Original Research Article

Estimation of Single Leaf Area ~~of Leaflets~~ of *Acacia mangium* Willd. ~~by means linear dimensions~~

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ABSTRACT

The objective of this study was to determine the best equation for estimating the leaf area of *Acacia mangium* Willd. from the linear dimensions of the leaflets of non-destructive form. For this, 476 leaflets of plants belonging to Lajeado farm were collected in the municipality of Ecoporanga, in the north of the State of Espírito Santo, Brazil. From each leaflet was determined the length (L) along the main midrib, the largest width (W), the product of the multiplication between the length and the width (LW) the observed leaf area (OLA). For the modeling, we used 382 leaflets in which OLA was the dependent variable in function of L, W or LW as independent variable, being adjusted the linear models of first degree, quadratic and power. For the validation, the values of L, W and LW of 94 leaflets were replaced in the equations obtained in the modeling thus obtaining the estimated leaf area (ELA). The means of ELA and OLA were compared by Student's t test at 5% probability. . It was also determined the mean absolute error (MAE), the root mean square error (RMSE) and Willmott's index d. In order to select the best equation, the following criteria were used: : not significant of the comparison of the means of ELA and OLA, values of MAE and RMSE with closer to zero and index d closer to one. The power model equation represented by $\mathrm{ELA} = 0.7946(\mathrm{LW})^{0.9727}$, is the most adequate to predict the leaf area of Acacia mangium Willd. quickly and non-destructively.

Comment [GV1]: What you call leaflets are actually the leaves since A. mangium has simple and not compound leaves.

Keywords: Acacia mangium Willd.*; modeling of leaf area; non-destructive method*

1. INTRODUCTION

~~The~~ *Acacia mangium* Willd. is a species of the family Leguminosae and subfamily Mimosoideae. This species is widely used in reforestation and recovery programs in areas with poor or degraded soils, such as slope and mining areas, as well as the production of wood, cellulose and charcoal [1]. ~~The~~ *A. mangium* Willd presents its leaf structure constituted by a leaflet, that is, an expanded portion of the petiole, being dilated and flattened, resembling the limb, which in general in this case is totally absent [2]. After the growth of the plant, the petiole dilates and the composite leaves fall, making this structure responsible for the photosynthetic function of the plant [3].

Comment [GV2]: Not clear.
In addition, A mangium bears compound leaves formed of leaflets in newly germinated plant. However, a few weeks following germination, the plants no longer produce these “true leaves”, since the leaf stalk and the main axis of the compound leaves flatten and are transformed into phyllodes.

The leaf area estimation helps to verify the photosynthetic surface, allows to obtain important indicators for the understanding of the plant responses to environmental factors [4]. According to Mota et al. [5], leaf area is an important indicator of the rates of CO_2 assimilation, O_2 release and transpiration, and plant vigor. This fact shows that the knowledge of the leaf area is important in the evaluation of the physiological state of a plant [6].

Comment [GV3]: Please add the fact that we usually need the leaf area in leaving plants though mon-destructive sampling.

There are several methods for the determination of leaf area that can be performed directly (destructive method), through automatic planimeters or indirect method (non-destructive method), through portable automatic planimeters, or mathematical models, using length and width of the leaf blade. Although accurate, direct methods are expensive and laborious, while the mathematical models allow faster assessments [7].

Based on the leaf dimensions of several species and without the destruction of the sample, several studies have reported the use of mathematical models to estimate leaf area [8,9,10,11,12,13,14]. With respect to *A. mangium* Willd., A non-destructive methodology for the determination of its leaf area is of great importance, since there are no mathematical equations in the literature that allow this measurement in the specie.

Thus, the objective of this study was to determine the best equation for estimating the leaf area of *A. mangium* Willd. from the linear dimensions of the leaflets of non-destructive form.

