Understanding Billbug Chemical Communication to Improve Management

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Objectives:

1) Characterize the response of bluegrass billbug and the hunting billbug to a known billbug aggregation pheromone ((S)-2-methyl-4-octanol).
2) Identifying species-specific volatile attractants that may be useful for monitoring and management.
3) Clarify the role of cuticular hydrocarbons in close-range mate recognition.

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Project Duration: 2 Years
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Summary Points

- Although neither hunting billbug nor bluegrass billbug adults were attracted to the putative aggregation pheromone (S)-2-methyl-4-octanol under field conditions, Y-tube olfactometry assays revealed that hunting billbug males are attracted to grass host volatiles while females responded to volatiles from male billbugs.
- Findings suggests that female billbugs may be responding to a male-produced volatile sex pheromone, the structure of which has not yet been determined.
- The cuticles of hunting and bluegrass billbug are coated with a series of aliphatic hydrocarbons that differ both between species and between sexes of a given species.
- Differences in cuticular hydrocarbon profiles could provide the basis for mate recognition between billbug species.
- Once the absolute configuration of both volatile and tactile chemical cues has been clarified, it may be possible to incorporate this knowledge into monitoring and management programs designed to manipulate the host- and mate-finding behavior of billbugs associated with turfgrass.

Summary

Behavioral chemicals, called semiochemicals, mediate insect behavior at multiple spatial scales. Semiochemicals that are active over longer distances include host-plant volatiles, sex pheromones or aggregation pheromones that are typically olfactory-perceived. These volatile stimuli are used by insects to find hosts, potential mates, or other insects of the same species. Close-range semiochemicals associated with mating behavior are typically non-volatile, long-chain cuticular hydrocarbons on the insect surface (Thornhill & Alcock 1983) and are usually

perceived through antennal contact. Despite the fact that sequential use of both volatile and contact semiochemicals for reproductive success has been documented in several insect species (Guarina et al. 2008, Hughes et al. 2015, Eliyahu 2008), the role of semiochemicals in directing the host- and mate-finding behavior of the billbug species complex associated with turfgrass has never been examined.

The long-term goal of this research is to understand the role of semiochemicals in orchestrating the host- and mate-finding behavior of billbugs associated with golf course turf. Our research efforts focused on characterizing the response of bluegrass and hunting billbugs to a known weevil aggregation pheromone (2-methyl-4-octanol) and determining the potential for semiochmicals to influence billbug dispersal and mate finding behavior. Efforts to understand these cues could lead to the development of novel, biologically-based monitoring and management strategies designed to manipulate billbug behavior.

To determine the extent to which hunting and bluegrass billbugs were attracted to the putative synthetic aggregation pheromone (2-methyl-4-octanol) (Zarbin et. al 2003), paired pitfall traps were monitored at several sites in West Lafayette, Indiana. One pitfall trap in each pair was baited with the pheromone lure. The number of males and females of each species captured in each trap was recorded. Because our results indicated that the number of captured billbugs of either species or sex did not differ between baited and un-baited traps at any time during the experiment, a binary choice y-tube olfactometry assay was designed to examine the attractiveness of several other biologically relevant odor sources.

Y-tube treatments were comprised of five combinations of hunting billbugs and above-ground Bermudagrass host-plant material that were all compared to a purified air control (Fig. 1). The response of hunting billbugs to the different odor sources varied depending on sex. Males positively responded to treatments containing Bermudagrass, but not to males or females of the same species (Fig. 2). In contrast, females positively responded to treatments containing males of the same species, but did not orient toward treatments containing only host-plant material or other females.

To examine the potential for cuticular hydrocarbons to mediate close-range mating behavior, we performed a short series of laboratory mating assays, and analyzed the cuticular hydrocarbons of male and female hunting and bluegrass billbugs. Because our mating assays with hunting billbug revealed behaviors consistent with the presence of behaviorally active cuticular compounds (Fig. 3), whole body hexane extracts were characterized using gas chromatography/mass spectrometry. Results confirmed qualitative and quantitative chemical differences in the hydrocarbon profiles between hunting and bluegrass billbugs, and quantitative differences between the sexes of both species (Table 1).

