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SHORT NOTE

Leaf area determination by digital image analysis

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SUMMARY

The aim of this study was the development of a digital method evaluation using the Digital Determination of Areas (DDA) software aiming at the area determination of different image forms and sizes, in order to propose its use on the leaf area evaluation. Fifteen geometric figures with mathematically known area were submitted to the determination area through leaf area electronic integrator LI-COR 3100 (considered a standard method of leaf area analysis) against DDA with five replications. The obtained values were compared through variance analysis and Fisher's mean comparison test. The results were evaluated through Pearson correlations and polynomial regression. No significant differences were observed between the analysis methods, presenting correlation values around 0.99, regression equations interception values close to zero, angular coefficient close to one, and the determination coefficient close to 100%. Areas determined by the digital method using the DDA software did not differ from those obtained through the electronic integrator of leaf area, both being equal to those calculated mathematically. The digital approach using the DDA software can be recommended for the determination of leaf area, being a useful tool in this evaluation and, consequently, the leaf area index, the primary variable for the understanding of plant dynamics.

Additional keywords

Electronic integrator.
Li-Cor.
Plant physiology.
Software.

Determinação de área foliar por análise de imagens digitais

RESUMO

Foi avaliado o uso do software Determinador Digital de Áreas (DDA) na determinação da área de imagens de diferentes formas e tamanhos, visando subsidiar sua utilização para a avaliação de área foliar, propósito para o qual foi desenvolvido. Quinze figuras geométricas com área matematicamente conhecida foram submetidas à determinação de área através de um integrador eletrônico de área foliar LI-COR 3100 (considerado o método de análise padrão) e do método digital de determinação de áreas denominado Determinador Digital de Áreas, com cinco repetições. Os valores obtidos foram comparados por análise de variância e teste de comparação de médias de Fisher. Os resultados também foram submetidos à correlação de Pearson e a regressão polinomial. Não foram verificadas diferenças significativas entre os métodos, com valores de correlação da ordem de 0.99 e equações de regressão com valores de intercepto próximos de zero, coeficiente angular próximo de um e coeficiente de determinação perto de 100%. Áreas determinadas pelo método digital usando o software DDA não diferiram daquelas obtidas através do integrador eletrônico de área foliar, sendo ambas iguais àquelas calculadas matematicamente. O método digital usando o software DDA pode ser recomendado para a determinação de área foliar, sendo uma ferramenta útil nesta avaliação e, consequentemente, do índice de área foliar, a principal variável para o entendimento da dinâmica vegetal.

Palavras chave adicionais

Fisiologia vegetal.
Integrador eletrônico.
Li-Cor.
Software.

Information

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INTRODUCTION

The leaf is the main organ in the plant transpiration process, which is the why the knowledge of the leaf surface is useful for evaluating culture practices such as pruning, fertilizing, planting density, and pesticides application (Pereira *et al.*, 1997; Lucena *et al.,* 2011). Leaf area represents the light interception apparatus for photosynthesis, it is a feature used in analyzes of plant growth. From the leaf area estimation can be reached, among others, the ecophysiological variables such as specific leaf area, leaf area ratio, liquid assimilation rate, relative leaf growth rate, and leaf area index, which allows inferences about the photosynthetic efficiency, patterns of growth, and plants development due to genetic or environmental differences (Fonseca and Condé, 1994).

The leaf area index is defined as the ratio between the total area of one side of the leaves and the area of land occupied by them (Watson, 1947). Since its definition, the leaf area index has been considered as the main variable for understanding the plant dynamics, being a central point to processes such as light interception, gas exchange and other ecological aspects such as inter and intraspecific competition, sequestering carbon from the soil, conservation and biogeochemical cycles (Breda, 2003; Sbrissia and Silva, 2008), besides guide the plants forage defoliation management based on light interception. When there is interception around 95% of the incident light, it starts the process of senescence, with the death of a leaf for each new one, settling, from there the leaf area index where the accumulation of dry matter is in its most, providing interception of any incident light (Silva *et al.*, 2008).

