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Calibration of chlorophyll hand-held meter based on vineyard NDVI zones for estimation of leaf N content

Calibração do medidor portátil de clorofila com base em zonas de NDVI do vinhedo para a estimativa do contéudo de N na folha

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ABSTRACT
The monitoring of chlorophyll content in grapevine leaves allows us to evaluate their N status, assisting with the information for the decision make about nutrient rate and application time to the vineyard. The present work aimed to propose an easy-to-use procedure for the calibration of a chlorophyll hand-held meter based on the NDVI homogeneous zones in a vineyard for the device readings and leaf sampling. We evaluated the wine grape 'Chardonnay' growing under drip irrigation in a vineyard located in the Southeast region of Brazil. Readings of the relative chlorophyll indices (*a*, *b*, and total) were taken in situ four times throughout the 2019 growing season, with the device placed on two leaves of every 40 pre-selected target plants in two homogeneous zones of NDVI previously defined in the vineyard. Subsequently, the chlorophyll and leaf nitrogen contents were determined in laboratory to relate them to the chlorophyll meter readings through generalized estimation equations. The chlorophyll meter is capable of estimating the levels of chlorophyll *a*, *b* and total by the models generated with an error of 0.98, 0.58, and 1.47 $\mu g\ ml^{-1}\ cm^{-2}$ for calibration and of 1.03, 0.67, and 1.49 $\mu g\ ml^{-1}\ cm^{-2}$ for prediction, respectively. The functions developed for the leaf N content present calibration error of 1.49 $g\ kg^{-1}$ and prediction error of 3.39 $g\ kg^{-1}$, but capable of providing an estimate when error is less than the amplitude of nitrogen sufficiency.

Index terms: *Vitis vinifera* L.; wine grapevine; drip irrigation; precision viticulture.

RESUMO
O monitoramento do teor de clorofila nas folhas da videira permite a avaliação do estado do nitrogênio nesse orgão da planta, o que pode auxiliar na definição da quantidade e do momento de aplicação deste nutriente ao vinhedo. O presente trabalho teve como objetivo propor um procedimento de fácil utilização para a calibração do medidor de clorofila portátil baseado em zonas de NDVI em um vinhedo para medidas com o equipamento e coleta de amostras de folha. Avaliamos o cultivo da videira para vinho 'Chardonnay' irrigada por gotejamento em um vinhedo localizado no Sudeste do Brasil. As leituras dos índices relativos de clorofila (*a*, *b* e total) foram feitas in situ quatro vezes durante o estádio de maturação do ciclo de produção de uvas em 2019, com o dispositivo colocado em duas folhas de cada 40 plantas pré-selecionadas em duas zonas homogêneas de NDVI previamente definidas. Posteriormente, os teores de clorofila e nitrogênio foram determinados em laboratório para relacioná-los com as leituras do medidor por meio de equações de estimativas generalizadas. O equipamento é capaz de estimar os níveis de clorofila *a*, *b* e total pelos modelos com erro de 0,98, 0,58 e 1,47 $\mu g\ ml^{-1}\ cm^{-2}$ (calibração) e de 1,03, 0,67 e 1,49 $\mu g\ ml^{-1}\ cm^{-2}$ (predição), respectivamente. As funções para o teor de N foliar apresentam erro de calibração de 1,49 $g\ kg^{-1}$ e erro de predição de 3,39 $g\ kg^{-1}$, mas fornecem uma estimativa quando o erro é menor que a amplitude de suficiência de nitrogênio.

Termos para indexação: *Vitis vinifera* L.; videira para vinho; irrigação por gotejamento; viticultura de precisão.

INTRODUCTION

Nitrogen (N) plays critical roles in plant growth and development since it is the most abundant mineral nutrient in plants, serving as a primary constituent of many essential compounds, such as nucleotides, nucleic acids, amino acids, proteins, chlrophyll (Chl), hormones, and secondary metabolites (Keller, 2010; Xu; Fan; Miller, 2012; Arrobas et al., 2014; O'Brien et al., 2016). In C_3 plants, such as grapevine, 75 % of leaf N is located in the chloroplast and a high percentage is associated with the Chl molecules (Lawlor, 2002) because each molecule ($C_{55}H_{72}O_5N_4Mg$) of these photosynthesis pigments contains four N atoms.

In grapevines, N is generally the fourth most abundant element preceded by hydrogen, carbon, and oxygen, although calcium can exceed N in vines grown on calcareous, high pH soils (Keller, 2010). The impact of nitrogen on grape composition and grape quality is often the combination of its direct effect on vine metabolism and indirect effects linked to its strong influence on vigor and yield (Poni et al., 2018). N composition of grape is mainly affected by soil properties and climate conditions within the vineyard, variety, rootstock and agricultural practices such as fertilization, cover crop, disease control, irrigation and leaf removal (Gutiérrez-Gamboa et al., 2020).

