Title: Remote Hyperspectral Imaging of Grapevine Leafroll-associated Virus 3 in Cabernet Sauvignon
Vineyards

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Abstract

Grapevine leafroll disease (GLD) is considered to be one of the most economically destructive viral diseases of grapevines worldwide, resulting in reduced vine vigor, yield reductions, and poor fruit quality. Five pathogens (grapevine leafroll-associated viruses) are recognized as causal agents of this disease, of which *Grapevine leafroll-associated virus 3* (GLRaV-3) is the most common. Although there is no cure for the disease, management strategies (including vigilant removal of infected vines) can drastically limit its spread and economic impact. Comprehensive mapping (total population sampling) of disease presence on the ground is cumbersome and cost prohibitive in many situations. In addition, the practice of identifying diseased vines requires individuals to be highly trained to recognize symptoms. Compared to more traditional detection methods, airborne hyperspectral imaging offers a potentially valuable alternative for monitoring the disease that is cost effective, reliable and automatable. This study tests the use of hyperspectral imaging to aid in the management of GLD.

Over the span of two years we monitored five Cabernet Sauvignon vineyards: ground surveys recorded the incidence of visual symptoms of disease in the field during the same months in which hyperspectral maps recorded disease incidence from the air. A customized Geographic Information System (GIS) methodology was developed to compare the visual symptoms to the results of the hyperspectral imaging technique. For a select number of vines, disease incidence was then confirmed by laboratory assays. On average, detection sensitivity was 94.1%, with a range of 88% to greater than 99% per vineyard. Various vineyard-specific factors appear to compromise detection sensitivity. Overall, our results show that remote hyperspectral imaging of GLRaV-3 infected Cabernet Sauvignon vineyards can be a useful and cost-effective approach to mapping diseased vines. Future studies should focus on the use of this tool for detecting GLRaV-3 in other grape varieties, as well as other grapevine pathogens.

Keywords: Wine grape; Grapevine Leafroll Disease; Grapevine leafroll-associated virus; Hyperspectral Imaging; Virus Detection

1. Introduction

Grapevine leafroll disease (GLD) is an economically damaging disease occurring in most grape-growing region worldwide (Almeida et al., 2013). The disease is not typically lethal, but results in significant economic loss from a combination of factors: Impaired photosynthetic activity (Bertamini et al., 2005) may lead to decreased fruit quality, pigmentation, and delayed maturity, as well as yield reductions of up to 40% (Goheen and Cook, 1959; Pearson et al., 1988; Woodrum et al., 1984) and economic impacts from \$29,902 to \$226,405 per hectare in California (Ricketts et al., 2015). Of the serologically distinct viruses known collectively as grapevine leafroll-associated viruses (GLRaV), GLRaV-3 is the predominant species of concern (Almeida et al., 2013; Martelli et al., 2012). Leaf symptoms may include a variety-dependent reddening or yellowing of the interveinal area, as well as a downward rolling of the margins of the leaf blade (Krake and others, 1993). Documented increases in disease incidence in coastal vineyards (Golino et al., 2008) have elevated the issue to a high management priority for California growers. The vectors of GLRaV-3 include five mealybug species common in California vineyards: grape (*Pseudococcus maritimus*), obscure (*Ps. viburni*), longtailed (*Ps. longispinus*), vine (*Planococcus ficus*) and Gill's (*Ferrisia gilli*) mealybugs (Golino et al., 2002; Tanne et al., 1989; Wistrom et al., 2016). The role of other mealybug and scale species in transmission of GLRaV-3 in California has not been quantified (Belli et al., 1994; Petersen and Charles, 1997).

Studies in South Africa, Israel, New Zealand, and California have shown that spread of GLRaV-3 is spatially correlated (patchy), indicating that the dominant mechanism of dispersal operates over a short distance (Charles et al., 2009; Habili and Nutter Jr, 1997) thereby emphasizing the importance of vine removal to disease management (Almeida et al., 2013). Simulated control strategies by Sokolsky et al. (2013) demonstrated that vine removal with or without insecticide treatment to neighboring vines has the potential to significantly decelerate rates of disease spread over a 7-year period.

The first step in a vine removal program is to locate the diseased vines within the vineyard. Because visual symptoms of GLD are variable across grapevine varieties and locations, and can be confused with

other biotic and abiotic maladies, personnel responsible for identifying diseased vines must be highly trained. Since symptoms tend to develop most clearly in the fall, coinciding with the busy harvest period, it can be challenging to visually inspect all acreage within the optimal period for disease expression. Because decisions on control strategies often depend on an accurate estimation of disease incidence within a vineyard, improvements in the ability to locate and quantify diseased vines can have major impacts on the practice of disease management.

