Engenharia Agrícola

ISSN: 1809-4430 (on-line)

www.engenhariaagricola.org.br

Doi: http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v39nep74-84/2019

Special Issue: Precision Agriculture

TEMPORAL VARIATION AND SPATIAL DISTRIBUTION OF RELATIVE INDICES OF LEAF CHLOROPHYLL IN GRAPEVINE cv. CHARDONNAY

Bruno R. S. Costa[1], Henrique Oldoni[1], Wendell A. Silva[1], Roberto L. Martins[1], Luís H. Bassoi[2*]

[2*]Corresponding author. Embrapa Instrumentação/ São Carlos - SP, Brasil.
E-mail: luis.bassoi@embrapa.br | ORCID ID: https://orcid.org/0000-0001-9469-8953

KEYWORDS

Vitis vinifera L., photosynthetic pigments, portable meter.

ABSTRACT

Variation in the spatial distribution of leaf chlorophyll content associated with the progression of the phenological cycle of crops may occur in cultivated areas as a result of the variability of environmental conditions and of the intrinsic properties of the plants. The objective of the present study was to model the trend in variation and assess the temporal stability of index of chlorophyll a, b, and total chlorophyll (Chl_a, Chl_b, and Chl_t, respectively), and to characterize the spatial distribution of Chl_t index in grapevine cv. 'Chardonnay'. The assessments consisted of *in situ* measurements made with a portable meter in a commercial vineyard located in the municipality of Espírito Santo do Pinhal, state of São Paulo, Brazil, in the period between flowering and fruit maturation. Descriptive statistics were applied to the indices and regression models were fitted to ascertain the relationship of their mean variation with time. The temporal stability of Chl_t index was estimated using Spearman's rank correlation analysis and thematic maps were created using geostatistical analysis and spatial estimation by ordinary kriging. The Chl_b and Chl_t indices were non-linearly associated with cycle progression and their decrease after the start of maturation was estimated. The temporal stability of the Chl_t index was low and variation in its spatial distribution was observed over the assessed period.

INTRODUCTION

Chlorophylls are green pigments specialized for the absorption of sunlight, mostly in the blue and red regions of the visible spectrum used by plants to convert into chemical energy. In addition to chlorophyll a, other pigments are involved in photosynthesis, including chlorophyll b, carotenoids, and phycobilins, the latter three known as accessory pigments. Although chlorophylls are the most abundant pigments in plants, they undergo degradation as a result of environmental factors (light, solar radiation, and air temperature) and intrinsic factors such as metabolization during leaf senescence (Streit et al., 2005).

Chlorophyll content gives a measure of leaf nitrogen because there is a close association between the pigment and the nutrient. Moreover, considering that chlorophyll concentration varies with the stages of plant development, its estimation in the field in different phenological stages of a crop may give the farmer information about the nutritional status of the plants that allows them to make decisions related to the monitoring and management of agricultural activities (Costa et al., 2015; Elarab et al., 2015, Arantes et al., 2016). In addition, the monitoring of chlorophyll may be used as an indication of the plants' water status (Silva et al., 2014). Thus, considering the importance of photosynthetic pigments in cultivated species and its relationship with the remaining production factors, the measurement of its content may be considered as relevant information to support crop management.

The quantification of chlorophyll content is usually performed using destructive methods such as UV and visible spectroscopy and high performance liquid chromatography, which require the conversion of leaf extract absorbance to values of pigment concentration through the use of standard published equations (Richardson et al., 2002; Prado-Cabrero et al., 2016). These are time-consuming, laborious methods that depend on sturdy equipment; in addition, they destroy the

[1] FCA UNESP/ Botucatu - SP, Brasil

Area Editor: Fabio Henrique Rojo Baio
Received in: 3-7-2019
Accepted in: 6-7-2019

available leaf samples, thereby precluding consecutive evaluations of the same plant material (Dey et al., 2016).

However, there is the alternative of non-destructive optical methods. They are based on the use of hand-held meters that give immediate readings of the intensity of the green color of the leaves that are related to relative leaf chlorophyll content. This method allows repeated evaluations in the same site, to perform a larger number of measurements, and it simplifies the study of pigment dynamics throughout the crop cycle. Moreover, intensive monitoring using these instruments enables the characterization of spatial variability of chlorophyll content in cultivated areas.

The use of instruments and sensors that allow the intensive and continuous monitoring of variables of interest associated with spatial analysis techniques allow the adoption of precision agriculture (PA). In this context, the use of hand-held meters to determine leaf chlorophyll content and its relationship with leaf nitrogen content was shown by Taskos et al. (2015). This demonstrates the applicability of these meters in the assessment of the variability of plant nutritional status and helps to make decisions regarding the potential implementation of variable rate management, a concept that is a part of PA.

The objective of the present study was to fit regression models to describe the temporal variability of the relative index of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (Chl_t) obtained with the use of a portable meter, and to conduct the assessment of the temporal stability and mapping of Chl_t index in a commercial Chardonnay vineyard, in the period between the onset of flowering and full fruit maturation.

MATERIAL AND METHODS

The study was conducted in a commercial wine grape orchard (*Vitis vinifera* L.), cv. 'Chardonnay', with a total area of 1.10 ha, located in the municipality of Espírito Santo do Pinhal, state of São Paulo, Brazil (coordinates: 22º 10' 49.1" S and 46º 44' 28.4" W; mean altitude: 875 m). The total area of the vineyard under study was divided into two subareas, namely Area 1 and Area 2, which covered 0.60 and 0.50 ha, respectively. The region's climate was classified as B3rB'3a' (Rolim et al., 2007), which was characterized as mesothermal, with absent or reduced water deficit and a sum of reference evapotranspiration varying between 855.0 and 997.0 mm per year. The soil in the experimental area was classified as eutrophic Tb Haplic Cambisol (Santos et al., 2018).

The spacing adopted in both sub-areas of the vineyard was 2.50 m between rows and 1.00 m between plants. The grapevines were grafted on Paulsen 1103 rootstock, grown in a vertical espalier system, and trained using the unilateral Royat cordon system. Moreover, the grapevines were subjected to double pruning management (Dias et al., 2017) with the aim of taking the crop into autumn and winter seasons when the climate conditions are more favorable for fruit maturation. Vineyard irrigation was performed using a drip system, with one lateral drip line per plant row and two emitters per plant, placed 0.50 m apart and with an average flow of 1.88 L h^{-1}. The criterion adopted for the irrigation management was the replacement of crop evapotranspiration (ETc, mm day^{-1}).

