Estimate of soybean defoliation via digital image processing in software¹

Estimativa na desfolha da soja via processamento digital de imagens em software

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ABSTRACT - This study aimed to develop and validate the digital image processing software to quantify leaf coverage, employing the correlation of defoliation values and NDVI with various gradients of defoliation severity of the Asian soybean rust pathosystem. The digital images were obtained from the experiment conducted in 2013/2014. To conduct the experiment, 4 treatments (3 replicates) were adopted, considering the useful floor area of each plot (4 linear meters, 3 lines spaced at 0.45 m). The gradients of defoliation were obtained by treatment with fungicide to control Asian soybean rust. The quantification of the disease severity was performed through diagrammatic scale. The NDVI values were obtained using the GreenSeeker®, conducting the equipment above the plants. The digital photos were obtained in three heights and subsequently processed in software. Then the defoliation sampling was held in 10 plants through treatment. The image processing data correlated with defoliation (94.22%) and with NDVI (89.27%), and we also observed the correlation of defoliation with NDVI (96.12%). These data suggests the use of digital images as an alternative to quantify the vegetation cover, with the advantage of being a dynamic and fast method that does not require experience from the assessor to quantify soybean defoliation.

Key words: *Phakopsora pachyrhizi*. NDVI. Reflectance. Leaf coverage. RGB.

Palavras-chave: Phakopsora pachyhizi. NDVI. Reflectancia. Cobertura foliar. RGB.

RESUMO - Este estudo teve como objetivo desenvolver e validar o software de processamento digital de imagens para quantificar a cobertura foliar, empregando a correlação dos valores de desfolha e NDVI com vários gradientes de severidade do patossistema ferrugem asiática da soja. As imagens digitais foram obtidas a partir do experimento realizado em 2013/2014. Para a realização do experimento, foram adotados 4 tratamentos (3 repetições), considerando a área útil de cada parcela (4 metros lineares, 3 linhas espaçadas a 0,45 m). Os gradientes de desfolha foram obtidos por tratamento com fungicida para controlar a ferrugem asiática da soja. A quantificação da gravidade da doença foi realizada por meio de escala diagramática. Os valores do NDVI foram obtidos com o GreenSeeker®, conduzindo o equipamento acima das plantas. As fotos digitais foram obtidas em três alturas e posteriormente processadas em software. Em seguida, a amostragem de desfolha foi realizada em 10 plantas por tratamento. Os dados do processamento de imagem correlacionaram-se com a desfolha (94,22%) e com o NDVI (89,27%), e também observa-se a correlação da desfolha com o NDVI (96,12%). Esses dados sugerem o uso de imagens digitais como uma alternativa para quantificar a cobertura vegetal, com a vantagem de ser um método dinâmico e rápido, que não requer experiência do avaliador para quantificar a desfoliação da soja.

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INTRODUCTION

Asian soybean rust, caused by the fungus *Phakopsora* pachyrhizi Syd. & P.Syd, promotes early defoliation of the crop (GARCÉS-FIALLOS et al., 2018). It is considered the most destructive soybean leaf disease and influences soybean yield (HOSSAIN; YAMANAKA, 2019). The application of fungicide is the primary method to control the disease and has been useful to reduce losses in productivity, because it decreases the levels of early defoliation, which contributes to the crop grain yield (BEYER et al., 2019; CRUZ et al., 2014; INAYATI; YUSNAWAN, 2016), however, it increases considerably the costs of production (YAMANAKA et al., 2016).

The quantification of plant diseases is a fundamental part for the correct interpretation of control and epidemiology studies. The decision about which type of measure to use will depend on the characteristics of the disease itself and on the time available for the assessment (MARTINELLI *et al.*, 2015).

Defoliation has been used in the field to estimate the effect of disease control methods, especially in assessments at the end of the crop cycle, when it presents correlation with the productivity (GLIER *et al.*, 2015). Nevertheless, not always the methods to estimate the defoliation are accurate and/or fast, they depend on the experience and skill of the observer and present a certain level of subjectivity (LIANG; KIRK; GREENE, 2018), however, good results have been obtained with measurements of NDVI – Normalized Difference Vegetation Index (CRUSIOL *et al.*, 2017; DE LA CASA *et al.*, 2018; GUAN; NUTTER JR., 2002).

