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Non-destructive equations to estimate the leaf area of *Styrax pohlii* and *Styrax ferrugineus*

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(With 2 figures)

Abstract

We developed linear equations to predict the leaf area (LA) of the species *Styrax pohlii* and *Styrax ferrugineus* using the width (W) and length (L) leaf dimensions. For both species the linear regression (Y=α+*b*X) using LA as a dependent variable *vs.* W × L as an independent variable was more efficient than linear regressions using L, W, L^2 and W^2 as independent variables. Therefore, the LA of *S. pohlii* can be estimated with the equation LA=0.582+0.683WL, while the LA of *S. ferrugineus* follows the equation LA=–0.666+0.704WL.

Keywords: Brazilian savanna, Styracaceae, validation, regression analysis, linear models.

Equações lineares para estimativa de área foliar de *Styrax pohlii* e *Styrax ferrugineus*

Resumo

Foram determinadas equações lineares para estimar a área foliar (AF) de *Styrax pohlii* e *Styrax ferrugineus* utilizando dimensões do limbo foliar (C – comprimento, L – largura). O modelo linear (Y=α+*b*X), utilizando AF *vs.* C × L, foi mais eficiente que os modelos lineares utilizando C, L, C^2 e L^2 como variáveis independentes na determinação da área foliar de *S. pohlii* e *S. ferrugineus*. Assim, a AF de *S. pohlii* pode ser estimada pelo modelo AF=0,582+0,683CL e a AF de *S. ferrugineus* pode ser estimada pelo modelo AF=–0,666+0,704CL.

Palavras chave: Cerrado, Styracaceae, validação, regressão, modelos lineares.

1. Introduction

The Brazilian savanna (Cerrado) is comprised of a mosaic of biomes, from savanna vegetation to gallery forests (Batalha, 2011; Pinheiro and Monteiro, 2010) that originally covered 21% of the Brazilian territory (Souza and Habermann, 2012). Because of human activities (mainly agriculture), the Cerrado has been drastically fragmented, and less than 34% of its original area still remains (Klink and Machado, 2005). In order to preserve these biomes, it is necessary to understand the physiology, phenology and ecology of the largest number of species. *Styrax pohlii* and *Styrax ferrugineus* have been used as model species to understand the differences between congeneric species from riparian forests and savanna formations in the peripheral region of the Cerrado, and also as model species that give ecophysiological responses, which are essential to understand their occurrences in the southern Cerrado areas (Habermann and Bressan, 2011; Habermann et al., 2011; Kissmann et al., 2012).

Like for crops and weeds, the leaf area (LA) of Cerrado's species is a good trait that can contribute to physiological studies of these plants (Rouphael et al., 2006). There are several methods to measure the LA (*e.g.* leaf area meter, blueprinting, and photographing), but all of these methods are time consuming, require the excision of leaves from the plants and do not allow the same leaves to be measured later (Rouphael et al., 2010).

There are several methods to estimate the LA using the width and length dimensions. The most common equations are based on general linear (Carvalho et al., 2011a), simple linear (Carvalho et al., 2011b), square (Severino et al., 2007) and exponential regressions (Bianco et al., 2005). The best model for estimating LA through equations works better when the measures to be taken can be easily and correctly identified and the equations are based on only one or few measurements (Severino et al., 2007).

The aim of this research was to develop two simple, fast, and non-destructive equations in order to estimate the leaf area of two Cerrado species, *Styrax pohlii* (riparian forest specie) and *Styrax ferrugineus* (savanna specie) based on the hypothesis that linear regression can be used to determine LA of these species using width and length dimensions.

2. Material and Methods

Three-year-old plants of *Styrax pohlii* and *Styrax ferrugineus* were cultivated in 100L-pots containing Cerrado soil (oxisoil) in the experimental garden of the Instituto de Biociências - UNESP, Rio Claro, Brazil. In the rainy season of 2010, we randomly collected 100 leaves (comprising the whole canopy) from 4 plants of each species.

Immediately after sampling, the leaves were stored in plastic bags to prevent leaf dehydration. We measured the maximum leaf width (W, cm), length (L, cm) and the area (cm^2) of each leaf (LA) by a portable area mater (*LI-COR* – *LI*-3000A, Inc., Lincoln, NE, USA) (Rouphael et al., 2010). The leaf length was measured from the lamina tip to the petiole point of insertion, along the lamina's midrib. The leaf width was measured from end-to-end between the widest lobes of the lamina perpendicular to the lamina's midrib (Rouphael et al., 2010).

The data on (LA, W, L, W^2, L^2 and W×L) were submitted to a Shapiro-Wilk test to verify the normal distribution of each variable. The relationship between LA (dependent variable) and L, W, L^2, W^2, and W × L (independent variables) were tested using a general linear model ($Y=\alpha+bX$) (Rouphael et al., 2010, 2006). The normality of the residual distribution of the all linear equations was tested using a Shapiro-Wilk test (Carvalho et al., 2011a).

To validate the equations with the highest R^2 (equation n°5 and n°10 in Table 1), we used 100 additional leaf samples, randomly collected from 4-5 adult plants of each species, during the same season. We determined the LA, L and W by the same procedures previously described, and performed a new regression for each selected model, correlating the observed leaf area (OLA = observed leaf area measured with an area meter) with the predicted leaf area (PLA = $\alpha+b$WL constant from model 10 for *S. ferrugineus* and from model 5 for *S. pohlii*) and performed a Spearman-Rank correlation (p=0.05) between OLA *and* PLA. The normality of the residual distribution of the validate equations was tested using a Shapiro-Wilk test.

