Developing an Early-Season Monitoring System for Leaffooted Bug on Almond

Project No.: **15-ENTO14-Tollerup**

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Objectives:

1. Develop indicators that provide an early-season mechanism for estimating leaffooted bug (LFB) population densities.
   a. Continue work to determine minimum temperature survival threshold for LFB.
2. Develop an efficient and effective sampling method for LFB on almond.
   a. Monitor overwintering aggregations of LFB and movement of LFB into almond.
   b. Determine effectiveness of color sticky traps as a method to monitor early-season dispersion of LFB into almond.
3. Determine if host-plat volatiles play a role in LFB aggregation behavior.
   a. Evaluate and determine if specific host-plant volatiles can be employed for monitoring LFB.

Interpretive Summary:

Although a sporadic pest, leaffooted bug (LFB) infestations can have a substantial negative impact on nut yield and quality (Daane, et al. 2007). In this study, we aimed to improve the understanding of how LFB moves from overwintering sites into almond and improve early-season LFB monitoring tools. Results of this study indicate that cold winter temperatures that historically occur in the San Joaquin Valley can have a substantial negative impact on overwintering LFB populations. Survival of leaffooted bug significantly decreases beginning at a low temperature of 26.6°F and exposure period of six hours. Albeit, additional work is needed, our data suggests that the occurrence of cold winter temperatures can affect the relative pest pressure of LFB on almond during the spring. Having such information, will aid growers and pest control advisors in making more efficient LFB management decisions.

Leaffooted bug aggregations and stink bug infestations were not found in sufficient numbers to fully address objective 2. However, in an effort to address the objective, we located large LFB aggregations in pomegranate in November 2015. Monitoring began in Mid-February and the number of individuals in aggregations averaged 33.4 ± 28.3. More detailed aggregation data were not collected due to unusually warm weather during mid and late February causing dispersal much earlier than expected.

We monitored adjacent citrus, and olive crops and found that LFB did not move into those crops. Also we intensely monitored orchards with adjacent planting of almond and citrus; and almond, citrus, pomegranate, and pistachio and observed no LFB in any of the crops except pomegranate. Interestingly, it appears that some LFB remained in pomegranate and successfully produced a generation.

In an effort to develop an early-season monitoring tool, we evaluated clear, green, red, yellow, and white colored sticky traps. The traps were placed in almond, pistachio, and pomegranate orchards in Kern County and monitored from mid-March to early June. The sticky trap colors that we evaluated did not attract nor trap any LFB and thus would not provide an effective monitoring method of adults moving into almond from overwintering sites.

As a preliminary study, we baited clear sticky traps with Peterson navel orangeworm bait. The Peterson bait (PB) is comprised of whole-ground pistachio (WGP) contained in a small mesh bag. Leaffooted bug accumulated on the PB but only on the clear sticky traps in pomegranate. And, although both adults and nymphs were observed, only nymphs became trapped in the adhesive and thus such baited sticky traps would not provide an effective monitoring tool.

Our objective to determine if a plant volatile or volatiles can be used to attract LFB and /or has a role in facilitating LFB aggregations is part of a long-term project. In our initial experiments we evaluated if the volatiles from almond, corn, olive, peanut, and walnut oils attracted nymphs and adults. None of the oils evaluated attracted either of the life stages at any detectable level.

Based on our results of baiting clear sticky traps with PB, we evaluated additional plant material, whole almonds (WA), whole-ground almond (WGA), and whole peanut (WP). Baits were tested in modified navel orangeworm traps (Trécé, Adair, Oklahoma) (**Figure 1**). The idea was that gravid females would be attracted to the bait and lay eggs on the trap close to the food source provided in the trap. In pomegranate, we counted approximately 2-fold and 7-fold more adults on the WGA – baited traps than on the WA or PB-baited traps respectively. And although females did not lay eggs on the modified NOW egg traps, the concept of using a plant volatile lure to monitor LFB and LFB egg-laying activity as they move into almond during early spring holds promise and should be investigated further.

