Research Note

Nondestructive Estimation of Leaf Chlorophyll Content in Grapes

Mark Steele,[1,2] Anatoly A. Gitelson,[1]* and Donald Rundquist[1]

Abstract: Leaf chlorophyll content provides valuable information about the physiological status of plants, and there is a need for accurate, efficient, practical methodologies to estimate this biophysical parameter. Reflectance measurement is a means of quickly and nondestructively assessing, in situ, the chlorophyll content in leaves. The objective of this study was to develop a precise, efficient, nondestructive technique to estimate leaf total chlorophyll (Chl) content in grapes. A relationship was established between Chl content and the red-edge chlorophyll index, based on reflectances in the red-edge (710–720 nm) and near-infrared (755–765 nm) spectral ranges, and the algorithm for Chl retrieval was calibrated. The accuracy of Chl prediction using an independent data set, containing sampled leaves from three field-grown grape cultivars (Edelweiss, Saint Croix, and DeChaunac), was evaluated with no re-parameterization (adjustment of the coefficients) after initial calibration. Although Chl in the validation data set was widely variable, from 3 to 506 mg m^{-2}, the calibrated algorithm was capable of accurately predicting grape leaf Chl with RMSE <30 mg m^{-2}. Such an approach has potential for developing simple hand-held field instrumentation for accurate nondestructive Chl estimation and in analyzing digital airborne or satellite imagery to assist in vineyard management decision making.

Key words: chlorophyll, grapes, leaves, nondestructive, reflectance

The chlorophylls, Chl a and Chl b, are virtually essential pigments for the conversion of light energy to stored chemical energy. The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content (Monteith 1972, Foyer et al. 1982). In addition, Chl gives an indirect estimation of the nutrient status because much of the leaf nitrogen is incorporated in Chl (Filella et al. 1995). Furthermore, leaf Chl content is closely related to plant stress and senescence (Hendry et al. 1987, Merzlyak and Gitelson 1995, Peñuelas and Filella 1998, Merzlyak et al. 1999). In grapevines, Chl relates to leaf age, when age is less than 60 days, as well as net photosynthesis in the leaf (Poni et al. 1994). However, the same authors concluded that caution is needed when trying to use Chl as an indicator of photosynthetic capacity for grapevine leaves of varying age.

Traditionally used wet chemical pigment analysis includes leaf extraction with organic solvents and spectrophotometric determination in solution (Lichtenthaler 1987). Recently, alternative solutions have been developed for analyzing leaf pigments by optical methods that are nondestructive, inexpensive, rapid, and applicable in a field setting (Buschmann and Nagel 1993, Gitelson and Merzlyak 1994, Markwell et al. 1995, Gitelson et al. 2003). The methods are based on numerical transformations (i.e., vegetation indices) derived from spectral reflectance or absorbance. Such spectral indices may provide the viticulturist with an efficient, nondestructive method of monitoring Chl content.

Much work has been done on nondestructive estimation of leaf Chl in species of plants other than grapes, including maple, chestnut, and beech (Gitelson and Merzlyak 1994, 1996), eucalyptus (Datt 1999), maize and soybeans (Gitelson et al. 2005), and paper birch (Richardson et al. 2002). The latter evaluated the performance of the optical methods, which are based on the absorbance or reflectance of light at certain wavelengths by intact leaves, and concluded that the noninvasive optical methods provided reliable estimates of leaf Chl. However, across the range of Chl content studied (4–455 mg m^{-2}), some reflectance indices consistently outperformed two commercially available hand-held Chl absorbance meters: the CCM-200 (OptiSciences, Tyngsboro, MA), and the SPAD-502 (Minolta Camera, Osaka, Japan). Two particular reflectance indices outperformed several others that were tested (Gitelson and Merzlyak 1994):

$$\text{Red-edge NDVI} = (\rho_{750}-\rho_{705})/(\rho_{750}+\rho_{705}) \quad (1)$$

$$\text{Summed red-edge index} = \sum_{n=705}^{750} (\rho_n / \rho_{705}) - 1 \quad (2)$$

The SPAD-502 has adequate sensitivity to Chl contents in grape leaves when the pigment content is less than 300 mg m^{-2} (Steele et al. 2008). Above that level, however, the accuracy of the instrument diminished considerably.

[1]Center for Land Management Information Technologies, School of Natural Resources, University of Nebraska–Lincoln, Lincoln, NE 68583; and [2]Agricultural Research and Development Center, University of Nebraska–Lincoln, Ithaca, NE 68033.

