Chemical Ecology

Electroantennogram Responses and Field Trapping of Asian Cockroach (Dictyoptera: Blattellidae) with Blattellaquinone, Sex Pheromone of the German Cockroach (Dictyoptera: Blattellidae)

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ABSTRACT The Asian cockroach, *Blattella asahinai* Mizukubo, first introduced to Florida in 1986, has been spreading throughout the southeastern United States. Populations can reach extremely high densities and cause damage to crops as well as become a nuisance in residential settings. Because the German cockroach, *Blattella germanica* L., is its closest extant relative, we characterized the *B. asahinai* male response to blattellaquinone, the sex pheromone of the German cockroach, in an effort to develop monitoring tools for *B. asahinai*. Electroantennogram (EAG) analysis was conducted on *B. asahinai* and *B. germanica* males and females, and revealed that the antennae of males of both species responded significantly more to blattellaquinone than females, and in both males and females absolute EAG responses of *B. asahinai* were greater than in *B. germanica* males and females, respectively. However, normalized male EAG response curves and ED_{50} values (effective dose to elicit 50% of maximal response) did not differ significantly between the two species. Results of field trapping experiments demonstrated that male *B. asahinai* were more attracted to blattellaquinone than any other life stage, and 10 µg of blattellaquinone attracted the most males. These results suggest that blattellaquinone or a similar compound might be a component of the sex pheromone of *B. asahinai* females.

KEY WORDS *Blattella asahinai*, *Blattella germanica*, sex pheromone

The Asian cockroach, *Blattella asahinai* Mizukubo, first described in 1981 in Okinawa, Japan (Mizukubo 1981), was introduced into Florida in 1986 (Roth 1986) and has since been spreading throughout Florida and the southeastern United States along major highway routes to Alabama, Georgia, South Carolina, and Texas (Koehler 1999, Sitthicharoenchai 2002, Pfannenstiel et al. 2008, Snoddy and Appel 2008). Populations of the Asian cockroach can reach high densities, and adults often enter homes when attracted to incandescent lights (Brenner et al. 1988). In addition to being a nuisance in residential settings, *B. asahinai* has been found in densities of up to 54,000 per acre in strawberries, and it damages fruit by creating excavations (Price and Nagle 2008). The Asian cockroach has also been observed to feed on parasitized brown citrus aphids in citrus groves (Persad and Hoy 2004), preventing the emergence of adult parasitoids. However, this invasive species has been observed to perform beneficial functions, such as feeding on the eggs of lepidopteran pests in soybean (Pfannenstiel et al. 2008).

The closest extant relative of the Asian cockroach is the German cockroach, *Blattella germanica* L. (Roth 1985). The two species are very difficult to differentiate using external morphology (Roth 1986, Lawless 1999),

but can be easily distinguished by their ecology and behavior (Brenner et al. 1988). Unlike the German cockroach, which lives exclusively indoors and cannot fly, the Asian cockroach lives outdoors where it inhabits leaf litter, and can fly readily (Brenner et al. 1988).

Females of many cockroach species, including several pest species, produce volatile sex pheromones that attract males (review: Gemeno and Schal 2004). These pheromones have been used to detect the presence of pest cockroach species, monitor population levels, and enhance attractiveness of traps containing insecticide (Bell et al. 1984, Liang et al. 1998). The major component of the female volatile sex pheromone of the German cockroach, blattellaquinone, attracts German cockroach males in olfactometer assays and lures those males to traps in the field (Nojima et al. 2005). Closely related species sometimes use similar or identical components in their sex pheromones, resulting in their coattraction to pheromone-baited traps (Elkinton et al. 2010, Eliyahu et al. 2012). Because *B. asahinai* is most closely related to *B. germanica* and the two species hybridize in the laboratory (Roth 1986, Ross 1992), it is possible that *B. asahinai* males may respond to blattellaquinone.

