Estimating leaf area of basil cultivars through linear dimensions of leaves

João Everthon da Silva Ribeiro[1], Jackson Silva Nóbrega[2], Ester dos Santos Coêlho[3], Thiago Jardelino Dias[2], Marlenildo Ferreira Melo[3]*

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ABSTRACT

Ocimum basilicum L. (basil) is an annual herb belonging to the Lamiaceae family that has economic importance for many regions around the world. Thus, ecophysiological studies are needed to assess this species growth and dispersal. This work aimed to obtain equations from regression models that meaningfully estimate the leaf area of basil cultivars using linear dimensions of leaves. For this purpose, 300 leaves from 'Italiano Roxo' and 500 leaves from 'Folha Fina' cultivar were collected from plants cultivated in a greenhouse. Then, the length, width, and leaf area of each leaf were measured, and product of length by width were calculated. The equations were adjusted using the simple linear, linear without intercept, quadratic, cubic, power, and exponential regression models. Criteria for selecting the best equations were highest determination coefficient and Willmott's agreement index, lowest Akaike information criterion and root mean square error, and BIAS index closest to zero. All the equations fitted using the product of length by width (L.W) can estimate the leaf area of basil cultivars. Thus, basil leaf area can be estimated through a non-destructive method using linear dimensions of leaves. However, the equation $w = 0.8175*LW^{0.9307}$ is the most suitable for 'Italiano Roxo' and $w = 0.6335*LW$ for 'Folha Fina'.

Keywords: *Ocimum basilicum*; biometry; allometric equations; Lamiaceae; leaf blade.

INTRODUCTION

Ocimum basilicum L., popularly known as basil, and as *manjericão*, *alfavaca*, *basílico* and *alfavaca-cheirosa* in Brazil, is an annual herb belonging to the Lamiaceae family. Reaching 30 to 60 cm in height under ideal environmental conditions (Minami *et al*., 2007), the plant is native to India and other Asian regions and cultivated in several countries, where the raw material is used to produce essential oils rich in linalool (Pinheiro *et al*., 2017). In addition to having biological properties, such as antibacterial and insecticide, the essential oil is used as flavoring and condiment in the food and pharmaceutical industry (Luz *et al*., 2009; Machado *et al*., 2012). Also, basil is used in all countries for culinary, medicinal, ornamental, and aromatic purposes (Hussain *et al*., 2008). In traditional medicine, basil leaves are freshly consumed or after infusion as analgesic, soothing, expectorant, invigorating, sedative, and tonic (Ribeiro *et al*., 2014; Sakurai *et al*., 2016).

Many basil varieties have been exploited commercially for presenting desirable characteristics, such as high essential oil content and greater biomass production. 'Italiano Roxo' (*Ocimum basilicum* var. *purpurascens* Benth.) is a basil cultivar that grows to 50 and 60 cm in height, and produces greenish-purple leaves, long inflorescences, and erect stem (Kamada *et al*., 1999). 'Folha Fina' (*Ocimum basilicum* var. *minimum* L.) has an erect and branched stem, grows to 40 and 50 cm in height, and produces short and white inflorescences (Matos, 2002).

Given the importance of this species, studies on its growth, physiology, development, reproduction, and propagation are of great interest. Leaf area measurement

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[1] Instituto Nacional do Semiárido, Campina Grande, Paraíba, Brazil. j.everthon@hotmail.com
[2] Universidade Federal da Paraíba, Areia, Paraíba, Brazil. jacksonnobrega@hotmail.com; thiagojardelinodias@gmail.com
[3] Universidade Federal Rural do Semi-Árido, Mossoró, Rio Grande do Norte, Brazil. estersantos12@hotmail.com; marlenildo-melo@hotmail.com
*Corresponding author: j.everthon@hotmail.com

is of fundamental importance because leaves are responsible for multiple functions in plants, such as light interception and absorption for photosynthetic processes, gas exchange, and stomatal opening, thus directly affecting the plant biomass production (Spann & Heerema, 2010; Taiz *et al*., 2017).

Leaf area can be determined by different methods, classified according to Marshall (1968) as direct and indirect, or destructive and non-destructive. Direct methods (destructive or not) are simple to measure but cost time and labor, in addition to being unfeasible for endangered species and plants in the early stages of development, and because it requires plants to be destroyed (Mota *et al*., 2014). On the other hand, indirect (non-destructive) methods allow quick and accurate evaluations, permitting successive measurements on the same plant, based on regression models using leaf dimensions (length and width), without destroying the sample (Pompelli *et al*., 2012; Sousa & Amaral, 2015; Ribeiro *et al*., 2019a).

