Developing Ambient Almond Orchard Volatile Mixtures for Navel Orangeworm Bioassay Analyses

Project No.: **08-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; and, using this information to develop a synthetic blend that mimics the major VOCs emitted for use in lab-based bioassays, and as a possible component to improve navel orangeworm mating disruption or trap lures. Steps to do this include:

1. Obtain duplicated samplings of ambient almond orchard volatiles via Tenax absorption.
2. Determine the identity and relative concentrations of the major volatile organic compounds (VOCs) in the ambient orchard bouquet via gas chromatography (GC-MS) analyses
3. Develop a minimum number of volatiles necessary for a corresponding synthetic blend. This blend will be utilized in these research laboratories during electroantennogram (EAG) or wind tunnel studies of NOW
4. Make available via publication the successful volatile mixture for other researchers to utilize for NOW studies

Interpretive Summary:

A Venturi-based large-scale ambient volatile collection (LSAVC) system was developed and implemented during the 2008 almond growing season. The volatile organic compound (VOC) emissions of two geographically different almond orchards were collected at two separate dates and analyzed via gas chromatography-mass spectroscopy (GC-MS). The concentrations of ambient orchard VOCs were determined and the major VOC components were formulated into blends. Several blends were evaluated for their ability to elicit responses from the antennae of the navel orangeworm (NOW) using an electroantennogram (EAG) detector.

Materials and Methods:

Volatile Collections: Each LSAVC system employed an independent on-site, Venturi-based vacuum that allowed an in-line Tenax cartridge (25 cm × 3 cm, 25 g of absorbent) to collect VOCs from the ambient orchard air. The vacuum was generated by a large cylinder of compressed air (232 cu. ft., 2300 psi) that delivered air through the Venturi. The Tenax cartridge and Venturi single-stage adapter were encased in a wooden box, 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. The air cylinder was safely secured with a chain to the post. Experiments were run continuously for 10-35 days with exchange of gas cylinders occurring every 2-3 days, depending upon flow rate, nominally set at 1500 mL/min. VOCs were collected from Nickels Soil Laboratory almond orchard in Arbuckle, CA and from a Paramount Farming Company almond orchard in Lost Hills, CA.

Volatile Desorption and Analysis: Absorbed volatiles were desorbed via published methods used by this laboratory (*1*) 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 (*2*). 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 navel orangeworm 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:

Each experiment utilized three collection systems: the first box, for control VOCs, was located on the outside, upwind edge of the orchard and collected extraneous VOCs from neighboring orchards, other commodities, homes, and/or businesses; the remaining two boxes were placed several rows apart, alongside similar cultivars, and employed to verify reproducibility of results. An example box placement is shown in **Figure 1**, and a summary of experiments run, dates and length of collection, cultivars present in the orchards, and location of orchards are provided in **Table 1**.

The first objective had one critical aim: to demonstrate that the prototype collection system yielded sufficient data during the trial runs to justify the method as being viable, reproducible, and yielding of accurate and compulsory data. One major obstacle, though it *did not* ultimately impede success of the first objective, was the unfortunate, numerous wildfires that scourged much of Northern California for a portion of the collection times. The runs affected are identified with asterisks in **Table 1**; however, the Nickels orchard was affected more than the Lost Hills orchard. A second obstacle, one that has been solved, was an incident where the caps of the Tenax cartridges were accidentally left in place after orchard spraying; thus, VOC collections were only limited to 10 days.

