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CHEMICAL DEFENSE IN THE STINK BUG *Cosmopepla bimaculata*

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Abstract—Adult *Cosmopepla bimaculata* discharge a volatile secretion from paired ventral metathoracic glands (MTG) when disturbed. Collected volatiles were similar in both sexes and consisted of *n*-tridecane (67%), (*E*)-2-decenal (12%), (*E*)-2-decenyl acetate (12%), (*E*)-2-hexenal (3%), hexyl acetate (2%), *n*-dodecane (2%), a tridecene isomer (1%), and *n*-undecane, *n*-tetradecane, and *n*-pentadecane (all <1%). In addition, undisturbed males produced a novel insect compound, (*E*)-8-heneicosene, whose function is unknown. The MTG secretion emerges as an enlarging droplet, which is held in place by a cuticular projection and a pleural scent area consisting of specialized rough cuticle surrounding the gland opening. Insects can selectively discharge from either the right or left gland or both glands simultaneously, can control the amount of fluid ejected, and can resorb the ejected secretion droplet back into the gland reservoir. In feeding trials, killdeer (*Charadrius vociferus*), starlings (*Sturnus vulgaris*), robins (*Turdus migratorius*), and anole lizards (*Anolis carolinensis*) rejected or demonstrated aversion to feeding on the bugs. Furthermore, bugs that lacked the secretion were more susceptible to predation than bugs with secretion, suggesting that the secretion functions in defense against predators.

Key Words—*Cosmopepla bimaculata*, Pentatomidae, chemical defense, *n*-tridecane, (*E*)-2-decenal, (*E*)-2-decenyl acetate, (*E*)-2-hexenal, hexyl acetate, *n*-dodecane, *n*-undecane, *n*-tetradecane, pentadecane, (*E*)-8-heneicosene.

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INTRODUCTION

Stink bugs (Heteroptera: Pentatomidae) are well known for the odorous volatiles they emit when molested (Blum, 1981; Aldrich, 1988). These secretions typically contain mixtures of *n*-alkanes, alkenyl acetates, alkenols, and alkenals that primarily function in defense against predators (Staddon, 1979; Blum, 1981; Aldrich, 1988) or as attractants and sex pheromones (Aldrich, 1995, 1996; McBrien and Millar, 1999).

Cosmopepla bimaculata (Thomas) is a 5- to 7-mm long pentatomid found throughout most of the United States, northern Mexico, and southern Canada (Blatchley, 1926). In southern Illinois (USA), adults emerge from overwintering sites in early May and have been collected as late as mid-September in scattered groups of about 15, on plants in pastures and open areas (Esselbaugh, 1946; Fish and Alcock, 1973; McPherson, 1976). Adults are aposematic with red stripes on a shiny black background. In central Illinois, nymphs are also mildly aposematic with early instars being black and reddish orange and later instars exhibiting black patterns on a background that can range from cream or pale yellow to pink or yellow–orange (Decoursey and Esselbaugh, 1962). Both adults and nymphs emit an odorous secretion when squeezed, suggesting that the secretion functions in chemical defense against predators. In this paper we identify the chemical components and glandular source of the secretion, investigate the stimuli that elicit secretion expulsion, examine the palatability of *C. bimaculata* against various predators, and test the hypothesis that the secretion acts to deter predators.

METHODS AND MATERIALS

Insect Collection and Care

Adult *C. bimaculata* were collected from obedient plant, *Physostegia virginiana* (L.) Benth. (Lamiaceae), and lousewort, *Pedicularis canadensis* L. (Scrophulariaceae), in and near Normal, Illinois, from mid-June through early October. Thereafter, the bugs were maintained until needed in clear plastic containers (5 liters) at 25°C under a 14L : 10D photoperiod, and fed daily with fresh green beans, *Phaseolus vulgaris* L.

Chemical Analysis

Volatile Collection and Analysis. Thirty *C. bimaculata* adults were separated by sex with the aid of a microscope. Individuals were then squeezed and quickly placed into individual Hewlett-Packard autosampler vials (glass, 12 × 32 mm, sealed with an 11-mm Teflon-lined septum and aluminum crimp cap). For

controls, undisturbed adult males and females were also individually isolated in autosampler vials. Sampling of released volatiles was by solid-phase microextraction (SPME). The fiber coating was 100 μm poly(dimethylsiloxane). SPME equipment was obtained from Supelco (Bellefonte, Pennsylvania). To sample, we inserted the sheath of the SPME through the septum, then extended the fiber, exposing it to the volatiles for 30–45 min. Volatiles were then injected into a GC for analysis (Hewlett-Packard 5890 Series II, equipped with an HP Chemstation, a split/splitless injector run in splitless mode, and a flame ionization detector). Duration of SPME injection was 30 sec, and the purge valve was opened after 30 sec. The DB-5 capillary column (J & W Scientific, Folsom, California) was 30 m $\times$ 0.25 mm ID and had a 1.0-μm film thickness. The GC oven was held at 50°C for 1 min, then raised to 250°C at 10°C/min, and then held at 250°C for 6 min. Inlet temperature was 200°C and the detector temperature was 250°C. Relative amounts of compounds emitted by the bugs were calculated from SPME-GC peak areas, after correcting for differences in fiber sensitivity (Bartelt, 1997).

