Remote Image and Leaf Reflectance Analysis to Evaluate the Impact of Environmental Stress on Grape Canopy Metabolism

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SUMMARY. Research suggests that blackleaf (a leaf disorder in grape, *Vitis labrusca* L.) is induced by high levels of ultra violet (UV) radiation and overall light intensity, resulting in color changes (purple-brown-black) for sun-exposed leaves of the outer canopy, and a corresponding >50% reduction in photosynthesis. Metabolic indicators (photosynthesis and leaf water potential), percent blackleaf expression, and full spectrum leaf reflectance were mapped within vineyards using global positioning system (GPS) and digital remotely-sensed images. Each image and data record was stored as an attribute associated with a specific vine location within a geographical information system (GIS). Spatial maps were created from the GIS coverages to graphically present the progression of blackleaf across vineyards throughout the season. Analysis included summary statistics such as minimum, maximum, and variation of green reflectance, within a vineyard by image capture date. Additionally, geostatistics were used to model the degree of similarity between blackleaf values as a function of their spatial location. Remote-image analysis indicated a decrease in percent greenness of about 45% between July and August, which was related to a decrease in photosynthesis and an increase in blackleaf symptom expression within the canopy. Examination of full spectral leaf reflectance indicated differences at specific wavelengths for grape leaves exposed to UV or water-deficit stress. This work suggests that remote-image and leaf spectral reflectance analysis may be a strong tool for monitoring changes in metabolism associated with plant stress.

A central goal of site-specific or precision agriculture is to adjust management inputs to meet the needs of areas within a given site, instead of managing the entire site based on an average of the most and/or least productive areas.

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To use site-specific management practices, producers must deal with variation which occurs within any field, orchard or vineyard. Monitoring differences in plant environmental stress within a site, is a tool that determines needed inputs as well as a key component to implementation of site-specific management. Development of methods to quantify how environmental stresses contribute to crop variation within a site, and thereby adversely impact crop productivity are needed to utilize this method.

Blackleaf is a leaf disorder that can occur in vineyards throughout the world, but commonly occurs in 'Concord' grapevines growing in the Columbia Basin, of central Washington. Due to its negative impact on fruit ripening and overall stress to grapevines, it has been identified as the top production/physiology research priority in juice grapes in the northwestern U.S. (Northwest Center for Small Fruit Research, 1997). The disorder is characterized by an initial discoloration (purple-brown-black) of sun-exposed leaves of the outer canopy. Visual symptoms are traditionally apparent on leaves on the outer canopy in late July and early August and may progress to successive canopy layers by harvest (September to October). In extreme cases, the entire canopy can be affected, resulting in defoliation of the vine. Blackleaf was reported to be related to low petiole and/or leaf potassium concentration (Clore and Woodbridge, 1963; Shaulis, 1954), although the correlative relationship was highly variable. Tremendous differences in symptom expression between blackleaf and classic potassium deficiency in grape occur not only in terms of timing and location of leaf symptoms, but also in location of symptom expression within the canopy and on individual shoots (Malstrom, 1970). Blackleaf has been recently related to light dependent loss of chlorophyll and >50% reduction in photosynthetic rate (Lang et al., 1998; Smithyman, 1999), thus diminishing the capacity of blackleaf-affected leaves to produce sugars and ripen fruits. Additionally, recent work has verified that leaf potassium contents are within or above recommended levels in blackleaf-affected vines (Smithyman, 1999), adding further evidence to discount the existing dogma. Instead, a significant seasonal decline in chlorophyll was

found, suggesting permanent damage to the photosystem (Lang et al., 1998). In continuing studies, blackleaf symptom development was most strongly associated with a combination of high light intensity, UV-B irradiation, and water deficit (Smithyman, 1999). With more than 20,000 acres (8,100 ha) of 'Concord' currently under production in the Columbia Basin, blackleaf is a disorder which negatively influences the yield and quality of 'Concord' grapes in the largest juice production area within the United States. Characterization of the intensity and the spatial variation of blackleaf symptoms within and between vineyards would be required to objectively assess blackleaf impact on vine productivity within and among vineyards. Before the present work, no objective method was established for quantifying the distribution of blackleaf in a specific vineyard. Thus, determining the impact of blackleaf in the Columbia Basin production area was not economically possible due to the amount of acreage to be evaluated and the variation in severity of symptom expression within and among different vineyards.

