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Cover Page Footnote

We thank Danielle Craig and Garrett Price for field assistance. We are grateful for the cooperation of superintendents and staff at Purdue Sports Turf and William H. Daniel Turfgrass Research and Diagnostic Center. We are grateful for the cooperation of Dr. Ian Kaplan and Ulianova Vidal Gómez for assistance with the olfactometry methods. Dr. Juli Carrillo, Dr. Laura Ingwell, and Dr. Elizabeth Long provided helpful insights on earlier versions of this manuscript. Dr. Diane Silcox Reynolds provided helpful insight and was integral in developing the initial questions during the A. Duffy's undergraduate research project. Funding was provided by the United States Golf Association (2015-10-525).

Hunting Billbug *Sphenophorus venatus* (Coleoptera: Curculionidae: Dryophthorinae) Adult Feeding Preference and Attraction to Warm- and Cool-Season Turfgrasses

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Abstract

The hunting billbug *Sphenophorus venatus* Say (Coleoptera: Curculionidae: Dryophothorinae) is a generalist stem-boring pest on warm- and cool-season grasses. The objectives of this work were to (1) investigate adult feeding preference for four common turfgrass host species and (2) explore whether adults are attracted to the volatile odors emitted by these grasses. In laboratory feeding assays, *S. venatus* adults preferred zoysiagrass *Zoysia japonica* Steud 'Meyer' over all other species tested: Bermudagrass *Cynodon dactylon* 'Patriot', Kentucky bluegrass *Poa pratensis* 'Barron', and creeping bentgrass *Agrostis stolonifera* 'Penncross'. In y-tube olfactometry assays, only males were attracted to bermudagrass. Our findings suggest that although *S. venatus* odor recognition potentially influence dispersal and host-seeking behavior, it may not ultimately determine feeding preference, where more close-range cues may be important. Further investigations on the mechanisms that cause variation in these behaviors could aid in the pursuit of more sustainable management techniques, such as strategic plant species selection and development of synthetic semiochemical lures for monitoring and trapping.

Keywords: olfactometry, integrated pest management

Beetles in the genus *Sphenophorus* Schönherr, commonly referred to as billbugs, are stem-boring, cosmopolitan pests of small grain crops and grasses (Vaurie 1951). A particularly problematic billbug species in urban environments is the hunting billbug, *Sphenophorus venatus* Say (Coleoptera: Curculionidae: Dryophthorinae), which causes severe injury to warm-season (C-4) grasses, such as zoysiagrass (*Zoysia* spp. Willd.) and bermudagrass (*Cynodon* spp. Pers.) (Doskocil and Brandenburg 2012, Huang and Buss 2013, Reynolds et al. 2016), but also damages cool-season (C-3) grasses, such as tall fescue (*Festuca arundinacea* Schreb.), Kentucky bluegrass (*Poa pratensis* L.), and creeping bentgrass (*Agrostis stolonifera* L. var. *palustris* Huds.) (Johnson-Cicalese and Funk 1990, Johnson-Cicalese et al. 1990, Vittum et al. 1999). Because of their host breadth, the hunting billbug is a particularly problematic pest in lawns and other urban greenspaces where both warm- and cool-season turfgrasses are grown (Milesi et al. 2005), and synthetic insecticides are often used to control their damage (Williamson et al. 2013). Because of public concern surrounding the use of chemical pesticides in these environments (Johnson et al. 2013), billbugs are one of a handful of insects that persist as a major obstacle for municipalities looking to reduce chemical inputs and create more sustainable recreational spaces. Adult billbugs chew notches in grass tillers and then females oviposit directly into the tiller (Williamson et al. 2013), thus infestations of the damaging larval stage are driven largely by adult host preferences. Understanding adult feeding behavior could help promote sustainability in urban turfgrass systems by identifying grass species that are less attractive to this pest and that, in turn, require fewer pesticides. We conducted two laboratory studies to 1) investigate the feeding preference of hunting billbug adults for warm- vs. cool-season grasses and 2) explore whether hunting billbug adults are attracted to the odors of different grass species.