GreenSeeker® is an instrument that provides the NDVI, whose interpretation offers quick information that aims at nutritional conditions, physiological state, stress and potential yield of crops (AMADO *et al.*, 2017; JUNGES; FONTANA; LAMPUGNANI, 2019; VIAN *et al.*, 2018). This commercial active optical sensor works at the wavelength of the red (660 nm) and of the near infrared light (770 nm), it measures the light reflected by the plants and automatically calculates the NDVI (DE LA CASA *et al.*, 2018; ORIMOLOYE *et al.*, 2019).

The reflectance can detect variations in the leaf area of plants attacked by diseases and the behavior of the reflectance measures these are relevant information for productivity forecast (CAO *et al.*, 2015; ZHANG *et al.*, 2017), in precision agriculture for the application of fertilizers (ALI; IBRAHIN; MAHMOUD, 2018; RONGTING *et al.*, 2017) and for early detection of lesions in the soybean crop using herbicides (ZHAO *et al.*, 2014).

However, spectral sensing for assessing plant vigor in crops is limited by the strong soil background reflection

(PREY; VON BLOH; SCHMIDHALTER, 2018). Despite the good results obtained with the radiometry, the equipment is still expensive.

The analysis of digital images on computer allows the direct assessment of severity, and can also be used in other areas of research, such as in the assessment of the leaf area. The analyses through digital images have low cost, and can cost less than a tenth of the value of a leaf area measurement equipment (DE LA CASA *et al.*, 2018; MICHELS *et al.*, 2019).

Thus, the aim of this study was to validate the use of digital images processed in software as an alternative to quantify the defoliation caused by Asian soybean rust.

MATERIAL AND METHODS

The experiment was conducted at the State University of Londrina (UEL), in the municipality of Londrina, Paraná, at 23°19'40,92" of South latitude and 51°12'19.20" of West longitude and 560 m of altitude, in 2013/14. Soybean seeding was performed in 12/05/2013 and the cultivar used was the M 6410 IPRO.

To conduct the experiment, four treatments were used in areas of 12 m of length by 12 lines of width, with 0.45 m spacing between them (Figure 1), higher than that used by Crusiol *et al.* (2017) and Prey, Von Bloh and Schmidhalter (2018). Three subareas were used as repetitions and laid down within each area, where the data were carried out. The treatments intended to provide the defoliation gradient caused by the Asian soybean rust, where the treatment without application of fungicide was considered witness; in the control treatment, six sprays were held (30; 45; 60; 75; 90 and 105 days after emergence); also, there were four spraying treatments (60; 75; 90 and 105 days after germination) and three sprays (75; 90 and 105 days after emergence).

The fungicide used for the gradient of intensity of Asian soybean rust was the commercial mixture of Pyraclostrobin+Epoxiconazol (66.5 + 25 g i.a. ha⁻¹), by spray volume of 200 L ha⁻¹ plus mineral oil as a vehicle, at a dose of 500 mL ha⁻¹ (MICHELS *et al.*, 2016).

Through diagrammatic scale, developed by Godoy, Koga and Canteri (2006), the quantification of severity was conducted, defined as the percentage of leaf area covered with symptoms of the disease. The assessments started after the development of the first pustules and repeated weekly until the total defoliation of the crop. The assessments were carried out in 12 trefoils (four of the lower third, four of the middle third, and four of the top third per plot), according to the methodology proposed by Hikishima *et al.* (2010).

Figure 1 - Experimental design with treatments: witness, without application of fungicides (P0); control with 6 applications (P6); with 4 applications (P4); and with 3 applications (P3). The area highlighted represents an experimental unit of 12×5.4 meters, identifying its useful floor area of 10×4.5 meters

		-		
P0	P3	P6	P4	
P3	P4	P0	P6	
P0	P6	P3	P4	
P4	P3	P6	P0	

NDVI readings were held weekly after the first fungicide application, using the portable equipment GreenSeeker[®], RT100 model, of NTech. Three readings were performed by subarea, passing the equipment on the useful floor area, 0.80 m above the top of the plants, as recommended by the manufacturer. The readings were held in the morning, between 09:00 h and 12:00 h (CRUSIOL *et al.*, 2017).