There are different methods for the leaf area estimation, and most cited those are based on electronic meters, planimeter techniques or relationships between leaf size and leaf area (Lima *et al.*, 2008; Sbrissia and Silva, 2008, Sousa *et al*., 2015, Cargnelutti Filho *et al*., 2015). These have always been complex and expensive options. After the computer graphics advent, the use of images to determine the leaf area has become more common and simple, when using design software or specific type of CAD (Computer Aided Design). Several studies have suggested the use of CAD applications for determining the leaf area, such as Vieira Júnior *et al.* (2006) for maize, Godoy *et al.* (2007) to orange and Dombroski *et al.* (2010) for pine. These methods, when using digital photographs, can have drawbacks as the correct resolution determination used, besides the camera distance and camera tilt over the leaves. Other methods require the perimeter of the leaf to be drawn or the leaf needs to be accompanied with a scale for reference to be digitally evaluated.

Based on this fact, was created the DDA (Digital Determination of Areas), a software capable of recognizing images of leaves, and calculate based on its contour and size, the total area of leaves. Therefore, after harvest, leaves can be scanned in a flatbed scanner and the images can be analyzed to obtain the area, through DDA software (Ferreira *et al.*, 2008; Ferreira *et al*., 2009). The same process has been effective in determining rib-eye areas (Ferreira *et al.*, 2012), its second functionality. However, experiments to assess its performance in determining areas of images with different shapes which can demonstrate its use in the determination of leaf area were not conducted until this work.

This study aimed to evaluate the digital method through DDA software to determine areas of different images forms and sizes, to support its use in leaf area assessment, one of the primary purpose for which it was developed. We believe that the DDA is more useful regarding response speed and with the same accuracy than most of the options available for the measurement task of leaf area.

MATERIAL AND METHODS

For evaluations, we used fifteen geometrical figures of different shapes, cut on legal paper weighing 75 g/m², with an area known for mathematical determination, such as circles with 3.75, 4.83, and 6.49 cm²; squares with 7.84, 12.25, and 17.64 cm²; triangles with 10, 15, and 21 cm²; rectangles with 6, 10, and 20 cm², and trapezoids with 6, 13.5, and 21 cm². Figures were submitted five times to the determination of their areas through leaf area electronic integrator (LAEI) LI-COR 3100 (considered the standard method of analysis) and the proposed digital method of determining areas called DDA – Digital Determination of Areas (Ferreira *et al.*, 2008) in a total of 75 evaluations. During the second method implementation, figures were scanned in a flatbed scanner using the 200 DPI resolution and images were saved in monochrome bitmap file format (Ferreira *et al.*, 2008). After this procedure, the aforementioned software determined the area of each figure. We used a completely randomized design with five repetitions, and the obtained values were compared by variance analysis and Fisher's least significant difference (LSD) comparison test ($p<0.05$). The results were submitted to the Pearson correlation and polynomial regression using the SAS statistical software (version 9.0).

RESULTS AND DISCUSSION

The correlation between the proposed digital method (DDA) and the mathematical measurements was high **(figure 1)**, with no significant differences ($p>0.05$) between the areas obtained by these methods in any of geometric images analyzed **(table I)**. Being

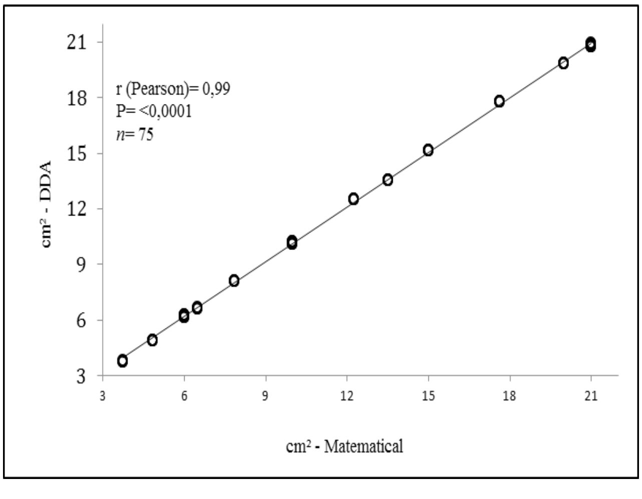

Figure 1. Correlation between the areas mathematically determined and the corresponding measurements by the Digital Determination of Areas – DDA (Correlação entre as áreas determinadas matematicamente e as mensurações correspondentes pelo Determinador Digital de Áreas – DDA).

