Another Look at Heromonal or Related Attractants for Leaffooted Bugs (*Leptoglossus* spp.) Infesting California Nut Crops

Project No.: **16-ENTO18-Millar/Daane**

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Objectives:

1. To field test the attraction of adults (*L. clypealis* and *L. zonatus*) of both sexes to caged males and caged females, under both summer and fall conditions, with the goal of verifying which sex is attractive, and whether the attraction is seasonal.
2. To identify/verify the metathoracic gland contents of both sexes (the likely alarm and defense compounds), and the contents of the male-specific ventral abdominal glands, to provide baseline data on the gland secretions produced by adults, some of which might be involved in attraction during different periods of the year.
3. To analyze headspace volatiles of sexually immature adults, and sexually mature adults, both virgin and mated, under long-day summer conditions when adults would typically be feeding and mating, and under short-day fall conditions, when adults would typically be forming over-wintering aggregations. Our goal here would be to look for compounds in addition to the defensive compounds and the known ventral abdominal gland compounds (VAG), which might be potential pheromone components.
4. To test potential attractant pheromone components in laboratory and field bioassays.
5. To analyze the cuticular hydrocarbons of adults of both sexes. These compounds may assist in helping to keep overwintering aggregations of bugs together.
6. To check sexually mature bugs of both sexes to produce vibrational signals that might also be involved in attraction of one sex to the other.

Interpretive Summary:

Work during the report period has been primarily focused on identification, synthesis, and preliminary testing of the group of compounds that seem to be most likely to be aggregation pheromone components for *L. zonatus*, i.e., the compounds which are produced by sexually mature summer-form males at their peak period of reproductive activity. We have all but one of the likely components identified, and work is progressing on identifying the remaining

compound, which is a compound new to science. Completing the identification and synthesis of this compound will be a major focus for the next few months. The field bioassays of a three-component blend, even without the remaining EAD-active compound, suggest that the blend is at least somewhat attractive to *L. zonatus*, i.e., that we are on the right track. There are good indications that *L. clypealis* may be using similar chemistry, but we need healthy populations of summer-form adults to be able to verify this.

Work on the comparative analyses of the cuticular hydrocarbons has been partially completed for both species. For *L. zonatus*, there were marked differences between the hydrocarbon profiles of the winter and summer-forms reared in the laboratory, and this only remains to be verified by analysis of winter and summer-forms collected directly from the field, to verify that the differences seen are not simply an artefact due to the unnatural conditions under which the insects are reared in the laboratory. Parallel analyses have been done with *L. clypealis*, but the results need to be re-checked with individuals from healthy laboratory colonies, or better-with individuals collected directly from the field.

In parallel with the chemistry work, Co-PI Wilson has been conducting a series of field trials aimed at optimizing the trap parameters. These trials have produced two useful results. First, the crossvane panel traps are by far the most effective trap to use for leaffooted bugs, even without an attractant bait. Secondly, their efficacy can be further improved by coating them with fluon to more effectively trap and retain the insects. Trials with different colored traps were indeterminate because of low bug populations, and so will be repeated over the remainder of the season.

Materials and Methods:

Insects:
Colonies of *L. zonatus* were started at UC Riverside from individuals shipped to us from Daane, and since then have been reinvigorated when possible by additional insects collected around Riverside, and by additional specimens from Daane and Wilson. The summer-form adults of this species mated, and so several successive generations have been maintained in the laboratory. Two laboratory colonies of *L. clypealis* were finally started from ~200 individuals hand collected from an overwintering population discovered in a shed at the Sweeney Granite Mountains Desert Research Center in October 2017.

Laboratory colonies of both species are maintained on a diet of organic zucchini, green beans, sweet corn, mandarin oranges, juniper, and raw peanuts and sunflower seeds. Adults and nymphs are housed together and are separated out as necessary for experiments.

Colonies are cycled back and forth between summer and winter forms by changing the light: dark schedule, i.e., adults were driven into and held in winter form at 15ºC by maintaining them under short day conditions (10L:14D), or into summer mode by holding under 14L:10D lighting conditions, and 21ºC.

Analysis of Defensive Secretions and Adult Bug Gland Contents:
The defensive compounds released by adult bugs were collected by shaking individuals in closed glass containers to induce them to discharge their defensive compounds. The

headspace of the containers was then sampled using solid phase microextraction devices, which were thermally desorbed directly into the injection port of a coupled gas chromatograph-mass spectrometer (GC-MS). Compounds were tentatively identified by matching with database spectra and/or interpretation of the spectra, followed by matching GC retention times and mass spectra with those of authentic standards.

To analyze the contents of the VAG, the glands were dissected out of sexually mature adult males and extracted in hexane for 20 min. Extracts were then analyzed by GC-MS. In parallel, contents of the VAG were also collected by "milking" adult males by gently squeezing the abdomen and collecting the resulting droplets of liquid in a 50 µL capillary tube, with multiple anesthetized males. The pooled liquid was then extracted with hexane, and the extracts were analyzed by GC-MS. The results of the "milking" technique was the same as the full gland dissection, so the "milking" method was adopted so that males could be kept alive for other experiments.

Collection of volatiles produced by adult insects:
For both species, volatiles were collected from both summer and winter form male and female bugs at multiple physiological stages. For both sexes, individuals were separated during the last nymphal instar and after eclosion to the adults, volatiles were collected from sexually immature (less than 10 d after eclosing to adults), and sexually mature individuals (more than 16 d after the final moult to adults). Then each male and female could mate, and the individuals were separated for another round of volatile collections. This allowed us to follow single individuals through the progression of physiological stages, and track differences in volatiles released. After the post-mating aerations were carried out, adults were used for cuticular hydrocarbon analyses (see below) or gland contents analyses (see above). Group aerations (10 individuals per chamber) using sexually mature and mated males or females were also carried out.

For collection of volatiles, adult bugs were very carefully transferred to half-pint canning jars with screw cap metal lids, with the lids fitted with Swagelok unions to allow attachment of air inlets and outlets. Organic green beans were provided for nutrition. The chambers were swept with clean air, with the volatiles trapped on an activated charcoal collector placed on the outlet. The trapped volatiles were eluted from the collectors with methylene chloride and analyzed by GC-MS. Multiple replicates were run for each sex, for summer form and winter form individuals, for different ages spanning sexually immature through sexually mature, and for each mating status (virgin or mated). In addition, volatiles were collected from single bugs, and from same-sex groups of bugs.

