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Aggregation Behavior of the Southern Chinch Bug (Hemiptera: Blissidae)

KARLA M. ADDESSO,¹ HEATHER J. MCAUSLANE,² AND RON CHERRY²

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ABSTRACT The southern chinch bug, *Blissus insularis* Barber, forms dense, multigenerational aggregations in St. Augustinegrass lawns leading to grass death from sap feeding. We conducted laboratory bioassays to better understand the signals responsible for the formation and maintenance of southern chinch bug aggregations. In small arena assays, chinch bugs demonstrated a stronger aggregation response over time and aggregated more often on or beneath St. Augustinegrass leaf blades than on or under artificial leaf-like shelters constructed from white or green paper. In Y-tube olfactometer assays, bugs of different age and sex were attracted to volatiles from mixed-sex chinch bug aggregations and showed particular attraction to groups of adult female chinch bugs. Adult males and nymphs were also attracted to adult males. Nymphs were attracted to nymphs and were also more attracted to aggregation volatiles when they could see bugs in the arm of the Y-tube. Adult males were more attracted to short-winged than long-winged adults, while females and nymphs demonstrated no preference. All bugs were attracted to St. Augustinegrass volatiles when presented alone, but only males preferred the odor of grass over odor released from a chinch bug mixed-sex aggregation. When presented with a choice of grass and grass + aggregation volatiles, males preferred the combined treatment. The results of these assays suggest that a complex combination of life stage, sex, as well as plant and insect-derived signals influence chinch bug aggregation behavior.

KEY WORDS *Blissus insularis* Barber, aggregation, pheromones, host-plant volatiles, Y-tube olfactometer

For some species, the benefits of high-population densities outweigh the costs of competition, and these species are often found to be highly aggregated on a spatial scale. The potential evolutionary advantages of such a life history strategy are many and may include a reduction in predation risk, increased ability to exploit hosts, environmental conditioning, greater mate choice opportunities, or a combination of such effects (Stephens and Sutherland 1999, Biedermann 2003, Wertheim et al. 2005). In the case of highly aggregated species, it is assumed at least one component of an individual's fitness exhibits positive density dependence and that the strength of the density or fitness relationship is great enough to select for an aggregative lifestyle (Courchamp et al. 2009).

Insects in several orders maintain nonsocial gregarious populations, often through a complex array of chemical, physical, and behavioral signals (Wertheim et al. 2005). While research has been conducted on the use of auditory (Fletcher 2008, Wijenberg et al. 2008) and visual cues (Reisenman 2000, Strom et al. 2001) in insect aggregation behavior, the major focus has been

on identifying insect aggregation pheromones. Aggregation pheromones are of particular interest as management tools for pest insects. These pheromones act to draw conspecifics together and/or maintain aggregations and can be found in the orders Blattodea, Collembola, Dermaptera, Orthoptera, Diptera, Coleoptera, Lepidoptera, Hemiptera, Hymenoptera, Thysanoptera, and Thysanura (Wertheim et al. 2005).

The southern chinch bug, *Blissus insularis* Barber (Hemiptera: Blissidae) is an insect pest species that forms high density aggregations. These bugs, particularly in large aggregations, have a pungent, sweet-smelling odor (K. M. Adesso, personal observation) suggesting volatile compounds are released by the insects. The southern chinch bug is a relative of several other pest chinch bug species, including the common (*B. l. leucopterus* Say), hairy (*B. l. hirtus* Montandon), and western (*B. occidentalis* Barber) chinch bugs. All of these insects are pests of various grass species in North America and the Caribbean (Sweet 2000). Southern chinch bug is a problem species in the southern United States where it feeds on the phloem of warm season grasses. Its preferred host plant is St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze for which it is the major insect pest (Crocker 1993), but its feeding range also includes species of torpedo grass (*Panicum repens* L.), pangola grass (*Digitaria decumbens* Stent.), zoysiagrass (*Zoysia* spp.), Bermuda grass

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(*Cynodon dactylon* L.), centipede grass (*Eremochla ophiuroides* (Monroe) Hack), and bahiagrass (*Paspalum notatum* Flugge.) (Sweet 2000). Southern chinch bug feeding damage results in eventual yellowing of the grass followed by plant death. An infestation is recognizable as a brown dead patch with a yellow border that expands as the aggregation moves outward to infest new plants. Aggregation densities can be very high and drop off rapidly within a short distance. Cherry (2001b) found a few dozen chinch bugs on dead grass at the center of the infestations, $\approx 1,500$ on the yellowing grass at the edge of the infestation and 2–3 individuals 5 m away in healthy grass. Like many of its congeners, this species exhibits a long (macropterous) and short (brachypterous) wing polymorphism. Long-winged adults occur more frequently when population density is high, specifically during the summer and fall months (Cherry 2001c), however, the behavioral and ecological consequence of this polymorphism remains unclear as long-winged morphs are rarely observed engaging in flight in the field.

