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

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

Project Leader: John J. Beck
USDA-ARS
Western Regional Research Center
800 Buchanan St.
Albany, CA
(510) 559-6154
john.beck@ars.usda.gov

Project Cooperators and Personnel:
Bradley S. Higbee, Research Entomologist, Paramount Farming Company
Gloria B. Merrill, Chemist, USDA-ARS
Douglas M. Light, Research Entomologist, USDA-ARS
Klaus Dragull, Research Biologist, USDA-ARS

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 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, and will allow both the development of more effective lures for NOW, as well as a method for overall orchard air emissions relative to geographical locales by:

1) Optimizing and implementing a facile large-scale VOC collection system capable efficient and reproducible ambient orchard analyses;
2) Producing a VOC collection method applicable to any agricultural commodity;
3) Collecting ambient almond and pistachio VOCs from select geographical locales, and characterizing the relative amounts and make-up of the constituents from the different geographies;
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. An example of NOW impact is the increase in the number of almond containers rejected by the European Union (EU) due to aflatoxin contamination. In 2003 five containers were rejected; however, in 2007 over 60 shipments were rejected. This led EU authorities to consider the implementation of "special measures" against California almonds. The pistachio industry has also had a large number of shipments rejected by the EU due to aflatoxin contamination. Hence, control of NOW continues to be a top priority of the California tree nut industry.

There are numerous reports in the literature on both volatile and non-volatile composition of various parts of some almond cultivars: for instance, proteins and fatty acids from kernels, fatty acids and triterpenoids from hulls; and, VOCs extracted via steam distillation of dried almond hulls, almond oil, and *ex situ* VOCs of whole damaged and undamaged almonds. Nonetheless, ***the VOC emission of almond orchards has not been studied over the course of an entire growing season***. This aspect is particularly relevant to research concerning NOW.

The discovery of an efficacious attractant for NOW monitoring/trapping has remained elusive despite breakthroughs with the pheromone, the pheromone blend, long-chain fatty acids, use of almond press cake, 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. 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. 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, but rather a reasonable EAG response that suggests a basal interest in the individual VOC or bouquet.

To further explore the presence and role of BSVs in tree nut orchards, a prototype, Venturi-based large-scale ambient volatile collection (LSAVC) system was developed and implemented in the 2008 growing season and at varying phenological stages of almond growth. Briefly, results from the 2008 study provided thirteen VOCs that were

tested via EAG bioassay. Seven VOCs were identified as being persistent in the Lost Hills collections in May and July. Six of these VOCs were also present in the Nickel's collections. The seven persistent volatiles have been subjected to EAG analyses: all of the seven VOCs elicited a positive response from both female and male NOW greater than the negative control, with four of the seven eliciting moderate responses from the female, and one of the seven eliciting a modest response from the male NOW. These results corroborate the hypothesis of a background adjuvant assisting the NOW to focus attention on the location of an appropriate VOC attractant.

Obstacles encountered with the 2008 prototype were primarily logistical, the chief barrier being the exorbitant cost of the air cylinders and associated demurrage. Scheduling of tank maintenance and changing, particularly in relatively remote areas, posed a problem to the orchard managers and associated personnel. Finally, due to the numerous changes in air cylinders, the delicate settings of air flow were occasionally, but inadvertently, altered.

Despite the logistical obstacles, sufficient data was procured during the trial runs of the prototype *to confirm the method as being viable, reproducible, and yielding of accurate and compulsory data*. Portions of this data were recently disseminated at an International venue for scientists investigating plant volatiles, and a conference for entomologists.

The LSAVC system utilized for the 2009 growing season was optimized to address all of the obstacles encountered previously. Ten optimized LSAVC systems were built and implemented over various phenological stages in both almonds and pistachios. An improved pump system translated to collection times being substantially shortened, thus allowed for tighter phenological VOC emission slices. Occasional monitoring of the flow rates in the field showed deviations of less then +/- 0.25 L/min. Moreover, the increased flow rates permitted movement of over 50,000 L of air during a collection time of one week. The total number of collections, dates, and degree dates from almonds is highlighted in **Table 1**.

Table 1. Ambient Orchard Volatile Collections for 2009 Growing Season

Collection Number	Location[a]	Collection Days		Total # Days	Degree Days[b]
		Start	End		
1A	Kern County	4/23	5/5	12	482-587
1B	Colusa County	5/12	5/18	6	593-719
2A	Kern County	6/30	7/7	7	1586-1732
2B	Colusa County	6/22	7/2	10	1241-1448
3A	Kern County	7/7	7/15	8	1752-1930
3B	Colusa County	7/17	7/23	6	1768-1908
4A	Kern County	8/11	8/21	10	2592-2817
4B	Colusa County	7/27	7/30	3	1993-2056

[a] Kern County = Lost Hills, CA, Paramount Farming; Colusa County = Arbuckle, CA, Nickels Soil Laboratory

[b] DD accumulation from Jan. 1, 2009 for navel orangeworm and corresponding locations

The 2009 VOC collections met with minimal obstacles, the primary being the alignment of degree days between the two collection sites. The UC IPM Online – Statewide Integrated Pest Management Program site was used to plan collection dates and in general the site provided a good timetable; however, future collection time planning will necessitate the prudent use of more up to date calculations as weather fluctuations hampered accurate long-term planning. A second obstacle encountered was equipment issues early in the collection time periods, which interfered with collections 1A and 1B. The issue was resolved and not encountered again. One final obstacle that will require better planning for future collections is the application of necessary sprays and requires the LSAVC system to be temporarily shut down; however, the researchers benefited enormously from ranch personnel cooperation.

In the upcoming months, research on this project will include continuation of VOC desorption from Tenax cartridges, data analysis, VOC identification and quantification, comparison of geographical data, comparison to pistachio VOC collections, and bioassay of identified VOCs and VOC blends.