Green color meters, also known as Chl meters, are portable devices that provide readings proportional to the levels of chlorophyll *a* (Chl*a*) and chlorophyll *b* (Chl*b*) present in the leaves, based on the signals from receptors (photodiode sensors) which convert photoelectric radiation into analog signals. Chl meters measure leaf absorbance at red and near-infrared wavelengths as chlorophylls strongly absorb the red light. The use of hand-held Chl meters makes possible to estimate both the leaf Chl and N contents through the fitting of models, providing a simple, low cost, fast and non-destructive field measurement, saving time and labor (Parry; Blonquist Jr; Bugbee, 2014; Kalaji et al., 2017; Brito et al., 2011; Rigon et al., 2013; Xiong et al., 2015; Costa et al., 2019; Walker et al., 2021).

Leaf Chl and N concentrations have been found to be directly related to Chl device readings for guava and mango (Shaahan; El-Sayed; El-Nour, 1999), citrus (Jifon; Syvertsen; Whaley, 2005), coffee (Netto et al., 2005; Reis et al., 2009), grapevine (Taskos et al., 2015; Filimon; Rotatu; Filimon, 2016; Yang et al., 2021), corn (Hurtado et al., 2010; Xiong et al., 2015; Kalaji et al., 2017), cotton (Brito et al., 2011; Xiong et al., 2015), papaya (Castro et al., 2014), soybean (Xiong et al., 2015), coconut (Hebbar et al., 2016), wheat (Uddling et al., 2007; Shah; Houborg; McCabe, 2017), tomato (Xiong et al., 2015; Kalaji et al., 2017), rice (Xiong et al., 2015; Zhang et l., 2017), sweet pepper (Padilla et al., 2018), peanut (Xiong et al., 2015) and potato (Uddling et al., 2007), amomg others.

However, the relationship between the device readings and Chl content has been found to vary widely among species, in some cases even within a same species (Uddling et al., 2007; Parry; Blonquist Jr; Bugbee, 2014; Xiong et al., 2015) In grapevine, Shaahan, El-Sayed and El-Nour (1999), Taskos et al. (2015) and Filimon, Rotatu and Filimon (2016) reported a linear relationship between Chl content and device readings. However, different procedures were used. The first authors used twenty readings (each one as an average of 30 measurements) versus leaf Chl and N concentrations determined in laboratory, while the second authors used different N fertilizer rates (0, 60 and 120 kg N ha^{-1}) to fit an equation. The last-mentioned authors sampled leaves for Chl and N analysis and made devices readings throughout the growing season.

Leaf chlorophyll content decreases after flowering as the leaves age, but increasing N rate application can reduce the rate of chlorophyll degradation and delayed leaf senescence. Difference in total leaf chlorophyll content as consequence of application of different N rates reached 24% after veraison (Keller; Kummer; Vasconcelos, 2001).

Precision viticulture requires the characterization of the spatio-temporal variability of the vineyard status to design the appropriate management for each area (Rey-Caramés et al., 2016). These authors reported that the assessment of the spatio-temporal variability of key vegetative components, such as leaf Chl and N contents, is of great importance to carry out a well-founded and differentiated vegetative management of the vineyard for precision agriculture purposes.

In this sense, the present work aimed to propose a procedure to define calibration and validation of models using homogeneous zones delimited from normalized difference vegetation index (NDVI) for choosing points in the vineyard to make readings with a hand-held Chl meter and sampling leaves to determination of Chl and N contents in laboratory. As far as we know, there are no published data analyzing calibration and validation of models of hand-held Chl meter based on vineyard's spatial variability.

MATERIAL AND METHODS

Experimental site

The study was carried out in the municipality of Espirito Santo do Pinhal, state of São Paulo, Brazil (22° 10' 49.1" S, 46° 44' 28.4" W, altitude 875 m). Grapevine (*Vitis vinifera* L.) 'Chardonnay' grafted onto 'Paulsen 1103' (*Vitis berlandieri* x *Vitis rupestres*) rootstock was planted in 2008 in a spacing of 2.5 m between rows and 1.0 m between plants in two contiguous areas, labeled 1 and 2 (Figure 1) with 0.6 and 0.5 ha, respectively. Soil in area 1 was categorized according to the U.S. Soil Taxonomy (Soil Survey Staff, 2014) as a complex of Inceptisols and Entisols (Orthents) while soil in area 2 was classified as an association of the same soil types (Oldoni et al., 2021). The soil layers of 0-0.2 m and 0.2-0.4 m presented, respectively, 379 and 441g kg^{-1} of clay; 125 and 129 g kg^{-1} silt; 494 and 430 g kg^{-1} sand.

