
Another look at pheromonal or related attractants for leaffooted bugs (*Leptoglossus* spp.) infesting California nut crops

Project No.: ENT018.Millar

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A. Summary

The main goal of this project is to identify attractant pheromones for leaffooted bugs (*Leptoglossus zonatus* and *L. clypealis*) that are pests of California nut crops. Prior to 2020, we had identified 8 of the 9 compounds produced by sexually mature male *L. zonatus*, i.e., compounds which are the most likely to be part of a pheromonal attractant. After two years of effort, in May 2020, we were finally able to identify the 9th and final compound produced by male bugs. This compound is new to science, and we are developing a synthesis of the compound to provide materials for lab and field bioassays in 2021. Work in 2020 was slowed by the closure of our research laboratories for three months due to COVID protocols.

In parallel, we have tested and optimized traps for leaffooted bugs. Multiply replicated field trials determined that hanging cross-vane panel traps were most effective, with yellow and blue traps resulting in the highest captures.

B. Objectives

Objective(s)	Milestones and deliverables associated to the objective
1. Finish identification and synthesis of the 9th and final compound produced by summerform male LZ (Millar)	Identification and synthesis of all candidate attractant compounds for summerform LZ
2. Bioassay the reconstructed blend of compounds produced by summerform male LZ (Wilson)	Verification of bioactivity of candidate attractants
3. Identify, synthesize, and bioassay the analogous blend of compounds produced by summerform male LC (Millar)	Identification and synthesis of all candidate attractant compounds for summerform LC
4. Bioassay summer- and winterform LZ and LC against each other, to verify that cuticular hydrocarbons are involved in maintaining overwintering aggregations (Millar)	Assessment of what role cuticular hydrocarbons play in maintaining overwintering aggregations of LZ and LC
5. Continue optimizing trap characteristics, including color, with and without attractant lures (Wilson)	Optimized, cost effective traps for monitoring leaffooted bugs in nut crops
6. Test attraction of LZ to infrared radiation (Wilson)	Assess a possible alternate means of attracting leaffooted bugs to traps

D. Results and Discussion

Objective 1. Finish identification and synthesis of the 9th and final compound produced by summerform male *L. zonatus* (LZ) (Millar)

In December 2019, we combined almost 100 extracts from separate cohorts of male *L. zonatus*, and by a combination of sequential chromatographic steps, isolated a larger sample of the 9th and final compound produced by sexually mature males. A series of different types of nuclear magnetic resonance (NMR) spectra were obtained from the purified compound, and in May 2020, we were finally able to conclusively identify this compound as a bicyclic sesquiterpene hydrocarbon, including the specific stereoisomer out of eight possible stereoisomers. Once the UCR labs reopened in mid-June after a 3 month Covid shutdown, a quick and dirty synthesis was carried out, aimed at producing enough material (a few milligrams) for us to unambiguously confirm the structure. We are currently developing an optimized synthesis to produce gram quantities of the compound for bioassays. We already have 7 of the 8 other compounds synthesized or purchased. We need to repeat the synthesis of one other compound to produce enough material for bioassays. Thus, by April, we anticipate having all of the 9 compounds available for testing in lab and field bioassays.

Objective 2. Bioassay the reconstructed blend of compounds produced by summerform male LZ (Wilson)

No work was possible on this objective because we had not finished the syntheses of all the compounds required for bioassays.

Objective 3. Identify, synthesize, and bioassay the analogous blend of compounds produced by summerform male *L. clypealis* (LC) (Millar)

We have identified all the compounds produced by sexually mature, summerform LC, some of which are the same as those produced by male LZ. We should have compounds available for bioassays in 2021 if populations of LC can be located. For the past several years, LC has been difficult to find because its numbers have been so low, despite its being the dominant leaf-footed bug in California nut crops 20 years ago.

Objective 4. Bioassay summer- and winterform LZ and LC against each other, to verify that cuticular hydrocarbons are involved in maintaining overwintering aggregations (Millar)

We have determined that the cuticular hydrocarbons that are used by the bugs in the close-range recognition of sex and species vary seasonally, particularly for female bugs, and we hypothesize that these changes may be involved in the formation and maintenance of overwintering aggregations. In 2019, our first set of bioassays that tested the responses of summerform males and females to winterform males and females and vice versa, were not successful, we suspect due to the chambers in which the bioassays were conducted being too small so that the bugs did not behave naturally. We intended to repeat these bioassays in 2020, but the shutdown of almost all research at UCR in March forced us to kill our leaf-footed bug colonies. Thus, we have rescheduled these bioassays for 2021 at Kearney Ag Center, where Co-PI Wilson has large and healthy colonies of leaf-footed bugs to work with. If our hypothesis is correct, and males respond differently to summerform versus winterform females, then we will attempt to identify the specific compounds that mediate this response.