Analysis of extracts of volatiles from males and females of both species:
To narrow down which compounds in the extracts may be potential pheromone components, gas chromatography coupled with electroantennographic detection (GC-EAD) was utilized. Thus, live bug antennae were suspended between a pair of electrodes, and the antennae were then challenged with the individual pure compounds in extracts as they eluted off the GC. Analogous trials were conducted with standards, to verify that the standards also equivalent responses from the antennae. In total, 33 separate GC-EAD runs were carried out using antennae from female *L. zonatus*. These analyses demonstrated that several compounds in

the adult male volatile blend consistently elicited responses from the antennae. We then focused our attention on identifying these compounds as a priority.

Compounds in extracts were identified using a combination of microchemical and spectroscopic techniques (mass and microbore NMR spectrometry). Some compounds were tentatively identified by matches with database spectra, and the identifications were then confirmed by matching GC retention times and mass spectra with those of authentic standards. Some standards were commercially available (□-caryophyllene, farnesene), others were isolated in small quantities from samples of commercial essential oils (□- and □-bergamotene), and sesquiphellandrene was available from previous work in Millar's group. To obtain sufficient material for field trials, two syntheses of □-bergamotene were carried out by Millar's group, using the synthetic route developed by Kulkarni et al. (1988).

However, one of the compounds that elicited a large response from antennae of females in GC-EAD trials appears to be a compound new to science, and we were not able to identify it by comparison of its mass spectra with either database spectra or the spectra of standards on hand. Consequently, we had to collect and purify enough of the compound to analyze it by NMR spectrometry. Thus, volatiles were collected from groups of 10 sexually mature adult *L. zonatus* held in modified mason jars along with green beans and almonds as food sources. These aerations were carried out for 48 hr. at a time, with a total of 75 separate aerations carried out from January through April 2018. The resulting extracts were all analyzed using GC-MS, and samples with a significant amount of the target compound were identified (55 of the 75 extracts in total). These 55 samples were combined and carefully concentrated by slowly evaporating the solvent under nitrogen. This concentrate was then fractionated sequentially by liquid chromatography on silica gel, followed by preparative gas chromatography. The resulting pure compound was rinsed from the collection tube into a micro vial, and then transferred into a microbore NMR tube, and sent to the UCR Analytical Chemistry Facility for NMR spectrometry. The residue adhering to the walls of the micro vial was then used for additional microchemical tests to try and obtain more information about the possible functional groups present.

Analysis of Cuticular Hydrocarbons of Adult Bugs of Both Species:
To examine potential difference in the cuticular hydrocarbon profiles of male and female adults in the winter and summer phases, adults from the rearing colonies and from field collections were freeze killed, then individually extracted in hexane for 1 min. The resulting extracts were analyzed by GC-MS.

Field bioassays:
Influence of Trap Type, Trap Coating, and Trap Color:
A field study to compare trap types for leaffooted bugs was carried out from September 7 – October 20, 2017. Five traps were evaluated – (1) uni-trap (Alpha Scents), (2) clear sticky trap (Trécé), (3) hanging cross-vane panel trap (Alpha Scents), (4) a 2-ft pyramid trap and (5) a 4-ft pyramid trap. The collecting receptacle in the uni-traps and hanging-panel traps contained 450 ml of a 10% dish soap solution. Each trap type was paired with each of three different bait treatments that included (1) 50 g almond meal + crude almond oil (10% by weight), (2) 50 g pomegranate (approximately ¼ wedge) and (3) a no bait control. The almond and pomegranate baits were each placed in a 4 x 6-inch organdy mesh bag that was hung from the

top of the clear sticky trap, hung from the center of the hanging-panel trap, placed inside the collection receptacle at the top of the pyramid traps, or placed in a small plastic deli container inside the uni-trap. In total there were 15 unique trap x bait treatments (5 traps x 3 baits = 15 treatments).

The trap x bait combinations were evaluated using a replicated complete block design at three separate field sites in Fresno and Tulare counties that included a pomegranate, pistachio and olive orchard with known leaffooted bug pressure. There were five replicates at each site and each replicate contained all 15 treatments. Traps and baits were setup at the three field sites on September 7, 20 and 22. Traps were checked every 2 weeks until October 20 (two or three 2-week sampling periods). At each check, the baits and sticky traps were replaced, and the 10% dish soap solution was refreshed.

After determining that the cross-vane panel traps were by far the most effective trap design, a second trial was done to test the effects of coating trap surfaces, the collecting funnel below the crossed vanes of the traps, and the collection jar with the Teflon-based lubricant fluon. Fluon: water dilutions of 1:0, 1:1, 1:4, and 1:8 (100%, 50%, 25%, 12.5% fluon, respectively) were applied to panel traps. Trap catch was compared to a no fluon control. Traps were setup adjacent to an unsprayed pomegranate hedgerow with high LFB populations. A randomized complete block design with 5 replicates was used and traps were checked every 2 weeks between Oct. 30 – Dec. 4, 2017.

In a third trial, different colors of the crossvane panel trap were compared. Treatments included black, blue, green, red, white, and yellow traps. No fluon was applied to the traps, because it distorts the trap color (the fluon leaves a faint white coating on the trap). The different colored traps were set up along an untreated pomegranate hedgerow with high LFB populations. A randomized complete block design with 5 replicates was used and traps were checked every 2 weeks between Dec. 4-18, 2017.

<u>Pheromone Field Assay 1 – Almonds (April 24 – May 17, 2018)</u>
Field sites consisted of mature (> 4th leaf) almond blocks (min. 20 acres) with visible or reported leaffooted bug damage. Three sites were used in this study (1 block per site) and each site was > 1 km apart. In each block, 5 replicate pairs of traps with and without the candidate pheromone blend were set up along the edge of the block (2 traps/pair x 5 pairs/site = 10 traps/site). Paired traps were 10 m apart and each replicate pair was 50-100 m apart. Traps were hung along the berm between trees from a 6 ft tall, L-shaped rebar post. All traps were coated with fluon and the collecting receptacle was filled with 500 ml of a 10% dish soap solution. Traps were setup on April 24 and then checked every 7 days until May 17 (3 rounds of sampling), at which point the study was terminated. Traps were baited with rubber septa impregnated with hexane solutions of the test compounds, or hexane alone for controls.

