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# Addressing the Complexity and Diversity of Agricultural Plant Volatiles: A Call for the Integration of Laboratory- and Field-Based Analyses

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**ABSTRACT:** As the sophistication and sensitivity of chemical instrumentation increase, so does the number of applications. Correspondingly, new questions and opportunities for systems previously studied also arise. As with most plants, the emission of volatiles from agricultural products is complex and varies among commodities. Volatiles are indicative of characteristics such as food quality, cultivar type, phenological stage, and biotic and abiotic stressors; thus, their systematic and accurate evaluation is important. Early volatile analyses entailed removal of the sample matrix in question, transport to the laboratory, and subsequent investigation. More recently, scientists are moving the laboratory to the field to obtain realistic emission patterns of the sample in its natural environment. This perspective proposes that a methodical and collaborative approach to the complex relationship between volatiles and agricultural commodities and their various phenological stages, oxidative degradation products, and fungal contamination is needed in order to fully comprehend the sample and associated relationships as a whole. These methodical approaches should incorporate both in situ and ex situ investigations of the sample. Ultimately, there exists an opportunity for development of methodologies that integrate both laboratory- and field-based collection of volatiles to explore and address the complex biological interactions of agricultural systems.

**KEYWORDS:** *agricultural, ambient, ex situ, fungal, host plant, insect pest, in situ, plant−insect, semiochemicals, volatiles*

## INTRODUCTION

In terms of chemical analyses of biological systems there seems to be a disparity between what is detected from a sample matrix in the laboratory environment versus what is detected when the same sample is intact and in its natural environment, e.g., fruit removed for study vs fruit on a branch.[1] This is not to say that one method is better than the other or that one provides more information. However, each has its limitations: the laboratory experiment (e.g., ex situ or in vitro) does not truly reflect the system as a whole and removes the sample out of its contextual environment; whereas the field experiment (e.g., in situ or ambient) may not yield detailed information, incorporates many variables, or may simply be impractical to study. Despite their limitations, both approaches are extremely important to the general body of knowledge for each specific sample and the associated analytes.[1,2]

Agriculturally related projects typically begin as an observation of the grower or commodity group, which relates it to a researcher, who then studies the sample matrix under sterile, controlled conditions. Inevitably though, a large percentage of successful laboratory-based results do not translate to success under field conditions, which are typically harsh, multivariable, and extremely complex. It thus becomes necessary to integrate these methods, i.e., to start with an observation in the field; reproduce the observation under in situ conditions, but mindful of the conditions (time of day, irrigation status, phenology); and to follow it with intensive laboratory studies to determine what experimental conditions to pursue in order to address the objective of the investigation. What becomes important is translation of the results from the dissected laboratory experiment to field conditions and thus provide usable information for researcher and grower alike.

To ensure the successful transfer of laboratory results to the field planning for the analysis of volatiles from agricultural products requires consideration of numerous variables. These include, but are not limited to, the following: vegetative or fruit; developmental stage of the sample (phenology); geographical location; cultivar and the possibility of several cultivars in one orchard; neighboring commodities; oxidative or thermal degradation products of the sample, analyte, or precursors; natural occurrence of fungi and their associated volatiles; and residual maintenance sprays. The objective of any investigation ultimately dictates what method for volatile collection should be used as well as the type of instrument to separate and characterize the volatile components. To assist with experimental design a number of reports can be consulted for recent developments in methods and techniques for analyses of volatiles and the associated data typically generated.[2−6] It should be noted that smaller agricultural commodities can be transferred to greenhouse conditions for subsequent volatile analysis. This minimizes the influence of external factors, increases access by researchers, and allows for field conditions to be mimicked more closely. Unfortunately for some crops this option is not practical.

> *"Always design a thing by considering it in its next larger context..." Eliel Saarinen, Architect*

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This perspective is designed to highlight the importance of considering the sample to be studied in the greater context of the agricultural setting, as well as the in-depth, focused investigation of an isolated portion of the sample as a whole, and, furthermore, to design collaborative experiments to include both laboratory- and field-based conditions and thus ensure that the former results translate efficiently to the field and thereby provide the answers growers need.

The following topics are examples of some areas of plant volatile investigations that could potentially benefit from integrated analyses, as well as demonstrate the diversity and complexity of plant volatiles. The listed items are introductions only, and the associated references are not comprehensive. It should be noted that there are instances of researchers incorporating both laboratory- and field-based experiments;[7,8] however, utilization of this type of approach for agrarian issues may benefit agricultural researchers and growers alike.

