Near-infrared spectroscopy for the evaluation and prediction of injuries associated with the attack of *Thaumastocoris peregrinus* (Carpintero & Dellapé) in *Eucalyptus camaldulensis* (Dehnh)

Espectroscopia de infravermelho próximo para a avaliação e predição de injúrias associadas ao ataque de *Thaumastocoris peregrinus* (Carpintero & Dellapé) em *Eucalyptus camaldulensis* (Dehnh)

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Resumo

Injúrias causadas por agentes estressores provocam mudanças na eficiência fotossintética das plantas, detectáveis por meio de análises ecofisiológicas. A espectroscopia de infravermelho próximo (NIRS - Near-infrared Reflectance Spectroscopy) é uma alternativa viável para a avaliação do estado ecofisiológico de plantas. Este estudo objetivou investigar o uso da técnica NIRS, aliada a métodos de calibração multivariada para avaliar a ecofisiologia de *Eucalyptus camaldulensis* atacados por *Thaumastocoris peregrinus*. Para isso, plantas foram mantidas em sala climatizada, foram infestados pelo inseto. Durante 13 dias, a cada 48 horas, foram medidas na superfície das folhas atacadas, a fluorescência inicial (F_o) e a fluorescência máxima (F_m) para a determinação da eficiência quântica potencial do Fotossistema II (F_v/F_m) e a taxa de transporte de elétrons (ETR) com o auxílio de um fluorímetro, o teor relativo de clorofila, por meio de um clorofilômetro e o teor de umidade foliar, sendo seus espectros obtidos em um espectrofotômetro de infravermelho próximo para o desenvolvimento dos modelos. O método utilizado foi o de mínimos quadrados parciais e a validação foi cruzada. Após a calibração e validação, foi feita a previsão dos modelos onde os resultados foram avaliados por meio do coeficiente de correlação e erro quadrático médio. Na etapa de previsão, registrou-se boa correlação e menor erro quadrático médio para as variáveis F_v/F_m , ETR e teor de umidade. Os resultados indicam que a técnica NIRS pode ser empregada para a caracterização e predição de danos associados ao ataque do percevejo bronzeado.

Palavras-chave: Percevejo bronzeado, Ecofisiologia, Espectroscopia, Infravermelho próximo.

Abstract

Injuries caused by stressor agents cause changes in the photosynthetic efficiency of plants, detectable by means of ecophysiological analysis. Near-infrared spectroscopy (NIRS - Near-infrared Reflectance Spectroscopy) is a viable alternative for evaluating the ecophysiological state of plants. This study aimed to investigate the use of the NIRS technique allied to multivariate calibration methods to evaluate the ecophysiology of *Eucalyptus camaldulensis* attacked by *Thaumastocoris peregrinus*. To do this, plants were maintained in an acclimatized room and infested by the insect. During 13 days, at every 48 hours, the initial fluorescence (F_o) and the maximum fluorescence (F_m) were measured on the surface of the attacked leaves to determine the potential quantum efficiency of Photosystem II (F_v/F_m) and the electron transport rate (ETR) with the aid of a fluorometer, the relative chlorophyll content, by means of a chlorophyll meter, and the foliar moisture content, its spectra being obtained in a near-infrared spectrophotometer to develop the models. The method used was that of partial least squares and the validation was crossed. After the calibration and validation, model prediction was performed where the results were evaluated through the correlation coefficient and mean square error. In the prediction step, there was good correlation and lower

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mean square error for the variables F_v/F_m , ETR and moisture content. The results indicate that the NIRS technique may be employed for the characterization and prediction of damages associated with the attack of the bronze bug.

Keywords: Bronze bug, Ecophysiology, Spectroscopy, Near infrared.

INTRODUCTION

Thaumastocoris peregrinus (Carpintero & Dellapé) is a pest insect of *Eucalyptus* spp. that has great potential for damage. The insect's sucking feeding habit causes the silvering (chlorosis) of leaves that, during the infestation period, begin displaying a bronze aspect, subsequently drying and falling off, leading to the reduction of the photosynthetic area of the plant (JACOBS; NESER, 2005; WILCKEN et al., 2010).