Regression models for estimating leaf area have been used by several authors in other species, such as *Capsicum annuum L*. (Padrón *et al*., 2016), *Smallanthus sonchifolius* (Poepp.) H. Rob. (Erlacher *et al*., 2016), *Salvia hispanica* L. (Mack *et al*., 2017), *Theobroma cacao* L. (Salazar *et al*., 2018), *Erythroxylum citrifolium* A.St.-Hil. (Ribeiro *et al*., 2019a), *Psychotria carthagenensis* Jacq. and *Psychotria hoffmannseggiana* (Willd. ex Schult.) Müll.Arg. (Ribeiro *et al*., 2019b), *Erythroxylum simonis* Plowman (Ribeiro *et al*., 2018), *Mesosphaerum suaveolens* (L.) Kuntze (Ribeiro *et al*., 2020a), and *Erythroxylum pauferrense* Plowman (Ribeiro *et al*., 2020b). Therefore, this work aimed to obtain equations from regression models that meaningfully estimate leaf area of basil cultivars (‘Italiano Roxo’ and ‘Folha Fina’) through linear dimensions of leaves.

MATERIAL AND METHODS

The experiment was carried out under greenhouse at the Center for Agrarian Sciences, Department of Phytotechnics and Environmental Sciences, Federal University of Paraíba, Campus II, Areia city, Paraíba state, Brazil (6°58’1.3" S, 35°42’49.09" O, 400 to 600 m altitude), where the climate is As type, hot and humid with autumn-winter rains (Alvares *et al*., 2013). During the experimental period, the average temperature was 28.4 °C and relative humidity was 54.8%, which were monitored using a digital thermo-hygrometer (MT-241A, Minipa).

Basil seeds from ‘Italiano Roxo’ and ‘Folha Fina’ cultivars were purchased at the local market. Then, seedlings were produced in polyethylene bags with 1.3 dm^3 capacity filled with a substrate composed of latosol, washed sand, and tanned cattle manure at the 3:1:1 ratio (Table 1).

At 55 days after planting, beginning of flowering, 300 leaves from ‘Italiano Roxo’ and 500 leaves from ‘Folha Fina’, of different sizes and shapes, were randomly collected. Only healthy leaves without injuries caused by pests, diseases, and other factors were selected. Then, the leaves were packed in plastic bags and transported to Plant Ecology Laboratory, at Federal University of Paraíba, Campus II. At the laboratory, the maximum length (L, cm) and width (W, cm) (Figure 1) of each leaf were measured using a millimetric ruler. Then, the product of length by width (L.W, cm^2) was calculated. Also, the real leaf area (LA, cm^2) was determined by digital photocopies obtained using a scanner (P-215II, Canon), and the images were analyzed in ImageJ® v.1.51j8 (Powerful Image Analysis) software.

A descriptive analysis was performed to determine the minimum, maximum, mean, amplitude, median, variance, standard deviation, standard error, and coefficient of variation of L, W, LW, and LA. Then, equations for estimating the leaf area were adjusted using the simple linear, linear without intercept (0.0), quadratic, cubic, power, and exponential regression models (Table 2). Subsequently, the equations that meaningfully estimated leaf area of the basil cultivars were selected by checking the highest determination coefficient (R^2) and Willmott’s agreement index (*d*) (Willmott, 1981) (Equation 1), lowest Akaike information criterion (AIC) (Akaike, 1974) (Equation 2) and root mean square error (RMSE) (Janssen & Heuberger, 1995) (Equation 3), and BIAS index closest to zero (Leite & Andrade, 2002) (Equation 4). Statistical analyzes were performed in R® v.4.0.0 software (R Core Team, 2020), using the package *hydroGOF* (Zambrano-Bigiarini, 2020).

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

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

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \quad (3)$$

$$BIAS = \frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{n}(y_i)} \quad (4)$$

Where $\hat{y}_i$ is the estimated leaf area, y_i is the observed leaf area, $\bar{y}_i$ is the mean of the observed values ($\hat{y}'_i = \hat{y}_i - \bar{y}$; $y'_i = y_i$; $\bar{y}_i$), $L(x\backslash\hat{\theta})$ is the maximum likelihood function that is defined as the product of the density function, *p* is the number of coefficients in the equation, and *n* is the number of observations.

