Understanding Aggregation Behavior of the Leaffooted plant bug *Leptoglossus zonatus*

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OBJECTIVES

- Determine which factors result in aggregation and/or attraction of *L. zonatus* under lab conditions
- Determine which factors result in aggregation and/or attraction of *L. zonatus* under field conditions
- Determine which factors result in dispersal from aggregations of *L. zonatus* under lab and field conditions

INTRODUCTION

Background:

Leaffooted bugs (*Leptoglossus* species) are large, seed-feeding insects that move from native host plants to crops such as almonds, pistachios, and pomegranates. Their long mouthparts pierce through almonds, feeding on developing kernels (Fig. 1a). In the early stages of almond development this feeding can cause gummosis or sap on developing almonds (Fig. 1b), indicating the bugs have been feeding; early season feeding also causes the almonds to discontinue growth and drop from the tree. Bug feeding later in the season can impact the kernel, with strikes or lines on the almond kernel.

Fig. 1a. *L. zonatus* feeding

Fig. 1b. Gummosis

When substantial gummosis or almond drop is observed, insecticide applications are often applied for insect control. However, by the time the damage is observed, the insects may have already dispersed. Management of these insects may be more effective through monitoring with traps to detect insects as they move into an orchard before damage is significant, or perhaps to trap insects late in the season.

Fig. 2 a) *L. clypealis*

2b) *L. zonatus*

During the last few years, we worked with collaborators to collect leaffooted bugs throughout the central valley of California. We determined there are two species of leaffooted bugs infesting almond orchards (Figs. 2a,b)(Joyce et al. 2017). In addition, there are two genetic types of *L. zonatus,* one more related to a type found in south america. *L. zonatus* (above right) is about twice as large as *L. clypealis.* We also conducted a field-cage study for 2 years (Fig. 3), and found that *L. zonatus* caused significantly more damage than *L. clypealis* on almonds.

Fig. 3. Field-cage study of feeding damage by leaffooted bugs.

Aggregation Behavior

The leaffooted bug, *L. zonatus,* has an aggregation behavior which might be exploited for insect management. As eggs hatch, nymphs cluster together and tend to remain in groups. Adult *L. zonatus* are observed in aggregations as well.

Fig. 4a. *L. zonatus* nymphs cluster together; Fig. 4b. *L. zonatus* adults on pomegranate Fig. 4c. *L. zonatus* adult winter aggregation

Previous studies describe *Leptoglossus* species including *Leptoglossus zonatus* aggregating in winter, under tree bark, in buildings and in the interior of trees (McPherson et al. 1990). As temperatures warm up in the spring, *Leptoglossus* spp. have been observed dispersing from aggregations (Blatt and Borden 1996, Daane et al. 2008). Understanding the factors or cues which contribute to the ***formation*** of aggregations, and also the factors or cues which contribute to ***dispersal*** from aggregations, will help us understand the timing of when we may see *L. zonatus* arrive into fields, which can help us anticipate the need for management.

Objective 1

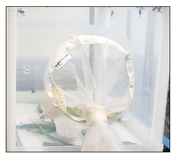
Objective 1: Determine which factors result in aggregation and/or attraction of *L. zonatus* under lab conditions.

Part 1a: Determine the Age of Adult Sexually Maturity

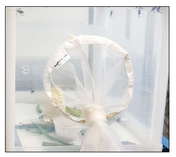
-Observed mating in groups of 2week, 4wk and 6 wk-old unmated adults to determine age of sexually maturity.

-*L. zonatus* adults at least 4 weeks of age mated.

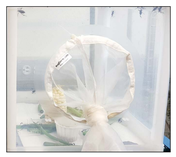
-Then, 4 wk. males & females used in wind tunnel trials.



2 week old adults



4 week old adults



6 week old adults

Part 1b. Attraction of Adult *L. zonatus* bioassays in Wind Tunnel

Lab colonies of *L. zonatus* are maintained year round, with additional insects collected from the field when they are abundant.

