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ALARM PHEROMONE SYSTEM OF LEAF-FOOTED BUG *Leptoglossus zonatus* (Heteroptera: Coreidae)

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Abstract—The alarm pheromone system of *Leptoglossus zonatus* (Dallas) adults was shown to be composed of hexyl acetate, hexanol, hexanal, and hexanoic acid. Single components tested in the field elicited dispersive behavior of over 70% of adults. 2-(*E*)-Hexenal, found in the secretion of nymphs, but not in the exudates of adults, was also active against adults. In addition, first-instar nymphs responded to the four components of the alarm pheromone of adults as well as to 2-(*E*)-hexenal, a component of their own alarm pheromone system. Adults and nymphs possess different alarm pheromone systems, which are not specific to their own life stage. That hemipteran alarm pheromone systems are not species-specific was supported by the fact that both adult and nymph *L. zonatus* responded to butanoic acid, an alarm pheromone of Alydidae, which was not found in this Coreidae species.

Key Words—*Leptoglossus zonatus*, hexyl acetate, hexanol, hexanal, hexanoic acid, 2-(*E*)-hexenal, 4-oxo-hex-2-en-1-al, alarm pheromone, Heteroptera, Coreidae.

INTRODUCTION

In many parts of the world, leaf-footed bugs (Coreidae) are important agricultural pests. In California, for example, *Leptoglossus clypealis* and *L. occidentalis* are the most important bugs implicated in the pistachio epicarp lesion,

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causing considerable crop losses. *L. zonatus* (Dallas) is reported as a pest of corn in Brazil (Panizzi, 1989) and may feed on several other economically important plants, such as cotton, citrus, peach, sorghum, and watermelon (Allen, 1969; Solomon and Froeschner, 1981; Schaefer and Mitchell, 1983).

The conspicuous sexual activity of *L. zonatus* observed in corn fields in Londrina, Paraná, Brazil (latitude 23°11'S, longitude 51°11'W) prompted us to investigate its sexual behavior, with emphasis on the chemical cues involved in communication. However, as with other hemipterans (Leal and Kadozawa, 1992a; Aldrich et al., 1987), the presence of large amounts of defensive secretions in the extracts makes the task of isolating sex or aggregation pheromones a difficult challenge. Volatiles (either airborne or whole-body extract) collected from insects possessing sex, aggregation, and alarm pheromones would not elicit attraction. This fact is substantiated by the case of the bean bug, *Riptortus clavatus* (Alydidae), which possesses an aggregation pheromone (Leal et al., 1993) whose active chemicals were masked by an alarm pheromone (Leal and Kadozawa, 1992b). In order to overcome this obstacle, the alarm pheromone system was studied first, before the final goal of identifying the chemicals involved in the aggregation behavior of the insect could be achieved.

In this first report on the chemical ecology of *L. zonatus*, we describe the alarm pheromone system of adults, which was demonstrated in the field to elicit dispersive behavior of adults, even those engaged in copulatory behavior. Aggregated nymphs, although possessing a remarkably different secretion, also responded to the alarm pheromone of adults.

METHODS AND MATERIALS

Insects. Leptoglossus zonatus adults were collected in corn fields in the Londrina area (EMBRAPA/CNPSo Field Experiment Station) and kept in cages (0.5 × 0.5 × 0.5 m) in the laboratory. Corn ears were used as food and potted soybean plants were introduced in the cages to host the insects. Egg masses obtained were placed in an environmental chamber (25 ± 1°C, 60 ± 5% relative humidity, and 14 : 10 hr light–dark photoperiod). Early instar nymphs to be used in bioassays were kept in Petri dishes, and remaining nymphs were returned to the rearing cages.

Extraction. Defensive secretions were extracted from eight groups (four to five individuals per group) of laboratory-raised male and female insects (8, 11, or 14 days old) and from two groups of field-collected males and females (unknown age). The insects were immersed in hexane for 3 min, the extract was filtered while transferring to 5-ml ampules, and these were ice-cooled, sealed, and shipped to Japan, where the samples were chemically analyzed.

Chemical Analysis. GC analysis was performed in a Hewlett-Packard 5890

gas chromatograph either in splitless (injector 210°C) or cold on column injection mode. The column, either HP-1 (12 m or 25 m × 0.2 mm; 0.33 μm) or DB-Wax (30 m × 0.25 mm; 0.25 μm), was operated at 50°C (or 40°C) for 1 min, programmed at 4°C/min to 180°C, held at this temperature for 1 min, programmed again at 10°C/min to 210°C, and held at this temperature for 20 min [50(1)-180(1)/4-230(20)/10 or 40(1)-180(1)/4-230(20)/10]. Gas chromatography-mass spectrometry (GC-MS) analyses were done on a Hewlett-Packard 5891 mass selective detector, EI mode at 70 eV, splitless injection, and equipped with a DB-wax column (as in GC) operated at 50(1)-180(1)/4-230(20)/10.