Control methods for southern chinch bug include proper mowing height (Trenholm et al. 2011), water and fertilization regimes, and the use of chinch bug resistant varieties (Buss 2010). Ants, spiders, and various predaceous bugs are all found within or around chinch bug infestations and have been suggested as natural controls of chinch bug populations (Reinert 1978). While investigating the interactions between southern chinch bugs and its predators, Cherry (2001a) and Cherry and Nagata (2005) found that although red imported fire ants (*Solenopsis invicta* Buren) and big-eyed bugs (*Geocoris* spp.) were present in high numbers, neither predator population suppressed chinch bug outbreaks. Development of southern chinch bug resistant St. Augustinegrass cultivars such as 'Floritam,' 'FX-10,' and, more recently 'Captiva,' aided in suppression of this pest; however, southern chinch bug populations in Florida and Texas have effectively overcome host-plant resistance in Floritam (Busey and Center 1987, Cherry and Nagata 1997, Rangasamy et al. 2006, Reinert 2008) and Captiva (Reinert et al. 2011). Unfortunately, the only way to prevent plant death on susceptible plants once an outbreak has begun is through the application of pesticides. Pesticides kill nymphs and adults, but eggs remain unaffected, requiring multiple pesticide applications for effective population control (Buss 2010). Heavy pesticide use in areas such as southern Florida has led to the development of pesticide resistance (Cherry and Nagata 2005, 2007). As a result, many common pesticides, such as bifenthrin, deltamethrin, lambda-cyhalothrin, and imidacloprid, are now ineffective against *B. insularis* in these areas.

There are no published studies on the aggregation behavior of species within the genus *Blissus*, despite the importance of this behavior to the destructiveness of these pests. We investigated physical and chemical cues and signals contributing to the formation and maintenance of *B. insularis* aggregations. In the following experiments we investigated the importance of visual and thigmotactic cues on initiation of aggrega-

tion, the relative attractiveness of volatiles from different wing morphs and life stages of the bug, and the attractiveness of St. Augustinegrass in small-arena and Y-tube assays.

Materials and Methods

Insects and Plants. Southern chinch bugs used in these experiments were collected from St. Augustinegrass lawns in Alachua Co., Gainesville, FL, and county-wide collection in Palm Beach County, FL to maximize genetic diversity of test insects. We chose Floritam St. Augustinegrass for insect maintenance and all of our experiments because it is the most common turf variety in Florida accounting for 69% of all St. Augustinegrass sod production in a 2003 survey (Haydu et al. 2005), and is a good host for southern chinch bug now that they have overcome its resistance. The chinch bugs were kept in white plastic buckets (30-cm diameter and 36-cm height) containing potted Floritam St. Augustinegrass under a photoperiod of 14:10 (L:D) h at an average temperature of 25–27°C and 40% RH. The grass in the buckets was watered and replaced with uninfested potted plants as necessary. Grass was grown in a greenhouse (30°C and 60% RH) in 12-cm diameter pots in Metro-Mix 200 (Sun Gro Horticulture, Bellevue, WA). Plants were watered daily and fertilized using Osmocote 14–14–14 slow release pellets (The Scotts Company, Marysville, OH). New pots of grass were initiated from nodes of old plants, and each new pot contained six newly transplanted nodes. In all of the following arena and Y-tube assays 'male' and 'female' refer to adult chinch bugs and 'nymphs' refers to a mix of bugs in their fourth and fifth instars of unknown sex (unable to sex nymphs).

Small-Arena Bioassays. In the first arena assay, the strength of the aggregation response by nymphs, males and females was investigated. Assay arenas were conducted in 9-cm diameter plastic petri dishes (Fisher, Pittsburgh, PA) with a moistened 7-cm diameter filter paper placed in the center to maintain humidity. The assay room was maintained at 25–27°C and 40% RH.

Ten nymphal, adult male or adult female chinch bugs were released into the arena and distributed 1-cm apart from one another with a fine tipped paintbrush. Twelve replications for each life stage or sex were performed and two characteristics of aggregation strength were recorded after 5 and 90 min. The first value was the number of groups formed by the chinch bugs in which insects were within 1 cm of each other (hereafter referred to as *aggregation #*). An 'aggregation' here may have contained anywhere from 2 to 10 individuals, therefore, aggregation strength increased as *aggregation #* decreased. The second characteristic recorded for each replicate was the largest number of individuals in an aggregation (hereafter referred to as *maximum aggregation*). This assay was conducted under light and in darkness to address the importance of visual cues in the formation of aggregations. For light assays, light was provided by a fluorescent light fixture

(four Sylvania Cool White high output 85-W bulbs) suspended 70 cm above the table surface.