The measurement of the relative index of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and chlorophyll a+b or total (Chl_t) were made *in situ* in leaves of georeferenced plants using a ClorofiLOG portable meter (model CFL 1030, Falker Automação Agrícola, Porto Alegre, RS, Brazil). This instrument emits light at three wavelengths, 635 nm and 660 nm in the red region and 880 nm in the near infrared region (Barbieri Júnior et al., 2012). The emitted radiation is transmitted by the leaf and reaches a receiver that gives measurements proportional to chlorophyll absorbance in a dimensionless unit. Georeferencing of the plants from which the leaves were selected to measure the indices of chlorophyll were collected using HiPer GGD (TOPCON, Pleasanton, CA, USA) GNSS (Global Navigation Satellite System) receivers with RTK (Real Time Kinematic) centimeter correction.

A systematic sampling system was used to perform measurements at regular intervals of three plants along the planted rows, in interspersed rows, resulting in a grid composed of 346 and 303 sampling points in Area 1 and Area 2, respectively. The limits of both areas and the points in the sampling grids used for the *in situ* measurement of the Chl_a, Chl_b, and Chl_t indices in Chardonnay grapevine leaves using the described portable meter are shown in Figure 1.

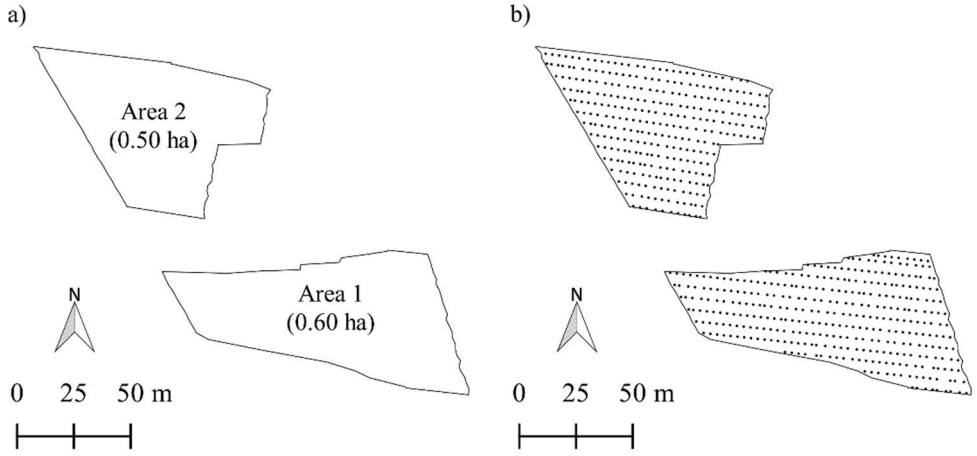

FIGURE 1. Limits and size of the experimental areas (Area 1 and Area 2) (a) and points of the sampling grids (b).

The assessments were made during the 2017 grapevine cycle, which was initiated with the pruning on January 4, 2017 and ended with the harvest on May 16, 2017, thus lasting for a total of 132 days. The measurements of the chlorophyll indices were performed on seven occasions during the 2017 cycle (at 41, 57, 64, 78, 85, 99, and 120 days after pruning - DAP). The onset of flowering and of fruit maturation occurred at 37 and 92 DAP, respectively.

The chlorophyll indices were measured in two leaves per plant, positioned opposite the bunches (in the assessments conducted up to defoliation in the region of the bunches, at 76 DAP, for greater exposure of the bunches to the sun) and in the middle third of the grapevine canopy (in the assessments starting from 76 DAP). The criterion for the selection of the plant material to be analyzed was fully expanded leaves with healthy appearance.

Data analysis initially consisted of the application of descriptive statistics to the sets of values obtained for Chl_a, Chl_b, and Chl_t indices for each assessment date, which resulted in the estimation of measures of central tendency, dispersion, and distribution pattern. Still in the exploratory analysis phase, the dispersion of data points around the mean was classified based on the coefficient of variation (CV), which was classified as low (CV ≤ 15%), moderate (15 < CV ≤ 35%), or high (CV > 35%) according to the categories proposed by Wilding (1985).

The assessment of the temporal variability of Chl_a, Chl_b, and Chl_t indices was performed by fitting regression models to predict and describe the variation trend of the mean values of the indices over time. The models were fitted according to the pattern of experimental data layout in a scatterplot, and the coefficients of these functions were estimated using the least squares method.

The temporal stability of the Chl_t index, measured in the various sampling points in both vineyards, was assessed through analysis of correlation between the values obtained in the same sites on different dates, according to Spearman's rank coefficient (r_s), calculated using [eq. (1)]:

$$r_s = 1 - 6\sum_{i=1}^{n}\left[R\left(X_{i,j}\right) - R\left(X_{i,j'}\right)\right]^2 / n(n^2 - 1) \quad (1)$$

Where:

n is the number of sampling points of the Chl_t value in each area;

$X_{i,j}$ and $X_{i,j'}$ are the Chl_t values measured in sampling point i on dates j and j', in this order,

$R(X_{i,j})$ and $R(X_{i,j'})$ are the ranks of $X_{i,j}$ and $X_{i,j'}$ among all the values obtained on dates j and j', respectively. The value of this coefficient varies from 1 to –1, which represent a perfect positive and negative association between ranks. Values of r_s close to 0 and 1 were indicative of the greater and lower magnitude, respectively, of the temporal stability of the Chl_t index between the two assessment dates. The descriptive statistical analysis and the regression analysis of the Chl_a, Chl_b, and Chl_t data, as well as the assessment of the temporal stability of the Chl_t index based on the r_s, were performed using the R software version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria, 2018).

In addition, geostatistical analysis was applied only to the Chl_t data set for each assessment date to evaluate the spatial variability of chlorophyll content by estimating empirical semivariograms and fitting theoretical models to the latter. Spherical, exponential, and Gaussian theoretical models were tested to fit the semivariograms. The criterion used to select the best theoretical model to characterize the spatial dependence of the Chl_t data was the value of the root-mean-squared error (RMSE) that resulted from the cross validation. Thus, the selected theoretical models were those with a lower RMSE. In addition, the modeling of the semivariograms showed the existence and correction of potential anisotropic effects associated with the spatial variability of the Chl_t index, as per the method proposed by Oliver & Webster (2014). Once the models of the theoretical semivariograms were defined, the degree of spatial dependence was determined based on the spatial dependence index (SDI) proposed by Seidel & Oliveira (2014), calculated independently for each model. Considering the spherical, exponential, or Gaussian models, respectively, the spatial structure of Chl_t index variation represented by spatial dependence was classified as strong (SDI > 15%, SDI > 13%, and SDI > 20%), moderate (7% < SDI ≤ 15%, 6% < SDI ≤ 13%, and 9% < SDI ≤ 20%), or weak (SDI < 7%, SDI < 6%, and SDI < 9%) (Seidel & Oliveira, 2016).

