Journal of Integrated Pest Management 7: 1-10 is available online at: https://doi.org/10.1093/jipm/pmw004.

CHAPTER II

DEVELOPING A DEGREE-DAY MODEL TO PREDCT BILLBUG (COLEOPTERA: CURCULIONIDAE) SEASONAL ACTIVITY IN UTAH AND IDAHO TURFGRASS[2]

Abstract

Billbugs are native pests of turfgrass throughout North America, primarily managed with preventive, calendar-based insecticide applications. An existing degree-day model (lower development threshold of 10°C, biofix 1 March) developed in the eastern U.S. for bluegrass billbug, *Sphenophorus parvulus* (Gyllenhal), may not accurately predict adult billbug activity in the western U.S., where billbugs occur as a species complex. The objectives of this study were 1) to track billbug phenology and species composition in managed Utah and Idaho turfgrass, and 2) to evaluate model parameters that best predict billbug activity, including those of the existing bluegrass billbug model. Tracking billbugs with linear pitfall traps at two sites each in Utah and Idaho, we confirmed a complex of three univoltine species damaging turfgrass consisting of (in descending order of abundance) bluegrass billbug, hunting billbug (*S. venatus vestitus* Chittenden), and Rocky Mountain billbug (*S. cicatristriatus* Fabraeus). This complex was active from February through mid-October, with peak activity in mid-June. Based on linear regression analysis, we found that the existing bluegrass billbug model was not robust in predicting billbug activity in Utah and Idaho. Instead, the model that best predicts adult activity of the billbug complex accumulates degree-days above 3°C

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[2] Dupuy, Madeleine M., Powell, James A., and Ricardo A. Ramirez

after 13 January. This model predicts adult activity levels important for management within 11 days of observed activity at 77% of sites. In conjunction with outreach and cooperative networking, this predictive degree-day model may assist end-users to better time monitoring efforts and insecticide applications against billbug pests in Utah and Idaho by predicting adult activity.

Key words: phenology, integrated pest management, bluegrass billbug, hunting billbug, Rocky Mountain billbug

Billbugs (Coleoptera: Curculionidae: *Sphenophorus* spp.) are turfgrass pests throughout North America (Dupuy and Ramirez 2016). In the United States, there are at least ten species of billbugs damaging turfgrass (Held and Potter 2012), and they are univoltine in most northern and western parts of the country (Kindler and Spomer 1986, Rondon and Walenta 2008), though partial second generations are common (Johnson-Cicalese et al. 1990, Richmond and Duffy 2015). As adults emerge from overwintering sites in spring, females mate and lay eggs in the stems of turfgrass, where they hatch and larvae feed until they are too large to remain in the stem. Larvae then drop to the soil where they continue feeding on the roots and crown of the plant (Johnson-Cicalese et al. 1990). Feeding by larvae results in expanding, yellow-brown patches of turf and eventual plant death (Dupuy and Ramirez 2016). Billbugs are especially problematic in Utah and Idaho, because their damage can be exacerbated by drought stress (Dupuy and Ramirez 2016). Most billbug research to-date has been conducted in the eastern United States, while very little is known about billbugs in the western United States.

Univoltine billbugs have traditionally been managed by preventive, early-spring applications of surface insecticides (e.g., pyrethroids) targeting adults emerging from overwintering sites, but because residual activity of these insecticides is brief (7-10 days) and subsequent billbug life stages are protected within stems (eggs, early instars) or in soil (later instars), timing is critical (Shetlar and Andon 2012). Another billbug management strategy involves preventive applications of systemic, long-residual insecticides targeting early instars feeding in stems (Watschke et al. 2013, Richmond 2015). The cost of insecticides for billbug management ranges from $12 to $114 per acre, and often, follow-up applications are required to achieve desired billbug suppression (Dupuy and Ramirez 2016, P. Stokes, personal communication). Systemic insecticides, such as the neonicotinoids and anthranilic diamides, are most effective against early instars, but they may also have activity against adults by direct contact and ingestion (Shetlar and Andon 2012, Watschke et al. 2013, Richmond 2015, Reynolds and Brandenburg 2015). Thus, early (spring) applications of systemic insecticides may suppress both adults and larvae (Richmond 2015), while surface insecticides applied at this time suppress adults (Watschke et al. 2013). One suggested strategy is that 30% of cumulative adult billbug emergence is the latest a preventive application will be effective (Watschke et al. 2013). There is also evidence that later (summer) applications of neonicotinoids can effectively suppress billbug activity (Baxendale et al. 1999, Pierson et al. 2008, Doskocil et al. 2012). Reynolds and Brandenburg (2015) suggest a second strategy that insecticide applications for hunting billbug occur at peak adult activity, or 50% of cumulative adult billbug activity.

Management decisions for turf pests tend to be based on past experience and judgement rather than specific action thresholds because traditional economic metrics of field crops (e.g., yield loss) do not translate readily to the turfgrass system (Held and Potter 2012). Preventive applications of systemic insecticides are applied regardless of whether a pest reaches an economically or aesthetically damaging level, an approach that is inherently not aligned with the goals of IPM (McCarty and Elliot 1994). Since strategies for billbug management are available in the literature, monitoring is important for preventive and curative applications because dates of first emergence from overwintering sites, mating, oviposition, egg hatch, and damage may occur at different times every year due to differences in weather patterns (McMaster and Wilhelm 1997). By predicting when certain life stages will be present based on heat unit accumulation, degree-day models provide a means to time monitoring more effectively (Brandenburg 2004) and to avoid calendar-based spraying and inefficient insecticide use (e.g., applications that are too early or too late and miss susceptible life-stages).

The degree-day approach relies on the assumption that insect development is directly related to ambient temperature and that higher temperatures result in increased growth rates, up to a certain temperature threshold (Higley et al. 1986). Heat units accumulate from a biofix—a starting date or biological event, such as first flight—every 24 h that the temperature is above a pre-determined lower development threshold (LDT; a temperature below which the insect does not develop) for a particular species (Higley et al. 1986). To develop a predictive model for stage-specific activity, observed pest seasonal activity and degree-day accumulations are matched (Bechinski et al. 1990, Knutson and Muegge 2010).

Degree-day models have been developed for several turf pests, including, but not limited to, black turfgrass ataenius (*Ataenius spretulus* Haldeman) (Wegner and Niemczyk 1981), masked chafer (*Cyclocephala pasadenae* Casey) (Blanco and Hernandez 2006), and more recently, annual bluegrass weevil (*Listronotus maculicolis* Dietz) (Syngenta 2015a). End-users (e.g., golf course superintendents and other turfgrass professionals) can access the annual bluegrass weevil model through an online program called WeevilTrak, which allows independent researchers and golf course superintendents throughout the eastern U.S. to track degree-days at different sites and share monitoring and treatment data(Syngenta 2015a). Currently, more than 2,300 golf courses are signed up for WeevilTrak (M. LaFleur, personal communication). This type of region-wide validation and cooperative networking is essential for getting degree-day models to end-users (Gelernter 1995).

A degree-day model for bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) was developed in Ohio using the average method of calculation, a 1 March biofix, and a LDT of 10°C (hereafter referred to as the "Ohio model") (Watschke et al. 2013). This model and recommendations based on degree-day accumulations are available to turf managers online, where 343 users are currently signed up to receive billbug alerts (MSU 2017, K. Frank, personal communication). The parameters used for the Ohio model may be applicable in other temperate regions, however, many factors differ in the northern Intermountain West (including Utah and Idaho) including elevation (e.g., Columbus, Ohio 275 m; Boise, Idaho 832 m; Logan, Utah 1382 m) and annual precipitation (e.g., 99.0 cm, 47.3 cm, and 50.1 cm, respectively) (NCEI 2011). Geographic location, in particular, can affect many insect life history traits, such as developmental rate, critical photoperiod, and diapause intensity (Masaki 1972, 1979, Danilevsky 1965, Bradshaw and Lounibos 1977). For these reasons, models for particular pests are commonly adapted and validated for different geographic locations. For example, Knight (2007) adapted a codling moth model for eastern Washington originally developed in Michigan (Brunner et al. 1982). Given these factors that may affect the predictive ability of the Ohio model in Utah and Idaho and the lack of basic knowledge on billbugs in the western United

States, we tracked billbug species composition and phenology and validated a degree-day model for billbugs in Utah and Idaho to improve timing of monitoring and insecticide applications. The objectives of this study were 1) to determine the phenology and species composition of billbugs in managed Utah and Idaho turfgrass, and 2) to evaluate model parameters (biofix and LDT), including those of the Ohio model, that best predict billbug activity in Utah and Idaho.

Materials and Methods

Study location. We surveyed four golf courses in Utah and Idaho with known billbug infestations. These sites were Logan Golf and Country Club in Logan, UT (subsequently referred to as “Logan”; est. 1931; 41.7447, -111.7890), South Mountain Golf Course in Draper, UT (“Draper”; est. 1998; 40.5010, -111.8500), Crane Creek Country Club in Boise, ID (“Boise”; est. 1963; 43.6514, -116.1883), and SpurWing Country Club in Meridian, ID (“Meridian”; est. 1995; 43.6680, -116.4286). The roughs of all courses had established Kentucky bluegrass (*Poa pratensis* L.) and were sprinkler irrigated. The predominant soil texture in roughs at all courses was a silty loam. During the study, private country clubs (Logan, Boise, and Meridian) spot-treated with applications of clothianidin or imidacloprid (Arena 50 WDG at 8oz per acre and Criterion 2F at 20 oz per acre, respectively) once or twice per year, targeted at early instars. Draper was a public course that did not apply insecticides for insect pests throughout the entire survey due to a minimal budget for pest management.

Billbug phenology and species composition. *Adults.* To sample for ground-active adult billbugs, we placed six linear pitfall traps composed of a 1m length of PVC pipe with one end connected to a collection cup and housed within an irrigation box (as described in Dupuy and Ramirez 2016) in roughs where billbug damage was observed at each course (24 traps total with six traps at each of four courses). Traps were placed no closer than 100 m apart. Logan was sampled weekly from 13 May-15 October 2013, 20 March-10 October 2014, and 25 February-23 October 2015. Meridian and Boise were sampled weekly from 15 May-24 October 2013 and 26 April-27 September 2014. Draper was added in 2014 as an additional Utah site, providing two course sites each in Utah and Idaho, and was sampled weekly from 29 March-9 October 2014 and biweekly from 17 April-17 October 2015. This sampling scheme provided nine independent site-years of adult billbug activity data. Adults were collected and brought back to the lab for identification following Johnson-Cicalese et al. (1990).

Immature stages. We sampled for immature billbug life stages using a 10.5 cm diameter golf course cup cutter (Lever Action Hole Cutter, Par Aide Products Co., Lino Lakes, MN) to take soil core samples to a depth of approximately 15 cm. This depth captured both larvae in the thatch and upper soil layers and pupae, which are often found deeper in the soil (Shetlar et al. 2012). Ten soil core samples were taken every other week at each course in areas of observed billbug damage, within a 50 m radius of each pitfall trap. Immature-stage sampling occurred throughout the duration of pitfall trap sampling for adults at each site. Soil from the samples was broken up by hand in the laboratory to search for soil-dwelling later instars and pupae. All grass stems from the samples were dissected individually with a fine blade under a stereomicroscope (Leica MZ6/M60, Leica Microsystems, Buffalo Grove, IL) to search for eggs and early instars within stems.

Immature stages were not identified to species because no external morphological features are currently known to distinguish species from one another (Johnson-Cicalese et al. 1990). Therefore, individuals from these samples were combined within stage-categories to describe the general presence of each life stage (eggs, early instars in stems, late instars in soil, and pupae).

Degree-day model development. Field collections at Logan in 2014 and 2015 provided the best approximation of 100% of billbug adult seasonal activity out of any of the sites because samples captured the earliest (20 March and 25 February) and latest activity (10 October and 23 October). Consequently, Logan data from 2014 and 2015 were combined and used for model development, while sampling data from all other sites and years were used for validation. Survey data of adult billbug activity were used for model development because effective methods of rearing billbugs under laboratory conditions to determine temperature development thresholds have not been established (Johnson-Cicalese and Funk 1990, Rondon and Walenta 2008). Though larvae are the damaging stage, adults were used to develop the model because they were more abundant and easy to monitor compared to larvae, which require time-consuming and destructive monitoring methods to locate (i.e., soil cores). Additionally, we did not observe any reduction in adult activity after insecticide applications were made against larvae (4 and 24 July 2014, 8 June and 8 July 2015 at Logan; Fig. 1B-C). Bluegrass, hunting (*S. venatus vestitus* Chittenden), and Rocky Mountain (*S. cicatristriatus* Fabraeus) billbug adults co-existed in Utah and Idaho, and therefore model development involved the activity of all 3 species combined.

Ambient air temperature data were gathered from weather stations located on each golf course, as only one weather station provided soil temperature data (Boise). Air temperature data are more relevant in predicting adult stages, which live aboveground, and are more readily available to turf managers, which may allow for widespread implementation of the model (Ahmad 1979). The Draper weather station failed to log data from August 2014 through April 2015. We filled in missing weather data from a nearby station in Murray, UT, (40.6313, -111.9200) that was on the same side of the Salt Lake Valley (east) and at a similar elevation to the Draper site.

Degree-day calculations. We calculated degree-day accumulations for Logan in 2014 and 2015 using the Ohio model parameters (average calculation method, a 1 March biofix, and a 10^{o}C LDT). We calculated degree-days in Microsoft Excel (2016, Microsoft Corp., Redmond, WA) with the formula

$$DD = \sum max \left[\frac{(T_{max}+T_{min})}{2}\right] - LDT, 0,$$

where summation is the sum of degree days beginning at the biofix and ending at the date of last billbug collections (23 October), T_{max} is the maximum ambient air temperature (^{o}C), and T_{min} is the minimum ambient air temperature (^{o}C) over a 24-hour period (McMaster and Wilhelm 1997).

In addition to degree-days calculated using the Ohio model parameters, we tested twelve unique parameter (biofix/LDT) combinations to determine the best-fit model for Utah and Idaho. We evaluated three biofixes including 1 March (standard), 1 February, and 13 January. The early biofix dates were chosen because the earliest recorded activity began 25 February and the earliest temperature data log occurred at the Logan weather station on 13 January 2014. The four tested LDTs were 3, 5, 7, and 10^{o}C. We chose these particular biofixes and LDTs because Utah and Idaho billbugs were active when ambient air temperatures were below 10^{o}C. There is support in the literature for using these methods of testing various biofix dates and lower development thresholds when an empirical biofix or LDT from lab trials is absent (Umble and Fisher 2000, Naves and de Sousa 2009, Akotsen-Mensah et al. 2011, Doddala et al. 2013, Hanson et al. 2015). Though many degree-day models also include an upper development threshold (UDT), a temperature above which insect development plateaus or stops, we chose not to include this parameter for several reasons. The Ohio model does not include an UDT, and effective methods for rearing billbugs under lab conditions to establish empirical development thresholds have not been developed (Johnson-Cicalese and Funk 1990, Rondon and Walenta 2008). Lastly, models with fewer parameters are more likely to be adopted for use (Pruess 1983).

We used the DegDay program (v. 1.01, Snyder 2005; Excel v. 2016, Microsoft Corp., Redmond, WA) single-sine method to calculate degree-day accumulations for each biofix/LDT parameter combination with daily high and low ambient air temperatures at the Logan site in 2014 and 2015. The single-sine method assumes that the temperature curve is symmetric around the maximum air temperature and that the variation in daily temperatures follows a sine function closely. Estimation of area under the curve (and above the LDT) using sine waves offer a better approximation of the curvilinear behavior of temperature than the average method, which estimates the area using rectangles (Allen 1976, Roltsch et al. 1999, Caicedo et al. 2012). We chose the single-sine method because it provides more accurate estimates of degree-days and it is the best method for spring accumulations (Pruess 1983), the critical period during which adult billbugs are active and preventive billbug treatment is applied (Reynolds and Brandenburg 2015, Dupuy and Ramirez 2016).

Choosing a best-fit model. To predict adult billbugs with each parameter combination, we matched respective degree-days with the calendar date on which pitfall samples were collected to determine the number of active adult billbugs over a range of corresponding degree-day values. Pitfall trap captures showed a skewed-right activity distribution over the course of a full season (Fig. 1A-I). When plotted on logarithmic scales, the skewness was removed, suggesting use of a log-normal distribution, which we later confirmed using formal regression techniques.

Adult billbug activity over the course of a season was predicted using $\textit{Predicted billbugs} = \textit{Total billbugs} \times F_x(z)$, where *Total billbugs* was the total number of adult billbugs collected from pitfall traps at Logan in 2014 and 2015 (2,979 billbugs), and $F_x(z)$ was the standard normal cumulative distribution function calculated by the "NORMSDIST" function (Excel v. 2016, Microsoft Corp. Redmond, WA). "NORMSDIST" returns the probability that the observed value of a standard normal random variable will be less than or equal to z, where

$$z = \left[\frac{(log_{10}(DD_x)\text{-}log_{10}(MDD))}{log_{10}(SD)}\right].$$

Here DD_x = a specific degree-day at which billbugs were collected, *MDD* = the median degree-day by which 50% of billbugs were active, and *SD* = standard deviation of degree-days. Calculating the standard normal cumulative distribution of z predicted a proportion of the total number of billbugs that were active by DD_x. Multiplying $F_x(z)$ by *Total billbugs* (2,979 billbugs) resulted in a predicted number of active billbugs by DD_x.

We chose the best fit model out of the 12 unique single-sine biofix and LDT combinations plus the Ohio model by selecting the combination with the smallest sum squared error between billbugs observed in the field and billbugs predicted by the model (Smith and Rose 1995),

$$\text{SSE}= \sum(\textit{Predicted billbugs}\text{-}\textit{Observed billbugs})^2.$$

A smaller SSE value indicates less departure of predictions from observations.

Degree-day model validation. We validated the model with the smallest SSE (best fit model) by using corresponding degree-day accumulations to calculate predicted billbugs for seven independent site-years (Logan 2013, Draper 2014 and 2015, Boise and Meridian 2013 and 2014) plus the two individual site-years used in model development (Logan 2014 and 2015; as opposed to the combined data used to develop the model). This provided nine validation datasets. This validation process using data from both the model development site and independent sites was similar to methods described by Akotsen-Mensah et al. (2011) and Bechinski et al. (1990). We also used the Ohio model to calculate degree-days and predicted adult billbug activity for the nine validation datasets, to determine whether the Ohio model was valid in Utah and Idaho.

Using MDD and SD derived from model development data, we repeated the above-described process for obtaining predicted billbugs using adult collection data from Draper in 2014 and for individual datasets from Logan in 2014 and 2015 (complete datasets, beginning collections in February or March). For datasets that did not account for 100% of billbug activity (“incomplete” datasets began collections later than March; Logan 2013, Draper 2015, Boise and Meridian 2013 and 2014), the process had to be adjusted because *Total billbugs* in the model development formula must account for billbug activity over an entire season (February or March through October). Thus, we prorated predictions using the proportional amount of activity observed. Specifically, we predicted a number of billbugs for each incomplete dataset using the equation $\mathit{Predicted\ billbugs} = [\mathit{total\ billbugs} \times w] / T$. Here, *total billbugs* was the total number of billbugs collected at a particular site and year, and $w = [F_x(z)]_a - [F_x(z)]_b$, or the difference between $F_x(z)$ at degree-day *a* and $F_x(z)$ at the degree-day corresponding to the previous collection week, degree-day *b*, which gives a proportion of predicted activity over the course of a week. *T* was the sum of all *w* over the (incomplete) collection period. In other words, *T* was a predicted proportion of billbug activity captured over the course of collections (e.g., we began collections 26 April at Boise in 2014, and T=0.91. Thus, the model predicted that we captured 91% of total billbug activity for this site and year.). $F_x(z)$ and *z* were as previously described, using MDD and SD from the best fit model or the Ohio model. The original collection data from the Ohio model were not available (Watschke et al. 2013), so here, MDD and SD were derived from our collection data, but using the Ohio model’s parameters (1 March biofix, 10^{o}C LDT, and average method calculation).

We used simple linear regression (PROC REG, SAS Studio University Edition 9.4) to obtain the coefficient of determination between observed and predicted billbugs to quantify and standardize goodness-of-fit among the validation datasets (Smith and Rose

1995). A coefficient of determination close to one indicates a strong relationship between observed and predicted billbug activity values.

Validating management strategies. Although our model was based on adult activity, billbug management strategies target both adults and larvae. The literature provides recommended strategies for timing insecticide treatments against both life stages at 30% and 50% of adult billbug activity (Watschke et al. 2013, Richmond 2015, Reynolds and Brandenburg 2015). Therefore, we used 30% and 50% of adult billbug activity to test how well the best-fit model predicts potential management timing. For each validation dataset we determined when 30% of activity was predicted by the model as an accumulation of days beginning 1 January (predicted). Then we determined what day 30% of activity was observed based on billbug collections at each site (observed). For those datasets that began later than February or March and thus did not capture all early season activity, we incorporated a correction factor based on the model-predicted proportion of missing data (i.e., in the *Predicted billbugs* formula for model validation, if $T = 0.91$, the model predicts that 9% of billbug activity is missing from our observations). Thus, we added a predicted number of missing billbugs to the total number of observed billbugs. From the data with the correction factor, we calculated the number of billbugs that constituted 30% of activity for each dataset over the season, representing an observation. We then compared the difference in days between predictions and observations at each site. The process was repeated for 50% billbug activity predictions.

Additional analyses. Managers may be more likely to adopt an average method model, given the simplicity of degree-day calculations without a complicated formula or special program (Pruess 1983). Thus, we calculated degree-days with the average method for the biofix and LDT of the chosen best-fit model. Management strategy predictions based on the single-sine method of calculation were assessed for goodness-of-fit when degree-days are calculated with the average method. The management strategy validation process was repeated with average method degree-days.

The Ohio model was developed for bluegrass billbug, but Utah and Idaho have multiple co-occurring species that may contribute to the Ohio model being unreliable. We conducted a separate analysis to evaluate the ability of both the Ohio and the best-fit model to predict bluegrass billbug activity in Utah and Idaho. We calculated new MDD and SD for each model based on bluegrass billbug collections at Logan in 2014 and 2015 (model development datasets), and used the bluegrass-only MDD and SD to predict bluegrass billbug activity. We then compared predicted bluegrass billbug activity to observed bluegrass billbug activity using SSE.

Results and Discussion

Billbug phenology and species composition. ***Adults.*** There was a complex of three billbug species co-occurring in Utah and Idaho turfgrass: bluegrass billbug (58.1% of total capture across all years and sites), hunting billbug (34.3%), and Rocky Mountain billbug (7.6%) (Table 1). Bluegrass billbug was the most captured species in 66% of site-years (Table 1), excluding Draper in 2015 and Meridian in 2013 and 2014, where hunting billbug was more abundant (Fig. 1 E, H, and I). Adults were present in pitfall traps as early as 25 February (1 bluegrass billbug total at Logan 2015; Fig. 1 C). By mid-March, 1-4 adults per trap at Logan 2014 and 2015 were consistently being captured (Fig. 1 B-

C). Activity increased through mid-June, when it peaked (6-50 billbugs per trap, representing the lowest and highest peaks at Meridian in 2014, the site-year with the least billbug activity, and Logan in 2013, the site-year with the most billbug activity, respectively) and then decreased and remained low from August until late October (<2 billbugs per trap). Rocky Mountain billbug populations were consistently low throughout the season (<1 billbug per trap, on average; Fig. 1 A-I), suggesting that bluegrass and hunting billbugs may be the most damaging species, based on their relative abundance in collections. Abiotic factors, such as cold, rainy weather, appeared to impact adult billbug activity, as indicated by low collection numbers at Logan on 20 June, 2014, when 1.75 cm of precipitation fell and average air temperatures ranged from 7.6-14.9^{o}C in the week prior to collection (Fig. 1B). The species complex in Utah and Idaho is similar to complexes in other areas of the western and northeastern U.S. in which both bluegrass and hunting billbug are present (Johnson-Cicalese et al. 1990, Walenta et al. 2004, Sutherland 2006). In Utah and Idaho, rank abundance of bluegrass or hunting billbug varied by site and year.

Immature stages. Immature billbugs were less abundant in our surveys than adults. The majority of eggs were found in May and June (0.6-1.3 eggs per sample; Fig. 2A), when adults were most active in our surveys (Fig. 1A-I). Early instars in stems were most common in mid-May through mid-June (up to 0.5 larvae per sample; Fig. 2D), while larvae in the soil were prevalent from mid-June through September (0.4-0.7 larvae per sample; Fig. 2A-B). The presence of large larvae in the soil both late and early in the season indicates a potential partial second generation, where later instars of some species may overwinter in the soil (Fig. 2A-C). Partial second generations have been reported for billbugs in Indiana and North Carolina (Doskocil et al. 2012, Richmond and Duffy 2015) and are suspected in New Jersey and Ohio (Johnson-Cicalese et al. 1990), however more research is needed to confirm the presence of overwintering larvae in Utah and Idaho.

Doskocil and Brandenburg (2012) report that most hunting billbug larvae are found 5-10 cm beneath the soil surface in North Carolina clay loams and fine sandy loams. While we also found larvae at this sampling depth, Reynolds and Brandenburg (2015) reported that hunting billbug larvae may be found up to 23 cm beneath the soil surface, though no data are provided on proportions of larvae than can be found at this depth. Although this may be a factor contributing to low recovery of larvae, we are confident that our sampling depth of 15 cm captured the majority of larvae because it is consistent with other reported depths at which billbug larvae can be found (Shetlar et al. 2012).

