



Leaf area estimation for *Psychotria carthagenensis* and *Psychotria hoffmannseggiana* as a function of linear leaf dimensions

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ABSTRACT. Determining leaf area is important for studies involving plant growth and development. The aim of the present study was to obtain models for estimating leaf area of *Psychotria carthagenensis* and *Psychotria hoffmannseggiana* using linear measurements of leaf blades (length and width). Two hundred leaf blades of each species were collected in Parque Estadual Mata do Pau-Ferro in the municipality of Areia, Paraíba, Northeast Brazil. The equations evaluated for producing potential models included the following: linear, quadratic, potential and exponential. The criteria used to determine the best model(s) were as follows: 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 ratio close to zero. All evaluated models satisfactorily estimated leaf area for the two species, but the equation $\hat{y} = 0.6373 * LW^{0.9804}$ was the most appropriate for *P. carthagenensis*, while $\hat{y} = 0.6235 * LW^{0.9712}$ was the most appropriate for *P. hoffmannseggiana*.

Keywords: biometry; non-destructive method; modeling; Rubiaceae.

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Introduction

Psychotria carthagenensis Jacq. is a shrub measuring 2–3 m in height. It is widely distributed in the Neotropical Region, occurring from Costa Rica to Argentina. In Brazil the species occurs from the state of Pará in the north to the state of Rio Grande do Sul in the south, and is found in a variety of habitats at elevations ranging from sea level to 600 m (Vitarelli & Santos, 2009; Taylor & Zappi, 2015). The species is highly significant for the conservation of available ecological resources because it serves as a source of food for fauna throughout the year, which disperse seeds (Blendinger et al., 2015).

Psychotria hoffmannseggiana [(Willd. ex Schult.) Müll.Arg.] is a subshrub measuring 30–60 cm in height. It is distributed throughout Central America and from Cuba to Paraguay (Delprete, 2010); it occurs in all regions of Brazil (Taylor & Zappi, 2015). The species is found in the Amazon, Cerrado, and Atlantic Forest biomes in habitats at elevations from 100 to 1500 m (Margalho, Rocha, & Secco, 2009). In the Northeast Region of the country it occurs in Atlantic Forest physiognomies including moist forests and restingas. The species has great importance in the region as a food source for fauna, mainly birds, which disperse seeds, and for the conservation and management of disturbed sites, such as moist forests (Schupp, 1993).

Due to the importance of these species, ecophysiological studies related to growth, development, reproduction, and propagation are needed. Leaf area is the most important parameter for determining plant growth (Benincasa, 1988), since leaves perform several functions for plants, including the interception and absorption of light for photosynthesis, and gas exchange and transpiration (Taiz, Zeiger, Møller, & Murphy, 2017). Taiz et al. (2017) described leaf area as one of the most difficult parameters to measure because it requires expensive equipment and/or destructive techniques.

Several methods exist for measuring leaf area, including those that are destructive, non-destructive, direct, or indirect (Marshall, 1968). Direct methods may or may not be destructive, while all indirect methods are non-

destructive. Destructive methods are generally considered simple and accurate, but by their nature they destroy leaves in the process and require more time and labor (Schmildt, Hueso, & Cuevas, 2014).

The use of non-destructive methods has been gaining importance for studies of plant populations because they allow quick and accurate successive leaf area measurements of the same individual (Bosco, Bergamaschi, Cardoso, Paula, & Casamali, 2012). The development of regression models allows leaf area to be estimated from linear dimensions of the foliar limb (length and width), and thus represents a simple non-destructive method for obtaining this parameter in the most diverse field conditions (Pompelli et al., 2012). In this context, regression models (non-destructive method) used to estimate leaf area are developed based on direct measurements of true leaf area using digitalized images of the collected leaves (destructive method). Several researchers have used such models to estimate leaf area for forest species [*Merremia aegyptia* (L.) Urb. (Assis, Linhares, Souza, Pereira, & Almeida, 2015) and *Juglans regia* L. (Keramatlou, Sharifani, Sabouri, Alizadeh, & Kamkar, 2015)] and agricultural species [*Litchi chinensis* Sonn. (Oliveira, Silva, Costa, Schmildt, & Vitória, 2017) and *Annona cherimola* Miller (Schmildt, Hueso, Pinillos, Stellfeldt, & Cuevas, 2017)].

