Heliyon 10 (2024) e26819

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Prediction of leaf nitrogen in sugarcane (*Saccharum* spp.) by Vis-NIR-SWIR spectroradiometry

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A R T I C L E I N F O

Keywords:
Remote sensing
Nitrogen fertilization
Spectral reflectance
Cross validation

A B S T R A C T

Nitrogen is one of the essential nutrients for the production of agricultural crops, participating in a complex interaction among soil, plant and the atmosphere. Therefore, its monitoring is important both economically and environmentally. The aim of this work was to estimate the leaf nitrogen contents in sugarcane from hyperspectral reflectance data during different vegetative stages of the plant. The assessments were performed from an experiment designed in completely randomized blocks, with increasing nitrogen doses (0, 60, 120 and 180 kg ha^{-1}). The acquisition of the spectral data occurred at different stages of crop development (67, 99, 144, 164, 200, 228, 255 and 313 days after cutting; DAC). In the laboratory, the hyperspectral responses of the leaves and the Leaf Nitrogen Contents (LNC) were obtained. The hyperspectral data and the LNC values were used to generate spectral models employing the technique of Partial Least Squares Regression (PLSR) Analysis, also with the calculation of the spectral bands of greatest relevance, by the Variable Importance in Projection (VIP). In general, the increase in LNC promoted a smaller reflectance in all wavelengths in the visible (400–680 nm). Acceptable models were obtained ($R^2 > 0.70$ and RMSE <1.41 g kg^{-1}), the most robust of which were those generated from spectra in the visible (400–680 nm) and red-edge (680–750 nm), with values of $R^2 > 0.81$ and RMSE <1.24 g kg^{-1}. An independent validation, leave-one-date-out cross validation (LOOCV), was performed using data from other collections, which confirmed the robustness and the possibility of LNC prediction in new data sets, derived, for instance, from samplings subsequent to the period of study.

1. Introduction

Since Brazil is one of the major sugarcane producers in the world, having doubled its production in the past decades [1], it is believed that this agricultural activity will continue to expand, aiming to meet the increase in the demand for bioenergy [2]. Currently,

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https://doi.org/10.1016/j.heliyon.2024.e26819
Received 11 August 2023; Received in revised form 24 January 2024; Accepted 20 February 2024
Available online 21 February 2024
2405-8440/

among the aspects that limit the increase in sugarcane productivity in Brazil, one of the most important is the nutritional status of nitrogen in the crop, as well as its correct management [3]. Besides promoting positive effects on growth, agronomic parameters and sugar contents [3,4], nitrogen is also one of the primary regulators of several physiological processes in the leaf, such as photosynthesis [5,6]. Conversely, the excessive use of nitrogen fertilizers harms the environment and, consequently, human health [7,8], besides impacting the costs of agricultural production [9]. Therefore, the optimization of the amount of nitrogen fertilizers in sugarcane crops is necessary to mitigate the negative impacts that are generated.

One of the alternatives with a great potential to optimize nitrogen fertilization is reflectance spectroscopy. This is a non-destructive technology which presents lower cost and fast data acquisition [10]. Furthermore, it is already used to evaluate the nitrogen status (N) in cultures such as rice [11]; tea leaves [9]; cotton [12]; apple [13]; soybean, tepary bean and mothbean [14] and spinach [15]. In general, these studies have shown good performance of Vis-NIR-SWIR spectroradiometry in estimating parameters related to agricultural crops, since N is closely related to the visible (400–700 nm) and red-edge (670–780 nm) bands [16,17].

By hyperspectral data, different statistical techniques have been employed to estimate N. Currently, among the most used are the linear analysis models, and the partial least squares regression (PLSR) is the most common. PLSR has good results in N prediction for ryegrass and barley ($R^2 > 0.80$ and RMSE <0.34 [18]), sugarcane ($R^2 = 0.85$ [19]) and apple trees ($R^2 = 0.6$ [13]). Differently from other crops, there are few studies considering the use of spectroradiometry for the evaluation of leaf contents in sugarcane, and they are even more scarce for the Brazilian conditions [16,20]. Conversely, the existing works indicate a great potential of the technique [16,17,21]. Despite presenting promising perspectives, most of these studies have directed their efforts to the quantification of the N content in only one vegetative stage of the crop. This can be considered an error, since the concentration of N in the sugarcane leaf exhibits variations according to crop development [22]. Therefore, estimating the nitrogen contents at different growth stages can provide the producer with critical time and spatial information, which may assist decision makers in monitoring their crops and in the management of agricultural operations aiming at maximizing production.

Therefore, this study aims at performing a detailed evaluation on the potential of the Vis-NIR-SWIR spectroradiometry for the prediction of leaf nitrogen. To better understand the robustness of the technique, the models generated were used in N prediction in periods different from that of the calibration. In other words, the performance of the model was assessed in different phenological stages and climate conditions from those considered in the calibration. This validation is here called leave-one-date-out cross validation, and aims at simulating conditions that are closer to the reality in the field, in which pre-calibrated models would be employed to predict N under unknown conditions. Thus, it is hoped to obtain insights about the influence of external factors on N prediction by spectroradiometry, as well as discuss the methodological limitations that still need to be overcome.