Imaging and field spectroscopy provide a potentially valuable alternative for efficiently mapping the incidence and spread of disease symptoms within vineyards and grapevine nurseries. When vegetation is under stress (water deficiency, nutrient or disease stress) the characteristic spectral features are altered in a predictable fashion, led by the decline of the infrared reflectance (Knippling, 1970). The use of an imaging spectrometer allows for detailed mapping of small spectral changes occurring in the plant due to specific disease or stress conditions. In the case of GLRaV-3-infected vines, spectrometer measurements of red varieties have demonstrated that both asymptomatic and symptomatic infected vines can be distinguished spectrally from healthy vines with the largest changes occurring around the green peak (near 550 nm) and around the “red edge” (red and near-infrared between 600-750 nm) (Naidu et al., 2009).

Imaging spectrometer (‘hyperspectral’) technologies have been used for a broad range of measurements including agricultural research and inspection (Eismann, 2012), remote mapping of minerals on the earth and other planets (Vane et al., 1993), atmospheric and environmental monitoring (Clark et al., 1995) as well as medical and defense applications (Eismann, 2012). Thermal, multispectral and hyperspectral domains have been used to assess water stress resulting from *Verticillium* wilt in olive (Calderón et al., 2013). Spectral sensors have also been applied to assess the presence of diseases of sugar beet, barley, wheat, tomato, apple and others, as reviewed by Mahlein (2016). Within vineyards specifically, hyperspectral technologies may be applied to estimate leaf biochemistry to indicate vine stress and nutritional deficiencies (Zarco-Tejada et al., 2005). The advantage of hyperspectral over multispectral sensors is that the former collect many more wavelengths of data, often over a broader spectral range (350

to 2,500 nm) and narrow spectral resolution (below 1 nm) (Steiner et al. 2008). In 2013 and 2014, we applied the results of Naidu et al. (2009) to remotely map the location of diseased vines in vineyards using an airborne imaging spectrometer. Remote detection is non-destructive, efficient, and relatively inexpensive. Hyperspectral data acquired with an airborne instrument can provide continuous measurements and mapping of vineyard blocks that are not possible to obtain using field sampling techniques. However, to date the robustness of this technique had not been verified with visual estimation of disease by ground surveys on a vineyard-wide scale.

Our objective in this study was to develop and test an analytical process to evaluate the reliability of hyperspectral airborne imaging to remotely detect the incidence of the GLRaV-3 at the vine level. Specifically, we aimed to (1) determine an optimal radius (“buffer distance”) for the pixel zone around the vine that would maximize detection sensitivity by the hyperspectral image; (2) understand the accuracy of the hyperspectral detection as compared to the ground survey (visual estimation); (3) evaluate seasonal changes in image quality to identify a key period for detection; and (4) assess incidence and likelihood of potential discrepancies in image quality caused by factors such as vine health, mixed virus infections, etc. The availability of a reproducible, efficient method—such as hyperspectral airborne imagery—to remotely detect and quantify disease at the vineyard scale has great potential for improving disease management by increasing the adoption of vine removal practices and thereby reducing economic impacts associated with grapevine leafroll disease.

2. Methods

2.1. Hyperspectral Imagery & Detection Methods

To best capture the foliar symptoms of GLRaV-3, which are apparent during late summer and fall, imaging data for three subject vineyards were collected in October of 2013. In 2014, two additional vineyard sites were added to the study and imaging data for all five of the subject vineyards were collected on two distinct days (9/29/2014 and 10/13/2014). All five of the subject vineyards are planted to

Vitis vinifera cv. Cabernet Sauvignon, and are located in Napa Valley, California; they differ in vine and row spacing, vine density, and total acreage planted (Table 1).

Using a Cessna T182 equipped with a Headwall Photonics VNIR E Series Hyperspectral camera, vineyards were flown at altitudes between ~2,000 and 4,000 feet (610-1,219 meters) above the ground. This imaging spectrometer captures a wavelength range 400-1000 nm at 0.65 nm spectral resolution using 1600 cross track pixels and is able to acquire data at speeds above 100 frames/ second. To optimize visibility of disease symptoms in the vineyard, the camera was tilted along the direction of flight opposite the rows. The resulting spatial resolution of ~0.25 to 0.50 meter per pixel allowed for the analysis of pure pixels of healthy and infected vines, soil and ground vegetation components.

