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Allometric models for non-destructive leaf area estimation of *Jatropha curcas*

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ABSTRACT

We evaluated the current used allometric models and proposed a reliable and accurate model using non-destructive measurements of leaf length (L) and/or width (W) for estimating the leaf area of the Jatropha plant (*Jatropha curcas* L.). For model construction, a total of 1200 leaves were randomly selected from different levels of the tree canopies and encompassed the full spectrum of measurable leaf sizes (0.19–367 cm^2). Power models better fit the Jatropha leaf area (LA) compared to linear models; however, when the LW was used, the intercept was removed from the linear model to precisely estimate LA. To validate these models, two independent data sets of 300 leaves were used. We demonstrate that the currently used models are biased and underestimate the area of Jatropha leaves. We developed an unbiased, single power model ($Y = x^{\beta 1}$) based on two leaf dimensions [$LA = (LW)^{0.9660}$] with high precision and accuracy as well as a random dispersion pattern of the residuals. The model may be simplified by using only one leaf dimension ($Y = x^{\beta 1}$), $LA = L^{1.9644}$ or $LA = W^{1.8929}$. However, when either W or L alone was used as the single leaf dimension, the power model predicted the LA with good accuracy at the expense heteroscedastic residual dispersion behavior.

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1. Introduction

Jatropha curcas L. is a multipurpose, drought resistant, and perennial eudicotyledon plant belonging to Euphorbiaceae family [1]. It has gained importance for the production of biodiesel [2,3] because their seeds contain between 25 and 32% oil [4]. Seed production ranges from about 0.4 to over 12 t $ha^{-1} y^{-1}$ after five years of growth [5–7]. In addition, Jatropha plants present a potential for soil erosion control, mainly in arid environments [8]. The native distributional of the species is Mexico, Central America, Brazil, Bolivia, Peru, Argentina and Paraguay [6,7,9], although it currently has a pantropical distribution. Until now, *Jatropha curcas* has been an undomesticated plant, although first breeding programs are recently published [10,11]. *Jatropha* plants can grow without irrigation in a broad spectrum of rainfall regimes from 250 to up to 3000 mm per year [6,12]. Jatropha trees grows well on poor stony soils, and, therefore, it is recommended for cultivation on degraded soils [1] in tropical and subtropical regions of the world [13].

Leaf area (LA) is a key variable for most agronomic and physiological studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration and responses to fertilizers and irrigation [14]. Plant yield and quality are affected by photosynthesis and transpiration rate, which are closely related to LA; making LA is a key variable in

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most developed models to simulate carbon and water dynamics [15,16]. Precisely determination of LA is necessary because LA is a fundamental component of crop growth models [17,18]. To evaluate continuous changes in LA and the subsequent growth, a modeling approach is essential [18]. Moreover, accurate estimation of LA is thus crucial to understanding and modeling ecosystem function [19].

New instruments, tools and machines such as hand scanners and laser optic apparatuses have been developed for LA measurements. These tools are expensive and complex devices for basic and simple studies [20]. Allometry establishes quantitative relations between some key characteristic dimensions (usually fairly easy to measure; *e.g.*, length, width, diameter) and other properties not easily determinate directly (*e.g.*, leaf area, mass, volume) [18]. Therefore, models for the non-destructive prediction of the leaf area, *i.e.* allometry, are useful tools for researchers in horticultural experiments. For example, such models enable researchers to measure leaf area on the same plants during the plant growth period and that may reduce variability in the experiments [21]. The use of simple linear measurement for predicting the LA of horticultural plants eliminates the need for costly leaf area meters [19,22,23]. A modeling approach involving linear relationships between LA and one or more dimensions of the leaf is a cheap, rapid, reliable and a non-destructive alternative for an accurate LA measurement [19]. Non-destructive methods do not require leaves to be detached and reduce the variability associated with destructive sampling procedures, which is important because measurements must be repeated during the plant's growth period [23,24]. Somehow, the development of statistical regression models from linear leaf measurements to predicting total or individual leaf area has been shown to be very useful in studying plant growth and development [16,20,21,24–27].

However, in many studies the adequacy of the assumptions model to estimate the LA has not been carefully examined. In this regard, small or minor violations of the underlying assumptions can invalidate the inferences drawn from the analysis [28]. There are two published methods to estimate LA from leaf dimensions of the *J. curcas* [16,29]. Severino and coworkers [29] estimated a power model in which the LA (dependent variable) can be calculated by the equation $LA = 0.84\,(WL)^{0.99}$, where WL is the product of the leaf length (L) and width (W) (independent variables). Another equation also proposed by those authors is $LA = W^{1.87}$ [29]. Achten and coworkers [16] proposed another allometric model to predict leaf area of Jatropha [*i.e.*, $LA = 0.803\,(WL)^{0.985}$]. However, these equations were chosen based on the highest coefficient of determination value (R^2) [29] and/or calculated F and residual plots, without assessing their accuracy [16]. Thus, a simple, rigorously tested, and accurate model for LA estimation of the Jatropha leaf area is necessary. In this study, we evaluate the current used models [16,29] and propose a reliable and accurate models using non-destructive measurements of L and/or W for estimating the LA of the *Jatropha curcas* L.

