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

Project No.: **17-ENTO8-Joyce**

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Objectives:

1. Determine which factors/cues result in formation of aggregations or attraction of *L. zonatus* under lab conditions
2. Determine which factors/cues result in formation of aggregations or attraction of *L. zonatus* in the field
3. Determine which factors/cues result in dispersal of *L. zonatus* from aggregations under lab and field conditions

Interpretive Summary:

Leptoglossus zonatus are leaffooted plant bugs which feed on seeds and occasionally cause damage to almonds. There is an interest to develop a trap or monitoring device for these insects. Although they are large insects, they are elusive and difficult to detect in orchards until damage is detected. The objectives of this project include investigating attractants for *L. zonatus* under laboratory and field conditions, and to determine the factors which are associated with the formation of and dispersal from aggregations. Attraction of *L. zonatus* to odors was investigated in the lab. Previously in this project, males were tested for their attraction to females, and females were tested for their attraction to males. Females appeared equally attracted to males and controls, while males showed a preference for attraction to females. Males responded more rapidly to female odors than to controls, while females responded at similar rates to odor sources from males or controls. This year, we focused on the attraction of adult male and female *L. zonatus* to odors associated with mating pairs of adult *L. zonatus*. This year, we also varied the number of mating pairs in the dual choice assays. We found that to date, odors associated with mating pairs are most attractive to adult male *L. zonatus*. Adult female *L. zonatus* were equally attracted to controls and odors associated with mating pairs. These odors are now being further tested with traps, and volatile collection is underway. Further results will be presented at the Almond Board Conference in December 2018.

Materials and Methods:

Objective 1: Determine which factors/cues result in formation of aggregations or attraction of *L. zonatus* under lab conditions.

There is a need to develop an attractant or trap for *L. zonatus*, to monitor the abundance of this insect in the field. Understanding when the insect is abundant, or being able to trap and kill the insect, or to disrupt its mating, would help to prevent these insects from causing significant crop damage.

To investigate what attracts male or female *L. zonatus*, we have been conducting dual choice odor tests in a low velocity wind tunnel (2m x .5m x .5m) in the lab using virgin adult males and females from a lab colony. As previously described, large colonies of *L. zonatus* were maintained year-round in the lab and fed a diet of corn, green beans, and raw seeds, so that large numbers of insects were available for experiments.

Trials to test attraction to different odor combinations were run in the lab (25ºC, summer light conditions, 15 hr. light: 9 hr. dark) in the wind tunnel. Insects were standardized in age to compare the response to different odors. Every week, new cages of newly emerged virgin males and females were isolated to produce a constant supply of 1-week old cohorts of virgin adults for behavior experiments.

Preliminary trials were run using virgin adult females and males of different ages to investigate the age at which adults were sexually mature and attracted to male or female odors. This was previously described, and we found that using four-week-old virgin adults provided experiments with sexually mature insects, so the response to pheromones could be tested.

Previously, we examined whether adult female *L. zonatus* are more attracted to either the odor of males or to a control odor. These dual choice odor tests were run in the wind tunnel throughout the day under summer conditions. The test odors consisted of an almond branch with almonds and 10 adult males while the control odor was an almond branch with almonds. For each of these tests, five adult females were released from a platform downwind of the odor sources. Females were observed for 30 minutes as they responded and moved toward the two odor sources. After every two replicates, the wind tunnel was cleaned. The odor sources were replaced with new material (new branches and insects). In addition, the positions of the odor sources in the wind tunnel were alternated between every two trials.

The second question (previously examined) was to address whether adult male *L. zonatus* are more attracted to the odor of sexually mature adult females or to a control odor. Dual choice odor tests were run in the wind tunnel throughout the day under summer conditions. The test odors similarly consisted of an almond branch with almonds and 10 adult females and the control odor was an almond branch with almonds. For each of these tests, five adult males were released from a platform downwind of the odor sources. Adult males were observed for 30 minutes as they responded to the two odors. After every two replicates, the wind tunnel was cleaned. The odor sources were replaced with new material (new branches and insects). In addition, the positions of the odor sources in the wind tunnel were alternated between every two trials.

For both above experiments, a stopwatch was used to record the time elapsed until each insect left the platform, the time elapsed until an insect landed on an odor source, and the time spent on each odor source, and the number of landings which occurred on the test odor and control odor.

Thirty replicates were performed for each of these experiments. Previously results were presented for these experiments, but they are included in this report as well (**Figure 1-6**).

