ESTIMATION OF LEAF AREA OF *Erythroxylum citrifolium* FROM LINEAR LEAF DIMENSIONS

ESTIMATIVA DA ÁREA FOLIAR DE Erythroxylum citrifolium *A PARTIR DE DIMENSÕES LINEARES DA FOLHA*

João Everthon da Silva RIBEIRO[1,*]; Ester dos Santos COÊLHO[1]; Francisco Romário Andrade FIGUEIREDO[2]; Sérgio de Faria LOPES[3]; Manoel Bandeira de ALBUQUERQUE[1]

1. Universidade Federal da Paraíba, Centro de Ciências Agrárias, Areia, Paraíba, Brasil. j.everthon@hotmail.com*; 2. Universidade Federal Rural do Semi-Árido, Mossoró, Rio Grande do Norte, Brasil; 3. Universidade Estadual da Paraíba, Departamento de Biologia, Campina Grande, Paraíba, Brasil.

ABSTRACT: *Erythroxylum citrifolium* is a neotropical plant species recorded in all regions of Brazil. Determining leaf area is of fundamental importance to studies related to plant propagation and growth. The objective was to obtain an equation to estimate the leaf area of *E. citrifolium* from linear dimensions of the leaf blade (length and width). A total of 200 leaf blades were collected in Parque Estadual Mata do Pau-Ferro in the municipality of Areia, state of Paraíba, Northeast Brazil. The models evaluated were: linear, linear without intercept, quadratic, cubic, power and exponential. The best model was determined by the criteria of: high coefficient of determination (R^2), low root mean square error (RMSE), low Akaike information criterion (AIC), high Willmott concordance index (*d*) and a BIAS index close to zero. All of the models constructed satisfactorily estimated the leaf area of *E. citrifolium*, with coefficients of determination above 0.9050, but the power model using the product between length and width (L*W) $\hat{y} = 0.5966 * LW^{1.0181}$ was the best, with the highest values of R^2 and *d*, low values of RMSE and AIC, and a BIAS index closest to zero.

KEYWORDS: Biometry. Non-destructive method. Allometric equations. Erythroxylaceae.

INTRODUCTION

Erythroxylum citrifolium A.St.-Hil. (Erythroxylaceae), commonly known as cumixá, is a Neotropical plant species distributed throughout Central and South America from Mexico to Southern Brazil, with records in all regions of Brazil (PLOWMAN; HENSOLD, 2004; LOIOLA et al., 2007). It is found in the most varied types of vegetation, but mainly in remnants of humid forests of the Atlantic Forest biome and in mountain forests known as Brejos de Altitude (LOIOLA; COSTA-LIMA, 2015). This species is used as a medicinal plant and has anti-inflammatory properties for the treatment of bronchitis and other respiratory diseases (GONZÁLEZ-GUEVARA et al., 2004). The branches and leaves have several pharmacological substances, including an important antimicrobial that may help in the treatment of herpes simplex virus type 1 (HSV-1) and human immunodeficiency virus (HIV) (HOZUMI et al., 1995; MATSUSEA et al., 1999; GONZÁLEZ-GUEVARA et al., 2004; DAN; CASTELLAR, 2015).

Due to the importance of this species, are increasingly needed physiological studies related to the parameters of plant growth, development and productivity. Among these studies, the measurement of leaf area is fundamental, and perhaps the most important parameter in the evaluation of plant growth and development (CANDIDO et al., 2013). However, leaf area is a difficult variable to measure due to the fact that it requires the use of expensive devices and destructive methods (CARGNELUTTI FILHO et al., 2015a).