After the fitting of the theoretical models, the ordinary kriging method of geostatistical estimation was used for spatial prediction and generation of thematic maps for Chl_t index (Oliver & Webster, 2014). The estimation of the Chl_t index in non-measured sites in both areas and in each assessment date by kriging allowed modeling of the distribution and spatial variability of Chl_t index across the phenological stages identified in the assessed period of the grapevine cycle. Subsequently, the estimated Chl_t data were classified into three distinct categories or regionalization, including high values (Class 1), intermediate values (Class 2), and low values (Class 3) of total chlorophyll content, assuming an ordinal scale as measurement of the variable in question. This categorization was made considering the natural breaks method, or Jenks optimization method, as the rule for the classification of the interpolation results. The geostatistical analysis described in this study was performed using the GS+ software version 7.0 (Gamma Design Software, Michigan, USA, 2004), and the thematic maps were created using the QGIS software version 2.18.17 (QGIS Development Team, 2016).

Finally, the comparison between the categorical maps of the Chl_t index obtained on subsequent dates was made based on the quantification of the level of spatial association between the regionalization of this variable, and the V-measure (V_1), a cluster evaluation measure, was used according to Nowosad & Stepinski (2018) (adapted). The values of this global measure of association vary between 0 and 1 and indicate a higher or lower spatial agreement, respectively, between the categories represented in the maps. In addition, the magnitude of the overlap between the regionalization of two consecutive Chl_t index maps was estimated using the Mapcurves algorithm proposed by Hargrove et al. (2006), based on the calculation of the goodness of fit (GOF) for measuring the coincidence between its categories. The highest possible score for GOF is 1, which indicates an exact coincidence between the compared maps. Both mentioned analyses were performed using the R software version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria, 2018).

RESULTS AND DISCUSSION

The normality of the distribution of the chlorophyll index data on all assessment dates was tested using the similarity between measures of central tendency (mean and median), in addition to coefficient of skewness (Cs) close to zero. The variability of the data obtained on all assessment dates and in both areas was classified according to the values of CV (low when CV ≤ 15% or moderate when 15 < CV ≤ 35%).

Given the CV value (21.97%), the lowest homogeneity of the data distribution in Area 1 was related to the measurements of the Chl_B made at 41 DAP, i.e. at the onset of flowering, although variability was classified as moderate. In this area, the lowest CV (5.73%) was related to the measurement of the Chl_a index performed at 120 DAP at the end of the assessment period near full fruit maturation, 12 days before the harvest. In Area 2, the set of data with the highest dispersion was also related to the Chl_b value at 41 DAP, which was associated with a CV of 20.70% and resulted in moderate variability. In this area, the lowest relative variation of the data sets was similar to that obtained in Area 1 regarding the index with the lowest variability, because it was also related to the obtained Chl_a index but at 85 DAP before the onset of maturation, assuming a CV value of 4.31%.

TABLE 1. Descriptive statistics of the relative index of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (Chl_t) measured in Chardonnay grapevine leaves on different days after pruning (DAP).

DAP	index	n	Mean	Med	Min	Max	s	CV (%)	Ck	Cs
					Area 1					
	Chl_a	346	22.88	22.68	12.55	32.05	3.37	14.71	-0.216	-0.056
41	Chl_b	346	5.96	5.95	2.95	9.75	1.31	21.97	-0.577	0.271
	Chl_t	346	28.85	28.80	15.50	40.65	4.50	15.59	-0.322	0.013
	Chl_a	343	26.65	26.95	14.85	31.90	2.33	8.75	2.600	-0.991
57	Chl_b	343	9.03	9.10	3.95	14.75	1.62	17.98	-0.015	-0.033
	Chl_t	343	35.67	36.05	20.25	46.65	3.85	10.78	0.921	-0.507
	Chl_a	344	29.91	30.40	21.05	47.50	2.62	8.77	6.333	0.533
64	Chl_b	344	9.64	9.60	1.60	20.10	1.74	18.08	4.195	0.444
	Chl_t	344	39.55	39.93	26.80	55.20	3.83	9.69	1.329	-0.014
	Chl_a	346	32.16	32.25	25.35	37.55	1.93	5.99	0.405	-0.483
78	Chl_b	346	10.03	10.05	5.95	19.00	1.29	12.90	6.166	0.889
	Chl_t	346	42.19	42.60	32.30	51.30	2.85	6.76	0.655	-0.500
	Chl_a	344	31.23	31.50	25.20	34.50	1.54	4.94	1.369	-0.960
85	Chl_b	344	10.13	10.20	3.25	14.15	1.42	14.03	0.898	-0.245
	Chl_t	344	41.36	41.43	32.20	48.55	2.66	6.42	0.550	-0.550
	Chl_a	346	30.12	30.15	25.65	34.35	1.56	5.17	-0.049	-0.008
99	Chl_b	346	10.06	10.00	6.70	15.05	1.27	12.62	0.440	0.400
	Chl_t	346	40.18	40.05	32.90	48.70	2.65	6.60	0.157	0.110
	Chl_a	344	32.62	32.80	23.15	38.05	1.87	5.73	1.813	-0.475
120	Chl_b	344	9.40	9.40	5.55	16.20	1.26	13.43	2.196	0.427
	Chl_t	344	42.02	42.30	29.75	50.50	2.87	6.84	0.949	-0.394
					Area 2					
	Chl_a	303	22.05	22.15	13.40	29.35	2.94	13.32	0.110	-0.197
41	Chl_b	303	6.44	6.50	2.95	11.15	1.33	20.70	0.443	0.234
	Chl_t	303	28.49	28.60	16.75	39.85	4.21	14.76	0.049	-0.087
	Chl_a	303	28.34	28.60	20.40	32.95	2.24	7.90	0.316	-0.597
57	Chl_b	303	8.67	8.55	5.20	12.50	1.34	15.43	-0.112	0.128
	Chl_t	303	37.00	37.35	26.35	45.45	3.18	8.60	0.671	-0.515
	Chl_a	303	30.98	31.30	16.20	38.65	2.31	7.45	6.149	-1.334
64	Chl_b	303	9.03	9.05	4.40	14.00	1.36	15.07	1.906	-0.008
	Chl_t	303	40.01	40.55	21.80	50.70	3.44	8.59	3.472	-0.932
	Chl_a	301	32.26	32.25	26.80	37.75	1.51	4.67	1.431	0.045
78	Chl_b	301	10.14	10.05	5.45	15.80	1.27	12.49	1.844	0.463
	Chl_t	301	42.40	42.30	33.85	53.35	2.51	5.91	2.198	0.478
	Chl_a	299	31.12	31.15	26.10	37.35	1.34	4.31	1.902	0.004
85	Chl_b	299	9.88	9.85	7.10	13.45	1.11	11.19	0.058	0.189
	Chl_t	299	41.00	41.00	33.20	49.10	2.20	5.38	0.959	-0.060
	Chl_a	302	32.25	32.20	23.95	38.15	1.73	5.36	1.785	-0.214
99	Chl_b	302	11.07	10.95	6.00	16.95	1.26	11.39	2.219	0.482
	Chl_t	302	43.33	43.45	29.95	52.80	2.78	6.41	1.950	-0.132
	Chl_a	302	34.56	34.65	28.80	38.95	1.62	4.68	0.315	-0.273
120	Chl_b	302	9.94	9.80	5.70	16.50	1.16	11.67	3.788	0.807
	Chl_t	302	44.49	44.50	35.50	51.50	2.54	5.71	0.496	-0.199

n: number of sampled plants; Med: median; Min: minimum value; Max: maximum value; s: standard deviation; CV: coefficient of variation; Ck: coefficient of kurtosis; Cs: coefficient of skewness.

