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Allometric models for non-destructive leaf area estimation in *Eugenia uniflora* (L.)

Modelos alométricos para la estimación no destructiva del área foliar en *Eugenia uniflora* (L.)

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Abstract

We aimed to propose a reliable and accurate model using non-destructive measurements of leaf length (L) and/or width (W) for estimating leaf area (LA) of Surinam cherry (*Eugenia uniflora* L.). For model construction, 560 leaves were randomly sampled from different levels of the tree canopies and encompassed the full spectrum of measurable leaf sizes. Power models better fit *E. uniflora* leaf area than linear models; but, among of then, the best fit were made when product of the L and W (LW) were used. To validate these models, independent data set of 156 leaves were used. Thus, we developed a single power model ($Y_i = \beta_0 x^{\beta 1}$) [LA = 0.685 $(LW)^{0.989}$; standard errors: β_0 = 0.014, β_1 = 0.005; R^2_a = 0.997] with high precision and accuracy, random dispersal pattern of residuals and unbiased. A simpler linear model [LA = 0.094 + (LW * 0.655); standard errors: β_0 = 0.025, β_1 = 0.001; R^2_a = 0.998] also described here to estimate leaf area of *E. uniflora*, which are as good as the first. The simplicity of the latter model may be relevant in field studies, as it does not demand high precision or expensive instruments.

Keywords: Surinam cherry; estimate model; leaf length; leaf width

Resumen

Nuestro objetivo fue proponer un modelo confiable y preciso utilizando mediciones no destructivas de la longitud de la hoja (L) y / o el ancho (W) para estimar el área foliar (LA) de la cereza de Surinam (*Eugenia uniflora* L.). Para la construcción del modelo, se tomaron 560 hojas al azar de diferentes niveles de las copas de los árboles y abarcaron todo el espectro de tamaños de hojas medibles. Los modelos exponenciales se ajustan mejor al área foliar de *E. uniflora* que los modelos lineales; el mejor ajuste se realizó cuando se usaron productos de L y W (LW). Para validar estos modelos, se utilizaron conjuntos de datos independientes de 156 hojas. Por lo tanto, desarrollamos un único modelo de potencia ($Y_i = \beta_0 x^{\beta 1}$) [LA = 0.685 $(LW)^{0.989}$; error estándar: β_0 = 0.014, β_1 = 0.005; R^2_a = 0.997] con alta precisión y exactitud, patrón de dispersión aleatorio de residuales e imparcial. Un modelo lineal más simple [LA = 0.094 + (LW * 0.655); error estándar: β_0 = 0.025, β_1 = 0.001; R^2_a = 0.998] también se describe aquí para estimar el área foliar de *E. uniflora*, que es tan buena como la primera. La simplicidad de este último modelo puede ser relevante en los estudios de campo, ya que no exige instrumentos costosos o de alta precisión.

Palabras llave: cereza de Surinam; modelo de estimación; longitud de la hoja; ancho de la hoja

Introduction

Leaf area (LA) is one of the six most important traits that drive plant form and function (Díaz *et al.*, 2016). This descriptor has been widely used to describe a range of variables including growth, productivity, photosynthetic efficiency, soil characteristics including salinity and acidity, transfer and exchange of heat, carbon, nutrients and water, which in turn affect plant yield (Cristofori *et al.*, 2007; Pompelli *et al.*, 2012). Thus, a correct determination of the LA becomes even more important in crop species, since the leaf is the organ of greater influence with the environment and it is through this that the agronomic studies are based on the important decision making.

Directly measure of LA of individuals is both, laborious, expensive as well as a time consuming task and often constrained by logistical factors. Leaf area is traditionally quantified by direct methods, which are destructively or obtained through high-cost equipment, such as AM350 portable leaf area meter (ADC BioScientific Ltd., Hoddesdon, UK). With the intensification of modeling techniques, numerous studies have proposed allometric models to predict the LA of different species (Blanco and Folegatti, 2005; Antunes *et al.*, 2008; Pompelli *et al.*, 2012; Keramatlou *et al.*, 2015; Liu *et al.*, 2017). Hence,

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simple linear measurements like leaf length (L), leaf width (W) are used in allometric equations to predict the leaf area (Peksen, 2007). Given the fact that *Eugenia uniflora* is a commercial crop species, non-destructive methods such as allometry are best suited for measure leaf area because preserve the leaf on the plant instead destructive methods (Cristofori *et al.*, 2007). This paper describes, for first time allometric equations to predict leaf area of *Eugenia uniflora* checking the accuracy and perform an unbiased tool for use in land and agronomic studies.