Materials and Methods:

Objective 1. Estimating population densities
We continued conducting a series of low-temperature growth chamber experiments using adult LFB. A single replication consisted of 10 individuals at approximately a 50:50 male / female ratio placed in an arena constructed from a 540 ml container (FABRI-KAL Corp, Greenville, South Carolina) plastic food cup with a nylon screen were placed over the top. In this series, we evaluated LFB survival after being exposed to temperature treatments of ~5, 10.4, 15.8, 21.6, and 45° F for periods of 2, 3, 4, or 6 hours. We replicated each treatment six times.

Objective 2a. Developing sampling methods
Originally we aimed to monitor five almond orchard sites located in Kern, Fresno, and Butte counties for LFB and additional large bug species. We did not locate aggregated populations of leaffooted bug as well as infestations of stink bug species, consequently we could not fully

address the objective. As an alternative to this objective we located large aggregated populations at two pomegranate orchards, both in Tulare County. At the Lindcove Station, site 1, the pomegranate orchard bordered citrus. At site 2, the pomegranates bordered citrus, olive, and newly-planted pistachio. Thirty-second visual counts of aggregations on 18 pomegranate trees began in February. The movement of LFB from the aggregations into the associated citrus and olive crops was monitored at least every 10 to 14 days. We monitored the citrus and olive orchards neighboring the LFB aggregations at sites 1 and 2 on 15 dates between late March and mid-July using 30-sec per tree visual counts. We monitored on both the outer and inner portion of tree canopies and therefore modified the sampling protocol. The original method called for from using 8-foot bamboo poles to knock the branches in the upper canopy and counting the number of individuals that flew.

Also, we began monitoring LFB at two additional sites located in Fresno and Madera counties, sites 3 and 4 respectively. Site 3 consisted of 60 acres of almond, with a history of LFB damage and approximately 40 acres of citrus. The site also borders riparian habitat and a large eucalyptus grove. Site 4 consisted of almond (60 ac), citrus (20 ac), pomegranate (40 ac), and pistachio (80 ac).

Beginning in March, we monitored at sites 1, 2, and 3 bi-weekly within the different crops. At site 3, the riparian / almond and eucalyptus / almond interfaces also were monitored. Monitoring at site 4 started during June. At all sites, monitoring continued through September of 2016. Monitoring consisted of 30-sec visual searches at mid-canopy (5 – 6 ft.) from the soil surface) and upper-canopy. We visually searched 10 or 20 trees among four tree-rows separated by at least 100 ft. At four locations in each of the almond orchards approximately 50 nuts beneath 10 randomly selected trees were collected and examined. In addition, 50 in situ nuts on 10 almond trees per location were examined.

Objective 2 b.

We conducted trials to evaluate clear, green, red, yellow and white sticky traps constructed from 6 x 8 in pieces of Plexiglas painted with Krylon (Cleveland, Ohio) spray paint. A randomized complete block design was used to evaluate the most effective trap color; traps were hung at about 5.5 ft. (height was not evaluated as originally proposed). Five blocks were established in each of a pomegranate and almond orchards.

As a preliminary study, we baited 15 of the clear sticky traps, five in each crop with Peterson female navel orangeworm bait (PB) consisting of proprietary ingredients, and whole-ground pistachio mummies contained in a small mesh bag.

Objective 3. Evaluating plant volatiles

Laboratory no-choice bioassays were used to determine if oils of almond, avocado, coconut, corn, olive, peanut, or walnut attracted adult and nymph LFB. Bioassays consisted of placing three adult and three second or third instar nymphs in an arena constructed from an 8 x 8 x 8 aluminum cage enclosed with 20 x 20 aluminum mesh (BioQuip products, Rancho Dominguez, California). One inch pieces of cotton wick were soaked in each oil and presented to the LFB in the arena. Observations were made every 15 min for a period of one hour. The bioassay experiment was terminated after one replication of each oil due to an absence of response.