RESULTS AND DISCUSSION

The length (L) of the ‘Italiano Roxo’ (IR) leaves varied from 1.100 to 10.738 cm, with 4.133 cm on average and 9.638 cm amplitude, while width (W) varied from 0.493 to

6.066 cm, with 2.391 cm on average and 5.573 cm amplitude. The product of length by width (L.W) ranged from 0.634 to 65.137 cm², with 12.229 cm² on average and 64.503 cm² amplitude, and real leaf area (LA) ranged from 0.256 to 37.933 cm², with 8.179 cm² on average and 37.677 cm² amplitude (Table 3). In turn, the length of ‘Folha Fina’ (FF) leaves differed from 0.403 to 3.495 cm, 1.663 cm on average and 3.092 cm amplitude, whereas width (W) ranged between 0.180 and 2.294 cm, 0.901 cm on average and 2.114 cm amplitude. The product length by width (L.W) varied from 0.079 to 7.889 cm², 1.774 cm² on average and 7.810 cm² amplitude. Finally, real leaf area (LA) was from 0.063 to 4.640 cm², 1.154 cm² on average and 4.577 cm² amplitude (Table 3).

Regarding variability in basil leaf dimensions, the lowest coefficients of variation were those from length (46.1% for IR and 41.21% for FF) and width (54.8% for IR and 48.61% for FF), whereas the highest coefficients of variation were those from the product of length by width (97.4% for IR and 89.90% for FF) and real leaf area (91.5% for IR and 85.69% for FF) (Table 3). High values of amplitude, standard deviation, standard error, and coefficient of variation are of fundamental importance for studies aimed at estimating leaf area from regression models, allowing measurements on leaves of different sizes and plants on different phenological stages (Pezzini *et al*., 2018). Therefore, the number of leaves used in the present study was adequate for estimating the basil leaf area through linear dimensions of leaves. Other studies also reported high variability in product of length by width (LW) and real leaf area (LA) as compared to L and W values (Leite *et al*., 2017; Ribeiro *et al*., 2018; Ribeiro *et al*., 2020c; Ribeiro *et al*., 2020d).

Regarding leaf size classes, 32.3% of ‘Italiano Roxo’ leaf area (n = 300) was in the range of 0.25 and 3.00 cm², and 37.4% of ‘Folha Fina’ leaf area was in the range of 0.51 and 1.00 cm², which shows these cultivars have high leaf area variation (Figure 2).

The regression models and allometric equations obtained from the relationship between real leaf area (w) and linear dimensions of leaf blades (L, W, and L.W) are shown in Table 4. The determination coefficients (R^2) were greater than 0.87, indicating that at least 87% of the variation in leaf area was explained by the equations adjusted using linear dimensions of leaves (Table 4).

The equations adjusted using the product of leaf length by width (LW) showed satisfactory assumptions for estimating leaf area, best fitting all the regression models (Assis *et al*., 2015; Oliveira *et al*., 2017; Lucena *et*

Table 1: Chemical characterization of the substrate used in the experiment

pH	OM	P	K^+	Ca^{2+}	Mg^{2+}	Na^+	BS	$H^+ + Al^{3+}$	Al^{3+}	CEC
	$g\ kg^{-1}$	$mg\ kg^{-3}$		$cmol_c\ dm^{-3}$						
7.8	22.2	85.5	693.6	2.9	1.59	0.23	6.5	0.0	0.0	6.5

OM: organic matter; BS: base saturation; CEC: cation exchange capacity.