<u>Pheromone Field Assay 2 – Pistachio (July 10 – July 31, 2018)</u>
The field site consisted of a mature (> 4th leaf) 2-acre pistachio block with significant leaffooted bug pressure at the Kearney Ag. Research and Extension Center. Five sets of paired traps with and without pheromone (10 traps total) were set up in the orchard interior. Paired traps were 10 m apart and each replicate pair was 18 m apart. Traps were hung along the berm between trees from a 6 ft tall, L-shaped rebar post. All traps were coated with fluon and the

collecting receptacle was filled with 500 ml of a 10% dish soap solution. Traps were setup on July 10 and checked weekly until July 31 (3 rounds of sampling), at which point the study was terminated.

For both trials, trap catch data were analyzed using general linear models with Poisson distribution and replicate nested within site nested within date as random effects. A full model was constructed using the response variable "Lure" (Pheromone or Control) and compared against a null model.

Field Assays Testing Different Spatial Arrays of Traps in Almond, Pistachio, and Pomegranate (March 24 – October 1, 2018)

Individual blocks of almond, pistachio, and pomegranate were selected at 3 sites in Madera and Fresno counties. The three sites were 38-81 km apart and the three blocks within each individual site were 0-2 km apart (most of them were adjacent to each other). In each block at each site, four sets of paired traps were set up at the block edge (0-5 m from edge) and interior (100 m from edge). Where possible, these four "edge/interior" transects were set up along the north, east, south, and west sides of each block. When more than one transect needed to be located along the same side of the block, the transects were separated by at least 100 m. All traps were coated with fluon and did not contain a bait. The collecting receptacle was filled with 500 ml of a 10% dish soap solution. Traps were initially setup on March 29 and then checked approximately once per month and will be checked until the end of the season (i.e. October).

Results and Discussion:

Insects:
The project overall has been more difficult and has progressed more slowly than we had hoped, primarily due to the difficulty in obtaining bugs to work with. As described, we were not able to establish a colony of our second study species, *L. clypealis*, until the end of the first year of the project in fall, 2017. Since then, despite numerous people actively searching for or looking out for any other populations of *L. clypealis*, none have been located. The situation is exacerbated by the difficulty in rearing *L. clypealis* in the laboratory. Despite focused efforts by trained staff with substantial experience in rearing insects, the laboratory populations decline and die out.

Leptoglossus zonatus has proven to be more amenable to collecting and laboratory rearing, but even with this species, collections have been sporadic and unpredictable. As a result, with both species, we have delayed doing experiments to test attraction to caged live insects (Objective 1), because we just have not had sufficiently large numbers of insects to work with. We hope to address this objective over the next few weeks as populations of bugs build up in the field.

L. zonatus:
The original colony was started from adults received from Kent Daane in October 2016. The adults in this colony were initially in the "winter" condition and were kept at a 10:14 light: dark schedule. All data on overwintering individuals of this species were collected at this time. Around January 1, 2017 it was noted that the adults had begun mating and had stopped

aggregating in the typical winter clusters, thus this colony was no long considered to be in the overwintering condition and has now been re-classified as being in the "summer" condition, and successive generations of adults have been successfully reared in this colony. This colony has been enriched whenever possible with *L. zonatus* caught in the field to maintain genetic diversity.

Between January – March 2018, multiple attempts were made to locate and sample overwintering aggregations of *L. zonatus*, but no aggregations were found and thus no samples were collected for analysis of cuticular lipids or egg loads. Additional attempts to locate newly formed overwintering aggregations will be made this fall/winter.

Until very recently, summer-form adults also proved to be hard to find. However, populations have been increasing in a pistachio orchard at the Kearney Ag. Center, enabling collection and shipment to UC Riverside of two groups of bugs on July 11 and July 19, for evaluation of their cuticular lipids and rejuvenation of the laboratory colonies.

L. clypealis

Two colonies of *Leptoglossus clypealis* were started from roughly 200+ individuals collected at the Sweeney Granite Mountains Desert Research Center in mid-October 2017. These insects appeared to be entering the "winter" condition and when brought back to the laboratory were kept under a 10:14 light: dark schedule and at a temperature of 15°C. Tests on winter-form *L. clypealis* were carried out using this colony. In December 2017, 30 individuals (1:1 ratio of males: females) were removed from this colony and kept under these winter conditions, while in January 2018 the main colony was slowly moved into a 14:10 light: dark cycle, and a temperature of 24°C. Individuals in the smaller, cool temperature colony remained in winter aggregations well into April 2018 and this was considered to still be a winter colony during this time. The colony under the warm, long-day conditions was considered a summer colony for carrying out experiments on individuals. Unlike the *L. zonatus* colony, these colonies have done poorly in the laboratory. The initial generation of adults mated and laid eggs within the cages, and a 1st generation of nymphs was generated, however few of these nymphs reached adulthood, and as the initial generation of adults expired the colony has declined and the bugs are obviously of poor quality. To try and avoid this problem, experimentation with different food sources will continue when new individuals are field collected so that the laboratory colonies can be restarted and hopefully maintained in better condition.

To date, the bugs collected from the Sweeney Granite Mountains site have been the only ones found in any numbers by any of the project collaborators, or the network of contacts of the project team. All the graduate students at UCR, many of whom collect insects or conduct field work around southern California, have been asked to collect any that they see, or to alert us to any populations that they find. Nevertheless, even this large group of trained personnel have not found more than a handful of individuals, suggesting that for reasons unknown, *L. clypealis* has become uncommon. The same situation seems to be true in the Central Valley because Wilson, Daane and their wide network of contacts, have not found any populations over a period of many months.

Analyses of Bug Defensive Compounds:
Results from the analyses of the defensive compounds from *L. zonatus* were reported in the 2017 report. Similar multiply replicated analyses of defensive compounds from *L. clypealis* have not yet been carried out because the laboratory colony is currently in such bad shape. Once we obtain fresh infusions of bugs, this gap will be filled in. However, this is not a major priority, because we had previously reported on the defensive compounds from this species (Wang and Millar 2000), and so repeating the analyses is largely just a matter of being careful to make sure that we did not miss anything the first time.