The aims of this study were to 1) characterize and compare the antennal responses to blattellaquinone of *B. asahinai* and *B. germanica* males and females using electroantennogram (EAG), and 2) to determine if

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blattellaquinone is effective in attracting *B. asahinai* to traps in the field. If blattellaquinone is attractive to *B. asahinai* in the field, it may be a useful tool for detection and monitoring population levels.

Materials and Methods

Insects. *B. germanica* were taken from the lab colony (Orlando Normal = American Cyanamid strain, collected >60 yr ago in Florida; in our culture since 1989) and provided continuously with rat chow (Lab-Diet 5001, PMI Nutrition International, Brentwood, MO) and water. Cockroaches were raised in an incubator under a photoperiod of 12:12 (L:D) h at 27°C and 40–70% relative humidity (RH).

B. asahinai were collected from a recently established field population on the North Carolina State University main campus in Raleigh, NC, and maintained in the lab in an incubator with a photoperiod of 14:10 (L:D) h at 27°C and 45–55% RH. Cockroaches were provided continuously with rat chow and water.

Electroantennogram. Adult male and female *B. asahinai* of unknown ages and 7–9-d-old adult male and female *B. germanica* were obtained from the lab colonies. Males and females of each species were isolated in the laboratory for 3 d. An antenna was excised above the scape and the first distal segment of the antennal tip was removed. The base of the antenna was mounted into a pulled glass micropipette containing *B. germanica* saline solution (Kurtti and Brooks 1976) and a gold wire electrode connected to ground. The antenna tip was mounted into a pulled glass micropipette containing *B. germanica* saline and a gold wire electrode connected to a high impedance universal single ended probe (Syntech, Kirchzarten, Germany). An IDAC-2 amplifier (Syntech) was coupled to a PC and EAG Pro Version 1.1 (Syntech) for data acquisition, display, and analysis.

A stock blattellaquinone (synthesized per Nojima et al. 2005) solution was dissolved in dichloromethane and serially diluted in hexane in decade steps from 10 µg/µl to 100 pg/µl. Ten microliters of each solution was applied to filter paper strips (Whatman number 1, GE Healthcare Bio-Sciences, Pittsburgh, PA) and allowed to evaporate. Ten microliters of hexane served as negative control. Filter papers were loaded into glass Pasteur pipettes and presented to each antenna in ascending order of dosage. A hexane control was presented first followed by blattellaquinone doses, and then hexane again. A 60-s recovery time was allowed between stimuli. Two *B. asahinai* and two *B. germanica* antennae were tested with each filter paper set, alternating each species. The species presented with odorants first alternated each time a new set of filter papers was made. Ten antennae were tested for each species and each sex.

Medical-grade breathing air from a tank was routed through flow regulators, humidifiers, and a three-way electronic valve that controlled air delivery to the stimulus in the Pasteur pipette. The total flow rate was 650 ml/min, and 400 ml/min were diverted to the stimulus pipette for 0.3 s to create a 2-ml stimulus puff.

The stimulus and general airflow were recombined in an 8-mm-id stainless-steel tube that delivered the stimulus to the antenna. The antenna was held 2–3 mm from the end of the tube.

Field Trapping. Blattellaquinone (100 µl of 0.01 µg/µl and 0.1 µg/µl in hexane) was loaded into rubber septa (11-mm sleeve stopper, Wheaton, Millville, NJ) and allowed to evaporate to create 1 µg and 10 µg lures. Control rubber septa lures were loaded with 100 µl of hexane. Lo-line traps (B&G, Jackson, GA) were used to trap *B. asahinai* in the field. Traps were placed ∼50 cm (low traps) or 135 cm (high traps) above the ground on trees and were attached using push pins. One trap of each of three treatments (control, 1 µg, and 10 µg) was placed at each site, with a total of five replicates for low traps and three replicates for high traps. Traps were placed at least 7 m apart at a total of six sites, and sites were between 15 and 80 m apart. Traps were placed at ∼5 p.m. on 23 August 2011 on a clear night with little rain and were collected at ∼8:30 a.m. on 24 August 2011. Trapping was done in wooded areas with dense leaf litter on the North Carolina State University main campus (Raleigh, NC) along railroad tracks that run through campus between (−78.67086, 35.78490833) and (−78.66815333, 35.78394667).