This year (2017-2018), we refined these experiments to ask the questions "Are males attracted to mating pairs more than a control?" and to ask "Are females attracted to mating pairs more than a control? The goal was to find which odor source produced the strongest response in male and female adult *L. zonatus.* The odor with the strongest response is a good candidate to pursue for volatile collection. We also wanted a strong bioassay, so we will know how to test response of adults if a synthetic lure (odor) becomes available. We were also interested in whether using more mating pairs in the experiment would produce a stronger response than we observed previously with only male or female odors. A similar experimental setup was used in 2017-2018 as described previously for 2016-2017. Dual choice odor tests were conducted in a wind tunnel in the lab. Insects were raised in the lab year-round with the diet and conditions as described above. Each week, newly emerged adults were setup into one-week old cohorts, so that we could standardize the insect age for testing in odor choice experiments. Again, we used two odor sources in the wind tunnel, and then placed test insects on a platform and observed their flight response. The test insects released on the platform, and observed as they flew to an odor source, were either four-week-old, unmated virgin adult male *L. zonatus*, or four-week-old, unmated virgin adult female *L. zonatus*. As previously described, we recorded the time elapsed from the start of the experiment until an insect flew toward the odors, the number of first landings on each odor (control or experimental odor), the time spent on the odor sources, and we examined the percent of insects responding in the experiment.

First, we tested whether males were more responsive to females or 5 mating pairs, and subsequently we tested whether males responded more to a control odor vs. odors associated with five mating pairs. Approximately 30 trials of these experiments were run.

Next, we tested whether an even larger number of mating pairs might produce a higher landing rate. We increased the number of mating pairs from 5 to 15 mating pairs (vs. a control odor), and there was a trend of increased numbers of males landing on the experimental odor source (odor of mating pairs). The number of mating pairs in the test odor was then increased to 20 mating pairs for the next experiment.

We tested whether odors associated with 20 mating pairs of adult *L. zonatus* were more attractive compared to a control odor. This experiment was tested separately for male adult virgin *L. zonatus*, and for adult virgin female *L. zonatus.* Experimental conditions were similar to those described above, except the two odor sources varied from those previously tested.

Objective 2: Determine which factors/cues result in formation of aggregations or attraction of *L. zonatus* in the field

Formation of *L. zonatus* aggregations/ attraction in the field: The most attractive combinations of odors found in the wind tunnel tests above will be tested in the field to determine if these odors can be used for attracting and trapping *L. zonatus*. We hoped to test these in Fall 2017, but instead we decided to further pinpoint the odor source most attractive to the bugs. Odor are now being tested in the wind tunnel (just bugs and traps), and in the field. Many *L. zonatus* are often seen at harvest time. These insects may be disturbed from trees at harvest during tree shaking, as they are often observed on the ground with almonds at harvest. To test attraction of *L. zonatus* in the field, we will hang 3-4 traps with mating pairs, and compare the landing rate on these traps vs. control traps with no odor. Previously trap materials and several candidate sticky materials were identified. This is important because adult leaffooted bugs are large and difficult to stick in traps. Data from insects and traps will be analyzed using a Chi-square test to determine if equal number of insects are attracted to each odor source or not.

Objective 3: Determine which environmental factors/cues result in dispersal from aggregations of *L. zonatus* in the lab and field.

Lab Observations of Dispersal from Aggregations: Preliminary studies were conducted to determine the importance of light and temperature in the formation and dispersal from aggregations of *L. zonatus*. Previously, aggregations of *L. zonatus* were field collected. The aggregations consisted of insects which were observed in a tight cluster, aggregated tightly in layers. The aggregations were collected and transported to the lab in large cages, which disturbed the aggregations. The non-aggregated insects moved freely around the cages for several days, but then the aggregations reformed in the cages. The aggregations remained together for approximately two weeks at lab temperature (25ºC) and summer light conditions. We suspect that once the insects form aggregations under winter conditions, they then need to be exposed to a certain number of hours at a warm temperature to begin leaving their aggregations. It is also possible that day length plays an important role in inducing the diapause-like aggregation behavior that is observed in winter. In preliminary trials, cages with *L. zonatus* were placed in a room maintained at 25ºC with short days (9 hr. light: 15 hr. dark), to explore whether several weeks of exposure to short days and long nights might contribute to the formation of aggregations. After several weeks under these conditions, none of the insects formed aggregations. These preliminary experiments suggest that temperature may be the critical factor in the dispersal from overwintering aggregations.

For this objective, we had hoped to have field cages of aggregations, and watch them disperse in spring of 2018, to relate dispersal to temperature changes. However, an extremely long winter of cold and rain prevented us from conducting this experiment. We felt that conducting the experiment would kill many of the insect colonies that we had, and insect would be better used to finish the wind tunnel work.

