









Leaf area estimation of squash 'Brasileirinha' by leaf dimensions

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ABSTRACT: The objectives of this work were estimate the leaf area of squash 'Brasileirinha' by linear dimensions of the leaves and check models available in the literature. An experiment was conducted in the 2015/16 sowing season. Were collected 500 leaves and in each one, were measured the length (L), width (W) and length×width product (LW) and determined the real leaf area (LA). Then, 400 leaves were separated to generate models of the leaf area (LA) as a function of linear dimension (L, W or LW) of squash. The remaining 100 leaves were used for the validation of models. A second experiment was conducted in the 2016/17 sowing season. Were collected 250 leaves, used only for the validation of the models of the first experiment. There is collinearity between L and W and, therefore, models using the LW product are not recommended. The model $LA=0.5482W^2 + 0.0680W$ ($R^2=0.9867$) is adequate for leaf area estimation of squash 'Brasileirinha'.

Key words: Cucurbita moschata, image processing, non-destructive method, mathematical models.

Estimação da área foliar de abobrinha 'Brasileirinha' por dimensões foliares

RESUMO: Os objetivos deste trabalho foram estimar a área foliar de abobrinha 'Brasileirinha' por dimensões lineares das folhas e testar modelos disponíveis na literatura. Foi conduzido um experimento na safra 2015/16 sendo coletadas 500 folhas. Em cada folha foram mensurados comprimento (L), largura (W), calculado produto comprimento×largura (LW) e determinada a área foliar real (LA). Depois, 400 folhas foram separadas para a geração de modelos da área foliar real (LA) em função da dimensão linear (L, W ou LW) de abobrinha. As demais 100 folhas foram utilizadas na validação dos modelos. Um segundo experimento foi conduzido na safra 2016/17, no qual foram coletadas 250 folhas utilizadas na validação dos modelos gerados no primeiro experimento. Existe colinearidade entre L e W e, por isso, os modelos que utilizam o produto LW não são recomendados. O modelo $LA=0,5482W^2+0,0680W$ ($R^2=0,9867$) é adequado para a estimação de área foliar de abobrinha 'Brasileirinha'.

Palavras-chave: Cucurbita moschata, processamento de imagens, método não destrutivo, modelos matemáticos.

INTRODUCTION

The squash 'Brasileirinha' is a cultivar of *Cucurbita moschata* species, which presents bicolor fruits utilized for ornamental and fresh consumption purposes, containing beta and alpha-carotene, and lutein (BOITEUX et al., 2007). According to the authors, the cultivar was originated from the cross between the Mocinha cultivar and an access of bicolor fruits with peel featuring remarkable bicolor coloring (yellow in the insertion area and green in the distal position of the fruit). Furthermore, according to BOITEUX et al. (2007), plants from this cultivar

show rusticity, indeterminate and prostrate growth, and retuse shaped leaves, with toothed leaf margin and discrete or absence hairiness.

Leaf area is often utilized for measuring plant growth, being directly related to photosynthesis and transpiration rate, among other physiological processes. In this sense, BLANCO & FOLEGATTI (2005) highlighted that the leaf area is a key variable in studies of plant growth, light interception, photosynthetic efficiency, evapotranspiration, and fertilizers and irrigation responses. As stated in FAVARIN et al. (2002), leaf area is used as yield indicator and it can be useful for crop technical

evaluations, as in sowing density, irrigation, fertilization, and application of agrochemicals.

Direct or indirect methods can be used to measure leaf area of a particular crop. Among the indirect methods, there are mathematical models that relate leaf area with the leaf linear dimensions, such as length, width, or the product of both. In this method, initially the linear dimension's measurements of a set of leaves and their respective real leaf areas are performed for subsequent generation of models which enable the prediction of the real leaf area as a function of the linear dimensions. Computational resources that allow evaluating intact and damaged leaves can be used in order to determine the real leaf area (VIEIRA JÚNIOR et al., 2006). Furthermore, digital image analysis has been identified as an effective way of replacing the standard LI-COR® method (ADAMI et al., 2008).

