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

Project No.: **10-ENTO4-Beck**

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Objectives:

To collect and identify ambient volatile emissions of almond orchards over the course of a growing season. Using this information a synthetic blend that mimics the major volatiles 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 volatile collection system. These systems allow the development of new and/or more effective lures for navel orangeworm (NOW) by:

1) Optimizing and implementing a facile large-scale volatile 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 volatiles from select geographical locales;
4) Evaluating efficacy of collected volatiles in bioassays on NOW, including electroantennogram (EAG) and field trapping;
5) Formulating a synthetic blend of background volatiles for use in lab-based NOW bioassays (in conjunction with current or future NOW attractant volatiles);
6) If discovered from ambient orchard analyses, isolating and identifying new NOW attractant volatile 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] 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 electroantennogram (EAG) bioassay suggested possible kairomonal-type behaviour from several of the collected volatiles.[11,19] For the purpose of this study, these volatiles 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,[12] but rather a reasonable EAG response that suggests a basal interest in the individual volatile or bouquet. A recent investigation by Liu *et al.* demonstrated the affinity of adult NOW moth olfactory proteins toward one of the discovered BSV components.[20]

To further explore the presence and role of BSVs in tree nut orchards the optimized large-scale ambient orchard volatile collection (LSAVC) 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 (**Table 1**).[11,21] The third year of this project, and subject of this final report, focused on the completion of data analysis of the 2010 volatile collections, their quantification, and associated EAG studies.

Table 1
Ambient almond orchard volatile amounts from Kern County, California collected during the 2009 growing season.

#	Compound ID	DB-Wax[a]			Ambient almond				Avg	s.e.
		RT	RI		Volatile amounts ($ng\,m^{-3}$)[b]					
			Calc'd	Lit	Collection 1	Collection 2	Collection 3	Collection 4		
1	Hexanal	6.49	1077	1077	26.8	49.3	31.1	23.3	32.6	5.8
2	Undecane	6.76	1088	1100	0.0	0.0	0.0	7.0	1.8	1.8
3	Cumene	8.77	1167	1168	3.0	5.3	0.0	3.5	3.0	1.1
4	Heptanal	9.11	1180	1180	12.2	13.1	11.4	13.1	12.4	0.4
5	Limonene	9.48	1194	1195	3.7	0.6	0.0	0.0	1.1	0.9
6	p-Cymene	11.59	1266	1264	1.8	4.8	4.5	6.9	4.5	1.0
7	Octanal	12.17	1285	1284	78.1	108.2	49.6	50.1	71.5	13.9
8	Nonanal	15.42	1390	1389	237.4	338.4	161.2	169.4	226.6	41.0
9	Acetic acid	17.29	1451	1447	11.1	3.9	13.9	11.2	10.0	2.1
10	Decanal	18.65	1495	1495	3.8	0.0	18.1	21.7	10.9	5.3
11	Benzaldehyde	19.20	1515	1516	306.8	165.3	306.6	1971.5	687.5	429.3
12	Benzonitrile	21.63	1595	1597	3.9	1.7	3.5	10.0	4.8	1.8
13	γ-Pentanolactone	21.78	1601	1600	3.4	8.1	10.8	6.2	7.1	1.5
14	Methyl benzoate	22.19	1615	1616	7.7	9.3	14.8	7.0	9.7	1.8
15	Sabina ketone[c]	22.51	1626	n/a	12.6	0.0	5.3	3.2	5.3	2.7
16	Phenylacetaldehyde	22.70	1633	1636	11.9	10.4	19.0	25.9	16.8	3.6
17	Acetophenone	22.98	1642	1645	151.5	224.8	263.8	355.4	248.9	42.5
18	Ethyl benzoate	23.51	1661	1661	51.9	59.7	23.3	31.8	41.7	8.5
19	Salicylaldehyde	23.72	1668	1673	5.4	7.2	5.3	9.8	6.9	1.1
20	γ-Hexanolactone	24.39	1691	1699	4.3	9.6	13.9	13.5	10.3	2.2
21	Naphthalene	25.44	1730	1734	0.0	0.0	1.4	3.0	1.1	0.7
22	Methyl salicylate	26.46	1767	1771	122.7	191.7	76.7	77.5	117.2	27.1
23	1-Methylnaphthalene	29.35	1876	1884	0.0	11.8	14.8	0.0	6.6	3.9
24	Phenol	32.48	2002	2000	74.7	83.7	74.4	87.8	80.2	3.4
25	p-Anisaldehyde	32.84	2017	2024	3.5	0.0	0.0	11.3	3.7	2.7
	Collection dates				4/23–5/5	6/30–7/7	7/7–7/15	8/11–8/21		
	Relative nut phenology				kernel filling	hull split[d]	hull split[d,e]	hull split[f]		

[a] RI calculated relative to *n*-alkanes on DB-Wax and compared to literature and internally generated data base values.
[b] Ambient volatile amount calculated using total analyzed relative amount of each volatile per volume of air collected (total number of minutes × flow rate for each Tenax cartridge).
[c] Tentative assignment, compound not available for authentication.
[d] Primarily relative to Nonpareil.
[e] Start of hull split for pollenizers.
[f] Primarily relative to pollenizers, late for Nonpareil.

