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Allometric equations for estimating the leaf area of *Thespesia populnea* by linear dimensions of leaf blades

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ABSTRACT – *Thespesia populnea* (L.) Sol. ex Corrêa is a tree species used for medicinal and ornamental purposes in different regions of the world. The determination of the leaf area is importance in studies related to the growth, physiology, and reproduction of the species. Therefore, the objective of the research was to obtain an allometric equation to estimate the leaf area of *T. populnea* using linear dimensions of the leaf blades. 300 leaf blades were collected in fragments of restinga forest, located in the municipality of Canguaretama, state of Rio Grande do Norte, Brazil. The models used were: linear, linear without intercept, quadratic, cubic, power and exponential. The choice of the best equation was based on the determination coefficient (R^2), Pearson's linear correlation coefficient (r), Willmott's agreement index (*d*), CS index (CS), Akaike information criterion (AIC), error mean absolute (MAE) and root of the mean square error (RMSE). The equations obtained with the product between length and width (LW) can estimate the leaf area of *T. populnea*, however the equation $\hat{y} = 0.69 * LW^{1.01}$ using the power model was the most indicated to accurately estimate the leaf area of the species (R^2=0.9962; r=0.9953; *d*=0.997; CS=0.993; AIC=1198.1; MAE=1.363; RMSE=2.107).

Keywords: biometry, Malvaceae, non-destructive method, regression models.

RESUMO – **Equações alométricas para estimativa da área foliar de *Thespesia populnea* a partir de dimensões lineares dos limbos foliares**. *Thespesia populnea* (L.) Sol. ex Corrêa é uma espécie arbórea utilizada para fins medicinais e ornamentais em diversas regiões do mundo. A determinação da área foliar é importante para estudos relacionados ao crescimento, fisiologia e reprodução das espécies. Diante disso, o objetivo da pesquisa foi obter uma equação alométrica para estimativa da área foliar de *T. populnea* utilizando dimensões lineares dos limbos foliares. Foram coletados 300 limbos foliares em fragmentos de floresta de restinga, localizados no município de Canguaretama, estado do Rio Grande do Norte, Brasil. Os modelos utilizados foram: linear, linear sem intercepto, quadrático, cúbico, potência e exponencial. A escolha da melhor equação foi baseada no coeficiente de determinação (R^2), coeficiente de correlação linear de Pearson (r), índice de concordância de Willmott (*d*), índice CS (CS), critério de informação de Akaike (AIC), erro absoluto médio (MAE) e raiz do erro quadrático médio (RMSE). As equações obtidas com o produto entre comprimento e largura (LW) podem estimar a área foliar de *T. populnea*, porém a equação $\hat{y} = 0{,}69*LW^{1{,}01}$ utilizando o modelo potência foi a mais indicada para estimar com precisão a área foliar da espécie (R^2=0,9962; r=0,9953; *d*=0,997; CS=0,993; AIC=1198,1; MAE=1,363; RMSE=2,107).

Palavras-chave: biometria, Malvaceae, método não destrutivo, modelos de regressão.

INTRODUCTION

Species native to tropical Asia, *Thespesia populnea* (L.) Sol. ex Corrêa is an arboreal species (5 to 12 m high) belonging to the Malvaceae family and popularly known as cotton-of-the-beach, cotton-of-the-coast, and cotton-of-the-para. It is cultivated in various parts of the world and widely distributed in tropical and subtropical regions, mainly in coastal areas worldwide, especially near mangroves (Santos & Fabricante 2018). Despite being an exotic species, in Brazil, it is also found in mixed settlements, being widely used for landscape purposes, such as the ornamentation of parks, avenues, and squares (Santos *et al*. 2018), in addition to being considered one of the potential sources for pulp production (Kathirselvam *et al*. 2019). It is an essential plant for medicinal use with several pharmacological properties. It can be used as an anti-inflammatory, analgesic, antipyretic, antidiarrheal, hepatoprotective, antioxidant, and wound healing, in addition to having antiproliferative activity against cancer (Nirmal *et al*. 2015, Lindamulage & Soysa 2016, Hiteksha & Mamta 2017, Savithramma *et al*. 2017, Rangani *et al*. 2018).