Results and Discussion:

<u>Objective 1: Attraction of *L. zonatus* adults to odors in the laboratory.</u>
Females were tested in the wind tunnel for attraction to adult male *L. zonatus*. There were 51 females that landed on an odor source. Of these females, 27/51 (53%) landed on the branch with males, while 24/51 (47%) landed on the control branch (**Figure 1**). Males were also tested for their response to female odors or to a control. There was a total of 47 males that landed on the treatment or the control. Of these males, 27/47 (57%) landed on the branch with females, and 18/47 (38%) landed on the control (**Figure 2**).

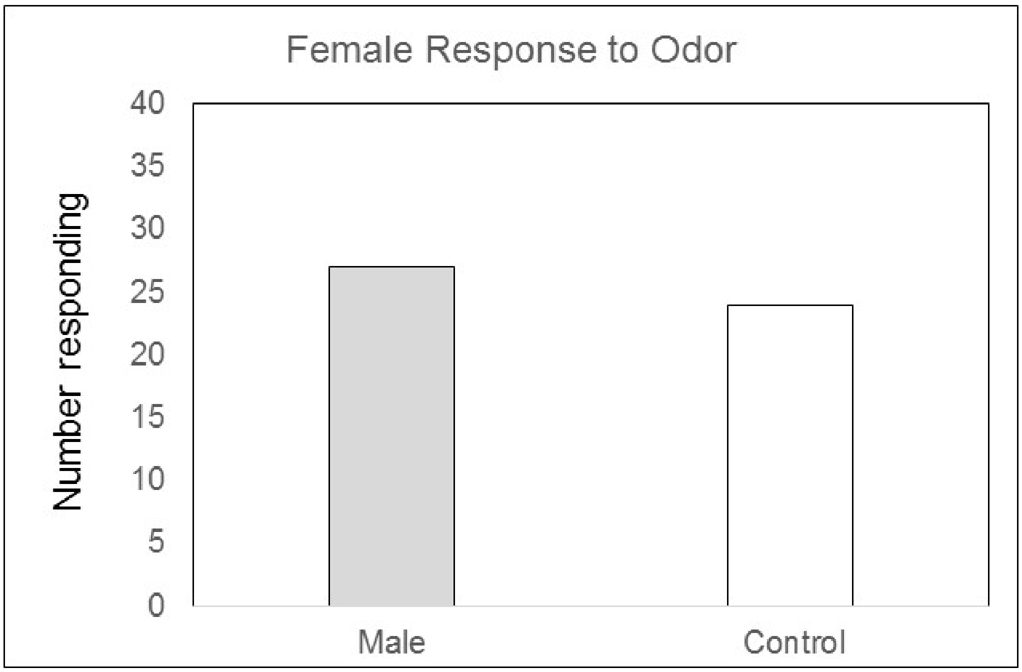

Figure 1. The number of females landing on males or a control.

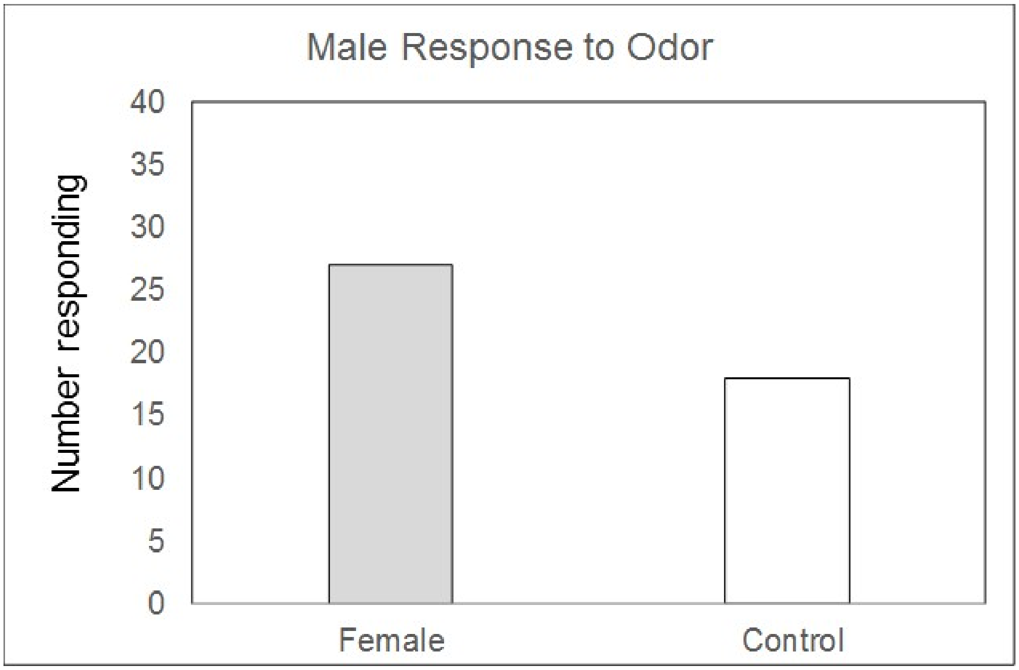

Figure 2. The number of males landing on females or a control.

