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Estimation Leaf Area by Composite Leaves of *Canavalia rosea* Seedlings Through Linear Dimensions From Last Leaflet

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Abstract

The objective of this work was to propose models of equations from measurements of the linear dimensions of the last leaflet for the estimation of the leaf area of the composite leaves of *Canavalia rosea*. For this purpose, 441 composite leaves of 198 seedlings were used, 45 days after sowing, produced in nursery and belonging to the Federal University of Espírito Santo, Campus São Mateus, located in the municipality of São Mateus, North of the State of Espírito Santo, Brazil. The length (L) along the main midrib and the maximum leaf width (W) of the last leaflet of each composite leaf, as well as the leaf area of all leaflets, were measured. Subsequently, it was determined the product of the multiplication of the length with the width (LW) and leaf area observed (OLA) from the sum of leaf area of leaflets in front of these measures were adjusted linear and non-linear equations of linear first degree, quadratic and power models, where, OLA was used as a dependent variable in function of L, W and LW as independent variable. Based on the models tested, we obtained equations for the estimated leaf area (ELA). The mean values of ELA and OLA were compared by Student's t test 5% probability. The mean absolute error (MAE), the root mean square error (RMSE) and the Willmott d index, were determined as criteria for validation. The best adjusted equation was chosen through the non-significant values in the comparison of the means of ELA and OLA, values of MAE and RMSE closer to zero, value of the index d near the unitary and higher values of R^2. Thus, the leaf area of the composite leaf of *C. rosea* seedlings can be estimated by the power model represented by equation ELA = 2.2951 $(LW)^{0.9474}$ quickly, easily and non-destructively.

Keywords: *Canavalia rosea*, mathematical models, non-destructive methods

1. Introduction

Canavalia rosea is a species of restinga, belonging to the family Leguminosae, popularly known as beach beans (Vatanparast, 2010). Its distribution occurs in all tropical and subtropical coastal areas of the world. Its importance varies from food consumption, until the use as fodder, fertilizers, bioactive compounds, pharmaceutical industry, soil establishment and erosion control (Sridhar & Bhagya, 2007; Mohajer, Taha, Mohamed, & Razak, 2017).

Due to the intense relationship between the leaves and the physiological processes, it is essential to estimate the leaf area in a simple and precise manner in ecological and agronomic studies, involving plant growth and development, light interception, photosynthetic efficiency, evapotranspiration and responses related to fertilizers and irrigation (Blanco & Folegatti, 2005; Spann & Heerema, 2010).

There are different ways of determining the leaf area of a plant, ranging from direct methods to indirect methods (Carvalho, Toebe, Tartaglio, Bandeira, & Tambara, 2017). In the vast majority, direct methods are destructive and expensive because they require specific electronic meters (Pompelli et al., 2012). The indirect methods, are more simple and faster in their execution, besides being non-destructive, allowing successive measurements to be made over time (Gamiely, Randle, Milks, & Smittle, 1991; Spann & Heerema, 2010).

An of the most used methods for indirect leaf area determination is through predictive mathematical equation models of the actual leaf area as a function of linear leaf dimensions and their respective combinations (Gamiely et al., 1991; Blanco & Folegatti, 2005). However, it is necessary to adjust mathematical models for each species of interest (Spann & Heerema, 2010).

In this way, are notorious the great number of works involving mathematical equations as non-destructive method to estimate the leaf area in a wide range of vegetal species as *Cucumis sativus* L. (Cho, S. Oh, M. M. Oh, & Son, 2007), *Helianthus annuus* L. (Rouphael, Colla, Fanasca, & Karam, 2007), *Jatropha curcas* (Pompelli et al., 2012), *Vitis vinífera* L. (Buttaro, Rouphael, Rivera, Colla, & Gonnella, 2015), *Coffea canephora* (Schmildt, Amaral, Santos, & Schmildt, 2015), *Rosa hybrida* L. (Costa, Pôças, & Cunha, 2016), *Crotalaria juncea* (Carvalho et al., 2017), *Prunus armeniaca* L. (Cirillo et al., 2017), *Litchi chinensis* Sonn (Oliveira, Silva, Costa, Schmildt, & Vitória, 2017), guava (Vitória et al., 2018), *Carica papaya* L. (Oliveira et al., 2019) and *Plectranthus barbatus* Andrews (Ribeiro et al., 2019).

