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Raman Spectroscopy to Detect and Measure NOW Pheromones

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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 effectively help reducing 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 ones and, in the case of monitoring, how efficaciously the insect follows the diffusing plumes and how those evolve from emission points, especially across large blocks and at plot borders.

We proposed to evaluate the use of enhanced Raman Spectroscopy (RS) for the detection of synthetic and natural NOW pheromones and, were that successful, use it in the future to measure pheromone diffusion in orchards. This year we continued Year 1 work, targeting two main objectives: (1) *Use Raman to Detect Pheromones and (2) Use Raman to Measure Pheromone Diffusion*. In Year 1 we were able to generate the Raman signatures for the main chemicals in synthetic pheromones, and now in Year 2 we have been able to provide a clear distinction of such signatures in mixed samples via Principal Component Analysis (PCA), validating RS for detecting emissions in more realistic conditions. In Year 2, we also continued our preparative efforts in measuring any release from dispensers or lures and setup some of our first "orchard in a box" experiments for diffusion studies. Given the low concentrations of pheromone release, we invested in boosting detection capacity by implementing two approaches: (1) preconcentrate samples with Solid Phase MicroExtraction (SPME) fibers[3] following GC-MS approaches applied to Raman and (2) pursue Surface Enhanced RS (SERS) to enhance the overall optical signals.

Funding from the CPRB was only approved within LLNL in May 2021, causing delays that were further accentuated by the lingering COVID-19 crisis, since laboratories had limited access until July 2021. During this downtime we requested and received DoE and State approval to work with live NOW moths for future experiments in collaboration with Dr. Wilson at UC Riverside. Despite all these constraints, we generated new and promising data on Raman selectivity and on pathways to enhancing its sensitivity for concentration-dependent diffusion studies of synthetic vs. natural pheromones.

Results

Since we had demonstrated the ability to detect Raman signals for various concentrations of what are accepted as most critical components in pheromone, i.e. ((Z,Z)-11,13-Hexadecadienal, (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene, and (E,Z)-7,9-Deocedadien-1-yl Acetate[1,2`], this year we extended our Raman exploration to mixtures diluted in hexane or acetone. Our Raman system works in reflection, and comprises a 785nm, 30mW power source with a built-in spectrometer with ~10cm^{-1} resolution. The portable gun aims at the core of a built-in vial holder where the vials with the chemicals, or lures/dispensers/moths, can be set. This configuration offers the best focusing of the laser beam on the samples and thus best Signal-to-Noise ratio. The mixtures clearly show variations in the spectrum from a single component (Figure 1A) which were also analyzed by PCA (Figure 1B) that can help isolate the response of each mixture. A database of such mixtures will be built for reference.

Our preliminary test with various lures contained in vials were not conclusive, likely because of the extremely low concentration they release and leakages through the vials. We then moved on to mating disruption dispensers, since they have 10-100x higher emission rates and we are currently testing them.