Analysis of Volatiles Produced by Adult Bugs in Different Physiological States:
L. zonatus:
As mentioned in last year's report, sexually mature summer-form male *L. zonatus* sex-specifically released a blend of sesquiterpene hydrocarbons that are not released by adult females, sexually immature summer-form males, or winter-form males. These compounds are entirely distinct from the defensive secretions released by both males and females, and the compounds found in the extracts of the male ventral abdominal gland (VAG).

As a first step in identifying which compounds in the summer-form extracts from males might be attractant pheromone components, the extracts were analyzed by coupled GC-EAD. These analyses showed that several compounds in the extracts elicited responses from antennae of females (**Figure 1**). These and several other compounds have now been identified as nonanal, decanal, alpha-cis-bergamotene, beta-caryophyllene, (*E*)-beta-farnesene, and sesquiphellandrene, and the major compound, beta-*cis*-bergamotene, has been synthesized in quantities sufficient to carry out preliminary bioassays (see below).

However, there is one remaining unknown compound (peak E in **Figure 1**)), which elicited consistent strong responses from bug antennae in GC-EAD assays. This compound appears to be new to science; it could not be identified by matching with any database spectra, or spectra from anything from our library of standards. Thus, a few micrograms of this unknown compound were purified by preparative gas chromatography from a combined sample from 55 separate collections of headspace volatiles from sexually mature summer-form males and subjected to microbore NMR spectrometry in June and again in July 2018. This provided enough information to be able to narrow down the likely structural possibilities to four possible structures. Of those four, two are deemed more likely. To further narrow this list, hopefully down to a single structure, we will begin making model compounds for comparison of their spectra with those of the insect-produced compound.

It also must be mentioned that a manuscript is in press from Paulo Zarbin's group in Curitiba, Brazil, describing possible male-produced aggregation pheromone components from a population of *L. zonatus* on southern Brazil. Somewhat surprisingly, the compound identities are largely the same as we have found, but the ratios reported from this population are distinctly different than those that we have found in our exhaustively replicated analyses of volatiles produced by summer-form male *L. zonatus* from California. However, a recent paper reported that there are genetic differences between *L. zonatus* from South America and California (Joyce et al. 2017), and this may be reflected in the profiles of volatiles produced by the male bugs.

L. clypealis:

Our efforts to work with *L. clypealis* were hampered by the long delay in finding a field population with which to work and start laboratory colonies. We were finally able to collect and start a colony in October 2017. Volatiles were collected from both “winter” and “summer” form *L. clypealis* males and females. Aerations of winter-form adults were carried out on males and females when they were initially brought into the laboratory from Sweeney Granite Mountains Desert Research Center, as well as with individuals drawn from winter aggregations once the colony had become established in the laboratory. Since all individuals aerated were already adults at the time of collection, their mating status and sexual maturity could not be determined, but it is likely that all individuals were sexually mature since they would have become adults last autumn. Adults were aerated either as individuals, or in groups of five or ten. Five aerations of females alone, 7 of males alone, and 5 of mixed sexes were carried out. The resulting extracts were analyzed using GC-MS. All aerations of winter-form adults, regardless of sex, consistently contained only two compounds, readily identified as hexanal and hexyl acetate, both of which are typical components of the *Leptoglossus* defensive secretions.

Aerations of summer-form adults were carried out after the colony was transitioned to summer conditions and mating and egg-laying by most individuals was observed. Males (13 groups) and females (4 groups) were aerated separately in groups of 10 for 24-72 hr. Again, because all these adults had been collected from the field, the mating status and sexual maturity of the individuals could not be determined. However, the copious mating and egg-laying observed in the cage indicated that most if not all individuals were likely sexually mature and mated.

Analysis of the resulting extracts showed that extracts from females contained only the two defensive compounds hexanal and hexyl acetate, in similar ratios to those seen in the winter aerations. In contrast, group aerations of summer-form males contained a consistent blend of compounds that were not found from the winter-form males (**Figure 2**). In contrast to *L. zonatus*, the major compound in the male *L. clypealis* blend was not beta-bergamotene, but benzyl alcohol, a previously identified compound from the ventral abdominal glands. Male *L. clypealis* also produced several of the same compounds as *L. zonatus* (including the three previously mentioned sesquiterpenes, and the unidentified compound), but these compounds were present at low levels. However, the low levels of these compounds should be interpreted with caution because by the time these samples of volatiles were collected, the *L. clypealis* colony was fading fast, so the males were likely of very poor quality. Overall, because the sesquiterpene compounds were produced only by summer-form males, and they are like those produced by the *L. zonatus* congeners, it seems virtually certain that they likely function in both species as a male-produced aggregation pheromone.

Analysis of Cuticular Hydrocarbons

L. zonatus:

The results of analyses of the cuticular hydrocarbons of summer- and winter-form adults of *L. zonatus* from the laboratory colonies were described in our 2017 report. Briefly, there were significant differences between the two physiological stages, with the extracts from the two forms appearing to have qualitative and quantitative differences. However, these differences need to be confirmed with extracts from freshly collected summer- and winter-form individuals, which we have not yet been able to obtain.

L. clypealis:
For *L. clypealis,* hexane extracts of adults in both the winter and summer phases of the laboratory colony established last October were analyzed. Winter phase individuals included 13 females and 13 males that were collected initially from the field population. An additional 4 females and 4 males were also collected from wintering aggregations in the lab colony in mid-December 2017. Once the colony had been moved into the summer phase, 4 females and 4 males were collected for extraction. The results are shown in (**Figures 3 – 5).** In contrast to what was seen with *L. zonatus*, there were no obvious differences between the summer and winter-form adults. However, as mentioned above regarding the male-produced volatiles, these results must be interpreted with caution because of the dubious quality of the insects used to prepare the extracts, especially the summer-form insects from the colony that was dying out. These analyses will be repeated with field collected summer- and winter-form adults as soon as we or our collaborators can find field populations.

