FungiSense

Agrichemical Remote Sensing to Improve Sustainable Grape Disease Management

Katie Gold (PPPMB)
Yu Jiang (Horticulture), Lance Cadle-Davidson (USDA-ARS GGRU).

Overview: With the pervasive dominance of technology in our daily lives, it has not yet been fully adopted and integrated into daily viticulture practices. Fungicides play a vital role in New York grape production, yet their overuse carries financial burdens and risks of resistance. If New York growers could monitor fungicide activity non-destructively, they could reduce usage and costs through more targeted applications and extended intervals between treatments. Hyperspectral imaging (HSI), merging imaging and spectroscopy, offers a promising approach by capturing and analyzing a wide spectrum of information from various environments. It utilizes wavelengths ranging from the visible to the shortwave infrared (VSWIR, 400–2,400 nm). Each pixel in a hyperspectral image contains a wide spectrum of data based on the camera's specifications, allowing for precise identification and analysis of an object's physicochemical composition through their unique spectral signatures. The lab's previous postdoc, Dr. Nikita Gambir, employed handheld hyperspectral spectroscopy to detect commercial formulations of metrafenone, Bacillus mycoides isolate J (BmJ), and sulfur on Chardonnay grapevines in vineyard or greenhouse settings. The results demonstrated that the treatments could be differentiated from the untreated control with an accuracy of 73.06% for metrafenone, 67.76% for BmJ, and 94.10% for sulfur, as published in the Journal of Phytopathology (2024) in an article titled "Non-Destructive Monitoring of Foliar Fungicide Efficacy with Hyperspectral Sensing in Grapevine." While these preliminary results were promising for the detection of fungicides using hyperspectral spectroscopy, achieving a high-accuracy model for any fungicide requires large, highly precise, and high-resolution HSI data to train machine learning- or deep learning-based algorithms. Consequently, in 2023, we shifted the focus of our study to develop a robot designed to automate the collection of high-resolution HSI data in a controlled laboratory environment. We developed an automated hyperspectral sensing system named Hyperbird which can scan 351 sample in 4 hours which enables us developing fungicide quantification models. We evaluated the robot on sulfur treated leaves and able to differentiate untreated leaves from sulfur treated leaves with AUC of 100%.

The Project advancement in 2023*:*

The objective of this project was the detection and quantification of fungicides on grapevine leaves in a non-destructive way, aiming to assist farmers with the optimal timing for fungicide application. A state-of-the-art study by a former postdoc of our lab, Gambir et al. (2024), demonstrated that hyperspectral sensing can accurately differentiate leaves treated with sulfur, metrafenone, and BmJ, achieving accuracies of 97.85%, 73.06%, and 94.10%, respectively. While their findings confirm that hyperspectral sensing can detect fungicides on grapevine leaves, they also highlight the necessity for developing specific machine learning models for each fungicide to be effectively applied in the field. Developing these models requires a significant amount of high-resolution data. Consequently, the primary focus in 2023 was on the development of an automated hyperspectral sensing system. This advancement is crucial for accelerating the model development process for different fungicides, thereby facilitating the quicker adoption of this technology in the field.

HyperBird, The automotive hyperspectral imaging system.

The HyperBird is a custom robotic stage designed for hyperspectral line-scan imaging of 1cm leaf disc samples, which captures spectral data to generate hypercube images. This device features a mobile platform that fits a custom-designed 320 x 260 x 20 mm acrylic sample tray, capable of holding up to 351 samples. It operates along the X, Y, and Z axes to switch between samples and focus, using a fixed camera setup. The imaging is performed by the MSV-500 spectral camera from Middleton Spectral Vision, with a spectral range of 400 to 1000 nm across 931 bins and a spatial resolution of 2100 pixels across 13.65 mm. The camera's capabilities are enhanced by an optical setup with a 1.2x magnification rate, offering a field of view of 11.5mm and a 57 mm working distance, where each pixel covers about 5.5 μm^2 of the sample area.
Critical to the setup is a custom cold LED light source comprising two EFFI-Flex-HIS-X2 lights by Effilux, emitting a balanced light spectrum suitable for the camera's sensor. These lights, equipped with HSI, IR 910, and IR 970 LEDs, project light in a thin line shape optimal for line-scan imaging. The imaging process utilizes 4x spectral binning to condense the camera's spectral bins from 931 to 233, enhancing the resolution to 2.58 nm per bin. The camera operates in a 16-bit low-noise mode at a 50 frames per second rate, thanks to a 20 ms exposure time and a global shutter mode, ensuring minimal overhead and consistent sample imaging. The hyperbird produces 2.5 GB hyperspectral cube with 233 bands, measuring 22092196 pixels for each sample. An in house python scrip thas been prepared to process the samples to segment the leaf disk from background and generates a CSV file compiling spectral averages across all bands for downstream analysis (**Fig 1**).