Degree-day model development. *Choosing a best-fit model.* Use of a logarithmic transformation to degree-days in calculations was supported by a regression of observed $\log_{10}$ degree-days (calculated with the best-fit model) pertaining to observed billbug activity against a simulated set of normal $\log_{10}$ degree-days (DATA step, $\mu=2.931$, $\sigma=0.2346$; PROC REG, $r^2= 0.95$ SAS Studio University Edition 9.4).

Sum squared error values (SSE) for each of the twelve biofix/LDT combinations plus the Ohio model parameters indicate that the combination with the strongest agreement between observed and predicted billbugs was a biofix of 13 January and an LDT of 3^{o}C (SSE=565,192; Table 2). The best-fit model is hereafter referred to as the

Utah-Idaho model. The Ohio model did not predict billbug activity as well as the Utah-Idaho model (Ohio SSE (896,785)>Utah-Idaho SSE (565,192); Table 2). Sum squared error increased with later biofixes and warmer LDTs, supporting a model with an early biofix and cooler LDT as the best-fit model for Utah and Idaho (e.g., single-sine model with 1 March biofix and 10°C LDT, SSE = 4,006,186; Table 2).

Degree-day model validation. The Utah-Idaho model was able to predict billbug activity with $r^2 \geq 0.70$ for eight of the nine validation datasets and showed better predictive accuracy than the Ohio model five of nine times (Table 3). In the other four instances, it showed similar predictive accuracy to the Ohio model (Table 3). Specifically, the Ohio model showed poor predictive accuracy ($r^2<0.70$) in Logan 2013 ($r^2=0.24$), Boise 2013 ($r^2=0.47$), and Meridian 2014 ($r^2=0.66$), indicating that the Ohio model was not robust in predicting adult billbug activity at all sites in Utah and Idaho. The Utah-Idaho model was a better predictor of billbug activity at these sites ($r^2= 0.82$, 0.70, and 0.70 for Logan 2013, Boise 2013, and Meridian 2014, respectively) and was more consistent and robust in predicting billbugs in Utah and Idaho (Table 3).

The weak predictive ability of both models at Draper in 2015 (Utah-Idaho $r^2=0.18$; Ohio $r^2= 0.26$) may have resulted from a change in sampling. In an attempt to comply with a restricted schedule at the Draper course, we collected pitfall trap samples every other week at Draper in 2015 instead of weekly as at all other sites and years. Assigning two weeks' worth of billbug activity to one degree-day value may skew the activity distribution such that it may no longer satisfy the log-normal distribution of the model.

Validating management strategies. The Utah-Idaho model predicted 30% of adult billbug activity to occur at 548±1 $DD_{3^{o}C}$, while peak activity (50% adult activity) occurred at 796±1 $DD_{3^{o}C}$. Previous work finds degree-day predictions to be acceptable when deviation from observations is within a 5-10% range (18-37 days) (Higley et al. 1986, Naves and de Sousa 2009). However, we find a deviation of <3% (<11 days) to be more acceptable for billbug management. The deviation of all predictions by the Utah-Idaho model were within 0-10% of observations (average error of 10 days) for the 30% of adult activity strategy (Fig. 3A). The deviation of seven of the nine predictions (including Logan 2013, 2014, and 2015, and Boise, and Meridian 2013 and 2014) ranged within 0-2% of observations (average error of 4 days). For the 50% adult activity strategy, the deviation of all predictions were within 0-9% of observations (average error of 8.11 days; Figure 4B). Predictions for the same seven sites described above were within 0-2% of observations (average error of 3.43 days). The majority of this error occurs when model predictions for billbug activity were earlier than observed billbug activity (Fig. 3A-B). Errors in this direction are compatible with billbug management strategies using long-residual, systemic insecticides, since these products can last for several weeks up to a few months, depending on the active ingredient and environmental factors including water, organic matter, and ultra-violet light (Potter 1998, Held and Potter 2012, Tofangsazi et al. 2015). Preventive treatments targeting adults with pyrethroids should also be compatible, as prediction errors (excluding the Draper site) are smaller than windows of residual activity (average error of 4 days versus residual activity of 7-10 days).

The Utah-Idaho model had r^2=0.97 at Draper in 2014, but it did not perform well at Draper in 2015 (r^2=0.18). Additionally, Draper was the only site for which predictions for each management strategy deviated greater than 3% from observations in both 2014 and 2015. For the 30% strategy, predictions occurred 23 and 39 days earlier than observed billbug activity in 2014 and 2015, respectively. For the 50% strategy, predictions occurred 16 and 33 days earlier than observed billbug activity in 2014 and 2015, respectively. Although the model is robust for other sites in Utah and Idaho, the high error of strategy predictions at Draper highlights the importance of combining model predictions with continued monitoring, especially as the model is being newly tested at different sites (Brandenburg 2004, Held and Potter 2012). Given the limited site-years involved in model development, it is possible that the model may not account for all possible variability in weather conditions and diverse landscapes at certain sites and years. Future research should aim to strengthen and validate these management strategies at more sites throughout the region.

The Utah-Idaho model predicts billbug activity to begin when 38±1 $DD_{3}{}^{o}{}_{C}$ have accumulated, providing managers a time point to begin monitoring for billbugs in Utah and Idaho. Linear pitfall traps used in this study and other pitfall trap types can assist managers in monitoring billbugs (Dupuy and Ramirez 2016). Previous work has recommended preventive insecticide applications using pyrethroids, neonicotinoids, or anthranilic diamides targeted at adults or early instar larvae be applied after first observed adult billbug activity and before 30% activity (Watschke et al. 2013, Richmond 2015). Model predictions become especially important if using pyrethroids to target adults before they lay eggs , as pyrethroids have short residual activity (7-10 days), and subsequent life stages are protected within stems and in soil (Shetlar and Andon 2012). Based on the Utah-Idaho model, these preventive applications would correspond to degree-day accumulations before 548±1 $DD_{3}{}^{o}{}_{C}$.

Neonicotinoid insecticide applications may be effective for late-season management as a curative measure at or before peak activity (50% adult billbug activity) (Baxendale et al. 1999, Pierson et al. 2008, Doskocil et al. 2012). Therefore, the degree-day accumulations for the Utah-Idaho model correspond with applications on or before 796±1 $DD_{3}{}^{o}{}_{C}$. Pyrethroids and trichlorfon may be other curative options at this time (Buss 2001), but trichlorfon is not effective in high pH (>7.0) soils which are common in Utah and Idaho (Chapman and Cole 1982, Cox and Koenig 2010). Peak adult activity may be too late to use an anthranilic diamide, such as chlorantraniliprole, given its low water solubility (1.02 mg/L at 20^{o}C) (Syngenta 2015b, Reynolds and Brandenburg 2015). However, cyantraniliprole, a more water-soluble diamide active ingredient (14,000 mg/L at 20^{o}C) (Syngenta 2015c), may be effective at peak adult activity (Van Dyke 2016). These management strategies are based on the available literature and have not been tested in Utah and Idaho. Validation of these management strategies through both research and cooperation with turfgrass managers in the region should be the goal moving forward (i.e., building a cooperative network similar to WeevilTrak).

Additional analyses. Predictions based on the single-sine method of calculation were assessed for goodness-of-fit when degree-days are calculated with the average method. For the 30% strategy, deviation of predictions from observations was within 0.2-

8.5% of days (average error of 8.11 days). The average method degree-days also yielded large discrepancies between observations and predictions at Draper. When these outliers were removed, predictions of the other seven site-years were within 0.2-3.3% of observations (average error of 3.71 days). For the 50% strategy, deviation of predictions from observations was within 0-8.2% of days (average error of 5.89 days). Interestingly, here the average method improved predictions for Draper in 2014 (7 day difference between observation and prediction), but not in 2015 (30 day difference), suggesting that average method calculations may offer better predictions for some site-years. Removing Draper 2015 as an outlier, predictions of the other eight site-years were within 0-1.9% of observations (average error of 2.88 days). These differences between predictions and observations are similar to what is seen for single-sine degree-day calculations. Thus, turf managers may use the average method to calculate degree-days, which can be done using a spreadsheet with the formula $DD = \sum max\left[\frac{(T_{max}+T_{min})}{2}\right] - LDT, 0$, and predictions based on the single-sine method (30% activity occurs at 548±1 $\mathrm{DD}_{3^{\circ}\mathrm{C}}$, 50% occurs at 796±1 $\mathrm{DD}_{3^{\circ}\mathrm{C}}$) should translate with little noticeable error.

Evaluating bluegrass billbug only, the Utah-Idaho model had a much smaller sum squared error (SSE=153,701) than the Ohio model (SSE=3,218,387) suggesting that the biofix and LDT of the Ohio model may be responsible for its inconsistent performance in Utah and Idaho, rather than activity of the species complex.

Conclusions. Our study demonstrates the importance of adjusting predictive models for specific regional areas. The standard biofix and LDT for temperate regions (1 March, 10°C), do not apply in the temperate states of Utah and Idaho. Older models, such as the Ohio model, and biofixes may no longer be applicable, as climate change (i.e., warmer spring temperatures) can result in degree-day accumulations before current biofixes are set or may even alter the degree-day requirements of an insect (Chen et al. 2015).

Similar to WeevilTrak, the Utah-Idaho model is available to turfgrass managers in Utah at https://climate.usurf.usu.edu/traps/ (Gillies et al. 2017). This application provides managers with a way to select a local weather station that calculates degree-days based on validated model parameters. By having an automated system that calculates accumulated degree-days, turf managers can be alerted if management action is recommended (e.g., at 38±1 $\mathrm{DD}_{3}{}^{\mathrm{o}}{}_{\mathrm{C}}$ managers should start monitoring; nearing 548±1 $\mathrm{DD}_{3}{}^{\mathrm{o}}{}_{\mathrm{C}}$ consider a preventive application; 796±1 $\mathrm{DD}_{3}{}^{\mathrm{o}}{}_{\mathrm{C}}$ last chance for effective curative application). Managers can use these alerts in combination with their continued monitoring data to determine if application is warranted based on billbug activity at their specific site. Managers in the region (e.g., Idaho) where local weather stations are not available through the Utah TRAPs site can track degree-days in a spreadsheet using the average method of calculation and weather data from a local station. The next steps to ensure adoption of the model include spreading the model to turfgrass managers in the region through extension and working with these managers to conduct research to confirm or adjust predicted management strategies.

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Disclaimers

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

Table 1. Total number of adult billbugs for each of three species collected for the sites and years indicated (6 traps at each site).

	All sites all years	Logan 2013-2015	Draper 2014-2015	Boise 2013-2014	Meridian 2013-2014
Bluegrass	4356	3087	250	815	204
Hunting	2572	1710	224	359	279
Rocky Mtn.	569	193	140	82	155
Total	7497	4990	614	1256	638

Table 2. Differences in median degree-day, standard deviation of degree-days, degree-day accumulations, and sum squared error (SSE) of each biofix/LDT combination using model development data from Logan 2014 and 2015.**

Biofix	Lower development threshold (ºC)	Median degree-day ($\log_{10}(DD_x)$)	Standard deviation of $\log_{10}$ degree-days	Cumulative degree-days from biofix through 23 October	Sum squared error (SSE)
January 13	3	2.901	0.297	3025.67	565,192
January 13*	3*	2.931*	0.265*	3208.86*	n/a
January 13†	3†	2.922†	0.279†	3025.67†	153,701†
January 13	5	2.760	0.373	2507.50	967,971
January 13	7	2.600	0.441	2066.22	1,147,227
January 13	10	2.236	0.767	1520.92	4,067,077
February 1	3	2.900	0.297	3025.67	568,518
February 1	5	2.759	0.373	2507.50	967,695
February 1	7	2.600	0.441	2066.40	1,147,433
February 1	10	2.236	0.767	1520.92	4,070,714
March 1	3	2.864	0.333	2987.33	744,167
March 1	5	2.735	0.404	2497.25	1,321,280
March 1	7	2.661	0.410	2497.25	954,263
March 1	10	2.232	0.768	1518.25	4,006,186
March 1*	10*	2.377*	0.508*	1616.42*	896,785*
March 1*†	10*†	2.502*†	0.720*†	1518.25*†	3,218,387*†

** Smaller SSE indicates better predictive ability of model parameters and was our measure for choosing the best fit model with which we continued validation.

*indicates that the average method of calculation was used to calculate degree-days

†indicates parameters were calculated with bluegrass billbug collection data only (note: SSE values may be smaller for these parameters because SSE is proportional to dataset size, and bluegrass billbug collection data was a set of smaller numbers than the datasets used for three-species model development.)

Table 3. Coefficient of determination (r^2) values showing strength of the relationship between observed billbugs and billbugs predicted by the model for both the Utah-Idaho model (13 January, 3ºC) and the Ohio model (1 March, 10ºC). [†]

Year	Intermountain West model				Ohio model			
	Logan	**Draper**	**Boise**	**Meridian**	**Logan**	**Draper**	**Boise**	**Meridian**
2013	0.8183	--	0.7040	0.9039	0.2376	--	0.4698	0.8728
2014	0.9986*	0.9695	0.7107	0.7036	0.9937*	0.9802	0.8158	0.6616
2015	0.9928*	0.1756	--	--	0.9950*	0.2639	--	--

[†] The predictive ability of each model was validated on seven independent data sets as well as the individual data sets that were collectively used to formulate each model (Logan 2014 and 2015), indicated by an asterisk (*) within the table.

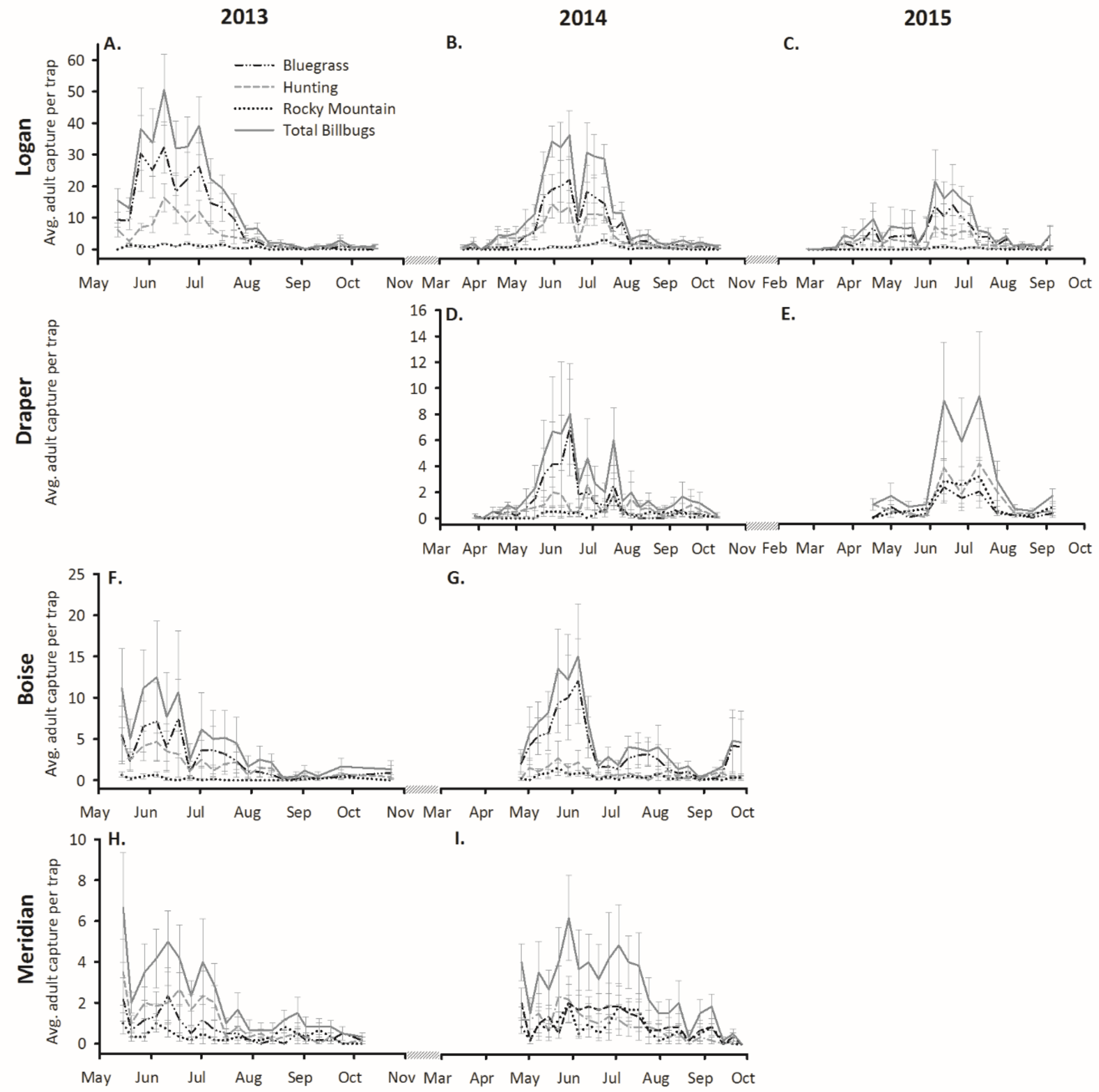

Fig. 1 A-I. Seasonal activity of adult billbugs from pitfall trap captures in Utah and Idaho at each site and year of collection.

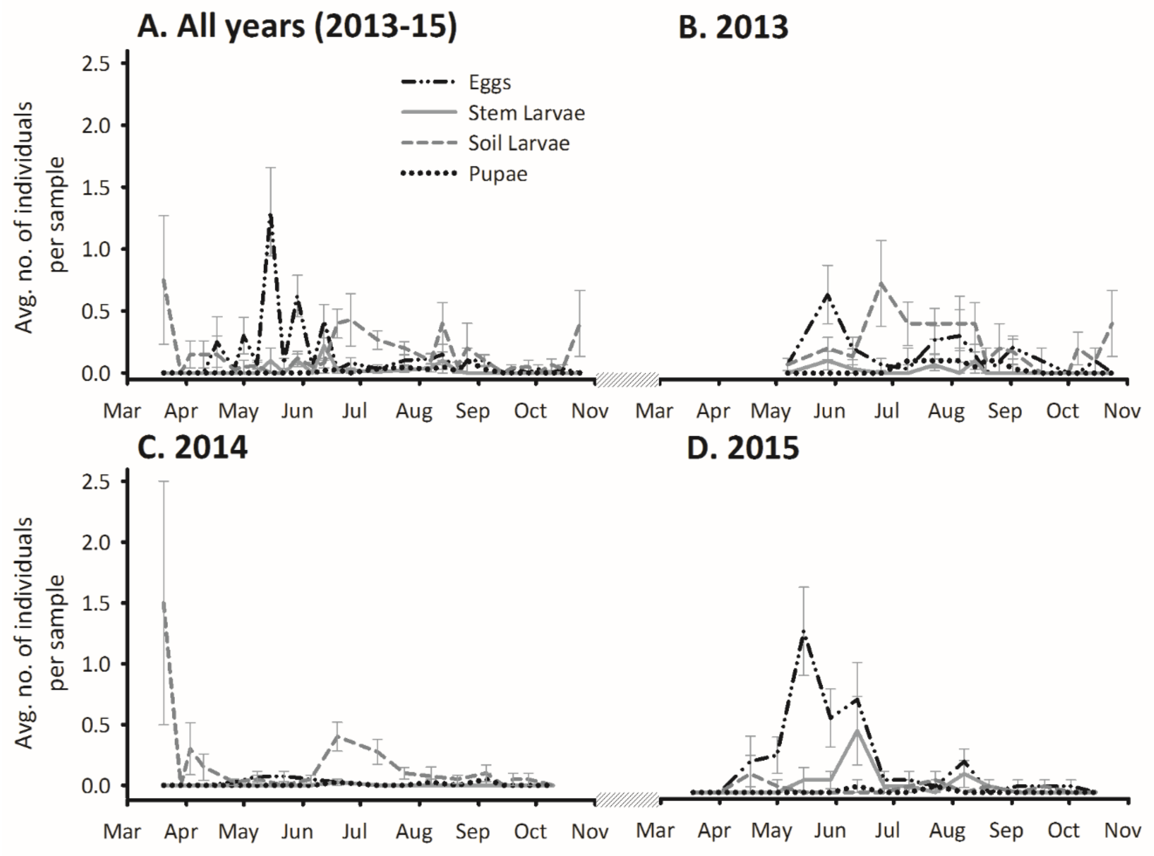

Fig. 2. Immature billbug seasonal activity in Utah and Idaho for (A) all sites and all years of surveys, (B) 2013 surveys in Logan, Boise, and Meridian, (C) 2014 surveys in Logan, Draper, Boise, and Meridian, and (D) 2015 surveys in Utah (Logan and Draper).

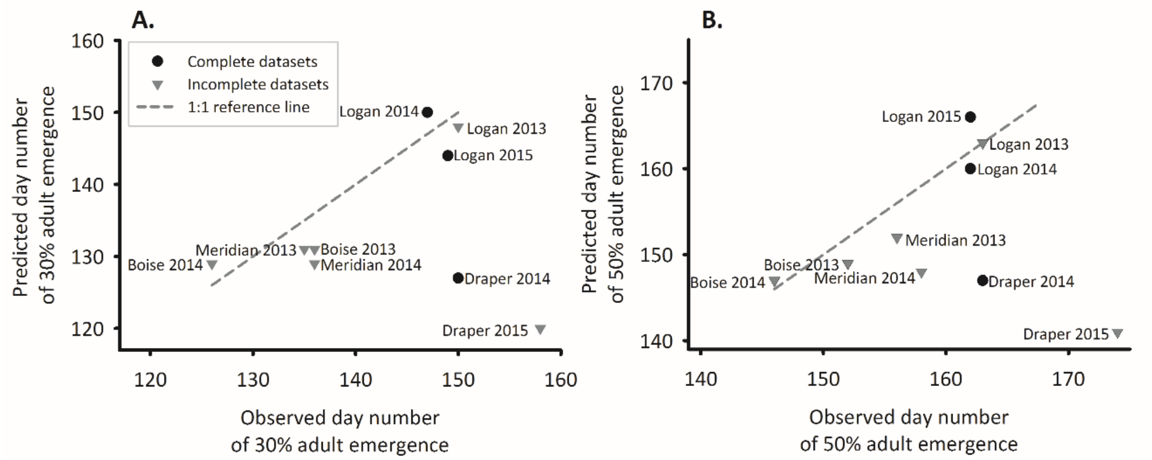

Fig. 3. Complete datasets are represented by circles. Incomplete datasets are represented by triangles, and observations for these datasets are a combination of observation and prediction. A one-to-one relationship is represented by the dashed line. The closer each point is to the dashed line, the closer the model prediction is to the field observation. Day numbers are a count of days beginning 1 January. (A) Predictions of 30% adult emergence were within 7 days of observed 30% of adult emergence at all sites and years except for Draper in 2014 and 2015. (B) Predictions of 50% of adult emergence were within 10 days of observed 50% of adult emergence at all sites and years except for Draper in 2014 and 2015.

CHAPTER III

EVALUATING RECOMMENDED MANAGEMENT TIMINGS AGAINST BILLBUGS (COLEOPTERA: DRYOPTHORIDAE) USING A REGIONAL INTERMOUNTAIN WEST DEGREE-DAY MODEL[3]

Abstract

Billbugs (Coleoptera: Dryophthoridae: *Sphenophorus* spp.) are a damaging turfgrass pest typically managed with preventive applications of systemic, long-residual insecticides including neonicotinoids and anthranilic diamides. In the Intermountain West, a degree-day model for adult billbug activity was developed to assist in management timing. Currently, management timing recommendations result from eastern U.S. research on billbugs, where preventive and curative treatments are recommended before 30% of adult activity and at peak adult activity, respectively. However, it is not clear whether these timings are effective for Intermountain West billbugs. We used two systemic insecticides, Acelepryn® and Merit®, and two bioinsecticides, Grandevo® and Venerate®, which contain bacteria and their fermentation products, to determine whether preventive and curative insecticide application timings predicted by the Utah-Idaho degree-day model were effective against Intermountain West billbugs. In addition, data from a pesticide applicator were used to determine how model-predicted management timings compare to traditional, calendar-based management strategies. Acelepryn® was most effective against billbugs out of all products tested, especially at the preventive timing, while bioinsecticides were not effective at either timing. It is possible that a single

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[3] Dupuy, Madeleine M., Adam Van Dyke, and Ricardo A. Ramirez

application of bioinsecticides was not sufficient to provide billbug suppression. Utah-Idaho model predictions appeared sufficient to time preventive and curative applications of systemic, long-residual insecticides. Instances where a pesticide applicator treated within model-predicted timings were also effective. Therefore, application timings as recommended in the eastern U.S. should be adopted within the Utah-Idaho degree-day model, contributing to an integrated approach to billbug management in the Intermountain West.

Key words: predictive modelling, integrated pest management, bluegrass billbug, hunting billbug, Rocky Mountain billbug

Introduction

Billbugs (Coleoptera: Dryophthoridae: *Sphenophorus* spp.) are turfgrass pests throughout North America, whose larvae damage and kill turf by feeding in stems and on the roots and crown of the plant (Dupuy and Ramirez, 2016). In the Intermountain West, bluegrass (*S. parvulus* Gyllenhal), hunting (*S. venatus vestitus* Chittenden), and Rocky Mountain billbugs (*S. cicatristriatus* Fabraeus) make up the species complex that infests cool-season turfgrasses (Dupuy et al., 2017). Billbugs are typically managed with preventive applications of systemic, long-residual insecticides; however, curative management strategies may also be used (Shetlar and Andon, 2012a; Watschke et al., 2013; Richmond, 2015; Reynolds and Brandenburg, 2015). Because damaging billbug larval stages are cryptic within stems and in soil, preventive and curative management strategies are typically applied when ground-active adults are present (Cranshaw and Zimmerman, 2014). Recommendations for billbug management in the eastern U.S. include applying preventive treatments before 30% of cumulative adult billbug activity is observed (Watschke et al., 2013) and applying curative treatments at peak, or 50% of cumulative adult activity (Reynolds and Brandenburg, 2015). The 30% management timing recommendation was associated with a predictive degree-day model for bluegrass billbug developed in Ohio (Watschke et al., 2013), however given regional differences in billbug activity and species, this model was not robust to reliably predict billbug activity in the Intermountain West (Dupuy et al., 2017). Having a regional predictive model with associated monitoring and application timings can assist turf managers currently relying on calendar-based sprays (Dupuy and Ramirez, 2016).

