Early Detection of Leaffooted Plant Bugs and Stinkbugs in Almond Orchards

Project No.: 15-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 almond 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 than in pomegranate, while *L. zonatus* was the dominant species in pomegranate, but in 2014 and 2015 became abundant in almonds and pistachios as well. 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. In 2014, both *L. zonatus* and *L. clypealis* were caged on almond branches to determine damage levels. In 2015, only *L. zonatus* were abundant enough for field-cage studies. The 2014 control branches had a low rate of almond 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. In 2015, a similar pattern of almond vulnerability to LFPB feeding was observed. The final damage from *L. zonatus* to Fritz and Nonpareil was about twice the level observed in 2014. 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 three species of stinkbugs which 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 1, Table 1**). We identified the leaffooted bugs to species, and looked at the abundance by host plant.

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). Methods were described in more detail in the 2015 report, but a short data summary will be provided here in the Results section.

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

The goal of this objective was to simultaneously investigate nut drop, the gummosis response, and damage which occurs 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. The field cage damage study was conducted in Merced County in Winton and Merced from March through mid-August 2014, and was repeated in March-August 2015.

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* (3 female/ 2 male) 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. Thicker or thinner hull width could influence vulnerability to leaffooted bug feeding.

Field-cage setup in 2015:
A few variations were needed as we ran the field-cage study in 2015. We had three experimental treatments, which were a) controls, b) mechanically damaged-punctured almonds and c) *L. zonatus* feeding in field cages. We did not have the fourth experimental treatment, *L. clypealis* feeding on branches, as we did not have the colony in 2015. In 2015, four almond varieties were included, rather than five. In 2014, we had Fritz, Nonpareil, Monterey, Carmel and Sonora, but the data from Sonora was not complete, due to a shortage of both leaffooted bug species. In 2015, we removed Sonora from the field–cage study and used Fritz, Nonpareil, Monterey and Carmel varieties. Growers asked if we could continue cage-feeding studies past May, the time when almonds were too hard to puncture with a pin, in order to test whether the almonds are really too hard for bugs to feed on and damage. In 2015, the field-cages with *L. zonatus* feeding were run a few additional weeks and continued into June.

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. The 2014 allowed comparison of feeding damage from both leaffooted bug species, while 2015 measured damage from *L. zonatus*.

Data Collection-Part 2 Final Damage Assessment

After bugs fed for 3-5 days, the insects were removed but field cages remained on the branches until harvest. Just before harvest, we use our field-caged branches to conduct a final assessment of almond nut drop and damage in our 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 the following parameters; 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.

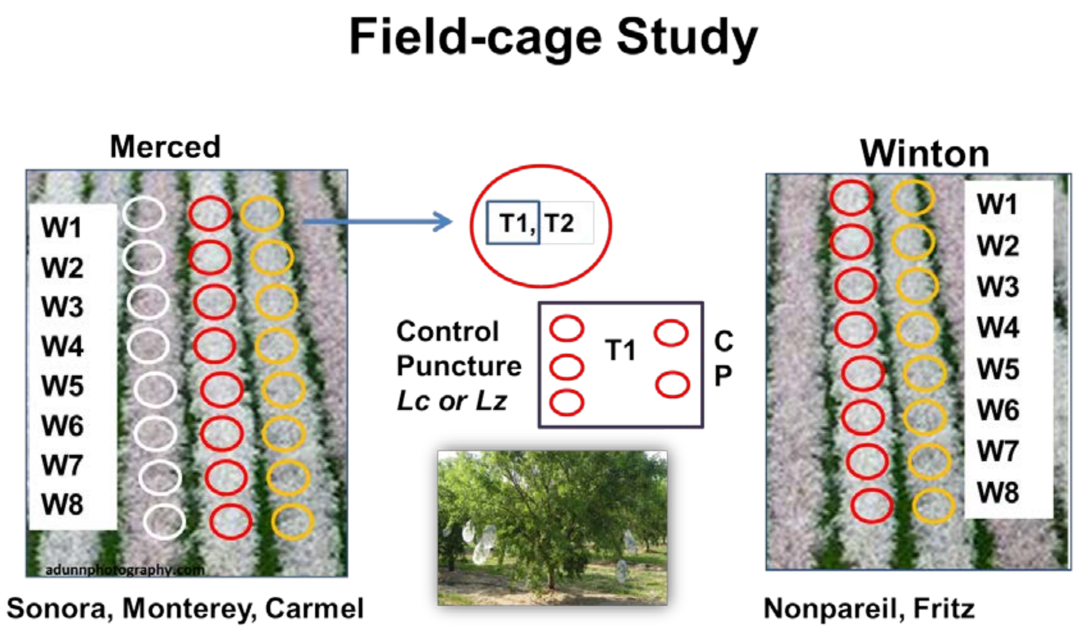

Figure 1. Set up field-cage study to assess adult leaffooted bug feeding damage

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, many of which are included in **Figure 2** and **Table 1**. In 2014, 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 was not expected; *Leptoglossus zonatus* was observed in almonds and pistachio. We did not find any individuals of the leaffooted bug *L. occidentalis* which has 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 its head.