The leaves subjected to stressor agents may trigger a complex and interactive set of molecular and physiological responses, including effects on photosynthesis and the different underlying mechanisms (NABITY et al., 2009). By means of ecophysiological studies, it is possible to understand the different responses of plants to environmental variations, as well as the capacity of adaptation to different stress conditions, including the presence of insects (BOWN et al., 2002). In this sense, measurements of foliar chlorophyll content and chlorophyll fluorescence are key tools, often used to investigate the response of plants to injuries caused by the feeding habit of insects (BOUNFOUR et al., 2002; BURD; ELLIOTT, 1996; IATROU et al., 1995).

In the near-infrared region, the optical properties of the vegetation are determined by the existing discontinuities between cell walls and by intercellular air spaces in the internal structure of the leaves; and these are closely related with their physiological state (BLACKBURN, 1998; MIRIK et al., 2007; PEÑUELAS; FILELLA, 1998). Diseased tissues tend to present lower reflectance in the infrared region in relation to healthy tissues, allowing the detection and quantification of symptoms (WEST et al., 2003). The amount of energy reflected on this wavelength is related to the plants' photosynthetic activity and this region is often employed in studies related to plant diseases (SANTOS JUNIOR et al., 2002).

The fast and predictive observation of symptoms is key for evaluating injuries and for detecting plants in areas attacked by pest insects. The NIRS (Near-infrared Reflectance Spectroscopy) technique is becoming a viable alternative, employed without losses in quality and in the specificities of the evaluations, in addition to being simple, fast, non-destructive and non-invasive, wherein its precision depends mainly on the accuracy of the method used as reference (VIANA et al., 2010).

This work had as objective to verify whether there is a correlation between spectral responses in the near-infrared region of the electromagnetic spectrum and the photosynthetic variables of leaves attacked by the bronze bug.

MATERIAL AND METHODS

The ecophysiological analyses were performed in an acclimatized room at the Integrated Center for the Propagation of Forest Species (CIPEF), located in the Department of Forest Engineering and the reflectance spectroscopic analyses in the near infrared (NIRS) were performed in the Laboratory of Microvinification and Quality of Fermented Beverage (LabVin), located in the installations of the Chemistry Department, both located at the JK Campus of the Federal University of Vales do Jequitinhonha e Mucuri, in Diamantina, Minas Gerais.

For the ecophysiological study, 10 plants of *E. camaldulensis* of approximately 2.5 years of age were employed, kept in an acclimatized room ($25 \pm 2^\circ\text{C}$, relative humidity of $60 \pm 5\%$ and photoperiod of 12 hours) under a stream of $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetically active photons. After a period of seven days for acclimatization, insects collected in a commercial plantation were transferred to the plants, simulating a natural bronze bug attack.

The evaluations were performed on the surface of expanded leaves with significant presence of insects and located in the middle third of the plant. The relative content of total chlorophyll and the chlorophyll *a* fluorescence variables: initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), potential quantum efficiency of Photosystem II (F_v/F_m) and the maximum electron transport rate (ETR) were evaluated at every 48 hours, during 14 days.

The chlorophyll *a* fluorescence variables were determined using a portable PAM fluorometer (JUNIOR-PAM, Heinz Walz GmbH). To perform the readings, magnetic leaf clips, connected to the fluorometer, were placed in the median region, on the adaxial side of the foliar limb. The relative total chlorophyll content of the evaluated leaves was quantified through the Soil Analysis Development chlorophyll meter (SPAD-502, Minolta, Japan). The evaluations were done in two points on each one of the sides of the leaves' central vein, generating a mean value.

The leaves were collected, washed with distilled water and subjected to spectrophotometric analyses, carried out by the spectrophotometer of the FEMTO brand, model NIR 900PLS. In this device, the leaves were accommodated in a glass cuvette for sample reading, having its entire adaxial surface evaluated (base and tips of the leaves). A spectral interval at the wavelength between 1100 to 2500 nm was employed. The device's operation mode was in Reflection and the reading was provided in Log 1/R.

After the completion of the readings, the leaves were weighed and subjected to drying in an oven at 60°C to calculate the percentage of foliar moisture.

The first collection, comprised of 10 foliar samples, was performed before insect infestation and the other six collections, comprised of five foliar samples each, were performed after the beginning of the infestation process. For the construction of models for the analysis of the ecophysiological characteristics, 40 foliar Eucalyptus samples were employed. These samples were measured in two regions (base and tips of the leaves), generating the 80 spectra used for model calibration and validation.