Materials and Methods:

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.[19] 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.

Results and Discussion:

Table 1 provides a list of compounds detected in the 2009 volatile collections. The 2010 collections demonstrated similar compositions, but in higher resolution in the form of greater number of volatiles detected and more specific ratios. The greater resolution for the 2010 collections will allows researchers to draw some initial conclusions regarding emission trends. For example, the average values (Avg) shown in **Table 1** provide a quick reference for relatively high amount of volatiles (a large Avg value), and the standard error (s.e.) describes either consistent or transient emission over the four collections. For instance, a low s.e. may indicate consistent emission of that volatile (*e.g.,* heptanal with an average emission of 12.4 ng m^{-3} ± 0.4); whereas a larger s.e. may indicate either an upward or downward trend in volatile emission (*e.g.,* phenylacetaldehyde, which increases over time, with an average emission of 16.8 ng m^{-3} ± 3.6).

In addition to separation and identification via GC-MS, the 2010 collections will be quantified via gas chromatography-flame ionization detector (GC-FID) to determine the emitted ratios of orchard components. When correlated to the EAG responses of the individual components, blends can be formulated that are more consistent with natural emission ratios.

Collections to date for 2010 have concluded and total 22 – 11 duplicated in Kern County and 11 matching duplicated collections in Colusa County. Data analysis of 2010 results and comparison to pistachio orchard emissions are near completion. EAG responses of all

components has been concluded (n=4), and blends, in part based on this and other projects, are being investigated by EAG bioassays and field trapping studies.

Current investigations on the fourth and final year of the funded project include completion of quantification via GC-FID, data analysis, EAG response studies for both almond and pistachio orchard volatiles, completion of field trapping studies, and combination of blends with known attractants for increased efficacy.

Status of Objectives:

1) The large-scale volatile collection system was successfully implemented for the 2009 and 2010 growing seasons;
2) The LSAVC can be applied to theoretically any agricultural commodity and has been successfully applied to collection of pistachio volatiles;
3) Ambient almond and pistachio volatiles were successfully collected in 2009 and 2010 growing seasons and from north and south Central Valley locations;
4) EAG responses of all individual components of collected volatiles has been successfully accomplished; select volatiles are currently being used in field trapping trials;
5) A synthetic blend of background volatiles for use in lab-based NOW bioassays is currently under development (based on EAG responses and multicomponent blends);
6) EAG responses of planned binary/ternary/quaternary blends may reveal new NOW attractant volatile candidates; and,
7) Successful conclusion of field trapping results may result in obtaining technology transfer potential lures.

Research Effort Recent Publications:

Beck, J.J.; Higbee, B.S.; Gee, W.S.; Dragull, K. Ambient orchard volatiles from California almonds. *Phytochem. Lett.* **2011**, *4*, 199-202.

Beck, J.J.; Mahoney, N.E.; Cook, D.; Gee, W.S. Volatile analysis of ground almonds contaminated with naturally occurring fungi. *J. Agric. Food Chem.* **2011**, *59*, 6180-6187.

Beck, J.J.; Higbee, B.S.; Gee, W.S.; Merrill, G.B.; Hayashi, J.M.; Light, D.M. Kairomone-based blend as an attractant for male and female navel orangeworm (*Amyelois transitella*) in almond orchards. In preparation.

References Cited:

(1) Campbell, B.C.; Molyneux, R.J.; Schatzki, T.F. Current research on reducing pre- and post-harvest aflatoxin contamination of U.S. almonds, pistachio, and walnut. *J. Toxicol. Toxin Rev.* **2003**, *22*, 225-266.

(2) Robens, J.; Cardwell, W. The costs of mycotoxin management to the USA: management of aflatoxins in the United States. *J. Toxicol. Toxin Rev.* **2003**, *22*, 143-156.

(3) Ahrens, S.; Venkatachalam, M.; Mistry, A.M.; Lapsley, K.; Sathe, S.K. Almond (*Prunus dulcis* L.) protein quality. *Plant Foods Hum. Nutr.* **2005**, *60*, 123-128.

