Pheromones as an alternative to insecticide control for an agricultural insect pest, *Leptoglossus zonatus* (Family: Coreidae)

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Introduction

The insect pest, *Leptoglossus zonatus* and Leaffooted Bugs

Leptoglossus zonatus is an insect in the Family Coreidae of the order Hemiptera. This bug is polyphagous and will eat a variety of plants and plant parts. It is known to feed on many plants including oranges, avocados, cotton, corn, and tomatoes, among a wide variety of other crops (Grimm 2010). Insects in this genus feed by piercing fruits or seeds with their rostrum and sucking the fluids within. The immature stages of the insect, called nymphs, develop through five stages (instars). These nymphs have leaf-like extensions on the tibia, and hence the source of the common name of the genus 'Leaffooted bugs' (Fig. 1). The adults overwinter in areas such as inside fruit of abandoned fruit crops like oranges, and in buildings such as barns (Fig. 2). In warm locations such as Florida, California, or in the tropics, they are found year-round (Fadamiro 2011).

L. zonatus is found in the southern United States as far east as Florida. It has been observed in the southwest of the United States, including California and in the central valley where agriculture is most prevalent. It occurs in Central America and the northern portions of South America. This insect is considered an agricultural pest. When it feeds on commercial fruits, they leave the fruits discolored and malformed or even cause the fruit to abort entirely (Fadarimo 2009). They are also capable of infecting fruits such as oranges with the yeast

Nematospora coryli which causes the internal fruit to suffer from dry rot. The damage from these bugs causes fruits to taste poor if they are even edible and leaves spots permanently on the rinds and skins of fruits which makes them much harder to sell if they even make it to market (Buss 2005). Another reason these insects are a pest is that Leaffooted bugs are generally long lived compared to many other insects. Adults live for an average of four weeks in summer and can overwinter in aggregations and survive through winter (Millar 2000). Maturation from the egg to the adult phase usually spans around 30 to 40 days (Millar 2000). One study found this insect had a 98% hatch rate, and a 75.6% survival rate of nymphs which became adults, thus high survival rate could contribute to this insect becoming a highly abundant and a pest (Fadamiro 2009). In addition to the above, leaffooted bugs are generally large insects, and they are difficult to kill with many common insecticides that work to control other insects.

Insect Control with Organophosphates: Appeal and Drawbacks

One group of pesticides used to control *Leptoglossus* are organophosphates. Organophosphates target acetylcholinesterase, which is an enzyme that inhibits the breaking down of acetylcholine, a neurotransmitter involved in nerve signal transmission and muscle movement. This results in neurons being continually stimulated, and leads to muscle twitching and seizures; finally, it can cause the organism to slowly become paralyzed or result in death. One organophosphate insecticide called Chlorpyrifos has been used for years for insect control, and it is currently being reviewed by the EPA for continued agricultural use (Reuters 2017). Organophosphates not only repel targeted pests but also beneficial insects at times. For example, *Cycloneda sanguinea*, *Orius insidiosus*, and *Chauliognathus flavipes*, important biological control insects used to reduce populations of aphids, have been shown to be repelled by the

organophosphates (Alves 2016). Organophosphates are not species specific, so they have the same neurological effects on beneficial insects as they do on pest insects and on humans.

Organophosphates and Child Health

One concern of organophosphate insecticides is the impact on children's health in agricultural locations. Chlorpyrifos was banned for domestic use in 2001 after several studies revealed that residues remain for a long time, and the insecticide has an impact on the health of children. Gurunathan (1998) published a study regarding how long the residue remains on toys. The study found that when a room was treated with chlorpyrifos and plastic or cloth toys were indirectly exposed, the insecticide residue could take as long as two weeks to fully degrade into a more harmless state (Ahmed et. al., 1998).

Researchers have further investigated the relationship between chlorpyrifos and childrens' health, especially in agricultural areas, among impoverished neighborhoods, and within minority groups (Fenske 1995; Berkowitz 1999; Barr 2007). As of 1999, about 5 million people in the United States worked in agriculture (Bradman 1995). Farmworkers, families that live directly on farms, and children within agricultural areas are the populations most exposed to chlorpyrifos. Potential exposures for these children include consuming contaminated produce, drift from nearby farms, ingesting contaminated breast milk from a mother exposed to chlorpyrifos, playing in fields treated with chlorpyrifos, and through a parent working in agriculture can accidentally transport residue home (Bradman et. al., 1999). In addition, there is also concern of the impact of chlorpyrifos exposure on neonates. With the growing usage of these pesticides, the health effects have become more apparent.

A Potential Alternative to Insecticides, Insect-Produced Pheromones

Pheromones are chemical substances excreted from insects and used for communication among insects (Karlson 1959). When a pheromone is excreted, another insect will sense it, often with their antennae. One type of pheromones are insect sex pheromones, which are well studied in moths and also known from insects called Hemiptera (true bugs), the order which leaffooted bugs belong to. Depending on the species, either male or female adults will excrete a pheromone to attract the opposite sex. As of 1975, 60 species of moths had been discovered to use sex pheromones including the females of genus *Ostrinia* (moths) (Barrion 1980). By 2005, over 200 nonsocial arthropod species in over 51 families belonging to 12 different orders have been recorded as producing aggregation pheromones (Baalen et.al., 2005). Pheromones are used in agriculture to attract, trap, and kill insects, as an environmentally and healthy alternative to insecticides. They are currently primarily used for moths (Barrion 1998) but have been used for stink bugs (classified in the same order as leaffooted bugs) on a small personal garden level successfully (Hogmire 2006). They are natural products and can be formulated to be used used by organic farmers. Pheromones can be used to attract insects into a trap and kill them, and they can also be used for mating disruption in the field.