Raw hyperspectral data were calibrated to units of radiance based on measurements of a Labsphere® uniform source system with National institute of standards and technology (NIST) traceable standards. The hyperspectral images were then orthorectified based on inertial and GPS data collected during the flight using customized software written in the commercial Interactive Data Language (IDL) software. The resulting georeferenced image cubes were then calibrated to units of reflectance using Modtran-based atmospheric correction software available in the commercial Environment for Visualizing Images (ENVI) software. Diseased vines were identified by applying an analysis method to the nearly 1,000 wavelengths collected by the hyperspectral system that involves comparisons to spectral libraries of diseased and healthy vines. This method involves a spectral de-mixing approach that compares reflectance signatures of diseased and healthy vines to measurements acquired by the hyperspectral camera, based upon leaf reflectance spectra specific to GLRaV-3 (Naidu et al. 2009). Spectral signatures representing non-vine components (e.g. soils, shadows and vines with unrelated symptoms) were also used to differentiate the leafroll-infected vines, as were methods to isolate the signatures of unknown background components. The resulting image is continuous and represents a relative probability that a given pixel contains the GLRAV-3 signature (as well as the pixel's spectral distance from materials that are identified as not being the signature of interest). Results were evaluated based on identification of spatial patterns characteristic of a vectored disease. In some cases spectral library-based method were further augmented based on the

inclusion of additional components observed in the reflectance calibrated image. The absolute geolocation accuracy of the hyperspectral results was further improved through alignment to 2011 1-foot (0.3 meter) ortho-imagery available for Napa County.

2.2. Ground Surveys & Disease Testing

In each vineyard, ground surveys recorded the incidence of visual symptoms of disease at the vine-level on single visits to each vineyard in September of both 2013 and 2014 (Table 1). In 2015, all vines showing a conflict between ground and image detection (as described in *Statistical Analysis*) were revisited and visually evaluated to explain possible sources of disagreement. Plant tissue samples were assayed for grapevine leafroll-associated virus 1, -2, -3, -4, -7, grapevine vitiviruses (GVA, GVB), and grapevine red blotch-associated virus (GRBaV). These assays were conducted for a minimum of 60 vines (30 symptomatic and 30 asymptomatic) from each vineyard. Samples from each study vine were collected during the dormant seasons of both 2013-14 and 2014-15, and consisted of four to six, 15 to 20 cm sections of basal canes per vine. Phloem extracts were prepared according to the GES protocol of Osman et al. (2006; 2012). GLRaV-1 and GLRaV-3 were tested with the enzyme-linked immunosorbent assay (ELISA). ELISA reagents for GLRaV-1 and GLRaV-3 were products of Bioreba (Switzerland) and Agri-Analysis (Davis, CA), respectively. The remaining grapevine leafroll-associated viruses, as well as GVA and GVB were tested with the one-step reverse transcription-polymerase chain reaction (RT-PCR) method described in Osman et al. (2006; 2012). GRBaV was tested with conventional polymerase chain reaction (PCR) method using the primers of Rwahnih et al. (2013). All PCR primers were custom synthesized by IDT DNA Technologies (Coralville, IA).

2.3. GIS Data Creation

To compare field data to the hyperspectral images, a customized geoprocessing tool was created in ArcGIS 10.3 to generate GIS point files with accurate row and vine spacing. Vine points were georegistered to 2011 1-foot (0.3 meter) ortho-imagery available for Napa County. We performed a preliminary analysis to determine the optimal circular buffer size by which to calculate zonal statistics for

each vine. We determined that the buffer should be large enough to include symptoms that are primarily near the vine head, but also leave space between vines to minimize overlap and isolate the individual vine being assessed. For this study, 14 different buffer widths were tested, including 0.5 foot (0.15 meter) increments between 1- and 4-feet (0.3 – 1.2 meters), and 10 percent increments of vine spacing between 50% and 100%, with 75% as mid-point. Based on this analysis, we selected a standard buffer size as a percentage of the overall space occupied by each individual vine, a value that varied between vineyards. We then calculated zonal statistics for all pixels of hyperspectral imagery located within the buffer zone associated with each vine.

