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Pheromone Detection by Raman Spectroscopy

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Pheromone Detection by Raman Spectroscopy

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ABSTRACT

Raman spectroscopy has allowed for the acquisition of Raman signatures for pheromone components, or chemicals, which are found in the pheromones of crop key pests: (Z, Z)-11,13-Hexadecadienal, (Z, Z, Z, Z, Z) and (3,6,9,12,15)-Tricosapentene for Naval Orange Worm, NOW, moth affecting tree nuts, and (E, Z)-7,9-Docedadien-1-yl Acetate (European Grapevine, EVG, moth). Raman signatures were successfully extracted, and peaks were assigned to different vibration and rotational modes for each primary chemical. Notably, 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. The sensitivity of the system's ability to detect these chemicals at very low concentrations was evaluated. Starting with 100% pure concentrated chemicals, series of dilutions were performed, testing various concentrations down to 0.1%, in both ethanol and acetone. Similar trends and peaks were observed, but with different intensities, the solvent being the only difference. Characterization of singular signatures standalone and in mixtures is conducted using Principal Component Analysis (PCA) as a way to ease separation. Principal component analysis is a multivariate data analysis technique, which allows for the better understanding of numerous factors which affect the spectral variation across different samples. A new set of axes, or principal components, are aligned with the maximum directions of variance inside of a data set. Scores, loadings, and residuals become the new matrices which are created through his method of analysis. By using the principal component analysis technique complex Raman spectra were successfully interpreted by revealing compounds and differences between each sample.

Keywords: pheromone, Raman spectroscopy, principal component analysis

1. INTRODUCTION

1.1 Insect Pheromones

Insect pheromones have become the future of pest control because of their non-toxic and non-persistent properties. Semiochemicals are released by many different species as a means of intra- and interspecific communication[1]. Pheromones contain chemical compounds which are released by insects in order to attract mating partners across long distances in complete darkness, without the use of any audible signals. Synthetic forms of pheromones have been used in many cases as the essential component of monitoring and/or mating disruption of key pests of agricultural crops, specifically the navel orangeworm (Amyelois transitella) NOW in California tree nuts[2]. A very minimal quantity of synthetic pheromones is needed for both monitoring and mating disruption to attract or confuse males. There are many commercially available mating disruption products available for NOW. Recent studies have demonstrated that they can effectively reduce the damage to crops caused by NOW. However, mating disruption is not fully established due to the variation across different products and target species. A better understanding of how synthetic pheromones compete with natural pheromone is necessary. For example, in the case of monitoring, it's questionable how efficiently the insects follow the diffusing plumes, specifically across large blocks and at plot borders. Whether an aerosol puffer for mating disruption or lure used 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.

1.2 Raman Spectroscopy

Raman spectroscopy analyzes molecular vibrations and can provide high molecular specificity. This type of spectroscopy relies on the inelastic scattering of monochromatic light which comes from a laser source. When the monochromatic light penetrates a sample, some light inclines to scatter in the same frequency of the incident light, this is known as Rayleigh scattering. Some light that scatters in different frequencies is known as Raman scattering. The different in frequency

between the incident light and scattered light heavily depends on the vibrational frequency of the molecular bonds found in the sample[3]. Raman spectroscopy can offer a means to detect synthetic pheromone because of its analytical properties and recent technological advancements. In combination with nanostructured fiber probes, Raman spectroscopy has a demonstrated capability to detect volatiles at extremely low concentrations, such as pheromones[4]. By developing 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 can then be used in a basic experiment (i.e., "orchard in a box") to quantify pheromone diffusion in a very stable environment. In this work we present our research towards identifying the Raman signatures of key pheromone compounds and demonstrate the detecting capability in mixtures given the specificity of the optical spectroscopy approach.

The Raman system used, works in reflection, and comprises of a 785nm, 30mW power source with a built-spectrometer of approximately $10cm^{-1}$ resolution. The portable gun aims at the core of a built-in vial holder where the vials with the liquid pheromones can be set. This configuration offers the best focusing of the laser beam on the samples and thus best signal-to-noise ratio.

