LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

LLNL-TR-818106

Annual Report on Raman Spectroscopy to Detect and Measure NOW Pheromones

T. Bond, S. SAhota , H. Wilson

January 6, 2021

Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

ANNUAL REPORT
Raman Spectroscopy to Detect and Measure NOW Pheromones

Authors: **Tiziana Bond**, PhD, Senior Staff Engineer, Center of Micro and Nano Technology, LLNL; **Sarah Sahota,** Junior Staff, LLNL**; Houston Wilson,** Asst. Coop. Extension Specialist, Kearney Agricultural Research and Extension Center, Dept. Entomology, UC Riverside

Introduction

Insect sex pheromones are chemical compounds that insects release to attract their partners over distances of hundreds of meters or even kilometers, in complete darkness and without any audible signals. Use of synthetic forms of key compounds have in some cases become an essential component of monitoring and/or managing key pests of agricultural crops, including navel orangeworm (*Amyelois transitella*) (NOW) in California tree nuts. These pheromone-based strategies can include monitoring, mating disruption, mass trapping, attract-and-kill and push-pull [1-3]. There are currently multiple commercially available mating disruption products available for NOW and recent studies have demonstrated that they can be effectively used to reduce crop damage by this pest [4]. Just how mating disruption works is not fully established and likely varies across products and target species [5]. For instance, the extent to which synthetic pheromones compete with natural pheromone is not well understood, or in the case of monitoring, how efficaciously the insect follows the diffusing plumes, especially across large blocks and at plot borders. Furthermore, there may be specific conditions under which poor or impeded diffusion of synthetic pheromone diminishes the disruption effect, which could result in some males effectively locating females for reproduction. At the same time, many commercially available synthetic lures are also available, and growers have been effectively using them to track population development – although the attractive range of these lures remains unclear. That is, while pheromone lures can attract many moths, the relationship between trap capture and local populations, much less crop damage, remains unclear.

Regardless of the emission source (aerosol puffers, meso emitters, lures etc.), we currently lack the ability to fully understand how these synthetic pheromone compounds diffuse away from their point-source of emission – and subsequently how this might affect the efficacy of mating disruption and/or the accuracy of monitoring efforts. Within this context, Raman spectroscopy may offer a means to detect synthetic pheromone because of its analytical properties and recent technological advancements [6]. In combination with nanostructured probes, Raman spectroscopy has a demonstrated capability to detect volatiles at extremely low concentrations [7]. Last year, we proposed a 2-year effort to evaluate the use of Raman for the detection of synthetic and natural NOW pheromones and, were that to be successful, use this tool in subsequent years to measure pheromone diffusion in orchards.

We have structured the effort to meet two objectives:

- *Objective 1 – Use Raman to Detect Pheromone (Year 1)*
- *Objective 2 – Use Raman to Measure Pheromone Diffusion (Year 2)*

<u>Objective 1 – Use Raman to Detect Pheromone</u>

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344; LLNL-TR-818106

Despite COVID-19 setbacks and constraints, we have been able to generate interesting and promising new data for the basic pheromone compounds, establishing a solid base for subsequent lure and aerosol studies.

Our Year 1 efforts have focused on Objective 1, testing the ability of Raman spectroscopy to detect emissions from synthetic pheromone lures and aerosol dispensers for mating disruption. We initially tried to do this with commercial lures and aerosol dispensers, but after some initial tests proved inconclusive we decided to focus on investigating the signatures of the primary active chemicals alone. That is, we pursued Raman calibration of the basic chemical components of the most commonly used lures and dispensers, as this information does not exist in the scientific literature, to the best of our knowledge.

We acquired the basic chemicals that compose a few lures, such as Trece L2L: (Z,Z)-11,13-Hexadecadienal; (Z,E)-11,13-Hexadecadienol; (3Z,6Z,9Z,12Z,15Z)-Tricosapentaene (E,Z)-7,9-Docedadien-1-yl Acetate [8,9], along with the same chemical samples from other companies, such as Alpha Scents. This second effort was successful, as we were able to extract the Raman signatures and assign peaks to different vibration and rotational modes for each primary chemical, which is completely new information for the pheromones under study (Figure 1). Notably, we found that the signatures of these primary chemicals are very unique, which not only confirms the ability of Raman to detect these chemicals, but to also differentiate between them.

We next went on to evaluate the sensitivity of our systems to detect these chemicals at very low concentration. Starting with 100% pure concentrated chemicals, we performed a series of dilutions, testing various concentrations down to 0.1%, in both ethanol (Trece) and acetone or hexane (Alpha Scents). Some of the results are summarized below in Figure 2 for the Trece samples. We have observed similar trends and peaks for the Alpha Scents sets but with different intensities, the solvent being the only difference. Our current analysis leads to believe that the acetone peaks overlap differently with the chemical peaks than ethanol, modifying the intensity of chemical specific peaks.