Given the importance of *T. populnea*, ecophysiological studies are needed to evaluate the species' growth, development, and reproduction. Among the studies, the determination of leaf area is one of the most critical analyses for plants, being essential for estimating growth and physiological parameters such as leaf area index, net

CO_2 assimilation rate, specific leaf area, and leaf area ratio, as well as being used to evaluate plants affected by biotic and abiotic factors (Taiz *et al*. 2017).

Currently, leaf area is determined from methods classified as direct or indirect, destructive and non-destructive (Toebe *et al*. 2019). The destructive methods allow more straightforward evaluations but require more time and labor in the analyses, in addition to presenting disadvantages such as the impracticability of research on plants with a limited number of leaves, such as endangered species or in early stages of growth, which can cause irreversible damage and death of plants (Mota *et al*. 2014). On the other hand, indirect and non-destructive methods present greater speed and accuracy in plant evaluations under adverse environmental conditions, allowing successive measurements in a given space-time from the use of regression equations, using the linear dimensions of leaf limbs, including width and length, or a relationship between these variables, without destroying samples (Ribeiro *et al*. 2018, Cemek *et al*. 2020).

Regression models to estimate leaf area from linear leaf dimensions were used in other studies with tree species, such as *Schinus terebinthifolius* Raddi ($LA = -2.6646 + 2.2124 * W + 1.3953 * W^2$) (Oliveira *et al*. 2019a), *Bertholletia excelsa* Bonpl. [$LA = (0.8743 * LW^{0.9790}) -1.84$] (Bouvié *et al*. 2020), *Moringa oleifera* Lam. ($LA = 0.035 + 0.720 * LW$) (Macário *et al*. 2020), *Ceiba glaziovii* (Kuntze) K. Schum. ($LA = 0.4549 * LW$) (Ribeiro *et al*. 2020a), and *Tabebuia impetiginosa* Mart. ($LA = 8.7772 + 2.3840 * LW$) (Santos *et al*. 2020). Therefore, the objective of the research was to obtain an allometric equation to estimate the leaf area of *T. populnea* using linear dimensions of leaf limbs.

MATERIAL AND METHODS

The study was carried out in fragments of restinga forest located in the municipality of Canguaretama, in the microregion of the South Coast of Potiguar and mesoregion of the East Potiguar, of the state of Rio Grande do Norte, in the Northeast region of Brazil (6°22′54.77″S and 35°8’18.05”W) (Fig. 1). The region has an altitude of 5m, average air temperature around 26°C and annual rainfall of approximately 1,360 mm (Climate-Data 2021). The region's climate is classified as As, being hot and humid tropical, with autumn-winter rains (Alvares *et al*. 2013).

In the forest fragments, 300 leaf limbs were collected randomly, of different sizes and shapes, in adult trees of *T. populnea*, considering the expanded leaves, free of direct and indirect damage and without the influence of abiotic factors. The leaves were packed in plastic bags and later allocated in thermal containers with the addition of ice to mitigate water loss by transpiration. Then, the collected material was transferred and sent to the Plant Ecology Laboratory, belonging to the Federal University of Paraíba, Campus II, Areia, Paraíba, Brazil.