2. MATERIAL AND METHODS

The study was carried out with leaflets of *Acacia mangium* Willd, collected from trees belonging to Lajeado farm, in the municipality of Ecoporanga, North of the State of Espírito Santo, Brazil, located at latitude 18° 22' 44.4" South and 40° 49' 22.4'' west longitude. The climate of the region according to Köppen is classified as tropical humid type AW, with dry winter and summer rains [15]. A total of 476 leaflets were collected at various stages of development of plants aged 8 to 10 years at four cardinal points and packed in plastic bags.

After the collection, in the laboratory, of each leaflet the length (L, in cm) along the main midrib and the largest width (W, in cm) were measured, both with the aid of a millimeter graduated rule (Fig. 1). ~~The product of the multiplication between length and width (LW, in cm2) was also determined.~~ Afterwards, all leaflets were scanned with HP Deskjet F2540® flatbed scanner and the images were saved in Tag Image File Format (TIFF) format with 300 dpi resolution. Then, the images were processed through ImageJ® software [16], from which the observed leaf area (OLA, in cm²) of each leaflet was obtained. The values of the descriptive statistics of maximum, minimum, mean, amplitude, standard deviation (SD) and coefficient of variation (CV) for L, W, ~~LW and~~ OLA were determined. The product of the multiplication between length and width (LW, in cm2) was also determined.

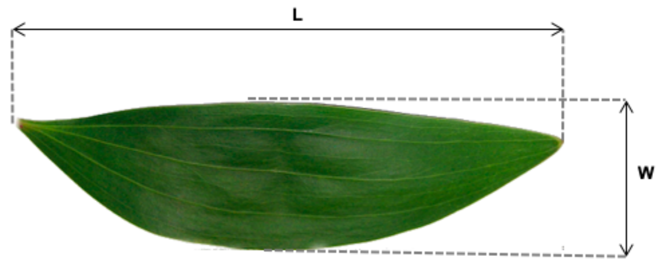

Fig. 1. Representation of the length (L) along the midrib and the maximum width (W) of leaflets of *Acacia mangium* Willd.

Comment [GV4]: It would be worthwhile to provide more information about the experiment mainly the sampling design.
For example, how many trees were sampled?
Are those trees randomly selected or their choice based on specific spatial design?
About the stages you are referring to, are there seedlings, saplings, trees? This is important since only earlier stage of plant germinated bear "true leave", at later stage only are present phyllodes.

Comment [GV5]: Hardly understandable, please rephrase.

Comment [GV6]: Please see my comment above.

Comment [GV7]: Are you sure this is a leaflet and not a phyllode.

For the modeling, we used 382 leaflets in which OLA was the dependent variable as a function of L, W or LW as independent variable, being adjusted the linear models of first degree, quadratic and power whose representation can be seen in table 1, totalizing nine equations in the estimation of the leaf area of *Acacia mangium* Willd.

Table 1. ~~Denomination and representation of equation m~~Models of estimation ~~of~~adjusted ~~for~~ leaf area ~~estimation. of leaflets~~ of *Acacia mangium* Willd using leaf dimensions as length (L), width (W), and their product (LW), respectively~~.~~

Denomination	Representation
Linear	$\mathrm{ELA} = \hat{\beta}_0 + \hat{\beta}_1 x$
Quadratic	$\mathrm{ELA} = \hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2$
Power	$\mathrm{ELA} = \hat{\beta}_0 x^{\hat{\beta}_1}$

For the validation of ELA, ~~the~~ L, W and LW values of 94 separate leaflets for this purpose were substituted in the equations obtained in the modeling~~, thus obtaining the estimated leaf area (ELA, in cm2)~~. Using the Student t test at 5% probability, the means of ELA and OLA were compared. It was also determined the mean absolute error (MAE), the root mean square error (RMSE) and Willmott's index d [17], for all equations, by means of ~~expressions~~ equations 1, 2 and 3.

$$\mathrm{MAE} = \frac{\sum_{i=1}^{n} |\mathrm{ELA} - \mathrm{OLA}|}{n} \quad (1)$$

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

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

In that, ELA are the estimated values of leaf area by the proposed equations; OLA are the observed leaf area values; $\overline{\mathrm{OLA}}$ is the average of the leaf area values observed; n is the number of leaflets used in validation, n = 94 in the present study.