3. Results

The leaf area (LA) of *S. pohlii* varied from 15.0 cm^2 to 51.6 cm^2 (average = 28.8 cm^2), the length (L) of leaves of this species ranged from 7.5 cm to 12.0 cm (average = 9.7 cm), and the width (W) from 2.7 cm to 6.0 cm (average = 4.3 cm). For *S. ferrugineus*, the LA varied from 11.5 cm^2 to 62.4 cm^2 (average = 35.5 cm^2), the L ranged from 6.8 cm to 14.4 cm (average = 10.7 cm), and the W from 2.5 cm to 6.2 cm (average = 4.7 cm). We noticed that both species have oblongs leaves that statistically differed for LA, L and W ($p<0.001$) (data not shown).

For both species, the best combination of the highest R^2 (>0.97) and most significant *p* residuals was observed in linear regression (equations n° 5 and 10 in Table 1) with WL as an independent variable (Table 1). For the validation of the equations we determined the PLA of each species. For *S. pohlii* the PLA was determined by using the equation LA=0.582+0.683WL (equation n° 5 in Table 1) and for *S. ferrugineus* the PLA was determined by using the equation LA=–0.666+0.704WL (equation n° 10 in Table 1). The correlation between PLA and OLA for both species was significant when tested by the Spearman-Rank correlation model (*S. pohlii* r_s = 0.999 and for *S. ferrugineus* r_s = 0.982). We also observed good correlation in the relationship between PLA and OLA, after we performed a new linear correlation (Figures 1 and 2). In both cases, we obtained R^2 > 0.97, suggesting that the models that were selected may be used with good precision to determine the LA with a non-destructive method.

4. Discussion

We selected equations number 5 and number 10 because they presented the highest R^2, which must be the selective criterion, as proposed by Carvalho et al. (2011b). Many studies have been reported and propose non-destructive equations to estimate the growth of leaves in crops

Table 1. Statistics and parameter estimates from linear regression models for leaf area estimation.

	***Styrax pohlii* (n=95)**		
Equation No.	**$Y=\alpha+\beta X$**	**R^2*****	**p residuals***
1	LA= –20.942+11.999W	0.906	0.023
2	LA= –29.091+6.048L	0.822	0.004
3	LA= 6.685+1.267W	0.900	0.272
4	LA= 2.884+0.281L^2	0.818	0.001
5	LA= 0.582+0.683WL	0.981	0.401
	***Styrax ferrugineus* (n=96)**		
6	LA= –11.771+9.446W	0.798	0.602
7	LA= –15.652+4.592L	0.577	0.032
8	LA= 8.429+1.081W^2	0.805	0.229
9	LA= 6.760+0.232L^2	0.577	0.030
10	LA= –0.666+0.704WL	0.972	0.774

*Residual normality distribution using a Shapiro-Wilk test. ***Significant linear coefficients ($p<0.001$).

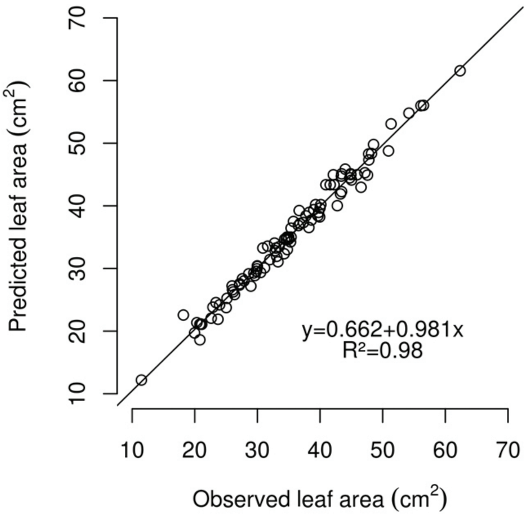

Figure 1. Predicted leaf area (PLA=0.582+0.683WL) plotted against the observed leaf area (LA determined by area mater) for *Styrax pohlii* (n=100).

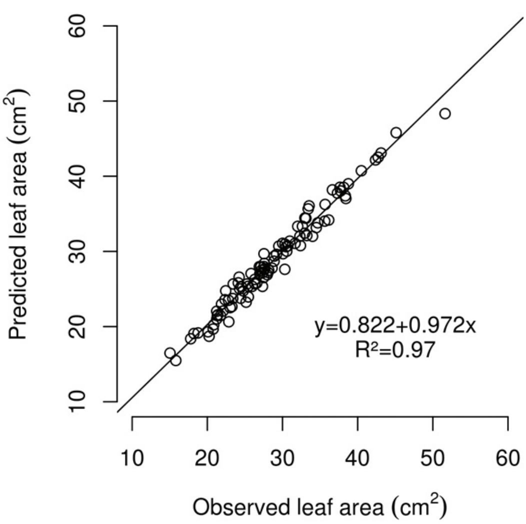

Figure 2. Predicted leaf area (PLA=–0.666+0.704WL) plotted against the observed leaf area (LA determined by area mater) for *Styrax ferrugineus* (n=100).

(Tsialtas and Maslaris, 2005; Peksen, 2007; Olfati et al., 2010; Rouphael et al., 2010) and weeds (Carvalho et al., 2011a; Carvalho et al., 2011b). However, specifically for Cerrado woody species, there are not any models like the one proposed in the present paper, and consequently, experiments have to be conducted in a way that leaves must be excised. Therefore, when using destructive methods, it is not possible to make successive measurements on the same leaf (Olfati et al., 2010), and it would not allow accurate observation of leaf growth using the same plant, but different plant samples.

We were able to accurately measure the leaf area of *S. pohlii* by using the LA=0.582+0.683WL equation, and the same accurate measurement was possible when using the LA=–0.666+0.704WL equation for *S. ferrugineus*. These models can provide the LA estimations with great accuracy, excluding the necessity of leaf excisions and/or expensive equipments (e.g., leaf area meter or digital cameras with image-measurement softwares).

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