PEAR PEST MANAGEMENT RESEARCH FUND PROJECT REPORT: 2007

Title: **Chemical and Acoustic Communication in Boxelder Bug, *Leptocoris* (=*Boisea*) spp. (Hemiptera: Rhopalidae)**

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Abstract:

In 2006, we had focused our attention on experiments to determine if box elder bugs might use either chemical or acoustic signals for communicating with each other. We found no evidence for use of acoustic signals, but there were indications that bugs might use volatile chemical signals. Thus, work in 2007 focused on trying to corroborate these preliminary results. We then collected and analysed volatile chemicals from sexually mature bugs, using gas chromatography-electroantennogram detection (GC-EAD) to locate compounds in extracts that might be pheromonal compounds, followed by GC-mass spectrometry (GC-MS) to identify compounds from the extracts. Approximately 35 different samples of headspace odors were collected from cohorts of virgin male or female bugs on both a natural host (ash seeds and twigs) and a nonnatural but acceptable food source (green beans). A group of compounds consistently appeared in volatiles from both sexes in relatively large amounts, and the structures of these have been identified. However, these did not elicit strong responses from the insects' antennae. In contrast, four trace compounds in the extracts from females elicited strong responses from antennae of males. Three of these four compounds have been identified. Work of the coming months will focus on a) completing identifications of the compounds mentioned above, including determination of the chirality of those compounds which are chiral, b) completion and collation of reciprocal GC-EAD analyses of antennae of female bugs challenged with extracts from males, and c) reconstruction of blends of these compounds for bioassays.

Introduction:

Box elder bugs (*Leptocoris* or *Boisea* spp.) are chronic pests of pears in pear orchards in riparian areas of California (Anonymous 1991). During the growing season, the bugs are often found in groups or aggregations in the field, and they also overwinter in large aggregations in warm or sheltered places, including invading homes. Despite the fact that they are relatively common and easy to find, little is known about their basic reproductive biology and behavior, and particularly, about the cues that mediate the formation and maintenance of their aggregations. Even the bugs' general reproductive biology is relatively poorly known, other than basic life history information such as the number of nymphal instars (5) and the number of generations per year (2 in California) (Anonymous 1991). So far, there have been no detailed investigations of chemical, visual, or acoustic signals that these bugs might use for communication with each other, particularly for attraction of males to females (or vice versa), or even general attraction of conspecifics to form aggregations. It is clear that these signals must exist, because males and females find and recognize each other for mating. Thus, our project goal was to investigate what types of signals these bugs might be using for communication, and if possible, to identify these signals and the roles that they play in the bugs' life history, with the hope of exploiting and manipulating these signals for sampling or control of these insects. In work done in 2006, we found no evidence of any acoustic communication, but we did find indications that chemical signals might be used. Thus, our 2007 project objectives were:

1. To identify and synthesize any attractive components produced by box elder bugs of both sexes.
2. To verify the activity of the attractant components in laboratory bioassays conducted under controlled conditions.
3. To determine the spectrum of activity of the attractants, in relation to the age and physiological status of adult bugs.

Plans and Procedures:

Insects.

Project cooperator Lucia Varella provided several shipments of overwintering insects in early spring. Some of these insects were used to restart our colonies, while the remainder were kept in cold storage and brought out in batches through late spring and early summer to provide fresh cohorts of reproductively active bugs. However, as the year progressed through late summer and fall, we found that the colony had finally become self-sustaining, with ongoing mating and oviposition activity, so that eggs could be collected at biweekly intervals for starting fresh cohorts. This is in direct contrast with 2006, when we had great difficulty in inducing bugs to mate and oviposit, despite the fact that the adults lived for several months. Currently, the colony is being maintained on a diet of green beans, raw sunflower seeds, and alfalfa bouquets.

Y-tube Bioassays

Y-tube bioassays were used to test for possible attractants produced by sexually mature virgin adults of either sex. The Y-tube was constructed of 2 inch diameter glass tubing, with ground glass joints for attachment of fittings and hose connections. For testing odors from live bugs on food, a single-sex group of virgin insects on food was placed in a glass cylinder, and clean air was passed through the cylinder and into one arm of the olfactometer. Air passed through a control cylinder containing only food was piped into the other arm of the olfactometer. All possible combinations were tested, i.e., males and females as odor sources, and males or females as responding insects. Insects were tested one at a time, with the apparatus being cleaned with hot water and acetone, then baked in a drying oven, after each insect. Several Y-tubes were used in rolling sequence, i.e., while some were being cleaned, others were in use.