2015: *L. zonatus* was the dominant leaffooted bug on all three plants. It was not reported in the IPM pages for almonds or pistachios prior to 2013, so it may now be more abundant. *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. *L. zonatus* has a distinct aggregation behavior which has not been observed for *L. clypealis*.

Figure 2. Map of many of the Leaffooted bug collection sites in California

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

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		

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 (Joyce et al. 2014). Mitochondrial DNA confirms the presence of two genetically divergent lineages of *L. zonatus*, both found on pomegranate. The two types of *L. zonatus* are morphologically similar, they appear identical and are 'cryptic species'. In addition, we are using mitochondrial DNA to examine the genetic diversity of three stinkbugs *Acrosternum hilarae* and *Chlorachroa uhleri*, and *Chlorachroa sayi.*

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 that began after April 28 in any variety. Cages with feeding by the other leaffooted bug, *L. zonatus* had 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.

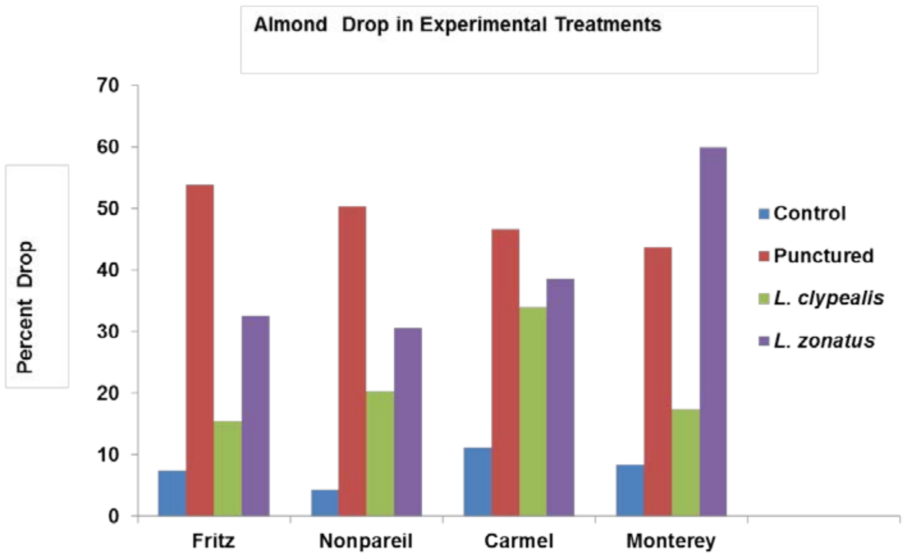

Figure 3. Total Nut Drop in Almond Varieties in 2014, with two species of Leaffooted bugs feeding