Based on results from the colored sticky trap evaluation, we deployed a modified trap design constructed from a Trécé navel orangeworm trap with a 9.75" wood shish kabob skewer attached to the side with zip ties. Leaffooted bug often lay eggs along small branches; we attached the skewer in order to provide an egg-laying surface. Traps contained either Peterson bait (PB) consisting of whole-ground mummy pistachio, whole-ground almond (WGA), or whole-ground pistachio (WGP). We deployed 24 sets of three traps (PB, WGA, and WGP) at site 2 in late Feb in pomegranate. At site 3, eight sets of four traps (WGA, WGP, whole-almond (WA), and a blank) were deployed in almond at mid-April. In June, four sets of three traps (WGA, WGP, and ground-peanut (GP) were deployed in almond and pistachio.

Results and Discussion:

Objective 1. Estimating population densities

Our results indicate cold winter temperatures that periodically occur in the San Joaquin Valley can have a substantial negative impact on overwintering leaffooted bug (LFB) populations. Survival of LFB significantly decreases beginning at a low temperature of 26.6°F and exposure period of six hours (**Figure 2**.). No significant decrease, although occurred at exposure periods tested below six hours at both 26.6 and 23°F. An error occurred in the survival data collection procedure at 23.0°F and exposure period of six hours, consequently we did not determine if LFB survival at those parameters decreased similarly to 26.6°F. At 21.2°F, LFB survival decreased to below 50% for each of the exposure periods tested (**Figure 2**). A small percentage of LFB survived at 15.8°F exposed for four hours and as low as 1.4°F exposed for two hours (**Figure 2**). These results support that winter temperature data provide information for predicting the relative pest pressure of LFB on almond during the spring. Our findings concur with those of Daane, et al. (2007). They observed aggregations of LFB in the field on citrus and found that at approximately 27°F survival in overwintering aggregations decreased considerably. They did not, however address the question of exposure period. The caveat to interpreting our results is that actual survival temperature and / or exposure period thresholds in the field could vary slightly up or downward due to micro habitats that occur within large LFB aggregations as well as the environment immediately surrounding aggregations.

Objective 2. Developing sampling methods

At mid-February, mean ± SD aggregation size on 15 pomegranate trees at site 2 equaled 33.4 ± 28.3 individuals. By the following scheduled sample date of March 3, only individual, non-aggregated LFB, were observed consequently numbers of LFB in aggregations could not be counted. Unseasonably warm temperatures occurred during weeks 8 (Feb 14), 9 (Feb 21), and 10 (Feb 28) with mean maximum temperatures reaching approximately 70, 72, and 72°F respectively with maximum temperatures of just under 80°F. Most probably the warm temperatures provided a major cue to disperse.

Interestingly LFB did not move from the pomegranate into the citrus or olive, we sampled over 540 trees over 14 sample dates at site 2; and 440 citrus and 440 almond trees over six sample dates at site 3, and observed essentially no LFB. At site 3, sampling was conducted starting at mid-April to mid-July in almond and citrus, as well as bordering riparian environments and eucalyptus groves. At site 2, a low number of LFB, however remained in the pomegranate reaching (mean ± SD) 2.8 ± 3.7 in early Apr to 0.4 ± 0.9 per tree in late July.

Given the absence of LFB in any of the crops other than pomegranate we could not adequately address this objective. The University of California Statewide IPM program recommends monitoring for LFB by visually inspecting for gummosis on dropped nuts during March through May (Zalom, et al. 2015). Given the cryptic (hit and run) and sporadic nature of this pest, this species is best monitored for using the UC guidelines until a lure and trap system is developed.

From mid-March to late April and over the six sample dates, we did not trap any adult or nymph LFB on the clear, green, red, yellow, or white colored sticky traps. Albeit, the question whether LFB use color as a visual cue and such a cue can be exploited to develop a monitoring trap should not be completely dismissed. Several examples exist of colored insect traps (Bian, et al. 2014, Maxwell 1968, Muhammad, et al. 2013). Moreover, observational data strongly suggest that LFB preferentially aggregate on red, dark red (nearly black), and fungus-infested (dark gray to black) pomegranate fruit.