Table I. Area (cm²) ± standard deviations determined through mathematical model, using the Leaf Area Electronic Integrator - LAEI, and through the Digital Determination of Areas - DDA (Áreas (cm²) e desvio padrão, determinadas matematicamente, pelo Integrador Eletrônico de Área Foliar - LAEI e pelo Determinador Digital de Áreas - DDA).

Figure	Method			LSD
	Mathematical	DDA	LAEI	
Circle	5.0227±1.17[a]	5.1047±1.21[a]	5.2053±1.20[a]	0.879
Square	12.577±4.15[a]	12.803±4.10[a]	12.922±4.10[a]	3.0327
Triangle	15.333±6.34[a]	15.424±6.13[a]	15.638±5.97[a]	3.3315
Rectangle	12.000±4.65[a]	12.043±4.52[a]	12.177±4.38[a]	4.4132
Trapezoid	13.500±6.09[a]	13.540±5.94[a]	13.477±5.92[a]	4.5325
Average	11.686±5.90[a]	11.783±5.81[a]	11.884±5.75[a]	3.2378

Values followed by the same letter, on the line, do not differ significantly from Fisher's test (p<0.05).

the exact mathematical determination without variation between repetitions, there was no dispersion of the points on the horizontal axis (the axis of areas obtained mathematically) of the correlation chart. Taking into account the vertical axis (the areas obtained by the DDA method), the dispersion indicated slight variations in data, primarily from about 11 cm². However, because these variations are of low magnitude, no significant differences were found between the two evaluated methods.

The correlation between the DDA results and the leaf area electronic integrator (LAEI) was also high **(figure 2)** and no present significant differences (p>0.05) between the areas obtained by these methods in any of the geometries analyzed **(table I)**. However, it can be seen that in some places there was much scatter in the horizontal axis (axis of areas obtained by LAEI), indicating that the area measurements, when determined by the electronic integrator are subject to greater variation than when determined by digital method - DDA **(figure 2)**.

The correlation between mathematical and LAEI measurements **(figure 3)**, despite its high value, there was also data dispersion along the horizontal axis (the axis of areas obtained by LAEI), indicating greater variation in data obtained by LAEI - considered the standard method for determining leaf area. However it is important to note that despite these variations, no significant differences were observed (p>0.05) between the determined areas by LAEI and the mathematical calculation **(table I)**. LAEI estimated the average area of the circles, squares, triangles, and rectangles, respectively 0.1826, 0.345, 0.305 and 0.177 cm² greater, and the trapezoidal area in the 0.023 cm² lower. Values that are below the minimum significant difference in the used statistical test **(table II)**, which by its characteristics is very sensitive in detecting differences between experimental groups.

Considering the above-discussed results, it can be inferred that the digital method using the DDA software demonstrates accuracy and precision in determining areas of different shapes and number of vertices, and it may also be used for the leaf area determination. Regression equations between methods with interception values close zero, the angular coefficient close a determination coefficient of 100% **(table II)**, as cited by Tavares-Júnior *et al*. (2002) and Godoy *et al*. (2007), they confirm the result. The results of this study also are in line with those obtained by Ferreira *et al*. (2012).

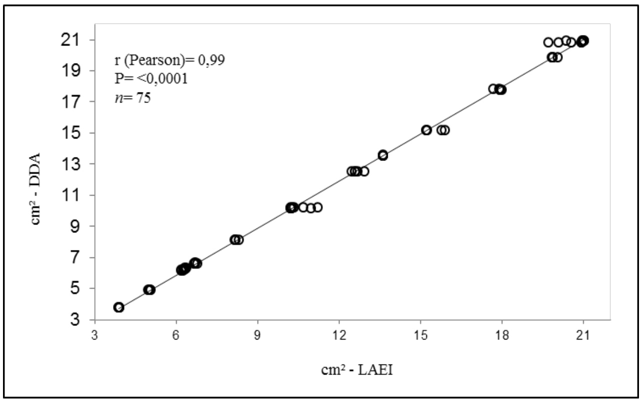

Figure 2. Correlation between the areas determined by the Leaf Area Electronic Integrator - LAEI and your corresponding measurements by Digital Determination of Areas – DDA (Correlações entre as áreas determinadas pelo Integrador Eletrônico de Área Foliar - LAEI e suas mensurações correspondentes pelo Determinador Digital de Áreas - DDA).

