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# Improving Trapping and Mating Disruption of the Navel Orangeworm

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

**Project Leader:** Walter S. Leal  
Department of Entomology  
UC Davis  
One Shields Ave.  
Davis, CA 95616  
(530) 752-7755  
e-mail: wsleal@ucdavis.edu

**Project Cooperators and Personnel:**

Frank G. Zalom, Zain Syed, Ruben Palma, Zhao Liu,  
UC Davis  
Franz J. A. Niederholzer, UCCE - Sutter/Yuba Counties  
David Wilson, Structural Biology Facility, UC Davis  
James B. Ames, Department of Chemistry, UC Davis

**Objectives:**

- 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 test new formulations in areas of high populations
- 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 a library of “easy-to-register” pheromones by single sensillum recordings and by electroantennographic detection (EAD)
- Conduct exploratory field test of new mating disruption blends
- 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 primarily controlled during the growing season with pyrethroid and insect growth regulators, but alternative methods of control are sorely needed. 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 goal of this project is the development of chemically stable, alternative blends for monitoring and controlling by mating disruption populations of the navel orangeworm. 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 determined by X-ray crystallography the structure of the pheromone-binding protein, AtrPB1, unbound and bound to two constituents of the sex pheromone, as well as the backbone solution structure by nuclear magnetic resonance (NMR). In addition, we have examined the electrophysiological and behavioral responses of female moths to kairomone we have previously identified from navel oranges. Moreover, examination of volatiles emitted from almonds as well as infected and healthy walnuts led to the discovery of another compound with potential application for trapping gravid females.

## Materials and Methods:

*Structural Biology:* Purified AtrOBP was crystallized at room temperature by the hanging drop vapor diffusion method. Drops containing 1  $\mu$ l of protein solution (30 mg/ml) were mixed with 1  $\mu$ l of the precipitant solution and suspended over a reservoir containing the precipitant solution 1.6M sodium citrate pH 6.5. Crystals used in data collection were transferred into the viscous cryoprotectant paratone-N and flash-cooled in a stream of liquid nitrogen at 110 K.

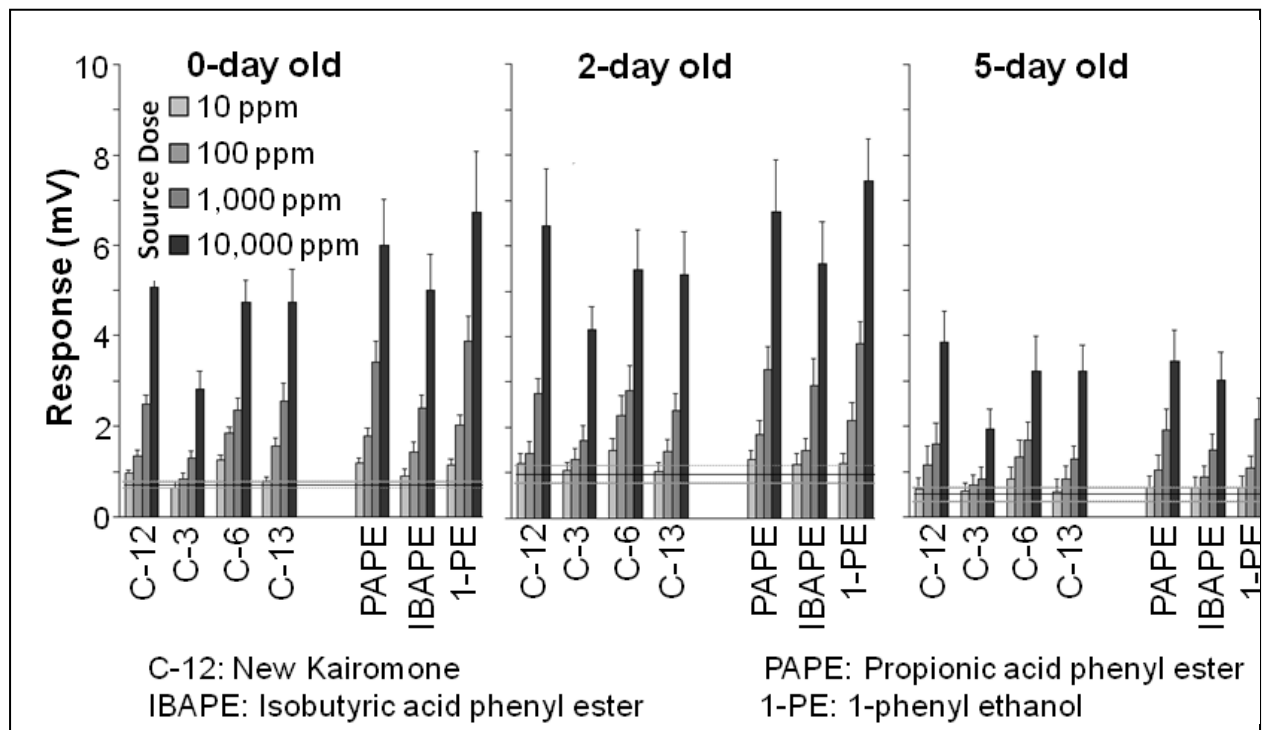
*Electrophysiological and Behavioral Studies:* Single-sensillum recordings, electroantennogram (EAG) and gas chromatography with electroantennographic detection (GC-EAD) was done as previously reported. Freshly emerged 15 females and 20 males were kept in ca. 15x15x6 cm plastic lunch boxes lined with paper towels for 2 nights to facilitate mating. After that, moths were transferred to a white mesh cloth cage 120 x 120 x 120cm. A clean 90 mm ID glass Petri dish holding a filter paper added with 10  $\mu$ l (10  $\mu$ g/ $\mu$ l) solution was placed at the center of a larger (D = 150mm) Petri dish. A manifold 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. Chemicals were diluted in paraffin oil to obtain a slow release in the behavioral experiments