2. Materials and methods

The experiment was carried out in two experimental cultivations of the Jatropha in the Atlantic rain forest region (09° 28 S; 35° 51′ W, 39 m a.s.l.) and a semi-arid region (09° 32′ S; 36° 38′ W, 240 m a.s.l.) in June 2008 (rain season) and February 2009 (drought season). Each Jatropha plantation had plants that were at least 5 years old obtained from seed bank, encompassing a wide genetic variability. The spacing between plants was 2 × 2 m, summing up about 2500 plants per ha. Each plant received 170 g of phosphorus as ordinary superphosphate, only at planting. Gypsum was used to equilibrate the rates of $S\text{-}SO_4$ carried by the P fertilizer. The N and K_2O rates were 350 and 170 g per plants, applied as urea and potassium chloride, respectively. These applications were once time per year in the rainy season.

For model construction, a total of 1200 healthy leaves were harvested in rainy ($n = 600$) and drought season ($n = 600$) at the two places described above. The leaves were randomly sampled from different parts of the plants and measured to develop the best fitting model to predict the Jatropha leaf area. The maximum leaf length (L) (from lamina tip to the point of the petiole intersection to the midrib) and leaf width (W) (the widest linear length perpendicular to the midrib) were measured to the nearest of 0.1 cm, as proposed to rose [30]. The leaves were then scanned using a scanner (Genius 1200 × 1200 dpi) and imagens were analyzed using the Image-Pro® Plus software (version 4.5, Media Cybernetics, Silver Spring, USA). The leaves encompassed the broadest range as possible. The minimum leaf area sampled was 0.19 cm^2 and maximum was 366.98 cm^2 (Table 1).

Several linear and non-linear regression models using L and/or W dimensions and observed LA were run for each rain regime and experimental place. The equality of a set of linear regression models was examined using the test for model identity (slopes and intercepts) [31]. Here, we present only power and linear relationships because they better fit the Jatropha LA than other tested models. Statistical criteria for model selection were based on the F-test, coefficient of determination, stability and standard error of estimates and dispersion pattern of residuals. These criteria allowed us to evaluate the occurrence of bias and the precision and accuracy of the models [32].

Table 1 – Means ± standard deviations (SD), minimum (min) and maximum (max) values for the leaf length (L) and width (W) and leaf area (LA) of the *Jatropha curcas* L.

Water regimes	L (cm)			W (cm)			LA (cm^2)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Rain	8.42 ± 5.05	0.5	20.6	8.94 ± 5.77	0.5	23.6	86.09 ± 91.70	0.19	366.98
Drought	8.11 ± 3.55	0.7	18.4	8.86 ± 4.15	0.6	20.5	73.18 ± 54.86	0.31	321.03

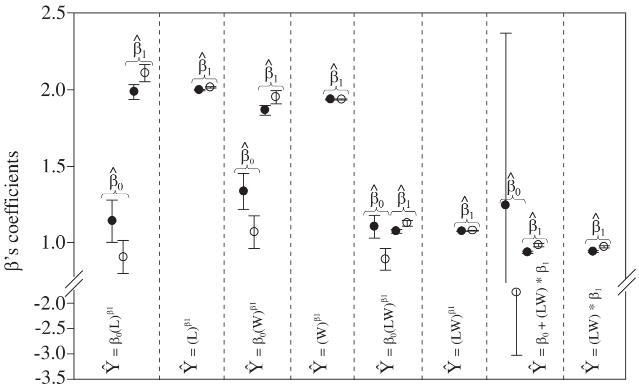

Fig. 1 – Statistical comparison of the β_0 and β_1 coefficients estimated using different models in distinct water regimes. Rain (black circles; $n = 600$), drought (white circles; $n = 600$). Vertical bars denote 99% confidence interval for β coefficients means.

For model validation, distinct leaves were used from different Jatropha plants growing in drought (March 2009) and rainy (July 2009) seasons in same conditions of those used in model construction. Two sets of the 300 leaf samples were measured as described in the calibration model. For validation procedure, the previous selected models were re-estimated with new, validating samples and the data was compared to calibrating models. Estimated leaf area (ELA) obtained by using the model was plotted against observed leaf area (OLA). To compare the ELA to OLA, graphical procedures described by Bland and Altman [33] were used.

All the statistical analyses were performed using the software Statistica version 8.0 (StatSoft, Tulsa, OK, USA), DataFit version 8.0.32 (Oakdale Engineering, Oakdale, PA, USA) and Minitab 14 (Minitab Inc., State College, PA, USA).

