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Article

NON-DESTRUCTIVE MODEL TO PREDICT *Commelina diffusa* LEAF AREA

Modelo Não Destrutivo para Estimar a Área Foliar de ***Commelina diffusa***

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ABSTRACT - Leaf length (L), leaf width (W), and leaf area (LA) were measured from 100 leaves aiming to determine a simple linear equation (Y=a*X) to predict the leaf area of *Commelina diffusa*, an important weed infesting annual and perennial crops in Brazil and worldwide. Results indicate the equation LA=0.7*LW reliably estimates the leaf area of *C. diffusa*, after correlating LA with LW, and then validating that equation by analyzing four new 25-leaf samples.

Keywords: plant growth, biometry, non-destructive method, mathematical model, climbing dayflower.

*RESUMO - Comprimento da folha (C), largura da folha (L) e área foliar (AF) foram medidos em 100 folhas com o objetivo de determinar uma equação linear simples (Y=a*X) para estimar a área foliar de Commelina diffusa, importante planta daninha que infesta culturas anuais e perenes no Brasil e no mundo. Os resultados indicam que a equação AF=0,7*CL estima confiavelmente a área foliar de* ***C. diffusa****, após correlacionar AF com CL e, em seguida, proceder à validação da equação analisando quatro novas amostras de 25 folhas.*

Palavras-chave: crescimento de plantas, biometria, método não destrutivo, modelo matemático, trapoeraba.

INTRODUCTION

Leaf area measurement is one of the most common parameters evaluated in a greenhouse and in field ecophysiological studies (Wang and Zhang, 2012). Accurate measurements of leaf area in field experiments may be time-consuming and generally requires the use of expensive equipment (e.g. portable leaf area meters) (Souza and Amaral, 2015; Souza et al., 2015). In addition, most of the techniques used to estimate leaf area require excising leaves from plants and scanning them in the lab, interfering in physiological and phenological responses because of canopy reduction (Chabot and Hicks, 1982). Thus, the excision of leaves can interfere in the results of other experiments that share the same group of plants (Souza and Amaral, 2015). Therefore, equations derived from non-destructive models, when available, represent a free and fast method that can be used *in situ*, providing leaf area estimates with no leaf excisions (Norman and Campbell, 1989).

The use of non-destructive models to estimate the leaf area has been used to understand the ecophysiology of many crops and weeds (Souza and Amaral, 2015; Tartaglia et al., 2016, and many others). Linear models based on length

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and width leaf measurements have been considered the most simple and efficient models to estimate the leaf area of some species (Demirsoy and Lang, 2010; Carvalho et al., 2011a; Giuffrida et al., 2011; Wang and Zhang, 2012; Souza and Amaral, 2015; Souza et al., 2015). In addition, for a better practical use, researchers can choose the simple linear model (Y=a*X), considering the product of length and width as the variable "X" (Carvalho et al., 2011b, c).

The objective of this research was to determine a simple linear equation (Y=a*X) to predict the leaf area of *Commelina diffusa*, an important weed infesting annual and perennial crops in Brazil and worldwide, as a function of leaf length and leaf width.

MATERIAL AND METHODS

This research was carried out in two steps. First, a general linear equation to estimate the leaf area of *C. diffusa* was determined. Next, the equation was validated.

Determining the equation, 100 leaves with no injury caused by insects, fungi or weather conditions were randomly gathered from *C. diffusa* plants in the flowering stage. Leaves were collected in a variety of ecosystems (annual and perennial crops, non-agricultural fields and urban conditions) in Lages, SC, Brazil, in March 2015, while taking into account several ecological conditions where the species grows.

Leaf length through midrib (L), leaf maximum width perpendicular to midrib (W) and leaf area (LA) were measured by using a Portable Area Meter (Licor, Mod. L1-3000, USA), and then the product of L and W (hereafter designed as LW) was calculated.

Subsequently, a linear regression analysis (Y=a*X), assuming Y as LA and X as LW was made. In addition, the Kolmogorov-Smirnov test was performed to verify the normality of regression residuals and the Spearman-Rank test was used to analyze the correlation between LW and LA (Carvalho et al., 2011a). Complementary, measured and estimated leaf area data were plotted in boxplot graphs and compared by a paired-t test. Moreover, the Spearman-Rank test was performed to analyze the correlation between measured and estimated leaf area and the Kolmogorov-Smirnov test was applied to check the normality of residuals from the correlation test (Carvalho et al., 2011a). For all tests, a probability of error was set at 1%.