The relative variability of the Chl_a, Chl_b, and Chl_t indices was higher in Area 1 than in Area 2 on all assessment dates. Therefore, even adjacent areas in a vineyard, assessed in the same period of the cycle, cultivated with a single grapevine cultivar, and subjected to the same agricultural practices exhibit different levels of variability regarding the relative content of photosynthetic pigments throughout the period between flowering and fruit maturation.

Moreover, in Areas 1 and 2 the variability of the Chl_a, Chl_b, and Chl_t indices followed a similar pattern according to the progression of the grapevine cycle. This was evidenced by the high CV values obtained in the first evaluations, soon after the onset of flowering. These values subsequently decreased and present smaller differences between each other starting from 78 DAP, before the onset of maturation. These results indicate the existence of a lower variability of photosynthetic pigment content in grapevine leaves during periods close to and after the onset of fruit maturation, a phenological stage called veraison, which was identified at 92 DAP in the assessed cycle. According to Keller (2015), this stage is marked by the decrease in berry firmness and the increase in fruit sugar content and is followed by a quick change in fruit color, i.e., from green to red in red grape cultivars and from green to yellow in white grape cultivars, which is the case for the Chardonnay cultivar.

It was inferred that the variation in the mean values of the chlorophyll indices assessed throughout the growth cycle of Chardonnay grapevine, from the phase that follows flowering onset until maturation (Figure 2), exhibited a decreasing quadratic trend. Thus, given the distribution of the mean values of the chlorophyll indices during the assessment period, non-linear models, more specifically quadratic functions, were fitted to the temporal series because they provided a satisfactory description of the trend of the means of the three indices throughout that interval of the grapevine cycle. In addition, this type of function was selected because it was the simplest form of representing the relationship between the considered variables.

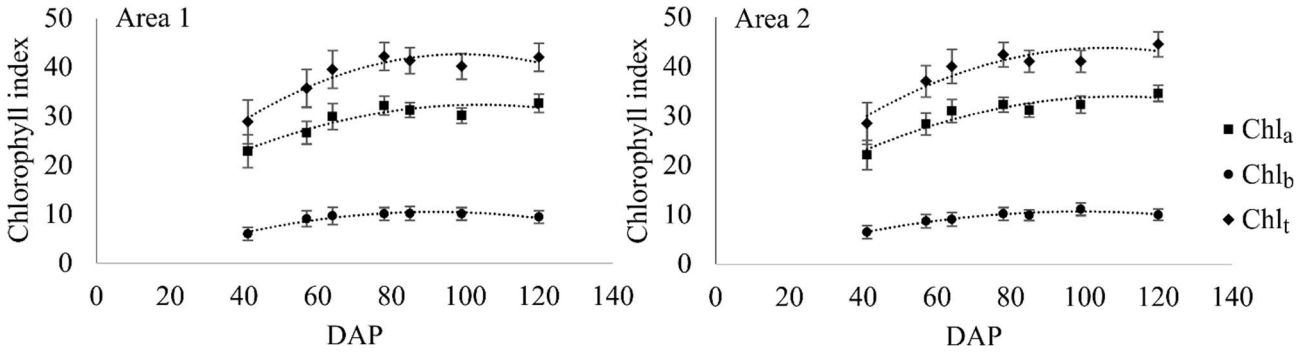

FIGURE 2. Mean values of the relative index of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (Chl_t) measured in Chardonnay grapevine leaves on different days after pruning (DAP) and trend lines obtained using polynomial regression models.

According to the analysis of variance for the regression models (Table 2), the F test confirmed the existence of a significant non-linear relationship between the chlorophyll indices measured in both areas and the phenological stages of the grapevine crop, represented by days after pruning, assuming a significance level of 5% ($\alpha = 0.05$). However, after the polynomial regression equations for Chl_a index variation were calculated, the quadratic effect was deemed significant only for Chl_b and Chl_t indices, assuming $\alpha = 0.05$. High (close to 1) coefficients of determination (adjusted r^2) fitted to the variation of Chl_b and Chl_t indices confirmed the models' quality of fit. Thus, most of the variation of the Chl_b and Chl_t indices in Chardonnay grapevine leaves occurs as a response to crop cycle progression and this relationship can be described with a 2nd order polynomial function. Because there was no evidence that the fitted polynomial regression models were able to describe the variation trend of the Chl_a index with grapevine cycle development, it was assumed that chlorophyll content tended to increase in Chardonnay grapevine leaves until the considered assessment date (120 DAP), i.e. even after the onset of fruit maturation.

TABLE 2. Parameters of the polynomial regression equations fitted to describe the mean variation trend of the relative index of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (Chl_t) measured in Chardonnay grapevine leaves on different days after pruning (DAP).

Index	b_0	b_1	b_2	Pr (>F)	r^2	Adjusted r^2
			Area 1			
Chl_a	7.6383	0.4749	-0.0023	0.0172	0.8690	0.8035
Pr (> t)	0.2597	0.0357	0.0709			
Chl_b	-2.9834	0.2936	-0.0016	0.0037	0.9393	0.9090
Pr (> t)	0.1509	0.0026	0.0040			
Chl_t	4.6549	0.7685	-0.0039	0.0080	0.9106	0.8659
Pr (> t)	0.5242	0.0118	0.0223			
			Area 2			
Chl_a	6.4299	0.5029	-0.0023	0.0108	0.8963	0.8444
Pr (> t)	0.3488	0.0340	0.0774			
Chl_b	-1.4495	0.2443	-0.0012	0.0017	0.9594	0.9390
Pr (> t)	0.3555	0.0026	0.0052			
Chl_t	4.9805	0.7472	-0.0035	0.0037	0.9390	0.9085
Pr (> t)	0.4695	0.0102	0.0242			

b_0, b_1, and b_2: estimated parameters of the quadratic regression model $Y_i = b_0 + b_1X + b_2X_i^2 + e_i$; r: coefficient of multiple correlation; r^2: coefficient of determination; adjusted r^2: adjusted coefficient of determination.