Mathematical models of the real leaf area as a function of the leaf linear dimensions may be generated, validated, and applied in field measurements at different plant development and growth stages in a nondestructive way with low cost and high precision. In this sense, models have been developed for fruit trees, vegetables and ornamentals crops such as cucumber (BLANCO & FOLEGATTI, 2003; 2005; CHO et al., 2007), tomato (BLANCO & FOLEGATTI, 2003), squash (*Cucurbita pepo* L.) 'Afródite' (ROUPHAEL et al., 2006), hazelnut (CRISTOFORI et al., 2007), melon (LOPES et al., 2007), favabean (PEKSEN, 2007), kiwi (MENDOZA-DE GYVES et al., 2007), small fruits (FALLOVO et al., 2008), ginger (KANDIANNAN et al., 2009), bedding plants (GIUFFRIDA et al., 2011), squash (*Cucurbita moschata*) 'Japonesa' (GRECCO et al., 2011), snap beans (TOEBE et al., 2012), *Vitis vinifera* L. (BUTTARO et al., 2015), *Plumeria rubra* L. (FASCELLA et al., 2015) and apricot cultivars (CIRILLO et al., 2017). Other crops of agricultural, and commercial interest as coffee (FAVARIN et al., 2002; ANTUNES et al., 2008), maize (VIEIRA JÚNIOR et al., 2006), soybean (ADAMI et al., 2008), jatropha (POMPELLI et al., 2012), gladiolus (SCHWAB et al., 2014), Persian walnut (KERAMATLOU et al., 2015), and jack bean (CARGNELUTTI FILHO et al., 2015) were also studied to generate models of leaf area estimation.

Leaf shape is a specific morphological trait of each plant species and the ratio between linear dimensions and leaf area depends on the amount of indentations in the edge of leaf blade, among other factors (PINTO et al., 2008). As plant species and even cultivars within the same species have certain trait patterns of leaf morphology, generating specific

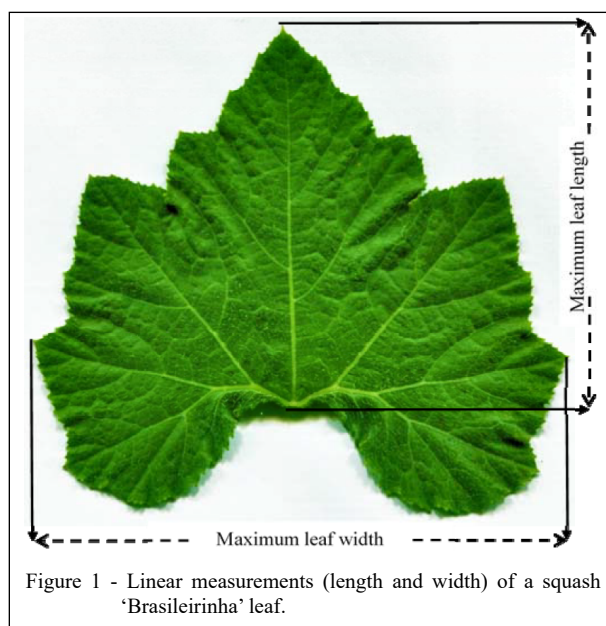
models of leaf area estimation is required. Thus, this research aimed to estimate the leaf area of squash 'Brasileirinha' as a function of linear dimensions of leaves and check models available in the literature.

MATERIALS AND METHODS

Two experiments were carried out with squash (*Cucurbita moschata*) Brasileirinha cultivar, in experimental area located at latitude of 29°09'S, longitude of 56°33'W, and altitude of 74 m. According to Köppen climate classification, the climate of the region is Cfa, subtropical humid. The type of soil is classified as Haplic Plinthosol (SANTOS et al., 2013). In the experimental area, two sites with 20 m long, 1.20 m wide, and 0.25 m tall were prepared. Liming was carried out in these sites to increase the pH=6.0 and subsequent fertilizers incorporation, according to soil analysis and recommendations for squash (CQFS, 2004), with 30 kg ha⁻¹ of N, 180 kg ha⁻¹ of P₂O₅, and 130 kg ha⁻¹ of K₂O as basic fertilization and 30 kg ha⁻¹ of N as topdressing fertilization.

In the first experiment, 'Brasileirinha' squash seeds were sown on 12/Sept/2015 in expanded polystyrene trays with 72 cells using MacPlant® commercial substrate and maintained in a protected environment with periodic irrigations. Seedlings were transplanted on 05/Oct/2015, when the seedlings had three expanded leaves at 23 days after sowing, in two interspersed rows, with spacing of 0.80 m between plants and 1.50 m between rows, totaling 13 plants in a row and 12 plants on the other row, 25 plants per plot, totaling 50 plants. In the second experiment, squash seeds were sown on 26/Oct/2016 and transplanted on 23/Nov/2016, at 28 days after sowing. The cultural practices were carried out uniformly across the experimental area and irrigation was carried out with a drip irrigation system in both experiments.