Mass spectra were obtained on a Hewlett-Packard 5973 MSD, with sample introduction through a DB-1 capillary column (15 m $\times$ 0.25 mm ID with 0.1-μm film thickness, temperature held at 50°C for 1 min, then raised to 250°C at 10°C/min, and then held at 250°C for 6 min). To analyze larger volatiles [i.e., (*E*)-8-heneicosene DMDS derivative] the final temperature was set at 300°C.

Because electron impact mass spectra make interpretation of double bond positions in hydrocarbons unreliable, the heneicosene found in adult males was derivatized with dimethyl disulfide (DMDS) so that the double-bond location could be determined. Five dead males were extracted by covering them with 2.5 ml of hexane and crushing with a glass rod. To purify the hydrocarbons, the extract was filtered, reduced in volume under N_2 (to concentrate before derivitization), and then applied to a 3- $\times$ 0.5-cm column of silica gel. The hydrocarbons were eluted with hexane. The DMDS adduct was then synthesized and the mass spectrum of this derivative was interpreted according to the procedure of Carlson et al. (1989).

Chemicals Used. We purchased *n*-undecane, *n*-dodecane, *n*-tridecane, *n*-tetradecane, and *n*-pentadecane (all 99%) from Sigma Chemical Co. (St. Louis, Missouri); (*E*)-2-hexenal (99%) and (*E*)-2-hexenol (96%) from Aldrich Chemical Co. (Milwaukee, Wisconsin); and (*E*)-2-decenal from Lancaster Synthesis (Windham, New Hampshire). (*E*)-8- and (*Z*)-8-Heneicosenes were available from a previous investigation (Bartelt and Jackson, 1984).

We synthesized two esters that were not obtained commercially, (*E*)-2-decenyl acetate and hexyl acetate. To make the former, (*E*)-2-decen-1-ol was first prepared by reducing (*E*)-2-decenal with $LiAlH_4$. The aldehyde (3 g in 10 ml dry ether) was added dropwise to 10 ml of a stirred 1 M solution of $LiAlH_4$ in ether under N_2 at 0°C. The temperature was not allowed to exceed 15°C. When the addition was complete, the solution was warmed to room temperature and

stirred for 2 hr. Then 2 g of $MgSO_4 \cdot 7H_2O$ was added to decompose the excess reagent and to release the alcohol. The solution was filtered, and the solvent was evaporated in vacuo. (*E*)-2-Decenyl acetate was prepared by allowing a mixture of (*E*)-2-decen-1-ol (1.1 g), acetic anhydride (1.1 g), triethylamine (1.5 ml), and 4-(dimethylamino)pyridine (0.075 g) to stand for 24 hr at room temperature. Ether and 2 N HCl were added, and the organic phase was washed with saturated $NaHCO_3$, dried over $MgSO_4$, and the solvent evaporated in vacuo (Höfle et al., 1978). The resulting product, (*E*)-2-decenyl acetate, was >97% pure by GC. We used the same procedure to prepare hexyl acetate (>95% pure product) from hexanol. All synthetic compounds gave satisfactory mass spectra.

Behavioral Response Towards Threats

We used a 3-mm-diam. wooden dowel to examine the response of *C. bimaculata* to threats. Ten wild adults were tested in the field on a warm sunny day (33–35°C) by (1) moving the tip of the dowel at a rate of approximately 2 cm/sec several times to within 0.5 cm of the insect, then (2) poking the insect with the dowel, and finally, (3) grabbing the insect and gently rolling it between the thumb and forefinger. The resulting behaviors were recorded. In addition, secretion discharge in nymphs and adults and the deterrent quality of the secretion were tested by placing bugs on our tongues and pressing them against our palates.

Predator Feeding Trials

Killdeer. A 1-week-old *Charadrius vociferus* L. was reared in the laboratory on a diet of arthropods, earthworms, snails, fish, and fruit. At about four weeks of age, when the bird could feed by itself and discriminate among prey (i.e., began to prefer some prey items over others), it was tested with the following method: The bird was given a control third-instar *Acheta domesticus* (L.) cricket to demonstrate hunger. Then, *C. bimaculata* individuals were sequentially offered until the bird no longer consumed the bugs. When this occurred, the bird was then offered another control cricket to verify that the bird was still hungry. If the control was eaten, then another bug was offered. Uneaten prey were removed 4 min after being placed in the cage with the bird. The experiment ended when the bird continued to eat the controls, but repeatedly rejected the bugs.

