

## Identification, Electroantennogram Screening, and Field Bioassays of Volatile Chemicals from *Lygus hesperus* Knight (Heteroptera: Miridae)

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**Hsiao-Yung Ho and Jocelyn G. Millar (2002)** Identification, electroantennogram screening, and field bioassays of volatile chemicals from *Lygus hesperus* Knight (Heteroptera: Miridae). *Zoological Studies* 41(3): 311-320. Volatile chemicals released by live virgin female and male *Lygus hesperus* were analyzed by gas chromatography (GC), coupled GC-mass spectrometry (GC/MS), and GC-electroantennographic detection (GC-EAD). In total, 17 compounds were identified in headspace extracts and extracts of metathoracic glands, with hexyl butyrate and (*E*)-2-hexenyl butyrate being the major components. No qualitative differences between female and male bugs were found. Quantitatively, females produced larger amounts of compounds than did males. There were also no qualitative differences in the antennal responses of female and male bugs to bug extracts, and only small differences in the responses of male and female antennae to standardized doses of compounds from extracts in electroantennogram (EAG) analyses. In field bioassays, neither nymphs nor adult bugs of either sex were attracted to any of the 120 possible binary combinations of 16 of the 17 compounds identified in the aeration extracts. <http://www.sinica.edu.tw/zool/zoolstud/41.3/311.pdf>

**Key words:** Hexyl butyrate, *E*2-hexenyl butyrate, Metathoracic gland, Attractant, Pheromone.

Plant bugs in the genus *Lygus* were described as early as 1833, with approximately 43 currently known *Lygus* species in the world, of which at least 9 species are of economic importance in North America, Europe, and Asia (Kelton 1975). *Lygus* bugs feed on the meristematic and developing reproductive tissues of their hosts. Several types of damage result, including the shedding of buds, blooms, and fruiting bodies, destruction of seeds or ovules, deformation of fruit, initiation of secondary vegetative growth, and necrotic spotting of fruits (Debolt and Patana 1985).

*L. hesperus* is the most-abundant species in western North America, including California (Clancy and Pierce 1966, Kelton 1975). In California alone, damage to crops such as strawberries, apples, pears, and cotton has been estimated at many millions of dollars annually (Kelton 1975). Despite its importance as a pest, methods

for sampling and monitoring *L. hesperus* populations are relatively primitive, relying on beating tray or sweep-net sampling, which are both time and labor intensive. Furthermore, sweep-net sampling of delicate crops such as strawberries is not practical because of damage to the berries (Zalom et al. 1993). Monitoring efforts would be aided tremendously if attractant-baited traps specific for *Lygus* bugs were available.

In the field, it has been observed that male bugs are attracted to virgin females (Strong et al. 1970, Graham 1987, McLaughlin 1996, HY Ho pers. observ.). In addition to *L. hesperus*, at least 10 other mirid species have been shown to use female-produced sex pheromones, including *L. lineolaris*, *L. desertinus*, and *L. elisus* (Graham 1987 1988), the cocoa capsid *Distantiella theobroma* (King 1973), the cocoa mirid *Helopeltis clavifer* (Smith 1977), the green apple bug *Lygocoris communis* (Boivin and Stewart 1982), the apple brown

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bug, *Attractotomus mali* (Smith and Gaul 1994), the mullein bug, *Campylomma verbasci* (Smith et al. 1991), *Phytocoris californicus* (Millar and Rice 1998), and *Phytocoris relativus* (Millar et al. 1997). However, pheromone blends have been identified only for the latter 3 species.

The aim of the work described herein was to identify possible sex pheromone components for *Lygus hesperus*. In particular, we describe the identification of a number of compounds from sexually mature virgin adults of both sexes, and the testing of these compounds in both electroantennograms and field bioassays.

## MATERIALS AND METHODS

### Insect rearing

A colony of *Lygus hesperus* was established from bugs collected by sweep-netting in an alfalfa plot at the University of California, Riverside Agricultural Operations, Riverside, CA. Bugs were reared at 24°C and 60%-75% relative humidity, on a diet of organically grown green beans, navel orangeworm eggs, and raw sunflower seeds. Eggs were laid in the green beans, which were removed every 3 d and transferred to 1.9-L cardboard ice-cream cartons. Eggs hatched in about 6 d, and nymphs were kept in the carton until they reached adulthood, with fresh food added every other day. Bugs were maintained under a regime of 17:7 (L:D), provided by fluorescent lights.

