Volatiles in the Dorsal Abdominal Glands and Exuviae of *Leptocoris abdominalis* and *Leptocoris augur* (Heteroptera: Rhopalidae)

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ABSTRACT Compounds in the dorsal abdominal glands (DAGs) of adults and nymphs, and exuviae of two scentless plant bugs *Leptocoris abdominalis* (F.) and *Leptocoris augur* (F.) (Heteroptera: Rhopalidae), were studied by gas chromatography-mass spectrometry. For both species, two dorsal abdominal glands (median and posterior glands) are found in the nymphs, whereas in adults only the posterior gland is functional and the median gland is reduced. Monoterpenes are the major compounds in posterior glands for both species. For *L. abdominalis* β-pinene is most abundant monoterpene and for *L. augur*, limonene is most abundant monoterpene. Alkenals and alkenoic acids were found in median glands of both species. There was no sexual dimorphism in the morphology and the contents of the DAGs of adults. Analysis of the compounds found in the exuvia indicated that components in the median gland were shed with the exuvia. Quantitative analysis of the major monoterpenes and alkenals in the DAGs of different ages of *L. abdominalis* supported the observation that components in the posterior gland were retained in the insect after each molting. Possible functions of the components in the DAGs are discussed.

KEY WORDS pinene, alkenal, dorsal abdominal gland, exuviae, limonene

Every spring in Taiwan, thousands of red bugs (Heteroptera) gather on and under the flamegold tree, *Koelreuteria henryi* Dummer (Sapindaceae) (Chaw and Liu 2002), feeding on the seeds. These red bugs are the scentless plant bug *Leptocoris abdominalis* (F.). Because the flamegold trees are often cultivated along streets and in parks, the large numbers of bugs sometimes cause panic. It was our purpose to identify sex or aggregation pheromones and use them as attractants to control these bugs. However, in the process of handling the bugs, we found that the smell of the crushed exuviae was very different from the smell of disturbed bugs. The bugs produce a pleasant smell when disturbed, but the irritating smell of crushed exuviae was that of defensive compounds usually found in stink bugs (Pentatomidae). Thus, chemical analysis of the compounds in the exuviae and glands of the bug was carried out. Contents in the dorsal abdominal glands (DAGs) and exuviae of *Leptocoris augur* (F.), a closely related scentless plant bug, frequently found in southern Taiwan feeding on seeds of balloon vine, *Cardiospermum halicacabum* L., also are analyzed.

Aldrich (1988) reported that *Leptocoris isolatus* (F.) has a reduced median DAG and a functional posterior DAG in adults and the same is true for *Boisea* (=*Leptocoris*) species (Aldrich et al. 1990, Aldrich 1995). The present article reports on the compounds found in the dorsal abdominal glands of nymphs and adults and in exuviae of *L. abdominalis* and *L. augur*. As far as we are aware, this is the first report on the volatiles from exuviae of scentless plant bugs.

Materials and Methods

Insects. A colony of *L. abdominalis* was started from bugs collected on flamegold trees on the Academia Sinica campus during early March 2003. The bugs were reared on the seeds of the flamegold tree supplemented with sugar water (40%) under a photoperiod of 14:10 (L:D) h regime with temperature controlled at 25 ± 2°C and relative humidity at 70%. Several hundred bugs, including nymphs and adults were collected and brought into the laboratory. A colony of *L. augur* was started from insects collected in the southern part of Taiwan around balloon vine. *L. augur* bugs were reared under the same conditions as for *L. abdominalis* except that *L. augur* was fed on balloon vine seed.

Extraction of Dorsal Abdominal Gland Contents of Adults and Nymphs. The extraction procedure was similar to the extraction of DAG contents described by Ho and Millar (2001) with modification as follows. After the DAGs were exposed, the median and posterior glands were cut off with scissors together with the tergites (terga 4–6) and then put in 50 μl of pentane in a 200-μl glass insert. The contents were then crushed with a drawn-out glass microcapillary

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Table 1. Total amount (in nanograms per bug) of components in dorsal abdominal gland of *L. abdominalis* (mean ± SD)