European Starling. A 3-week-old *Sturnus vulgaris* L. was reared in the laboratory on a diet of arthropods, fruit, and cat food. When the bird showed strong food discrimination patterns (i.e., began to prefer some foods over others), it was tested. The feeding trials were conducted in the same way as for *C. vociferus*.

American Robin. A 1-week-old *Turdus migratorius* L. was reared in the laboratory on a diet of arthropods, fruit, and cat food. When the bird was able to discriminate between food items and feed without assistance, it was tested as above, except that dewinged adult house flies (*Musca domestica* L.) were used

as controls and, after six bugs were consumed, flies and bugs were alternated as prey. We recorded number of prey attacked, number of prey consumed, and time to attack (the time from introduction of the insect until the bird pecked at or lowered its head toward the insect).

Green Anoles. Four adult *Anolis carolinensis* Voigt, obtained from a local pet shop, were maintained for two weeks at 26°C with surplus food (second to fourth instar *A. domesticus*) and water. The anoles were isolated and starved for two days prior to testing. Each anole was tested individually in its home container by introducing a series of adult *C. bimaculata*. Small, second to fourth instar *A. domesticus* crickets served as control prey.

To begin each test, an anole was initially offered a cricket to verify hunger. Next, *C. bimaculata* adults were sequentially offered until the anole refused to attack them. This two-step procedure was repeated until the anole continually rejected *C. bimaculata*, yet continually ate controls. Following this first trial, the anoles were starved for 48 hr and then tested again using the same procedure.

Test of Secretion Function

To test the hypothesis that the metathoracic gland secretion of adult *C. bimaculata* serves a defensive function, we compared the response of lizard predators to milked vs. unmilked bugs. Five adult green anoles were each offered a series of adult *C. bimaculata* that had been milked of their secretion by repeatedly squeezing and washing them under water. Five other adult anoles were offered unmilked bugs. Each anole was tested in its home cage and was given a series of *C. bimaculata* until it failed to consume three successive bugs. Prey were offered at about 4-min intervals, and each of the 10 trials was completed within 10–55 min. We noted aversive behaviors and the total number of prey eaten for each anole.

Source of Volatiles

We examined *C. bimaculata* adults and nymphs under a dissecting microscope to determine the presence and location of external gland orifices. We then tested four locations as the possible source of the bugs' secretion: tip of beak, anus, metathoracic gland (MTG), and dorsal abdominal gland (DAG). Seven adult males and seven adult females were chilled to 10°C. Five of each sex were glued ventral side up onto cardstock. The remaining four adults were dewinged with small scissors and glued ventral side down. All bugs were then allowed to warm to room temperature, viewed under a dissecting microscope, and stroked, poked, or pinched with tweezers to elicit expulsion of the secretion. Slivers of filter paper (ca. 3.0 mm^2) were then placed on either the MTG opening, dorsal abdomen, beak, or anus to absorb any ejected fluids. We conducted similar experiments with fourth and fifth instars.

RESULTS

Chemical Analyses

Both male and female *C. bimaculata* produced a blend of at least 11 different compounds when agitated. Nine of the compounds yielded good-quality mass spectra that were tentatively identified by matching to library spectra. These identifications were confirmed when commercial or synthetic standards gave identical mass spectra and GC retention times (Figure 1A, Table 1). The relative compositions of the identified volatiles were not significantly different between males and females (two-sample t test). Pooled over both sexes ($N = 18$), the mean percentages and their standard errors for the compounds were: *n*-tridecane, 67.4% ± 1.4; (*E*)-2-decenal, 12.2% ± 1.5; (*E*)-2-decenyl acetate, 11.5% ±