Objective 3. Evaluating plant volatiles

No detectable attractiveness of almond, corn, olive, peanut, or walnut oil was observed by nymphs or adults in laboratory no-choice bioassays. One reason for the oils not being attractive may be that the processing alters or eliminates attractive volatiles. In future studies, we plan to modify bioassay experiments and evaluate additional compounds.

Peterson bait, WGA, and WGP attracted both nymphs and adults when placed in pomegranate trees at site 2 (**Table 1**). Starting in early April, adult LFB began accumulating on the modified NOW traps. On all sample dates adult LFB were observed on each of the baits (**Figure 3**). On the first two sample dates in April, only adults were observed and a numerically greater number were attracted to WGA, than either WGP, or PB. Although LFB did not lay eggs on any of the baited wooden skewers, first instar nymphs were observed on 18 and 25 April, and 2 May on WGA, WGP, and PB-baited traps respectively. The greatest number of first through fourth instar nymphs were observed on WGA, followed by WGP and PB (**Figure 3**).

It is interesting that LFB were observed on baited traps only in pomegranate at site 2. We hypothesize that the attractive area around the trap is small and LFB must come in close proximity before detecting the food source. The relatively large number of LFB at the site, compared to the other sites increased the likelihood that a trap would be found.

This area of research should continue; in an additional study we dissected 424 overwintering LFB collected between 28 January and 24 March and determined that approximately 50% of females enter aggregations in the fall mated. This implies that once they disperse in the spring they potentially start laying eggs. If an attractive plant volatile can be found, it can be used as a lure in a trap designed to encourage egg-laying. Thus the system could be used in almond as an early-season monitoring tool and potential egg trap.

Each of the baits were tested by placing them in navel orangeworm traps (Trécé, Adair, Oklahoma) modified by attaching a wooden skewer to the side in order to provide an egg-laying surface (**Figure 1**). In February of 2016, we placed, WA, WGA, and WGP – baited traps in almond and pomegranate. In pomegranate, WGA – baited traps attracted all LFB life stages and attracted significantly more LFB than WA or WGP. Females, although, did not lay eggs on the skewers in great enough numbers to be useful as a monitoring tool.

Conclusion

The cryptic, hit and run nature and the sporadic occurrence of infestations makes leaffooted bug a very challenging pest to manage. Currently, our monitoring tools are limited to recommendations of visual inspection for damaged nuts, provided by the University of California IPM Guidelines. Leaffooted bug monitoring recommendations are limited, however in that they do not provide a tool for detecting LFB at the time they begin moving into almond in the early spring. Rather, LFB damage has already occurred before this time. This study has shown that adult LFB arrest on (appear to be attracted to and aggregate) on modified NOW egg traps filled with WGA. Our continuing research will focus on determining if a plant volatile is involved in this behavior. And moreover if any identified volatiles can be exploited as a lure for more effectively monitoring LFB early in the season.

Figure 1. Modified navel orangeworm Trécé egg trap filled with whole-ground almond deployed in pomegranate. Trap has both adult and second instar nymphs on it. A wooden shisha kabob skewer was attached to the side in order to encourage egg laying by adult females.

Table 1. Mean leaffooted bug for 30-second counts on pomegranate and pistachio. Counts associated with traps baited with various baits.

Date	Site 1 Tulare Co Pomegranates mean	± SD	Site 2 Tulare Co Pomegranates mean	± SD	Kearney Fresno Co Pistachios mean	± SD
29-Mar			0.90	2.71		
31-Mar	0					
4-Apr	0.78	1.14	2.60	3.01	0	
11-Apr	0.28	0.51	1.28	1.58	0	
18-Apr	0.55	0.78	2.78	3.68	0	
25-Apr	0.20	0.41	0	0		
2-May	1.70	1.88			0	
9-May	0.30	0.76	0.80	1.32	0	
17-May	0.28	0.51	0.25	0.59	0	
18-May						
7-Jun			0.40	0.78	0	
13-Jun						
14-Jun			0.98	1.70	0	
27-Jun	0.20	0.56	0.03	0.16	0	
1-Jul						
5-Jul					5.10	8.93
6-Jul			0.18	0.45		
11-Jul			0.18	0.45		
18-Jul					0.65	1.09
19-Jul			0.4	0.88		

