

## Predicting leaffooted bug outbreaks to improve control

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### Summary:

In spring 2006, many almond and pistachio orchards had significant crop loss from adult leaffooted bugs (*Leptoglossus* species). In the 2007 crop season, we investigated leaffooted bug biology in order to develop more predictive assessment of winter populations and potential spring damage. Our specific objectives were to (1) develop sampling protocols for winter and spring populations of leaffooted bug; (2) investigate overwintering cues that result in leaffooted bug aggregation, and determine if these aggregations can be manipulated for monitoring or control; and (3) determine the potential number of leaffooted bug generations per year, and when the adult population begins and ends its overwintering period.

Winter samples were taken in almond, pistachio and pomegranate orchards. By late fall, no nymphs were found in the nut orchards, whereas large numbers of nymphs were found in the pomegranate orchard. Adult leaffooted bugs were observed migrating from the orchards to winter shelters, to form aggregations, in November. Average aggregation size was 12.5 adults; the aggregations were usually in a warm area, such as the sunny side of the citrus tree, or a protected area, such as underneath the bark of a palm tree. Low temperatures in December killed all of the leaffooted bugs located in exposed aggregations (e.g., citrus branch) while those in protected aggregations (e.g., palm tree) survived. Movement back into the nut orchards began in late March and by the first week of April damage nuts were found. Sampling for damaged nuts was far more effective than searching for adult bugs.

Our attempt to manipulate winter aggregations by providing artificial shelters was not successful. Laboratory work suggested that aggregations were formed when evening temperatures cooled were near or below 5°C (41°F). Other cues that promote aggregations or that are used by the adult bugs to fly to an aggregation site were not determined and will be the subject of further studies.

At ambient temperatures (Fresno County) there were three generations per year. The overwintering adults began depositing eggs in mid-March, with maximum production between April and mid-May. Egg hatch was as long as 45 days (April to May) and as short as 5 days (August). Egg production ceased in October, with the onset of cooler temperatures and shorter day-lengths. These data are similar to the 2006 trial and strongly suggest there are only three annual generations. What is critically important for nut and pomegranate growers is the number of adults vs. nymphs going into the overwintering period. Overwintered adults survived with or without food during the entire winter period, while small and large nymphs were unable to survive the winter, regardless of the food supplied. These results are discussed with respect to overwinter mortality and the size of the leaffooted bug population in spring time.

## Introduction

Many Hemiptera, or true bugs, are capable of causing direct damage to almond or pistachio nuts. Most of these pests are present early in season, especially the "small" bugs, which include several species of Miridae and Rhopalidae (e.g., *Lygus*) (Daane et al. 2005). Small bug feeding results in considerable nut drop and epicarp lesion damage in April and early May, before shell hardening; however, in most years this damage is negated by plant compensation. The second group is composed of species of Pentatomidae (the stink bugs) and Coreidae (leaffooted bugs). These "big or large bugs," especially the leaffooted bug, can cause the same damage as their smaller relatives during the first half of the season. More important for nut damage levels is that after the shell begins to harden in May, they can still penetrate the shell and cause damage to nutmeat, although the amount of penetration and damage is significantly lower than in the early- or mid-season periods (Daane et al. 2005).

In spring 2006, many California almond and pistachio orchards had significant crop loss from one particular large bug group and stage – adult leaffooted bugs (Daane et al. 2006). The occurrence of damaging leaffooted bug populations covered a wide geographic range, including nut producing regions from the northern, western, and southern Central Valley. However, leaffooted bug densities and damage varied considerably, with some growers reporting significant bug densities and more than 50% crop loss, while other growers reported little crop damage. Regardless, spring 2006 was the worst leaffooted bug year over the past 20 years. High levels of damage resulted, in part, because many growers did not realize until after damage occurred that large leaffooted bug populations had entered their orchards.

In the 2007 crop season, we investigated leaffooted bug biology in order to develop more predictive assessment of winter populations and potential spring damage. Our specific objectives were to (1) develop sampling protocols for winter and spring populations of leaffooted bug; (2) investigate overwintering cues that result in leaffooted bug aggregation, and determine if these aggregations can be manipulated for monitoring or control; and (3) determine the potential number of leaffooted bug generations per year, and when the adult population begins and ends its overwintering period.

## Procedures

*1) Sampling winter and spring populations.* Different types of pest sampling methods are used to predict pest densities. The most common is a direct count of individuals on the plant leaves or branches, caught in pheromone or sticky traps, or collected via sweep nets or beating trays. These methods are not very effective for spring-time leaffooted bug populations, which move into the orchard as adults (Daane et al. 2006) and are difficult to sample (Daane et al. 2000). To develop better sampling protocols for winter and spring periods, we surveyed orchards and observed the number, density and winter survival of leaffooted bug populations. We report here on winter 2006/07 data, as the study is ongoing for the current 2007/08 season.