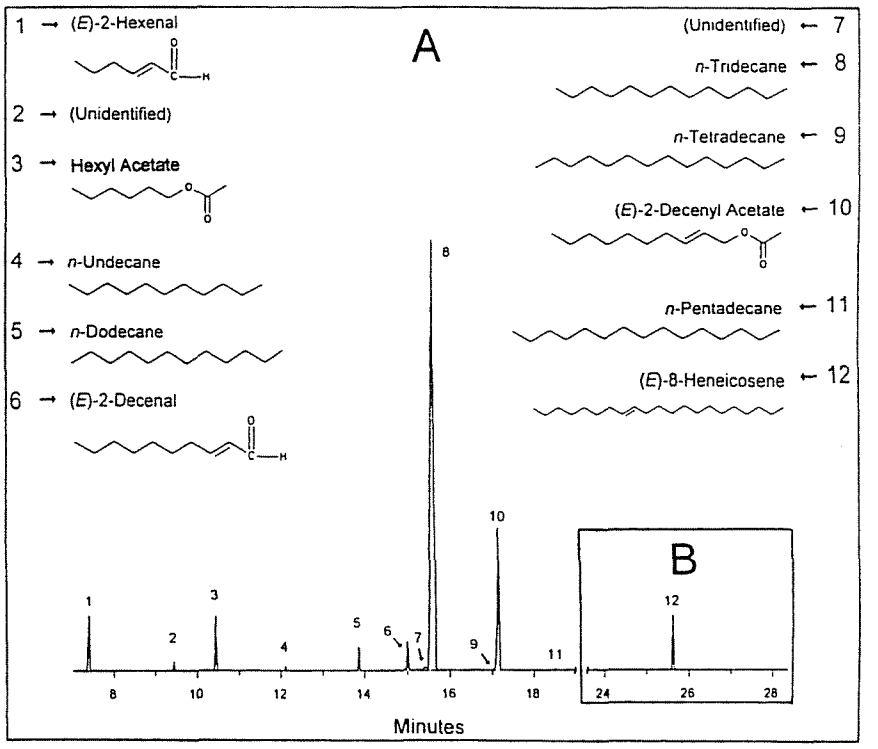

FIG. 1. (A) Gas chromatogram of volatile emissions from disturbed adult female *Cosmopepla bimaculata*. (B) Volatile emission from a non-disturbed adult male *C. bimaculata*. Peak numbers correspond to those in Table 1.

TABLE 1. CHEMICALS IDENTIFIED IN *C. bimaculata* SECRETIONS

GC Peak	Identification	Major MS ions[a] (percentages)
1	(*E*)-2-hexenal	98 (M+, 26), 83 (68), 69 (83), 57 (49), 55 (90), 42 (60), 41 (100), 39 (69)
2	(Unidentified)	112 (M+, 15), 97 (1), 83 (100), 69 (2), 57 (19), 55 (61), 39 (5)
3	Hexyl Acetate	144 (M+, NOT SEEN), 101 (2), 84 (22), 73 (10), 69 (19), 61 (24), 56 (49), 55 (24), 43 (100)
4	*n*-Undecane	156 (M+, 10), 85 (38), 71 (61), 57 (100), 43 (83), 41 (43)
5	*n*-Dodecane	170 (M+, 8), 85 (46), 71 (74), 57 (100), 43 (88), 41 (55)
6	(*E*)-2-Decenal	154 (M+, 0.4), 83 (61), 70 (88), 69 (54), 57 (64), 55 (92), 43 (100), 41 (100), 39 (67)
7	?-Tridecene	182 (M+, 8), 111 (19), 97 (46), 83 (63), 69 (78), 55 (100), 43 (86), 41 (92)
8	*n*-Tridecane	184 (M+, 7), 85 (48), 71 (77), 57 (100), 43 (99), 41 (83)
9	*n*-Tetradecane	198 (M+, 8), 85 (52), 71 (76), 57 (100), 43 (86), 41 (46)
10	(*E*)-2-Decenyl Acetate	198 (M+, 0.1), 156 (14), 110 (22), 96 (26), 95 (19), 82 (24), 81 (26), 68 (21), 67 (28), 55 (29), 54 (34), 43 (100), 41 (25)
11	*n*-Pentadecane	212 (M+, 6), 85 (46), 71 (69), 57 (100), 43 (70), 41 (34)
12[b]	(*E*)-8-Heneicosene	294 (M+, 21), 111 (50), 97 (90), 83 (97), 71 (46), 70 (51), 69 (88), 57 (84), 56 (50), 55 (100), 43 (88), 41 (70)

[a]Major MS ions (*m/z*), in order of decreasing *m/z*.
[b]Compound detected only from some male samples (see text).

1.0; (*E*)-2-hexenal, 3.3% ± 0.6; hexyl acetate, 2.2% ± 1.0; *n*-dodecane, 1.7% ± 0.1; ?-tridecene, 1.4% ± 0.8; *n*-undecane, 0.2% ± 0.02; *n*-tetradecane, 0.1% ± 0.02; *n*-pentadecane, 0.07% ± 0.01.

As suggested by the standard errors (above), the proportions of individual secretion components varied from bug to bug, particularly with some of the lower-molecular-weight compounds. For example, in some samples, compound 2 was more abundant than compound 1 or 3, whereas in other samples, compound 2 could not be detected. Additional compounds not depicted in Figure 1 occasionally appeared in the glandular secretions of *C. bimaculata*. For example, we tentatively identified small concentrations of (*E*)-2-octenal in some adult male and female secretions.