According to the simulated values obtained using the regression equations that represent the trend lines of leaf pigment content variation over time, the highest means expected for the Chl_b and Chl_t indices in Area 1 would be obtained approximately at 92 and 99 DAP, respectively. In Area 2, the Chl_b and Chl_t indices would reach the highest simulated values at 99 and 106 DAP, respectively. In both cases, maximum values of chlorophyll b and total chlorophyll contents were estimated to occur straight after the onset of fruit maturation. In line with this finding, Filimon et al. (2016) stated that the fruit maturation phase may be considered the starting point of leaf senescence in grapevines. Other authors (Maia & Piedade, 2002; Zhang et al., 2008; Paula et al., 2015) also obtained results indicating chlorophyll content variation according to the phenological stages in other plant species, in a pattern similar to that reported in that study. Specifically in wine grape cultivars, chlorophyll content variation in leaves of Pinot Noir with leaf age and position in the plant has been described by Bertamini & Nedunchezhian (2002).

In addition to the natural pattern of variation of chlorophyll content over time, the effect of other variables on the relative chlorophyll indices was inferred from the variation of the mean values obtained in the period in question, compared with the values simulated by the trend lines fitted by the least squares method. Therefore, in addition to leaf age, plant nitrogen (N) dynamics stands out as one of the factors that may explain pigment content variation, because this element is directly related to chlorophyll content. According to Arrobas et al. (2014), the reduction in N content throughout the grapevine cycle can also be attributed to the remobilization of this nutrient to the plant's perennial structures, as well as loss to the atmosphere. According to the previously mentioned authors, this is evidenced by the comparison between the concentration of N in chlorotic leaves and in green leaves and by the concomitant increase in N content in the woody parts of the plant.

Another possible explanation for the temporal variation in photosynthetic pigment content is the pattern of N demand by the grapevine throughout its development cycle. This demand tends to be greater between the phases of budding and flowering, with a gradual increase in the supply of N from the soil during flowering, fruiting, and the first stage of berry growth; this supply may be even greater after the onset of maturation (Keller, 2015). The effect of nitrogen fertilization on chlorophyll content and the positive correlation between the relative chlorophyll index and leaf N content in grapevine cultivars was observed by Tecchio et al. (2011) and Taskos et al. (2015). In addition, effects on the relative index of leaf chlorophyll in table grape cultivars in response to the variation in fertilizer application rate, including sources of N, P_2O_5, and K_2O, have been shown by Andrade et al. (2017).

Considering only the Chl_t results, because this index is the sum the remaining indices, there was a modest temporal stability of total chlorophyll content in the assessed period in both areas. This was inferred from the low values of r_s (close to zero) obtained in the comparison between all pairs of assessment dates (Table 3). Despite the values of r_s, the most significant correlation occurred before the onset of fruit maturation, between 64 and 78 DAP in Area 1 and between 78 and 85 DAP in a period nearer to the veraison stage in Area 2.

TABLE 3. Spearman's coefficient of correlation (r_s) for the association between the index of total chlorophyll (Chl_t) measured in Chardonnay grapevine leaves on different days after pruning (DAP).

Area 1							
	41 DAP	57 DAP	64 DAP	78 DAP	85 DAP	99 DAP	120 DAP
41 DAP	1						
57 DAP	0.179 **	1					
64 DAP	0.137 *	0.326 **	1				
78 DAP	0.134 *	0.160 **	0.335 **	1			
85 DAP	0.208 **	0.201 **	0.258 **	0.255 **	1		
99 DAP	0.214 **	0.095 ns	0.084 ns	0.193 **	0.288 **	1	
120 DAP	0.176 **	0.154 **	0.208 **	0.242 **	0.245 **	0.302 **	1
Area 2							
	41 DAP	57 DAP	64 DAP	78 DAP	85 DAP	99 DAP	120 DAP
41 DAP	1						
57 DAP	0.048 ns	1					
64 DAP	0.098 ns	0.177 **	1				
78 DAP	0.172 **	0.096 ns	0.111 ns	1			
85 DAP	0.097 ns	0.150 **	0.226 **	0.381 **	1		
99 DAP	0.122 *	0.171 **	0.229 **	0.209 **	0.255 **	1	
120 DAP	-0.034 ns	0.084 ns	-0.003 ns	0.068 ns	0.047 ns	0.060 ns	1

Results of the statistical significance test: *: significant at 5% ($p < 0.05$); **: significant at 1% ($p < 0.01$); ns: non-significant.

The analysis of the spatial variation of the Chl_t index showed different patterns of distribution of total chlorophyll content according to DAP. As is shown in Table 4, the model that best fitted the majority of experimental semivariograms was the exponential model, whereas the spherical model was only used for Chl_t data of the measurements made at 41 DAP (Area 1) and at 78 and 85 DAP (Area 2). The geostatistical analysis of the Chl_t data showed the presence of geometric anisotropy (Oliver & Webster, 2014) only in Area 1 at 64, 78, and 85 DAP, with longer range of spatial dependence (greater continuity) in the 75º, 90º, and 90º directions, respectively.

In Area 1, the variation in the range of spatial dependence of the data was greater when the two first assessment dates were compared, with variogram amplitude results of 55.10 and 5.20 m, respectively. In the remaining assessment days in the same area, the range of spatial dependence was between 16.59 and 45.78 m. This indicated that spatial dependence of the Chl_t data in Area 1 was more stable starting from 64 DAP, i.e. there was a consolidation of the spatial continuity of chlorophyll content represented by the Chl_t, a result that is in line with the coefficient r_s (Table 3).

In Area 2, the values of maximum range of spatial dependence on all assessment dates were lower than those of the semivariograms fitted to the sets of data for Area 1, varying between 5.20 and 17.46 m at 78 and 57 DAP, respectively. Given the magnitude of the values of this parameter, it was inferred that using measurements made in a higher sampling density would improve spatial inference of the Chl_t index in Area 2, because the range of spatial dependence represented the distance at which maximum variability was identified (Grego & Oliveira, 2015).

TABLE 4. Models of the experimental semivariograms and their respective fitting parameters from the data of the index of total chlorophyll (Chl_t) measured in Chardonnay grapevine leaves on different days after pruning (DAP).