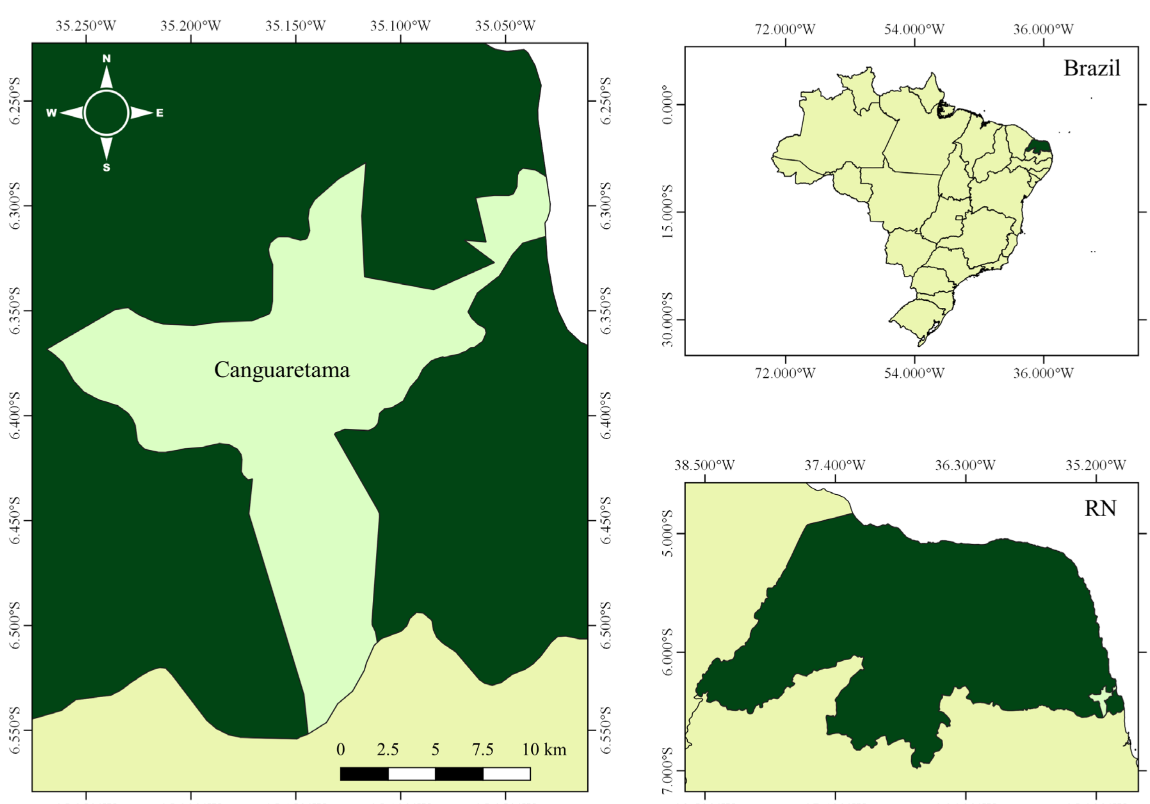

Figure 1. Geographical location of the municipality of Canguaretama, state of Rio Grande do Norte, Northeastern Brazil.

For each leaf, the maximum length (L) and maximum width (W) (Fig. 2) were calculated using a ruler graduated in millimeters. Then the following products were calculated between the parameters: maximum length x maximum width (LW), maximum length x maximum length (LL), and maximum width x maximum width (WW). The determination of the observed leaf area (LA) for each leaf was performed from the digitization of the leaves with a flatbed scanner, in which later the images were contrasted and analyzed with the aid of the ImageJ® (*Powerful Image Analysis*) software (Ribeiro *et al*. 2018).

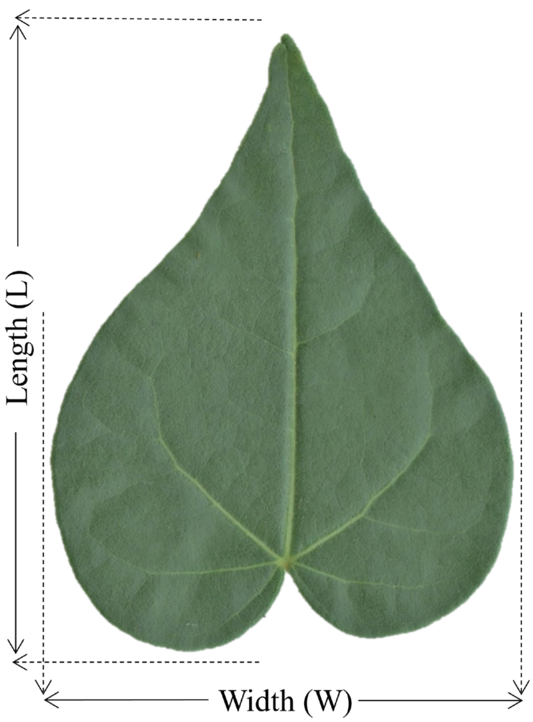

Figure 2. Linear leaf dimensions [maximum length (L) and maximum width (W)] used to estimate the leaf area of *Thespesia populnea*.

From the data of L, W, LW, LL, WW, and LA of the 300 leaf limbs, a descriptive analysis of the data was performed, being determined the minimum, maximum and mean values; amplitude, median, variance, standard deviation, standard error, coefficient of variation (CV), asymmetry and kurtosis for each parameter evaluated. The Shapiro-Wilk test to verify the normality of the data was used (Shapiro & Wilk 1965).