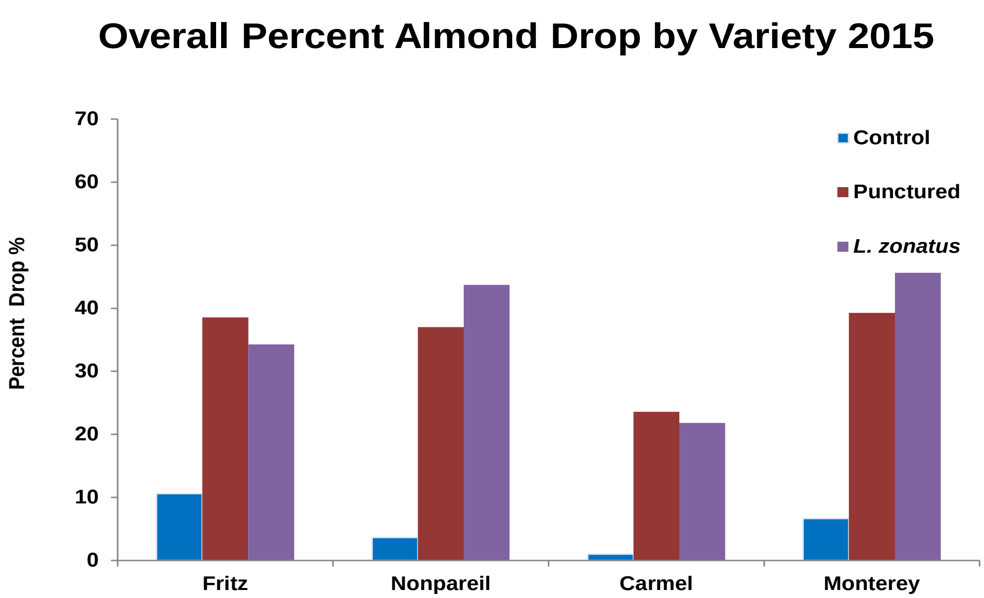

Figure 4. Total Nut Drop in Almond Varieties in 2015 with *L. zonatus* feeding

2015** Field-cage study:* ***Part 1 Nut Drop

We included graphs from both years for comparison. In 2015, only *L. zonatus* was available to assess feeding damage (**Figure 4**). Control cages in 2015 had similar levels of almond drop to 2014, about 5-10%. Punctured almonds in 2015 had 30-40% drop, which was lower than in 2014 when 40-50% dropped from trees. Comparing the almond drop for *L. zonatus* between years, 2014 and 2015 almond drop were relatively similar for Fritz and Nonpareil, where *L. zonatus* feeding resulted in 30-40% almond drop in cages. The results for Carmel and Monterey varied more between the two years. In 2015, about half the number of almonds dropped from Carmel and Monterey as occurred in 2014 (**Figure 4**). In 2015, about 20% almond drop occurred in Carmel and 40% in Monterey.

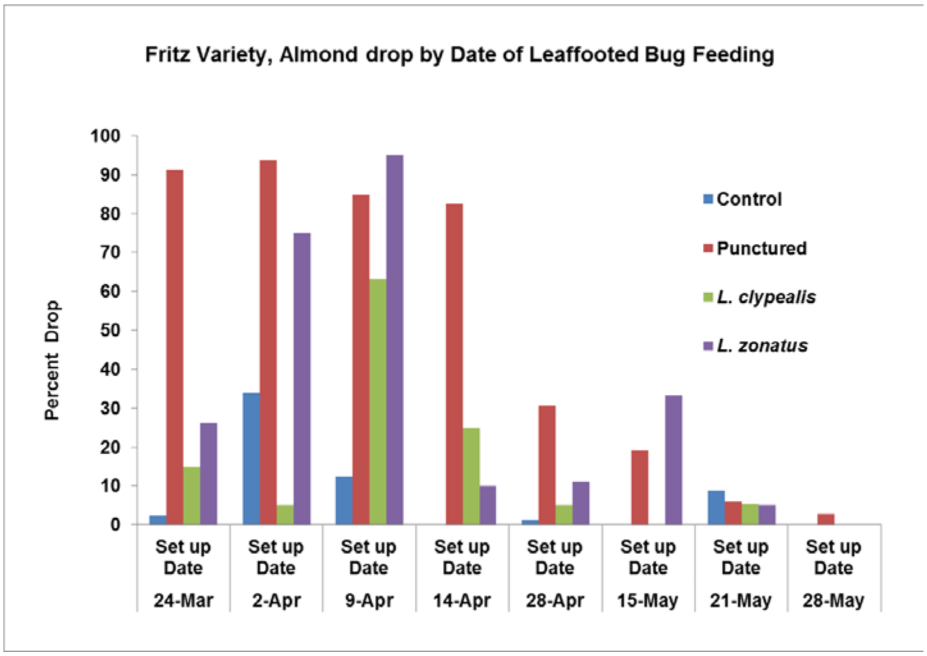

Figure 5. Dates in 2014 for almond branches that were exposed to bug feeding in Fritz.