Material and Methods

For model construction, 560 healthy leaves were collected at least 20 healthy plants naturally grown at Caetés Ecological Station, Paulista, Pernambuco, Brazil (7º55'28"S; 34º56'02"O; 88 m.a.s.l.) in April 2018 (end of the growing season). To validate the model, an independent data set of 156 leaves were sampled randomly from different levels of the tree canopy, removed from the branches and taken to the laboratory. The maximum leaf length (L) (from lamina tip to the point of the petiole intersection to the midrib) and leaf width (W) (the widest linear length perpendicular to the midrib) were measured to the nearest of 0.001 cm. The leaves were scanned using a scanner (Epson 1200 *x* 1200 dpi) and images were analyzed using the Image-Pro® Plus software (2001). The leaves encompassed the broadest range as possible. The minimum leaf area sampled was 0.17 cm^2 and maximum was 72.40 cm^2 (Table 1).

Nine theoretical models (more widely used in the literature) were tested, based on different combinations between the components of LA (dependent variable) and respective values of L and W (independent variables). The equations were deduced by the principle of parsimony (Steel and Penny, 2000), and thus from the "simplest", or an "optimal" description of the data. All equations were adjusted following the linear simple, modified linear (from exclude β_0) and power models (more information, see Table 2). All parameters of each model were obtained using DataFit version 8.0.32 (Oakdale Engineering, 2002). The statistical criteria used to select the models were based on (*i*) the analysis of variance (F test, $P < 0.001$), (*ii*) adjusted coefficient of determination (R^2_a), (*iii*) mean squared error (MSE), (*iv*) Student's t-test ($P < 0.001$) for absolute mean of errors with confidence intervals (Cumming *et al.*, 2007), (*v*) dispersion pattern of residuals in percentage terms (%) and the best relationship (major R^2_a) between observed leaf area and estimated leaf area of the independent data set used to validating equations. The dispersion of the residues were observed in the total sample set, both in small leaves and in larger leaves. The hypothesis of normality of the errors were evaluated, so that heteroscedasticity was considered a reason for model disqualification. These procedures allowed us to assess the occurrence of bias and accuracy in all proposed models (Walther and Moore, 2005).

Results and Discussion

All nine developed equations (Table 3) presented good predictors of the *E. uniflora* leaf area, since R^2_a was always higher than 0.8; coefficient higher than those proposed to some crop plants (Cristofori *et al.*, 2007; Peksen, 2007; Kumar, 2009; Souza and Amaral, 2015) and within the range of those proposed by others (Blanco and Folegatti, 2005; Antunes *et al.*, 2008; Demirsoy, 2009; Pompelli *et al.*, 2012; Shabani and Sepaskhah, 2017). Thus, at first glance, all proposed equations should be able to predict with accuracy the leaf area of *E. uniflora*. However, when we analyzed the deviation between estimated leaf area and observed leaf area (Fig. 1), we demonstrate that equations #4, #5, and #8 were biased, because a significant underestimation of LA. In this case, the underestimation can lead for 53.1% of the estimated leaf area (using equation #8) to 93.2% of the estimated leaf area (using equation #4). In similar manner, the equation #6 can lead to overestimation leaf area in about 19.1% of the leaves. All these equations had an estimated significant difference from zero (biased) and were excluded

Table 1. Means ± standard deviations (SD), minimum (min), maximum (max) values for the leaf length (L), width (W), and leaf area (LA) of the *Eugenia uniflora* L.