Estimates of regression equations were performed using the following statistical models: simple linear, simple linear without intercept (0.0), quadratic, cubic, power, and exponential, whose description of each model is presented in Table 1.

Table 1. Regression models used to estimate leaf area of *Thespesia populnea*.

Model	Equation
Linear	$\hat{y} = \beta_0 + \beta_1 * \chi + \varepsilon_i$
Linear (0.0)	$\hat{y} = \beta_1 * \chi + \varepsilon_i$
Quadratic	$\hat{y} = \beta_0 + \beta_1 * \chi + \beta_2 * \chi^2 + \varepsilon_i$
Cubic	$\hat{y} = \beta_0 + \beta_1 * \chi + \beta_2 * \chi^2 + \beta_3 * \chi^3 + \varepsilon_i$
Power	$\hat{y} = \beta_0 * \chi^{\beta_1} + \varepsilon_i$
Exponential	$\hat{y} = \beta_0 * {\beta_1}^{\chi} + \varepsilon_i$

$\hat{y}$: leaf area; χ: linear dimensions.

The best equation to accurately estimate the leaf area of *T. populnea* was chosen from the highest coefficient of determination (R^2), Pearson's linear correlation coefficient (r), Willmott agreement index (*d*) (equation 1) and CS index (CS) (equation 2); and lower Akaike information criterion (AIC) (equation 3), mean absolute error (MAE) (equation 4) and root mean square error (RMSE) (equation 5) (Akaike 1974, Willmott *et al.*1981, Janssen & Heuberger 1995, Camargo & Sentelhas 1997). Statistical data analyses were performed with the software R® v.4.0.0 (R Core Team 2020).

$$d = 1 - \frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{\sum_{i=1}^{n} (|\hat{y}'_i| + |y'_i|)^2} \quad (1)$$

$$CS = r \times d \quad (2)$$

$$AIC = -2 \ln L(x \backslash \hat{\theta}) + 2(p) \quad (3)$$

$$MAE = \frac{\sum_{i=1}^{n} |\hat{y}i - yi|}{n} \quad (4)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}i - yi)^2}{n}} \quad (5)$$

were: is the estimated leaf area; is the observed leaf area; is the mean of the observed values; ; is the maximum likelihood function, defined as the product of the density function; is the number of parameters in the template; is the number of observations.

RESULTS

The length (L) of the 300 leaf limbs of *T. populnea* ranged from 0.763 to 11.822 cm, with an average of 5.788 ± 2.632 cm, with an amplitude of 11.059 cm; the width (W) varied between 0.282 and 10.832 cm, with a mean value of 5.331 ± 2.612 cm and 10.550 cm of amplitude; the product between length and width (LW) presented values ranging from 0.215 to 139.760 cm², with an average of 37.412 ± 30.085 cm² and total amplitude of 116.279 cm²; the product between length and length (LL) presented values ranging from 0.582 to 139.760 cm², mean of 35.210 ± 29.115 cm² and 139.178 cm² of total amplitude; the values of the product between width and width (WW) ranged from 0.080 to 117.332 cm², with an average of 35.210 ± 29.115 cm² and amplitude of 117.252 cm². The observed leaf area (LA) presented values ranging from 0.144 to 81.744 cm², a mean of 26.806 ± 21.886 cm², and a total amplitude of 81.600 cm² (Table 2).

Regarding the variability of data from 300 leaf limbs, the lowest coefficients of variation (CV) were recorded for length and width, with 45.47 and 48.99%, respectively (Table 2). The coefficients of variation of the products between length and width (80.41%), length and length (79.61%), width and width (82.69%), and observed leaf area (81.65%) were approximately twice as high as the CV of L and W (Table 2).

The kurtosis coefficients (k) of each leaf parameter were lower than the normal distribution ($k < 3$), indicating a platykurtic distribution. The Shapiro-Wilk normality test showed that the data analyzed did not fit a normal distribution (Table 2). This departure from the normal distribution in all parameters (L, W, LW, LL, WW, and LA) occurred due to the deviations in the asymmetry and kurtosis coefficients (Table 2).