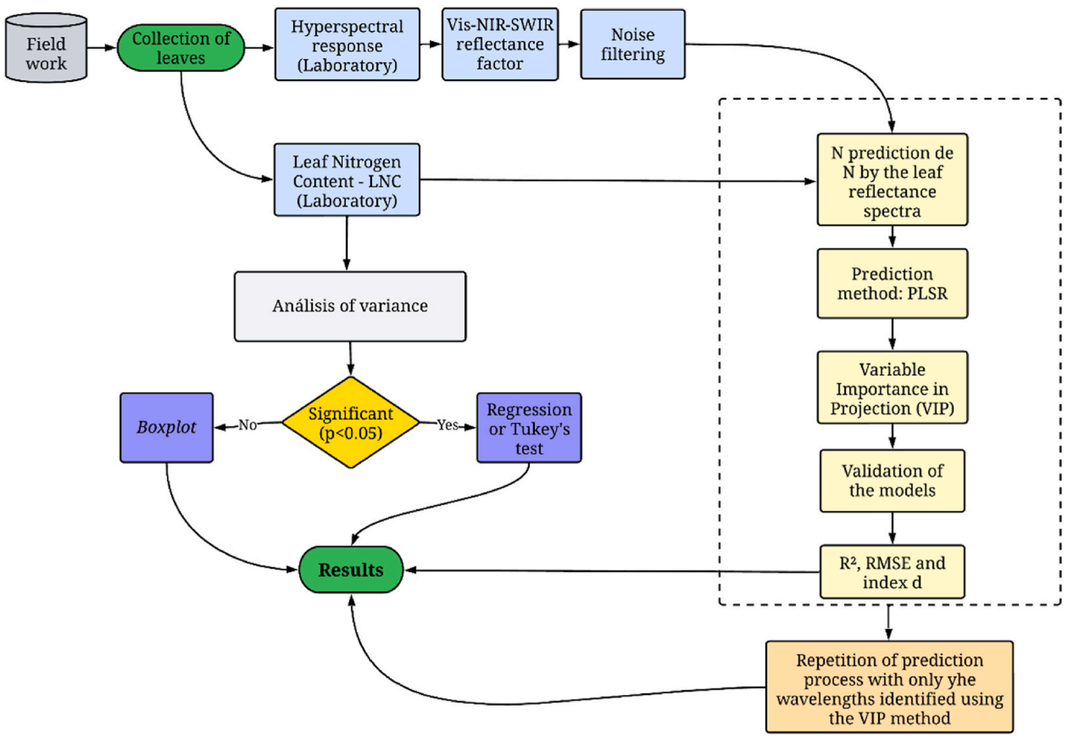

Fig. 1. Flowchart of the proposed method.

2. Materials and methods

The method employed in this work presents four main steps (Fig. 1), namely: (i) collection of leaf samples; (ii) acquisition of the spectral curve of the leaf samples; (iii) laboratory analysis of the plant tissues to obtain LNC; and (iv) application of the models for N prediction in sugarcane.

2.1. Description of the experiment

The experiment was installed in the municipality of Piracicaba, São Paulo, Brazil, between the geographic coordinates 22°41′02″ and 22°41′04″ of South latitude and 47°38′44″ and 47°38′42″ of West longitude. The soil present in the area was classified as a very clayey Red-Yellow Argisol (PVA), whereas the climate in the region corresponds to the humid subtropical (CWa), with average annual rainfall inferior to 1400 mm, with rainy summer and dry winter [23].

A survey was performed to verify the monthly rainfall distribution for the harvest 2014/2015 and the historical or normal average rainfall in the period from 1973 to 2015. The information was obtained by the meteorological station of the Luiz de Queiroz College of Agriculture - ESALQ, located at approximately 3 km of the experimental area.

The variety used in the experiment was IAC 95–5000, which is characterized by high agricultural production, good erect growth, excellent ratoon sprouting, good tillering and closing between rows, without falling off and flowering, besides being resistant to the main diseases and presenting optimum results under conditions of water deficiency [24].

The experiment was conducted throughout the harvest 2014/15, when the culture was in the first ratoon cycle (second cut). It was installed in the year 2013, with an area of approximately 0.5 ha. The adopted design was in completely randomized blocks with 4 nitrogen doses and 28 plots, composed of five lines of sugarcane (15 m in length) and spaced in 1.5 m, with the three central rows considered as the useful area, discarding the borders at each end. The N doses used were 0, 60, 120 and 180 kg ha^{-1}, with urea as N source, being applied manually on the straw right after cutting the sugarcane. The cultural traits, such as pH correction and fertilization, followed the standard adopted by the sugarcane production system for the region, according to the need of the crop after routine analysis for soil fertility.

2.2. Sampling of the leaf material

The field visits were performed on the dates 67, 99, 144, 164, 200, 228, 255 and 313 DAC, totalizing eight collections. The collection of the leaf material, for further laboratory analysis, was performed using ten leaves per plot. The assessments were performed in the middle third of the first leaf completely expanded from the apex of the crop. After removal, the leaves were stored in plastic bags and transported in thermal boxes with ice to the geoprocessing laboratory for the spectral readings, without direct contact between the leaves and the ice. This technique was adopted to preserve the turgidity and the spectral properties of the leaves [25,26].

2.3. Acquisition of sugarcane leaf spectral reflectance

In the laboratory, the spectral reflectance was obtained, using the spectroradiometer ASD FieldSpec FR Spectroradiometer® (ASD – Analytical Spectral Devices Inc., Boulder, CO, USA). The sensor operates in the spectral range from 350 to 2500 nm, with spectral resolution of 1.4 nm from 350 to 1050 nm and 2 nm from 1050 to 2500 nm. To obtain the spectral reads of the leaves, the probe Leaf Clip® (ASD-Analytical Spectral Devices Inc., Boulder, CO, USA) was attached to the device. Leaf Clip® can maintain the same intensity of light and orthogonal incidence in all reads, thus acting as a totally controlled method [27]. The calibration of the device was performed after reading ten leaves, using the Lambertian surface inserted in Leaf Clip® as reference.

2.4. Determination of the leaf nitrogen content (LNC)

After obtaining the spectral reads of the leaves, they were sent to the laboratory of leaf analysis to obtain the mean value of the LNC per plot. The process was repeated for each of the eight dates of sampling in the field. The leaves that went through the spectroradiometric process were separated per plot, being primarily washed in running water, followed by distilled water with detergent, and then only distilled water. Subsequently, they were placed in paper bags for drying in an oven with forced ventilation at 65 °C, until constant weight was reached. After drying, the samples were ground for the determination of the LNC. The chemical analyses for the acquisition of the LNC were determined in the extracts obtained by the sulfuric digestion using the semimicro Kjeldahl method [28].

2.5. Pre-processing of the spectra

The reflectance data were pre-processed, aiming at correcting inconsistencies in the reads caused by external factors, such as, for instance, noise, environmental variations (moisture and temperature) or even the scattering of light [18].

The pre-processing of the data occurred by three steps, according to the following sequence: (i) Removal of the wavelengths of 350–450 nm and 2000–2500 nm, ranges in the spectrum which presented large concentrations of noise, which has already been observed in other works [29,30]; (ii) The data were transformed using the logarithmic function (Log(1/R)), which is a mathematical technique for the transformation of spectral reflectance data [31–34]; (iii) Application of the Savitzky-Golay (SG) filter [35], with 3-point smoothing and second order polynomial [32].