### Collection of insect-produced chemicals

Sixty to 70 sexed, virgin adult bugs and ~5 green beans were put into a 1-L cylindrical glass chamber consisting of 2 halves sealed together with an O-ring joint, and with small ground-glass joints on each end for attachment of air lines. The chamber was lined with a screen for the bugs to perch on. Humidified, charcoal-filtered air was drawn through the chamber by house vacuum (1 L/min). Entrained bug volatiles were collected on activated-charcoal traps made from 4-mm-i.d. glass tubes loaded with a 4-cm bed of 80-100-mesh activated-charcoal, precleaned by thermal desorption at 200°C overnight under a slow flow of nitrogen. Aerations were conducted for 48 h, using 3 to 7-d-old bugs. The collectors were eluted with ~1.5 ml of pentane, and extracts were stored in glass vials in a freezer until needed for experiments.

### Aeration of individual insects

A sexed, virgin adult *Lygus hesperus* was put in a screw-cap 20-ml glass scintillation vial (Fisher Scientific #03-337-4) together with a piece of a green bean (about 0.5 cm long). Two holes were made in the cap of the vial to accommodate an activated-charcoal inlet air filter, and a volatiles collector made from a disposable glass Pasteur pipette loaded with a 2-mm bed of activated-charcoal held in place by glass-wool plugs. The collector was connected to house vacuum, and air was pulled through the vial at 1 L/min. Individual bugs of different ages (1-11 d) were aerated for 24 h. The collectors were eluted with pentane (~160 µl), and extracts were stored in glass vials in a freezer.

### Metathoracic gland extraction

Both female and male bugs have well-developed metathoracic glands, which were dissected out after the bugs had been put in a freezer for 30 min. The dissected glands were briefly soaked in a few microliters of pentane. Extracts were analyzed by gas chromatography (GC) and coupled gas chromatography-mass spectrometry (GC-MS) as described below.

### Collection of volatile chemicals from squashed insects

In case pheromone components are produced in glands other than the metathoracic glands, volatiles were collected from squashed whole bugs. Virgin adult bugs were squashed between filter paper sandwiched between aluminum foil (5 bugs on a 5 by 2-cm piece of filter paper). The filter paper and aluminum foil were then placed in a scintillation vial and aerated as described above for 20-22 h. The collected volatiles were eluted with 200 µl of ethyl acetate. Mature virgin female and male bugs, and newly emerged, sexually immature adult female and male bugs were squashed and aerated separately.

### Aeration of squashed bugs spiked with heptyl butyrate

Virgin sexually mature female bugs were anesthetized with CO<sub>2</sub>, and 1 µl of a solution of heptyl butyrate (80 µg/µl in acetone) was placed on the abdomen. The bugs were then squashed between filter paper sandwiched between alu-

minum foil, and the volatiles were collected and eluted as described above. Mature and virgin female and male bugs were treated and squashed separately.

### Analyses of bug extracts

Extracts were concentrated by evaporation under a gentle stream of nitrogen as required, and analyzed by gas chromatography in splitless mode on a DB-17 column (20 m x 0.32 mm i.d.), with a temperature program of 40°C for 1 min, then increasing by 5°C/min to 250°C. Helium was used as the carrier gas, and compounds were detected with a flame ionization detector. Extracts were also analyzed by coupled GC/MS, using a DB5-MS column (20 m x 0.2 mm i.d.) programmed at 40°C/1 min, then increasing by 10°C/min to 250°C. Compounds were identified by matching the retention times on both columns and mass spectra with those of authentic standards, which were purchased from commercial sources.

For group aeration extracts, the relative amounts of each component were calculated. For individual aeration extracts, an internal standard (dodecane) was added so that the absolute quantity of the major component in the extracts could be calculated as the amount per bug per aeration hour.

### Coupled gas chromatography-electroantennographic detector (GC-EAD)

GC-EAD analyses were carried out using male bug antennae. A bug's abdomen and legs were snipped off, and the head and thorax were mounted on a micromanipulator. The tip of 1 antenna was snipped off, and a recording electrode was placed over the cut tip. The reference electrode was inserted into the head at the base of the antenna. Saline-filled glass capillary electrodes were used, which were connected to a custom-built amplifier with chloridized silver wires. The saline solution (Visser 1979) contained glucose (354 mM), KCl (6.4 mM), KH<sub>2</sub>PO<sub>4</sub> (20 mM), MgCl<sub>2</sub> (12 mM), CaCl<sub>2</sub> (1 mM), NaCl (12 mM), and KOH (9.6 mM). The pH was adjusted to 6.5 with NaOH and HCl as required.