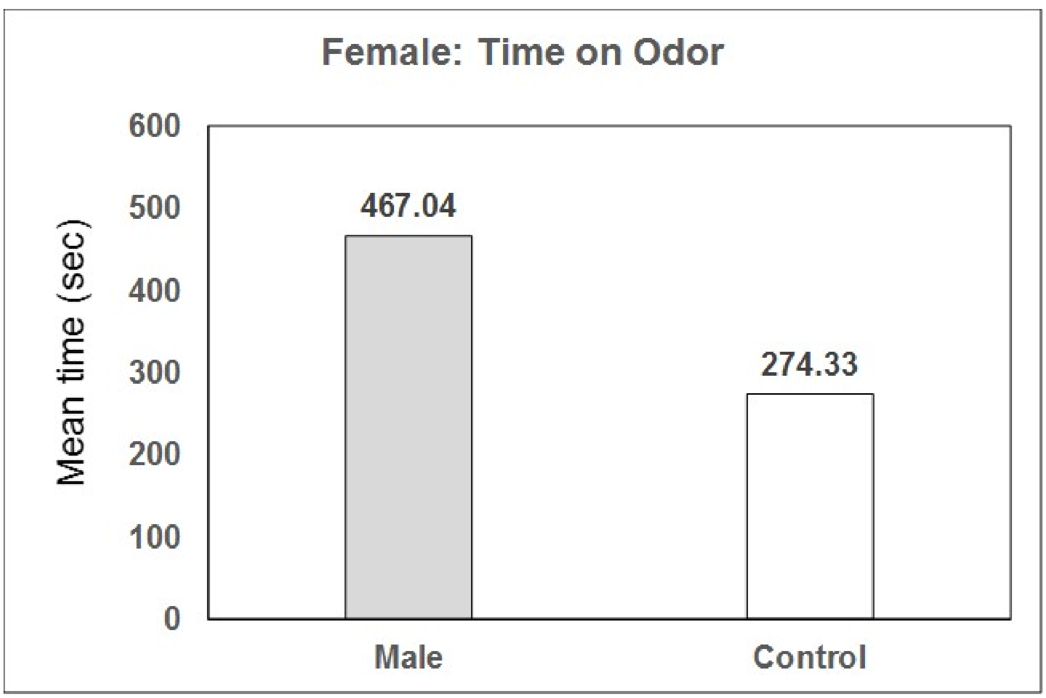

Figure 3. Mean time a female spent on odor source (males vs. control)

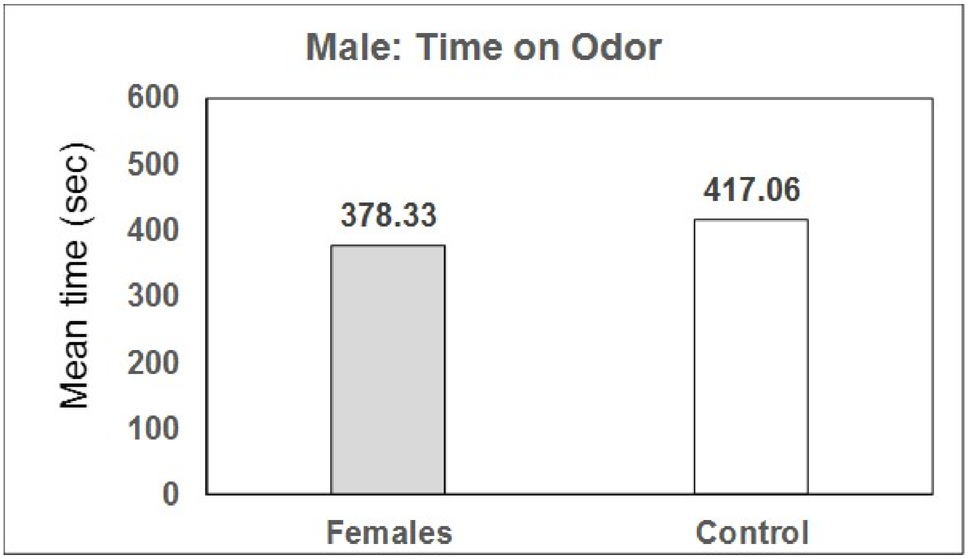

Figure 4. Mean time a male spent on odor source (females vs. control)

Each female spent an average of 467.04 sec on a cage with males, and 274.33 sec on cages with control branches (**Figure 3**). Trials testing response of males found they spent an average time of 378.33 sec on cages with females, and 417.06 sec on control cages (**Figure 4**).