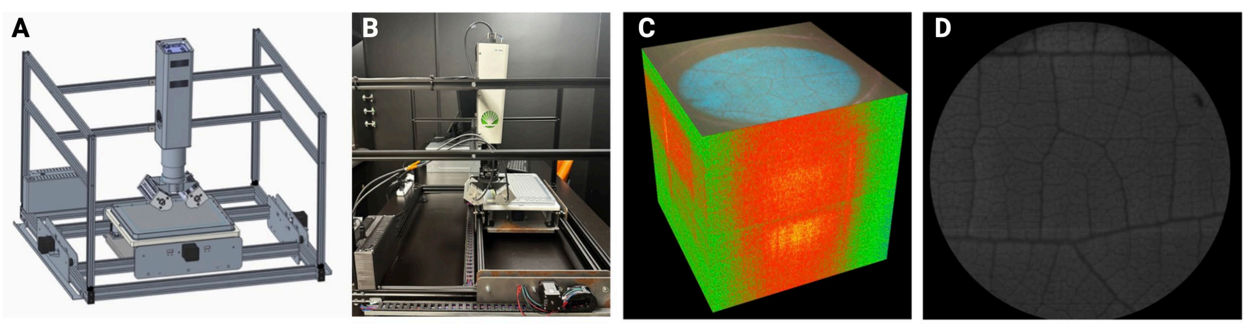

Figure 1. A, scheme of HyperBird structure. B, Reasl photo of HyperBird Structure of HyperBird consisting of a Middleton Spectral Vision MSV-500 camera with a spectral range of 400-1000 nm, optics for magnification, and a customized LED-based light source for balanced high intensity illumination. The platform scans 1cm leaf discs on a 351 well tray with the rate of 40s/sample, generating hypercube spectral images. C, a raw hyperspectral cube without segmenting the leaf disk. D, the segmented leaf disk at band 880 nm.

To explore the potential applications of Hyperbird, an experiment was conducted on 'Chardonnay' leaves treated three different fungicides including Flint, Quadris and microthiol. By the writing of the report we have analyzed just data fro microthiol which the report will be focused on. Leaves treated with different concentrations of microthiol (sulfur) including zero, low (10g/L), medium (20g/L), and high (40g/L), all mixed with Silwet L-17 (2.5ml/L). After being submerged to ensure saturation, leaves were processed post four hours; two 8mm leaf discs were cut from each using a cork borer and placed in a 351-well tray with 0.7% water agar, with each well, being 1 cm in diameter (**Fig 2**).

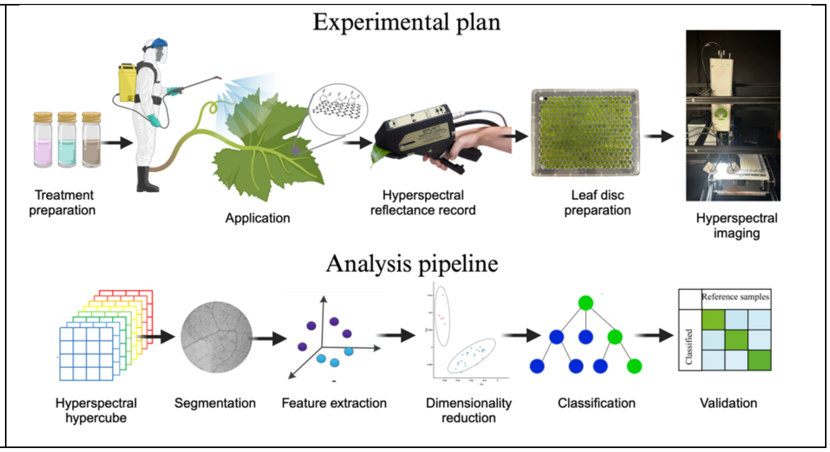

Figure 2. The figure shows the brief pipeline of the applying different fungicides to the leaves and loading in the HyperBird for data collection. The second row is the data analysis path starting with segmenting leaf disk from hyperspectral cube and extracting the feature for model preparation.

The collected images were processed into numerical data and used for analysis involved principal component (PC) analysis, revealing a significant variance in spectral data with PC1 accounting for 93.05% and PC2 for 6.40%, indicating a clear differentiation in the data pattern, especially noticeable in the PCA plot where control, low, medium, and high sulfur-treated samples showed distinct groupings. Further analysis using Permutational Multivariate Analysis of Variance (PERMANOVA) on microthiol treated samples distinguished by sulfur treatment levels showed significant differences in leaf spectral properties (pseudo-F = 14.261084, p = 0.001, with 999 permutations). Post hoc comparisons highlighted significant disparities among the groups, notably between control and treated groups, with varying degrees of significance. The analysis evidenced substantial impact of sulfur concentration on spectral characteristics, though medium and high-sulfur treatments did not significantly differ from each other (pseudo-F = 0.17, corrected p = 1.000), underlining the nuanced effects of sulfur treatment on leaf properties (**Fig 3**).