However, studies involving species of Brazilian restinga are scarce, in addition, the determination of the leaf area of the composite leaves can be laborious and time consuming due to the large number of leaflets that need to be measured. Thus, the objective of this study was to propose mathematical equations using measurements of the linear dimensions of the last leaflet for the estimation of the leaf area of the composite leaves of *C. rosea*.

2. Method

For the accomplishment of the present study were used 441 leaves composed of 198 *Canavalia rosea* seedlings obtained in nursery,belonging to the Federal University of Espírito Santo, Campus São Mateus, located in the municipality of São Mateus, North of the State of Espírito Santo, Brazil, with geographic coordinates of 18º40′36″ south latitude and 39º51′35″ longitude East. The climate of the region according to the classification of köppen is tropical type AW (tropical humid), with rains in summer and dry winter (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2014).

The seeds used were obtained from the Socio-Cultural and Environmental Center José Bahia, located in the municipality of São Mateus, North of the State of Espírito Santo, Brazil. For the preparation of the seedlings was used a tube with volume of 290 cm^3 filled with substrate based on sand, clay and coconut fiber. The irrigation was done by a localized sprinkler system with an 8 mm d^{-1} irrigation depth and a 1 hour irrigation frequency, distributed in four 15 minute applications. The seedlings were kept in a greenhouse covered with sombrite® screen of 30% shading until they were completed 45 days after sowing.

In the laboratory, the last leaflet of the 441 composite leaves of *C. rosea* was measured. Each composite sheet was scanned in HP Deskjet F4280® Scanner and the images saved in TIF format with 75 dpi, each sheet was composed of 3 leaflets. The length (L) along the main midrib and the maximum width (W) were measured in cm of the last leaflet of each composite leaf (Figure 1), as well as the leaf area of all leaflets with the help of the ImageJ® program (Schindelin, Rueden, Hiner, & Eliceiri, 2015). The product of length and width (LW) was obtained by multiplying L and W and observed leaf area (OLA, in cm^2) from the sum of leaf area of leaflets.

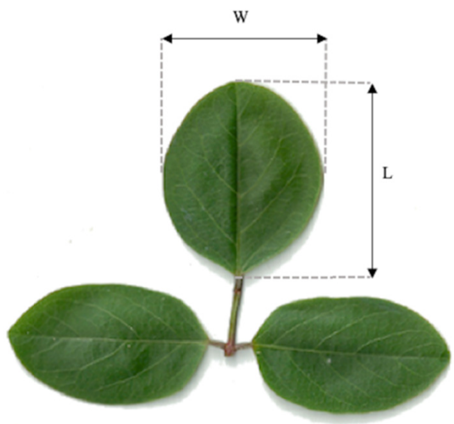

Figure 1. Representation of the length (L) along the midrib and the maximum leaf width (W) of the last leaflet of leaves of *Canavalia rosea* seedlings

Data were submitted to descriptive statistics analysis, obtaining the minimum, maximum, mean, amplitude and coefficient of variation (CV) values. For the estimation of the leaf area of the composite leaves of *C. rosea* through the dimensions of the last leaflet, nine modeling equations were obtained from the sample of 396 composite leaves. For this purpose, the first degree linear models represented by $ELA = \hat{\beta}_0 + \hat{\beta}_1 x$, quadratic represented by $ELA = \hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2$, and power represented by $ELA = \hat{\beta}_0 x^{\hat{\beta}_1}$, in which OLA was used with dependent variable in function of L, W and LW as independent variables (x).