DAP	Model	Nugget effect	Level	Range (m)	SDI (%)	RMSE
			Area 1			
41	Spherical	18.09	20.66	55.10	3.61^W	4.39
57	Exponential	9.71	14.89	5.20	0.80^W	3.93
64	Exponential	7.74	16.25	20.00	4.66^W	3.77
78	Exponential	4.57	9.36	45.78	10.42^M	2.64
85	Exponential	4.32	7.68	24.30	4.73^W	2.55
99	Exponential	3.52	7.23	21.90	4.99^W	2.45
120	Exponential	4.23	8.27	16.59	3.61^W	2.82
			Area 2			
41	Exponential	9.21	18.15	6.56	1.98^W	4.28
57	Exponential	7.34	10.26	17.46	3.05^W	3.13
64	Exponential	6.74	9.83	12.02	2.32^W	3.12
78	Spherical	0.16	6.01	5.20	3.68^W	2.64
85	Spherical	1.96	4.65	5.62	2.36^W	2.29
99	Exponential	2.64	7.49	5.83	2.32^W	2.76
120	Exponential	3.07	6.32	6.11	1.93^W	2.56

SDI: spatial dependence index (Seidel & Oliveira, 2014); W, M, and S: weak, moderate and strong spatial dependence, respectively (Seidel & Oliveira, 2016). RMSE: root mean squared error.

In the semivariograms for Area 1, spatial dependence of the Chl_t index was classified as weak on all assessment dates, with the exception of 78 DAP when it was considered moderate. Spatial dependence associated with the semivariograms of the Chl_t index measured in Area 2 was classified as weak on all assessment dates. Because the results of SDI were low throughout the evaluated period, it was not possible to identify the phase of the cycle in which the results of kriging, and therefore of Chl_t index mapping, had the lowest uncertainty. Thus, this result showed the heterogeneity of the spatial distribution of total chlorophyll content in Chardonnay grapevines within the same vineyard. Despite the results obtained for the degree of spatial dependence of the Chl_t index, the validation of the semivariogram fitting models was confirmed by the value of the root-mean-squared error (RMSE) in all assessment dates, with the lowest values obtained at 99 DAP (2.55 in Area 1) and at 85 DAP (2.29 in Area 2).

Figures 3 and 4 show the thematic maps of spatial distribution of the Chl_t index in Areas 1 and 2, respectively, based on the classification of the interpolated values of Chl_t into three categories. In the maps, regions with dark colors show higher estimated values of Chl_t (Class 1), whereas lighter colors indicate sites with lower values of Chl_t (Class 3), according to the values obtained on each assessment date.

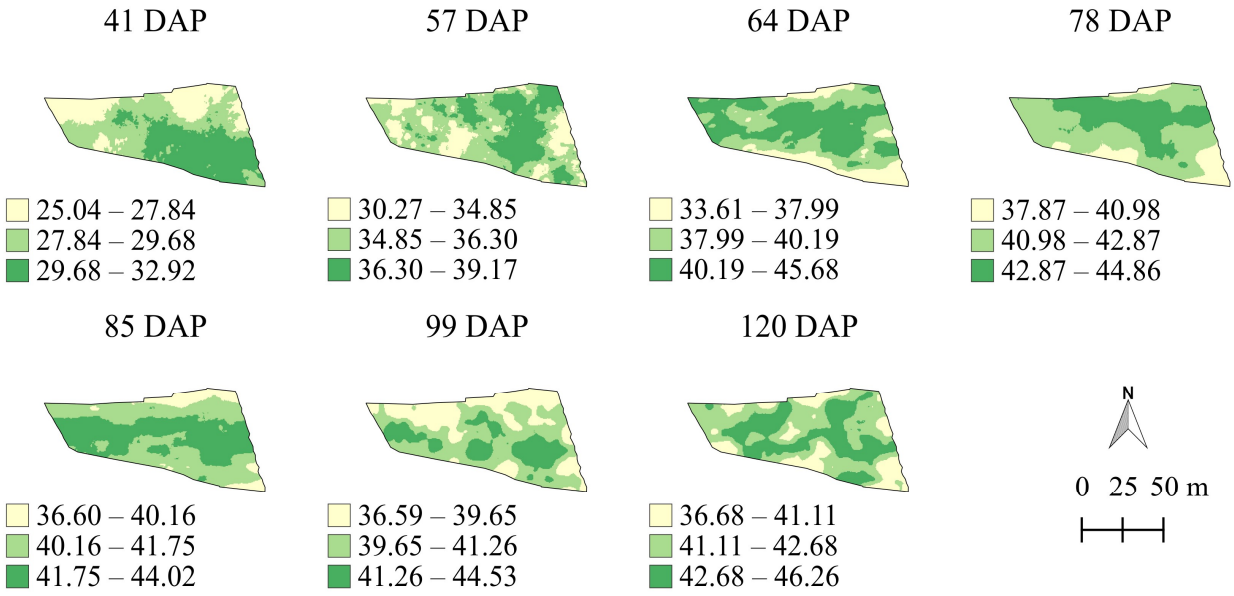

FIGURE 3. Spatial distribution of the index of total chlorophyll (Chl_t) in Chardonnay grapevine leaves estimated on different days after pruning (DAP) in Area 1 and clustered into three categories.

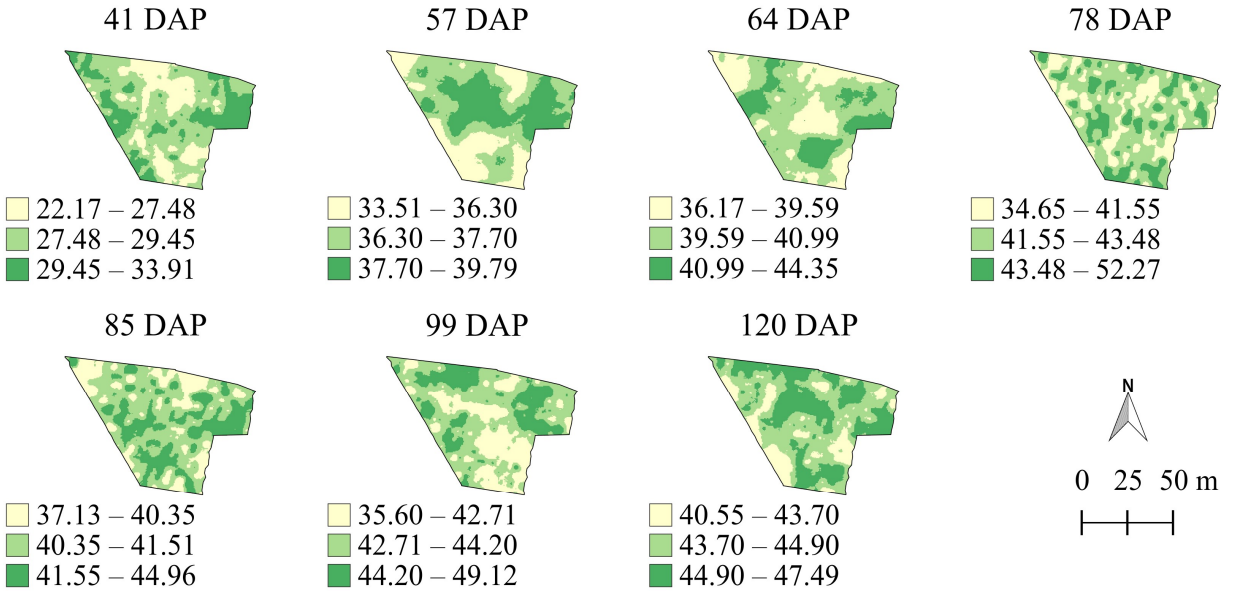

FIGURE 4. Spatial distribution of the index of total chlorophyll (Chl_t) in Chardonnay grapevine leaves estimated on different days after pruning (DAP) in Area 2 and clustered into three categories.