We are currently working on how to deconvolve the solvent contributions, using post processing and techniques such as Principal Component Analysis. The latter will also be very important in the study of mixtures for which we have initiated some experiments as shown in Figure 3, where we report on the signals for 2-chemical mixes, noting that we can distinguish the specific peaks of each chemical but that some of them also appear to be influenced by the companion chemical. Following the deconvolution of 2-chemical mixes, we will investigate more complex mixtures.

Given the successful outcome in the first part of Year 1 (our funding actually turned on in May), we now intend to continue our evaluation of Raman spectroscopy and provide results on Raman-based pheromone diffusion using our "Orchard in a box" emulated environment, the latter being the main focus in Year 2. In fact, we are now ready to perform tests with lures and dispensers being acquired (e.g. lures Trece L2L/L2H, Suterra BioLure;, PacificBioControl ISOMATE CTT) to follow with more aggressive aerosols (Suterra Puffer NOW ACE). We will also receive natural pheromone samples from live moths collected by Dr. Houston Wilson at UC Riverside. We are considering validating our experiments with electroantennography (hopefully in collaboration with USDA/ARS in Albany, CA).

<u>Objective 2 – Use Raman to Measure Pheromone Diffusion</u>

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344; LLNL-TR-818106

With the detection system in hand, we are targeting our efforts towards a small-scale pheromone diffusion experiment (i.e. the "the orchard in a box"). Here, we will use an enclosed experimental unit (1 cubic-meter dimension) that we built in Year 1 and insert a few Raman probes or substrates spatially arranged around the central pheromone source, which will then be used to map the concentration and diffusion coefficient within the volume of the box as the pheromone is released. The lures/aerosols will also be arranged in a box containing the Raman gun (Figure 4) and the environment will be tested at regular sampling times and at different positions of the lures/puffers to investigate the capability to measure the diffusion of the emitted pheromones in this other way. Finally, if time permits, initial testing with Surface Enhanced Raman will be carried out as well.

Using this information, we can then begin to understand how pheromone diffusion might work under orchard conditions. This "orchard in a box" experiment is just a starting point to understand pheromone diffusion, and actual diffusion models that incorporate the true complexity of orchard structure and environmental conditions will require significantly more work. What we are doing here is simply a starting point for a proof-of-concept effort, and if our Year 2 efforts are successful, we plan to leverage these data into a larger proposal to USDA AFRI to continue the development of this new technology and apply it in orchards. For example, future efforts will target the use of nanostructured substrates and probes to enhance excitement of the backscattered Raman signal [6,7]. With the configuration shown in Figure 4, we will explore the implementation of optical fiber to demonstrate the additional capability of fiber enabling practical deployment of Raman detection in the fields. The fiber would be eventually integrated with the Raman gun for use under field conditions.

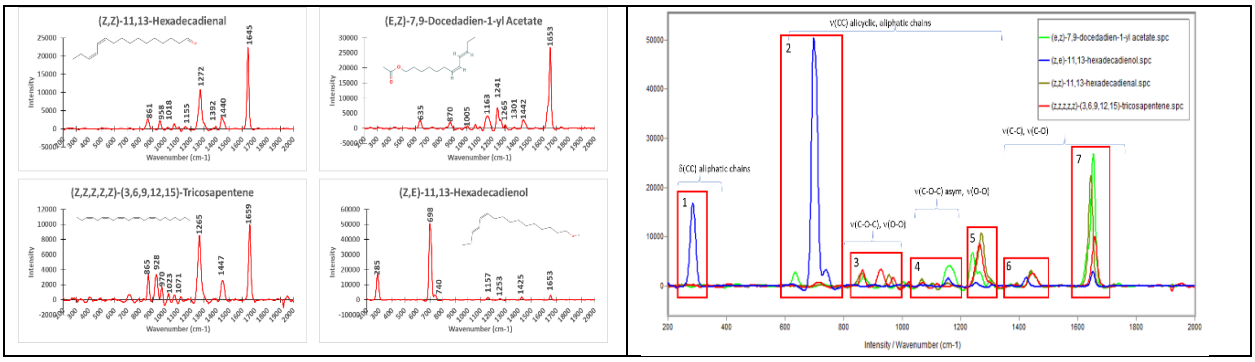
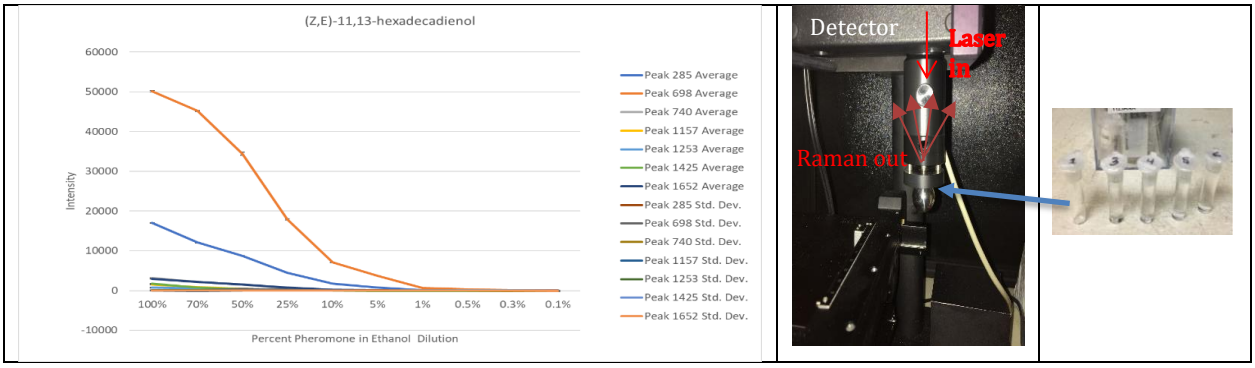