How Pheromone Traps Work

Pheromones, if found and characterized for *L. zonatus*, would be used to trap these insects in lieu of insecticide applications. There is substantial evidence of using pheromones to control other Hemiptera (true bug) insect pests (Dimeglio 2015). Pheromone traps come in four main varieties. Delta traps are triangular tents with an adhesive paper within it. Delta traps are primarily used with moths, as are wing traps. The third trap type are called mass capture traps,

which are designed to catch as many varieties of insects as possible. The final kind of trap, the stink bug trap, is the focus of work with Hemiptera (true bugs). This trap is built with a cone or fins leading up to a tube (Fig. 3). This tube releases pheromones to draw in bugs which crawl up the fin or cone to reach the tube. When they enter the tube, they cannot exit and die of dehydration. These traps are environmentally friendly and can attract both sexes of a species depending on the pheromone used. One aspect studied for these traps is the effect of coloration regarding attracting the bugs (Khrimian 2012).

Are there pheromones in Leaffooted bugs?

Insects in the genus *Leptoglossus*, the leaffooted bugs, use chemical signals to communicate (Blatt 1998). These pheromones are not specific to a life stage and are found in both nymphs and adults (Blatt 1998). *Leptoglossus* has been shown to use three main types of pheromones. Alarm pheromones are released when an insect feels threatened (Blatt 1998, Panizzi 2004). For the species *Leptoglossus zonatus*, a defensive chemical is released that contains a strong scent somewhat similar to a citrus odor to deter predators while sending a warning signal to nearby bugs. This pheromone is most often found in juvenile stages (Aldrich 1988). Another pheromone type is the aggregation pheromone, which can also be released while feeding. It causes more insects to gather, leaving the group less susceptible to predators and able to find more food (Gries 1998). The aggregation pheromones for *L. zonatus* are not yet known. Finally, there are sex pheromones; one sex releases sexual pheromones to attract another. These emissions may also attract competition for mates. Tachinid flies, a type of parasitic insect, have

learned to identify and follow these scents produced by bugs to use them as egg-laying substrates (Aldrich 1988).

In *Leptoglossus* as a whole, the literature suggests that females seem to be attracted by the pheromones of the males, rather than males being attracted to the odors of females. Studies both in the lab and out in the field have determined this. The presence of host plants could have influenced field experiment results, however (Yasuda 1998). Most mating pairs will copulate multiple times at close intervals if not interrupted. Male *L. clypealis* can display courtship behaviors as early as eight days after their final molt into adulthood (Millar 2000). Studies have also shown that mating pairs in general are sexually attractive to male *Leptoglossus* to the point that they will try to copulate with a female while she is copulating with another male (Aldrich 1976). The presence of food especially seems to impact pheromone production (Landolt et al. 1997). As *L. australis* eat, they release more pheromones that lead to aggregation. Males also seem to release more sex pheromones when they are eating, attracting more females (Yasuda 1998).

Investigating Pheromones

The behaviors induced by the pheromones can be measured using many methods, including using live insects for experiments or using synthetic pheromone lures. For example bioassay tests in one study examined the odors produced in isolated pairs and found there was a significant attraction from the females to the males (Wang and Millar 2000). While Millar's group used live insects to produce the pheromones, Dimeglio's team (2015) used synthetic male pheromone to lure in live *Murgantia histrionica*. This team caught their bugs in a research field of brassicaceous crops (Dimeglio 2015). Another study by Hogmire and Leskey (2006) tested

pheromone traps rather than using live bugs to produce pheromones. Pyramid traps were constructed similar to those available for commercial use and compared to controls without the pheromones. These traps were placed in an orchard of “Rome” apple trees and measured for fourteen weeks for the presence of both living and dead Pentatomidae (Hogmire 2006). Currently, there are no known effective leaffooted bug traps. Since this insect is a pest and organophosphates are one of the primary controls which are effective, this study seeks to investigate whether *L. zonatus* pheromones might be an alternative control strategy.

Study Objectives

The objectives of this study are to investigate whether there was evidence for male or female produced pheromones in the locally abundant agricultural pest species, *Leptoglossus zonatus*. If pheromones attract adult *L. zonatus*, the chemicals associated with attraction of adult males or females can be chemically characterized and used to produce pheromone traps for farmers. Availability of a pheromone trap would provide an alternative control for these insects, as it could replace the use of the organophosphate insecticide currently used. Finding an alternative natural control is crucial. The specific objectives were the following: 1) to determine the age of sexual maturity for adult male and female *L. zonatus*, so the correct age could be used in behavior attraction studies and 2) investigate in a wind tunnel whether adult males or females were attracted to odors produced by other adult males or females (i.e. pheromones). It is hypothesized that the females will be at least 2 weeks old before mating occurs and 2) females in the wind tunnel will be more attracted to males, than males are to females.

Materials and Methods

Raising the Colony of *Leptoglossus zonatus*

In the laboratory, there were colonies of *L. zonatus* that were previously collected from multiple locations in California and reared in the laboratory for approximately 5 generations. Occasionally new insects were captured in the field and added to the colonies to increase the genetic diversity of the lab colonies. One of the large colonies used for the studies I undertook was originally collected in Lost Hills, California, in the southern San Joaquin Valley, CA. Colonies were housed in large mesh cages (1 m x 0.5 m x 0.5 m) with a 1-gallon size *Thuja occidentalis* tree which served as habitat and a water source for the insects. Insects were fed a handful of green beans and corn, and organic seeds. Twice a week, bugs were provided with additional food.

From these colonies, I removed nymphs (young insect stages) in the fifth (last) instar stage, one instar away from adulthood, and kept them separated from the main colony. As new adults emerged, the adults were identified as male or female, and then placed into separate cages, one for males, one for females. All males who emerged within a week were housed together, as were females with each other. Each week, new cages (cohorts) of emerging males and females were set up. This provided a constant source of unmated male and female adults of known ages for experiments. In addition, males and females were kept in separate rooms to prevent them from being exposed to any odors (pheromones) from the opposite sex prior to the experiments which were run.

Determining the Age of Sexual Maturity

Prior to testing whether males or females were attracted to odors of the opposite sex (pheromones), we assessed what age the insects became sexually mature, and might be attracted to the opposite sex. This helped us standardize future experiments, using insects of a known age for all experiments.