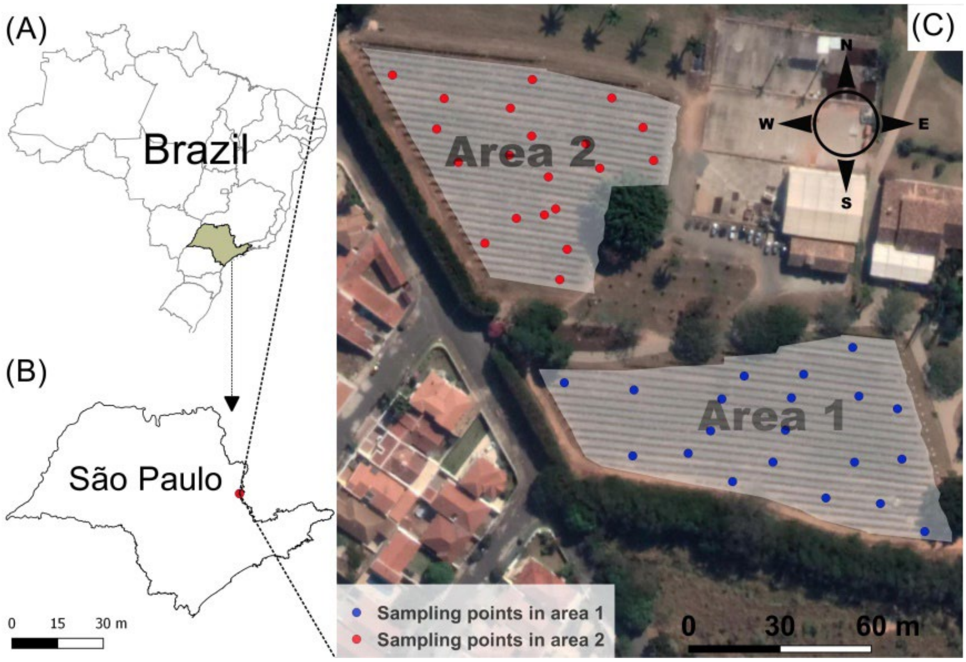


Figure 1: Location of the study area in Brazil (A), in the state of São Paulo (B) and arrangement of the vineyard in area 1 and area 2 (C).

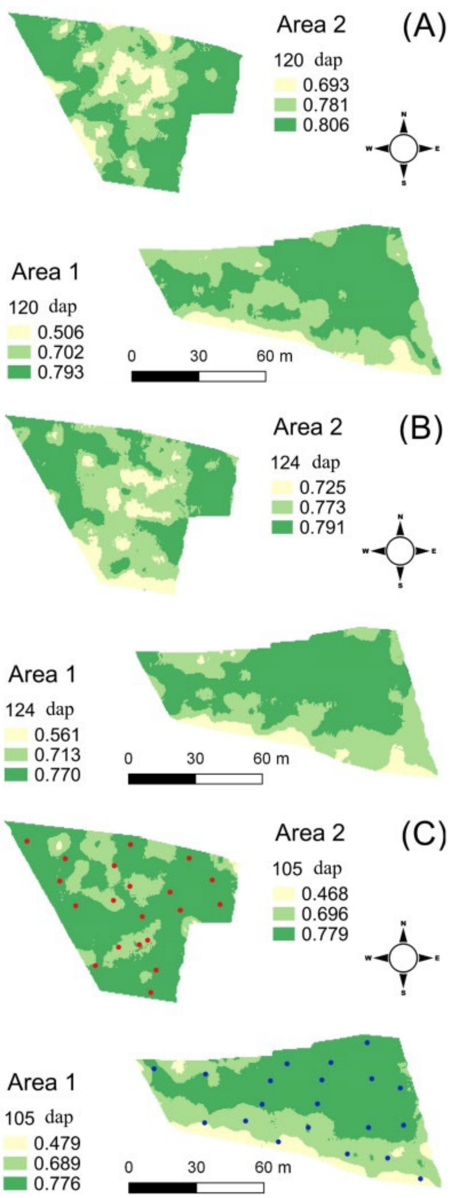
Plants were grown in a vertical trellis system (east-west oriented) and trained on a unilateral Royat cordon. Annual double pruning (Favero et al., 2011; Souza et al., 2015; Dias et al., 2017) was performed in this vineyard, with plant formation pruning carried out in July and August (winter) and pruning performed in January (summer) for grape production. Plants were irrigated using a drip system, with one lateral line for each row of vines and one emitter every 0.5 m (two emitters per vine) with a mean flow rate of 1.8 and 2.0 L h^{-1} in areas 1 and 2, respectively. In 2019 gowing season, fertigation was performed in sprouting phase, applying only ammonium nitrate fertilizer at 2, 3, 11 and 23 days after prunig (dap) at a rate of 12.6, 36.0, 36.0 and 36.0 $kg.ha^{-1}$, respectively.

According to Köppen's classification, the local climate is Cwa, which refers to humid subtropical climates with dry winters and hot summers (Alvares et al., 2013).

Homogeneous zones

The spatial distribution of the normalized difference vegetation index (NDVI) in this vineyard was determined by Oldoni et al. (2021) in 2017 and 2018 growing seasons (Figure 2 A, B), and those authors reported that it was positively correlated with vine's vegetative vigor. In that ocasion, a Crop Circle ACS-430 active canopy sensor was used to collect reflectance data on wavelengths of 670 (red) and 780 nm (near infrared) for the determination of NDVI. The assessments were made by walking through all the vines in the vineyard with a view from the lowest point at a height of ~ 0.30 m from the top of the canopy with ten acquisitions per second. This height provided a bean width of ~ 0.25 m (calculated based on ~ 0.82 times the distance between the sensor and the target for a field-of-view ~ 40-45 degrees, according to the ACS-430 manufacturer's manual) projected parallel to the plant row. Rover and base GNSS (Global Navigation Satellite System) receivers were used for georeferencing reflectance data, which enabled increased real-time positioning accuracy using the RTK (Real Time Kinematic) method.