Data Analysis. EAG responses (in millivolts) were obtained from the EAG Pro software as the maximum EAG amplitude. Analysis of differences in absolute EAG responses between species was carried out using SAS version 9.4 (PROC GLM, SAS Institute 2013b). Base-10 logarithms of the millivolt EAG response were taken in order to normalize the data for two-way ANOVA. The General Linear Models (GLM) procedure was used to find the least squares means with the Tukey adjustment for multiple comparisons. Analysis was run separately for males and females. Analysis of differences in absolute EAG responses within each species was carried out using JMP 11 Pro (SAS Institute 2013a). Millivolt responses were log transformed and analyzed by one-way ANOVA with a Tukey HSD test to compare means. Analysis was run separately for each species and sex.

The millivolt EAG responses of males were normalized relative to the response to the hexane control for each replicate. SAS version 9.4 was used to fit a four-parameter logistic model to the data using the NLIN procedure. A separate model was fit for each species, and ED_{50} values (effective dose to elicit 50% of maximal response) were derived.

Field trapping data were analyzed using JMP 11 Pro. A GLM with Poisson distribution was fit to the data. Differences among trap position, dose, and life stage were determined using the Contrast platform command in a pairwise manner by setting the contrast levels to 1 and −1, respectively, for the pair being compared.

Results

Electroantennogram Responses. Antennae of both species responded to blattellaquinone in a dose-dependent manner with two clear patterns: as expected

for a sex pheromone, male antennae were much more responsive than female antennae, and surprisingly the absolute EAG responses of *B. asahinai* males were higher than *B. germanica* males at all doses of blattellaquinone as well as the hexane control (Fig. 1). However, differences in absolute male EAG response between the two species were significant only at the doses of 0.001 μg ($P = 0.047$), 0.01 μg ($P = 0.026$), and 0.1 μg ($P = 0.008$; two-way ANOVA $F = 34.29$; df = 13,126; $P < 0.0001$; Fig. 1A). The dose–response curves indicated significant differences in EAG responses among doses for *B. asahinai* male antennae ($F = 19.68$; df = 6,63; $P < 0.0001$) and *B. germanica* male antennae ($F = 57.58$; df = 6,63; $P < 0.0001$; Fig. 1A). In *B. asahinai* males, the response to 10 and 100 μg doses was significantly higher than all other doses except 1 μg. In *B. germanica* males, the response to 10 and 100 μg doses was significantly higher than to all other doses. Males of both species did not respond significantly to doses <1 μg.

After the male EAG responses were normalized relative to their respective hexane controls, the logistic fit dose–response curves of the two species were similar. The estimated ED_{50} of blattellaquinone for *B. asahinai* males was 0.94 μg, which did not differ significantly from the blattellaquinone ED_{50} for *B. germanica* males (1.27 μg; Fig. 1B; Table 1). The estimated slopes for *B. asahinai* (1.02) and *B. germanica* (1.33) were also similar, suggesting that the two normalized curves did not differ significantly.

Female antennae of both species responded much less to blattellaquinone than male antennae, but as in males, the absolute EAG responses of *B. asahinai* females were higher than *B. germanica* females to the hexane control ($P = 0.018$; Fig. 1C) and to all doses except 10 μg (0.001 μg, $P = 0.001$; 0.01 μg, $P = 0.030$; 0.1 μg, $P = 0.0002$; 1 μg, $P = 0.002$; and 100 μg, $P = 0.007$; two-way ANOVA $F = 16.27$; df = 13,126; $P < 0.0001$; Fig. 1C). The dose–response curves had significant differences in EAG responses among doses for *B. asahinai* female antennae ($F = 10.37$; df = 6,63; $P < 0.0001$) and *B. germanica* female antennae ($F = 6.82$; df = 6,63; $P < 0.0001$; Fig. 1C). The highest doses (10 and 100 μg) elicited significantly greater responses in *B. asahinai* and *B. germanica* females than all other doses except 1 μg. Females of both species did not respond significantly to doses <10 μg blattellaquinone.