Phase 1: Preliminary Observations. To assess the age of sexual maturation for *Leptoglossus zonatus*, we set up four plastic containers (1-pint size) with a green bean and a single pair (male and female) of unmated adult *L. zonatus*. The four containers each held an adult pair of a different age class, either one week, two weeks, three weeks, or four weeks of age, with age being determined by counting from when they emerged as adults (explained in prior section).

To begin with, the four groups were observed every hour to record whether mating was observed in any of the four insect pairs (1-4 weeks of age). Mating activity was counted only if copulation occurred where the pair stood abdomen to abdomen. Additional notes were taken based on rejections or other aggregation activity. The pairs were checked upon hourly from 10 am to 5 pm each weekday for two weeks. To determine if mating occurred at night, a time lapse GoPro video camera was set up to capture footage overnight. The time-lapse camera took a video clip every thirty seconds. The GoPro was set up to observe the four-week-old group because they showed the most activity. The next day, the footage was collected and reviewed. The room temperature was 26° C. Light was also regulated within the room with the lights timed to 14h L:10h D. Data was collected on a spreadsheet using yes or no to indicate whether mating was observed.

Dissections to Examine Egg Development. After the two weeks, females in the four-week-old cages were removed and frozen for later dissection, to determine if eggs had begun development. The dissection was done using a dissecting microscope and a petri dish filled with water. The females were submerged in water and cut open along the abdomen with scissors. Pins were used to delicately move internal organs in the abdomen and search for eggs. The number of developing and fully developed eggs were recorded on a data sheet. Developing eggs appeared greener while developed eggs were brown

Experimental Determination of the Age of Sexual Maturity of Adult *L. zonatus.*

Few matings were observed in the preliminary observations described above. We modified the setup to observe mating behavior in larger cages, and to include several pairs of adult *L. zonatus*. We considered what we had observed in nature and in agricultural fields and came to the realization that insects were typically seen *mating in aggregations* in the field. Perhaps with groups of insects (several pairs of adults), we might be more likely to observe a larger number of matings.

To assess the age of sexually mature adults, three age groups of insects were used. We compared the mating frequency in groups of unmated adults that were 2 weeks, 4 weeks, and 6 weeks of age. A plastic cage (29 x 29 x 29 cm) (Bioquip, Rancho Dominguez, CA) was used to house five virgin males and five virgin females of each age group. Unmated insects were used for the experiments, to determine whether unmated sexually mature adults use a pheromone (odor) to attract the opposite sex. From the preliminary experiments described in the previous section, we had several observations of 3-4 week old insects mating, so we wanted to compare mating frequency in this age group (4 weeks), along with mating frequency in a younger age (2 week) and an older age group (6 week). Within each bug dorm (mating cage), a paper towel was

placed on the bottom of the cage with a handful of washed and dried green beans and one half of a corn cob which served as a food and moisture source. The food was replaced on average twice a week.

There were 3 cages set up each week, one each with the pairs of 2-week, 4-week, or 6-week-old adult insect pairs. Each week, for four weeks, an additional set of 3 cages (one with 2-week, 4-week, and 6-week-old insects) was set up. By the end of setup, this led to a total of 12 cages to observe.

Data Recorded:

At least twice per week, all cages were observed. Observations were made every hour from 12-5 on each weekday. For each observation, the number of pairs mating in each cage was recorded. As mating continued and eggs were laid, the date the eggs were first observed was recorded, as well as the date the first eggs hatched. Once eggs hatched, the instars of the nymphs were recorded (first, second, third, fourth, fifth) to estimate developmental time to the adult stage. When the last developmental stage of F1s were observed, parental adults were lightly marked on their thorax using pink nail polish to distinguish them from the newly emerged adults. As parental adults were found dead, the sex and date were recorded. This allowed the tracking of the approximate time of each instar and their lifespan.

For examining the age of sexually maturity, data were recorded for the following;

1. The number of days elapsed from the start of the experiment until the first mating pair was observed, for the 2, 4, and 6-week old *L. zonatus* cages. The mean, standard deviation, and range for number of days until mating was observed for each age group.
2. Data were recorded for the number of matings observed within the first six days of observations for the 2 week, 4 week, and 6 week old cages. For each age group, the mean, standard deviation, and range were determined.
3. The number of days elapsed from when the first mating was observed until egg laying (oviposition) began in a cage for each of the 12 cages. The mean, standard deviation, and range from smallest to largest for the 3 age groups was determined.
4. The number of days of adult longevity for each of the 12 insect cages. The mean, standard deviation, and range were determined.

Observing Adult Attraction to Pheromones in the Wind Tunnel

One of the most efficient ways to test for evidence of pheromones is to observed insects in a wind tunnel. A wind tunnel consists of a rectangular or cylindrical cage in the middle, with clean filtered air entering at one end and existing at the other end. A fan produces a current, which helps any pheromones or odors drift to the test subjects (Fig. 4). In the wind tunnel, bugs of one gender are put in an isolated mesh container or on a plant while the other gender is let loose within the tunnel (Fig.4). The experiment tests to see if the opposite sex will fly against the wind to reach the pheromones the wind carries (Kainoh 2011).

Wind Tunnel Trials

To test for the presence of male or female produced sex pheromones and their potential to attract or trap *Leptoglossus zonatus*, a wind tunnel was employed.

Experimental setup

General setup: Before added materials to the wind tunnel, the tunnel is wiped down with a damp paper towel with soap and water, and wiped clean, to remove any old remaining odors from previous trials. An anemometer (wind meter) was used to measure the wind speed to make sure the wind current was consistent in every part of the wind tunnel. After two trials, the wind tunnel is again cleaned to remove any odors. At the end of the trials, the fan is run for ten minutes to help clear out any residual odor.

Preparing test odors: Two mason jars with almond branches were placed within the wind tunnel an equal distance from a metal platform where insects would be released to test their response to odors (Fig. 4). The almond branches serve as a substrate for the test insects. The almond branches were collected by visiting local almond orchards and were washed off prior to use to remove any dust or other contaminants.