Collinearity between L and W was tested by using the variance inflation factor (VIF) (Marquardt, 1970) and the tolerance value (T) (Gill, 1986). If VIF was larger than 10 or if T was smaller than 0.1, one of the variables (L or W) should be excluded from the model (Cristofori et al., 2007; Souza et al., 2015). We verified VIF<10 (2.406) and T>0.1 (0.416) from a 100 leaf sample, indicating L and W could be both included in the model to predict the leaf area of *C. diffusa*.

Validating the equation, four new samples of 25 leaves were gathered and characterized as described above. For each sample, the Spearman-Rank test was performed to analyze the correlation between measured and estimated leaf area and the Kolmogorov-Smirnov test was applied to verify the normality of residuals from the correlation test (Carvalho et al., 2011a). For all tests, the probability of error was set at 1%.

RESULTS AND DISCUSSION

Leaf area of *C. diffusa* may be estimated as a function of LW. There was a high correlation between LW and LA (r=0.988, P<0.001), hence leaf area of *C. diffusa* could be calculated by a simple linear regression (Y=a*X) (Figure 1). The same figure shows the equation parameter "a" (0.6989) and the normal distribution of residuals (d=0.072, P=0.6636). High correlation between LW and LA and a normal distribution of regression residuals, in addition to a high coefficient of determination, are required for an accurate model (Carvalho et al., 2011a; Souza and Amaral, 2015; Souza et al., 2015). Thus, the results indicate the equation LA=0.6989*LW (R^2=0.968) significantly expressed the correlation between LW and LA.

For a better practical use, the approximated equation (Y=0.7000*X) was tested to estimate leaf area. At first, there were no differences between the mean value of the measured leaf area (6.53 cm^2) and the estimated leaf area (6.53 cm^2) for equation Y=0.7000*X, as we observed for equation Y=0.6989*X (Figure 2). Next, there was a high correlation between measured and estimated leaf area by the equation Y=0.7000*X (r=0.984, P<0.001) and a normal distribution of residuals (d=0.0858, P=0.4361) (Figure 3). As discussed above, the results indicate the equation LA=0.7000*LW also significantly expressed the correlation between LW and LA, hence it could be used to predict the leaf area of *C. diffusa*.