In fall 2006, almond, pistachio, and pomegranate orchards were selected that had histories of leaffooted bug damage. The five sites monitored were located in Madera and Tulare counties;

each site was surrounded by potential overwintering locations for the leaffooted bug (foothills, eucalyptus trees, palm trees, a citrus orchard).

November and December leaffooted bug populations were assessed in the orchards by visually surveying 100 trees for a 1 minute period. Observations were made by circling each tree and recording the number of leaffooted bug adults, and randomly checking 10 nuts for damage, indicated by sap exuding from a feeding wound. These observations were made at eye level. A wooden pole (4 ft long) was used to beat the higher branches, causing the leaffooted adults to take flight, for a second visual survey of each tree. This was only effective when temperatures were high (70°F).

After documenting winter leaffooted bug populations in the cash crop, the surrounding area was searched for possible overwintering aggregations, such as Italian cypress, Eucalyptus, barns, palm trees, citrus trees, etc. Once found, these sites searched and leaffooted bug aggregations were marked for repeated visits to record the number of leaffooted bugs per aggregation. Observations of these aggregations were taken every 2-3 weeks during the winter.

2) *Winter aggregations.* Leaffooted bugs often spend the winter period in aggregations of 5-2000 adults, clumped together in a sheltered area. Along with monitoring field sites for these aggregations, we also attempted to manipulate aggregations of leaffooted bug adults in three orchards.



Photo 1. Screened cage with artificial aggregation of adult leaffooted bugs, provided with food (peanuts) and water. The aggregations were either: females only, males only, or both sexes.



Photo 2. Wooden shelter, which housed the artificial leaffooted bug aggregations, were placed in orchards to help determine if adult leaffooted bug dispersion and aggregations could be manipulated

In the 2006/07 winter trial, we housed 20 leaffooted bugs in wire cages, provisioned with food (peanuts) and water (photo 1). These artificial aggregations were then placed in an artificial shelter, a “leaffooted bug overwintering chamber,” constructed of four 1 × 2 ft. plywood sections stacked on each other with a tapered 0.5 to 1 inch spacing between the sheets (photo 2). The goal was to create a protected location where the bait (other leaffooted bugs) would attract resident

leaffooted bugs to form overwintering aggregations. The baits tested were groups of caged leaffooted bugs: (i) adult males and females, (ii) adult males only, and (iii) adult females only, as well as a (iv) no leaffooted bug control. Twelve overwintering chambers were placed in each of three fields, with 3 chambers for each treatment. The chambers were placed on trees at a height of 0.5 to 1.5 m. The treatments were randomly assigned to the chambers. The chambers were checked each month (November to January) for resident leaffooted bugs that moved into the chambers.

In the second study, we used large (10 × 12 ft.) cages to manipulate leaffooted bug aggregations. In each of the four corners of the cage a potted tree was placed as a “substrate” for the leaffooted bugs to form an aggregation upon. We then added to an artificial aggregation of caged leaffooted bug adults to one of the trees, using either (i) males and females, (ii) only males, or (iii) only females. In the center of the large cage, leaffooted bugs were released in the morning and their movement was monitored at 1100 and 1600 hrs on the release day and then at 0800 and 1100 hrs on the following day. The trial was repeated every other day to produce eight replicates. For each trial, the position of the caged leaffooted bugs was randomly changed.

**3) Leaffooted bug seasonal development.** We utilized small cages to collect information on leaffooted bug development. At the Kearney Agricultural Center, adult leaffooted bugs were collected during the winter. This cohort was placed into small tree cages placed outside and held at ambient temperatures. The leaffooted bugs were fed peanuts and provided with an Italian cypress and fresh green beans as food and structural support. During each egg-laying period, additional cages were established for each consecutive generation produced. We then determined the longevity of the adults, their egg-laying period, and the number and length of each successive generation.



Photo 3. Container with adult leaffooted bug females with food (green beans and peanuts) and water (cotton wick).