A regional degree-day model was developed for the Intermountain West billbug complex in Utah and Idaho, providing turf managers with a tool to track cumulative degree-days and predict billbug adult activity (Dupuy et al., 2017). Although this regional model is available to turf managers (https://climate.usu.edu/traps/), current management timings are based on literature from the eastern U.S. (Watschke et al. 2013, Reynolds and Brandenburg 2015). Specifically, the Utah-Idaho model predicts that 30% of adult billbug activity, or the last chance to apply preventive treatments, will occur at 548 $DD_3{}^o{}_C$, and curative timing, or 50% of adult billbug activity, is predicted to occur at 796 $DD_3{}^o{}_C$ (Dupuy et al., 2017). While these calculations are easily attainable from the regional model with local weather data each season, the application timings based on eastern U.S. management recommendations have not been evaluated for efficacy against Intermountain West billbugs.

Billbugs have traditionally been managed by preventive, early-spring applications of surface insecticides (e.g., pyrethroids) targeting adults emerging from overwintering sites, but because residual activity of these insecticides is brief (7–10 d) and subsequent billbug life stages are protected within stems (eggs, early instars) or in soil (later instars), timing is difficult (Shetlar and Andon, 2012a; Dupuy et al., 2017). Alternatively, preventive applications of systemic, long-residual insecticides targeting early instars feeding in stems are more flexible in timing and have gained traction as the preferred billbug management strategy (Watschke et al., 2013; Richmond, 2015). Systemic insecticides, such as neonicotinoids and anthranilic diamides, are most effective against early instars, but they may also have activity against adults by direct contact and ingestion (Shetlar and Andon, 2012a; Watschke et al., 2013; Richmond, 2015; Reynolds and Brandenburg, 2015). However, the continued availability and efficacy of these synthetic products in turf is not guaranteed, especially neonicotinoids, which are facing mounting public scrutiny over non-target effects (Gross, 2013; Larson et al., 2013; Dupuy and Ramirez, 2016). Alternative options for billbug control include the bioinsecticides Grandevo® and Venerate® XC (Marrone Bioinnovations, Davis, CA). These products contain bacteria and their fermentation products, which are toxic to certain insects (MBI, 2013, 2017). Because both of these products work by contact and/or ingestion and neither has systemic activity, billbug life stages that are likely to come into contact with these products include soil-dwelling late-instar larvae and surface-active adults, but not early instar larvae within stems. Like pyrethroids, timing is likely critical for the efficacy of these short-residual products (MBI, 2013, 2017).

To determine whether eastern U.S. management timing recommendations are effective against Intermountain West billbugs as predicted by the Utah-Idaho degree-day model, we first field-tested four insecticides, including the conventional turf insecticides Merit® (neonicotinoid) and Acelypryn® (anthranilic diamide), and two bioinsecticides, Grandevo® and Venerate®, against resident billbug populations at the preventive and the curative application timings. We also tested these insecticides at pre- and post-billbug presence timing in the greenhouse, to isolate their effects on billbug life stages. Additionally, we compared our data and model-predicted timings to those of a commercial pesticide applicator making applications against billbugs to determine how model predictions compare with traditional calendar-based applications.

Materials and Methods

Field assays: Insecticide applications at model-predicted management times

Field trials were conducted May-September of 2016 and 2017 at Utah State University’s Greenville Research Farm in North Logan, UT, in an established stand of Kentucky bluegrass (*Poa pratensis* L.) planted as sod in 2002. The native soil at Greenville was a silty loam, and a native population of bluegrass, hunting, and Rocky Mountain billbugs have been monitored and identified at this site since 2013. Fifty 3×3 m plots were established with a 1 m buffer between each plot to represent each experimental unit. Each plot was randomly assigned to a treatment time (preventive or curative application timing) and one of four insecticide treatments or an untreated control, resulting in five replicates for each time-treatment combination. Insecticides included the systemics Merit® 75 WP (AI: imidacloprid, Bayer Environmental Science, Research Triangle PK, NC) and Acelepryn® SC (AI: chlorantraniliprole, Syngenta Crop Protection, LLC, Greensboro, NC) and the contact bioinsecticides Grandevo® WDG and Venerate® XC (Marrone Bioinnovations, Davis, CA). Insecticides were applied at label rates for billbugs (Table 1) in 700 mL of water with a CO_2 sprayer (50 psi) and irrigated into the soil with sprinklers for 30 minutes (approx. 0.45 cm) on each application date.

Table 1. Label rates for billbug control and insecticide application rates per plot.

Insecticide	**Rate on label**	**Amount applied per 3 x 3 m plot** (in 700mL of water)
Acelepryn® SC	1.02 l/ ha (14 fl oz/ ac)	9.19 mL
Merit® 75 WP	4.9 g/ 93 m^2 (3.5 tsp/1000 ft^2)	0.47 g
Grandevo® WDG	3.36 kg/ ha (3 lbs/ ac)	3.02 g
Venerate® XC	0.438 l/ ha (6 fl oz /ac)	0.39 mL

To determine the preventive (before 30% of adult billbug activity) and curative (at 50% of adult billbug activity) application timings, predicted to occur at accumulated 548 and 796 $DD_3{}^o{}_C$, respectively, weather data were gathered from an on-site weather station (41.7664, -111.8103; Rotronic HC2S3, Rotronic, Hauppage, NY) accessed through the Utah AgWeather network (climate.usurf.edu), and degree-days were calculated using model parameters (13 January biofix, 3 ^{o}C lower development threshold, single-sine method of calculation) in the DegDay program (v. 1.01, Snyder 2005; Excel v. 2016, Microsoft Corp.). The preventive treatment was applied on 17 May in 2016 at 502 $DD_3{}^o{}_C$ and 12 May in 2017 at 479 $DD_3{}^o{}_C$. The curative treatment was applied on 14 June in 2016 at 754 $DD_3{}^o{}_C$ and 8 June in 2017 at 799 $DD_3{}^o{}_C$. Field conditions at the time of each treatment were: 17 May 2016—air temperature 12.22 °C, overcast, calm; 14 June 2016—air temperature 17.78 °C, clear, calm; 12 May 2017—air temperature 22.22 °C, clear, calm; 8 June 2017—air temperature 23.89 °C, clear, winds NNW at 4.6 mph.

The turfgrass in experimental plots was maintained at a height of 7.62 cm (3 in). Plots were sprinkler irrigated to replace 80% of reference evapotranspiration each time 80% of actual evapotranspiration exceeded 1.27 cm (0.5 in) (Rain Bird ET Manager, Rain Bird, Corp., Azsusa, CA). Soil moisture was recorded weekly in each plot with a

FieldScout TDR 100 Soil Moisture Meter (Spectrum Technologies, Aurora, IL) and was maintained between 22 and 38% volumetric water content average in all plots throughout the course of the experiments.

We installed pitfall traps in the center of each plot to track the activity of billbug adults throughout the trial. Traps consisted of a pair of nested 16 oz plastic cups dug into the ground such that the lip of the top cup was flush with the soil surface. Pitfall samples were collected weekly beginning the week after each insecticide application. Billbug adults were counted and identified to species according to keys in Johnson-Cicalese (1990).

To sample for billbug life stages in soil and in stems, one 10-cm diameter soil core to a depth of 15 cm was collected in each plot with a standard golf cup cutter (Par Aide, Lino Lakes, MN) in each plot. In 2016, soil core samples were collected weekly beginning 1 week after pesticide application for 3 weeks, then bi-weekly for 8 more weeks, for a total of 7 soil core samples per plot. In 2017, soil core samples were taken weekly for 5 weeks beginning 1 week after treatment, then bi-weekly for 8 more weeks, for a total of 9 soil core samples per plot. Grass stems from soil core samples were cut at the soil surface (retaining crown) and were stored in the freezer (-13.9^{o}C) before processing. We dissected each stem individually under a stereomicroscope using a fine blade to search for stem-dwelling eggs and early-instar larvae. We counted the number of eggs found in stems of soil core samples as a measure of oviposition in response to insecticide treatments. We also recorded all evidence of billbug presence in stems, including eggs, hatched eggs (chorion only), larvae, larval exuvia, and frass to further determine if insecticide treatments affected presence and activity of billbug life stages. Immature stages were not identified to species, as no external morphological features are currently known to distinguish species from one another (Johnson-Cicalese et al., 1990).

Soil from core samples was stored at 4^{o}C no more than two weeks before processing. We broke up soil by hand to manually search for soil-dwelling late instar larvae and pupae. Whether a recovered larva was dead or alive at the time of recovery was recorded as a measure of mortality. Larvae found in stems were added to counts of larvae from soil for an overall number of larvae in each soil core sample.

To assess overall damage in plots over time, weekly normalized difference vegetation index (NDVI) readings were recorded with a FieldScout TCM 500 Turf Color Meter (Spectrum Technologies, Inc. Aurora, IL). Each week three readings in each plot were recorded and averaged to determine the overall NDVI value.

Statistics

Each season (2016 and 2017) was analyzed separately. Because of differences in sampling dates, preventively- and curatively-treated plots were analyzed separately from one another and compared only to controls that were sampled at the same dates. Count responses (adults in pitfall traps, oviposition, overall evidence of billbugs in stems, stem and soil larvae) were analyzed using a generalized linear mixed model with repeated measures (proc glimmix, SAS Studio University Edition 9.4). Treatment (control, Acelepryn®, Merit®, Grandevo®, and Venerate®) and date of sampling were fixed effects in the model. Random effects included date of sampling (to account for inherent correlation among multiple observations from the same date of sampling), with subject as plot code (assigned identifier unique to each replicate) nested within treatment. We used a spatial power covariance structure to account for weekly versus bi-weekly sampling within the same data set. The model used a Poisson distribution, the LaPlace estimation method (adds a small number to each count to ensure that each response has a nonzero probability of occurring within each class), and the design-adjusted MBN estimator to provide better error estimates for small sample sizes to reduce type I error. Billbug adults were not separated by species in analyses because they occur and damage turfgrass as a complex in the Intermountain West, the Utah-Idaho model accounts for the species complex, and we wanted to assess the efficacy of management timing on the species complex.

In addition to analyzing counts of stem and soil larvae in each treatment for statistical significance compared to controls, we also calculated the percent reduction in total number of larvae found in all plots of a particular treatment over all dates compared to the total number of larvae found in control plots over all sampling dates. Previous studies assessing insecticide treatments against billbugs have been considered to provide excellent control if they provided ≥80% control of billbug larvae (Shetlar et al., 2000; Toda et al., 2008; Stamm et al., 2014; Van Dyke, 2016).

Larval mortality data were analyzed as a sum of overall mortality (number of dead larvae/total number of larvae found) of larvae found within a plot (replicate), thus date was not a factor. We analyzed these binomially distributed data with a generalized linear mixed model, with treatment and application time (preventive or curative) as fixed effects and intercept as the random effect with subject as plot code nested within treatment and application time. Mortality was assessed only for those samples in which soil larvae were found.

NDVI damage data were analyzed with a generalized linear mixed model with repeated measures (proc glimmix, SAS Studio University Edition 9.4). Treatment (control, Acelepryn®, Merit®, Venerate®, and Grandevo®) and date of sampling were fixed effects, and date of sampling was the random effect, with subject as plot code nested within treatment. NDVI data were collected weekly throughout the experiment; thus, we used the first-order autoregressive structure (considers correlations to be highest for time-adjacent times, with decreasing correlation with increasing distance between time points). We used the second order Kenward-Roger denominator degrees of freedom calculation to provide improved F approximations for small sample sizes.

Comparison to calendar-based insecticide applications for billbug management in the Intermountain West

In order to determine how applications based on model predictions compare to those made by a pesticide applicator in Utah on a calendar-basis, we assessed efficacy data from spray trials performed by Professional Turfgrass Solutions, LLC (Van Dyke, 2016). We applied model-calculated degree-day values to application timings using historical weather data, and compared success of applications made near model-predicted degree-day timings to applications that did not align with model-predictions. In 2013, applicator trials were conducted at Greenville Research Farm (maintained as described previously). In 2014-2017, applicator trials were conducted on a fairway at Logan Golf and Country Club (intensively managed Kentucky bluegrass; est. 1931; 41.7447, -111.7890). Plots were 1.52×3.05 m with a 0.305 m buffer between plots, and there were 4-5 replicates for each insecticide treatment. Success of applications was assessed on the basis of billbug larval density in soil 4-11 weeks after treatment, as measured by 3-5 soil core samples (10-cm diameter) taken from each plot and averaged within treatments. Products tested by the pesticide applicator included Acelepryn® and Merit® as well as Ference® (AI: cyantraniliprole, class: anthranilic diamide; Syngenta Crop Protection, LLC, Greensboro, NC), Meridian® (AI: thiamethoxam, class: neonicotinoid; Syngenta Crop Protection, LLC, Greensboro, NC), a Meridian® + Ference® tank mix, and Arena® (AI: clothianidin, class: neonicotinoid; Valent BioSciences, LLC, Libertyville, IL). Here, we regarded any application with ≥80% control of billbug larvae compared to untreated controls as successful billbug suppression.

Greenhouse assays: Effects of insecticide applications pre- and post-billbug introduction on early billbug life stages

To isolate effects of insecticide treatments on billbug life stages, in 2016, we tested insecticides on greenhouse plants pre- and post-introduction of billbug mating pairs in pots. Kentucky bluegrass was planted from seed in 16.51×17.78 cm pots (6.5×7 in; 0.21 g/pot). The bottom 2.54 cm (1 in) of each pot was filled with gravel for drainage.

The remainder of the pot was filled with sieved (5 mm mesh) and sterilized silty loam topsoil collected from Greenville Research Farm. Turfgrass was grown in the greenhouse for 12 weeks at 23°C, 14:10 L:D, and 37% RH, before treatments were applied. Turfgrass was watered from above three times weekly and was maintained at a height of 7.62 cm (3 in).

Insecticides included the systemics Merit® 75 WP and Acelepryn® SC and the contact bioinsecticides Grandevo® WDG and Venerate® XC. Pots were randomly assigned to an insecticide treatment or control and pre- or post-billbug introduction timing. Applications were made pre- and post-billbug introduction and did not correspond to degree-day accumulations as in field experiments. There were three replicates (pots) for each time-treatment combination and six control pots. Insecticides were applied at label rates for billbugs in 200 mL of water (Table 1). In pre-billbug introduction treatments, insecticides were applied first, then one week following application, two hunting billbug mating pairs (2 male and 2 female adults) were added to each pot. Simultaneously, in post-billbug introduction treatments, 2 hunting billbug mating pairs (2 male and 2 female adults) were added to their respective pots (without insecticides). Adult billbugs used in greenhouse assays were field-collected in linear pitfall traps (as described in Dupuy and Ramirez, 2016) at Logan Golf and Country Club in Logan, UT. Billbugs were identified to species and sexed according to keys in Johnson-Cicalese (1990). Hunting billbug adults were separated and stored by sex in petri dishes with moistened cotton wicks at 4°C no longer than two weeks prior to use in experiments. To prevent billbug escape from experimental pots, a cylindrical cage made from plastic transparency sheets 21.59 cm (8.5 in) in height and 16.51 cm (6.5 in) in diameter was affixed into each pot.

Mating pairs for pre- and post- introduction treatments were allowed to mate and lay eggs in pots for two weeks before removal. Twenty-four hours after removal of mating pairs, the post-billbug insecticide treatments were applied. Two weeks after post-billbug treatments, all plants were destructively sampled. Soil was broken up by hand in the laboratory to search for pupae and late instar larvae and to assess mortality of late instar larvae. Grass stems were frozen until dissection with a fine blade under a stereo microscope to search for eggs, early instar larvae, and other signs of billbug presence (including frass, chorions, and larval exuviae).

Statistics

Count data from stems (eggs and all evidence of billbug presence in stems) from this four (Acelepryn®, Merit®, Grandevo®, or Venerate®) × two (application time, pre- or post-billbug) factorial plus control were analyzed with a generalized linear model. A “factor” variable was specified with factor=0 representing control and factor=1 representing treatments other than control in order to compare control treatments with the otherwise complete factorial. Low recovery of soil larvae precluded statistical analysis; therefore, a descriptive approach was used to report mortality rates.

Results

Field assays: Insecticide applications at model-predicted management times

Activity of adult billbugs

In 2016, the preventive application timing (502 DD_{3^oC}, approaching 30% of adult billbug activity) had significantly fewer adult billbugs in plots treated with Acelepryn® (27.5% reduction) (t=13.33, df=300, P<0.0001), Merit® (8.6% reduction) (t=5.82, df=300, P<0.0001), and Grandevo® (25.9% reduction) (t=6.89, df=300, P<0.0001) compared to control (no insecticide) plots (Fig. 1A). Date also had a significant effect (F=22.54, df=14, 300, P<0.0001), apparently resulting froma decrease in billbug populations later in the season (Fig. 1A). There was no date × treatment interaction (F=0.43, df=51, 300, P=0.9998). The curative application timing (754 DD_{3^oC}, representing 50% of cumulative adult billbug activity) showed no effect of insecticides on billbug adult counts compared to controls (F=0.19, df=4, 300, P=0.9453), by date (F=1.42, df=14, 300, P=0.1406), or the date × treatment interaction (F=0.62, df=54, 300, P=0.9824) (Fig. 1B).

In 2017, for both preventive (479 DD_{3^oC}) and curative (799 DD_{3^oC}) applications, insecticides did not affect adult billbug counts compared to controls (F=0.24, df=4, 360, P=0.9162 and F=0.74, df=4, 360, P=0.5650, respectively). Date had a significant effect on capture of billbug adults in pitfall traps (F=2.73, df=17, 360, P=0.0003), and billbug adults were most active in preventively-treated plots 16 June-14 July (Fig. 1C). At the curative application time, adult billbugs were most active at approximately the same range of dates (*F*=2.07, *df*=17, 360, *P*=0.0077), indicating that insecticide treatment did not alter adult billbug activity (Fig. 1D). The date × treatment interaction was not significant for either preventive or curative treatments (*F*=0.54, *df*=68, 360, *P*=0.9986 and *F*=0.48, *df*=68, 360, *P*=0.9998, respectively).

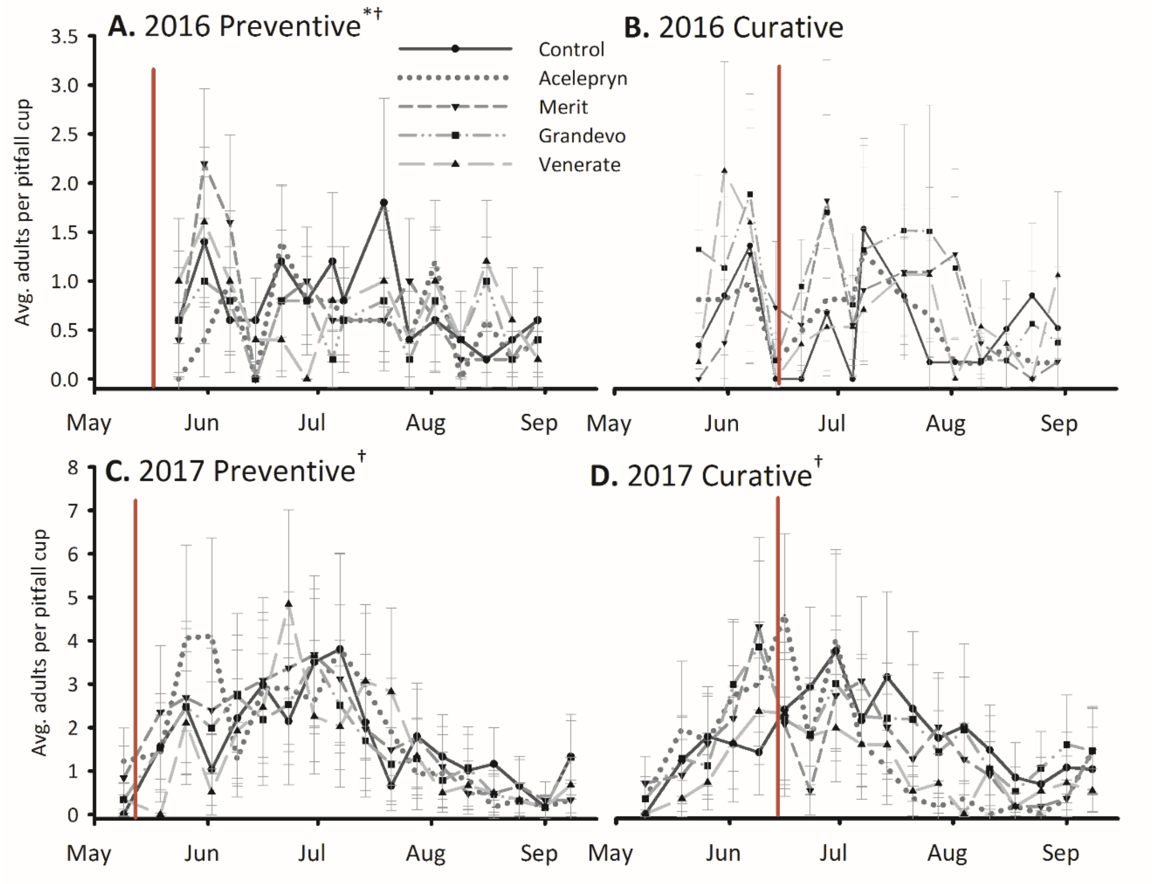

Figure 1. The activity of adult billbugs over time in treated field plots, as captured by pitfall traps in the center of each plot. The red line indicates the date that treatments were applied. An asterisk (*) in the title represents a significant main effect of treatment, † represents a significant main effect of date, and ** indicates a significant date × treatment interaction.

Oviposition

In 2016, there were significantly fewer eggs found in Acelepryn® (*t*=9.03, *df*=140, *P*<0.0001) and Grandevo® (*t*=9.67, *df*=140, *P*<0.0001) treated plots compared to controls when applications were at the recommended preventive timing, corresponding to reduced adult activity in these plots. Merit®, which only had 8.6% reduced adult activity compared to controls, did not have significantly lower oviposition (*t*=0.05, *df*=140, *P*=0.9581). Venerate® treated plots also had similar egg counts compared to controls (*t*=0.02, *df*=140, *P*=0.9820). There were significant differences in eggs found through the season (*F*=26.34, *df*=6, 140, *P*<0.0001), and most eggs were found 11 weeks after treatments (WAT) were applied (2 August 2016). There was no interaction between date and treatment (*F*=0.92, *df*=18, 140, *P*=0.5546) (Fig. 2A). Curative application timing showed similar egg counts among insecticide treatments and controls (*F*=0.04, *df*=4, 139, *P*=0.9969), and eggs differed through the season (*F*=3.59, *df*=6, 139, *P*=0.0025). Most eggs were found 5 WAT (19 July 2016). There was no interaction between date and treatment (*F*=0.17, *df*=20, 139, *P*=1.0000) (Fig. 2B).

In 2017, insecticide treatments at the preventive application timing had similar egg counts compared to controls (*F*=0.34, *df*=4, 180, *P*=0.8523), and eggs differed throughout the season (*F*=2.79, *df*=8, 180, *P*=0.0063). Specifically, most eggs were found 2 WAT (26 May) and steadily declined thereafter. There was no interaction between treatment and date (*F*=0.59, *df*=32, 180, *P*=0.9621) (Fig. 2C). At the curative application time, the date × treatment interaction was significant (*F*=136.87, *df*=24, 180, *P*<0.0001). The interaction was apparently driven by eggs being lowest in Venerate® treated plots 11

August-8 September and in Acelepryn® treated plots on 25 August, but counts were similar among all treatments for all other sampling dates (Fig. 2D).

