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Attractants for Navel Orangeworm

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Introduction. The Navel Orangeworm (NOW), *Amyelois transitella*, is a commercial pest of a number of crops (walnuts, figs, etc) and the most serious pest of almond and pistachios in California. NOW is primarily controlled with organophosphate (OP) and pyrethroid insecticides, but alternative methods of control are sorely needed given the regulations regarding applications of OP and secondary pest problems caused by pyrethroids. Sex pheromones and other semiochemicals are invaluable tools for monitoring and control of insect pest populations. When these chemicals, produced by insects (for their own communication), are accurately identified and synthesized, they may be employed in IPM strategies to monitor populations and determine treatment timing or to reduce populations by mass trapping, lure-and-kill, or mating disruption. A number of economically important lepidopteran species have been successfully controlled using synthetic sex pheromone in mating disruption. For the successful application of pheromones in mating disruption, it is essential that the synthetic and natural sex pheromone be identical, i.e., the chemicals emitted by female to lure males must be accurately identified. Minimal modifications in the chemical structures of pheromones render them completely inactive. Largely, the pheromone systems of moths contain multiple constituents in a precisely defined ratio. Despite the tremendous efforts by leading scientists in the field of chemical ecology over two decades, only one single constituent (Z11Z13-16Al) has been identified from the NOW pheromone system (Coffelt et al. 1979), thus, suggesting that a complete identification may be beyond the current chemical ecology approaches. We have taken a molecular-based strategy in which potential pheromone constituents and attractants are screened with proteins involved in the reception of pheromones. The reception of pheromones and other semiochemicals in insect antennae is initiated by binding to pheromone-binding

proteins (PBPs) or odorant-binding proteins (OBPs) that transport the hydrophobic (water insoluble) pheromones through the aqueous environment inside the antennae towards the pheromone receptors (Leal 2003, 2005b, a).

The ultimate goal of this project is the discovery of new attractants that may lead to improved pheromone systems for monitoring and controlling NOW populations. To reach this goal we have previously (1) isolated odorant- and pheromone-binding proteins from NOW, (2) obtained amino acid sequences of the isolated OBPs and PBPs, and (3) cloned the cDNAs (genes) encoding these OBPs and PBPs. We have now completed the goals for Y2 of this project, namely, (i) the construction of recombinant vectors for the cloned OBPs and PBPs, (ii) development of expression systems for NOW OBPs and PBPs, and (iii) production, characterization, and purification of recombinant OBPs and PBPs. In addition, we have already started performing the goals established for Year-3, i.e., development of a binding assay for the screening of attractants.

Results. We have developed expression systems for odorant- and pheromone-binding proteins identified in the year one of this project, namely, AtraPBP1, AtraPBP2-F102, AtraPBP2-Y102, AtraGOBP, and AtraCSP. These recombinant proteins have been overexpressed in LB medium using transformed BL21(DE3) cells. Proteins in the periplasmic fractions were extracted by three cycles of freeze-and-thaw, and purified by ion-exchange chromatography (DEAE, Mono-Q) and gel filtration (Sepharose). Fractions were analyzed by SDS gel electrophoresis and liquid chromatography-mass spectrometry. Pure protein fractions (>98%) were desalted, lyophilized and stored at -80°C until use. We have also developed a novel binding assay (Leal et al. 2005a) based on the separation by a centrifugal filter device of bound and free ligands. After incubation of a test compound and an OBP or PBP (under the desired conditions of temperature, pH, ionic strength, etc), the free ligand was filtered out of the mixture, whereas the ligated protein is retained by the filter's membrane. Then the ligand is released from the protein by lowering the pH, extracted by an organic solvent with an internal standard, and analyzed by gas chromatography-

mass spectrometry (for identification) and/or gas chromatography for quantification. Using this novel binding assay, we have demonstrated that the putative pheromone-binding protein AtraPBP1 is indeed a pheromone-binding protein (Fig. 1).