Leaf area can be determined by direct or indirect methods, which are classified as destructive and non-destructive respectively (MALAGI et al., 2010). Destructive methods are simple and precise, but require a lot of time and cause the total destruction of vegetal mass (MOTA et al., 2014). On the other hand, non-destructive methods allow for numerous evaluations of the same plant with speed and precision, and without destroying the sample (MOTA et al., 2014). One such indirect method is estimating leaf area by means of regression equations using actual leaf area as a function of leaf parameters (length and width), which are directly related to leaf surface (ZHANG; PAN, 2011). This non-destructive method has been used innumerous studies, both for cultivated species (POMPELLI et al., 2012; SILVA et al., 2013; FRANCISCO et al., 2014; BUTTARO et al., 2015; GANESHAMURTHY et al., 2016; OLIVEIRA et

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al., 2017; CARVALHO et al., 2017) and forest species (POMPELLI et al., 2012; SILVA et al., 2013; QUEIROZ et al., 2013; ASSIS et al., 2015; KERAMATLOU et al., 2015; RIBEIRO et al., 2018a; RIBEIRO et al., 2019). Thus, the objective was to obtain an equation for estimating the leaf area of *Erythroxylum citrifolium* from linear dimensional parameters of leaf blades.

MATERIAL AND METHODS

Study area

The study was undertaken in Parque Estadual Mata do Pau-Ferro, in the municipality of Areia located in the micro-region of Brejo and mesoregion of Agreste Paraibano, state of Paraíba (PB), Northeast Region of Brazil (6°58'12"S 35°42'15"W) (Figure 1). The climate of the region is tropical and classified as Aw (PELL et al., 2007), which is characterized as hot and humid with autumn-winter rains. The mean annual temperature is 22 ºC and the mean annual rainfall is 1,400 mm (RIBEIRO et al., 2018b). The elevation of the region varies between 400 and 600 meters.

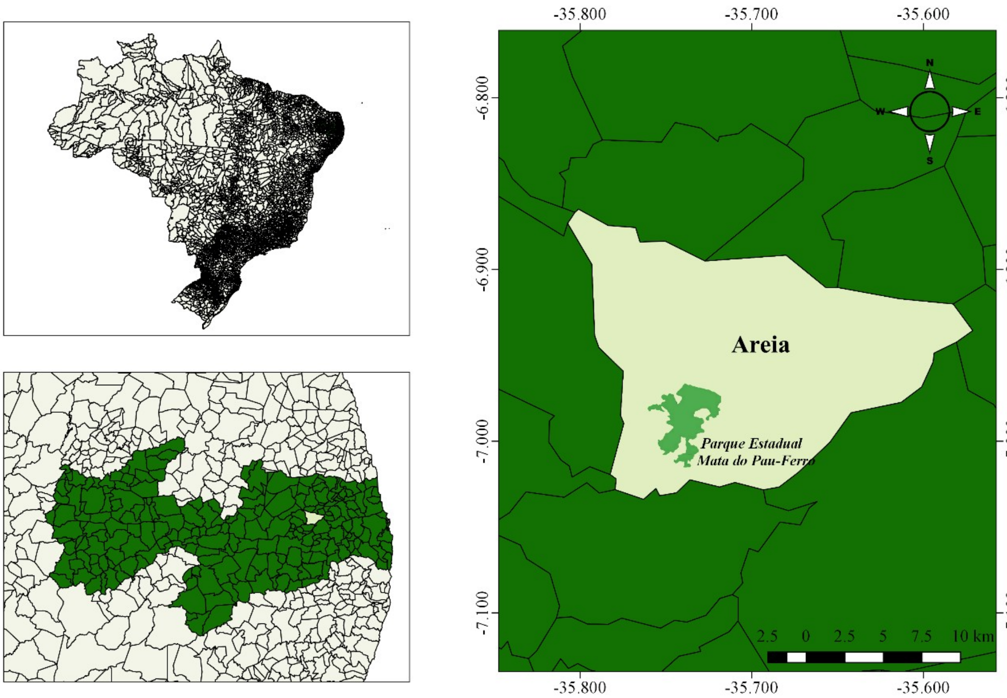

Figure 1. Geographic location of Parque Estadual Mata do Pau-Ferro, municipality of Areia, state of Paraíba Northeast Brazil.