To test the importance of thigmotactic cues to the initiation of an aggregation, an assay was conducted where insects were provided with a shelter of *St. Augustinegrass* leaf blades or artificial leaf-like shelters. The shelter assay was conducted following the methods described above under light. Groups of 10 chinch bugs were provided with a shelter under no-choice conditions: a 6-cm leaf blade of *St. Augustinegrass* (≈ 1 -cm width), a 6×1 cm piece of white filter paper, or a 6×1 cm piece of green construction paper. No attempt was made to match the color of the paper to the color of *St. Augustinegrass* because *St. Augustinegrass* ranges in color from yellow to dark green, based on fertilization. Green paper represented a plant-like visual cue in the absence of volatile chemical cues. The paper shelters were folded lengthwise and placed with the crease side up to provide a hiding place for the chinch bugs. Leaves were similarly oriented so that chinch bugs could hide beneath the leaf. Twelve replications for each life stage or sex were performed and the total number of bugs aggregating on or under the leaf blade or artificial shelters was recorded after 5 and 90 min. Arena assay data for both experiments were analyzed by paired *t*-tests for across time period comparisons within treatments (PROC TTEST; SAS Institute 2006) and with MANOVA (PROC GLM) for comparisons between treatments and sexes at repeated time intervals (5 and 90 min).

Y-Tube Olfactometer Bioassays. Two glass Y-tube olfactometers (Analytical Research Systems, Gainesville, FL) resting horizontally on a padded bench were used in the experiments. Compressed air was pushed through a charcoal filter and humidified by bubbling through water before separating into two glass chambers 21 cm long \times 3 cm diameter (=volatile source chambers). Teflon tubing (60-cm in length) connected volatile source chambers to each arm of the olfactometers and airflow was regulated at 250 ml/min using four inline flow meters (Manostat, New York, NY). The Y-tubes (12 cm common tube, 10 cm arms, 2.5 cm internal diameter) were placed 70 cm below fluorescent lights (four Sylvania Cool White high output 85-W bulbs). Adult chinch bugs used in the following assays were sexed the afternoon prior and maintained separately in $10 \times 10 \times 8$ cm clear containers with a moist paper towel and with Floratam *St. Augustinegrass* in 10-cm floral aquatubes (Wholesale-Floral.com, Buffalo, NY). The experiments were conducted from 0900 hours to 1400 hours. Treatment insects and/or plants were allowed to acclimate in the volatile source chamber for 10 min before assays were conducted. For each assay, individual bugs were placed into the inlet of the Y-tube and given 15 min to make a decision. The chinch bugs were considered to have made a decision once they crossed 5 cm into an arm of the Y-tube and those that did not make a decision after the allotted time were recorded as no choice. Bugs were assayed until 50 males, 50 females, and 50 nymphs had responded. After every five successful choice replicates, the tubes were switched so

the right and left side airflows were reversed to avoid directional bias. Y-tubes were rinsed with ethanol and dried following every 10 replicates to prevent build-up of any possible cues from previous walkers and between male, female, and nymph treatments to avoid conflicting odor sources within the Y-tube. At the end of each day, Y-tubes and glass chambers were washed with soap and water, rinsed with ethanol, dried with paper towels and placed in an oven at 250°C overnight to ensure removal of any residue.

Four assays were conducted to investigate southern chinch bug attraction to conspecific volatiles. In all of the following assays, an aggregation was composed of 20 randomly selected males, females, and fourth and fifth instars, unless stated otherwise. In the first assay, we investigated attraction of males, females, and fourth and fifth instars to an aggregation. The bugs were given a choice between filtered air and aggregation volatiles. In the second assay, we tested whether a visual cue of chinch bug presence would increase attraction to the aggregation volatiles. Volatiles from an aggregation were presented simultaneously in both arms of the Y-tube, while one arm contained five dead southern chinch bugs placed at the half-way point of the arm (5 cm). The dead chinch bugs had been killed in a freezer and desiccated in a drying oven for a minimum of 15 h at 40°C before the assay to remove living aggregation odors and approximate the condition of desiccated chinch bug cadavers in the field. In a third assay, attraction to volatiles from 20 males, 20 females, or 20 nymphs were tested against clean air to identify differences in attraction by sex and life stage. In the fourth assay, 20 short-winged insects (10 male and 10 female) were placed in one chamber and 20 long-winged insects (10 male and 10 female) were placed in the opposite chamber.

Three assays were conducted to investigate the influence of plant volatiles and a combination of plant and bug volatiles in attraction. Cuttings of Floratam *St. Augustinegrass* in aquatubes were used in all of the following assays (three stolons, each with three nodes). Cuttings were replaced periodically if they began to wilt. In the first assay, attraction to volatiles from grass cuttings and filtered air were compared. In the second assay, bugs were given a choice between volatiles from an aggregation and volatiles from grass. In the final assay, volatiles from a combination of grass cuttings and an aggregation were tested against volatiles from grass alone. All Y-tube assays were analyzed using chi-squared analysis for categorical data where the null hypothesis was a 50:50 ratio (Proc Freq; SAS Institute 2006).

