Early Detection of Leaffooted Plant Bugs and Stinkbugs in Almond Orchards

Project No.: **14-ENTO8-Joyce**

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Objectives:

1. Conduct a survey to determine which species of stink bugs and leaffooted plant bugs are most abundant in almonds throughout the year and investigate their alternate host plants.
2. Conduct a mechanical damage experiment on almonds to determine natural nut drop, to determine the relationship between almond age and the gummosis response, and investigate the level of feeding damage to almond kernels by live feeding leaffooted bug adults.

Interpretive Summary:

Stink bugs (Pentatomidae) and Leaffooted plant bugs (*Leptoglossus spp.,* LFPBs) feed on almonds and can result in economic losses. There is currently no early warning detection system in almonds for these insects. Often the first sign of bug feeding is when either nut drop or a condition called gummosis occurs, and by the time this symptom appears the bugs may have already left the field. The first objective of this project was to survey the different species of stink bugs and leaffooted plant bugs present in almond orchards and on alternate host plants throughout the year. We used molecular markers to determine the number of species or strains of each species. Two species of leaffooted bugs were abundant, *Leptoglsosus clypealis*, and *Leptoglossus zonatus*. *L. clypealis* was more abundant in almonds and pistachio, while *L. zonatus* was the dominant species in pomegranate. The second objective was to conduct a mechanical damage study in the field which would simulate bug feeding on almonds, in order to observe the level of nut drop and gummosis on a number of almond varieties over the course of the entire almond growing season. Live bugs were caged on branches with almonds to compare feeding damage to mechanical damage. Control branches had a low rate of nut drop of 5-10%. Mechanically damaged almonds (punctured) had about

50% of almonds fall from branches. Cages where *L. clypealis* or *L. zonatus* fed had from 30-60% almond drop. Cages where leaffooted bugs fed on or before April 15th had more almonds drop than did cages with bugs feeding at later dates. Punctured almonds and almonds exposed to bug feeding took at least two weeks to fall from branches. Leaffooted bugs may have left the orchard by the time the nut drop is observed, and insecticide applications at this time may be too late to control leaffooted bugs. The long term goal is to develop an early detection monitoring system for these insects (such as a trap or pheromones) and to develop action thresholds, in order to improve timing of controls and to reduce losses.

Materials and Methods:

1. Survey Leaffooted bugs and Stinkbugs, Molecular Identification of Species/ Strains

Our first goal was to determine which species of leaffooted bugs were abundant, and whether there may be cryptic species or host plant associated strains. Two species of leaffooted plant bugs (*Leptoglossus clypealis* and *L. occidentalis*) have been reported to be damaging to almonds (Daane et al. 2008), and the three species of stinkbugs reported from almonds are *Acrosternum hilare* (the green soldier bug), *Thyanta pallidovirens* (the red shouldered stink bug), and *Chlorochroa uhleri* (the green plant bug). Understanding which species are most abundant in almonds and investigating the movement of stink bugs and leaffooted plant bugs (LFPB) among host plants throughout the year could help target management efforts.

We first emphasized examining species diversity of leaffooted bugs. Insects were collected and obtained from collaborators throughout the Central Valley from almonds, pistachios and pomegranates (**Figure 1b, Table 1**). We identified the leaffooted bugs to species, and looked at the abundance by host plant (**Figure 2**).

Approximately 300 leaffooted plant bug adults were collected or obtained from collaborators and were stored in a freezer for DNA studies, to examine the species composition and whether there are cryptic species or biotypes (McPherson et al. 1990; Vos et al. 1995; Joyce et al. 2010; Park et al. 2011). 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. Results from some genetic work (DNA) were presented on the Almond Conference Poster in 2014, but we will mention some highlights here in the results section.

In addition, we have used another DNA technique called mitochondrial DNA to look at the number of leaffooted bug species. This allows a second molecular method to confirm our earlier results. Finally, we have been using mitochondrial DNA to search for cryptic species of two species of green stinkbugs, *Acrosternum hilarea* and *Chlorochroa uhleri*. This work is ongoing but it will be presented at the Almond Conference in 2015.

Objective 2: Field-cage study to investigate nut drop, gummosis and leaffooted bug feeding damage.