*Corresponding author (email: agitelson2@unl.edu)

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Unfortunately, this decrease in sensitivity takes place in the range of Chl that is typical for green vegetation (above 300 mg m^{-2}); thus, it prevents using a SPAD meter for accurate measurement of Chl in healthy vegetation and for early warning (previsual) of plant stress.

All the vegetation indices described in this paper are based on the relationship between leaf reflectance ρ and the inherent optical properties; namely, absorption α and scattering β coefficients:

$$\rho = \beta/(\alpha+\beta) \quad (3)$$

A conceptual model that uses three discrete spectral bands to estimate the content of plant pigments such as total chlorophyll, anthocyanin, and carotenoids was developed recently (Gitelson et al. 2003, 2006). The model relates the pigment of interest and leaf reflectance $\rho_{\lambda i}$ in three spectral bands λ_i:

$$\text{Pigment content} \propto \alpha_{\text{pigment}} = (\rho_{\lambda 1}^{-1} - \rho_{\lambda 2}^{-1})\times\rho_{\lambda 3} \quad (4)$$

where α_{pigment} is the absorption coefficient of the pigment of interest. λ_1 is the spectral band where reflectance is maximally sensitive to absorption by the pigment of interest, but it also is affected by absorption of other pigments as well as leaf scattering. For removing the effects of both absorption by those other pigments and scattering (β in the denominator of Eq. 3) on reflectance at λ_1, reciprocal reflectance in the second spectral band λ_2 is used. In this spectral band, reflectance should be minimally influenced by absorption related to the pigment of interest (i.e., Chl); however, absorption by other pigments has the same level as in band λ_1. The difference $(\rho_{\lambda 1}^{-1} - \rho_{\lambda 2}^{-1})$ relates to absorption by the pigment of interest but is also affected by leaf scattering (β in the numerator of Eq. 3). Thus, for leaves with different scattering (because of different leaf thickness, density, or surface properties), the $(\rho_{\lambda 1}^{-1} - \rho_{\lambda 2}^{-1})$ will be different for the same content of a pigment of interest. To remove the effect of variability in leaf scattering, the third band $\rho_{\lambda 3}$ has been used. Reflectance at that spectral location should be sensitive to leaf scattering and invariant with respect to absorption by pigments. For Chl estimation, the λ_1 could be located either in the green (around 550 nm) or red-edge (around 700 nm) spectral regions while both λ_2 and λ_3 should be located in the near-infrared (NIR) region (Gitelson et al. 2003, 2006).

The three-band model of Eq. 4 could be applied for Chl estimation in one of two ways, depending on which λ_1 was selected. Therefore, chlorophyll indices (CI) have been suggested in the forms (Gitelson et al. 2003, 2006):

$$CI_{\text{green}} = \rho_{\text{NIR}}/\rho_{\text{green}}-1 \quad (5)$$

$$CI_{\text{red edge}} = \rho_{\text{NIR}}/\rho_{\text{red edge}}-1 \quad (6)$$

CI_{green} was found to be an accurate measure of Chl content *only in leaves that do not contain anthocyanin* (Gitelson et al. 2006). Anthocyanin absorbs in situ around 550 nm (Gitelson et al. 2001); thus, if $\rho_{\lambda 1}$ is located in the green band near 550 nm, the index will be affected by absorption of both anthocyanin and Chl, causing significant overestimation of the Chl content. So, for Chl estimation in anthocyanin-containing leaves, use of the $CI_{\text{red edge}}$ was suggested (Gitelson et al. 2006).

The goal of the current study was to investigate the performance of a reflectance-based nondestructive technique to estimate Chl in grape leaves that may contain anthocyanin and specifically to: (1) identify the optimal position of spectral bands and their widths as they relate to the red-edge chlorophyll index, $CI_{\text{red edge}}$, intended for accurate Chl content estimation; (2) establish the relationship between the $CI_{\text{red edge}}$ and Chl content measured analytically and calibrate the algorithm for Chl estimation; (3) validate the algorithm using an independent data set for three grape cultivars investigated, thus determining the accuracy of Chl-content prediction without re-parameterization (adjustment of the coefficients) after initial calibration; and (4) evaluate the performance of other vegetation indices used to measure Chl in various plant species.

Materials and Methods

Selected grape cultivars. Three grape cultivars were investigated: Edelweiss, St. Croix, and DeChaunac. Edelweiss, a white *Vitis labrusca* cross between Minnesota 78 and Ontario cultivars, was introduced in 1980 and is known as a vigorous vine resistant to foliage diseases and is cold hardy to temperatures of -34°C. St. Croix, a red *Vitis riparia* cross between ES-283 and ES-193, was introduced in 1981 and is a vigorous vine with known resistance to black rot and is cold hardy to temperatures of -35°C. DeChaunac, a red French-American hybrid introduced into Canada in 1946, is a hybrid of Seibel 5163 and Seibel 793. The vine is vigorous and more disease resistant than other French hybrids and is cold hardy to temperatures of -26°C.