Odors were collected from single-sex cohorts of virgin bugs on food by placing the bugs and food in glass aeration chambers swept with clean air, with the volatiles trapped on activated charcoal traps. Control aerations were made with chambers containing only food. After eluting the trapped volatiles with methylene chloride, the extracts were tested in Y-tube bioassays. Data from all bioassays was analyzed with t-tests.

Analysis of extracts.

Each extract initially was analyzed by gas chromatography to get a general idea of the profile of volatiles it contained. Most extracts were then further analyzed by coupled gas chromatography-electroantennogram detection (GC-EAD) to locate compounds in the extracts that elicited responses from bug antennae. Because the EAD analyses were noisy, replicates of these GC-EAD assays were then lined up and compared, to identify peaks that consistently elicited antennal responses. Selected extracts were then analyzed further by GC-mass spectrometry to tentatively identify compounds in the extracts. Identities were confirmed by matching with authentic standards.

Results

1. Basic reproductive behaviors.

Our breeding colony of box elder bugs was rejuvenated with a large infusion of overwintering adults from project cooperator Lucia Varella in early spring. As in 2006, the long-lived adult insects proved to be relatively easy to maintain in the laboratory, but initially difficult to induce to reproduce. However, through the fall of this year, the colony seems to have broken through this bottleneck; mating and oviposition in the colony are now almost continuous, allowing us to rear fresh cohorts of adult bugs for experiments and bioassays as needed. This should be a significant benefit, because previously the only way that we could get fresh cohorts of reproductively active bugs was to chill adults for extended periods, simulating the overwintering period. The do not yet know why the colony has changed, but it may be related to diet. We had been rearing the colony on ash twigs and seeds, and this foliage, cut through the different seasons of the year, may have provided cues to the insects for timing their reproductive cycles. For convenience, we are now rearing the bugs on a typical generic phytophagous bug diet of green beans, raw sunflower seeds, and alfalfa bouquets, and this more neutral nutrition, with minimal or no seasonal changes in chemistry, may have resulted in the change in reproductive patterns that we are seeing.

It may also now become possible to look at the various parameters of mating behavior, such as the sexual maturation period, the duration and frequency of mating, and the sex initiating courtship and mating behaviors.

2. Bioassays with various combinations of bug and food odors.

Our initial bioassays using live bugs on food as odor sources had indicated quite strong attraction of males to odors of both males and females (Table 1, tests 2 and 3 respectively), suggesting that both sexual and aggregative interactions might be mediated by volatile chemical signals. However, the results of further Y-tube bioassays that tested the activity of extracts of bug volatiles versus the volatiles of food or versus solvent controls were either indeterminate, or even negative, possibly due to extracts being tested at doses that were too high. Because of these inconsistencies between the initial, highly promising bioassays using live bugs as odor sources and these later results with extracts, further bioassays were put on hold until we could get a better idea of the compounds in the various extracts and their amounts. Furthermore, we wanted to use the GC-electroantennogram technique to provide an indication as the compounds in the extracts to which the bugs might be most sensitive, i.e., the ones which elicited the largest responses from bug antennae. These results are described in the next section.

Table 1. Results of Y-tube bioassays testing the responses of male and female bugs to various odor sources or controls.

Test #	Responder	CHOICES		Results	P value
1	Female	Green beans+males	Green beans	19/11	0.14
2	Male	Green beans+males	Green beans	26/3	4.36E-05
3	Male	Green beans+females	Green beans	23/6	0.003
4	Female	Green beans+females	Green beans	20/10	0.068
5	Female	Solvent control	Female extract	10/17	0.18
6	Male	Solvent control	Female extract	18/9	0.083
7	Female	Solvent control	Male extract	19/8	0.034
8	Male	Solvent control	Male extract	17/10	0.18
9	Female	Solvent control	Male extract	15/5	0.025
10	Male	Solvent control	Male extract	16/4	0.007
11	Male	Food fed on by males	Fresh food + live males	6/14	0.074
12	Female	Food fed on by males	Fresh food + live males	13/7	0.18
13	Female	Food fed on by females	Fresh food + live females	9/11	0.65
14	Male	Food fed on by females	Fresh food + live females	10/10	1
15	Male	Food fed on by males	Fresh food	8/12	0.37
16	Female	Food fed on by males	Fresh food	8/12	0.37