Compound	Position[a]	DAG of nymph (fifth instar, $n = 4$)	DAG of adult	
			Male (9 d old, $n = 3$)	Female (9 d old, $n = 3$)
(*E*)-2-Hexenal	M	5,500 ± 900	0	0
(*E*)-2-Hexenoic acid[b]	M	640 ± 580	0	0
4-oxo-(*E*)-2-Hexenal[b]	M	12 ± 5	0	0
(*E*)-2-Octenal	M	540 ± 150	0	0
(*E*)-2-Octenoic acid[b]	M	112 ± 67	0	0
4-oxo-(*E*)-2-Octenal[b]	M	750 ± 150	0	0
α-Pinene	P	96 ± 29	134 ± 52	190 ± 46
Camphene	P	62 ± 29	81 ± 32	116 ± 22
β-Pinene	P	28,100 ± 11,100	24,500 ± 9,700	34,700 ± 5,400
Myrcene	P	350 ± 182	380 ± 150	520 ± 89
α-Terpinene	P	26 ± 14	37 ± 22	56 ± 15
Limonene	P	1,680 ± 690	2,860 ± 2310	2,770 ± 480
β-Phellandrene	P	138 ± 65	57 ± 26	79 ± 11
γ-Terpinene	P	70 ± 36	133 ± 65	216 ± 39
Ocimene	P	530 ± 260	460 ± 230	628 ± 40
Terpinolene	P	480 ± 350	680 ± 470	1,260 ± 150

[a] M, means median gland; P, posterior gland.
[b] These four compounds are not detected in every bug examined.

tube. These extracts were used for coupled gas chromatography-mass spectrometry (GC/MS) analysis. Each bug was extracted and analyzed individually, and benzophenone was added as an internal standard for quantitative analysis. Median and posterior glands were dissected separately and stored in different containers when contents in the glands needed to be analyzed separately. Median and posterior glands are referred to as glands between terga 4 and 5 and 5 and 6 respectively (Davidová-Vilímová et al. 2000).

Extraction of Contents in Exuviae. Exuviae were collected from the rearing cups every morning, a couple hours after being shed. The exuviae was crushed in 50 μl of pentane with a drawn-out glass microcapillary tube to release the contents in the glands in the exuviae.

Analysis of Extracts. Extracts were analyzed by splitless GC-MS with a Thermo Quest Trace GC, interfaced with Finnigan Trace Mass spectrometer (electron impact ionization, 70 eV). The GC was held at 40°C for 10 min and then programmed at 10°C/min to 250°C, with injector and transfer line temperatures kept at 200 and 250°C, respectively. DB-1 (30 m × 0.32 mm i.d., J & W Scientific, Folsom, CA) and DB-23 columns (30 m × 0.25 mm ID) were used, with helium as the carrier gas.

Quantification. GC/MS was used for the quantification of the components in the glands. Instrument responses to chemical standards and the internal standard benzophenone were determined by coinjecting equal amounts (10 ng) of the compounds into the GC/MS. The ratios of the peak areas in the reconstructed ion chromatograms were used for future calculations of the amount of each component in the glands. If pure standard compounds were not available, the instrument response of a chemical with similar structure was used for calculations.

Chemical Standards. (+)-α-Pinene, camphene, (+)-β-pinene, (−)-β-pinene, myrcene, α-terpinene, limonene, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-hexenoic acid, and (*E*)-2-octenoic acid

Table 2. Total amount (in nanograms per bug) of components in dorsal abdominal gland of *L. augur* (mean ± SD)

Compound	Position[a]	DAG of nymph (fifth instar, $n = 2$)	DAG of adult	
			Female ($n = 4$)	Male ($n = 11$)
(*E*)-2-Hexenal	M	181 ± 69	0	0
(*E*)-2-Hexenoic acid	M	12 ± 16	0	0
4-oxo-(*E*)-2-Hexenal	M	0	0	0
(*E*)-2-Octenal	M	700 ± 450	0	0
(*E*)-2-Octenoic acid	M	29 ± 16	0	0
4-oxo-(*E*)-2-Octenal	M	110 ± 330	0	0
α-Pinene	P	17 ± 25	8 ± 9	19 ± 23
Camphene	P	4 ± 5	2 ± 3	7 ± 10
β-Pinene	P	1,380 ± 1,560	890 ± 550	1,700 ± 1,500
Myrcene	P	156 ± 184	85 ± 50	150 ± 120
α-Terpinene	P	54 ± 64	38 ± 26	57 ± 34
Limonene	P	9,700 ± 9,300	7,200 ± 3950	10,200 ± 5,400
Phellandrene	P	2 ± 3	0	1 ± 3
γ-Terpinene	P	240 ± 290	71 ± 43	140 ± 110
Ocimene	P	410 ± 430	240 ± 150	410 ± 290
Terpinolene	P	1,450 ± 1,870	100 ± 80	480 ± 730

[a] M, median gland; P posterior gland.