2.4. Statistical Analysis

The zonal statistics were subjected to a regression analysis to segment the remaining image into homogeneous categories of infected and non-infected vines. We compared visual mapping to the hyperspectral image for each study vineyard using a CART (Classification and Regression Tree) algorithm. Regression trees function by binary recursive partitioning of a dataset into mutually exclusive and exhaustive subsets using combinations of explanatory variables; that is, they group similar data and distinguish these groups from other, dissimilar groups (Crawley, 2007; De'ath and Fabricius, 2000). In this study, presence of disease as documented by the ground survey acted as the response variable (binary: GLRaV-3 /no GLRaV-3), and the zonal statistics of the hyperspectral image operated as explanatory variables. Thus, we were able to assign each vine within the study vineyards to one of three categories: GLRaV-3 -diseased, non- GLRaV-3 diseased, or conflict (See Figure 2). Vines were assigned to the first two categories when ground data and imaging results matched across all imaging dates. Vines were assigned to the conflict category if they were not consistently identified as diseased or healthy for one or more of the imaging dates. Both the hyperspectral image processing and the CART analysis were run separately for each vineyard and each respective imaging date.

2.5. Subsampling Analysis

For the first phase of the CART analysis, ground survey data from all vines within each study vineyard was used as an input into the model. However this level of ground mapping effort is not practical outside of research situations. To test the robustness of the technique using a subset of ground-surveyed data we ran a subsampling study for multiple scenarios with varying spatial distribution in each of the vineyards. As a precursory analysis we tested 6 subsampling scenarios in vineyard F. The two most promising results—a selection of 40 random vines located throughout the vineyard or a selection of 40 contiguous vines within a single vineyard row—were then applied as a test for the remaining vineyards in the study. The subsamples of 40 vines represented approximately (0.63%- 1.80%) of the total vines within each vineyard. In a limited capacity, we also explored the effects of vine density and varying spatial distribution of infection on detection capabilities.

3. Results

In 2013, we analyzed a total of 12,159 vines over three study sites, and in 2014, we analyzed a total of 21,501 vines over five study sites (Table 1). Grapevine leafroll disease incidence rates ranged from 5.8 to 80.35% of the total vines in the vineyard.

3.1. Ground Detection and Laboratory Analysis

Laboratory analysis of sampled vines revealed that the accuracy of ground detection of symptomatic and non-symptomatic vines by a trained expert is high for most sites (Table 2). Out of a total of 347 vines tested, laboratory assays detected GLRaV-3 in 178 vines; of those, the ground surveys identified 176 (98.9%) as diseased. Laboratory assays did not detect GLRaV-3 in 169 vines; of those, the ground surveys identified 161 (94.8%) as non-diseased. In the 4.7% of cases where there was a disagreement between the test results and ground detection of GLRaV-3 negative vines, test results showed another virus present in the vine; for example vines were infected with *Grapevine leafroll-associated virus 2* (GLRaV-2) rather than GLRaV-3. In addition, test results indicate that some vines are infected with

multiple viruses, including grapevine red blotch-associated virus (GRBaV), GLRaV-2 and/or 3, *Grapevine virus A* and/or *Grapevine virus B*.

3.2. Buffer size

The results of the buffer analysis show that beyond a buffer size that is 50% of the vine spacing, there is little difference in detection sensitivity ($<0.1\%$). We observed that the optimal buffer size for calculating single-vine zonal statistics is most stable between 70% and 80% of the vine spacing (average 99.5% match for all vines). Within this range there are tradeoffs between over- and under-detection (in other words, a vine may be detected as diseased in the hyperspectral image, but shows no symptoms on the ground or a vine showing symptoms on the ground may go undetected in the hyperspectral image). With this in mind, we selected a buffer size of 75% of the vine spacing as the standard buffer size for generating the single-vine zonal statistics to drive the remaining CART analyses.

Figure 1. Example of results of a buffer analysis used to determine optimal range for calculating zonal statistics around a vine.

3.3. Statistical Analysis

We defined a successful detection as the number of vines for which airborne imaging and ground survey results are consistent across imaging dates, whether the vine was leafroll-diseased or healthy. Our average rate of successful detection is 94.1%, so an average of fewer than 6% of vines were misidentified either by ground survey or spectral detection.

We observed more variability in detection accuracy between vineyard sites than between imaging dates (Figure 3). Depending on the vineyard site, successful detection rates ranged between 88.8% (Vineyard M) to greater than 99% (Vineyard T). Across dates, Vineyard T consistently proved to be the site with the highest rate of agreement between ground and aerial images (99.5%-99.8%). In this vineyard, disease signatures on the ground are quite clear because only two viral pathogens are present: GLRaV-3 and GRBaV. This is also the youngest vineyard (planted 1996) of all five vineyards in the study. In contrast, Vineyard M, which showed less agreement between the ground and aerial surveys (88.8%-90.4%), has multiple viruses present, often in mixed infections within a single vine. This is also the oldest vineyard in the study (planted 1945) and includes a significant number of vines that are almost dead and exhibit a small canopy. The other three vineyard sites in the study showed comparable detection rates between 92% and 95.4%.