The validation of the data was based on the sample of 45 separate composite leaves for this purpose. All values of L, W and LW were substituted in the equations adjusted for modeling, obtaining the estimated leaf area (ELA), in cm^2. A simple linear equation model was then fitted for each proposed model, where ELA was used dependent variable in function of OLA. Subsequently, the mean values of ELA and OLA were compared by Student's t test with a 5% probability. The mean absolute error (MAE), the root mean square error (RMSE) and the Willmott d index (Willmott, 1981), for all equations were also determined by Equations 1, 2 and 3.

$$EAM = \frac{1}{n-1} \sum_{i=1}^{n} \left| ELA - OLA \right| \quad (1)$$

$$RQME = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (ELA - OLA)^2} \quad (2)$$

$$d = 1 - \left[\frac{\sum_{i=1}^{n} (ELA_i - OLA_i)^2}{\sum_{i=1}^{n} \left(\left| ELA_i - \overline{OLA} \right| + \left| OLA_i - \overline{OLA} \right| \right)^2} \right] \quad (3)$$

where, ELA, are the values of the estimated leaf area; OLA, values of leaf area observed; n the number of leaves contained in the sample for validation, where n = 45, in the present study.

The criteria used to determine the best model for the estimation of the *C. rosea* leaf area were based on the non-significant values of the comparison of the means of ELA and OLA, values of MAE and RMSE closer to zero and index d closest to the unit. All the statistical analyzes were performed in the software R (R Core Team, 2018), through the data package ExpDes.pt version 1.2 (Ferreira, Cavalcanti, & Nogueira, 2018).

3. Results and Discussion

The leafs sampled for model adjustment and data validation presented a high amplitude for length (L), width (W), product of length with width (LW) and leaf area observed (Table 1). The high amplitude can be proven due to the high variability of the data, surpassing the 22% for all the characteristics. These values are considered according to the classification of Pimentel-Gomes (2009) as high or very high. However, this high variability is fundamental in obtaining mathematical models, since the selected equation allows to estimate the leaf area for leafs of various sizes, always respecting the limits of the values analyzed (Oliveira et al., 2017).

Note that the L, W, LW and OLA values of the sampling used for the validation are within the range of the values used to perform the modeling. This finding is important because according to Levine, Stephan, and Szabat (2017), the values used for validation can not extrapolate the values used for the modeling adjustments.

Table 1. Minimum, maximum, average, amplitude and coefficient of variation (CV) values of the variables: length (L); width (W); product of the length and width (LW) and observed leaf area (OLA) of leaves of *Canavalia rosea* seedlings

Variable	Unit	Minimum	Maximum	Average	Amplitude	CV (%)
396 leaves were used for modeling						
L	cm	1.58	8.15	4.96	6.57	25.02
W	cm	1.13	6.44	4.25	5.31	27.45
LW	cm^2	1.92	51.90	22.49	49.98	44.83
OLA	cm^2	3.50	96.06	43.57	92.56	44.02
45 leaves for validation						
L	cm	1.94	7.42	5.21	5.50	22.35
W	cm	1.34	6.26	4.51	4.92	24.49
LW	cm^2	2.61	45.26	24.64	42.65	39.52
OLA	cm^2	4.33	88.08	47.64	83.75	38.55

Note. Through the scatter diagram (Figure 2) that there was a linear and non-linear association for OLA as a function of L, W, and LW. This behavior was observed by Carvalho et al. (2017) studying the leaf area estimation for *Crotalaria juncea,* suggesting that linear and non-linear models should be generated and tested in these cases. Thus, in the present study, linear model of first degree, quadratic and power were adjusted for each analyzed dimension.