Table 3. Total amount (in nanograms per bug) of components in the exuviae of *L. abdominalis* (mean ± SD)

Compound	Exuviae (fifth instar to adult, $n = 3$)
β-Pinene	164 ± 82
(*E*)-2-Hexenal	3,930 ± 1540
3-Hepten-2-one	12 ± 14
(*E*)-2-Heptenal	8 ± 5
(*E*)-2-Octenal	680 ± 400
4-oxo-(*E*)-2-Hexenal	25 ± 16
4-oxo-(*E*)-2-Octenal	430 ± 210

Table 4. Total amount (in nanogram per bug) of components in the exuviae of *L. augur* (mean ± SD)

Compound	Fifth instar to adult ($n = 2$)
Limonene	0
(*E*)-2-Hexenal	840 ± 660
3-Hepten-2-one	0
(*E*)-2-Heptenal	0
(*E*)-2-Octenal	1,490 ± 870
4-oxo-(*E*)-2-Hexenal	0
4-oxo-(*E*)-2-Octenal	83 ± 71

were purchased from Aldrich Chemical Co. (Milwaukee, WI). 3-Hepten-2-one was from TCI (Tokyo, Japan). Ocimene, γ-terpinene, and terpinolene were from Fluka (Basel, Switzerland). 4-oxo-(*E*)-2-Hexenal and 4-oxo-(*E*)-2-octenal were gifts from J. G. Millar (University of California, Riverside, CA).

Aeration of Calm Bugs and Disturbed Bugs. Nymphs and adults were confined in 300-ml aeration chambers. The setup was similar to aerations described by Ho and Millar (2002). For calm conditions, the bugs were introduced into the chamber gently and then the chamber was aerated for 2 h without shaking. For disturbed bugs, the chamber was shaken with the bugs in the chamber for 10 s every 20 min for 2 h. The volatiles collected were extracted with pentane, concentrated under a stream of nitrogen, and then subjected to GC/MS analysis. Five to six fifth instars or adults were used for this experiment.

Results

Components in DAGs of Adults and Nymphs of *L. abdominalis* and *L. augur*. There was no apparent dimorphism in the morphology of the DAGs of females and males. Components of DAG extracts were identified by comparison of the mass spectral data and retention times of the unknowns with those of authentic samples. The amounts and identities of the components in DAGs of *L. abdominalis* and *L. augur* are shown in Tables 1 and 2. Qualitatively, both bugs have similar (*E*)-2-alkenals, 4-oxo-(*E*)-2-alkenals and (*E*)-2-alkenoic acids in the median gland and monoterpenes in the posterior gland. The major components were different between the two species. (*E*)-2-Hexenal was most abundant in the median gland of *L. abdominalis*, whereas (*E*)-2-octenal was most abundant for *L. augur*. In the posterior gland, β-pinene was the most abundant for *L. abdominalis*, whereas, for *L. augur*, limonene was most abundant. The median gland is vestigial in adults.

Components in Exuviae of *L. abdominalis* and *L. augur*. The amount and identity of each component in exuviae of *L. abdominalis* and *L. augur* are shown in Tables 3 and 4. Compounds found in the exuviae were mainly from the median gland of nymphs. Although there was some β-pinene in the exuviae of *L. abdominalis*, the amount was very little (164 ng) compared with the amount in the posterior gland of nymphs (28,121 ng), and β-pinene was not found in some bugs. This difference might be due to contam-