Bug extracts were injected for GC, and the GC column effluent was split equally with a glass press-fit Y-connector between the GC detector and a heated transfer line (250°C) which emptied the effluent into a humidified airstream (300 ml/min) which was passed over the antennal preparation.

Antennal signals and the GC detector signals were simultaneously recorded on a pair of HP 3396 recording integrators.

### Electroantennogram (EAG) screening of chemicals identified in insect extracts

EAG responses of female and male antennae from bugs of different ages (1, 8, and 18-d-old) to 15 chemicals identified from aeration extracts were recorded, with 8 replicates for each age group. The 15 chemicals are listed in table 1, except that (*E*)-4-oxo-2-hexenal was not available at the time of the test, and that hexanol was used as the standard. Ten microliters of acetone solutions (10 µg/µl, 100 µg of chemical) of the test chemicals were adsorbed on a piece of filter paper (1 by 2 cm), which was then inserted into a Pasteur pipette. A 2.5-ml puff of air was blown through the pipette into an air stream flowing over the antennal preparation. In this experiment, an entire antenna was carefully pulled off from the head of a just-killed *Lygus* bug, and the tip of the antennae was cut off. The base of the antenna was mounted on a ground electrode, and the cut tip was placed in contact with a recording electrode. Test chemicals were puffed over the antenna in random order, with about 30 s between puffs. Responses were quantified versus the response of the antenna to a standard, i.e., hexanol, with the hexanol response being arbitrarily assigned as 1 unit. The mean responses of males and females of a particular age group were compared using Student *t*-tests.

### Field tests

Field tests were conducted in an alfalfa plot at UC Riverside Agricultural Operations. In summer 1996, chemicals were loaded as hexane solutions (100 mg/ml; dose used was 20 µl, or 2 mg of test compound) onto 11-mm gray rubber septa (The West, Lititz, PA), and placed in sticky triangular Delta traps (Tréce, Salinas, CA). Treatments were spaced about 5 m apart. Each treatment was replicated 3 times, bugs trapped were counted on 3 successive days, and the entire experiment was repeated twice. Treatments used in this bioassay consisted of different blends of the major components found in the aeration extracts (see below). As a positive control, virgin females (5/trap, with a green bean for food; 2 replicates) were placed in small screen cages inside traps.

In summer 1997, field screening trials were

carried out using all possible binary combinations of 16 chemicals (a total of 120 combinations) found in the aeration extraction. The ratio of the 2 components in each blend was the ratio found from group aeration extracts. The 16 chemicals tested are those listed in table 1, except for (*E*)-4-oxo-2-hexenal, which was not available at the time the tests were run. Chemicals were dissolved in acetone, and a total of 250 µg of the blend of 2 compounds was loaded on gray rubber septa. Eleven Delta traps were set up every 2 d in the alfalfa field, with 2 replicates of 5 different treatments and 1 control (acetone on an 11-mm gray rubber septa). Each trap was separated by about 5 m. Traps were set out at about 1900 h and checked every day at around 1900 h. The entire group of 120 different binary combinations of chemicals was tested from mid-June until mid-August.

## RESULTS

### Analyses of aeration and metathoracic gland extracts from bugs

The compounds identified in the group aeration extracts, and the metathoracic gland contents from both female and male bugs are listed in table 1. Both male and female bugs produced the same compounds, and there appeared to be no sex-specific compounds. Extracts from the metathoracic glands contained fewer compounds, again with no qualitative sex-specific differences between extracts.

Chemicals labeled with a superscript (<sup>b</sup>) in table 1 are compounds which elicited electroantennogram responses in male antennae. The series of butyrates (butyl-, pentyl-, heptyl-, and octyl-) was not detected from *Lygus* bugs in a previous report (Aldrich et al. 1988).

