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Leaf Area Estimation of *Palicourea racemosa* (Aubl.) Borhidi from Linear Measurements

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Abstract

Estimating leaf area is essential to evaluate vegetal growth. Our study sought to obtain statistical models to allow the leaf area estimation of *Palicourea racemosa*, considering its length (L) and width (W). For such purpose, we collected 200 leaves of this species in the State Park Mata do Pau-Ferro, in the municipality of Areia, Paraíba, Northeast of Brazil. The regression models used were: linear, linear without intercept, power and exponential. The choice of the best equation was based on the values of the coefficient of determination (R^2), root mean square error (RMSE), Akaike information criterion (AIC), Willmott concordance index (*d*) and BIAS ratio. All linear and power models may be used to measure the *P. racemosa* leaf area; however, the power model $LA = 0.609*(L.W)^{0.995}$ is the most recommended to estimate this species' leaf area.

Keywords: biometry, modeling, non-destructive method, leaf dimension, Rubiaceae.

1. INTRODUCTION AND OBJECTIVES

Palicourea racemosa (Aubl.) Borhidi is a neotropical species that belongs to the Rubiaceae family. It is a shrub that can reach 4 meters high. Relatively abundant and easy to identify on the field, its distribution occurs from South of Mexico to South America (Fajardo-Gutiérrez et al., 2012). Species of this family have great economic importance, since they are explored for food and ornamental purposes, as well as in the pharmaceutical industry (Coelho et al., 2006). According to Calixto et al. (2016), this kind of plants has several utilities in the traditional medicine due to its pharmacological properties, especially regarding to neurodegenerative diseases.

Performing ecological studies involving aspects of propagation, growth, and development of *Palicourea racemosa* is necessary due to this species importance. Among these studies, the leaf area measuring is agronomically and physiologically important, being possibly considered as the most important variable in the vegetal growth evaluation (Benincasa, 1988; Bianco et al., 1983; Bianco et al., 2007).

We can use several methods to estimate the leaf area of a given plant: destructive or non-destructive and direct or indirect methods (Marshall, 1968). Destructive methods demand more effort because they require the removal of a part of the plant (Leite et al., 2017; Taiz et al., 2017), which may represent a limiting factor depending on the species with which we are working or even on the number of plants we are using in the sample. These methods sometimes hinder the measurement, including cases involving endangered species, species in life stages that do not allow the collection of leaves, small species with a reduced number of leaves, and species with big leaf blades.

Non-destructive indirect methods are performed through regression equations to estimate the leaf area between the measured leaf area and the leaf linear parameters with extreme precision, being considered a much easier and faster procedure, without destructing the sample (Lima et al., 2012; Lizaso et al., 2003). In this context, the regression models employed to estimate leaf areas (non-destructive method) are based on the image digitalization technique of the collected

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leaves (destructive method). Such non-destructive method helps the researchers to monitor the standard growth of plants in different seasons and environments. This non-destructive technique has stood out when compared with all other techniques used to measure leaf areas due to its practicability and high precision (Pompelli et al., 2012). The non-destructive method was successfully employed both with growth (Erlacher et al., 2016; Hinnah et al., 2014; Schwab et al., 2014; Schmildt et al., 2015) and with forest species (Cabezas-Gutiérrez et al., 2009; Keramatlou et al., 2015; Monteiro et al., 2017; Mota et al., 2014; Ribeiro, Barbosa, & Albuquerque, 2018; Silva et al., 2013).

Therefore, our study sought to determine the regression equations that would allow us to estimate the leaf area of *Palicourea racemosa* (Aubl.) Borhidi based on the its linear dimensions.

2. MATERIALS AND METHODS

This study was developed in the State Park Mata do Pau-Ferro, located at 5 km west from the municipality of Areia, at the microregion of Brejo and mesoregion of the Agreste Paraibano (6° 58’ 12” S and 35° 42’ 15” W) (State of Paraíba, Northeast of Brazil), as shown in Figure 1. This region has an altitude that ranges from 400 and 600 meters, an annual average temperature of 22 °C (Ribeiro, Barbosa, Lopes et al., 2018), and tropical climate, classified as Aw – hot and moist with autumn-winter rains, according to Peel et al. (2007).