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. 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 2014, we expanded the field cage damage study to include 5 varieties of almonds which were Nonpareil, Fritz, Sonora, Monterey, and Carmel. The field cage damage study was conducted in Merced County in Winton and Merced from March through mid-August 2014, and is also being replicated this year 2015 (**Figure 1a**).

There are four comparisons (treatments) in this field study. 1) Caged control branches with almonds to examine natural nut drop 2) Caged branches with mechanically punctured almonds 3) Caged branches with live adult feeding *L. clypealis,* enclosed in the cage for 4-6 days, and 4) Caged branches with live *L. zonatus* adults feeding in the cage for 4-6 days. The four treatments were setup each week for 8 weeks. Control branches in each variety consisted of 4 individually caged branches (replicates) 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 consisted of caged adult *L. zonatus* (3female/2male) feeding for 4-6 days which were then removed. New branches were set up weekly until the almond shell was too hard to be punctured by a pin, at which time (in 2014) we assumed almonds were no longer susceptible to bug feeding. In addition to setting up these four experiments/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. This gave us a measure of the almond size each week of experimental setups, and also hull width. Thicker or thinner hull width could influence vulnerability to leaffooted bug feeding.

Data Collection-Part 1 Nut Drop

Each week, after we setup new cages, the cages which were setup on previous weeks were checked to determine the number of almonds that had dropped off the branches in the different treatments. This data shows 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. We also determined how quickly nut drop occurs after mechanical puncturing or 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. Finally, we will learn whether one of the bug species, *L. clypealis* or *L. zonatus* can cause more damage to the different almonds varieties.

Data Collection-Part 2 Final Damage Assessment
Just before harvest, we use our field-caged branches to conduct a final assessment of almond nut drop and damage in our four experimental treatments. We counted the number of almonds remaining on all branches in the study. For each control branch and each branch with mechanically damaged almonds, we took a subsample of four nuts to the lab to assess 1. Strikes on the hull 2) strikes on the nut 3) nut damage and 4) shriveled almonds. For the branches that were caged with adult leaffooted plant bugs, all remaining almonds were removed to assess the same damage parameters.

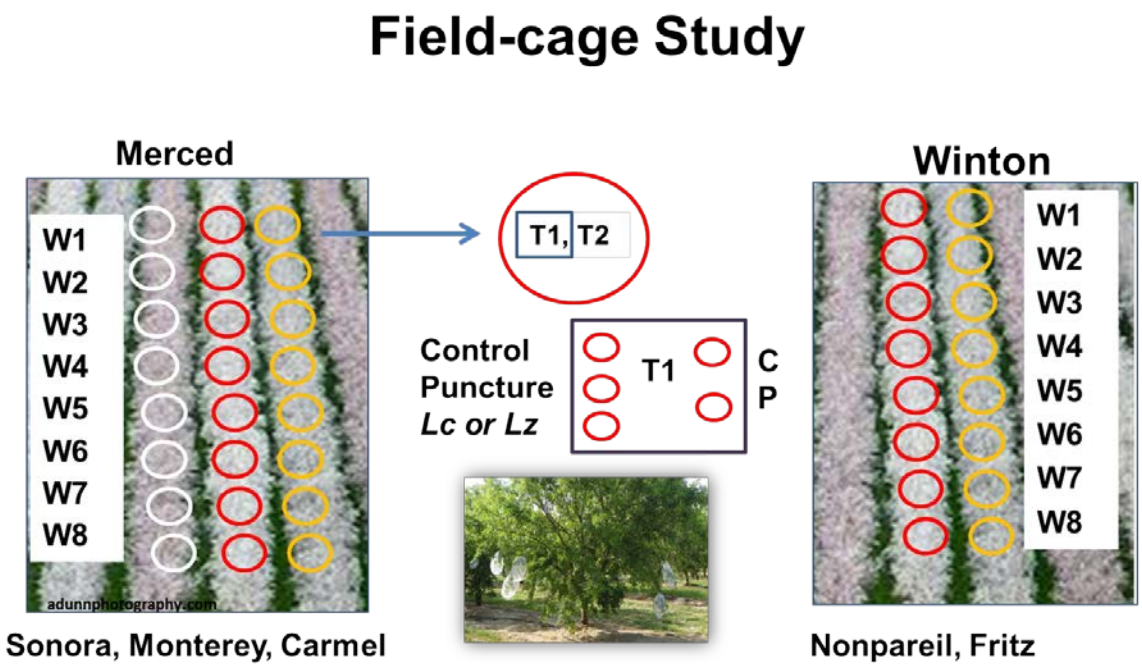

Figure 1a. Set up for field-cage study to assess adult leaffooted bug feeding damage