Amounts of the major volatile component (n-

**Table 1.** Percent composition of aeration and metathoracic gland extracts of sexed virgin adults of *Lygus hesperus* reared in groups, relative to the most abundant compound, hexyl butyrate (mean ± SD)<sup>a</sup>

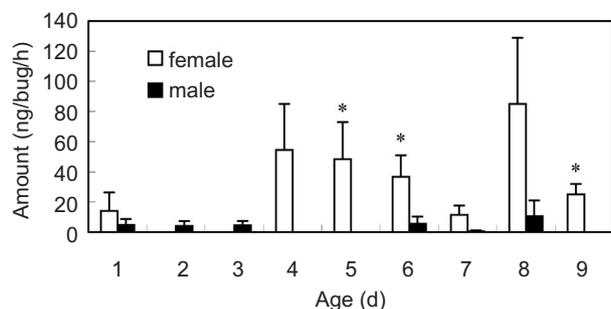
Compound	Female aeration (n = 3)	Male aeration (n = 3)	Female glands (n = 6)	Male glands (n = 4)
Hexanol <sup>b,c</sup>	9.90 ± 3.00	9.90 ± 5.00	1.20 ± 1.50	1.90 ± 0.80
( <i>E</i> )-2-Hexenol <sup>b,c</sup>	0.50 ± 0.30	0.30 ± 0.40	0	0
( <i>E</i> )-2-Hexenal <sup>b,c</sup>	10.00 ± 3.50	1.60 ± 1.60	2.90 ± 3.20	1.90 ± 1.40
Butyl butyrate <sup>b</sup>	0.07 ± 0.04	0.13 ± 0.23	0	0
Pentyl butyrate <sup>b</sup>	0.35 ± 0.12	0.27 ± 0.03	0	0
Hexyl butyrate <sup>b</sup>	100	100	100	100
Heptyl butyrate <sup>b</sup>	0.04 ± 0.01	0.02 ± 0.02	0	0
Octyl butyrate <sup>b</sup>	0.02 ± 0.01	0.08 ± 0.06	0	0
( <i>E</i> )-2-Hexenyl butyrate <sup>b</sup>	4.50 ± 0.15	4.00 ± 0.02	7.60 ± 5.70	4.00 ± 1.30
Hexyl acetate	0.28 ± 0.06	0.31 ± 0.25	0	0
( <i>Z</i> )-3-Hexenyl acetate	0.43 ± 0.49	1.40 ± 1.50	0	0
( <i>E</i> )-4-Oxo-2-hexenal <sup>b</sup>	1.16 ± 0.03	0.05 ± 0.10	0	0
Indole	0.09 ± 0.03	0.14 ± 0.10	0	0
Hexyl ( <i>E</i> )-2-butenate	0.06 ± 0.02	0.05 ± 0.02	0	0
Linalool <sup>c</sup>	1.00 ± 0.50	4.00 ± 1.30	0	0
Nonanal	0.20 ± 0.50	0.50 ± 1.00	0	0

<sup>a</sup>Percentages listed in aeration extracts are only estimations because no experiments were carried out to check for compounds breaking through traps.

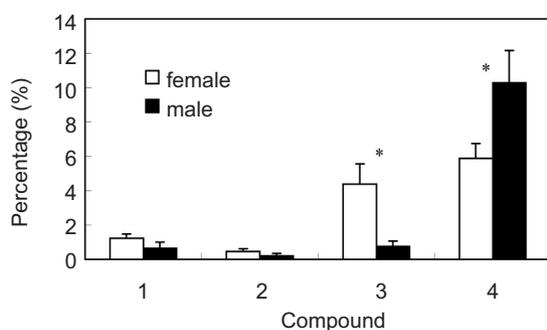
<sup>b</sup>These chemicals elicited electroantennogram responses from male antennae.

<sup>c</sup>These compounds were also found in the green bean aeration extract.

hexyl butyrate) produced by individual female and male bugs differed (Fig. 1), with females generally producing more than males, and with females having significantly higher production rates for the 5-, 6-, and 9-d-old age groups. There were no signifi-



**Fig. 1.** Amount of hexyl butyrate (mean  $\pm$  SE) from aeration extracts of adult *Lygus hesperus* of different ages, with bugs aerated individually ( $n = 6$ ). Asterisks indicate a significant difference between sexes ( $t$ -test,  $p < 0.05$ ).