Field Bioassays:
Evaluation of Different Trap Types:
In this experiment, the primary species recovered was *L. zonatus*. A few *L. clypealis* were recovered as well, but densities were too low to compare catches between the various traps/baits. The cross-vane panel trap (**Figure 6**) was by far the most effective for trapping *L. zonatus* (**Figure 7**) which was the dominant species at all field sites. The almond and pomegranate baits did not appear to have any effect on trap catch. Consequently, the bugs may be attracted by the vertical silhouette of the trap, or its dark color or contrast against the background, or some combination. Whatever the case, these results suggest that even without an attractant bait, the panel traps may be useful in detecting the immigration of bug populations into traps.

Evaluation of Coating Crossvane Panel Traps with Fluon:
Coating traps with the Teflon-based lubricant fluon (**Figure 8**) had a marked effect on trapping efficacy, with all traps coated with fluon catching/retaining far more bugs than untreated control traps. All dilutions of fluon, down to the 8:1 dilution (12.5%) appeared to be effective (**Figure 9**).

Evaluation of Different Trap Colors:
The results from this experiment were indeterminate because by the time it was deployed in early December, field populations of bugs had declined to very low levels, and there were no significant differences among any of the treatments (data not shown). Consequently, this trial will be repeated this summer or fall.

Trials with Different Arrangements of Cross-vane Panel Traps:
This study is still in progress. To date, *L. zonatus* have not been consistently recovered from any crop or location within a crop (**Figure 10**). A more complete analysis will be carried out at the end of the sampling program (October).

Structure of Life-stages and Egg Loads:
The intention of this subobjective was to regularly sample *L. zonatus* populations throughout the year to make observations on life-stages and female egg loads. However, abundance of *L.*

zonatus has been generally low so far this year and thus it has been very difficult to locate populations to sample as part of this effort. As such, no data towards this objective are presented here.

Preliminary Bioassays with Possible Pheromone Blend:
Based on the analyses of the volatiles produced by sexually mature summer-form *L. zonatus*, we prepared a reconstructed blend containing synthesized beta-*cis*-bergamotene and sesquiphellandrene, and (*E*)-beta-farnesene. The test solution of compounds was loaded onto rubber septa at a dose of 1 mg per lure of the major compound (beta-*cis*-bergamotene), and proportionate amounts of the two minor components.

Trap captures in the first trial in almonds were too low to be meaningful, due to low population levels in the test orchard (data not shown). The second, more recent trail in pistachio (**Figure 11**) did result in more *L. zonatus* in the traps with the pheromone blend, but preliminary analysis did not find the difference to be significant ($P = 0.09$). This trial is on-going and scheduled to conclude on July 31.

Results from the almond trial may have been skewed due to low pest pressure. We are more confident in the findings from the trial in pistachio, where there was at least a trend towards more *L. zonatus* in the traps baited with pheromone.

Research Effort Recent Publications:

No publications have been submitted because no part of this research is sufficiently advanced or complete to warrant submission of manuscripts.

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List of figures:

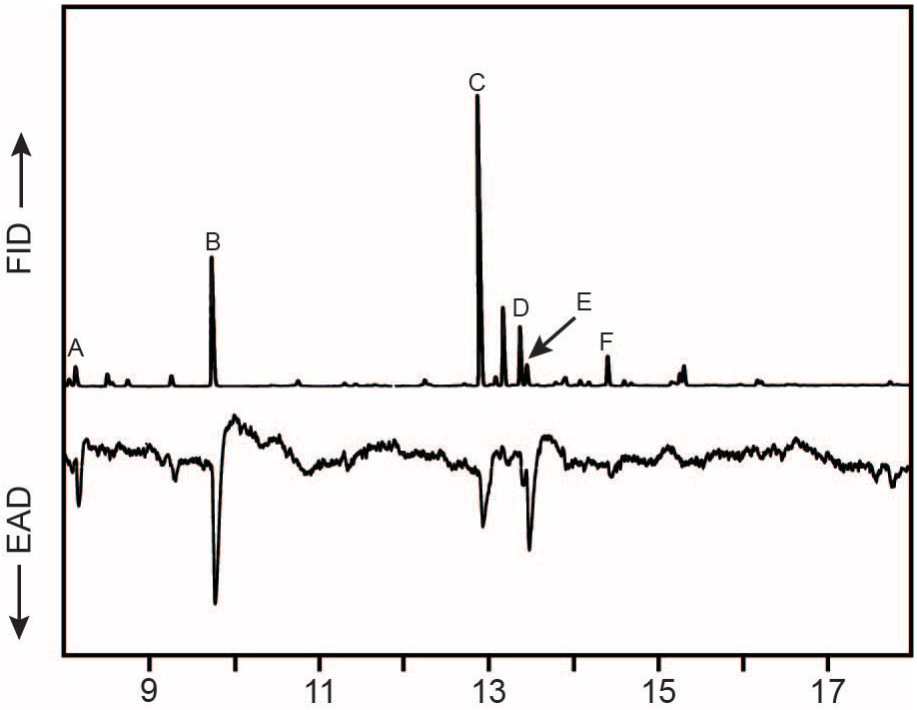

Figure 1. Gas chromatography-electroantennographic detection (GC-EAD) of a *L. zonatus* male extract vs. a female *L. zonatus* antenna. The top trace shows the GC profile of compounds released by summer-form sexually mature adult male *L. zonatus*. The bottom, inverted trace shows the responses elicited from a female *L. zonatus* antenna by each of the compounds. Compound identities: **A**) nonanal, **B**) decanal, **C**) alpha-cis-bergamotene, **D**) (*E*)-beta-farnesene, **E**) unidentified compound, **F**) sesquiphellandrene.