Data collection

For the collection of leaf area data, 200 leaf blades of different shapes and sizes were selected from individual plants in a matrix of *Erythroxylum citrifolium*, considering only leaves that did not exhibit damage caused by external factors, such as climate, pests and diseases (SCHMILDT et al., 2014). The leaf blades were packed in a box with cold water to avoid water loss and taken to the Plant Ecology Laboratory (Universidade Federal da Paraíba). The length (L) and width (W) of each leaf blade (Figure 2) were measured using a graduated ruler, and their product calculated (L*W). In order to determine true leaf area, the leaf blades were scanned with a reference scale, the images contrasted using the ImageJ® Software and the true area of the leaf blades measured (JADOSKI et al. (2012).

To choose the best equation for estimating leaf area of *E. citrifolium*, regression studies were performed employing the following statistic models: linear ($\hat{y}= a + bx$), linear without intercept ($\hat{y}= bx$), quadratic ($\hat{y}= a+ bx + c x^2$), cubic ($\hat{y}= a+ bx + cx^2 + dx^3$), power ($\hat{y}= ax^b$), and exponential ($\hat{y}= ab^x$), in which the dependent variable ŷ estimates leaf area (LA) as a function of x (length, width or the product between length and width).

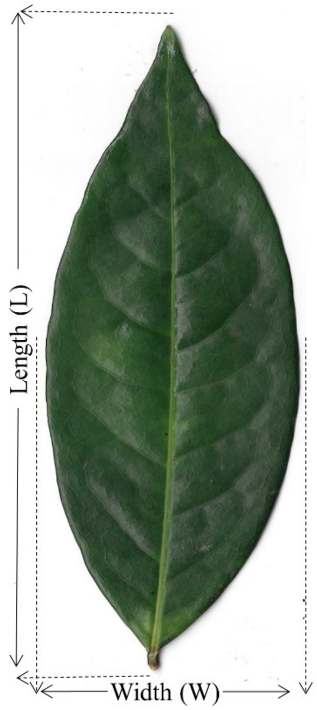

Figure 2. Length (L) and width (W) of a leaf blade of *Erythroxylum citrifolium* used for leaf area estimation.

The criteria used to choose the best model for estimating leaf area of *E. citrifolium* were the coefficient of determination (R^2), root mean square error (RMSE) (JANSSEN; HEUBERGER, 1995), Akaike Information Criterion (AIC) (FLORIANO et al., 2006), Willmott concordance index (*d*) (WILLMOTT et al., 1985), and BIAS index (LEITE; ANDRADE, 2002). The best model was considered the one with greatest values of R^2 and d, lowest values of RMSE and AIC, and a BIAS index closest to zero. The statistical analyses were performed with the software R® v.3.4.3 (R CORE TEAM, 2018), using the 'hydroGOF' statistical package.

RESULTS AND DISCUSSION

The leaf blades of *E. citrifolium* varied in length (L) from 1.20 to 17.91 (cm), with a mean of 8.60 cm, and width (W) from 0.43 to 6.13 cm, with a mean of 2.82 cm. True leaf area (LA) varied from 0.40 to 70.44 cm² with a mean of 18.85 cm² (Table 1).

Table 1. Minimum, maximum, median, standard deviation, standard error and coefficient of variation for length (L), width (W), the product between length and width (L*W) and leaf area (LA) of 200 leaf blades of *Erythroxylum citrifolium*,

Descriptive statistical	L (cm)	W (cm)	L*W (cm²)	LA (cm²)
Minimum	1.20	0.43	0.62	0.40
Maximum	17.91	6.13	109.11	70.44
Mean	8.60	2.82	29.53	18.85
Median	8.00	2.68	21.62	13.58
Standard deviation	4.08	1.32	25.32	16.42
Standard error	0.29	0.09	1.79	1.16
C.V. (%)	47.43	46.62	85.75	87.14

