PEAR PEST MANAGEMENT RESEARCH FUND PROJECT REPORT: 2008

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

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Abstract:

We have finally developed a strain of box elder bugs that will reproduce year round in laboratory colonies; in previous years, research had been limited by colonies ceasing to reproduce and/or dying out over the winter, despite being maintained under long-day, summertime conditions.

In previous years, we had shown that acoustic signals are not used by box elder bugs for communication. Thus, in 2008, we concentrated our efforts on possible chemical signals. We collected volatiles repeatedly from a variety of sources, including virgin box elder bugs of both sexes on food substrates, food damaged by bug feeding, and bug excreta, with appropriate controls. In all these collections, we have not seen any reproducible sex-specific compounds produced by either sex, although there may be quantitative differences in the amounts of volatiles produced by females. Extracts were analysed by coupled gas chromatography-electroantennogram detection, but we have not found any obvious differences in the patterns or sizes of antennal responses from antennae of either males or females. Thus, at this point we have no strong leads for chemical attractants for these insects. Nevertheless, we will carry out field bioassays with blends of the compounds that produced the largest antennal responses, and with blends of the major insect-produced chemicals this coming spring to determine whether one or both sexes are attracted by any of the compounds identified to date.

Introduction:

Box elder bugs are chronic pests of pears in some parts of California, particularly near riparian areas (Anonymous 1991). These brightly colored bugs are often found in aggregations in the field, and they are well known from their habit of forming large, overwintering aggregations in sheltered sites, including homes and garages. When the PPMRF encouraged us to initiate this project, nothing was known about chemical or other types of signals that these insects might use for communication, including the signals that mediate the formation of aggregations in the field or in overwintering sites. Similarly, relatively little is known about the bugs' general reproductive biology and behavior, other than basic information such as the number of nymphal instars (5) and the number of generations per year (2 in California) (Anonymous 1991). To date, investigations of chemical, visual, or acoustic signals that these bugs might use for crucial functions such as mate location and recognition have not been carried out. Thus, our project goal was to attempt to determine what types of signals these bugs might be using for communication, with the aim of exploiting these signals for sampling and/or control purposes Our overall project objectives were:

1. To document basic reproductive parameters in this species.
2. To determine whether these insects use long distance pheromones to bring the sexes together for mating.
3. To collect, identify, synthesize, and test any chemicals that these bugs use for long-range attraction and communication.
4. To determine whether these insects use acoustic signals for communication.
5. If vibrational signals are used, to record and characterize the signals produced by each sex, and determine the behavioral context in which they are produced
6. To determine whether chemical signals may be involved in the formation or maintenance of overwintering aggregations.

Procedures:

Insects.

Our bug colonies were restarted more than a year ago with material from overwintering aggregations provided by cooperator Lucia Varela. Bugs are reared on a standard diet of green beans and raw nuts. Eggs are removed from the general breeding colony, and reared in same-age cohorts in plastic containers with screen lids. Upon emergence, adults are segregated by sex, and held in single-sex cohorts supplied with food and water until used in experiments.

Collections and analyses of extracts of volatiles

Cohorts of sexually mature virgin males and females were transferred to glass aeration chambers, and were aerated continuously for periods of several weeks. Clean air was passed through the chambers, and the insect-produced and food volatiles were trapped on collectors of activated charcoal. The collectors were changed twice a week, and the collected volatiles were washed off the activated charcoal with methylene chloride. The resulting extracts were analyzed first by gas chromatography, then by coupled gas chromatography-electroantennogram detection and mass spectrometry. Aeration extracts were prepared from the following materials, with each extract replicated multiple times:

1. Sexually mature virgin females on food (beans and sunflower seeds).
2. Sexually mature virgin males on food.
3. Undamaged beans and sunflower seed controls.
4. Beans and sunflower seeds fed on by virgin females.
5. Beans and sunflower seeds fed on by virgin males.
6. Filter papers with excreta from virgin males.
7. Filter papers with excreta from virgin females.
8. Filter paper controls.
9. Blank aeration chamber controls.

Compounds were tentatively identified by interpretation of mass spectra, comparisons of retention characteristics with those of standards, and matchups with database spectra.