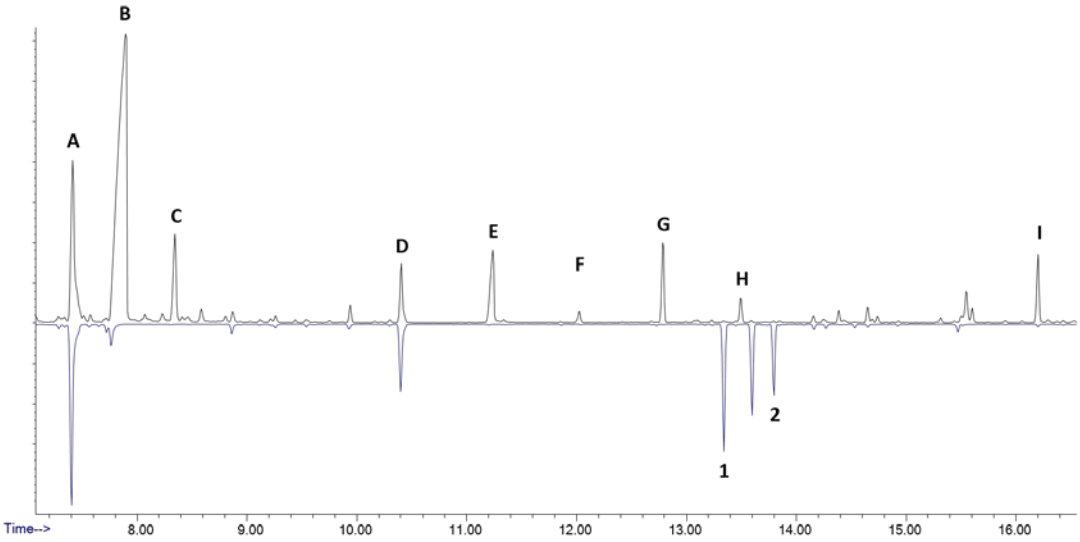

Figure 2. Gas chromatograms showing volatiles from summer-form *L. clypealis* males (top, **black line**), and *L. zonatus* males (bottom, **blue**) line. *Leptoglossus clypealis* compound identities: **A**) hexyl acetate, **B**) benzyl alcohol, **C**) 1-octanol, **D**) decanal, **E**) unidentified, **F**) methyl geranate, **G**) geranyl acetate, **H**) β-caryophyllene, **I**) unidentified. *Leptoglossus zonatus* compound identities: alpha-cis-bergamotene, and (*E*)-beta-farnesene.

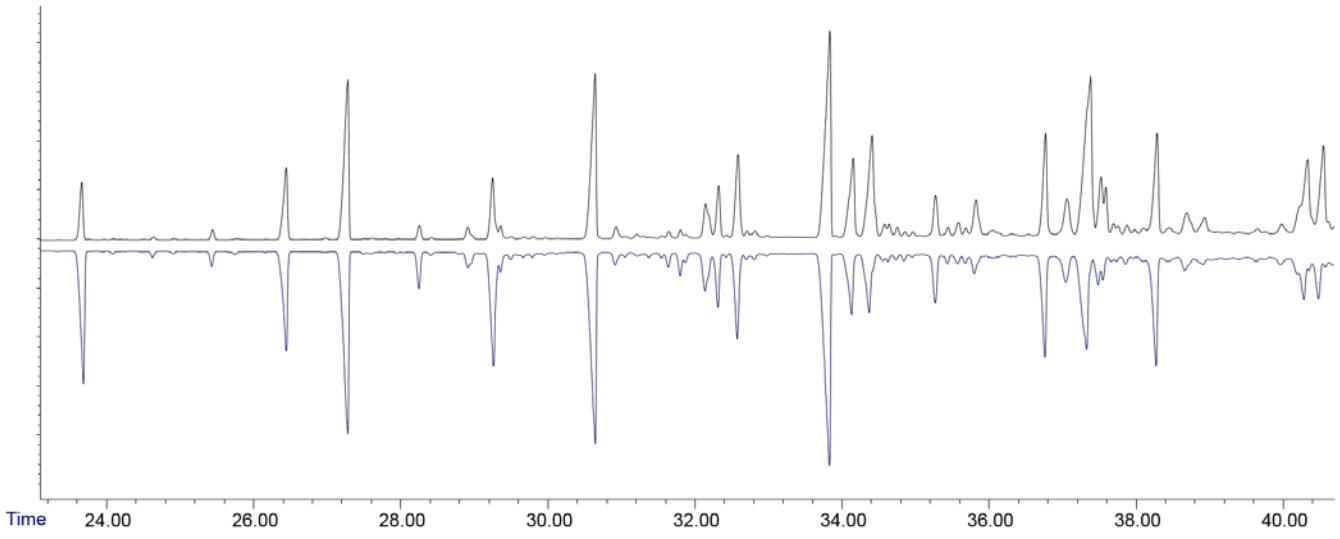

Figure 3. Gas chromatograms showing the cuticular hydrocarbon profiles of a laboratory summer male (**top**) and female (**bottom**) *L. clypealis*.

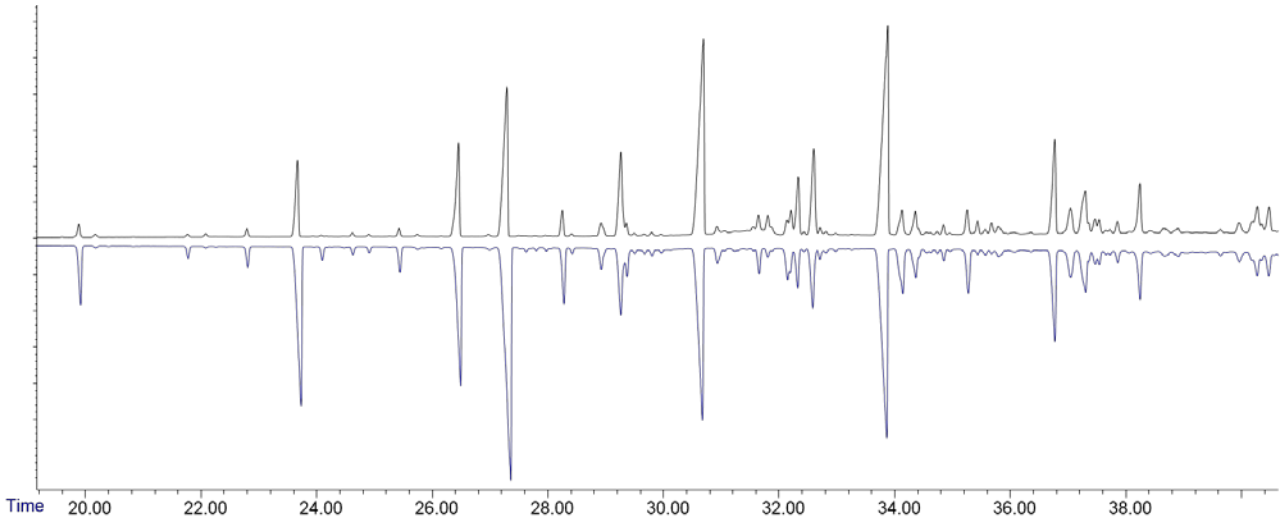

Figure 4. Gas chromatograms showing the cuticular hydrocarbon profiles of winter male (**top**) and female (**bottom**) *L. clypealis*.