The aim of the present study was to obtain models that satisfactorily estimate leaf area of *Psychotria carthagenensis* and *Psychotria hoffmannseggiana* using linear dimensions of the foliar limb.

Material and methods

The study was conducted in Parque Estadual Mata do Pau-Ferro (6°58'12" S and 35°42'15" W) located in the municipality of Areia, in the microregion of Brejo Paraibano and mesoregion of Agreste Paraibano (Paraíba, Brazil) (Figure 1). The climate of the area is classified as Aw according to Peel, Finlayson, and McMahon (2007), which is tropical wet and dry with autumn-winter rains, and an average annual temperature of 22°C (Ribeiro, Barbosa, Lopes, Pereira, & Albuquerque, 2018a). Elevations of the area range between 400 and 600 m.

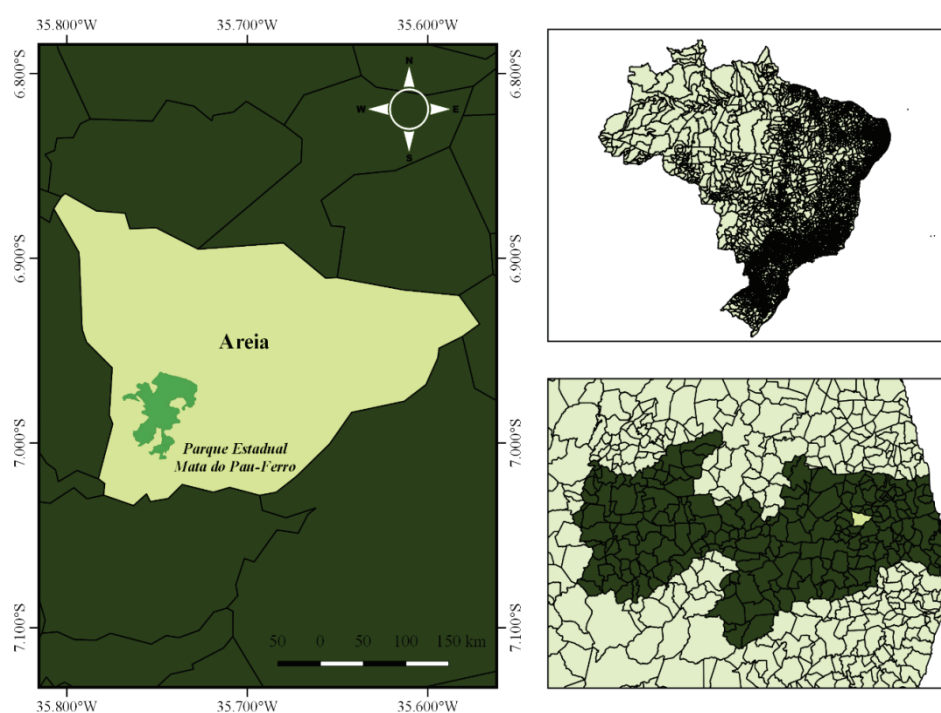


Figure 1. Geographic location of Parque Estadual Mata do Pau-Ferro in the municipality of Areia, state of Paraíba, Brazil.