L (cm)			W (cm)			LA (cm^2)		
Mean ± SD	Min	Max	Mean ± D	Min	Max	Mean ± SD	Min	Max
3.55 ± 2.32	0.72	14.31	1.97 ±1.42	0.27	7.62	5.83 ± 10.73	0.17	72.40

Table 2. Statistical models and equations to predict leaf area as a function of linear dimensions of leaves

(Model #1)	Linear	$Y_i = \beta_0 + \beta_1 * \text{Length} + \varepsilon_i$
(Model #2)	Linear	$Y_i = \beta_0 + \beta_1 * \text{Width} + \varepsilon_i$
(Model #3)	Linear	$Y_i = \beta_0 + \beta 1 * (\text{Length}* \text{Width}) + \varepsilon_i$
(Model #4)	Linear without intercept	$Y_i = \beta_1 * \text{Length} + \varepsilon_i$
(Model #5)	Linear without intercept	$Y_i = \beta_1 * \text{Width} + \varepsilon_i$
(Model #6)	Linear without intercept	$Y_i = \beta_1 *(\text{Length}* \text{Width}) + \varepsilon_i$
(Model #7)	Power	$Y_i = \beta_0 * \text{Length}^{\beta_1} + \varepsilon_i$
(Model #8)	Power	$Y_i = \beta_0 * \text{Width}^{\beta_1} + \varepsilon_i$
(Model #9)	Power	$Y_i = \beta_0 * (\text{Length}*\text{Width})^{\beta_1} + \varepsilon_i$

Y_i = leaf area, β_0 and β_1 = model coefficients and = random error from the further analysis as recommended by Antunes *et al.* (2008) for coffee trees, by Pompelli *et al.* (2012) for purging-nut trees and by Yadav *et al.* (2007); all agronomic species.

With the disqualification of equations #4, #5, #6 and #8, five equations remained to be analyzed in more details. Thus, a deep analysis of relationship between estimated leaf area and dispersal pattern of residuals, revealed that equations #1 lead to an overestimation of 34.5% and #2 to an underestimation of 36.2% of the leaves (when considering relative errors ≥ 40%). A possible source of error must be due to negative values of β_0, that in these equations were bigger than -7.4. Higher values of β_0 were previously (Blanco and Folegatti, 2005; Cristofori *et al.*, 2007; Antunes *et al.*, 2008; Pompelli *et al.*, 2012; Schmildt *et al.*, 2015) used to disqualification the allometric equations, because such equations return negative values of LA (*i.e.*, invalid biological condition).

The heteroscedasticity of residuals of model #7, was used to disqualify it, because this model lead to overestimation or underestimation on 21.2% of the leaves (when considering relative errors ≥ 20%). The biased pattern of residuals showed in equations #1, #2 and #7 are as large as the smaller sampled leaves. So, we can only recommended the use of these equations when a stratification of the leaf size classes is performed, simultaneously checking the dispersion pattern of the residues in all classes of leaves, as suggested by others (Walther and Moore, 2005; Antunes *et al.*, 2008; Zuur *et al.*, 2010; Pompelli *et al.*, 2012). However, this "solution", sometimes becomes laborious and impractical, despite the ease of adjustment and operation of this type of model.

From nine initial proposed models, only two models (#3 and #9) were entirety approved. As shows in Fig. 2, the equations #3 lead to overestimation some leaf areas. Therefore, this overestimation is lower than 4%, which cannot invalidate this equation. In this issue, we argue that the best equation is equation #9, made with power model. Even if the model #7 has been previously disqualified, we can verify that it presents an excellent fit curve ($R^2_a = 0.985$) between linear dimensions of leaves and observed leaf area (Fig. 3B). Models using single leaf dimension power model incorporating L or W may be an interesting option because