**Fig. 2.** Relative percentage of minor compounds mean  $\pm$  SE to hexyl butyrate, the major compound, for female and male bugs from individual aerations ( $n = 10$ ) for all age groups. Compounds: 1 = hexanol, 2 = (*E*)-2-hexenol, 3 = (*E*)-2-hexenal, 4 = (*E*)-2-hexenyl butyrate. Asterisks indicate a significant difference between sexes ( $t$ -test,  $p < 0.05$ ).

cant differences in the amounts of hexanol (compound 1) and (*E*)-2-hexenol (compound 2) produced by individual males and females relative to the major component (Fig. 2). The relative percentage of (*E*)-2-hexenal (compound 3) was significantly higher in extracts from females than males. Conversely, males produced a higher relative percentage of (*E*)-2-hexenyl butyrate than did females.

### Analysis of volatiles from squashed bugs

There were no sex-specific differences in aeration extracts from mature female and male bugs squashed on filter paper. The major components in the extraction were qualitatively similar to those found in the aeration extracts from live bugs, except that there was considerably more hexanol in squashed-bug samples (Table 2). Hexanol was 400% and 519% the amount of hexyl butyrate in squashed females and males, respectively, whereas it was only about 10% relative to hexyl butyrate in the aeration extracts from live females and males. Absolute amounts of compounds in squashed-bug extracts were not determined.

### Aeration of squashed bugs spiked with heptyl butyrate

Analysis of aeration extracts of mature female and male bugs spiked with heptyl butyrate and then squashed on filter paper revealed a large quantity of heptanol (spiked males, 252% relative to heptyl butyrate) and hexanol (164% relative to hexyl butyrate). In samples from spiked females, the relative amount of heptanol to heptyl butyrate was 56%, and hexanol to hexyl butyrate was 89%. Because the extracts from unspiked bugs con-

**Table 2.** Analysis of volatiles collected from squashed, sexually mature and immature *Lygus hesperus* (% relative to hexyl butyrate) (Mean  $\pm$  SD)<sup>a</sup>

Compound	Female aeration ( $n = 3$ )	Male aeration ( $n = 3$ )	Female glands ( $n = 6$ )	Male glands ( $n = 4$ )
Hexanol	400.0 $\pm$ 173.0	519.0 $\pm$ 256.0	529.0	140.0
( <i>E</i> )-2-Hexenol	14.6 $\pm$ 5.4	21.2 $\pm$ 14.8	53.0	40.0
( <i>E</i> )-2-Hexenal	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2		
Hexyl butyrate	100	100	100	100
( <i>E</i> )-2-Hexenyl butyrate	2.1 $\pm$ 0.4	1.97 $\pm$ 0.8	4	6.6

<sup>a</sup>The absolute amounts of compounds in the squashed-bug extracts were not measured, but the ratio of the GC peak area of hexyl butyrate in female and male extracts was about 2 to 1.

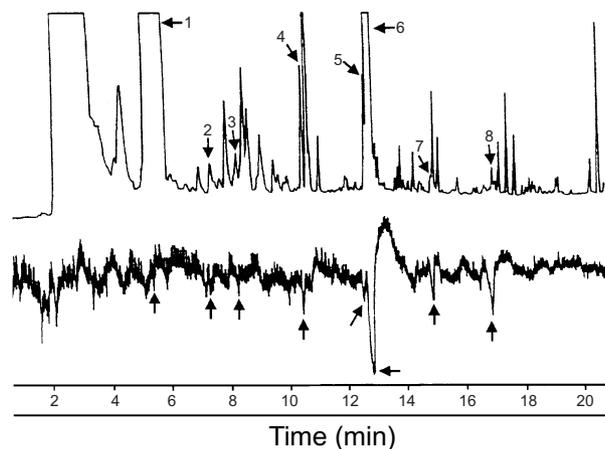
tained no heptanol, these results demonstrate that extensive hydrolysis of esters occurred in the squashedbug samples.

### GC-EAD analysis of aeration extracts

GC-EAD analysis of group aeration extracts of male and female bugs revealed 9 compounds which consistently elicited antennal responses from both females and males (Fig. 3) (Peak #1 contained 2 compounds). These 9 compounds were (*E*)-2-hexenol, (*E*)-2-hexenal, *n*-butyl butyrate, *n*-pentyl butyrate, *n*-hexyl butyrate, *n*-heptyl butyrate, *n*-octyl butyrate, (*E*)-2-hexenyl butyrate, and (*E*)-4-oxo-2-hexenal. Overall, the signals were comparatively weak, and there were no obvious differences in the responses of antennae between males and females.