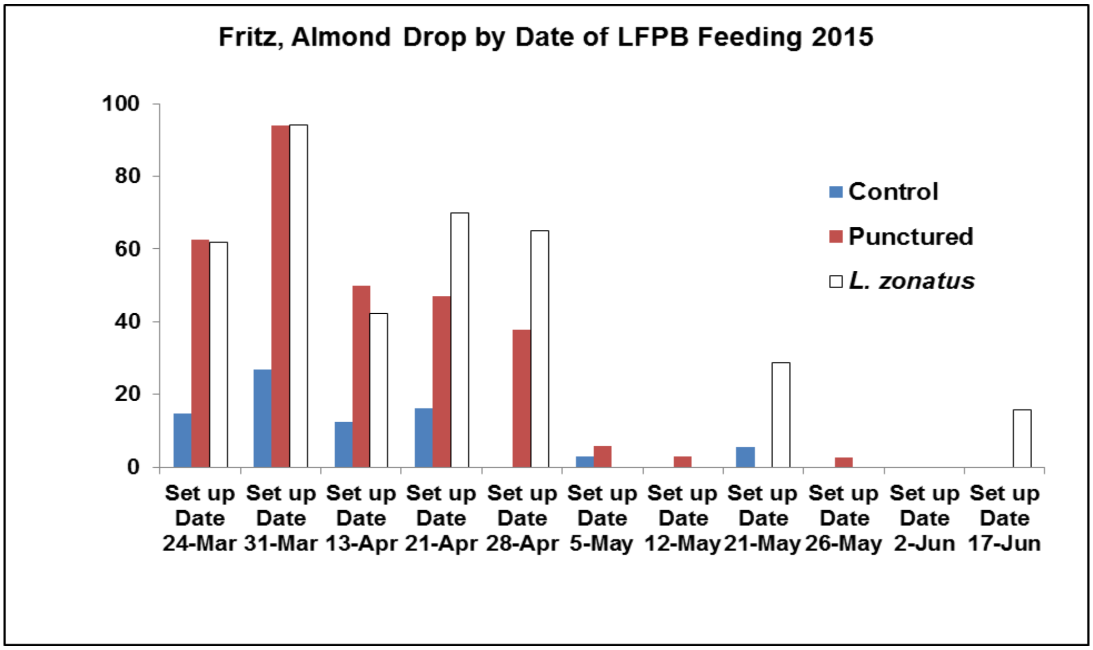

Figure 6 Dates in 2015 for almond branches that were exposed to bug feeding in Fritz.

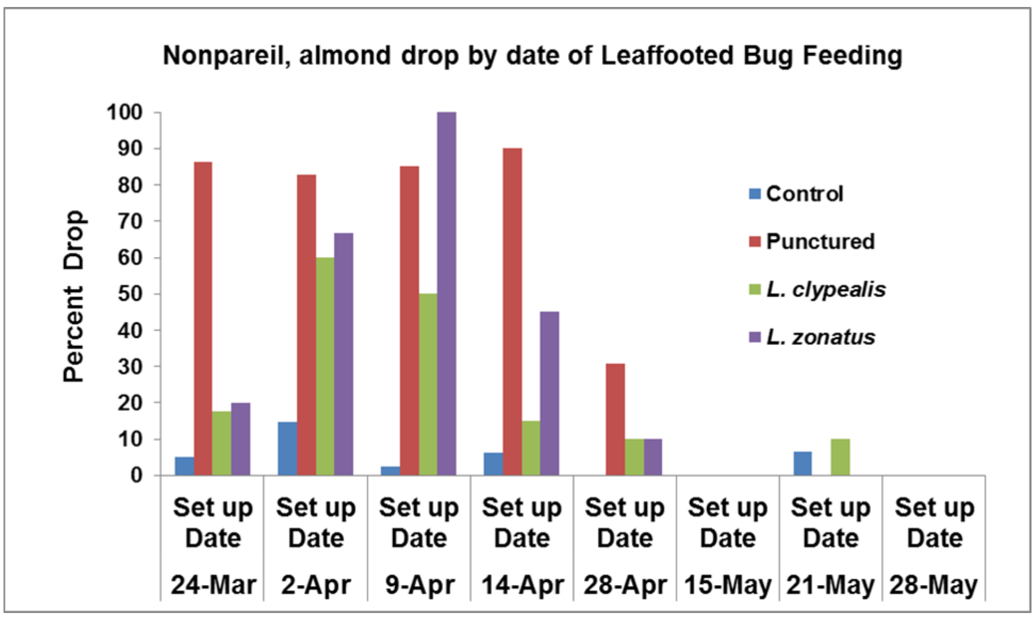

Figure 7. Dates that 2014 for almond branches were exposed to bug feeding in Nonpareil.