Descriptive statistical analysis was performed, and outliers (mean ± three times the standard deviation) were removed from the NDVI data set of each measurement. Subsequently, geostatistical tools were used to interpolate the NDVI data, generated by automated semivariogram modeling and kriging within a moving window procedure in 5 × 5 m blocks and a moving window of, at most, 200 neighboring points for calculating experimental semivariograms, which were automatically adjusted using the exponential model. Geostatistical procedures were performed using the Vesper software (Variogram Estimation and Spatial Prediction Plus Error, version 1.62).



Red and blue points are the poitns of leaf sampling and device readings.

Figure 2: Spatial distribution of the normalized difference vegetation index (NDVI) measured at 120, 124 and 105 days after pruning (dap), respectively in 2017 (A), 2018 (B) and 2019 (C) growing seasons of grapevine 'Chardonnay'.

The interpolated NDVI data were classified into three classes of values using the Jenks natural breaks optimization method. The class with the highest value of NDVI was considered as a high NDVI zone, and the two remaining classes with lower values were considered as low NDVI zones due to the low territorial extension of the class with the lowest values.

In 2019, measuremnts of NDVI were also performed at 105 dap following the procedure described by Oldoni et al. (2021) in the two earlier years. Vines maintained the NDVI distribution pattern after the ripening phase in 2019 (Figure 2C) as previously observed. Thus, the NDVI zones guided the choice of locations for data collection in the 2019 growing season.

Hand-held device readings and laboratory analysis

Readings with the hand-held chlorophyll meter ClorofiLOG CFL 1030 (Falker, Porto Alegre, Brazil) were performed on 40 plants in 2019 (Figure 1 C) on February 7 (31 dap - flowering), February 22 (46 dap - lead-shot sized berries), March 21 (73 dap - pea-sized berries) and April 22 (105 dap - begining of maturation or veraison), following the Baggiolini scale (1952). In each plant, two fully expanded leaves were sellected, without any evidence of pest or disease attack, and readings were performed at two points on the adaxial face of each leaf. Subsequently, the two leaves were sampled and two discs with a diameter of 1.55 cm were collected from one leaf of each plant. Samples were put inside conical plastic tubes containing 2 mL of 96% ethanol (v v^{-1}) and stored in a thermal bag to protect against light and heat. Leaves were then stored in paper bags.

The determination of the total chlorophyll content (*a*+*b*) was performed by the spectrophotometric method (Eijckelhoff; Dekker, 1997). The collected discs were macerated in 2 mL of 96% ethanol (v v^{-1}) in a mortar, under low light conditions, as described by Aidar et al. (2010). The extracts obtained were stored in conical tubes and centrifuged at 3,000 rpm for 5 minutes. The supernatant was analyzed in a Biotek Epoch2 microplate spectrophotometer at wavelengths 649 and 665 nm.

The extinction coefficients (Equations 1 and 2) were used for the quantification of pigments as reported by Lichtenthaler and Wellburn (1983):

$$\mathrm{Chl}a = 13.95\ .\ A_{665} - 6.88\ .\ A_{649} \quad (1)$$

$$\mathrm{Chl}b = 24.96\ .\ A_{649} - 7.32\ .\ A_{665} \quad (2)$$

where: Chl*a* and Chl*b* are, respectively, content (µg ml^{-1}) of chlorophyll *a* and *b*, and A_{665} and A_{649} are, respectively, the absorbance values measured at the wavelengths of 665 and 649 nm.

Chl*a* and Chl*b* were determined based on the leaf disk area (LD*a*, cm^2) and considering the volume of ethanol plus macered leaf disk (V*e*, mL) used in the extraction. Thus, the results obtained were expressed in μg.mL.cm^{-2} by Equations 3 and 4:

$$\mathrm{Chl}a = (\mathrm{Chl}a \ . \ \mathrm{V}e) \ / \ \mathrm{LD}a \quad (3)$$

$$\mathrm{Chl}b = (\mathrm{Chl}b \ . \ \mathrm{V}e) \ / \ \mathrm{LD}a \quad (4)$$

The two sampled leaves from each plant were used to calibrate the chlorophyll meter for leaf nitrogen content. Among the 40 plants sampled per date (160 in total), nitrogen contents were determined in 10 plants per date (40 in total). Leaves were selected based on the relative chlorophyll index (RCI) provided by the hand-held meter, i. e., leaves with the five highest and the five lowest values of each sampling date were selected and dried in a forced air oven at 65°C, grinded in a knife mill and passed through a 2 mm mesh sieve. Samples were weighed on an analytical balance (0.00001 g) in aliquots of approximately 0.0095 g and then encapsulated with tin sheets.

A carbon, hydrogen and nitrogen elemental analyzer model Perkin-Elmer CHN 2400 was used to determine leaf nitrogen contents using the procedure described by Merlini et al. (2017). Samples were decomposed in oxygen-rich cells with high temperature generating gases which were dragged by helium gas to the thermal conductivity conductor for determination of N content (%), within a detection limit of <0.03%.