### Electroantennogram screening of chemicals identified in insect extracts

The results of EAG tests of responsiveness of antennae from male and female bugs of different age groups to compounds identified from aeration extracts are shown in figure 4a-c. The highest responses from antennae of both sexes, for all age groups, were elicited by the series of butyrate esters from butyl butyrate to octyl butyrate, as well



**Fig. 3.** Coupled gas chromatogram-electroantennogram responses of a male antenna to an aeration extract from a female *Lygus hesperus*. Top trace: gas chromatogram; bottom trace: electroantennogram. Compounds: 1 = (*E*)-2-hexenal and (*E*)-2-hexenol; 2 = (*E*)-4-oxo-2-hexenal; 3 = butyl butyrate; 4 = pentyl butyrate; 5 = (*E*)-2-hexenyl butyrate; 6 = hexyl butyrate; 7 = heptyl butyrate; 8 = octyl butyrate. Arrows indicate EAG responses to the numbered peaks. A DB-5 column was used.

as by (*E*)-2-hexenyl butyrate. There were no consistent patterns of differences in responses of the sexes across the 3 age groups. For 1-d-old bugs, male antennae gave significantly higher responses to (*E*)-2-hexenal, indole, butyl butyrate, pentyl butyrate, and hexyl butyrate than did female antennae. For 8-d-old bugs, male antennae gave higher responses to hexyl butyrate than did those of females, whereas female antennae gave higher responses to nonanal. For 18-d-old bugs, male antennae responded more to pentyl butyrate and octyl butyrate than did female antennae. The EAG responses of all age groups were combined (Fig. 4d) to see whether any further information could be obtained. Overall, male antennae responded more to hexyl (*E*)-2-butenolate, butyl butyrate, hexyl butyrate, and (*E*)-2-hexenyl butyrate, whereas female antennae responded more to nonanal than did male antennae.

### Field tests

Field tests conducted in 1996 with C<sub>4</sub>-C<sub>8</sub> butyrate esters, (*E*)-2-hexenyl butyrate, and hexanol as single components, with binary blends of different ratios of hexyl butyrate and (*E*)-2-hexenyl butyrate, and with 4 multi-component mixtures (Table 3) were not successful, with only a single male *L. hesperus* being trapped. A larger field screening trial in summer 1997, testing all possible binary blends of 16 compounds identified from aeration extracts of females, in the ratios found in the extracts, was also unsuccessful, with no indication of attraction of male bugs to any of the lure blends tested (data not shown).

### DISCUSSION

One of the methods used to determine potential sex pheromone components in insects is to examine differences between chemical profiles of female and male extracts, looking specifically for sex-specific compounds. Here with *L. hesperus*, no sex-specific compounds were found in aeration extracts, extracts of dissected metathoracic glands, or in volatiles collected from squashed insects. However, quantitative analysis of the extracts revealed that there were differences in the amounts of compounds produced by female and male bugs.

