

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

Project No.: 07-ENT05-Leal/Zalom

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Objectives:

1. Collect large number of females of the ten-lined June beetle from almond orchards
2. Extract natural pheromone from field-collected females and re-isolate the active fraction
3. Isolate pheromone to purity by preparative fraction collection
4. Determine the chemical structure of the pheromone by GC-MS, GC-FTIR, and micro-analytical chemistry
5. Synthesize new pheromone
6. Develop pheromone traps with slow-release devices (dispensers)
7. Field evaluations and exploratory tests for mass trapping

Interpretive Summary:

The ultimate goal of this project is to develop pheromone-based strategies for monitoring (trapping) and possibly controlling populations of the Ten-Lined June Beetle (TLJB). Females of the TLJB do not fly but attract males with a very potent sex pheromone emitted while they emerge out of the soil. Mated females return to the soil to lay large numbers of eggs which generate the progeny. The immature larvae, also called grubs, flourish by voraciously feeding on roots of almonds and other commercial trees. Grubs submerge so deep in the soil that they are essentially out of reach of the conventional pesticide treatments. The major opportunity for effective control of TLJB populations is to interrupt the cycle when adults come out of the soil for mating. This window of opportunity is limited to a few minutes during the daily mating activity in summer. A synthetic sex pheromone will allow monitoring the exact time for treatment

and/or open the door for controlling by mass trapping males. Our initial goals are (1) to obtain a large sample of field collected females, (2) extract the natural sex pheromone, (3) re-isolate the active compound, and (4) identify the chemical structure of the sex pheromone. Once the chemical structure is known, we will (5) synthesize the sex pheromone, (6) incorporate the synthetic compound in devices that will allow a long-lasting formulation for field applications, and (7) evaluate the feasibility of the synthetic sex pheromone for trapping and controlling populations of this insect pest. While the potency of the sex pheromone raises high expectation for practical applications, the amounts produced by females are so small that makes chemical identification a challenging task, particularly given the unusual chemical structure. This year we have beefed up our logistics for capturing females (Fig. 1) so as to generate material for chemical characterization.

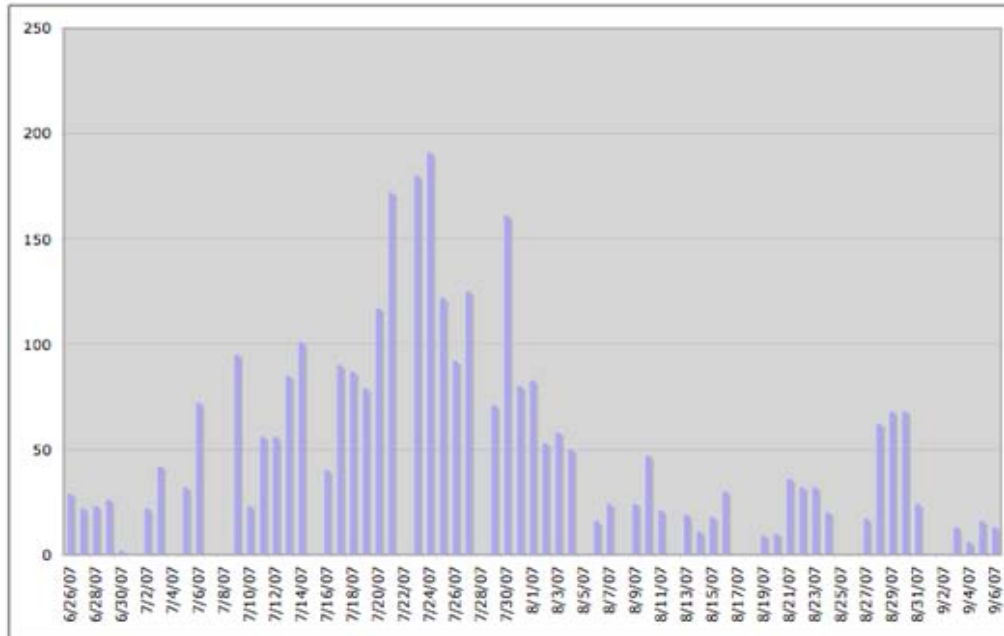
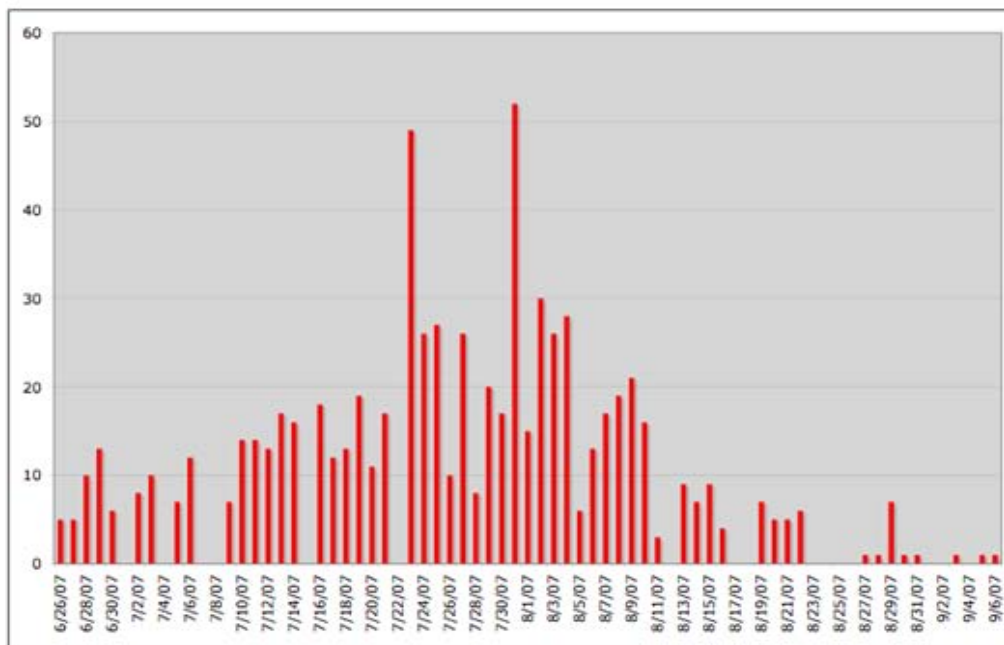
A**B**