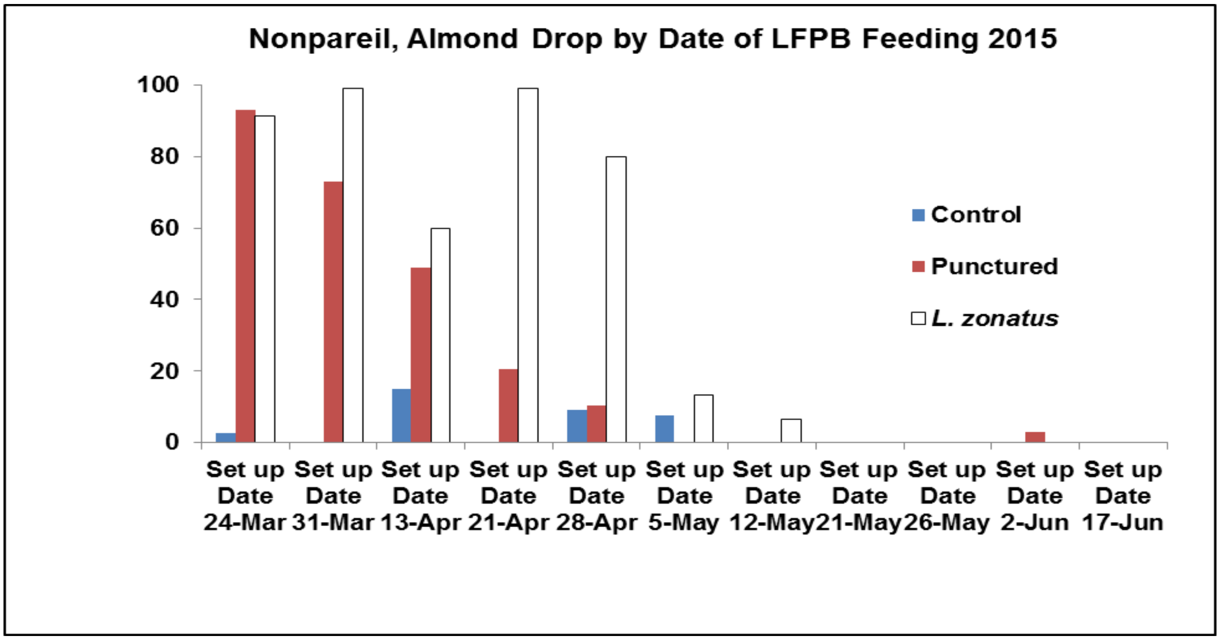

Figure 8. Dates that 2015 for almond branches were exposed to bug feeding in Nonpareil.

Time from Exposure to Bug Feeding until Nut Drop on branches

Comparing 2014 and 2015

Fritz: Figure 5 and 6 show the date the experimental cages for the treatments that were set up in Fritz for 2014 (**Figure 5**) and 2015 (**Figure 6**) (See page above for Fritz graphs). The experiment was set up this way so that we could examine the vulnerability of different age almonds to leaffooted bug feeding. In 2014, for 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. In 2015, the feeding damage from *L. zonatus* on Fritz generally had a similar pattern of damage. From late March through late April, *L. zonatus* feeding resulted in significant almond drop, which was highest on March 31 and April 21. The almonds matured in the field faster in 2015 than in 2014. In the Fritz controls, almost no almond drop occurred after April 28th, and it was difficult to mechanically puncture the almonds after this date as well. However, *L. zonatus* feeding after the shell hardening date could result in some almond drop, which was observed when bugs were allowed to feed in field-cages on May 21 and June 17.

Nonpareil is compared between 2014 and 2015 in **Figures 7 and 8**. In 2014, almonds also had the highest drop from bug feeding on April 2 and 9 (**Figure 7**). The general pattern of susceptibility to LFPB feeding was from late March to about May 1. After May 1, almost no almonds dropped in controls or from puncturing or bug feeding. Even LFPBs that were caged in June on nonpareil did not result in any almond drop.

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 2016.