Variation in the linear dimensions of *E. citrifolium* leaf blades showed that length and width had the low values for the coefficient of variation, while greater variability was observed for their product (L*W) and leaf area (Table 1). This greater variability is fundamental for the elaboration of regression models that estimate leaf area from linear measurements because it allows applicability to leaves of different shapes and sizes (CARGNELUTTI FILHO et al., 2012). Other studies have likewise found greater variability for the product between length and width (L*W) compared to length (L) and width (W) in leaves of *Canavalia ensiformis* (TOEBE et al., 2012), *Brassica napus* (CARGNELUTTI FILHO et al., 2015a), *Cajanus cajan* (CARGNELUTTI FILHO et al., 2015b) and *Passiflora edulis* (SCHMILDT et al., 2016).

According to dispersion diagrams for length, width, product between length and width and leaf area of *E. citrifolium,* patterns of association of data adjusted by linear and non-linear models (Figure 3) can be observed corroborating other studies (CARGNELUTTI FILHO et al., 2012; CARGNELUTTI FILHO et al., 2015a, b).

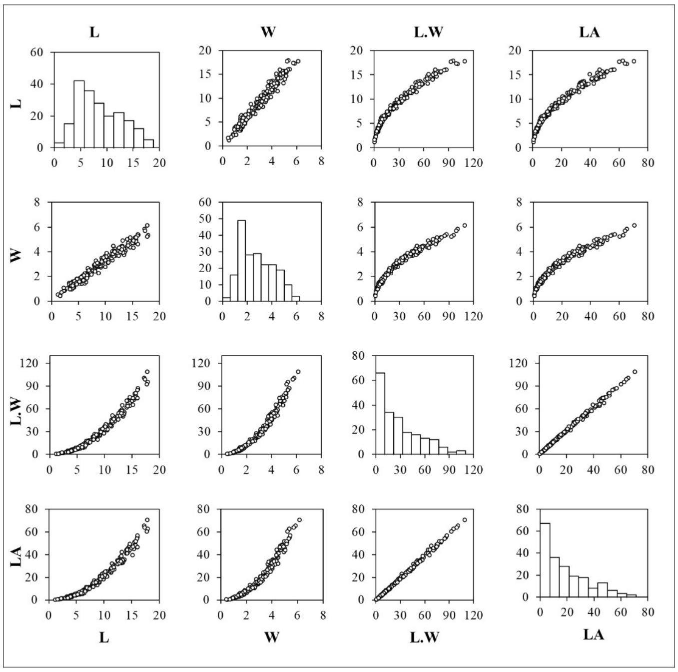

Figure 3. Frequency histogram and dispersion graph of length (L), width (W), product between length and width (L*W) and true leaf area (LA), in 200 leaf blades of *Erythroxylum citrifolium*.

The equations obtained for the adjusted models relating true leaf area (LA) with length (L), width (W) and product between length and width (L*W) (Table 2). All equations produce satisfactory estimations of *E. citrifolium* leaf area, since the coefficients of determination (R^2) were all above 0.905 (Table 2), indicating that at least 90.50% of the variation in *E. citrifolium* leaf area is explained by the proposed models using linear leaf blade parameters.

Comparing the equations that used L or W, it is verified that the product dependencies between length and width (L*W) are the most recommended for estimating leaf area, similar to that observed by Hinnah et al. (2014). Similar results have been recorded for other forest species, such as *Amburana cearenses*, *Caesalpinia ferrea* and *Caesalpinia pyramidalis* (SILVA et al., 2013), *Acrocomia aculeata* (MOTA et al., 2014) and *Merremia aegyptia* (ASSIS et al., 2015). Such results were also observed for cultivated species, such as *Ananas comosus* (FRANCISCO et al., 2014), *Vigna unguiculata* (OLIVEIRA et al., 2015), *Prunus persica* (SACHET et al., 2015), *Smallanthus sonchifolius* (ERLACHER et al., 2016) and *Litchi chinensis* (OLIVEIRA et al., 2017).