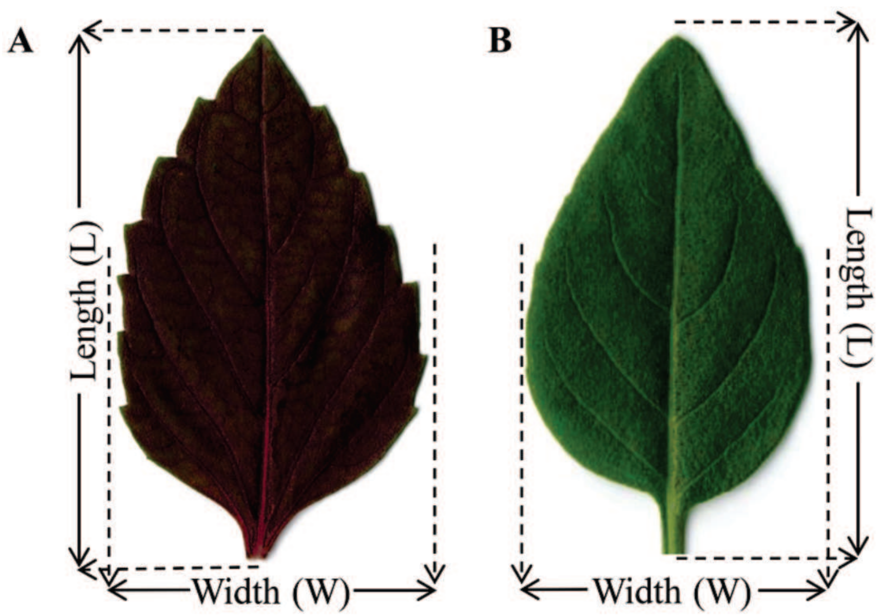

Figure 1: Linear dimensions [length (L) and width (W)] of leaves of ‘Italiano Roxo’ (A) and ‘Folha Fina’ (B) basil cultivars used to estimate leaf area.

al., 2018; Ribeiro *et al.*, 2018). Except for the equation adjusted using the exponential model, which showed best indexes when just using leaf width, as was also reported by Silva *et al.* (2017).

Following the criteria for selecting the equations that meaningfully estimated the leaf area of basil cultivars through linear dimensions of leaves, it was found that the power model and linear model without intercept, both fitted using the product of length by width, were the equations that meaningfully estimated the leaf area of 'Italiano Roxo' and 'Folha Fina' basil cultivars, respectively. These equations showed the highest R^2 (0.9945 and 0.9894) and *d* (0.9979 and 0.9942), lowest RMSE (0.695 and 0.150) and AIC (609.6 and 76.9), and BIAS index closest to zero (-0.0064 and 0.0301) (Table 4). Therefore, the equation $w = 0.8175*LW^{0.9307}$ is the most suitable for estimating the leaf

Table 2: Models and equations used to estimate leaf area of basil through linear dimensions of leaves

Model	Equation
Linear	$\hat{y} = \beta_0 + \beta_1 * L + \varepsilon_i$
Linear	$\hat{y} = \beta_0 + \beta_1 * W + \varepsilon_i$
Linear	$\hat{y} = \beta_0 + \beta_1 * LW + \varepsilon_i$
Linear without intercept (0.0)	$\hat{y} = \beta_1 * LW + \varepsilon_i$
Quadratic	$\hat{y} = \beta_0 + \beta_1 * L + \beta_2 * L^2 + \varepsilon_i$
Quadratic	$\hat{y} = \beta_0 + \beta_1 * W + \beta_2 * W^2 + \varepsilon_i$
Quadratic	$\hat{y} = \beta_0 + \beta_1 * LW + \beta_2 * LW^2 + \varepsilon_i$
Cubic	$\hat{y} = \beta_0 + \beta_1 * L + \beta_2 * L^2 + \beta_3 * L^3 + \varepsilon_i$
Cubic	$\hat{y} = \beta_0 + \beta_1 * W + \beta_2 * W^2 + \beta_3 * W^3 + \varepsilon_i$
Cubic	$\hat{y} = \beta_0 + \beta_1 * LW + \beta_2 * LW^2 + \beta_3 * LW^3 + \varepsilon_i$
Power	$\hat{y} = \beta_0 * L^{\beta 1} + \varepsilon_i$
Power	$\hat{y} = \beta_0 * W^{\beta 1} + \varepsilon_i$
Power	$\hat{y} = \beta_0 * LW^{\beta 1} + \varepsilon_i$
Exponential	$\hat{y} = \beta_0 * \beta_1^{L} + \varepsilon_i$
Exponential	$\hat{y} = \beta_0 * \beta_1^{W} + \varepsilon_i$
Exponential	$\hat{y} = \beta_0 * \beta_1^{LW} + \varepsilon_i$

Table 3: Minimum, maximum, mean, amplitude, median, variance, standard deviation, standard error, and coefficient of variation of length (L), width (W), product of length by width (L.W), and leaf area (LA) of leaf blades of 'Italiano Roxo' and 'Folha Fina' basil cultivars