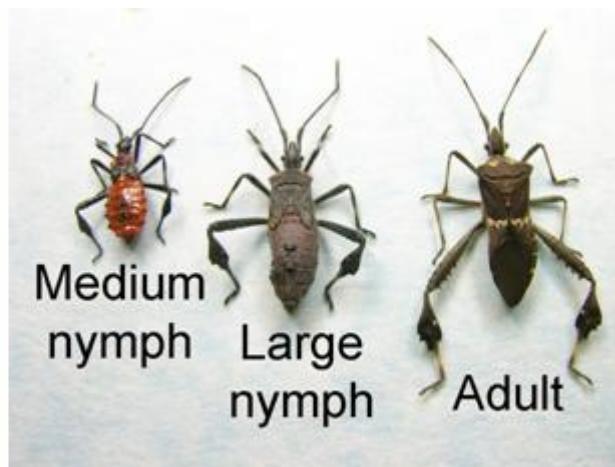


Photo 4. Different sizes of leaffooted bugs tested for overwinter survival and development.

To determine the number of eggs produced per female under ambient temperatures (Fresno County), 20 newly molted females were mated and isolated in plastic containers (0.5 L), provisioned with water, peanuts and fresh green beans (photo 3). The number of clusters, eggs per cluster, and adult female longevity were recorded every 1-4 days.

To determine if all stages can overwinter – with or without food – medium and large nymphs and adults (Photo 4) were collected in early winter, placed in containers provisioned with food (beans and peanuts), and held outside (Fresno Co.) from December 2006 to April 2007.

## Results and Discussion

**1) Field samples.** November (2006) visual samples of 100 orchard trees (1 min. per tree) found no leaffooted bug aggregations, and only isolated adults. No nymphs were found in the nut orchards, whereas large numbers of nymphs were found in the pomegranate orchard.

No adult leaffooted bugs were observed forming aggregations in or near the almond and pistachio fields monitored, and monitoring at these sites was discontinued.

Adult leaffooted bugs were observed in the pomegranate field in November, with more than 60% of the trees surveyed with leaffooted bug nymphs or adults. Adults were observed leaving the pomegranate field in November as well, and forming aggregations under the bark of nearby Eucalyptus and palm trees, as well as on the foliage of citrus trees in an adjacent commercial field. The citrus field was monitored for winter aggregations. The number of aggregations found per 100 citrus trees searched ranged from 12.5 (11 January 2007) to 4.0 (18 January 2007) when including both live and dead (on plant or ground) leaffooted bugs.

In November, there were several aggregates of 15 or more adults tightly clustered on the terminals of citrus branches. The aggregations were usually on the sunny side of the tree and towards the outside of the tree. Leaffooted bug populations (aggregations) on citrus ranged in size from 1 to 34. These aggregations were marked for repeated sampling. The average size (leaffooted bugs per aggregation) varied over the sampling period from  $12.2 \pm 3.2$  (30 November 2006) to  $1.8 \pm 0.6$  (11 January 2007).

After each period of low temperature from December through January, the leaffooted bug populations dropped in density and we observed dead bugs on the ground, below the aggregations on the citrus above. After the hard freeze in mid-January, no live leaffooted bugs were observed on the citrus (Figure 1).

After the hard January freeze, no live leaffooted bugs were found in the citrus orchard. However, these aggregations were exposed, typically on the exterior branches, where the low temperatures were extreme. A further search of the region found live leaffooted bugs in aggregations in the more protected regions – under the bark of the palm trees and Eucalyptus trees.

On 26 March 2007 we resumed monitoring in the almond, pistachio and pomegranate orchards. For the nut crops, the original search method (100 trees, 1 min. per tree) was not efficient. After noting that most damaged nuts and leaffooted bugs were on the south (warmer) facing side of the tree, a faster and more efficient sampling technique was developed. Walking down the rows, a 15 second was conducted on only the south facing side, looking initially for damaged nuts, which could have been from many different bug species. However, we found that leaffooted bugs were

commonly still next to damaged nuts as it was still early in the season and relatively cool. Commonly, the leaf footed bugs were observed mating or feeding on nuts, without moving far from other damaged nuts. This proved more effective and we were able to document the period of increased bug activity. On 2 April, five adults and 10 damaged nuts were found in three fields; on 11 April we found 24 adults and over 100 damaged nuts were found (most were in at one site). The surrounding citrus orchards were sampled at the same time but no bugs were observed, suggesting that the migration into nut crops because at the end of March to the beginning of April, under 2007 conditions.