Field sampling of leaves. Ninety-three leaves were sampled during three field campaigns: (1) 11 Aug 2005, 31 Edelweiss leaves; (2) 7 Sep 2005, 21 DeChaunac leaves; (3) 7 Oct 2005, 22 St. Croix leaves and 19 Edelweiss leaves. Individual leaves were selected based on various levels of greenness and to ensure a range in color from dark green to yellow. The leaves studied were relatively young, primarily between 10 and 90 days old. Selected leaves were cut from the canopy, immediately sealed in a plastic bag with a small amount of water, and placed in a cooler with ice. When the coloration of the entire leaf was not uniform (as especially occurred during and after veraison), areas of homogeneous pigmentation on each leaf were identified and delineated with a permanent marker.

Reflectance measurements. The spectral reflectance of sampled leaves was measured for each of the three grape cultivars noted above using a clip with a bifurcated fiber optic attached to a USB2000 radiometer and an LS-1 tungsten halogen light source (Ocean Optics, Dunedin, FL). The radiometer uses a charged coupled device to measure radiance with a spectral sampling of ~1.5 nm across 2024 individual spectral channels ranging from 350–1010 nm in wavelength. The instrument has a 12-bit radiometric resolution; thus it records levels of radiance

ranging from 0 to 4095. The light uses a regulated power supply and a tungsten halogen filament bulb burning at 3100 K to output a steady beam of light with a spectral range between 260 and 2500 nm.

The plastic leaf clip, used to position the fiber against individual grapevine leaves, consisted of a black polyvinyl chloride attachment and a 2.3-mm diam bifurcated glass fiber optic. The clip held each leaf at a 60° angle to the fiber to reduce specular reflectance from the leaf surface. The clip also held a black foam background, with a nominal reflectance of 3% within the spectral range of the instrument, which the leaf was placed on during spectral sampling. The low background reflectance minimized extraneous reflected light transmitted through the leaf.

The radiometer was calibrated prior to each data-collection session using a Labsphere Spectralon reference panel (North Sutton, NH) with a nominal reflectance of 99% between 250 and 2500 nm. The reference panel was held tightly against the fiber optic, and a spectral scan was recorded. The sensor was operated using the CALMIT Data Acquisition Program. The reflectance spectrum was calculated as a ratio of leaf radiance to the radiance of the calibration standard at wavelength λ.

Six reflectance measurements were acquired within the marked homogeneous area of each leaf. Locations of the measured spots throughout the entire marked area were carefully superimposed on the leaf to acquire an accurate representation of the marked area reflectance. The average of the six scans per sample was calculated to establish a single representative reflectance spectrum per leaf.

Pigment extraction. After collection of reflectance measurements for each sample, two or three 1-cm diam discs were cut from the marked area using a standard leaf punch. Discs were removed from the same areas on the leaf where reflectance was measured. The punched disks were weighed and ground in an 80% aqueous acetone solution using a mortar and pestle. The tissue was ground until the pulp turned white and all pigments were suspended in the solution. The resulting homogenate was centrifuged in test tubes for 6 min. Absorption spectra of the solution were recorded using a Cary Spectrophotometer (Palo Alto, CA), which was configured to measure absorption of the sample at 1 nm intervals between 400 and 800 nm. Chl a and Chl b as well as carotenoid contents were calculated from the spectra using coefficients described by Porra et al. (1989).

Vegetation indices. The performance of the vegetation indices (VI) was tested using the sampled spectral reflectance from grape leaves, with the results being compared to the Chl contents as determined in the wet lab. The following five vegetation indices were examined:

(1) The simple ratio (SR), developed by Jordan (1969):

$$SR = \rho_{NIR}/\rho_{red} \quad (7)$$

where ρ_{NIR} is reflectance at NIR band and ρ_{red} is reflectance in the red range. The SR uses the reflectance at the red Chl absorption band, referenced to the NIR band to estimate the content of that pigment. Centers of bands at 680 nm (red) and 800 nm (NIR) with width 10 nm were used, as suggested by Blackburn (1998).

(2) The widely used normalized difference vegetation index (NDVI) developed by Rouse et al. (1974):

$$NDVI = (\rho_{NIR}-\rho_{red})/(\rho_{NIR}+\rho_{red}) \quad (8)$$

(3) The enhanced vegetation index (EVI), developed by Huete et al. (1997), was intended to increase sensitivity to moderate to high vegetation density, thus, Chl content:

$$EVI = 2.5\times(\rho_{NIR}-\rho_{red})/(\rho_{NIR} + 6\rho_{red}-7.5\rho_{blue} + 1) \quad (9)$$

where ρ_{blue} is the reflectance in the blue range of the spectrum 470–490 nm.