The scatterplots between length (L), width (W), the product between length and width (LW), the product between length and length (LL), the product between width and width (WW), and observed leaf area (LA), indicated patterns of data association, with adjustments of linear and nonlinear models (Fig. 3).

Regarding the percentage frequency distribution of the 300 leaf limbs of *T. populnea* about leaf area (LA) size classes, it was observed that 48% of the observed leaf area is within the range between 0.14 and 20.0 cm², indicating that the species present wide variability in leaf size (Table 3).

The allometric equations and regression models obtained from the relationship between the observed leaf area (ŷ) and the linear dimensions of the leaf limbs () (L, W, LW, LL, WW, and LA) are presented in Table 4. The equation obtained with the power model using the product between length and width (LW) [$\hat{y} = 0.69 * LW^{1.01}$] presented the best criteria to estimate the leaf area of *T. populnea* from linear leaf dimensions, obtaining the highest values of the coefficient of determination ($R^2 = 0.9962$), Pearson's linear correlation coefficient ($r = 0.9953$), Willmott agreement index ($d = 0.997$) and CS index (CS = 0.993), and lower values of the Akaike information criterion (AIC = 1198.1), absolute mean error (MAE = 1.363) and root of the mean square error (RMSE = 2.107) (Table 4). Regarding the model's fit, little data dispersion was observed, confirming that the equation $\hat{y} = 0.69 * LW^{1.01}$ can satisfactorily estimate the leaf area of *T. populnea* (Fig.4).

The estimation of the leaf area of *T. populnea* from the proposed equation showed a satisfactory relationship with the observed leaf area, in which the coefficient of determination (R^2) was 0.9906 (Fig. 5).

Table 2. Descriptive analysis of length (L), width (W), the product between length and width (LW), the product between length and length (LL), the product between width and width (WW), and observed leaf area (LA) of 300 leaf limbs of *Thespesia populnea*.

Descriptive statistics	L	W	LW	LL	WW	LA
Minimum	0.763	0.282	0.215	0.582	0.080	0.144
Maximum	11.822	10.832	116.494	139.760	117.332	81.744
Mean	5.788	5.331	37.412	40.395	35.210	26.806
Amplitude	11.059	10.550	116.279	139.178	117.252	81.600
Median	5.550	5.265	29.192	30.803	27.720	20.599
Variance	6.925	6.820	905.078	1034.200	847.703	478.975
Standard deviation	2.632	2.612	30.085	32.159	29.115	21.886
Standard error	0.159	0.158	1.814	1.939	1.756	1.320
CV	45.47	48.99	80.41	79.61	82.69	81.65
Asymmetry	0.127	0.054	0.767	0.812	0.820	0.781
Kurtosis $^{+3}$	2.119	2.134	2.570	2.743	2.757	2.589
Shapiro-Wilk	0.0002**	0.0003**	<0.0001**	<0.0001**	<0.0001**	<0.0001**