Bioassay. Alarm pheromonal activity was investigated both in the lab and in a corn field at the EMBRAPA/CNPSo, Brazil. Samples were prepared by loading Pasteur pipets with strips of filter paper containing the candidate chemicals. One microliter of a 10 μg/μl solution was loaded on the filter paper and the solvent was evaporated for 30 sec; the control was prepared in a similar manner by loading the filter paper with solvent (hexane) only. Silicone bulbs (3 ml) were fixed on the pipets and the narrow tips were brought close (2–3 cm) to the test insects. For each test, air inside the control pipet was puffed three times on the body of the insects, then experiments were identically performed with pipets containing the samples. Recovery tests were done in the laboratory by puffing air in the same way out of sample pipets on small pieces of glass wool, containing 1 μg of an internal standard, hexyl isovalerate. The glass wool was washed with 100 μl of hexane, the extract concentrated, and the recovery rate was quantified by GC and GC-MS.

Statistical Analysis. Data comparing dispersive behavior of bugs elicited by sample and control were analyzed by a contingency table using JMP software (version 2) (Anonymous, 1989).

RESULTS AND DISCUSSION

GC and GC-MS analyses revealed that whole-body extracts of *L. zonatus* adults contained mainly hexanal, hexanol, hexyl acetate, and hexanoic acid. Except for hexanoic acid, these chemicals were previously found in the secretion of two other species, *L. oppositus* and *L. clypealis* (Aldrich and Yonke, 1975). The proportions of the four constituents varied from sample to sample, but hexyl acetate (27.1 ± 19.0 μg/bug) and hexanal (22.2 ± 20.1 μg/bug) were the major components. Only very small amounts of hexanoic acid were found in some samples (Figure 1), but it was the major component of others (average 9.3 ± 11.2 μg/bug), whereas hexanol (1.8 ± 1.3 μg/bug) was a minor component. These chemicals are secreted by the metathoracic scent gland, whose glandular cells are segregated from the reservoir wall into primary and secondary accessory glands. Variations in the proportions are due in part to compartmentalized bio-

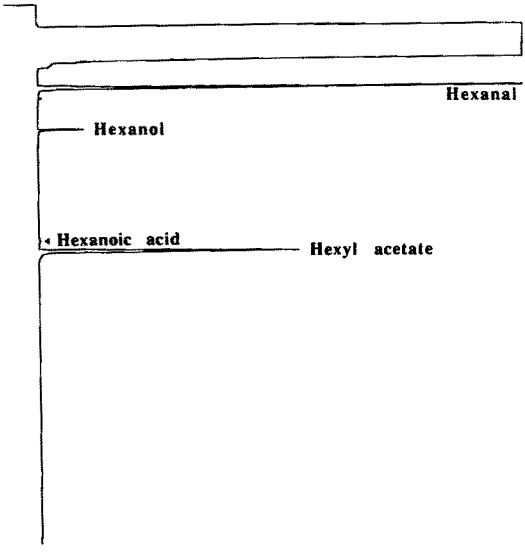

FIG. 1. Gas chromatogram of whole-body extract of *L. zonatus* adults obtained on a 12-m HP-1 capillary column operated at 40(1)-180(1)/4-230(20)/10. (Retention times: hexanal, 2.58 min; hexanol, 3.82 min; hexanoic acid, 7.24 min; hexyl acetate, 7.59 min).

synthesis. Hexyl acetate is secreted by the primary accessory glands into the reservoir, where esterase and dehydrogenase enzymes, apparently secreted by the secondary accessory glands, cleave the ester and oxidize the resulting alcohol (Aldrich, 1988). Nevertheless, it is not clear why insects of the same sex and nearly the same age possessed the four components in such different proportions.

In order to quantify the chemical stimuli during the bioassays, we investigated the amount of chemicals released during puffs from Pasteur pipets. Although the recovery rate changed from chemical to chemical, it was very low for all compounds (0.1–1%). Small recovery rates were also found by Todd and Baker (1993) in similar experiments. We found that for hexyl acetate, for example, out of 10 μg (ca. 0.4 individual-equivalent) loaded on a strip of filter paper, only 0.5% (50 ng on average) was effectively blown out of the pipet in one puff. The amount of alarm pheromone released by a bug in response to an environmental hazard is not accurately known, but it is certainly higher than the effective amount released in one puff under our experimental conditions (pipet loaded with 10 μg) and probably much less than the amount obtained by whole-body extraction.

Preliminary bioassays in the field showed that adults dispersed in response

to a mixture of hexyl acetate and hexanoic acid. Of 31 individuals tested, only one responded to puffs from the control, whereas 24 responded to puffs of the synthetic mixture. Over 50% of the bugs responded by flying away, and those engaged in copulatory behavior stopped mating and dispersed. Although only few nymphs of third, fourth and fifth instars were found in the field during the tests (January 19–21, 1993), their response was very clear: 0% to the control and 75–100% to the synthetic mixture.