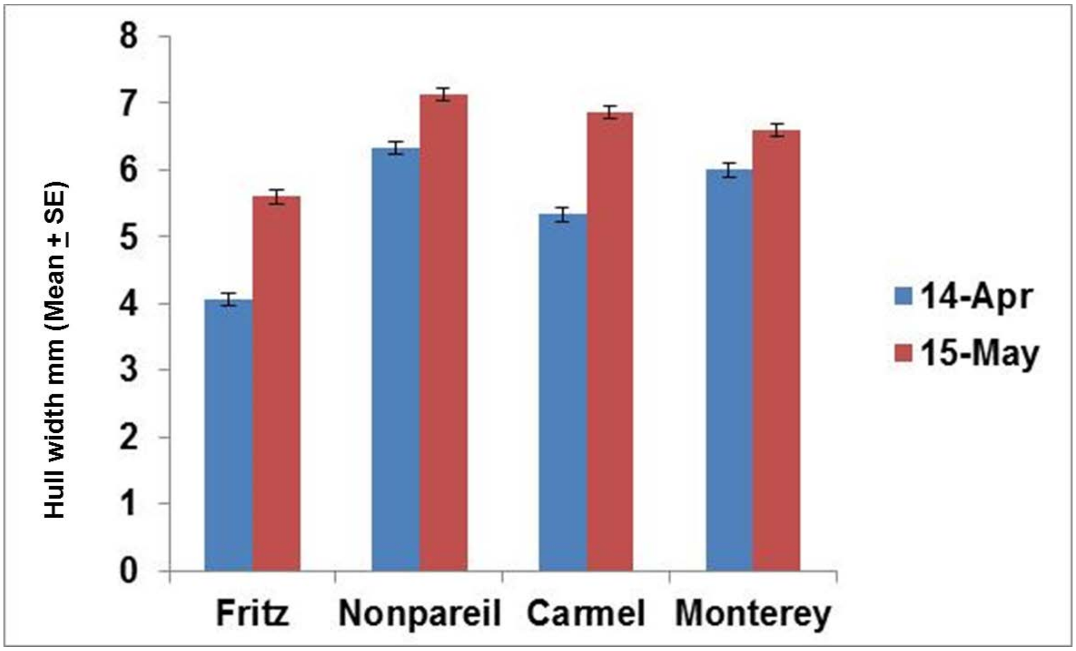

Figure 9. Data from 2014 field cage study. Almond hull width at two of the dates bug feeding occurred by *L. clypealis* and *L. zonatus.*

Hull Width, 2014 compared to 2015:

For 2014, we found that there was a significant difference in the hull width of Fritz and Nonpareil (**Figure 9**). However, they were both vulnerable to leaffooted bug feeding until around April 15th. Carmel and Monterey varieties had thinner hulls than Nonpareil earlier in the season, yet they remained vulnerable to leaffooted bug feeding for several weeks after Nonpareil and Fritz. The 2015 hull width comparisons are shown in **Figure 10**. As seen in 2014, there is a significant increase in hull width over the course of a month. Hull width was measured every week that samples were set up, but is shown here on two sample dates for simplicity. In 2014 and 2015, Fritz had the thinnest hull width (**Figure 9 and 10**). Nonpareil had a thicker hull overall than Fritz for both years. Monterey and Nonpareil did not vary much between 2014 and 2015. However, the Carmel hull widths were thicker in 2015 than in 2014. Less almond drop was observed in Carmel in 2015 than in 2014, perhaps partially due to the thicker hull widths of the almonds.

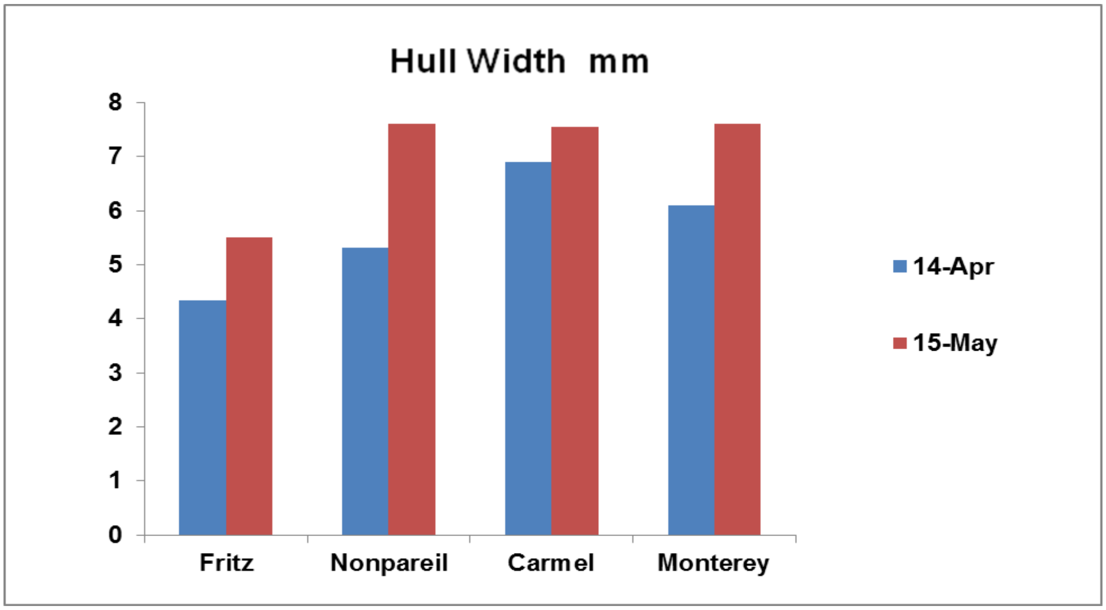

Figure 10. Data from 2015 field cage study. Almond hull width at two of the dates of bug feeding by *L. zonatus.*

Part 2 Final Damage Assessment

2014 and 2015 Field-cage study: We measured the final damage to the almonds in the field cage at harvest time. We took a subsample of nuts from each branch. Hull strikes, nut damage, nut strikes and nut shriveling were recorded. For 2014, for Fritz almonds, control nuts had no evidence of hull or nut strikes (**Figure 11**), and less than 5% had nut damage or were shriveled. In bug feeding treatments on Fritz, almond damage and nut strikes occurred in about 15% of almonds with *L. clypealis* feeding and about 25% of almonds with *L. zonatus* feeding, with about 5% shriveled in both bug feeding treatments (**Figure 11**). In 2015, the final assessment of Fritz found twice as much nut damage, nut strikes (up to 40%) and shriveled almonds (~10%) as occurred in 2014 (**Figure 12**).