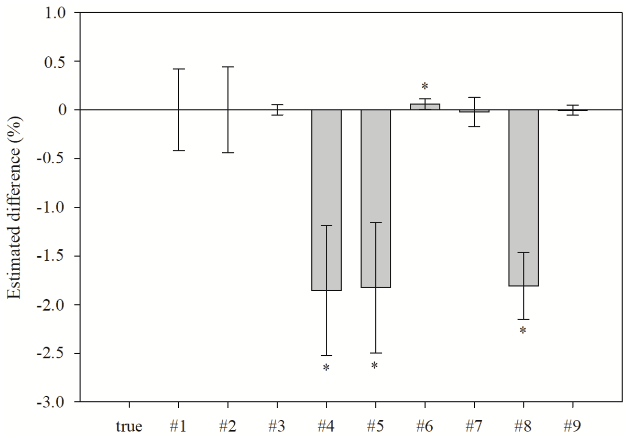

Figure 1. Statistical analysis of the deviation of the estimated area from the observed area for an individual leaf. Leaf area for *Eugenia uniflora* was estimated using several models in which β_0 and β_1 are coefficients. Vertical bars denote means and spreads denote 99% confidence intervals of the difference. Numbers below the graph denote model numbers (see further details in the Table 1). Asterisks in the bars denote a biased model.

it requires measurement of only one leaf dimension, thus simplifying measurement procedures (Blanco and Folegatti, 2005; Cristofori *et al.*, 2007; Antunes *et al.*, 2008; Pompelli *et al.*, 2012). Because of this, we prefer to keep this equation to next step of validation.

When we pooled the relationship between observed leaf area and linear estimated leaf area of the independent sample leaves (Fig. 3, right panel), we verified that equations #3, #7, and #9 returns good fit curves, showing the coefficient of determination above of 0.960. However, as suggested above, the equation #7 returns the lesser determination coefficient of all, besides being present the lesser *P* value (P = 0.186) than others ($P \geq 0.674$).

Finally, we argue that from nine initial proposed models, only two models (Model #3 and #9) can provide an unbiased estimation of leaf area using the linear dimensions of leaves. These models were approved in all statistical analysis and then are able to use without errors, both in field and in greenhouses evaluations. However, among then, the

Table 3. Statistical models, regression coefficients (β_0, β_1), degrees of freedom of residuals (R-d.f.), mean squared error (MSE), coefficients of determination adjusted for the degrees of freedom (R^2_{adj}) and *P* value as a function of linear dimensions of leaves.

Models	Coefficients		R-d.f.	MSE	R^2_{adj}	P
	β_0	β_1				
#1	-7.641	3.854	352	9.311	0.884	<0.001
#2	-7.418	6.834	352	10.207	0.873	<0.001
#3	0.094	0.655	352	0.141	0.998	<0.001
#4	-	2.353	353	23.516	0.819	<0.001
#5	-	4.221	353	23.770	0.818	<0.001
#6	-	0.658	353	0.146	0.996	<0.001
#7	0.407	1.944	352	1.166	0.985	<0.001
#8	2.343	1.591	352	6.198	0.923	<0.001
#9	0.685	0.989	352	0.136	0.997	<0.001

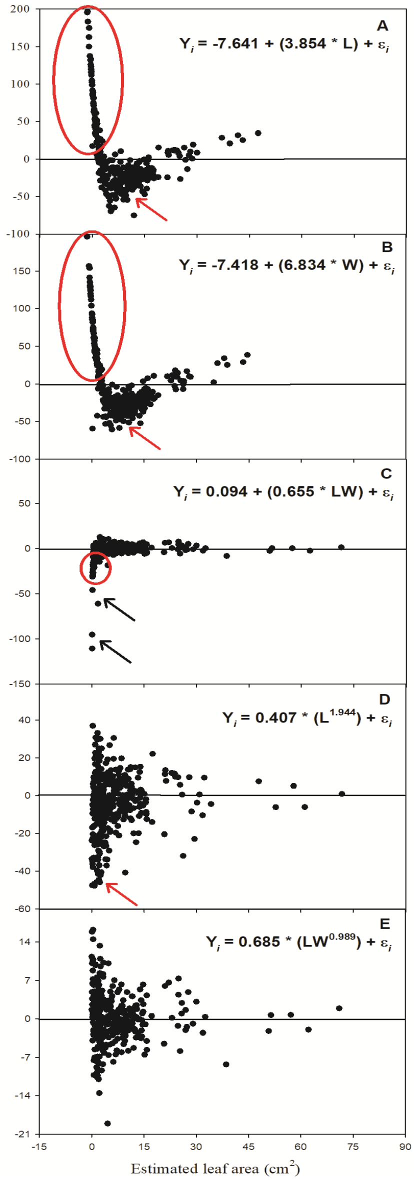

Figure 2. The relationship between estimated leaf area and dispersal pattern of residuals to each selected equations. Red oval shape denote strong underestimated (A, B) or overestimated (C) leaf area. Red arrows denote strong biased estimated leaf areas, mainly in the smaller leaves, while black arrows denote slightly skewed estimated leaf areas. See further details in the text.