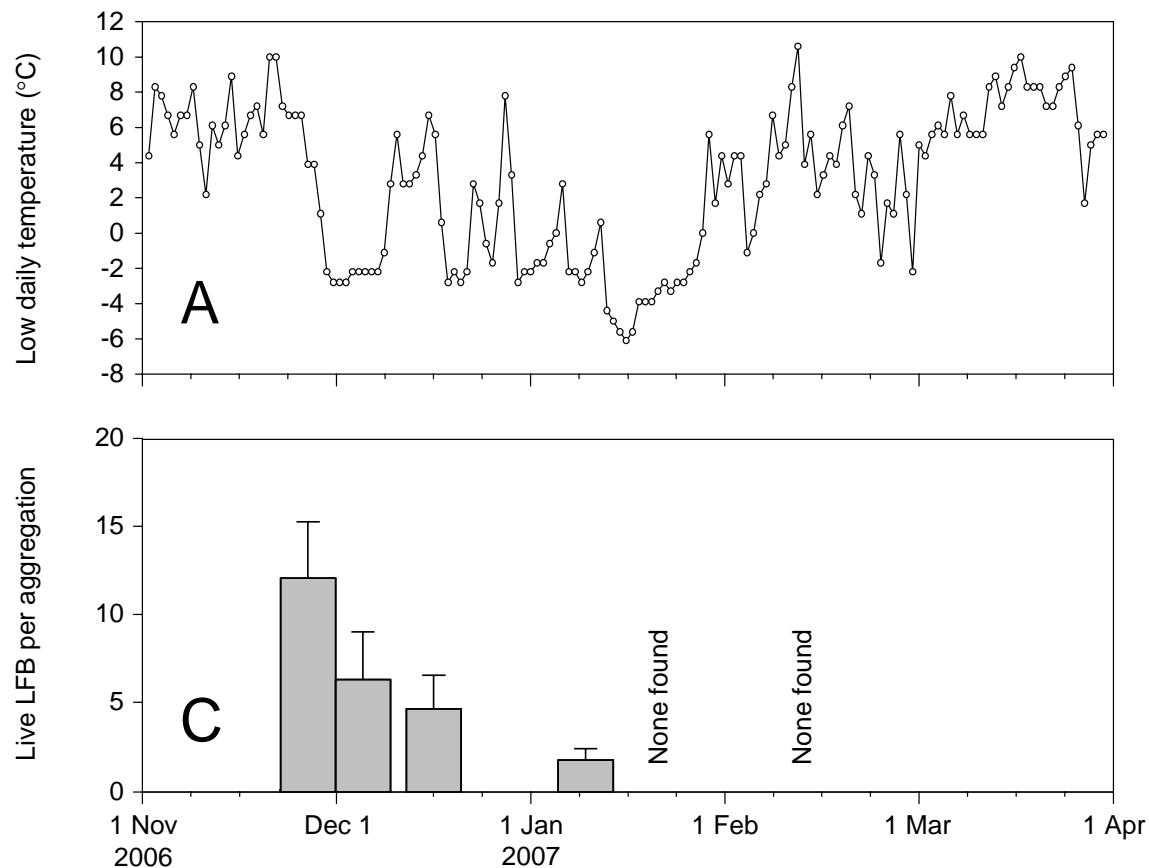


Figure 1. (A) Low winter (2006/07) temperatures for Porterville (Tulare County) and (B) the number of live leaffooted bugs per aggregation site in a citrus orchard monitored from November 2006 through March 2007. Results show a drop in live leaffooted bugs after the hard freeze in mid-January.

The results provide strong evidence that the large and damaging leaffooted bug populations in spring 2006 were the result of a large fall 2005 population that had lower than normal winter mortality (2005/06) as a result of mild winter temperatures. The results also raise other important questions.

First, what caused so many of the bug aggregations to form on the exposed citrus branches, where there was less protection from temperature extremes? The high daily November and December temperatures provided plenty of opportunity for the adults to move from the branch terminals to more protected locations inside the tree, or to even fly to better shelters. However, once the aggregations formed in November, leaffooted bugs tended to remain right where they were. Individuals not in aggregations often did not move to find and join a nearby aggregation. Second, can the winter mortality be used to predict spring populations? In February 2007, we predicted that spring leaffooted populations would be average or lower than average. This prediction appears to have been valid, based on grower reports of 2007 insect damage. Currently, we are testing whether bug densities can be monitored during the winter in various locations throughout the state in order to provide an “early warning” for years with high bug densities. The accumulated data may show a simple correlation to winter temperatures, which can be used as a predictive guide rather than field monitoring.

Third, can these aggregations be manipulated? We suspect that leaffooted bug aggregations form in response to a combination of (i) a food source, (ii) a shelter, (iii) pheromone, (iv) vibration or sound cues (such as the call of a cricket) and/or (v) low temperatures. Understanding these cues and the bug response may help develop programs that can manipulate aggregations for control, such as attract and kill, or monitoring. We note here that (pheromone work should be done by a pheromone chemist, and Dr. Jocelyn Millar (UC Riverside) has done extensive work with bug pheromones and vibration cues.