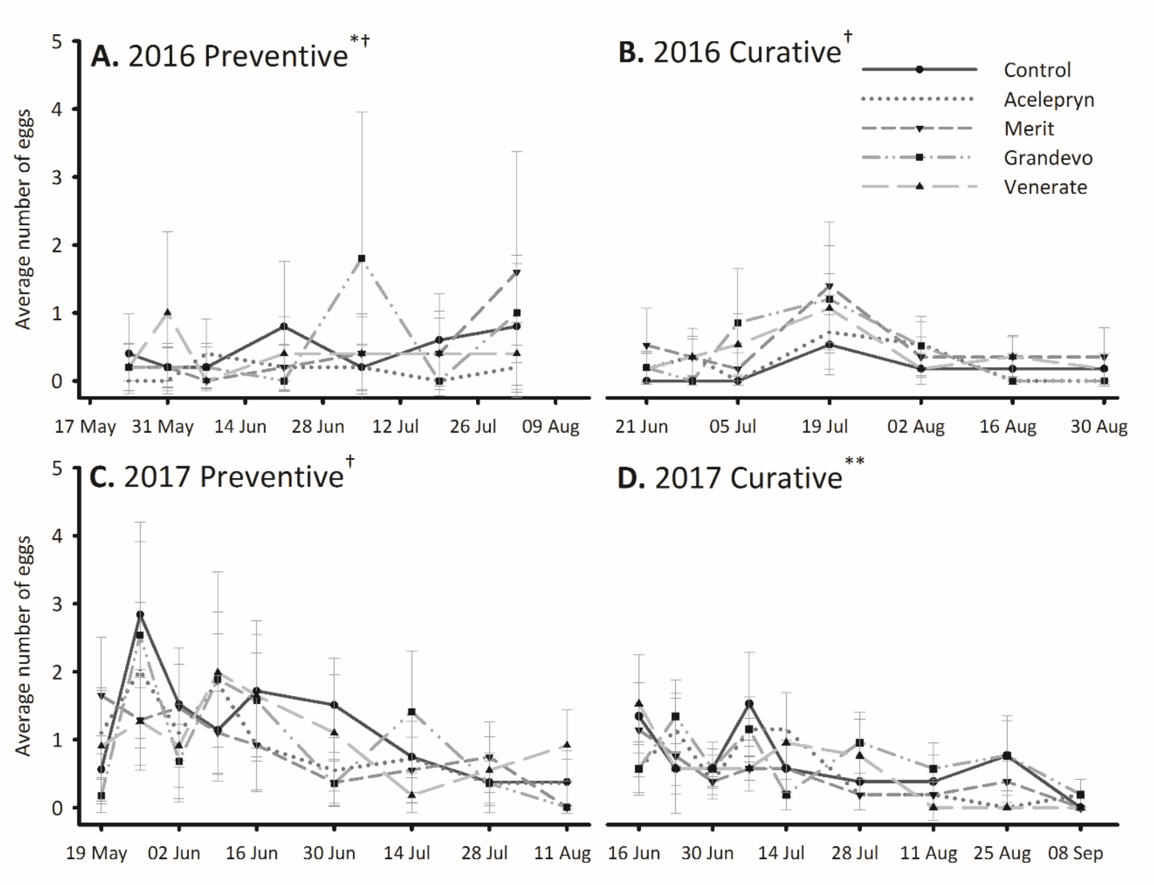

Figure 2. Average number of billbug eggs found over sampling dates in grass stems from treated field plots for (A) preventive treatments in 2016, (B) curative treatments in 2016, (C), preventive treatments in 2017, and (D) curative treatments in 2017. An asterisk (*) in the title represents a significant main effect of treatment, † represents a significant main effect of date, and ** indicates a significant date × treatment interaction.

Overall evidence of billbugs in stems

In 2016, at the preventive application timing, no treatments were significantly different from controls in terms of overall evidence of billbugs found in stems (*F*=0.22, *df*=4, 140, *P*=0.9241), nor did overall evidence change over date (*F*=0.26, *df*=6, 180, *P*=0.9550) (Fig. 3A). There was no interaction between date and treatment (*F*=0.96, *df*=24, 180, *P*=0.5166). At the curative application timing, Acelepryn® treated plots had, on average, significantly lower evidence of billbugs in stems than controls (98.5% reduction) (*t*=5.58, *df*=140, *P*<0.0001). No other treatments were different from controls (Fig. 3B). Number of stems with evidence of billbugs changed over time (*F*=24.11, *df*=6, 140, *P*<0.0001) and was highest 5 WAT (19 July 2016). There was no interaction between date and treatment (*F*=0.70, *df*=23, 140, *P*=0.8384) (Fig. 3B).

In 2017, no treatments had significantly less evidence of billbugs compared to controls at either the preventive (*F*=0.73, *df*=4, 180, *P*=0.5703) or curative (*F*=0.97, *df*=4, 180, *P*=0.4248) application timings (Fig. 3B). Number of stems with billbug evidence changed over date for preventive treatments (*F*=5.23, *df*=8, 180, *P*<0.0001) and curative treatments (*F*=6.21, *df*=8, 180, *P*<0.0001), and the number of stems with billbug evidence appeared to be highest 5 and 1 WAT, respectively, on 16 June (Fig. 3C-D). There was no interaction between date and treatment for either application timing (preventive: *F*=1.38, *df*=32, 180, *P*=0.1000; curative: *F*=0.99, *df*=32, 180, *P*=0.4833).

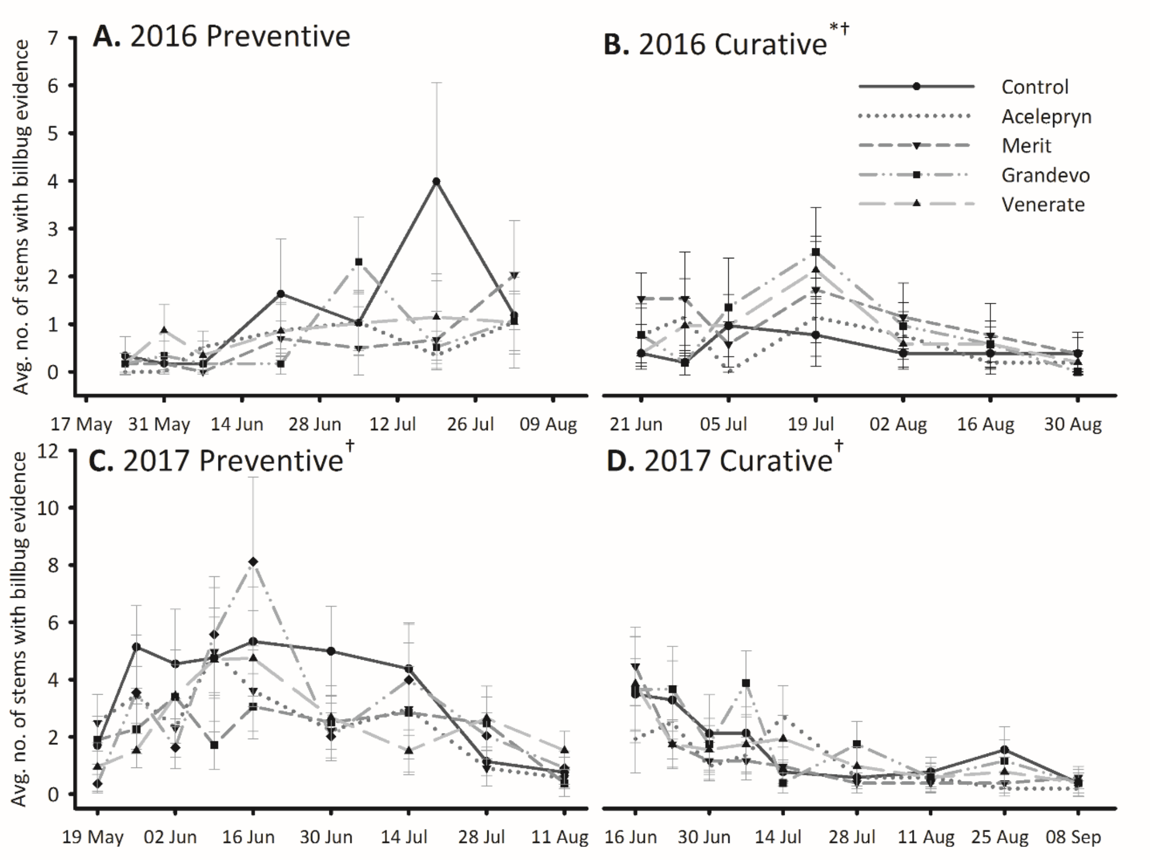

Figure 3. Average number of grass stems showing evidence of billbug activity (eggs, egg shells, larvae, larval exuvia, and frass) found over sampling dates from treated field plots for (A) preventive treatments in 2016, (B) curative treatments in 2016, (C), preventive treatments in 2017, and (D) curative treatments in 2017. An asterisk (*) in the title represents a significant main effect of treatment, † represents a significant main effect of date, and ** indicates a significant date × treatment interaction.

Presence of larvae in stems and soil

In 2016 at the preventive application timing, Acelepryn® provided 95% control of larvae, Merit® provided 86% control, Grandevo® provided 52% control, and Venerate® appeared to provide no control (Table 2). However, larval counts did not differ statistically among treatments (F=0.01, df=4, 140, P=0.9998). Larval abundance changed over time (F=9.88E30, df=6, 140, P<0.0001) and most larvae were found 7-9 WAT (5 and 19 July 2016). There was no interaction between date and treatment (F=0.19, df=11, 140, P=0.9981) (Fig. 4A). However, a trend showed that when larval numbers increased in controls, Grandevo®, and Venerate® plots, they remained low in Acelepryn® and Merit® plots (Fig. 4A). At the curative application timing, Acelepryn® provided 70% control of larvae, Merit® provided 65% control, Grandevo® provided no control, and Venerate® provided 74% control (Table 2). The date × treatment interaction was significant (F=28.51, df=15, 140, P<0.0001), and appeared to be driven by controls having significantly more larvae than treated plots 5 WAT (19 July 2016) (Fig. 4B). Again Acelepryn® and Merit® plots seemed to maintain lower numbers of larvae over time than controls, especially 3-5 WAT (5-19 July) (Fig. 4B). Numbers of larvae were similar for all treatments at other sampling dates.

In 2017, at the preventive application tiing, Acelepryn® provided 65% percent control of larvae, Merit® provided 72% control, Grandevo® provided 28% control, and Venerate® provided 44% control (Table 2). The date × treatment interaction was significant at the preventive application timing (F=59.36, df=25, 180, P<0.0001), and controls had significantly more larvae than treated plots 7 WAT (30 Jun 2017) (Fig. 4C). Additionally, Acelepryn® and Merit® treated plots tended to have lower numbers of larvae over time than control, Grandevo®, and Venerate® plots. At the curative application timing, Acelepryn® provided 90% percent control of larvae, Merit® provided 72% control, Grandevo® provided 0% control, and Venerate® provided 22% control (Table 2). Overall, there were fewer larvae in Acelepryn® treated plots compared to controls, but it depended on date, and differences were only seen 1, 3, 4, and 7-9 WAT (16 June, 30 June, 7 July, and 28 July-11 August) (*F*=29.55, *df*=22, 180, *P*<0.0001) (Fig. 4D).

Table 2. Raw totals and percent control of larvae found in stems and soil of all plots of each treatment/application time combination, across all dates of sampling.

	2016 Preventive		**2016 Curative**		**2017 Preventive**		**2017 Curative**	
	Total Larvae	% Control	Total Larvae	% Control	Total Larvae	% Control	Total Larvae	% Control
Control	23	--	23	--	75	--	68	--
Acelepryn®	1	95	7	70	26	65	7	90
Merit®	3	86	8	65	21	72	19	72
Grandevo®	11	52	24	0	54	28	83	0
Venerate®	23	0	6	74	42	44	53	22

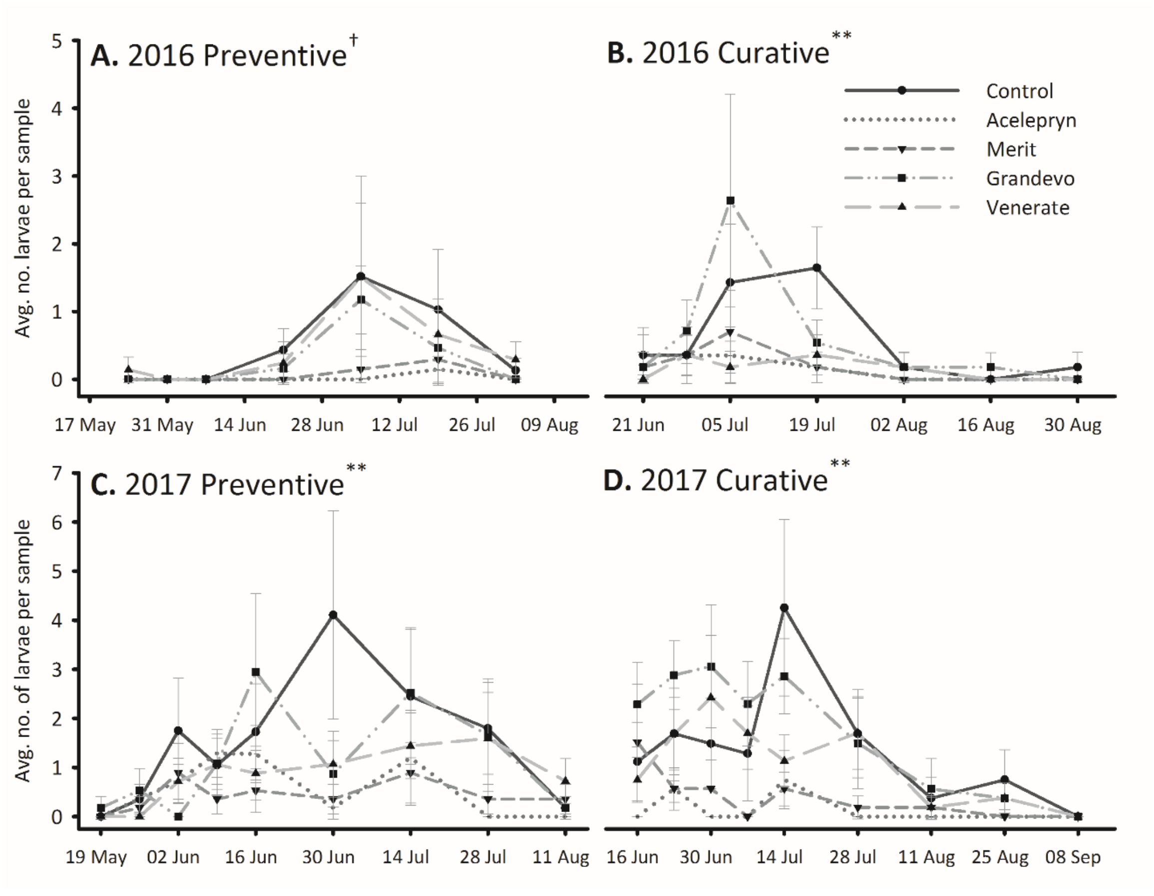

Figure 4. Average number of larvae found in soil and in stems over sampling dates in treated field plots for (A) preventive treatments in 2016, (B) curative treatments in 2016, (C), preventive treatments in 2017, and (D) curative treatments in 2017. An asterisk (*) in the title represents a significant main effect of treatment, † represents a significant main effect of date, and ** indicates a significant date × treatment interaction.

Mortality of soil larvae

In 2016, the mortality of soil larvae was not different among treatments (*F*=0.15, *df*=4, 26, *P*=0.9595), nor was it different between the two application timings (*F*=0.25, *df*=1, 26, *P*=0.6181). However, mortality was highest (99.8%) in plots treated with Acelepryn® at the preventive application timing, compared to 55% mortality of larvae in control plots (Fig. 5A). In 2017, the mortality of soil larvae was not different among treatments (*F*=0.33, *df*=4, 34, *P*=0.8547), nor was it different between the two application timings (*F*=0.06, *df*=1, 34, *P*=0.8073) (Fig. 5B).

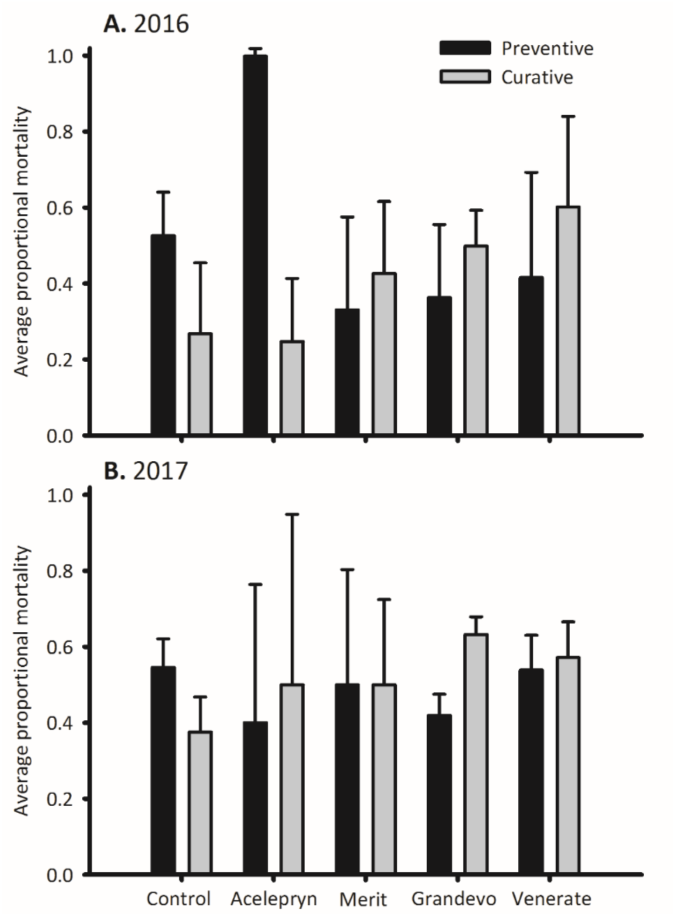

Figure 5. Proportion of soil larvae that were dead at the time of processing. No treatments were significantly different from controls at either application timing, in either year.

Turfgrass damage (NDVI)

In 2016, at the preventive application timing, NDVI was not different in any treatments compared to controls. Over time (date of sampling), NDVI first increased in all plots, then declined (F=20.40, df=14, 95.28, P<0.0001) (Fig. 6A). There was no interaction between date and treatment (F=0.76, df=56, 154.2, P=0.8787). At the curative application timing, no treatments caused significant differences in NDVI compared to controls (F=1.87, df=4, 40.25, P=0.1351). NDVI declined rapidly after treatment was made, regardless of treatment (F=17.52, df=14, 92.32, P<0.0001) (Fig. 6B). There was no interaction between date and treatment (F=1.02, df=56, 150.4, P=0.4593).

In 2017, at the preventive application timing, NDVI was higher in Acelepryn® treated plots than in control plots (4.3% increase in average NDVI) (t=-2.23, df=28.12, P=0.0341). No other treatments were different from controls. NDVI showed a gradual decline over time in all plots (F=7.46, df=17, 28.12, P<0.0001). There was no interaction between date and treatment (F=1.12, df=68, 176.4, P=0.2829), though a trend shows that NDVI appeared to decline more in controls and less in treated plots, especially those treated with Acelepryn® (Fig. 6C). At the curative application timing, NDVI was not different in any treatments compared to controls (F=1.64, df=4, 20.81, P=0.2014). Again, NDVI shows a gradual decline over time, regardless of treatment (F=5.96, df=17, 81.85, P<0.0001). A trend shows that NDVI appeared to remain higher in Acelepryn® and Merit® treated plots (4.7% and 5.2% increase compared to controls, respectively) than in

Venerate® or Grandevo® treated or control plots, but the date × treatment interaction was not significant (*F*=0.89, *df*=68, 147, *P*=0.7045) (Fig. 6D).

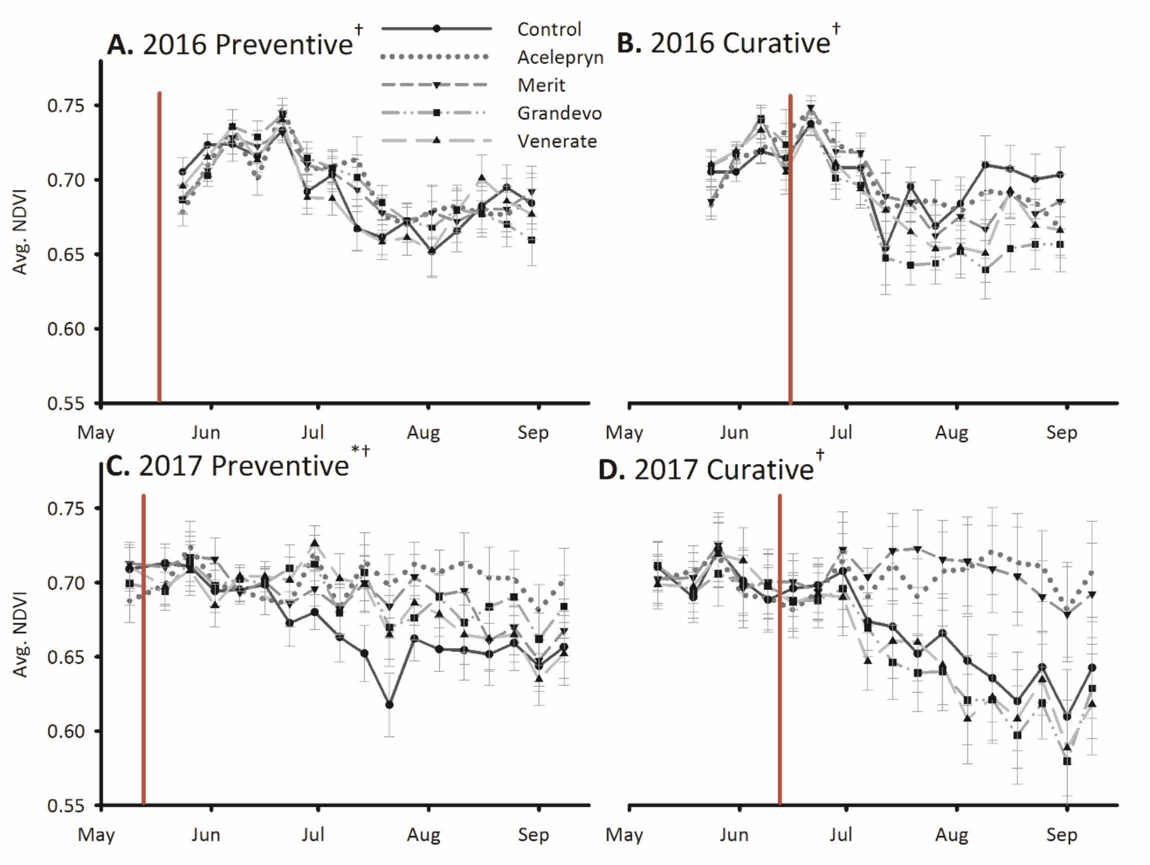

Figure 6. Average NDVI in treated field plots over time (date). The red line indicates the date that treatments were applied. An asterisk (*) in the title represents a significant main effect of treatment, † represents a significant main effect of date, and ** indicates a significant date × treatment interaction.

Comparison to calendar-based insecticide applications for billbug management in the Intermountain West

Insecticides applied by Professional Turfgrass Solutions, LLC appeared to result in reduced larvae compared to controls (Fig. 7), with some application times showing more reduction in larval densities than others. Applications made on 12 May 2016 and 19 May 2017, corresponding to 461 and 534 DD$_{3}{}^{o}{}_{C}$, respectively, fall within the range of degree-days at which one might apply treatment when following the model's preventive treatment recommendation (i.e., treatment before 30% of adult billbug activity has occurred, or before 548 DD$_{3}{}^{o}{}_{C}$). At 461 DD$_{3}{}^{o}{}_{C,}$ products included Acelepryn®, which resulted in 100% control of billbug larvae, Merit®, which resulted in 95% control, and Meridian®, which resulted in 87% control (Fig. 7). At 534 DD$_{3}{}^{o}{}_{C}$, Acelepryn® resulted in 100% control of billbug larvae. The closest application timing to the model predicted curative timing (i.e., 796 DD$_{3}{}^{o}{}_{C}$, at 50% of adult billbug emergence) occurred on 5 June 2015 at 837 DD$_{3}{}^{o}{}_{C}$. Only Ference® was tested at this time, and it resulted in 99% control of billbug larvae (Fig. 7). Treatments applied earlier than model recommendations at 380 DD$_{3}{}^{o}{}_{C}$ (5 May 2014), resulted in 100% and 93% control of billbug larvae by Acelepryn® and the Meridian® + Ference® tank mix, respectively. Treatments at 424 DD$_{3}{}^{o}{}_{C}$ (17 May 2013) resulted in only 37% control by Acelepryn®, 95% control by Ference®, and 71% control by Meridian®. "Rescue" treatments applied later than model recommendations at 1339 DD$_{3}{}^{o}{}_{C}$ (7 July 2016) resulted in 74% control by Ference®, 37% control by Meridian®, and 81% control by Arena®. Therefore, timings outside of model-predicted recommendations were the only timings with some unsuccessful treatment results, however, earlier timings at 380 DD_{3^oC} resulted in adequate control of billbug larvae with Acelepryn® and the Meridian® + Ference® tank mix.

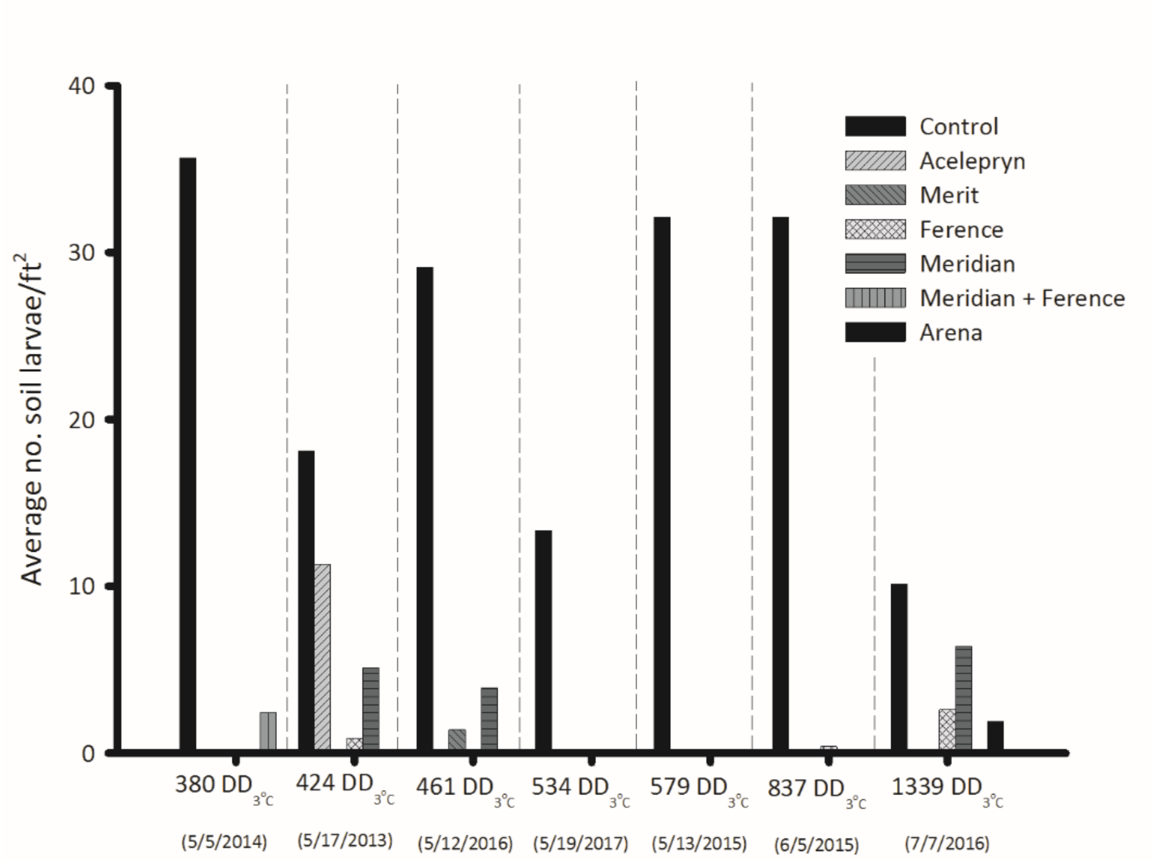

Figure 7. Results of commercial applicator trials conducted by Professional Turfgrass Solutions, LLC. The x-axis shows accumulated degree days (beginning January 13th at 3°C) at each application date, calculated with historical weather data. Not all products were tested on all dates. Bars not shown for products which resulted in 0 larvae/ft^2: Acelepryn at 380, 461, 534, and 579 DD_{3^oC}; Ference at 579 DD_{3^oC}; and Arena at 424 DD_{3^oC}.