Results

Small-Arena Bioassays. In the aggregation assay, southern chinch bug aggregation # decreased (and therefore aggregation strength increased) over time for nymphs and males under light and darkness (Table 1). Female aggregation strength increased over time under darkness, but not light. When the aggregation # for the three age or sex classes were compared, males

Table 1. Aggregations formed in arenas containing 10 southern chinch bugs (mean \pm SEM) under light and dark conditions ($n = 12$)

Sex	Treatment	Aggregation # ^a				Maximum aggregation # ^b			
		5 min	90 min	Paired <i>t</i>	<i>P</i> value ^c	5 min	90 min	Paired <i>t</i>	<i>P</i> value
Nymph	Light	4.9 (\pm 0.5)	3.6 (\pm 0.4)	3.2	0.0082	4.8 (\pm 0.6)	5.6 (\pm 0.5)	1.1	0.2829
	Dark	7.2 (\pm 0.4)	4.8 (\pm 0.6)	4.7	0.0007	2.9 (\pm 0.5)	4.7 (\pm 0.6)	2.5	0.0316
	<i>t</i> -test ^d	3.4	1.9			2.5	1.1		
	<i>P</i> value	0.0027	0.0739			0.0211	0.2830		
Male	Light	4.8 (\pm 0.3)	2.8 (\pm 0.4)	4.2	0.0015	4.8 (\pm 0.4)	7.2 (\pm 0.6)	3.2	0.0083
	Dark	5.8 (\pm 0.6)	4.1 (\pm 0.4)	3.7	0.0035	3.9 (\pm 0.6)	5.3 (\pm 0.5)	0.9	0.4078
	<i>t</i> -test	1.5	2.5			1.1	2.2		
	<i>P</i> value	0.1473	0.0198			0.2814	0.0354		
Female	Light	5.7 (\pm 0.7)	4.7 (\pm 0.7)	1.2	0.2412	4.2 (\pm 0.7)	5.2 (\pm 0.9)	3.0	0.0126
	Dark	6.0 (\pm 0.4)	4.3 (\pm 0.4)	2.8	0.0172	4.3 (\pm 0.4)	5.2 (\pm 0.4)	2.0	0.0673
	<i>t</i> -test	0.4	0.4			0.1	0.0		
	<i>P</i> value	0.6848	0.6859			0.9214	1.0		

^a An 'aggregation' can contain 2-10 insects. As aggregation # decreases, aggregation strength increases.

^b The largest size aggregation formed in each petri dish.

^c *df* for paired-*t*-test = 11.

^d *df* for *t*-test = 22.

had a stronger aggregation response than females under light ($F = 3.86$; $df = 2, 69$; $P = 0.0259$; male-female $t = 2.74$; $df = 1$; $P = 0.0079$) but there were no significant differences in any of the other comparisons. Under darkness there was no difference between age or sex classes ($F = 2.91$; $df = 2, 69$; $P = 0.0615$). The maximum number of bugs per group increased over time for nymphs under darkness and males and females under light (Table 1). When the *maximum aggregation* value for the three age or sex classes were compared, there was no difference under light ($F = 2.00$; $df = 2, 66$; $P = 0.1440$) or darkness ($F = 1.93$; $df = 2, 66$; $P = 0.1535$). Nymphs aggregated faster at 5 min under light than darkness as observed by the smaller aggregation number ($t = 3.4$; $df = 22$; $P = 0.0027$) and larger maximum aggregation size ($t = 2.5$; $df = 22$; $P = 0.0211$). Males displayed a light effect at 90 min, with larger (*maximum aggregation*, $t = 2.2$; $df = 22$; $P = 0.0354$), fewer (*aggregation number*, $t = 2.5$; $df = 22$; $P = 0.0198$) aggregations in the light treatment. There was no effect of light on female aggregation number or maximum aggregation size at 5 or 90 min.

In the no-choice shelter assay, more nymphs, males, and females aggregated on grass than on artificial leaf blades at 90 min, but at 5 min only female aggregations were larger on grass (Table 2). Aggregation on grass blades increased over time for both nymphs and males, but not for females. There was no change in aggregation response in green and white artificial shelter treatments for any of the age or sex classes over time. Aggregation on or under artificial leaf-like shelters was low, with most chinch bugs observed gathering in other parts of the petri dish.

