Developing Ambient Almond Orchard Volatile Mixtures for Navel Orangeworm Bioassay Analyses

Project No.: **08-ENTO4-Beck**

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Objectives:

1. Obtain three duplicate samplings of ambient almond orchard volatiles via Tenax absorption. The three samplings will correlate to flight times of Navel Orangeworm (NOW), mid April, late June, and mid September.
2. Determine the identity and relative concentrations of the major volatile organic compounds (VOCs) in the ambient orchard bouquet via GC-MS analyses. The VOCs will be grouped as average of all three runs as well as according to the flight of NOW.
3. Develop a minimum number of volatiles necessary for a corresponding synthetic blend. This blend will be utilized in these research laboratories during puff or EAG studies of NOW.
4. Make available via publication the successful volatile mixture for other researchers to utilize for NOW studies.

Interpretive Summary:

Research on attractants for navel orrangeworm (NOW) monitoring and lure and trap purposes has not yet yielded a commercially viable and dependable product. In the laboratory setting, NOW puff (a volatile sample passed across the insect) or electroantennogram (EAG) studies typically utilize laboratory ambient air as the background volatile. This presents the problem that ambient laboratory air is not providing an appropriate bouquet of VOCs that would normally be perceived by the NOW in an almond orchard environment. Consequently, any potential VOC puff the NOW receives from researchers may not elicit an appropriate response due to the

absence of typical “orchard” volatiles, thus researchers may be missing key signals from the NOW.

Although the non-quantified VOC make-up of almond orchards has been reported, and the VOC composition of removed almonds has been investigated, the specific, quantified, ambient almond grove VOC composition has not been investigated. This proposed research will provide current and future NOW bioassay investigations with a quantified analysis of ambient almond grove volatiles, thus offering a realistic VOC composition as the background effluent to test potential NOW attractants.

A large-scale, Tenax-based, ambient VOC collection system was developed and implemented in May of the 2008 almond growing season. Each system employed an independent on-site, Venturi-based vacuum that allowed an in-line Tenax cartridge 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 is a hollow glass cylinder (25 cm × 3 cm) containing a sand-like absorbent capable of capturing VOCs as they pass through the cartridge. The Tenax cartridge and Venturi single-stage adapter are encased in a wooden box with a screened-in bottom and attached to a wooden post five feet off the ground, located in the tree-line and near the canopy. The air cylinder was secured with a chain to the post. For ambient VOC collection, the experiments were run continuously for 14-35 days. The gas cylinders were exchanged every 2-3 days, depending upon the flow rate, typically set at 15-20 mL/min.

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, or businesses; the other two boxes were placed several rows apart, but next to similar cultivars, and were employed to verify reproducibility of results. **Table 1** provides a summary of experiments run, dates and length of collection, cultivars present in the orchards, and location of orchards.

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	30	NP:MO:CA	2:1:1	NP	PFC
2	6/24/08	8/5/08	35*	NP:MO:CA	2:1:1	NP	PFC
3	6/5/08	7/1/08	26	NP:MO:CA:AL	4:2:1:1	NP	NK
4	7/1/08	8/14/08	14*	NP:MO:CA:AL	4:2:1:1	NP	NK

[a] Almond varities 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: PFC = Kern county, Paramount Farming Co.; NK = Colusa county, Nickels Research Laboratory.

The first objective of this project 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 Kern county orchard.

Once collected onto the Tenax medium, the VOCs are desorbed and analyzed via gas chromatography/mass spectroscopy (GC-MS). Preliminary data analysis of the VOCs collected in the experiments listed in Table 1 show very consistent emission of *ca.* eight VOCs, four of which are most likely aldehydes, a common functional group on many volatiles found in nature. However, these volatiles are found predominantly as the corresponding carboxylic acids, the oxidized version of aldehydes. The oxidation of aldehydes is most likely due to air as it passes through the Tenax, past the absorbed volatiles. To test this theory, a small amount of benzaldehyde was placed on a clean cartridge of Tenax, the cartridge placed in an oven at 35 ^{o}C, and a flow of air passed through it for four weeks. Desorption of the benzaldehyde and subsequent GC-MS analysis to determine if conversion took place is currently pending. The identities and relative amounts of the specific VOCs will be disclosed after verification of the VOCs from all experiments and EAG analyses have been performed.

The goals of objective #1, as listed above, have been primarily successful. Obstacles encountered, but amendable in subsequent analyses, were 1) capping of the Tenax during orchard spraying, but inadvertently left on for the remainder of the analysis thus, that particular experiment was null (#4, **Table 1**); 2) spraying in the orchards resulted in the temporary shutting down of the collection systems. This affected experiments #2 and #4; and, as previously mentioned, 3) the large amount of smoke during the fires in Northern California may have introduced unwanted VOCs. Not all of the Tenax cartridges have been desorbed and evaluated for VOC content. Appropriate analyses are imminent.

The completion of objective #2 is necessary before the goals outlined in objectives 3 and 4 can be concluded. Once all cartridges from the VOC collection experiments have been analyzed, work on objective #3 will be performed and completed via comparison of VOCs content from each experiment. Data will also be evaluated for any correlation to NOW flights.

Plans for completion of objective #3 include electroantennogram bioassay of the VOCs noted in all experiments from objective #2. The VOCs will be evaluated individually as well as in mixtures that mimic the relative abundances noted in the GC-MS experiments. VOCs that elicit EAG responses will also be combined and evaluated for EAG efficacy, but with minimal number of VOCs. This optimized mixture will be used for future EAG studies and assessed for use with existing and future attractants for NOW.

An expected timeline for the achievement of the remaining objectives is as follows: November and December, finish desorption of all Tenax cartridges and complete data analysis; December-February, EAG analyses of all VOCs, both individually and as blends; March, write-up results in a formal report and begin optimization of VOC

collection method for use in future studies. It is planned that the method will be applied to first reproduce results from the 2008 growing season, and second, used for almond orchards in varying locales. Results will then be compared to the Kern and Colusa counties noted in **Table 1**. It should be noted that this method, once optimized, will be applicable to other commodities, and other insect pests of CA, as well as nationally, or internationally.

Any successful results from the above experimentation will undergo appropriate technology transfer of method and dissemination of results to germane researchers, agricultural end-users, and future Almond Board Conferences.