Improving Trapping and Mating Disruption of the Navel Orangeworm

Project No.: **08-ENTO2-Leal/Zalom**

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Objectives:

1. Improve Formulation for Trapping Males
   - Determine Optimal Ratio of Constituents
   - Determine Minimal Constituents Required for Maximal Attraction
   - Determine Optimum Amount of Pheromone per Trap
   - Develop Stable and Long-Lasting Formulations
   - Field Evaluate New Formulations in Areas of High Populations
2. Develop Alternative Multi-Component Blends for Mating Disruption
   - *Determine three-dimensional structures of NOW pheromone-binding proteins*
   - *Explore a molecular-based design of parapheromones*
   - Screen by Single Sensillum Recordings & EAD a library of “easy-to-register” pheromones
   - Conduct exploratory field test of new mating disruption blends
3. Development of Female Attractants
   - *Express odorant-binding proteins (OBPs) previously isolated from female antennae*
   - *Screen potential ligands based on affinity to female OBPs*
   - *Test potential female attractants by sensory physiology and trapping*

Interpretive Summary:

The Navel Orangeworm (NOW), *Amyelois transitella*, is the most serious insect pest of almond in California. NOW is controlled by thorough postharvest orchard sanitation along with applications of organophospate (OP) and pyrethroid insecticides. Given the regulations regarding applications of OPs and secondary pest problem caused by pyrethroids, alternative methods of control are highly desirable. Pheromones offer an environmentally-friendly alternative to control insect populations. Indeed, a number of economically important lepidoteran insect pests have been successfully controlled by using pheromones in mating disruption. Pheromones may also be employed in IPM strategies to monitor population levels and determine treatment timing. The major long-term goals of this project is the development of chemically stable, alternative blends for monitoring and controlling by mating disruption populations of the NOW. Throughout this project we focus our efforts on minimizing the number of constituents and the development of kairomones from natural sources and parapheromones from a molecular strategy based on binding affinity to odorant-binding proteins. This year we have focused our efforts on Objectives 2 and 3 (see above). We have determined by X-ray crystallography the structure of the pheromone-binding protein, AtraPBP1, unbound and bound to two constituents of the sex pheromone, as well as the backbone solution structure by NMR. In addition, we have examined the electrophysiological and behavioral responses of female moths to a kairomone, which we have previously identified from navel oranges. Moreover, examination of volatile emitted from almonds as well as infected and healthy walnuts led to the discovery of another compound with potential application for trapping gravid females.

To explore the development of alternative parapheremones to replace unstable, costly and registration-challenging constituents of the sex pheromone system, we are studying structural biology of the major pheromone-binding protein from NOW, AtraPBP1. We have expressed the protein in a heterologous system, and obtained crystals of AtraPBP1 bound to the major constituent of the sex pheromone, Z11,Z13-16Ald and a major secondary constituent, Z11,Z13-16OH. These crystals diffract to high resolution, and we have already determined the basic crystallographic parameters. There are important structural differences between the protein bound to the major and secondary compounds. These structural features may lead to the design of parapheromones. Simultaneously, we have obtained the backbone structure of the solution structure of AtraPBP1 at low pH by Nuclear Magnetic Resonance (NMR) (Xu et al., Biomol. NMR Assign., http://www.springerlink.com/content/n281271575405l30/fulltext.pdf and are now obtaining the refinements of this structure.

Using the same heterologous expression system we employed to obtain AtraPBP1 for structural studies, we have expressed odorant-binding proteins previously isolated from female antennae. With a previously developed binding assay, we are now screening potential oviposition attractants based on their affinity to female AtraOBPs.

We have tested the electrophysiological and behavioral responses of female NOW to a novel kairomone we have identified last year. Previously, we have demonstrated that

female antennae are endowed with olfactory receptor neurons (ORNs) more sensitive to the newly discovered kairomone than phenyl propionate, which is a “gold standard” oviposition attractant. We have now examined the effect of female age on the response of these female ORNs to the new kairomone. Surprisingly, female moths respond to this compound with high sensitivity from the day of emergence until day-4, with noticeable decrease in response only on day-5. Behavioral responses in indoor assays mirrored electrophysiological responses, with female moth laying significantly more eggs in arenas impregnated with the new kairomone than with the gold standard, phenyl propionate (Fig. 1). [Freshly emerged 15 females and 20 males were kept in *ca*. 15x15x6 cm plastic lunch boxes lined with paper towels for 2 nights to facilitate mating. After that, moths were transferred to a white mesh cloth cage 120 x 120 x 120 cm. A clean 90 mm ID glass Petri dish holding a filter paper added with 10 µl (10 µg/µl) solution was placed at the center of a larger (ID, 150 mm) Petri dish. Chemicals were diluted in paraffin oil to obtain a slow release in the behavioral experiments A white towel paper disc of appropriate diameter was laid inside the bigger Petri dish and added with 45 ml water. This insured a humid environment to lure moths to oviposit. For the control, only the same amount of paraffin oil was added on the filter paper.]