Figure 1. (*Left*) Raman spectra for the four main chemicals showing peaks that are singularly associated with each chemical, confirming clear distinction among them. (*Right*) Overlay of all spectra with assigned vibrations.



This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344; LLNL-TR-818106

Figure 2. (*Left*) Concentration trend for (Z,E)-11,13-hexadecadienol for several peaks with dilutions in ethanol from 100% to 0.1%. (*Center*) The Raman gun holder and the vials that are positioned inside the holder. (*Right*) Four samples and reference empty vials that are placed into the Raman gun

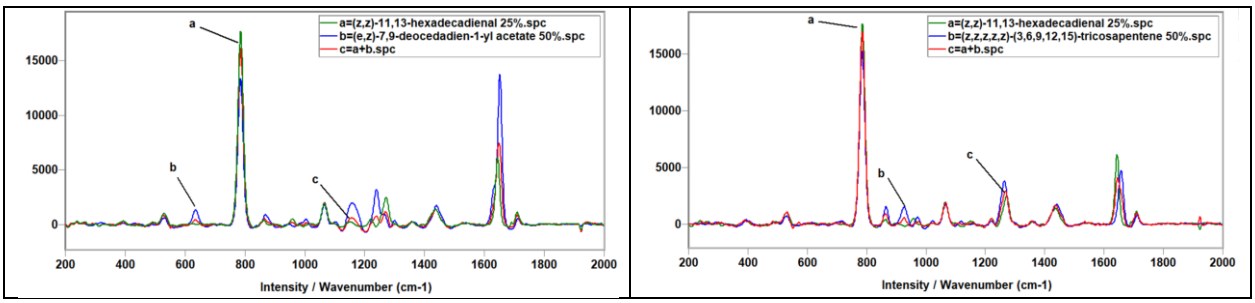


Figure 3. Spectra of mixed samples: (*Left*) 150uL of 25% of (Z,Z)-11,13-Hexadecadienal and 150uL of 50% of (E,Z)-7,9-Deocedadien-1-yl Acetate (*Right*) 150uL of 25% of (Z,Z)-11,13-Hexadecadienal and 150uL of 50% of (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene.