The comparison of the categorical maps of the Chl_t index obtained on subsequent assessment dates (Table 5) showed that values of V_1 close to zero obtained in both areas and intervals indicated a weak spatial association between the regionalization of total chlorophyll content. Low values of GOF also indicated a modest degree of spatial association between the pairs of categorical maps of the Chl_t index estimated throughout the assessment period. However, considering the two mentioned criteria, the strongest associations were observed between the thematic maps for the intervals between 64 and 78 DAP (Area 1) and between 78 and 85 DAP (Area 2), which were shown to be more similar, a finding that is in line with the higher temporal stability estimated using Spearman's rank correlation analysis (Table 3).

TABLE 5. Measurements of spatial association obtained from the comparison between the mapping of the total chlorophyll (Chl_t) index measured in Chardonnay grapevine leaves on different days after pruning (DAP).

Area 1						
Parameter	41-57 DAP	57-64 DAP	64-78 DAP	78-85 DAP	85-99 DAP	99-120 DAP
V_1	0.01	0.01	0.17	0.09	0.13	0.04
GOF	0.35	0.35	0.45	0.41	0.44	0.36
Area 2						
Parameter	41-57 DAP	57-64 DAP	64-78 DAP	78-85 DAP	85-99 DAP	99-120 DAP
V_1	0.01	0.02	<0.01	0.04	0.02	0.01
GOF	0.34	0.35	0.33	0.35	0.34	0.35

V_1: V-measure; GOF: goodness of fit.

In Area 1, the similarity between the Chl_t index maps was lower in the comparison of the three first pairs of assessments than in the comparison of the following assessments, and it decreased again at the end of the period in question. From 64 DAP until 85 DAP, there was a concentration of high and intermediate values (Class 1 and Class 2) of Chl_t index in the central region and a concentration of low values (Class 3) in the upper and lower limits, which resulted in similar patterns of Chl_t index spatial distribution in this period. Thus, in Area 1, the spatial distribution of the classes of this attribute varied less during these intervals than in the remaining intervals.

According to the V_1 and GOF values obtained for Area 2, the similarity between the maps was low and stable throughout the assessed period, given the pattern of spatial distribution of the regionalization of the Chl_t index, characterized by the instability of the layout of the classes of total chlorophyll content in the area and of the variation of the patterns obtained on each occasion. Moreover, although it was possible to identify classes of Chl_t index in this vineyard, they exhibited excessive dispersion and fragmentation resulting in smaller adjacent areas, which may preclude the use of this spatial analysis for the specific management of the area.

Considering only Area 1, the categorical map of Chl_t index obtained between 64 and 78 DAP and between 85 and 99 DAP, especially, may serve to guide the use of the portable meter for monitoring the nutritional status of the plant regarding N content in regions of interest within the vineyard. This is possible because the measurement given by this instrument correlates with leaf N content. These results were also related to the determination of the optimal phase for leaf sampling for the inference of the nutritional status of the grapevine, because the sampling timing affects the diagnosis of the concentration of mineral nutrients in the plant (Arrobas et al., 2014). In grapevine crops, it is more appropriate to perform nutritional assessment in samples of leaves collected during full flowering and at the onset of fruit maturation. Thus, assuming that the onset of maturation in the evaluated cycle occurred at 92 DAP, spatial analysis of the Chl_t index performed in the interval between 64 and 99 DAP could have been used to delimit more regular sampling regions regarding total chlorophyll content in Chardonnay grapevine leaves. The determination of leaf N content performed through the measurement of Chl_t index (according to its spatial variability and during the adequate period) together with soil fertility analysis may be useful to define areas for variable rate fertility management, thereby increasing the efficiency of fertilizer recommendations in the vineyard.

CONCLUSIONS

The mean variation of the Chl_b and Chl_t indices in Chardonnay grapevine leaves throughout its cycle may be represented by the quadratic trend used to estimate the reduction in the content of chlorophyll b and total chlorophyll after the onset of fruit maturation. In contrast, high mean values of Chl_a index are still detected after the onset of this stage.

The Chl_t index of Chardonnay grapevine exhibited a low temporal and spatial stability in the period between flowering and full fruit maturation in geographically close vineyards under similar cultivation and management conditions. However, the period that preceded the onset of fruit maturation was associated with a higher temporal stability of indirect measures of total chlorophyll content, with a higher similarity between consecutive categorical mappings of this variable.

ACKNOWLEDGEMENTS

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for providing a study grant, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing a study grant and financial support, and the Vinícola Guaspari for providing the study area and support during research work.

REFERENCES

Andrade VPM, Silva JAB, Souza JSC, Oliveira FF, Simões WL (2017) Physiological characteristics of grapevine under irrigation and fertilization management. Pesquisa Agropecuária Tropical 47(4):390-398. DOI: http://dx.doi.org/10.1590/1983-40632017v4748640

Arantes AM, Donato SLR, Siqueira DL, Amorim EP, Rodrigues Filho V (2016) Chlorophyll index for real-time prediction of nutritional status of 'Prata' banana. Revista Brasileira de Engenharia Agrícola e Ambiental 20(2):99-106. DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v20n2p99-106

Arrobas M, Ferreira IQ, Freitas S, Verdial J, Rodrigues MA (2014) Guidelines for fertilizer use in vineyards based on nutrient content of grapevine parts. Scientia Horticulturae 172:191-198. DOI: 10.1016/j.scienta.2014.04.016

Barbieri Júnior E, Rossiello ROP, Silva RVMM, Ribeiro RC, Morenz MJF (2012) Um novo clorofilômetro para estimar os teores de clorofila em folhas do capim Tifton 85. Ciência Rural 42(12):2242-2245. DOI: https://dx.doi.org/10.1590/S0103-84782012005000109

Bertamini M, Nedunchezhian N (2002) Leaf age effects on chlorophyll, Rubisco, photosynthetic electron transport activities and thylakoid membrane protein in field grown grapevine leaves. Journal of Plant Physiology 159(7):799-803. DOI: https://doi.org/10.1078/0176-1617-0597