** Significant at 1% probability.

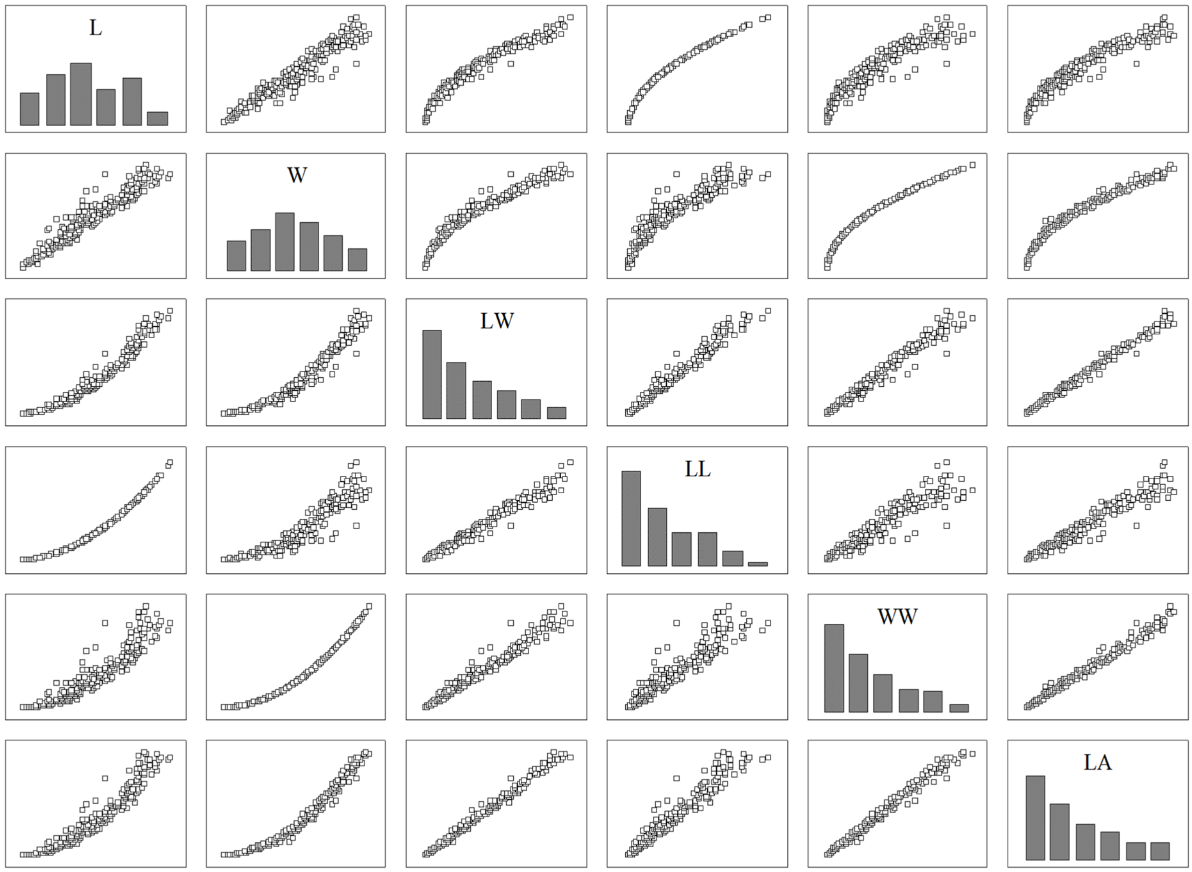

Figure 3. Histograms and scatter plots between length (L), width (W), the product between length and width (LW), the product between length and length (LL), the product between width and width (WW), and observed leaf area (LA) of 300 leaf limbs of *Thespesia populnea*.

Table 3. Size classes of the observed leaf area of 300 leaf limbs of *Thespesia populnea*.

Size classes LA (cm²)	%
[0.14 - 10.01]	26.91
[10.01 - 20.0]	21.09
[20.01 - 30.0]	15.27
[30.01 - 40.0]	9.82
[40.01 - 50.0]	9.82
[50.01 - 60.0]	6.18
[60.01 - 70.0]	5.82
[70.01 - 82.0]	5.09

DISCUSSION

The high values of total amplitude, standard deviation, standard error, and coefficient of variation show a more significant variability of leaf data, being of fundamental importance for studies related to the use of regression equations for estimating the leaf area of species. The data's high variability allows the use of regression models on leaves of different shapes and sizes at different stages of plant development (Pezzini *et al*. 2018). Thus, the present study's sample size (number of leaves = 300) is adequate for estimating the leaf area of *T. populnea* using linear dimensions of the leaf limbs. Other research has also recorded greater variability for the product between length and width (Gomes *et al*. 2020), the product between length and length (Ribeiro *et al*. 2020b), the product between width and width (Oliveira *et al*. 2019b), and observed leaf area (Donato *et al*. 2020). The kurtosis coefficients of the variables of the present study showed that the frequency distribution was flatter than the normal distribution (Ribeiro *et al*. 2020a).

In the present study, the deviations observed in the values of the asymmetry and kurtosis coefficients were of fundamental importance for the lower adequacy of the leaf data to the normal distribution (Ribeiro *et al*. 2020b). Such behavior, associated with the adjustments of linear and nonlinear models for the variables analyzed, has been proven by studies by other researchers (Cargnelutti Filho *et al*. 2015, Carvalho *et al*. 2017).

Table 4. Models, equations, coefficient of determination (R^2), Pearson's linear correlation coefficient (r), Willmott agreement index (d), CS index (CS), Akaike information criterion (AIC), mean absolute error (MAE) and root mean square error (RMSE) of 300 leaf limbs of Thespesia populnea.