(4) The red-edge normalized difference vegetation index (red-edge NDVI) was developed to enhance sensitivity to moderate to high Chl (Gitelson and Merzlyak 1994):

$$\text{Red-edge NDVI} = (\rho_{NIR}-\rho_{red\ edge})/(\rho_{NIR}+\rho_{red\ edge}) \quad (10)$$

where $\rho_{red\ edge}$ is the reflectance at the red-edge range 710–720 nm and ρ_{NIR} is reflectance in the range 755–765 nm.

(5) The red-edge chlorophyll index, $CI_{red\ edge}$, described in Eq. 6 with $\rho_{red\ edge}$ in the red-edge range 710–720 nm and ρ_{NIR} in the range 755–765 nm.

The accuracy of Chl estimation and sensitivity of each index to Chl content was assessed by noise equivalent (NE) calculated as:

$$NE\ \Delta Chl = RMSE(VI\ vs.\ Chl)/[d(VI)/d(Chl)] \quad (11)$$

where RMSE(VI vs. Chl) is root mean square error (RMSE) of the relationship between the vegetation index selected (VI) and Chl, and d(VI)/d(Chl) is the first derivative of VI with respect to Chl. Noise equivalent defined in this way allows the direct comparison among different VIs, with different scales and dynamic ranges (Viña and Gitelson 2005).

Calibration and validation. Reflectance spectra and the corresponding analytically measured Chl contents were split into two groups: a calibration and a validation subset. All samples were combined and sorted from low to high Chl contents. Odd-numbered samples were assigned to the calibration subset and even-numbered samples were assigned to the validation subset. $CI_{red\ edge}$ values were calculated from reflectance data. The $CI_{red\ edge}$ from the calibration subset was regressed against the corresponding measured Chl contents to calibrate the algorithm. The algorithm was then used to predict Chl contents with reflectance values from the validation data set. The predicted Chl content was compared to measured Chl content and both RMSE and NE were calculated.

Results and Discussion

Chlorophyll content. Laboratory analytical Chl extraction of 93 leaves yielded a broad range of pigment values, ranging from 3.01 to 515.27 mg m^{-2} (Table 1). The range of Chl was comparable to those observed in other studies (Gitelson and Merzlyak 1994, Sims and Gamon 2002, Richardson et al. 2002).

Reflectance spectra. Leaf reflectance has several specific spectral features (Figure 1). Minimal reflectance values are in the blue range (400–500 nm) where both chlorophylls and carotenoids absorb and in the red range around 670 nm where only Chl absorbs. In the green range (530–600 nm), carotenoids do not absorb and absorption by both chlorophylls (-*a* and -*b*) is minimal but still important, especially in green to dark-green leaves, resulting in a peak of reflectance in the green range. Beyond 680 nm, an increase in reflectance occurred because of a decrease in Chl absorption and an increase in leaf scattering. This increase is quite sharp in leaves with moderate to high Chl (spectra near the bottom of the graph) that absorbs strongly in the red range (~670 nm) and scatters light in the NIR range. In leaves with low Chl (spectra near the top of the graph), light absorption in the red range is not so strong and the slope of increase in reflectance toward longer wavelengths is smaller. In this so-called red-edge range (700–740 nm), reflectance in leaves with different Chl content varies widely. In the NIR range (beyond 750 nm), reflectance reaches maximum values affecting by leaf scattering (i.e., leaf thickness and structure).

Leaf reflectance in the visible range of the spectrum (400–700 nm) decreases with increasing leaf Chl content (Figure 1). Spectra near the top of the graphic represent leaves with low Chl, while spectra near the bottom represent leaves with moderate to high Chl. However, the rate of this decrease is very different in the blue, green, red and red-edge regions (Figure 2), where reflectances in these ranges are plotted versus total Chl content in leaves. In the blue range, reflectance of the yellow leaves is ~20% and declines sharply with an increase in Chl up to 100 mg m^{-2} (slightly green and yellow-green leaves). Then, when Chl increases from 100 to greater than 500 mg m^{-2} and, thus, leaf color changes from yellow-green or slightly-green to dark-green, the blue reflectance remains very low (below 5%) and is virtually insensitive to leaf Chl. In the red range, ~670 nm, reflectance of yellow leaves is ~40%, and then it decreases noticeably with an increase in Chl. However, as Chl exceeds 150 mg m^{-2} (slightly-green and yellow-green leaves), the red reflectance does not change much with further Chl increase, remaining virtually invariant to Chl content above 150 mg m^{-2}. Only reflectances in the green and the red-edge ranges are sensitive to Chl variation in yellow through slightly-green to dark-green leaves (Figure 2). Reflectance in the NIR range is high and varies randomly around 50% mainly because of variation in leaf internal structure and thickness, and it does not depend on Chl content.