## APPLICATIONS FOR VOLATILES IN AGRICULTURE

The collection and identification of volatiles from agricultural commodities is an important field of study for several reasons. One significant example is volatiles as chemical cues for insects. Chemical communication between plants and insects is greatly regarded in the chemical ecology and entomological literature,[9,10] and the collection, identification, and role of volatiles are extremely important. A closely related topic is the use of host plant volatiles to attract insect pests of agricultural commodities.[11−14] Host plant volatiles can also be used to help disrupt mating of insects in an orchard,[14] for integrated pest management,[15] as banker plant systems,[16] or for other biological control.[17]

Volatiles also play an important role in cultivar distinctions. If only the end product (consumed food) is of interest, then the matter of integration of laboratory- and field-based experimentation may be inconsequential since volatile analyses would be postharvest and performed under controlled conditions. However, if cultivar volatile differences have field implications such as cultivar resistance to infestations[18] or detection of plant diseases,[3,4] there is the potential for incorporation of new methodologies such as portable MS or GC−MS systems[19,20] for integration of laboratory- and field-based experimentation. It should be noted that the use of solid-phase microextraction (SPME) for field analysis of plant volatiles is also an option; however, this type of volatile collection can be limited to qualitative analysis[2] or comparisons. Concerns of adsorbed volatile loss from the SPME fiber during storage between field collection and laboratory analysis can be assuaged by placing bagged SPME cartridges over ice[21] or in sealed culture tubes and over dry ice.[8] Numerous examples of plant, insect, and microbe volatile analyses are described in a review concerning SPME,[22] in which the authors support the idea of SPME being a powerful tool for field analyses when combined with portable detector devices.

Advances are being made in the use of microbe-produced volatiles and change in host plant emission as an early warning for contamination of agricultural commodities.[3,4] Similarly, biosensors and chemical sensors have been investigated[23,24] and the use of host plant emissions may have a role to play for volatiles that signal changes in stored fruit quality, foodborne pathogens or toxins, and detection of pesticide residues. A review by Ruiz-Altisent et al.[24] provides detailed information on numerous types of sensors and also lists the need for a portable detection device for field and other preharvest applications.

Genetic engineering of agricultural commodities has modified the emission of volatiles in order to augment plant defenses, attract beneficial insects, or improve odor quality.[25,26] For instance, downregulation of limonene synthase in orange plants resulted in marked resistance to microbes in addition to decreased attraction of the citrus pest medfly.[27] Examples such as this highlight the importance of plant volatile emissions and the need to accurately delineate the roles of the volatiles under proper laboratory and field conditions.

Analysis of fungal volatiles for food flavor has a long history, but there are other microbial interactions, their volatile emissions, and associations to agricultural commodities that are relevant. For instance, the volatile emission from arbuscular mycorrhizal host plants has been investigated.[28] In their report, the authors discussed the complex tritrophic relationship between the host plant, mycorrhizal effects, herbivore predators, and the need to investigate more thoroughly these interactions for agricultural applications. The tritrophic interactions of host plants, insects, and bacterial-[29] or fungal-produced volatiles[30] have many agricultural implications such as attraction or reduction of pest insects,[31,32] as well as analytical challenges.

Other examples of microbe-produced volatiles and their relation to agriculture are covered in germane reviews.[22,33] Nonetheless, there is opportunity for the integration of microbial volatile analyses in the laboratory and field environment as researchers investigate ways to make agricultural commodities sustainable and their management more environmentally friendly.[34]

## TOWARD THE INTEGRATION OF LABORATORY AND FIELD ANALYSES

The investigation of host plant volatiles that exhibit semiochemical characteristics toward an insect pest is offered as an example of the need to combine both laboratory and field analysis. The navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae), is a major insect pest to almonds and pistachios in California.[35] *A. transitella* larvae cause feeding damage to kernels as well as introduce the aflatoxigenic fungi, *Apergillus flavus* and *Aspergillus parasiticus*. Current efforts for control and monitoring of *A. transitella* are insufficient for commercial purposes. To address the need for efficacious monitoring of *A. transitella* our laboratories have examined almond host plant volatiles for potential attractants. In our laboratory's 2008 report[36] on the volatile emission of damaged and undamaged almonds fungal-related volatiles were noted as a result of the ex situ method employed for volatile collection. While the detected volatiles provided insight into the emission of damaged and fungal-contaminated almonds, it was realized that the amount and stage of fungal growth did not represent the odor-bouquet typically encountered by the insect pest. This observation led to the utilization of an in situ method of volatile collection from almonds,[35] which yielded additional volatile components to consider as potential semiochemical candidates. More important was the incorporation of the laboratory-based electroantennographic (EAG) bioassay to evaluate the potential biological activity of the volatiles detected. It is important to note that some type of screening bioassay is necessary to reduce the high number of volatiles detected to a manageable number.

Instances of a single host plant volatile being the active semiochemical for insects are rare, and multicomponent blends are typically the norm. Thus, it is important to screen the typically large number of volatiles collected from a host plant via some type of laboratory-based bioassay. For example, the combined number of volatile components from the in situ and ex situ experiments described above[35,36] was ca. 50 compounds. The use of EAG to screen the identified components decreased the number of volatiles of major interest to ca. 10−12. It should be noted that the use of a laboratory-based bioassay to assess the bioactivity of the identified volatile components is dependent upon the insect and its response to these types of assays. Additionally, these types of bioassays typically require that the compounds of interest are commercially available or can be readily synthesized.

