Synthesis and Field Evaluation of the Sex Pheromone from the Ten-Lined June Beetle

Project No.: **09-ENTO5-Leal**

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Objectives:

- Identification and synthesis of the sex pheromone of the ten-lined June beetle, *Polyphylla sobrina*
- Formulation of synthetic sex pheromone
- Developing the use of sex pheromones for detection, monitoring, and risk assessment
- Exploring the use of sex pheromones for mass trapping and/or mating disruption
- Reverse chemical ecology approach for the development of scarab beetle attractants

Interpretive Summary:

Sex pheromones and other semiochemicals are invaluable tools in insect control programs. These green chemicals can be employed in integrated pest management (IPM) programs for monitoring established populations and to reduce and optimize insecticide sprays as well as for detection and survey programs for exotic species. To implement an effective IPM approach, it is critical to know which pest species are present (detection), and whether or not their population densities warrant control (monitoring and risk assessment). Detection of the presence or absence of a pest species is often a concern in quarantine when an invasive species is expanding its range, and area-wide action can be taken to limit or prevent that spread when early colonizers are first detected. Sex pheromones are also used for direct control of insect populations in mass trapping, mating disruption, and attract and kill. The objective of

mass trapping is to reduce pest density without other intervention. If the trapped stage is the damaging stage of the insect, the effectiveness of mass trapping could be proportional to the number of insects removed.

The cornerstone of successful IPM programs is the identification and synthesis of sex pheromones of insect pests. Here, we aim at identifying and synthesizing the sex pheromones of ten-lined June beetle (TLJB), *Polyphylla sobrina,* a chronic agricultural pest problem where it occurs. This season we attempted to re-isolate the sex pheromone by extracting field-collected females and pooling fractions containing trace amounts of the natural product. Unfortunately, the flight activity was very low this year; we similar efforts from previous years we were able to collect only 280 females. Previously, we have isolated the sex pheromone in 4% fractions (with further purification by Gerstel's GC-Preparative Fraction Collection). We pooled the satellite fractions (3% and 5%), which were leftovers from previous years and kept at -80^{o}C, combined with new extracts, and re-isolated the pheromone using gas chromatography-mass spec (GC-MS) to identify the active peak. With this newly isolated sample, we obtained additional insights on the molecular structure of the sex pheromone. Clearly, proton exchange took place when the isolated sample was re-dissolved in deuterated methanol, CD_3OD. The increase in mass-to-charge ratios (m/z) of various fragments strongly suggests that the pheromone molecule has one free hydroxy group. Comparison with the original MS indicates shifts in m/z 283→284, 241→242, and 171→172 (base peak). Although this new information alone is not enough for structure elucidation, it adds new key pieces to the puzzle, the most important being the occurrence of a free OH group. The polarity of this hydroxy group might be stabilized by intramolecular hydrogen bonding, as indicated by the low polarity of the compound (difference in Kotatz indexes, ∆polar - non-polar, 295). This low polarity is consistent with elution from silica gel column with 4% ether in hexane. Unfortunately, we could not collect enough beetles to generate the amount required for structure elucidation. Thus, we explored a contingency plan.

Recently, we devised a "reverse chemical ecology" approach to be used in combination with conventional chemical ecology approaches to tackle challenging problems, such as the identification of unusual moth pheromones [1] and effective mosquito oviposition attractants [2]. In the reverse chemical ecology approach olfactory proteins are isolated, cloned, expressed, and employed in binding assays to screen for potential attractants and generate "lead" compounds. Simply put, structure elucidation of sex pheromones and other naturally-occurring chemicals is a puzzle composed of pieces of information derived mostly from spectral data and chemical derivatizations. The reverse chemical ecology approach generates additional pieces of molecular-based information, i.e., affinity of related compounds to an olfactory protein. To employ this reverse chemical ecology approach in the case of the ten-lined June beetle we isolated, cloned, and expressed a pheromone-binding protein expressed specifically in the antennae of this species, which we named PsobPBP. We have constructed an expression cassette,

pET22b●PsobPBP, and the protein was over-expressed in LB medium using transformed BL21 (DE3) cells. Proteins in the periplasmic fraction were extracted by freeze-and-thaw and purified by ion-exchange chromatography (DEAE and MonoQ) and gel filtration (Sepharose) to generate pure samples (**Figure 1**). That the recombinant protein is identical to the native PBP was determined by gel electrophoresis, protein sequencing, and mass spectrometry. Migration of native PsobPBP and recPsobPBP in native PAGE (**Figure 2**), the N-terminal sequence of recPsobPBP, obtained by Edman degradation (MSEEME), and molecular mass, obtained by LC-ESI/MS (**Figure 3**), confirmed that recPsobPBP is identical to the native PBP. We examined the secondary structure of recPsobPBP by circular dichroism (CD). As indicated by the maximum at 192 nm and the two minima at 210 and 219 nm (**Figure 4**), PsobPBP is an α helix-rich protein. This type of secondary structure is a common feature of pheromone-binding proteins.

