AES/CE Workgroup/Department:

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: **March 2009 through February 2010** Anticipated Duration of Project **2 Years**

Project Leader: **John J. Beck** Location **USDA-ARS, Plant Mycotoxin Research**

Cooperating Personnel **Glory B. Merrill, Douglas M. Light**

Project Title: **Ambient orchard and on-tree volatile collection system for monitoring and detection of attractants for navel orangeworm**

Keywords
Commodity(s) **NOW lure; attractant VOCs** Relevant AES/CE Project No. _____

Problem and its Significance:

The navel orangeworm (NOW) is a major insect pest of California tree nuts. Its feeding damage lowers nut kernel quality resulting in considerable 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 is presently a significant food safety problem due to its carcinogenic and teratogenic attributes. Hence, control of NOW has now become one of the top priorities of the CA tree nut industry. Despite having a natural defense against *Aspergillus flavus*, walnuts are still susceptible to NOW damage. The California Walnut Board Entomology Research Group recommended that control and monitoring of NOW be considered a top research priority. This proposal will directly address the use of natural attractants from California tree nut orchards for monitoring of NOW. Currently the ability to detect and monitor NOW populations suffers from the lack of dependable and accurate semiochemical lures.

Objectives:

Obtain sufficient funding in order to construct several optimized volatile organic compound (VOC) systems. These systems would allow the development of new and more effective lures for NOW by:

1) Optimizing and implementing a large-scale volatile organic compound (VOC) collection system capable of both ambient orchard and on-tree (*in-situ*) analyses;
2) Producing a method that is applicable to any agricultural commodity;
3) Collecting ambient almond, pistachio, and walnut VOCs from select geographical locales;
4) Evaluating efficacy of collected VOCs in bioassays on NOW, including electroantennogram (EAG), flight tunnel, and field trapping;
5) Formulating a synthetic blend of background VOCs for use in subsequent EAG, flight tunnel bioassays, and field trapping studies in conjunction with current or future NOW attractant VOCs;
6) If discovered, 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.

Plans and Procedures:

Several studies have investigated the various non-volatile and volatile components of an assortment of plant parts from tree nuts; however, the volatile organic compound (VOC) emission of any one single cultivar, or the VOC emission of several cultivars in one orchard, has *not* been studied over the course of an entire growing season. This aspect is particularly relevant to research concerning the navel orangeworm moth (NOW), a major insect pest of almond, pistachios, and walnuts. *The identification of particular VOCs, or their potential relationship to NOW flights, has not been addressed*.

My laboratory currently utilizes several volatile organic compound (VOC) collection techniques for both in-lab and in-field applications. Some of the in-field techniques have been developed as a result of research agreements with Paramount Farming Company and the Almond Board of California (ABC). Research based upon tree nuts and tree nut volatiles has led to the development of a small-scale on-tree (*in-situ*) method for collecting VOCs. The method utilizes a small, inert Teflon bag with two ports for analyses and allows for the immediate collection of VOCs of multiple tree nuts *in-situ*. However, one critical drawback to this *in-situ* method is that it employs solid-phase microextraction (SPME) as the VOC collection medium. Because the adsorption of VOCs on the SPME is equilibrium-based, long transportation times between field collection sites and laboratory analysis results in loss of minor VOCs. Although minor, these compounds may be critical as natural attractants to NOW. A second drawback of SPME is that it is an analytical technique only, and collects extremely small amounts of VOCs. Thus, any subsequent analyses beyond separation, such as electroantennogram (EAG) detection, or olfactory choice studies, are not possible.

As a remedy, my lab developed a prototypic VOC collection method (**Figure 1)** capable of performing ambient, large scale, in-field analyses, as well as single cultivar, *in situ* analyses (leaf and/or nut). The prototype employs an independent on-site, Venturi-based vacuum system that allows an in-line Tenax cartridge to collect VOCs from the ambient orchard air or single cultivar. The vacuum is generated by a large cylinder of compressed air (232 cu. ft., 2300 psi) delivering 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, and located in the tree-line and near the canopy. The air cylinder is secured with a chain to the post. For ambient VOC collection, the system runs for 22-30 days, day and night. The gas cylinder is exchanged every 2-3 days, depending upon the flow rate. Each experiment utilizes three collection systems: the first box, for control VOCs, is located on the outside, upwind edge of the orchard and collects extraneous VOCs from neighboring orchards, other commodities, homes, or businesses. The other two boxes are placed several rows apart, but next to similar cultivars. Two boxes are employed as replicates to show reproducibility of results.