Typically, dual choice trials are run in the wind tunnel. This means that attraction to two odors is tested at the same time. For example, a test of two odors could consist of comparing the attraction of a bug to odors of females on a plant vs. attraction to a control odor (plant only), or attraction to odors of males on a plant compared to attraction to a control odor (plant only). Both branches are covered in a mesh to keep insects contained on the plant while the attraction to odors is tested.

Releasing test insects: After initial set up of insects and plants in the wind tunnel, the insect test subjects are placed on a platform which is downwind from the odor. From that time,

they are free to move, fly, or crawl around inside the wind tunnel. Over the course of 30 minutes, the actions of the insects released on the platform were recorded and observed. For example, the time elapsed until the insects left the test platform was recorded (sec). The first odor they land on is recorded, and their final location in the wind tunnel at the end of the test is recorded. Time spent on different odor sources is similarly recorded. After the trial concluded, the test subjects and insect odor sources were returned to the larger lab colony, and insects are not reused in future experiments.

Other test details: For these trials, the positioning of the odor-producing bugs was switched from the left to the right (alternated) in the wind tunnel after every two trials. This helped to remove any positional bias in the behavior experiments. *Preliminary observations and modifications*: Lab temperatures were relatively cold from an insect's perspective, so we had to increase the room temperature of the experiment to 80F.

Experiment 1: Are females attracted to male odors more than a control odor?

Hypothesis: Male *L. zonatus* will be more attracted to female *L. zonatus* than to a control

Experiment 2: Are male *L. zonatus* attracted more to female odors than a control odor?

Hypothesis: Female *L. zonatus* will attract the male *L. zonatus* more than a control odor.

Experiment 3: Are males more attracted to mating pairs than a control odor?

Hypothesis: *L. zonatus* mating pairs will attract the male *L. zonatus* more than a control.

Data collection and Analysis: Data collected included the time elapsed for each insect to leave the test platform (in other words, how long until an insect flew from the platform), the first

landing choice of each insect, and the final odor choice of each test insect was recorded as well. At least 20 replicates were run for each experiment.

Results

Age of Sexually Maturity

For each age group (2 wk, 4 wk, 6 wk), the mean number of days elapsed from the start of the experiment until mating was observed was determined (Table 1). For the 2-week-old cages, an average of 7.33 $\pm$ 0.58 d passed until mating was observed. For the 4-week-old cages, a mean of 3.5 $\pm$ 3 d elapsed until the first mating was seen. Similarly, it took an average of 3.75 $\pm$ 1.26 d until a mating pair was observed in the 6-week old cages.

For each age group (2 wk, 4 wk, 6wk), the mean number of matings observed over the first six observation days in the study was determined (Table 2). For the 2-week-old cages, an average of 5.00 $\pm$ 2.16 matings was observed. For the 4-week-old cages, a mean of 20.00 + 13.74 matings occurred. Finally, an average of 24.50 $\pm$ 12.29 matings were observed in the 6-week old cages.

To determine if one age group had a significantly larger number of matings than the other age groups, a mean comparison was run. A nonparametric Analysis of Variance (ANOVA) test was used, called the Kruskall-Wallis test. If the overall P value was <0.05, the test was considered significant overall. If the overall test was significant, then a pairwise comparison was run to examine which pairs of means were significantly different. Again, if the P-value was <0.05, the pairwise test was considered significant.

Egg Development and Oviposition

For each age group, the mean number of days elapsed from mating until oviposition was observed was calculated. For the 2-week-old cages, the first oviposition was observed after an average of 16.50 $\pm$ 5.80 d. For the 4-week-old cages, a mean of 13.00 ± 3.46 days passed until eggs were oviposited on the cage (Table 3). Finally, an average of 12.50 $\pm$ 3.11 days elapsed until oviposition was observed in the 6-week old cages. When all observations were combined, the mean number of days until eggs were observed was 14.00+ 4.31 days.

Adult Longevity

For each age group, the mean longevity of adults after being introduced into the experimental cage was calculated (Table 4). For the 2-week-old cage, adults lived an average of 73.57 $\pm$ 19.86 d. For the 4-week-old cage, adults lived an average of 60.75 + 31.72 days, and finally, an average of 50.62 $\pm$ 31.72 days longevity was observed for adults in the 6 week old cages. When all observations were combined, the mean longevity of adults was 61.13 $\pm$ 28.42 days.

Testing for Evidence of Pheromones in the Wind Tunnel

For each experiment, the number of landings on the test odor was compared to the number of landings on the control odor (Table 5). Males had landed more frequently on the female odors than on the controls ($p=0.04$) (Fig. 5), while there was no significant attraction of females to the odors associated with males ($p=0.70$) (Fig.6). Finally, males were more attracted to the odors associated with mating pairs than the control ($p=0.0077$) (Fig.7).

The time spent by males and females on each odor source was determined, and compared within each dual choice test. In Experiment 1, the mean time spent by males on the branch with females was 284.89 $\pm$ 77.27 sec, while the time spent on the control was 331.70 $\pm$ 106.38 sec ($p > 0.05$)(Fig.8). In Experiment 2, the mean time spent by females on the branch with males was 487.80 $\pm$ 94.61 sec, while the time spent on the control was 299.00 $\pm$ 85.79 sec (Fig. 9). The difference in time spent on these two odor sources was not statistically significant (Table 6, $P>0.05$). Finally, in Experiment 3, the mean time spent by males on the branch with mating pairs was 382.76 $\pm$ 121.32 sec, while the time spent on the control was 175.78 $\pm$ 113.83 sec (Fig.10). These durations were statistically significant ($P=0.04$). (Table 6).

Discussion

The approximate age of sexually maturity of *L. zonatus* adults within this experiment was 3 to 4 weeks. In Millar's and Wang's trial, a related species, *L. clypealis*, was found to be sexually mature at approximately 8 days. (2000) With this knowledge of the life cycle of *L. zonatus*, this information helps to determine which age insects should be used in behavioral experiments. After mating, it took about 14 days until oviposition occurred. This is helpful to know, as one day when pheromone traps are used for this species, they may attract and trap adult insects before they have laid eggs in the field.