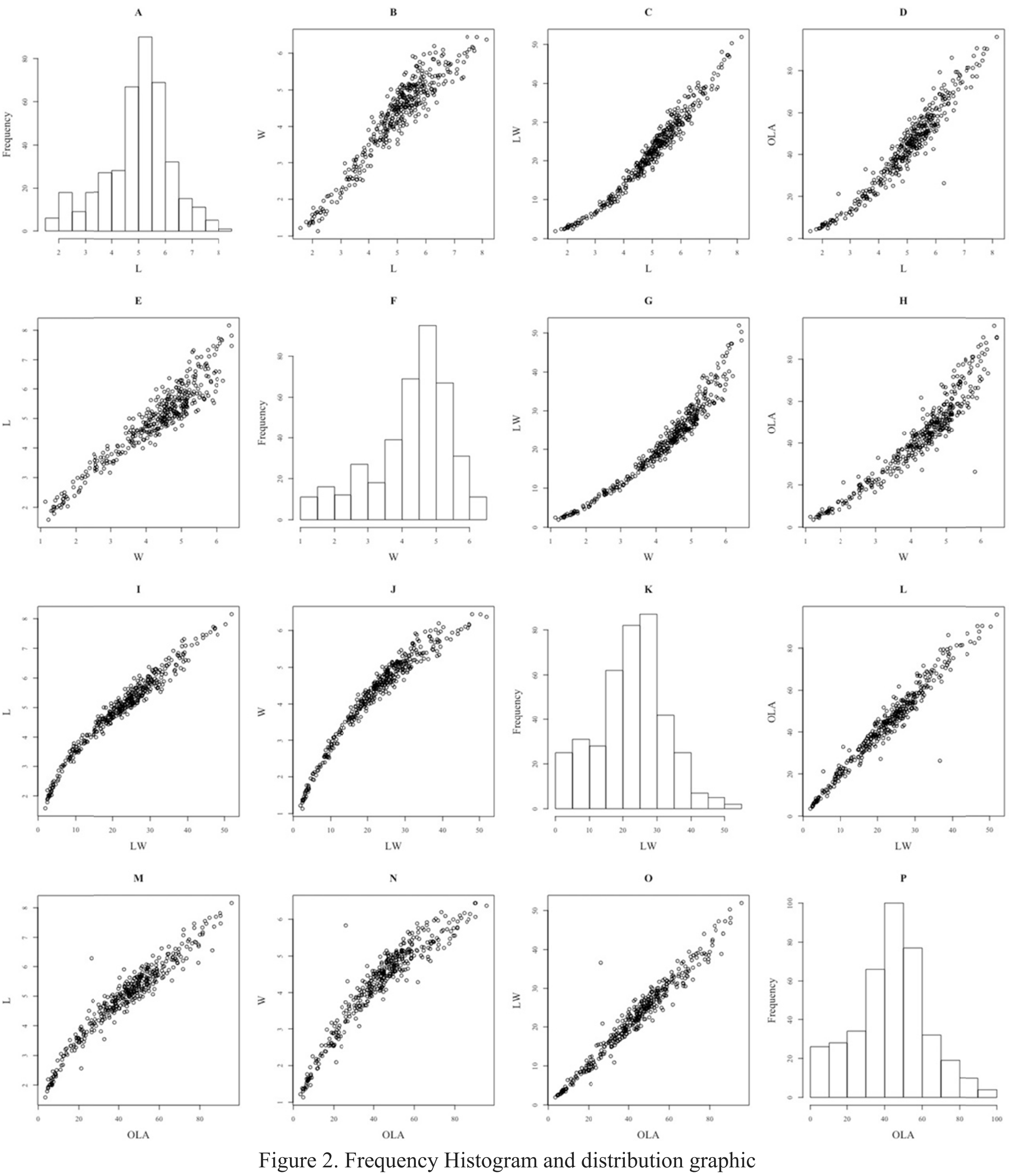

Figure 2. Frequency Histogram and distribution graphic

Table 2 shows the equations of the *C. rosea* leaf area estimation models. Note that the best adjustments were using LW as independent variable, with R^2 greater than 0.95. Note also that the equations based on OLA as dependent variable in function of L and W did not present a good fit. Lower values of R^2 based on only one measure as independent variables were also found by Buttaro et al. (2015) and Oliveira et al. (2017) noting that these variables do not have a good relationship with OLA. However, as suggested by Antunes, Pompelli, Carretero, and Damatta (2008), mathematical equations should not be chosen only by the higher value of R^2

because this practice can cause erroneous estimations of the leaf area. In this way, the validation of the models through appropriate methods becomes indispensable to estimate the leaf area with precision (Fascella, Darwich, & Rouphael, 2013).