Materials and Methods

Insect and Plant Material. *S. venatus* adults were collected from bermudagrass *C. dactylon* 'Patriot' athletic fields in West Lafayette, IN from May to October 2015 using pitfall traps or hand collecting at night. Billbugs were separated by sex (Johnson-Cicalese et al. 1990) and maintained in a growth chamber (25–27 °C; 78–85% RH; 10:14 (L:D)) in plastic deli cups (12 cm diameter) with a moist paper towel and clippings of bermudagrass host-plant material until used in bioassays (12 to 48 hours). Individuals were used only once, and the same individuals were not used in both feeding choice and olfactometry assays. Two cool-season grasses, Kentucky bluegrass (*P. pratensis* 'Park') and creeping bentgrass (*A. stolonifera* 'Penncross'), and two warm-season grasses, bermudagrass (*C. dactylon* 'Patriot') and zoysiagrass (*Z. japonica* 'Meyer'), from plots at the Purdue University W.H. Daniel Center for Turfgrass Research and Diagnostics were used for plant material in both assays.

Feeding choice assays. Adult feeding preference was tested using a series of petri-dish bioassays adapted from Richmond (1999) and Johnson-Cicalese and Funk (1990). The bioassays incorporated two cool-season grasses, Kentucky bluegrass (*P. pratensis* 'Park') and creeping bentgrass (*A. stolonifera* 'Penncross'), and two warm-season grasses, bermudagrass (*C. dactylon* 'Patriot') and zoysiagrass (*Z. japonica* 'Meyer'). Binary choice treatments included: 1) bermudagrass vs. Kentucky bluegrass, 2) bermudagrass vs. bentgrass, 3) zoysiagrass vs. Kentucky bluegrass, 4) zoysiagrass vs. bentgrass, and 5) bermudagrass vs. zoysiagrass. Treatments encompassed all combinations of warm- vs. cool-season grasses as well as a comparison of the two warm-season grasses. Each treatment was replicated 20 times for males and 20 times for females.

Adult billbugs were placed individually in 5 cm petri dishes lined with moist filter paper and isolated with no food for 24 hours before providing fresh grass tillers. One tiller of each grass species was provided to each billbug and petri dishes were arranged on the bench in a randomized complete block design. Feeding scars were used as an indicator of preference (Richmond 1999) and were counted using an Olympus IX51 dissecting microscope after 24, 48, and 72 hours. New grass tillers were provided at the beginning of each 24-hour interval. The influence of grass species and insect sex on variation in the mean number of feeding scars were tested for each of the binary-choice treatments using analysis of variance (ANOVA) followed by Tukey's HSD (Statistica 13, Dell Inc. 2016).

Olfactometry. The behavioral response of *S. venatus* adults to the odors from four grass species (Kentucky bluegrass, creeping bentgrass, bermudagrass, and zoysiagrass) was tested using a y-tube olfactometer (8 cm and 12 cm arms; 2 cm diameter, round glass joints; Analytical Research Systems, Gainesville, FL), a well-developed and widely used method for quantifying insect responses to plant odors. Odor stimuli were delivered by pushing purified air through an activated charcoal filter and split into two air steams (1 L/min) which were blown through a glass container (14 cm long, 2 cm diameter) that was either empty (purified air control) or contained 5 grams of above-ground plant clippings from one of the four grass species. The odors were then delivered to one of the arms of the olfactometer. Glassware was washed using 1% diluted Alconox soap, rinsed with acetone, and baked in an oven for ~10 minutes at 200 °C between observations. Each odor treatment was compared to a purified air control and fresh odor sources were used for no longer than 30 minutes (McGraw et al. 2011). All observations were made between 9:00 PM and 6:00 AM using a red headlamp. Based on Duffy et al. (2018), a billbug was considered responsive if it walked upwind in the 12-cm base arm and then turned to walk 2 cm into one of the 8–cm arm corresponding with an odor source (treatment or control) and remained within that arm for at least one minute. Billbugs not making a choice between the two odor treatment arms within ten minutes were considered non-responsive (McGraw et al. 2011, Duffy et al. 2018). Each individual billbug was used once, and treatment arms were switched after each replicate to control for directional bias. Treatments were replicated until 20 responsive male and female adults were observed. A Chi-square goodness of fit test was used to test the null hypothesis that *S. venatus* adults showed no preference for the host-plant odor source vs. purified air control (Statistica 13, Dell Inc. 2016).