Based on the highly visible symptoms associated with blackleaf, the disorder appeared to have excellent potential as a model for integrating site-specific horticulture management of canopy quality, with remote sensing and GPS technology. Images collected from a point not in direct contact with an object of interest (remotely sensed images) provide a broad look at patterns which are difficult to decipher at field level, due to the scale on which they occur. Remotely sensed images can be collected from a variety of elevations (platforms) including: passive aerial devices (kite or helium blimp); fixed-wing aircraft at different altitudes; or satellites, depending on the appropriate scale and technical needs of the analysis (Harris et al., 1996; Wilkie and Finn, 1996). In the work presented here, remotely sensed image capture was combined with geostatistical analysis to examine the directional trends and spatial dependency of blackleaf in two vineyards throughout the 1998 growing season. Additionally, a goal of this analysis was to further exploration of the spatial relationship of blackleaf to early season environmental stress patterns within the vineyard, to provide a means for growers to monitor and quantify the

disorder, before visual symptom development. Capture of remotely sensed imagery, has potential to provide real time information to identify variation within the production site. Timeliness of stress detection is needed for coupling site specific management strategies with immediate alterations of inputs to prevent negative impacts on yield or quality. Application of site specific management inputs before symptom expression may then avoid adverse impact on both crop quality and yield, while reducing inputs to meet goals of environmentally sound stewardship of agricultural land.

Materials and methods

STUDY SITE DESCRIPTION AND DATA COLLECTION INCORPORATING GLOBAL POSITION TECHNOLOGY. Data were collected in the Yakima Valley of eastern Washington, from a commercial 'Concord' vineyard (Dorsey) and a 'Concord' research vineyard (H6) located at the Washington State University, Irrigated Agricultural Research and Extension Center (IAREC), at Prosser, Wash. In each vineyard, two blocks of vines were designated which received standard commercial nutrient and water management. Each block contained a minimum of 50 vines, to insure adequate sampling for spatial analysis of each vineyard. At each vine to be sampled, geographic coordinates were collected using a Trimble Global Positioning System (Trimble Navigation Ltd., Sunnyvale, Calif.). Coordinates were collected [2.3 ft (0.7 m) real time and 1.6 ft (50 cm) $\pm$ 1 ppm postprocessing accuracy] in the universal transverse mercator (UTM) projection system.

REMOTE-IMAGE CAPTURE. During the 1996 and 1997 growing seasons, remote-image capture was tested using helium blimp [50 to 140 ft (15.3 to 42.7 m) altitude] and fixed-wing aircraft [1,000 ft (305 m) altitude] platforms using 35 mm color (Ektachrome and Kodachrome, Eastman Kodak Co., Rochester, N.Y.) and infrared (color and black-and-white) slide film. The two image capture platforms did not provide necessary image replication or sufficient image resolution, respectively. Based on evaluation of these image capture tests, ground-based digital images were collected above each sample vine during the 1998 growing season. A four-wheel drive vehicle was used to move through the vineyard and serve as a platform on

Fig. 1. (top) Remotely captured digital image of 'Concord' canopy with blackleaf disorder, standard 18% gray photographic card and black fabric. The latter two items were used for calibration of light intensity, hue, and canopy area within the image. (middle) Induced blackleaf on 'Concord' exposed to ultra violet light and the same image with an overlay of green leaf areas selected by SigmaScan image analysis computer software (bottom) which place a color layer (light green in this example) over all areas within the image which fall within the defined hue and saturation thresholds.

which to stand to capture images, with a camera (D-500L; Olympus, Melville, N.Y.) directly over the top of each canopy. Images were captured at three key phenological stages: fruit set (late June to early-July), veraison, or the beginning of fruit ripening (August), and harvest (September to October). Images were archived for later analysis at the end of the growing season.

GROUND-BASED METABOLIC DATA.

