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Raman Spectroscopy to Detect and Measure NOW Pheromones ("Orchard in a box")

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Raman Spectroscopy to Detect and Measure NOW Pheromones

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Introduction

Insect sex pheromones are chemical compounds that insects release to attract mates 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. 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. How mating disruption works is not fully established and likely varies across products and target species. For instance, it is not well understood how synthetic pheromones compete with natural pheromones and, in the case of monitoring, how efficaciously the insect follows the diffusing plumes, especially across large blocks and at plot borders. Whether an aerosol puffer is used for mating disruption or lure for monitoring population development, 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. In response to these unknowns, Raman spectroscopy may offer a way to detect synthetic pheromone because of its analytical properties and recent technological advancements. In combination with nanostructured probes, RS has demonstrated the capability to detect volatiles at extremely low concentrations. We proposed 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 Pheromones.

Objective 2–Use Raman to Measure Pheromone Diffusion.

Our initial efforts have focused on *Objective 1,* on testing the ability of Raman spectroscopy to detect emissions from synthetic pheromone lures. We have acquired lures and aerosols, some of which were provided by Dr. Houston Wilson at UC Riverside. At first, we pursued Raman calibration of the basic components of the most used lures as it does not exist in the scientific literature, to the best of our knowledge. Secondly, we have performed analysis of how the lures basic component signatures differ from each other, which defines the capability of Raman to detect and differentiate one pheromone from another. Our other study has targeted the sensitivity of our systems and we have planned for this year to evaluate the quality of different substrates to register the signal of the various pheromones' sources (i.e. lure vs. aerosol emission). Due to the COVID-19 crisis, the experiments planned for 2020 have suffered a large setback because of mandatory closure of the laboratories from the beginning of March until mid-July, with limited access afterwards due to controlled shifts and use of the lab equipment. Despite all these constraints, we believe we have been able to generate some interesting and promising new data for the basic pheromone chemical components, establishing a solid base for subsequent lure/aerosol studies.

Results

Our system consists of a Raman gun at 785nm with ~30-50mW power shooting into a built-in vial holder where the vials with the chemicals or the lure can be properly set. This configuration offers the best focusing of the laser beam on the samples and thus best Signal-to-Noise Ratio (SNR). We have acquired the basic chemicals that compose a few lures we have identified as used in the fields such as Trece L2L: (Z,Z)--11,1311,13--hexadecadienal hexadecadienal ;(Z,Z)--11,1311,13—hexadecadienolhexadecadienol; (Z,E)Vial #5: (Z,E)--11,1311,13—hexadecadienolhexadecadienol, (3Z,6Z,9Z,12Z,15Z)—tricosatricosapentaenepentaene. [1,2]. We have analyzed the same samples also from other companies as we well (i.e. Alpha Scents). For all of them we have extracted the Raman signatures and assigned the peak

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to different vibration and rotational modes, which is completely new information for the pheromones under study (Figure 1). We have also carried experiments to understand the ability to detect low concentrations. Starting with 100% pure chemicals we have been doing dilution experiment, testing various concentrations down to 0.1%, in ethanol or acetone. The results are summarized below.