Results and Discussion:

Results Objective 1. Survey of Leaffooted bugs and Stinkbugs, Molecular identification of Species/ Strains

Leaffooted bugs were obtained from sites through the Central Valley on almonds, pistachios and pomegranates (**Figure 1b, Table 1**). We found the dominant species of leaffooted bug in almonds was *Leptoglossus clypealis*. We also found that pistachios harbored *L. clypealis* as expected. However, a second species of leaffooted bug was found in almonds and pistachios which were not expected; *Leptoglossus zonatus* was observed in almonds and pistachio. We did not find any individuals of the leaffooted bug *L. occidentalis,* which have been previously reported on these crops. The newly observed species, *L. zonatus*, is very distinct. *L. clypealis* has a pointed clypeus, a spine-like nose at the front of its head. It looks very different than *L. zonatus*, which has two prominent yellow/orange spots on the prothorax behind is head (**Figure 2**). *L. zonatus* was not reported in the IPM pages for almonds or pistachios prior to 2013. *L. zonatus* is a much larger insect, about twice the size of *L. clypealis*. *L. zonatus* may cause more damage to almonds than *L. clypealis*, since it is a larger bug with longer mouthparts. Finally, pomegranate from both Northern and Southern California had predominantly *L. zonatus* populations. *L. zonatus* has a distinct aggregation behavior which has not been observed for *L. clypealis*.

Figure 1b. Map of Leaffooted bug collection sites in California

Table 1. Leaffooted bug species and host plants associated with collections in **Figure 1b**.

L. zonatus		*L. clypealis*	
Site Collected	Host Plant	Site Collected	Host Plant
1. Chico	Pomegranate	2. Manteca	Almond
4. Delhi	Almond	3. Merced	Unknown
5. Gustine	Pomegranate	7. Le Grand	Pistachio
6. Gustine	Almond	11. Lost Hills	Pistachio
8. Lost Hills	Pomegranate	17.Bakersfield	Pistachio
9. Lost Hills	Pistachio		
10. Lost Hills	Pomegranate		
12. McKittrick	Almond		
13. McKittrick	Pistachio		
14. Bakersfield	Pomegranate		
15. McFarland	Pomegranate		
16. McFarland	Pistachio		

