PEAR PEST MANAGEMENT RESEARCH FUND PROJECT REPORT: 2006

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

Principal Investigator: **Jocelyn G. Millar**, Professor, Dept. of Entomology, University of California, Riverside CA 92521.
FAX: 951 827 3086. Email: jocelyn.millar@ucr.edu

Cooperator: **Lucia Varella**, IPM Advisor, North Coast

Abstract:

Box elder bugs proved to be easy to maintain and lived a long time in the laboratory as adults, but it was remarkably difficult to induce the adults to break reproductive diapause. We found no evidence of acoustic or vibrational signals being used for communication. However, male bugs were strongly attracted to odors of live males or females in two-choice Y-tube olfactometer bioassays; females appeared to be less strongly attracted. Bioassays using live bugs in cages were not successful: the test bugs simply aggregated at the top of the cage. The profile of odors collected from males and females appeared to be similar, and the antennae of males and females showed the same pattern of relatively weak responses to components in the odor extracts. Thus, the formation of the aggregations of bugs seen in the field and in the laboratory may be mediated by species-specific but not sex-specific blends of insect-produced odors. The detailed analysis and reconstruction of these blends will be the focus of work in the coming year.

Introduction:

Boxelder bugs (*Leptocoris* or *Boisea* spp.) are chronic pests of pears in some parts of California, particularly near riparian areas (Anonymous 1991). These insects tend to be found in clumped distributions in the field, and they are well known from their large, overwintering aggregations, in which they often invade houses and create a nuisance for homeowners. Virtually nothing is known about the cues that the insect use to form and maintain aggregations in the field, or the overwintering aggregations. Similarly, few details of the bugs' general reproductive biology and behavior appear to be 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). In particular, there have been no investigations of chemical, visual, or acoustic signals that these bugs might use for communication with each other, particularly for reproductive purposes such as attraction of males to females (or vice versa), and short range recognition of each sex by contact pheromones or other types of signals. It is clear that these signals must exist, because males and females come together and recognize each other as being of the right species and sex before mating. Thus, our project goal was to conduct some preliminary experiments to determine what types of signals these bugs might be using for communication. Identification of these signals and the roles that they play in the bugs' life history may reveal weaknesses that we can be exploit and manipulated for sampling or control of these sporadic pests. Thus, our overall project objectives were:

1. To document the basic parameters of reproductive behaviors 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.

It must be emphasized that we did not expect to accomplish all these objectives within one year. Rather, our objective for the first, pilot year of this project was to determine whether there was evidence of any type of chemical or acoustic signals that could possibly be exploited for sampling or control of box elder bugs. If such evidence were found, this would serve as the basis for further more in-depth work to fully identify, reconstruct, and test these signals for manipulating box elder bug populations. Here, we report the progress that has been made in addressing these objectives.

<u>Plans and Procedures:</u>

<u>Insects.</u>

A starter colony of insects was obtained from Lucia Varella in early March. The eggs laid by this initial cohort of insects were reared through to adults, and maintained in sleeve cages on maple and ash branches. Late stage nymphs were removed from the breeding colonies and segregated until they emerged as adults. Upon emergence, adults were segregated by sex, and held in single-sex cohorts on host plants until used in experiments. Adults were then subjected to various temperature and light regimes to determine which set of conditions triggered sexual activity and reproductive behavior, i.e., physiological stages when one sex or the other might emit pheromones.

<u>Bioassays for Insect-Produced Signals</u>

Sexually mature adults may use either chemical signals or acoustic signals to bring the sexes together, so bioassays were carried out in several different ways, as follows:

1. To check for <u>any</u> type of attraction over a distance, sexually mature virgin females were placed in a small sleeve cage on one branch of an ash twig with seeds, with sexually mature males in another sleeve cage on a different twig. The twigs with cages were placed in the back corners of a 1 meter square cage, and a group of sexually mature males or females were released into the cage. The cage was checked at intervals, when the number of insects on one or the other of the sleeve cages was recorded.
2. To test for possible attractive pheromones, experiments were also conducted with Y-tube olfactometers, using live insects as odor sources. Thus, a single-sex group of virgin insects on an ash twig 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 an ash twig 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.
3. Odors were collected from single-sex cohorts of virgin bugs on fresh ash foliage by placing the bugs and foliate in glass aeration chambers swept with clean air,

with the volatiles trapped on activated charcoal traps. Control aerations were made with chambers containing only foliage. After eluting the trapped volatiles with methylene chloride, the extracts were analyzed by gas chromatography and coupled gas chromatography-electroantennogram detection to locate compounds in the extracts that elicited responses from bug antennae.

4. Adult bugs were tested for any evidence of vibrational signals that might be used over intermediate to short ranges, analogous to the vibrational signals that other families of true bugs are known to use (e.g., McBrien et al., 2002; McBrien and Millar, 2003; Cokl et al., 2004). Recordings were made from insects placed on the membrane of a midrange loudspeaker, which serves as a sensitive and accurate transducer of acoustic and vibrational signals. Recordings were made from bugs for continuous periods of 12 hours of both light and dark, from 12:00 to 0:00 and 0:00 to 12:00 hr, using multiple replicates of single bugs, mixed sex pairs, or groups of 3 males and 3 females.

Results

Basic reproductive behaviors. A breeding colony of box elder bugs was started on March 2, from overwintering adults collected in vineyards by cooperator Lucia Varela. Under the long-day laboratory conditions, the bugs rapidly broke reproductive diapause and began ovipositing, with the first adults of the new generation appearing in mid-April. The immatures and adult bugs fed readily on fresh ash and maple branches, and the distribution of bugs within the cages was always nonrandom, i.e., the bugs showed a strong tendency to aggregate, particularly in the top corners of the cages.