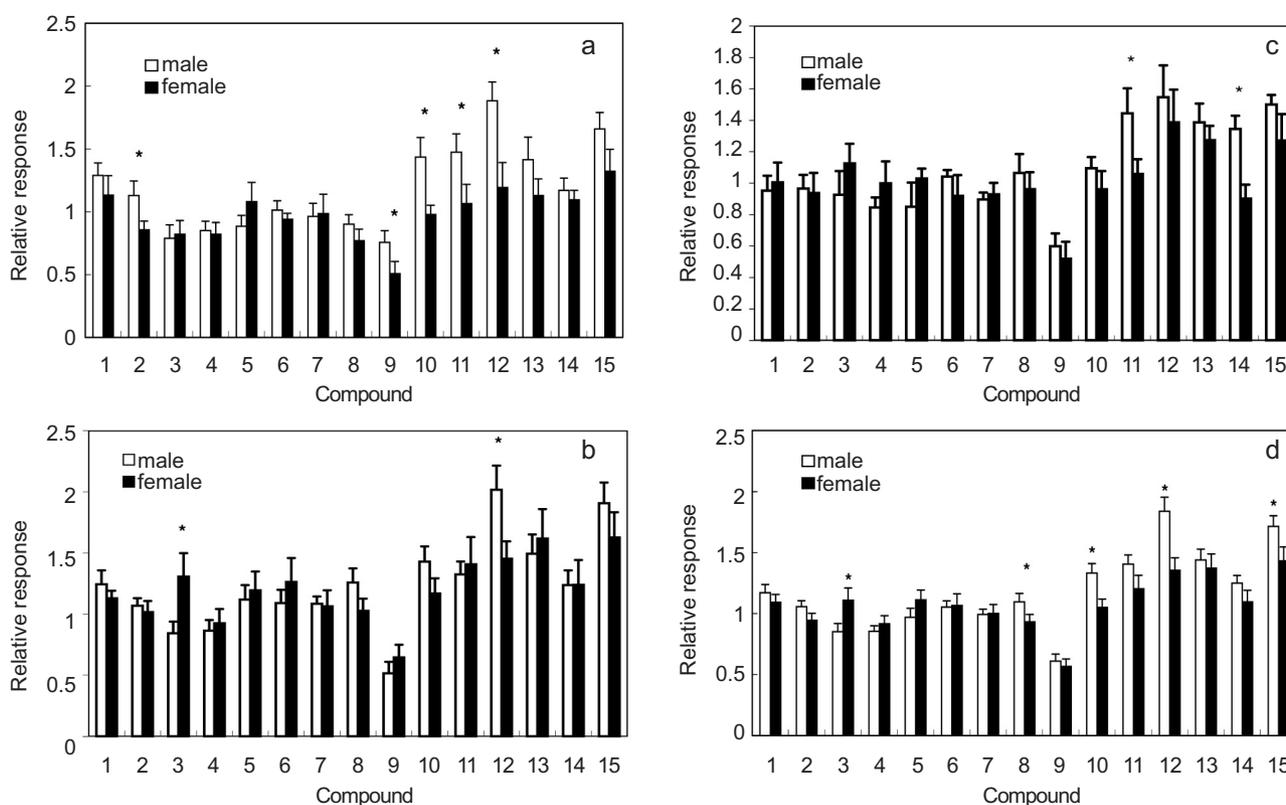
The experiment in which squashed bugs were aerated was carried out with the idea that the contents of all glands in the bugs, not just the

metathoracic glands, would be released. In particular, these insects are small, and it is not easy to locate and dissect small glands, so by collecting volatiles from squashed bugs, we hoped to discover compounds produced by glands that we had not found by dissection. There were no qualitative differences in the volatiles collected from squashed female and male bugs. However, the ratio of hexanol to hexyl butyrate (~4-5:1) was much higher than that found in aeration extracts from live bugs (~1:10) or from metathoracic gland extracts. An additional experiment in which bugs were spiked with heptyl butyrate before squashing, followed by the detection of large amounts of heptanol in the resulting extracts, indicated that the high levels of hexanol were probably artefactual, and were the result of hydrolysis of hexyl butyrate during the aeration period.

The possibility of compounds breaking through the collectors during the collection periods was not evaluated during the aerations, so the

amounts and relative percentages of the various components in the aeration extracts may slightly differ from the amounts actually released. However, the purpose of this work was to look for sex-specific compounds, or large differences in the ratios of components between males and females, and these factors, if present, would have been obvious, even if some of the more-volatile components had broken through the collectors during aeration.

The GC-EAD experiments indicated that the antennae were most sensitive to butyrate esters in the aeration extract, and in particular, responses were obtained to some minor ester compounds that had not been reported previously by Aldrich et al. (1988), such as trace amounts of butyl butyrate, pentyl butyrate, heptyl butyrate, and octyl butyrate. Because the butyrates elicited relatively strong responses from male antennae, exploratory field trials using the butyrates, singly or in combinations, were conducted in summer 1996.



**Fig. 4.** Electroantennogram responses (mean  $\pm$  SE) of *Lygus hesperus* of different ages to 15 compounds identified from aeration extracts. Compounds: 1 = (*E*)-2-hexenol; 2 = (*E*)-2-hexenal; 3 = nonanal; 4 = tridecane; 5 = linalool; 6 = hexyl acetate; 7 = (*Z*)-3-hexenyl acetate; 8 = hexyl (*E*)-2-butenate; 9 = indole; 10 = butyl butyrate; 11 = pentyl butyrate; 12 = hexyl butyrate; 13 = heptyl butyrate; 14 = octyl butyrate; 15 = (*E*)-2-hexenyl butyrate. Responses were measured relative to the response obtained with a hexanol standard. Asterisks indicate compounds for which there was a significant difference between the sexes ( $t$ -test,  $p < 0.05$ ). 4a: 1d-old; 4b: 8d-old; 4c: 18d-old; 4d: all ages combined.