Greenhouse assays: Effects of insecticide applications pre- and post-billbug introduction on early billbug life stages

In greenhouse experiments, no treatments were significantly different from the untreated controls in terms of number of eggs found in stems at either pre- or post-billbug introduction application timings (*F*=1.59, *df*=3, 21, *P*=0.2215). However, trends showed that oviposition was lower in pots treated pre-billbug introduction with Acelepryn® and Merit®, which had 0 eggs compared to 7 eggs in controls (Grandevo® pots had 5 eggs and Venerate® pots had 1 egg). There was no trend in reduced oviposition in pots treated post-billbug introduction with the same insecticides (Fig. 8A). The same trends were seen when assessing all evidence of billbug activity in stems (*F*=1.04, *df*=3, 21, *P*=0.3953) (Fig. 8B). A total of four larvae were found in soil from 2016 greenhouse experiments. All four larvae were found in Grandevo® treatments—three in one preventively treated pot, and the remaining larva in a curatively treated pot. All larvae were alive at the time of sampling.

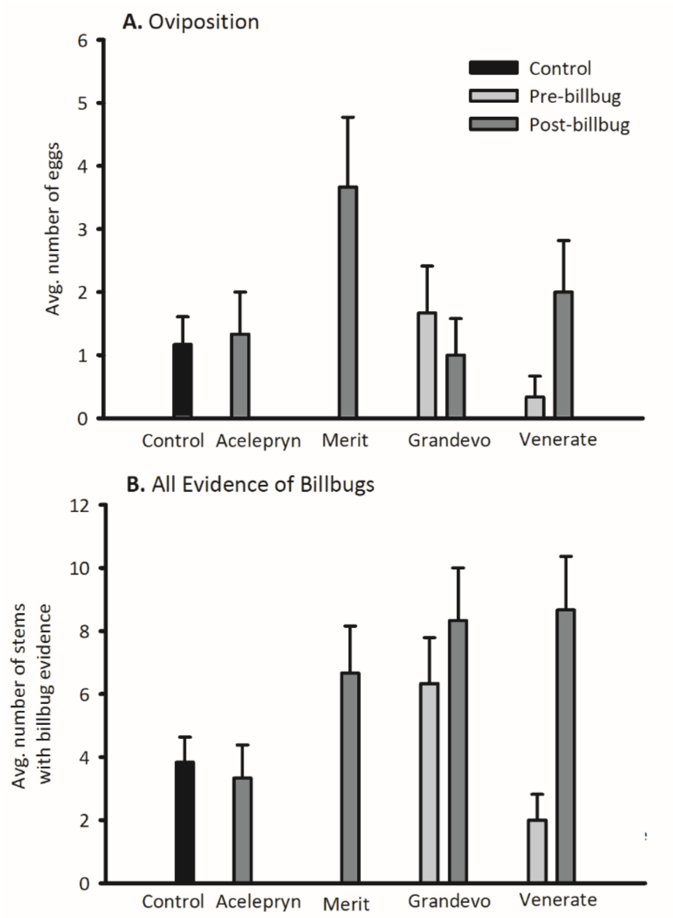

Figure 8. Average number of billbug eggs found in grass stems (A) and average number of stems showing evidence of billbug activity (eggs, egg shells, larvae, larval exuvia, and frass) (B) from greenhouse experiments. No treatments were significantly different from controls for either measure.

Discussion

In turf insect management, one of the primary measures of success is a decrease in larval populations, as larvae are typically the damaging stage for many turf pests, including billbugs (Shetlar et al., 2000; Stamm et al., 2014; Van Dyke, 2016). Therefore, the presence of billbug larvae in stems and in soil was our most informative response variable. Larval counts in samples were significantly affected by preventive insecticide treatments over time (date of sampling) in 2017, but in both years the trend over time showed that when larval counts increased in control, Grandevo®, and Venerate® plots, larval counts remained low in Acelepryn® and Merit® plots. In 2016, preventive applications of Acelepryn® and Merit® showed ≥80% reduction in billbug larvae compared to controls. In 2017, Acelepryn® treated plots were significantly less damaged than control plots (Fig. 6C). Results of applications of neonicotinoids and anthranilic diamides by Professional Turfgrass Solutions, LLC within the preventive model recommendation were also successful, with ≥80% reduction in larvae compared to controls for all products tested, including Acelepryn® and Merit®. The effective use of neonicotinoids and anthranilic diamides in preventive billbug management has been well-supported in the literature (Heller et al., 2008, 2009; Toda et al., 2008; Shetlar and Andon, 2012; Van Dyke, 2016). These results suggest that the preventive timing based on eastern U.S. recommendations as predicted by the degree-day model (i.e., apply before 30% of adult billbugs emergence, or 548 $DD_{3^{o}C}$) was an effective time to apply these products to suppress the damaging billbug larval stage over time.

Interestingly, treatments made by Professional Turfgrass Solutions, LLC at 424 DD$_{3}{}^{o}{}_{C}$, which fell on 17 May 2013, were not successful for Acelepryn® (38% control) or Meridian® (71% control). Because this treatment was made >100 DD$_{3}{}^{o}{}_{C}$ before the model-predicted last chance to treat preventively (in 2013, 424 DD$_{3}{}^{o}{}_{C}$ was two full weeks before 548 DD$_{3}{}^{o}{}_{C}$ had accumulated), this timing is unlikely to be within a range at which one might treat if following model predictions for preventive timing. However, applications made at 380 DD$_{3}{}^{o}{}_{C}$ with Acelepryn® and a Meridian®+Ference® tank mix were successful. Applications at 424 DD$_{3}{}^{o}{}_{C}$ occurred at the Greenville site in 2013, while all other trials performed by Professional Turfgrass Solutions, LLC, including those at 380 DD$_{3}{}^{o}{}_{C,}$ occurred at Logan Golf and Country Club. Applications made at the Logan Golf and Country Club fairway site primarily showed levels of larval control that were higher than we saw in trials at Greenville (Table 2, Fig. 7). Differences in management between these two sites may have contributed to higher success of applications at Logan Golf and Country Club. Specifically, a thicker thatch layer at Greenville may have intercepted and reduced efficacy of insecticide applications (Van Dyke, 2016). This highlights the importance thatch management as a component of integrated management, as thatch can impact insecticide efficacy (Lickfeldt and Branham, 1994; Raturi et al., 2003). Because the model recommended preventive timing is a “treat before” recommendation, how far in advance to treat remains open to interpretation. Given that applications made by Professional Turfgrass Solutions, LLC at 380 DD$_{3}{}^{o}{}_{C}$ were effective, it may be possible that applications >100 DD$_{3}{}^{o}{}_{C}$ away from the last chance to treat at 548 DD$_{3}{}^{o}{}_{C}$ can be effective with certain products and appropriate site management (e.g., thatch management). More research is needed to determine how far in advance of 548 DD_{3^oC} preventive treatments can be effective, and ideally a range of degree-days between which preventive treatments are most effective will be established.

The curative management time predicted by the Utah-Idaho degree-day model, as based on eastern U.S. billbug management recommendations (i.e., treatment at peak or 50% of adult billbug activity, 796 DD_{3^oC}) also appears to be an effective time to apply certain insecticides against billbugs. As in preventively treated plots, larvae remained lower in plots treated curatively with Acelepryn® or Merit® than in controls (Fig. 4B,D). Particularly, in both years, Acelepryn® treated plots maintained significantly lower numbers of larvae over time compared to control, Grandevo®, and Venerate® plots. Acelepryn® treatment also resulted in ≥80% reduction of billbug larvae compared to controls in 2017. Though not reaching target control, Merit® provided 72% control of billbug larvae at the curative application timing both years, and other studies report the effectiveness of Merit® at a curative application timing (Shetlar and Andon, 2012b; Reynolds and Brandenburg, 2015). Additionally, plots treated curatively with Acelepryn® and Merit® in 2017 seemed to sustain less damage over time (Fig. 6D). Further, what is considered a "rescue" treatment by a pesticide applicator (Professional Turfgrass Solutions, LLC) (1339 DD_{3^oC}) was much later than our model recommendation for a curative treatment (796 DD_{3^oC}). An anthranilic diamide (Ference®) and a neonicotinoid (Meridian®) tested at the rescue time did not result in ≥80% reduction in billbug larvae. However, applications of Acelepryn® and Ference® (chlorantraniliprole and cyantraniliprole, respectively) made near the model-predicted curative timing at 837

DD_{3^oC} resulted in 100% reduction of billbug larvae compared to controls. This suggests that our curative timing at 796 DD_{3^oC} may be more appropriate for billbug management than traditional "rescue" timing, especially when using products with low water solubility, such as chlorantraniliprole (Reynolds and Brandenburg, 2015; Van Dyke, 2016; Dupuy et al., 2017). These results support that anthranilic diamides are effective when applied at the curative application timing predicted by the degree-day model, and other studies have shown that neonicotinoids, including imidacloprid (Merit®), are also effective at a curative timing (Shetlar and Andon, 2012b; Reynolds and Brandenburg, 2015).

Unfortunately, Grandevo® and Venerate® did not result in adequate billbug control at either application timing. It was expected that the curative application timing would be effective for Venerate®, which contains heat-killed *Burkholderia* spp. strain A396 cells and fermentation products. Compounds produced by this bacterium can degrade the insect exoskeleton and interfere with molting when contacted or ingested (MBI, 2017). This insect growth regulator activity and contact/ingestion action suggested that Venerate® was likely to affect soil-dwelling larvae, though it may also degrade the exoskeleton of adults. However, Venerate® was not significantly different from controls for any responses, nor did it provide ≥80% reduction of billbug larvae. Grandevo® contains *Chromobacterium subtsugae* and its fermentation products, which work by ingestion, suggesting that it may be effective against soil larvae (MBI, 2013). However, Grandevo® also did not reduce larvae compared to controls at either application timing. Previous trials with these products against billbugs have yielded variable results.

Grandevo® has provided anywhere from 35-80% control of billbug larvae and pupae while Venerate® has provided from 9-93% control, without correlation of higher doses to increased control for either products (Shetlar and Andon, 2014; Stamm et al., 2014). Both trials assessed a single application timing. Many bioinsecticides require multiple applications because of issues with quick degradation (Glare et al., 2012). In fact, the specimen labels for Grandevo® and Venerate® (MBI, 2013, 2017) recommend application on a 3-10 day interval until pest pressure is alleviated. Thus, even when timing applications precisely according to model predictions, a single application may not be adequate to control billbugs. Interval timings and most effective number of applications based on degree-day model predictions targeting soil larvae should be assessed for these products. Another factor contributing to variable efficacy of bioinsecticides may be the rate of post-application irrigation. Our post-application irrigation rate of 0.45 cm was ideal for systemic products targeting billbugs (Koppenhöfer, 2016), but bioinsecticides that have contact action against soil pests typically require deeper watering (e.g., 1.27 cm) to reach the target zone, especially at sites with thicker thatch (Koppenhöfer, 2016; Phyllom, 2017).

Not only can insecticides have direct impacts by suppressing damaging larval stages, they can also have indirect effects by altering pest behavior. For instance, anthranilic diamides have been shown to have repellent and oviposition-reducing effects on insects in other systems (Tiwari and Stelinski, 2013; Bielza and Guillen, 2014), and Grandevo® is purported to repel, act as an anti-feedant, and reduce reproduction of insects when ingested (MBI, 2013). In addition to well-known sub-lethal effects on honeybees, imidacloprid (Merit®) has also been shown to have behavioral effects on aphids and Japanese beetles (*Popillia japonica* Newman) (Nauen, 1995; Boiteau and Osborn, 1997; George et al., 2017; Fischer et al., 2014). Indeed, our results showed some indirect effects of products. In 2016, Acelepryn® and Grandevo® applied preventively significantly reduced the activity of adults in treated plots compared to controls, accompanied by a resulting reduction in oviposition. Merit® plots also had an 8.6% reduction in adult activity (compared to 27.5 or 25.9% reduction for Acelepryn® and Grandevo®, respectively), which, though significantly different from controls, was apparently not enough to result in a reduction in oviposition. However, results from greenhouse assays show that Acelepryn® and Merit®, but not Grandevo®, were effective at reducing oviposition when applied pre-billbug introduction. These results indicate some support for repellent effects against billbugs of Acelepryn®, Merit®, and Grandevo® when applied preventively.

Differences between seasons (2016 and 2017) can have major impacts on resident billbug populations. In 2016, billbugs were much less abundant than in 2017 (Table 2, Fig. 1 A-D, Fig. 4 A-D). One of the challenges of working with billbugs in research is that effective methods of rearing billbugs under laboratory conditions have not been established (Johnson-Cicalese and Funk, 1990; Rondon and Walenta, 2008), and attempts to introduce billbugs into experimental field plots have been unsuccessful (MMD, unpublished data, 2014). In part, the difficulties associated with billbug rearing were one reason conclusions were difficult to draw from greenhouse assays. Replication was limited by the availability of field-collected billbug mating pairs, especially females, which are less abundant than males (MMD, personal observation). Strategizing to find heavily infested areas is key to successful research relying on resident populations.

Conclusions

Our results combined with data from Professional Turfgrass Solutions, LLC support that eastern U.S. recommendations were effective timings to apply preventive and curative insecticide treatments against Intermountain West billbugs with synthetic, systemic products including neonicotinoids and anthranilic diamides. It is likely that a single application, even when precisely timed with susceptible billbug life stages according to model predictions, is insufficient to provide adequate billbug control for either bioinsecticide. Future research should focus on timing interval applications of these bioinsecticides with model predictions. However, the recommendations from the eastern U.S. for preventive and curative billbug management timing should be adopted within the Utah-Idaho degree-day model for billbug management using synthetic, systemic products.

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CHAPTER IV

CONSUMPTIVE AND NON-CONSUMPTIVE EFFECTS OF PREDATORY ARTHROPODS ON BILLBUG (COLEOPTERA: DRYOPHTHORIDAE) PESTS IN TURFGRASS[4]

Abstract

Generalist predators affect pest populations through direct consumption or by non-consumptive effects, whereby predators induce changes in prey behavior which represent a cost to prey. A diverse community of predatory arthropods has been described in turfgrass, contributing to the direct mortality of pests including black cutworm, fall armyworm, and Japanese beetle. Billbugs are a major pest of turfgrass in the Intermountain West, but the composition of the local predatory arthropod community and whether predators aid in billbug suppression through consumptive or non-consumptive effects is unknown. First, we catalogued the predatory arthropod community on Utah and Idaho golf courses using linear pitfall traps. Then, we assessed adult billbug consumption by resident predators in the field. Using a series of lab assays, we assessed the most abundant predators' consumption of billbug life stages, including adults, sentinel waxworm larvae at varied soil depths, and eggs in turf stems. Finally, we assessed the non-consumptive effects of these abundant predators on adult billbug activity (mating, oviposition, thanatosis). We found that the predatory arthropod community consisted primarily of carabids (*Pterostichus melanarius, Harpalus* sp., *Amara aenea,* and

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Anisodactylus sp.) and spiders (lycosids), representing 60% and 28% of all predators, respectively. In the field and in lab assays, adult billbug mortality from predation was generally low at <6%. While predators readily consumed sentinel larvae in petri dish arenas, larvae escaped predation at 1 cm soil depth. The greatest consumptive effects of predators were on billbug eggs, with *Anisodactlylus* sp. feeding on 46% of eggs. Predator exposure reduced overall billbug activity by 56%, and for hunting billbugs, specifically, reduced mating activity by 28%. Our new understanding of the consumptive and non-consumptive effects of predators on billbugs supports the importance of conservation biocontrol in turfgrass and assists in planning for enhancement of specific predators.

Keywords Bluegrass billbug, hunting billbug, integrated pest management, natural enemies, trait-mediated interactions

1. **Introduction**

The role of generalist predators in natural pest control is best known as direct, consumptive effects on herbivorous pests. However, more recent work has found that predators also decrease herbivory and pest outbreaks via a myriad of indirect, non-consumptive effects. Non-consumptive effects range from density-mediated, whereby predators cause trophic cascades, thereby indirectly affecting pest densities, to trait-mediated, whereby predators may induce costs by stimulating prey defensive behaviors (Preisser et al., 2005; Prasad and Snyder, 2006). Trait-mediated effects have recently gained attention as researchers have recognized their importance in pest management systems. Trait-mediated non-consumptive effects occur when defensive behavior of prey species in the presence of predators, such as reduced foraging effort, results in lost feeding time, and thus, higher plant productivity and reduced fitness of pests (Schmitz, 1997; Janssen et al., 1998; Preisser et al., 2005). For example, spotted cucumber beetles significantly reduced feeding in response to the presence of the generalist wolf spider, *Tigrosa (Hogna) helluo* (Walckenaer) (Snyder and Wise, 2000). Furthermore, the presence of predators can also impact mating and oviposition behavior of prey species with the possibility of a negative impact on prey demographics (Sih et al., 1990; Blaustein et al., 2004; Preisser et al., 2005). The impact of predator intimidation on prey demographics, a trait-mediated effect, has been found to be as strong as that of direct consumption (Preisser et al., 2005).

The predatory arthropod community in turfgrass is diverse and has been well-documented in the eastern United States, but a catalogue of predators from the western

U.S. is lacking. Common predatory taxa in eastern turf systems include spiders (Arachnida: Araneae), ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae), and ants (Hymenoptera: Formicidae) (Bixby-Brosi and Potter, 2012). The effects of predators on turfgrass pests has been studied primarily through assessment of conservation biological control strategies, such as bunchgrass strips, naturalized roughs, and plant diversification. These strategies enhance predatory arthropod abundance in turf ecosystems (Frank and Shrewsbury, 2004; Bixby-Brosi and Potter, 2012; Dobbs and Potter, 2016), however, assessments of whether conservation biocontrol strategies actually result in increased predation rates show mixed results. Dobbs and Potter (2016) found no direct spillover of predation from naturalized roughs to nearby mowed areas, and Braman et al. (2002) showed consistently high rates of predation on fall armyworm (*Spodoptera frugiperda* J.E. Smith) eggs and larvae and Japanese beetle eggs regardless of whether surrounding borders were composed of wildflowers or mulch. Conversely, Frank and Shrewsbury (2004) showed that conservation strips increased predator activity and instances of predation on black cutworm (*Agrotis ipsilon* Hufnagel) larvae on adjacent fairways. These studies have assessed consumptive effects only, and there is a gap in the knowledge regarding potentially important non-consumptive effects of predators in turfgrass.

Billbugs (Coleoptera: Dryophthoridae: *Sphenophorus* spp.) are a major pest of turfgrass in the Intermountain West, where bluegrass (*S. parvulus* Gyllenhal), hunting (*S. venatus vestitus* Chittenden), and Rocky Mountain billbugs (*S. cicatristriatus* Fabraeus) damage turfgrass as a complex (Dupuy et al., 2017). Adult billbugs are ground active, rarely fly, and females lay eggs in turfgrass stems. The damaging larval stage feeds within stems and on roots belowground. Insecticides that are commonly used against turf pests, like neonicotinoids, have been shown to harm existing populations of predatory arthropods and interfere with their ability to provide natural pest suppression (Terry et al., 1993; Kunkel et al., 2001; Peck, 2009a, b; Peck and Olmstead, 2010; Larson et al., 2014).

The ability of common generalist predators in turf to feed on billbug life stages and rates of direct predation on billbugs in the field are currently unknown. Billbug adults are known to feign death when threatened (thanatosis) (Kindler and Spomer, 1986), and this reduction in activity could have important implications for turf health and billbug fitness. However, the extent to which thanatosis is induced by generalist predators and other defensive behaviors of billbugs are unknown, and the non-consumptive effects of generalist predators have not been assessed for billbugs or any other turfgrass pest.

The objectives of this research were 1) to document the predatory arthropod community in Intermountain West turfgrass, 2) to determine the consumptive effects of predators on billbug adults in the field and all life stages in the lab, and 3) to determine non-consumptive effects of predators on billbug adult behavior, mating, and oviposition. We surveyed for generalist predators at four golf courses in Utah and Idaho using linear pitfall traps. We conducted field trials to determine rates of predation on billbug adults by resident populations of generalist predators in the field and assessed the most common generalist predators from surveys for their ability to directly feed on billbug adults and cryptic life stages in laboratory assays. We also documented non-consumptive effects of predators on billbug behavior in laboratory assays.

2. **Material and methods**

2.1 Predatory arthropod community composition

2.1.1 Location of survey sites

We surveyed four golf courses in Utah and Idaho: Logan Golf and Country Club in Logan, UT (subsequently referred to as “Logan”; est. 1931; 41.7447, -111.7890), South Mountain Golf Course in Draper, UT (“Draper”; est. 1998; 40.5010, -111.8500), Crane Creek Country Club in Boise, ID (“Boise”; est. 1963; 43.6514, -116.1883), and SpurWing Country Club in Meridian, ID (“Meridian”; est. 1995; 43.6680, -116.4286). The roughs of all courses were sprinkler irrigated and planted with Kentucky bluegrass (*Poa pratensis* L.). The predominant soil texture in roughs at all courses was a silty loam. During the study, private country clubs (Logan, Boise, and Meridian) spot-treated with applications of clothianidin or imidacloprid targeted at early instar billbugs (Arena 50 WDG at 8oz per acre and Criterion 2F at 20 oz per acre, respectively) once or twice per year. Draper was a public course that did not apply insecticides for insect pests throughout the entire survey due to a minimal budget for pest management.

2.1.2 Sampling methods

We placed six linear pitfall traps (Dupuy and Ramirez, 2016) in roughs at each course, for a total of 24 pitfall traps. Traps were placed no closer than 100 m to each other. Logan was sampled weekly from 13 May-15 October 2013, 20 March-10 October 2014, and 25 February-23 October 2015. Meridian and Boise were sampled weekly from 15 May-24 October 2013 and 26 April-27 September 2014. Draper was sampled weekly from 29 March-9 October 2014 and biweekly from 17 April-17 October 2015. All predatory arthropods were pinned and identified to genus or subgenus (beetles) or stored in 70% ethanol and identified to family (spiders) according to keys in Arnett et al., 2002 and Ubick et al., 2004, respectively. Beetles were identified to species when the species was evident based on notes on Nearctic genera in the key.

2.2 Collection and maintenance of arthropods common to field and lab experiments

Bluegrass and hunting billbugs used in experiments were field collected in linear pitfall traps and cup pitfall traps (consisting of two nested plastic cups, dug into the ground such that the top cup was flush with the soil surface) at Logan Golf and Country Club and Utah State University's (USU) Greenville Research Farm (subsequently referred to as "Greenville") in North Logan, UT (41.7661, -111.8107) in turf. Billbugs were identified to species and sexed according to keys in Johnson-Cicalese (1990) and stored by sex and species at 4 ^{o}C in petri dishes with moistened cotton wicks.

All predatory arthropods used in experiments were field collected from the same pitfall traps as billbugs and additional cup pitfall traps in alfalfa at Greenville. Predators were collected weekly and stored singly in vials at 4 ^{o}C with a moistened cotton wick. Predators were starved and used in assays within one month of collection. Before use in assays, predators were moved to 25 ^{o}C for 24 h. Major taxa of predators identified in section *2.1* were used in laboratory assays and included the ground beetles *Pterostichus (Morphnosoma) melanarius* (hereafter referred to as *P. melanarius*), *Harpalus (Pseudoophonus)* morpho-type A (hereafter referred to as *Harpalus* sp.), *Anisodactylus (Anadaptus)* morpho-type C (hereafter referred to as *Anisodactylus* sp.), and *Amara (sensu stricto) aenea* (hereafter referred to as *A. aenea*) and wolf spiders in the family

Lycosidae (Table 1). All lab experiments were conducted at Utah State University in Logan, UT.

