FungiSense

Agrichemical Remote Sensing to Improve Sustainable Grape Disease Management

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Overview: While the development of digital viticulture tools has been growing, advances in digital disease management have lagged. Fungicides are crucial tools for NY grape production, but overuse has environmental, financial, and pathogen resistance consequences. If NY grape growers had a way to non-destructively monitor for plant protection status via in-field fungicide activity, they could reduce fungicide usage and expenditure by maximizing product and lengthening application intervals. Imaging spectroscopy (aka hyperspectral sensing) can quantify chemistry in soil, rock, and vegetation based on the way light interacts with chemical bonds. In 2020 & 2021, we conducted a series of spectral campaigns whose preliminary findings affirmed our hypothesis that the principles that allow us to quantify environmental chemistry with imaging spectroscopy transfer to synthetic fungicide detection. The overarching goal of our Venture funded work is to assess the scalability of fungicide detection with hyperspectral sensing. We hypothesized that we could quantify fungicide activity in vineyards based on the interaction of light with surface residue and chemical bonds. In 2022, Venture funding allowed us to conduct a more thorough analysis of these data, including a direct comparison between fungicide prediction to hyperspectral. These findings were written up into a manuscript entitled, “Non-destructive monitoring of foliar fungicide efficacy with hyperspectral sensing,” and submitted to an APS society journal *Phytofrontiers* special issue on plant disease detection (Gambir et al, *accepted).* Before leaving the Gold Lab to take on a Senior Scientist role at FMC, postdoc Dr. Nikita Gambhir presented on this work at APS Plant Health conference 2022 and was awarded an honorable mention for the APS Schroth Face of the Future Award. Recently, Dr. Saeed Hosseinzadeh joined the Gold Lab as a postdoc and has taken leadership of this project. We are excited for him to bring his expertise in multi-omic approaches and enthusiasm for digital agriculture to this project and summarize his intended research focus herein.

Findings from Gambhir et al. *accepted:* The goal of this research was to examine if hyperspectral sensing in the visible to shortwave infrared range (VSWIR, 400–2,400 nm) could be used to monitor synthetic and biofungicide efficacy in grapevine over time. Commercial formulations of metrafenone, *Bacillus mycoides* isolate J *(BmJ),* and sulfur were applied on Chardonnay grapevines in vineyard or greenhouse settings. Foliar reflectance was measured with handheld hyperspectral spectroradiometers at multiple days post application. Fungicide efficacy was estimated as a proxy for fungicide residue and disease control measured with the Blackbird microscopy imaging robot. We found that spectra of untreated and fungicide-treated leaves could be differentiated with moderate to high accuracy, with the highest accuracy being 97.85% for sulfur treated leaves at 2 DPA. 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. The spectral regions important for discriminating treatments were different for different fungicides, indicating that their chemical fingerprints could be detected using spectroscopy. Fungicide efficacy could be predicted as a function of disease severity for metrafenone (R^2 = 0.38) and as a function of disease severity and residue concentration for sulfur with moderate accuracy (R^2 = 0.36 and R^2 = 0.42 respectively). Generally, we found prediction accuracy of fungicide efficacy to be the highest at the earliest timepoints (R^2 = 0.54). *BmJ* treatment impacted foliar physiology by enhancing leaf mass/area and reducing nitrogen and total phenolic content as estimated from spectral reflectance. *Bmj* controls disease by inducing plant defenses. Plant physiological response as estimated by spectral data corroborated that the *BmJ*-treated plants were reallocating resources to defense responses. Overall, our results suggest that spectroscopy can be used to monitor fungicide efficacy in grapevine; however, prediction accuracy depends on the fungicide and the time of assessment. Results suggest that hyperspectral sensing can be used to monitor in-situ fungicide efficacy and prediction accuracy depends on the fungicide and the time-point measured. The ability to monitor in-situ fungicide efficacy could facilitate more strategic fungicide applications and promote sustainable grapevine protection.