## Results and Discussion:

*Structural Biology and Prospecting of Novel Attractants/Parapheromones:* To explore the development of alternative parapheromones 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., 2008) and are now obtaining the refinements of this structure.

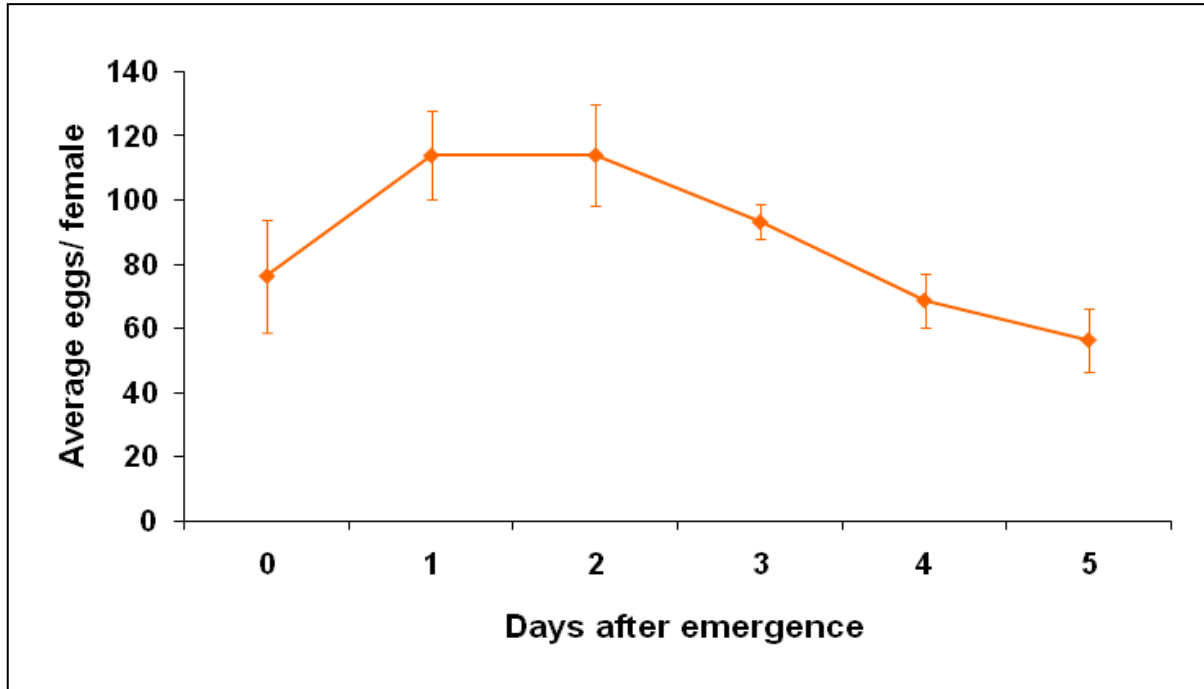
Using the same heterologous expression system we employed to obtain AtraPBP1 for structural studies, we have expressed odorant-binding proteins previously isolated from female antennae. With a previously developed binding assay, we are now screening potential oviposition attractants based on their affinity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

