Early Detection of Leaffooted Plant Bugs in Almond Orchards

Project No.: **13-ENTO8-Joyce**

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Objectives:

1. Determine the species composition of leaffooted plant bugs on almonds and alternate host plants.

2. Conduct a field cage study on almonds to explore the relationship between almond age and variety with a) natural levels of nut drop, b) mechanical damage and the gummosis response, c) damage which occurs from *Leptoglossus clypealis* and *L. zonatus.*

Interpretive Summary:

Several species of leaffooted plant bugs feed on almonds which results in nut drop and almond kernel damage. These insects can be difficult to detect in the field prior to observing damage such as nut drop or gummosis (sap) appearing on the almond hull. Ideally, an early detection trap such as a pheromone trap would be developed to help monitor these insects. The first objective of our work was to determine which species of leaffooted bugs were abundant in almonds and on two alternate host plants (pistachios and pomegranates). *Leptoglossus clypealis* was found on almonds and pistachios, and a second unexpected species *L. zonatus* was collected from pistachios and pomegranates. Genetic evidences suggest *L. zonatus* may actually consist of two species or biotypes, and this work is still in progress. The results have IPM implications, as we need to determine whether *L. zonatus* feeds on almonds or whether the primary leaffooted bug pest in almonds is *L. clypealis*.

Our second objective is a field-cage study which is being run to investigate the relationship between almond variety and the age of the nut through the growing season with four parameters, 1) the natural nut drop that occurs on each variety, 2) the gummosisresponse which occurs on mechanically damaged almonds, 3) the levels of kernel damage caused by feeding adult *L. clypealis* and 4) the levels of kernel damage caused by feeding adult *L. zonatus*. The final data from the field cage study is currently being collected (just prior to harvest). The summary of the field cage study results will be presented at the 2014 Almond Conference.

Materials and Methods:

Objective 1- Determine the species composition of leaffooted plant bugs on almonds and alternate host plants**.** Leaffooted plant bugs have been collected from almonds, pistachios and pomegranates from the northern, central and southern Central Valley. Many of these collections were obtained with the help of collaborators. Adult *Leptoglossus clypealis* from Manteca and Ripon were used to start a laboratory colony, and adult *L. zonatus* were obtained from field collections in southern California and also used to start a colony. Both colonies were needed in order to have live adult insects for the field cage study described below in Objective 2. The colonies are being maintained in insect rearing cages on a diet of green beans, corn on the cob, raw peanuts, raw sunflower seeds, raw pine nuts, raw pumpkin seeds and arborvitae plants. Colonies of bugs will be maintained year round.

Approximately 300 leaffooted plant bug adults were collected or obtained from collaborators and were stored in a freezer for use in DNA studies to examine the species composition on almonds and alternate crop host plants. We will determine which species are abundant and whether there are cryptic species or biotypes (McPherson et al. 1990; Vos et al. 1995; Joyce et al. 2010; Park et al. 2011). A long term goal is to develop a monitoring device or trap such as a pheromone trap. It is important to understand which species are present and which are abundant before initiating work related to pheromones, since pheromones can be species specific (Aldrich et al. 1979; Yasuda 1998; Wang and Millar 2000). *Leptoglossus* spp. can be difficult to find by sampling in the field, and the ability to detect adult *Leptoglossus* spp. in the field with traps before almond damage is visible could help time the application of insecticides or reduce their use (Daane 2007; Daane et al. 2008).

DNA was extracted from the thorax of male leaffooted plant bugs using the Qiagen Dneasy Blood and Tissue Kit using standard tissue protocols and a 1 hour incubation. Amplified fragment length polymorphisms (AFLPs) were developed (Vos et al. 1995). Samples were randomized on two 96-well plates. Two primer combination were used (M-CAT, E-ACT; M-CAC, E-ACG) to produce fragments for comparison. Samples were run on a 3730 Genetic Analyzer at UC Berkeley. Genemapper 3.9 software was used to determine the presence or absence of each allele. Nei's genetic distance was calculated and used to generate a neighbor joining tree using Phylip 3.65 to examine genetic similarity of individuals.