1.3 Principal Component Analysis

Chemometrics combines statistics and logic in order to design or select best measurement procedures and experiments. It allows for the extraction of maximum relevant chemical information by analyzing chemical data and helps in the understanding of chemical systems[5]. Principal component analysis, or PCA, is a multivariate data analysis tool that represents a multivariate data table as smaller set of variables or summary indices. This statistical analysis tool allows for the observation of trends and outliers in a data set and can help uncover correlations between observation and variables. The analysis helps reduce the dimensionality of a multi–dimensional data set comprising a large number of variables, while retaining those characteristics of the original data set that contribute most to its variance. PCA is an orthogonal linear transformation of the original data into a new basis with new coordinates (Principal Components – PCs) that are mutually orthogonal and ordered such that the first few variables exhibit the greatest amount of variation, and the remaining may be disregarded (thus allowing for a massive reduction of data – with no relevant loss in information)[6]. It provides a clear distinction of Raman signatures in mixed samples of pheromones, strengthening conclusions on the ability of Raman spectroscopy for detecting emissions in more realistic conditions.

2. EXPERIMENTAL METHODS

The following key pheromones NOW constituents[7] were obtained from Alpha Scents Inc: (Z,Z)-11,13-Hexadecadienal, (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene, and (E,Z)-7,9-Docedadien-1-yl Acetate. Concentration studies at 100%, 70%, 50%, 25%, 10%, 5%, 1%, 0.5%, 0.3%, and 0.1% were performed on the individual pheromones. Both ethanol and acetone were used separately to dilute the pheromones. Synthetic pheromone samples used for the principal component analysis were diluted with acetone down to 50% and 25% concentrations. Mixtures were then created of the various synthetic pheromone concentrations (Table 1). 100% (Z,Z)-11,13-Hexadecadienal was mixed with 100% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene. This mixture was then further diluted to 50%. 100% (Z,Z)-11,13-Hexadecadienal was mixed with 100% (E,Z)-7,9-Docedadien-1-yl Acetate. This mixture was then further diluted to 50%. The 50% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene was mixed with 50% (E,Z)-7,9-Docedadien-1-yl Acetate. Lastly, the mixture of 100% (Z,Z)-11,13-Hexadecadienal and 100% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene was combined with the mixture of 100% (Z,Z)-11,13-Hexadecadienal and 100% (E,Z)-7,9-Docedadien-1-yl Acetate. Raman spectroscopy was conducted using the DeltaNu's Inspector Raman Hand-Held Field Analysis Spectrometer with a 120mW 785nm laser. Raman spectra were obtained on all of the pure samples, dilutions, and mixtures. Spectra were analyzed using Thermo Fisher Scientific's GRAMS AI software.