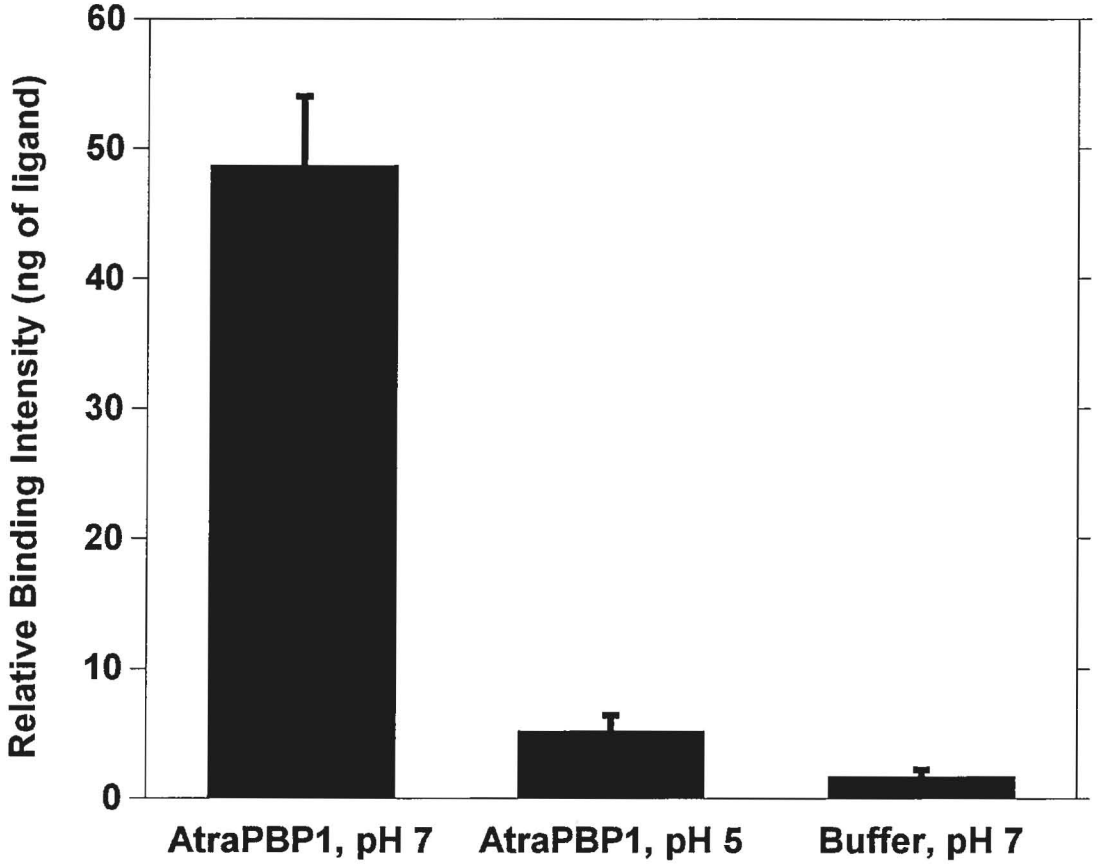

Figure1. Binding of the only known pheromone of the navel orangeworm, (Z,Z)-11,13-hexadecadienal at pH 7. Control experiments (buffer, pH 7) did not differ significantly from those with the olfactory protein at pH 5, indicating that there is no binding at low pH.

AtraPBP1 showed high affinity for the known pheromone, (Z,Z)-11,13-hexadecadienal (Coffelt et al. 1979) at the pH of the sensillar lymph (pH 7), but no binding activity at low pH, as expected for a pheromone-binding protein (Leal 2004a, 2003, 2004b).

Having validated AtraPBP1 as a molecular target, we used our binding assays to screen for other potential attractants for the navel orangeworm. In these tests, we observed that AtraPBP1 binds to (Z,Z)-11,13-hexadecadienyl acetate (NOWOAc, in short) with the same apparent affinity as that observed for (Z,Z)-11,13-

hexadecadienal (ZZNOW, in short) (Fig. 2). Interestingly, the binding ability of NOWAc was observed even before this semiochemical was detected in the pheromone glands of virgin females and characterized as a behavioral antagonist (Leal et al. 2005b).