Table 2. Equation with linear adjustment of first degree, quadratic and power and its respective coefficient of determination (R2) using the observed leaf area (OLA) as dependent variable, as a function of length (L), width (W), product of length with width (LW) of leaves composed of seedlings of *Canavalia rosea*

Model	Equation	R^2
Linear	$ELA = -30.1381 + 14.8531(L)$	0.9246
Linear	$ELA = -22.2932 + 15.4773(W)$	0.8890
Linear	$ELA = 1.76473 + 1.85898(LW)$	0.9552
Quadratic	$ELA = -13.8766 + 7.3285(L) + 0.8056(L)^2$	0.9331
Quadratic	$ELA = 0.07305 + 2.20261(W) + 1.75252(W)^2$	0.9114
Quadratic	$ELA = 0.791287 + 1.967282(LW) - 0.002408(LW)^2$	0.9555
Power	$ELA = 2.3289(L)^{1.8030}$	0.9268
Power	$ELA = 3.1761(W)^{1.7720}$	0.9121
Power	$ELA = 2.2951(LW)^{0.9474}$	0.9555

After the validation of the data based on the 45 leaves sampling of *C. rosea*, it was verified that of the nine equations, those that use LW as independent variable presented higher values of R^2, being the equation power model slightly higher than the linear models of first degree and quadratic. This same equation was the with the highest R^2 value in the modeling adjustment, giving a better correlation between OLA and ELA (Table 3).

Note in Figure 3 that models involving a single measurement were less acceptable in the *C. rosea leaf* area estimate due to its lower R^2 values. In addition, the equations based on L and W individually presented MAE and RMSE values that were farther from zero and values of the Willmott d index more distinct to one, when compared the models using LW (Table 3), these criteria should be used to select the model that best fits the leaf area estimate (Oliveira et al., 2017). It was also verified that the comparative means of OLA and ELA did not statistically differ by Student's t test at 5% of probability in all proposed models (Table 3), attesting a good similarity of the means of OLA and ELA.