We collected 400 healthy leaves (i.e., not damaged by external factors, such as diseases and pests, *sensu* Schmildt et al., 2014) of different shapes and sizes: 200 leaves of *P. carthagenensis* and 200 leaves of *P. hoffmannseggiana*. The leaves were packed in plastic bags and sent to the Laboratory of Plant Ecology (Universidade Federal da Paraíba, Campus II) to measure their maximum length (L) and maximum width (W) (Figure 2), using a graduated ruler. The leaves were then scanned using a flatbed scanner with an established scale to determine their 'true' leaf area (LA) using *ImageJ*® software (U.S. National Institutes of

Health, USA) with increased contrast to better delimit the area to be measured (Ribeiro, Barbosa, & Albuquerque, 2018b).

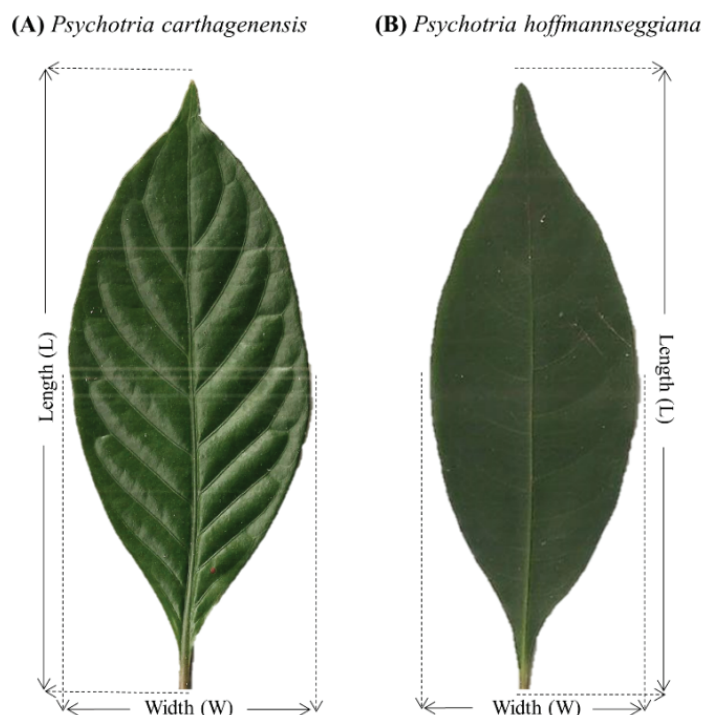


Figure 2. Maximum length (L) and width (W) of leaf blades of (A) *Psychotria carthagenensis* and (B) *Psychotria hoffmannseggiana* used to estimate leaf area.

The following equations were evaluated for as models for estimating leaf area as a function of the linear dimensions (length and width) of a leaf: linear ($\hat{y} = a + bx$), quadratic ($\hat{y} = a + bx + cx^2$), potential ($\hat{y} = ax^b$), and exponential ($\hat{y} = ab^x$). The dependent variable ($\hat{y}$) estimates leaf area as a function of the independent variable(s) (x), which were length (L), width (W), and/or their product (length x width).

The criteria used to determine the model(s) that best estimates leaf area of *P. carthagenensis* and *P. hoffmannseggiana* as a function of the linear dimensions of the leaf limb (L, W and / or L x W) were: coefficient of determination (R^2), root-mean-square error (RMSE), Akaike information criterion (AIC), Willmott concordance index (d) and the BIAS ratio. The best models were considered those with highest R^2 and d , lower values of RMSE and AIC, and a BIAS ratio close to zero. All statistical analyses were performed using R® v.3.4.3 (R Core Team, 2017).

Results and discussion

The leaf blades of *P. carthagenensis* had a mean length (L) of 14.52 cm (range 5.81 – 28.16 cm) a mean width (W) of 5.24 cm (1.79 – 10.32 cm), and a mean leaf area (LA) of 48.25 cm² (6.54 – 153.26 cm²) (Table 1). The leaf blades of *P. hoffmannseggiana* had a mean length of 5.93 cm (1.03 – 8.74 cm), a mean width of 2.05 cm (0.42 – 3.32 cm), and a mean leaf area of 7.43 cm² (0.27 – 15.54 cm²) (Table 1).