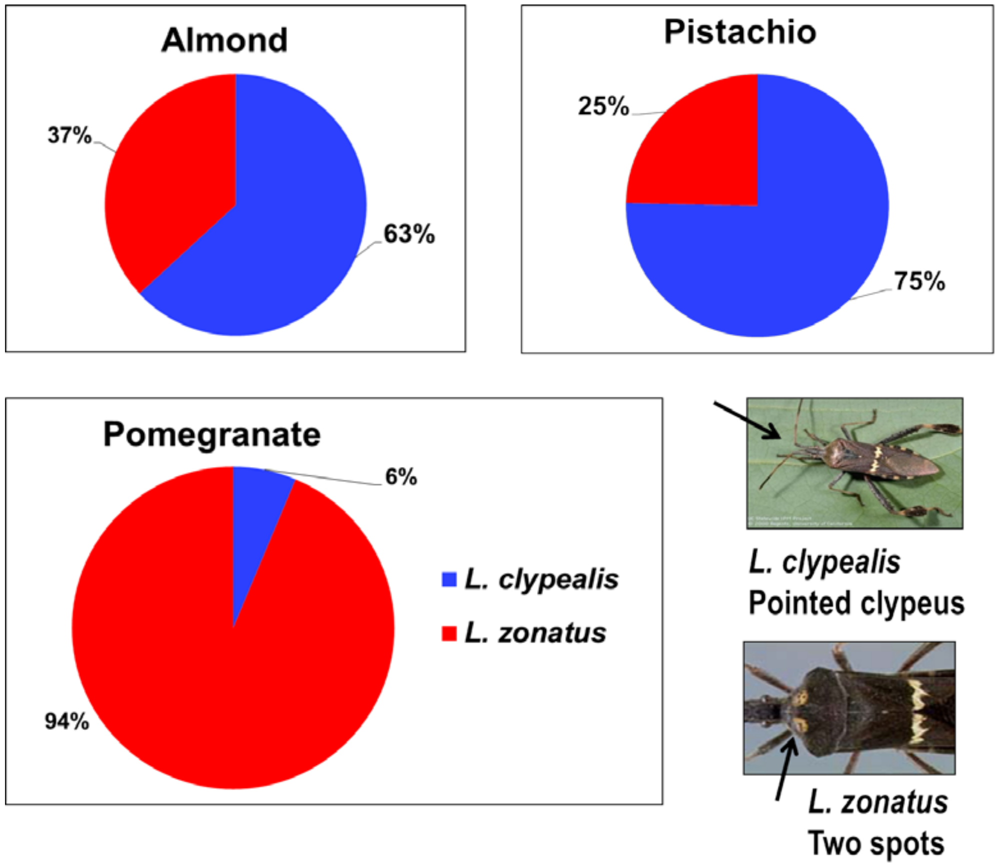

Figure 2. The percent of each leaffooted bug species found on host plants.

DNA was extracted from adult *L. clypealis and L. zonatus.* Using molecular markers (AFLPs) I found gene flow among the *L. clypealis* populations on almonds and pistachios, suggesting that *L. clypealis* moves between almonds and pistachios and both populations interbreed (Joyce et al. 2013, 2014). It is helpful for management to know that *L. clypealis* is indeed moving between the two host plants and interbreeding. No host plant strains or cryptic species were found for *L. clypealis*. For *L. zonatus*, we used the same type of molecular markers (AFLPS) and found there were two strains of *L. zonatus*. This is important, as different strains or biotypes of insects can differ in their susceptibility to parasitoids, can vary in their host plant preferences, may have different environmental adaptations such as adaptation to drought/dry climates or wet climates, and may use different pheromones to communicate. The genetic results are summarized in brief here, since they were presented on the 2014 Almond Conference poster (Joyce et al. 2014).

We have continued the work with *L. clypealis* and *L. zonatus* using mitochondrial DNA to examine genetic diversity. In addition, we are using mitochondrial DNA to examine the genetic diversity of two stinkbugs *Acrosternum hilarae* and *Chlorachroa uhleri*. We will report on the results from mitochondrial DNA at the Almond Conference in 2015.

Results Objective 2: Field-cage study to investigate nut drop, gummosis and leaffooted bug feeding damage.

2014 Field-cage study: Part 1 Nut Drop

First we measured total nut drop over the entire study (**Figure 3**). For the caged control branches, the natural nut drop ranged from 4.5% in Nonpareil to 11.2% in Carmel (**Figure 3**). There were a few days that more nut drop was observed, which were April 21, May 7 and June 19 (no graph shown). The branches with punctured almonds were the experimental treatment with the highest rates of nut drop, 44% Fritz to 53% in Monterey (**Figure 3**). Almonds took at least 2 weeks to fall off trees after they were punctured. Finally, cages with adult leaffooted *L. clypealis* had nut drop that ranged from 15% in Fritz to 34% in Carmel. Almonds took at least 2 weeks to fall off branches after *L. clypealis* feeding, and few almonds fell from feeding cages began after April 28 in any variety. Feeding by the other leaffooted bug *L. zonatus* had cages with almost twice as much nut drop. Nut drop from feeding caged *L. zonatus* ranged from 30% in Nonpareil to 60% in Carmel. Nut drop mostly occurred 3 weeks after bug feeding for *L. zonatus*. Few nuts fell from feeding cages started after May 15. We began the study including Sonora, but early on we ran short of adult bugs for the feeding trials so we did not include Sonora data comparisons here.