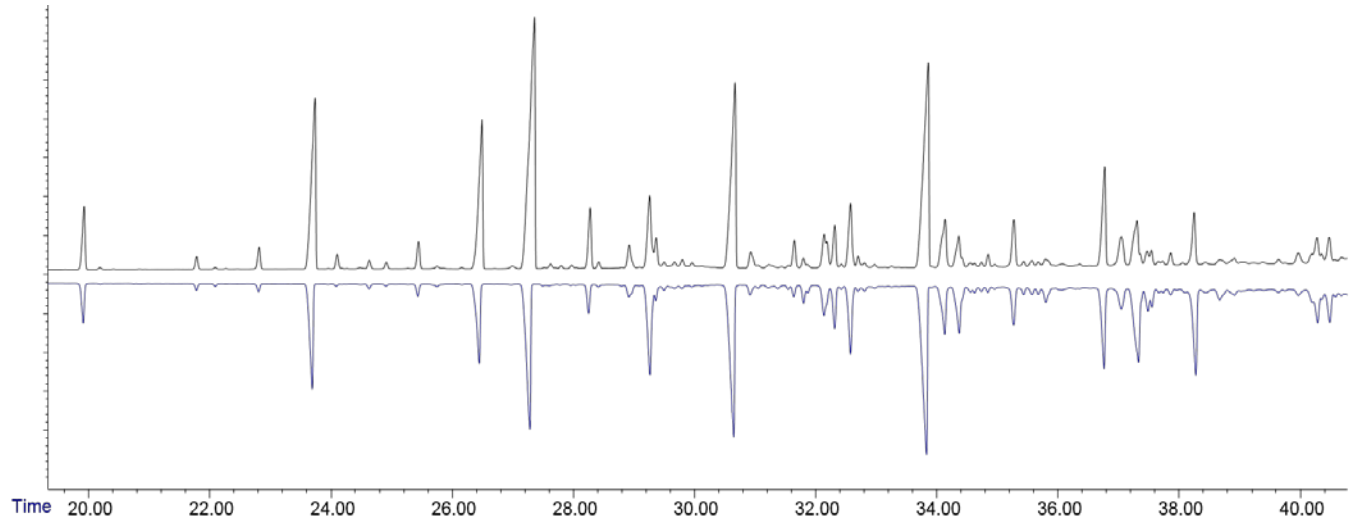

Figure 5. Gas chromatograms showing the cuticular hydrocarbon profiles of winter female (**top**) and summer female (**bottom**) *L. clypealis*

Figure 6. Cross-vane panel trap suspended from 6 ft rebar post

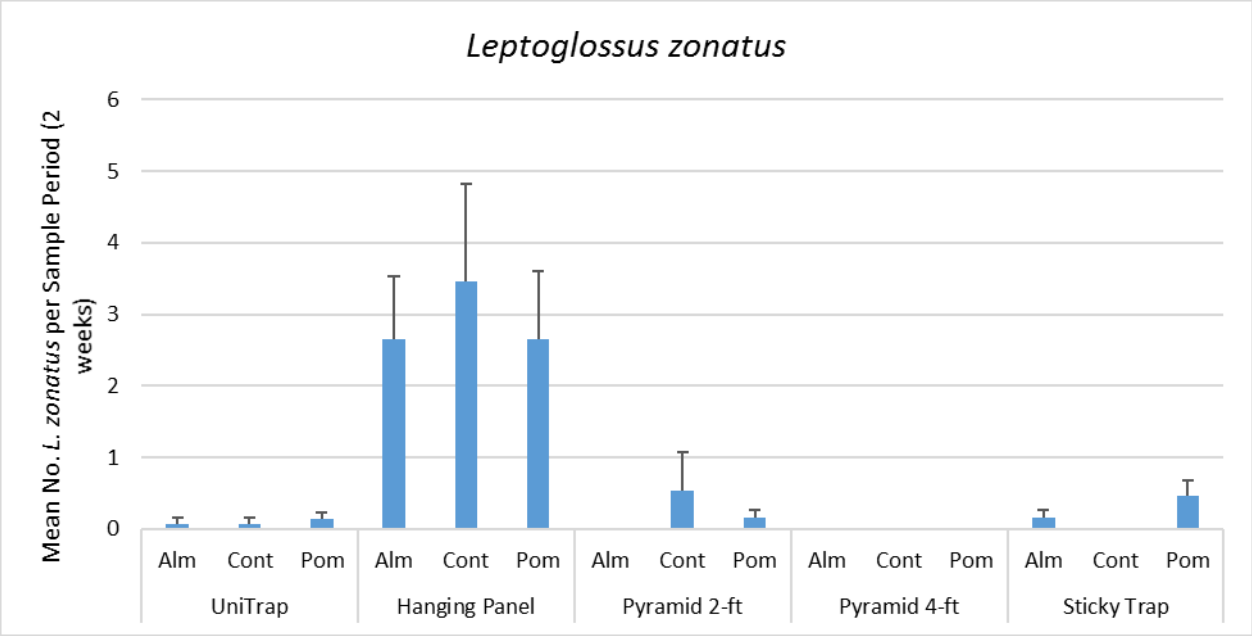

Figure 7. Numbers of *L. zonatus* caught in various trap designs, in almonds, pistachios, and pomegranates.