Photosynthetic rate, xylem water potential and visual estimates of blackleaf development based on mesophyll browning (MB) and epidermal discoloration (ED) were measured to examine the relationship between changes in plant metabolism and blackleaf development. Net CO₂ exchange of fully expanded sun leaves, under ambient vineyard conditions, was measured using an infrared gas analyzer (model LCA-2; Analytical Development Co., Hoddesdon, England). Vine water status was measured using a pressure bomb (Plant Moisture Stress Instrument Co., Corvallis, Ore.) to determine xylem water potential. Fully expanded leaves were selected and xylem water potential was measured after tissue reached equilibrium, as described by Smithyman (1999). Blackleaf symptom development was assessed (Smithyman, 1999) on leaves removed from the same canopy location at which photosynthesis and xylem water potential were measured. Each leaf was examined under 10× magnification with illumination under the field of view. The percentage of MB and ED within five leaf blade positions were determined. As a check of visual assessment, blackleaf symptoms were also analyzed using digital images (D-500L; Olympus) from 50 leaves using SigmaScan Pro 2.0 (SPSS Inc., Chicago, Ill.) image analysis package. There was a strong linear relationship ($r^2 >$

0.81) between visual and computer analyzed assessment of blackleaf. Visual examinations of blackleaf (MB and ED), photosynthetic rate and xylem water potentials were not per-

formed on the H6 vineyard due to limitations in equipment and personnel availability.

Ground-based portable spectroradiometric measurements were used to

Fig. 2. Percent image greenness, epidermal discoloration, and mesophyll browning (top) and xylem water potential and photosynthetic efficiency (middle) in the 'Concord' (*Vitis labrusca*) commercial vineyard (Dorsey) during the 1998 growing season. Percent greenness in the research vineyard during the 1998 growing season (bottom).

obtain leaf reflectance measurements of blackleaf-affected and unaffected leaves. A portable FieldSpec FR (Analytical Spectral Devices Inc., Boulder, Col.) spectroradiometer provided in situ measurements in 1-nm increments from 450 to 2500 nm to create a full spectral curve library for comparison with the corresponding metabolic status of the grape leaf. Spectral libraries were analyzed to select a number of optimum bands (parameters derived from the bands) that best fit the range of plant conditions associated with plant stress indicators. Based on evidence that blackleaf symptom development (MB and ED) was

closely associated with UV-B and water-deficit stress (Smithyman, 1999), leaves from greenhouse grown 'Concord' plants maintained under well watered or deficit-water conditions, were exposed to continuous or sequential durations of UV-B irradiation ranging from 10 to 30 min (10 min; 10 + 10 min; 20 min continuous exposure; 10 + 10 + 10 min; and 30 min continuous exposure). Spectroradiometric measurements of these leaves were used to determine whether unique spectral reflectance curves could be identified in order to differentiate one stress from the other under greenhouse conditions.

IMAGE ANALYSIS. Individual digital canopy images were analyzed using SigmaScan Pro 4.00.23 (SPSS Inc., Chicago, Ill.) commercial image analysis software. To calibrate distance and area within each image, a 10 × 20 cm (4 × 8 inch) standard photographic gray card (18% gray, Delta1/CPM, Dallas, Texas), with half the area covered by a flat black cloth was used. The gray/black card was laid on top of the canopy to be included in each image (Fig. 1). In cases in which insufficient card area was visible for calibration, image area was measured in pixel units rather than square centimeters.

Using the SigmaScan Define by Color tool, all areas of green leaves within the image were selected. Green was used due to the similarity of blackleaf discoloration to shadow, stems, or dark earth. Thus, using green provided greater accuracy in determining all leaf area which remained green (percent greenness) and allowed for calculation of the decrease in greenness through the season, as blackleaf developed. Preliminary color calibrations and threshold tests were completed on several sample images to determine correct hue and saturation levels, so as to consistently separate leaf greenness from the rest of the image. For images which may have had significantly different lighting characteristics, due to changes in solar radiation during image capture, hue and saturation were re-calibrated by comparison of the calibration card between the image and all others. The SigmaScan Define by Color tool placed a color layer over all areas within the image which fell within the defined hue and saturation thresholds (Fig. 2A and B). Selected threshold areas were visually verified to insure the layer adequately captured leaves and not areas within the images which were not of interest. The

Fig. 3. Spatial maps of percent greenness in the 'Concord' research vineyard (H6) for July (top), August (middle), and September (bottom) during the 1998 growing season created from analysis of ground-based digital images. Distance is reported in meters (1 m = 3.3 ft).