**2) Winter aggregations.** The attempt to manipulate field populations of leaffooted bugs to form winter aggregations in artificial shelters was not successful. Result found few resident leaffooted bugs were attracted to the artificial aggregations and no true aggregations of resident bugs were formed.

The obvious conclusion is that our artificial shelters and/or treatments were not attractive; this, however, may be inaccurate. It is as likely that the shelter were placed into the field too late as we observed, while establishing the plots, that much of the dispersing leaffooted bug population moved in October and early November. For this reason, we believe the adult dispersion from almond, pistachio and pomegranate orchards may have occurred before we set up the chambers and that, once formed, adult leaffooted bugs simply remained in these original aggregations. Another possibility is that our artificial shelters lack some key ingredient in the formation of aggregations. The conclusion that we can not make is that the cues tested (leaffooted bug males and females, males only, and females only; and the shelter and food only) are not involved with formation of winter aggregations.

In the second study, we used large (10 × 12 foot) cages to manipulate leaffooted bug aggregations. In each of the four corners of the cage a potted tree was placed as a “substrate” for the leaffooted bugs to form an aggregation upon. We then added to one of the trees an artificial aggregation of caged leaffooted bug adults.

Results from the 2006/07 winter trials were similar to the field trial – freely released leaffooted bugs did move towards the aggregation cage in any of the trials. As before, we suspect that

either the design is flawed, or that the trial was conducted too late in the season (February). This design was repeated in winter 2007/08 and while the trial is ongoing, we have observed aggregations form, although not always near the caged leaffooted bugs.

In summary, aggregation cues might be a combination of changing climate, from summer to fall, as well as an insect cue, such as an aggregation pheromone, sound, or vibration cue (or a combination of many cues). To date, our experiments have not, however, provided shown what these cues might be. We note that the aggregations observed in the cages were formed in the early morning hours and, as the temperatures warmed each day, the insects would begin to break from the aggregation and move about the cage. Eventually, the aggregations formed and did not bread apart.

**3) Leaffooted bug seasonal development.** There were three generations per year. Through the cage study, we investigated the longevity and population dynamics of leaffooted bug held at ambient temperatures (Fresno County) and supplied a constant diet of food (green beans, peanuts, Italian cypress) and water. In each generation there was a large population increase, more than the cages and provided food could support and, for that reason, there were 1000s of leaffooted bugs produced and 1000s that died.

Figure 2 shows the “average cumulative production” for each life stage (a more complete data analysis will be completed after the project is completed). Twenty-five female overwintering adults were used to begin the trial, and this is the only group that is shown as actual density rather than cumulative density (Fig. 2A). The overwintering adults began depositing eggs 15 March, with maximum production between 1 April and 15 May 2007. Some of the overwintered adults survived until the cages were cleaned out in July, although egg mass production ceased in late June. Each egg mass contained about 15 individual eggs. Nymphs hatched within 20 days of egg production. Development from 1<sup>st</sup> to 5<sup>th</sup> instar stages required about 45 days during the spring period, with most adults produced between 5 and 20 June.

A portion of the adults produced from the first generation were used to begin the next series of cage trials. These adults lived, on average, from mid June to mid August, producing eggs during this period, with peak production in July. Nymphs hatched from late June through August, almost all becoming adults before mid August. A portion of the adults produced were used to begin the next series of cages (Fig. 2C). These 2<sup>nd</sup> generation adults began to produce egg masses during the first week of August and, while they remained alive in fall, the ceased egg production at the beginning of October. The nymphs hatched began mid August and continued into December. Nymphs from the earlier egg hatching period (August to September) became adults from early September through October. Many of the nymphs from the smaller egg hatching period in October never developed to adults. The 3<sup>rd</sup> generation adults produced were placed in cages to being the fourth development set, however, to date (15 December 2007) none of these have produced an egg mass.

These data are similar to the 2006 trial and strongly suggest there are only three annual generations. What is critically important for nut and pomegranate growers is the number of adults going into the overwintering period. The mild fall conditions in 2007 provided enough