Figure 8. Close-up of a crossvane panel trap coated with fluon. The hanging basket in the center of the trap contains the pheromone lure

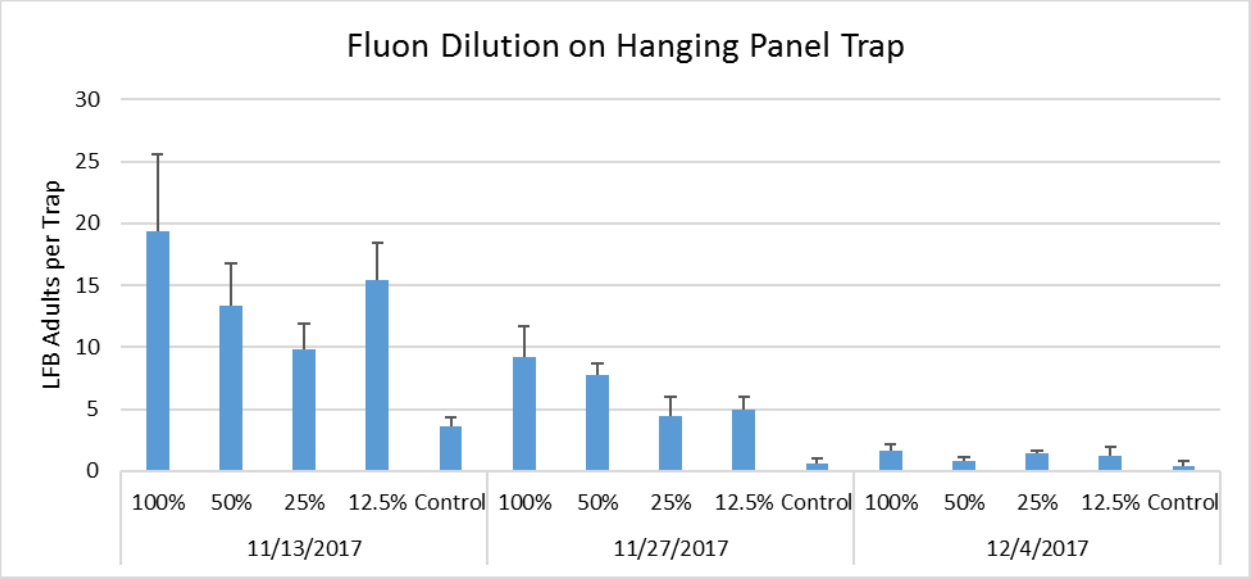

Figure 9. Numbers of *L. zonatus* caught in crossvane panel traps with and without treatment with fluon.

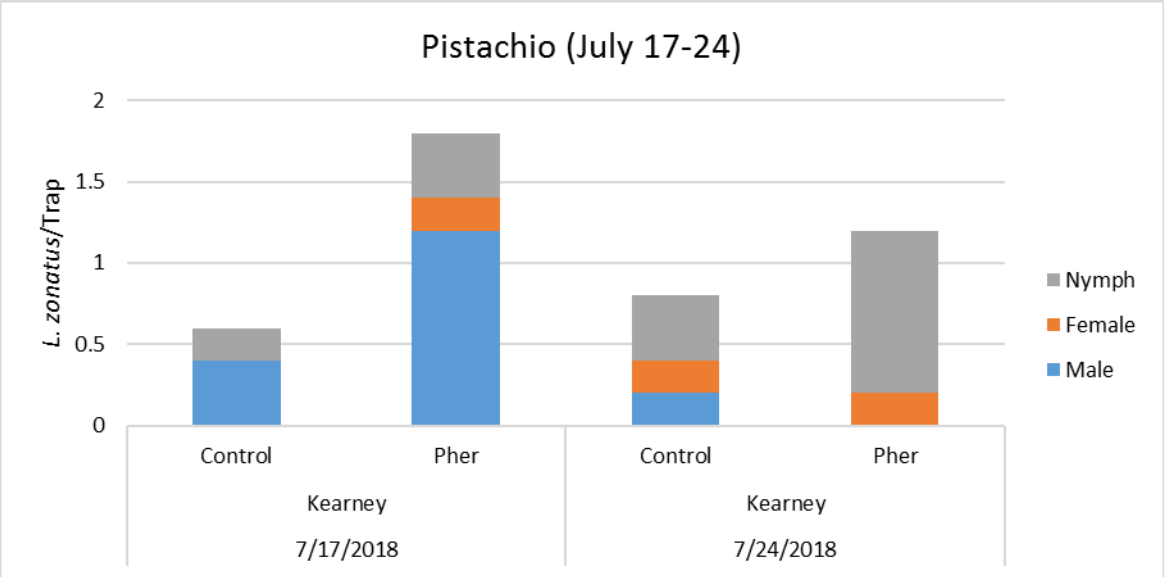

Figure 10. Trap captures of *L. zonatus* in crossvane panel traps at the edge and interior of almond, pistachio, and pomegranate orchards.

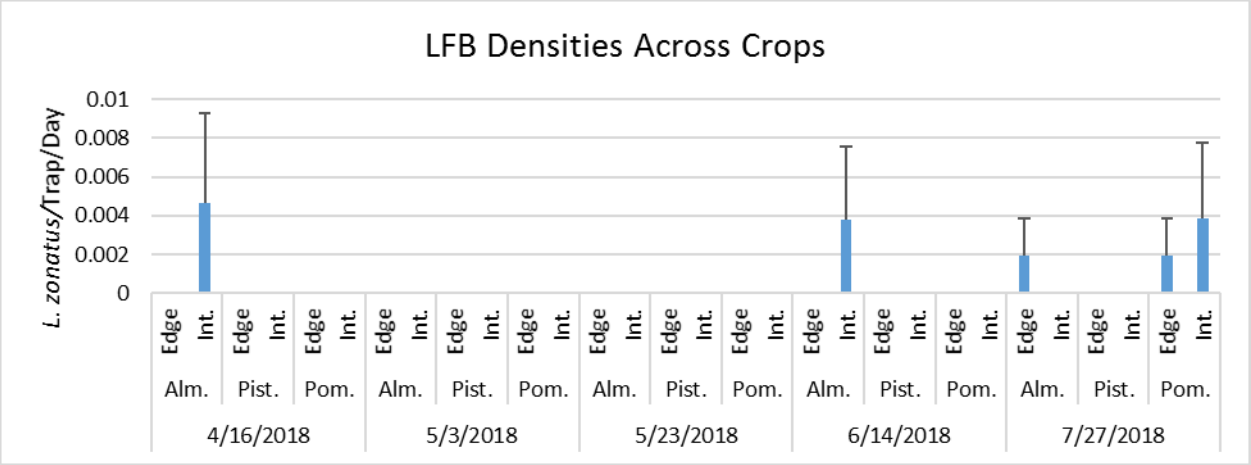

Figure 11. Numbers of *L. zonatus* caught in crossvane panel traps baited with a test pheromone blend, in pistachios.