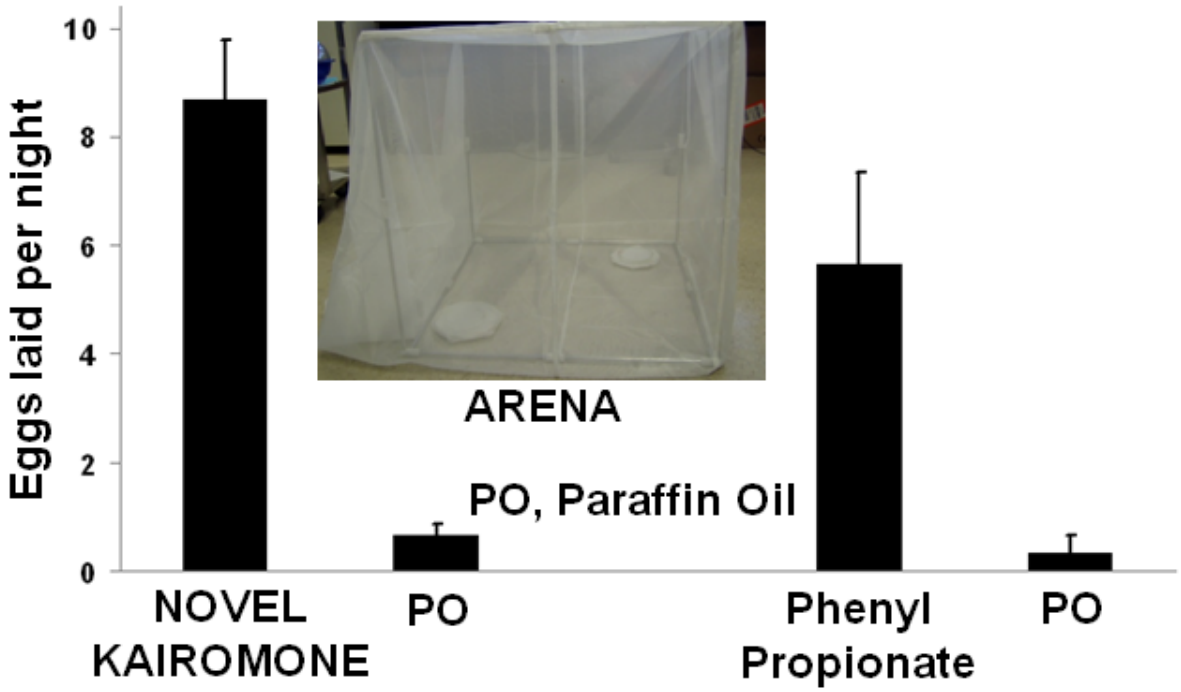

Fig. 1 Behavioral reposnes of NOW femals to kairomones in a two-choice oviposition arena (inset).

These results encouraged us to prospect for additional plant-derived compounds with potential application as oviposition attractants. In an attempt to find new attractants we have compared the gas chromatographic profiles of healthy walnuts with those of blight infested, which are consider to attract more female to oviposit. Identification of putative attractants is underway.

We have analyzed by gas chromatography coupled with an electroantennographic detector using female antennae as the sensing element the airborne volatiles collected from almonds. Interestingly, the strongest EAD response (Fig. 2) was observed with a compound which was not the most abundant compound in the chromatogram. We have already obtained synthetic samples of the compounds and conducted preliminary electrophysiological and behavioral tests. Preliminary indoor bioassays (using the same arena as in Fig. 1) showed that NOW gravid females preferentially lay eggs in arena impregnated with this almond-derived kairomone. In addition, female antennae responded to the synthetic compound in GC-EAD assays with high sensitivity.

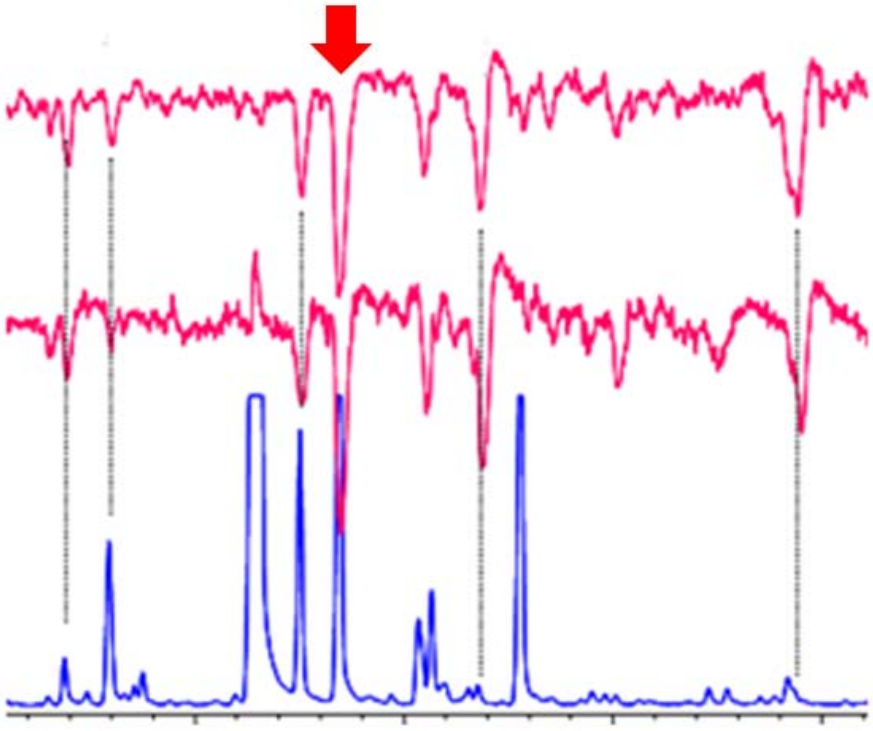

Fig. 2 GC-EAD traces obtained with airborne volatile collections from almonds. GC response with FID (bottom). EAD responses (2 traces) with female antennae (top). Arrow highlights a novel kairomone.

The previously reported kairomone, which is now demonstrated to be an oviposition attractant (Fig. 1), was first identified as an EAD-active compound from airborne volatile collections from navel oranges. By contrast, this newly identified putative kairomone (Fig. 2) was identified from airborne volatile collections from almonds.

Although both kairomones may have potential applications, the navel orange-derived kairomone has the advantage of not competing with natural sources in almond orchards.