Y-Tube Olfactometer Bioassays. In the first assay, males, females, and nymphs all oriented preferentially to aggregation volatiles over air (Fig. 1A). Males and nymphs were highly attracted to the aggregation; 74% of males and nymphs preferred aggregation volatiles ($\chi^2_{(1)} = 11.52$; $P = 0.0007$), while only 66% of females did ($\chi^2_{(1)} = 5.12$; $P = 0.0237$). In the second assay (Fig. 1B), males and females did not discriminate between the aggregation volatile treatments with or without visual and olfactory cues of dead chinch bug presence (males, $\chi^2_{(1)} = 2.00$, $P = 0.1573$; females, $\chi^2_{(1)} = 1.28$,

Table 2. Number of southern chinch bugs (out of 10) aggregating under natural or artificial shelters (mean \pm SEM) in arenas under light conditions in no-choice tests ($n = 12$)

Sex	Shelter	5 min	90 min	Paired <i>t</i>	<i>P</i> value ^a
Nymph	Grass	0.8 (\pm 0.3)	2.3 (\pm 0.6) ^{a,b}	2.24	0.0469
	White paper	0.7 (\pm 0.3)	0.4 (\pm 0.3) ^b	1.91	0.0819
	Green paper	1.1 (\pm 0.5)	1.0 (\pm 0.5) ^b	0.25	0.8088
	<i>F</i> -test	0.32	3.99		
	<i>P</i> value ^c	0.7318	0.0282		
Male	Grass	1.8 (\pm 0.4) ^a	4.5 (\pm 0.7) ^a	4.58	0.0008
	White paper	0.5 (\pm 0.2) ^b	0.6 (\pm 0.3) ^c	0.20	0.8451
	Green paper	1.3 (\pm 0.5) ^{ab}	1.9 (\pm 0.5) ^b	1.34	0.2072
	<i>F</i> -test	3.11	13.44		
	<i>P</i> value	0.0580	<0.0001		
Female	Grass	2.9 (\pm 0.7) ^a	3.7 (\pm 0.6) ^a	1.33	0.2118
	White paper	1.0 (\pm 0.5) ^b	1.6 (\pm 0.8) ^b	1.47	0.1708
	Green paper	0.8 (\pm 0.2) ^b	0.8 (\pm 0.2) ^b	0.36	0.7227
	<i>F</i> -test	5.07	6.78		
	<i>P</i> value	0.0120	0.0034		

^a *df* for paired *t*-tests = 11.

^b Aggregation values for each sex at 5 or 90 min followed by different lowercase letters are significantly different (LSD).

^c *df* for *F*-tests = 2, 33.