Extensive variation in the dimensions of the leaf blades of *P. carthagenensis* and *P. hoffmannseggiana*, resulted in lower values for the coefficient of variation for length and width, and greater variability in their product (LxW) and leaf area (Table 1). This abundant variability of the data is of fundamental importance for the construction of statistical models that estimate leaf area because it will favor models that can be used for small, medium, and large leaves (Cargnelutti Filho, Toebe, Burin, Fick, & Casarotto, 2012).

Previous studies have also found greater variability for the product between the length and width (LxW) of leaves than in the values of length and width themselves (Cargnelutti Filho, Toebe, Alves, Burin, & Kleinpaul, 2015a; Cargnelutti Filho, Toebe, Alves, & Burin, 2015b; Schmildt, Negrís, Cesana Junior, Schmildt, & Alexandre, 2016a).

Table 2 shows the results of the evaluation of the regression models relating length (L), width (W), and their product (LxW) to leaf area (LA). All models satisfactory estimated leaf area for *P. carthagenensis* and *P.*

hoffmannseggiana, with coefficients of determination (R^2) being above 0.8874, which indicates that 88.74% of the observed variation in leaf area of the species is explained by the proposed model using linear dimensions of the leaf blade.

Table 1. Minimum, maximum, and mean values and coefficient of variation (CV) of length (L), width (W), length x width (LxW) and leaf area (LA) for 200 leaf blades of *Psychotria carthagenensis* and for 200 leaf blades of *Psychotria hoffmannseggiana*.

<i>Psychotria carthagenensis</i>				
	L (cm)	W (cm)	LxW (cm ²)	LA (cm ²)
Minimum	5.81	1.79	10.90	6.54
Maximum	28.16	10.32	268.16	153.26
Mean	14.52	5.24	82.84	48.25
C.V. (%)	29.22	32.01	59.09	58.10
<i>Psychotria hoffmannseggiana</i>				
	L (cm)	W (cm)	LxW (cm ²)	LA (cm ²)
Minimum	1.03	0.42	0.43	0.27
Maximum	8.74	3.32	27.85	15.54
Mean	5.93	2.05	12.86	7.43
C.V. (%)	23.91	25.74	41.59	40.75

Table 2. Models used to estimate leaf area (cm²) of *Psychotria carthagenensis* and *Psychotria hoffmannseggiana* using linear measurements of length (L), width (W), and their product (LxW). Criteria for selecting the best model are also provided: coefficient of determination (R^2), Akaike information criterion (AIC), root-mean-square error (RMSE), Willmott concordance index (d), and BIAS ratio.