3. Results

Individualized models for each rain regime was estimated (Fig. 1). Separate regression models that estimate LA from L, W and the LW product did not differ significantly ($\alpha = 0.05$) among rain regimes for tested models developed here (data not shown). Therefore, data for these rain regimes were pooled and single regression models were fit to the combined data (Table 2). Regression analysis showed that there were strong relationships between LA and L, W, and the product of L and W, which were good parameters that explain the major part of total variation for leaf area. The coefficient of determination adjusted for the degrees of freedom (R^2_a) for all tested models was highly significant, exceeding 96.8% (Table 2). Accurate analysis of model deviation showed that power models [$LA = 0.84\ (LW)^{0.99}$] and [$LA = (W)^{1.87}$] [29] or [$LA = 0.803\ (LW)^{0.985}$] [16] have good precision but were biased, which lead to a significant underestimation of LA. The model developed by Achten et al. [16] underestimate, in average, the LA in about 10.99%, while the models developed by Severino et al. [29] underestimate, in average, the LA in 4.54 or 5.76% depending on used equation.

Table 2 – Statistical models, regression coefficients, standard errors of estimates (SE), coefficients of determination adjusted for the degrees of freedom (R^2), degrees of freedom of residuals (R-d.f.), residual sum of squares (R-SS), calculated F (F_{calc}), *P* value, and equations of leaf area as a function of linear dimensions of leaves (length, L, and width, W) of the *Jatropha curcas* L.

Model	Coefficients	SE	R^2_a	R-d.f	R-SS	F_{calc}	P	Estimator of LA (Ŷ)
$Y = \beta_0 x^{\beta 1} e_i$ (1)	$\beta_0 = 1.0671$	0.0369	0.9683	1198	6,670,904	36,599	< 0.0001	$\hat{Y} = 1.0671^*(L)^{1.9400}$
	$\beta_1 = 1.9400$	0.0131						
$Y = x^{\beta 1} e_i$ (2)	$\beta_1 = 1.9644$	0.0014	0.9682	1199	6,670,234	36,514	< 0.0001	$\hat{Y} = (L)^{1.9644}$
$Y = \beta_0 x^{\beta 1} e_i$ (3)	$\beta_0 = 1.0966$	0.0290	0.9819	1198	6,764,639	65,029	< 0.0001	$\hat{Y} = 1.0966^*(W)^{1.8595}$
	$\beta_1 = 1.8595$	0.0096						
$Y = x^{\beta 1} e_i$ (4)	$\beta_1 = 1.8929$	0.0010	0.9817	1199	6,763,359	64,410	< 0.0001	$\hat{Y} = (W)^{1.8929}$
$Y = \beta_0 x^{\beta 1} e_i$ (5)	$\beta_0 = 0.9798$	0.0207	0.9888	1198	6,812,203	105,909	< 0.0001	$\hat{Y} = 0.9798^*(L^*W)^{0.9698}$
	$\beta_1 = 0.9698$	0.0039						
$Y = x^{\beta 1} e_i$ (6)	$\beta_1 = 0.9660$	0.0004	0.9888	1199	6,812,140	105,911	< 0.0001	$\hat{Y} = (L^*W)^{0.9660}$
$Y = \beta_0 + \beta_1 x + e_i$ (7)	$\beta_0 = 0.8592$	0.3424	0.9883	1198	6,808,746	101,311	< 0.0001	$\hat{Y} = 0.8592 + 0.8282^*(L^*W)$
	$\beta_1 = 0.8282$	0.0026						
$Y = \beta_1 x + e_i$ (8)	$\beta_1 = 0.8329$	0.0018	0.9883	1199	6,808,323	100,859	< 0.0001	$\hat{Y} = 0.8329^*(L^*W)$
$Y^{\#} = \beta_0 x^{\beta 1}\ e_i$	$\beta_0 = 0.803$	0.040	0.9897	87	–	8992.1	< 0.0001	$\hat{Y} = 0.803^*(L^*W)^{0.985}$
	$\beta_1 = 0.985$	0.010						
$Y^* = \beta_0 x^{\beta 1}\ e_i$	$\beta_0 = 0.84$	–	0.98	248	–	–	–	$\hat{Y} = 0.84^*(L^*W)^{0.99}$
	$\beta_1 = 0.99$	–						
$Y^* = x^{\beta 1}\ e_i$	$\beta_1 = 1.87$	–	0.97	248	–	–	–	$\hat{Y} = (W)^{1.87}$

[#] and [*] refers to equations proposed by Achten et al. [11] and Severino et al. [24], respectively.