Regression equations were adjusted relating the spectral information with the results of ecophysiological analyses. To evaluate the prediction ability of the constructed models, 10 plants were once again infested by the bronze bug, simulating the insect's attack, in the same previously mentioned conditions. In the same way, another seven leaf collections were performed, considering the same duration and symptoms of the bronze bug attack over time. These leaves were subjected to analyses through the spectrophotometer and the ecophysiological variables were estimated based on the previously obtained reflectance values.

The ecophysiological analysis were evaluated by the Main Components Regression method. The multivariate calibration method used was the regression of Partial Least Squares. To evaluate the efficiency of the calibration models, the root mean square error (RMSE) values were calculated, which express model accuracy by the proximity between the values calculated by the model and the values obtained by the analytical method.

The results obtained in the prediction step were analyzed by comparing the results obtained using the NIRS technique and those using conventional ecophysiological analyses methods. These were evaluated by means of correlation coefficients at 5% of significance, generated between the results of the two methodologies employed.

RESULTS AND DISCUSSION

From the ecophysiological data analyzed, a main component analysis was performed to verify the interrelation between these variables (Figure 1).

Figure 1 shows the graph of the scores for the two main components. In this graph, it is possible to detect the relations between the variables and the duration of infestation. The highest values for the relative chlorophyll content, Fv/Fm and ETR variables were registered at zero time, when the plants had not been infested yet, and decreased over the infestation period. In contrast, at the end of the infestation period, increases in the values of Fo and Fm were observed. The values recorded for the foliar moisture variable also decreased during the time of exposure of the plants to the insects' attack.

The response to a stressful condition can change the foliar chlorophyll content (COSTA et al., 2001; FANIZZA et al., 1991; SAMDUR et al., 2000), causing decreases in the plant's photosynthetic capacity. Moreover, the occurrence of chlorosis on the leaves is associated to the feeding habit of sap-sucking insects (NI et al., 2001). The chlorosis symptoms, observed in Eucalyptus leaves, indicate that the succivorous feeding habit of *T. peregrinus* might have affected the chlorophyll, causing the progressive loss of the photosynthetic rate. The reductions in the foliar chlorophyll content throughout the infestation period are possibly associated with the type of injury caused by the bronze bug. These reflect on the chlorophyll *a* fluorescence emission kinetics and may explain the behavior of the variables Fo , Fm , Fv/Fm , ETR .

Figure 1. Graph of PC1 and PC2 weights for the 6 observed variables: Maximum fluorescence (Fm); Initial fluorescence (Fo), Quantum efficiency of PSII (Fv/Fm), Leaf moisture (Moisture); Relative chlorophyll content (Chlorophyll) and electron transport rate (ETR).

Figura 1. Gráfico dos pesos de PC1 e PC2 para as 6 variáveis observadas: Fluorescência máxima (Fm); Fluorescência inicial (Fo), Eficiência quântica do FSII (Fv/Fm), Umidade foliar (Umidade); Teor relativo de clorofila (Clorofila) e Taxa de transporte de elétrons (ETR).

The inactivation and, or, the impairment of the reaction centers of Photosystem II (FSII) can be evidenced by the *Fv/Fm* ratio. Stress factors may affect the working of the FSII, reducing the values observed for *Fv/Fm* (KRAUSE; WEIS, 1991). Generally, plants having the photosynthetic apparatus in perfect conditions present values of *Fv/Fm* ranging between 0.75 and 0.85, whereas values below 0.75 may indicate damage to the plants' photosynthetic apparatus (BOLHÀR-NORDENKAMPF et al., 1989). The decreases in the values recorded for this variable over the infestation period indicate that the injuries caused by the bronze bug attack may have compromised the photosynthetic efficiency of *E. camaldulensis*.

In addition to the reduction in the photochemical efficiency of FSII, the bronze bug attack also caused greater decreases in ETR values in infested plants over the infestation period, suggesting that the presence of the insect in the leaves altered the fluorescence due to the damages caused in the photosystems. Welter (1989) and Zangerl *et al.* (2002) also reported the occurrence of losses in the quantum yield and on the electron transport rates of plants attacked by phyllophagous insects.