<i>Psychotria carthagenensis</i>							
x ¹	Equation	R ²	AIC	RMSE	d	BIAS	Model
L	Linear	0.9138	1415.83	8.2124	0.97698	0.04879	$\hat{y} = -43.4465 + 6.3122 * L$
W	Linear	0.9497	1308.03	6.2723	0.98693	0.04883	$\hat{y} = -37.1041 + 16.2755 * W$
LxW	Linear	0.9949	848.39	1.9879	0.99873	-0.00586	$\hat{y} = 0.9281 + 0.5713 * LW$
L	Quadratic	0.9459	1324.52	6.5036	0.98592	0.01248	$\hat{y} = -0.2551 + 0.00006 * L + 0.2067 * L^2$
W	Quadratic	0.9713	1197.85	4.7384	0.99266	0.03734	$\hat{y} = -6.1207 + 4.0952 * W + 1.0853 * W^2$
LxW	Quadratic	0.9950	847.09	1.9762	0.99874	-0.00806	$\hat{y} = 0.2985 + 0.5866 * LW - 0.0001 * LW^2$
L	Potential	0.9459	1322.48	6.5031	0.98591	-0.00752	$\hat{y} = 0.2198 * L^{1.9850}$
W	Potential	0.9712	1196.28	4.7435	0.99262	-0.06068	$\hat{y} = 2.2865 * W^{1.7984}$
LxW	Potential	0.9950	846.05	1.9715	0.99875	-0.00305	$\hat{y} = 0.6373 * LW^{0.9804}$
L	Exponential	0.9195	1409.46	8.0827	0.97665	-0.71425	$\hat{y} = 8.8819 * 1.1160^L$
W	Exponential	0.9392	1356.04	7.0722	0.98237	-0.73463	$\hat{y} = 10.6035 * 1.3111^W$
LxW	Exponential	0.9392	1470.50	7.0722	0.98237	-0.73463	$\hat{y} = 24.0849 * 1.0077^{LW}$
<i>Psychotria hoffmannseggiana</i>							
x ¹	Equation	R ²	AIC	RMSE	d	BIAS	Model
L	Linear	0.8874	578.91	1.0134	0.96935	0.03740	$\hat{y} = -4.5017 + 2.0124 * L$
W	Linear	0.9165	519.23	0.8730	0.97777	-0.07072	$\hat{y} = -3.8318 + 5.4796 * W$
LxW	Linear	0.9924	38.74	0.2626	0.99809	-0.02056	$\hat{y} = 0.1769 + 0.5641 * LW$
L	Quadratic	0.9317	480.88	0.7892	0.98205	0.06184	$\hat{y} = 0.4724 - 0.1197 * L + 0.2064 * L^2$
W	Quadratic	0.9378	462.30	0.7534	0.98371	0.03551	$\hat{y} = -0.7465 + 1.8467 * W + 0.9730 * W^2$
LxW	Quadratic	0.9925	38.35	0.2610	0.99812	-0.00577	$\hat{y} = 0.0823 + 0.5815 * LW - 0.0006 * LW^2$
L	Potential	0.9314	480.30	0.7920	0.98212	0.01354	$\hat{y} = 0.2102 * L^{1.9722}$
W	Potential	0.9377	460.64	0.7540	0.98360	-0.00593	$\hat{y} = 2.1148 * W^{1.6913}$
LxW	Potential	0.9925	36.58	0.2612	0.99812	0.00104	$\hat{y} = 0.6235 * LW^{0.9712}$
L	Exponential	0.9276	492.70	0.8170	0.98021	-0.02867	$\hat{y} = 1.0479 * 1.3722^L$
W	Exponential	0.9128	533.44	0.9045	0.97488	-0.05139	$\hat{y} = 1.5026 * 2.1091^W$
LxW	Exponential	0.9128	516.62	0.9045	0.97488	-0.05139	$\hat{y} = 3.0808 * 1.066^{LW}$

Dispersion diagrams between length (L) (cm), width (W) (cm), their product (LxW) (cm²) and leaf area (LA) (cm²) for *P. carthagenensis* and *P. hoffmannseggiana* indicate patterns that fit linear and non-linear models (Figure 3A and B), thus corroborating the results of Cargnelutti Filho et al. (2012) (*Raphanus sativus* L.) and Cargnelutti Filho et al. (2015a; 2015b) (*Brassica napus* L. and *Cajanus cajan* L.).

The models that used the product of length and width were better at estimating leaf area than those that used the two measurements separately (Hinnah et al., 2014). Similar results have been reported for other forest species, such as *Amburana Cearenses* A. C. Smith, *Caesalpinia ferrea* Mart. and *Caesalpinia pyramidalis* Tul. (Silva, Queiroz, & Neto, 2013), *Combretum leprosum* Mart. (Candido, Coelho, Maia, Cunha, & Silva, 2013), *Acrocomia aculeata* Jacq. (Mota, Leite, & Cano, 2014), *Merremia aegyptia* (L.) Urban (Assis, Linhares, Souza, Pereira, &