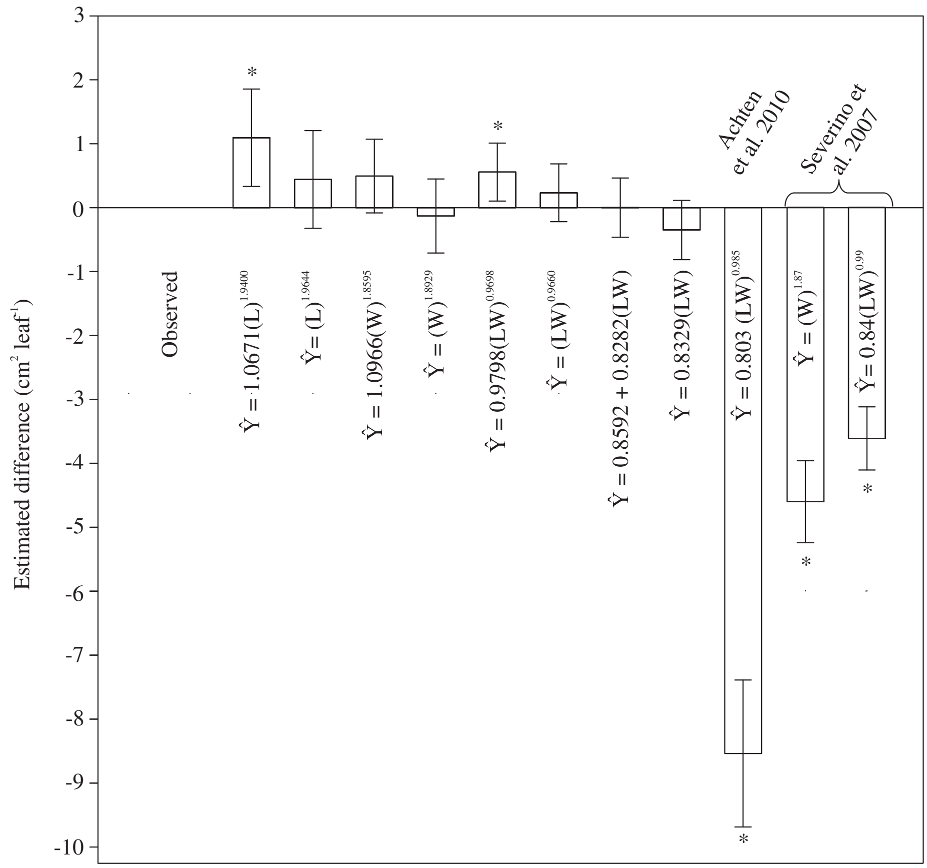

Fig. 2 – Statistical analysis of the deviation of the estimated area from the observed area for an individual leaf. Leaf area for the purging nut (*Jatropha curcas* L.) was estimated using several models in which β_0 and β_1 are coefficients. Vertical bars denote means and spreads denote 95% confidence intervals of the difference. L, length; W, width (see further details in the text).

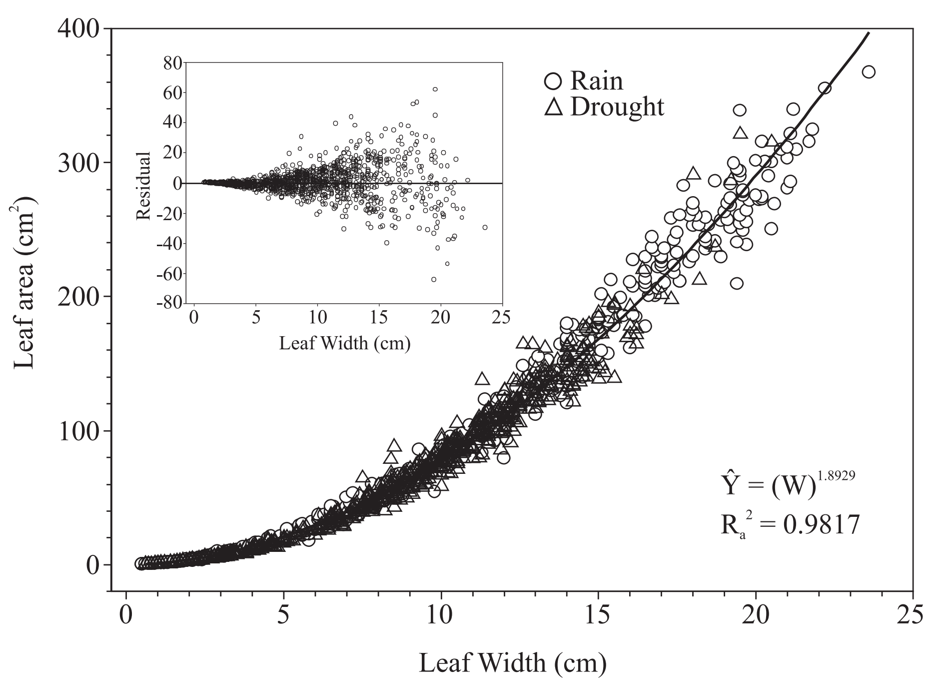

Fig. 3 – The relationship between estimated leaf area and leaf width (W) for purging nut (*Jatropha curcas* L.) using a power model. In the insets, dispersion pattern of residuals relative to the model $Y = (W)^{\beta 1}$.