3. RESULTS AND DISCUSSION

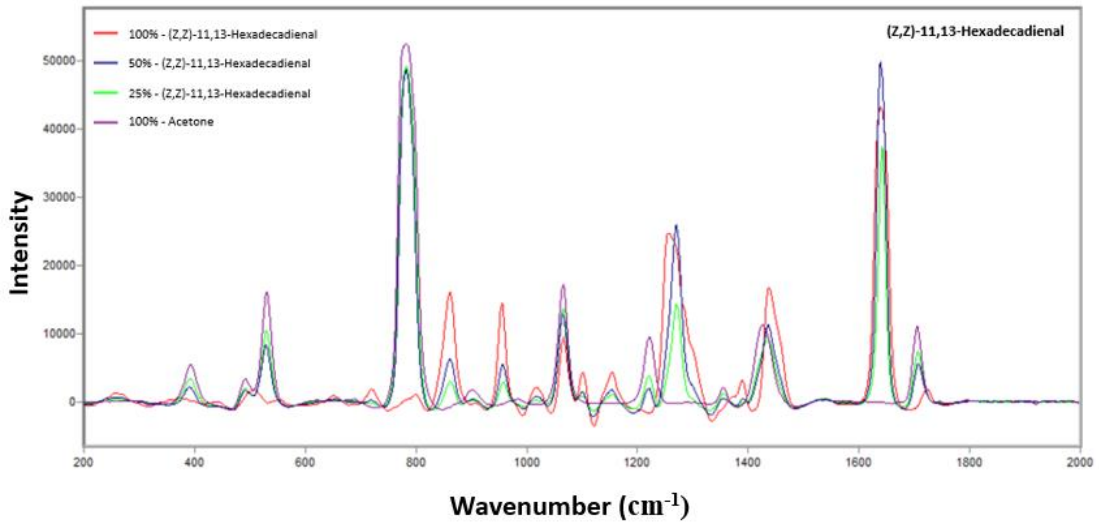
3.1 Raman Spectroscopy

Chemical signatures acquired of the synthetic pheromones: (Z,Z)-11,13-Hexadecadienal, (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene, and (E,Z)-7,9-Docedadien-1-yl Acetate show that Raman spectroscopy has the capability to detect and differentiate between pheromones (Figure 1). The dilutions are clearly visible in the Raman spectrum of each synthetic pheromone. As the pheromone concentration decreases, the significant peaks associated with the acetone increases and those associated with the synthetic pheromones decrease. The intensity of the peaks can be seen decreasing from 100%, 50%, and then to 25%. Raman spectroscopy shows that the dilutions were made accurately. Some peaks overlap with the

acetone peaks, causing additional variability in the peak intensities. Overall, it's very clear which peaks correspond to each pheromone, and which correspond to the acetone. Where peaks overlap a weighted analysis can also be exploited for extracting more information.

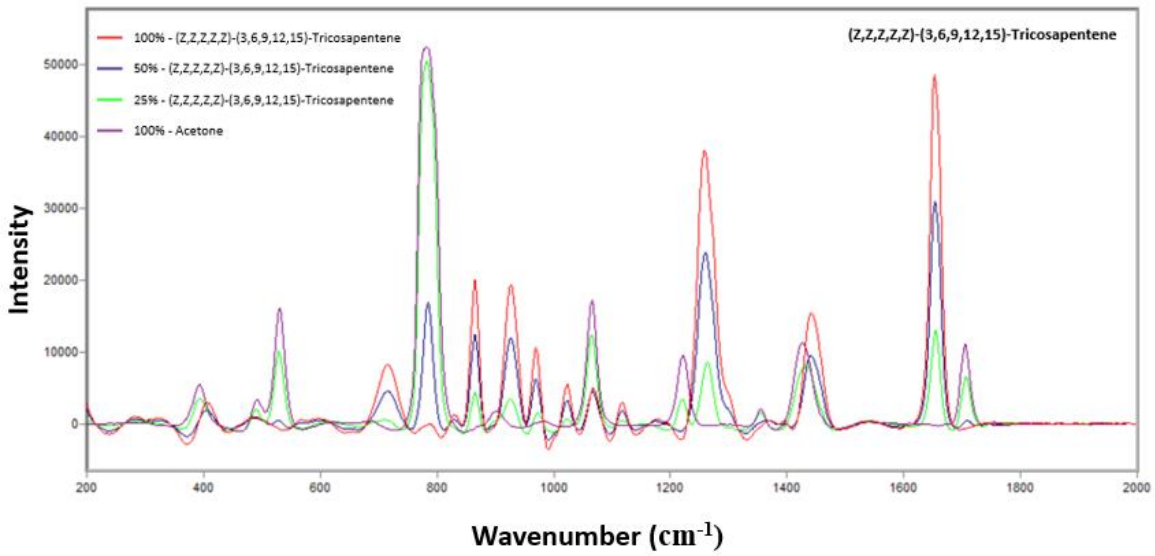
Table 1: Dilutions and Mixtures of synthetic pheromones.

	Pheromone	Pheromone Volume (µ)	Acetone Volume (µ)	Total Volume (µ)
100T	100% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene	300	0	300
50T	50% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene	150	150	300
25T	25% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene	75	225	300
100H	100% (Z,Z)-11,13-Hexadecadienal	300	0	300
50H	50% (Z,Z)-11,13-Hexadecadienal	150	150	300
25H	25% (Z,Z)-11,13-Hexadecadienal	75	225	300
100D	100% (E,Z)-7,9-Docedadien-1-yl Acetate	300	0	300
50D	50% (E,Z)-7,9-Docedadien-1-yl Acetate	150	150	300
25D	25% (E,Z)-7,9-Docedadien-1-yl Acetate	75	225	300
H+D	25% (Z,Z)-11,13-Hexadecadienal + 50% (E,Z)-7,9-Docedadien-1-yl Acetate			
(H+D)50	25% (Z,Z)-11,13-Hexadecadienal + 50% (E,Z)-7,9-Docedadien-1-yl Acetate			
H+T	25% (Z,Z)-11,13-Hexadecadienal + 50% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene	150ul of 25% H and 150ul of 50% T		
(H+T)50	25% (Z,Z)-11,13-Hexadecadienal + 50% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene	150ul of H+T and 150ul of 100% Acetone		
50T+50D	50% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene + 50% (E,Z)-7,9-Docedadien-1-yl Acetate	150ul of 50% T and 150ul of 50% D		
HT+HD	25% (Z,Z)-11,13-Hexadecadienal + 50% (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene + 25% (Z,Z)-11,13-Hexadecadienal + 50% (E,Z)-7,9-Docedadien-1-yl Acetate	150ul of HT and 150ul of HD		
100A	100% Acetone			



a.

b.