Objective 5. Continue optimizing trap characteristics, including color, with and without attractant lures (Wilson)

An initial field study in 2017 identified the cross-vane hanging-panel trap ('panel trap') as an effective trap for LZ. Subsequent studies in 2018 and 2019 found that trap catch was improved by coating the traps with fluon lubricant, and use of a yellow or blue trap. Additional field trials with unbaited panel traps placed at the edge and interior of large almond orchards found that unbaited traps were of little use in a standard commercial setting, thus reinforcing the need for an effective lure to go with this newly identified trap.

Objective 6. Test attraction of LZ to infrared radiation (Wilson)

A previous study found that a related species, *Leptoglossus occidentalis*, was able to perceive differences in background infrared radiation ('IR') as a means to locate hosts (Takacs et al. 2010). LZ appear to have similar IR detection structures and so an experiment was initiated to evaluate this. Unfortunately, the graduate student recruited for this project was unable to join our lab group due to COVID-19 restrictions, and so this objective has been put on hold for the foreseeable future. The idea here is that developing nuts may have a slightly higher temperature relative to foliage due to high rates of metabolism as nuts grows and develop, and LZ could use this IR cue to locate host nuts.

E. Outreach Activities

1. Please describe outreach activities including the event description (date, location, topic of the presentation, aprox number of participants and type of audience)

Date	Talk/Event	Location/Audience
Nov. 16, 2017	"Orchard Trapping for Leaf-footed Bugs" Pistachio Short Course	Visalia, CA 75-100 growers/PCAs
June 13, 2018	"Pheromone and Related Attractants for Leaf-footed Bugs in California Nut Crops" Symposium PB-ESA Annual Meeting	Reno, NV 20 scientists/students
Sep. 13, 2018	"Pheromone and related attractants for leaf-footed bugs in California nut crops" CAPCA Education	Bakersfield, CA 80-90 PCAs
Dec. 6, 2018	"Leaf-footed Bug Pheromones and Related Attractants" Almond Board California Annual Conference	Sacramento, CA 100 growers/PCAs/industry
Jan. 9, 2019	"Pheromonal and Related Attracts for LFB in CA" Orchard Pest and Disease Management Conference	Portland, OR 60 extension/faculty
Apr. 17, 2019	"LFB Pheromones and Related Attractants" IPM Breakfast, UCCE North San Joaquin Valley	Modesto, CA 45 growers/PCAs

F. Materials and Methods:

Colonies of LZ and LC were started from field-collected bugs, and were maintained in large cages in the laboratory on organic green beans, sunflower seeds, and cypress berries.

Headspace odors were collected from cohorts of summer- and winterform LZ of both sexes by holding cohorts of bugs in glass chambers swept with clean air, collecting the volatiles on charcoal traps. Traps were eluted with solvent, and the resulting extracts were combined and fractionated by liquid and gas chromatography. Purified compounds were identified by a combination of mass and NMR spectrometry, and microchemical tests for specific functional groups. Identified compounds were synthesized by standard organic chemistry methods; co-PI Millar's research group has several decades of experience with this type of work. As mentioned above, no laboratory work was possible at UCR from mid-March to mid-June 2020 due to Covid shutdowns, and ongoing lab work has been slowed by social distancing requirements.

All trap studies were conducted in commercial almond, pistachio and pomegranate orchards in Fresno, Tulare, Kings, and Madera counties, as well as in experiment orchards at the Kearney Ag. Center in Parlier, CA. Panel traps included 500 ml of collecting solution (10% biodegradable soap and water). In all studies traps were monitoring weekly. Trap comparison studies utilized a randomized complete-block design with 5-7 replicates.

As mentioned, Objective 6 to evaluate LZ response to IR was unsuccessful due to COVID-19 barriers that prevented recruitment of a qualified graduate student to conduct this work.

G. Publications that emerged from this work

Wilson, H., J. J. Maccarro, K. M. Daane. 2020. "Optimizing trap characteristics to monitor the leaffooted bug *Leptoglossus zonatus* (Heteroptera: Coreidae) in orchards" *Insects* 11(6), 358.

2. Manuscript in prep: Identification and synthesis of leptotriene, a novel sesquiterpene from male leaffooted bugs. To be submitted to Journal of Natural Products