Field Trial. Traps were attached to tree trunks at two heights, 50 cm (low traps) and 135 cm (high traps) above the ground (Fig. 2). The highest mean number of *B. asahinai* captured in traps was males at the 10 μg dose of blattellaquinone, both in the high and low trap positions (Fig. 3). The GLM fit to the trapping data was highly significant ($\chi^2 = 134.67$; df = 17; $P < 0.0001$). Pairwise comparisons of each life stage captured at each dose indicated that significantly more males were captured at the 10 μg dose than almost any other life stage and dose ($P < 0.0001$ for most). However, male trap catch at the 10 μg dose high positioned traps was not significantly more than nymphs captured by the 10 μg dose low traps ($P = 0.075$). Additionally, although more males at the 10 μg dose were captured
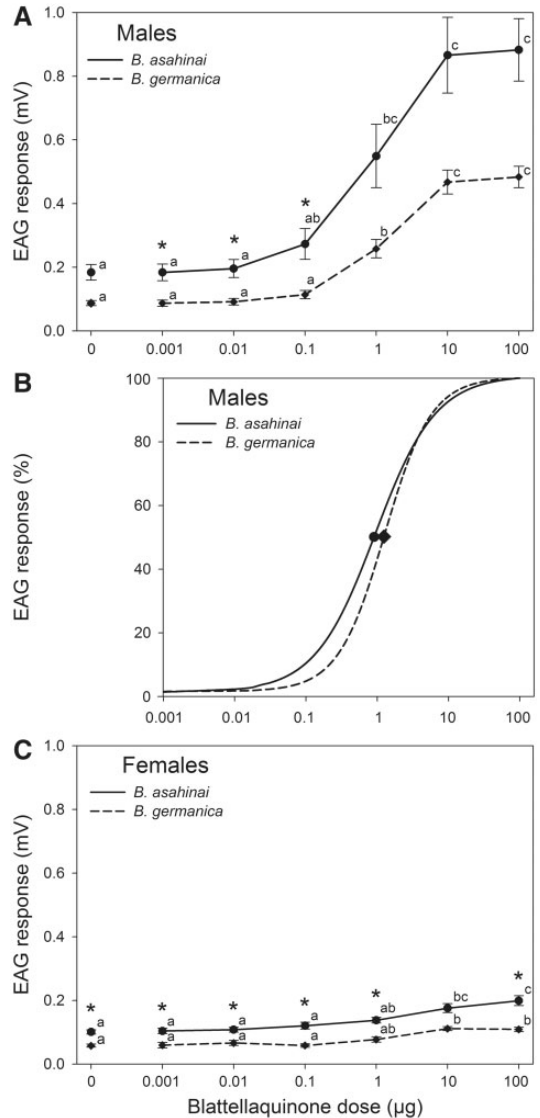

Fig. 1. Mean (±SE) EAG responses of *B. asahinai* and *B. germanica* to blattellaquinone applied to filter paper. (A) Absolute male response; (B) Normalized EAG responses for males; (C) Absolute female response. In (A) and (C), the peak EAG amplitude is shown in millivolts. Doses with the same letter are not significantly different within each species for each sex. Asterisks denote a significant difference at a given dose between species for each sex. Sample size was 10 antennae for each species and sex. In (B), responses were normalized as percentages relative to the hexane control for each antenna. The ED_{50} values, denoted by symbols, and the slope of the curve for each species are not significantly different between species (see Table 1). Curves were generated with four-parameter logistic models.

in traps at the low position than the high position, the difference in mean trap catch for males was not significant ($P = 0.093$). Few nymphs and females were captured, and only in the low traps at 50 cm.