Italiano Roxo (n = 300 leaf blades)				
Statistics	**L**	**W**	**L.W**	**LA**
Minimum	1.100	0.493	0.634	0.256
Maximum	10.738	6.066	65.137	37.933
Mean	4.133	2.391	12.229	8.179
Amplitude	9.638	5.573	64.503	37.677
Median	3.883	2.179	8.394	5.933
Variance	3.631	1.717	141.969	56.047
Standard deviation	1.906	1.310	11.915	7.486
Standard error	0.113	0.078	0.707	0.444
Coefficient of variation	46.10	54.80	97.40	91.50
Folha Fina (n = 500 leaf blades)				
Statistics	**L**	**W**	**L.W**	**LA**
Minimum	0.403	0.180	0.079	0.063
Maximum	3.495	2.294	7.889	4.640
Mean	1.663	0.901	1.774	1.154
Amplitude	3.092	2.114	7.810	4.577
Median	1.494	0.776	1.150	0.787
Variance	0.469	0.192	2.545	0.9778
Standard deviation	0.685	0.438	1.595	0.9888
Standard error	0.031	0.020	0.073	0.0451
Coefficient of variation	41.21	48.61	89.90	85.69

area of 'Italian Roxo', and the equation w = 0.6335*LW for 'Folha Fina' (Table 4).

Despite the linear patterns, the power regression model was the best adjustment for predicting 'Italiano Roxo' leaf area, which was also recommended for other species, such as *Vigna unguiculata* (L.) Walp. (Oliveira *et al*., 2015), *Theobroma cacao L*. (Schmildt *et al*., 2017), *Stizolobium cinereum* Piper & Tracy (Cargnelutti Filho *et al*., 2018), and *Manihot esculenta* Crantz (Guimarães *et al*., 2019). In turn, the linear model indicated to estimate 'Folha Fina' leaf area was also recommended for species such as *Moringa oleifera* Lamarck (Macário *et al*., 2020), *Allium cepa* L. (Córcoles *et al*., 2015), and *Commelina difusa* Burm.f. (Carvalho *et al*., 2017).

According to the proposed equations to estimate the basil cultivars leaf area, data showed low dispersion from the regression line in the scatterplot and residues were homogenously distributed, showing that variances were homogeneous, and residues were normally distributed (Figure 3A and B).

Leaf area estimated by the proposed equations was positively correlated with real leaf area, with determination coefficients (R^2) of 0.9913 and 0.9894, showing the high quality of the adjustments (Figure 4A and B). Therefore,