*Electrophysiological and Behavioral Studies:* We recorded electrophysiological and behavioral responses of female NOW to a novel kairomone we have identified last year (see 2007-08 Final Report and 2008 Proceedings). Previously, we have demonstrated that female antennae are endowed with olfactory receptor neurons (ORNs) more sensitive to the newly discovered kairomone (C-12) than phenyl propionate, which is known as a NOW attractant. We have now examined the effect of female age on the response of these female ORNs to the new (C-12) and previously known kairomones (**Figure 1**).



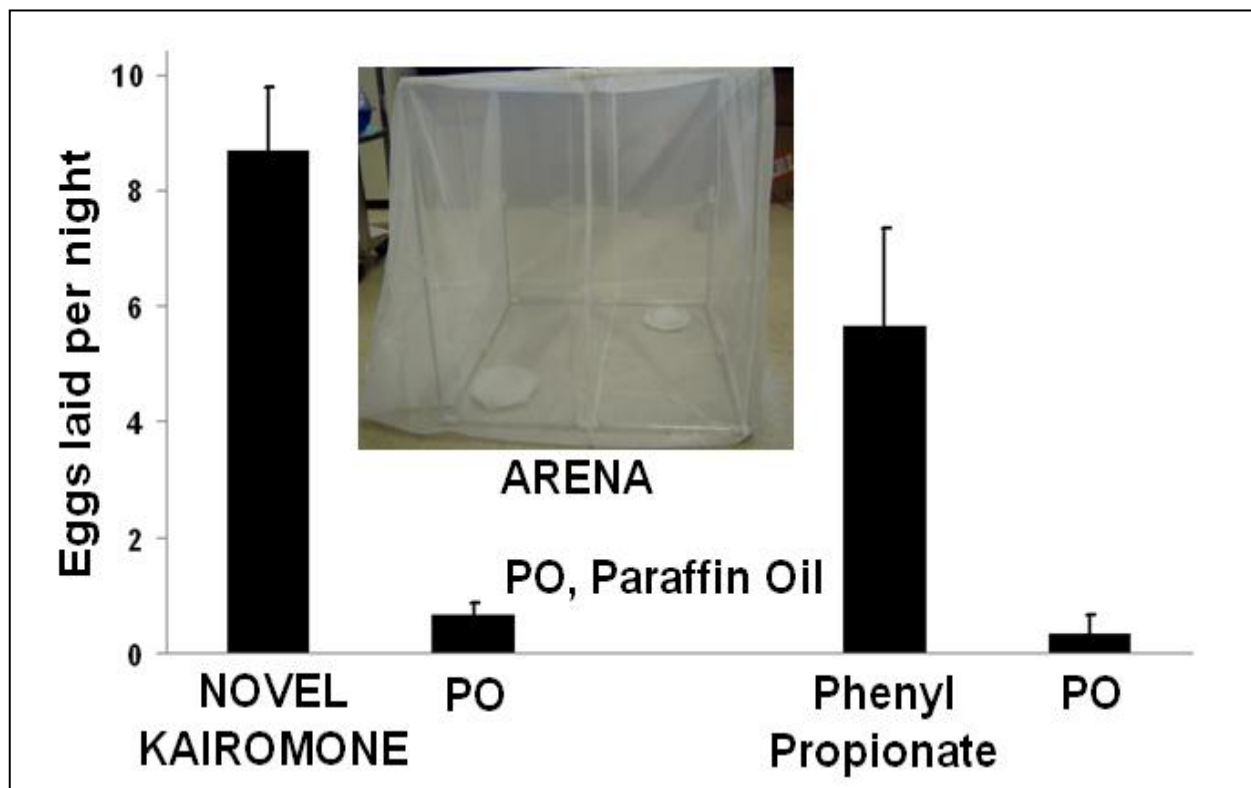
**Figure 1.** Age dependence of female NOW response to a novel and known kairomones.