However, the new generation of bugs showed no signs of reproductive activity in the main colony. Furthermore, multiply replicated pairs of bugs held in cages with host plant materials for food were videotaped continuously for 15 days after the final moult with a time-lapse video recorder, and none mated or even showed any sign of interest in each other. Even after 2 months as adults under long-day light and temperature conditions, adults showed no signs of mating, and no eggs were produced.

In case there was a requirement for dispersal before beginning reproductive activities, mature adults, >14 days after the final moult, were suspended by fine threads glued to the thorax to induce them to fly. Suspended bugs flew readily for extended periods, but after flying, they still showed no sign of reproductive activity when placed together again in mixed sex pairs. Thus, it appears that dispersal is not a prerequisite to mating activity in this species.

We then subjected cohorts of adults to cold treatments of varying duration, to determine whether a period of cold was required for the adults to break reproductive diapause. Thus, the first cohort was held in a cold room at ~8ºC for 35 days (June 9-July 14), then returned to the environmental chamber where the main colony was kept under long-day temperature and light conditions. Pairs of bugs from this chilled cohort still showed no sign of mating activity.

A second cohort of mature adults was chilled for 66 days (June 9-August 14), then returned to the rearing room. No mating activity was detected for another month, until Sept. 13, when the first pair was observed to mate, and from then one, a pair was occasionally seen mating. A few eggs were also laid, but most of these proved to be nonviable. A third cohort of bugs that was chilled from July 3-August 28 behaved similarly, with a few bugs starting to mate a few weeks after being brought out of the cold room.

Because of the difficulty in breaking the reproductive diapause, it was not possible to carry out experiments looking at the various parameters of mating behavior, such as the sexual maturation period, the duration and frequency of mating, or the sex initiating courtship and mating behaviors.

Collection and analysis of volatiles produced by adults of both sexes. Headspace odors were collected continuously for periods of several weeks from cohorts of sexually mature virgin adult bugs of both sexes on host plant material at intervals from March to October. These odor collections included cohorts of bugs that had and had not been subjected to cold treatments. Analysis of the extracts by gas chromatography showed no obvious differences between the odor profiles of each sex, and no obvious differences between bugs either before or after cold treatments. Similarly, coupled gas chromatography-electroantennogram analyses of the extracts, using antennae from live males or females as detectors, showed that antennae from both sexes responded weakly to the same compounds in the extracts, i.e., there was no sign of sex specificity in either the production or the antennal response to volatiles produced by adult bugs.

Bioassays using live bugs as odor sources. In the first set of bioassays, using live sexually mature virgin bugs caged on host plant material in a screen cage as odor sources, bugs of either sex showed no sign of attraction to the caged bugs. Most of the test bugs simply eventually formed aggregations at the top of the cage.

Y-tube olfactometer bioassays using live virgin insects confined in glass chambers with food as odor sources, versus a food control, were more successful (Table 1). Males responded strongly to odors from either other males or females, whereas the response of females was weaker. In these bioassays, test bugs released into the Y-tube could not see the bugs being used as the odor source, i.e., visual cues were excluded, so any attraction must have been due to either chemical cues, or possibly acoustic cues transmitted through the tubing and glassware. However, the possibility of acoustic cues causing the responses seen seems unlikely, based on other experiments reported below. Thus, these experiments provided the first evidence that the observed aggregative behavior of this species may be mediated by volatile chemical cues produced by both sexes.

Table 1. Responses of sexually mature, virgin adult boxelder bugs to odors from live insects on ash twigs, versus odors from ash twigs alone. Each bioassay was run with a single insect at a time, with a maximum 2 minute test period for each bug. Test insects were used only once. *** = P < 0.001; ** = P < 0.01.

	Responding males		Responding females	
Stimulus comparison	Treatment	Control	Treatment	Control
Males on food vs food	26***	3	19	11
Females on food vs food	23**	6	20	10

Experiments looking for acoustic signals. Experiments were conducted periodically with cohorts of sexually mature adult bugs from April through August, using individual insects, mixed sex pairs, and groups of 3 males and 3 females (>30 replicates of each). Insects were placed on a loudspeaker membrane, and recordings of sound and vibrational signals were carried out continuously for 12 hour periods (0:00 to 12:00 or 12:00 to 0:00, with the photophase from 06:00 to 20:00, so that recording sessions included periods of both light and dark). The insects were simultaneously videotaped with a time-lapse recorder. There was no sign of any acoustic or vibrational signaling, and the only sounds and vibrations recorded were due to the bugs moving around on the membrane. Thus, it seems unlikely that acoustic or vibrational signals form an important part of the communication system of this species.

References cited:

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

Čokl, A., Janez Presern, Meta Virant-Doberlet, Glen J. Bagwell, and Jocelyn G. Millar. 2004. Vibratory Signals of the Harlequin Bug and their Transmission through Plants. Physiol. Entomol. 29:372-380.

McBrien, H.L., A. Cokl, and JG. Millar. 2002. Comparison of Substrate-Borne Vibrational Signals of Two Congeneric Stink Bug Species, *Thyanta pallidovirens* (Stal) and *T. custator accerra* McAtee (Heteroptera: Pentatomidae). J. Insect Behavior 15: 715-738.

McBrien, H.L., J. G. Millar. 2003. Substrate-Borne Vibrational Signalling in the Consperse Stink Bug, *Euschistus conspersus*. The Canadian Entomologist 135: 555-567.

Yoder, K.M. and W.H. Robinson. 1990. Seasonal abundance and habits of boxelder bug, *Boisea trivittata* (Say) in an urban environment. Proc. Entomol. Soc. Wash. 92:802-807.