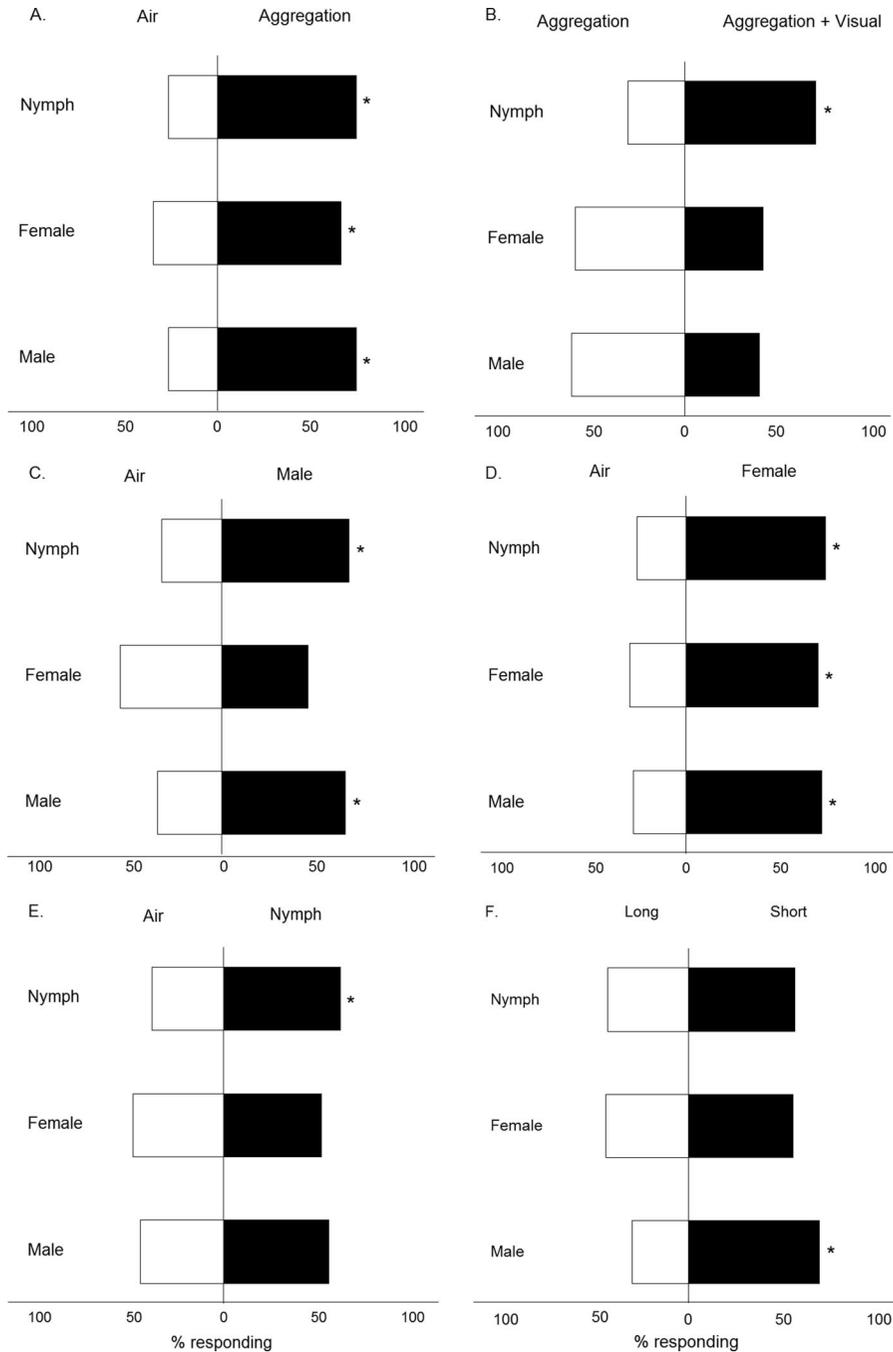


Fig. 1. Percentage response of adult males, females, and fourth or fifth stage nymphs to volatiles from (A) an aggregation of mixed sex and age versus air, (B) an aggregation versus aggregation + visual cues of chinch bug presence, (C) air versus male bugs, (D) air versus female bugs, (E) air versus fourth or fifth stage nymphs, and (F) long versus short-winged adults. Asterisk indicates a chi-squared P value of ≤ 0.05 .

$P = 0.2579$). Nymphs, however, chose the treatment arm containing dead bug cues more often than the arm with volatiles alone ($\chi^2_{(1)} = 8.0, P = 0.0047$; visual + aggregation treatment = 70%).

In the third assay, females did not show preferential attraction to male-produced volatiles compared with

air ($\chi^2_{(1)} = 0.64, P = 0.4237$) (Fig. 1C), whereas males and nymphs were attracted to male aggregations (male, 66%, $\chi^2_{(1)} = 10.24, P = 0.0014$; nymph, $\chi^2_{(1)} = 12.96, P = 0.0003$). When chinch bugs were presented with female-produced volatiles (Fig. 1D), >70% of males, females and nymphs chose the arm bearing the

female aggregations compared with the air-only arm; (female, $\chi^2_{(1)} = 16.0$, $P < 0.0001$; male, $\chi^2_{(1)} = 19.36$, $P < 0.0001$; and nymph, $\chi^2_{(1)} = 23.04$, $P < 0.0001$). When presented with nymph volatiles or air (Fig. 1E), males and females showed no preference (male, $\chi^2_{(1)} = 1.44$, $P = 0.2301$; female, $\chi^2_{(1)} = 0.16$, $P = 0.6892$). Nymphs, however, were attracted to other nymphs (nymph, $\chi^2_{(1)} = 5.76$, $P = 0.0164$). In the fourth assay (Fig. 1F), where bugs were given a choice between volatiles from long and short-winged adult chinch bugs, males strongly preferred volatiles released by short-winged bugs to that of the long-winged bugs ($\chi^2_{(1)} = 16$; $P = 0.0001$) while females ($\chi^2_{(1)} = 1.44$; $P = 0.2301$) and nymphs ($\chi^2_{(1)} = 1.80$; $P = 0.1802$) did not discriminate between volatiles from the two morphs.

In the first of the plant volatile assays, when offered grass volatiles or purified air, males, females, and nymphs all preferred grass volatiles (Fig. 2A) (females, $\chi^2_{(1)} = 14.29$, $P = 0.0002$; males, $\chi^2_{(1)} = 16.65$, $P = 0.0001$; and nymphs, $\chi^2_{(1)} = 7.84$, $P = 0.0051$). In the second assay, females ($\chi^2_{(1)} = 0.64$, $P = 0.4237$) and nymphs ($\chi^2_{(1)} = 0$, $P = 1.0$) showed no preference for aggregation or grass volatiles while males preferred grass volatiles over the aggregation ($\chi^2_{(1)} = 31.36$, $P < 0.0001$; Fig. 2B). In the final assay, however, only males preferred grass and aggregation over grass alone ($\chi^2_{(1)} = 23.04$, $P < 0.0001$; Fig. 2C). Females ($\chi^2_{(1)} = 0$; $P = 1.0$) and nymphs ($\chi^2_{(1)} = 1.96$; $P = 0.1615$) showed no preference for the combined treatment over grass alone.

In each of the aforementioned assays, a total of 50 responders were used in the analysis. Individuals who demonstrated no preference were not considered in the analysis. Across all experiments, an average of 12 ± 2 males, 13 ± 2 females, and 15 ± 2 nymphs failed to walk. Males and females had the lowest failure rates in the whole aggregation (seven males and six females) and female aggregation experiments (seven males and five females). Nymphs had the lowest failure rates in the whole aggregation and nymph aggregation assays where eight and nine chinch bugs failed to respond, respectively. The highest failure rate for both males and females was in the long versus short-winged assay (21 males and 21 females failed to respond). Nymphs performed the worst in the air versus grass assay with 24 failing to respond.