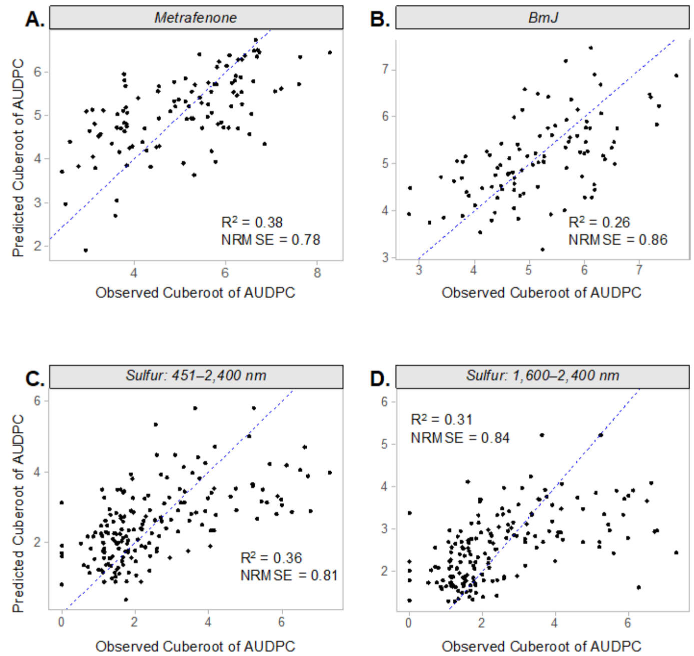
Table 1: Differentiation among spectra of fungicide treated vs untreated leaves at 2 to 10 days post-application (DPA) as estimated by Permutational Multivariate Analysis of Variance (PERMANOVA) and Partial Least Square Discriminant Analysis (PLS-DA).

Fungicide	**DPA**	**n**	**PERMANOVA *p-value***	**PLS-DA**		
				Accuracy (%)	**Kappa**	**Comp.**
Metrafenone	2	159	0.46	72.91	0.46	11
	5	153	**0.001**[a]	77.47	0.55	13
	7	160	**0.017**	71.79	0.44	8
	All	472	**0.007**	73.06	0.46	15
Bacillus mycoides isolateJ *(BmJ)*	2	158	0.47	69.99	0.39	9
	5	157	**0.001**	71.69	0.43	8
	7	156	**0.001**	72.14	0.44	7
	All	471	**0.002**	67.76	0.35	8
Sulfur[b]	3	120	0.18	97.85	0.94	12
	6	118	0.46	95.22	0.86	15
	10	118	**0.001**	95.73	0.89	13
	All	356	**0.001**	94.10	0.84	16
Sulfur[b] (Spectra above 1,600 nm only)	3	120	0.12	93.73	0.82	9
	6	118	0.43	90.55	0.73	10
	10	118	**0.001**	92.92	0.81	10
	All	356	**0.001**	88.42	0.67	11

[a]*p-values* in bold are significant at α = 0.05

[b]Sulfur experiments were performed on 3, 6, 10 DPA instead of 2, 5, 7 DPA so that experiments are performed on weekdays

Fig. 1. Partial Least Square Regression (PLS-R) between cuberoot of Area Under the Disease Progress Curve (AUDPC) and spectral reflectance for A) Metrafenone, B) *Bacillus mycoides* isolate J *(BmJ)*, and Kernel PLS-R between cuberoot of AUDPC and spectral reflectance for C) Sulfur using 451-2,400 nm wavelength range and D) Sulfur using 1,600-2,400 nm wavelength range. The R^2 and Normalized Root Mean Square Error (RMSE) values for the PLS-R models are mentioned on the plots. The blue dashed line is a reference line with slope = 1.



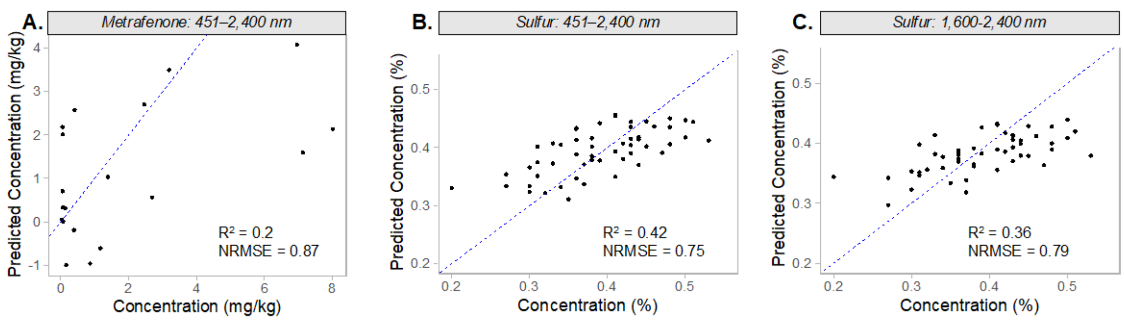


Fig. 2. Partial Least Square Regression (PLS-R) between fungicide concentration and spectral reflectance for A) Metrafenone (accuracy impeded by a 0-heavy dataset and low sample number); B) Sulfur using 451-2,400 nm wavelength range; and C) Sulfur using 1,600-2,400 nm wavelength range. The R^2 and Normalized Root Mean Square Error (NRMSE) values for the PLS-R models are mentioned on the lower-right corner. The blue dashed line is a reference line with slope = 1.