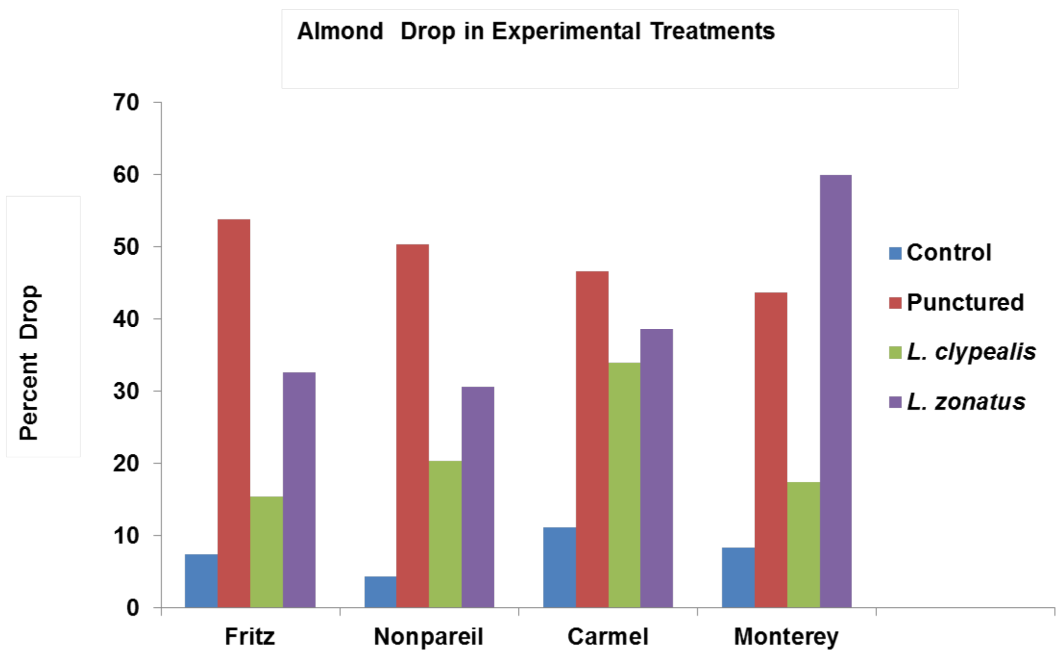

Figure 3. Total Nut Drop in 4 Almond Varieties in 2014, with two species of Leaffooted bugs feeding

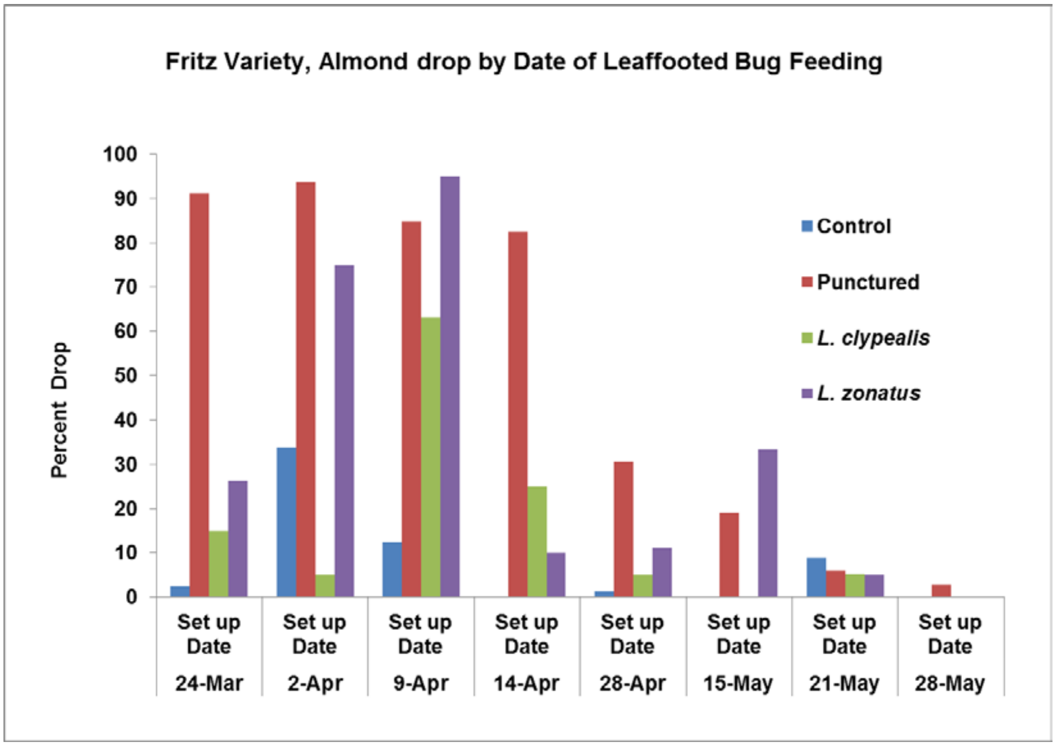

Figure 4. Dates almond branches were exposed to bug feeding in Fritz.