The images were taken with a Lumix camera, DMC-FS3 model of 8.1 MP (ISO 1600 to 6400), of Panasonic. The camera was positioned perpendicularly to the plants, with the aid of a rod fixed to the ground, in three heights (1.20 m; 2.00 m and 3.00 m) (PREY; VON BLOH; SCHMIDHALTER, 2018). The images were obtained in three subareas within each treatment, transferred to a CPU and processed in the Leaf Coverage Analyzer Software (LCAS) (MARQUES *et al.*, 2018).

The LCAS was used to quantify leaf coverage (%) by counting the green pixels in a digital photo and was responsible for determining the covering degree through the analysis of the RGB components of the digital images, it was developed in programming language Borland C++ Builder 6.0, compatible with the platform Windows 32 bits.

The estimate of defoliation was performed through destructive collection, in which 10 plants of each treatment (out of the useful floor area) were extracted, we counted the leaves on the main stem, on the secondary stems, fallen leaves on the main stem and fallen leaves on the secondary stems.

The Pearson correlation coefficient and the comparison of means by Scott-Knott at 5% of significance were adopted as statistical methods.

RESULTS AND DISCUSSION

The digital images were obtained in three heights and then processed in the LCAS for leaf coverage (Figure 2). When performing comparison analysis of means by the Scott-Knott test at 5% of significance, by comparing the values in different heights, we obtained statistical equality (Table 1), i.e. the height did not influence the reading of the leaf coverage obtained through digital image processing, unlike that found by Crusiol *et al.* (2018).

Figure 3 shows the behavior of the leaf coverage obtained by digital images processed in software (A), of NDVI (B), the number of fallen leaves (C), disease severity (D), and the number of leaves (E) during the time of data collection of treatments with 6; 4; 3 and 0 sprays, similar behaviors were observed between the methods in the course of time. It is noteworthy that the defoliation of plants occurs due to diseases, but also due to natural plant senescence.

Using the Pearson correlation coefficient, we obtained values above 0.72 between defoliation and the other items assessed: NDVI, leaf coverage obtained through digital images and processed in the LCAS (Table 2) and the disease severity, in treatments with different gradients of Asian soybean rust.

Mean values of 0.893 were found in the relation between NDVI and the number of leaves. NDVI can provide estimates of defoliation accurately and consistently, confirming claims by Ritchie and Bednarz (2005), who developed studies with beans. Guan and Nutter Jr. (2002) state that in studies with alfalfa,

Figure 2 - Digital images in the heights 1.20 m (a), 2.00 (b) and 3.00 m (c) and subsequently processed in software for determination of soybean leaf coverage (d, e and f) where the greenish pixels were highlighted

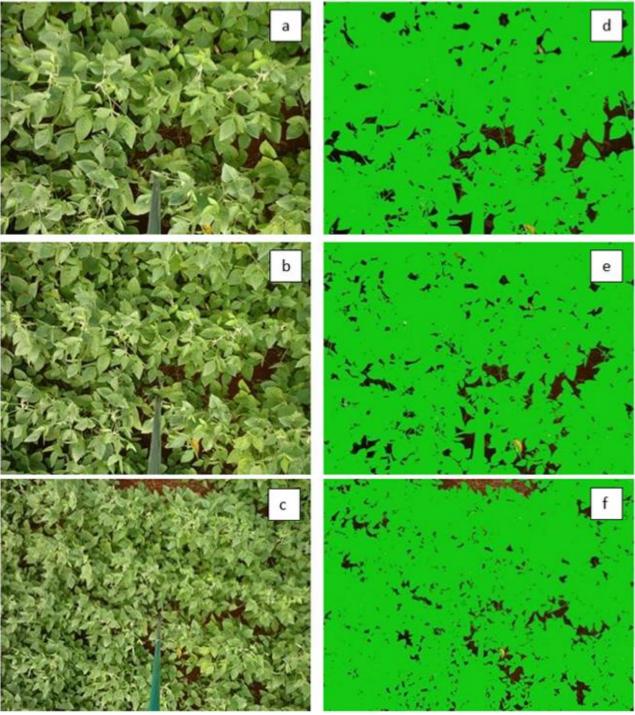


Table 1 - Means comparison by Scott-Knott test, where equal letters indicate equality at 5% significance, by comparing leaf coverage obtained through digital image processing in the heights 1.20; 2.00 and 3.00 m in treatments without spraying, with 6 sprays; 4 sprays and 3 sprays, according to Days After Emergency (DAE) and their corresponding phenological stages