Discussion

Chinch bugs formed aggregations within minutes of being placed in a petri dish and this aggregation behavior increased over time resulting in fewer aggregations with larger numbers of insects per aggregation. This was the result of solitary bugs joining previously formed aggregations and smaller groups of bugs coalescing into larger aggregations. When bugs aggregated, it was not uncommon for them to climb on top of each other, forming small piles of bugs—a phenomenon observed in colony plants as well (K. M. Adesso, personal observation). These aggregation behaviors were observed in the presence and absence of light,

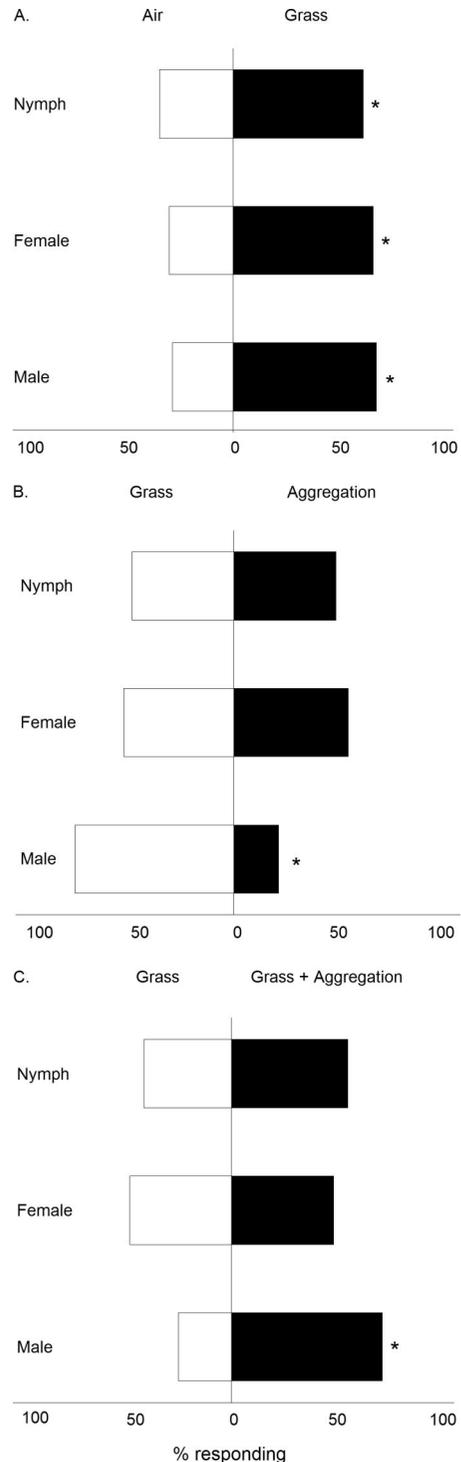


Fig. 2. Percentage response of adult males, females, and fourth or fifth stage nymphs to volatiles from (A) air versus grass, (B) grass versus aggregation, and (C) grass versus grass + aggregation. Asterisk indicates a chi-squared P value of ≤ 0.05 .

suggesting visual cues are not required for the formation of aggregations. While light is not essential for the formation of aggregations, visual cues may be important for nymphs and males as these two groups had larger, fewer aggregations under light conditions than under dark conditions. Additionally, thigmotactic stimuli alone do not appear sufficient to stimulate aggregation. In the field, chinch bugs are often found hiding in groups within the leaf sheaths of grass, but the presence of a paper shelter did not appear to play a significant role in the formation of aggregations as the bugs were not any more likely to spend time on or under the shelter than in the remainder of the dish. Leaf blades, however, were sheltered under more often than paper shelters suggesting a role of host plant cues in addition to conspecific cues in aggregation formation.

Conspecific cues clearly play a role in southern chinch bug attraction as evinced in both the arena and Y-tube bioassays. Many hemiptera are known to assemble in large aggregations at least once in their lifetime and these aggregations are often aided by the production of aggregation pheromones (Wertheim et al. 2005). At present, no pheromones in the family Blissidae have been chemically identified, but pheromones of other hemipteran families such as Lygaeidae, Miridae, and Coreidae have been investigated to a much greater extent (The Pherobase 2011). Our results suggest that adults of both sexes may produce aggregation pheromones as is the case in other species, such as the boxelder bug, *Boisea rubrolineata* Barber (Schwarz and Gries 2010), though attraction to females appears to be stronger than attraction to males, as is the case with the tarnished plant bug, *Lygus rugulipennis* Poppius (Glinwood et al. 2003). The lack of a strong female attraction to males may be because of the release of sex-specific pheromones by males. This result, however, does not prove that females are never attracted to males. The insects used in this study were field collected and a large proportion may have mated before the bioassays, decreasing their sexual responsiveness to males as is the case for a wide range of insects, including hemipterans such as the rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Yamane et al. 2011), and the whitefly predator, *Macrolophus caliginosus* Wagner (Gemeno et al. 2007). There remains the possibility that unmated females would be attracted to male bugs. Preliminary analysis of chinch bug headspace revealed the presence of unique compounds in male and female southern chinch bug headspace in addition to a suite of common compounds, making it possible for males and females to differentiate between one another on the basis of olfaction alone (K. M. Adesso, personal observation).