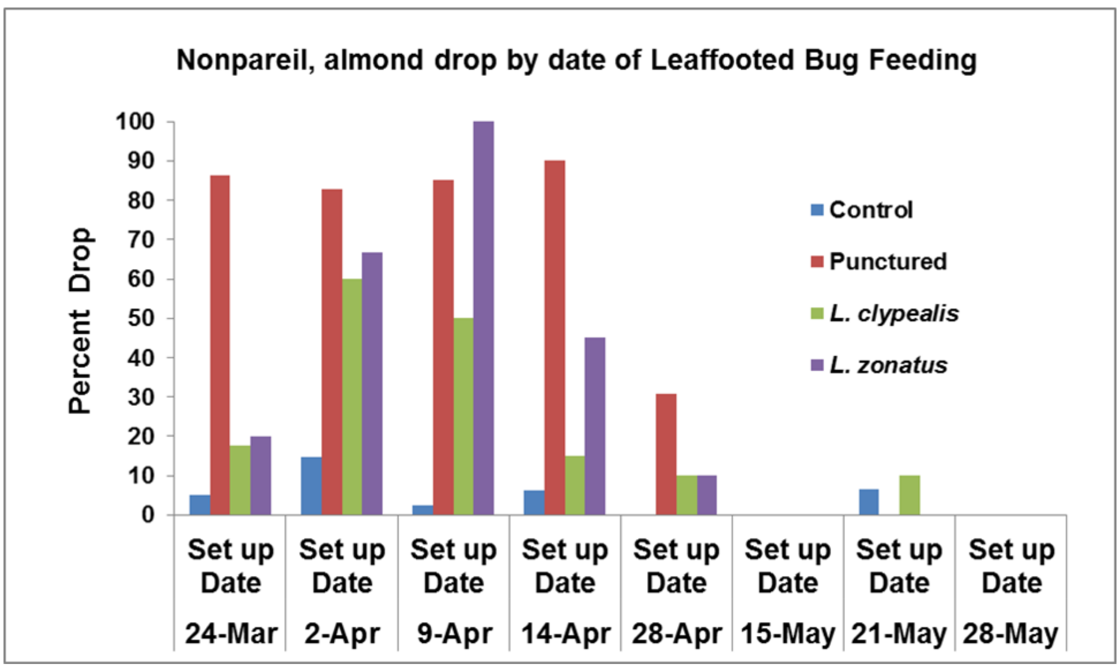

Figure 5. Dates that branches were exposed to bug feeding in Nonpareil.

Time from Exposure to Bug Feeding until Nut Drop on branches
Figure 4 and **5** show the date the experimental cages for the four treatments were set up in Fritz and Nonpareil. We included these two varieties here since Nonpareil is an early maturing variety and Fritz is a late maturing variety. In addition, they were growing in the same orchard under the same conditions. Data will be presented for Monterey and Carmel varieties at the Almond Conference in 2015.

The experiment was set up this way so that we could examine the vulnerability of different age almonds to leaffooted bug feeding. In Fritz, the week of April 9th had the highest nut drop due to leaffooted bug feeding (**Figure 4**). Nearly 90% of almonds dropped in *L. zonatus* feeding cages, while nut drop was high from *L. clypealis* on April 2nd. By April 15th, the nut drop due to leaffooted bug feeding decreased and stayed low through almond shell hardening. For Nonpareil, almonds also had the highest drop from bug feeding on April 2 and 9 (**Figure 5**). Carmel and Monterey graphs will be presented at the almond conference. The window of vulnerability from leaffooted bug feeding was a bit longer in Carmel and Monterey, through May 14th (not shown here).