For the selection of the equation that best estimate the leaf area of leaflets of *Acacia mangium* Willd. in function of L, W or LW, the following criteria were used: not significant of the comparison of the means of ELA and OLA, values of MAE and RMSE with closer to zero and index d closer to one. The statistical analyzes were performed with the aid of software R [18], with scripts developed for the ExpDes.pt version 1.2 package [19].

3. RESULTS AND DISCUSSION

The analysis of the descriptive statistics of the characteristics under study is present in table 2. Note that there was high amplitude of the sample data in all the characteristics, and the values of the sample used for modeling presented values higher than the sample used for

Comment [GV8]: 476 sampled – 382 used for the modeling, what about 94 remaining?

Comment [GV9]: To make it easy to follow, please give the meaning of x in the the equations of the Tab. 1, by indicate that each equation is run for each of independent variable (L, W, LW), which make 9 models at all. Hence x stands for L, W and LW. Not giving this precision make it difficult to follow.

You should also indicate how did you determine the intercept β_0 and the estimates β_1 and β_2. I understand that they are estimated by running 1) OLA = $\hat{\beta}_0 + \hat{\beta}_1 x$, 2) OLA = $\hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2$, and 3) OLA = $\hat{\beta}_0 x^{\hat{\beta}_1}$. Note that we use at this step OLA, to determine the estimate $\hat{\beta}_0, \hat{\beta}_1$ *and* $\hat{\beta}_2$.
In a second step, knowing $\hat{\beta}_0, \hat{\beta}_1$ *and* $\hat{\beta}_2$ from the first step, we run the same equations the same equations having ELA as dependent variable and measured L, W, and LW as independent variables.
The final step is to compare OLA and ELA to see how good are the predictions of the models.

Comment [GV10]: Which equation, tab. 1? ELA need clarification in the explanatory variable (x) used to estimate it (see comments in tab. 1) validation. This, according to Levine et al. [20] is adequate since the measures used in the validation should not extrapolate the measures used to adjust the equations.

In relation to the standard deviation (SD) and the coefficient of variation (CV) obtained, it is noted that the LW characteristic presented the highest values in both the sample used for the modeling and in the validation sample. High values of these measurements are important in studies aiming at the determination of mathematical equations of modeling of the leaf area, since it indicates the use of leaves with different sizes, corresponding to all the phenological stages of the species, suggesting that these equations can be used throughout the cycle.

Table 2. Descriptive statistics with value minimum, maximum, mean, amplitude, standard deviation (SD) and coefficient of variation (CV) of the variables: length (L); width (W); product of the length and width (LW) and observed leaf area (OLA) of leaflets of *Acacia mangium* Willd.

Variable	Unit	Minimum	Maximum	Mean	Amplitude	SD	CV (%)
382 leaflets were used for modeling							
L	cm	10.00	23.40	15.85	13.40	2.44	15.44
W	cm	2.80	7.70	4.71	4.90	0.97	20.62
LW	cm^2	33.00	150.80	75.34	117.80	21.53	28.58
OLA	cm^2	22.34	99.85	53.12	77.51	15.02	28.27
94 leaflets for validation							
L	cm	10.90	22.70	16.83	11.80	2.01	11.95
W	cm	2.70	5.70	3.88	3.00	0.64	16.63
LW	cm^2	36.71	122.58	66.06	85.87	16.86	25.52
OLA	cm^2	26.53	88.04	47.05	61.51	12.15	25.82

In fig. 2, it is possible to notice that there is a linear and non-linear association between L, W and LW and OLA, in this way the linear mathematical equations of first degree, quadratic and power were adjusted for the estimation of the leaf area of *Acacia mangium* Willd. According to Toebe et al. [11], these three models are reliable, presenting high predictive efficiency and high reliability, being used with precision in the estimation of leaf area of several crops, without the necessity of the destruction of the leaves. Corroborating this assertion, several authors have tested and adjusted these models for other species, such as *Crotalaria juncea* [8], *Litchi chinensis* Sonn. [9], *Artocarpus heterophyllus* [10], *Cucurbita moschata* [11], *Pennisetum glaucum* [12] and *Plectranthus barbatus* Andrews [13].