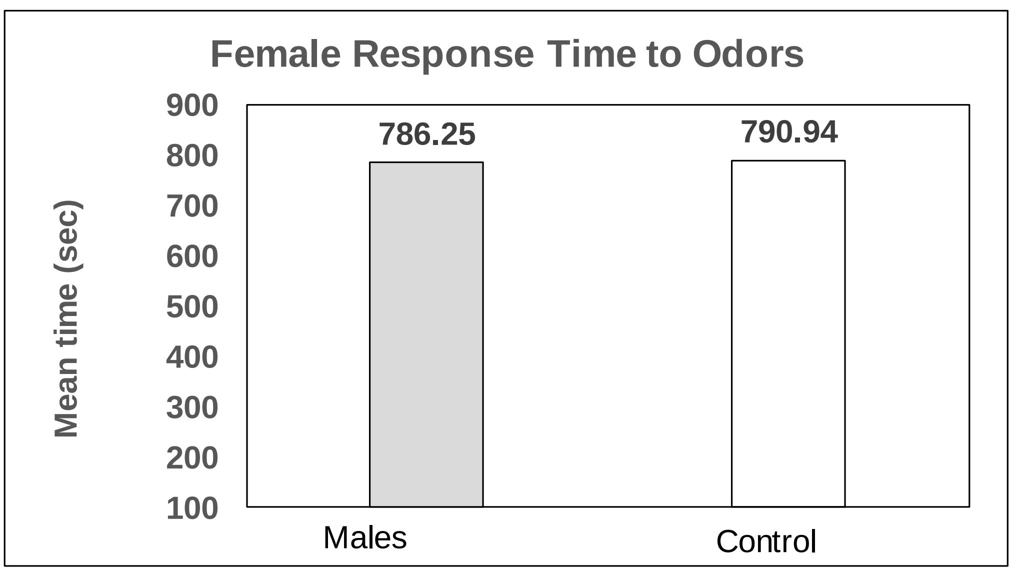

Figure 5. Mean female response time to Male or Control odors.