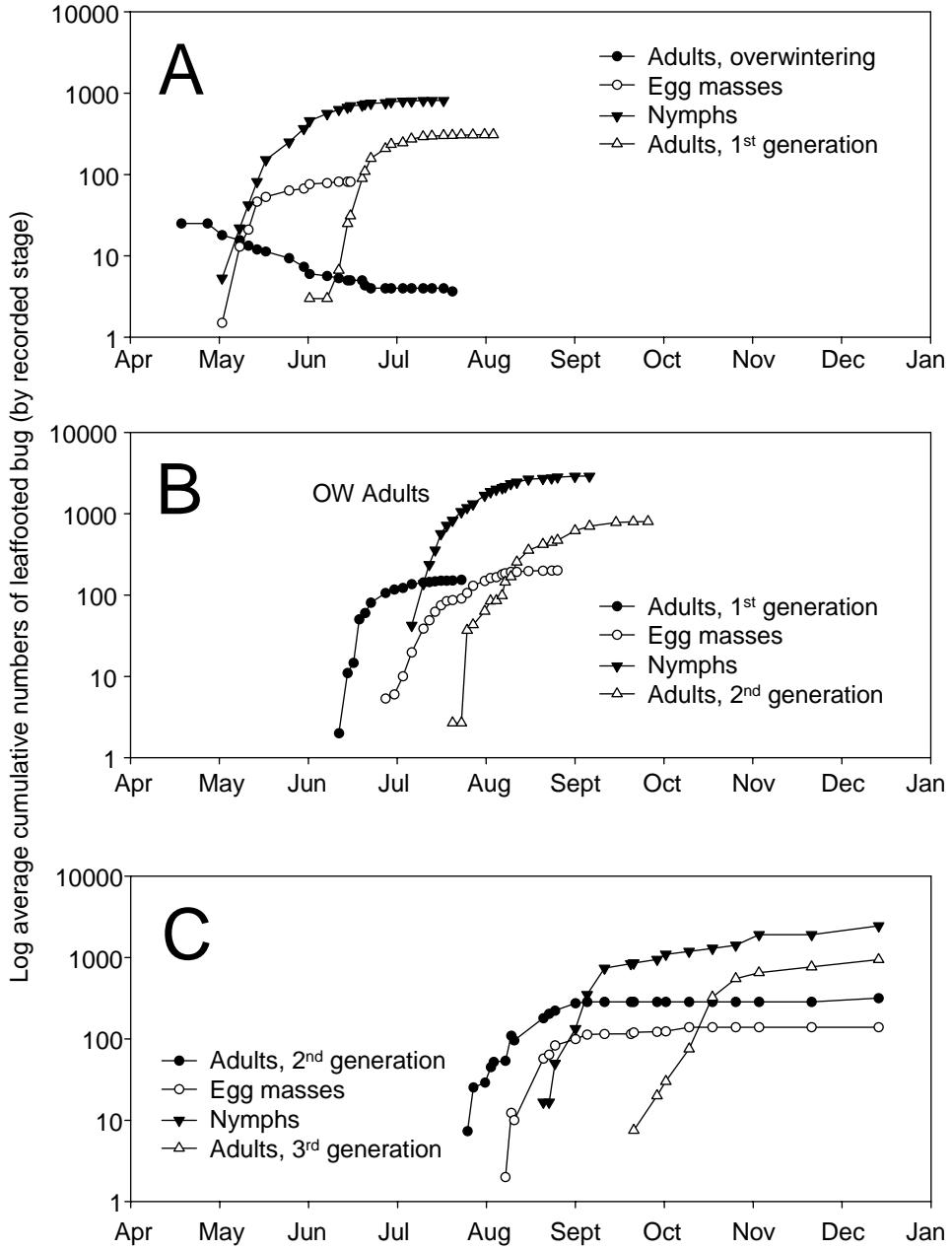


Figure 2. The average “cumulative” leaffooted bug density by development stage for each of three 2007 generations. The bugs were held in cages and provided food (green beans, peanuts and Italian cypress) and water. Note that the average number of overwintering females (Fig. 1A) is provided, showing the gradual decrease in their density.

warm temperatures for many of the 2<sup>nd</sup> generation adults to deposit egg masses in August and September, for those eggs to hatch during September, and for the resulting nymphs to develop to the adult stage. In contrast, we observed that many of the late developing nymphs in fall 2006 did not make it to the adult stage. These individuals would have been unable to fly to a shelter and form aggregations during the winter – and would most certainly have died. For that reason,

we see a larger potential overwintering leaffooted bug population in 2007/08. Low winter temperatures from December through February will determine the spring population size.

The overlap of leaffooted bug stages makes insecticides applications based on short residual insecticides very difficult because there are always adults that can migrate into the orchard. Typically, control is based on April or May applications of chlorpyrifos (e.g., Lorsban), pyrethroids, or permethrin to kill overwintering adults that have migrated into the orchard. The biggest concern with these products is the potential to flare spider mites later in the season. In general, control in June is not needed because populations of overwintering adults have declined and most nymphs are too small to penetrate into the kernel.