SigmaScan Measure Defined tool was used to calculate defined threshold, calibration card, and total image areas. Percent greenness was calculated as $\% \text{ green} = 100 \times [\text{area of green leaves} / (\text{total image area} - \text{card area})]$.

STATISTICAL ANALYSIS. Summary statistics (minimum, maximum, average, SE, variance and skew) were calculated by vineyard and sampling dates for a total of six data sets (two vineyards, three sampling dates each) to examine the development and variation of blackleaf (measured by decline in percent greenness) over the season. The decline in percent greenness was also compared with ground verification data already described. Spatial maps of percent greenness were created for each vineyard and sampling date using the GPS coordinates of each sampled vine and Arc/Info 7.2.1 Geographic Information System (Environmental Systems Research Institute, Inc., Redlands, Calif.) to graphically represent progression of blackleaf visual symptom development throughout the season. Each image number was associated with a (X, Y) coordinate pair, with its respective percent greenness value as an attribute within the GIS coverage. Data files were imported to Arc/Info Generate, allowing the creation of a coverage map of each vineyard which was defined for UTM projection (ProjectDefine). ArcView GIS 3.1 (Environmental Systems Research Institute, Inc., Redlands, Calif.) software was used to display and map the resulting coverages of percent greenness by sampling date for each vineyard. Variowin (Public Domain Version 2.2) geostatistics software (Pannatier, 1996) was used to create variogram surfaces to model the degree of similarity between blackleaf symptoms, as a function of their spatial location. Directional trends (anisotropy) were tested in all directions (omnidirectional) and in the direction of the long axis of the sample block. Lag spacings of 6.6 to 9.8 ft (2 to 3 m) provided the resolution needed to examine the spatial structure of the data.

Results and discussion

Handheld, above canopy, digital images provided the necessary resolution to indicate distinct areas of visual blackleaf symptom expression by ED on individual leaves within each canopy (Fig. 1). ED symptom expression was visible within a few days of veraison (15 Aug. 1998). In the Dorsey vine-

Fig. 4. Full spectroradiometric leaf reflectance curves ('Concord') from 450 to 4250 nm of leaves from greenhouse grown plants maintained at water-deficit (top) status or well-watered (bottom) and exposed to 10 min, 10 + 10 min, 20 min continuous, 10 + 10 + 10 min or 30 min continuous ultra violet (UV) radiation. The difference in leaf reflectance between control and UV and/or water-deficit plants is reported as sensitivity and are not presented with other units. Differences in leaf reflectance for water-deficit + UV radiation (top) grape plants were significant at 580, 670, 1420 and 1933 nm. Differences in leaf reflectance for well-watered + UV radiation (bottom) grape plants were significant only at 570, 680, and 1925 nm.

yard, MB was visible under 10× magnification several weeks before ED (Fig. 3A). By harvest in September, MB and ED had affected 79% and 52% of the canopy in the Dorsey vineyard, respectively. In comparison, the peak in photosynthetic rate occurred in August near veraison (Fig. 3B). During the same interval water potential declined (Fig. 3B). Lowest photosynthetic rates

were measured near harvest, when over half the canopy exhibited distinct visual ED blackleaf symptoms (Fig. 3A and B). The same pattern in diminished photosynthetic activity has been previously observed for both ED and visually green leaf tissue of blackleaf-affected leaves (Lang et al., 1998).