We collected 200 leaves of matrix plants of *P. racemosa*, without damages caused by external factors such as pests and diseases. Leaves of different shapes and sizes were sampled, stored in plastic bags, and taken to the Laboratory of Vegetal Ecology (Universidade Federal da Paraíba, Campus II) to measure their length (L) and width (W), and then the real leaf area (LA) was estimated. The length (L) was measured with a graduated ruler next to the main vein, not including the petiole, as the width (W) was measured perpendicularly to the main vein, as shown in Figure 2. To determine the leaf area, all leaf blades were digitalized with the assistance of a table scanner, to which a given scale was added, as shown in Figure 3. Then, we used the software ImageJ® (Powerful Image Analysis) of public domain to estimate the leaf area.

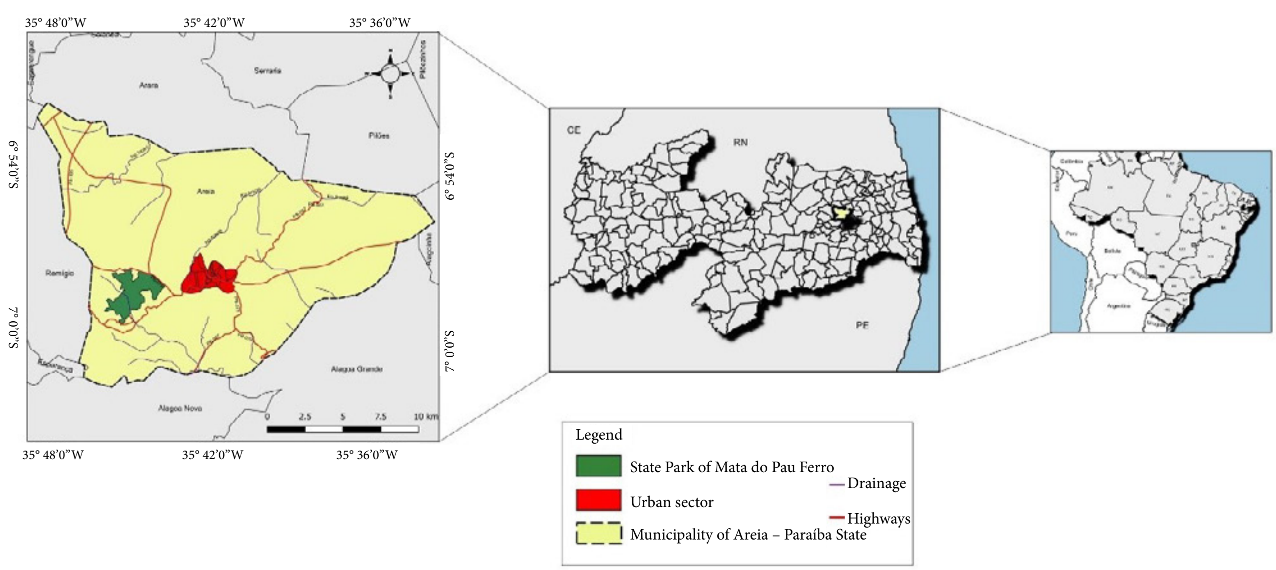

Figure 1. Geographic location of the State Park of Mata do Pau-Ferro, municipality of Areia, State of Paraíba, Brazil.

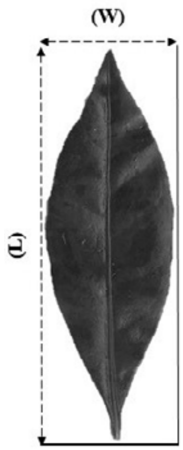

Figure 2. Length (L) and width (W) of the leaf of *Palicourea racemosa*, used to estimate the leaf area.