We grouped med-sulfur and high-sulfur samples together due to insignificant differences, forming two groups for modeling. Using a random forest model, we achieved a 100% precision and AUC with 66.7% recall in differentiating control from sulfur-treated groups. A comparison of low-sulfur with med/high-sulfur groups resulted in 66.7% precision, 80% recall, and 90% AUC. We identified the top 10 discriminative bands for both control vs. sulfur-treated (758, 789, 768, 881, 950, 942, 955, 771, 760, 792 nm) and low-sulfur vs. med/high-sulfur treatments (979, 771, 950, 942, 958, 974, 953, 776, 758, 768 nm), with bands 768, 771, 942, 950, and 758 nm being crucial for both models.

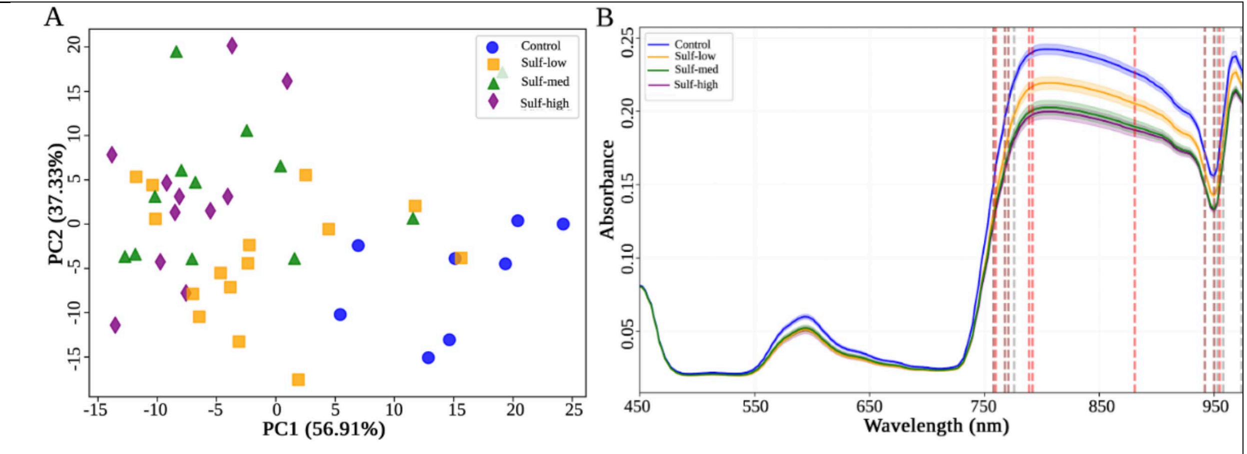

Figure 3. A, PCA indicates clear separation between control samples and sulfur-treated ones, with leaves exposed to medium and high sulfur concentrations merging. In contrast, low sulfur treatments are distinctly separated, supported by PERMANOVA. B, Spectral analysis, using a random forest model, identifies red vertical lines as crucial for differentiating control from sulfur-treated leaves, and gray lines for distinguishing low-sulfur treatments from others.

Our plan for 2024. Over the past two years, we have demonstrated the applicability of hyperspectral sensing for the detection and quantification of fungicides. Subsequently, we developed an automated robot, enabling us to collect high-resolution hyperspectral data to develop models for various fungicides. In 2024, our first task will be to prepare a manuscript introducing HyperBird to the scientific community as a unique hyperspectral imaging robot. This tool aims to facilitate collaboration among scientists.

Our second focus will be on analyzing data collected from Quadris and Flint treated samples to measure HyperBird's capability in estimating their concentration. Concurrently, we are preparing and measuring the actual concentration of fungicides on leaves using mass spectrometry analysis. This measurement will aid in developing models not only to classify fungicide-treated and untreated leaves but also to quantify the concentrations of fungicides on the leaves.

Fungicides exhibit the highest concentration at the time of application, and this concentration gradually decreases to the minimum inhibitory concentration (MIC), below which a fungicide no longer provides full protection against pathogens. The duration before reaching the MIC varies based on environmental factors such as rain, humidity, and sunlight radiation. The optimal time for reapplication is just before the concentration falls below the MIC, assuming the presence of a conducive environment for the pathogen. Due to the variability in degradation rates, the ability to detect low concentrations, as demonstrated by our study, could lead to reduced pesticide usage. Another goal for this year's study is to identify the minimum inhibitory concentration and the minimum detectable concentration by HyperBird, and to compare them. These data points for each fungicide will assist us in developing a new model to advise farmers on the optimal timing for fungicide application.