The role of single components on the dispersive behavior of adults was further investigated in the field with synthetic chemicals. The test compounds were the metathoracic gland constituents: hexyl acetate, hexanoic acid, hexanal, and hexanol; 2-(*E*)-hexenal, a common alarm pheromone of stink bugs (Leal and Kadosawa, 1992a and references therein; Lockwood and Story, 1987; Kou et al., 1989), and butyric acid, a constituent of Alydidae species (Aldrich and Yonke, 1975; Leal and Panizzi, unpublished).

The responses to *L. zonatus* exudates (hexyl acetate, hexanoic acid, hexanal, and hexanol) were the strongest (>70%), whereas butyric acid gave the poorest response (<40%). 2-(*E*)-Hexenal also elicited a response in adults, even though this chemical was not found in the secretion of adults (Figure 2), but only in nymph exudates (see below).

It has been demonstrated by means of EAG measurements that a minimum chain length of six carbons and a terminal carbonyl group were required for optimal alarm pheromonal activity of the bedbug *Cimex lectularius*; however, a Δ^2-double bond was not essential (Levinson et al., 1974). Conversely, it can be speculated that 2-(*E*)-hexenal mimics hexanal, one of the alarm pheromone constituents of *L. zonatus* adults. Similarly, the response of *L. zonatus* elicited

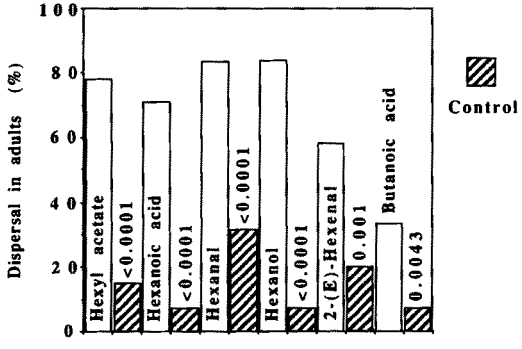

FIG. 2. Dispersal of *L. zonatus* adults ($N = 30$) in a corn field elicited by single components of the alarm pheromone system and related compounds. The results of Pearson chi-square tests ($P > \chi^2$) for each pair (sample × control) are given on the top of the control bars.

by butanoic acid may be explained by its similarity to hexanoic acid. Therefore, alarm pheromones of hemipterans are of little species specificity.

A prerequisite for the evolution of alarm pheromones is the evolution of group living (Nault and Phelan, 1984). Aggregations of *L. zonatus* adults are considerably more dispersed than those of first-instar nymphs. Nevertheless, the secretion of the former causes conspecific adults to disperse. As pointed out by Blum (1985), the evolution of chemical releasers of alarm behavior appears to have occurred in arthropods belonging to disparate taxa that, during at least one life stage, form groups in which the individuals are near enough to each other to rapidly communicate.

The alarm pheromone of adults and related compounds tested in the lab on first-instar nymphs gathered on Petri dish covers showed that dispersal due to the puffs of control was less pronounced in nymphs than in adults (Figure 3). 2-(*E*)-Hexenal elicited the greatest dispersal, only rivaled by hexanal. In fact, 2-(*E*)-hexenal was found in whole-body extracts of all nymph stages along with 4-oxo-hex-2-enal. These compounds were previously found in the secretions of *L. opposítus* and *L. clypealis* (Aldrich and Yonke, 1975).

The fact that, as a general rule, the exocrine chemistry of heteropteran nymphs is distinct from that of the adults (Aldrich and Yonke, 1975) suggests the existence of different alarm pheromone and defensive systems in adults and nymphs of true bugs. Our findings support this hypothesis in the case of the alarm pheromone systems of *L. zonatus* adults and nymphs. Similarly, nymphs of the bean bug, *R. clavatus*, responded to the alarm pheromone of adults, 2-(*E*)-hexenyl hexanoate (Leal and Kadozawa, 1992b), a chemical that was not found in the secretion of any nymphal stage (Leal, unpublished). Interestingly,

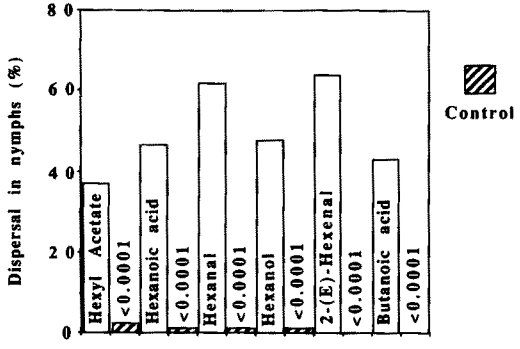

FIG. 3. Dispersal of first-instar nymphs of *L. zonatus* ($N = 44$) in a laboratory bioassay, elicited by the same chemicals tested against adult insects. The figures on the top of control bars are the $P > \chi^2$ according to the Pearson χ^2 tests applied to each pair (sample × control).

fifth-instar nymphs, found to be the most gregarious stage of the bean bug in the field (H. Higuchi, personal communication), displayed the strongest response to 2-(*E*)-hexenyl hexanoate (Leal and Kadozawa, unpublished).

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