Comment [GV11]: The tab. 2 only is not enough to determine the linear relationships between L, W, and LW.
You should statistically test it by using correlation, regression or variance inflation factor.

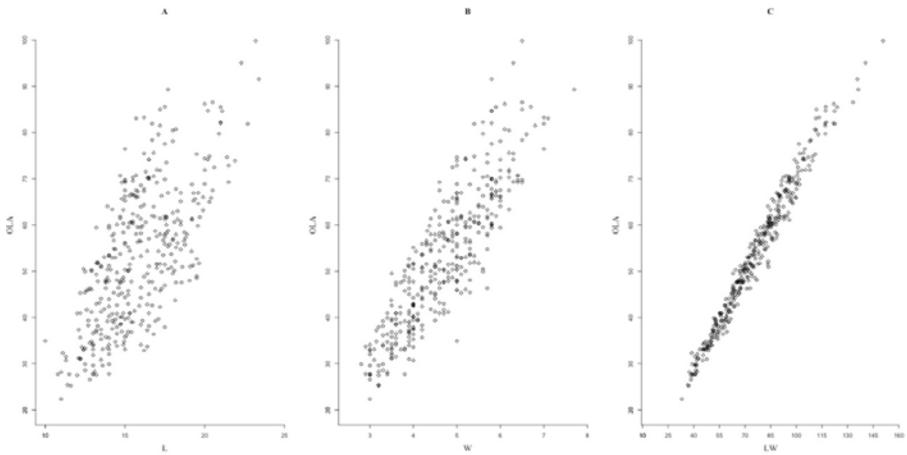

Fig. 2. Scatter plot of the actual leaf area in relation to length (L) (A), width (W) (B) and product of length with width (LW) (C).

Comment [GV12]: I really don't understand what the point of plotting OLA with L, W, and LW respectively. What do you want to demonstrate here?
To my view, plotting ELA (for each of the 9 equations) against their corresponding OLA is enough to determinate the type of relationship and how good are the models tested. There is no point plotting the independent variables.

Table 3 describes the nine models of equations generated for the estimation of the leaf area of *Acacia mangium* Willd. through the linear dimensions of the leaf surface. Note that among those models that were generated with only a linear dimension, the coefficient of determination (R^2) values were very low, less than 0.47 for the length and not greater than 0.76 for the width. For this reason, these equation models were not adequate for estimating the leaf area of *Acacia mangium* Willd. The low correlation of these characteristics (L and W) with observed leaf area (OLA) can be related due to the irregular shape that the leaflets present (Fig. 1), which may lead to erroneous estimations of leaf area when used individually.

Comment [GV13]: Actually this Tab. 3 should be the start of your analysis (i.e. replacing tab 1 above) since more consistent with your study objective as specified in L105-107.

On the other hand, the equations based on LW presented the highest values R^2, surpassing 0.97, which according to Pompelli et al. [14] shows good accuracy of the models if the selection criterion were only the high values of R^2. However, in order to choose the best adjusted equation, it should not only be based on the values of R^2 because there may be underestimation of the leaf area leading to imprecise measurements using the equations used.

Table 3. Equation with linear adjustment of first degree, quadratic and power and its respective coefficient of determination (R^2) using the observed leaf area (OLA) as dependent variable, in function of length (L), width (W), product of length with width (LW) of leaflets of *Acacia mangium* Willd.