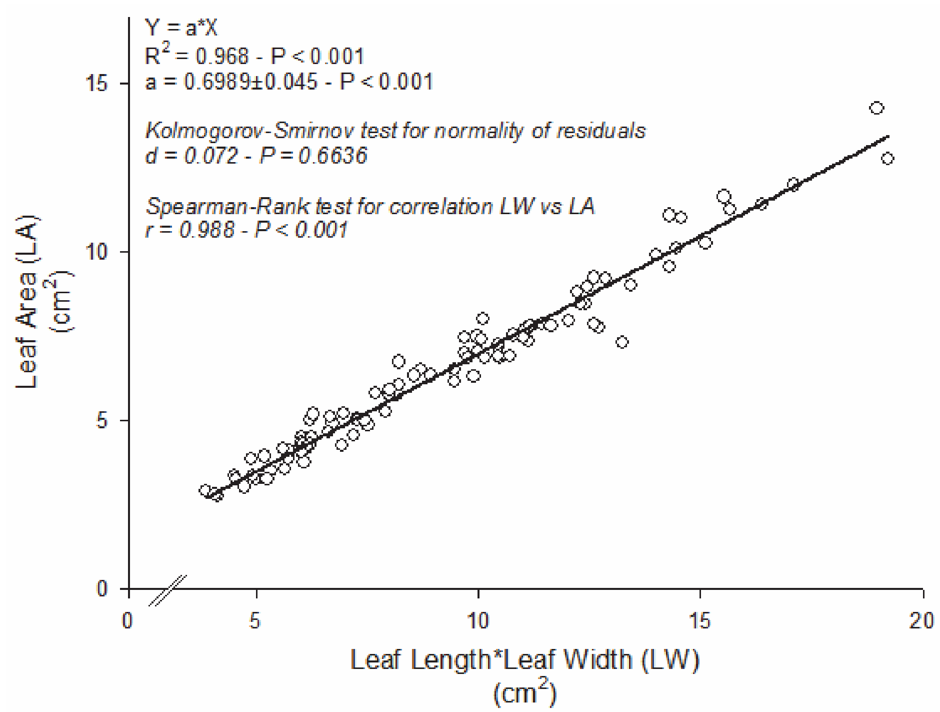

Figure 1 - Linear regression between leaf area and the product of leaf length and leaf width from a sample of 100 leaves of *Commelina diffusa*, and a summary of normality and correlation tests.

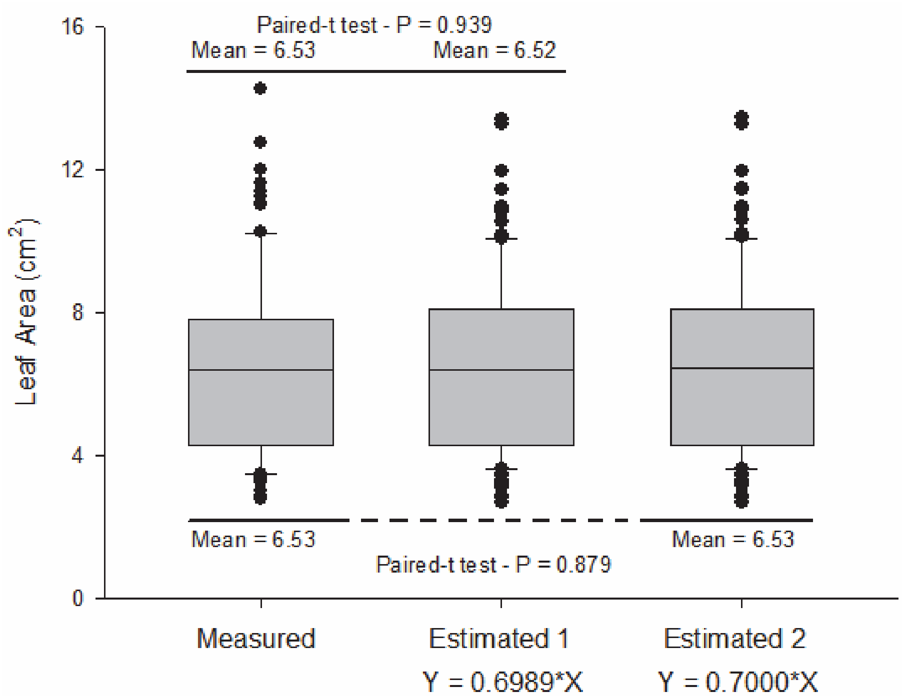

Figure 2 - Boxplots of measured and estimated leaf area from a sample of 100 leaves of *Commelina diffusa*.