Almeida, 2015), and for cultivated species, such as *Ananas comosus* L. (Merrill) (Francisco, Diotto, Folegatti, Silva, & Piedade, 2014), *Vigna unguiculata* (L.) (Oliveira et al., 2015), *Mangifera indica* L. (Silva, Cabanez, Mendonça, Pereira, & Amaral, 2015), *Prunus persica* (L.) (Sachet, Penso, Pertille, Guerrezii, & Citadin, 2015), *Smallanthus sonchifolius* (Poepp.) H. Rob. (Erlacher, Oliveira, Fialho, Silva, & Carvalho, 2016), *Macadamia integrifolia* Maiden & Betche (Schmidt et al., 2016b) and *Litchi chinensis* Sonn. (Oliveira et al., 2017).

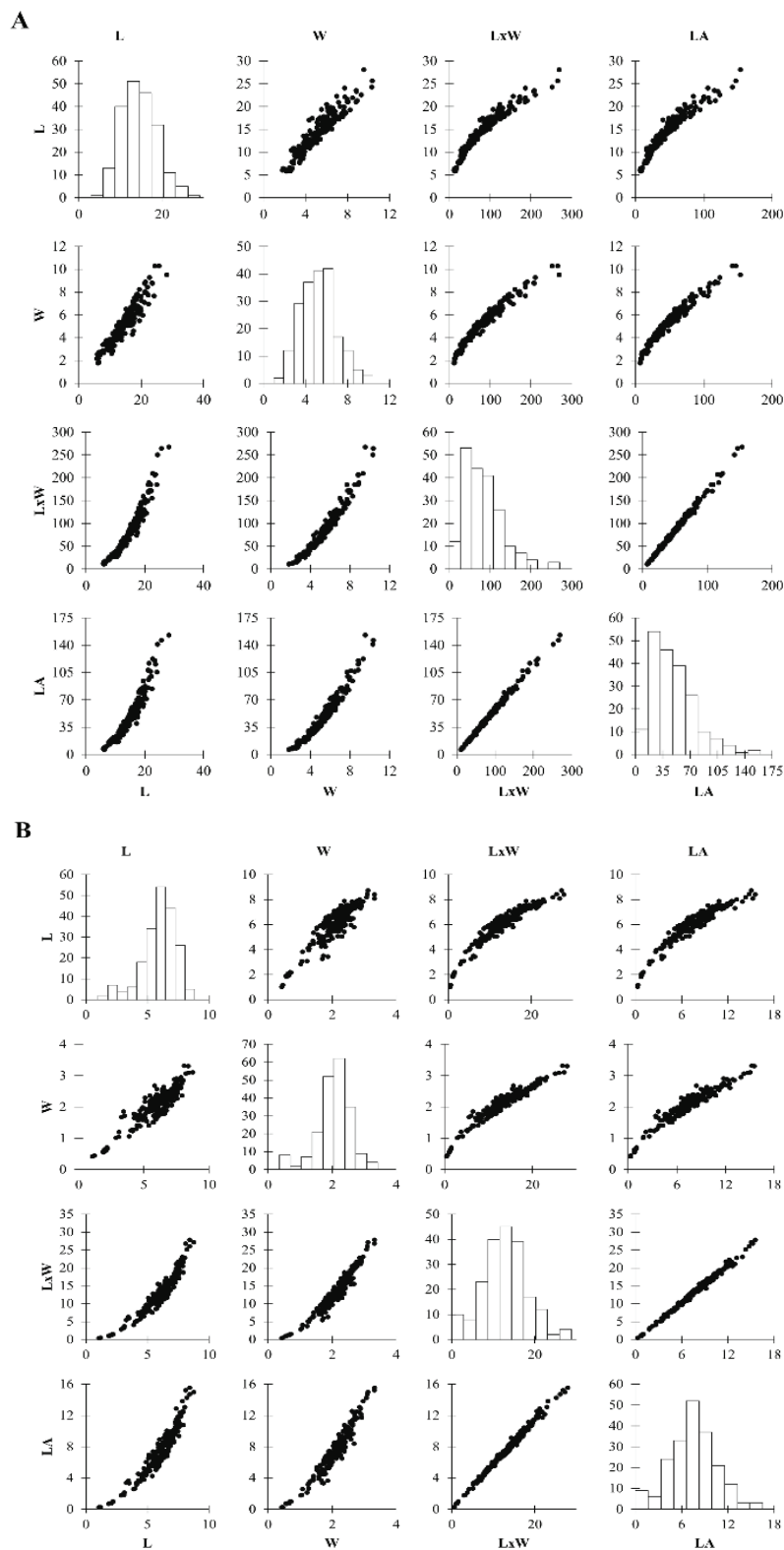


Figure 3. Frequency histogram (diagonally) and scatter plots between length (L), width (W), the product between length and width (LxW) and the true leaf area (LA) of 200 leaf blades of (A) *Psychotria carthagenensis* and 200 leaf blades of (B) *Psychotria hoffmannseggiana*.

For both studied species, a potential model that used the product of length and width (LxW) had the highest R^2 (0.9950 and 0.9925) and d (0.99875 and 0.99812), lower values for RMSE (1.9715 and 0.2612) and AIC (846.05 and 36.58), and a BIAS ratio closer to zero (-0.00305 and 0.00104) (Table 2). Thus, based on the criteria used, the model $\hat{y} = 0.6373 * LW^{0.9804}$ is the best for estimating leaf area of *P. carthagenensis*, while the model $\hat{y} = 0.6235 * LW^{0.9712}$ is best for estimating the leaf area of *P. hoffmannseggiana*. These models presented the best adjustments, coinciding with studies in other species (Schmidt et al., 2016a; Tartaglia et al., 2016; Leite, Lucena, Sá Júnior, & Cruz, 2017; Schmidt et al., 2017).

The data revealed little dispersion in relation to the adjustment curve, indicating that the models mentioned above satisfactorily estimate the true leaf area of *P. carthagenensis* and *P. hoffmannseggiana* (Figure 4A and B). The models for estimating the leaf area of *P. carthagenensis* ($\hat{y} = 0.6373 * LW^{0.9804}$) and *P. hoffmannseggiana* ($\hat{y} = 0.6235 * LW^{0.9712}$) were satisfactory close at estimating true leaf area, with coefficients of determination of 0.9950 and 0.9925 (Figure 5A and B). The models identified by the present study may contribute to ecological studies that involve the growth, development, and propagation of these two species, since leaf area is fundamental parameter for research in these areas.