With the exception of Vineyard H, four out of five sites showed slightly improved results for the October imagery date as compared to the September imagery date in 2014. Successful detection across sites averaged 94.0% in September versus an average of 94.4% in October – a 0.4% increase.

Figure 2. Visual representation of results of regression tree analysis comparing visual mapping to hyperspectral imaging for *Vitis vinifera* cv. Cabernet Sauvignon vineyard in Napa County (data are presented in numerical format in Figure 3)

Figure 3. Results of regression tree analysis based on data from all vines. Each vine in all vineyards was assigned to one of three categories: “agree, non-GLRaV-3” and “agree, GLRaV-3” when the ground data and imaging results matched for all imaging dates. Vines were categorized as “Image/Ground conflict” when they were not consistently identified as diseased or healthy.

3.4. Subsampling Analysis

The results of the subsampling analysis showed comparable or slightly lower results to the full sample analysis (Figure 4). In two of the five vineyards (Vineyard M & Vineyard F) subsampling had little to no measurable effect on overall accuracy. In Vineyards T and H, subsampling reduced accuracy by 0.5% and 3%, respectively; this was equivalent to a total of 38 and 116 vines. We observed the greatest measurable reduction in accuracy due to subsampling in Vineyard P, where there was a 3.3% decrease (a difference of 210 vines).

Of the two methods used in the subset analysis, the selection of 40 contiguous vines within a single row shows comparable, or in some cases better, results than the random selection of 40 vines. Given the convenience of sampling contiguous vines of mixed infection in one row as compared to locating dozens of vines scattered throughout the vineyard, it appears this technique would be preferred. Although not reported here, we observed that factors such as vine density, disease incidence and spatial distribution of diseased vines can affect the accuracy of subsampling.

Figure 4. Results of regression tree analysis based on subsampled data. Each vine in all vineyards was assigned to one of three categories: “agree, non-GLRaV-3” and “agree, GLRaV-3” when the ground data and imaging results matched for all imaging dates. Vines were categorized as “Image/Ground conflict” when they were not consistently identified as diseased or healthy.

3.5. Sources of conflict

Vines designated as “conflict” were not consistently identified as diseased or healthy for one or more of the imaging dates. In September 2015, we observed all conflict vines in ground surveys of four of the five study vineyards; we did not include vineyard H because all diseased vines were removed in spring 2015 as part of the management program. We noted several factors that may compromise our ability to detect leafroll-diseased vines (Table 3). In our study vineyards, reductions in canopy size stemming from old age or poor vine health compromised our ability to detect diseased vines from the air even though symptoms were perceptible in ground surveys (Vineyard T (27.9%) and Vineyard M (27.5%)). And in the opposite situation, overly vigorous canopies that overlap adjacent vines, particularly when younger vines

are inter- or replanted within a mature vineyard, can also affect detection efficiency (Vineyard P (3.2%)), although not to the degree that reductions in canopy size did. In some instances (Vineyard F (17.0%) and Vineyard M (24.8%)), the lack of strong symptoms, i.e. “light symptoms” complicated detection (Figure 5). The presence of another disease or vines infected with multiple diseases (Vineyard T (21.3%) & other vineyards (ca. 8.5%)) also affected detection. Most notably, the presence of GRBaV in vines explained 3.5% - 8.2% of conflict vines across sites. This is not surprising, given that symptoms of grapevine red blotch disease are similar to those of leafroll disease (Sudarshana et al., 2015). Other viruses such as GLRaV-2, *Grapevine virus A* and *Grapevine virus B* were also present in some sites, as were Pierce’s Disease (*Xylella fastidiosa*) and the dieback and stunting symptoms typically associated with trunk disease pathogens such as *Eutypa lata* and species in the family *Botryosphaeriaceae*. In the majority of instances (52.6% of cases), inspection of the vines revealed apparently healthy or diseased vines that were not identified as such in airborne images. Given that our ground surveys did not uncover a possible explanation, we suggest that interpretation of the sensor data could account for these discrepancies. A closer look at the subset of vines that were labeled a conflict as a result of interpretation of the sensor data shows that the majority of these conflicts were attributable to false positives on the part of the image sensor (i.e. over-sensitivity). One exception was in Vineyard P, which had more cases of false negatives, likely due to the close spacing of vines in this vineyard and subsequent canopy overlap. Additionally, false negative results were more common in ground surveys than false positives. Our survey of conflict vines took place in 2015, one year after the original mapping was completed, suggesting that the false negative results may be indicative of the sensor’s ability to detect the presence of the virus early, before visual symptoms become apparent. Thus there could be an application for early disease detection, although multiple variables may need to be applied to improve the accuracy of detection for both symptomatic and asymptomatic infected vines (Naidu et al. 2009).