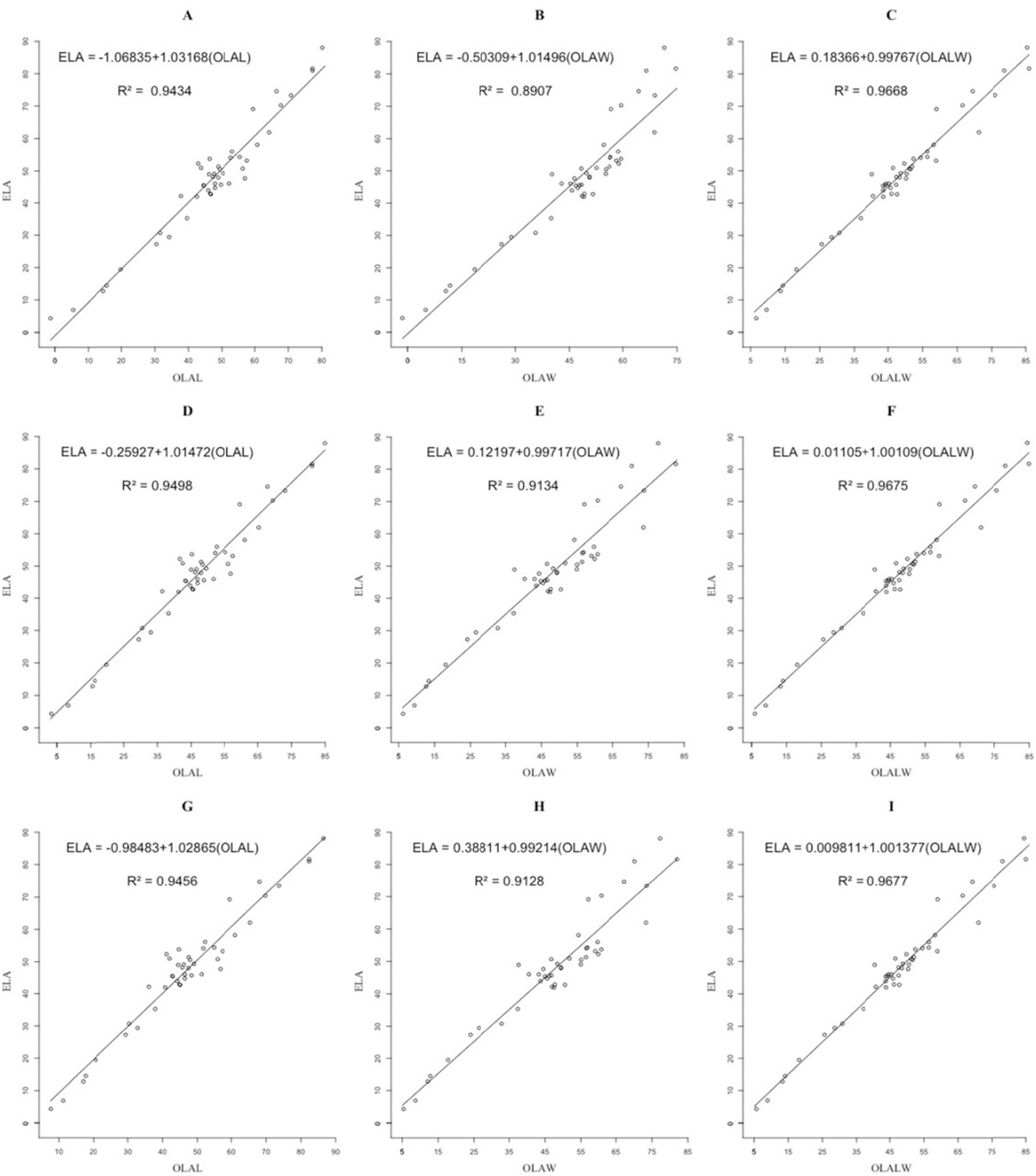

Figure 2. First degree linear adjustment validation equation and its respective determination coefficient (R^2) using the estimated leaf area (ELA) as the dependent variable obtained by first degree linear modeling equations (A, B and C), quadratic (D, E and F) and power (G, H and I), as a function of leaf length observed (OLAL), width (OLAW) and length product with width (OLALW) of *Canavalia rosea* leaf

Table 3. Observed leaf area (OLA) and estimated leaf area (ELA) of linear equations of first degree, quadratic and potential for the independent variables length (L), width (W) and product of length and width (LW), besides the value of p, mean absolute error (MAE), root mean square error (RMSE) and Willmott d index of leaves composed of *Canavalia rosea* seedlings used for validation

Model	Variable	OLA	ELA	*p** value	MAE	RMSE	d
Linear	L		47.2185	0.9098	3.6051	4.4253	0.9844
Linear	W		47.4392	0.956	4.8565	6.0831	0.9698
Linear	LW		47.5732	0.9849	2.3970	3.3462	0.9915
Quadratic	L		47.2105	0.9090	3.1609	4.1481	0.9866
Quadratic	W	47.6460	47.6589	0.9973	4.2078	5.4048	0.9769
Quadratic	LW		47.5830	0.9870	2.3798	3.3126	0.9916
Power	L		47.2762	0.9221	3.4032	4.3306	0.9852
Power	W		47.6322	0.9971	4.2236	5.4267	0.9768
Power	LW		47.5707	0.9844	2.3601	3.3047	0.9917

Note. **P* values higher than 0.05 indicate that the observed leaf area (OLA) and the estimated leaf area (ELA) do not differ by Student t-test.

The use of only a linear dimension in practice is easier to execute, making the work less costly. However, these models are adjusted only for specific cases, being less precise for most species (Espindula et al., 2018). Therefore, models using the relation of linear dimensions as found in this study are notoriously used as reported for several species as *Jatropha curcas* (Pompelli et al., 2012), *Vitis vinífera* L. (Buttaro et al., 2015), *Coffea canephora* (Schmildt et al., 2015), *Crotalaria juncea* (Carvalho et al., 2017) and *Litchi chinensis* Sonn (Oliveira et al., 2017).

Thus, the power model equation represented by $ELA = 2.2951(LW)^{0.9474}$ based on the product of length and width as independent variable, can be used to estimate the leaf area of *Canavalia rosea* with better precision. These variables can be measured quickly, without the need to use specific and costly equipment, and after the establishment of the model, the mathematical equations can be used for further research without the obligation of destroying new leafs for the establishment of new models (Spann & Heerema, 2010).

6. Conclusion

It is possible to conclude that the leaf area of *Canavalia rosea* seedlings can be measured with better precision through the product of the length multiplication along the main midrib with the maximum width of the leaf (LW) of the last leaflet, from the model power represented by the equation $ELA = 2.2951(LW)^{0.9474}$, quickly, easily and non-destructively.

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