Identifications were confirmed by comparisons of mass spectra and retention times of aeration extract compounds with those of authentic standards.

Results

Insect rearing.

Over the past year, we finally have overcome the problem of getting the bugs to continue to reproduce over the winter, so that we can have bug colonies and reproductively active bugs to work with year-round. In the first years of the project, the seasonality of the bugs was a major problem because in late summer and fall, the bugs would stop breeding and go into reproductive diapause, even though they were being held under constant, long-day temperature and light-dark conditions (16L,8D). In the past, we had tried all kinds of manipulations of light (different light sources, different day lengths) and temperature conditions (constant temperatures, daily fluctuating temperatures, different chilling periods for different amounts of time) to either prevent the bugs going into reproductive diapause, or if they had gone into diapause, to induce them to become reproductively active again. Most of these manipulations were unsuccessful. We speculate that the colony may have gone through a rearing bottleneck and has now been selected for a strain that does not require diapause. Alternatively, in previous years we had reared the bug colonies on some of their natural hosts (ash and maple foliage and seeds). Because these host materials were collected from mature trees growing under natural conditions, the bugs may have cued their reproductive physiology to seasonal changes in the host plant chemistry. In contrast, for the past year, we have been feeding the bug colonies with a standardized diet of commercial organically grown green beans and raw sunflower seeds and almonds, which probably have little or not seasonal variation in their chemistries. This standardized diet, when combined with continuous long-day environmental conditions in the rearing rooms may be preventing the bugs from going into winter reproductive diapause. Furthermore, it is clear that this standardized diet is more than adequate to support normal reproduction and behaviors because the colony is thriving, and at any given time during daylight hours, numerous pairs of bugs can be observed mating. Under the laboratory rearing conditions, eggs hatch in about 5 days, bugs complete their development in ~3-4 weeks, and the adults become sexually mature and commence mating after about 1 week. Adults are relatively long-lived, feeding and mating for 2 months or more under the long-day laboratory conditions.

Collection and analysis of volatiles. We have now collected, extracted, and compared the profiles of headspace odors from a variety of sources, including a) cohorts of sexually mature virgin bugs on food (green beans and raw sunflower seeds), b) undamaged food, c) food damaged by bugs of both sexes, d) excreta from both sexes, and system blanks and controls. In total, 85 different extracts have been analyzed by GC, with subsets of these extracts then analyzed further by GC-EAD and/or GC-MS. From these analyses, several generalizations emerged:

1. The odor profiles of virgin male or female bugs aerated on food appear to be qualitatively similar (Fig. 1), although cohorts of females appear to produce larger amounts of odors than similar numbers of males.
2. In GC-EAD analyses, bug antennae responded most strongly to several minor compounds in the extracts, and gave small responses to the most abundant components in the extracts.
3. We did not see any obvious differences in the profiles of responses of antennae from males or females, further suggesting that these bugs do not produce sex-specific volatile pheromones.

We have identified most of the components in the extracts, and we plan to follow up with field bioassays in spring and early summer of 2009. We will go directly to field bioassays, rather than experimenting with laboratory bioassays first, because the field is “where the rubber meets the road”, i.e., the blends of compounds will only be useful to growers if they are attractive in the field, regardless of whether we can show responses under the unnatural conditions of lab bioassays, where there are few competing stimuli. We will prepare and test blends using two approaches. First, we will prepare blends of the components that elicited the largest responses from bug antennae, despite the fact that these were only minor components in the extracts overall. Nevertheless, because the antennae are clearly very sensitive to these compounds, they are possible candidates for attractants. In an alternative approach, we will prepare and bioassay blends of the most abundant compounds in the extracts, based on the idea that these are produced in higher quantities by females than males.

Furthermore, during our studies with the pheromones of cerambycid beetles over the past field season, we discovered that small resealable plastic bags are much more effective as release devices than rubber septa for very volatile chemicals such as those produced by box elder bugs. Thus, all field trials will be carried out using these bags as release devices.