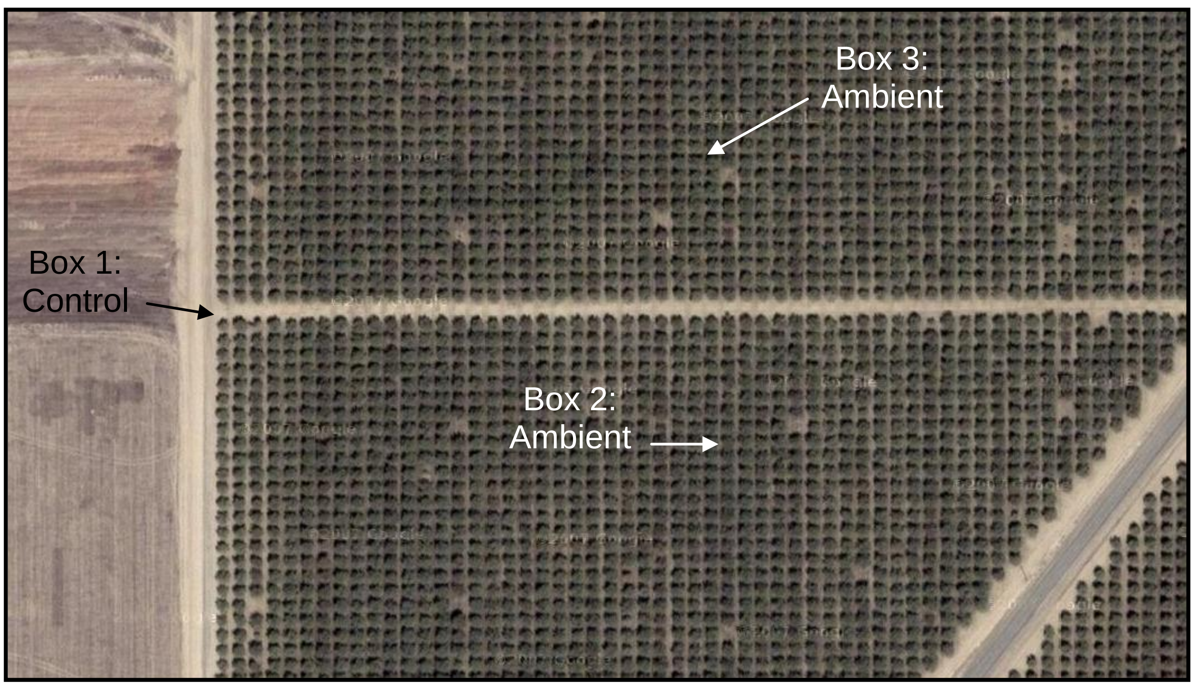

Figure 1. Example of collection box placements

Regardless of the major obstacles associated with the development of the LSAVC system, compulsory data was obtained and possible trends were noted. These possible trends will be discussed presently; however, more importantly they served to set priorities for the ensuing years of investigation of ambient VOCs.

Table 1. Summary of VOC Collection Experiments.

	Experiment Dates			Cultivars[a]			
Expt #	**Start**	**End**	**Total Days**	**Mix**	**Ratio**	**Next to**	**Location[b]**
1	5/6/08	6/10/08	10-30	NP:MO:CA	2:1:1	NP	Kern
2	6/5/08	7/1/08	26	NP:MO:CA:AL	4:2:1:1	NP	Colusa
3	6/24/08	8/5/08	21-35*	NP:MO:CA	2:1:1	NP	Kern
4	7/1/08	7/10/08	10*	NP:MO:CA:AL	4:2:1:1	NP	Colusa

[a] Almond varieties within orchard analyzed, variety ratio, and variety the collection box was next to.
CA = Carmel, MO = Monterey, NP = Nonpareil, AL = Aldrich

[b] Orchard location of VOC collection experiment: Kern = Kern county, Lost Hills, Paramount Farming Co.; Colusa = Colusa county, Arbuckle, Nickels Research Laboratory.

Analysis of **Table 2** provides some similarities in VOC emission between Kern and Colusa counties as well as the VOCs emission at different degree days. To align as closely as possible the phenological development of the almonds in Kern County with the almonds in Colusa County, a biofix date of January 1, 2008 was chosen and the VOC collection dates with overlapping degree days compared. Regions and time periods with the same letter have the approximate same crop / navel orangeworm phonological age.

Research toward the second objective produced five compounds that were consistently present (**Table 2**) in all four collections, but in varying concentrations. The variations between concentrations of the same experiment were most likely due to inconsistent flow rates and the continual changing of air cylinders to run the Venturi-based vacuum. These problems were importunate throughout this year's investigation, but have been solved for current and future collections. Some other notable variations were due to necessary equipment movement, and thus shorter collection times.

Table 2. Relative VOC Concentrations per Region and Time Period.