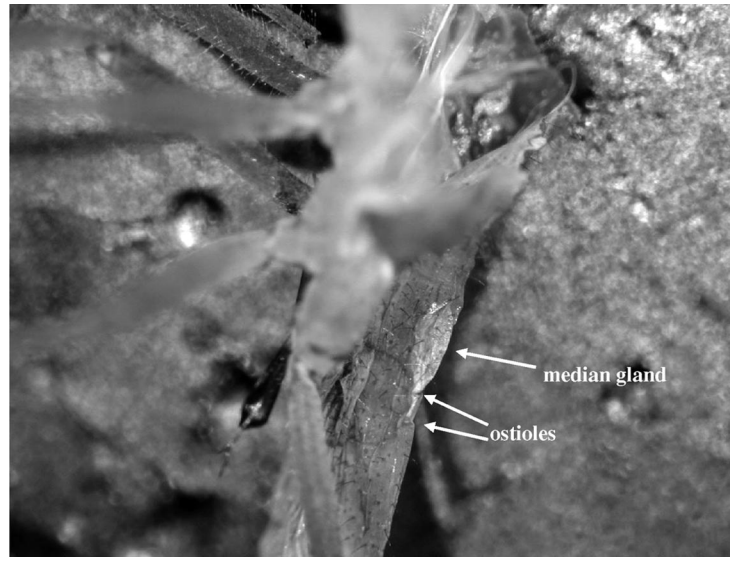

Fig. 1. Picture of exuvia of *L. abdominalis*.

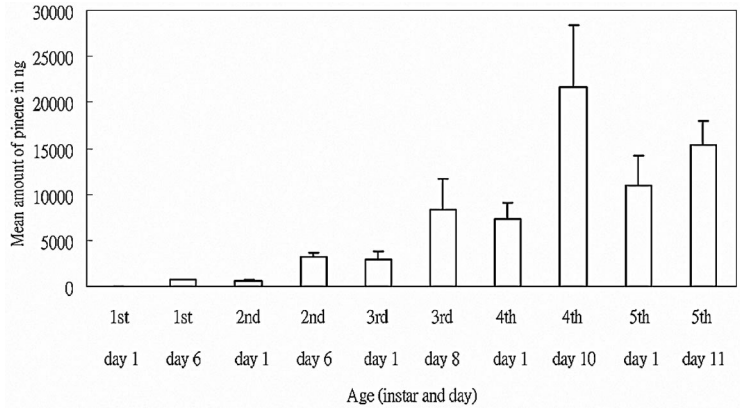

Fig. 2. Amount of β-pinene in DAG of nymphs at the age of first day and last day of each instar of *L. abdominalis* (n = 5; mean ± SE).

ination of the exuviae from the bugs in the rearing container. Comparison of the compounds found in the DAG of nymphs and exuviae revealed that the alkenoic acids were present only in the median gland but not in the exuviae, and the alkenoic acids were not found in every bug examined. A picture of the exuviae of *L. abdominalis* (Fig. 1) indicates a full median gland and an empty posterior gland.

Quantitative Analysis of β-Pinene and (*E*)-2-Octenal in the DAGs of Nymphs of *L. abdominalis*. To further prove that median gland is shed with the exuviae and posterior gland is retained in the molting process, the amounts of β-pinene and (*E*)-2-octenal in DAGs of nymphs at the first day and the last day of each instar were determined and are shown in Figs. 2 and 3. In Fig. 2, the amount of β-pinene increases steadily as the nymphs grew, with the amount in the last day of an instar close to that of the first day of the next instar. This result indicates that monoterpenes are retained in the posterior gland. In Fig. 3, the amount of (*E*)-2-octenal in the median gland in the first day of each instar is much less than the amount in the last day of the previous instar.

Aeration Extracts of Calm and Disturbed Bugs. Aeration extracts of calm and disturbed nymphs and adults were collected and analyzed as shown in Table 5. The results showed that calm bugs released very little of the compounds in the gland. However, disturbed nymphs released large amount of monoterpenes together with some of the alkenals. The relative ratio of monoterpenes to alkenals in the aeration extracts of disturbed nymphs is comparable with the relative amount of the components found in the glands of nymphs. These results indicate that the contents in both the median gland and the posterior gland are released at the same time when disturbed.