2.3 Consumptive effects

2.3.1 Field assay: Direct consumption of adult billbugs by resident predators

To test rates of predation on adult billbugs in the field by resident predator populations, trials were conducted at Greenville on 17 June 2016, 6 July 2016, 16 August 2016, 21 June 2017, 18 July 2017, and 10 August 2017 and in a rough at Logan, on 21 June 2016, 21 June 2017, 19 July 2017, and 10 August 2017. Hunting and bluegrass billbug males were tethered with a 17.78 cm (7 in) microfilament (Umpqua nylon tippet 7X; Umpqua Feather Merchants, Louisville, CO) around the abdomen between the mid- and hind legs to a 10.16 cm (4 in) nail. Four 10.97 m (36 ft) transects were randomly placed at each site, two for each species of billbug, and a nail with a tethered billbug was placed along a transect every 0.91 meters (3 ft). In 2016 and 2017, respectively, 25 and 24 billbugs of each species were placed at each site at each date. Tethers were collected after 24 h, and billbug survivorship and signs of predation were recorded. Signs of predation included tethers with missing billbugs and microfilament that appeared torn or frayed, insect residue (haemolymph) left on the microfilament, billbug remains found in the turfgrass nearby, or dead billbugs with body parts missing still attached to tethers. Predation was not recorded in instances where billbugs were missing and none of the described signs were present.

Binomial field predation data (0=survival; 1=predation event) were analyzed with logistic regression (proc logistic, SAS Studio University Edition 9.4) to determine effects of site, date, and billbug species (fixed effects) on probability of predation. Firth's Penalized Likelihood option was utilized to address bias of parameter estimates for binomial response data.

2.3.2 Lab assay: Direct consumption of billbug mating pairs by the most abundant predators

Field assays were only able to show mortality of adult billbugs from suspected predation, therefore we also conducted laboratory assays using the most abundant predators identified in field surveys (section *2.1*). Bluegrass or hunting billbug mating pairs (one male and one female) were placed in arenas with a predator from one major taxon to determine the consumptive effects of the most abundant predators on billbug adults. An arena consisted of a sterile, 90 mm diameter petri dish and filter paper moistened with distilled water. Billbugs were acclimated in arenas for 30 min. before adding an individual predator, either *P. melanarius, Harpalus* sp., *Anisodactylus* sp., *A. aenea*, or a lycosid spider. In control arenas, to measure natural mortality of billbugs, a sterilized gravel rock approximately 15 × 5 mm was introduced in place of a predator. Mortality of billbugs was assessed 24 h after the predator was added to the arena. Filter paper was re-moistened with distilled water after 3 h, then again after 6 h. Number of replicate trials for each billbug species/predator taxon combination was determined by the availability of live predators. This resulted in 3 trials each for bluegrass and hunting billbugs with *A. aenea,* the most limiting predator; 8 with *Harpalus* sp.; 10 with *Anisodactylus* sp.; 10 with lycosids; and 13 (bluegrass billbugs) or 18 (hunting billbugs) with *P. melanarius,* the most abundant predator. There were 8 control trials for bluegrass billbugs and 12 controls for hunting billbugs.

Binomial mortality data (0=survival; 1=predation event) were analyzed with logistic regression (proc logistic, SAS Studio University Edition 9.4) to determine effects of predator and billbug species (fixed effects) on probability of predation. Firth's Penalized Likelihood option was utilized to address bias of parameter estimates for binomial response data. Only those predator taxa that caused billbug mortality were included in the analysis.

2.3.3 Lab assay: Direct consumption of billbug males by the most abundant predators

Identical arenas were also assessed with single males to determine if predators were more likely to attack or consume billbugs without the influence of two individuals. Males were used instead of females because they were more abundant and available from pitfall traps. Methods were identical to those used for mating pairs. Because of predator availability constraints, single males were assessed only with *P. melanarius* and *Harpalus* sp. We conducted 6 and 7 trial replicates with *Harpalus* sp., 10 and 6 replicates with *P. melanarius,* and 10 and 6 controls with bluegrass and hunting billbugs, respectively.

Binomial mortality data (0=survival; 1=predation event) were analyzed with logistic regression (proc logistic, SAS Studio University Edition 9.4) to determine effects of predator and billbug species (fixed effects) on probability of predation. Firth's

Penalized Likelihood option was utilized to address bias of parameter estimates for binomial response data.

2.3.4 Lab assay: Direct consumption of sentinel waxworm larvae by the most abundant predators at varied soil depths

We conducted lab bioassays using 4th instar *Galleria mellonella* (L.) (waxworms) as sentinel prey (purchased from Speedy Worm, Alexandria, MN) to assess the ability of common generalist predators to locate and feed on billbug larvae in soil (late instars). We used a sentinel prey rather than billbug larvae because effective methods of rearing billbugs under laboratory conditions have not been well-established (Johnson-Cicalese and Funk, 1990; Rondon and Walenta, 2008), and local turf infestations were not dense enough to efficiently field-collect billbug larvae. Additionally, working with field-collected larvae presents other challenges in determining mortality such as infection with pathogens. Waxworms are larger than billbug larvae, so if predators are capable of feeding on waxworms, we assume they are also capable of feeding on smaller billbug larvae.

One waxworm was buried in a 16-oz clear plastic cup (Dart Container Corp. Mason, MI) filled with field-collected, silty loam soil at a depth of 1, 3, 5, or 10 cm, representative of the depths at which later instar billbug larvae are found in soil (Doskocil and Brandenburg, 2012; Shetlar et al., 2012). Soil was collected at Greenville and was sieved through 5 mm mesh and autoclaved for 45 minutes before use in assays. The top, inner 2.54 cm (1 in) of each cup was painted with Insect-a-Slip (BioQuip Products, Inc. Rancho Dominguez, CA) to prevent predator escape. A predator was introduced on the soil surface, then each cup was covered with a sterile, 10 cm diameter glass petri dish lid. Number of replicates for each depth/predator combination was based on availability of predators. There were 3 replicates for *A. aenea* at each depth, 6 replicates for *Harpalus* sp., 7 replicates for *Anisodactylus* sp., 27 replicates for *P. melanarius*, and 22 replicates for lycosids. There were 7 control (waxworm with no predator) replicates at each depth to account for natural waxworm mortality during trials. Cups were placed on a laboratory benchtop at 25ºC, 15.7% RH, and 12:12 L:D cycle, and waxworms were recovered after 24 h. Position of waxworms and predators in the soil profile and mortality of waxworms due to predation were recorded.

Binomial larval predation data from cups was analyzed only for those predators and depths at which a predation event occurred and was compared to controls with logistic regression (proc logistic, SAS Studio University Edition 9.4). Because predation events occurred only at one depth, predator taxon was the sole fixed effect. Firth's Penalized Likelihood option was utilized to address bias of parameter estimates for binomial response data.

As an additional predator exposure control, given that larvae readily burrow in the soil, we used petri dish arenas (with moistened filter paper) with a single waxworm. This provided a method to determine whether predators were capable of feeding on waxworms on the same plane. We established 8 replicates with *P. melanarius*, 10 with *Harpalus* sp., 18 with *Anisodactylus* sp., 10 with *A. aenea*, and 8 with lycosids. We replaced waxworms in dishes as they were consumed (during the first 6 hours of the trial) and recorded mortality of waxworms and number of waxworms consumed by each predator after 24 h on laboratory bench tops (25°C, 15.7% RH, and 12:12 L:D cycle).

Binomial predation data on sentinel larvae in petri dishes was analyzed with logistic regression (proc logistic, SAS Studio University Edition 9.4), with predator taxon as the fixed effect. Firth's Penalized Likelihood option was utilized to address bias of parameter estimates for binomial response data.

2.3.5 Lab assay: Direct consumption of eggs in stems by the most abundant predators

To determine rates of consumption of billbug eggs, which are laid within turfgrass stems, we set up laboratory assays in which petri dish arenas (with moistened filter paper) contained a predator and a turf stem containing a concealed hunting billbug egg. Kentucky bluegrass seed was planted in the USU Research Greenhouse in Logan, UT and grown for 12 weeks before use in trials. Individual large stems (at least 4 cm tall and 1.5 mm thick) were plucked from pots no more than 1 h before use. To simulate billbug eggs that are protected within grass stems, we made a small incision in a stem with a fine blade. A billbug egg was then carefully inserted into the incision such that the egg was completely hidden within the stem. Billbug eggs were gathered from mated hunting billbug females that were left at 25 °C in a petri dish with moistened filter paper for approximately 72 h. Eggs were collected daily and stored at 4 °C in a 3 cm diameter petri dish with moistened filter paper until use. The lid of the petri dish was covered on the underside with Parafilm "M" (Bemis Company, Inc., Oshkosh, WI) to maintain relative humidity within the dish and reduce egg desiccation.

Predators were acclimated in arenas for 30 min. before stems with eggs were introduced. Arenas were placed on laboratory benchtops for 24 h (25°C, 15.7% RH, 12:12 L:D cycle), then stems were dissected, eggs were recovered, and signs of predator foraging on stems were recorded.

There were 16 replicates for *P. melanarius*, 12 replicates for *Harpalus* sp., 26 replicates for *Anisodactylus* sp., 6 replicates for *A. aenea*, and 10 replicates for lycosids. There were 14 control replicates with no predator to ensure eggs survived and were recoverable from stems after 24 h. Binomial egg predation data were analyzed with logistic regression (proc logistic, SAS Studio University Edition 9.4), with predator taxon as the fixed effect. Firth's Penalized Likelihood option was utilized to address bias of parameter estimates for binomial response data.

2.4 Non-consumptive effects

2.4.1 Assessment of billbug mating pair behavior in the presence of predators

Here we evaluated billbug behavior in petri dish arenas in the presence of predators without risk of consumption. Arenas consisted of a sterile, 90 mm diameter petri dish and filter paper moistened with distilled water. Each arena had a mating pair of either hunting or bluegrass billbugs. Predator treatments consisted of an introduction of individual *A. aenea, Harpalus sp., P. melanarius, Anisodactylus sp.*, or lycosid spiders, whose mouthparts were glued shut, rendering it incapable of killing or harming the billbugs, thereby isolating non-consumptive effects (Schmitz, 1998).

Observations on non-consumptive, behavioral responses to the presence of predators in each arena were made every one-half hour over 6 h, for a total of 12 observations per arena. It was recorded at each observation time whether both (2), one (1), or no (0) billbugs were active (multinomial response), whether or not billbugs were mating (binomial response), and number of billbug eggs that were laid in the arena (count response). The activity response included whether or not a billbug was moving at the time of the observation, which encompassed thanatosis responses. Thanatosis was not separated from general non-activity because there was no distinctive behavior change to differentiate it from simply not being active at limited observation time points.

The same behavioral responses described above were recorded in identical arenas assessing consumptive effects from section *2.3.2* in which unmanipulated predators (no glued mouthparts) were included with hunting and bluegrass billbug mating pairs, as well as control arenas. Glued and unglued predators were compared to one another to determine if unglued predator arenas could be included as replicates in analysis of billbug behavioral responses, to increase replication since number of trials we could conduct was limited by the availability of field-collected predators and billbugs. *Anisodactylus* sp. was the only predator to which billbugs responded differently to glued versus unglued predators (*F*=6.42, *df*=1, *P*=0.0124), indicating that this predator changed its behavior in response to the glued mouthparts treatment. Therefore, *Anisodactylus* sp. was not included in the primary analysis, but replicates without glued mouthparts were analyzed for non-consumptive effects on their own. However, other arenas from consumptive laboratory assays containing *P. melanarius, Harpalus* sp., *A. aenea,* and lycosids (section *2.3.2*) were included as replicates in the analysis, except if the replicate had an instance of billbug mortality (7 arenas with mortality). Therefore, we had a total of 21 replicates with

P. melanarius for bluegrass billbugs and 24 replicates for hunting billbugs. With *Harpalus* sp. there were 11 replicates with bluegrass billbugs and 16 with hunting billbugs. We had 6 trial replicates for *A. aenea* and 20 for lycosids with each species of billbug. There were 8 control replicates for bluegrass billbugs and 12 controls for hunting billbugs.

Data were analyzed within a 4 (predator type minus *Anisodactylus* sp.) × 2 (mouthpart manipulation—glued or not) × 2 (billbug species—hunting or bluegrass) complete factorial design plus control using a generalized linear mixed model (proc glimmix, SAS Studio University Edition 9.4). Fixed factors included billbug species, predator taxon, and predator manipulation level (mouthparts glued or not), all (except billbug species) nested within control (0) vs. predator (1) factor levels. This factor nesting allowed us to compare controls, which had no mouthpart manipulation level, to the remaining factors. The random factor was the individual petri dish arena (replicate). Denominator degrees of freedom for multinomial and binomial response variables (billbug activity and mating) were calculated using the Satterthwaite method. Count data (eggs) were analyzed with a generalized linear model (proc glimmix, SAS Studio University Edition 9.4), using the LaPlace estimation and a Poisson distribution. We used contrasts of least square means to make pairwise comparisons between each model effect.

Unmanipulated *Anisodactylus* sp. replicates, given differences in behavior between predators with glued and unglued mouthparts, were compared to controls only using a generalized linear mixed model. Billbug species and predator taxon (*Anisodactylus* sp. vs. control) were fixed effects while the individual petri dish arena (replicate) was the random effect. Denominator degrees of freedom for multinomial and binomial response variables (billbug activity and mating) were calculated using the Satterthwaite method. Count data (eggs) were analyzed with a generalized linear model (proc glimmix, SAS Studio University Edition 9.4), using the LaPlace estimation and a Poisson distribution. We used contrasts of least square means to make pairwise comparisons between each model effect.

2.4.2 Assessment of adult billbug male behavior in the presence of predators

Identical arenas were also assessed with single males to determine whether billbug behavioral responses to predators were different when they were alone versus with a mate. Following the same protocol as previously described, billbug activity was recorded at each observation time (binomial response). Pooled with unmanipulated predators from section *2.3.3* (except arenas with mortality—2 arenas—and *Anisodactylus* sp. arenas), there were 10 control replicates with bluegrass billbug and 6 with hunting billbugs. For *Harpalus* sp., there were 10 replicates with bluegrass and 14 with hunting billbugs. With *P. melanarius* there were 20 replicates with bluegrass and 12 with hunting billbugs.

Single male arenas were analyzed for the binomial response variable of activity only. *Anisodactlyus* sp. was again assessed separately from other predator taxa. Both analyses were as described in section *2.4.1* (for activity response only).

3. **Results**

3.1 Predatory arthropod community composition

The community of ground-dwelling predatory arthropods on golf course turf in Utah and Idaho was comprised mainly of Carabidae (60%) and spiders (28%). Major taxa of carabids included *Amara aenea, Pterostichus melanarius, Anisodactylus (Anadaptus)* sp. morphotype-C, and *Harpalus (Pseudoophonus)* sp. morphotype-A, comprising 23, 21, 16, and 6% of total carabids, respectively (Table 1). Sixty-six percent of all spiders captured were Lycosidae. Both carabids and lycosids overlapped temporally with adult billbugs in the field during all three years of collections (Fig. 1A-C). *P. melanarius* and *Harpalus* sp. appear to be active later in the season compared to billbug adults (activity beginning late May-mid-June) while *A. aenea, Anisodactylus* sp., and lycosids are active during the same general time frame as billbug adults (March-September). Other largely predatory groups included Staphylinidae (8%), Elateridae (3%), and Histeridae (<1%) (Table 1). Non-predatory taxa caught in pitfall traps included Curculionidae (other than billbugs), Dermestidae, Silphidae, and Tenebrionidae (Table 1).

3.2 Consumptive effects

3.2.1 Field assay: Direct consumption of adult billbugs by resident predators

Twenty-nine of 488 (5.97%) billbugs tethered in field trials showed signs of suspected predation. No differences were seen among sites, dates, or billbug species (X^2= 11.3, *df*=11 *P*=0.4186). The variation in predation occurrence fluctuated from 8.2% in June, to 2.8% in July, and 6.2% in August but was not statistically different (Fig. 2A).

3.2.2 *Lab assay: Direct consumption of billbug mating pairs by the most abundant predators*

P. melanarius and *Harpalus* sp. were the only predators to cause mortality to adult billbugs. Neither predator caused significantly more mortality of billbugs than the other (X^2= 1.82, *df*=2, *P*=0.4024). *Harpalus* sp. consumed bluegrass billbugs at a calculated 31% (5 of 16) in arenas containing mating pairs (Fig. 2B). *P. melanarius* consumed 5.6% (2 of 36) of hunting billbugs in arenas containing mating pairs (Fig. 2B).

3.2.3 *Lab assay: Direct consumption of billbug males by the most abundant predators*

Neither predator caused significantly more mortality than the other (X^2= 0.68, *df*=2, *P*=0.7124). *Harpalus* sp. consumed 33% (2 of 6) of bluegrass billbugs in arenas containing single males. No individual male hunting billbugs were consumed by either *Harpalus* sp. or *P. melanarius* (Fig. 2B).

3.2.4 *Lab assay: Direct consumption of sentinel waxworm larvae by the most abundant predators at varied soil depths*

Predators readily consumed waxworms that were in soilless arenas (i.e., not buried), supporting both our use of waxworms as sentinel hosts and the success of our predator starvation method. *P. melanarius* consumed 90% (9 of 10) of waxworms, *Harpalus* sp. consumed 70% (7 of 10), *A. aenea* consumed 40% (4 of 10), *Anisodactylus* sp. consumed 50% (9 of 18), and lycosids consumed 62.5% (5 of 8) of waxworms (Fig. 2C). *P. melanarius* is the only predator that fed on more than one waxworm, with 2 of 8 individuals consuming 2 waxworms each. No predators fed on significantly more waxworms than other predators (X^2= 6.9094, *df*=4, *P*=0.1408). Predation of sentinel larvae declined lower in the soil profile. *P. melanarius* and lycosids were the only predators to locate and consume waxworms buried in soil, and only at a depth of 1 cm. No waxworms buried deeper than 1 cm were consumed by any predator. Lycosids consumed 4.5% of waxworms that were buried at 1 cm (1 of 22), and *P. melanarius* consumed 22% of waxworms that were buried at 1 cm (6 of 27) (Fig. 2C). No predators fed on significantly more waxworms than other predators (X^2= 2.8020, *df*=2, *P*=0.2464).

3.2.5 Lab assay: Direct consumption of eggs in stems by the most abundant predators

Harpalus sp. fed on eggs hidden in stems at a rate of 16.7% (2 in 12), *P. melanarius* at 31.25% (5 in 16), *A. aenea* at 33.33% (2 in 6), and *Anisodactylus* sp. at 46.15% (12 in 26) (Fig. 2D). One hundred percent of eggs were recovered from controls, and *Anisodactylus* sp. is the only predator with significantly less egg recovery than controls (*z*=-2.09, *P*=0.0366). The only predator that did not feed on eggs was Lycosidae.

3.3 Non-consumptive effects

3.3.1 Assessment of billbug mating pair behavior in the presence of predators

When billbug mating pairs, regardless of species, were in the presence of *A. aenea*, *Harpalus* sp., and lycosids their activity decreased by 56, 55, and 26%, respectively compared to controls (*F*=16.56, *df*=3, 129.3, *P*<0.0001). Billbug activity decreased by only 7% in the presence of *P. melanarius* and was not significantly different from controls (*t*=0.86, *df*=107.5, *P*=0.3899) (Fig. 3A). The interaction between predator taxon and billbug species was significant (*F*=4.51, *df*=3, 129.3, *P*=0.0048). This was driven by *A. aenea* reducing bluegrass billbug activity more than hunting billbug activity. Bluegrass billbug activity in the presence of *A. aenea* was significantly lower than bluegrass billbug activity in controls (*t*=4.78, *df*=133, *P*<0.0001), while hunting billbug activity in arenas with *A. aenea* was not significantly lower than controls (*t*=1.02, *df*=108.9, *P*=0.3104) (Fig. 3A). Bluegrass and hunting billbugs also responded differently to *Harpalus* sp.—bluegrass billbug reduced its activity more than hunting billbug (*t*=-2.04, *df*=116.8, *P*=0.0437), but both species had significantly lower activity than controls (bluegrass: *t*=5.07, *df*=115.7, *P*<0.0001; hunting: *t*=3.42, *df*=108, *P*=0.0009). In arenas with other predators, bluegrass and hunting billbugs had similar levels of activity (Fig. 3A). *Anisodactylus* sp. (unglued mouthparts) reduced the activity of billbug mating pairs compared to controls (*F*=13.38, *df*=1, 29.54, *P*=0.0010). There was no difference in the way bluegrass and hunting billbugs responded to *Anisodactylus* sp. (*F*=1.43, *df*=1, 29.54, *P*=0.2420) (Fig. 3A).

Bluegrass billbugs did not mate in petri dish arenas, therefore instances of mating were assessed for hunting billbugs only. Hunting billbugs exposed to predators mated significantly less compared to no-predator control treatments (*F*=9.87, *df*=1, 55.08, *P*=0.0027) (Fig. 3C). On average, mating activity was calculated at 31% of observations in controls, which was significantly more than the mating activity of billbugs exposed to *Harpalus* sp. (2.3%; *t*=3.39, *df*=71, *P*=0.0099) or *P. melanarius* (3.7%; *t*=2.96, *df*=71, *P*=0.0332) (Fig. 4B). However, hunting billbugs in the presence of *A. aenea* (13%; *t*=1.05, *df*=58.32, *P*=0.8306) and lycosids (7%; *t*=2.39, *df*=55.56, *P*=0.1312) were not statistically different from no-predator controls (Fig. 3C). Hunting billbugs also mated significantly less in the presence of *Anisodactylus* sp. compared to controls (*F*=5.99, *df*=1, 20, *P*=0.0238) (Fig.3C).

Egg counts were assessed for hunting billbug only because bluegrass billbugs did not lay eggs in petri dishes. On average, hunting billbugs deposited 0.58 eggs in no-predator controls, but the impact of predators on oviposition ranging from an average 0-0.2 eggs per replicate was not significantly different (*F*=0.09, *df*=3, 71, *P*=0.9648) (Fig. 3D). There was no difference in the number of eggs laid by hunting billbugs in the presence of *Anisodactylus* sp. compared to controls (*F*=0.37, *df*=1, 20, *P*=0.5485) (Fig. 3D).

3.3.2 Assessment of adult billbug male behavior in the presence of predators

Harpalus sp. and *P. melanarius* did not reduce the activity of males alone compared to controls, but billbug species responded differently to different treatments (*F*=7.34, *df*=1, 64, *P*=0.0086). Bluegrass billbugs were more active than hunting billbugs in both controls and arenas containing *P. melanarius*, but not in arenas containing *Harpalus* sp. (Fig. 3B).

4. **Discussion**

The turf predator community in Utah and Idaho is similar to predator communities in turf sites throughout North America (Cockfield and Potter, 1984; Braman and Pendley, 1993; Dobbs and Potter, 2014). *Pterostichus* spp., *A. aenea,* and lycosids, in particular, are ubiquitous in turfgrass (Cockfield and Potter, 1984; Braman and Pendley, 1993; Jo and Smitley, 2003). Typically, carabid activity was higher than that of lycosids, and lycosids were particularly sparse in pitfall traps during 2015 collections. In 2015, we collected only at Logan and Draper sites, and these are the sites with the lowest lycosid activity (Table 1). This and other differences among sites and years in predator community composition may be attributed to a number of factors including site age, temperature and precipitation, and landscape diversity. Draper and Meridian, the most recently established courses, generally had lower predatory arthropod activity and diversity (Table 1). Common generalist predators overlap temporally with billbugs, but their activity does not seem to correspond strongly to peaks of billbug activity, and they maintain low levels of activity without strong cyclical activity (Fig. 1A-C). This suggests that billbug adults are not a primary source of food for generalist predators. Carabids are typically univoltine, but adult longevity can exceed one season (up to four years) for certain, typically larger, species, including members of Harpalini and Pterostichini (Lovei and Sunderland, 1996). Lycosids often have biennial life cycles and females can be similarly long-lived (Pickavance, 2001), thus it is expected that populations of these long-lived adults are smaller than those of billbugs, which likely has implications for predator effects on billbugs in the field.

In an attempt to evaluate predation on billbug adults in the turf landscape, we were surprised to find that overall predation rates in the field were low (5.97%), particularly given the abundance and diversity of predators that are present in turf. It is important to note that billbugs may have been consumed by any potential predator that resided at turfgrass sites, including larger vertebrate animals like birds and toads. We were unable to determine specific predators involved with each predation event.

However, our lab assays pairing each predator taxon with billbug adults to evaluate direct consumption showed similar low level predation by the most abundant predators. Specifically, the larger carabids (*P. melanarius* and *Harpalus* sp.) were capable of subduing and consuming billbugs adults. Some species of large carabids and wolf spiders are known to feed on adults of Coleoptera, however, it is likely to be uncommon when alternate, soft-bodied prey is available (Lovei and Sunderland, 1996; Kromp, 1999; Rendon, 2016).

Generalist predators in turfgrass systems have been shown to have strong effects on surface-feeding larvae, such as black cutworm (Hong et al., 2011), and predators in assays were fully capable of feeding on sentinel larvae when “exposed at the surface” in petri dish controls (Fig. 2C). However, only *P. melanarius* and lycosids were capable of feeding on larvae buried in soil. Larval predation in soil was low, and though many predators showed an affinity for burrowing into the soil, most did not feed on waxworms. Larvae at depths below 1 cm appeared to escape predation, even when predators were found up to 5 cm deep in the soil profile. It is possible that predation of billbug larvae is much more challenging under field conditions given their small size compared to waxworms, protection of early instars within stems, and locations of root feeding larvae that are likely deeper than 1 cm (Doskocil and Brandenburg, 2012; Shetlar et al., 2012). We understand that in using waxworms as sentinel prey, we may be missing important factors such as billbug host cues or root volatiles from billbug feeding. Particularly, olfactory cues have been shown to be important for ground beetle species including *P. melanarius* and *Harpalus rufipes* (DeGeer) and the lycosid *Trochosa parthenus*

(Chamberlin) (Kielty et al., 1995; Punzo and Kukoyi, 1997). Therefore, it is possible that our laboratory assays underestimate predation upon soil larvae in the field, given that we used a sentinel prey to which predators may respond differently to than a prey they come into contact with in their native ecosystem.