In general, the power model that used the product of length and width (L*W) presented the highest values of R^2 (0.9979) and *d* (0.99950), lowest values of REMS (0.7506) and AIC (459.12), and a BIAS index of nearly zero (-0.0012) (Table 2). Thus, based on the criteria adopted, the estimation of *E. citrifolium* leaf area can be performed by the equation $\hat{y} = 0.5966 * LW^{1.0181}$.

Low dispersion of the data can be seen in the adjustment curve, indicating that equation ($\hat{y} = 0.5966 * LW^{1.0181}$), can explain *E. citrifolium* leaf area satisfactorily (Figure 4A and B). The power model using the product (L*W) has also been recommended for estimating the leaf area of other species, such as *Styrax pohlii* and *Styrax ferrugineus* (SOUZA et al., 2014), *Vigna unguiculata* (OLIVEIRA et al., 2015), *Passiflora edulis* (SCHMILDT et al., 2016), *Crotalaria juncea* (CARVALHO et al., 2017), *Urochloa mosambicensis* (LEITE et al., 2017), and *Psychotria carthagenensis* and *Psychotria hoffmannseggiana* (RIBEIRO et al., 2019).

Overall, our results hold great potential for ecophysiological studies, especially for forest species, because they allow the monitoring of leaf area in a given place and time, and thus help to understand the growth patterns of plants.

Table 2. Equations for estimating leaf area (cm^2) of *Erythroxylum citrifolium* with determination coefficients (R^2), Akaike information criterion (AIC), root mean square error (RMSE), Willmott concordance index (*d*) and BIAS index, using linear measures of length (L), width (W) and their product (L*W).

Model	x (1)	Equation	R²	AIC	RMSE	*d*	BIAS
Linear	L	$\hat{y} = - 14.8712 + 3.9194*L$	0.9483	1099.4	3.723	0.9865	0.0052
Linear	W	$\hat{y} = - 15.3209 + 12.1061*W$	0.9408	1126.6	3.985	0.9845	-0.0089
Linear	L*W	$\hat{y} = - 0.2848 + 0.6480*LW$	0.9979	463.0	0.758	0.9994	0.0022
Linear (0.0)	L*W	$\hat{y} = 0.6424*LW$	0.9977	472.6	0.758	0.9994	0.0024
Quadratic	L	$\hat{y} = 0.1976*L^2 + 0.1859*L - 0.6537$	0.9873	820.2	1.843	0.9968	0.0024
Quadratic	W	$\hat{y} = 2.0132*W^2 - 0.2615*W + 0.0799$	0.9840	866.6	2.070	0.9959	0.0029
Quadratic	L*W	$\hat{y} = 0.0001*LW^2 + 0.6362*LW - 0.1475$	0.9979	461.9	0.752	0.9994	0.0020
Cubic	L	$\hat{y} = - 0.0017*L^3 + 0.2465*L^2 - 0.2240*L + 0.3078$	0.9872	821.2	1.839	0.9968	0.0069
Cubic	W	$\hat{y} = - 0.0843*W^3 + 2.8179*W^2 - -2.5258*W + 1.8747$	0.9840	866.4	2.058	0.9960	0.0100
Cubic	L*W	$\hat{y} = - 0.000002*LW^3 + 0.0005*LW^2 + 0.6208*LW - 0.0392$	0.9979	462.8	0.751	0.9994	0.0015
Power	L	$\hat{y} = 0.2210 * L^{1.9760}$	0.9873	818.6	1.845	0.9967	-0.0414
Power	W	$\hat{y} = 1.8780 * W^{2.0276}$	0.9840	864.9	2.071	0.9959	-0.0198
Power	L*W	$\hat{y} = 0.5966 * LW^{1.0181}$	0.9979	459.1	0.750	0.9995	-0.0012
Exponential	L	$\hat{y} = 3.3942 * 1.1885^{L}$	0.9630	1051.3	3.301	0.9890	-0.6126
Exponential	W	$\hat{y} = 3.3715 * 1.7023^{W}$	0.9573	1081.1	3.557	0.9871	-0.6760
Exponential	L*W	$\hat{y} = 8.7128 * 1.0220^{LW}$	0.9051	1240.2	5.294	0.9695	-0.9390