The lifespan for the bugs was found to be approximately 61.3 days, while in Grimm and Somarriba's (2001) experiment with *L. zonatus*, the lifespan was shown to be approximately four weeks after emerging in summer and two to three months in winter. These are long lived bugs, and lifespan in the lab under ideal conditions may be longer than insects live in the field under natural conditions.

Wind tunnel results found the males where attracted to females, while previous studies of other Leptoglossus species found that females were attracted to males. In Yasuda's study (1998), it was indicated that the females were attracted to the male pheromone as expected. (1998) In an older study by Aldrich, Blum, and Duffey, related species *Leptoglossus phyllopus* was also shown to have its mating pairs attractive to males (1976). The wind tunnel test regarding the time males spent on plants with mating pairs was significant (Table 6, P=0.04) as was the time females spent on plants with males (Table 6, P=0.03). This suggests that the male pheromone attracts females, but the pheromones released while mating might also attract males. For the test regarding the number of landings, the males being attracted to female pheromones was significant (Table 5, P=0.04) and the males being attracted to mating pairs was especially significant (Table 5, P=0.0077). Between the number of landings and length of landings, the more important behavioral measure is the number of landings, which would mimic if more insects would be attracted to a hypothetical pheromone trap in the field. A number of behavioral comparisons remain to be observed, such as whether females are attracted to odors associated with mating pairs as well.

Future experiments should focus on what other factors might affect if the bugs are attracted to pheromones. One such factor is whether the type of plant being used as a food source affects the attraction. However, some are investigating the odors of almond and pistachio nuts as attractants with other insect species, such as the navel orangeworm moth's attraction to *Prunus dulcis*. (Beck 2009). Comparisons between other leaffooted bug food sources could be drawn to determine which food plant is most attractive to this insect. Another factor worth investigating is the time of year. Many insects have an active mating season and narrowing down to when they are most active can help determine when to leave traps out. In Blatt's 1998 paper, it was found

that the species *L. occidentalis* only responded strongly to pheromones in summer while barely responding in fall.

In Fadamiro and Xiao's 2009 study, they found that the actual lifespan of *L. zonatus* is affected by their food source. For example, the developmental period on *Zea mays* (maize/corn) was found to be about 42 days while in *Jatropha curcas* it was around 21 days. Within this study, all the colonies were fed a universal diet of corn, green beans, and mixed seeds and lived an average of 61.3 days. In Yasuda's 1998 research on *L. australis*, he observed sudden immigrations of the bugs to bitter gourds and loofahs as well as many landings on male-baited cages instead of on plants without bugs.

Eventually, efforts should be made to synthesizable the pheromones of this insect for field use, as an alternative to insecticides.

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Tables

Table 1. Number of days elapsed until first mating was observed in three different age groups (2 week, 4 week and 6 week old insects)

Age Group	Values (# of days)	Mean	Standard Deviation	St. Err	Range (Min-Max)
2-week-old cages	8, 7, 7	7.33	0.58	0.33	7-8
4-week-old cages	5, 7, 1, 1	3.50	3.00	1.50	1-7
6-week-old cages	5, 2, 4, 4	3.75	1.26	0.63	2-5

Table 2 The mean number of matings in six observations, for the three age groups (2 week, 4 week and 6 week old insects)

Age Group	Total number of matings	Mean	Standard Deviation	Range (Min-Max)
2-week-old cages	5, 6, 2, 7	5.00	2.16	2-7
4-week-old cages	29, 5, 34, 12	20.00	13.74	5-34
6-week-old cages	39, 24, 26, 9	24.50	12.29	9-39

Table 3. The number of days elapsed from the first mating until oviposition (egg laying)

Age Group in cages	Total number of days	Mean	Standard Deviation	Range (Min-Max)
2-week-old	**19, 21, 8, 18**	**16.50**	**5.80**	**8-21**
4-week-old	**14, 14, 16, 8**	**13.00**	**3.46**	**8-16**
6-week-old	**13, 8, 14, 15**	**12.50**	**3.11**	**8-15**
All ages combined	**19, 21, 8, 18 14, 14, 16, 8 13, 8, 14, 15**	**14.00**	**4.31**	**8-21**

Table 4. The longevity in days of the adults in the three experimental groups.

Age Group	Total number of days	Mean	Standard Deviation	Range (Min-Max)
2-week-old cages	**71, 33, 74, 74, 86, 81, 96**	**73.57**	**19.86**	**33-96**
4-week-old cages	**29, 74, 17, 72, 31, 81, 96, 86**	**60.75**	**31.72**	**17-96**
6-week-old cages	**71, 30, 23, 14, 86, 81, 81, 19**	**50.62**	**31.72**	**14-86**
All age groups combined		**61.13**	**28.42**	**14-96**

Table 5. Response to odors in the wind tunnel

	Odor Sources	Number of Landings on Test Odor	Number of Landings on Control	Chi-Square Value	P-value
Experiment 1: Are Males Attracted to Female Odors?	**Females vs. Control**	**33**	**23**	**4.35**	**0.04**
Experiment 2: Are Females attracted to Male Odors?	**Males vs. Control**	**27**	**25**	**0.16**	**0.70**
Experiment 3: Are Males attracted to Mating Pairs?	**Mating Pairs vs. Control**	**17**	**9**	**7.11**	**0.0077**

Table 6. Time spent on each odor source

	Odor Source	Mean time on Test Odor	Mean time on Control	Median Test-Test value	P-value
Experiment 1: Are Males Attracted to Female Odors?	**Females vs. Control**	**284.89 +77.266**	**331.70 + 106.38**	**Wilcoxon (check)**	**0.659 ns**
Experiment 2: Are Females attracted to Male Odors?	**Males vs. Control**	**487.80 + 94.61**	**299.00 + 85.79**	**wilcoxon 0.049**	**0.03 ** sig.**
Experiment 3: Are Males attracted to Mating Pairs?	**Mating Pairs vs. Control**	**382.76 + 121.32**	**175.78 +113.83**	**Wilcoxon 0.054**	**0.04 * sig**

Figures

Figure 1. Adult *L. zonatus*. Photograph by Natasha Wright, Florida Department of Agriculture and Consumer Services.