3. Collection and analysis of volatiles produced by adults of both sexes.

Collections of headspace odors were collected for periods of several weeks from cohorts of sexually mature virgin adult bugs of both sexes on host plant material, from March to August, for a total of approx. 15 and 20 different collections from males and females respectively, along with appropriate controls (i.e., headspace collections from food materials only). Each extract was analyzed by gas chromatography, and most extracts were also analyzed by coupled gas chromatography-electroantennogram detection. Examination of the GC and EAD traces from multiple extracts revealed the following points:

A. The major peaks in the extracts appeared to be common to both males and females (Fig. 1), but were not present in the volatiles from food alone (Fig. 2). Thus, it appears as though bugs produce a "species" odor, which may serve for general recognition, or may mediate the formation of the aggregations in which the insects are commonly found. The gross structures of these compounds have been identified, but they are chiral, i.e., they can exist in nonsuperimposable, mirror-image forms that are recognized as different compounds by biological receptor proteins. Over the next few weeks, we will be using a chiral stationary phase GC column to determine which form of each of these molecules is produced by each sex, and then reconstruct the blend from synthetic compounds so that it is available for bioassays.

B. Extracts from both males and females were analyzed by coupled GC-electroantennography, with a total of 47 different specimens being used for the analyses (some extracts were run several times). A typical GC-EAD trace is shown in Figure 3. Although the antennal signals were noisy, comparing the traces from a number of different runs revealed several consistent responses from antennae of male bugs to several trace components in extracts from virgin female bugs (Fig. 3). Three of these components have been identified, and the identification of the 4th compound is in progress. Once the identification is complete, we will reconstruct and test blends of the four compounds for their effects as possible attractants for both males and females.

We have also carried out the reciprocal analyses, i.e., testing the responses of antennae of females to extracts from males, but the results have not yet been collated and carefully compared to determine whether there are also male-produced compounds that consistently elicit antennal responses from females.

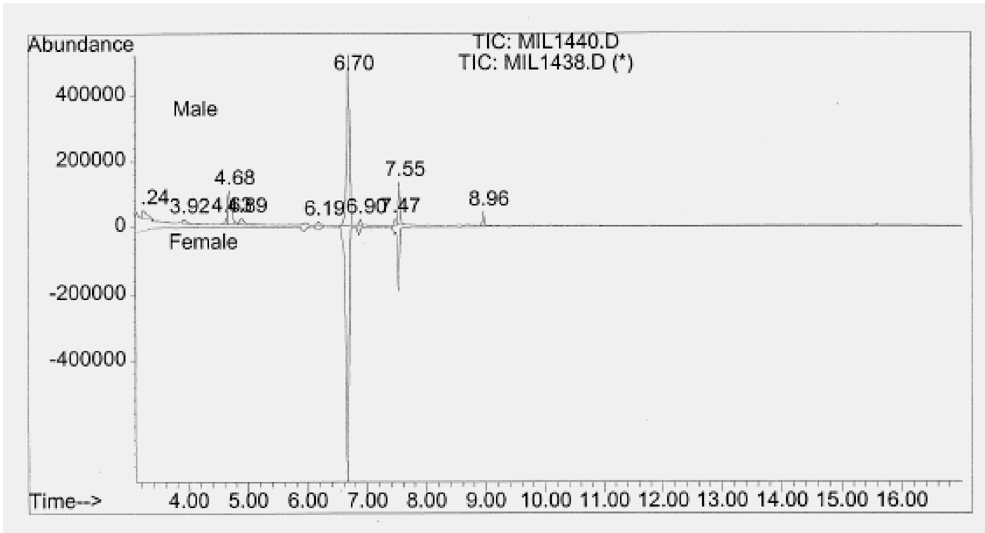

Figure 1. GC-MS traces comparing volatiles collected from virgin male (top trace) and virgin female (bottom, inverted trace) box elder bugs. Peaks between 3 and 5.5 min are from food (see Fig 2). Note that the profiles of compounds are otherwise quite similar.