At the completion of the experiment, the Tenax cartridges are removed, transported to the laboratory, and the VOCs desorbed using diethyl ether, a low boiling point organic solvent. The desorbed VOCs are then concentrated and subjected to a number of experiments/bioassays including: 1) gas chromatography/mass spectroscopy (GC-MS), the method of choice to analytically separate and identify the majority of VOCs; 2) EAG analysis, a method of using the antennae of NOW moths to identify VOCs that elicit responses from the excised antennae; 3) flight tunnel bioassay, an in-lab method to evaluate the efficacy of a VOC or mixture of VOCs to elicit responses from moths navigating a short tunnel with air flow; 4) GC coupled to an EAG detector (GC-EAD), a method used to separate and evaluate the individual components of a mixture of VOCs; and, 5) isolation and identification of minor components, the last stage of identifying active, but known compounds. My lab employs and maintains all of the aforementioned instrumentation/equipment.

The preliminary portion of the proposed project was partially funded by the ABC to develop a monitoring system of ambient VOCs in almond orchards of different geographical locales. The prototype system was tested in Kern and Colusa counties during the 2008 growing season. As of this writing, data from the experiments are being downloaded, analyzed, and categorized as to structure and phenological origin. Initial findings ***show the presence of VOCs that have exhibited positive EAG responses to both male and female NOW moths*** in prior experiments.

OBSTACLES ENCOUNTERED: Problems encountered with the scaled-up system have primarily been logistical, the chief barrier being the exorbitant costs of the air cylinders and associated demurrage. Scheduling of tank maintenance and changing, particularly in relatively remote areas, poses a problem to the orchard managers and associated personnel. Finally, due to the numerous changes in air cylinders, the delicate settings of air flow are 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*. This was verified by comparison of preliminary data to the previous year’s data, which utilized the small-scale SPME VOC collection method.

ADDRESSING THE OBSTACLES ENCOUNTERED AND TIMETABLE: To directly address these logistical obstacles, my lab has initiated the process of optimizing the method of VOC collection. The research objectives of this proposal are to *optimize the existing large-scale ambient VOC collection system prototype* and move forward with the following tasks:
1) Assemble nine independent, self-sustaining, and reliable large-scale VOC collection systems capable of ambient and/or *in-situ* analyses (**Figures 2 & 3**).
2) In year one of the project, three systems will be field tested in a single orchard of each commodity (nine boxes total). Adjustments or modifications can be made and implemented up to three- to four times in the first year's growing season.
3) Utilize the off-season to make final adjustments to the system and assemble a sufficient amount of units (total of 20 units) to perform multiple analyses in year two.
4) Utilize the off-season to analyze data and perform appropriate bioassays (i.e., EAG, flight-tunnel) on verified VOCs, and any necessary separations and/or isolations.
5) In the second year, implement several collection systems to perform analyses in walnut, pistachio, and almond orchards; either ambient and/or *in-situ*, in stages over the course of the growing season.
6) Utilize the remaining time in the second year to perform bioassays to determine which VOCs exhibit responses from NOW; and, compare/contrast VOC emissions of each cultivar.
7) Incorporate potential VOCs as background or adjuvant for existing attractants for NOW.

My lab is currently outfitted to perform all of the items listed above, and have the requisite knowledge and expertise to perform the necessary analyses. Devices have been investigated for the optimization of the collection system. The main focus will be to eliminate the need for air cylinders and enable the system to be completely independent – no adjustments required during the length of the experiment. Investigations have been initiated into the use of a small air pump, a portable battery source, and a solar panel on a telescoping rod attached to the main wood pole (see **Figure 2** and **Figure 3** for schematics of the proposed system).

ACQUISITION OF MATERIAL FOR TWENTY COLLECTION UNITS: The purpose of this proposal is to obtain funds to purchase requisite material and supplies for the collection boxes necessary to fulfill the stated objectives. Current projections estimate each box will require approximately $1,400. This includes (dollar figures are estimated): solar panel, pole, wood, and wire, $800; battery, $100; Tenax cartridge, $50; Tenax, $250; commercial-grade pump, $100; collection bag and tubes (for *in-situ*), $100. The requested amount will cover the costs of nine- to ten boxes, thus accomplishing the goals of the first year.

REPORTING OF RESULTS: All results will be disseminated via peer-reviewed journals (i.e., *Journal of Agricultural and Food Chemistry*) as well as presentations at the Walnut Research Conference and other commodity and professional (chemistry/agriculture) meetings. The USDA-ARS has a web site specifically for publications and select projects. Finally, the ARS has an in-house Office of Technology Transfer for the facilitation of information directly beneficial to the agricultural community.