Thus, there are common characteristics of the reflectance vs. Chl relationship in grape leaves: minimum sensitivity to Chl content in the blue between 400 and 500 nm and in the NIR; in leaves with moderate-to-high Chl (>200 mg m^{-2}), reflectance in the red region is not sensitive to Chl content; and the highest sensitivity of reflectance to Chl content is in the green from 530 to 590 nm and in the red edge around 700 nm (Figure 2). This finding is in accord with spectral features found in leaves from trees and crops (Chappelle et al. 1992, Gitelson and Merzlyak 1994). The relationship between the green and red-edge reflectance and Chl content was found to be hyperbolic, as is the case with tree leaves (Gitelson et al. 1996). Thus, the reciprocal of reflectance in these spectral bands was quite closely and linearly related to Chl content (not shown).

Model tuning. The optimal bands for use in the three-band model (Eq. 4) are determined by performing a cali-

Table 1 Chlorophyll content and number of samples (N) used in this study.

		Chlorophyll (mg m^{-2})			
Cultivars	**N**	**Min**	**Max**	**Mean**	**Median**
DeChaunac	21	53.52	515.27	235.25	211.26
Edelweiss 1	31	3.01	508.63	218.06	204.55
Edelweiss 2	19	4.25	417.88	178.85	159.47
St. Croix 2	22	5.59	505.47	214.54	180.54

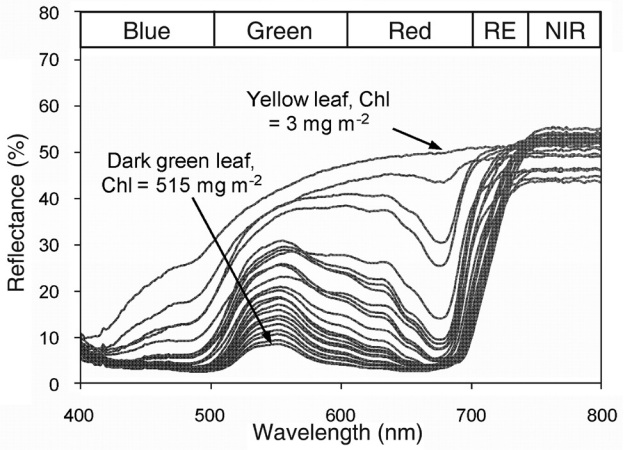


Figure 1 Selected mean reflectance spectra (average of six readings) with chlorophyll content from 3–515 mg m^{-2} of Edelweiss, Saint Croix, and DeChaunac sampled during the 2005 growing season. Top spectrum corresponds to minimal Chl while lowest spectrum corresponds to highest Chl in this data set.

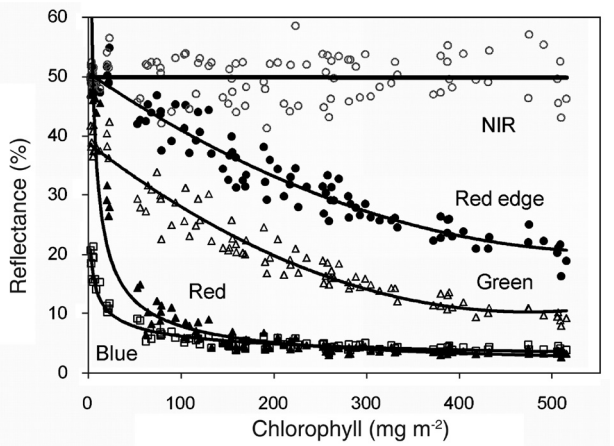


Figure 2 Reflectance of leaves in blue (450 nm), green (550 nm), red (670 nm), red-edge (715 nm), and NIR (760 nm) spectral regions plotted versus leaf Chl content. The highest sensitivity of reflectance to Chl content was in the green and in red-edge ranges of the spectrum.

bration for a continuous range of wavelengths from 400 to 800 nm (isolating one band at a time) and choosing each of the three bands according to a minimal RMSE of Chl estimation in the calibration data set (details in Gitelson et al. 2003, 2006). In grape leaves, this operation identified a wide range between 700 and 740 nm within the red-edge region as being suitable for λ_1 (Figure 3). The band between 760 and 800 nm, located in the NIR region, was the best for λ_2 and λ_3. Thus, the tuning procedure demonstrated that $CI_{red\ edge}$ (Eq. 6), with λ_1 = 700–740 nm and $\lambda_2 = \lambda_3$ = 760–800 nm, has minimal RMSE of Chl estimation and can be used for accurate Chl content determination. Given the commercial availability of inexpensive, quality detectors with bandwidths of 10 nm, a band centered at 715 nm was selected for λ_1 and a 10 nm band centered on 760 nm was chosen for λ_2 and λ_3.