Under ambient conditions (Fresno County), overwintered leaffooted bugs held outside produced egg masses over an 8 week period, from the end of March through mid June (Fig. 3). Adults produced early in the season (from the overwintered female offspring) produced eggs from late June to August. Second generation adults produced late in the season produced eggs for a shorter period in September. Third generation adults produced eggs for a similar period. Results from this experiment are suspect as adult longevity was very short, probably resulting from temperatures inside the plastic containers or lack of fresh food. The experiment shows that the overwintered adults live and produce eggs during the important March through May period, when nuts are most susceptible. Because they produce eggs during this period, they are most likely going to continue to feed – and it is the large adults rather than their offspring that can cause the most damage to nut crops.

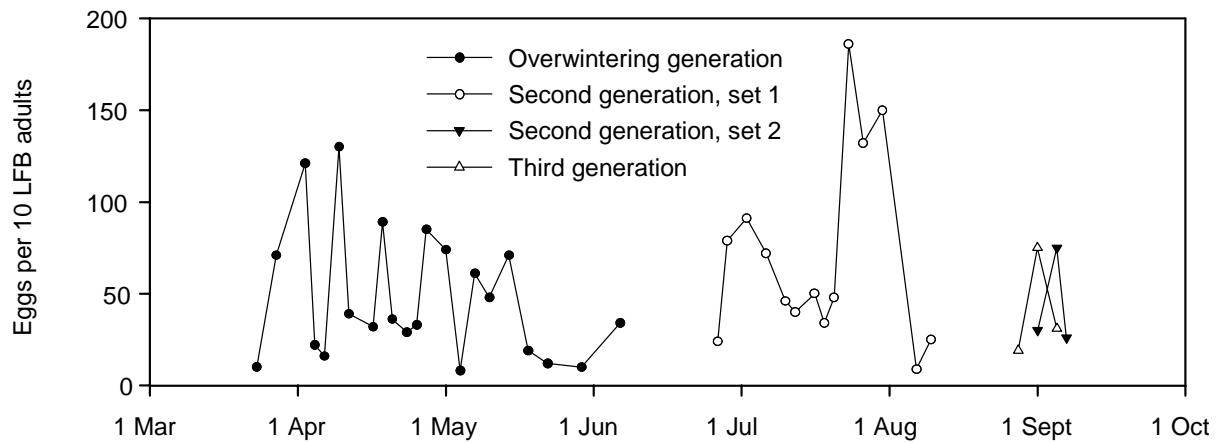


Figure 3. Egg production of overwintering, second generation and third generation leaffooted bugs held in plastic containers and at ambient weather conditions (Fresno County).

Overwintered adults survived the winter with or without food during the entire winter period, under ambient conditions in Fresno County (Fig. 4). Eggs were produced by adults both with and without food. Small and large nymphs were unable to survive the winter, regardless of the food supplied. These results suggest that the adults can overwinter in any protected shelter (e.g., protection from cold weather) even without food. This suggests that pump houses, barns, thrash

piles are all potential host sites. Without food, there was greater mortality of adults, but still 20% survived the winter period.