Objective 1

Part 1b. Attraction of Adult *L. zonatus* bioassays in Wind Tunnel

Cages of fifth instar nymphs of *L. zonatus* are set up weekly (Fig. 5a,b). New adults are identified and separated into male only or female only cages. Male only cages are maintained in a separate room from female only cages. Sexually mature virgin male and female adult *L. zonatus* were used for dual choice attraction bioassay trials in a wind tunnel. Lab trials were run using standard lab conditions, 25ºC and 14:10 L:D.

Preparing insects for bioassays

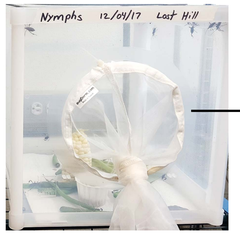


Fig. 5a. 5th instar *L. zonatus*. Any newly emerged, virgin male or female adults removed.

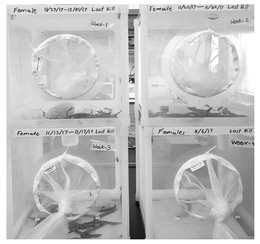
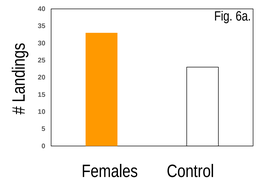


Fig. 5b. Cages such as this are used to isolate newly emerged virgin females in a separate room from male cages until trials.

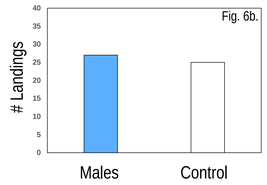
Comparisons run in the wind tunnel include the following:

1. Males attraction to Females vs. Control
2. Female attraction to Males vs. Control
3. Male attraction to Females vs. Males
4. Female attraction to Females vs. Males
5. Male attraction to Mating Pairs vs. Females
6. Male attraction to Mating Pairs vs. Controls

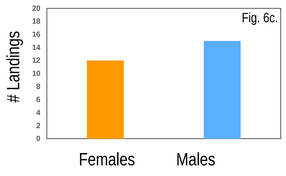
Objective 1-continued



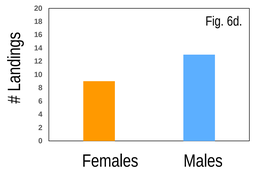
Male Attraction to Odors



Female Attraction to Odors



Male Attraction to Odors



Female Attraction to Odors

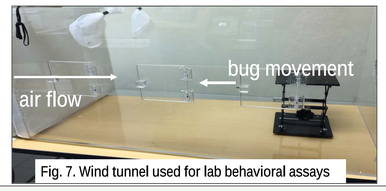
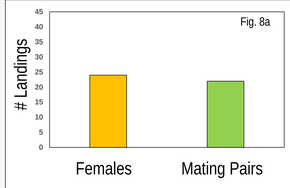
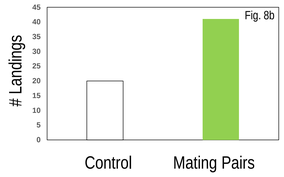


Fig. 7. Wind tunnel used for lab behavioral assays

Objective 1-Results



Male Attraction to Odors



Male Attraction to Odors

Trials first examined male attraction to female vs. a control, and female attraction to males vs. a control. Males were marginally more attracted to females than the control (Fig. 6a), while females were equally attracted to males and the control (Fig. 6b). In trials where males or females were given a choice of male or female odors (Fig. 6c,6d), neither was significantly more attractive. Another set of experiments involved giving males a choice of females vs. mating pairs (Fig. 8a) and mating pairs vs. a control (Fig. 8b). For males, mating pairs were more attractive than controls (Fig. 8b).

Objective 2

•Determine which factors result in aggregation and/or attraction of *L. zonatus* under field conditions

Factors which were found to influence attraction and aggregation in the lab will be tested for attraction in the field in spring.

Objective 3

Objective 3:

Determine which factors result in dispersal from aggregations of *L. zonatus* under lab and field conditions

In the spring with warming temperatures, aggregations of *L. zonatus* begin to disperse. We will observe aggregations in the field in field cages to determine the conditions which promote their dispersal.

Aggregation of *L. zonatus*

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