In relation to foliar moisture, it should be emphasized that this has a significant impact on photosynthesis and that, depending on the feeding habit of the insect, there may be a disturbance of the physical integrity of the leaves, allowing the loss of water (OSTLIE; PEDIGO, 1984, WELTER, 1989). In addition to the likely loss of photosynthetic tissue, the herbivory by *T. peregrinus* caused reductions in moisture content in eucalyptus leaves subjected to insect attack during the experimental period.

The calibration models acquired and the results observed between the real values of the eco-physiological variables, obtained by traditional methods, and the predicted values, obtained by spectrophotometric analyses, were plotted on correlation graphs for each of the variables (Figure 2).

Figure 2. Correlation between the reflectance values in the near infrared of *Eucalyptus camaldulensis* leaves, attacked by the bronze bug, measured in vivo and estimated by the adjusted model. (a) Moisture in eucalyptus leaves. (b) Relative foliar chlorophyll content. (c) Initial fluorescence (Fo). (d) Maximum fluorescence (Fm). (e) Quantum efficiency of FSII (Fv/Fm). (f) Electron transport rate (ETR). The values measured in vivo and used for model calibration are in blue and the predictive values after validation for the same model are plotted in red.

Figura 2. Correlação entre os valores de reflectância no infravermelho próximo de folhas de *Eucalyptus camaldulensis*, atacadas pelo percevejo bronzeado, medidos in vivo e estimados por meio do modelo ajustado. (a) Umidade em folhas de eucalipto. (b) Teor relativo de clorofila foliar. (c) Fluorescência inicial (Fo). (d) Fluorescência máxima (Fm). (e) Eficiência quântica do FSII (Fv/Fm). (f) Taxa de transporte de elétrons (ETR). Em azul estão os valores medidos in vivo e utilizados para a calibração do modelo e em vermelho são plotados os valores preditos após validação para o mesmo modelo.

The best correlations were observed for the foliar moisture variables, *Fv/Fm* and *ETR*. It should be emphasized that correlations close to 0.70 are expressive, since the standards employed for model construction are not simulated and, or pre-quantified, but are real samples, subject to natural interferences (SANTOS, 2011). The modeling prediction errors for these variables were lower than the prediction errors observed for the relative chlorophyll content, *Fo* and *Fm*.

The analysis of light reflectance by the leaves can be employed for evaluating the structure and the physiological state of a plant (CARTER; KNAPP, 2001; ZARCO-TEJADA et al., 2000). In general, the prediction of the morphophysiological variables of eucalyptus leaves infested by the bug presented linearity. This indicates that, for the set of samples evaluated, the values predicted by the models in NIR spectroscopy were correlated with actual values, obtained by conventional methods.

Using wave ranges of a hyperspectral sensor to generate standardized wave indices, employed for the prediction of damages associated to the bronze bug, Oumar et al. (2013) registered high correlations between the damages caused by the insect and the generated indices, calculated from the wavelengths in the near infrared region. The strong correlations were attributed to the physiological alterations observed in the leaves, by the stress on the physiological variables, stemming from the attack of *T. peregrinus*.

The prediction of morphophysiological characteristics of eucalyptus plants attacked by the bronze bug by means of NIR spectroscopy was successful. This technique is fast and non-destructive and has great potential to evaluate damages caused by the insect in the photosynthetic apparatus of leaves. In addition, this technique can be employed to obtain information on plant health and the level of infestation in crops where the bug is present. This can facilitate pest control and reduce monitoring costs, considering that the acquisition of NIR electromagnetic data can be performed using a drone, a low-cost unmanned vehicle, compared to the current means of monitoring.

CONCLUSÕES

The bronze bug attack affects the photosynthetic apparatus of *E. camaldulensis* leaves and can cause damages to its productivity.

The analysis of the ecophysiological variables allows the evaluation of damages to the photosynthetic apparatus of *E. camaldulensis* associated with the bronze bug attack.

The analysis of reflectance in the near-infrared region can be employed to evaluate damages to the photosynthetic apparatus of *E. camaldulensis* subjected to the bronze bug attack, as well as for monitoring the degree of infestation at field level, as a replacement for conventional methods.

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