Developing Ambient Almond Orchard Volatile Mixtures for Navel Orangeworm (NOW) Bioassay Analyses

Project No.: **09-ENTO4-Beck**

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Objectives:

To collect and identify ambient volatile organic compounds (VOCs) emitted by almond orchards over the course of a growing season. Using this information a synthetic blend that mimics the major VOCs emitted will be developed and used for lab-based bioassays, and as a possible agricultural adjuvant for existing trapping and mating disruption. The experiments will utilize an optimized VOC collection system. These systems allow the development of new and/or more effective lures for NOW by:

1) Optimizing and implementing a facile large-scale VOC collection system capable efficient and reproducible ambient orchard analyses;
2) Producing a method that is applicable to any agricultural commodity;
3) Collecting ambient almond and pistachio VOCs from select geographical locales;
4) Evaluating efficacy of collected VOCs in bioassays on NOW, including electroantennogram (EAG) and field trapping;
5) Formulating a synthetic blend of background VOCs for use in lab-based NOW bioassays (in conjunction with current or future NOW attractant VOCs);
6) If discovered from ambient orchard analyses, isolating and identifying new NOW attractant VOC candidates; and,
7) Obtaining technology transfer of method and disseminate results to germane researchers and agricultural end-users.

Interpretive Summary:

The navel orangeworm (NOW), *Amyelois transitella* (Walker), is an insect pest of California tree nuts. Its feeding damage lowers nut kernel quality resulting in extensive monetary loss to

growers, producers, and shippers. Moreover, NOW feeding damage directly contributes to aflatoxin contamination. Aflatoxin is a mycotoxin produced by *Aspergillus flavus*, a ubiquitous fungus in tree nut orchards, and represents a food safety problem due to its carcinogenic and teratogenic attributes.[1,2]

There are numerous reports in the literature on both volatile and non-volatile composition of various parts of some almond cultivars.[3-10] Nonetheless, ***the VOC emission of almond and pistachio orchards has not been studied over the course of an entire growing season***. This aspect is particularly relevant to research concerning NOW and the identification of any associated and relevant semiochemicals.

The discovery of an efficacious attractant for NOW monitoring/trapping has remained elusive despite breakthroughs with the pheromone,[11] the pheromone blend,[12-14] long-chain fatty acids,[15] use of almond meal, or caged virgin female NOW. The ability of an insect to locate the desired host plant is in part dependent upon its ability to detect a specific VOC (kairomone). As with the complex blend of NOW pheromone noted by Leal et al., a complex mixture of ubiquitous plant VOCs may be necessary to elicit an appropriate response from the insect to the host-plant.[16,17] Recent investigations of *in situ* ambient almond emission and corresponding NOW electroantennogram (EAG) bioassay suggested possible kairomonal-type behaviour from several of the collected VOCs.[18] For the purpose of this study, these VOCs are termed background signaling volatiles (BSVs) and are defined as ubiquitous volatiles from almonds that may act as obligatory cues to direct NOW towards key attractant(s). Hence, the BSVs need not demonstrate an EAG response greater than a specified attractant, such as the major aldehyde component of the female NOW pheromone,[11] but rather a reasonable EAG response that suggests a basal interest in the individual VOC or bouquet. A recent investigation by Liu *et al.* demonstrates the affinity of adult NOW moth olfactory proteins toward one of the discovered BSV components.[19]

Figure 1. 2009 AOVC System

To further explore the presence and role of BSVs in tree nut orchards a prototype large-scale ambient orchard volatile collection (AOVC) system was developed and implemented in the 2008 growing season and at varying phenological stages of almond growth. The results from this study have been reported.[20] The second year of this project, and subject of this final report, utilized an optimized AOVC system that was independent, maintained higher flow rates via a more efficient and consistent pump, provided higher resolution, and was solar-powered (**Figure 1**).

Ambient orchard volatile collections from the 2008 and 2009 growing season provided a blend (Blend A) that was taken forward for *in vitro* EAG and flight tunnel studies using female NOW. Results from the *in vitro* studies demonstrated Blend A elicited moderate EAG responses from female NOW. Additionally, flight tunnel studies showed Blend A stimulated ovipositional

behavior in female NOW. The EAG responses of Blend A support the idea of ambient volatiles exhibiting BSV behavior. Moreover, Blend A possesses the ability to stimulate female NOW *in vitro*.

Materials and Methods:

Volatile Collections: Each AOVC system employed an independent on-site, solar-powered, electric pump that allowed an in-line, metal Tenax® cartridge (2.5 cm × 6.5 cm, 5 g of absorbent) to collect VOCs from the ambient orchard air. The Tenax cartridge and pump/control center were housed in individual plastic boxes *ca.* five feet off the ground, with a screened-in bottom and attached to a wooden post located in the tree-line and near the canopy. Experiments were run continuously for 4-7 days with a flow rate set at 5 mL/min. VOCs were collected from Nickels Soil Laboratory almond orchard in Arbuckle, CA (Colusa County) and from a Paramount Farming Co. almond orchard in Lost Hills, CA (Kern County).