No volatiles were collected from nonagitated adult female *C. bimaculata*

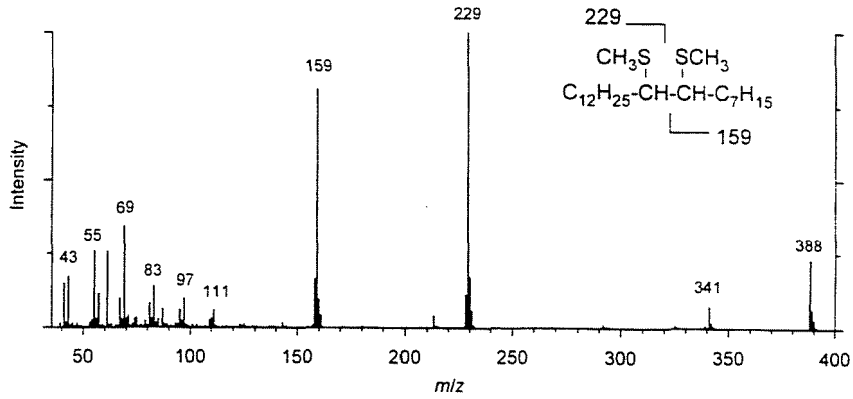

FIG. 2. Mass spectrum of the DMDS adduct of the insect-derived (*E*)-8-heneicosene, illustrating key peaks with m/z of 159 and 229.

but three of four undisturbed adult males (collected in early August 1997) produced one compound (Figure 1B). This compound was tentatively identified as a heneicosene isomer by its mass spectrum (Table 1). The double bond position (= 8) was then determined from the mass spectrum of the DMDS adduct, which had major fragment peaks at m/z 159 and 229 (Figure 2). The standard 8-heneicosene and its DMDS adduct gave corroborative mass spectra. Our synthetic standard 8-heneicosene contained both *E* (ca. 25%) and *Z* (ca. 75%) isomers, and their linear temperature programmed retention indexes, relative to *n*-alkanes, were 2078 and 2073, respectively, on the DB-1 column. The insect-derived compound matched the *E* isomer in GC retention, and this was confirmed by co-injection of insect-derived and standard samples. The amount of (*E*)-8-heneicosene was not determined because in October, when we analyzed the defensive secretions quantitatively, field-collected adults showed no traces of (*E*)-8-heneicosene.

Behavioral Response Towards Threats

Adult *C. bimaculata* showed little defensive behavior when initially approached with a 20-cm-long dowel. Only when the dowel was about 5 mm away from the bugs, or touching them, did they react by slowly crawling away from the dowel to the opposite side of the leaf or stem. A continued, gentle prodding of the bugs caused them to move away, hide within a node or flower, drop to the ground, or fly away, but failed to cause them to expel perceivable volatiles. The bugs secreted only after they were picked up and roughly handled. Other bugs secreted after being pinched on their antennae or legs. Adults and fourth and fifth instars that were placed in human mouths immediately secreted when mildly

TABLE 2. ADULT *Cosmopepla bimaculata* AND CONTROL PREY (CRICKETS OR HOUSE FLIES) CONSUMED BY BIRDS DURING ONE FEEDING TRIAL

	C. bimaculata		Controls	
Predator	Offered (N)	Eaten (N)	Offered (N)	Eaten (N)
Killdeer	5	1	6	6
Starling	4	0	5	5
Robin	12	8	12	12

squeezed between the tongue and palate, producing an instantaneous burning sensation and chemical taste that lingered for up to 20 min. This was followed by a slight localized numbness of the tongue, which lasted 1–2 hr. We were surprised at the intensity of the burning sensation and taste delivered by even small fourth instars. Newly hatched first instars produced no secretion or taste when placed on the tongue and gently squeezed. However, when chewed, the burning and taste characteristic of the secretion appeared, although less intense than in older nymphs.

Predator Feeding Trials

The killdeer readily attacked and consumed the first bug offered, which expelled its secretion, producing a strong odor. The bird then rejected the next four bugs, and would: (1) bob its head toward its prey as if to attack and then quickly withdraw without touching the prey; (2) step rapidly around the bug while pecking at the cage bottom near the bug; or (3) completely ignore the bug (this behavior was exhibited toward the last bug offered). All six cricket controls were immediately consumed by the bird, and, on three occasions, the bird stepped over a bug to get to a cricket (Table 2).

The starling showed strong aversion towards adult *C. bimaculata*. The bird quickly attacked the first bug offered, but immediately ejected the still-living bug out of its beak. The odor of the defensive secretion was strong. Five seconds later, the bird repeated these behaviors, then ignored all subsequent bugs, but eagerly consumed all crickets (Table 2).