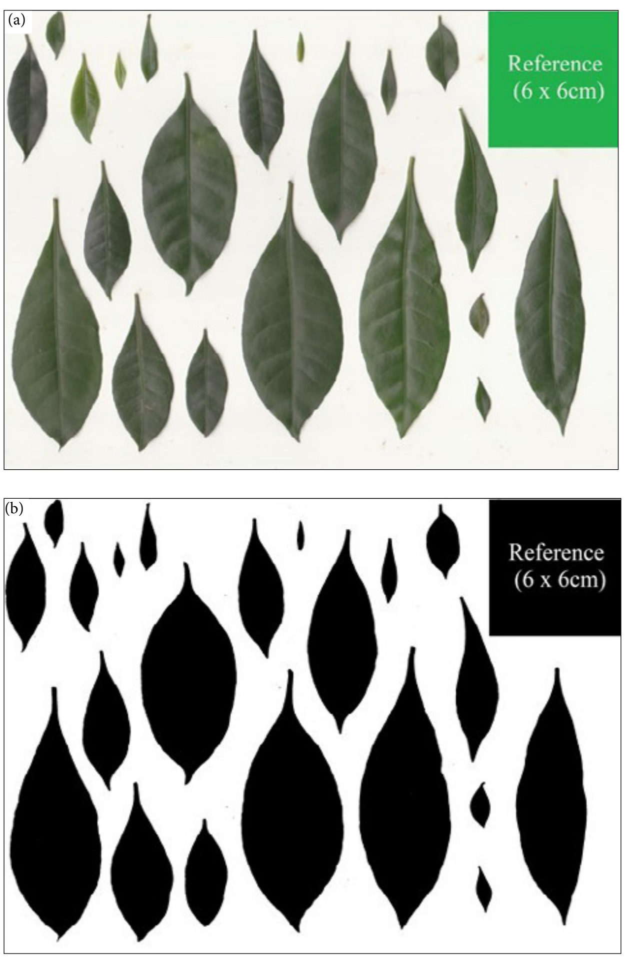

Figure 3. (a) *Palicourea racemosa* leaves of different sizes scanned for leaf area estimation using ImageJ software. (b) Scanned leaves with contrasting background used to determine leaf area using ImageJ software.

The following regression models were adjusted to choose the adjusted equation to estimate the leaf area of *P. racemosa*: linear, linear without intercept, power, and exponential, as shown in Table 1. The best equation to estimate the leaf area of *P. racemosa* for L (cm), W (cm) and/or L.W (cm^2) was chosen based on the coefficient of determination (R^2), root mean square error (RMSE), Akaike information criterion (AIC) (Floriano et al., 2006), Willmott concordance index (*d*) (Willmott et al., 1985), and BIAS ratio (Leite & Lima, 2002). The greater the values of R^2 and *d*, the lower the RMSE and AIC values, and the closer the BIAS is to zero, the better is the model. The statistical analyses were performed with the software R® v.3.4.3.

Table 1. Models used to estimate the leaf area of *Palicourea racemosa*.

Model	Formula
Linear	$LA = a + b*L$
Linear	$LA = a + b*W$
Linear	$LA = a + b*(L.W)$
Linear without intercept (0.0)	$LA = b*(L.W)$
Power	$LA = a*L^b$
Power	$LA = a*W^b$
Power	$LA = a*(L.W)^b$
Exponential	$LA = a*b^L$
Exponential	$LA = a*b^W$
Exponential	$LA = a*b^{(L.W)}$

3. RESULTS AND DISCUSSION

The *P. racemosa* leaves showed an average length of 6.96 cm, ranging from 0.73 to 15.31 cm; the width ranging from 0.26 to 5.30 cm, with mean values of 2.47 cm. The observed leaf area ranged from 0.13 to 49.01 cm^2, with an average of 12.59 cm^2, as shown Table 2.

Observing the wide range of leaf dimensions, a lower coefficient of variation were registered for length and width (CV = 47.1 and 48.7%, respectively), and greater variability for the product (L.W) and leaf area (LA) (CV = 83.7 and 83.6), also showed in Table 2. The wide variability of data is important to build statistical models, allowing their use with small, medium and big leaves. Some authors also noticed greatest variability for the product (L.W) when compared with the values of L and W (Cargnelutti Filho et al., 2015; Toebe et al., 2012). The dispersion diagrams, between L, W, L.W and LA, reveal association patterns of the data with the adjustment of non-linear and linear models, as shown in Figure 4, which agree with the study of Cargnelutti Filho et al. (2012).

Table 2. Minimum, maximum, mean, median, standard deviation, standard error, and coefficient of variation (CV) for length (L), width (W), the product of length and width (L.W), and leaf area (LA) of 200 leaves of *Palicourea racemosa*.