2.6. Statistical analysis and data processing

2.6.1. Partial least squares regression (PLSR)

The models for the prediction of the nitrogen contents were individually calibrated, for each date of collection. In parallel, a general prediction was tested, considering the data of the eight dates (67, 99, 144, 164, 200, 228, 255 and 313 DAC). The prediction of the nitrogen contents by the leaf spectral behavior was performed using the technique PLSR with the algorithm NIPALS. This is a multivariate analysis technique which can treat correlated independent variables (wavelengths) and relatively few observations, reduce them to a set of components, avoiding multicollinearity for the estimation of a set of dependent variables (LNC) [36]. During the calibration step, PLSR uses the information of the independent variables (spectra) and dependent variables (N contents), to generate new variables called latent variables (or factors). The aim of adjusting a model by PLSR is to find the smallest possible number of PLS factors necessary to explain the dependent variables.

To define which spectral bands were indeed relevant in the prediction, the Variable Importance in Projection (VIP) was calculated, according to Equation (1). The VIP indices are calculated for each spectral band, and the bands presenting VIP values above 0.8 were considered relevant. The whole processing described was performed using the program ParLeS 3.1 [37].

$$VIP_K(a) = k\sum_a w^2 ak\left(\frac{SSY_a}{SSY_t}\right) \tag{1}$$

where VIP_k (a) is the importance of the wavelength variable based on a model with factors *a* (PLSR components); Wak are the PLSR weights of the variables (wavelengths) in a PLSR factor; SSY_a represents the sum of squares of Y explained by a PLSR model with factors *a*, and SSY_t is the total sum of the squares of Y of the squares explained in all factors [38].

After identifying the spectral ranges of greater relevance in the prediction of N, a second set of models was calibrated, this time employing only the wavelengths that registered the greatest importance in the first edition. Subsequently, it was observed whether the values best adjusted, and how good the final model was in nitrogen prediction by the leaf spectra.

2.6.1.1. Validation of the models. During the calibration of the model, both the model with the optimum number of factors and its respective performance were defined. The best model presented the highest coefficient of determination (R^2) and the lowest root of the mean square error (RMSE), described in equations (2) and (3), respectively. It is important to highlight that R^2 represents the dispersion of the points in the line of regression of the best adjustment, and thus, it measures how good the regression model was in nitrogen prediction by the leaf spectra of the plant. To test the agreement of the prediction models, the Willmott index was used (d), which reflects the degree to which the measured data are accurately estimated by the predicted data [39], and is presented in equation (4).

$$R^2 = \frac{\left[\sum(\gamma_p - \overline{\gamma}_p)\bullet(\gamma_o - \overline{\gamma}_o)\right]^2}{\sum(\gamma_p - \overline{\gamma}_p)^2 \bullet \sum(\gamma_o - \overline{\gamma}_o)^2} \tag{2}$$

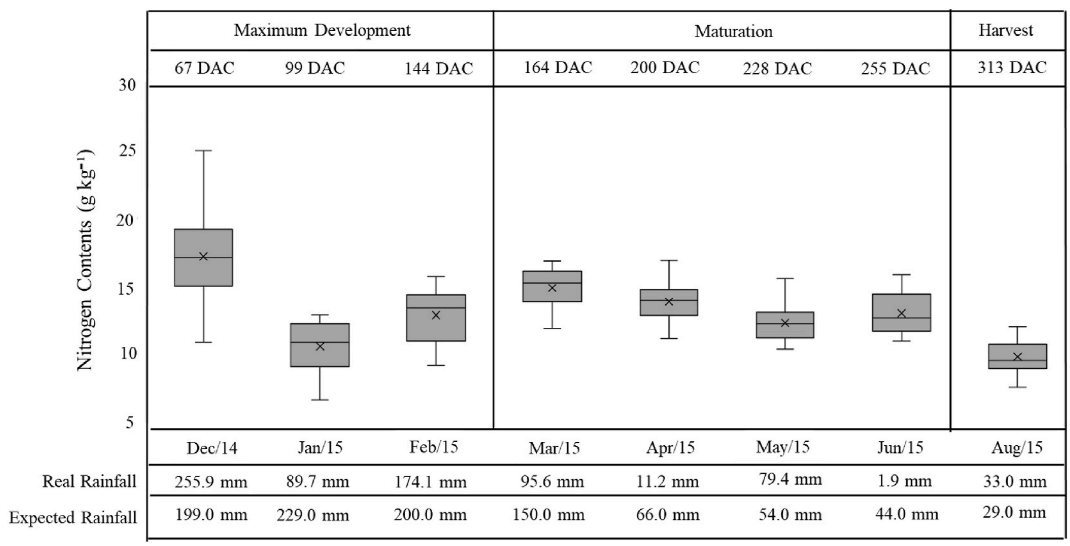

Fig. 2. Mean values of the leaf nitrogen contents in sugarcane for the dates 67, 99, 144, 164, 200, 228, 255 and 313 – DAC, monthly mean rainfall in the months of collection (Real Rainfall) and rainfall from a historical mean of 30 years (Expected Rainfall).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (\widehat{y_i} - y_i)^2}{n}} \tag{3}$$

$$d = 1 - \frac{\sum_{i=1}^{n} (Y_i - X_i)^2}{\sum_{i=1}^{n} (|Y_i - X| + |X_i - X|)^2} \tag{4}$$

Two validation strategies were performed, one of them the k-fold cross validation and the other by the recursive generation of new models using data from seven collections (dates) and validated with the remaining data (leave-one-date-out cross validation; LOOCV), which did not participate in the process of calibration. Thus, the possibility of estimating leaf N contents from spectra derived from a date different from those of the calibration was evaluated. Furthermore, to compare and evaluate the efficacy of the LOOCV technique, the holdout validation was tested, where 70% of the data were used for the prediction, and 30% for the validation. Thus, we tested which technique best adjusted to the spectral data. The performance of the models was evaluated by the values of R^2 and RMSE.

3. Result

3.1. Leaf nitrogen content

Fig. 2 shows the mean values of LNC and the mean values of the real and expected rainfall on the eight dates of assessment (67, 99, 144, 164, 200, 228, 255 and 313 DAC). Date 67 had the greatest nitrogen concentrations in the sugarcane leaf, whereas the last collection (313 DAC) had the smallest N contents (Fig. 2).