Of the eight developed models (Table 2), the best ones were determined according to selection criteria described in materials and methods. For the Jatropha LA estimation, equations (1) and (5) had an estimated difference significantly different from zero (biased) (Fig. 2) and, for this reason, were eliminated from further considerations. Equations (3) and (7) had low stability of the coefficients and high deviation of parameters estimators; however this deviation was not different from zero (Figs. 1 and 2). Nonetheless, power models using single leaf dimensions (shown only by W but also valid to L) and the dispersion pattern of residuals did not follow a Normal distribution (*i.e.*, a heteroscedastic behavior) (Fig. 3). Equation (7) also had good precision, but its bias led to an overestimation of LA in function of β_0, especially for small leaves (Fig 4A, inset).

The best estimation of LA of Jatropha leaf was obtained using the power model from LW [$LA = (LW)^{0.9660}$; Equation. (6)]. This model had a high R^2_a, accuracy and precision and lack of bias (Table 2; Figs. 1, 2 and 4B), and it could rigorously estimate LA regardless of leaf dimensions. Of the single-variable power models, those incorporating width (Equation. (4)) had greater coefficients of determination and smaller standard errors of estimates (SE) than models using length alone (Table 2). Therefore, a simplification of the LW power model was conducted with a single leaf dimension, either W ($LA = W^{1.8929}$) or L ($LA = L^{1.9464}$). When developing such a model, we noted that the residual scatter plot had heteroscedastic behavior (Fig. 3). In this context, the ordinary least squares method to estimate the model coefficients cannot be reliably used unless the corrective action is applied to remove the heteroscedasticity

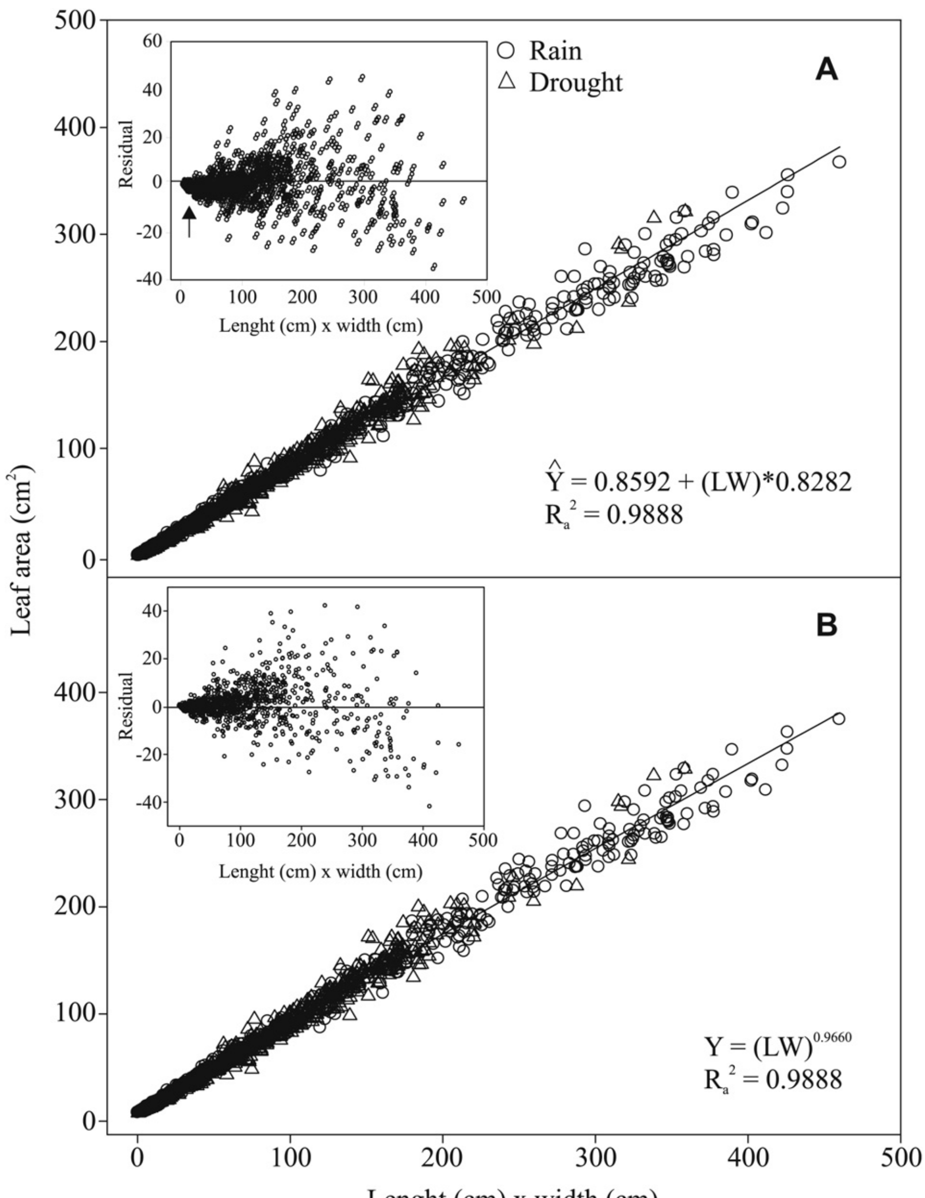

Fig. 4 – The relationship between estimated leaf area and leaf dimensions (L, length and W, width) for both seasons using a linear (A) and power (B) model. The analysis of dispersion pattern of residuals for the respective models is shown in the insets. Arrow indicates an overestimating area for small leaves when using the linear model (A, inset).