Figure 5. GLRaV-3 symptomatic vines with 1) strong symptoms, 2) light symptoms, and 3) an instance of light and strong symptoms of GLRaV-3, as virus spreading from vine to vine by vector.

4. Discussion

Our results validate the innovative use of hyperspectral airborne imaging technology to identify leafroll-diseased Cabernet Sauvignon grapevines. Because visual estimation of plant disease is resource intensive and limited by the skill set of the individual, newly developed technologies such as this are necessary to address current and future challenges in agriculture and to increase the sensitivity, precision and reliability of disease detection, as reviewed by Mahlein (2016). The rapid and reproducible airborne imaging technique tested here offers several advantages over more traditional detection methods. Further refinements of the technique have the potential to improve detection capabilities and increase adoption of this technology.

Laboratory analysis showed our visual estimation of disease to be very accurate (nearly 98%) for GLRaV-3 infected vines. Although the accuracy of ground surveys for detecting non-GLRaV-3 infected vines was high, it was likely compromised by multiple infections or infections with pathogens that exhibit symptoms similar to GLRaV-3 (for example GLRaV-2). In the case of this study, a highly trained expert

performed ground surveys; we might expect less accurate results from a lesser-trained individual, particularly in instances where multiple pathogens are present in a vineyard. Thus, in the absence of a highly trained professional, the benefits of airborne hyperspectral imaging may be even greater than what we have demonstrated in this study.

Between the five vineyard sites studied, successful detection of GLRaV-3 averaged 94.1%. We found there to be the most variation between vineyard sites rather than between imaging dates. This is likely due to specific nuances within the site that are contributing to conflicts between what can be detected in a ground survey versus an airborne hyperspectral image. Factors such as leaf inclination, illumination and surface texture (Gitelson et al., 2002; Jensen, 2007) that differ among vineyard sites due to grape variety, trellis system, and vineyard layout may result in spectral variations that affect sensor data and detection sensitivity. Within vineyard sites, we found only a small difference in detection rates between dates, with the mid-October date (two weeks later in the season) slightly better (avg. 0.4% increase in successful detections) than the late September date. This is likely because the foliar symptoms showed more strongly at the later date. However, the development of symptoms may be affected by vine cultivar, scion-rootstock combinations, and environmental factors, and can therefore vary between vineyards and growing seasons (Naidu et al. 2014). We demonstrated that airborne images could be collected during the fall season, once visual symptoms of GLD are clearly apparent and well distributed through the vine canopy. Future studies could explore the use of this technology during other times of the growing season, specifically as a tool for early detection. Given the apparent delay of one growing season between virus inoculation and symptom development (Blaisdell et al., 2016), detecting diseased vines before symptoms develop could facilitate early intervention (vine removal) that may affect disease progression. And results from our inspection of “conflict” vines suggest that there may be some value in applying this technology for early detection.

Several factors compromised our ability to detect diseased vines, including: reduced canopy size resulting from advanced vineyard age or diseases such as *Eutypa* and *Botryosphaeria* dieback or Pierce’s disease (*Xylella fastidiosa*); unclear symptom development, typically resulting from mixed viral infections; and