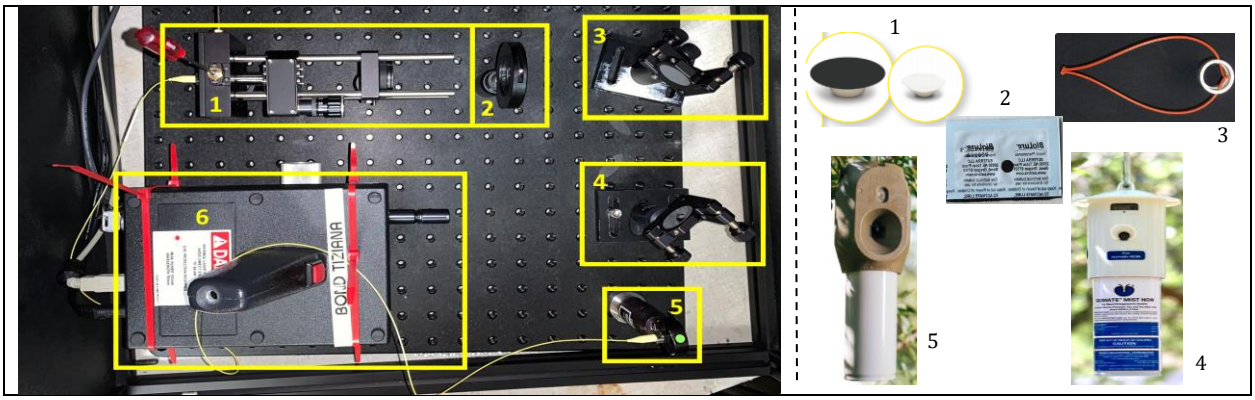


Figure 4. *(Left)*Top view of enclosed Raman system for first "Orchard in a box" experiment. (1) Optical Cage System; (2) Iris Diaphragm (model: Newport ID-1.0); (3) Kinematic Mirror Mount #2 (model: Thorlabs KM100); (4) Kinematic Mirror Mount #1 (model: Thorlabs KM100); (5) Lens Mount (model: Thorlabs LMR1); *(Right)*: examples of pest management tools that will be tested n the box (for low to fast release): (1) Trece L2L/L2H lures and (2) Suterra Biolure for detecting and monitoring; (3) PacificBioControl CTT dispenser, (4) Pacific BioControl ISOMATE Mist, and (5) Suterra Puffer used for mating disruption.

Projected Results

The goal of this project is to develop the use of Raman spectroscopy to detect synthetic and possibly natural NOW pheromones, which would have a variety of laboratory and field applications to improve our understanding of pheromone diffusion in orchards. Having demonstrated the ability of Raman to detect the primary chemical components of pheromone lures, we will now explore the use of this technology to monitor the diffusion of pheromones within a small, enclosed space. This would be eventually corroborated by basic mathematical diffusion models. In the long-term, these findings will provide the necessary proof-of-concept data to leverage this effort into a larger project funded by USDA AFRI or a similar institution.

Contingency Plan

In the event that Raman spectroscopy is inadequate to detect emission of pheromone from synthetic lures and aerosols, we will investigate alternate additional measurement routes,

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344; LLNL-TR-818106

including (1) attachment of aerosolized gold nano-tags to the pheromone and (2) chemical papers/metals that trap compounds and we detect with electrical pulses.

Potential Benefit to California Pistachio Growers

This project will develop the use of Raman spectroscopy to detect synthetic pheromone compounds emitted by trap lures and mating disruption products, as well as natural pheromone emitted from NOW adults. This detection capability will then be used in a basic experiment (i.e. "orchard in a box") to quantify pheromone diffusion in a very stable environment. Preliminary data on pheromone detection will be used to attract additional funding from additional agencies (e.g. USDA AFRI) to fully mature the technology. The specificity and sensitivity offered by this technique along with being portable, economic, and fast, could lead to the development of a tool for studying the spatial distribution of synthetic NOW pheromone in orchards. Furthermore, it may be possible to use this tool to detect natural NOW pheromone in orchards as well, which would afford some additional research opportunities into the ecology of NOW and behavior in orchards.

Literature

1) S. Tewari, T.C. Leskey, A. L. Nielsen, J.C.Piñero C. R.Rodriguez-Saona1,*Integrated Pest Management Current Concepts and Ecological Perspective*, Academic Press pp. 141-168, 2014.
2) W. S. Leal · A. L. Parra-Pedrazzoli · K.-E. Kaissling ·T. I. Morgan · F. G. Zalom · D. J. Pesak ·E. A. Dundulis · C. S. Burks · B. S. Higbee, "Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist", Naturwissenschaften v.92, pp,139–146, 2005
3) B.S. Higbee and C.S. Burks, "Effects of mating disruption treatments on navel orangeworm (lepidoptera: pyralidae) sexual communication and damage in almonds and pistachios, horticultural entomology", v. 101 pp.1633-1642,2008
4) Haviland, D. 2017 "Arthropod Pest Management in the Lower San Joaquin Valley" Project 17-ENTO6-Haviland, *2017.2018 Annual Research Report*, Almond Board of California
5) R.T. Cardé and A.K.Minks, "Control of Moth Pests by Mating Disruption: Successes and Constraints", Annual Review of Entomology, v.40, pp.559-585,1995
6) T.C. Bond, *Real-time, in-situ detection of volatile profiles for the prevention of aflatoxin fungal contamination in pistachios*. CPRB report 2017;
7) X. Yang et al, "Nanopillar array on a fiber facet for highly sensitive surface-enhanced Raman scattering", *Optics Express*, Vol. 20, pp. 24819-26, 2012; X. Yang et al., "High Sensitivity Multiplexed Gas Sensing by Raman Spectroscopy Using Photonic Crystal Fiber", *Sensor &Actuator B Chem.,* v. 176 , pp. 64– 68, 2012.
8) C. S. Burks, and C. Wilk, *Effect of storage of pheromone lures for Amyelois transitella: field performance and compound ratios*,, Florida Entomologist, Volume 100, No. 4,2017.
9) B. S. Higbee, C. S. Burks, and T. E. Larse, Demonstration and Characterization of a Persistent Pheromone Lure for the Navel Orangeworm, *Amyelois transitella (Lepidoptera: Pyralidae)*, Insects, 5, 2014.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344; LLNL-TR-818106