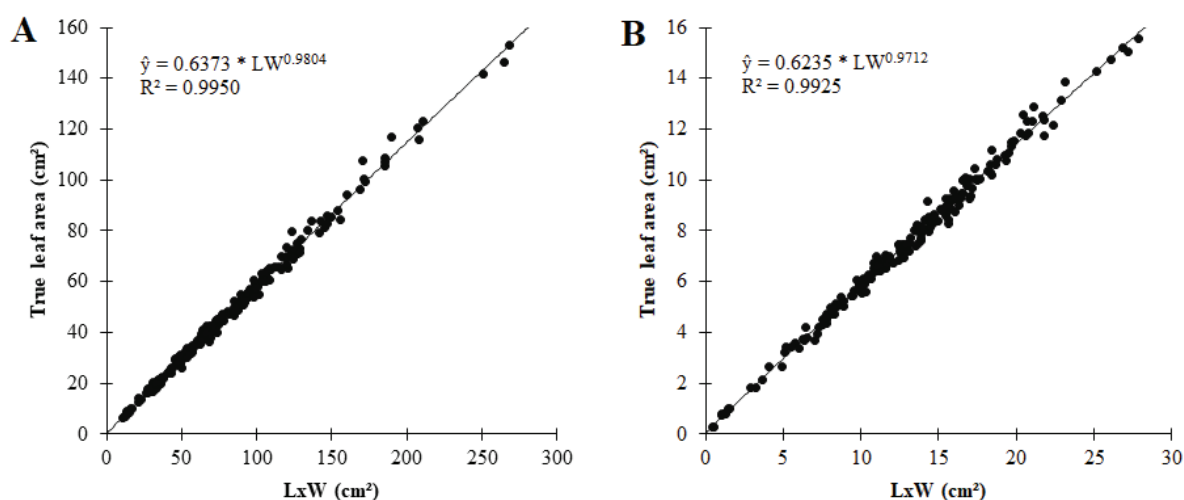


Figure 4. Variation in true leaf area of (A) *Psychotria carthagenensis* and (B) *Psychotria hoffmannseggiana*, as a function of the product of leaf blade length and width (LxW) using the models that were found to best estimate leaf area.

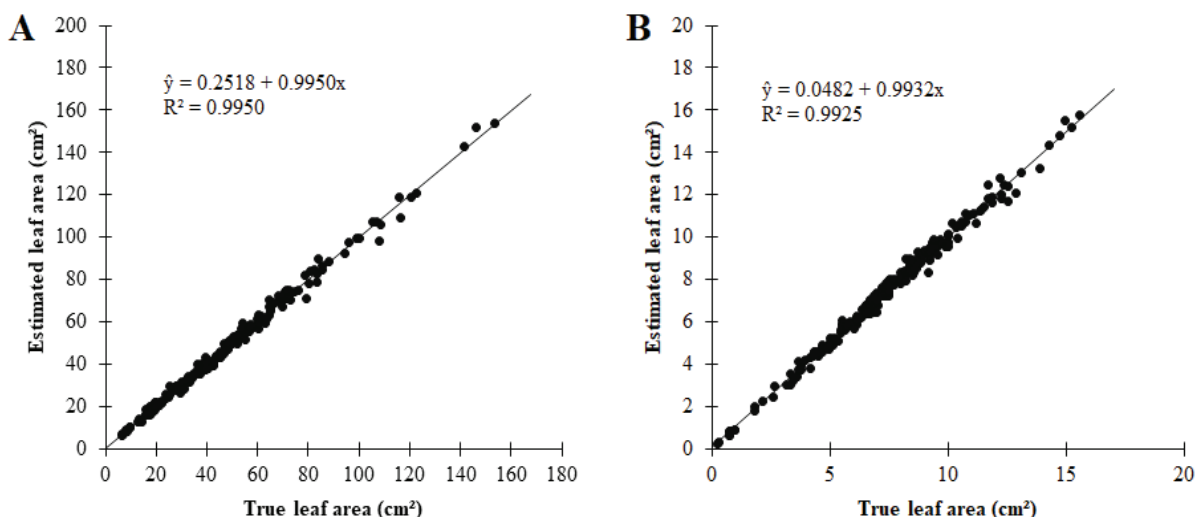


Figure 5. Variation of true leaf area and leaf area estimated by the models proposed for (A) *P. carthagenensis* $\hat{y} = 0.6373 * LW^{0.9804}$ and (B) *P. hoffmannseggiana* $\hat{y} = 0.6235 * LW^{0.9712}$.

Conclusion

Leaf area of *P. carthagenensis* and *P. hoffmannseggiana* can be satisfactory estimated by a non-destructive method that uses linear measurements of leaf blades.

The models found, which incorporate the product of length and width (LxW) sufficiently estimated leaf area for *P. carthagenensis* and *P. hoffmannseggiana*.

The most suitable model for estimating leaf area of *P. carthagenensis* was $\hat{y} = 0.6373 * LW^{0.9804}$, while that for *P. hoffmannseggiana* was $\hat{y} = 0.6235 * LW^{0.9712}$.

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