overlapping vine canopies. Both the oldest (Vineyard M, planted in 1945) and the youngest (Vineyard T, planted in 1996) vineyard had reduced canopies that were less visible in the remotely sensed image. This suggests that vineyard age matters less than the overall health of the vineyard. Detection was also compromised for vines with less distinct visual symptoms (“light symptoms”); the spectral signature of these vines may have been less pronounced and therefore more difficult to distinguish, as has been found for sugar beet leaves with lesser disease incidence (Mahlein et al., 2013). We attributed the greatest impact on detection capability to interpretation of the sensor data as has been demonstrated in other systems (Mahlein, 2016). Because the sensors are not directly measuring plant physiological parameters, but instead recording the resulting reflectance over a broad spectrum, techniques must be developed that are highly sensitive to interpret the sensor data accurately. Improvements in interpretation of the sensor data have the potential to increase the reliability and adoption of remote sensing technology for detection of plant diseases. Generating a robust library of the spectral changes in the vine resulting from GLRaV-3 infections across the wide range of growing conditions in California vineyards has the potential to improve our ability to interpret data and correctly attribute changes resulting from specific diseases or conditions. In addition, a larger contributing factor may be the lack of spectral libraries for all the other variations within different vineyards (e.g. other diseases, nutritional deficiencies, or other differences among vineyard sites, as described previously). More rigorous testing and development of vegetation indices, such as was completed for olive (Calderón et al., 2013) have the potential to improve imaging processing and detection capability. Our results are fairly consistent, repeatable, and reproducible and detection capabilities are high for Cabernet Sauvignon vineyards. Still, the number of conflict vines is significant from a management perspective (greater than 200 in some vineyards). Improvements in interpretation of the sensor data that are likely to develop over time as this technique is increasingly adopted have the potential to reduce the number of vines that are erroneously identified, thereby ensuring that diseased vines are removed and healthy ones remain in production.

The results of our subsample analysis show promise for greatly minimizing the ground effort needed to remotely assign individual vines into infected and non-infected categories, particularly in situations where

surveying all diseased vines is not practical. There are several factors, including vine density, disease incidence and spatial patterns that may affect the accuracy of subsampling efforts. For example, in vineyard H random vine selection initially gave better results than single row selection. However, when the row test was repeated on rows with differing spatial distribution of infection, results improved when selecting rows with clustered infected and non-infected vine as compared to rows with vines of more scattered infection. Additionally, in Vineyard T, there was an overall lower incidence of GLD as compared to the other vineyards in our study, and therefore fewer positive examples to choose from when data is subset. As a result, sub-sampling tended to overestimate the incidence of disease. Finally, in Vineyard T there was an overall greater number of vines in this vineyard, therefore selecting a larger sample of 80 vines (1.3% of the total) improved results measurably over the smaller sample size of 40 vines (0.6% of the total). Although we were able to identify a number of factors that affect the accuracy of subsampling, our analysis was fairly limited in scope and we were unable to test a full complement of combinations. We therefore suggest that factors comprising a subset be explored in greater detail before recommendations can be developed on the location and selection of vines for sub-sampling.

In this study, we focused on vineyards planted to *Vitis vinifera* cv. Cabernet Sauvignon; the most commonly planted grape variety in Napa County (46% of the total acreage, according to the 2015 Napa County Agricultural Crop Report) where the study vineyards are located. Future research could explore the use of this technology for other cultivars. In the course of this study, we conducted a precursory analysis of two blocks of differing cultivars (Merlot and Cabernet franc) neighboring one of our Cabernet Sauvignon study vineyards. Our preliminary results (not reported here) suggest that changes in spectral reflectance differ by cultivar (likely due to factors described previously) and therefore the technique will have to be optimized for each variety. Extending the use of this technique to asymptomatic hosts, or for early detection, would also be beneficial. For example, *V. vinifera* cv. Sauvignon Blanc does not express obvious visual symptoms of leafroll disease and the use of laboratory assays to identify infected vines is cost-prohibitive. Hybrids (such as *V. vinifera* x. *V. californica*), juice and muscadine grapes and rootstocks are also asymptomatic hosts of GLRaV-3 (Naidu et al., 2014). The ability to detect the

presence of the pathogen in asymptomatic hosts could reduce pathogen reservoirs that can impact management programs in surrounding commercial vineyard blocks.

Vigilant removal of diseased vines has been a successful approach to maintain healthy vineyards and reduce the long-term impacts of GLD (Bell, 2015; Pietersen et al., 2013). Airborne hyperspectral imaging conducted over consecutive years could be used to evaluate the rate of spread. In addition to identifying the location and number of infected vines, the maps can help locate the origin of the disease and direction of the spread, which can be useful in eliminating pathogen reservoirs.

5. Conclusion

Grapevine leafroll disease, with its rapid spread and negative impact on wine quality, is a major worldwide concern for grape growers. As multiple studies have shown, identification and removal of diseased vines is critical for effective disease management. For this project we tested the use of hyperspectral airborne imaging technology to remotely map leafroll diseased vines in commercial vineyards. Here we have demonstrated this technique to have a greater than 94% success rate of leafroll detection in Cabernet Sauvignon vineyards. These results illustrate that this technique can be especially useful where visual estimation of disease by a highly trained individual is not practical. Furthermore it offers several advantages over ground-based detection methods in that it is reliable, repeatable and automatable. Maps generated through this imaging technology can assist managers in the process of vine removal and quantification of disease incidence, as well as the identification of pathogen reservoirs. Furthermore, our validation of this technology should give researchers and end-users more confidence in the reliability of this tool to rapidly locate and quantify diseased vines.