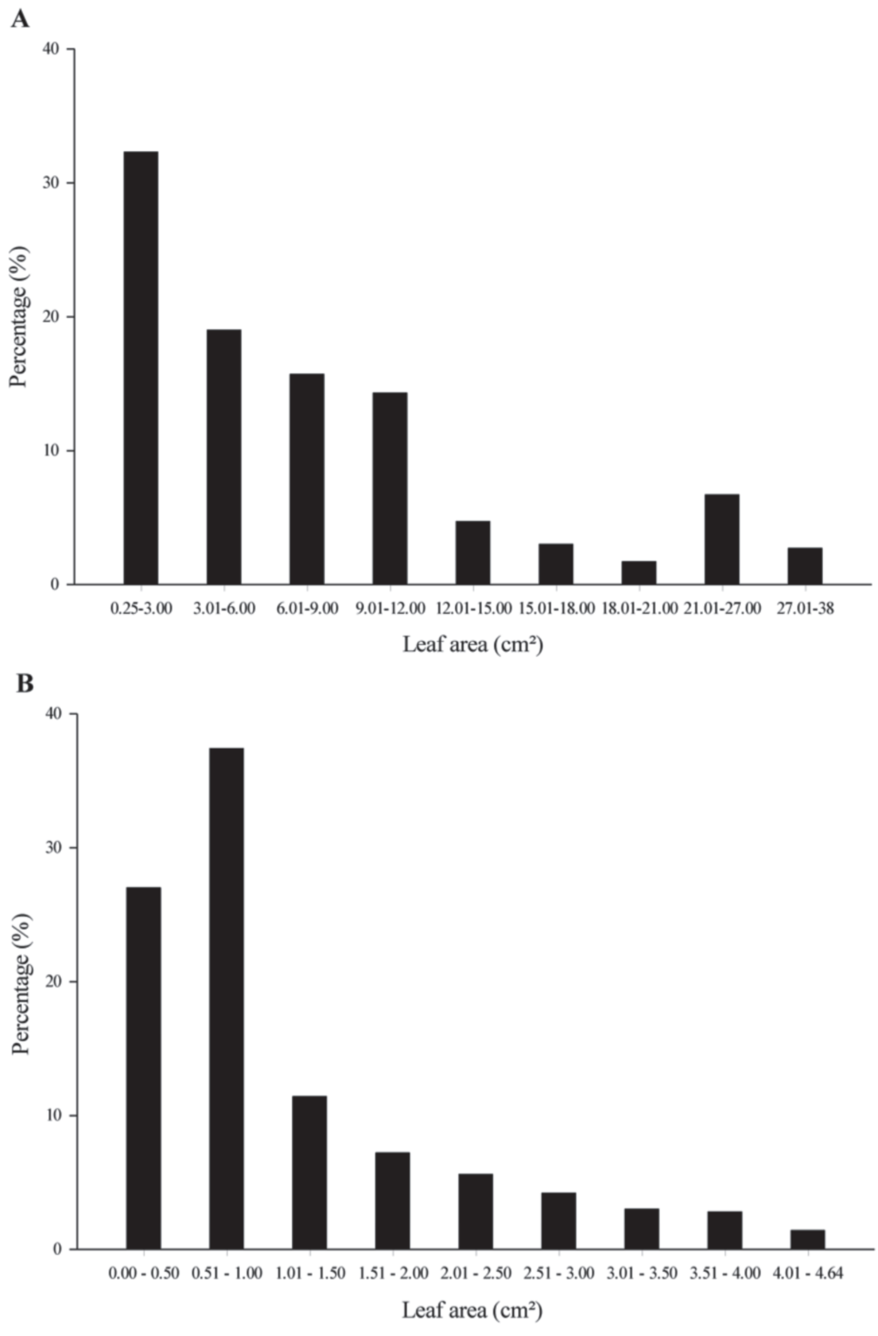

Figure 2: Percentage distribution of leaf area classes of 'Italiano Roxo' (A) and 'Folha Fina' (B) basil cultivars.

Table 4: Regression models, allometric equations, determination coefficient (R^2), Willmott's agreement index (*d*), Akaike information criterion (AIC), root mean square error (RMSE), and BIAS index of 300 leaves of 'Italiano Roxo' and 500 leaves of 'Folha Fina' basil cultivars