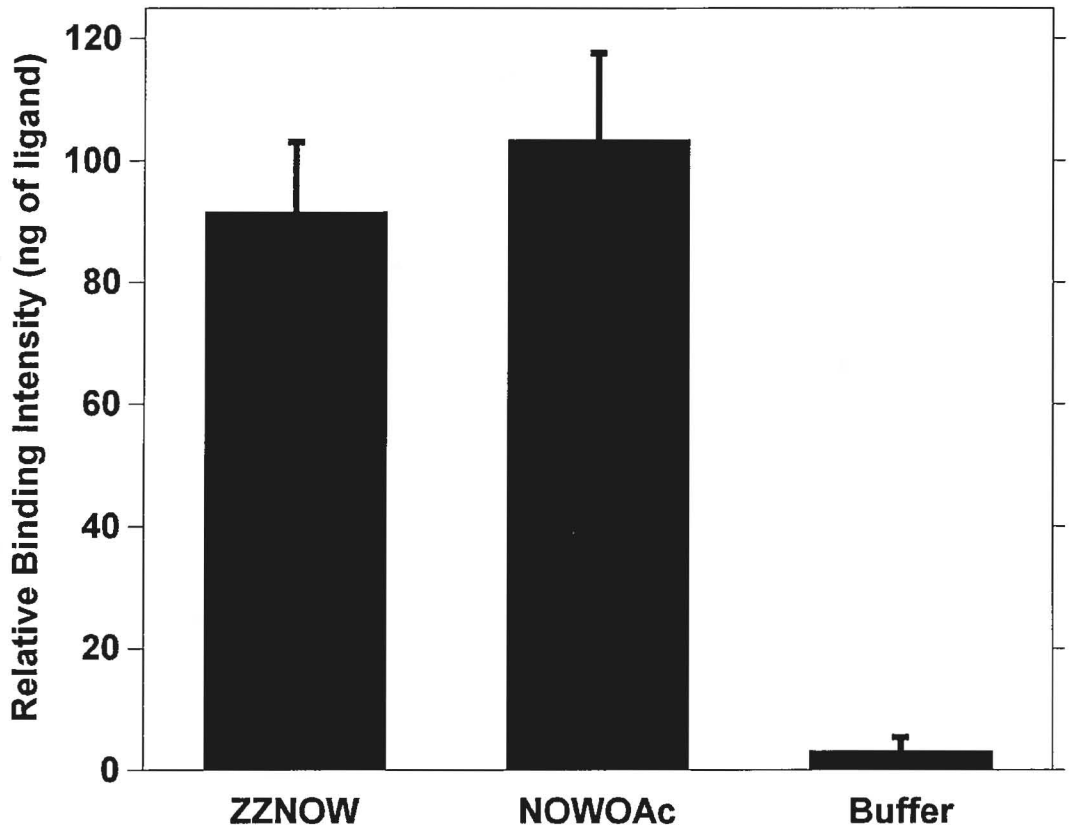

Figure 2. Binding of (Z,Z)-11,13-hexadecadienol and (Z,Z)-11,13-hexadecadienyl acetate to AtraPBP1 at pH 7. Only traces of these ligands were detected when protein was replaced by buffer.

In addition, we discovered another ligand, (Z,Z)-11,13-hexadecadienol (NOWOH, in short), which was later identified as one of the constituents of the sex pheromone of the navel orangeworm (Leal et al. 2005b). As demonstrated in competitive binding assays, in which ApolPBP1 was exposed to the three ligands at the same time, ApolPBP1 showed slightly higher preference for the behavioral antagonist (NOWOAc), whereas both ZZNOW and NOWOH showed apparently the same binding affinity (Fig. 3). These findings prompted us to carry out an independent thorough investigation of the pheromone chemistry of the navel orangeworm. With behavioral studies, single sensillum recording, and gas

chromatography couple to an electroantennographic detection (GC-EAD), we were able to identify the complete pheromone system of the navel orangeworm (Leal et al. 2005b).