(4) Kodad, O.; Socias I Company, R. Variability of oil content and of major fatty acid composition in almond (*Prunus amygdalus* Batsch) and its relationship with kernel quality. *J. Agric. Food Chem.* **2008**, *56*, 4096-4101.

(5) Takeoka, G.R.; Dao, L.T. Antioxidant constituents of almond [*Prunus dulcis* (Mill.) D.A. Webb] hulls. *J. Agric. Food Chem.* **2003**, *51*, 496-501.

(6) Takeoka, G.; Dao, L.; Teranishi, R.; Wong, R.; Flessa, S.; Harden, L.; Edwards, R. Identification of three triterpenoids in almond hulls. *J. Agric. Food Chem.* **2000**, *48*, 3437-3439.

(7) Buttery R.G.; Soderstrom, E.L.; Seifert, R.M.; Ling L.C.; Haddon, W.F. Components of almond hulls: possible navel orangeworm attractants and growth inhibitors. *J. Agric. Food Chem.* **1980**, *28*, 353-356.

(8) Buttery, R.G.; Seifert, R.M.; Haddon, W.F.; Lundin, R.E. 2-Hexyl-3-methylmaleic anhydride: an unusual volatile component of raisins and almonds hulls. *J. Agric. Food Chem.* **1980**, *28*, 1336-1338.

(9) Miraliakbari, H.; Shahidi, F. Oxidative stability of tree nut oils. *J. Agric. Food Chem.* **2008**, *56*, 4751-4759.

(10) Beck, J.J.; Higbee, B.S.; Merrill, G.B.; Roitman, J.N. Comparison of volatile emissions from undamaged and mechanically damaged almonds. *J. Sci. Food Agric.* **2008**, *88*, 1363-1368.

(11) Beck, J.J.; Higbee, B.S.; Gee, W.S.; Dragull, K. Ambient orchard volatiles from California almonds. *Phytochem. Lett.* **2011**, *4*, 199-202.

(12) Coffelt, J.A.; Vick, K.W.; Sonnet, P.E.; Doolittle, R.E. Isolation, identification, and synthesis of a female sex pheromone of the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). *J. Chem. Ecol.* **1979**, *5*, 955-966.

(13) Leal, W.S.; Parra-Pedrazzoli, A.L.; Kaissling, K.-E.; Morgan, T.I.; Zalom, F.G.; Pesak, D.J.; Dundulis, E.A.; Burks, C.S.; Higbee, B.S. Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. *Naturwissenschaften* **2005**, *92*, 139-146.

(14) Kuenen, L.P.S.; McElfresh, J.S.; Millar, J.G. Identification of critical secondary components of the sex pheromone of the navel orangeworm (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **2010**, *103*, 314-330.

(15) Kanno, H.; Kuenen, L.P.S.; Klingler, K.A.; Millar, J.G.; Carde, R.T. Attractiveness of a four-component pheromone blend to male navel orangeworm moths. *J. Chem. Ecol.* **2010**, *36*, 584-591.

(16) Phelan, P.L.; Roelofs, C.J.; Youngman, R.R.; Baker, T.C. Characterization of chemicals mediating ovipositional host-plant finding by *Amyelois transitella* females. *J. Chem. Ecol.* **1991**, *17*, 599-613.

(17) Bruce, J.J.A.; Wadhams, L.J.; Woodcock, C.M. Insect host location: a volatile situation. *Trends Plant Sci.* **2005**, *10*, 269-274.

(18) Schroder, R.; Hilker, M. The relevance of background odor in resource location by insects: a behavioral approach. *Bioscience* **2008**, *58*, 308-316.

(19) Beck, J.J.; Merrill, G.B.; Higbee, B.S.; Light, D.M.; Gee, W.S. *In situ* seasonal study of the volatile production of almonds (*Prunus dulcis*) var. 'nonpareil' and relationship to navel orangeworm. *J. Agric. Food Chem.* **2009**, *57*, 3749-3453.

(20) Liu, Z.; Vidal, D.M. Syed, Z.; Ishida, Y.; Leal, W.S. Pheromone binding to general odorant-binding proteins from the navel orangeworm. *J. Chem. Ecol.* **2010**, *36*, 787-794.

(21) Beck, J. J. Developing ambient almond orchard volatile mixtures for NOW bioassay analyses. 2008-2009 and 2009-2010 Final Report for the Almond Board of California. 08-ENT04-Beck and 09-ENT04-Beck. 07/19/2009 and 07/30/2010, respectively.