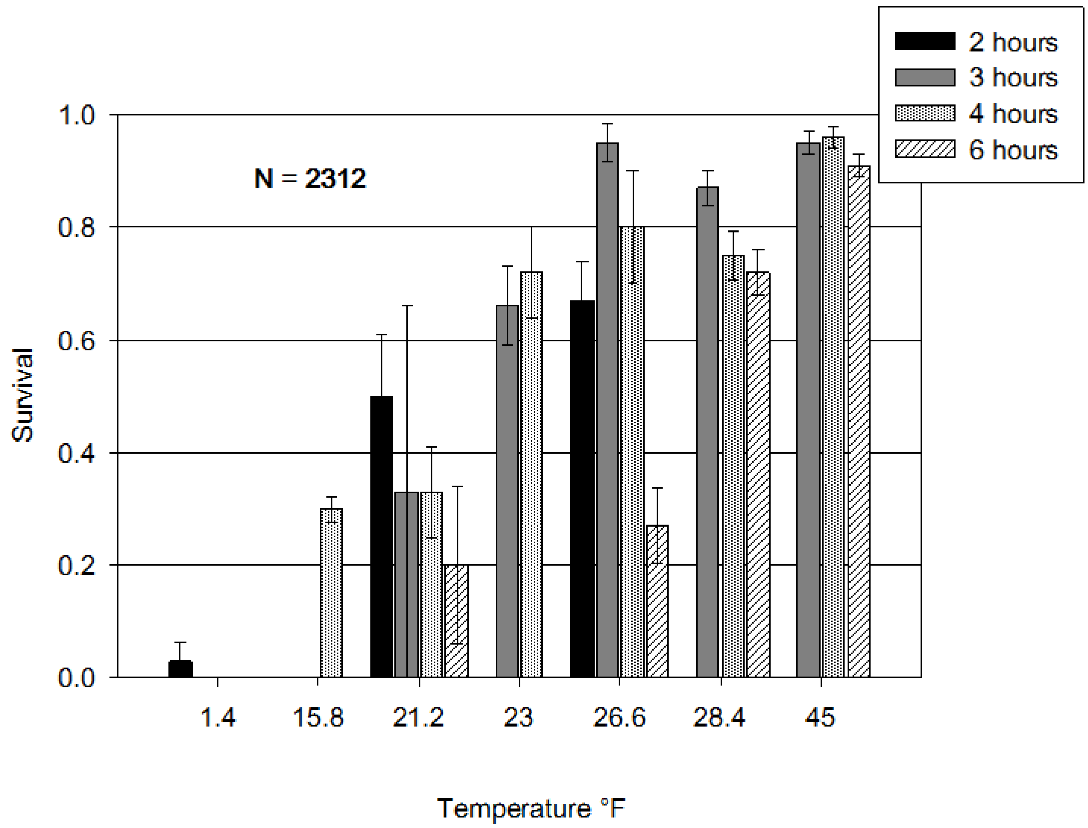

Figure 2. Mean survival of adult leaffooted exposed to cold temperatures for 2, 3, 4, or 6 hours. Leaffooted bug used in the experiment were collected in the field and maintained in a laboratory colony for no longer than 45 days.

