Ambient Almond Orchard Volatiles as Attractants for Navel Orangeworm (NOW) Monitoring

Project No.: **11-ENTO4-Beck**

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Objectives:

This project has two objectives: 1) to collect and identify ambient volatile emissions (odors) of almond orchards over the course of a growing season; to develop a synthetic blend that mimics the primary orchard odor components for laboratory-based bioassays; and 2) to develop an agricultural adjuvant, or additive, that could be used to enhance existing navel orangeworm trapping and mating disruption.

Interpretive Summary:

The navel orangeworm (NOW) 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. Aflatoxins are mycotoxins produced by *Aspergillus flavus* and *A. parasiticus*, ubiquitous fungi in tree nut orchards, and represent a grave food safety problem due to their 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.[3-11]Until recently; the volatile emission of almond[11] 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,[12] the pheromone blend,[13-15] long-chain fatty acids,[16] 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 volatile semiochemical (kairomone). As with the complex blend of NOW pheromone noted by Leal et al., a complex mixture of ubiquitous plant volatiles may be necessary to elicit an appropriate response from the insect to the host-plant.[17,18] Recent investigations of *in situ* ambient almond emission and corresponding NOW

electroantennographic (EAG) bioassays suggested possible kairomonal-type behaviour from several of the collected volatiles.[11,19]

To further explore the presence and role of these ambient volatiles from tree nut orchards an optimized large-scale ambient orchard volatile collection system was implemented in the 2010 growing season and at varying phenological stages of almond growth. The results from the exploratory 2008 and subsequent 2009 study have been reported.[11, 20] The third year of this project focused on the completion of data analysis of the 2010 volatile collections, their quantification, and associated EAG studies.[20] The fourth and final year of this project entailed evaluation of the blend via EAG and collaborative field trapping studies and comparison to almond meal, the current NOW monitoring standard.

Materials and Methods:

Electroantennographic Studies: The antennae of laboratory-reared, sexed NOW moths, *Amyelois transitella*, were excised, positioned on a fork electrode, and analyzed on an IDAC-4 acquisition controller electroantennogram (Syntech, Kirchzarten, Germany). The antennae were humidified with a stream of purified air at a flow rate of 200 mL/min. The individual compounds for EAG analysis (50 g) 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.

Field Trapping Studies: A randomized block design was used in almond orchards located in Kern County. Standard delta traps with glue liners were baited with: Nalgene bottles containing 200 mg of the host plant volatile blend; traps baited with almond meal (ca. 18.6 g) in standard egg traps as a female attractant standard; and, mesh cages containing three virgin females as a male attractant standard. Trap catches were collected weekly and fresh blend bottles placed in the traps. Each moth capture experiment comprised two one-week trapping intervals in 2011.

Results and Discussion:

Formulations of host plant volatiles from varying almond sources provided a blend (denoted host plant volatile blend) for the season-long field trapping study. The full report of the blend composition and results from the EAG field trapping studies will be provided in an upcoming peer-reviewed journal article.[21]A summary of the overall field trapping results of the Blend and comparison to almond meal and blank treatments is provided in **Table 1.**

The overall number of NOW captures is the averages of five two-week trapping intervals performed in Kern County and are comprised of both male and female adult moths. The host plant volatile blend was able to attract significantly more female NOW moths than the current standard, almond meal. Additionally, the ability of the host plant volatile blend to attract male NOW may have positive implications for monitoring of NOW

populations during mating disruption treatments. A CDFA Specialty Crop Block Grant[22] was recently awarded to John Beck and Brad Higbee for in-depth field trapping studies of the host plant volatile blend.

The volatiles in the host plant volatile blend currently undergoing optimization were comprised of almond hull split and damage volatiles. However, the collection system devised, optimized, and used for the ambient almond orchard volatile project played a significant role in the collection of the volatiles for the host plant volatile blend. Additionally, ambient volatile components are still undergoing investigations for use as a background blend that can be used in conjunction with other NOW attractants. A comprehensive EAG overview of all almond volatiles collected by these laboratories is being conducted and will be reported in the coming months.

Table 1. Mean captures of *A. transitella* (NOW) over five two-week treatments and reported as per trap per week.

Apr-Aug	**Treatment**		
Intervals	**Host Plant Volatile Blend**	**Almond Meal**	**Blank**
Overall	4.08 ± 0.89 a	0.53 ± 0.15 b	0.05 ± 0.04 b
Total Moths	155	20	2

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