Improving Trapping and Mating Disruption of the Navel Orangeworm

Project No.: **07-ENT02-Leal/Zalom**

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Objectives:

1. Improve Formulation for Trapping Males
   a. Determine Optimal Ratio of Constituents
   b. Determine Minimal Constituents Required for Maximal Attraction
   c. Determine Optimum Amount of Pheromone per Trap
   d. Develop Stable and Long-Lasting Formulations
   e. Field Evaluate New Formulations in Areas of High Populations
2. Develop Alternative Multi-Component Blends for Mating Disruption
   a. Determine the three-dimensional structures of NOW pheromone-binding proteins unbound and bound to pheromone constituents
   b. Explore a molecular-based design of parapheromones
   c. Screen by Single Sensillum Recordings & EAD a library of “easy-to-register” pheromones
   d. Conduct exploratory field test of new mating disruption blends

Interpretive Summary:

The most serious insect pest of almond in California is the Navel Orangeworm (NOW) for which alternative environmentally-friendly and effective methods of control are highly desirable. Largely, sex pheromones may be employed in IPM for monitoring moth

populations, determining treatment time, and consequently reducing insecticide sprays. In addition, moth populations may be effectively controlled by using sex pheromone in strategies like mating disruption. We are justifiably proud of our recent discovery of additional constituents of the pheromone system of the navel orangeworm, some/most of which are essential for moth trapping and mating disruption. Unfortunately, a few of the newly identified chemicals and key constituents pose formulation and regulatory challenges. Therefore, the major goal of this project is to develop chemically stable, alternative blends for NOW trapping and mating disruption. We focus both on minimizing the number of constituents in the pheromone-based formulations and the development of attractants derived from natural sources (kairomones) or related to the natural pheromones (parapheromones). This year we concentrated most of our efforts on the latter approach. We focused both on (i) providing the foundation for the molecular-based approach, and (ii) the screening potential attractants. Using gas chromatography with electroantennographic detection (GC-EAD) and single sensillum recording (SSR), we have discovered a number of potential attractants, which will be tested in the next season(s).

We have expressed a pheromone-binding protein from the navel orangeworm (AtraPBPP1) in bacteria, purified the recombinant protein, and co-crystallized it with pheromone constituents. In addition, we have generated AtraPBP1 samples labeled either with nitrogen-15 alone or with both carbon-13 and nitrogen-15 so that the structure of the unbound protein, named apo-ApolPBP1, can be analyzed by nuclear magnetic resonance (NMR). X-Ray crystallography and NMR studies will allow us to determine the three-dimensional structures of AtraPBP1 alone and bound to pheromones so as to lay the foundation for the design of parapheromones. Simultaneously, we are exploring the development of other attractants from natural sources that may improve attractancy of a less complex, more stable, and cost-effective pheromone blend.

Examining mixtures of plant-derived compounds, we have observed that both male and female antennae detect plant-derived semiochemicals (potential attractants) with nearly the same intensity (Fig. 1) in a GC-EAD assay.