Results

Feeding choice assays. For warm- vs. cool-season grass treatments, the interaction between grass species and billbug sex was not significant ($F \leq 1.57$; df = 1, 76; $P \geq 0.215$). Grass species did not have a significant effect on the mean number of adult feeding scars for bermudagrass vs. Kentucky bluegrass ($F = 0.03$; df = 1, 72; $P = 0.869$; Fig. 1A) or bermudagrass vs. bentgrass ($F = 0.30$; df = 1, 76; $P = 0.585$; Fig. 1B). Grass species did, however, affect the mean number of feeding scars observed for both the zoysiagrass vs. Kentucky bluegrass ($F = 10.81$; df = 1, 76; $P = 0.002$; Fig. 1C), and zoysiagrass vs. bentgrass treatments ($F = 34.70$; df = 1, 74; $P < 0.001$; Fig. 1D), with zoysiagrass having a higher amount of feeding in both cases. In addition, sex affected the number of feeding scars for the zoysiagrass vs. bentgrass treatment ($F = 15.33$; df = 1, 74; $P < 0.001$; Fig. 1D), with males feeding more on both grass species than females.

When given a choice between the two warm-season grasses, bermudagrass and zoysiagrass, the number of feeding scars

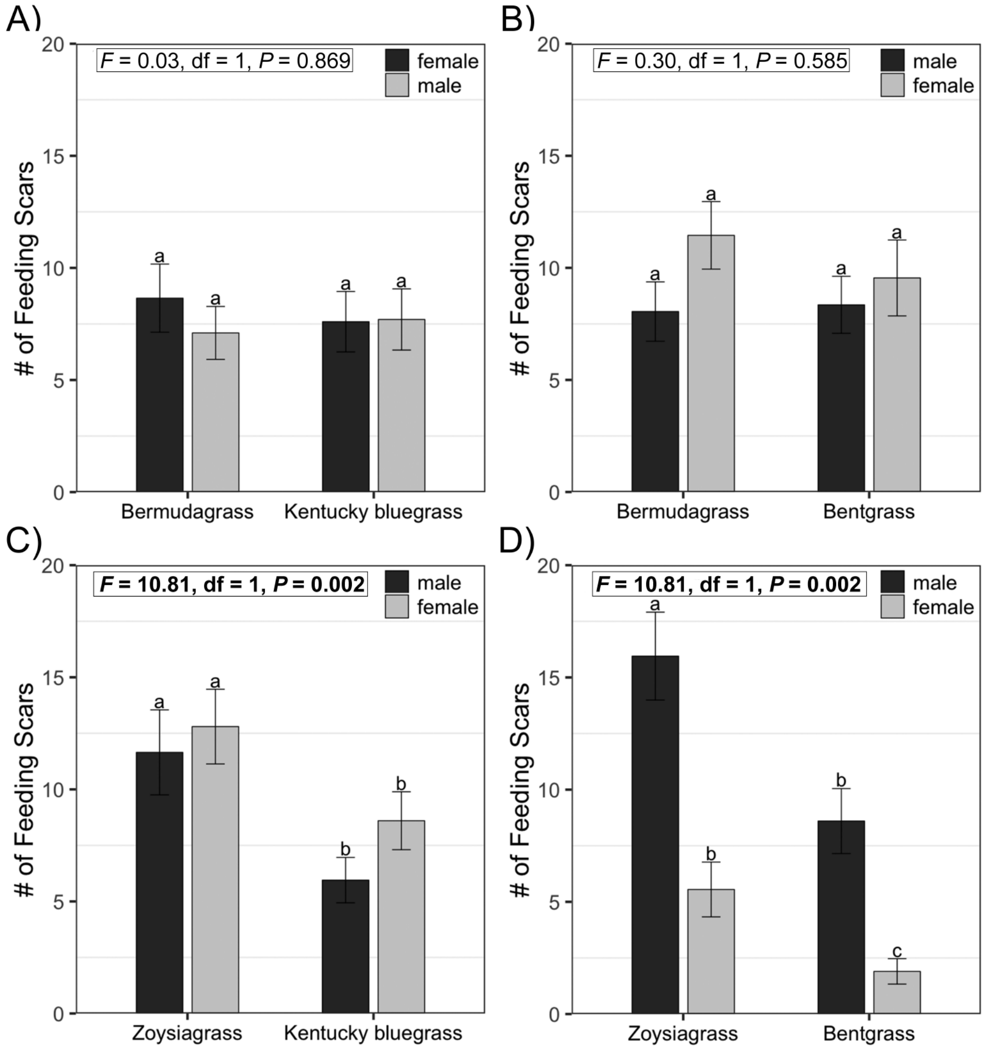

Figure 1. Number of hunting billbug *Sphenophorus venatus* Say feeding scars on warm-season turfgrass, bermudagrass (A and B) and zoysiagrass (C and D) vs. cool-season turfgrass, Kentucky bluegrass (A and C) and bentgrass (B and D) in laboratory choice bioassays (n = 40). Differences were tested with analysis of variance (ANOVA) followed by Tukey's HSD. Because there was no significant interaction between 'grass species' and 'sex', the F and p-values represent the effect of 'grass species' and significant effects ($P < 0.05$) are indicated in bold. Means with the same letter are not significantly different ($P > 0.05$).