Model	x	R^2	AIC	RMSE	*d*	BIAS	Equation
'Italiano Roxo'							
Linear	L	0.8997	1306.0	2.367	0.9730	0.2752	$\hat{y} = -7.224 + 3.727*L$
Linear	W	0.9463	1128.6	1.734	0.9860	0.2583	$\hat{y} = -5.110 + 5.558*W$
Linear	L.W	0.9903	640.6	0.737	0.9978	0.1883	$\hat{y} = 0.5325 + 0.6253*LW$
Linear (0.0)	L.W	0.9914	703.2	0.825	0.9970	0.1593	$\hat{y} = 0.6476*LW$
Quadratic	L	0.9515	1101.7	1.648	0.9874	0.1638	$\hat{y} = 0.2591 - 0.1629*L + 0.4149*L^2$
Quadratic	W	0.9686	978.0	1.327	0.9920	0.1508	$\hat{y} = -1.0851 + 2.0488*W + 0.5871*W^2$
Quadratic	L.W	0.9913	611.2	0.699	0.9976	-0.0242	$\hat{y} = 0.2126 + 0.6844*LW - 0.00138*LW^2$
Cubic	L	0.9550	1082.7	1.589	0.9884	0.1742	$\hat{y} = 4.0019 - 3.0819*L + 1.0576*L^2 - 0.0417*L^3$
Cubic	W	0.9686	979.8	1.326	0.9920	0.1629	$\hat{y} = -0.86917 + 1.7403*W + 0.7068*W^2 - 0.01315*W^3$
Cubic	L.W	0.9914	610.3	0.696	0.9978	-0.0086	$\hat{y} = 0.2482 + 0.6743*LW - 0.0008*LW^2 - 0.000007*LW^3$
Power	L	0.9514	1100.2	1.650	0.9874	0.0945	$\hat{y} = 0.3863*L^{2.0138}$
Power	W	0.9686	976.0	1.327	0.9919	0.1198	$\hat{y} = 1.699*W^{1.642}$
Power	L.W	0.9945	609.6	0.695	0.9979	-0.0064	$\hat{y} = 0.8175*LW^{0.9307}$
Exponential	L	0.9036	1323.1	2.440	0.9690	-0.4478	$\hat{y} = 1.8565*1.3794^{L}$
Exponential	W	0.9353	1197.4	1.957	0.9812	-0.2824	$\hat{y} = 2.178*1.605^{W}$
Exponential	L.W	0.9352	1438.0	1.957	0.9812	-0.2824	$\hat{y} = 4.669*1.040^{LW}$
'Folha Fina'							
Model	**x**	**R^2**	**AIC**	**RMSE**	***d***	**BIAS**	**Equation**
Linear	L	0.8798	253.3	0.342	0.9672	0.2614	$\hat{y} = -1.097 + 1.354*L$
Linear	W	0.9234	238.5	0.273	0.9798	0.2433	$\hat{y} = -0.8011 + 2.170*W$
Linear	L.W	0.9769	122.0	0.150	0.9941	0.1983	$\hat{y} = 0.067 + 0.613*LW$
Linear (0.0)	L.W	0.9894	76.9	0.150	0.9942	0.0301	$\hat{y} = 0.6335*LW$
Quadratic	L	0.9296	82.7	0.262	0.9815	0.1638	$\hat{y} = 0.1702 - 0.2030 *L + 0.4086*L^2$
Quadratic	W	0.9495	114.4	0.222	0.9870	0.1508	$\hat{y} = -0.0798 + 0.5636*W + 0.7235*W^2$
Quadratic	L.W	0.9769	152.4	0.156	0.9942	-0.0327	$\hat{y} = 0.0542 + 0.6279*LW - 0.0025*LW^2$
Cubic	L	0.9295	84.4	0.261	0.9815	0.1811	$\hat{y} = 0.24934 - 0.36038*L + 0.5005*L^2 - 0.0160*L^3$
Cubic	W	0.9505	85.7	0.219	0.9873	0.1832	$\hat{y} = 0.1747 - 0.3563 *W + 1.6694 *W^2 - 0.2819*W^3$
Cubic	L.W	0.9769	151.8	0.150	0.9938	-0.0965	$\hat{y} = 0.0364 + 0.6612*LW - 0.0158*LW^2 + 0.0014*LW^3$
Power	L	0.9293	85.1	0.263	0.9815	0.0896	$\hat{y} = 0.3465*L^{2.0321}$
Power	W	0.9502	83.1	0.221	0.9871	0.1255	$\hat{y} = 1.213*W^{1.693}$
Power	L.W	0.9769	452.3	0.150	0.9941	-0.0625	$\hat{y} = 0.6792*LW^{0.9488}$
Exponential	L	0.9132	193.4	0.294	0.9755	-0.0259	$\hat{y} = 0.2116*2.4684^{L}$
Exponential	W	0.9187	169.1	0.287	0.9765	-0.0333	$\hat{y} = 0.2982*3.7314^{W}$
Exponential	L.W	0.9188	310.0	0.287	0.9765	-0.0333	$\hat{y} = 0.5931 * 1.3627^{LW}$