References cited:

Anonymous. 1991. Integrated Pest Management for Apples and Pears. UCIPM, DANR Pub#3340

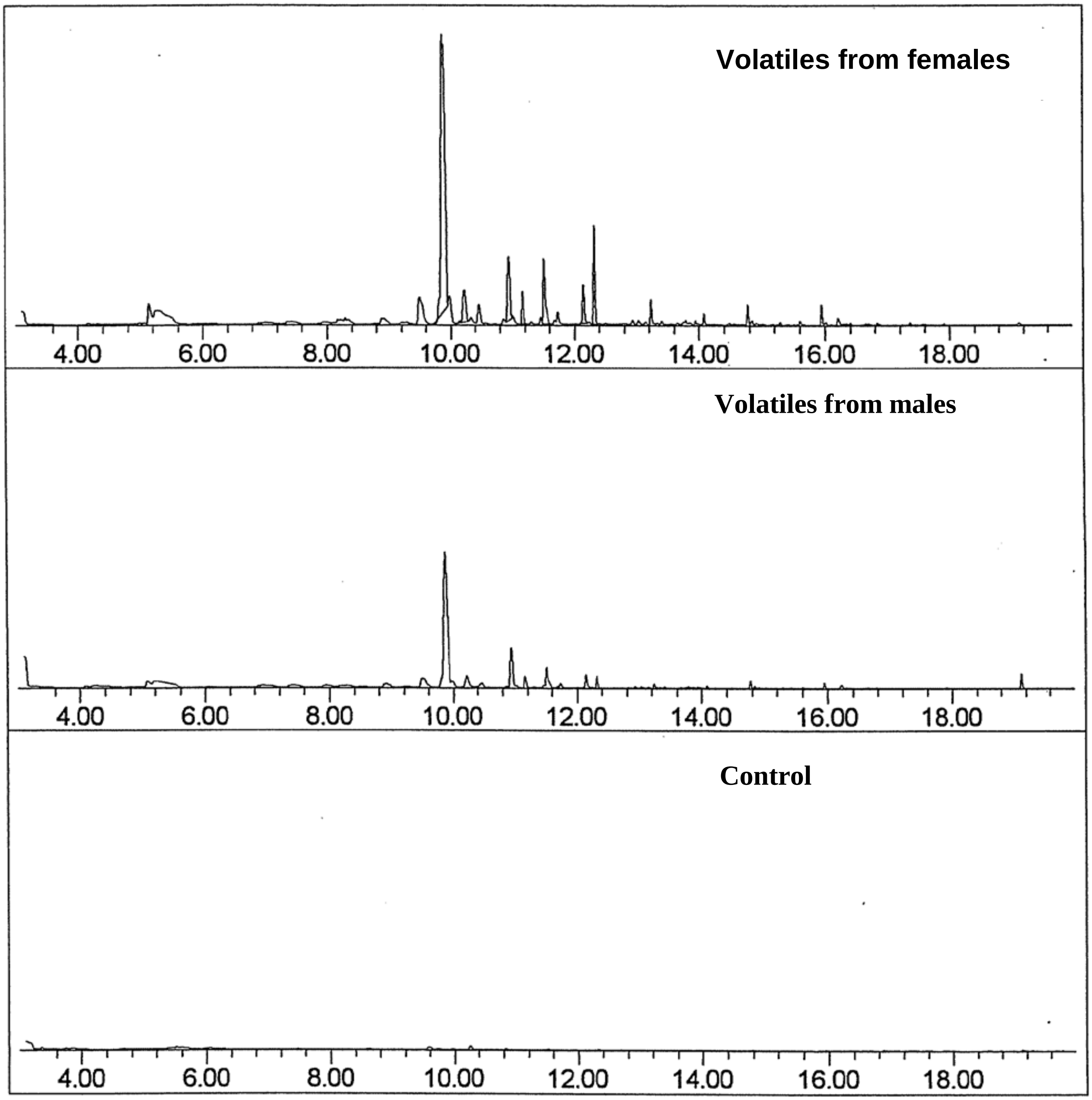

Figure 1. Volatiles collected from aeration of sexually mature, adult box elder bugs. Top, females on food (green beans and sunflower seeds); middle, males on food; bottom, food control.