	Length (cm)	Width (cm)	L.W (cm^2)	Leaf area (cm^2)
Minimum	0.73	0.26	0.19	0.13
Maximum	15.31	5.30	80.69	49.01
Mean	6.96	2.47	20.98	12.59
Median	6.86	2.37	15.92	9.50
Standard deviation	3.27	1.20	17.56	10.52
Standard error	0.23	0.08	1.24	0.74
CV (%)	47.06%	48.71%	83.70%	83.63%

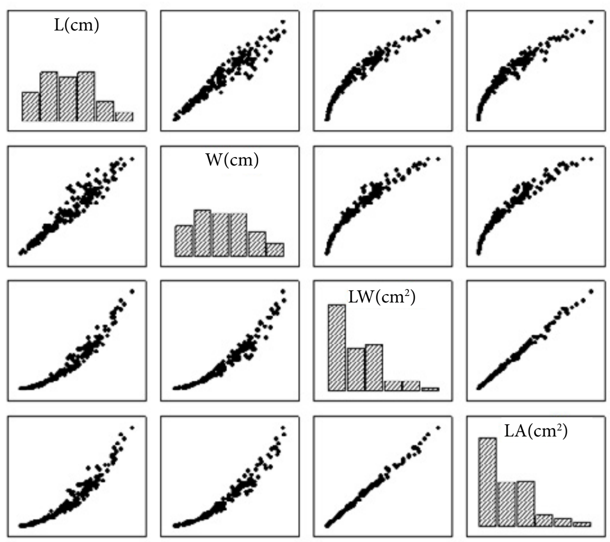

Figure 4. Frequency histogram and scatter plots between length (L), width (W), the product of length and width (L.W), and leaf area (LA) of 200 *Palicourea racemosa* leaves.

The results of the equations obtained from the linear, power and exponential regression models, using the length (L), width (W) and product (L.W), are shown in Table 3. According to the results, we noticed that the linear and power regression models satisfactorily estimate the area of *P. racemosa* leaf, with the coefficients of determination (R^2) presenting values over 0.91, indicating that at least 91% of the species' leaf area variations were explained by the proposed equations, using the leaf dimensional variables.

The coefficient of determination ranged from 0.783 to 0.998; with the greatest value obtained through the power model, using the product of length and width (L.W), while the lowest corresponded to the exponential model, in which the width (W) was used, as shown in Table 3. The AIC ranged from 412.82 to 1,522.80; with the greatest value resulting from the linear model, using the leaf length (L) and the lowest values corresponding to the power model, using the product (L.W). The RMSE values ranged from 0.6691 to 3.6979; with the greatest value corresponding to the exponential model, and the lowest value was obtained in the power model, both using the product (L.W). The Willmott concordance index (*d*) ranged from 0.9630 to 0.9990, with the greatest value obtained from the power model and the lowest corresponding to the exponential model, both using the product (L.W). The BIAS ratio ranged from 0.0041 to 0.6021, with the greatest value found in the exponential model and the lowest corresponding to the power model, both using the product (L.W). All values are shown in Table 3.

Generally, the power model, using the product of length and width (L.W), showed the greatest values of R^2 (0.9975) and *d* (0.9990), the lowest values of the RMSE (0.6691) and AIC (412.82), and BIAS rate closer to zero (0.0041), also shown in Table 3. Therefore, based on the used criteria, the power model $LA = 0.609^*(L.W)^{0.995}$, obtained from the dimension of the product (L.W), is the most recommended to estimate the leaf area of *P. racemosa*. There was little data dispersion regarding the adjusted curve, confirming that the equation $LA = 0.609^*(L.W)^{0.995}$ may satisfactorily explain the real leaf area of *P. racemosa*, as shown in Figure 5a. Although it has linear patterns, we confirmed that this power model showed the best adjustment, which was similar to studies made with other species (Antunes et al., 2008; Kandiannan et al., 2002; Maldaner et al., 2009; Misle et al., 2013; Pompelli et al., 2012; Souza et al., 2014; Tartaglia et al., 2016; Wang & Zhang, 2012; Williams & Martinson, 2003).

Models that depend on the product (L.W) were the most suitable to estimate the leaf area when compared with those that used solely L or W variables (Cargnelutti Filho et al., 2012; Hinnah et al., 2014), except for the power model. Similar results were found for other forest species, for example Schinopsis brasiliensis Engl. and *Tabebuia aurea* (Silva Manso) Benth. & Hook. f ex S. Moore (Queiroz et al., 2013), Acrocomia aculeata (Jacq.) Lodd. Mart. (Mota et al., 2014) and *Erythroxylum simonis* Plowman (Ribeiro, Barbosa, & Albuquerque, 2018), and also for agricultural species, the example of *Arachis hypogaea* L. (Cardozo et al., 2014) and Brassica napus L. (Tartaglia et al., 2016).