varied with grass species and billbug sex (grass species × sex interaction, $F = 16.08$; df = 1, 76; $P < 0.001$), with males feeding more on zoysiagrass than bermudagrass whereas females exhibited no preference between the two warm-season grass species (Fig. 2).

Olfactometry. Across all treatments, 79.7% (n = 192) of the billbugs tested were responsive and walked upwind in the y-tube olfactometer and made a choice between odor treatments. Adult *S. venatus* males were attracted to bermudagrass in y-tube olfactometry bioassays ($X^2 = 5.00$; df = 1; $P = 0.025$), with 75% (n = 20) of males choosing the arm with the odor treatment containing the bermudagrass host-plant material relative to a purified air control. In contrast, females were not attracted to bermudagrass ($X^2 = 0.80$; df = 1; $P = 0.317$). Neither males nor females were attracted to the odors from any of the other host species, zoysiagrass ($X^2 \leq 1.80$; df = 1; $P \geq 0.180$), Kentucky bluegrass

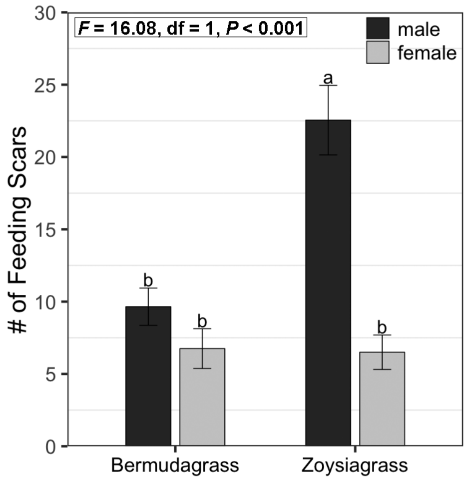

Figure 2. Number of hunting billbug, *Sphenophorus venatus* Say, feeding scars on bermudagrass vs. zoysiagrass from males (n = 20) and females (n = 20) in laboratory choice bioassays. Differences were tested with analysis of variance (ANOVA) followed by Tukey's HSD. *F* and *P*-value represent the interaction effect of 'grass species' × 'sex'. Means with the same letter are not significantly different ($P > 0.05$).

($X^2 \leq 0.80$; df = 1; $P \geq 0.371$), or bentgrass ($X^2 \leq 0.80$; df = 1; $P \geq 0.371$) compared to the purified air control.

