Understanding Aggregation Behavior of the Leaffooted Bug, *Leptoglossus zonatus*

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INTRODUCTION

The leaffooted bug species, *Leptoglossus zonatus*, is a large seed-feeding insect found on many host plants, including pistachio (McPherson et al. 1990). This species has been collected in California from Butte County to Kern County in the Central Valley and can be damaging to pistachios (Michailides 1989, Joyce et al 2014, 2017). Although there are at least two species of leaffooted bugs that occur in pistachios--*Leptoglossus clypealis* and *Leptoglossus zonatus*--*L. zonatus* is substantially larger than *L. clypealis*. A field-cage study of feeding damage from *L. zonatus* and *L. clypealis* on almonds found that *L. zonatus* was more damaging (Joyce et al. 2015). *L. zonatus* appears to have become more abundant in the last few years in California (Haviland 2007, Joyce et al. 2014). There is a need to develop traps or monitoring devices to detect the presence of this insect in orchards, before the damage from bug feeding is observed and before it causes economic damage. Although leaffooted bugs are large insects, they are difficult to monitor because they are elusive. Currently there is no pheromone or attractant available for monitoring or trapping this insect. The research in this ongoing study will contribute to understanding the biology and behavior of *L. zonatus* and the ability to trap or monitor this insect.

Attractants for leaffooted bugs could include pheromones, host plant volatiles or plant volatiles associated with bug feeding (Aldrich et al. 1979, Yasuda 1998, Wang and Miller 2000). A notable behavior of leaffooted bugs is the overwintering aggregations formed by adults, which then disperse in the spring as temperatures warm and day length increases (Daane et al. 2008). Semiochemicals, such as aggregation pheromones and sex pheromones, or host plant volatiles might eventually be manipulated for detection, monitoring or trapping tools and included as components of an IPM program. However, the aggregation behavior of *Leptoglossus* spp. in the Central Valley has been difficult to manipulate and may involve environmental or seasonal cues that need to be studied further (Daane 2007). We will investigate the relative attraction of volatiles to *L. zonatus*, and the environmental conditions that promote formation of aggregations and contribute to dispersal from aggregations as well. The ability to attract, detect and quantify adult *Leptoglossus* spp. in the field, before damage is observed in pistachios or in almonds, could improve timing of controls and could also reduce the use of preventative insecticide applications.

RESULTS

We continued to raise the insect *Leptoglossus zonatus* in the lab, during the year, so that insects were available for lab bioassays. Large numbers of insects were produced to conduct the studies described below, which were 1) to examine the age of sexually maturity of adult *L. zonatus*, 2) to examine attraction to volatile odor sources in the wind tunnel and 3) to investigate dispersal from aggregations.

Before experiments began, we had prepared cages of adult females and separate cages of males that were 1 week old, 2 weeks old, 3 weeks old, 4 weeks old, 5 weeks old, and 6 weeks old.

The age of sexually maturity of adult *L. zonatus* was investigated. We previously had an estimate of the age of the insects' sexually maturity of approximately 2 weeks. This year we ran experiments to pinpoint the age when *L. zonatus* adults would mate most rapidly. We compared the mating frequency of unmated adult male and female *L. zonatus,* for three different age categories. We set up cages with 2-week-old males and females, 4-week-old males and females and 6-week-old male and female insects. These insects were all observed at the same time each day to determine the mating frequency for 2-week-, 4-week-, and 6-week-old adults. We experimentally determined the age of sexual maturity for *L. zonatus* and found that 4-week-old and 6-week-old male/female pairs would mate most rapidly. For all experiments described for 2017, we used 4-week-old unmated male and female adult *L. zonatus* for investigating attraction to odor sources.

Adult insects that were 4-weeks old and unmated were used in behavioral bioassays in the wind tunnel. Six different experiments were run to examine whether males, females or combinations of males and females were more or less attractive than plant odors. Insects were only used for one behavioral trial to avoid pseudo-replication. Dual choice trials were run to compare the following: 1) the attraction of females to either 10 males vs. a control; 2) the attraction of females to either 10 females vs. a control; 3) the attraction of males to either 10 females vs. 10 males; 4) the attraction of females to either 10 females vs.10 males. The next set of experiments involved dual choice trials again, but one choice consisted of mating pairs of *L. zonatus*. The fifth experiment compared the attraction of males to either 5 mating female/male pairs vs. attraction to a control. The sixth and final experiment compared the attraction of males to 5 mating pairs of male/females vs.10 females.

Data recorded included the number of landings on each odor choice, the time spent on each odor, and the time from the start of the experiment until a test insect landed on an odor. At least 25 replicates were run for each of these experiments. Insects were observed for at least 15 minutes in experiments. In the wind tunnel, behavioral trials were continued to examine attraction of males and females to volatile odor sources. The results found that males were most attracted to females, which is consistent with our preliminary data reported last year. In experiment 2, females landed equally on either males or on the control. When males or females were offered a choice of males vs. females (experiments 3 and 4), there was no significant difference in landings on males vs. females. Finally, the attraction of males was tested to odors of females vs. mating pairs, and there was no significant difference in attraction. However, when males were tested to determine their response to odors associated with mating pairs vs. a control, males had nearly twice the number of landings on mating pairs as they did on the control. Odors associated with mating pairs will be further investigated as attractants.

Aggregations of leaffooted bugs could be influenced by changing light and temperature conditions in the spring and the fall. Last spring 2017, we investigated the effect of light and temperature on the formation of aggregations in the lab. Changing light conditions did not cause formation of aggregations; temperature may a critical factor in causing insects to join or disperse from aggregations. This spring, aggregations will be placed outdoors in field cages. Aggregations of known size will be monitored daily. We will determine the number of insects leaving each aggregation each day, and relate it to temperature.

CONCLUTIONS AND PRACTICAL APPLICATION

There are many potential volatile attractants for adult *L. zonatus*. This year, experiments included both insect odors and host plant odors. Results suggest that the most attractive odor source is associated with mating pairs of *L. zonatus.* This is being investigated further to pinpoint the most attractive age of mating pairs and the associated odors. Volatiles will be collected and attraction to volatiles will be tested. Traps using insect mating pairs as lures also will be tested in the field this spring.

Aggregation behavior is being monitored in the field this spring to relate dispersal to temperature. Information on temperature might be used in a model to predict when leaffooted bugs in aggregations would disperse and arrive in adjoining fields. This research will contribute to an early-detection system such as a trap for *Leptoglossus zonatus,* which is needed to develop an IPM program for this species.