Fig. 1. Numbers of male (A) and female (B) of the Ten-lined June Beetle sampled in Manteca in 2007. With almost daily trips during the flight season we were able to collect 3,173 males and 742 females.

During the last flight season, the PI, co-PI, and collaborators, including postdocs, undergraduate and graduate students, and volunteers took over 60 night trips to infested almond orchards near Manteca. We were able to capture 742 females (Fig. 1), the largest sample collected in one season thus far. Except for two days in which press coverage generated an additional number of volunteers and two peaks of captured females, manual catches of females are well correlated with capture of males in light traps. The major flight activity in 2007 took place in July and the beginning of August.

Last year we were able to obtain for the first time a mass spectrum that of the major constituent of the sex pheromone. We identified the active compound by gas chromatography linked to an electroantennographic detector, a technique named GC-EAD, and analyzed by gas chromatography-mass spectrometry (GC-MS) a sample purified from all extracts collected in previous years. Detailed analysis of the analytical data indicated that this sex pheromone has a chemical structure new to science. Therefore, more analytical data is necessary for structure elucidation. When enough samples are available the process of structure elucidation can be conducted by microderivatization followed by chemical analysis (GC-MS). We could subject the compound to chemical reaction (e.g.: hydrogenation) and examine the MS after the reaction. That way one can determine if there are unsaturations (in which case the starting material reacts) or not (starting material do not react). In addition, the mass spectral data after the reaction may facilitate structure elucidation because in some cases the product may be a known compound identifiable by MS only, and this hint leads to the identification of the original sex pheromone. Since the amount of pheromone is so small we have to develop new strategies to determine the novel structure.

We have re-isolated the sex pheromone of the TLJB and conducted chemical derivatizations, but monitored the reactions by GC-EAD. This non-conventional approach can be done with small amounts of sample and can add substantial information for structural elucidation, but lack the structural features of the product. For example, we have subjected the isolated sex pheromone of the ten-lined June beetle to hydrogenation. This is a chemical reaction that would indicate if the sex pheromone contains double bonds, which is, for example, a common feature of moth sex pheromones. We observed that EAD activity of the sex pheromone was retained after the hydrogenation thus indicating that the sex pheromone did not react and, consequently, the structure of the pheromone does not contain any double bonds. Likewise, we observed that the isolated pheromone does not undergo methylation which rules out a possible free acid. Although we have made significant progress towards structure elucidation we do not yet have enough information to propose an unambiguous structure for synthesis and confirmation. The limited amounts of pheromones produced by females and the novelty of the chemical structure are major obstacles. We have, however, developed new strategies that will certainly lead us to the completion of the work in the very near future. We are in the process of modifying the gas chromatograph-infrared instruments so as to allow minimal destructive measurement from small amount of samples.