Discussion

Information on hunting billbug feeding behavior is limited (Johnson-Cicalese and Funk 1990, Reinert et al. 2011, Huang and Buss 2013). This is the first study to investigate feeding preference of billbugs when provided a choice between different grass species, and the first to survey multiple species of both warm- and cool-season grass types. *Sphenophorus venatus*, displayed a preference for one specific warm-season species, zoysiagrass, feeding more on this species when compared to any of the other grass species examined. This initial study suggests that the other grasses may be less preferred habitats for this species, which may reduce the likelihood of damage and subsequently reduce reliance on synthetic insecticides. However, additional field experiments, such as measuring the feeding and damage rates within monoculture turf stands, should be conducted before drawing definitive conclusions on selecting these grasses as a management strategy. Future work should also include additional warm- and cool-season grass species to the ones tested in this study, as well as multiple genotypes or cultivars within those species (Reinert et al. 2011, Huang and Buss 2013).

Host-seeking behavior directed by plant odors serves as a more energy efficient strategy for locating preferred hosts than random foraging. The ability for *S. venatus* to orient towards odors was first documented by Duffy et al. (2018), but this study only tested a single grass species, bermudagrass. The present study assessed the attractiveness of four different grass species, Kentucky bluegrass, creeping bentgrass, bermudagrass, and zoysiagrass (*Z. japonica* 'Meyer'). We observed that male, but not female, hunting billbug adults were attracted to bermudagrass in the y-tube olfactometer. Surprisingly, billbugs were not attracted to any of the other grass species tested, even though zoysiagrass was their preferred host in feeding assays. These results are intriguing, as volatile attraction is known to be influenced by previous host exposure in other beetle species (Austel et al. 2014). The billbugs in this study were collected from a well-established population infesting a bermudagrass monoculture and were only attracted to bermudagrass in the olfactometer. It would be interesting to test for the olfactory responses of individuals from other populations and geographical regions which colonize different grass species and investigate if a pattern of requiring previous exposure to elicit attractiveness would emerge. Future research on intraspecific variation in the attraction to odors and the potential influence of feeding experience could provide insight into the utility of regional or even site specific semiochemical lures for monitoring and management, as well as the ability for these pests to colonize novel host plants. Lack of attraction towards the odors of any grasses by female hunting billbugs could be due to reliance on a putative male-produced pheromone hypothesized by Duffy et al. (2018). It would be interesting to investigate whether the addition of conspecifics alters the attractiveness of grass species in a future study. The observations from this study suggest that a species-specific blend of odors may be unique to bermudagrass and that this odor blend is particularly attractive to this population of *S. venatus* males. In pursuit of more sustainable monitoring and management techniques, such as synthetic semiochemical lures, future efforts should focus on isolating and identifying the specific volatile compounds or blend of compounds that elicited the observed attractiveness.

Our findings from both the feeding preference and olfactometry assays suggest plant selection may be a useful strategy in urban landscape managers' efforts to create more sustainable urban environments that are less vulnerable to insect pests. Although it appears male hunting billbugs are attracted to the odor of bermudagrass, they displayed a clear feeding preference for zoysiagrass. However, the cool-season grasses (Kentucky bluegrass and creeping bentgrass) were less preferred in both feeding and olfactometry assays by both males and females. Our findings imply that these grasses may be good candidates for creating urban habitats that are less attractive and less preferred by hunting billbugs. Field studies are still needed to test this hypothesis, but our findings suggest it may be possible to reduce the amount of feeding and resultant damage in urban greenspaces vulnerable to hunting billbug infestation by planting cool-season grasses, such as Kentucky bluegrass and creeping bentgrass.

Acknowledgments

We thank Danielle Craig and Garrett Price for field assistance. We are grateful for the cooperation of superintendents and staff at Purdue Sports Turf and William H. Daniel Turfgrass Research and Diagnostic Center. We are grateful for the cooperation of Dr. Ian Kaplan and Ulianova Vidal Gómez for assistance with the olfactometry methods. Dr. Juli Carrillo, Dr. Laura Ingwell, and Dr. Elizabeth Long provided helpful insights on earlier versions of this manuscript. Dr. Diane Silcox Reynolds provided helpful insight and was integral in developing the initial questions during the A. Duffy's undergraduate research project. Funding was provided by the United States Golf Association (2015-10-525).

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