The robin demonstrated mild aversion towards adult *C. bimaculata*. It attacked 11 of 12 bugs offered and consumed eight of these. In contrast, it attacked and consumed all 12 houseflies offered (Table 2). However, the robin took significantly longer to attack *C. bimaculata* than house flies (two sample t test, $df = 10$, $0.05 > P > 0.025$) (Table 3). During the trial, *T. migratorius* showed aversive behavior toward *C. bimaculata* such as ejecting bugs out of the beak and running toward newly introduced bugs and then turning away. Near the end of the trial, the bird was standing in the middle of the cage and was given a

TABLE 3. TIME (MEAN ± SE) FOR *T. migratorius* TO ATTACK *C. bimaculata* VS. HOUSE FLIES

	Time to attack (sec)	
Predator	*C. bimaculata*	*M. domestica*
Robin	40.6 ± 19.4 (N = 11)	2.3 ± 0.3 (N = 12)

choice between two bugs positioned in opposite corners of the cage and a house fly that was introduced in a third corner of the cage. The bird quickly ran toward one bug, turned and ran toward the other bug, and then turned and ran toward the control fly, which was promptly seized and consumed. The bird continued to ignore the two bugs crawling about the cage, but ate two additional flies.

One of the four anoles, upon sampling its first bug, vigorously threw the bug out of its mouth, wiped its snout on the bottom of the cage; then ignored all subsequent bugs, but readily consumed all controls offered. The other three anoles readily attacked and consumed the first bug offered, but became more hesitant with the second or third bug, and eventually ignored the fourth (Table 4). When attacked, the bugs emitted their strong, characteristic odor. When these same anoles were retested two days later, three refused to attack, one ate one *C. bimaculata*, yet all anoles eagerly attacked and consumed controls.

Test of Secretion

Five anoles that were offered *C. bimaculata* adults that lacked MTG secretion ate 5, 6, 6, 6, and 9 bugs, respectively ($\bar{X} \pm$ SE = 6.4 ± 0.68). Five other anoles that were given fully charged bugs at 1, 1, 2, 2, and 3 bugs, respectively ($\bar{X} \pm$ SE = 1.8 ± 0.37). The means are significantly different (t test, $P < 0.001$), suggesting that milked bugs were more palatable to anoles than unmilked bugs.

During these tests, anoles demonstrated aversion behaviors toward the

TABLE 4. ADULT *C. bimaculata* AND SECOND–THIRD INSTAR CRICKETS CONSUMED BY LIZARDS DURING TWO TRIALS SEPARATED BY TWO DAYS

	Day 1		Day 3	
Anole	*C. bimaculata* eaten (N)	*A. domesticus* eaten (N)	*C. bimaculata* eaten (N)	*A. domesticus* eaten (N)
1	3	5	0	3
2	3	3	0	3
3	0	4	0	4
4	3	2	1	4

charged bugs, but not the milked bugs. These aversive behaviors included spitting out the bug, backing away from the bug, wiping the head and mouth upon substrate, excessive mouthing before swallowing, and ignoring the bugs upon their introduction.

Source of Volatiles

All nymphal stages of *C. bimaculata* appeared to have three pairs of orifices on the dorsal abdomen corresponding to the DAGs of other Heteroptera. When fourth or fifth instars were squeezed by the antennae or legs, liquid emerged from the DAG orifices and spread over the surrounding cuticle. When absorbed onto slivers of paper, this liquid smelled similar to the adult defensive secretion. Nymphal stages lacked ventral MTG openings, and no liquid appeared on the venters of nymphs when molested. Occasionally liquids appeared at the mouth or anus of the disturbed nymphs and adults, but these liquids were odorless, suggesting that these structures were not the source of the defensive secretion.

Adults lacked discernible DAGs, and no liquid appeared on adult dorsums when adults were squeezed. However, adults of both sexes had paired MTG openings. Each orifice was surrounded by an extensive pleural scent area of dull, rough cuticle, which contrasted with the glossy, smooth cuticle, covering the remainder of the insect.

Various stimuli elicited secretion discharge from the MTGs. When adults (glued upside down to a piece of cardstock) were calm, they secreted only when strongly squeezed on an appendage or on the body. However, once agitated (by prior squeezing), adults would discharge when lightly stroked with a fine paintbrush on the eyes, antennae, thorax, or abdomen.

The insects could selectively secrete from either the left or right MTG and could control the volume of discharged secretion. When agitated adults were stroked on the right side, only the right gland discharged. Pressure applied to the head or abdomen caused both glands to discharge. When lightly stimulated, only a small droplet of secretion appeared at the gland orifice. When strongly stimulated (crushing an appendage) droplets larger than the insect's compound eyes emerged from the MTG orifices.

Discharge of secretion was usually accompanied by a telescoping of the abdomen into the rigid thoracic box. Secretion did not spray out, but emerged as an enlarging droplet. As the liquid flowed out of the MTG orifice, it formed a spherical droplet that was held in place by a cuticular projection at the gland orifice. Typically, droplets sat for 2–3 sec and then were drawn back into the insect's body. The pleural scent area appears to cause the droplet to bead up, and thus serves to hold the droplet in place. However, when agitated adults touched a leg to the droplet, it immediately wetted the cuticle of the leg and thorax by spreading over it.