With the exception of the first collection (67 DAC), in all others the N contents in the sugarcane leaves stayed below the reference values, between 18 and 25 g kg^{-1}. It is worth highlighting that the rainfall in the period of cultivation (harvest 2014/2015) was not regular. Therefore, in the first cycle of ratoon cane there were periods of water deficit in almost the whole vegetative growth phase from January to April 2015, making it difficult for the plants to absorb N (Fig. 2).

3.2. Visual analysis of the leaf spectrum

The spectral response of the leaves on dates 67, 99, 144, 164, 200, 228, 255 and 313 DAC was assembled from the highest to the lowest leaf N content. The spectral curves were generated between the wavelengths in the visible (450–680 nm), and the choice of these spectral bands derived from the fact that they were directly related to the N contents and the leaf pigments. Although the variation in the N contents in the leaves was, in general, small (Fig. 3), variations in the spectral curves were observed in relation to the N contents. Notably, the leaves with the highest N concentrations exhibited the highest absorption of electromagnetic energy in the

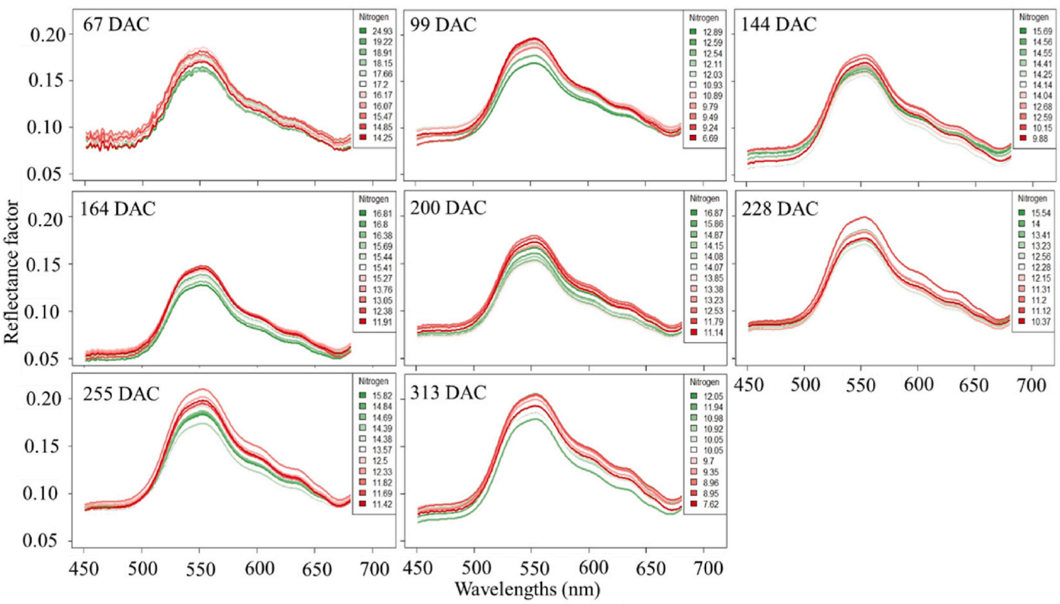

Fig. 3. Spectral curves of the sugarcane leaves in the wavelengths of visible (450–680 nm) on dates 67 DAC, 99 DAC, 144 DAC, 164 DAC, 200 DAC, 228 DAC, 255 DAC and 313 DAC, being assembled from the highest to the smallest leaf N content.

green range (550 nm), whereas those with lower N levels presented a higher reflectance in this same spectral range.

3.3. N prediction by the Vis-NIR-SWIR spectrum

The results obtained indicate that the models generated are at acceptable levels ($R^2 > 0.70$), including the one calibrated with the data from all collections (general), which presented R^2 of 0.72, RMSE of 1.33 and d index = 0.91. The models calibrated by collection date had a similar performance, with R^2 varying between 0.70 (99 DAC) and 0.90 (228 DAC) and RMSE between 0.41 (99 DAC) and 0.71 (144 DAC). Furthermore, the Willmott index registered values above 0.90 (99 DAC), indicating the models obtained a good precision. It is worth highlighting that, in the second collection (99 DAC), the values of R^2, "d" index and RMSE were 0.70, 0.90 and 1.07 g kg^{-1}, respectively, presenting the smallest precision among the prediction models (Fig. 4).

3.4. Variable Importance in Projection (VIP)

The VIP values were used to identify which wavelengths and spectral regions are more relevant for N prediction in sugarcane leaves (Fig. 5). In general, the most important ranges varied among the bands in the visible (450–680 nm), red-edge (680–750 nm) and some small bands of the mid-shortwave infrared (1360–1660 nm and 1780–2000 nm).

On date 144 DAC, for instance, the highest VIP values were identified in the region of visible (400–680 nm), being more pronounced in Green (520–580 nm) and Red-Edge (700–750 nm). This behavior was similar for dates 99, 164, 200, 228, 255 and 313 DAC, as well as for the general prediction. Conversely, the VIPs in the collections 99, 200, 228 and 255 DAC also registered pronounced peaks in the shortwave infrared region (1360–1660 nm and 1780–2000 nm), whereas the VIPs regarding 313 DAC (close to the harvest) presented a peak in the near-infrared (750–1360 nm) (Fig. 5).

The VIP values show the region of visible as more sensitive to N variations in the sugarcane leaf; additionally, the smaller the LNC observed, the smaller the contribution of the range of visible in the prediction and with sharper peaks in the wavelengths of green and red-edge (99, 200, 228 and 255 DAC).

3.5. Validation in the Vis-NIR models

To further study the potential of the ideal wavelengths for leaf N prediction in sugarcane, models of only the wavelengths in the visible (400–680 nm) and red-edge (680–750 nm) were generated, which were selected as the most effective wavelengths for leaf N prediction in sugarcane. The bands in the Vis-NIR range presented the greatest importance in the prediction of N in the sugarcane leaf (Fig. 5); therefore, new models were calibrated considering only these spectral ranges (Fig. 6).