Calibration and validation. $CI_{red\ edge}$ (Eq. 6) was calculated using average reflectances in spectral bands located at 710–720 nm and 755–765 nm for each of 49 reflectance spectra comprising the calibration data set. $CI_{red\ edge}$ was compared with analytically measured Chl content for these 49 leaves. Comparison yielded a linear relationship (Figure 4), with the resulting algorithm:

$$\text{Chl, mg m}^{-2} = 322.26 \times CI_{710\text{-}720;\ 755\text{-}765} + 29.97 \qquad (12)$$

The data fit the line very closely with a determination coefficient $r^2 > 0.96$ ($p < 0.001$) and RMSE of Chl estimation below 28 mg m^{-2}. Thus, the relationship between Chl content and $CI_{red\ edge}$ was established and the algorithm for Chl determination was calibrated (Eq. 12). To verify the algorithm, the validation subset of data was used. Average reflectance values in the bands 710–720 nm and 755–765 nm from the validation subset (44 leaves) were used to calculate predicted Chl content (Chl_{pred}) using Eq. 12. Then, these Chl_{pred} values were compared with analytically measured Chl_{meas} in leaves of the validation subset (Figure 5). The algorithm was capable of accurately predicting Chl content in the range from 3.8 to 506 mg m^{-2} with an RMSE < 29.6 mg m^{-2}.

Importantly, the algorithm (Eq. 12) as developed in this study was used for predicting Chl content in three grape cultivars with no re-parameterization of the coefficients. It shows that the algorithm does not require adjustment of coefficients and cultivar-specific calibration for Chl determination in different cultivars.

Performance of vegetation indices. Performances of the vegetation indices SR, NDVI, EVI, red-edge NDVI, and $CI_{red\ edge}$ in estimating total Chl content in 93 grapes leaves were compared (Figure 6). SR, NDVI, and EVI had a nonlinear asymptotic relationship with Chl. The sensitivity of NDVI and EVI to Chl content drops drastically when Chl exceeds 100 mg m^{-2}. The sensitivity of SR also decreases when Chl exceeds 200 mg m^{-2}. The relationship red-edge NDVI vs. Chl was much more linear with slight decrease in sensitivity to Chl exceeding 400 mg m^{-2}. $CI_{red\ edge}$ displayed a close linear relationship with Chl content, and there was no evidence of saturation by the index within the range of measured Chl content.

To further evaluate the accuracy of each index in Chl estimation, we calculated noise equivalent (NE) values for each index and plotted them against measured Chl

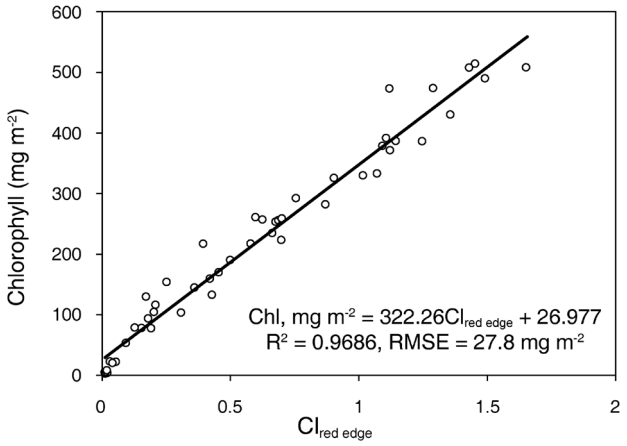


Figure 4 Chl content measured analytically in lab plotted versus $CI_{red\ edge}$ for calibration subset containing 49 leaves. Solid line is best-fit function.