DISCUSSION

Chemistry of C. bimaculata *Secretion*

Disturbed adult male and female *C. bimaculata* secrete a similar blend of at least 11 volatile compounds (two aldehydes, two esters, five alkanes, and two unidentified compounds). Approximately 92% of the secretion consisted of only three components: *n*-tridecane (68%), (*E*)-2-decenal (12%), and (*E*)-2-decenyl acetate (12%). The metathoracic gland (MTG) secretions of male and female *C. bimaculata* were nearly identical, other than the presence of (*E*)-8-heneicosene found in some males (which may or may not originate from the MTG).

Overall, the secretion of *C. bimaculata* contains typical pentatomid MTG compounds (Staddon et al., 1987; Aldrich, 1988, 1995; Nagnan et al., 1994; McBrien and Millar, 1999) and is strikingly similar to *Erthesina fullo*, *Lincus malevolus*, and *L. spurcus* (Kou et al., 1989; Nagnan et al., 1994). Of the nine components identified in the defensive secretion of both male and female adult *C. bimaculata*, seven [(*E*)-2-hexenal, (*E*)-2-decenal, and the C_{11}–C_{15} hydrocarbons] are common in the secretions of terrestrial arthropods. For example, (*E*)-2-hexenal exists in the secretions of cockroaches (Farine et al., 1997), beetles (Tschinkel, 1975), ants (Crewe et al., 1972), and many true bugs (Aldrich, 1988; Leal et al., 1994), and (*E*)-2-decenal has been identified in cockroaches (Wallbank and Waterhouse, 1970), beetles (Tschinkel, 1975), and various heteropterans (Farine et al., 1992; Aldrich et al., 1993). Likewise, *n*-tridecane occurs in thrips (Suzuki et al., 1989), ants (Bellas and Hölldobler, 1985), mites (Kuwahara et al., 1991), moths (Severson et al., 1991), and numerous Heteroptera (Farine et al., 1992; Aldrich et al., 1993). In contrast, two of the compounds in the defensive secretion of adult *C. bimaculata* [hexyl acetate and (*E*)-2-decenyl acetate] are generally found only within the Heteroptera (Blum, 1981; Surender et al., 1987; Aldrich, 1988; Gunawardena and Bandumathie, 1993; Leal et al., 1994; Millar et al., 1997; Millar and Rice, 1998).

The (*E*)-8-heneicosene isolated from males early in the season is interesting because, to our knowledge, this is the first *E* configuration for a natural insect alkene. However, similarly structured C_{21} cuticular hydrocarbon isomers [e.g., (*Z*)-10-heneicosene] have been found in the Diptera and Coleoptera (Bartelt et al., 1986; Peschke and Metzler, 1986).

Function of Secretion

Our results suggest that the secretion of *C. bimaculata* functions in antipredator defense because: (1) The secretion is ejected in response to disturbance. (2) Some secretion components [(*E*)-2-hexenal, *n*-undecane, *n*-dodecane, and *n*-tridecane] are known toxins, irritants, or repellents (Blum, 1981; Whitman et al., 1990). (3) Bugs lacking the secretion were more susceptible to predation

than bugs with secretion. (4) The secretion composition is similar in both sexes, suggesting that the secretion is not used as a sexual pheromone. (5) The bugs exhibit traits commonly associated with chemically defended insects (Pasteels et al., 1983; Guilford, 1990; Vulinec, 1990) such as conspicuous coloration, gregariousness, diurnal activity, and poor locomotory capability (e.g., when approached, they do not readily fly and can easily be caught with the fingers). (6) All the predators we tested developed some sort of food aversion towards the bugs: The starling refused to consume a single *C. bimaculata*; the killdeer ate one, but rejected all others; the anoles ate them initially, but rejected them during the second trial; and the robin exhibited strong attack hesitancy. In addition, these predators displayed various aversive, conflict, or displacement behaviors (Gustavson, 1977), such as ejecting bugs from their mouths, wiping their mouths on the floor as if to clean off irritating chemicals, reversing or interrupting predation in mid-attack, or running rapidly about the cage pecking near, but not at, the bug. These behaviors strongly imply that the bugs were distasteful.