Surprisingly, female moths respond to the new C-12 kairomone with high sensitivity from the day of emergence until day-4, with noticeable decrease in response on day-5 (**Figure 1**). We then examined NOW egg laying behavior, which is important not only for the development of oviposition attractants, but also for mating disruption strategies. Peak of egg-laying was reached 1-2 days after emergence, but NOW females continued to lay eggs even 5 days after emergence (**Figure 2**).



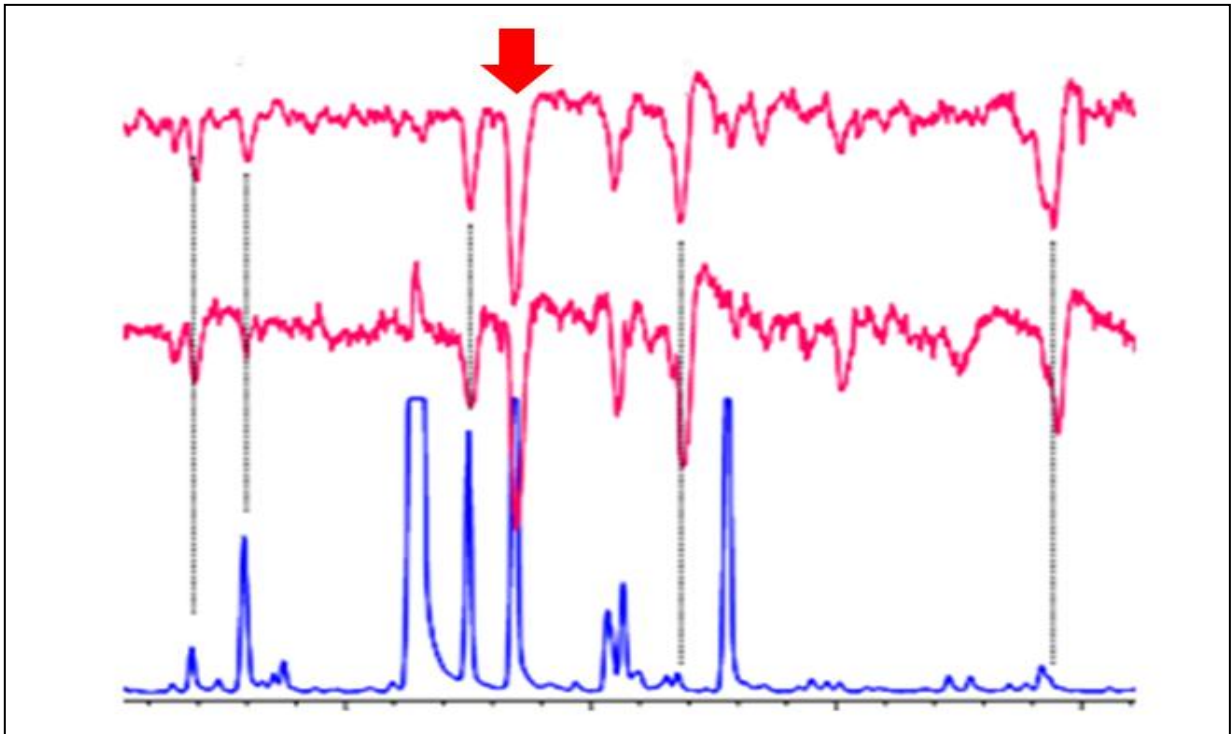
**Figure 2.** Effect of female age on egg laying by the navel orangeworm.  $N=6$

Next, we examined female response to the C-12 kairomone. Behavioral responses in indoor assays mirrored electrophysiological responses, with female moth laying significantly more eggs in arenas impregnated with the new C-12 kairomone than with a known attractant, phenyl propionate (**Figure 3**).