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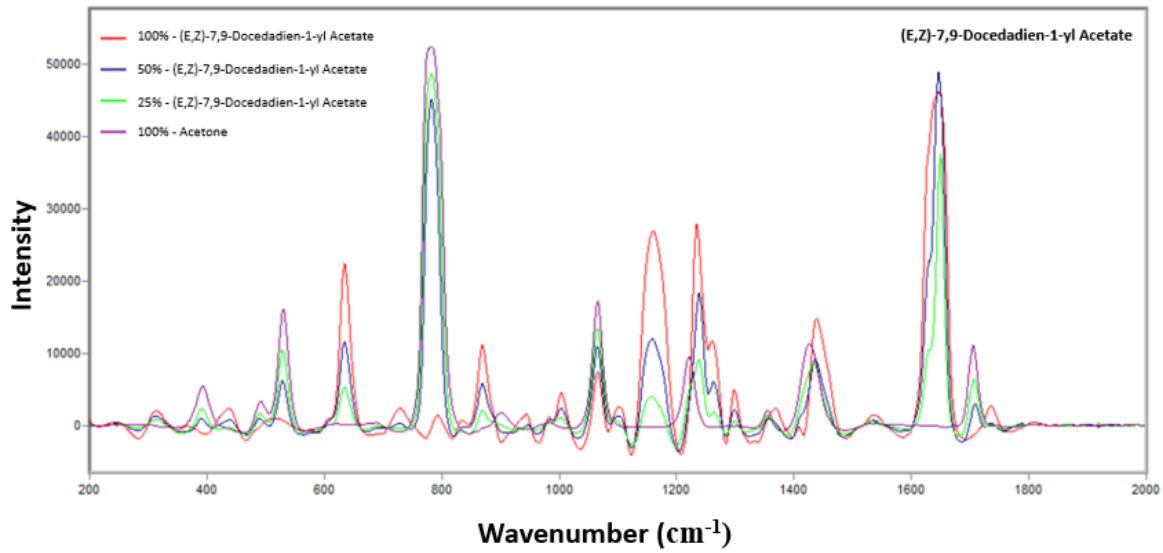

Figure 1: Raman spectra of (a) (Z,Z)-11,13-Hexadecadienal, (b) (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene, and (c) (E,Z)-7,9-Docedadien-1-yl Acetate at 100%, 50%, and 25% concentrations along with 100% acetone.

3.2 Concentration Studies

Sensitivity analysis was performed between 0.1 and 100% concentrations in different solvents (Figure 2). Series of dilutions were made. Various concentrations were tested down to 0.1%, in both ethanol and acetone. Similar trends and peaks were observed, but with different intensities, the solvent being the only difference. Five Raman measurements were taken of each concentration and then averaged. It was demonstrated that a large dynamic range can be covered, potentially expanding to lower than 0.1% concentration. Principal component analysis can further deconvolve the solvent contributions.

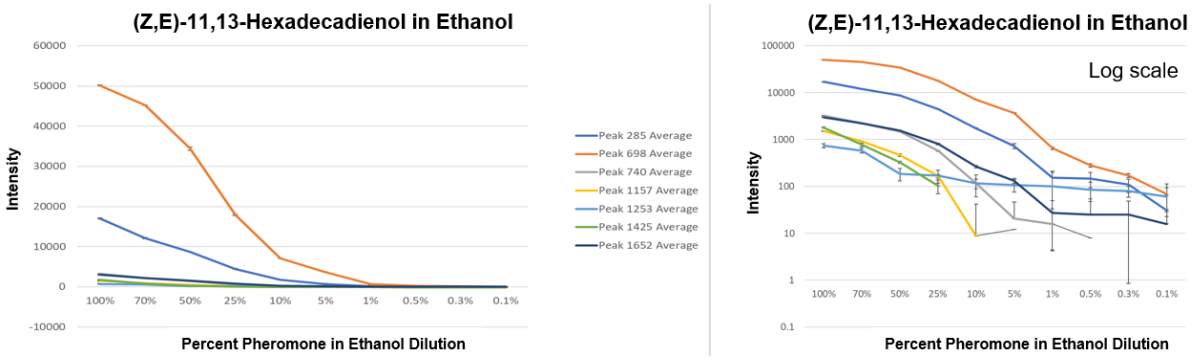

Figure 2: Trending Raman signature peaks of (Z,E)-11,13-Hexadecadienol with concentration.