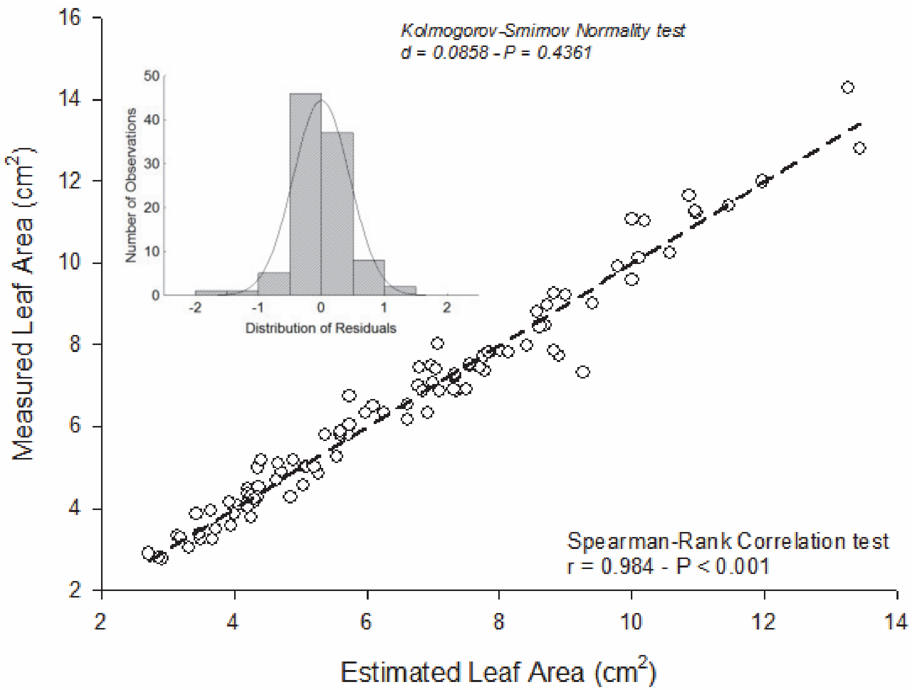

Figure 3 - Linear correlation between measured leaf area and estimated leaf area (by the equation Y=0.7000*X) from a sample of 100 leaves of *Commelina diffusa*, and a summary of normality and correlation tests.

Finally, the equation Y=0.7000*X was validated by testing the correlation between measured leaf area and estimated leaf area (Carvalho et al., 2011a; Souza and Amaral, 2015; Souza et al., 2015), from other four new samples of 25 leaves. The values of measured leaf area ranged from 6.18 cm^2 up to 6.67cm^2 while estimated leaf area, from 6.24 cm^2 up to 6.79 cm^2 (Table 1). In all cases, there were no differences between measured and estimated leaf area. In addition, there was a high correlation between measured leaf area and estimated leaf area ($0.978 \leq r \leq 0.991$, $P<0.001$) and a normal distribution of residuals ($0.1038 \leq d \leq 0.1689$, $0.4380 \leq P \leq 0.9383$) (Table 1). Thus, as discussed above, the results confirm that the equation LA=0.7000*LW significantly expresses the correlation between LW and LA; thus, it can be used to predict the leaf area of *C. diffusa*.

Non-destructive methods to predict leaf area are more interesting for use because there is no need to collect the plant, or parts of plants, for measurements (Bianco et al., 2011). The use of regression equations to predict plant leaf area is a simple, fast, accurate, and reliable method, allowing to monitor leaf growth and expansion from the same plant until the end of life cycle or experiment with no need to collect plant material (Gamiely et al., 1991). In addition, the simple linear regression should be preferred because it is easiear and more practical to use for leaf area estimation (Duarte et al., 2009). Moreover, it can predict leaf area from the starting point

Table 1 - Mean ± standard error of measured leaf area and estimated leaf area from four samples of 25 leaves of *Commelina diffusa*, and a summary of the Spearman-Rank correlation test, the Kolmogorov-Smirnov normality test and collinearity analysis

Sample	Measured	Estimated(1)	Correlation test(2)		Normality test(3)	
	(cm^2)	(cm^2)	r	d	d	P
1	6.61±0.41	6.45±0.49	0.978	0.1038	0.1038	<0.001
2	6.18±0.48	6.24±0.48	0.988	0.1171	0.1171	<0.001
3	6.67±0.58	6.65±0.58	0.991	0.1051	0.1051	<0.001
4	6.65±0.52	6.79±0.52	0.979	0.1689	0.1689	<0.001

(1) Y=0.7000*X, where Y represents leaf area and X is the product of leaf length and leaf width. (2) Correlation test between measured and estimated leaf areas. (3) Normality test for regression residuals.

0,0 (X,Y), considering the y-intercept is equal to 0. It is important because, if y-intercept is not 0 (generally negative), one may consider a possibility of negative values for leaf area. Thus, the models predicting y-intercept different from 0 do not adequately represent the biological behavior of the plant.

In a nutshell, leaf area (LA) of *C. diffusa* can be calculated as a function of the product of leaf length and leaf width (LW). The equation LA=0.7*LW reliably estimates the leaf area of *C. diffusa* in a simple and efficient manner. Leaf length and leaf width can be easily measured with a ruler, hence model is an important tool for ecophysiological studies of *C. diffusa* under field or greenhouse conditions. In addition, the use of that equation would enable researches to make non-destructive and repeat measurements in the same leaf, with no use of expensive electronic equipment.

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