Calibration and validation procedures

RCI values were related to the absolute N contents using the method of generalized estimating equations (GEE), proposed by Zeger and Liang (1986) and Liang and Zeger (1986), to estimating regression parameters, especially when data are autocorrelated. Models for the prediction of chlorophyll *a*, chlorophyll *b* and total (*a*+*b*) were fitted with linear, quadratic and interaction effects and the best models were selected. The Gaussian family and the AR (1) first-order autoregressive covariance structure were used in the GEE models.

Calibration models were developed from the data obtained in Area 1, and their validation was performed using data belonging to Area 2. As in the calibration for chlorophyll, all the aforementioned statistical analyzes were used in the calibration of the hand-held chlorophyll meter for leaf nitrogen content. Analyzes was performed using R software version 4.03 (R Core Team, 2021) and the "geepack" package developed by Højsgaard, Halekoh and Yan (2006).

RESULTS AND DISCUSSION

Calibration of hand-held chlorophyll meter to estimation of leaf chlorophyll content

The best adjusted models for predicting the content of leaf pigments in 'Chardonay' grapevine are presented in Table 1.

Table 1: Models for prediciting chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*) and total chlorophyll (Chl*t*) contents adjusted as function of chlorophyll relative indice (RCI) of Chl*a* and Chl*b* provided by the hand-held chlorophyll meter.

Model	R^2	RMSEP
Chl*a* = 1.893890 - 0.108625 Chl*a* + 0.007809 Chl*a*²	0.75	1.03
Chl*b* = -0.4544 + 0.3124 Chl*b*	0.67	0.67
Chl*t* = 7.551 - 0.93761 Chl*a* + 0.01137 Chl*a*² + 3.1296 Chl*b* - 0.11173 Chl*b*²	0.76	1.49

R^2: determination of coefficient; RMSEP: root mean square error of prediction.

Among the models obtained for predicting the chlorophyll contents (Table 2), only the model generated for the estimation of chlorophyll *b* showed linear behavior and absence of interaction.

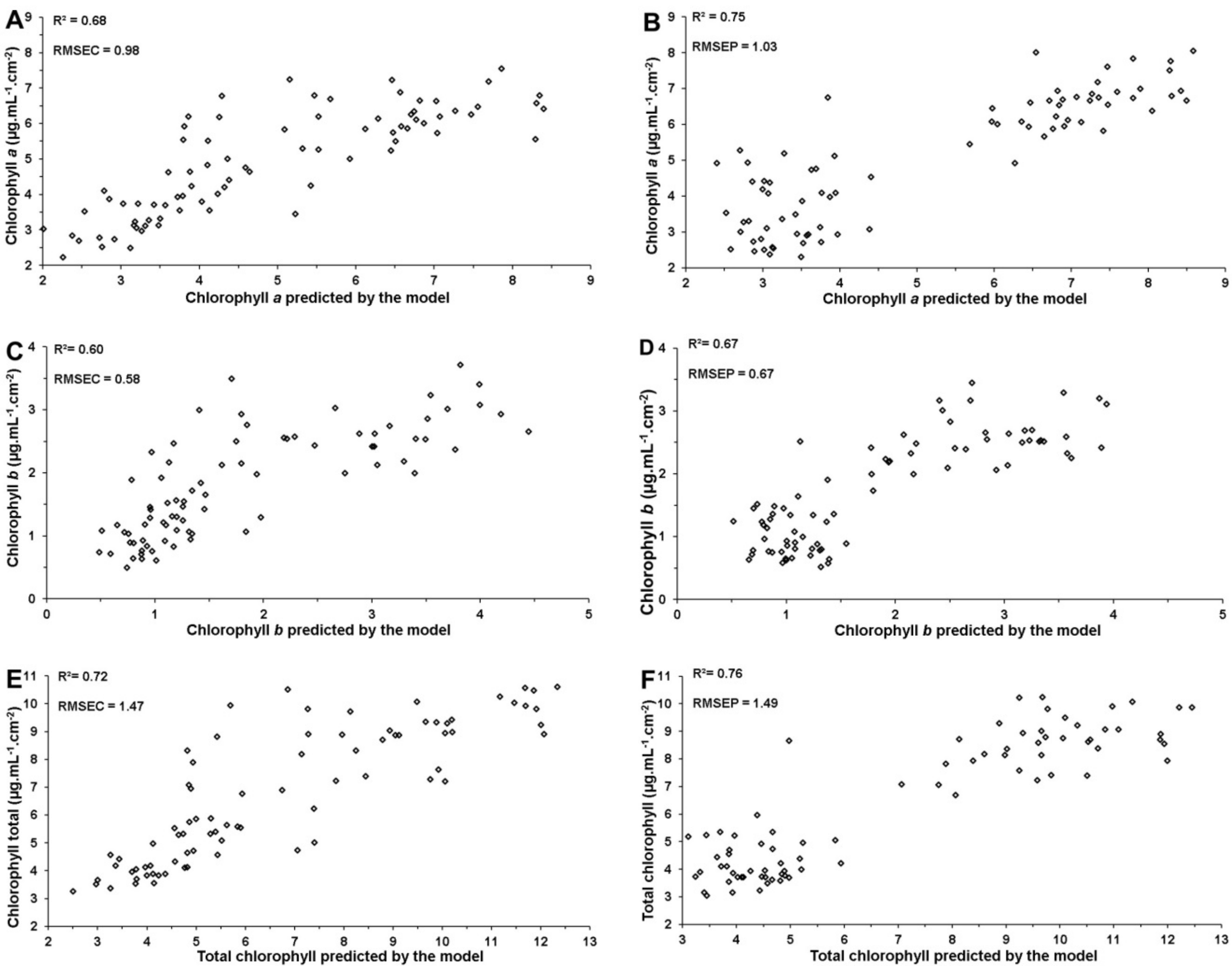
Table 2: Analysis of variance of the generalized estimation models, with AR(1) covariance structure and Gaussian family, adjusted as a function of relative chlorophyll (Chl) indices (RCI) provided by the hand-held chlorophyll meter.