Comment [GV14]: How did you obtain the estimates of all equations?
All the equation should be written first in their general form

Model	Equation	R^2
Linear	$\text{ELA} = -13.0938 + 4.1774\ (\text{L})$	0.4636
Linear	$\text{ELA} = -10.3455 + 13.4676(\text{W})$	0.7588
Linear	$\text{ELA} = 1.25971 + 0.68837(\text{LW})$	0.9741

Quadratic	$ELA = -34.77086 + 6.88965(L) - 0.08286(L)^2$	0.4654
Quadratic	$ELA = -14.6376 + 15.3323(W) - 0.1942(W)^2$	0.7590
Quadratic	$ELA = -2.3619848 + 0.7868752(LW) - 0.0006189(LW)^2$	0.9747
Power	$ELA = 1.7832\ (L)^{1.2273}$	0.4564
Power	$ELA = 8.3418(W)^{1.1913}$	0.7559
Power	$ELA = 0.7946(LW)^{0.9727}$	0.9741

When we analyzed the comparison of the means of OLA and ELA by the student t test ($p < 0.05$), we observed that for all the models generated from a single linear dimension (L or W), there were significant results, attesting that the predictive leaf area by the models is different from the actual leaf area of the plants, or this reason, these equations were not efficient in the estimation of the leaf area of *Acacia mangium* Willd (Table 4). Therefore, models based on only one linear dimension, be it length or width, should not be used, so these equations have been eliminated.

On the other hand, the leaf area estimated by the models based on combined length and width (LW) values was similar to the actual leaf area. Although the LW based linear of first degree, quadratic and power models showed good accuracy in the prediction of the leaf area of *Acacia mangium* Willd., With identical values of 0.9952 of the index d for all three models, the power model better met the criteria of MAE and RMSE with values closer to zero indicating more precision for this model.

Table 4. Observed leaf area (OLA) and estimated leaf area (ELA) of linear equations of first degree, quadratic and power 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 leaflets of Acacia mangium Willd. used for validation

Model	Variable	OLA	ELA	p* value	MAE	RMSE	d
Linear	L		57.2021	<0.05	10.9157	12.2060	0.7201
Linear	W		41.9274	<0.05	5.9906	7.6759	0.8676
Linear	LW		46.7367	0.8562	1.2802	1.6454	0.9952
Quadratic	L	47.0511	57.3705	<0.05	11.1342	12.4501	0.7079
Quadratic	W		41.8672	<0.05	6.0118	7.6599	0.87074
Quadratic	LW		46.7474	0.8622	1.3243	1.6590	0.9952
Power	L		57.1159	<0.05	10.8261	12.1207	0.7217

Power	W	42.0982	<0.05	5.9365	7.6756	0.8627
Power	LW	46.7806	0.8762	1.2794	1.6448	0.9952

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.

Thus, it is evident that the equations generated with LW presented better performance when compared to the equations based on only one dimension of the leaflet. Although the combined measures of L and W require more time to determine the leaf area of a species, this combination is the most used, due to the high precision of the generated models, reducing the error in the forecast [21]. Corroborating with this statement, in fact, the models involving the combination of linear measurements are notoriously reported as those that present better fit for several plant species as already observed for *Crotalaria juncea* [8], *Litchi chinensis Sonn*.[9], *Artocarpus heterophyllus* [10], and *Plectranthus barbatus* Andrews [13].

Comment [GV15]: Should tell why.

Comment [GV16]: Not adequate.

Therefore, we indicate the power model equation represented by $\mathrm{ELA} = 0.7946(\mathrm{LW})^{0.9727}$ as the best ~~most accurate to and~~ model to estimate ~~of~~ the leaf area of *Acacia mangium* Willd. ~~because it better meets the statistical criteria established in this study~~ (Fig. 3). Developing such models required destructive sampling~~It is worth mentioning that for the modeling adjustment it was necessary to destroy the leaflets,~~. ~~h~~However, once successfully fitted, ~~after the establishment and the verification of~~ the models can be used to non-destructively predict leaf area ~~the measurements of the dimensions of the leaf surface can be easily inferred~~ with only the aid of a simple equipment ~~, with for example~~ as a ruler~~, without the obligatory destruction of new leaflets~~.