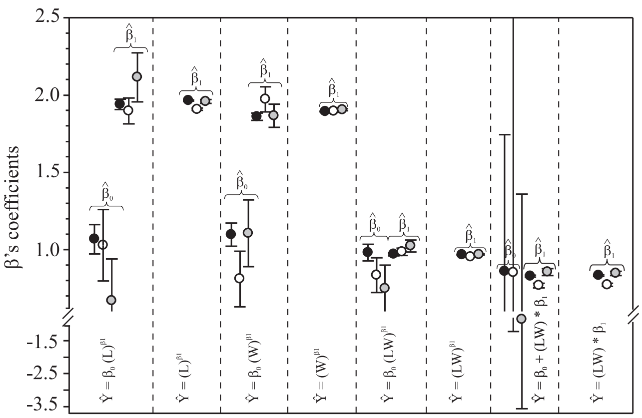

Fig. 5 – Statistical comparisons of the β_0 and β_1 coefficients at different models using the calibration (pooled both seasons; black circles; $n = 1200$) and the validations (drought; white circles or rainy; grey circles; $n = 300$) data sets. Vertical bars represent the 99% confidence intervals for the β coefficient means.

[28]. Transformation of data can be performed using logarithmic transformation to stabilize residual variance; however, the logarithmic can difficult the estimation processes. The developed power models based on single-dimension factors compared with the either LW power or linear models without intercept models showed a slight decrease in R^2_a and lower precision but had similar accuracy (Table 2, Fig. 1).

For validating such models, we re-estimated them with distinct samples (Fig. 5). Some models do not show a good stability of estimated parameters (different mean and/or high deviation) *i.e.*, Equations. (1), (3), (5) and (7). At the same time as, power models [$Y = X^{\beta1}$, where X = W, L or LW; Equations. (2), (4) and (6), respectively] or linear models without constants [$Y = X^*\beta_1$; Equation. (8)] had a high stability of estimated parameters.

In the model validation, correlation coefficients showed that there was a highly reliable relationship between ELA and OLA values (Fig. 6). Correlation coefficients between them for models using L, W or LW were 0.9728, 0.9890 and 0.9904, respectively (Fig. 6).

Because we calibrated the model using large number of sample, the minimum sample size for developing such a model was evaluated. This was performed using the Minitab software that randomly selected 10 samples for each predefined sample size, and the parameter β_1 were estimated using model ($Y = X^{\beta1}$) and compared to entire data set β_1 variability. It diminishes as increasing sample size Fig. 7. In fact, the indicated sample size varies with the error desired.

4. Discussion

In this paper, we described how LA can be accurately estimated from simple non-destructive measurements of Jatropha leaves, for which a linear and power model incorporating either the leaf L alone or both leaf L and W together were developed. We also demonstrated that the currently used linear allometric models for estimating the area of a Jatropha leaves [16,29] are inappropriate. The equations proposed by