Source of variation	Freedom degree	Chi square	p-valor
Chlorophyll *a*			
Chl*a*	1	190	< 0.001
Chl*a*²	1	3	0.082
Chlorophyll *b*			
Chl*b*	1	105	< 0.001
Total Chlorophyll			
Chl*a*	1	153.451	< 0.001
Chl*a*²	1	7.243	0.007
Chl*b*	1	8.699	0.003
Chl*b*²	1	3.471	0.063

Similarly to results described on Table 2, Filimon, Rotaru and Filimon (2016) obtained linear equations when assessing chlorophyll contents by non-destructive methods with the CCM-200 plus (Opti-Sciences, Tyngsboro, Mass).

The quadratic behavior of both models generated for the determination of Chl*a* and Chl*t* may be related to the greater contribution of the first pigment relation to the total value (Table 2). Chlorophylls are components of chloroplast membranes and occur in an approximately Chl*a* and Chl*b* ratio of 3:1 (Lichtenthaler et al., 1981). Photosystems (PS) I and II have Chl*a* and Chl*b* ratios of 4:1 and 1:2, respectively, and are responsible for 50 to 60% of the Chl*t* (Hopkins; Hüner, 2009). The degree of influence of each component in the model can be understood by observing the chi-square parameter (χ^2). Higher χ^2, higher is the influence the component in the model. In Chl*t* model the components that have the greatest influence are Chl*a* and Chl*b* followed by their respective quadratic effects. The effects are significantive since the probability values (p-value) are smaller than the estipulated significance level (0.05). However, in some models there are effects with probability at the limit of significance and for this reason it was decided to keep them in the model (Table 2).

The behavior of the calibration (Figure 3A, C and E) and validation models (Figure 3B, D and F) of leaf chlorophyll levels is explained by using a non-destructive method. The models presented for the chlorophyll contents showed a coefficient of determination of 76%, 75% and 67% for Chl*t*, Chl*a* and Chl*b*, respectively (Figure 3F, B and D).



R^2: coefficient of determination; RMSEC: root mean of square error of calibration; RMSEP: root mean of square error of predicting

Figure 3: Models of calibration and validation for predicting chlorophyll *a*, clhorophyll *b* and total chlorophyll contents in leaves from grapevines 'Chardonnay' based on relative chlorophyll indices (RCI) of chlorophyll *a* and *b* provided by the hand-held meter.

The determination of total chlorophyll is the sum of chlorophylls *a* and *b*, and its validation (Figure 3F) tends to present greater similarity with the major component´s validation, i.e., chlorophyll a (Figure 3B). In both figures the quadratic effect in those equations is observed too. A remarkable increase in similar proportions of R^2 and RMSE (RMSEC and RMSEP) can be seen in Figures 3A, B, E and F. R^2 values of calibration equations were 0.68 and 0.72, and those from validation equations were 0.75 and 0.76 for Chl*a* and Chl*t*, respectively. Similarly, RMSE values of calibration equations (0.98 and 1.47) and validation equations (1.03 and 1.49) were closed for chlorophyll *a* and total chlorophyll.

Calibration of SPAD-502 meter for predicting total chlorophyll in vines 'Cabernet Sauvignon' presented R^2 values ranging from 79% to 57% (Taskos et al., 2015), while a R^2 value of 92% for the calibration of CCM-200 Plus meter for table grapevines 'Gelu', 'Milcov', 'Cetinouia' and 'Napoca' (Filimon; Rotaru; Filimon, 2016). Model presented herein (Figure 3F) was developed with data presenting great variation due to the increase of leaf chlorophyll content throughout the growing season, differently from both reports previouly mentioned. Consequently, there was an increase in the distance between the trend lines (model equations) and the data generated by analysis. As the RCI (device readings) and the chlorophyll contents increase, the observed points moved away from the models, resulting in a gradual increase of prediction error. This data behavior is linked to changes in leaf tissue due to the grapevine's development. Leaf chlorophyll levels in grapevines reach their maximum concentration around the fifth or sixth week after bud burst, remaining practically unchanged until the beginning of senescence, when there is a gradual reduction of pigments (Keller, 2010). Even though our model is capable of predicting the total chlorophyll content in the leaves of grapevines 'Chardonnay'.