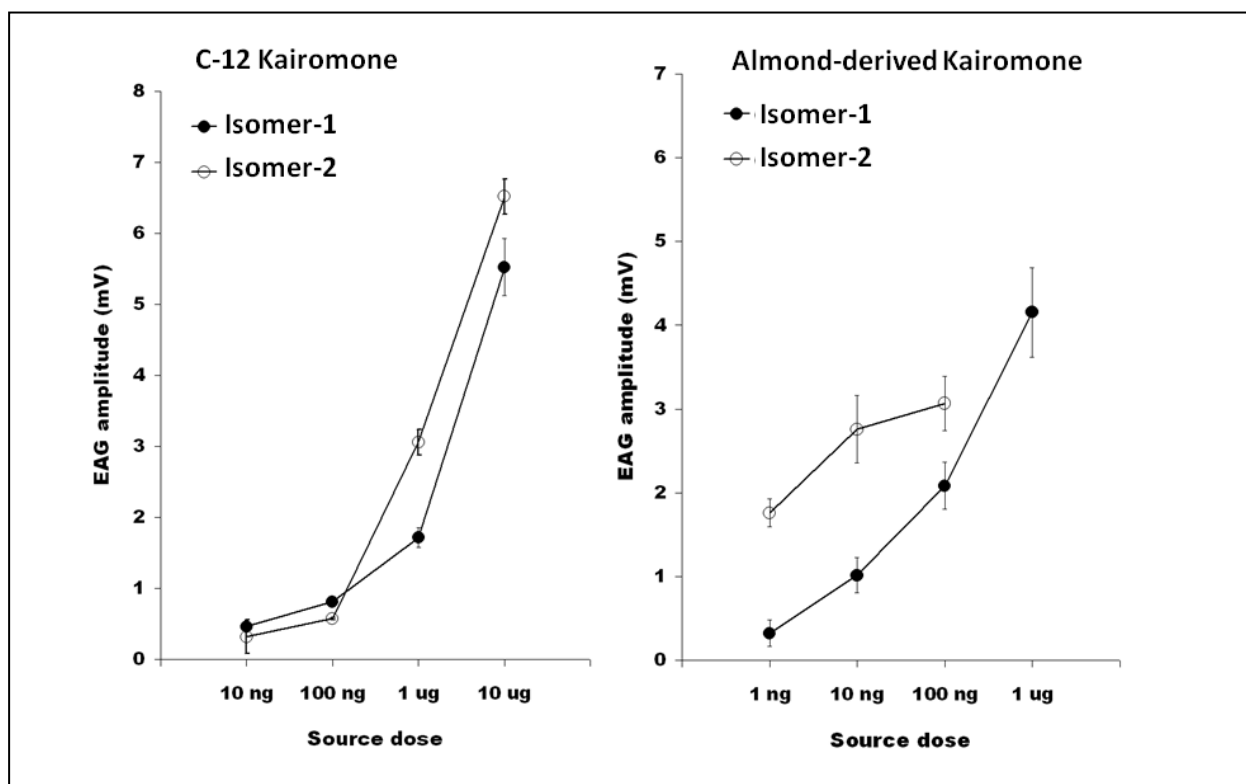
**Figure 3.** Behavioral responses of female moths to kairomones in a two-choice oviposition arena (inset).

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 considered 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 (**Figure 4**) 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 **Figure 3**) 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.



**Figure 4.** GC-EAD traces obtained with airborne volatile collections from almonds. GC responses with analytical detector (blue, FID). EAD responses (red) with female antennae (two traces). Arrow highlights an almond-derived kairomone.

Interestingly, both kairomones have isomers, which could not be separated by gas chromatography. We then examined the electrophysiological responses of female moths to the isomers of these newly identified kairomones by EAG with pure compounds. While olfactory receptor neurons in female antennae responded to both isomers of C-12 with almost the same sensitivity (**Figure 5, left**), there was a clear preference for the isomer-2 of the almond-derived kairomone (**Figure 5**). The isomer-1 of the almond-derived semiochemical required at least two order of magnitude higher doses to generate comparable physiological response. Future behavioral studies will determine if the isomer with lower sensitivity is beneficial, inhibitory or merely a diluent.



**Figure 5.** Antennal responses from olfactory receptor neurons in female antennae to isomers of two kairomones.

Both oviposition attractants have potential applications for management of NOW population, but the navel orange-derived kairomone, C-12, has the advantage of not competing with natural sources in almond orchards.

#### Recent Publications:

X. Xu, Y. Li, J. Rayo, Y. Ishida, W. S. Leal, J. B. Ames,  $^1\text{H}$ ,  $^{15}\text{N}$ , and  $^{13}\text{C}$  Chemical shift assignments of the navel orangeworm pheromone-binding protein-1 (Atra-PBP1). *Biomol NMR Assign* (2008) 2:105–106.