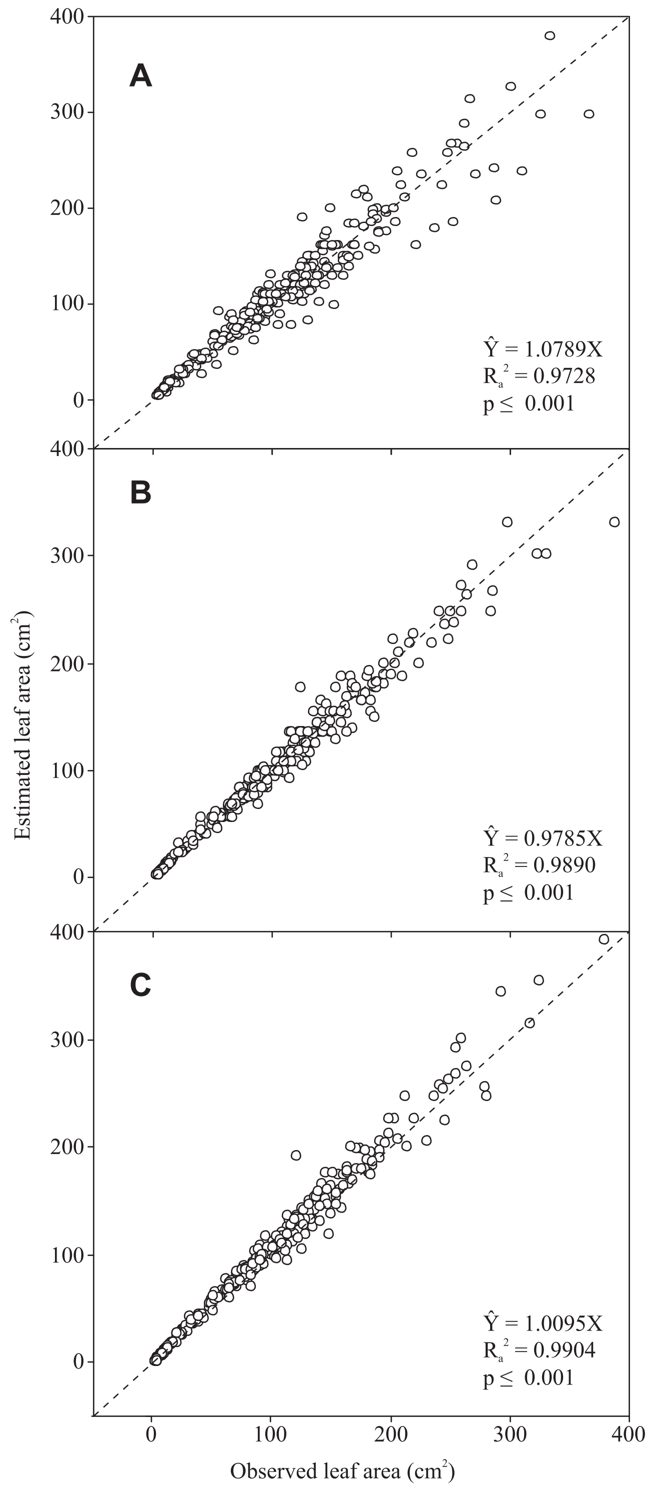

Fig. 6 – The relationship between estimated and observed leaf area for the purging nut (*Jatropha curcas* L.). Leaf area was estimated using power models to L [$\hat{Y} = (L)^{1.9644}$; A], W [$\hat{Y} = (W)^{1.8929}$; B] or LW [$\hat{Y} = (L^*W)^{0.9660}$; C] (for more details, see Table 1). Dotted line represents the 1:1 relationship. $n = 600$.

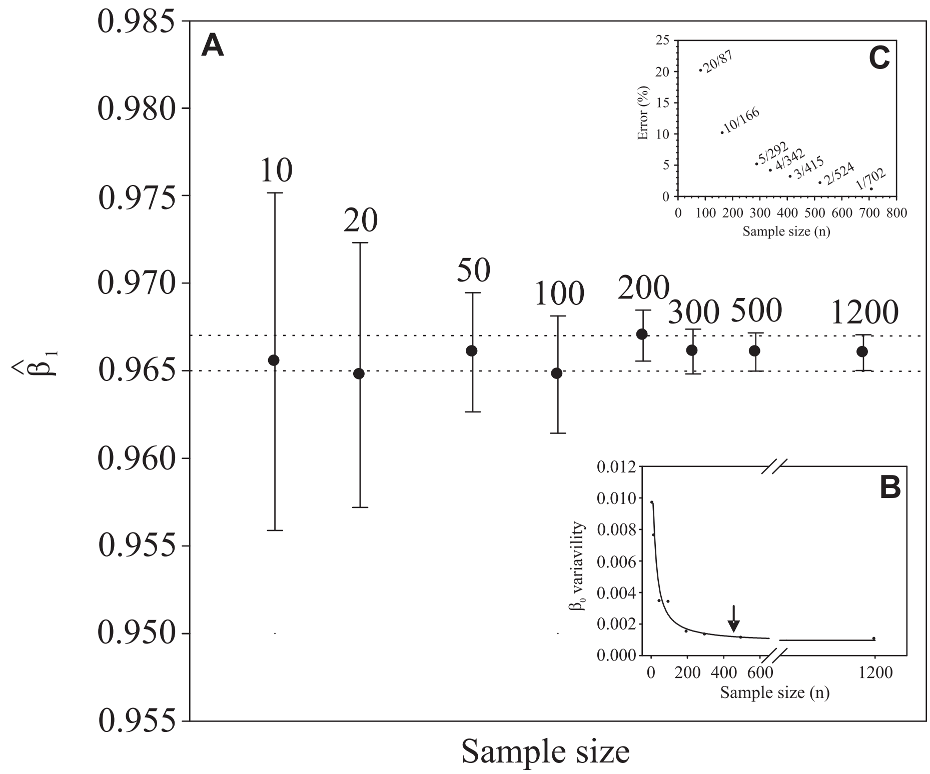

Fig. 7 – β_1 estimation in the model $Y = X^{\beta 1}$ as function of sample size. Vertical bars represent the 99% confidence intervals for the β_1 coefficient means. The numbers above the bars denote the sample size ($n = 10$) (A). The entire data set was composed by a sample of 1200 leaves. In the inset (bottom), 2nd order inverted polynomial regression for β_1 deviation as a function of leaf size showing the approximate minimum sample size (indicated by the arrow) to develop a regression model to reliably predict the area of purging nut (B). In the inset (top), estimated error in function of sample size. The numbers above dots denotes % of error/estimated sample size (C).