However, Costa et al. (2019) observed in the same Chardonnay vineyard that the chlorophyll content increased until the last evaluation at 120 dap in 2017 season, when maturation was completed. This divergent behavior can be explained by the climatic condition in which the vines evaluated by Costa et al. (2019) were exposed, since it is a tropical climate with high solar incidence during almost the entire growing season.

Both SPAD-502 and CCM-200 devices calibrated for predicting chlorophyll *a*, *b* and total chlrophyll presented a similar behavior as reported in this study, with estimates showing less precision as chlorophyll values increased (Richardson; Duigan; Berlyn, 2002). Calibration of ClorofiLog CFL 1030 device for use in soybean (*Glycine max* L.) crop presented similar error pattern (Rigon et al., 2016). Three devices' calibration (SPAD-502, atLEAF+, and MC-100) for predicting total chlorophyll content in two RCI ranges (0-40 and 40-80) in sweet pepper (*Capsicum annuum* L.) crop showed a drastic reduction in R^2 values (29-54% to 84-94%) and an increase in prediction errors (Padilla et al., 2018).

Furthermore, there is a tendency for the device to overestimate chlorophyll levels when measured on thicker leaves (Netto et al., 2005; Jifon; Syvertsen; Whaley, 2005). Grapevine leaves undergoing morphological changes as development proceeds become increasingly thicker until their maturity (Koundouras et al., 2009). A supposed interference of leaf thickness on chlrophyll measurements was reported in the evaluation of nitrogen productivity in vines 'Cabernet Sauvignon' and 'Xinomavro', since RCI did not follow the variations in chlorophyll contents determined in the laboratory (Taskos et al., 2015).

Another possible explanation is based on the distribution of chlorophyll levels in leaves with high pigment concentrations, which are less uniform when compared to leaves with low chlorophyll content (Terashima; Saeki, 1983). Absorbance meters (chlorophyll meters) have shown poor performance in estimating high levels of chlorophyll due to the relationship between the irregular distribution of chlorophyll and the 'sieve effect' (Monje; Bugbee, 1992).

A third cause of change in the data behavior is that at high levels of chlorophyll, much of the light (660 mm) is absorbed by the leaf leaving few photons to be measured by the device sensor, and therefore longer wavelengths could be used in order to improve the sensitivity of these portable devices (Richardson; Duigan; Berlyn, 2002).

In contrast, a linear pattern (R^2 equal to 0.37) was obtained with SPAD-502 calibration for RCI and leaf nitrogen content in grapevine 'Narince' (Dilek; Sabir, 2016). Linear relationships were also reported for rice (Zhang et al., 2017) and coconut (Hebbar et al., 2016) when comparing the responses of different cultivars to nitrogen fertilization. Similarly to our study, a quadratic pattern was found in the calibration of atLEAF+ device for the prediction of leaf nitrogen in sweet pepper crop (Padilla et al., 2018).

Taskos et al. (2015) found similar behaviors for SPAD-502 calibration with R^2 ranging from 0.44 to 0.74 and 0.15 to 0.52, respectively, for grapevines 'Cabernet Sauvignon' and 'Xinomavro'. It is worth mentioning that the models presented by those authors were not validated as in the present study.

Calibration of hand-held chlorophyll meter to leaf nitrogen estimative

Equation 5 about leaf N (R^2 equal to 0.26; RMSEP equal to 3.39) presented the best fit to predicting leaf nitrogen content in grapevines 'Chardonnay' as function of RCI of Chl*a* and Chl*b* (device readings) provided by the hand-held chlorophyll meter:

$$\text{Leaf N} = 59.355 - 4.1333*(\text{Chl}a) + 0.0686*(\text{Chl}a)^2 + + 6.1294*(\text{Chl}b) - 0.3079*(\text{Chl}b)^2 \quad (5)$$

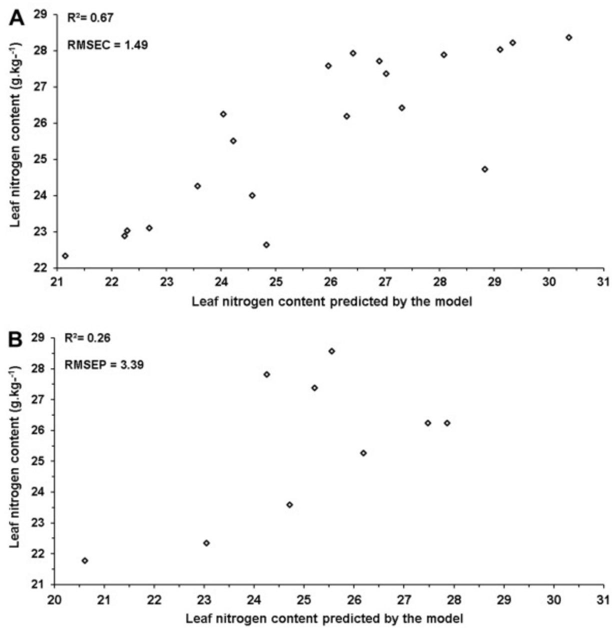
The behavior of the data was better represented by an ascending quadratic function (Table 3). The existing significance in the model components that represent the relationship between the variables is confirmed by means of the significative p-value, which is lower than the stipulated significance level (0.05), with the exception of the components $(\text{Chl}a)^2$ and Chl*b*. However, some components present probability at the limit of significance and for this reason it was decided to keep them in the model.