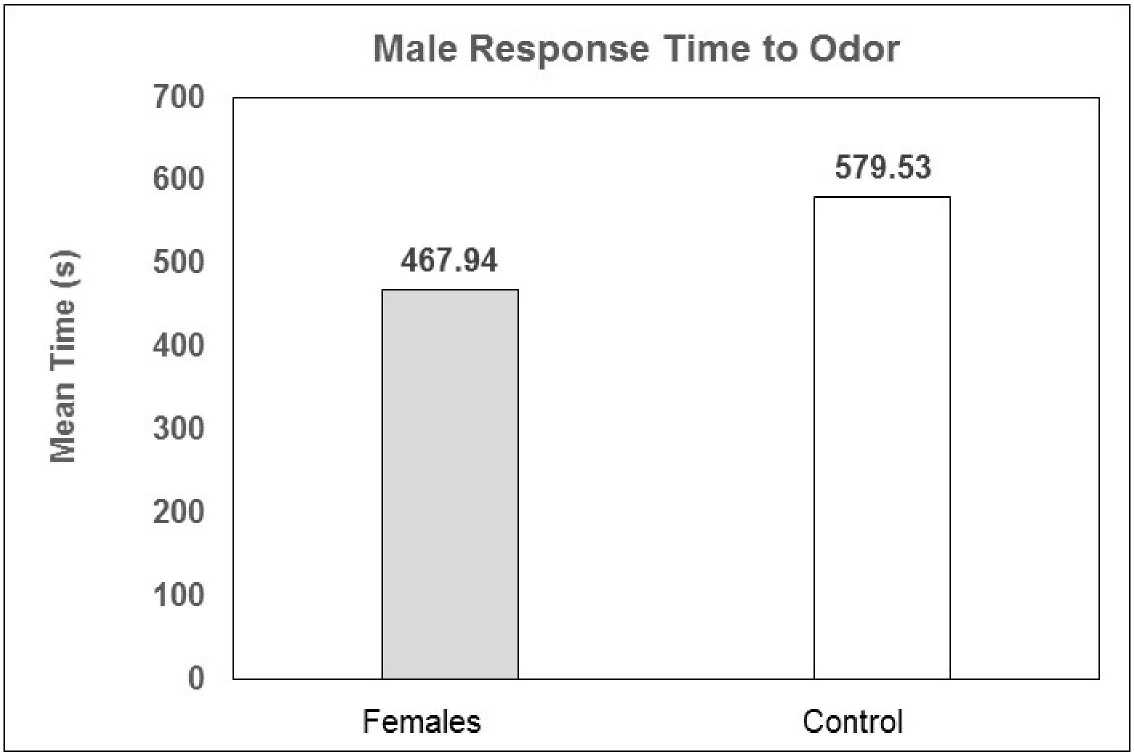

Figure 6. Mean male response time to Female or Control odors.

Females had an average response time of 786.85 sec to move from the starting platform onto the male odor source, and 790.94 sec to move on to the control odor source (**Figure 5**). Males responded more quickly to the female odor source (mean 467.94 sec), while taking an average of 579.53 sec to respond to the control odor (**Figure 6**).

Objective 1 (attraction in lab wind tunnel), results from 2017-2018: In the wind tunnel, the dual choice test which examined whether males were more attracted to the odor of 5 mating pairs vs. control, found a significantly higher number of males landed on the mating pairs than on the control (20 landings vs. 7) (**Figure 7**). The time each male stayed on the odor source was compared as well. Males spent an average of 337.2 sec on the mating pairs, and only 221.8 sec on the controls (**Figure 8**).

Additional tests in the wind tunnel were run to examine the attraction to males to 15 mating pairs vs. controls. A similar pattern was seen, where males landed more frequently on the mating pairs than on the control (data not shown), so the next experiment increased the number of mating pairs to 20.

Finally, dual choice trials were run in the wind tunnel, comparing the responses of adult, unmated, four-week old virgin males to two different odor sources, which were either 20 mating pairs on an almond branch, or a control (almond branch alone). Significantly more males (37) landed on the branch with the 20 mating pairs than the number of males that landed on the control branch (20 landed on control) (**Figure 9**). In addition, a similar experiment was run to test the response of female unmated adult *L. zonatus*, and their attraction to the same two odor sources. In the case of the adult females, there was a nearly equal response of females landing on both the control (17 landings) and the branch with 20 mating pairs (25 landings) (**Figure 10**).

In summary, all the trials which tested mating pairs vs. a control odor had quicker and higher response rates of insects flying toward the odors than in the previous trials conducted the previous year (or just male or just female odors).