(1) Linear dimensions: length (L) and width (W)

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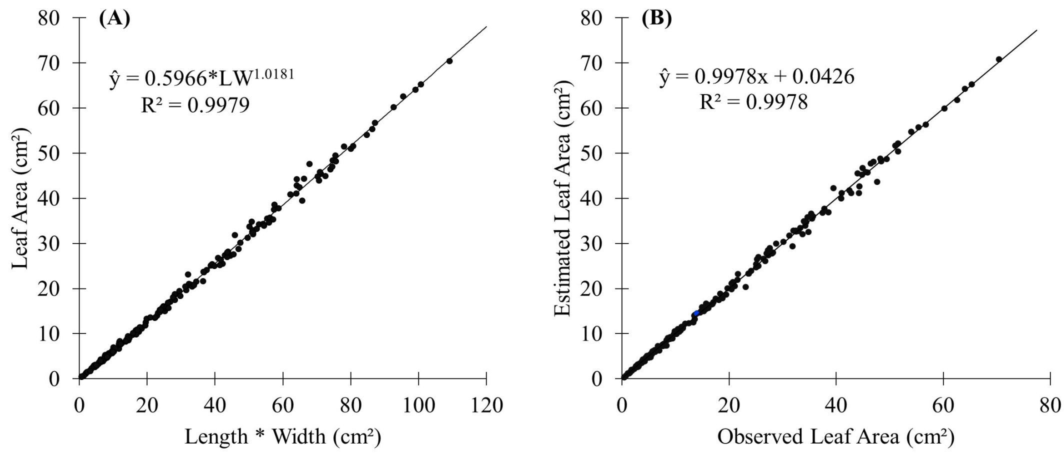

Figure 4. (A) True leaf area of *Erythroxylum citrifolium* as a function of the product of length and width (L*W) of leaf blades, according to the equation indicated for estimating the leaf area. (B) Relationship between true leaf area and leaf area estimated by the proposed equation.

CONCLUSIONS

The leaf area of *E. citrifolium* can be accurately estimated by the non-destructive method of using linear measurements of leaf blades.

The equations that used the product of length and width (L*W) were the most successful at estimating *E. citrifolium* leaf area, with the power model using the product (L*W) being the best.

The most suitable equation for estimating leaf area of *E. citrifolium* was $\hat{y} = 0.5966*LW^{1.0181}$.

RESUMO: *Erythroxylum citrifolium* é uma espécie de planta neotropical com registros em todas as regiões do Brasil. A determinação da área foliar é de fundamental importância em estudos relacionados a propagação e crescimento vegetal. O objetivo foi obter uma equação que permita estimar a área foliar de *E. citrifolium* a partir de dimensões lineares do limbo foliar (comprimento e largura). Foram coletados 200 limbos foliares no Parque Estadual Mata do Pau-Ferro, Areia, Paraíba, Nordeste do Brasil. Os modelos empregados foram: linear, linear sem intercepto, quadrático, cúbico, potencial e exponencial. Os critérios utilizados para escolher o melhor modelo, teve como base o maior coeficiente de determinação (R^2), menor raiz do quadrado médio do erro (RMSE), menor critério de informação de Akaike (AIC), maior índice de concordância de Willmott (*d*) e índice BIAS mais próximo de zero. Todos os modelos construídos podem estimar satisfatoriamente a área foliar de *E. citrifolium*, com coeficientes determinação acima de 0,9050, porém o modelo potencial utilizando o produto entre comprimento e largura (L*W) $\hat{y} = 0{,}5966 * LW^{1{,}0181}$ é o mais indicado, com os maiores valores de R^2 e *d*, menores valores de RMSE e AIC, e índice BIAS mais próximo de zero.

PALAVRAS-CHAVE: Biometria. Método não-destrutivo. Equações alométricas. Erythroxylaceae.

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