The models of prediction with Vis-NIR had a better performance ($R^2 > 0.83$ and RMSE <1.04) in all dates (Fig. 6), as well as the

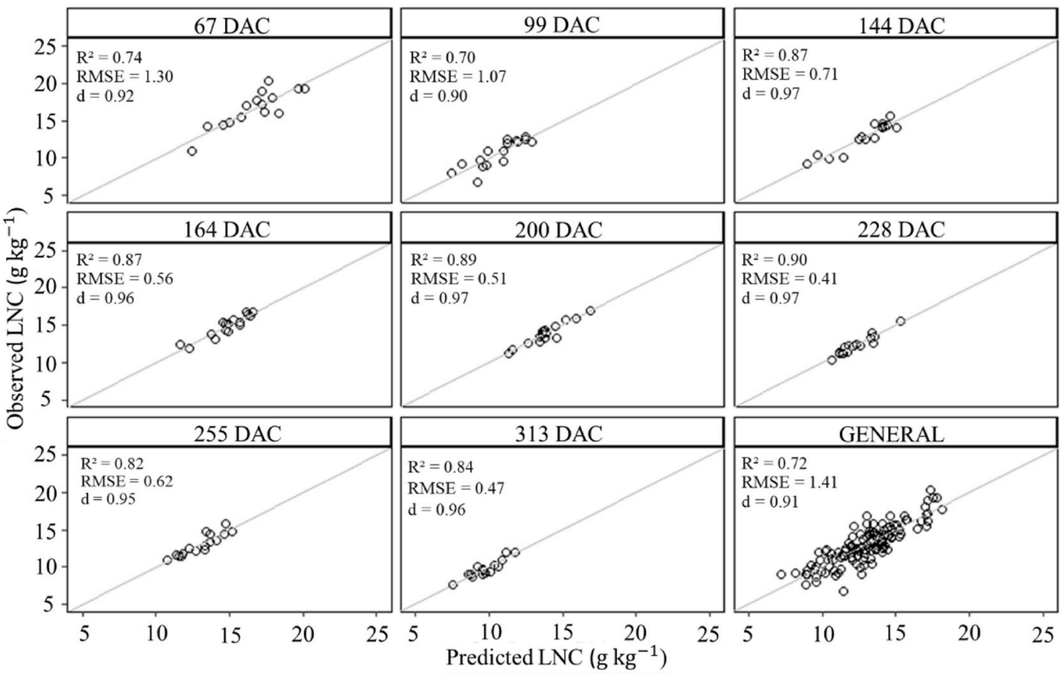

Fig. 4. Estimation of LNC by the sugarcane leaf spectra by the Partial Least Squares Regression - PLSR for the dates 67 DAC, 99 DAC, 144 DAC, 164 DAC, 200 DAC, 228 DAC, 255 DAC, 313 DAC and general. The values of R^2, RMSE and d were obtained from the results of the k-fold CV validation.

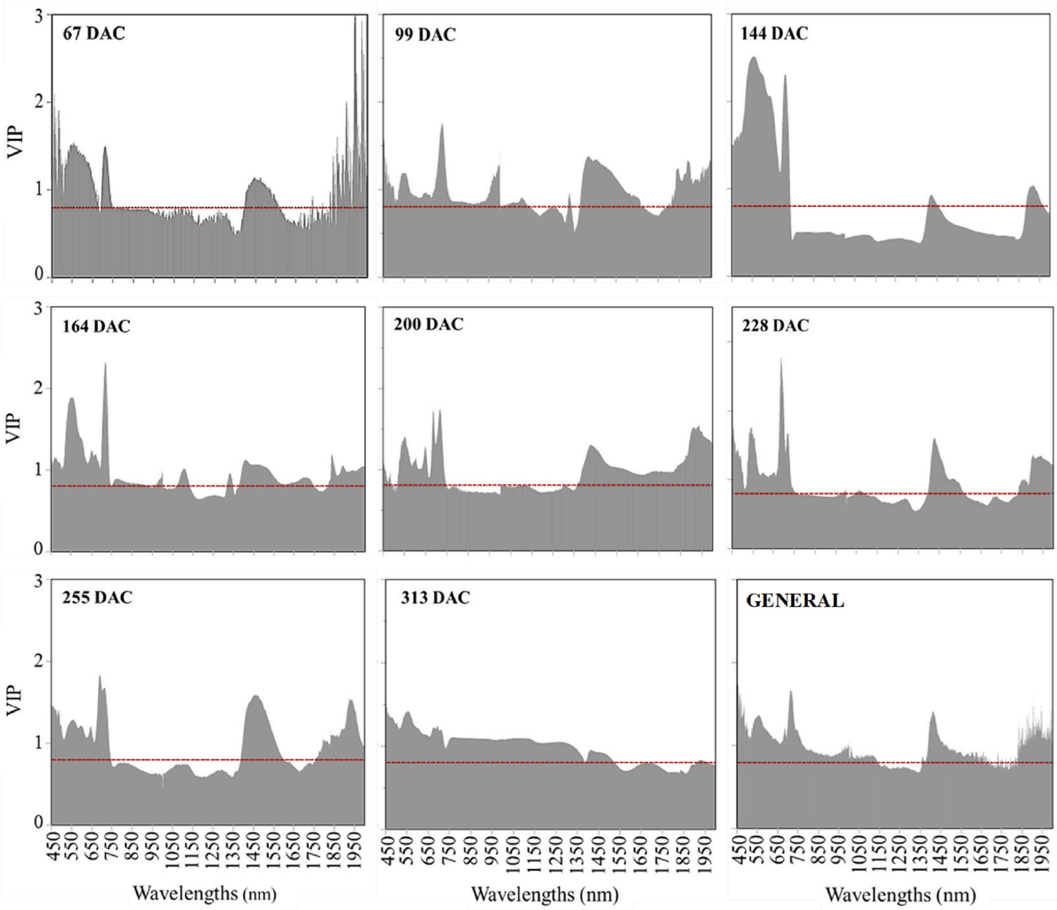

Fig. 5. Wavelengths of the greatest Variable Importance in Projection (VIP) according to the regression models during the prediction stage, considering as VIP values only the wavelengths above 0.8 (dotted line).

general prediction. The *d* index registered values at excellent levels (d > 0.94), indicating good accuracy.