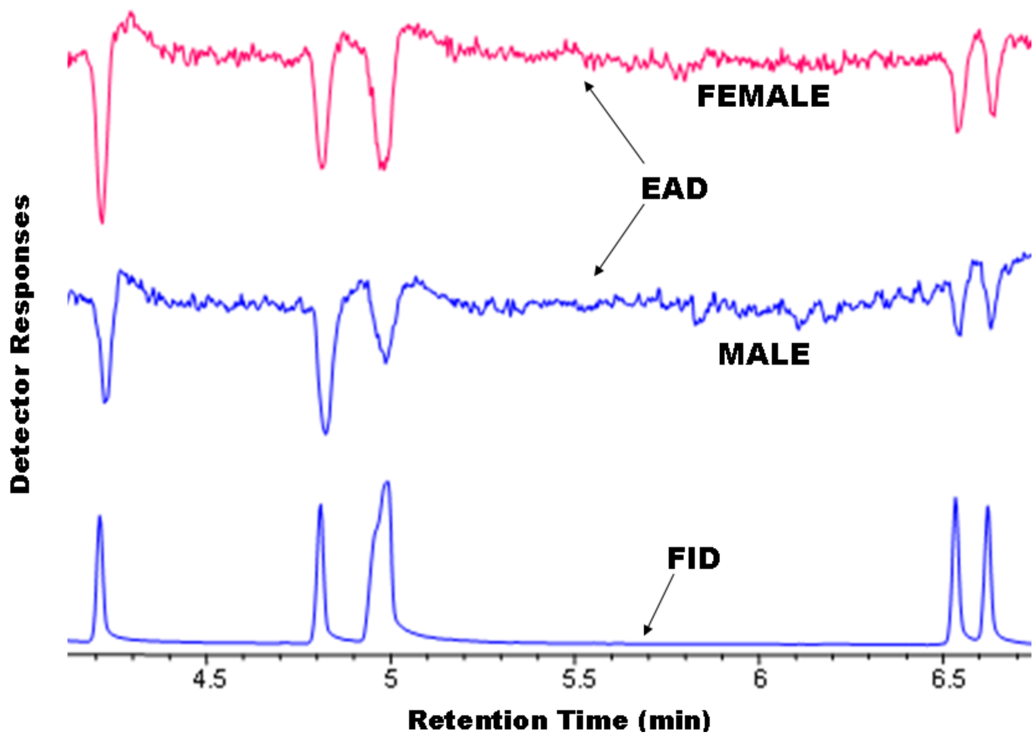

Fig. 1. Analysis of potential attractants for the navel orangeworm by coupled gas chromatography and electroantennographic detection (GC-EAD). Female (top trace) and male (middle trace) antennae were used as the sensing element. The lower trace is the standard detector response (FID) from the gas chromatograph.

We then employed a technique named electroantennogram (EAG) to focus on response of individual compounds of potential application for trapping and/or mating disruption. We recorded responses of male and female antennae to various biologically relevant compounds (Fig. 2), including the previously identified constituents of the sex pheromone (Leal and collaborators, Naturwissenschaften v. 92, p. 139, 2005) and attractants (Price et al, California Agriculture, p. 10, 1967), and new test compounds.

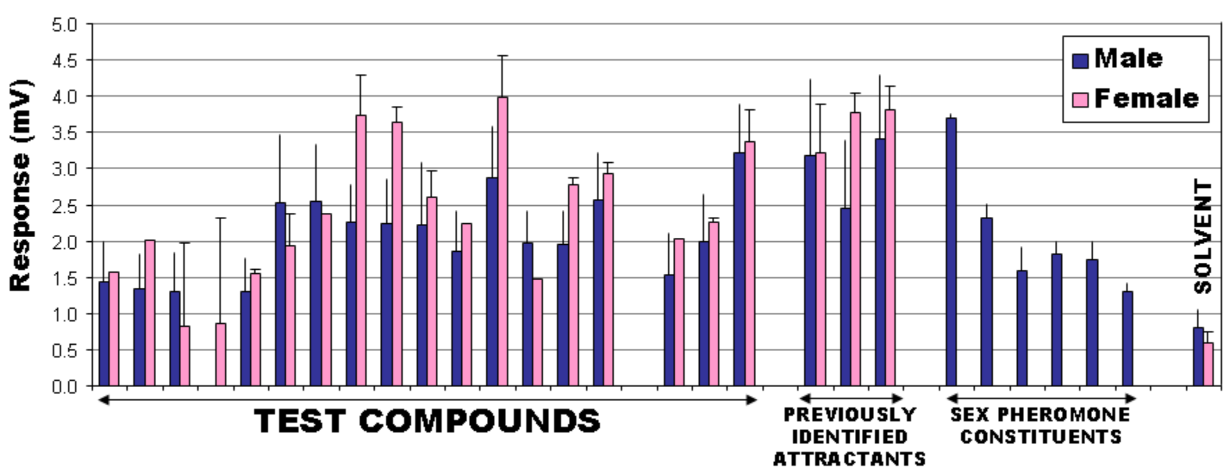

Fig. 2. EAG responses recorded from male and female antennae of the navel orangeworm when stimulated with various semiochemicals, including constituents of the female-produced sex pheromone (which are specific to males), previously identified attractants, and newly tested compounds.

A few of the newly identified plant-derived compound showed stronger or comparable response to previously identified attractants. Because both males and females responded (Fig. 2) to those test compounds with nearly the same high intensity, we assume they are physiologically relevant, possibly as attractant (kairomones). We then aimed at finding the single units (sensilla) detecting those attractants on the antennae.

We studied the morphology of the moth antennae by scanning electron microscopy (SEM) and identified a variety of sensillum types. Using single sensillum recordings, we then investigated the physiological properties of sensilla on the male antennae responding to sex pheromone. The pheromone-detecting sensilla have been identified and these decetors will be explored in future studies for the development of parapheromones.

Next, we generated a chemical mapping of the detectors on the female antennae. Our results showed that the auriculate sensilla (Fig. 3) houses two olfactory receptor neurons, one of them sensitive to phenyl propionate. This discovery is particularly important to us because this compound is a “gold standard attractant.” Attractancy of phenyl propionate was discovered by Price and collaborators (1967) and later confirmed in extensive field studies by Kunen and collaborators (California Pistachio Commission Report, 2004). Having identified the “attractant sensilla” we employed the same SSR technique to screen plant-derived compounds, some of which are produced by almonds and other crops infested by the navel orangeworm. These experiments led to the identification of multiple easy-to-register, inexpensive compounds, which showed stronger responses than the “gold standard” (Fig. 3).