The 2014 Nonpareil almonds had controls with low damage, 5% or less (**Figure 13**, see next page). The cages where bugs had fed in Nonpareil had 15-20% overall damage. The 2015 Nonpareil assessment of *L. zonatus* feeding damage found double the level of damage observed in 2014, with nearly 40% nut damage and nut strikes, and 20% shriveled (**Figure 14**).

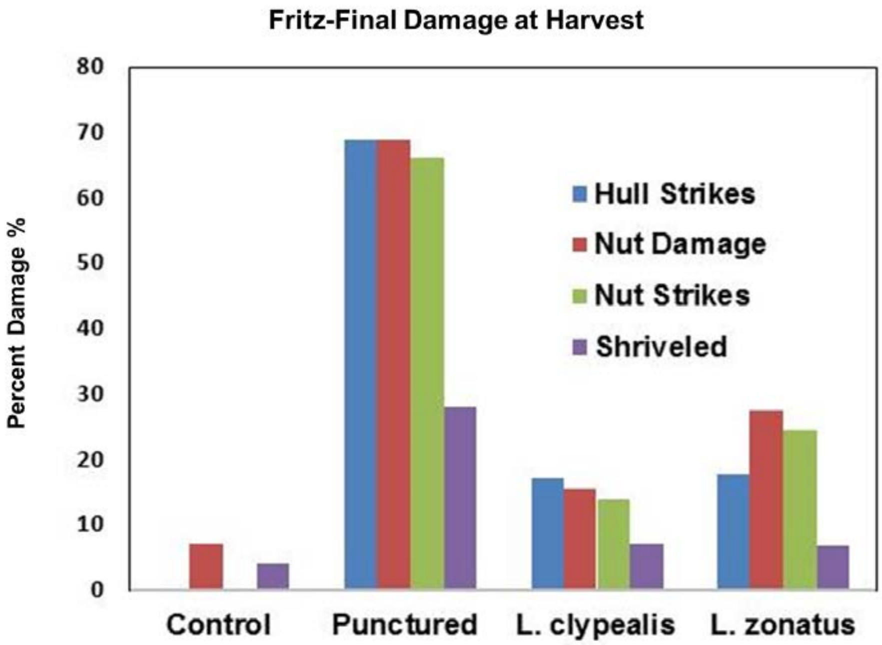

Figure 11. For 2014, the final Assessment of Feeding Damage at Harvest, Fritz Almonds

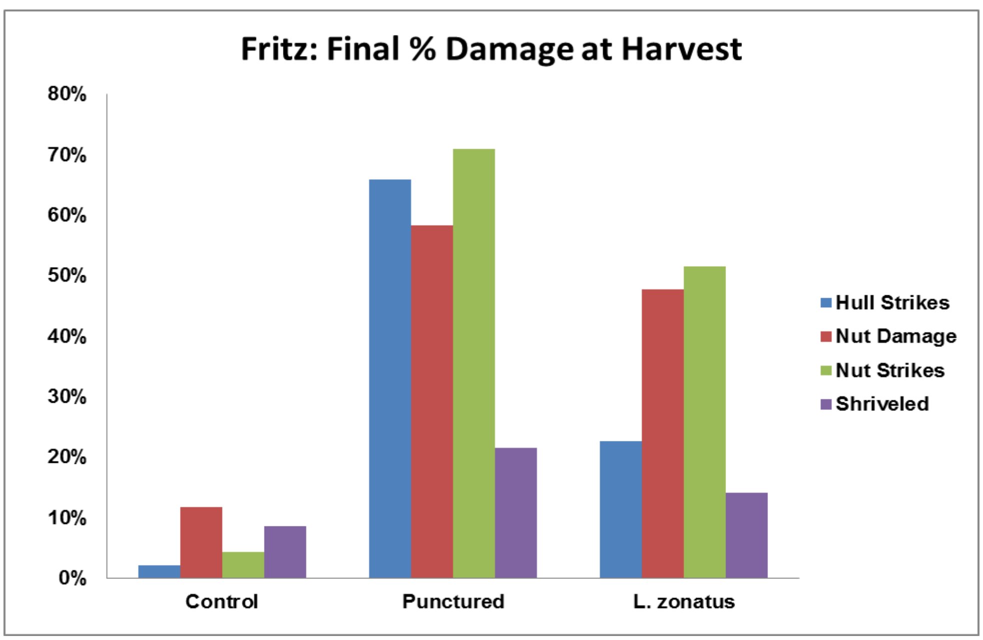

Figure 12. For 2015, the final Assessment of Feeding Damage at Harvest, Fritz Almonds