		Relative VOC Amounts (ng/day)[a]											
		Kern Expt #1			Colusa Expt #2			Kern Expt #3			Colusa Expt #4		
	Compound[b]	North	South	West (ctrl)	East	West	North (ctrl)	North	South	West (ctrl)	East	West	North (ctrl)
Constant	**1**	23.7	0.1	96.4	82.2	44.7	51.9	104.9	355.8	80.0	0.2	222.4	167.9
Constant	**2**	950.1	197.3	1693.6	72.0	64.6	34.5	865.9	1667.9	1224.4	216.6	1362.5	1017.6
Constant	**3**	53.9	11.2	57.1	20.7	14.8	14.4	0.3	295.3	75.1	100.1	131.8	67.9
Constant	**4**	52.2	0.1	172.6	100.9	110.1	49.9	82.4	159.1	83.7	112.8	83.4	66.5
Constant	**5**	43.7	23.3	78.0	162.6	80.5	60.0	76.3	519.9	135.0	171.0	265.1	124.2
Persistent	**6**							0.3	34.5	11.8	29.5	31.6	16.8
Persistent	**7**							38.4	206.8	50.7	40.9	55.7	87.4
Persistent	**8**				10.8	8.1	5.5	865.9	32.7	11.4	17.7	37.7	99.7
Persistent	**9**				28.6	0.0	9.6	38.4	20.8	0.2	0.2	53.9	3284.3
Transient	**10**				70.1	58.7	29.6						
Transient	**11**				63.2	41.6	10.4						
Transient	**12**				9.8	8.2	6.3						
Transient	**13**							104.9	144.6	32.5			
Degree Days[c]													
550-700		A	A	A									
840-1040		B		B	B	B	B						
1040-1320					C	C	C						
1340-1550								D	D	D	D	D	D
1570-2010								E	E	E			
2030-2370								F		F			

[a] Relative amounts of VOCs normalized to 15 μg cyclodecanone, internal standard, and divided by the number of days of collection for each Tenax cartridge

[b] VOC identified by comparison of retention indices and mass fragmentation patterns. Only four of fifteen transients shown.

[c] Degree days calculated to a biofix of January 1st, 2008; almond; navel orangeworm; for approximate correlation of phenological development stage
Regions and time periods with the same letter have the approximate same crop / navel orangeworm phonological age.

Compounds from the 'consistent' VOCs were formulated into several blends of varying ratios, which were then subjected to EAG and wind tunnel studies (WTS) with NOW. One blend demonstrated moderate values/responses in both the EAG and WTS corroborating the idea of a blend or series of background signaling volatiles (BSVs) for NOW (*2*). This formulation and other blends are being investigated.

It is interesting to note one set of results for the consistent VOCs. If the ng/day value for Kern #1 South is disregarded (10 d of collection with earlier degree days vs. 30 d for North and West), compound **2** is present in relatively large amounts when compared to the ng/day values for Colusa #2 (see 'B' blocks for 840-1040 degree days). This result implies there may be a geographical difference noted between orchards and/or the Colusa pollinator Aldrich contributed significant different VOC emissions, comparatively.

It should be noted that the 'C' and 'E' blocks of collection correspond to non-overlapping collections dates between experiments. Subsequent collections will not have these large gaps in collection overlap. **Table 1** allows for comparison of VOC emissions when blocks are shared between experiments (i.e.; Kern #3 can be partially compared to Colusa #4 since these experiments both have 'D' degree days).

Additionally, there were several 'persistent' and 'transient' VOCs unique and/or infrequent throughout the four collections. The presence of the persistent VOCs in the later degree days is suggestive of a phenological difference compared to the VOC emission of Kern #1 and Colusa #1. The Colusa County (experiment #2) orchard appears to have emitted higher number transient VOCs relative to the other experiments. The presence of compound **13** in Kern #3 may be due to the longer sampling time (into higher degree days). These VOCs are being investigated for significance to NOW.

While this research raises many questions, it undoubtedly provided enough compulsory data to validate the collection method, and more importantly provided guidelines for subsequent collections. The salient points of this research to date are:

1. a large-scale ambient volatile collection (LSAVC) system was implemented
2. the LSAVC provided usable data and demonstrated potential VOC differences in:
    a. phenological stages
    b. geographical locales
    c. varying pollinator cultivars within orchards
3. consistent VOCs were noted throughout the experiments
4. data suggests the consistent VOCs were dynamic throughout the season
5. several blends of the consistent VOCs were formulated and are undergoing further assays with NOW

There were several complications with the experiments, all of which have been fixed for subsequent analyses. Among them were: inconsistent flow rates between LSAVCs; inconsistent run times; and, the degree days between Kern and Colusa Counties were not aligned. Fortunately there was some incidental overlap of degree days that allowed some comparisons to be made.

Recent Publications:

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*, (8), 1363-1368.

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*, (9), 3749-3753.