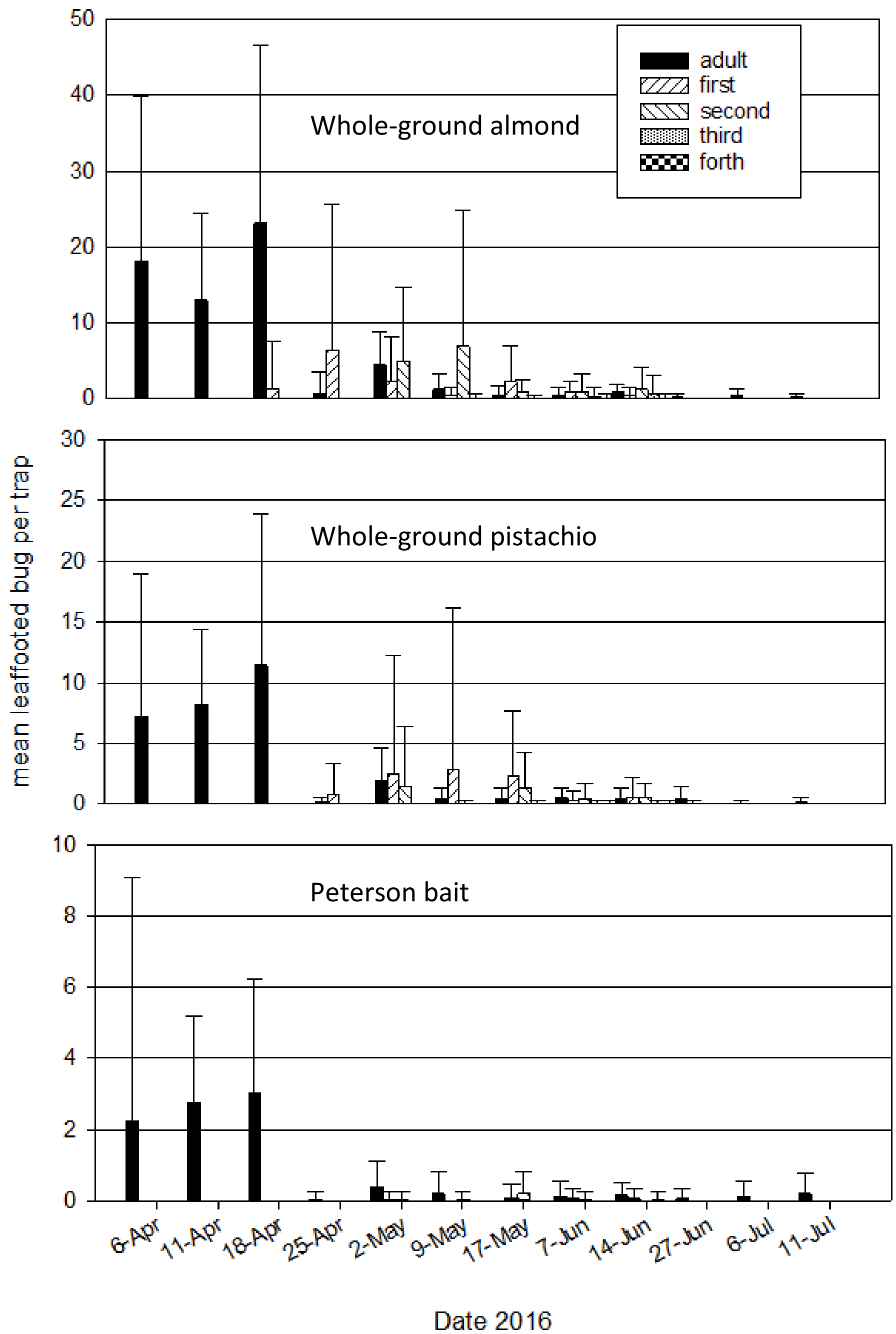

Figure 3. Mean number ± SD of adult, first, second, third, and fourth instar nymphs counted on modified navel orangeworm traps placed in pistachio and baited with three types of leaffooted bug host plant material.

Research Effort Recent Publications:

Tollerup, K. 2015. Leaffooted bug: What we know and what we need to know. CAPCA Advisor XVIII: 50-54.
Tollerup, K., and D. Haviland. 2015a. Be on the alert for leaffooted bug, pp. 1, Newsletter, Almond Board of California. Almond Board of California, http://www.almonds.com/newsletters/outlook/be-alert-leaffooted-bugs.
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Zalom, F., D. Haviland, E. Symmes, and K. Tollerup. 2015. UC IPM Pest Management Guidelines: Almond, Insect and Mites. University of California, Agriculture and Natural Resources, Publication 3431.