Figure 2. Adult *L. zonatus* on a damaged orange. Photograph by Ayanava Majumdar, Alabama Cooperative Extension System.

Figure 3. Finned *Pentatomidae* trap for household use. Photograph courtesy of Sterling International, Inc.

Figure 4. An example of a setup of the wind tunnel. Mesh was added to the plants afterward. Photo by Cassandra Strizak.

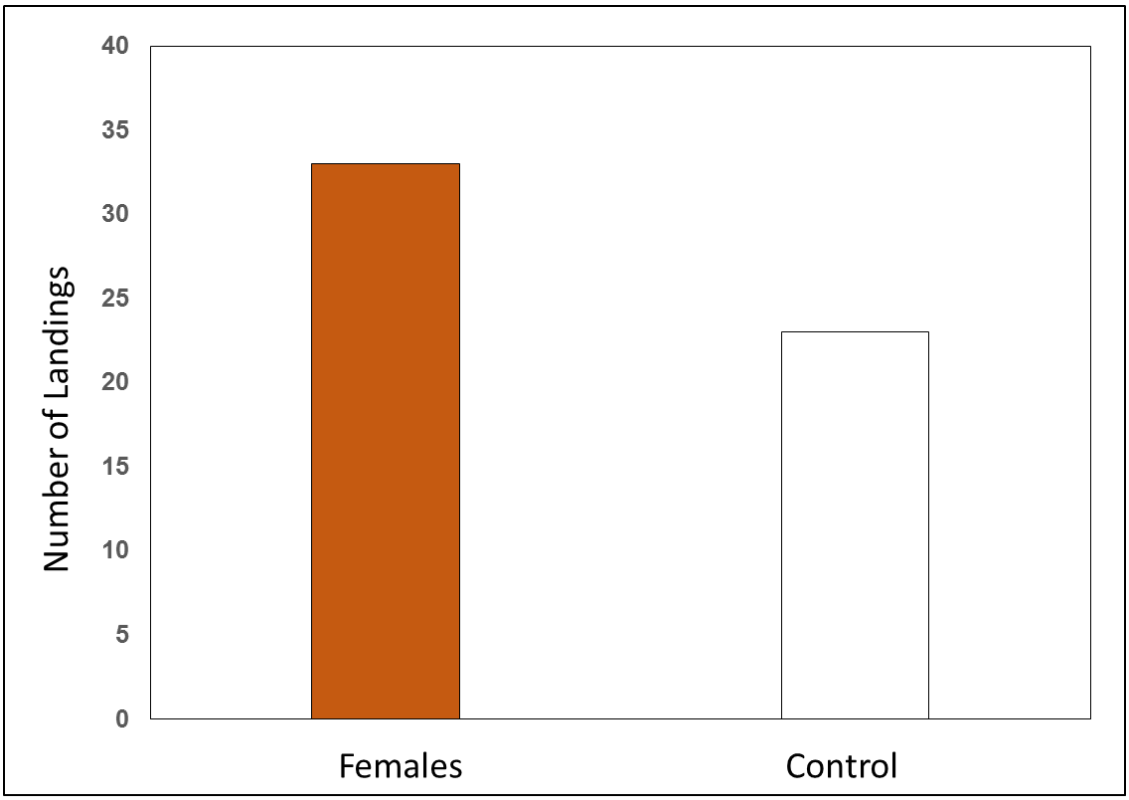

Fig. 5 Comparison of the number of male landings on branches with females versus control plants in the wind tunnel Experiment 1.

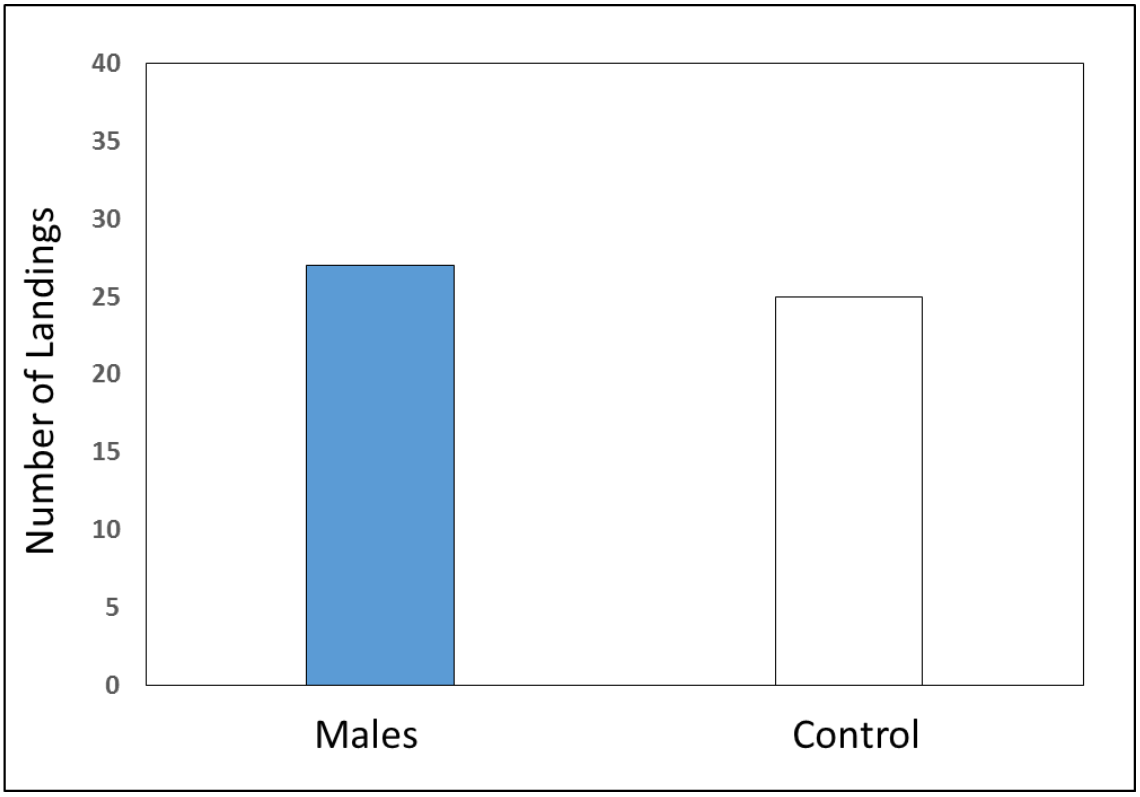

Fig. 6. Comparison of the number of landings by females on branches with males vs. branches of control plants in the wind tunnel Experiment 2.