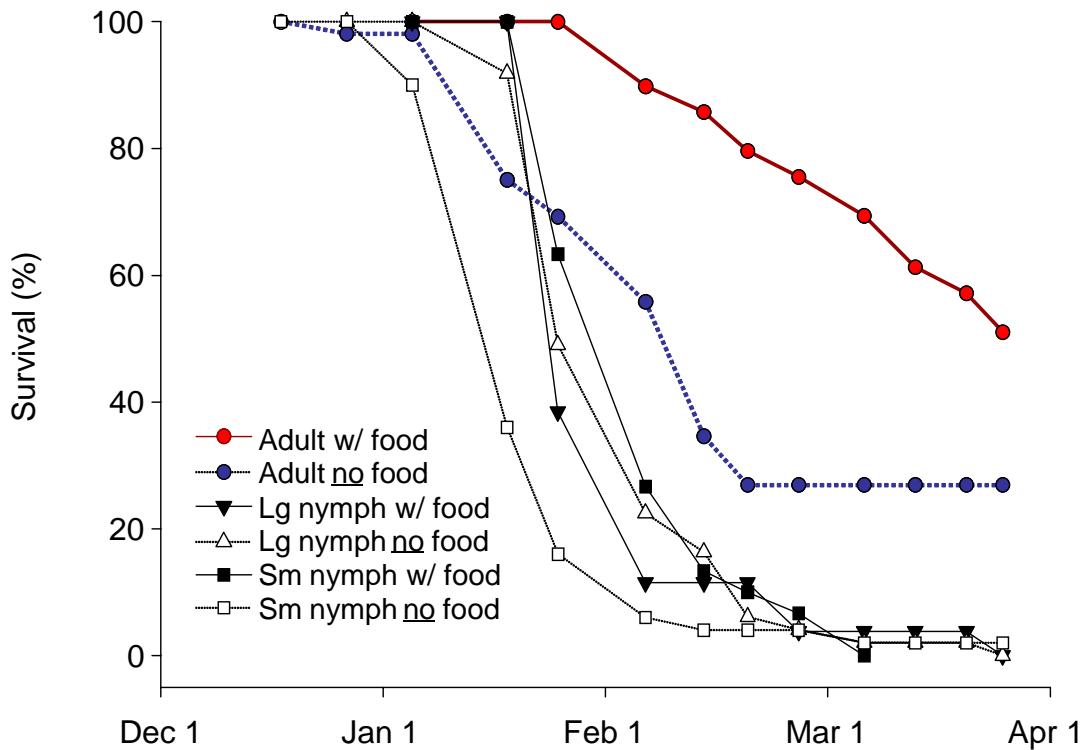


Figure 4. Overwintering survival of three states of the leaffooted bug (small nymphs, large nymphs and adults) held with and without food.

#### Conclusions and Practical Applications.

Our previous work (2000-2002) on the leaffooted bug showed that leaffooted bug feeding can cause a considerable number of dropped nuts early in the season (April), but that most of this damage is “compensated” for when the tree drops fewer nuts to set the crop load. Cage trials suggest that May to early June is a critical period for damage – when dropped nuts represent lost crop (after fruit set) and the damaged nuts that remain on the tree often have unmarketable nut meats (kernel necrosis or stigmatimycosis). Late season damage (late June to harvest) was more difficult to categorize. There was often no epicarp lesion to provide an outward signal of bug feeding, lesions that did form were often apparent only weeks after the initial damage occurred, and many of the punctures did not penetrate to the nut meat.

Our current work (2006-2007) is in response to the 2006 damage in pistachio and almonds? In most years, leaffooted bug populations do not reach damaging levels – we suspect from a combination of biotic (a small egg parasitoid) and abiotic (climate extremes) mortality. However,

many California almond and pistachio orchards had significant crop loss from adult leaffooted bugs in spring 2006, particularly from late March through early May.

Our work has shown that damage occurred from overwintering adults that move into the orchards from nearby winter aggregations, typically in protected areas, such as in woodpiles, barns, under the bark of eucalyptus trees, in cypress trees or juniper trees. They are very strong flyers and will disperse from the aggregation into the orchard in March and April.

Winter studies conducted in 2006 and 2007 suggest that a number of factors determine the size of the overwintering populations, but the three most important are:

- (i) The number of nymphs that develop to the adult stage in fall and early winter from eggs deposited by the second and third generation adults will determine the size of the overwintering populations. In years with a cold or wet fall period, a large number of nymphs will not make it to the adult stage and will die during the winter.
- (ii) Cold winter temperatures result in mortality of a majority of the overwintering leaffooted bugs. We suspect that cold spells of low evening temperatures near -5°C (23°F) kill most of the exposed leaffooted bugs.
- (iii) The abundance of overwintering shelters near the orchard will provide protection from cold temperatures. Growers should seek out potential shelters areas near their orchards and first check for leaffooted bugs to treat the overwintered populations, and consider removing or reducing these shelters.

What can be done to improve leaffooted bug control? A major issue is sampling the overwintered population to help predict potential damage. There are few effective sampling programs for adult leaffooted bugs in the pistachio or almond orchard (Daane *et al.* 2000). This is critical because the adults are strong flyers and can quickly move into the orchard in large numbers and cause significant damage in a short period of time. Our studies in winter 2006 and 2007 suggest that December through February samples of winter populations densities and age structure (nymphs vs. adults) can help to predict damage in springtime. We successfully predicted a low 2007 spring population of leaffooted bug and damage, and we will provide similar notice in February 2008, after finishing winter samples.

We suspect that the overwintered population forms aggregations, and these aggregations are in response to either: (i) a food source, (ii) a shelter, (iii) a pheromone response (such as a sex pheromone or an aggregation pheromone), or a (iv) calling signal (such as a vibration). We attempted to manipulate these aggregations to improve control options, such as attract and kill, or and sampling. A number of experiments were attempted, but we were unable to manipulate leaffooted bug behavior. Currently, evidence suggests that cold temperatures are an important cue for leaffooted bugs to cease egg-laying and begin to form aggregations. However, other cues would be needed to draw separate adults in from many different trees to form the tight aggregations, and we have not yet determined what those cues might be. These studies of winter aggregations and bug biology will continue.

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