Strategic Re-Directioning: For the 2023 season we are re-strategizing our scientific approach to focus on lab-based sensing for fungicide model optimization in anticipation of eventual field development with our Mjolnir hyperspectral drone. The covid-19 pandemic has had wide reaching impacts on technology manufacturing across all sectors. The UAV base to our Mjolnir sensor was unfortunately impacted by this. We discovered a critical malfunction in our UAV during research activities associated with this project in the 2022 season that required return to manufacturer to fix. These repairs are underway, and we are optimistic for their completion in time for 2023 field deployments. However, we cannot yet say with certainty when we will be able to deploy. Because of this, we are refocusing our efforts on model optimization with the fully functional hyperbird so that necessary progress can be made while we wait for our base to be calibrated and certified.

Dr. Hosseinzadeh's Plans for 2023: Our objectives for the 2023 season are to 1) collect foliar samples of grapevines treated with sulfur and/or strobilurin fungicides for assessment with handheld spectrometers and the hyperbird. We will validate fungicide concentration RP-C; 2) Analyze reflectance data and hyperbird imagery with the machine learning method Partial Least Squares Regression (PLSR) to evaluate how accurately multiple fungicides can be predicted from these data; and 3) use these hyperbird models to compare our estimates of fungicide longevity to labeled rates.

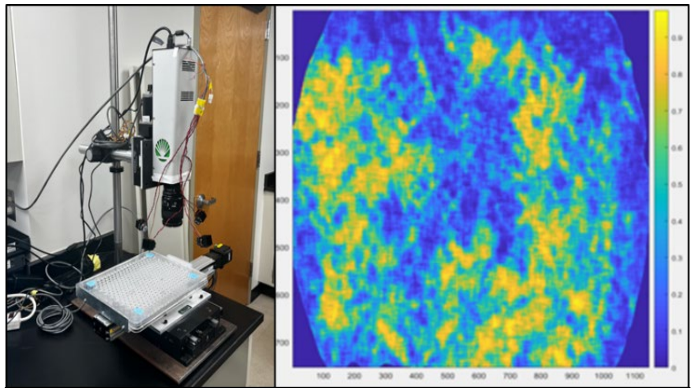


Fig. 3- (Left) The automated hyperspectral system can produce images at a 224x224 pixel resolution. (Right) An example image of powdery mildew detection from preliminary investigations.

The HYPERBIRD (**Fig 3**) is a hyperspectral imaging system based on a Cartesian robotic arm that integrates a line-scan VNIR hyperspectral camera (MSV500, Middleton Spectral Vision) with magnification, illumination, and an embedded computer. The custom imaging module can acquire line images at 200 frames per second, equivalent to 20 seconds per 10-mm leaf disk sample. We will conduct controlled studies to build foliar models for spectral detection of sulfur and strobilurin-class fungicides. Grapevine leaves will be spot inoculated with known quantities and volumes of fungicide (Warneke et al. 2021), processed into leaf discs, and monitored up to 21 days with the hyperbird (**Fig. 4**). Leaf disks will be sampled from varying distances from the point of fungicide deposition to examine systemic movement and dilution of fungicides. We will normalize to control, untreated leaf discs to account for foliar degradation over time. We will experiment with combination treatments of fungicide plus inoculation with *E. necator* populations of varying fungicide resistance backgrounds to assess how spectral profiles of fungicide-treated leaves changes in the presence of active infections. Infected leaf disc assays will be of shorter duration (~14 days) due to PM-induced foliar degradation. To measure true fungicide concentration, a sub-sample of monitored leaf discs over the course of the study will be freeze-dried, weighed, and then to be sent to AGQ Labs for reversed-phase chromatography to measure residue (Camino-Sanchez et al. 2011, Restrepo et al. 2014). We will employ vetted analytical techniques from Gambhir et al *in review* as well as explore alternate machine and deep learning-based approaches in collaboration with co-PI Yu Jiang.