Though the in situ investigation[35] was field-based and meant to provide a more realistic almond emission profile, the project was limited in that it evaluated the emission of almonds from a single cultivar. In contrast, a typical commercial almond orchard has several cultivars within an orchard. An additional limitation of the in situ study was the time of day that the volatiles were collected. Due to distances between the field site and laboratory, and the need to analyze the collected volatiles the same day (to minimize storage time of adsorbed volatiles on the SPME fiber), the volatiles were collected in the morning hours. Though important insight was gained from the volatiles identified from the in situ study, the emission profile was not representative of what the insect encounters since *A. transitella* are active in the evening hours.

Because the in situ study did not capture the characteristic semiochemical medium which the targeted insect pest typically encounters, attention was turned to the collection of ambient volatiles from a commercial almond orchard.[37] The ambient study provided a more accurate picture of the volatile bouquet present in the orchard. A limitation of the study was that the average diurnal volatile emissions were collected and reported versus delineation of the scotophase and photophase volatiles. In retrospect, the overall experiment would have benefited from the proper choice of volatile collection method that addressed these obstacles. However, the almond volatiles collected and identified were invaluable for the overall objective of the project.

A turning point in the research was the return to a concept learned from the laboratory-based ex situ study and the volatile output as a result of fungal contamination of the almonds. Attention was turned to the investigation of volatiles produced from almond kernels with naturally occurring fungi.[38] The almond samples investigated were unique in their unusual high level of naturally occurring aspergilli contamination. *Aspergillus flavus* and *A. parasiticus* are ubiquitous fungi in almond and pistachio orchards that when allowed to grow on the kernels can produce aflatoxins. The volatiles identified from this study provided more compounds (in addition to the volatiles identified in the ex situ, in situ, and ambient collections) for EAG bioassay and additional semiochemical candidates.

Interesting to note from these studies of almond host plant emissions was the disparity in volatile compositions between experiments despite the relatively same sample matrix being studied. While no one individual study outlined above provided a blend of components that was highly active by either EAG or field studies, the combination of EAG-active components from all of the studies provided the basis for a series of blends that have demonstrated the ability to attract adult *A. transitella*.[39] Moreover, from these studies it would appear the project progressed along a typical line of experimental design based on results from previous experiments. However, retrospection of the system as a whole—the host plant and its diurnal emission patterns, the insect and its behavioral patterns, and microbial populations and their volatile emissions while on the host plant—provided the basis for the chemical analysis of a complex system involving botany, entomology, and microbiology. As a consequence of the above trials, our laboratory is currently developing an experiment that will take place under field conditions, but will be measurable and reliable and provide confirmation of the outcomes and subsequent hypothesis as a result of studies noted above.

Implementation of an integrated approach is not a trivial matter, and each project will have unique obstacles. For instance, with insect-related volatile studies one of the biggest challenges will be a rapid and realistic laboratory-based bioassay to measure behavioral responses of insects to potential attractants or repellents. For projects investigating microbe emissions, a challenge will be the development of real-time field microbe detectors.[3,4] As an example, it is known that fungi produce different volatiles depending on the growth medium,[40] thus laboratory experiments should be designed to mimic field conditions to ensure that proper expectations are met in the field. To add further complication, the volatile output of a single fungus (or other microbe) can be different or influenced by the presence of other or multiple fungi, which is more typical of what is found in the field. A possible result of increased field analyses via real-time detection may be the change in chemotype profiling of samples being analyzed. Though perhaps not as sensitive or extensive as laboratory-based MS or nuclear magnetic resonance analyses, field-based volatile analysis may provide a different chemical phenotype profile of a sample because it keeps the sample intact and in its natural environment.

The challenge of producing safe agricultural products will remain at the forefront of scientific endeavors. Chemical analyses of agricultural products are only one important step in ensuring food safety, determining nutritional content, and measuring agronomic qualities,[41] and volatiles can play an important role in these agriculturally related issues. Despite the extreme complexity of the roles of volatiles and their production,[9,10,42−44] an integrated approach of laboratory- and field-based experiments can help delineate the intricate relationship between sample matrix function, analyte production, and subsequent chemical cues to other plants, insects, and microbes.

Perhaps this perspective is verification that scientists from several major disciplines—biologists, chemists, engineers, and entomologists—are needed to understand and solve the complex interactions of agricultural volatiles; and that we as scientists must form a “multi-faceted counterattack”[45] and develop concurrent methodical top-down (field experiments) and bottom-up (laboratory experiments) approaches for agricultural and food chemistry.[46] One important point to highlight is that this type of research is not possible without the critical input, observations, cooperation, and collaborative efforts of stakeholders, commodity groups, and growers. Other researchers have started to recognize the need to conduct both laboratory and field studies[7,10,47] to investigate complex plant−insect interactions; thus, this appeal to the chemistry community to contribute their expertise and assist in developing methods or technologies. An integrated and cooperative approach will help derive simplicity from complexity; as Winston Churchill once said, “Out of intense complexities intense simplicities emerge”.

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### Notes

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