3.6. Validation by LOOCV and holdout

At this stage, for the prediction process, two validation techniques were used: LOOCV (Fig. 7a) and holdout (Fig. 7b), divided into two steps: (i) using all wavelengths (450–2000 nm); and (ii) only the effective wavelengths (450–750 nm). In general, the performance of the prediction with the data in the range of visible and red-edge (450–750 nm) demonstrated the most satisfactory results in the two techniques, LOOCV ($R^2 = 0.68$, RMSE = 1.45 g kg^{-1}) and holdout ($R^2 = 0.68$, RMSE = 1.59 g kg^{-1}). In a comparison between the two validation methodologies employed, it was observed that the model derived from the technique holdout demonstrated a superior adjustment ($R^2 = 0.60$ and RMSE = 1.23 g kg^{-1}) when all wavelengths were considered (450–2000 nm). This scenario was reversed when the analysis was restricted to the wavelengths of 450–750 nm, resulting in an inferior R^2 (0.67) and superior RMSE (RMSE = 1.59 g kg^{-1}), compared to the LOOCV technique ($R^2 = 0.68$ and RMSE = 1.23 g kg^{-1}).

4. Discussion

4.1. Leaf nitrogen content in sugarcane

The N contents in the leaf stayed below the ideal range of 18 and 25 g kg^{-1} [40], except for the collection 67 DAC. In this stage, the culture was in the initial period of the vegetative development, which, according to the literature, is the stage in which sugarcane presents the greatest concentrations of N in the leaf, as well as large leaf area and photosynthesis rate [41,42]. On the other hand, the lowest LNC occurred in the last collection (313 DAC), a decrease which derived from the increase in sucrose contents [43].

The low LNC values occurred in the months of lower rainfall concentrations in the region, which contributed to a decrease in the absorption of N and other nutrients by the plant [44,45]. The incidence of water deficit in the periods between tillering and the beginning of the big growth may lead to an insufficient supply of nitrogen, given the restricted capacity of absorption by the plant [4].

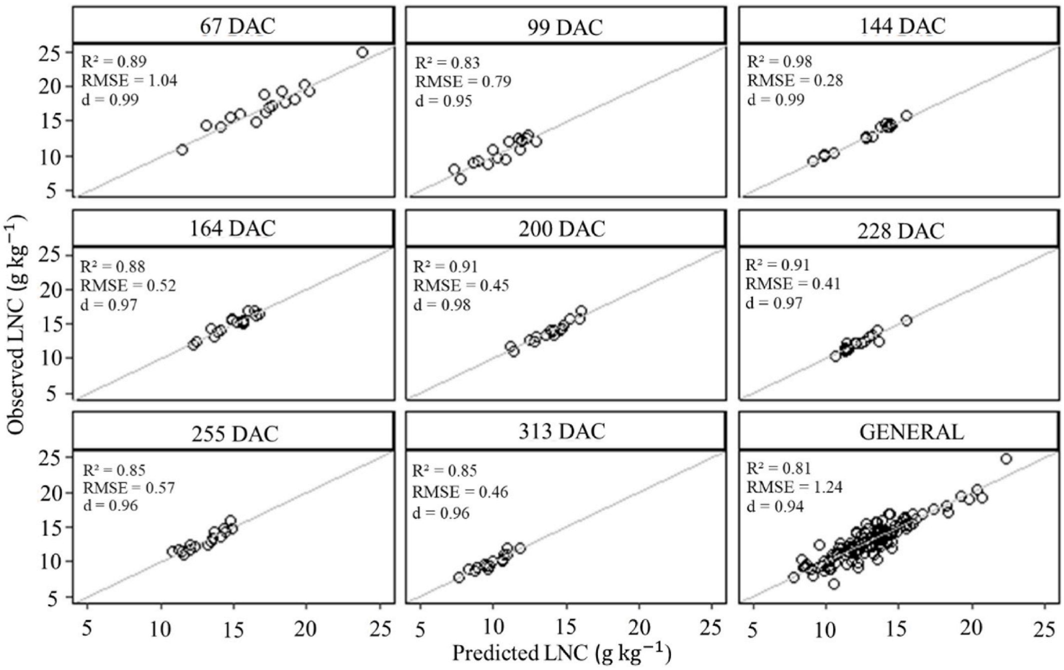

Fig. 6. Validation of the predicted N contents with Vis-NIR spectrum for the dates 67 DAC, 99 DAC, 144 DAC, 164 DAC, 200 DAC, 228 DAC, 255 DAC, 313 DAC and GENERAL. The values of R^2, RMSE and d were obtained from the results of the k-fold CV validation.

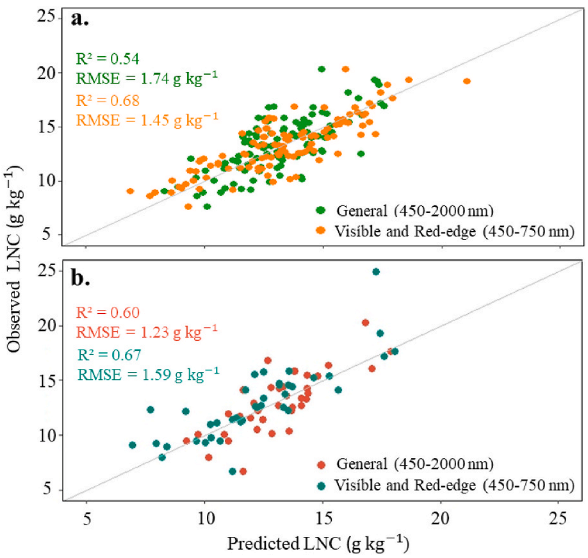

Fig. 7. Dispersion of the observed and predicted values for the Leaf Nitrogen Content - LNC in sugarcane from the general (450–2000 nm) and effective (400–750 nm) spectral data, using the validation technique LOOCV, with a validation with seven collections and a validation with only one which did not participate in the prediction (Fig. 7a.) and holdout, where 70% of the data were used for prediction and 30% for validation (Fig. 7b.).

Furthermore, there are severe reductions in the photosynthetically active area of sugarcane when subjected to water deficit [46,47].