Volatile Desorption and Analysis: Absorbed volatiles were desorbed via published methods used by this laboratory[10] and analyzed on both a DB-Wax and DB-1 column (60 m × 0.32 mm i.d. × 0.25 μm J&W Scientific, Folsom, CA) installed on two HP-6890 GCs coupled to HP-5973 mass selective detectors (Palo Alto, CA) using published methods.[18] NIST, Wiley, and internally generated databases were used for fragmentation pattern identification. The retention indices (RIs) were calculated using a homologous series of *n*-alkanes on the DB-Wax and DB-1 columns. VOC identifications were verified by injection of authentic samples and comparison to retention times of an internally-generated list of volatiles on identical columns.

Electroantennogram Bioassays: The antennae of laboratory-reared, sexed NOW moths, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), were excised, positioned on a fork electrode using electrode gel, and connected to an IDAC-4 acquisition controller electroantennogram using Syntech's PC-based software (Syntech, Kirchzarten, Germany). The antennae were humidified with a stream of purified air bubbled through distilled water at a flow rate of 200 mL/min. The individual compounds for EAG analysis (50 μg; 10 μL of a 5 μg/μL solution in pentane) were loaded onto oven-dried 0.25″ assay discs, allowed to air-dry for five minutes, inserted into 5.75″ Pasteur pipets and the ends temporarily capped with parafilm. The antennae were exposed to each compound by a two-second puff of air and the resulting response recorded. The antennal response was duplicated for each VOC with a one minute delay between puffs, with each run lasting no longer than 30 minutes from excision to completion of run on the antenna pair.

Flight Tunnel Bioassays: Laboratory-reared NOW larvae were allowed to pupate on NOW diet. The newly emerged NOW were removed from the rearing jars (1 gallon, clear) and placed in a separate 1 gallon jar containing only a vial of honey water and cotton. NOW, both male and female, were allowed to interact for a definitive number of days, removed, sexed, and the female NOW moths used for flight tunnel bioassays. Flight tunnel conditions: size, 1 × 1 × 3 m; air flow 60 m/sec; rotating two-sample bar at 0.5 rotation/min; black delta traps with sticky trap inserts on the bottom; almond meal (63 mg) placed in a scintillation vial and placed on sticky trap; Blend A (1 mg) placed in a septum and hung via a paperclip from the top of the delta trap. Moths captured and eggs deposited on the inside and outside of the delta trap were counted after one day. Females were checked for mating status at the conclusion of each experiment.

Results and Discussion:

A total of 16 large-scale collections were performed in 2009, four duplicated in Kern County and four matching duplicated collections in Colusa County (see 2009 Proceedings Project No. 09-ENTO4-Beck). Analysis of these collections via gas chromatography-mass spectroscopy (GC-MS) provided a series of tree emissions representative of an almond orchard. All compounds (greater than ~1% of highest peak relative abundance) identified were analyzed via EAG for baseline individual EAG responses (n = 2). The major components of these collections were identified, quantified, and several synthetic BSV candidate mixtures were developed and subjected to EAG analyses (n = 5). Additionally, the major component quantities were tracked over the course of the growing season and were suggestive of a dynamic ratio of BSVs. The components of the blends and the potential dynamic BSV ratios will be discussed in greater detail in a forthcoming peer-reviewed manuscript. One of the blend iterations demonstrated moderate EAG signals relative to typical EAG responses of male NOW to the main aldehyde pheromone component.[11] This BSV blend (termed Blend A for this report) was then analyzed via more replicates of EAG and concurrent flight tunnel studies.

Collections to date for 2010 will be soon concluding and will total 16 collections, eight duplicated in Kern County and eight matching duplicated collections in Colusa County. Data analysis of 2010 data and comparison to pistachio orchard emissions are underway.

Preliminary EAG studies suggested attention be turned to the analysis of mated vs. virgin female NOW moths. The flight tunnel studies also utilized mated and unmated females and interesting results were obtained from both Blend A vs. blank controls and Blend A vs. almond meal, the current standard female monitoring tool. Mated female moths exhibited ovipositional preference for the egg traps containing Blend A. However, the number of female captures was nearly equal for all comparisons. Again, a detailed analysis of the ovipositional preference will be discussed in the forthcoming manuscript.

Current investigations on the third and final year of the funded project include completion of volatile collections in both almond and pistachio orchards, analysis of data from both, formulation of new synthetic blends based on almond and/or pistachio analyses, *in vitro* bioassay of newly formulated synthetic blends, and combination of blends with known attractants for increased efficacy.

Research Effort Recent Publications:

Beck, J. J.; Light, D. M.; Higbee, B. S.; Dragull, K.; Gee, W. S. Ambient volatiles collected from almond orchards stimulate ovipositional behavior in female navel orangeworm *in vitro*. Manuscript in preparation 8/2010.

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