When percent greenness over the season was viewed via spatial vineyard

maps, created by analysis of ground-based digital images, the progression of blackleaf visual symptom development over the 1998 season was apparent. Early in the season (July), the digital images indicate a threshold quantity or area of green canopy for the H6 vineyard, within the field-of-view of the digital camera (Fig. 5A). Spatial maps of percent greenness in H6, based on analysis of ground-based digital images, indicated that within the H6 vineyard there was a progressive loss of greenness from July to August (Fig. 4A and B). Percent greenness significantly decreased by 35% and 48% from July to August 1998 in the Dorsey and H6 vineyards, respectively (Fig. 3A and C). Therefore, the amount of green area within the field of view was significantly reduced by veraison, during a time interval in which green canopy area would be expected to increase (Fig. 5B). Blackleaf affected canopy area continued to increase between August and September 1998, with clearly visible loss of canopy greenness (Fig. 4B and C). By harvest (September), well before normal leaf senescence, canopy greenness essentially disappeared (Fig. 5C). For the Dorsey vineyard, loss of greenness, as quantified by digital image analysis, was correlated with a 53% and 49% increase of MB and ED, respectively (Fig. 3A). The decline in greenness in the Dorsey vineyard also corresponded to between a 33% to 57% decrease in photosynthesis by September when compared to photosynthetic rates in July and August, respectively (Fig. 3C).

Previous work suggested that full spectroradiometric leaf reflectance measurements could be used to differentiate blackleaf-affected and unaffected 'Concord' leaf tissue (Perry et al., 2000). Full spectroradiometric leaf reflectance measurements indicated distinct changes at specific wavelengths for well-watered + UV-irradiated leaves (570, 680, and 1925 nm) which were similar to water-deficit + UV-irradiated leaves (580, 670, 1933 nm) (Fig. 5A and B), both of which were analyzed as a comparison to non-stressed plants (reported as sensitivity). An additional difference in leaf spectral reflectance was measured for leaves of water-deficit + UV-irradiated at the 1420 nm wave length, that did not occur in the leaf reflectance curves of control or well-watered + UV-irradiated plants (Fig. 5A and B). Thus,

these data suggest the two imposed stresses of UV-irradiation and water-deficit stress do not produce identical stress spectral reflectance curves, but rather could be separated by unique spectral signatures.

Spatial analysis of blackleaf development in the Dorsey and H6 vineyards indicated distinct spatial structure early in the season. Analysis of combined sample blocks of the Dorsey vineyard exhibited 39% of leaf greenness in July was dependent on location within the vineyard. In August, only the northeast block still had a spatial dependency of 38%. By September little spatial dependency (13% to 17%) could be found in either sample block of the vineyard. For the H6 vineyard spatial dependency of the two sample blocks ranged from 33% to 48% in July. As with the Dorsey vineyard, percent greenness in the H6 vineyard lost much of its spatial dependency in August. This suggests that the loss in spatial dependency of visual blackleaf symptom expression may in fact be the result of the disorder's pervasiveness throughout the vineyard by September. However, by September in the H6 vineyard the spatial dependency of the combined sample blocks in H6 was 49%. Thus additional analysis of the spatial relationship of blackleaf in 'Concord' vineyards will be necessary.

Conclusion

From this preliminary work we suggest that leaf spectral reflectance may in fact

be a strong tool for monitoring changes in metabolism associated with environmental stress. Geostatistics and spatial analysis of remote images appear to partially explain the variation of blackleaf symptom expression within vineyards early in the season, which is a critical time for assessment of plant stress. Continuing work will emphasize the testing of the unique leaf reflectance signatures as indicators of environmental stress, before visual symptom expression. The ability to pinpoint early season stress, when changes in vineyard management can alleviate stress conditions which result in negative impacts on fruit quality and yield holds potential as a key to the implementation of site-specific management practices. Continuing work within this system will focus on long-term monitoring of multiple locations to directly link biotic and abiotic plant stress (within the vineyard) with changes in canopy metabolism that influence overall canopy quality. Additionally, these techniques could monitor and detect changes in plant status over time within other site specific crop management systems, which may vary in scale from large field and orchard production to smaller production systems in nurseries and greenhouse operations. Such techniques hold potential for uses in the research and management of other ecological and physiological aspects, in natural ecosystems and production systems. By exploring remote sensing platforms which broaden the spatial content within image capture and/or spectral reflectance measurements, it would be possible to examine community dynamics and the impact of agricultural operations on those ecosystems.

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