Objective 2 - Investigating nut drop, gummosis and leaffooted plant bug feeding damage. Often the first evidence of leaffooted bug activity in an orchard is when ***nut drop*** occurs, or when nuts with ***gummosis*** are observed. Insecticide applications for leaffooted bugs may be based on observations of either of these symptoms. However, symptoms may not appear until a week or more after bug feeding, and by that time leaffooted bugs may have already dispersed.

The goal of this objective is to simultaneously investigate nut drop, the gummosis response, and damage which occur from adult leaffooted bugs feeding on almonds. Some almond varieties appear more susceptible to nut drop and damage from bug feeding (Haviland and Viveros 2006; Haviland 2007). In addition, we wanted to examine how the age of the developing almond impacts its susceptibility to feeding damage from leaffooted bugs. This

information will assist with developing action thresholds and control decisions for leaffooted bugs. In our first year of working with the Almond Board of California (2013), we started in May and ran replicates to examine nut drop and the gummosis response on Sonora and Monterey trees. This was preliminary data that helped us design a larger study for the 2014 field season (Joyce et al. 2013).

In 2014, we expanded the field cage damage study to include 5 varieties of almonds: Nonpareil, Fritz, Sonora, Monterey, and Carmel. The field cage damage study is currently being conducted in Merced County in Winton and Merced. This study will run from March 2014 through mid-August 2014, in order to follow the susceptibility of the almonds through their entire development. We are now removing the almonds in our study, just before the harvesting of each variety, in order to asses final damage in the treatments.

There are four treatments in this study.: 1) caged control branches with almonds; 2) caged branches with mechanically punctured almonds; 3) caged branches with live adult *L. clypealis,* enclosed in the cage for 4-6 days; and 4) caged branches with and different species, live *L. zonatus* adults, feeding in the cage for 4-6 days. Each week, on each of the five varieties, the four treatments were set up. Control branches in each variety consisted of 4 caged branches with 20 almonds each so that we could follow the natural nut drop on each variety through the growing season. This data is important to help determine whether some varieties have a large percentage of natural nut drop while others may not. The second treatment is the 'mechanical damage treatment', which also consisted of a weekly setup of 4 caged branches with 20 almonds each on each of the five varieties. Each almond was mechanically damaged using a #1 insect pin to puncture the almond kernel 4-5 times on each nut. After the almonds were punctured, we observed whether gummosis occurred immediately or not. A third treatment '*L. clypealis* feeding' consisted of caging a branch with 20 almonds and introducing 5 adult (3 female/2 male) *L. clypealis* leaffooted bugs which were allowed to feed 4-6 days and were then removed. The fourth treatment '*L. zonatus* feeding' was similar to the third, but was with a different species and consisted of caged adult *L. zonatus* adults (3 female/2 male) feeding for 4-6 days which were then removed. Within each of the five almond varieties, there are a minimum of 500 total almonds in the controls, 500 almonds in the mechanical damage treatment, and 500 almonds in the bug feeding treatments, being followed through the growing season. New branches were set up weekly until the almond shell was too hard to be punctured by a pin, and they were no longer susceptible to bug feeding. In addition to setting up these four comparisons each week, we also took a sample of 20 almonds from each variety to the lab to measure hull width, almond length and width.

Each week, cages were checked to determine the number of almonds that had dropped off the branches in the different treatments. Data will show when natural nut drop is highest during the growing season for each variety, and whether one variety has a higher percentage of natural nut drop than other varieties. Also, we will learn how quickly nut drop occurs after leaffooted bug adult feeding. In the treatments with adult leaffooted bugs feeding, we will also see the percent of almonds with gumming in each variety, and how many of those almonds have strikes on the hull or damage on the nut. Finally, we will learn whether *L. clypealis* or *L. zonatus* cause more damage to different almonds varieties.

Just before harvest, we are doing a final assessment of almond nut drop and damage. We will count the number of almonds remaining on all branches in the study. For each control branch and each branch with mechanically damaged almonds, we will take a subsample of four nuts to the lab to assess 1) presence or absence of gumming on hull 2) strikes on the hull 3) strikes on the nut and 4) damage to the nut. For the branches that were caged with adult leaffooted plant bugs, all remaining almonds will be removed to assess the same damage parameters. At this time (end of July), almonds are being removed and damage is being assessed.