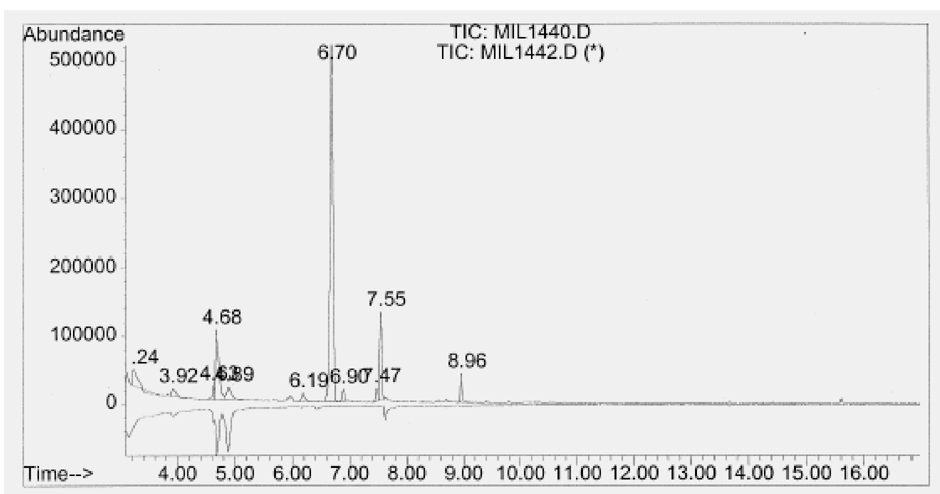

Figure 2. GC-MS traces comparing the volatiles collected from virgin male box elder bugs on green beans (top trace) and green beans alone (bottom, inverted trace), showing that the peaks between 3 and 5.5 min are food volatiles (see Fig 2).

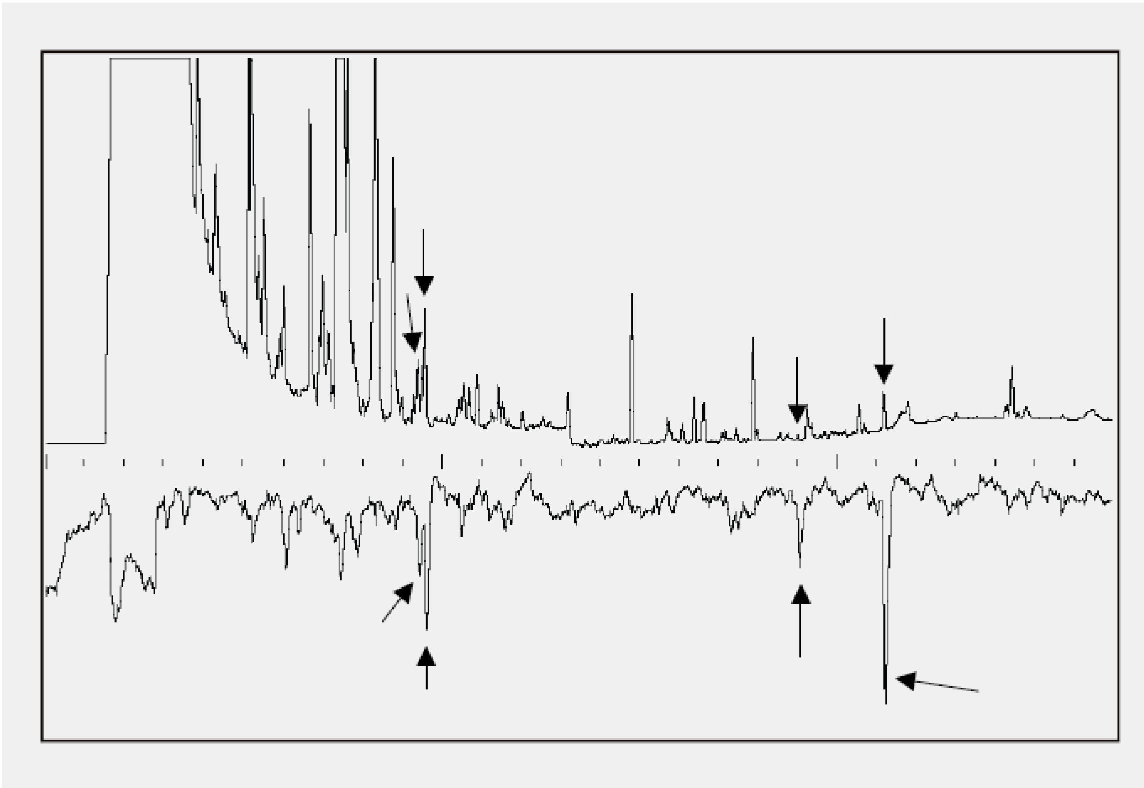

Figure 3. Coupled GC-electroantennogram of volatiles from virgin female box elder bugs. Top, GC detector response, expanded to show trace components; bottom, inverted trace, simultaneous response recorded from the antenna of a male bug. The 4 small to trace components that elicited consistent strong responses from the male antenna are indicated by arrows. The first two and the 4th have been identified, whereas the 3rd component is not yet known.

References cited:

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