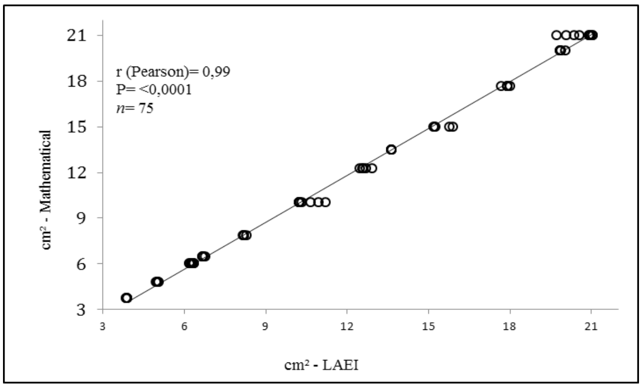

Figure 3. Correlation between the areas mathematically determined and its corresponding measurements by the Leaf Area Electronic Integrator – LAEI (Correlação entre as áreas determinadas matematicamente e suas mensurações correspondentes pelo Integrador Eletrônico de Área Foliar – LAEI).

Table II. Regression equation for the estimative of the area determined by the Digital Determination of Areas – DDA and mathematical calculation - MAT and Leaf Area Electronic Integrator – LAEI (Equações de regressão para estimativa das áreas determinadas através do Determinador Digital de Áreas – DDA, do cálculo matemático – MAT e do Integrador Eletrônico de Área Foliar – LAEI).

Equation of regression *	R^2	p > t Intercept	p > t Slope
DDA= -0.19458 + 1.00788 x LAEI	0.99	0.0061	<0.0001
DDA= 0.27985 + 0.98428 x MAT	0.99	<0.0001	<0.0001
MAT= -0.4742 + 1.0233 x LAEI	0.99	<0.0001	<0.0001

When the authors evaluated the DDA software in determining rib-eye area, they found a correlation of up to 0.98 ($p<0.0001$) and determination coefficients up to 0.97 using other measurement methods traditionally used in these types of assessments.

The absence of statistical differences when the methods were compared for the mean value of the area of the figures **(table I)**) indicates that the type of leaf or its shape will not interfere with the efficiency of the digital method using the DDA software. The mean value presented in **table I** considers all the geometric forms simultaneously, simulating the analysis of leaves with a different number of vertices, angles, and semicircles in their format. Thus, the forage species to be analyzed, regardless of the type of leaf presented, does not interfere with the results of the analysis. Since it is a method of destructive analysis, in which the leaves are removed from the plant, as well as in the electronic integrator and other destructive methods of leaf area analysis (Mielke *et al*., 1995; Sbrissia and Silva, 2008), the sward structure also does not interfere with the analysis process. A limitation that could be taken into account is the size of the analyzed leaf when analyzing large ones, to digitize them into smaller pieces that fit on the scanner screen. Also, leaves with a thick central vein may present problems regarding the inadequate closure of the scanner, allowing the entrance of light and, consequently, the appearance of black spots (noises) next to the scanned images, which would have their area computed as leaves, and overestimating the analysis.

Comparing methods for leaf area determination, using *Phaseolus vulgaris* leaves as a model, Reis *et al*. (2000) found no difference between the tested methods, and the use of either method depends on the equipment availability and the purpose of evaluation. Through statistical analysis using similar criteria to those adopted in this study, Tavares-Júnior *et al*. (2002) considered the digital method, replacing the leaf area electronic integrator by SIARCS software 3,0® to estimate the leaf area of *Coffea arabica*, as well as Flumignan *et al*. (2008) using a digital camera as a digital method. Meanwhile, Lucena *et al*. (2011) concluded, based on the highest coefficient of determination by the AM 300 portable integrator on *Malpighia emarginata* leaf area, estimation closer to the precision standard reference (images of leaves scanned on a table scanner along with millimeter ruler).

CONCLUSIONS

Areas determined by the digital method using the DDA software did not differ from those obtained through the electronic integrator leaf, and both of which are the same as those calculated mathematically.

The digital method using DDA software can be recommended for the leaf area determination as a useful tool in this type of evaluation. Such analysis is essential because the leaf area index consists of the primary variable for the understanding of plant dynamics and it guides the plants forage defoliation management based on light interception.

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