References Cited

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Figure 1. Y-tube olfactometry bioassays were used to characterize the response of hunting billbugs to plant and insect volatile organic compounds that may help billbugs locate both host-plants and mates.

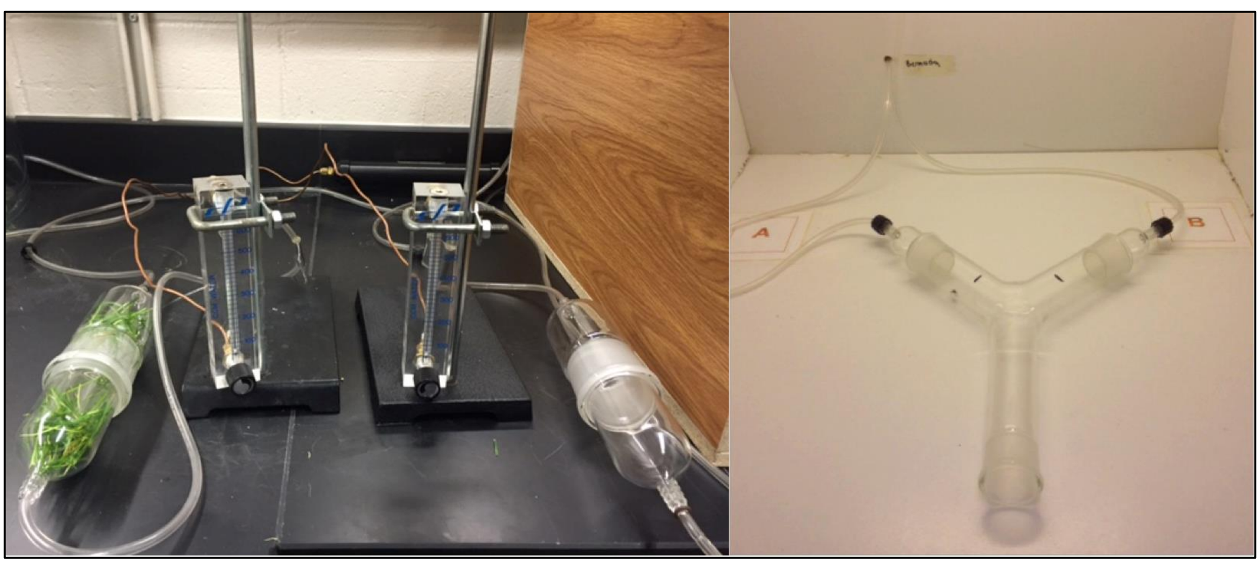

Figure 2. Percentage of hunting billbug adults responding to five combinations of other billbugs of the same species and/or host-plant material vs. purified air in a y-tube olfactometer bioassay. *P<0.05, **P<0.01 (Observed vs. Expected Chi-Square).