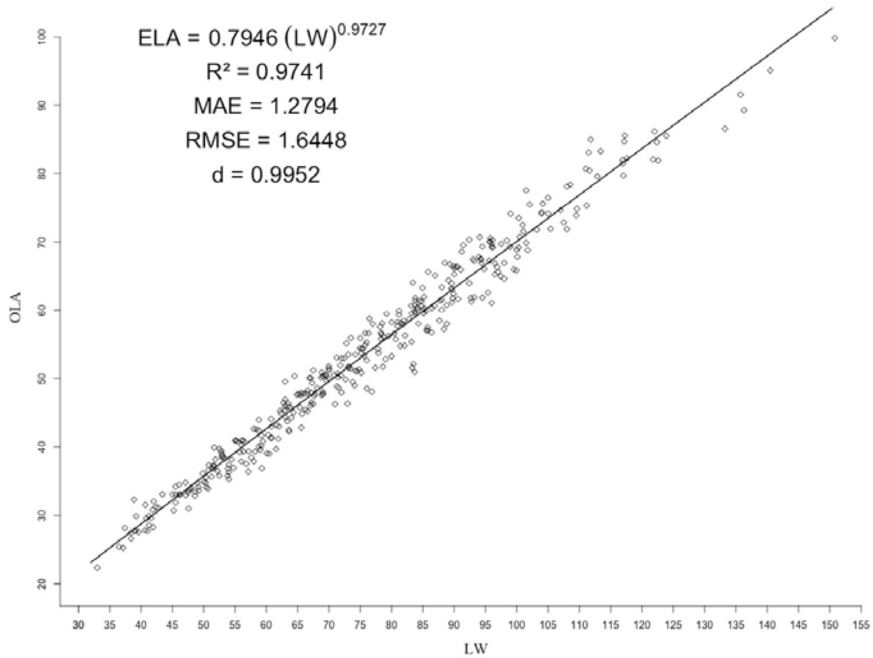

Fig. 3. Equation of power model, determination coefficient (R^2), the mean absolute error (MAE), the root mean square error (RMSE) and index d, using the foliar area observed (OLA) as dependent variable, in function of the product of the length and width (LW) of leaflets of *Acacia mangium* Willd.

Comment [GV17]: I would omit this Fig. because we don't see your point here.

4. CONCLUSION

~~The equation models generated with only a linear measure of the leaflet (L or W) were not adequate for the estimation of the leaf area of Acacia mangium Willd. for failing to meet the statistical parameters established in this study.~~

The power model equation represented by $\mathrm{ELA} = 0.7946(\mathrm{LW})^{0.9727}$, where LW is the multiplication of length and width measurements, is the most adequate to predict the leaf area of *Acacia mangium* Willd. quickly and non-destructively without the need for specific equipment.

Comment [GV18]: We still need for a caliper or a ruler to measure L and W in the field.

Comment [GV19]: Please briefly conclude on the potential application if such models.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rossi LMB, Azevedo CP, Souza CR. *Acacia mangium*. Manaus: Embrapa Amazônia Ocidental. 09-10. 2003

2. Vidal WN, Vidal MRR. Botânica organografia: quadros ilustrados de fanerógamas. Ed.UFV, v.4, p.78-80. 2006.

3. Sacinelli TS, Ribeiro Jr. ES, Dias LE, Lynch LS. Symptoms of nutritional deficiency in seedlings of *Acacia holosericea* submitted to absence of macronutrients. Revista Árvore. 2004; 28: 173-181. http://www.scielo.br/pdf/rarv/v28n2/20981.pdf

4. Lopes CM, Andrade I, Pedroso V, Martins S. Empirical models for leaf area estimation of the grapevine cv. Jaen. Ciência e Técnica Vitivinícola. 2004; 19(2): 61-75. http://www.scielo.mec.pt/pdf/ctv/v19n2/v19n2a02.pdf

5. Mota CS, Leite HG, Cano MAO.Equations to estimate leaf area of *Acrocomia aculeta* leaflets. Pesquisa Florestal Brasileira. 2014; 34(79): 217-224. http://doi.org/10.4336/2014.pfb.34.79.684

6. Partelli FL, Vieira HD, Detmann E, Campostrini E. Estimative of leaf foliar area of *coffea canephora* based on leaf length. Revista Ceres. 2016; 53(306): 204-210.