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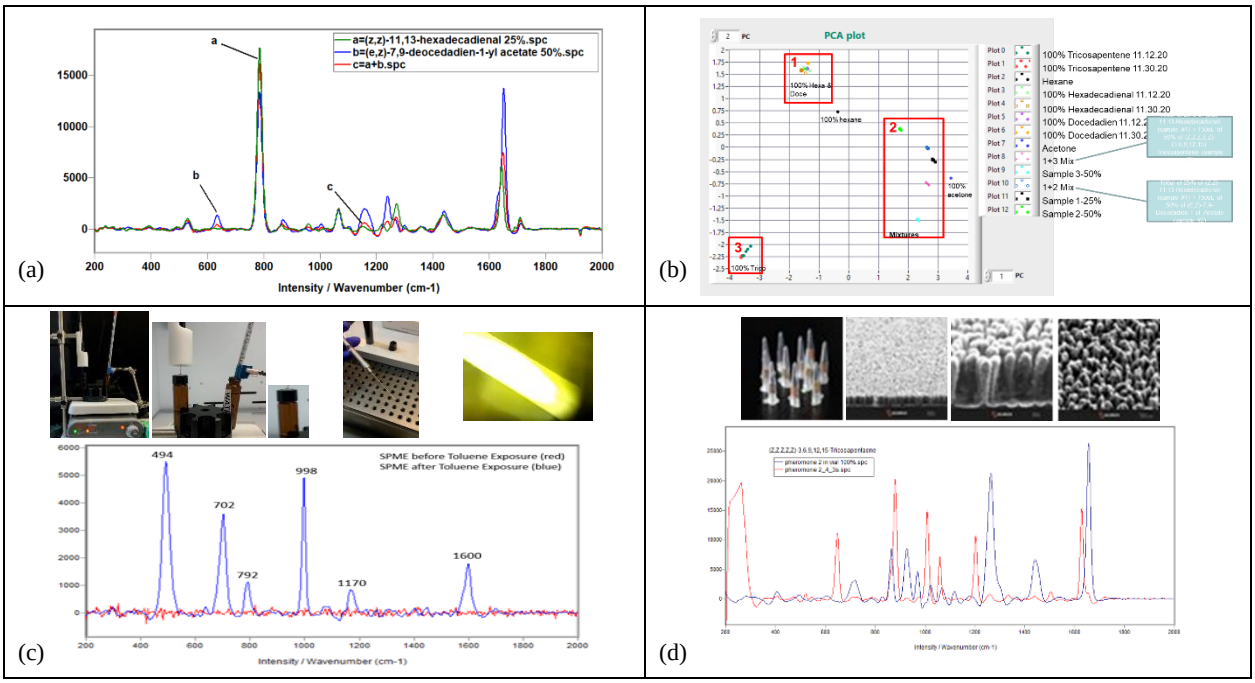

Figure 1. (a) Raman of single samples and their mixture: (mix #1) 150uL of 25% of (Z,Z)-11,13-Hexadecadienal & 150uL of 50% of (E,Z)-7,9-Deocedadien-1-yl Acetate; (b) PCA main components (p1,p2) plots showing isolation of single components and mixtures mix #1 and mix #2 (150uL of 25% of (Z,Z)-11,13-Hexadecadienal and 150uL of 50% of (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene); (c) *top:* SPME test setup: fibers exposed just to the headspace of the vial on hotplate, partially filled with toluene, *bottom*: Raman of SPME before (blue plot) and after (red plot) exposure to toluene for 5 minutes at 130C indicating successful uptake; (c) SERS (red) vs Raman (blue) of (Z,Z,Z,Z,Z)-3,6,9,12,15-Tricosapentaene on Ag nanosubstrate.

We have also increased sensitivity by using SPME to preconcentrate compounds. The SPME 50/30um DVB/CAR/PDMS, Stableflex 24Ga (Sigma-Aldrich) was first tested with toluene uptake and proved successful (Fig. 1C). The second test with the release of adsorbed toluene in another vial headspace did not provide any meaningful results, likely due to inadequate heating (<200C) to enable the release. We are now verifying headspace release and semiochemical uptake at >200C. We are also exploring SERS for increasing sensitivity[4] using nanopatterned leaning pillars, which introduce many hotspots that augment light shining onto and scattering back from the sample (preliminary results, Fig. 1D). The SERS peaks are shifted from bulk Raman since vibrational modes are likely affected by how molecules orientate on the substrate. Finally, we are improving setups for diffusion experiments, starting with emulating diffusion conditions (i.e. test for concentrations mapping). This will be verified by preparing lab samples or positioning lures, and exploiting SPME, SERS if possible.

Conclusion and Practical Applications

We are on the path to demonstrate RS to detect synthetic pheromone compounds emitted by trap lures, mating disruption products, and natural pheromone from NOW adults. This detection capability will be tested in an experiment (i.e. "orchard in a box") to quantify pheromone diffusion in a very stable environment. Preconcentrating via SPME or SERS might be key elements. Preliminary data on pheromone detection will be used to attract additional funding from Federal agencies to fully mature the technology. Ultimately, the goal of this work is to characterize the nature of pheromone diffusion through the orchard environment, which will contribute to improved use of mating disruption and monitoring NOW with pheromone lures, as well as provide new information on the chemical ecology of NOW pheromones.

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