However, bugs were not attracted to any of the baits tested. In a larger study in 1997, all possible binary combinations of the 16 most-abundant compounds in the aeration extracts were tested, again with no significant attraction of *L. hesperus* of either sex to any lure tested.

Because female and male bugs produce similar compounds which only appear to differ in the amounts produced (in individual aerations), EAG experiments were carried out to determine whether the antennae of males and females displayed sex-specific differences in sensitivities to specific compounds in aeration extracts. We found that male antennae gave significantly stronger responses to hexyl (*E*)-2-butenate, butyl butyrate, hexyl butyrate, and (*E*)-2-hexenyl butyrate than did female antennae, whereas female antennae responded better to nonanal, using data of all age groups combined (Fig. 4d).

The lack of qualitative differences between female and male volatiles is similar to what has been found previously with *L. hesperus* (Aldrich et al. 1988) and *L. lineolaris* (Gueldner and Parrott

1978). For *L. lineolaris*, chemical analysis of the contents of the metathoracic glands showed no difference between the sexes (Aldrich et al. 1988). Different formulations of esters were tested as *L. lineolaris* attractants in the field, but no sex pheromones were identified (Hedin et al. 1985). EAD responses of antennae of *L. lineolaris* to different chemicals (Chinta et al. 1994) were analyzed, and it was found that hexyl butyrate and (*E*)-2-hexenyl butyrate elicited greater EAG responses from males than from females, similar to our results with *L. hesperus*.

There was no clear pattern of ratios of compounds in extracts from males and females, nor are the relevance and biological functions of the newly identified trace components clear. As summarized by McBrien and Millar (1999), there are at least 3 possible factors for the repeated failures of a number of research groups to identify pheromones for *Lygus* species. These factors are (1) minimal differences in the chemical profiles of males and females, (2) the inability to produce consistently attractive extracts, and (3) the inability

**Table 3.** Composition (in %) of baits used in field bioassays in summer 1996. Each rubber septum lure was loaded with a total of 2 mg of compounds. Each treatment was replicated 3 times

Bait #	Butyl butyrate	Pentyl butyrate	Hexyl butyrate	( <i>E</i> )-2-Hexenyl butyrate	Heptyl butyrate	Octyl butyrate	Hexanol
LG-1	100						
LG-2		100					
LG-3			100				
LG-4				100			
LG-5					100		
LG-6						100	
LG-7							100
LG-8			99	1			
LG-9			97	3			
LG-10			96	4			
LG-11			95	5			
LG-12			94	6			
LG-13			90	10			
LG-14			75	25			
LG-15			50	50			
LG-16			25	75			
LG-17			10	90			
LG-18 <sup>a</sup>			82	9			9
LG-19 <sup>a</sup>	25	25			25	25	
LG-20	25	58.3			8.3	8.3	
LG-21	0.3	0.3	92.6	6.2	0.3	0.3	

<sup>a</sup>A single male *Lygus hesperus* was caught in one of the 3 traps using this bait.

to develop reproducible and reliable laboratory bioassays to guide fractionation of extracts. In this report, we have described the results of thorough chemical and electrophysiological analyses of bug extracts prepared in several different ways, and found only quantitative differences between sexes. Future attempts at identifying pheromones for *Lygus* bugs may be equally unsuccessful unless reliable bioassays can be developed and reproducibly attractive extracts can be prepared.

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## 盲椿 *Lygus hesperus* Knight 揮發性成分之鑑定，觸角電位篩選以及田間試驗

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本研究利用氣相層析質譜儀、氣相層析儀以及氣相層析觸角電位儀鑑定盲椿 *Lygus hesperus* 之揮發性成分，得知主要成分是丁酸己酯和丁酸己烯酯，其他尚包括丁酸戊酯、丁酸庚酯、丁酸辛酯、烯醛類及烷類等。發現雌蟲與雄蟲的成分在定性上沒有差異，定量上則雌蟲含量較高。觸角電位篩選所鑑定成分，雌雄觸角對這些成分的反應，差異不大。以鑑定得到的成分，組成各種誘餌，進行田間試驗，並未誘捕到 *Lygus hesperus*。

關鍵詞：盲椿 *Lygus hesperus*，丁酸己酯，丁酸己烯酯，氣相層析觸角電位法，田間試驗。

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