Costa JPR, Caputti GP, Galzerano L, Silva WL, Ruggieri AC, Malheiros EB (2015) Relative chlorophyll contents in the evaluation of the nutritional status of nitrogen from *Xaraes palisade* grass and determination of critical nitrogen sufficiency index. Acta Scientiarum 37(2):109-114. DOI: http://dx.doi.org/10.4025/actascianimsci.v37i2.24854

Dey AK, Sharma M, Meshram MR (2016) An analysis of leaf chlorophyll measurement method using chlorophyll meter and image processing technique. Procedia Computer Science 85:286-292. DOI: https://doi.org/10.1016/j.procs.2016.05.235

Dias FAN, Mota RV, Souza CR, Pimentel RAM, Souza LC, Souza AL, Regina MA (2017) Rootstock on vine performance and wine quality of 'Syrah' under double pruning management. Scientia Agricola 74(2):131-141. DOI: http://dx.doi.org/10.1590/1678-992x-2015-0384

Elarab M, Ticlavilca AM, Torres-Rua AF, Maslova I, McKee, M (2015) Estimating chlorophyll with thermal and broadband multispectral high resolution imagery from an unmanned aerial system using relevance vector machines for precision agriculture. International Journal of Applied Earth Observation and Geoinformation 43:32-42. DOI: http://doi.org/10.1016/j.jag.2015.03.017

Filimon RV, Rotaru L, Filimon RM (2016) Quantitative investigation of leaf photosynthetic pigments during annual biological cycle of Vitis vinifera L. table grape cultivars. South African Journal for Enology and Viticulture 37(1):1-14. DOI: http://dx.doi.org/10.21548/37-1-753

Grego CR, Oliveira RO (2015) Conceitos básicos da geoestatística. In: Oliveira RO, Grego CR, Brandão ZN. Geoestatística aplicada na Agricultura de Precisão utilizando o Vesper. Brasília, Embrapa, p41-62.

Hargrove WW, Hoffman FM, Hessburg PF (2006) Mapcurves: a quantitative method for comparing categorical maps. Journal of Geographical Systems 8(2):187-208. DOI: http://dx.doi.org/10.1007/s10109-006-0025-x

Keller M (2015) The science of grapevines: anatomy and physiology. San Diego, Academic Press, 2 ed. 508p.

Maia LA, Piedade MT (2002) Influência do pulso de inundação na fenologia foliar e conteúdo de clorofila em duas espécies da floresta de igapó da Amazônia Central do Brasil. Acta Amazonica 32(1):55-64. DOI: http://dx.doi.org/10.1590/1809-43922002321064

Nowosad J, Stepinski TF (2018) Spatial association between regionalizations using the information-theoretical V-measure. International Journal of Geographical Information Science 32(12):2386-2401. DOI: 10.1080/13658816.2018.1511794

Oliver MA, Webster R (2014) A tutorial guide to geostatistics: Computing and modelling variograms and kriging. Catena 113:56-69. DOI: http://doi.org/10.1016/j.catena.2013.09.006

Paula SM, Naves ER, Franco IM, Padua KJM, Silva KR, Fernandes WP, Oliveira DC, Lemos-Filho JP, Moreira ASFP (2015) Desempenho fotossintético de folhas jovens e maduras de *Vochysia cinnamomea* (*Vochysiaceae*). Bioscience Journal 31(2):591-600. DOI: http://dx.doi.org/10.14393/BJ-v31n2a2015-23474

Prado-Cabrero A, Beatty S, Howard A, Stack J, Bettin P, Nolan JM (2016) Assessment of lutein, zeaxanthin and meso-zeaxanthin concentrations in dietary supplements by chiral high-performance liquid chromatography. European Food Research and Technology 242:599-608. DOI: https://doi.org/10.1007/s0217-015-2569-9

Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153(1):185-194. DOI: https://doi.org/10.1046/j.0028-646X.2001.00289.x

Rolim GS, Camargo MBP, Lima DG, Moraes JFL (2007) Classificação climática de Köppen e de Thornthwaite e sua aplicabilidade na determinação de zonas agroclimáticas para o estado de São Paulo. Bragantia 66(4):711-720. DOI: http://dx.doi.org/10.1590/S0006-87052007000400022

Santos HG dos, Jacomine PKT, Anjos LHC dos, Oliveira VA de, Lumbreras JF, Coelho MR, Almeida JA de, Araujo Filho JC de, Oliveira JB de, Cunha TJF (2018) Sistema brasileiro de classificação de solos. Brasília, Embrapa, 5 ed. 356p.

Seidel EJ, Oliveira MS (2016) A classification for a geostatistical index of spatial dependence. Revista Brasileira de Ciência do Solo 40:1-10. DOI: http://dx.doi.org/10.1590/18069657rbcs20160007

Seidel EJ, Oliveira MS (2014) Novo índice geoestatístico para a mensuração da dependência espacial. Revista Brasileira de Ciência do Solo 38(3):699-705 DOI: http://dx.doi.org/10.1590/S0100- 06832014000300002

Silva MA, Santos CM, Vitorino HS, Rhein AFL (2014) Pigmentos fotossintéticos e índice SPAD como descritores de intensidade do estresse por deficiência hídrica em cana-de-açúcar. Bioscience Journal 30(1):173-181.

Streit NM, Canterle LP, Canto MW, Hecktheuer, LHH (2005) As clorofilas. Ciência Rural, 35(3):748-755. DOI: http://dx.doi.org/10.1590/S0103-84782005000300043

Taskos DG, Koundouras S, Stamatiadis S, Zioziou E, Nikolaou N, Karakioulakis K, Theodorou N, (2015) Using active canopy sensors and chlorophyll meters to estimate grapevine nitrogen status and productivity. Precision Agriculture 16:16-98. DOI: https://doi.org/10.1007/s11119-014-9363-8

Tecchio MA, Moura MF, Paioli-Pires EJ, Terra MM, Teixeira LAJ, Smarsi RC (2011) Teores foliares de nutrientes, índice relativo de clorofila e teores de nitrato e de potássio na seiva do pecíolo na videira 'Niágara Rosada'. Revista Brasileira de Fruticultura 33(2):649-659. DOI: https://dx.doi.org/10.1590/S0100-29452011005000058

Wilding LP (1985) Spatial variability: its documentation, accommodation, and implication to soil surveys. In: Nielsen DR, Bouma J (ed). Soil spatial variability. Pudoc, p166-194.

Zhang SB, Hu H, Li ZR (2008) Variation of photosynthetic capacity with leaf age in an alpine orchid, *Cypripedium flavum*. Acta Physiologiae Plantarum 30:381-388. DOI: http://dx.doi.org/10.1007/s11738-008-0135-9