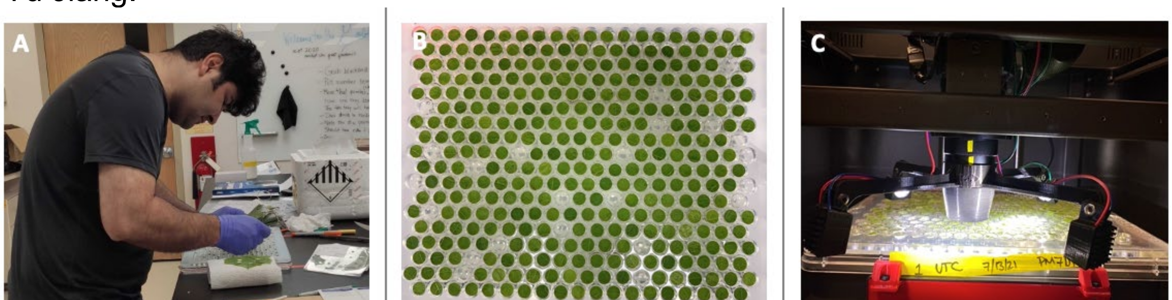


Figure 4: Quantifying both foliar fungicide concentration and disease control efficacy are essential for effective plant protection monitoring. Samples will be collected and processed into leaf discs by key personnel Dr. Saeed Hosseinzadeh (A), inoculated (B), and then imaged with either or both the Hyperbird and Blackbird imaging robots (C) over 9-11 days. A subset of leaf discs are preserved for precise fungicide concentration measurement.

Alignment with NASA Acres: In 2022, PI Gold and co-PI Jiang joined NASA Acres, a new NASA agriculture consortium dedicated to domestic food security. As part of this program, Gold and Jiang will build a proof of practice grape disease detection system for use with the forthcoming satellite Surface Biology and Geology (**Fig. 5**) based cross-scale hyperspectral imagery and powered by AI. Continued funding from the Venture Research Fund will allow us to continue our foundational research on fungicide activity sensing with so that we may one day build a second functionality for fungicide monitoring into this system. If grape growers could monitor both disease incidence *and* fungicide coverage in their vineyards, they could make more strategic and data driven decisions about crop protection, such as maximizing interval length, strategizing spray timing after weather events, and applying curative fungicides only when and where most needed.

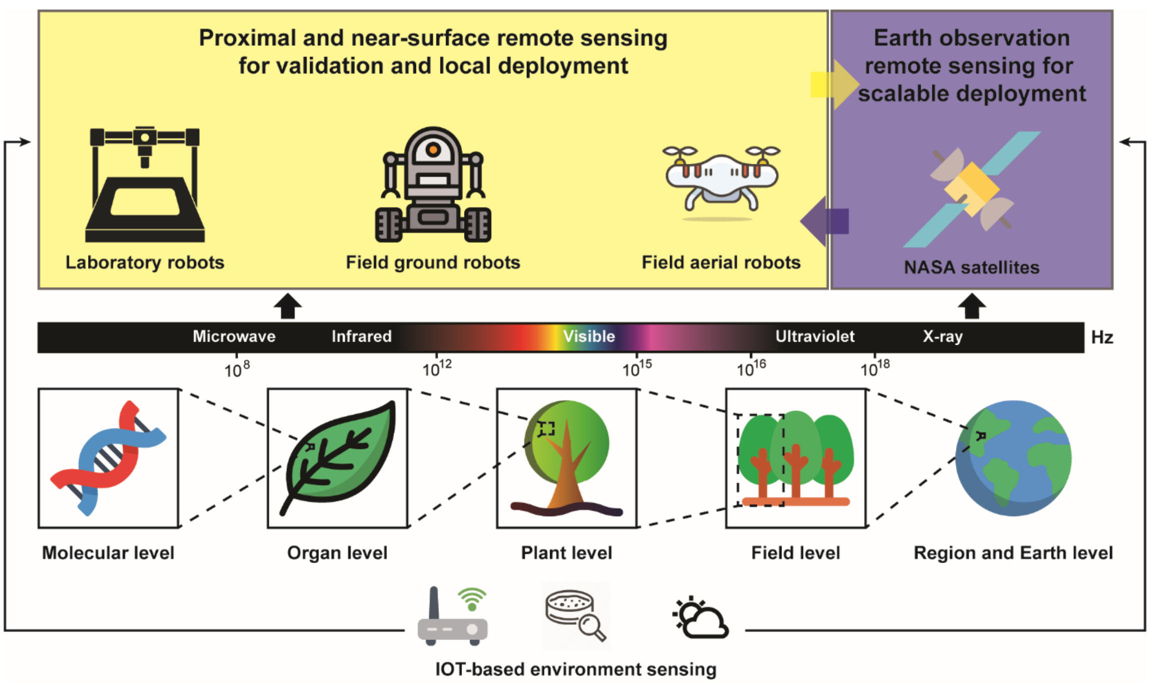


Figure 5: Diagram of the disease detection system being developed by Gold and Jiang as part of NASA Acres. This system will use proximal and near-surface hyperspectral sensing to calibrate and validate of spaceborne disease detection models.