Neither males nor females were attracted to nymphs in our assays, but nymphs were attracted to each other. This type of stage specific aggregation behavior has been observed in other hemipteran nymphs that feed in groups on plants, such as immature spittlebugs, *Neophilaenus albipennis* (F.) (Biere-mann 2003), and southern green stink bug, *Nezara viridula* (L.) (Lockwood and Story 1985). The gre-

garius feeding behavior of the immature stage may aid in overcoming plant defenses and/or decreasing predation risk. An attractive volatile signal specific to immatures is one method for attracting early instars to one another. Nymphs also responded positively to the presence of dead adults in the olfactory arm, suggesting they use additional cues related to adult chinch bugs in maintaining contact with the aggregation. Dead adults and younger nymphs are left behind in older areas of an infestation as the bulk of the aggregation migrates across a field. These dead adults may act as an additional aggregation cue for nymphs to follow. While the presence of dead adults enhanced attraction of nymphs to the aggregation pheromone, it is unclear whether the nymphs were responding to the visual cue offered by the presence of dead adults, olfactory cues provided by necromones, or a combination of the two.

Wing polymorphism also played a role in attraction, particularly for male attraction to adults. In the common and hairy chinch bugs, long-winged morphs are the migratory forms that seek out new host patches for utilization (Sweet 2000). Southern chinch bug, however, feeds on common lawn grass varieties and its primary dispersal method is walking (Buss 2010). Southern chinch bug has never been observed to fly in the laboratory or field but there is circumstantial evidence that flight is possible, as they were on one occasion observed in flooded rice fields in south Florida (R. Cherry, personal observation). Whether or not they can fly, the macropterous phenotype has fitness consequences for females. While there was no difference in overall body size between the two female morphs, Cherry and Wilson (2003) found that the dissected ovaries of long-winged females were less likely to contain eggs. In the same study, only 40% of long-winged females laid eggs during a 10-d trial period compared with 75% of short-winged females and those long-winged females laid fewer eggs than their short-winged sisters. Similar reductions in reproductive output were reported for long-winged females of the small brown planthopper (Mishiro et al. 1994), the firebug *Pyrrhocoris apterus* (Hodkova and Socha 2006), and the crickets *Gryllus rubens* Scudder (Mole and Zera 1993) and *Modicogryllus confirmatus* Walker (Tanaka 1993). As female reproductive fitness affects both females and their mates, we would expect males to choose mates that maximize their reproductive output. If short and long-winged females emit quantitative or qualitatively different odors, and these odors correlate with reproductive fitness traits, then the detection of these differences would be advantageous to males when selecting mates.

Chinch bugs are attracted to host plant volatiles, but the interaction of aggregation pheromones and plant volatiles is not straightforward. A growing body of literature suggests that the combination of insect-specific and host plant volatiles are often more attractive than either odor alone (Reddy and Guerrero 2004). In the case of the southern chinch bug, only males display a preference for grass combined with insect odors over grass alone, while females and nymphs are ambivalent.

Ambivalence in this context could be explained by competing interests: to remain close to the aggregation or to disperse to new oviposition or feeding sites. An alternate explanation is that the choices presented to females and nymphs in the Y-tube may not represent choices the bugs make in nature. Nymphs, for example, may rarely be presented with the choice of healthy grass or healthy grass with an aggregation, but rather a choice between the dying foliage on which they were laid and adjacent healthy foliage to which the aggregation has migrated (Cherry 2001b). Females, too, may be more likely to choose grass without insects present when food and oviposition sites become scarce, but not before. If males are the pioneers of the aggregation, it would explain their preference for grass volatiles over an aggregation without grass and it would be equally advantageous for males to seek out the combined odor of potential mates and healthy grass over healthy grass alone.

The purpose of our study was to gain a greater understanding of southern chinch bug aggregation behavior and suggest areas of further study. It is clear from the Y-tube studies that adult and nymphal southern chinch bugs exhibit an especially strong upwind response toward female odors. Additionally, nymphs appear to emit a unique odor attractive only to other nymphs. We suggest that further study into the composition of these odors will improve our understanding of how chemical communication influences the formation of southern chinch bug aggregations as well as its effects on nymph survival, mate selection, predation risk, and other fitness traits.

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