It is common for different predators to respond differently to the same prey (Whitman, 1988; Whitman et al., 1990), and in our study, each predator species responded differently to *C. bimaculata*. All of our predators were naive (had never encountered *C. bimaculata* before), and all eagerly attacked the first bug offered. However, subsequent predatory behaviors varied according to predator species; each was deterred, but apparently via a different mechanism. The starling rejected *C. bimaculata* almost immediately upon sampling, suggesting that rejection in this predator occurred via an immediate, stimulus–response reaction mediated by secretion–stimulation of peripheral chemosensilla in or near the mouth. Hence, with this predator, the secretion was strong enough to elicit ejection from the mouth before the bug was killed. This is significant because it implies individual selection for defensive attributes (Wiklund and Järvi, 1982; Sillén-Tullberg and Bryant, 1983; Engen et al., 1986; Guilford, 1990). In contrast, three of four anoles demonstrated strong feeding aversions only after the first day and only after consuming several bugs (Table 4). This suggests that aversion in some anoles is mediated in part by an internal physiological response, such as toxicosis, which occurs after the bugs are swallowed and requires time to develop. This type of predator response implies kin or group selection (Fisher, 1958; Harvey and Greenwood, 1978; Guilford, 1990) as the driving force for the evolution of chemical defense in *C. bimaculata*. Finally, the results with the robin suggest that *C. bimaculata* is only partially deterrent to this predator and would be eaten when alternative prey were absent.

It is not known how the various secretion components interact. However, bioassays conducted by Gunawardena and Herath (1991) have shown that two common components of pentatomid secretions [(*E*)-2-hexenal and *n*-tridecane] were more effective as repellents to insects when combined than when individually tested. Furthermore, they found that other *n*-alkanes, when combined with

(*E*)-2-hexenal, were not as effective deterrents towards other insects as *n*-tridecane. Hence, *n*-tridecane appears to be the optimal *n*-alkane to work synergistically with (*E*)-2-hexenal to repel insects. The other secretion components of the multicomponent blend of *C. bimaculata* may likewise function in an additive way.

In addition to its defensive role, the secretion of *C. bimaculata* may serve other functions. In the field, *C. bimaculata* are often highly clumped, which suggests that they may possess aggregation or sexual pheromones, as do other pentatomids (Aldrich, 1995, 1996; McBrien and Millar, 1999). Conversely, the secretion could function as an alarm pheromone. Indeed, adult *C. bimaculata* immediately dropped off plants at the approach of fingers contaminated with secretion. Conversely, the secretion could act against entomopathogenic fungi. In bioassays, the hemipteran exocrine products hexyl acetate, (*E*)-2-decenal, and tridecane have all exhibited fungicidal activity (Surender et al., 1987; Sosa-Gomez et al., 1997).

Secretion Ejection

Theory predicts and observations confirm that insects are frugal when using their valuable defensive secretions (Wallace and Blum, 1969; Pasteels et al., 1984; Whitman et al., 1990). For some insects, regeneration of lost defensive stores may require weeks or months, leaving the prey more susceptible to predation (Fescemyer and Mumma, 1983; Carrel, 1984; Whitman et al., 1992). Hence, arthropods are generally reluctant to discharge, often waiting until stimuli indicate a clear and immediate danger (Eisner et al., 1976; Blum, 1981; Whitman et al., 1991). In our trials, *C. bimaculata* adults did not readily expel their secretion: no discharge occurred in response to visual threats or to mild tactile stimulation. Insects discharged only after they were rolled in the fingers or squeezed. There may be an additional benefit in not discharging prematurely: secreting in a predator's mouth (which contains a high density of chemosensilla) might be a more effective predator deterrent than secreting in response to the approach or initial investigative touches by a potential predator.

That the bugs expelled secretion when squeezed by the antennae or legs and could selectively emit from just one gland shows that discharge is not passive, but under neural–muscular control; external pressure is not required to force the secretion out. Indeed, pentatomids are known to possess complex musculature facilitating secretion discharge from internal reservoirs to the outer surface of the insect (Staddon, 1979). The morphology of the plural scent area allows adults to expel and hold droplets of secretion, which, if danger passes, can be pulled back into the gland reservoir and later reused. Alternatively, when further threatened, a bug can quickly coat itself with a rapidly volatilizing protective film of secretion, by touching a leg to the droplet, causing it to spread immediately over the glossy, waxy cuticle.

Function of (E)*-8-Heneicosene*

(*E*)-8-Heneicosene was found in only males, suggesting its possible role as a male sex pheromone. Male stink bugs are well known to produce attractive pheromones (Aldrich, 1995; McBrien and Millar, 1999). The hypothesis that (*E*)-8-heneicosene serves a pheromonal role is supported by an anecdotal observation: adults overwinter, and mate and oviposit during the late spring to late-summer. We observed widespread copulations in early August when (*E*)-8-heneicosene was detected among field collected males. However, no copulations were observed in the same population consisting of newly eclosed adults collected in early October when there was no detection of (*E*)-8-heneicosene from field collected males. For now, both the glandular source and the function of (*E*)-8-heneicosene remains uncertain, requiring further research to establish its role in the life history of this insect.

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