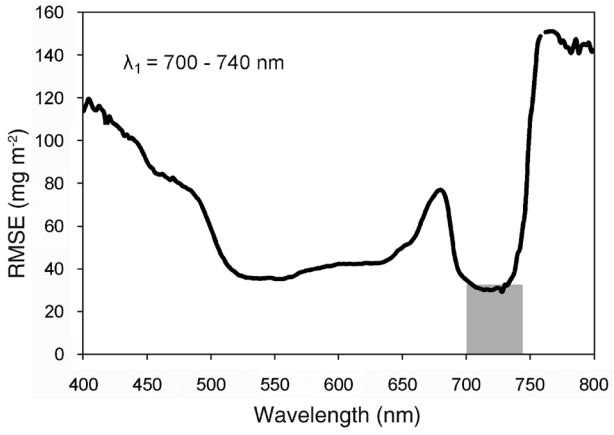


Figure 3 Root mean square error (RMSE) of Chl estimation using the three-band model (Eq. 4) for 49 leaves of the calibration subset indicating optimal location of λ_1 in the red-edge range between 700–740 nm.

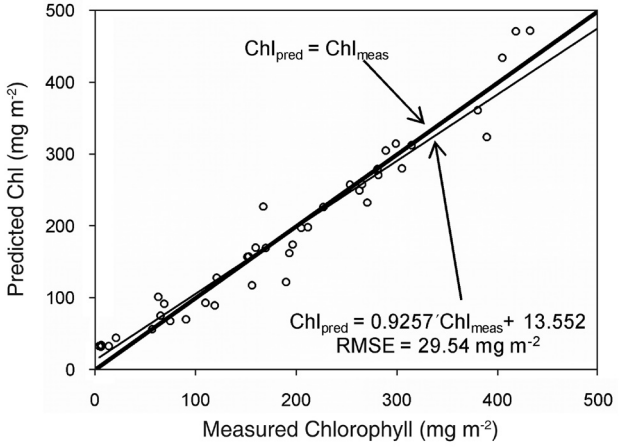
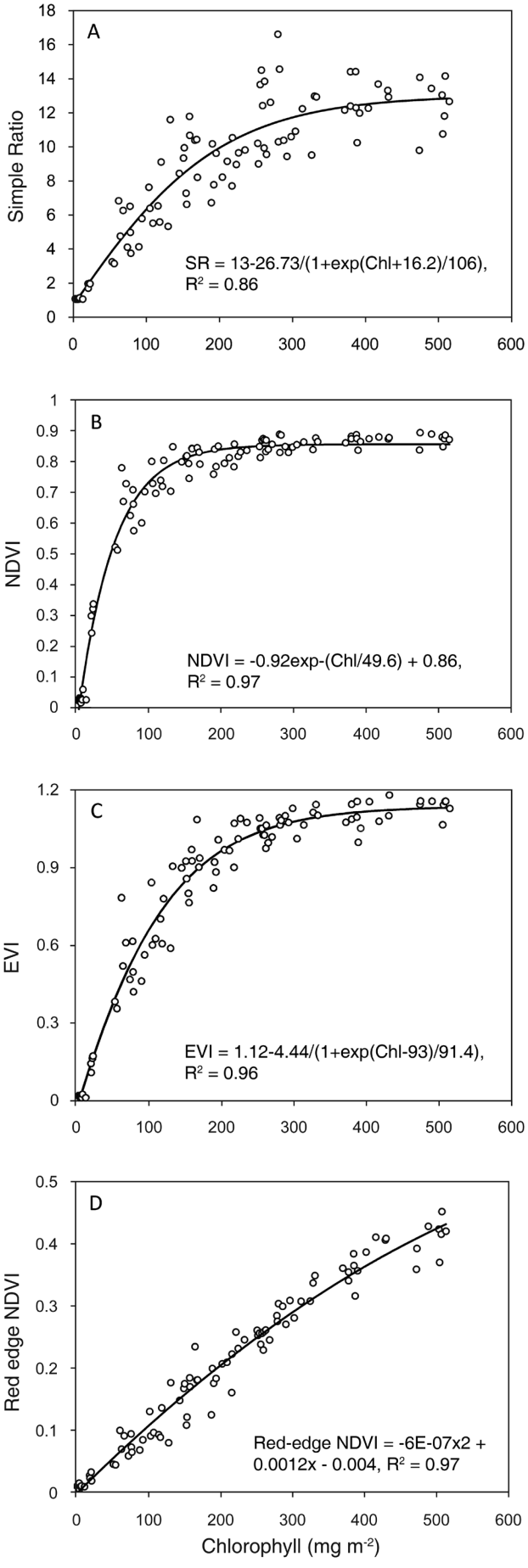


Figure 5 Chl content measured analytically in lab plotted versus Chl predicted by the algorithm (Eq. 12).