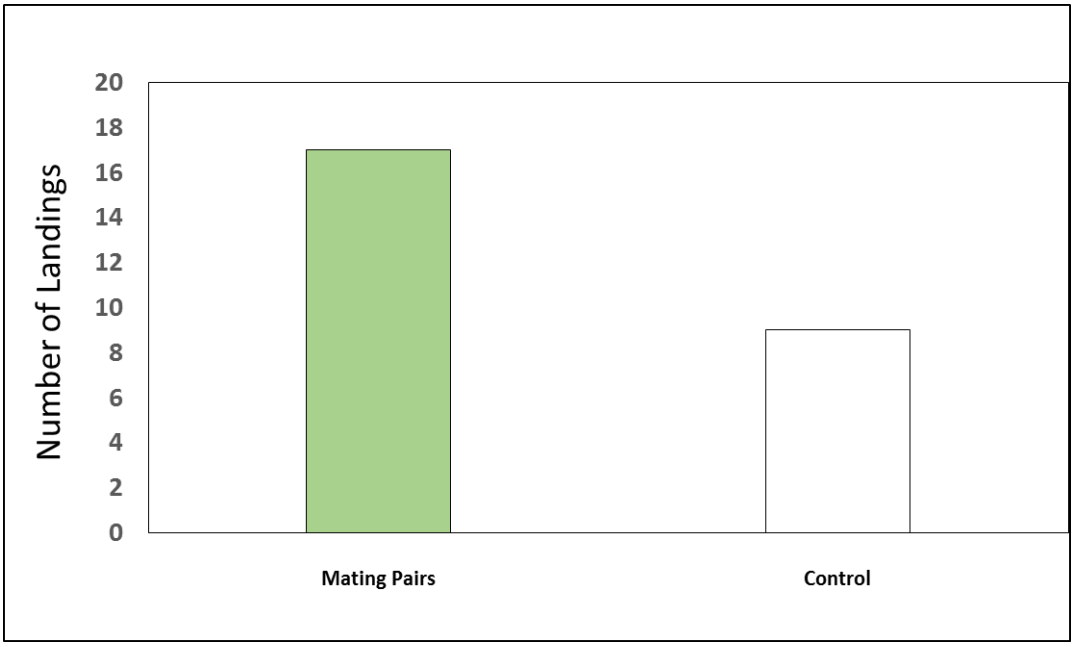

Fig. 7. Comparison of the number of landings by males on branches with mating pairs versus control plants in Experiment 3.

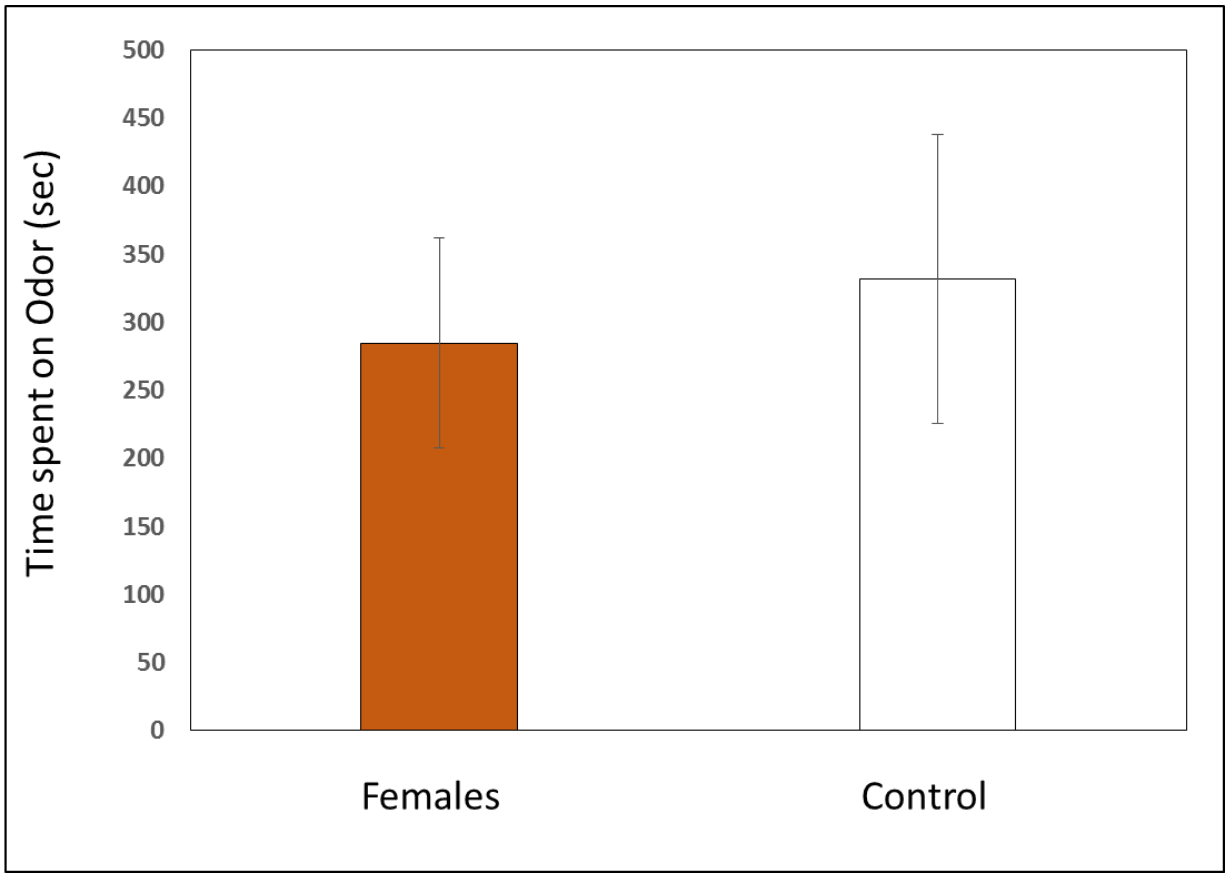

Fig. 8. Comparison of the time spent by males on branches with females vs. branch of control plants in the wind tunnel