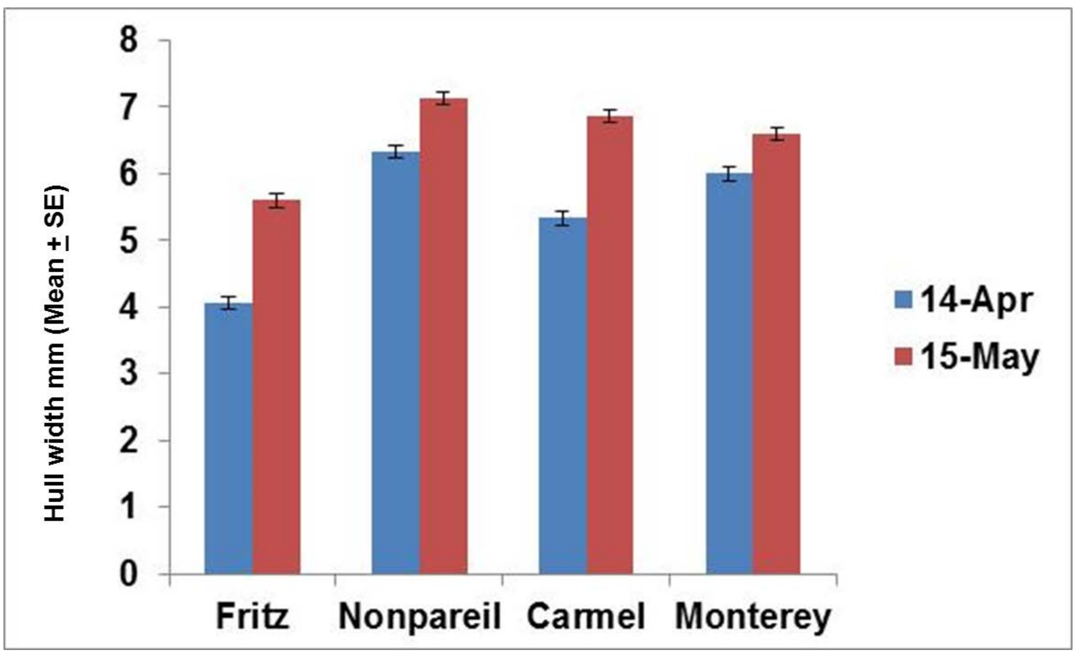

Figure 6. Almond hull width at two of the dates bug feeding occurred by *L. clypealis* and *L. zonatus.*

Interestingly, there is a significant difference in the hull width of Fritz and Nonpareil (**Figure 6**). However, they were both vulnerable to leaffooted bug feeding until around April 15th. Carmel and Monterey varieties had thinner hulls than Nonpareil, yet they remained vulnerable to leaffooted bug feeding for several weeks after Nonpareil and Fritz.

2014 Field-cage study: Part 2 Final Damage Assessment

We measured the final damage to the almonds in the field cage treatments just before almonds were harvested. We took a subsample of nuts from each branch. In some cases, no almonds remained on the branches where almonds had been punctured. Hull strikes, nut damage, nut strikes and nut shriveling were recorded. For Fritz almonds, control nuts had no evidence of hull or nut strikes (**Figure 7**). Less than 5% of Fritz almonds had nut damage or were shriveled. In bug feeding treatments on Fritz, at least twice the level of almond damage was observed for nut damage and shriveled nuts. In addition, hull strikes and nut strikes were observed in about 20% of almonds (**Figure 7**). For Nonpareil almonds, some of all types of damage were present in the controls at a very low level, about 5% or less (**Figure 8**). The cages where bugs had fed in Nonpareil had 15-20% overall damage, but the damage in Nonpareil by bug feeding was lower than where bugs had fed in Fritz. Final statistical analysis will determine whether the nut damage or percent shriveled nuts among varieties was significantly different among the four varieties. All data for the final damage level observed just before harvest will be presented at the Almond Conference 2015. We will also include the data from the field study from this year, which we are just starting to collect/harvest for data collection.