ADDITIONAL INFORMATION: The Principal Investigator (PI) of this project has recently been awarded a grant from USDA Headquarters for a postdoctoral research associate (PDRA). The funds from this award will partially fund a PDRA for two years. It is the intent of this PI to utilize the PDRA toward fulfillment of the goals set forth in this proposal. Additional external funds are being sought to fully support the PDRA for this project. Funds requested from the WRB are approximately 10% of the overall expected budget for this project. The scope of the project will utilize the current personnel in my lab (myself, one chemist, one PDRA, one technician) and a portion of Dr. Doug Light's lab. It is anticipated that one more technician will be necessary for the intensive field work in both years of the project. Additional funds will be sought for technical assistance.

Research utilizing the optimized collection method *will be continued into the ensuing years beyond the timeline of the grant* and will address germane VOC issues of California agricultural commodities. For example, the optimized collection system can address VOC issues such as: applicability to other insect pests, their pheromones, or other attractant semiochemicals; monitoring experiments; comparison of volatiles of experimental orchards (i.e., pruned, irrigation, new cultivars, plant stresses); and effects of VOCs, pheromones, herbicides, or pesticides from neighboring orchards of a different commodity.

Figure 1. Current large scale ambient VOC collection system.

Figure 2. Exploded view of the proposed large scale ambient VOC collection system utilizing solar energy for the vacuum source.

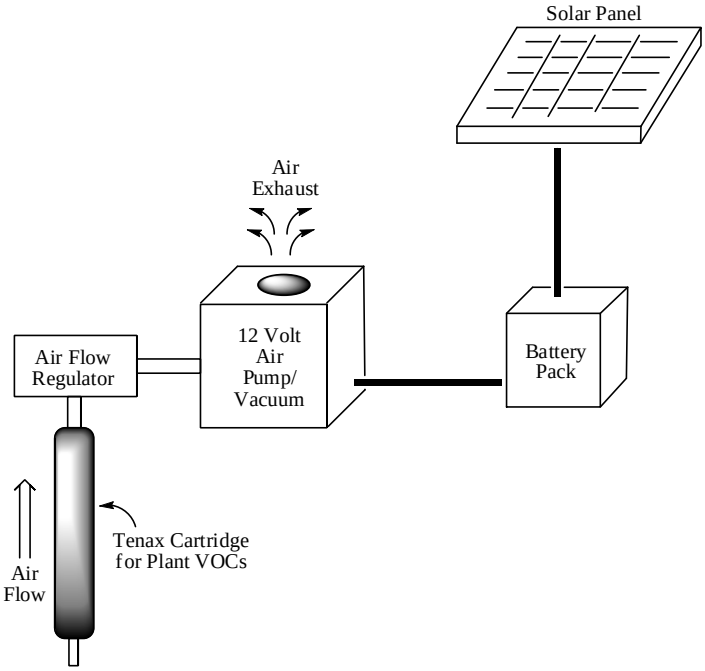
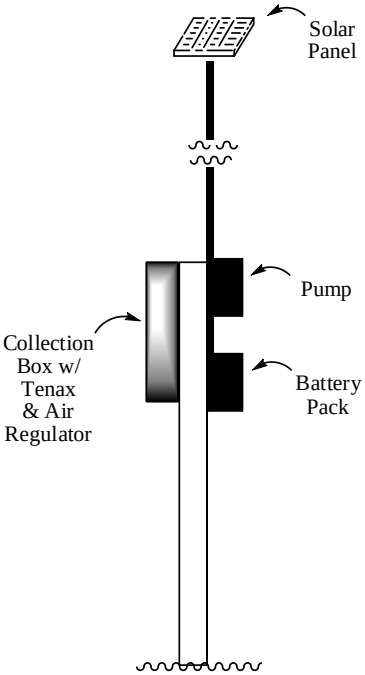


Figure 3. Illustration of collection system in the field (with telescoping solar panel).



BUDGET REQUEST

Funding Source				Budget Year
Salaries and Benefits				
Postdocs/RAs			$	0.00
SRAs			$	0.00
Lab/Field Assistance			$	0.00
Subtotal		Sub 2	$	0.00
Employee benefits		Sub 6	$	0.00
		SUBTOTAL	$	0.00
Supplies and Expenses		Sub 3	$	20,000.00
Equipment		Sub 4	$	0.00
Travel		Sub 5	$	0.00
		TOTAL	$	20,000.00

Department account number _____

_____ Date _____

Originator's Signature

COOPERATIVE EXTENSION — County Director _____ Date _____

Program Director _____ Date _____

AGRICULTURAL EXPERIMENT STATION — Department Chair _____ Date _____

LIAISON OFFICER _____ Date _____

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