7. Toebe M, Cargnelutti Filho A, Loose LH, Heldwein AB, Zanon AJ. Leaf area of snap bean (*Phaseolus vulgaris* L.) according to leaf dimensions. Semina: Ciências Agrárias; 33(1): 2491-2500, 2012. DOI: 10.5433/1679-0359.2012v33Supl1p2491

8. Carvalho JO, Toebe M, Tartaglio FL, Bandeira CT, Tambara AL. Leaf area estimation from linear measurements in different ages of *Crotalaria juncea* plants. Anais da Academia Brasileira de Ciências. 2017; 89 (3): 1851-1868. http://doi.org/10.1590/0001-3765201720170077

9. Oliveira PS, Silva W, Costa AAM, Schmildt ER, Vitória EL. Leaf area estimation in litchi by means of allometric relationships. Revista Brasileira de Fruticultura. 2017 39(Special): 1-6. http://doi.org/10.1590/0100-29452017403

10. Oliveira VS, Hell LR, Santos KTH, Pelegrini HR, Santos JSH, Oliveira GE, Nascimento AL, Santos GP, Schmildt O, Czepak MP, Arantes SD, Alexandre RS, Schmildt ER. Estimation of Leaf Area of Jackfruit Through Non-destructive Method. Journal of Agricultural Science. 2019; 11(6): 77- 85. https://doi.org/10.5539/jas.v11n6p77

11. Toebe M, Souza RR, Mello AC, Melo PJ, Segatto A, Castanha AC. Leaf area estimation of squash ‘Brasileirinha’ by leaf dimensions. Ciência Rural. 2019; 49(4): 1-11. http://dx.doi.org/10.1590/0103-8478cr20180932

12. Leite MLMV, Lucena LRR, Cruz MG, Sá Júnior EH, Simões VJLP. Leaf area estimate of *Pennisetum glaucum* by linear dimensions. Acta Scientiarum. Animal Sciences. 2019; 41: 1-7. http://dx.doi.org/10.4025/actascianimsci.v41i1.42808

13. Ribeiro AMS, Mundim DA, Mendonça DC, Santos KTH, Santos JSH, Oliveira VS, Santos GP, Rosa LVCA, Santana WR, Schmildt O, Vitória EL, Schmildt ER. Leaf Area Estimation of Garden Boldo From Linear Dimensions. Journal of Agricultural Science. 2019; 11(4): 461-469. https://doi.org/10.5539/jas.v10n12p272

14. Pompelli MF, Santos JNB, Santos MA. Estimating leaf area of Jatropha nana through non-destructive allometric models. AIMS Environmental Science. 2019; 6(2): 59–76. http://www.aimspress.com/journal/environmental

15. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift. 2014; 22(6): 711-728. https: //doi.org/10.1127/0941-2948/2013/0507

16. Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. The ImageJ Ecosystem: An Open Platform for Biomedical Image Analysis. Molecular Reproduction and Development. 2015; 82 (7-8); 518–529. https:// doi.org/10.1002/mrd.22489

17. Willmott CJ. On the validation of models. Physical Geography. 1981; 2(2): 184-194. https://doi.org/10.1080/02723646.1981.10642213

18. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. 2018. Vienna, Austria.

19. Ferreira EB, Cavalcanti PP, Nogueira DA. Package 'ExpDes.pt'. 2018.

20. Levine DM, Stephan DF, Szabat KA. Estatistic for managers using Microsoft Excel: Global edition (8th ed., p. 728). London: Person. 2017.

21. Blanco FF, Folegatti MV. Estimation of leaf area for greenhouse cucumber by linear measurements under salinity and grafting. Scientia Agricola. 2005; 62(4): 305-309 http://doi.org/10.1590/S0103-90162005000400001

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