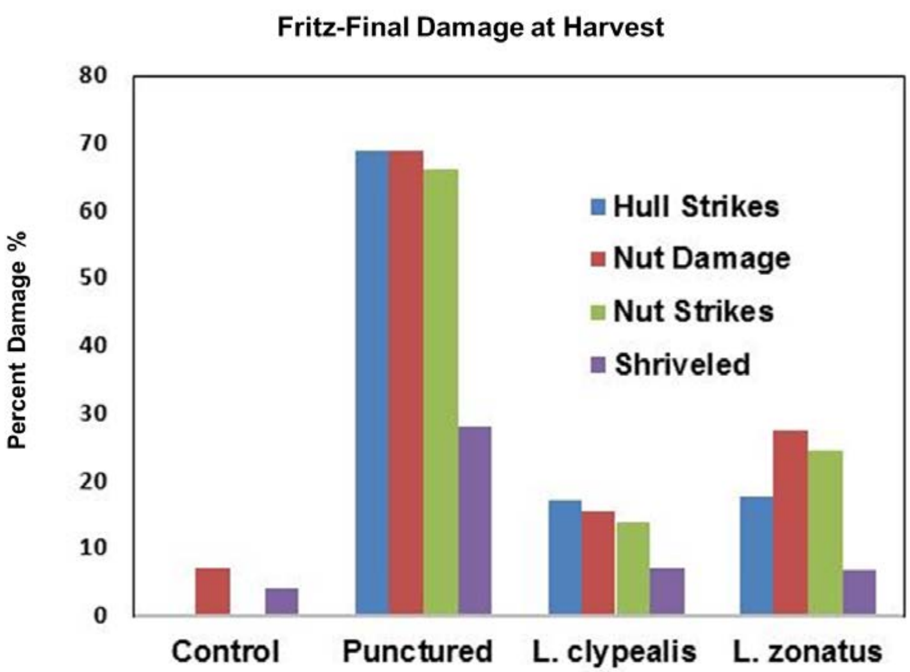

Figure 7. Final Assessment of Feeding Damage at Harvest-Fritz Almonds

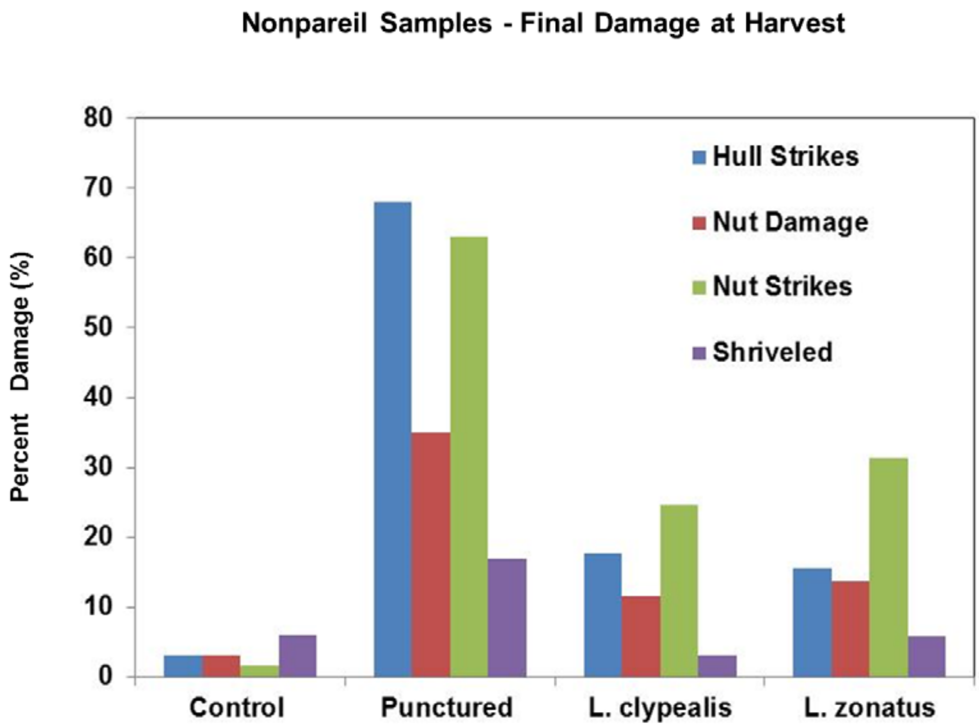

Figure 8. Final Assessment of Feeding Damage at Harvest-Nonpareil

Research Effort Recent Publications:

This work is intended to be for two articles. One article will focus on genetic diversity of leaffooted bugs in almonds and on alternate host plants. The second article will summarize the damage assessment from leaffooted bugs feeding on almonds.

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