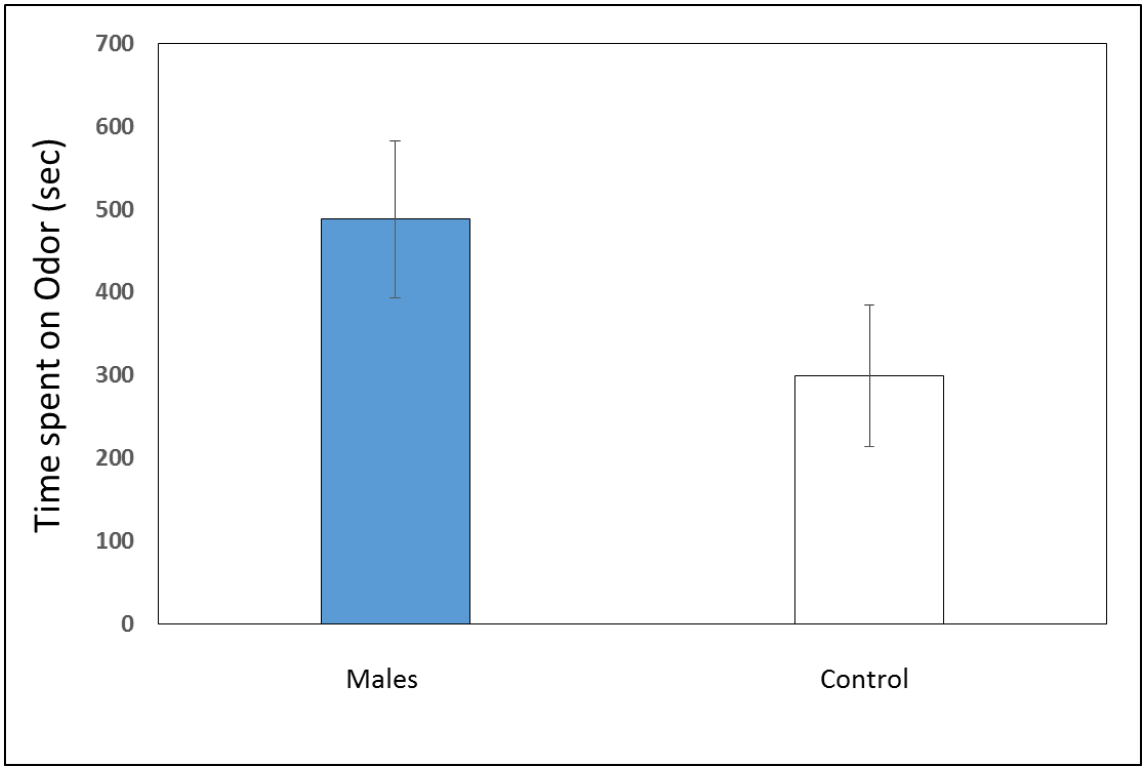

Fig. 9. Comparison of the time spent by females on the branches with males versus branches of control plants in Experiment 2.

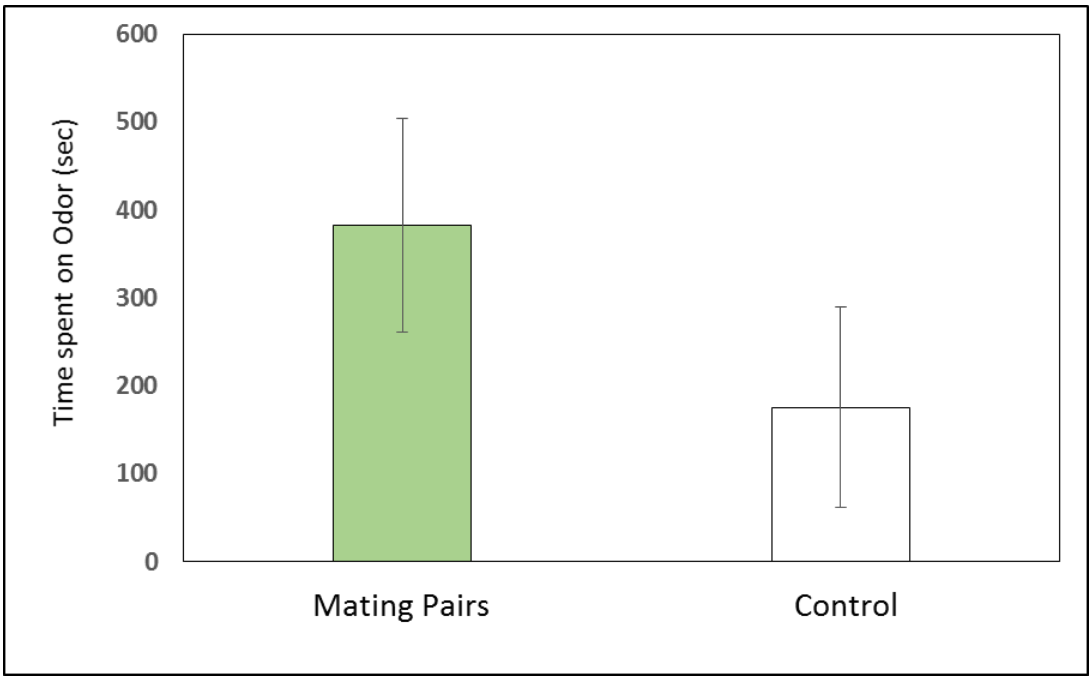

Fig. 10. Comparison of the time spent by males on branches with mating pairs versus control plants in Experiment 3.