Table 1. EAG responses of adult male *B. asahinai* and *B. germanica* antennae to blattellaquinone

Species	n	Slope ± SE (95% CL)	ED_{50} µg (95% CL)	F	P
B. asahinai	10	1.02 ± 0.51 (0.003, 2.045)	0.94 (0.118, 1.755)	39.83	<0.0001
B. germanica	10	1.33 ± 0.50 (0.325, 2.332)	1.27 (0.748, 1.789)	132.80	<0.0001

EAG responses were normalized relative to the hexane control response for each antenna, and four-parameter logistic models were fit to the data (PROC NLIN; SAS 2013).

Fig. 2. Blattellaquinone-baited Lo-line traps, low position (50 cm) and high position (135 cm) above the ground, in the field site at North Carolina State University, Raleigh, NC, August 2011.
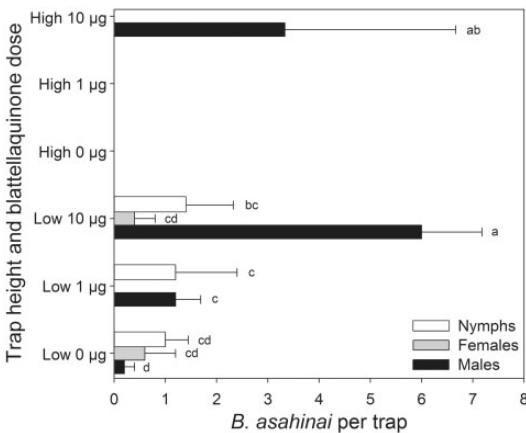

Fig. 3. Mean (±SE) *B. asahinai* captured in high (135 cm) and low (50 cm) positioned Lo-line traps, August 2011. Sample size is three for high traps and five for low traps. Bars indicate trap catches for nymphs, adult females, and adult males. Bars with the same letters are not significantly different. A GLM with Poisson distribution was fit to the data.

Discussion

Blattellaquinone is a major component of the volatile sex pheromone of *B. germanica* females that attracts males (Nojima et al. 2005). Because *B. germanica* is the closest extant relative of *B. asahinai* and the two species hybridize in the laboratory (Roth 1986, Ross 1992), we hypothesized that blattellaquinone might elicit EAG responses from the antennae of *B. asahinai* in a sex-specific manner. If so, we would also expect that blattellaquinone might attract males in the field and therefore could be developed as a tool to detect and monitor *B. asahinai* populations. A recent invasion and persistence over several years (Y.K.M., personal observation) of a highly localized *B. asahinai* population near railroad tracks and a construction site at North Carolina State University allowed us to test these hypotheses.

Antennae of adult male *B. asahinai* and *B. germanica* exhibited clear dose-dependent EAG responses to blattellaquinone (Fig. 1A). The antennae of adult females of both species also responded to blattellaquinone, but with much lower EAG responses than males

(Fig. 1C). These results show that the *B. germanica* sex pheromone also elicits sex-specific EAG responses from *B. asahinai* males. Surprisingly, the absolute EAG responses of *B. asahinai* males and females were significantly higher, respectively, than the EAG responses of male and female *B. germanica*. However, when male responses of both species were normalized to the respective hexane control responses, there was no significant difference in the ED_{50} values or slopes of normalized curves of the two species (Fig. 1B). Thus, both species have similar antennal responses to blattellaquinone, suggesting that blattellaquinone might be a component of the female volatile sex pheromone of *B. asahinai*. However, the reproductive biology and chemical ecology of *B. asahinai* have not been investigated, and it has not been observed whether females engage in calling behavior and emit a volatile pheromone, as do *B. germanica* females (Liang and Schal 1993a, b); if they do, the identification of this pheromone warrants further study.