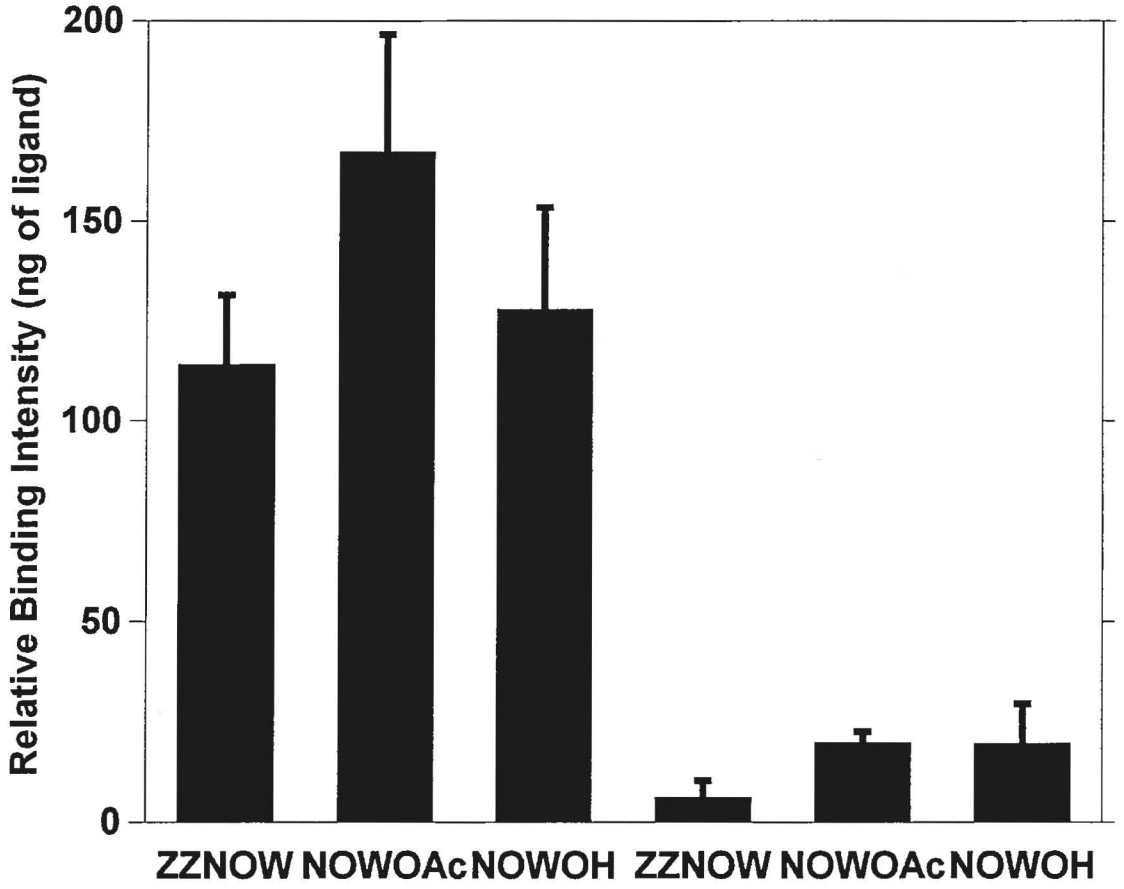

Figure 3. Competitive binding assays. The right side of the panel shows the testss with buffer only (controls).

Conclusions and practical applications. We have constructed recombinant vectors, developed expression systems for NOW pheromone-binding proteins, produced and purified recombinant olfactory proteins, developed a new binding assay, and validated AtraPBP1 as a molecular target. With these molecular tools, we obtained leading compounds, which were later identified in independent studies as essential constituents of the sex pheromone system. In summary, this project is leading directly and indirectly to better attractants and better trapping systems for the navel orangeworm as well as to improvements of mating disruption strategy for pest control.

References

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