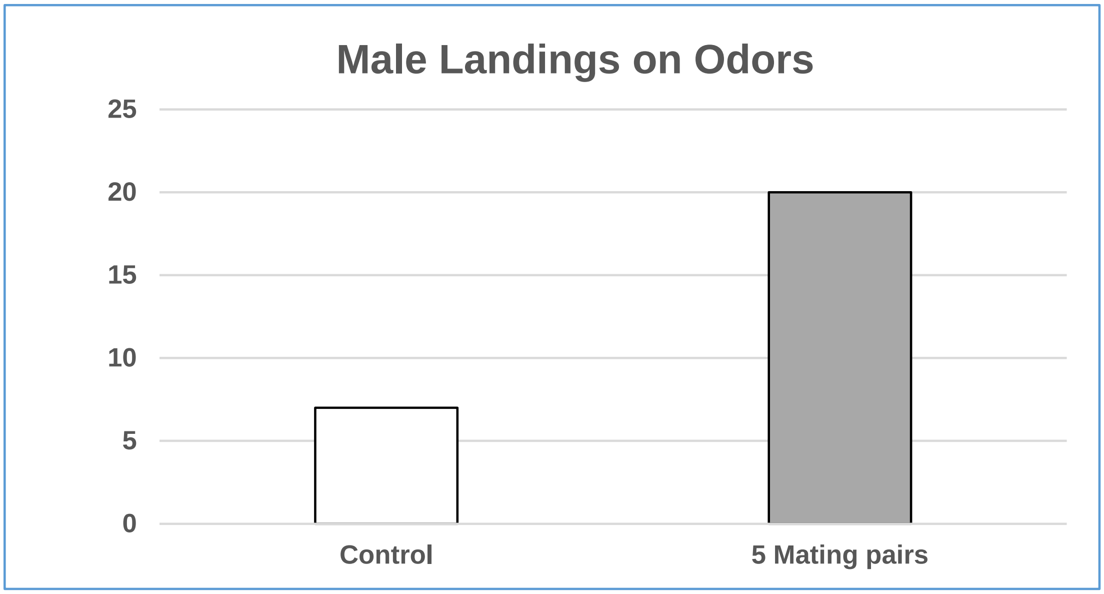

Figure 7 Male landings in choice trails of 5 mating pairs or control

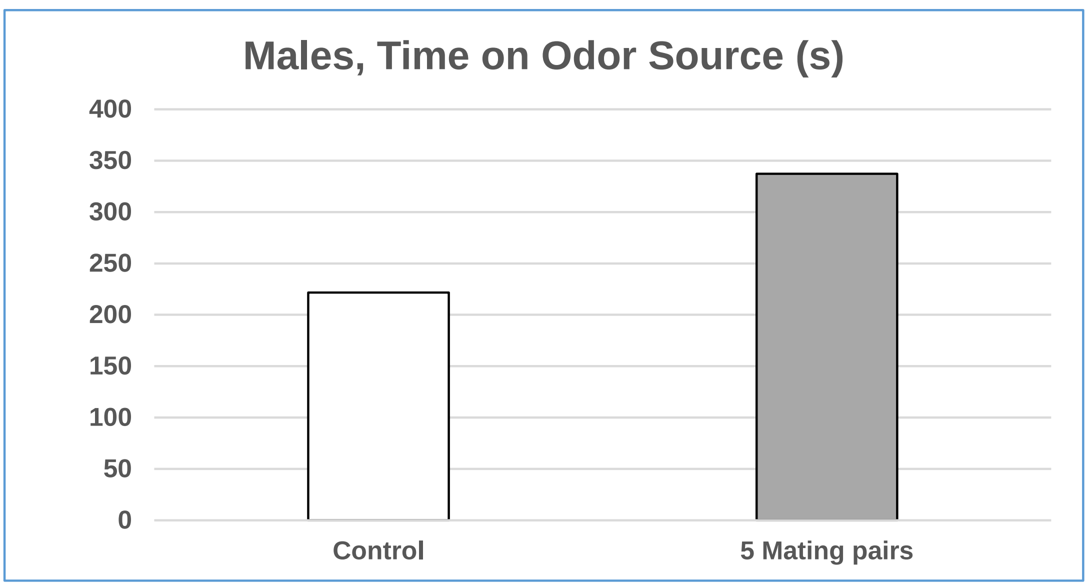

Figure 8. Male time spent (sec) on odor sources

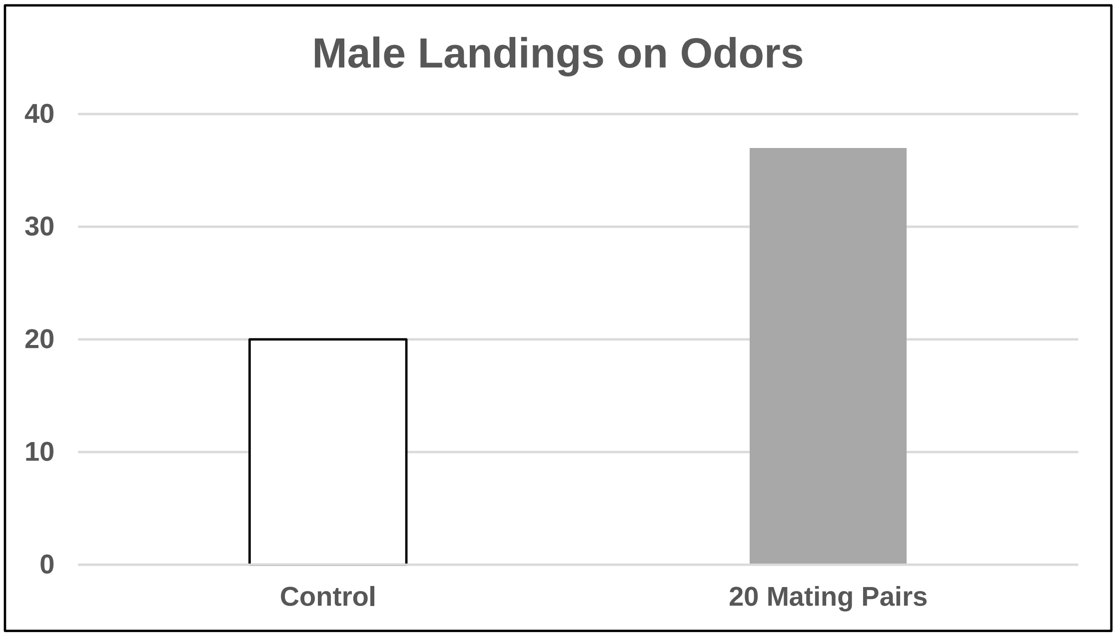

Figure 9. Male landings in choice trial of 20 mating pairs vs. control

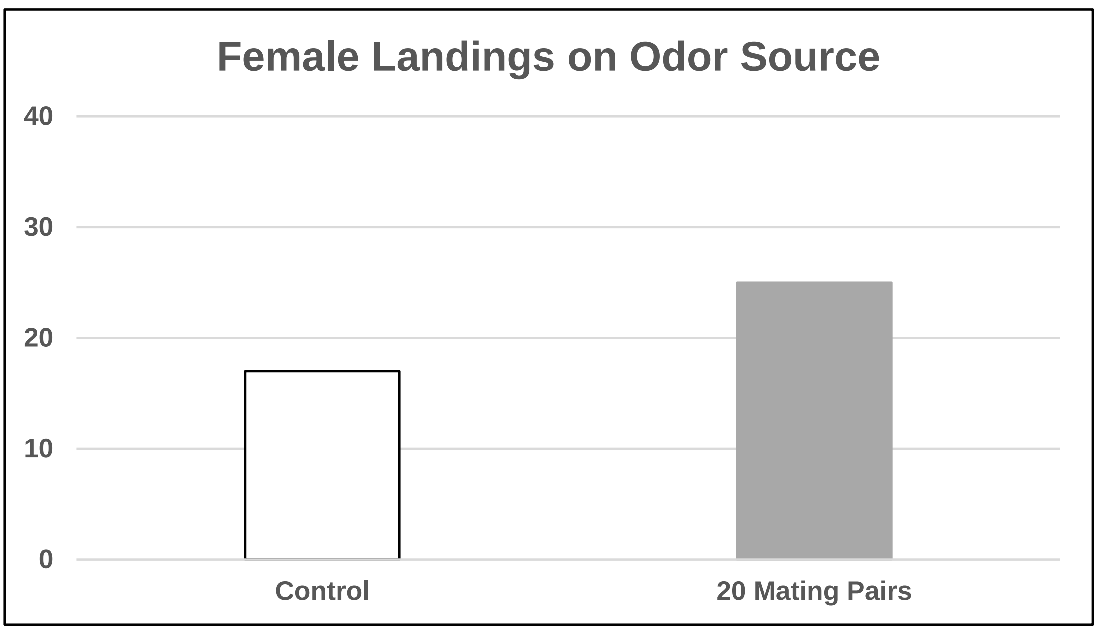

Figure 10. Female landings in choice trial of 20 mating pairs. vs. control

Discussion:

Objective 1: Previously we found that males landed more frequently on odors associated with females than on the control, while females were equally attracted to males or a control. Interestingly, males spent a similar amount of time on the female and control branches. Overall, males responded more quickly to the female odor than the control odor; females responded at similar rates to the male odor as they did to the control odor. In year two (2017-2018), we focused on the attraction of adult *L. zonatus* to mating pairs. We tested adult *L.*

zonatus with a shorter experimental test response time of 15 minutes. Odors associated with mating pairs were a more attractive odor source than controls to adult male *L. zonatus*. This odor source will be further pursued now as a potential attractant for trapping. Odors will be further tested using traps in the wind tunnel and field, and volatile collections will be made with the collaboration of a chemist/ chemical ecologist.

Objective 2: Attraction of *L. zonatus* to odors in the field will be now tested, as described at the end of Objective 1. Previously, effort focused on pinpointing the most attractive odor source. The insect colonies are prepared and ready for running these experiments. Results will be presented at the Almond Board Conference in December 2018.

Objective 3: Influence of temperature on aggregation behavior. Only preliminary data has been collected, which found that temperature was more important than light, in impacting dispersal and aggregation. This study would benefit from further field work in the spring.

Research Effort Recent Publications:

Joyce AL, Higbee BS, Haviland DR, Brailovsky H. 2017. Genetic variability of two leaffooted bugs, *Leptoglossus clypealis* and *L. zonatus* (Hemiptera: Coreidae) in the Central Valley of California. *Journal of Economic Entomology* 110: 2576-2589. https://doi.org/10.1093/jee/tox222

In preparation, Joyce AL, Robson L, Higbee B, Doll D, 2018. Field-cage studies to assess feeding damage from leaffooted bugs *Leptoglossus zonatus* and *L. clypealis*. *Journal of Economic Entomology*

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