4.2. *Influence of nitrogen on the hyperspectral reflectance of the sugarcane leaf*

The leaves that presented the highest N contents had the greatest absorption of electromagnetic energy in the wavelengths of green (550 nm), which demonstrates that LNC was directly related to the leaf pigments. This happened because the pigments present in the chloroplasts absorb the greatest part of light in the range of green, especially in the wavelengths above 530 nm [48]. Chlorophyll, in this case, participates in the interaction between the electromagnetic radiation and the internal components of the leaf, mainly influencing radiation absorption [48,49]. Therefore, leaves with high N content present a higher absorbance level. This occurs because of the high concentration of chlorophyll in the leaf tissues, that increase the interaction with the electromagnetic radiation [50].

In general, the wavelengths in the visible 450–680 nm are known for being sensitive to variations in leaf N, which demonstrates the LNC of sugarcane can be measured by reflectance spectroscopy, in agreement with previous works [16,51,52]. One of the main effects aused by the deficiency or variation in LNC in the leaves can be observed by the variation in chlorophyll and in the photosynthetic rate of the plants, factors that are directly related to leaf reflectance [53–56].

4.3. N prediction by leaf spectrum

In this study, sugarcane was used in its second production cycle, where prediction best adjusted to 200 and 228 DAC (Fig. 4). Reyes-Trujillo et al. (2021) employing the same prediction technique (PLSR), but with canopy reflectance values, highlight that N prediction in sugarcane leaves can be performed with the greatest precision in the culture at 90, 60 and 180 DAC, respectively.

The general laboratory model was reliable (Fig. 4, General model), with values of R^2 and RMSE of 0.72 and 1.41 g kg^{-1}, respectively, as well as the others for the analyzed dates. The values of $R^2 > 0.70$ obtained in this work can be considered promising, since, in the literature, similar works have obtained values of R^2 equal to 0.76 [57] and 0.81 [58] for LNC prediction. Reyes-Trujillo et al. (2021) preformed studies regarding the estimation of N concentration in sugarcane canopy from spectral data on leaf reflectance. The authors highlight they found prediction models at acceptable (R^2 between 0.50 and 0.75) and good (R^2 higher than 0.75) levels. Li et al. (2016) tested different methods for leaf nitrogen prediction in canola (*Brassica napus* L.) crop using spectral data. The results demonstrated that the technique PLSE employed in the leaf spectra after transformation by the first derivative produced the highest coefficient of determination (R^2 0.963) and the lowest RMSE (0.29 g kg^{-1}).

In general, the most important wavelengths for the prediction of leaf N were in accordance with the variations in the spectral curves (Fig. 5), where the region of visible, especially in the range of green (520–580 nm), demonstrated great sensitivity to changes in the leaf N contents. The spectral region of blue (450–500 nm) recorded a high participation in the predictions, which is interesting, since the region of blue is not commonly associated to the nutritional stress of the plant, generating situations for greater investigations. Nonetheless, these findings have already been mentioned in other studies [36,38].

Nevertheless, this aspect is flexible and varied according to the vegetative stage of the sugarcane crop. Except for the collections at 144 and 313 DAC, in the others, the mid-infrared at 1450 nm presented a great contribution in the prediction, and the spectral bands 1450 nm and 1950 nm are related to the water content in the leaf [59], this might be related to the concentrations of rainfall in the region of the experiment, since rainfalls did not occur regularly in the period of the collections, which might have influenced the process of N absorption by the plans [46]. Thus, LNC suffered variations and, consequently, the spectral curves and the models responded to these variations, as demonstrated in this study.

Therefore, identifying the limiting factors (water and nutrient deficit) at the moment of the spectral reads is of extreme importance for the adoption of new techniques that are non-destructive and sensitive to alterations in the genotype and phenotype of the culture. The results of this work indicate that VIS-NIR-SWIR spectroscopy can be a useful tool for decision making, since it could identify the variations in LNC at different collection dates. At dates 99, 200 and 255 DAC, for instance, a period in which there were low rainfall concentrations in the region, the spectral ranges of blue (450–500 nm) and mid-infrared (1360–1660 nm and 1780–2000 nm) had the greatest importance in the predictions. Water scarcity causes significant alterations in nutrient absorption by the cultures [60], with the consequence of reduced nitrogen metabolism, with a decrease in cell expansion [45]. Therefore, the region of the mid-infrared is influenced by the water contents inside the leaf, which, in turn, can be altered according to the amount of water available for the plant [61].

The data obtained near sugarcane harvest (313 DAC) presented again a spectral behavior different from previous dates, with a contribution of the spectra in the region of NIR and a pronounced decrease in the wavelengths of red-edge. This characteristic derives from the advanced maturation stage of the crop, a period in which there is a reduction in the N contents of the plant for the sucrose contents to increase [43]. Furthermore, the band of red-edge is linearly related to the concentration of N [58]. Since there was a decrease in the leaf N contents at 313 DAC, the values of VIP in this date were also lower.

4.4. Models generated from the effective wavelengths

The graph on VIP (Fig. 5) identified the wavelengths with the greatest importance in N prediction. According to the magnitudes, the regions of visible (450–680 nm) and red-edge (680–750 nm) were the most important spectral ranges. Therefore, the models generated with only the effective wavelengths (450–680 nm) presented the best performance ($R^2 > 0.81$, RMSE <1.24 g kg^{-1}, d index >0.94), which is in accordance with the literature [18,36,38]. Li et al. (2016) selected, by the VIP values, the spectral wavelengths at 432, 467, 519, 614, 772, 912 and 1072 nm to generate nitrogen prediction models with greater precision for the canola crop (*Brassica napus* L.); consequently, the new models achieved values of $R^2 > 0.86$, being considered acceptable. The VIP analysis identified the same spectral regions (visible and red-edge) as the most important (effective) for N prediction in apple tree, improving the MLR (Multiple Linear Regression) models when only the effective wavelengths were used ($R^2 = 0.78$ for the raw data and $R^2 = 0.77$ for the data transformed by the first derivative) [13].

This better adjustment of the models with the bands of visible and red-edge occurs because these spectral ranges are directly related to pigment absorption in the crops [38]. N predictions from chlorophyll indices, in this case, have been demonstrated as promising ($R^2 = 0.74$), a fact already expected, considering the strong correlation between the leaf N and the Chlorophyll contents [52,61]. Other studies indicate the spectral ranges of visible and red-edge as promising to be directly related to the leaf N content [16,20,55], which justifies the more satisfactory prediction results in this study.