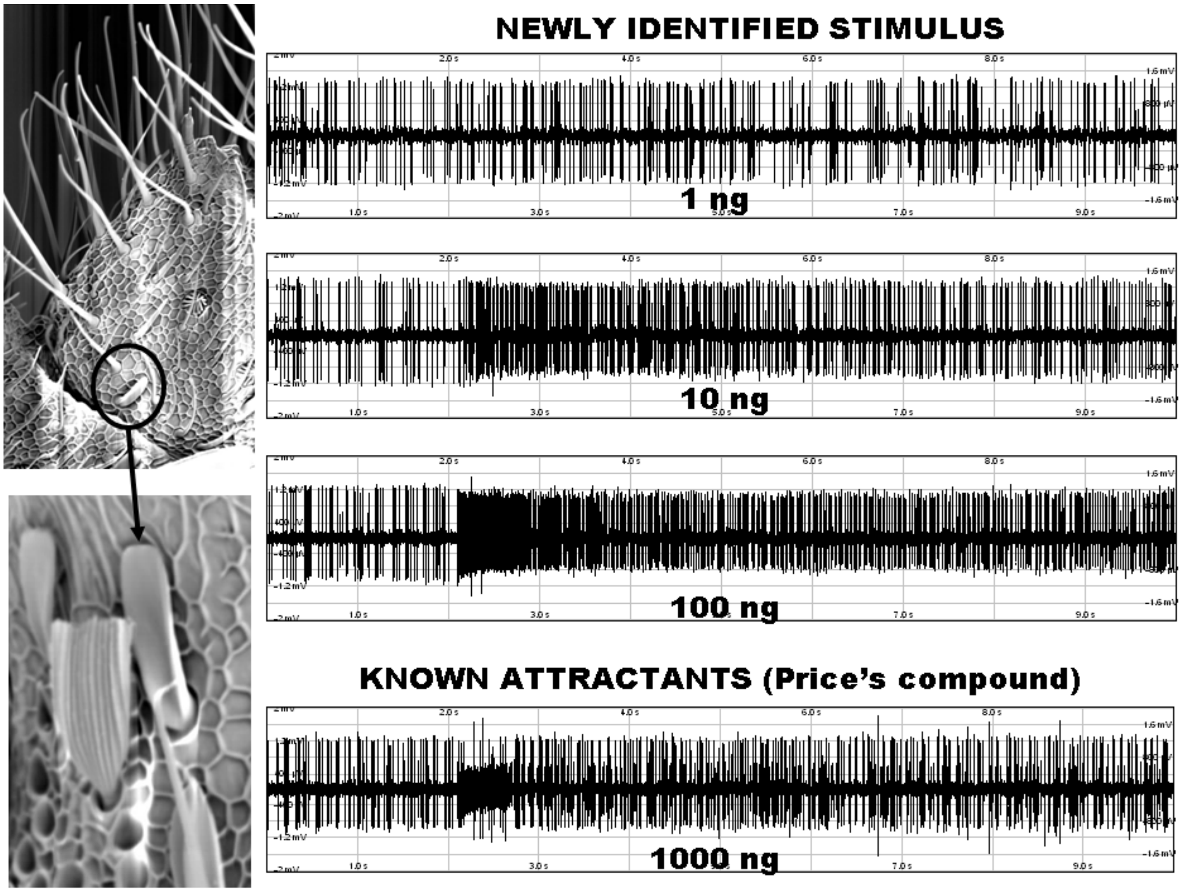

Fig. 3. Electrophysiological responses (SSR) recorded from an auriculate type of sensilla (highlighted on the left) on the antennae of the navel orangeworm. Upper traces show excitation of a cell with large spike amplitude by increasing doses of a newly identified compound. Bottom trace shows excitation by another cell housed in the same sensillum (small spike amplitude) elicited by an attractant, phenyl propionate, previously identified by Price and collaborators, and confirmed to be a potent attractant by Kunen and collaborators.

Although field tests are yet to be performed in the next season(s), these are potential attractants for the navel orangeworm. These compounds are detected by the “attractant sensilla” and showed a much higher sensitivity than a “gold standard.” For example, one of the newly identified compounds (Fig. 3) responded in a dose-dependent manner, with lower threshold and higher sensitivity than phenyl propionate. The newly identified, putative kairomones may be employed in combination with some constituents of the sex pheromone to improve male trapping. In addition, some of them may be employed as baits in oviposition traps.