Height 41 DAE V5 58 DAE R2 65 DAE R3 72 DAE R4 79 DAE R5 86 DAE R6 92 DAE R7 102 DAE R7-R8

Without Spraying

	Continuation Tabla 1							
1.20 m	75.17 a	88.48 a	83.30 a	76.17 a	82.67 a	89.90 a	45.29 a	4.82 a
2.00 m	77.73 a	89.51 a	83.37 a	77.49 a	82.59 a	90.22 a	43.55 a	4.35 a
3.00 m	78.96 a	91.54 a	83.39 a	78.23 a	89.62 a	92.38 a	40.35 a	3.44 a
6 Sprays								
1.20 m	78.42 a	90.09.a	92.70.a	79.55.a	89.06.a	92.87 a	67.31 a	21.06 a
2.00 m	80.44.a	89.52.a	89.92.a	80.51.a	89.75.a	93.40 a	64.69 a	20.20 a
3.00 m	84.77.a	91.21.a	89.05.a	81.19.a	90.73.a	94.56 a	65.10 a	18.48 a
4 Sprays								
1.20 m	94.44 a	99.15.a	94.54 a	87.90.a	91.36 a	96.96 a	61.80 a	17.82 a
2.00 m	93.98 a	99.25.a	95.57 a	88.81 a	92.98 a	97.94 a	64.11 a	17.90 a
3.00 m	94.23 a	99.45.a	95.14 a	90.27 a	93.69 a	98.33 a	66.69 a	16.47 a
3 Sprays								
1.20 m	84.61 a	87.81 a	78.75 a	78.82 a	80.34 a	84.36 a	48.84 a	7.93 a
2.00 m	83.08 a	88.41 a	80.13 a	79.61 a	80.46 a	86.82 a	49.75 a	7.84 a

81.97 a

83.92 a

86.20 a

NDVI presents higher correlation with the production if compared with visual analysis of defoliation. Xu and Katchova (2019) state that there is a relationship between productivity and NDVI in soybean, but not linear.

89.10 a

83.18 a

3.00 m

81.83 a

Between the variables, number of leaves and leaf coverage obtained through digital image processed in LCAS, the mean values were 0.942, showing, as well as in NDVI, the potential to replace the leaf visual coverage analysis using diagrammatic scale, which, according to Hirano *et al.* (2010), can present subjectivity in the estimates.

Defoliation in soybean crop occurs because of the natural crop senescence or prematurely as a result of the Asian soybean rust disease (GOELLNER *et al.*, 2010). The influence of natural crop senescence influenced the values of correlation found between defoliation and disease severity, which presented a mean of 0.78.

The correlation between the data of leaf coverage obtained through digital images processed in LCAS with: NDVI and disease severity (Table 3) was considered high (0.961 and -0.941, respectively), as well as the correlation between NDVI and disease severity (0.977).

Mean values of -0.941 were obtained through the Pearson Correlation between percentage of leaf coverage, from the processing of digital images via software, and the percentage of the Asian soybean rust disease was analyzed with the aid of diagrammatic scale (GODOY; KOGA; CANTERI, 2006). During the experiment (Table 3), the values were satisfactory as well as those presented by Martin *et al.* (2013) who used the ImageJ software (National Institutes of Health) and a leaf area integrator in study with beans. By correlating the disease severity with the NDVI values we obtained the mean of 0.977, concluding that there is high correlation between the two variables, corroborating with the results obtained by Hikishima *et al.* (2010).