3.3 Principal Component Analysis

Specificity of Raman signatures with principal component analysis is a very powerful tool to identify compounds. The analysis of principal components involves the transformation of possibly correlated variables into uncorrelated variables. This will reduce the dimensionality of the sample data and at the same time maintain their variability[8]. Raman signatures were measured for pheromones basic components, standalone, and in mixtures, exploiting multivariate analysis tools. Each mixed sample falls in the areas between the pure synthetic pheromones, closer to one or another depending on the dilutions and mixtures. Starting with 100% of the synthetic pheromone, the samples get into closer proximity of the acetone, due the increase in the amount of acetone in those samples (Figure 3). The 50% samples are found to be in between both the acetone and their 100% counterparts. As the concentrations of the samples decreases, they are found to be closer to the acetone. This multivariate analysis tool allows for the better discrimination of not only diluted samples but mixed samples as well. When principal component analysis is accurately and successfully completed, the first few principal components or PCs explain most of the variance found in the data[8]. By analyzing mixtures, it's possible to improve multivariate approaches for enhancing specificity. This database of mixtures and pure samples produces a calibration of semiochemicals via principal component analysis that can eventually help deconvolve unknown mixtures.

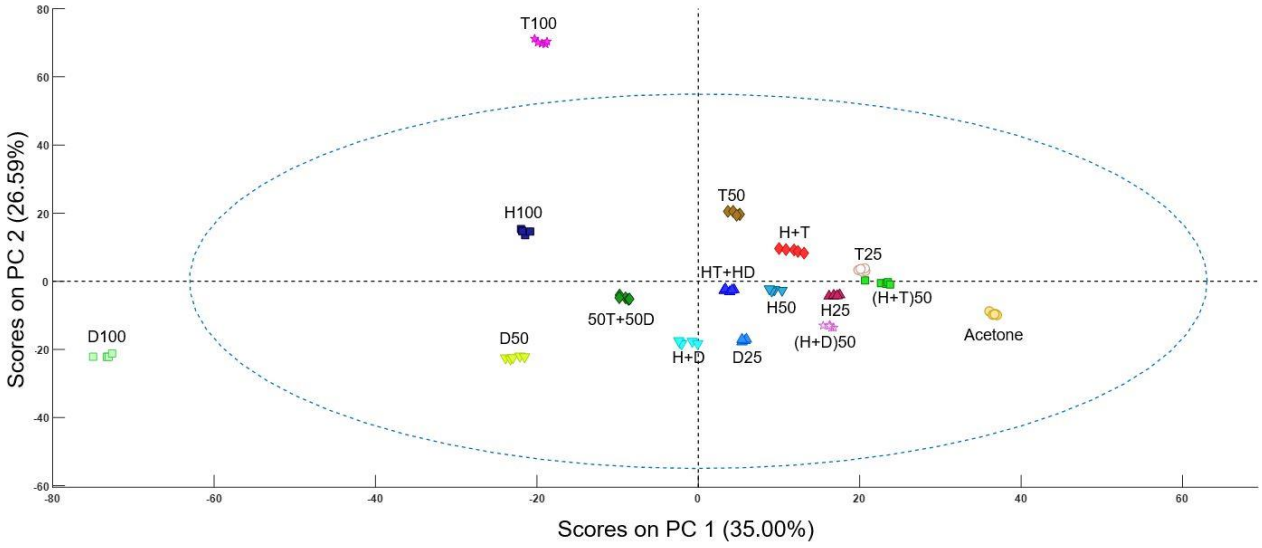

Figure 3: Principal component analysis plot of all pure samples, dilutions, and mixtures.