The estimated leaf area of *P. racemosa* obtained by the indicated model [$LA = 0.609^*(L.W)^{0.995}$] allows a satisfactory proximity of the real leaf area, since the coefficient of determination was 0.9959, as shown in Figure 5b. Therefore, the use of accurate equations for leaf area estimation of *P. racemosa* using linear measures of leaf blades (length and width) and their comparison with real leaf areas is a methodology as efficient as the destructive methods. Additionally, it is simpler and faster even under different field conditions or with limited technological resources (Pompelli et al., 2012).

Table 3. Regression models for estimating leaf area (cm²) of *Palicourea racemosa* with respective coefficient of determination (R^2), Akaike information criterion (AIC), root mean square error (RMSE), Willmott concordance index (*d*), and BIAS ratio, as a function of linear measurements of length (L), width (W), and the product between length and width (L.W).

Model	Estimated equation	R^2	AIC	RMSE	*d*	BIAS
Linear	LA = 8.754 + 3.065*L	0.9100	1,522.804	3.1514	0.9759	0.3151
Linear	LA = −8.235 + 8.425*W	0.9281	1,478.121	2.8183	0.9809	−0.2379
Linear	LA = 0.035 + 0.598*(L.W)	0.9960	903.240	0.6696	0.9989	−0.0147
Linear (0.0)	LA = 0.598*(L.W)	0.9960	901.236	0.6700	0.9989	−0.0148
Power	$LA = 0.219*L^{1.9857}$	0.9749	843.539	1.9639	0.9910	−0.0111
Power	$LA = 1.941*W^{1.8726}$	0.9809	807.169	1.7932	0.9925	0.0251
Power	$LA = 0.609*(L.W)^{0.995}$	0.9975	412.820	0.6691	0.9990	0.0041
Exponential	$LA = 2.384 * 1.233^{L}$	0.7828	960.685	2.6321	0.9827	0.4151
Exponential	$LA = 2.586 * 1.749^{W}$	0.7976	952.750	2.5804	0.9833	0.4139
Exponential	$LA = 6.187 * 1.029^{(L.W)}$	0.8694	1,096.683	3.6979	0.9630	0.6021

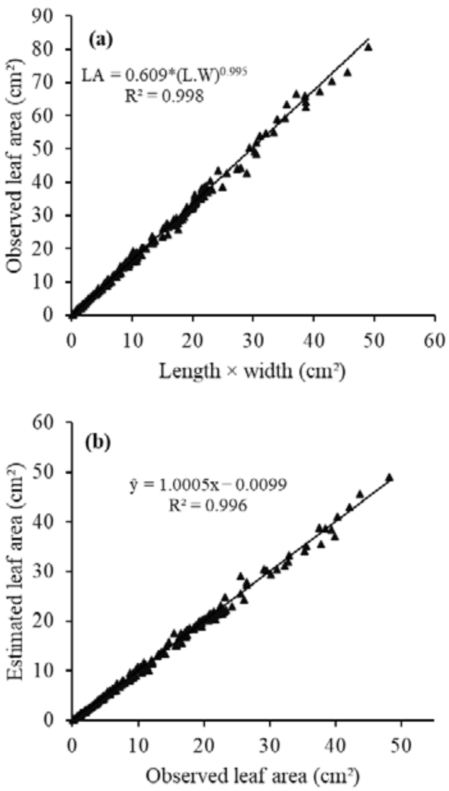

Figure 5. (a) Relation between real leaf area of *Palicourea racemosa* as a function of the product between length and width (L.W) of leaves, by the regression equation indicated to estimate the leaf area. (b) Relation between real leaf area and estimated leaf area by the regression equation $LA = 0.609*(L.W)^{0.995}$.

CONCLUSIONS

The linear and power models that used the product of length and width (L.W) may be used to accurately estimate the leaf area of *P. racemosa*.

The power model $LA = 0.609*(L.W)^{0.995}$ based on the product of length and width (L.W) is the most recommended to estimate the *P. racemosa* leaf area.

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