Table 3: Analysis of variance of the generalized estimation models, with AR(1) covariance structure and Gaussian family, adjusted as a function of leaf total chlorophyll (Chl) and nitrogen contents.

Source of variation	Freedom degree	Chi square	p-valor
Chl*a*	1	27.26	<0.001
Chl*a*²	1	0.09	0.77
Chl*b*	1	0.09	0.77
Chl*b*²	1	16.35	<0.001

The prediction model of leaf nitrogen content from the RCI (device readings) of Chl*a* and Chl*b* (Figure 4) presented R^2 value of 0.67 and 0.26 for calibration and validation equations, respectively, demonstrating is able to explain only 26% of the data variability. Chlorophyll measurements in 'Chardonnay' grapevine leaves by hand-held meter (SPAD 502) showed a moderately strong positive correlation to leaf N content in December, January and March of 2017/2018 growing season with R^2 values varying from 0.60 to 0.74, but the seasonal correlation was fairly weak ($R^2 < 0.45$). In December and February of 2018/2019 growing season these R^2 values decreased to 0.00 to 0.14, respectively (Walker et al., 2021).

By the other hand, values of RMSEC (1.49) and RMSEP (3.39) were the best results for the calibration and validation equations (Figure 4), and the difference between them demonstrate the need for model validation, since calibration alone is not enough to attest to its precision and accuracy. In respect of practical use of model, it is possible to affirm the existence of a certain degree of limitation. Brunetto et al. (2012), Taskos et al. (2015) and Walker et al. (2021) made the same recommendation. Depending on the amplitude of nutritional sufficiency adopted, the model may present an error (RMSEP) greater than the difference between the maximum and minimum limits. Even in this scenario, the model can be used as long as its error is considered, providing users with a good estimate of the vineyard's nitrogen status.



R^2: determination of coefficient; RMSEC: root mean square error of calibration; RMSEP: root mean square error of prediction

Figure 4: Models of calibration and validation for predicting leaf nitrogen content in grapevine 'Chardonnay' based on the relative chlorophyll indices (RCI) provided by the hand-held chlorophyll meter.

Nevertheless, there is a diverse information available in literature about the level of nitrogen sufficiency in grapevines: 25 to 27 g kg^{-1} (Malavolta; Vitti; Oliveira, 1997); 30 to 35 g.kg^{-1} (Terra et al., 2003); 20 and 23 g kg^{-1} (Spring; Verdenal, 2017); and 22.5 to 27.5 g kg^{-1} (Stefanello et al., 2021).

The high complexity existing in the relationship between nitrogen and the environment makes very difficult the understanding through a single mathematical model, thus requiring periodic modeling (Taskos et al., 2015). Grapevines accumulate the nutrients absorbed in one growing season and use them in the next one (Brunetto et al., 2006). About 33% of the nitrogen found in the biomass of branches and bunches are mobilized from the existing reserves in permanent structures of grapevines, predominantly from the roots (Roubelakis-Angelakis; Kliewer, 1992).

CONCLUSIONS

We have proposed a procedure for define calibration and validation equations of hand-held Chl meter based on spatial variabilty of NDVI in a drip irrigated vineyard of 'Chardonay' in Southeastern Brazil. The device is capable of estimating the levels of Chl *a*, *b* and total with a coefficient of determination of 0.68, 0.60, and 0.72 for calibration model, and 0.75, 0.67, and 0.76 for prediction model, respectively. Additionally, an error of 0.98, 0.58, and 1.47 μg ml^{-1} cm^{-2} for calibration model, and 1.03, 0.67, and 1.49 μg ml^{-1} cm^{-2} for prediction model, respectively, were found. As for the leaf N content, coefficient of determination was 0.67 for prediction model and 0.26 for calibration model, while error were 1.49 and 3.39 g kg^{-1}, respectively. Even in the face of its limitation hand-held meter is able to provide to the user the estimation of the leaf N status especially when operate at greater amplitudes of N sufficency.

AUTHOR CONTIBUTIONS

Conceptual idea: Silva, T.M.M.; Bassoi, L.H.; Methodology design: Silva, T.M.M.; Costa, B.R.S.; Oldoni, H.; Bassoi, L.H.; Data collection: Silva, T.M.M.; Costa, B.R.S.; Oldoni, H.; Bassoi, L.H.; Data analysis and interpretation: Silva, T.M.M.; Costa, B.R.S.; Oldoni, H.; Mitsuyuki, M.C.; Bassoi, L.H.; and Writing and editting: Silva, T.M.M.; Costa, B.R.S.; Oldoni, H.; Mitsuyuki, M.C.; Bassoi, L.H.

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