The greatest consumptive effects of generalist predators on billbugs resulted from carabids finding and consuming billbug eggs hidden in turf stems. Egg predation by carabids on several taxa of pests, especially dipterans, is well-known (Kromp, 1999), and egg predation has been shown to be important for turf pests including black cutworm and Japanese beetle (Lopez and Potter, 2000). Members of the genus *Anisodactylus* have been shown to feed on eggs of Colorado potato beetle (*Leptinotarsa decemlineata* Say) in commercial potato fields (Hazzard et al. 1991), so it is possible that *Anisodactylus* sp., which consumed nearly 50% of eggs in lab assays (Fig. 3D), may also feed on billbug eggs at high rates in the field. Interestingly, hunting billbugs did not lay fewer eggs in the presence of *Anisodactylus* sp. compared to controls, even though it represents the greatest risk to billbug eggs out of all the predators tested. This indicates that billbugs may not be able to assess risk to eggs by predators, regardless of predator type.

The strength of consumptive effects may be diminished in the field by several factors including the polyphagous nature of carabids, especially omnivory by genera like *Harpalus* that largely feed on plants and seeds (Kromp, 1999), and intraguild predation, which is commonly seen in ecosystems containing multiple species of carabids (Prasad and Snyder 2006). Additionally, the low activity of generalist predators compared to billbugs, as indicated by pitfall trap captures (Fig. 1A-C), suggests a disparity in population densities that may dilute predator impacts. Ideally, caged field experiments where densities of both billbug life stages and predators are manipulated could be used to confirm consumptive effects of generalist predators on billbug life stages in a more complex field setting. However, there are many challenges associated with such experiments. As previously mentioned, effective methods of rearing billbugs under laboratory conditions have not been established (Johnson-Cicalese and Funk, 1990; Rondon and Walenta, 2008), and previous attempts at seeding field cages with billbug mating pairs have been unsuccessful (MMD personal observation). Field-collecting enough predators and billbug life stages to complete such a field experiment with adequate replication is a major hurdle that may only be overcome when effective rearing techniques are established.

Generalist predators had non-consumptive effects on billbugs through significantly reducing activity levels of billbug mating pairs and mating activity compared to controls. We observed thanatosis and hiding under filter paper in arenas as defensive responses contributing to overall reduced activity. Similar impacts of predators are seen in other systems, where predator presence or predation risk has reduced prey activity in terms of foraging effort or mating or has influenced prey oviposition choices (Sih et al., 1990; Schmitz, 1997; Janssen et al., 1998; Blaustein et al., 2004; Preisser et al., 2005). Such effects have been shown to have positive impacts on plant productivity (Schmitz et al., 1997; Snyder and Wise, 2000). If responses to predators resulting in reduction of activity and mating are as strong in the field as they are in the laboratory, generalist predators could have important biological control benefits in the turf system despite low or unlikely direct consumption in a field setting, particularly against the adult stage.

Billbug mating pairs reduced their activity in the presence of all predators compared to controls, except for *P. melanarius*, suggesting that certain billbug defensive responses may be somewhat predator-specific. *P. melanarius* in assays on consumptive effects was observed attacking billbug mating pairs during 20% of observation times on average, and this aggressive behavior may have caused billbugs to attempt to escape rather than a typical thanatosis response. Running or walking away is a common defensive response to danger known in many different animals, including humans. In insects, specifically, walking away from predators has been observed in Colorado potato beetle larvae, *Enallagma* spp. damselflies (i.e., swimming away), pipevine caterpillars (*Battus philenor* L.), aphids (Hemiptera: Aphididae), among others (Stamp, 1986; McPeek, 1990; Gross, 1993; Ramirez et al., 2010). Additionally, walking away from a stimulus instead of a typical thanatosis response has been observed in other weevils, specifically *Cylas formicarius* (F.) (Miyatake, 2001). Though billbugs paired with *P. melanarius* had similar levels of activity compared to controls, the components of "activity" were different. Billbugs paired with *P. melanarius* had significantly reduced mating compared to controls (Fig. 3C). Thus, the presence of *P. melanarius* in turfgrass may still have important impacts on billbug fitness, as time spent avoiding predator attacks is time not spent mating.

There were differences in the way billbug species responded to different predators for both assays with mating pairs and assays with single males. In assays with mating pairs, bluegrass billbugs in the presence of *A. aenea* were significantly less active than bluegrass billbugs in controls, but hunting billbugs paired with *A. aenea* had no difference in activity compared to controls. This is possibly because *A. aenea* is smaller than hunting billbugs (6-8 mm vs. 7-9 mm, respectively), thus it may not pose the same threat, real or perceived, as it does to smaller bluegrass billbugs. Bluegrass billbugs also reduced their activity in the presence of *Harpalus* sp. more than hunting billbugs. This is potentially because of the same size/risk association as seen with *A. aenea,* however both species were significantly less active than their control counterparts (Fig. 3A). Activity of single males paired with *P. melanarius* or *Harpalus* sp. was not reduced compared to controls, and single bluegrass billbug males in controls and arenas with *P. melanarius* were significantly more active than their hunting billbug counterparts, suggesting that there are differences in the way billbug species respond to predators when in the presence of a conspecific of the opposite sex versus when they are alone. Olfactory cues of females influencing male behavior is well-known for insects and may play a role here (Shorey, 1973). Additionally, thanatosis has been shown to be more advantageous to animals, including certain insects, when in groups with conspecifics (Rogers and Simpson, 2014). Thus, the relative advantage of thanatosis-driven reductions in activity may also play a role in the differences between mating pair and single male billbug responses to predators.

In these assays we concentrated on the most abundant ground-active predators from pitfall trap captures because they are the most likely to come into contact with ground-active billbug adults. We are aware that ants are an important part of the turf predator community, however, our pitfall traps did not allow for successful ant captures, as mesh was large enough for them to escape through. It would be worthwhile to investigate ants within biological control programs for the suppression of billbugs, especially soil-dwelling larvae, as ants have been shown to be important for other larval turf pests (Lopez and Potter, 2000; Dobbs and Potter, 2014; Dobbs and Potter, 2016). Additionally, other, less abundant predators may have larger impacts on billbugs if conservation biological control specifically targets enhancing their populations. For instance, very large (~2.54 cm length) carabids found in low numbers in our pitfall traps, including *Carabus* sp., *Harpalus (Megapangus)* sp., and *Pterostichus (Metallophilus)* sp. (Table 1), are likely to be more adept at feeding on billbug adults than smaller, but more abundant carabid species. In fact, an informal laboratory pilot involving one individual of *Harpalus (Megapangus)* sp. and Rocky Mountain billbug adults, the largest of the Intermountain West species, showed that this large carabid was readily capable of consuming billbug adults in a petri dish arena (MMD, personal observation). Billbugs are also known to be host to Hymenopteran parasitioids including *Zavipio (Vipio) belfragei* (Cresson; Branconidae) reared from larvae and *Anaphes (Anaphoidea) calendrae* (Gahan; Myrmaridae) on eggs (Young 2002). Indeed, a Hymenopteran parasitoid has been observed in billbug eggs in the Intermountain West (MMD, personal observation). However, whether *A. calendrae* has extended its range from the previously known eastern U.S. or if the Intermountain West egg parasitoid is a different species is yet to be determined. Conservation biological control strategies such as flowering borders have been shown to enhance populations of parasitoids in turfgrass (Braman et al. 2002), and similar effects on billbug parasitoids and resulting impacts on billbug populations should be assessed.

5. Conclusions

Predators are abundant in Intermountain West turfgrass but have low levels of activity compared to those of billbugs. Conservation biocontrol strategies such as conservation strips and beetle banks have shown to enhance populations of predatory arthropods, including those assessed here (Frank and Shrewsbury, 2004; MacLeod et al., 2004; Dobbs and Potter, 2016). Support for conservation biological control of generalist predators as part of a billbug management program is strongest for direct consumption of billbug eggs and the non-consumptive impact of predators on billbug adult behavior through reductions in activity and mating, which may reduce billbug fitness and ultimately increase turf health. Future research should assess the efficacy of conservation biocontrol as it relates to suppression of billbugs in the field and specific strategies to enhance particularly lethal and threatening predator species in the turf system.

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Tables

Table 1. All non-billbug taxa captured in pitfall traps at 4 study sites in Utah and Idaho in 2013, 2014, and 2015. Taxa are listed in alphabetical order and are reported as total number of individuals at each site over the entire collection period. Relative abundance is reported as both proportions of taxa within their family and proportions of taxa out of all pitfall trap captures. Families containing predatory members are indicated with an asterisk (*).

	Logan Golf	**Draper**	**Meridian**	**Boise**	**All Sites**	**Relative Abundance (within family)**	**Relative Abundance (out of total)**
***CARABIDAE** (ALL)	**685**	**316**	**235**	**859**	**2095**		**0.4794**
Acupalpus meridianus	0	1	0	0	1	0.0005	0.0002
Agonum sp.	3	0	1	0	4	0.0019	0.0009
Amara (Bradytus) sp. A	61	47	2	3	113	0.0539	0.0259
Amara (Bradytus) sp. B	14	1	12	0	27	0.0129	0.0062
Amara (Bradytus) sp. C	0	66	4	0	70	0.0334	0.0160
Amara (Bradytus) sp. D	0	2	0	0	2	0.0010	0.0005
Amara (Bradytus) sp. E	0	3	2	18	23	0.0110	0.0053
Amara (Curtonotus) sp. A	1	11	1	1	14	0.0067	0.0032
Amara (Curtonotus) sp. B	0	0	1	0	1	0.0005	0.0002
Amara (sensu stricto) aenea	80	68	31	299	478	**0.2282**	**0.1094**
Amara (Zezea) sp.	0	0	1	0	1	0.0005	0.0002
Anisodactylus (Anadaptus) sp. A	40	10	31	100	181	0.0864	0.0414
Anisodactylus (Anadaptus) sp. B	14	5	3	7	29	0.0138	0.0066
Anisodactylus (Anadaptus) sp. C	192	19	18	98	327	**0.1561**	**0.0748**
Anisodactylus (sensu stricto) sp.	5	9	6	17	37	0.0177	0.0085
Bradycellus (Catharellus) leconti	0	0	0	1	1	0.0005	0.0002
Calathus sp.	0	0	0	1	1	0.0005	0.0002
Carabus sp.	0	13	0	1	14	0.0067	0.0032
Clivina fossor	30	12	16	8	66	0.0315	0.0151
Dicheirus piceus	0	0	3	3	6	0.0029	0.0014
Harpalus (Megapangus) sp.	1	0	0	0	1	0.0005	0.0002
Harpalus (Pseudoophonus) sp. A	87	3	5	41	136	**0.0649**	**0.0311**
Harpalus (Pseudoophonus) sp. B	25	13	0	6	44	0.0210	0.0101
Harpalus (Pseudoophonus) sp. C	12	1	0	4	17	0.0081	0.0039
Harpalus (sensu stricto) sp.	10	2	1	1	14	0.0067	0.0032
Loricera sp.	2	0	6	0	8	0.0038	0.0018
Pogonus sp.	1	0	1	0	2	0.0010	0.0005
Pterostichus (Metallophilus) sp.	0	0	0	1	1	0.0005	0.0002

Pterostichus (Morphnosoma) melanarius	106	1	81	249	437	**0.2086**	**0.1000**
Rhadine sp.	0	2	0	0	2	0.0010	0.0005
Selenophorus sp.	0	17	4	0	21	0.0100	0.0048
Stenolophus sp. A	1	6	3	0	10	0.0048	0.0023
Stenolophus sp. B	0	0	2	0	2	0.0010	0.0005
Synuchus sp.	0	4	0	0	4	0.0019	0.0009
CURCULIONIDAE (ALL)	**23**	**40**	**4**	**171**	**238**		**0.0545**
Barypeithes pellicudus	7	25	0	159	191	0.8025	0.0437
Centrinogyna sp.	2	2	0	0	4	0.0168	0.0009
Hypera zoilus	0	0	0	1	1	0.0042	0.0002
Listronotus sp.	4	0	0	0	4	0.0168	0.0009
Miloderoides sp.	0	1	0	0	1	0.0042	0.0002
Otiorhynchus ovatus	6	5	0	1	12	0.0504	0.0027
Peritelinus sp.	0	4	0	0	4	0.0168	0.0009
Sitona hespedulis	0	0	0	2	2	0.0084	0.0005
Sitona lineatus	4	3	4	8	19	0.0798	0.0043
DERMESTIDAE (ALL)	**8**	**0**	**1**	**39**	**48**		**0.0110**
Dermestes sp. A	7	0	0	39	39	0.8125	0.0089
Dermestes sp. B	1	0	0	0	0	0.0000	0.0000
Lepidocnemplatia sericea	0	0	1	0	1	0.0208	0.0002
***ELATERIDAE** (ALL)	**26**	**28**	**16**	**24**	**94**		**0.0215**
Aeolus sp. A	4	12	15	17	48	0.5106	0.0110
Aeolus sp. B	3	0	0	1	4	0.0426	0.0009
Conoderus sp.	19	15	1	6	41	0.4362	0.0094
Lanelater sp.	0	1	0	0	1	0.0106	0.0002
***HISTERIDAE** (ALL)	**0**	**1**	**0**	**18**	**19**		**0.0043**
Euspilotus sp.	0	0	0	**2**	2	0.1053	0.0005
Geomysaprinus sp.	0	1	0	**7**	8	0.4211	0.0018
Hister sp.	0	0	0	**4**	4	0.2105	0.0009
Hypococcus sp.	0	0	0	**2**	2	0.1053	0.0005
Margarinotus sp.	0	0	0	**1**	1	0.0526	0.0002
Unknown Histeridae	0	0	0	**2**	2	0.1053	0.0005
SCARABAEIDAE (ALL)	**277**	**79**	**32**	**18**	**406**		**0.0929**
Aphodius (Planolinoides) sp.	239	23	0	0	262	0.6453	0.0600
Aphodius (Chilothorax) distinctus	3	2	2	2	9	0.0222	0.0021
Aphodius (Dellacasiellus) sp.	0	0	2	6	8	0.0197	0.0018
Aphodius (Ballucus) sp.	1	0	0	1	2	0.0049	0.0005
Ataenius spretulus	34	43	14	9	100	0.2463	0.0229
Ataenius sp. A	0	11	0	0	11	0.0271	0.0025

Rhyssemus neglectus	0	0	14	0	14	0.0345	0.0032
SILPHIDAE (ALL)	**0**	**0**	**0**	**146**	**146**		**0.0334**
Heterosilpha sp.	0	0	0	140	140	0.9589	0.0320
Nicrophorus sp.	0	0	0	2	2	0.0137	0.0005
Thanatophilus lapponicus	0	0	0	4	4	0.0274	0.0009
***STAPHYLINIDAE** (ALL)	**191**	**11**	**9**	**66**	**277**		**0.0634**
Aleocharinae	24	2	1	4	31	0.1119	0.0071
Apocellus sp. A	64	0	0	0	64	0.2310	0.0146
Apocellus sp. B	4	0	0	15	19	0.0686	0.0043
Eriksonius sp.	0	0	0	2	2	0.0072	0.0005
Guaropterus fulgiclus	2	0	0	0	2	0.0072	0.0005
Ocypus sp.	4	4	2	30	40	0.1444	0.0092
Paederini	0	0	0	1	1	0.0036	0.0002
Philonthus sp. A	10	0	0	1	11	0.0397	0.0025
Philonthus sp. B	77	3	5	2	87	0.3141	0.0199
Pseudopsis sp.	0	0	1	0	1	0.0036	0.0002
Quedius sp. A	0	2	0	1	3	0.0108	0.0007
Quedius sp. B	0	0	0	1	1	0.0036	0.0002
Tachinus sp.	0	0	0	1	1	0.0036	0.0002
Tachyporus sp.	0	0	0	1	1	0.0036	0.0002
Tasgius sp.	1	0	0	5	6	0.0217	0.0014
Xantholini: *Hesperolinus* sp.	5	0	0	2	7	0.0253	0.0016
TENEBRIONIDAE (ALL)	**3**	**52**	**2**	**5**	**62**		**0.0142**
Blapstinus sp.	2	42	1	1	46	0.7419	0.0105
Coniontis sp.	0	5	0	0	5	0.0806	0.0011
Eleodes sp.	0	3	0	2	5	0.0806	0.0011
Neobaphion sp. A	1	1	0	0	2	0.0323	0.0005
Neobaphion sp. B	0	0	0	2	2	0.0323	0.0005
Tenebrio sp.	0	1	0	0	1	0.0161	0.0002
Trichoton sordidum	0	0	1	0	1	0.0161	0.0002
***ARANEAE** (ALL)	**110**	**197**	**357**	**321**	**985**		**0.2254**
Dysderidae	8	4	4	0	16	0.0162	0.0037
Gnaphosidae	20	54	62	23	159	0.1614	0.0364
Linyphiidae	14	8	12	9	43	0.0437	0.0098
Lycosidae	35	112	231	269	647	**0.6569**	**0.1481**
Opiliones	14	1	2	8	25	0.0457	0.0103
Salticidae	0	1	2	1	4	0.0254	0.0057
Theridiidae	2	0	0	0	2	0.0041	0.0009
Thomisidae	7	12	23	2	44	0.0020	0.0005
Not found/unable to ID	10	5	21	9	45	0.0447	0.0101

Figures

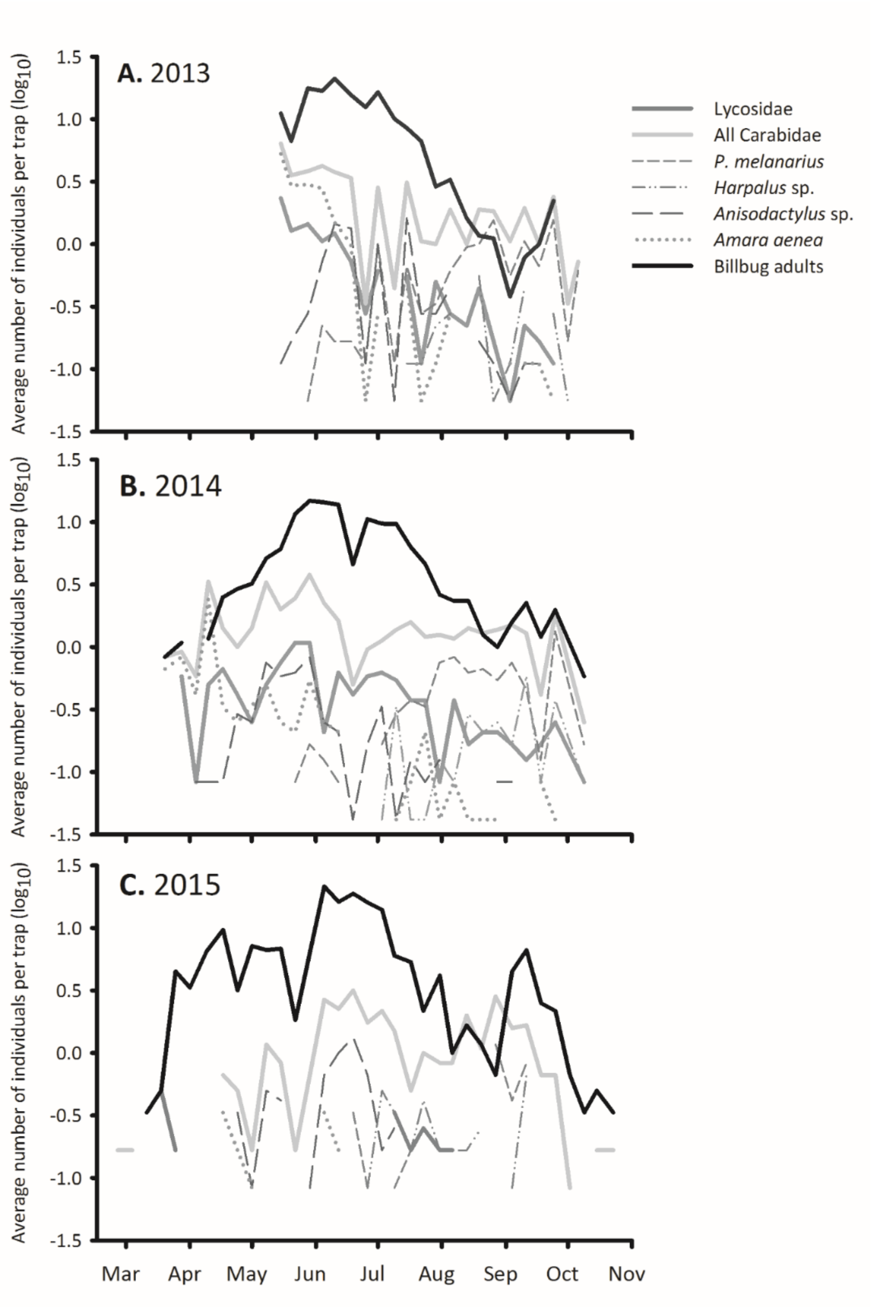


Figure 1. Seasonal activity of billbugs and generalist predatory arthropods in the Intermountain West for (A) 2013 surveys in Logan, Boise, and Meridian, (B) 2014 surveys in Logan, Draper, Boise, and Meridian, and (C) 2015 surveys in Utah (Logan and

Draper). Average captures per trap are plotted on the $\log_{10}$ scale to account for large disparity between levels of billbug activity (high) and levels of predatory arthropod activity (low).

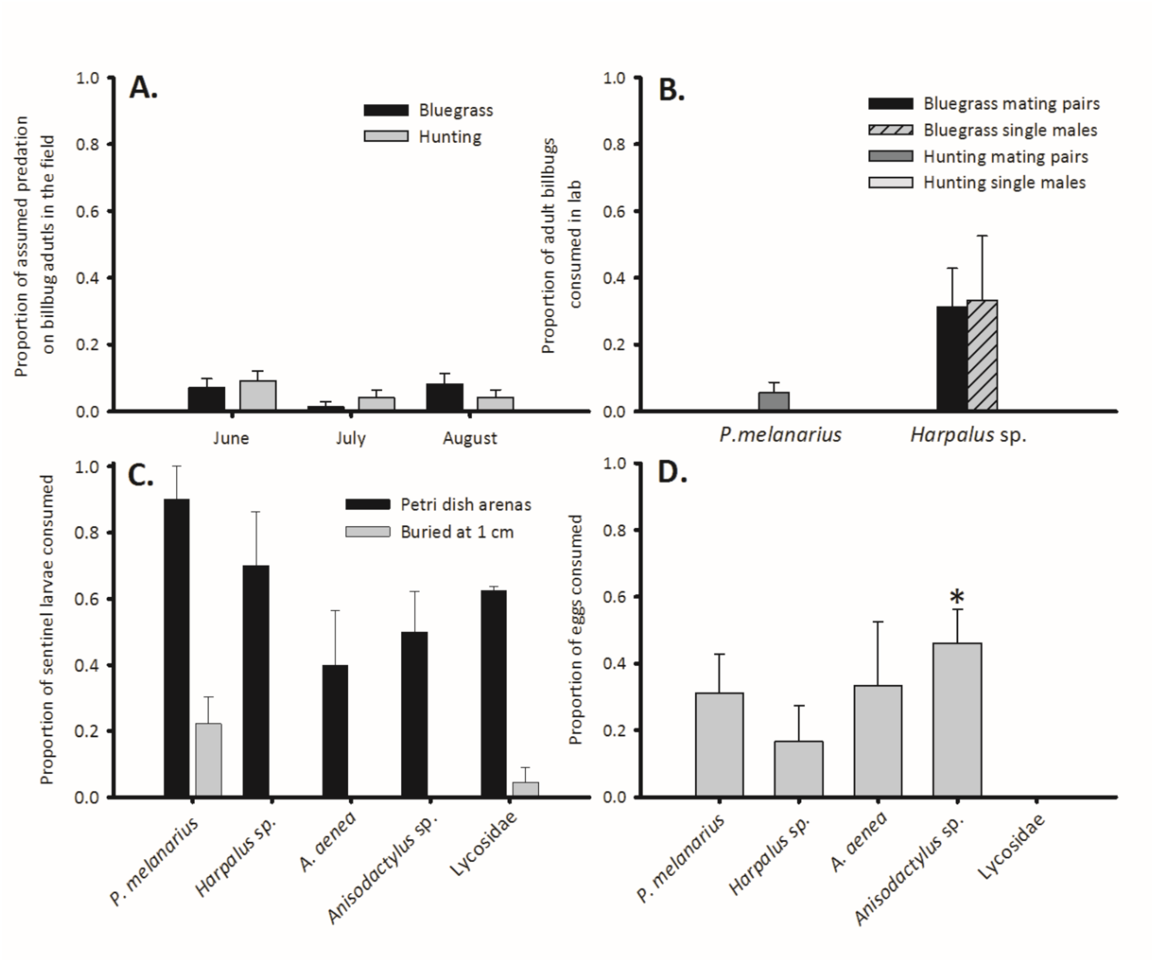


Figure 2. Consumptive effects of predators on billbug life stages including assumed predation of bluegrass (black bars) and hunting (grey bars) billbug adults in the field (A), consumption of bluegrass billbug mating pairs (black bars) and single males (grey bars with diagonal stripes) and hunting billbug mating pairs (dark grey bars) and single males (light grey bars, not shown because no hunting billbug single males were consumed) billbug adults in the lab (B), consumption of sentinel larvae in petri dishes (black bars) and buried at 1 cm in soil (grey bars) (C), and of eggs within stems in the lab (bar color has no meaning) (D). Bars with an asterisk (*) are significantly different from controls (not shown), where no consumption occurred.