The principal component analysis is further improved with hierarchal delineation and can be used to calibrate for the deconvolution of unknown detected profiles (Figure 4). Cluster analysis is used as a method to organize samples into a dendrogram. The samples with the greatest correlations are put together in a group and the samples with the least correlations have a wider separation. More specifically, two samples which have the greatest correlation are merged into a single synthetic sample. Then the samples which are left are searched for the largest correlation with the previous synthetic sample. This process is repeated until all of the samples have merged into a single sample. These determined correlations among samples are then articulated as a hierarchical tree. It's preferential to use cluster analysis because when the groups are created, the variance among all of the sample dataset is considered. Whereas in principal component analysis, only 60-90% variance is represented by the first few principal components[9]. The clusters of the principal component analysis plot are visually represented in a dendrogram. The dendrogram shows the hierarchical relationship between the pure samples, dilutions, mixtures, and acetone. It is created as an output from hierarchical clustering. The dendrogram easily allocates the samples into clusters to observe the data in a more organized way. The variance weighted distance between the cluster centers decreases for samples that are mixtures and those that are diluted with acetone compared to their 100% pure samples. The clusters of sample mixtures and dilutions are much closer to each other due to the addition of acetone. This dendrogram depicts a clear relationship between the samples.

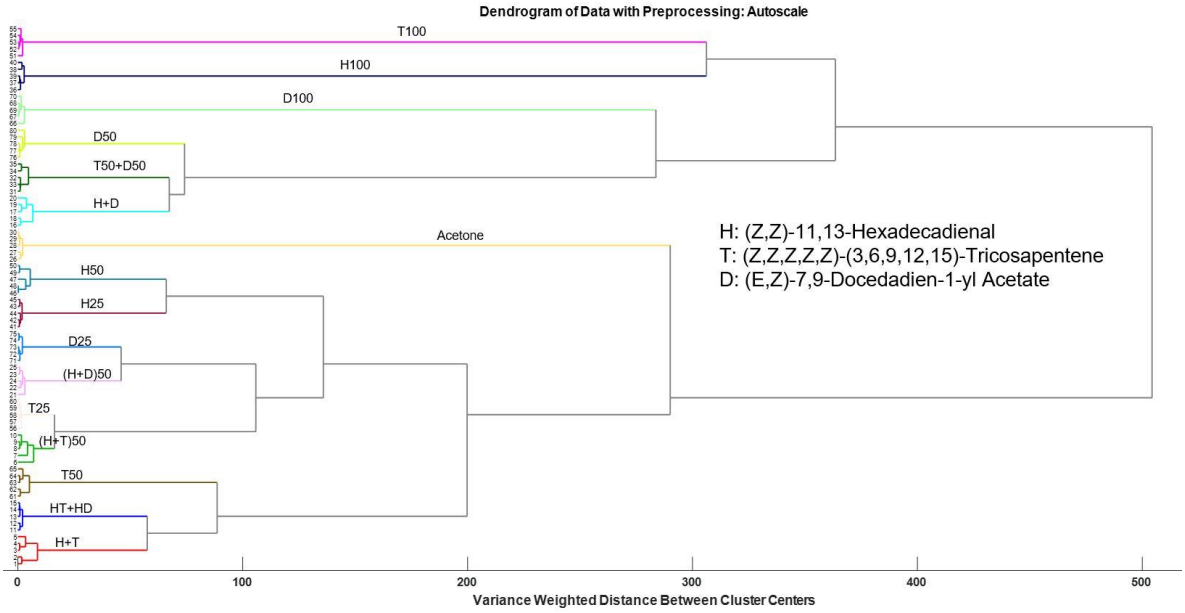

Figure 4: Dendrogram of all pure samples, dilutions, and mixtures.

4. CONCLUSION

The use of synthetic forms of key compounds have 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[10]. The characterization of these samples using Raman Spectroscopy in combination with principal component analysis has successfully identified Raman signatures of synthetic pheromones. Both of these tools together can allow for the identification of unknown compounds found in pheromones of moths. By developing Raman spectroscopy for monitoring the navel orangeworm population development it can allow for the optimization of trap distribution based on predicted and actual diffusion and can also map moth movements to in-situ concentration measurements. This method validates an enhanced Raman approach as a tool for pheromone emission profiles measurements. This study creates a pathway to demonstrate Raman spectroscopy to detect synthetic pheromone compounds emitted by trap lures and mating disruption products, as well as the natural pheromone emitted from navel orangeworm adults.

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