Model		R^2	r	d	CS	AIC	MAE	RMSE	Equation
Linear	L	0.9025	0.9502	0.973	0.925	1841.5	5.423	6.810	$\hat{y} = -18.93 + 7.90*L$
Linear	W	0.9308	0.9649	0.981	0.947	1747.1	4.733	5.736	$\hat{y} = -16.29 + 8.08*W$
Linear	LW	0.9906	0.9953	0.997	0.993	1198.6	1.374	2.116	$\hat{y} = -0.28 + 0.72*LW$
Linear (0.0)	LW	0.9906	0.9953	0.997	0.992	1200.2	1.395	2.122	$\hat{y} = 0.72*LW$
Linear	LL	0.9398	0.9696	0.984	0.954	1708.6	3.617	5.348	$\hat{y} = 0.15 + 0.66*LL$
Linear	WW	0.9868	0.9934	0.996	0.990	1292.6	1.639	2.510	$\hat{y} = 0.51 + 0.75*WW$
Quadratic	L	0.9402	0.9699	0.984	0.954	1707.9	3.616	5.321	$\hat{y} = -2.17 + 0.90*L + 0.59*L^2$
Quadratic	W	0.9868	0.9934	0.996	0.990	1292.3	1.629	2.500	$\hat{y} = -0.28 + 0.35*W + 0.72*W^2$
Quadratic	LW	0.9906	0.9953	0.997	0.993	1200.2	1.369	2.114	$\hat{y} = -0.29 + 0.72*LW - 0.000007*LW^2$
Quadratic	LL	0.9411	0.9703	0.984	0.955	1703.8	3.590	5.282	$\hat{y} = -1.14 + 0.74*LL - 0.0007*LL^2$
Quadratic	WW	0.9871	0.9936	0.996	0.990	1286.2	1.631	2.472	$\hat{y} = -0.09 + 0.79*WW - 0.0004*WW^2$
Cubic	L	0.9410	0.9704	0.984	0.955	1705.2	3.579	5.276	$\hat{y} = 2.02 - 2.17*L + 1.19*L^2 - 0.03*L^3$
Cubic	W	0.9872	0.9953	0.996	0.992	1285.9	1.645	2.462	$\hat{y} = 1.63 - 1.38*W + 1.10*W^2 - 0.02*W^3$
Cubic	LW	0.9878	0.9936	0.997	0.990	1198.2	1.369	2.114	$\hat{y} = 0.006 + 0.68*LW + 0.001*LW^2 - 0.000006*LW^3$
Cubic	LL	0.9425	0.9712	0.985	0.956	1698.2	3.554	5.210	$\hat{y} = 0.38 + 0.57*LL + 0.002*LL^2 - 0.00002*LL^3$
Cubic	WW	0.9906	0.9939	0.997	0.991	1272.1	1.583	2.401	$\hat{y} = 0.77 + 0.66*WW + 0.002*WW^2 - 0.00002*WW^3$
Power	L	0.9405	0.9698	0.984	0.954	1707.0	3.640	5.333	$\hat{y} = 0.74 * L^{1.95}$
Power	W	0.9870	0.9935	0.996	0.990	1288.9	1.622	2.493	$\hat{y} = 0.85 * W^{1.94}$
Power	LW	0.9962	0.9953	0.997	0.993	1198.1	1.363	2.107	$\hat{y} = 0.69 * LW^{1.01}$
Power	LL	0.9405	0.9698	0.984	0.954	1707.1	3.640	5.333	$\hat{y} = 0.74 * LL^{0.97}$
Power	WW	0.9870	0.9935	0.996	0.990	1288.9	1.622	2.493	$\hat{y} = 0.85 * WW^{0.97}$
Exponential	L	0.9116	0.9548	0.973	0.929	1829.2	5.049	6.660	$\hat{y} = 4.80 * 1.30^{L}$
Exponential	W	0.9632	0.9814	0.989	0.970	1601.9	3.576	4.405	$\hat{y} = 4.82 * 1.32^{W}$
Exponential	LW	0.9632	0.9814	0.989	0.970	1838.9	3.576	4.405	$\hat{y} = 11.36 * 1.02^{LW}$
Exponential	LL	0.9107	0.9543	0.972	0.927	2002.3	5.630	6.778	$\hat{y} = 12.40 * 1.02^{LL}$
Exponential	WW	0.8361	0.9144	0.945	0.864	1890.5	7.118	9.122	$\hat{y} = 12.09 * 1.02^{WW}$

χ: linear dimensions.