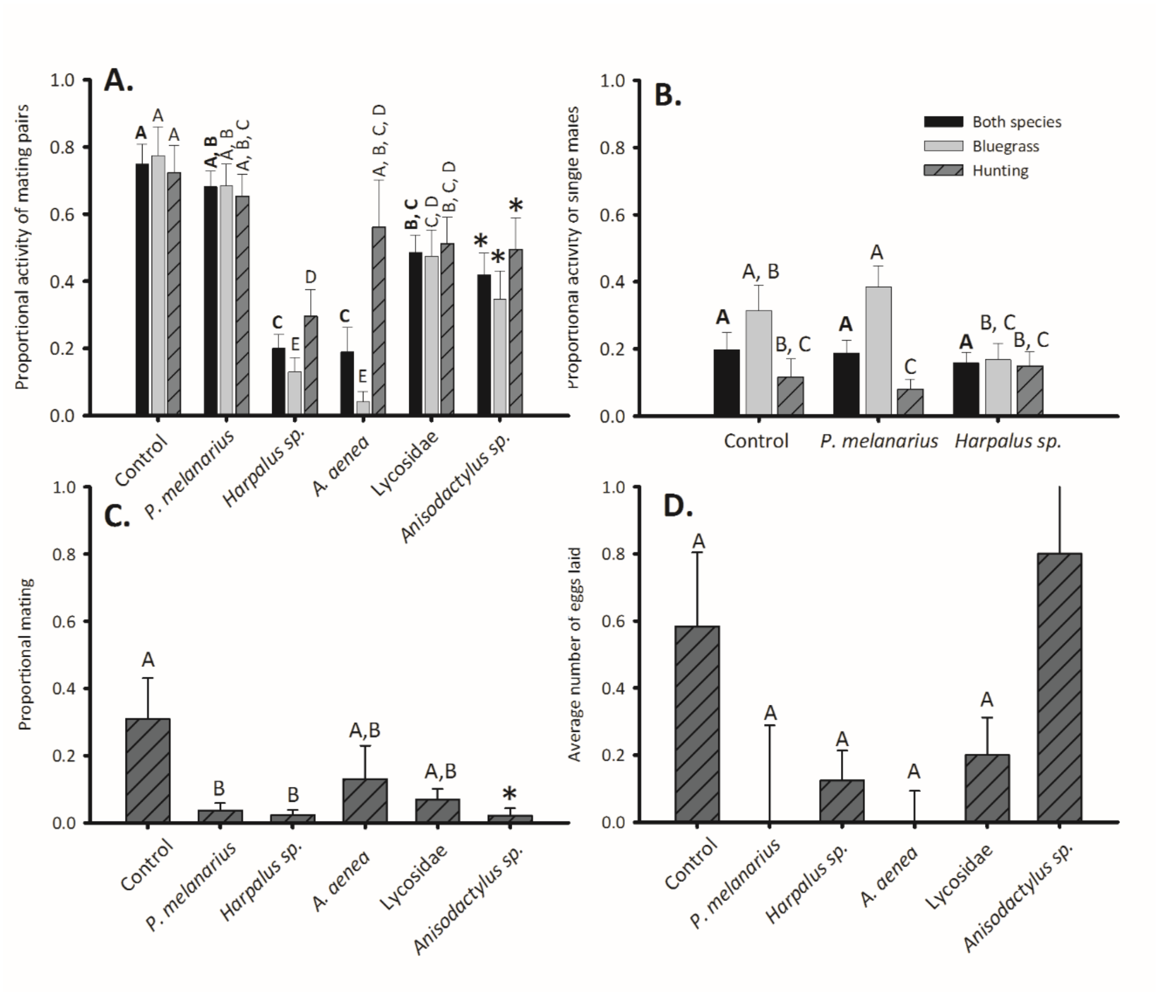


Figure 3. Non-consumptive effects of predators on the activity level of billbug mating pairs (A) and single male billbugs (B), hunting billbug mating activity (C), and hunting billbug oviposition (D) (note: average eggs laid were <0.001 in *P. melanarius* and *A. aenea* treatments). Main effects of predators on both billbug species combined are represented by black bars, bluegrass billbugs are represented by light grey bars, and hunting billbugs are represented by dark grey bars with diagonal stripes. Bars with the same letter within a graph are not significantly different from one another. Bars with a **bolded** letter within a graph are not significantly different from bars with the same

bolded letter (i.e., black bars for predator main effects on both species of billbug). Bars showing data from *Anisodactylus* sp. are significantly different from controls if they have an asterisk (*; separate analysis). Significance was determined according to post-hoc T-groupings at alpha=0.05.

CHAPTER V

GENERAL SUMMARY AND CONCLUSIONS

General summary

Billbugs are a major turfgrass pest in the Intermountain West; however, regional research and best management practices for this pest are lacking. Billbug management currently relies on preventive, calendar-based applications of systemic, long-residual insecticides, applied regardless of whether or not billbugs will be problematic. Commonly used insecticides against billbugs, including neonicotinoids, have been shown to harm beneficial insects such as pollinators and predators among other negative environmental impacts. However these predators may provide natural pest suppression services in the turf system. An integrated approach to billbug management involving several strategies such as monitoring, predictive modelling, and biological control, is necessary to ensure sustained, effective management of billbugs in the Intermountain West.

In my first study, I determined the seasonal activity of billbug life stages on golf courses in Utah and Idaho and used those data to develop a degree-day model. I found a complex of three species infesting Intermountain West turf, including bluegrass, hunting, and Rocky Mountain billbug. I found that a previously developed degree-day model for bluegrass billbugs in Ohio was not robust enough to predict the activity of the billbug complex in Utah and Idaho. The best fit model to predict adult billbug activity in Utah and Idaho accumulates degree-days above 3°C after 13 January.

In my second study, I evaluated eastern management timing recommendations, as predicted by the Utah-Idaho degree-day model. I evaluated both traditionally used turf insecticides, including neonicotinoids and anthranilic diamides, and newer, microbial-based bioinsecticides. I found that eastern management recommendations for preventive and curative timings were effective times to apply traditionally used synthetic turf insecticides, but bioinsecticides showed high variability in efficacy and may not be appropriate for single-application use.

In my third study, I investigated the potential impact of conservation biological control by resident populations of predatory arthropods on suppression of billbugs. I tracked the seasonal activity and community composition of predatory arthropods at golf courses in Utah and Idaho. I found the predatory arthropod community to be diverse and composed primarily of carabids and wolf spiders. Resident predators had low rates of direct consumption of billbugs in the field. I evaluated the impact of a few major taxa of generalist predators on billbug life stages in the lab. Predators had high rates of consumption on billbug eggs and affected billbug behavior by reducing both their general activity and mating.

Conclusions

These studies represent the foundation of an integrated approach to billbug management in the Intermountain West. With regional data on billbug phenology and a robust predictive model, turfgrass managers can begin to move away from calendar-based insecticide applications. In combination with monitoring, managers may use the degree-day model to more efficiently time insecticide applications with model predictions for preventive and curative billbug management, ideally reducing overall use of insecticides. Further, my research sheds light on the importance of resident populations of predatory arthropods in the potential natural suppression of billbugs. Particularly, important effects of predatory arthropods on billbugs support the development of conservation biological control strategies to enhance populations of effective predators in turfgrass. Ultimately, my research offers some practical and sustainable solutions to billbug management in Intermountain West turfgrass, while concurrently opening the doors for further investigation of integrated management strategies for billbugs.

APPENDICES

APPENDIX A

AUTHORSHIP AND CITATION OF PUBLISHED CHAPTERS

Chapter I:

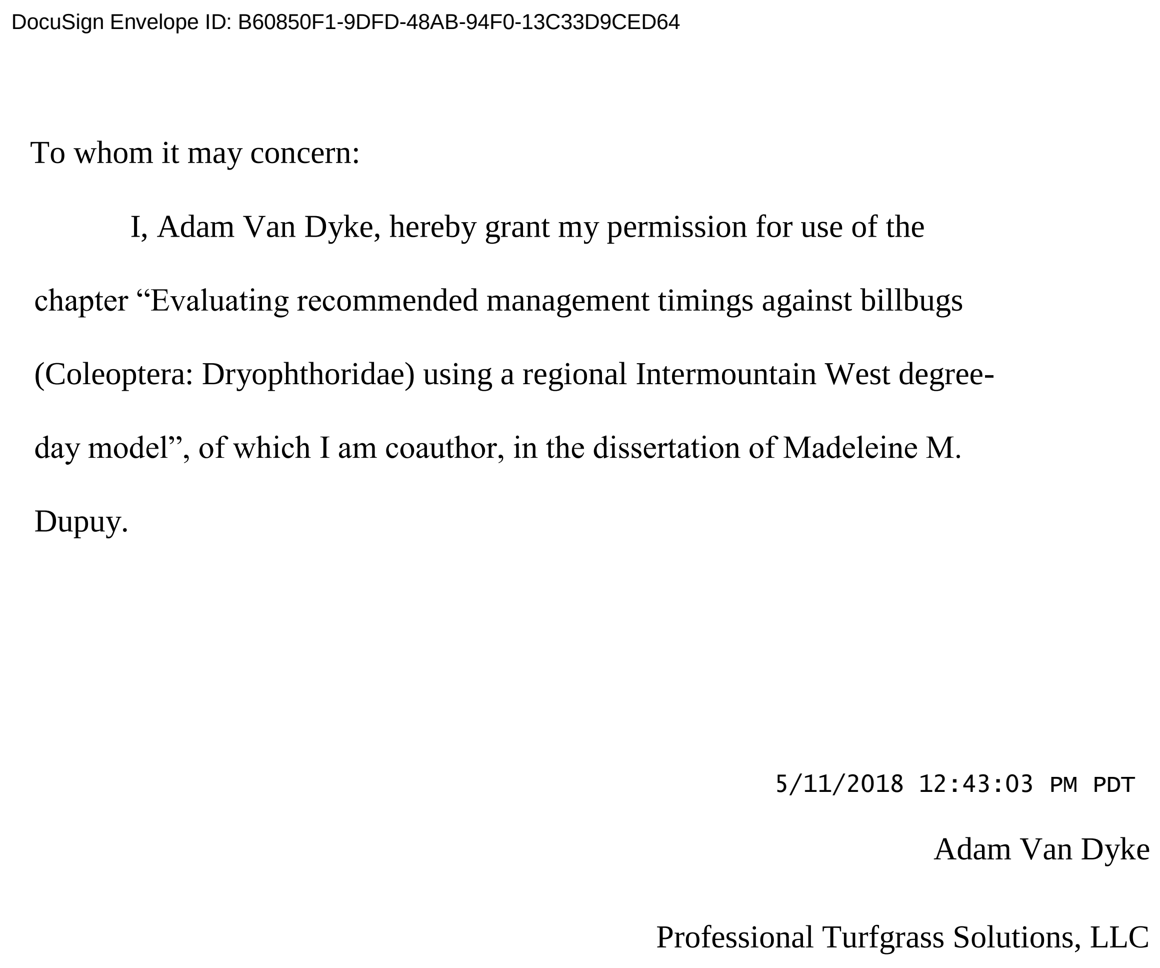
This is a pre-copyedited, author-produced version of an article accepted for publication in the *Journal of Integrated Pest Management* following peer review. The version of record **Dupuy, M.D. and R. A. Ramirez**. 2016. Biology and management of billbugs (Coleoptera: Curculionidae) in turfgrass. Journal of Integrated Pest Management 7: 1-10 is available online at: https://doi.org/10.1093/jipm/pmw004.

Chapter II:

This is a pre-copyedited, author-produced version of an article accepted for publication in the *Journal of Economic Entomology* following peer review. The version of record: **Dupuy, M.M., J.A. Powell, and R.A. Ramirez**. 2017. Developing a degree-day model to predict billbug (Coleoptera: Curculionidae) seasonal activity in Utah and Idaho turfgrass. Journal of Economic Entomology 110: 2180-2189 is available online at: https://doi.org/10.1093/jee/tox210)

APPENDIX B

LETTERS OF PERMISSION



DocuSign Envelope ID: B60850F1-9DFD-48AB-94F0-13C33D9CED64

To whom it may concern:

I, Adam Van Dyke, hereby grant my permission for use of the chapter “Evaluating recommended management timings against billbugs (Coleoptera: Dryophthoridae) using a regional Intermountain West degree-day model”, of which I am coauthor, in the dissertation of Madeleine M. Dupuy.

5/11/2018 12:43:03 PM PDT

Adam Van Dyke

Professional Turfgrass Solutions, LLC

Bryan Delp
R&D Regional Head, Lawn & Garden

Syngenta Crop Protection, LLC
2973 20th St.
Vero Beach, FL 32960
Bryan.Delp@syngenta.com Tel: 772 564 9012

May 11, 2018

Adam VanDyke
Professional Turfgrass
Solutions, LLC PO Box 95025
South Jordan, UT 84095

Sent Via E-Mail

RE: Agreement for Contract Research between Professional Turfgrass Solutions LLC (“**CRO**”) and Syngenta Crop Protection, LLC (“**Syngenta**”) effective February 14, 2012 (the “**Agreement**”)

Dear Mr. VanDyke,

Syngenta hereby grants CRO permission to share Work Product data from the project regarding billbugs (Coleoptera: Dryophthoridae) with Madeleine M. Dupuy solely for the purpose of use in her dissertation. Such permission shall not be deemed a waiver of future confidentiality or any other relevant provisions of the Agreement. All Work Product will remain Syngenta Proprietary Information and the sole property of Syngenta.

Capitalized terms used and not otherwise defined in this letter shall have the meanings given them in the Agreement.

Please do not hesitate to contact me if you have any questions..

Sincerely,

Bryan Delp
R&D Regional Head, Lawn and Garden

Madeleine M. Dupuy

Utah State University Department of Biology
5305 Old Main Hill| Logan, UT 84322 | madeleine.dupuy@usu.edu | (912)704-1418

EDUCATION

Utah State University | *Department of Biology*

Logan, UT **PhD:** Biology/Ecology
June 2013-April 2018 **Dissertation:** *Integrated Management of Billbugs (Coleoptera: Dryopthoridae) in the Intermountain West* with Dr. Ricardo Ramirez

- *Biology and Management of Billbugs (Coleoptera: Curculionidae) in Turfgrass*
- *Developing a Degree-Day Model to Predict Billbug (Coleoptera: Curculionidae) Seasonal Activity in Utah and Idaho Turfgrass*
- *Evaluating Recommended Management Timings Against Billbugs (Coleoptera: Dryophthoridae) Using a Regional Intermountain West Degree-Day Model*
- *Consumptive and Non-Consumptive Effects of Predatory Arthropods on Billbug (Coleoptera: Dryopthoridae) Pests in Turfgrass*

The University of Georgia | *Warnell School of Forestry and Natural Resources*

Athens, GA **BS in Forest Resources:** Natural Resources Recreation and Tourism
August 2009-May 2013 **Minor:** Ecology
Graduated Cum Laude: GPA 3.63
Senior Thesis: *Remnant Forests in Clear Cuts as Reservoirs of Ground Beetle Diversity* with Dr. Kamal Gandhi

TEACHING EXPERIENCE

Spring 2018 **BIOL 4430:** Introduction to Plant Pathology
Graduate Instructor

Fall 2017 **BIOL 1615:** General Biology for Science Majors, Lab Portion
Graduate Teaching Assistant

BIOL 3300: Introduction to Microbiology
Graduate Teaching Assistant (prep)

Spring 2017 **BIOL 4430:** Introduction to Plant Pathology
Graduate Teaching Assistant

BIOL 3300: Introduction to Microbiology
Graduate Teaching Assistant (prep)

Fall 2016	**BIOL 1615:** General Biology for Science Majors, Lab Portion *Graduate teaching assistant (prep)*
	BIOL 3300: Introduction to Microbiology *Graduate Teaching Assistant (prep)*
Spring 2016	**BIOL 4430:** Introduction to Plant Pathology *Graduate Teaching Assistant*
	BIOL 3300: Introduction to Microbiology *Graduate Teaching Assistant (prep)*
Fall 2015	**BIOL 1610:** General Biology *Graduate Teaching Assistant*

My graduate program has provided me with diverse teaching experience, both with hands on experience in the laboratory (BIOL 1610, 1615, and 4430) and traditional lecturing in the classroom (BIOL 4430). In spring of 2018, I served as Graduate Instructor for Introduction to Plant Pathology (BIOL 4430). In this role, I was responsible for designing lectures, exams, and a syllabus, updating lab activities, and managing a Graduate Teaching Assistant to run the lab portion. My role as a Graduate Teaching Assistant has primarily focused on introducing relevant background information to students before guiding them through laboratory exercises. In this capacity, I have worked both as a member of a team of teaching assistants, keeping information consistent among several lab sections (BIOL 1610 and 1615) and as the sole teaching assistant, developing my own introductory lectures and updating lab activities (BIOL 4430). As the preparatory assistant for the general microbiology labs, I was responsible for providing TAs with cultures and supplies they need for each week's lab activities. My assignment as preparatory assistant is BIOL 1615 in fall of 2016 required me to conduct a trial run of a new, several-weeks long experiment involving the diversity of endophytic fungi. From start to finish, I developed protocols, handouts, and online tools for students to use (see: USU Plant Tour—BNR and Beyond), and clear and concise introductory presentations for other teaching assistants to teach from.

PEER-REVIEWED PUBLICATIONS

Dupuy, M.M., J.A. Powell, and R.A. Ramirez, 2017. Developing a degree-day model to predict billbug (Coleoptera: Curculionidae) seasonal activity in Intermountain West turfgrass. Journal of Economic Entomology 110:5 p. 2180-2189.

Dupuy, M.M. and R.A. Ramirez, 2016. Biology and management of billbugs (Sphenophorus spp.) in turfgrass. Journal of Integrated Pest Management 7:1 p.1-10.

OTHER PUBLICATIONS

Dupuy, M.M., 2016. USU Plant Tour—BNR and Beyond. Built on "Campus Tree Tour" by Michael Kuhns, 2012. BIOL 1615 Course Materials, USU Department of Biology.

Dupuy, M.M., 2016. Turf Entomology 101. Turf IPM Advisory, Spring 2016. USU Extension.

Ramirez, R.A. and **M.M. Dupuy**, 2014. The Usual Suspects for Turf Insects & Research Results in 2013. Utah Pests News, Vol. VIII, Winter 2014. Utah Plant Pest Diagnostic Laboratory and USU Extension.

GRANT WRITING

Ramirez, R.A. and **M.M. Dupuy**, 2016. The contribution of predatory arthropods toward billbug suppression in Intermountain West turfgrass. Utah State University Extension Grants Program. Submitted: March 2016.

Received: $9,429.20

PROFESSIONAL PRESENTATIONS

Ramirez, R.A. and **M.M. Dupuy**. 2017. The contribution of predatory arthropods toward billbug suppression in Intermountain West turfgrass, 2017 Utah State University Extension Annual Conference, Logan, UT, 2 March.

Dupuy, M.M., 2017. A degree-day model for billbugs in Utah and Idaho turfgrass, 2017 Utah State University Extension Annual conference, Logan, UT, 1 March.

Dupuy, M.M., 2017. Insect Pathology. USU BIOL 4430 (Plant Pathology) guest lecture, Logan, UT, 23 February.

Dupuy, M.M., 2017. Bee flies! (Diptera: Bombyliidae). USU Entomology Club Bug of the Month lecture series, Logan, UT, 15 February.

Dupuy, M.M., 2016. Pretty Little Borers: Emerald Ash Borer (*Agrilus planipennis*). USU Entomology Club Bug of the Month lecture series, Logan, UT, 6 April.

Dupuy, M.M., 2016. Small Wonders Rulers: Our Insect Overlords. USU Entomology Club and Merrill-Cazier Library Present: Small Wonders: The Expansive World of Insects opening reception, Logan, UT, 7 February.

USU Entomology Club and Merrill-Cazier Library Present: Small Wonders: The Expansive World of Insects, a collaborative visual and educational display. On view in the Merrill-Cazier Library Atrium, Logan, UT, February through May 2016.

Ramirez, R.A. and **M.M. Dupuy**, 2015. Kill billbug: A degree-day model for billbugs (*Sphenophorus* spp.) in Intermountain West turf. Entomological Society of America 63rd Annual Meeting, Minneapolis, MN, 15-18 November.

Dupuy, M.M. and R.A. Ramirez, 2015. Tools for IPM: A degree-day model for billbugs (*Sphenophorus* spp.) in Intermountain West turf. Entomological Society of America 63rd Annual Meeting, Minneapolis, MN, 15-18 November.

Ramirez, R.A. and **M.M. Dupuy**, 2015. Poster: Tracking billbugs (*Sphenophorus* spp.) to improve IPM in Intermountain West turfgrass. 8th International IPM Symposium, Salt Lake City, UT, 23-26 March.

Dupuy, M.M. and R.A. Ramirez, 2014. Tracking billbugs (*Sphenophorus* spp.) and generalist predators to advance biocontrol in Intermountain West turfgrass. Entomological Society of America 62nd Annual Meeting, Portland, OR, 16-19 November.

Dupuy, M.M., Spears, L.R., and R.A. Ramirez, 2014. Poster: A survey of billbugs (*Sphenophorus* spp.) to improve turfgrass management in the Intermountain West. Entomological Society of America Pacific Branch 98th Annual Meeting, Tucson, AZ, 6-9 April.

Dupuy, M.M., Spears, L.R., and R.A. Ramirez, 2013. Poster: A survey of billbugs (*Sphenophorus* spp.) to improve turfgrass management in the Intermountain West. Entomological Society of America 61st Annual Meeting, Austin, TX, 10-13 November. 1st place President’s Prize, P-IE section.

Dupuy, M.M. and K.J.K. Gandhi, 2013. Poster: Remnant forests as reservoirs of ground beetle diversity. Annual Warnell Graduate Student Symposium, Athens, GA, March. 2nd place prize for undergraduate posters.

HONORS & AWARDS

USU Graduate Enhancement Award

\$4,000.00 *May 2015*	Recognizes 20 graduate students at USU from all departments for excellence in academics and contributions to the university and the local community.

ESA Student Travel Award

\$250.00 *March 2015*	Grants travel funds to the Entomological Society of America Pacific Branch meeting

Invited to Golden Key International Honour Society

November 2014	Recognizes top 15% of graduate students in all fields of study

Entomological Society of America 61st Annual Meeting

\$150.00 *November 2013*	Student poster 1st place President’s Prize, P-IE section

Warnell Graduate Student Symposium

\$50.00 *March 2013*	Undergraduate poster 2nd place prize

Warnell School of Forestry and Natural Resources

Fall 2010-Spring 2013	Dean’s List

HOPE Scholarship

Fall 2009-Spring 2013	Pays full tuition at UGA for in-state students maintaining at least a 3.0 GPA throughout study

LEADERSHIP AND OUTREACH ROLES

USU Entomology Club

Fall 2013-Present

President
May 2015-May 2016

- Started Bug of the Month lecture series, where one club member gives a short presentation about an interesting insect at each monthly club meeting
- Conceived, organized, and oversaw a collaborative exhibition for the general public with the USU Merrill-Cazier Library (Small Wonders: The Expansive World of Insects)
- Headed fundraising efforts at the 2015 ESA annual meeting in Minneapolis, MN, from which the club made >$800 profit from t-shirt and jewelry sales.

Secretary
May 2014-May 2015

- Participate in club outreach events and insect education for school groups and summer camps, USU undergraduate student body, and the Logan, UT community

Linnaean Games Team Member
Fall 2013-Spring 2015

- Competed at the Entomological Society of America Pacific Branch meeting in Tucson, Arizona, 2014

USU Biology Graduate Student Association

Active member,
Fall 2013-Present

- Participate in outreach with insect education at campus and community event

UGA Students for Environmental Action

Secretary, *Fall 2011*

Vice President
Spring 2012-Spring 2013

- Monthly community service and pragmatic initiatives to improve campus sustainability
- Conceive and head environmental initiatives and lead club members in completion of these projects

Natural Resources Recreation and Tourism Society

Member
Fall 2011-Spring 2013

- Outdoor community service, trail maintenance and clean-up, community outreach

OUTREACH EXPERIENCE

Consultation Field Visit

Willow Pond Park, *Murray, UT* *July 2015*	Visit to a park with turf problems to diagnose insect damage

USU Extension Services

Turf Field Day, Sandy, UT *Summer 2014*	Participate in outreach education of Intermountain West turfgrass managers

Turf Field Day, Logan, UT
Fall 2013

Entomological Society of America Pacific Branch

Check-in desk volunteer
April 2014

Athens-Clarke County Leisure Services

Summer Camp Counselor
Bear Hollow Zoo at Memorial Park, Athens, GA
Summer 2012

Project WILD Certification

Fall 2012

Project Learning Tree Certification

Fall 2012

PROFESSIONAL DEVELOPMENT

Active Learning — Empower Teaching Excellence Learning Circle

October-December 2017	USU Center for Innovative Design and Instruction 4-part workshop

Finding and Applying for Higher Education Jobs that Fit You

October 2017	USU College of Science 6-hour seminar

Tips for Teaching Undergraduate Courses

September 2017	USU Graduate Training Series 1.5-hour seminar

Getting Started as a Successful Proposal Writer and Academician

February 2015	USU College of Science 6-hour seminar

Great Smoky Mountains Field Course

GSMNP, North Carolina and Tennessee *August 2012*	Plant and animal ID, salamander and stream sampling techniques, local ecology

Tropical Ecology Maymester

Costa Rica *May-June2011*	Ecological research, plant and animal ID, local ecology and culture

PROFESSIONAL SOCIETIES

Entomological Society of America

Student member
September 2013-Present

USU Ecology Center

Active member
August 2013- Present