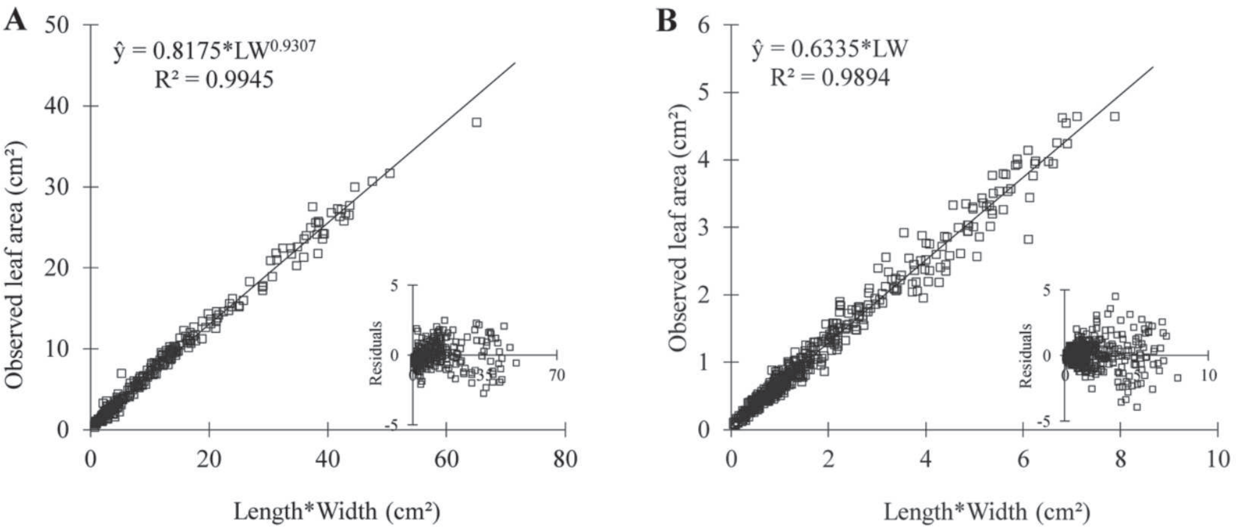

Figure 3: Variation in 'Italiano Roxo' (A) and 'Folha Fina' (B) real leaf areas as a function of the product of length by width (L.W) of leaf blades by the equations indicated to estimate leaf area. The residual dispersion is shown in the inserted chart.

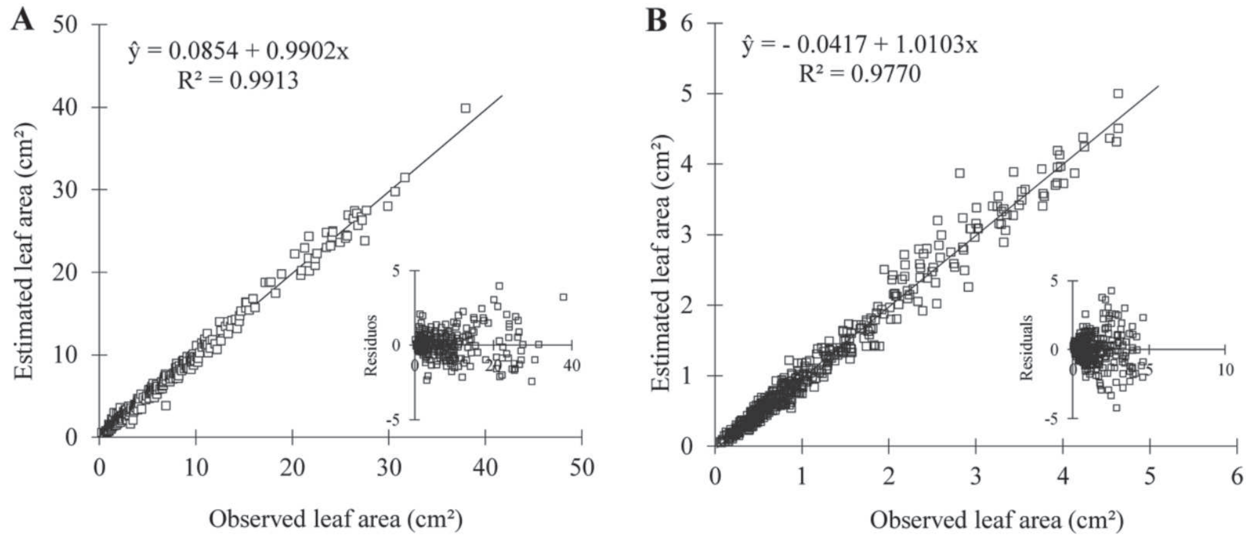

Figure 4: Relationship between observed leaf area and leaf area estimated by the proposed equations $w = 0.8175*LW^{0.9307}$ ('Italiano Roxo', A) and $w = 0.6335*LW$ ('Folha Fina', B). The residual dispersion is shown in the inserted chart.

the equations $w = 0.8175*LW^{0.9307}$ ('Italiano Roxo') and $w = 0.6335*LW$ ('Folha Fina') allow quickly and accurately estimate basil leaf area though the product of leaf length by width (L.W). Such equations confirm that using regression models allows a quick and precise leaf area estimation of basil cultivars ('Italiano Roxo' and 'Folha Fina') from linear dimensions of leaf limbs. Also, the proposed equations can be used to validate data obtained by leaf area meters.

CONCLUSIONS

Basil leaf area can be quickly and accurately estimated through a non-destructive method using linear dimensions of leaves.

Equations adjusted using the product of leaf length by width (L.W) can meaningfully estimate basil leaf area.

The equation $w = 0.8175*LW^{0.9307}$ adjusted using the power model (for 'Italiano Roxo' cultivar) and $w = 0.6335*LW$ adjusted using the linear model without intercept (for 'Folha Fina' cultivar) are the most suitable for estimating the leaf area basil cultivars.

The proposed equations can contribute to studies on basil growth, development, and physiology since leaf area estimation is of fundamental importance for these studies.

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