Future studies could expand upon our work to further explore the capabilities of this technology. For example, we expect that this technology could be refined to detect diseased vines in other cultivars, and potentially in asymptomatic hosts. We would also like to explore whether hand-held, tractor, ATV or unmanned aerial vehicle (UAV)-mounted cameras may improve our accuracy and detection capabilities, given that this would reduce the distance between the sensor and the object, thereby improving spatial

resolution and potential for disease detection (Mahlein et al., 2012). If the mounted camera was integrated into a system that automatically marked diseased vines (such as with spray paint) as they were detected, this would make it easier for vineyard managers to direct the removal of diseased vines. Our work has been almost exclusively in commercial production vineyards; if this imaging were proven reliable in a nursery setting, it could be used to improve a nursery's ability to supply disease-free plant material to growers. Finally, we would like to explore the potential for hyperspectral imaging to identify other diseases, since removal of diseased vines may also be an integral component of those disease management programs. Our results demonstrate the potential for applications of this technology, and future studies could expand upon our work to provide increasingly sophisticated, reliable tools for disease management in agricultural production systems.

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Vineyard	Vineyard Size Ac (Ha)	Number of Vines	Vine Spacing x Row spacing	Planting Date	Hyperspectral Imaging		Ground mapping	
					2013	2014	2013	2014
H	7.2 (2.9)	3,821	8 x 10 feet (2.4 x 3.0 m)	1974	Oct 02	Sep 29, Oct 13	Oct 15	Sep 29
M	4.1 (1.7)	2,218	8 x 10 feet (2.4 x 3.0 m)	1945	Oct 02	Sep 29, Oct 13	Sep 10	Aug 28
T	10.4 (4.2)	6,120	7.5' x 10' (2.3 x 3.0 m)	1996	Oct 02	Sep 29, Oct 13	Sep 30	Sep 22
P	10.3 (4.2)	6,390	6 x 12 feet (1.8 x 3.7 m)	1994	None	Sep 29, Oct 13	None	Oct 1, 2
F	4.6 (1.9)	2,952	7 x 10 feet (2.1 x 3.0 m)	1987	None	Sep 29, Oct 13	None	Sep 11

Table 1. Description of the study vineyards.

TESTED VINES SUMMARY	GLRaV-3 detected in laboratory assays	GLRaV-3 not detected in laboratory assays	Total
Ground Survey Agrees	176 (98.9%)	161 (95.3%)	337 (97.1%)
Ground Survey Disagrees	2 (1.1%)	8 (4.7%)	10 (3%)
Total	178	169	347

Table 2. Results of laboratory assays for *Grapevine leafroll-associated virus 3* (GLRaV-3).

CONFLICT RESULTS	M	T	P	F	AVG
# Conflict Vines	396	61	431	294	
Interpretation of sensor data	34.1%	36.1%	79.1%	61.2%	52.6%
Reduced canopy/Almost Dead	27.5%	27.9%	0.0%	1.0%	14.1%
Light Symptoms	24.7%	4.9%	5.1%	17.0%	12.9%
Other Disease (Single or Mixed Infection)	8.3%	21.3%	8.6%	8.5%	11.7%
Mixed Infection w LR3	0.0%	4.9%	1.6%	7.5%	3.5%
Young Vine	1.3%	1.6%	3.2%	1.0%	1.8%
Other	0.5%	1.6%	1.2%	2.7%	1.5%
No Data	3.5%	1.6%	1.2%	1.0%	1.8%

Table 3. Vines designated as “conflict” were not consistently identified as diseased or healthy for one or more of the imaging dates. In September 2015, we observed all conflict vines in ground surveys and noted several factors that may compromise our ability to detect leafroll-diseased vines.

Conflicts Resulting from Interpretation of Sensor Data	M	T	P	F
# Vines	125	22	326	153
Image false negative	13.6% } 81.6%	18.2% } 81.8%	62.6% } 77.9%	32.0% } 82.4%
Image false positive	68.0%	63.6%	15.3%	50.3%
Ground false negative	15.2% } 18.4%	18.2% } 18.2%	18.1% } 22.1%	13.1% } 17.6%
Ground false positive	3.2%	0.0%	4.0%	4.6%

Table 4. Breakdown of conflicts due to interpretation of sensor data.