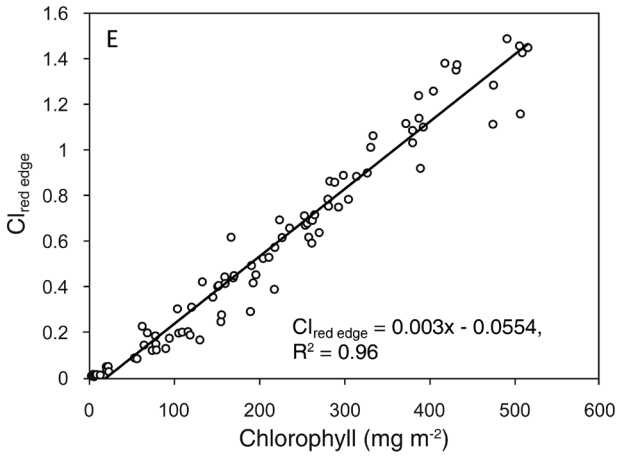


Figure 6 Vegetation indices plotted versus analytically measured Chl in 93 grape leaves, for five indices (**A**–**E**).

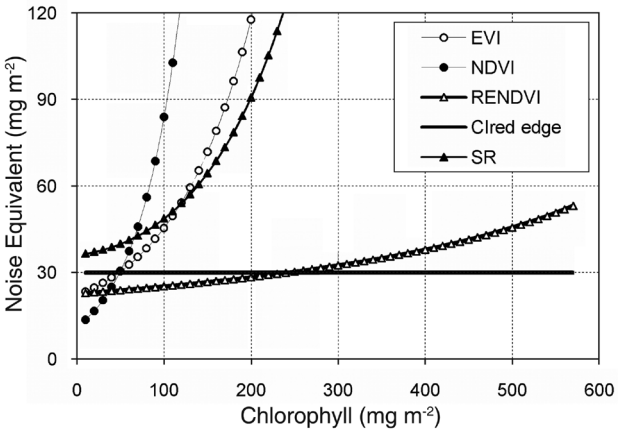


Figure 7 Noise equivalent of vegetation indices SR, NDVI, EVI, red-edge NDVI, and $CI_{red\ edge}$ plotted versus total chlorophyll in 93 grape leaves.

content (Figure 7). At very low Chl content, both NDVI and EVI have NE values of only 10 mg m^{-2} and 25 mg m^{-2}, respectively. However, NE increased exponentially with Chl content, reaching 200 mg m^{-2}, as Chl exceeded 130 mg m^{-2}. SR displayed NE of ~40 mg m^{-2} as Chl is less than 50 mg m^{-2}, but NE increased nearly exponentially exceeding 200 mg m^{-2} when Chl approached 300 mg m^{-2}. The red-edge NDVI showed NE values as low as 20 mg m^{-2} when Chl was less than 200 mg m^{-2} and gradually increased, reaching 45 mg m^{-2} at Chl = 500 mg m^{-2}. Finally, NE of the $CI_{red\ edge}$ has a constant value of 29.95 mg m^{-2} throughout the range of Chl from 3.8–506 mg m^{-2}.

Thus, the red reflectance, used in SR, NDVI, and EVI, is an effective indicator of Chl content below 200 mg m^{-2} (in slightly-green and yellow-green leaves). The high noise associated with SR for moderate-to-high Chl content is caused by extremely low and noisy (<3%) reflectance in the red range. The decline in sensitivity to moderate-to-high Chl content as displayed by NDVI (Eq. 8) is a result of the saturation of the red absorption and the magnitude of NIR reflectance is much higher than that of red reflectance (ρ_{red} <5%, while ρ_{NIR} >40%); so, the NDVI is governed mostly by ρ_{NIR}, which is not affected by Chl content

(Gitelson 2004). For moderate-to-high Chl content, the denominator of EVI (Eq. 9) became practically invariable and insensitive to Chl content. For Chl >200 mg m^{-2}, EVI $\propto (\rho_{NIR} - \rho_{red})$ and is governed mainly by ρ_{NIR}; that is, by leaf scattering and not Chl content.

The red-edge NDVI had much less noise than SR, NDVI, and EVI in the whole range of Chl variation. However, a slight decrease in sensitivity to Chl (increase in NE) could be seen for Chl >400 mg m^{-2}. It remains to be seen how this decline will affect the accuracy of Chl estimation when the pigment content exceeds 500 mg m^{-2}.

Conclusion

$CI_{red\ edge}$ has the lowest amount of noise in the whole range of Chl studied, and the developed algorithm proved to be robust regardless of the data set used and the grape cultivar. The algorithm yielded an RMSE of pigment prediction of less than 30 mg m^{-2} in the independent data set. $CI_{red\ edge}$ was validated with three cultivars of grapes, and it does not appear that the index is cultivar-specific among the tested varieties. Thus, the algorithm is likely to allow accurate Chl determination in *Vitis vinifera* vines.

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