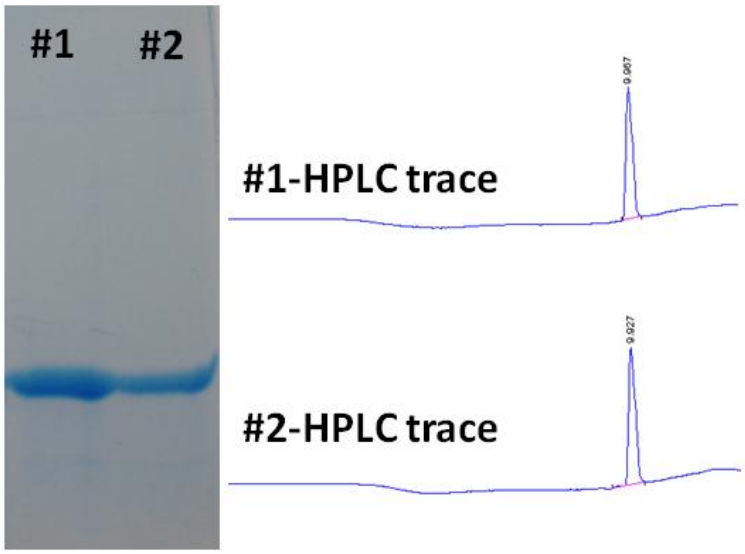

Figure 1. Pure recPsobPBP samples as indicated by SDS (left) and HPLC (right**).**

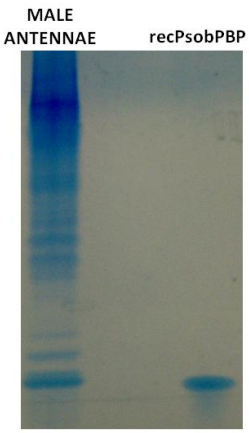

Figure 2. Native-PAGE (15%) analysis of native and recombinant proteins**.**

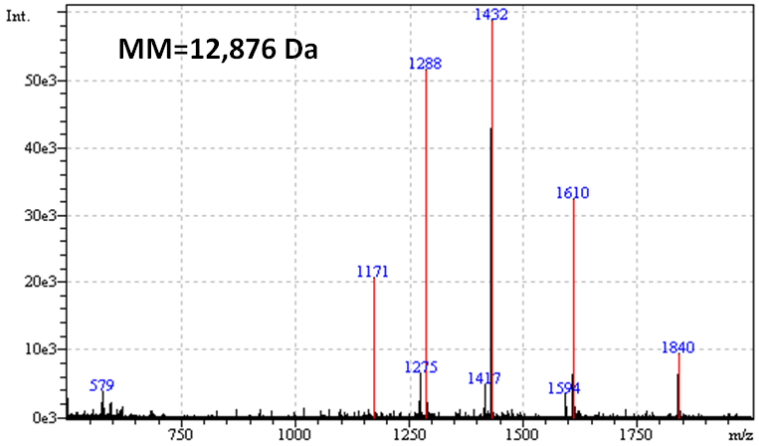

Figure 3. LC-ESI/MS data indicating that observed (12,876 Da) and calculated (12,875 Da) molecular masses are in agreement.

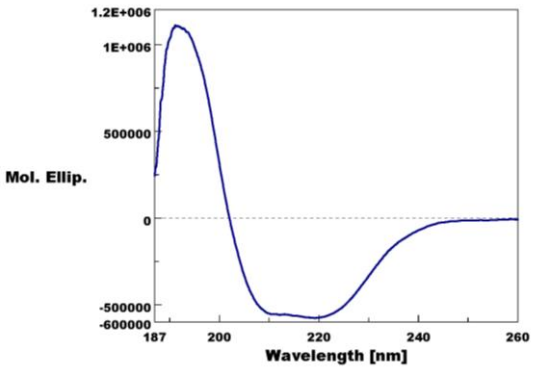

Figure 4. Far-UV CD spectrum of PsobPBP at pH 7

Next, we will use this recombinant protein as a molecular target in binding assays-oriented identification of lead compound(s), which will also be tested as possible attractants.

References:

Leal WS, Parra-Pedrazzoli AL, Kaissling KE, Morgan TI, Zalom FG, et al. (2005) Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. Naturwissenschaften 92: 139-146.

Leal WS, Barbosa RM, Xu W, Ishida Y, Syed Z, et al. (2008) Reverse and conventional chemical ecology approaches for the development of oviposition attractants for Culex mosquitoes. PLoS ONE 3: e3045.