Results and Discussion

Objective 1 - Determine the species composition of leaffooted plant bugs on almonds and alternate host plants**.** We found *L. clypealis* on both almonds and pistachios (**Figure 1**). This is the leaffooted bug which can be identified by a pronounced 'pointed nose' or clypeus. In 2013, this species was abundant on both almonds and pistachios in the mid-Central Valley (Manteca to southern Merced County) (Joyce et al. 2013). The aflp genetic markers showed that the individuals of this species collected were one large interbreeding population, likely moving between the two types of host plants. So far, *L. clypealis* has not been collected on pomegranates. It is likely that once *L. clypealis* can no longer feed on almonds due to the hardening of the almond shell these insects move into pistachios and feed on pistachios a bit later into the growing season.

Another species, *L. zonatus*, has been collected on pomegranates and pistachios (**Figure 1**). The aflp genetic markers have found that within *L. zonatus* there are two genotypes, or genetically different groups. Further work will investigate the level of genetic divergence to determine if these are two separate species. We will contact the entomological expert of this insect group for confirmation of our findings.

These results on host plant preference of *L. clypealis* and *L. zonatus* have important implications for IPM of leaffooted bugs. It has been presumed that leaffooted bugs (*L. clypealis*) overwinter in pomegranates, and then move in the spring into almonds. However, this may not be the case. *L. zonatus* may prefer to feed on pomegranate or pistachios, but not move into almonds or feed on almonds. Few *L. zonatus* were collected in almonds in 2013. Our second objective which we are currently examining in our field cage study is to determine the level of nut drop, gummosis and feeding damage from leaffooted bugs on different almond varieties through the growing season. One of the treatments in our field cage study (described below in more detail), was to cage live adults of the two leaffooted bug species, *L. clypealis* and *L. zonatus*, to examine the level of damage caused by bug feeding on almonds. Our field study will help shed light on whether *L. zonatus* is a threat to almonds, or perhaps *L. clypealis* is the dominant leaffooted bug pest in almonds.

Objective 2 - Investigating nut drop, gummosis and leaffooted plant bug feeding damage**.** Preliminary field cage damage trials were conducted in Merced County and in Bakersfield in 2013. Findings from Merced in 2013 showed that the natural level of nut drop in Sonora controls was ~10% while no natural nut drop was observed in Monterey almond controls. However, data obtained in 2013 was limited to a few replicates, as the study began in May (Joyce et al. 2013). It is important to determine which varieties have higher levels of natural nut drop so that it is not mistaken for plant bug or leaffooted bug feeding damage. The 2013 study

found that almonds exhibited the gummosis response immediately, and the sap (gum) remained on the hull until harvest. In the 2013 mechanical damage/puncture trials, all kernels that were punctured showed damage at harvest.

The 2014 field cage study is still in progress. The final data will be presented at the 2014 Almond Conference. For each of five varieties in the field cage damage study, we will determine for controls the cumulative mean nut drop per week, and total nut drop for the growing season. For the mechanically damaged almonds, we will also determine the nut drop per week after damage occurs, and total nut drop for the season. In addition, we will determine if the almond age affects whether or not the gummosis response occurs. Finally, in the cages with *L. clypealis* and *L. zonatus* adult bugs feeding, we will determine how the bug feeding impacts the five varieties. We will learn how long it takes from bug feeding until the almonds drop off the branches, and whether different ages or varieties of almonds exhibit the gummosis response. We will determine whether strikes are found on the hull and nut, and nut damage that results from either bug species feeding. This will tell us whether *L. clypealis* or *L. zonatus* is more damaging to almonds, and if feeding by one of these species results in a higher level of damage to the nuts.