Discussion

Components found in the two *Leptocoris* bugs investigated here were similar to those found previously

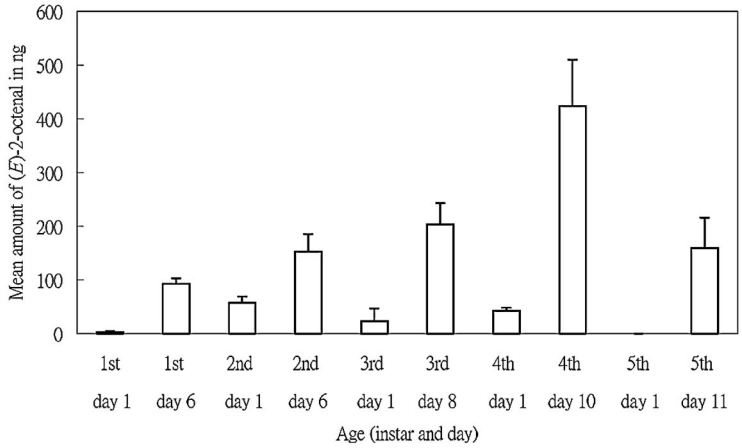

Fig. 3. Amount of (*E*)-2-octenal in DAG of nymphs at the age of first day and last day of each instar of *L. abdominalis* (n = 5; mean ± SE).

Table 5. Average amount (nanograms per bug per hour, mean ± SD) of compounds found in the aeration extracts of disturbed and calm nymphs and adults of *L. abdominalis*

Compound	Disturbed bugs			Calm bugs		
	Nymph (fifth instar, $n = 3$)	Male (7 d old, $n = 4$)	Female (7 d old, $n = 4$)	Nymph (fifth instar, $n = 3$)	Male (7 d old, $n = 5$)	Female (7 d old, $n = 3$)
α-Pinene	2 ± 2	20 ± 30	21 ± 5	11 ± 5	6 ± 1	4 ± 3
Camphene	1 ± 1	7 ± 14	8 ± 6	0 ± 1	1 ± 0	1 ± 1
β-Pinene	600 ± 240	400 ± 310	2,150 ± 1,180	37 ± 42	11 ± 9	7 ± 5
Myrcene	0	3 ± 4	19 ± 14	2 ± 1	1 ± 0	12 ± 6
Limonene	52 ± 17	110 ± 120	190 ± 50	12 ± 16	12 ± 4	0
(*E*)-2-Hexenal	33 ± 57	0	0	0	0	0
(*E*)-2-Octenal	0	0	0	0	0	0

for *Boisea* and *Leptocoris* species (Aldrich 1988, 1995; Aldrich et al. 1990). However, in this report alkenoic acids also were found from the median gland.

Examination of the glands and exuviae revealed that the bugs shed the contents of the median gland but not the components of the posterior gland at each molt. A possible reason for the retention of components in the posterior gland might be that the bugs are able to use the contents in the posterior gland immediately after molting, which gives better protection as suggested for a Peruvian stick insect, *Oreophoetes peruana* (Saussure) (order Phasmatodae), by Eisner et al. (1997). Pinene, limonene, myrcene, and other monoterpenes have been demonstrated to have defensive efficacy for the burrowing bug *Sehirus cinctus cinctus* (Heteroptera: Cydnidae) (Krall et al. 1997, Hick et al. 1999). Pinene serves as alarm/recruitment pheromones in termites (Roisin et al. 1990). So, we propose that the compounds in the posterior gland are used for defensive or alarm purposes.

Another observation here is that in the nymphal stage, the bugs accumulate components in the median gland after shedding the gland with the exuviae during each molt, but in the adult stage, the median gland is empty and not functional. Because the amount of the components in the median gland is much less than the amount of components in the posterior gland in the nymphal stage, it is possible that the median gland is ancestral character of the bug, considering that the median gland is vestigial in the adult stage. The results of aeration of disturbed and calm bugs indicate that contents in both median and posterior glands were released, so both glands might serve the same function in the nymphal stage.

However, another possibility is that the components (alkenals) in the median gland are used as aggregation pheromones. For example, (*E*)-2-hexenal and (*E*)-2-octenals are reported to be defensive allomones as well as alarm pheromones in Heteroptera (Blum 1996), and at low concentrations, they may promote aggregation as well (Blum 1996). It is possible that the compounds in the median gland and the exuviae are used as an aggregation pheromone such that the components in the exuviae can attract bugs of the same species without the risk of the compounds being used as a kairomone by natural enemies.

In summary, we found that two DAGs are present in the nymphal stage of *L. abdominalis* and *L. augur*, but only one gland is functional in the adult stage. We also reported here that one of the two DAGs shed with the exuviae and the contents of the shed gland increased through the nymphal stage after shedding, but not in the adult stage. The functions of two glands are currently under further investigation in our laboratory.

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