Deployed in Lo-line sticky traps, blattellaquinone attracted more male *B. asahinai* in the field than any other life stage. However, trap catch was low, despite numerous *B. asahinai* active at each of the collection sites (Y.K.M., personal observation). Two possible reasons for low catch include 1) low trap efficiency and 2) incomplete pheromone blend. In preliminary studies, we placed sticky traps at various heights on tree trunks, attached them to low stakes vertically and horizontally, and placed them horizontally on top of leaf litter. We also tested other styles of traps baited with blattellaquinone, including plastic food storage containers with sections of the sides removed, and plastic 2-liter soda bottles with the top third removed, inverted, and reinserted into the bottle to form funnel traps. Additionally, we tested food attractants such as peanut butter, but no trap and food attractant combination performed as well as blattellaquinone on Lo-line sticky traps. Higher doses of blattellaquinone, such as 100 and 1,000 μg were tested in preliminary trials, but did not yield significantly higher trap catches than the 10 μg dose. *Parcoblatta lata* (Brunner) males, which also fly like *B. asahinai* males, were attracted to a similar formulation of parcoblattalactone, the female sex pheromone, in sticky traps fastened vertically to tree trunks (Eliyahu et al. 2012). However, it is possible that another trap design or trap placement would be more efficacious at capturing *B. asahinai* males.

Low trap catch could more likely be attributed to an incomplete pheromone blend. In Lepidoptera, closely related moth species often share major sex pheromone components, which alone fail to attract males, and species specificity is encoded in a multicomponent sex pheromone blend (Sasaerila et al. 2000, Lelito et al. 2008). In contrast, for some cockroach species, including the German cockroach, brownbanded cockroach (*Supella longipalpa* F.), broad wood cockroach (*P. lata*), and American cockroach (*Periplaneta americana* L.), a single compound in the pheromone blend is sufficient to elicit the full behavioral response from males (Seelinger and Gagel 1985, Charlton et al. 1993, Nojima et al. 2005, Eliyahu et al. 2012, reviewed in Gemeno

and Schal 2004). Nevertheless, in some of these cockroach species males respond better to a blend of multiple sex pheromone components. For example, a complete sex pheromone blend of major component periplanone-B and minor component periplanone-A elicits optimal odor source localization responses in *P. americana* males (Seelinger and Gagel 1985). It is possible that female *B. asahinai* produce more than one behaviorally active pheromone compound. That blend may be necessary to achieve optimal attraction of males. Additionally, EAG alone cannot inform us whether blattellaquinone could be a major or minor component of the *B. asahinai* female sex pheromone. For example, *P. americana* males showed similar amplitude EAG responses to major component periplanone-B and minor component periplanone-A (Nishino and Manabe 1983, Yang et al. 1992). Further studies running female pygidium extracts on gas chromatography coupled to electroantennographic detection, and behavioral assays could elucidate the presence and role of minor components in the *B. asahinai* female sex pheromone.

Cockroach sex pheromones have not been as extensively studied as sex pheromones from other insect taxa. Interestingly, sex pheromones identified to date represent unique chemical structures across different cockroach taxa (review: Gemeno and Schal 2004). However, in the genus *Periplaneta*, several variations on the compound periplanone have been identified as volatile sex pheromones in several species (Gemeno and Schal 2004). No chemical ecology studies exist on olfactory sexual communication in *Blattella* species other than *B. germanica*, so it is possible that the major component of the *B. asahinai* female sex pheromone is closely related to blattellaquinone, such that blattellaquinone is sufficient to attract males in the field, but does not perform as well as the authentic *B. asahinai* female sex pheromone.

Our results indicate that *B. asahinai* males respond to blattellaquinone, the female sex pheromone of *B. germanica*. These results suggest that *B. asahinai* females might emit a similar sex pheromone and its proper identification could provide an attractive lure for this species and prove useful for detection and monitoring Asian cockroach populations. *Blattella* is a diverse genus with 51 species (Roth 1985, 1997) that include pest and nonpest species with diverse ecologies and reproductive biology traits. Elucidating their sex pheromones will facilitate a deeper understanding of species relationships within this important genus.

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