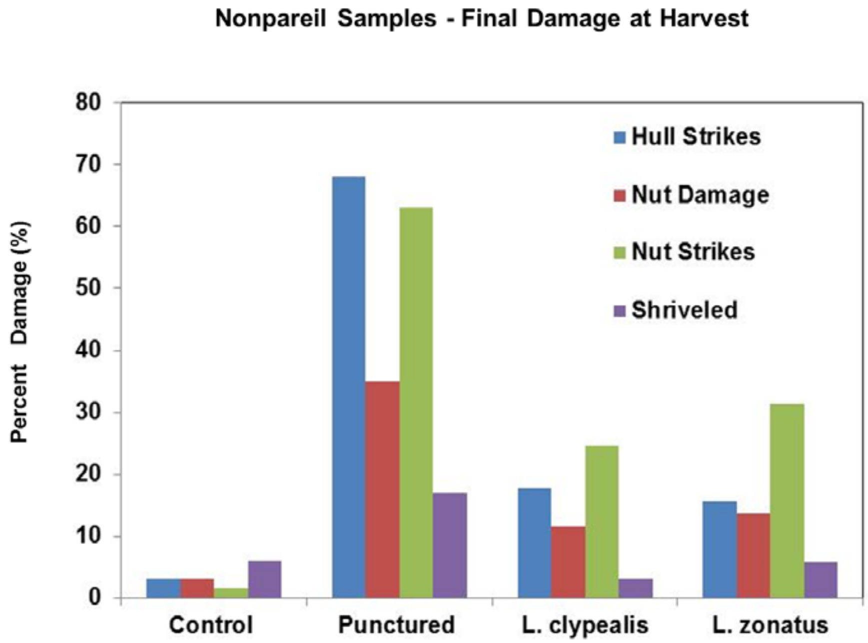

Figure 13. For 2014, the Final Assessment of Feeding Damage at Harvest-Nonpareil

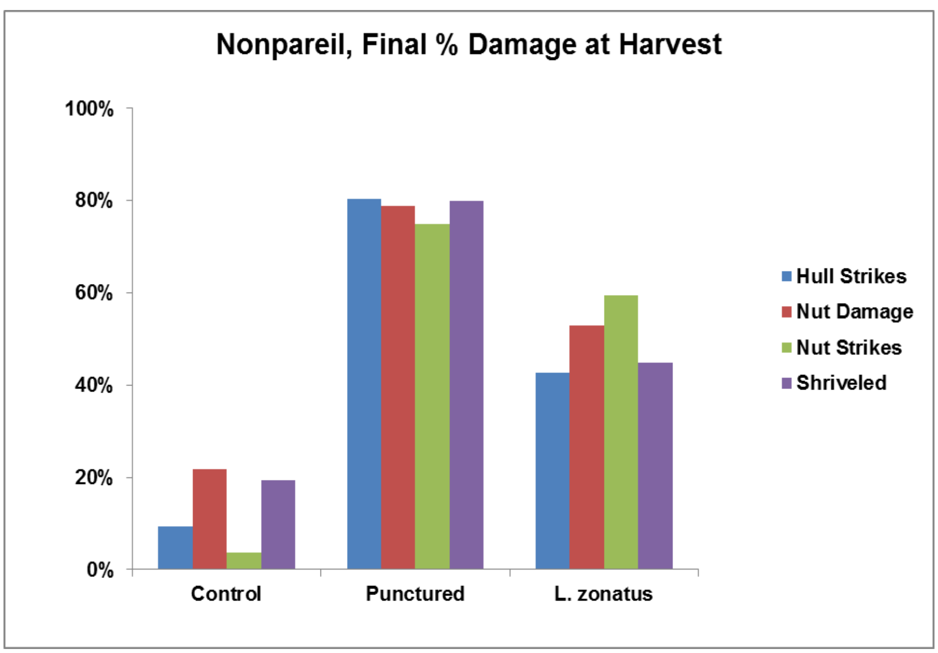

Figure 14. For 2015, the Final Assessment of Feeding Damage at Harvest-Nonpareil

Research Effort Recent Publications:

This work is intended to be for two articles. A manuscript on population genetic structure of *L. zonatus* and *L. clypealis* on almonds, pistachios and pomegranate is currently in preparation. The second article will summarize the damage assessment from leaffooted bugs feeding on almonds in 2014 and 2015.

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