equation #3 is simpler than equation #9, because it does not require more complex calculations. In the other hand, if the researcher has notion of the error merged in equation #7 and this may lead to an overestimation of approximately 21% of the estimated leaf area, this equation could also be an interesting option because it requires measurement of only one leaf dimension, simplifying measurement procedures (Blanco and Folegatti, 2005; Pompelli *et al.*, 2012), an important aspect specially in the field when a large number of leaves has to be monitored.

Conclusion

In this study, we developed a reliable and accurate equation to estimate the leaf area or *Eugenia uniflora* using non-destructive method. A power equation, type $Y_i = \beta_0 x^{\beta 1}$ [LA = 0.685 (LW)$^{0.989}$] was made. This equation may estimate

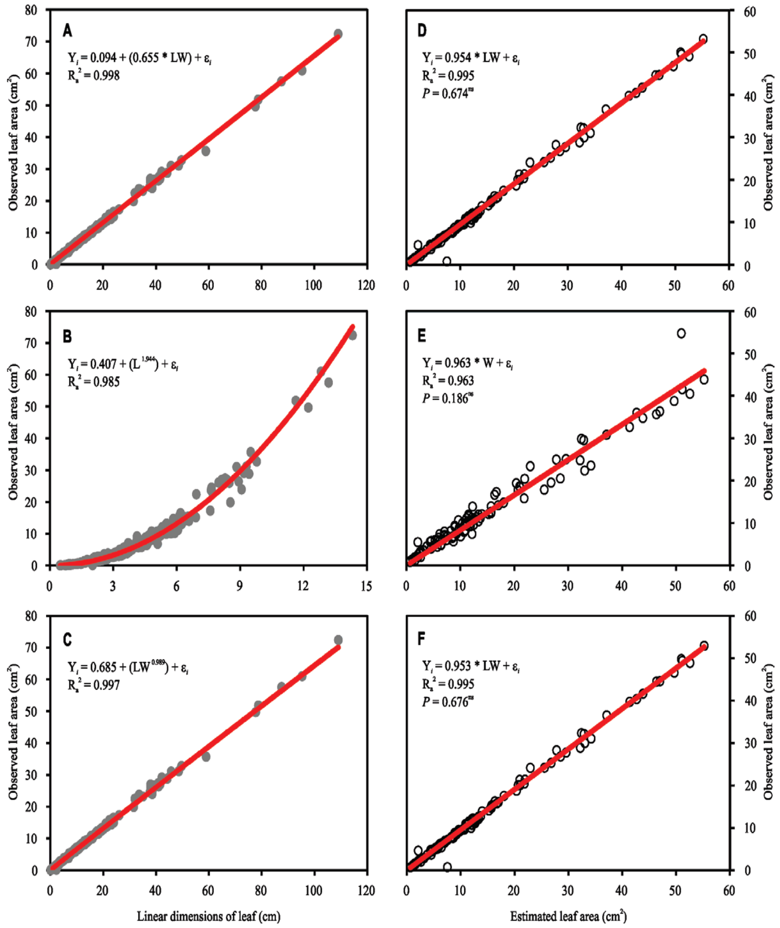

Figure 3. The relationship between observed leaf area and linear dimensions of leaf (A, B and C) or between estimated leaf area (D, E and F) for model #3 (A and D), model #7 (B and E) and model #9 (C and F). L, leaf length; LW, product of leaf length and leaf width; ns, not significant; R_a^2, coefficients of determination adjusted for the degrees of freedom.

the leaf area with 99.7% of accuracy. The simplification of this equation could be done using a linear equation [LA = 0.094 + (LW * 0.655) without loss of accuracy. This procedure should be less laborious because use a linear equation instead a power equation. This is the first study that describes with great accuracy an allometric equation to estimate the leaf area of *Eugenia uniflora*, showing all common mistakes in allometric equations published until now.

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