4.5. Leave-one-date-out cross validation

Another important aspect is to evaluate the potential of the calibrated models to predict sugarcane information regarding a period that has not been sampled. In practice, predictive models capable of describing the nutritional status of sugarcane under different conditions are sought, regardless of the phenological stage of the plant, or even the possible impacts of adverse conditions (e.g., variation of the water content in the leaf). Therefore, to represent the impact of these factors on the prediction, as well as to prove the robustness of the models generated in this work, we performed the leave-one-date-out (LOOCV). To compare and evaluate the efficacy of the LOOCV technique, the holdout validation was employed to test which technique best adjusted to the spectral data.

When the performance of the methods was compared (R^2 and RMSE) by the techniques of LOOCV (Fig. 7a.) and k-fold CV (Figs. 6 and 4), the values found by LOOCV were lower ($R^2 = 0.54$ and 0.68, for the ranges 450–2000 nm and 450–750 nm, respectively). According to [62], in some specific situations, LOOCV may present reductions in the performance of the models, because of the decrease in the size of the sample used for the test. Furthermore, it was observed that the model derived from the holdout technique demonstrated a superior adjustment ($R^2 = 0.60$ and RMSE = 1.23 g kg-1) when all wavelengths were considered (450–2000 nm). This scenario was reversed when the analysis of the wavelengths was restricted to 450–750 nm, resulting in an inferior R^2 (0.67) and superior RMSE (RMSE = 1.59 g kg-1), compared to the LOOCV technique ($R^2 = 0.68$ and RMSE = 1.23 g kg-1). Although the validation results were inferior in LOOCV in some cases, the performance of the models was satisfactory, thus confirming the robustness and the possibility of LNC prediction in several conditions and periods.

Sexton et al. (2021) also used the PLS technique for the prediction of N in tobacco leaf, and highlight a reduced performance ($R^2 = 0.35$) in the cross validation (leave-one-out) when all wavelengths were used (350–2500 nm), with increased performance of the model ($R^2 = 0.59$) when it was predicted using the wavelengths of the mid-infrared (1400–2500 nm). This technique was employed in other predictions and the results of the validation (leave-one-out) indicated that there are significant correlations between the estimated and observed values for the N contents in different cultures ($R^2 = 0.663$, RMSE = 0.577) [63]. Therefore, our results demonstrated that this technique of prediction by spectroradiometry is promising and has the potential for application in other cultivation environments and other cultivars, as long as representative data sets and robust prediction models are employed.

4.6. Limitations and perspectives

The absorption of electromagnetic radiation is directly related to components such as phenolic compounds, flavonoids, anthocyanins, carotenoids, and chlorophyll concentration [64]. Many of these compounds, such as chlorophyll, are biochemical indicators strictly related to the N content in the plants [6]. These interactions between N and the physiological compounds of the plants contribute to generate characteristic curves of leaf reflectance. In this work, we observed that there are characteristic spectral ranges for N (450–680 nm), in which plants with greater N contents reflect less in the visible band. Therefore, the selection of wavelengths characteristic to the response of N needs to be considered in research works with spectroradiometry by PLSR. Traditionally, the studies on nutrient prediction in leaves consider the whole spectrum (450–2500 nm) or, at most, the cuts are performed in the VIS-NIR range of 400–1300 nm, without a model or algorithm to assist in decision making. This can be considered as a deficiency, since we observed that when we worked only with the spectral ranges most related to the N contents, the predictive models improved. Therefore, the selection of variables before PLSR modeling may generate models in a simple, fast and precise way, allowing the acquisition of cheaper sensors or sensors with specific wavelengths, which do not need to operate in the whole VIS-NIR-SWIR range [65].

Future research works will be developed to explore two aspects that are still not considered in the N prediction works: i) To evaluate the hyperspectral responses of the sugarcane crop in regions geographically distinct from the calibration. In other words, we will investigate the feasibility and the efficacy of prediction models in areas characterized by climate conditions and soil types distinct from those included in the calibration process. ii) To incorporate as covariables in the models, in addition to spectral signatures, information associated to the sugarcane production environment, with emphasis to the variables related to the soil (pH, organic matter content and texture), climate conditions (rainfall, temperature, relative humidity and solar radiation) and topography (altitude, relief, inclination). Therefore, we will test different data sources to reinforce the prediction capacity, considering the diversity in planting.

5. Conclusion

The study on N prediction for sugarcane from all wavelengths (450–2000 nm) generated acceptable models ($R^2 > 0.70$ and RMSE <1.41 g kg-1). The region of visible (400–480 nm) and red-edge (680–750 nm) were the bands of greatest importance in the prediction of N by spectral data. In this case, clearly, the prediction models are improved ($R^2 > 0.81$ and RMSE <1.24 g kg^{-1}) from the use of only the visible and red-edge spectra for N prediction. In general, the rise in LNC because of the application of increasing nitrogen doses in the soil promoted a smaller reflectance in all wavelengths of the visible (400–680 nm). The wavelength of green (550 nm), in the descriptive analysis of the spectral curves, presented reduced reflectance with the greatest LNC.

The technique was promising and efficient in the prediction of nitrogen in the sugarcane crop. Nevertheless, new studies in controlled environments are recommended, in different periods and harvests, to evaluate whether the effects of prediction will remain with the R^2 and RMSE values at acceptable levels, testing the possibility of generating models from data from other harvests and cultivars. Therefore, the construction of PLS models considering N and hyperspectral reflectance at the leaf level, may contribute to a better understanding of nitrogen fertilization with the vegetative parameters of the culture, and new techniques can be developed to help the management of fertilization in more appropriate moments.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Peterson Ricardo Fiorio: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Carlos Augusto Alves Cardoso Silva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Formal analysis, Conceptualization. **Rodnei Rizzo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Formal analysis, Data curation, Conceptualization. **José Alexandre Melo Demattê:** Writing – review & editing, Writing – original draft, Visualization, Resources, Formal analysis, Conceptualization. **Ana Cláudia dos Santos Luciano:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Marcelo Andrade da Silva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

To the Luiz de Queiroz Agrarian Studies Foundation – FEALQ, for funding the publication of this work. To the State of São Paulo Research Foundation (FAPESP), for funding the project n. 2013/22435-9, to which this work belongs, and to the Research and Projects Financing (FINEP), which, by the project PROSENSAP, allowed the acquisition of the spectroradiometer used in this research.

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