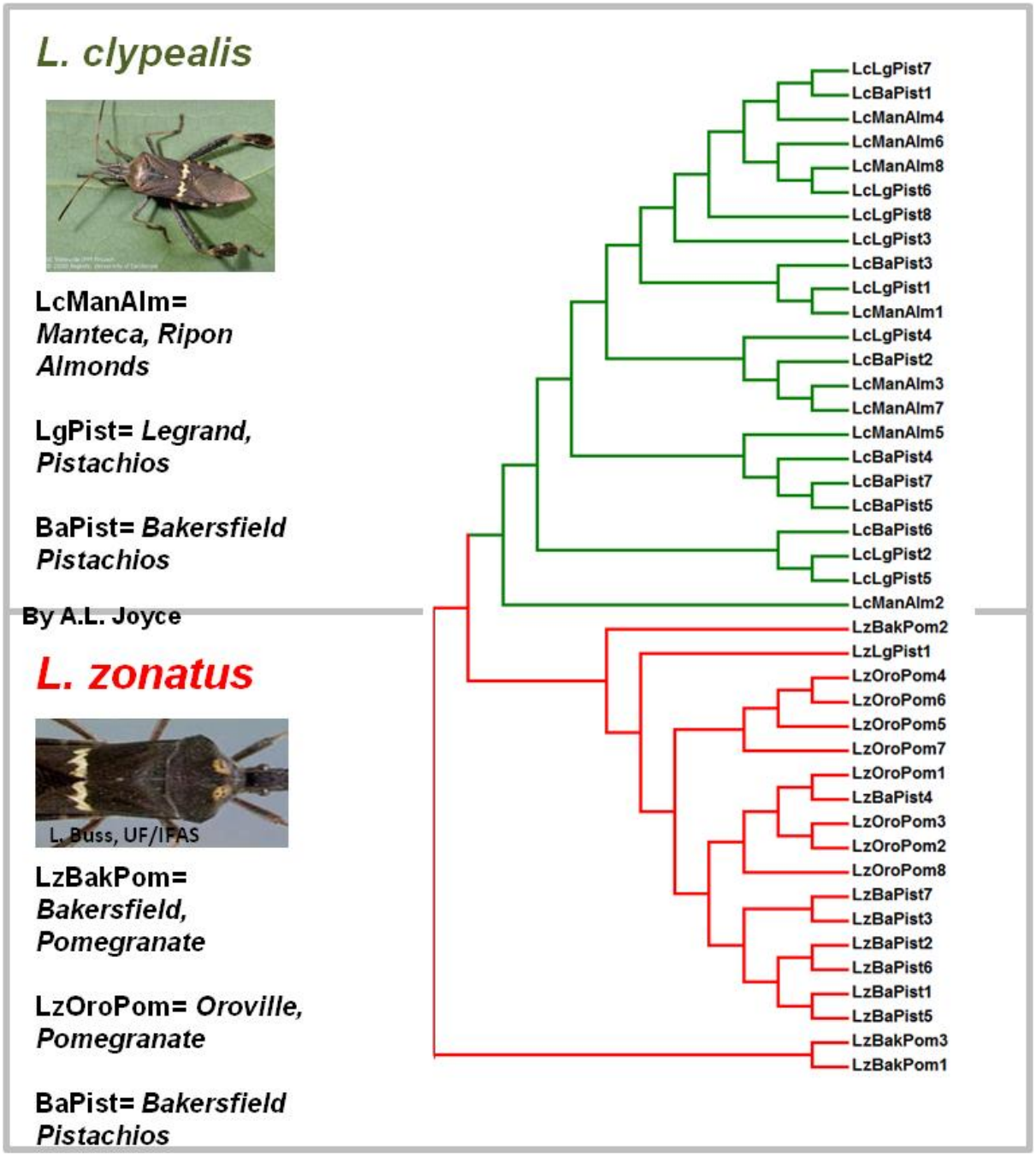

Figure 1. Leaffooted plant bug species collected from almonds, pistachios, and pomegranates in California. *L. clypealis* was collected on almonds and pistachios. *L. zonatus* was found on collections from pomegranates and pistachios.

Research Effort Recent Publications:

The research in this report is intended for two separate publications. One article will describe the species composition of the leaffooted plant bugs on almonds and alternate host plants. A separate article will be prepared to discuss the damage caused by the two species of leaffooted plant bugs feeding on different almond varieties.

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