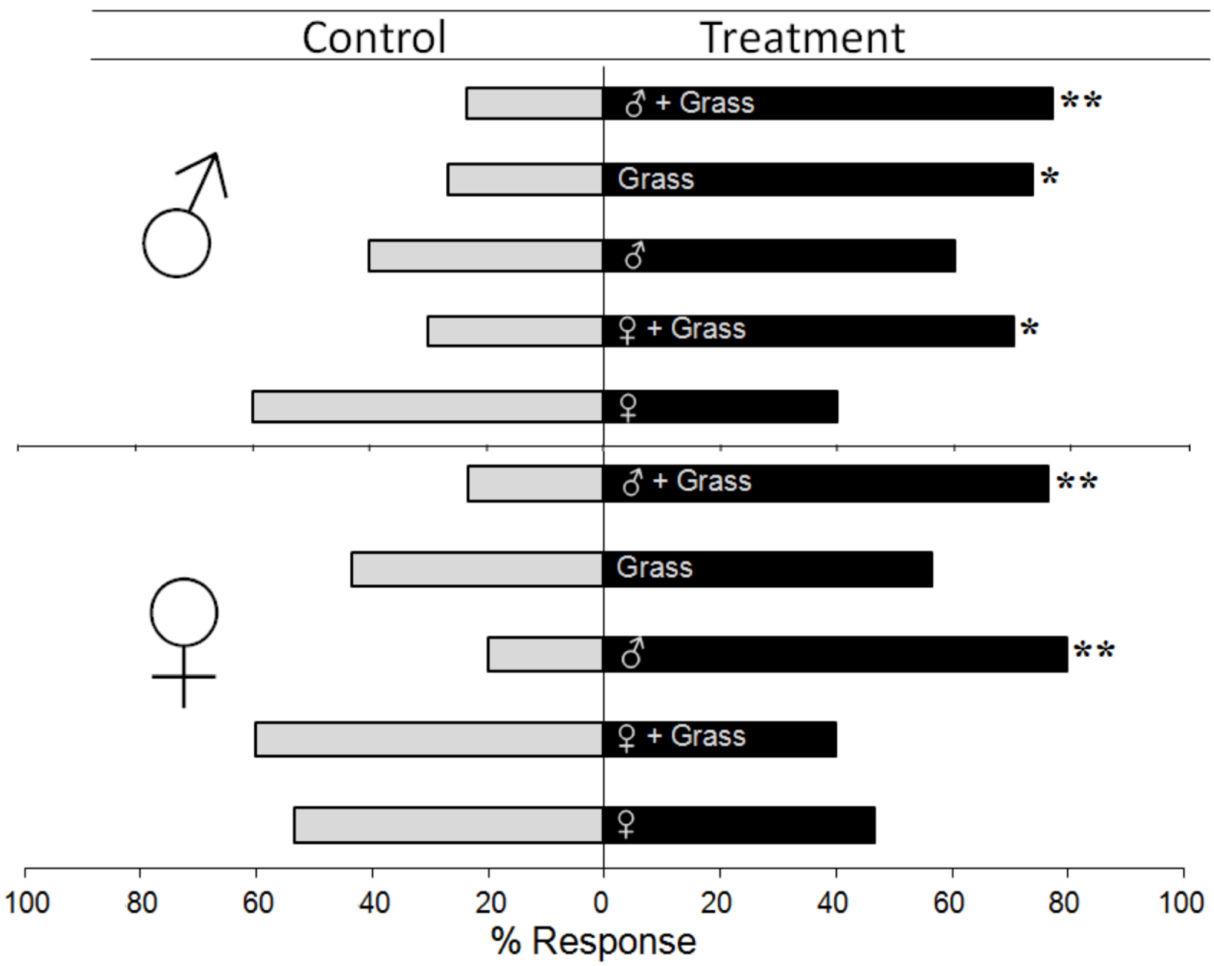

Figure 2. Because billbugs frequently occur as a complex of several closely-related species that overlap geographically and temporally, tactile chemical signals may be used to facilitate close-range mate recognition.

Table 1. Relative differences in the cuticular hydrocarbon profiles of hunting and bluegrass billbugs.

Peak #[a]	Retention Time (min)	Relative Proportion of Total Hydrocarbons[b]			
		Hunting Billbug		Bluegrass Billbug	
		Female	Male	Female	Male
1	22.35	a	a	a	a
2	22.91	ab	ab	b	a
3	23.11	a	a	a	a
4	23.63	ND	ND	b	a
5	23.85	ab	b	a	ab
6	24.48	b	a	c	b
7	24.60	a	a	a	a
8	25.18	a	a	a	a
9	25.26	ND	ND	a	b
10	25.33	ND	ND	a	b
11	25.45	a	a	a	a
12	27.3	a	a	b	b
13	27.36	a	a	ND	ND
14	27.40	ND	ND	a	a
15	27.53	ND	ND	a	a
16	27.66	a	a	a	a

[a] Peaks correspond with individual compounds identified through GC/MS.
[b] Differences in the relative abundances of cuticular hydrocarbons of hunting billbug males (n=10) vs. females (n=10) and bluegrass billbug males (n=8) vs. females (n=5) were tested with MANOVA followed by mean separation using Tukey's HSD test. Cells in the same row that contain the same letter are not significantly different ($\alpha<0.05$). ND=not detected.