Severino et al. [29] or Acthen et al. [16] to predict LA of the Jatropha are simple with relatively high R_a^2 and high precision, albeit these equations were developed using a small sample size [250 or 89 leaves, respectively to Severino et al. [29] or Acthen et al. [16]] and were not validated with a independent sample of leaves. Using a small sample, the estimation is not accurate enough (Fig. 7C), and therefore may result in an underestimation of LA (Fig. 2). It is worth to note that, in this paper, we used at least 1200 leaves to build the models and two sets of 300 leaves were used to validate these models. The amplitude of leaves used in this paper were 0.19–366.98 cm^2 compared to 7.33–215.92 cm^2 used in model proposed by Severino et al. [29]; these differences may be responsible for the lowering of R^2 (<0.98) and a false perception of accuracy [28]. Based on the fact that variance (and variability) diminishes with increasing sample size, we suggest, based on natural variability found for Jatropha leaves, which is feasible to reduce the sample size with some penalty of increasing estimated error. The minimum value of 415 leaves is necessary for reasonable error and good precision of LA estimation for Jatropha (Fig. 7). Our models showed that the equations proposed to estimate LA are reliable independent of leaf size. This finding is different from those reported by Severino et al. [29] and Oliveira and Santos [26] for Jatropha or vineyards leaves, respectively, in which the models are able to accurately calculate the leaf area only when the leaf reaches a certain developmental stage.

The power models based on leaf L or W was considered the most adequate for estimating LA in other perennial crops, such as black pepper [34], grapevine [35], dwarf coconut tree [36] and pecan [37]. Furthermore, when using the W in power model, there is no need to measure L; this is particularly noteworthy because W is meticulously measured using an imaginary perpendicular line to the leaf L and the fully expanded Jatropha leaves are not flat, both which can cause inaccurate measurements. These facts must be judiciously considered when measuring W to obtain reliable LA estimations using the LW model. This problem was also described by Torri et al. [37] in relation to leaf area estimation of pecan cultivars. Williams and Martinson [35] reported that single-variable models could avoid issues of collinearity between leaf W and L. When we use LW as variable, we took into consideration that it is one variable, *i.e.* the product of both dimensions.

We show that a single-dimension of either L or W had relatively high R_a^2 value and low residual sum of squares (R-SS) but a non-Normal residual scatter plot (Table 2, Fig. 3). These features can invalidate such models [28]. Notwithstanding, the models involving both dimensions had higher coefficients of determination than the models involving only one dimension.

Thus, the power model based on two leaf dimensions could be used to estimate LA with high precision and accuracy (Fig. 1), especially for $Y = (X)^{\beta 1}$, which is the best model for estimating leaf area compared to $Y = \beta_0(X)^{\beta 1}$. The combined absence of bias, homoscedastic residual scatter, high stability (low deviation and similar average values from calibration and validation) of estimated coefficients and the absence of other penalties observed in other models confidently estimates the coefficient means. Based on these analyses, we propose a model where $\hat{Y} = (LW)^{0.9660}$ for estimation of Jatropha leaf area. In other words, for such models, there is high probability that estimated parameters are closest to the true value. Irrespective of leaf measurements and developmental stages or growing environments, we demonstrated that LW power models $[Y = (LW)^{\beta 1}]$ are simple and robust non-destructive tools for leaf area estimation for the *Jatropha curcas* L.

The introduction of a constant (the intercept) in linear models creates difficulty in estimating LA [28], particularly for the smaller, expanding leaves (Fig. 4A), which can be safely neglected with increasing leaf size (Fig. 4A, inset). The bias can be corrected by the elimination of intercept (Equation. (8); Table 2). This method was used by Antunes et al. [19] to estimate leaf area for several genotypes of coffee plants. Because of this, polynomial models including the β_0 parameter (constant) are not adequate for estimating leaf area because the model is not biologically valid if β_0 is different from zero.

5. Conclusion

In this study, very close relationships were found between the actual leaf area and the predicted leaf area by the model. A rapid and simple model was developed to predict the leaf area for the Jatropha, $LA = (LW)^{0.9660}$. This model was chosen for their simplicity and capacity to produce results with the same level of accuracy as other more complex estimation models or expensive equipment. Dimensions of the leaves can be easily measured in the field, greenhouse and/or pod experiments. Use of this equation would enable researchers to make non-destructive or repeated measurements of the same leaves. The current model used to estimate leaf area of the Jatropha underestimate LA, thus, in this study, we propose a new method that more accurately estimates LA as well as propose reliable evaluation of sample size for this kind of study.

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