54.02 a

8.14 a

By analyzing the leaf coverage obtained by digital image processing software and correlating the values with the NDVI, in each experimental treatment, we observed mean values of 0.961 (Table 3), this association occurs due to the dependence of both techniques on the amount of structures plant chlorophylls. Zarate-Valdez et al. (2012) obtained results equally satisfactory between NDVI and satellite images in an almond orchard. With this high correlation we obtained, through potential regression, in scatterplot, the expression $y=186.1x^{3,1509}$ (Figure 4). With R² of 0.967 we obtained a result of greater accuracy when comparing it with the values of Hirano et al. (2010) who, in linear regression, obtained mean R² of 0.75 in the accuracy of estimates of defoliation when compared with the actual data of soybean defoliation caused by rust.

Figure 3 - Behavior of the leaf coverage obtained by digital images processed in software (a), NDVI (b) number of fallen leaves (c), disease severity (d) and number of leaves (e), in relation to treatments with different numbers of fungicide applications in the course of time (DAE – days after emergence)

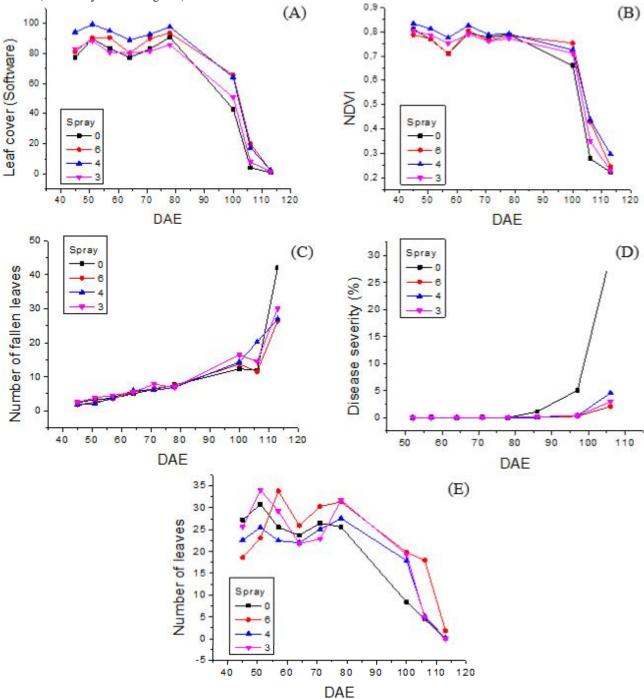


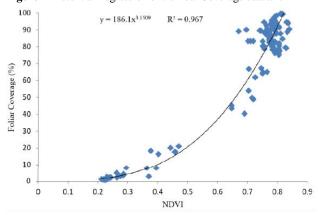
Table 2 - Results of the Pearson correlation coefficient between the data of number of leaves versus leaf coverage obtained through digital images processed in software and NDVI, in treatments with different gradients of Asian soybean rust

Spraying	6	4	3	0	Average
NDVI	$0.777 \pm 0,034$	$0.965 \pm 0,004$	$0.926 \pm 0,003$	$0.903 \pm 0,005$	0.893
Leaf Coverage	$0.856 \pm 0{,}015$	$0.987 \pm 0,002$	$0.956 \pm 0,002$	$0.970 \pm 0,005$	0.942

Table 3 - Results of the Pearson correlation coefficient between the leaf coverage data obtained through digital image processed in software versus NDVI and disease severity, in treatments with different gradients of Asian soybean rust

Treatment	6	4	3	0	Average
Disease	$-0.953 \pm 0,003$	-0.945 ± 0.010	-0.938 ± 0.010	$-0.927 \pm 0,009$	-0.941
NDVI	$0.948 \pm 0{,}007$	$0.975 \pm 0,008$	$0.967 \pm 0{,}011$	0.955 ± 0.010	0.961

Figure 4 - Potential Regression of the Leaf Coverage data and NDVI



CONCLUSIONS

- We concluded that it is possible to estimate the soybean defoliation caused by Asian rust through analysis of processed images via LCAS, reducing the interference of human error and increasing the dynamics of defoliation readings;
- The estimate of defoliation using digital image analysis
 processed via LCAS presents more precise results, i.e.
 with minor error when compared with analyses by
 diagrammatic scale.

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