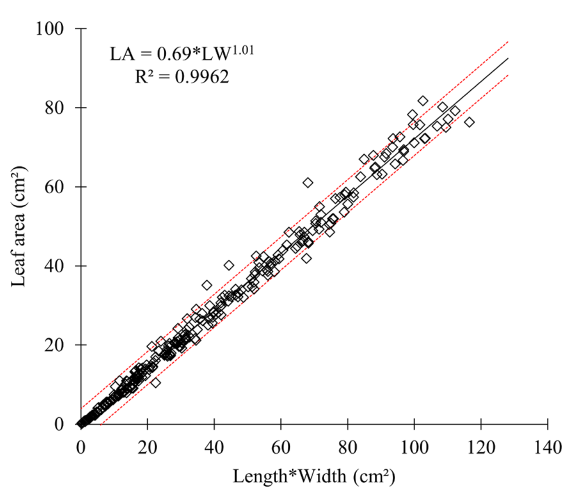

Figure 4. Relationship between the observed leaf area (LA) and the product between length and width (LW) of the leaf limbs of *Thespesia populnea*, using the equation $\hat{y} = 0.69 * LW^{1.01}$.

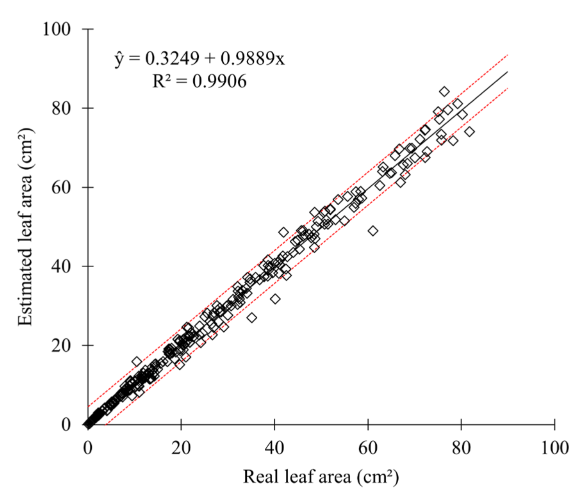

Figure 5. Relationship between observed leaf area and leaf area estimated by equation $\hat{y} = 0.69 * LW^{1.01}$.

The determination coefficients (R^2) of the equations obtained were higher than 0.83, indicating that at least 83% of the variations observed in the leaf areas of *T. populnea* were explained by the models constructed using linear dimensions of the leaf limbs. The allometric equations that were obtained from the product between length and width (LW), compared to those that employed L or W, provided the greatest adjustments to the regression models (Guimarães *et al*. 2019, Goergen *et al*. 2021, Lucena *et al*. 2021, Cargnelutti Filho *et al*. 2021, Toebe *et al*. 2021), as well as the best criteria to satisfactorily estimate the leaf area of *T. populnea*, except for the cubic model, in which the best equation was obtained using the product between length and length of leaf limbs (LL).

The power model was also indicated to accurately estimate the leaf area of other species, such as *Erythroxylum citrifolium* A.St.-Hil. [$LA = 0.5966 * (LW)^{1.0181}$] (Ribeiro *et al*. 2019a), *Palicourea racemosa* (Aubl.) Borhidi [$LA = 0.609 * (LW)^{0.995}$] (Ribeiro *et al*. 2020c), *Psychotria carthagenensis* Jacq. [$LA = 0.6373 * (LW)^{0.9804}$], and *Psychotria hoffmannseggiana* (Willd. ex Schult.) Müll. Arg. [$LA = 0.6235 * (LW)^{0.9712}$] (Ribeiro *et al*. 2019b).

Therefore, using an equation to estimate the leaf area of *T. populnea* from the product between the linear dimensions of the leaves (length and width) is a non-destructive method as efficient as the destructive methods. In addition, the method proposed in the present study presents greater simplicity and speed in the measurements in plants submitted to adverse field conditions or for research with low potential for technological resources.

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