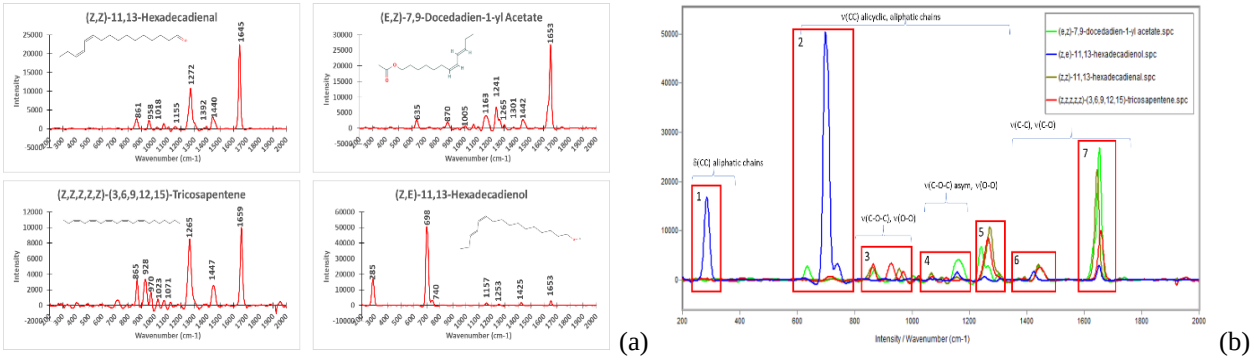

Figure 1. (a) Raman spectra for the four main chemicals showing the peaks that are singularly associated with each chemical thus confirming clear distinguishment among them. (b) Overlay of all spectra with assigned vibrations.

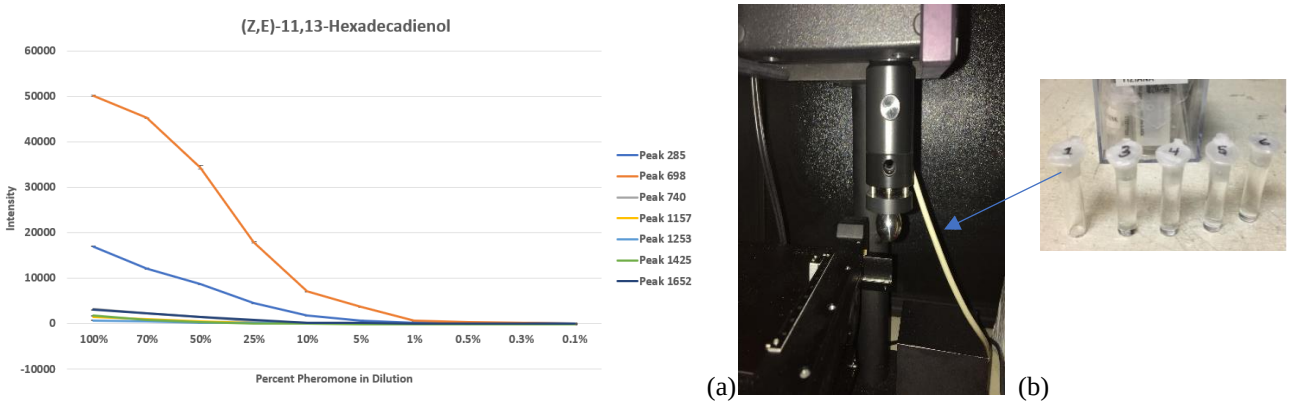

Figure 2. (a) Concentration trend for (Z,E)-11,13-hexadecadienol for several peaks with dilutions in ethanol from 100% to 0.1% (plot of mean with standard deviation over 5 measurements). (b) Picture of the Raman gun holder and of the vials that are positioned inside the holder, for the 4 sample s+ reference empty vials.

Initial testing with lures provided from Dr. Wilson were not conclusive mainly because of initial hardware and software hiccups that required us to modify our system setup, improve operation procedures, and upgrade the software. In the interim, as information is lacking in the literature, we focused on investigating the signatures of the active chemicals of interest and properly assign peaks, as shown. Therefore, we are now set to perform tests with puffers and new lures we are acquiring. Specifically, we are going to use lures from Trece (L2L/L2H) and Suterra (BioLure), and aerosols from Suterra (Puffer NOW ACE). Also, we expect to receive natural pheromones samples from Dr. Wilson. The sources will be arranged in a box containing the Raman gun and the environment will be tested at regular sampling times and at different positions of the lures/puffers to investigate the capability of measuring the diffusion of the emitted pheromones. Initial testing with Surface Enhanced Raman will be carried if time permits and we expect to optimize the limit of low detection.

Conclusion and Practical Applications

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) to fully mature the technology.

1. *Charles S. Burks, and Cristofer Wilk,* Effect of storage of pheromone lures for *Amyelois transitella*: field performance and compound ratios**, 2017** – Florida Entomologist – Volume 100, No. 4
2. Bradley S. Higbee, Charles S. Burks, and Thomas E. Larse, Demonstration and Characterization of a Persistent Pheromone Lure for the Navel Orangeworm, Amyelois transitella (Lepidoptera: Pyralidae) **2014**, Insects, 5