In the first experiment, in full female flowering and early fruiting at 72 days after transplantation, 500 leaves were collected randomly throughout the experimental area. In the second experiment, at 63 days after transplantation, 250 leaves were collected randomly throughout the experimental area. In each leaf, length (L) and width (W) were measured with a millimeter ruler (Figure 1). Thereafter, the length×width product (LW) was calculated and the real leaf area (LA) of each one of the 750 leaves was determined through digital images. For this, leaves were placed in sequence on the EPSON scanner, Perfection V33/V330 model and scanned with a resolution of 240dpi and 300dpi, respectively, in the first and



second experiment. Thereon, these digital images were processed with Digimizer v.4.5.2® software (MEDCALC SOFTWARE, 2018) for the real leaf area quantification. From the 500 measured leaves in first experiment, 400 leaves were randomly separated (80% of collected leaves) to generate models and 100 leaves (20% of collected leaves) to proceed the validation of the models. The 250 leaves collected in the second experiment were used only in the validation of the models generated in the first experiment.

For data of length, width, length×width product, and leaf area of leaves used for the generation and validation of models, measures of central tendency, dispersion, and distribution were calculated, normality was verified through the Kolmogorov-Smirnov test, and frequency histograms and scatter plots were constructed. Hereafter, real leaf area (LA) determined by image processing, was modeled in function of L or W and/or LW through the models: linear ($LA=a+bx$), quadratic ($LA=a+bx+cx^2$), and power ($LA=ax^b$), wherein these models, x is the linear dimension of the leaf (L, W or LW). In linear and quadratic models, the intercept was equals to zero (linear coefficient $a=0$), whereas when a linear dimension (L, W or LW) is zero, the estimated leaf area will also be zero, as indicated by SCHWAB et al. (2014).

In the models generated using the LW product of the leaf, was performed the diagnosis of collinearity based on the Variance Inflation

Factor: $VIF=1/(1 - r^2)$ and in the Tolerance $T=1/VIF$ (CRISTOFORI et al., 2007; FALLOVO et al., 2008; TOEBE & CARGNELUTTI FILHO, 2013; BUTTARO et al., 2015), where r^2 is the coefficient of determination of the linear regression between L and W. $VIF > 10$ and $T < 0.10$ is consider severe collinearity and the use of the two variables (length and width) is not recommended in the generation of the model. In this condition, one of the variables should be eliminated as described by CRISTOFORI et al. (2007), FALLOVO et al. (2008), TOEBE & CARGNELUTTI FILHO (2013) and BUTTARO et al. (2015).

Validation of leaf area estimation models was performed based on 100 values of leaf area estimated by the model (LAE_i) and 100 observed values (LA_i) in first experiment and based on 250 LAE_i and 250 LA_i in second experiment. In each model, a simple linear regression ($LAE_i=a+bLA_i$) of leaf area estimated by the model (dependent variable) in function of the observed leaf area (independent variable) was adjusted. The hypotheses $H_0: a=0$ versus $H_1: a \neq 0$ and $H_0: b=1$ versus $H_1: b \neq 1$ were tested through the Student t-test at 5% probability. Following, the linear correlation coefficients of Pearson (r) and determination (R^2) between LAE_i e LA_i were calculated. For each model, mean absolute error (MAE), root mean square error (RMSE) and the index d (WILLMOTT, 1981) were calculated, as detailed by TOEBE et al. (2012). After, the model proposed by GRECCO et al. (2011) for squash

(*Cucurbita moschata*) ‘Japanese’ was tested, being held the replacement of slope and linear coefficients in relation to the original proposal of the authors and the validated model was $LA=6.7940+0.8259LW$. The model $LA=4.77+0.61W^2$ was also tested, as proposed for squash (*Cucurbita pepo* L.) ‘Afrodite’ by ROUPHAEL et al. (2006).

In order to select the leaf area estimation models for squash ‘Brasileirinha’, the following criteria were utilized: linear coefficient not different of zero, slope coefficient not different from one, linear correlation coefficients of Pearson and determination coefficient closer to one, mean absolute error and root mean square error closer to zero and d index closer to one (TOEBE et al., 2012). Statistical analyzes were performed using Microsoft Office Excel® application and Statistica 12.0® software (STATSOFT, 2015).

RESULTS AND DISCUSSION

The period of days for full flowering and early fruit was greater than the period reported by BOITEUX et al. (2007) in the first experiment, which may be due to the growing region, the low luminosity and high rainfall rates of the 2015/2016 growing season in southern Brazil, under *El niño* weather conditions. In the second experiment at 2016/2017 growing season, the full flowering and early fruit was similar with the reported by BOITEUX et al. (2007). Mean and median values were similar to each other for all measured variables (length, width, length×width product, and leaf area) for generation and for the validation of the models based in data from the two experiments (Table 1), indicating adequate data distribution. Furthermore, only small deviations of the data regarding to asymmetry ($-0.78 \leq \text{assimetry} \leq 0.41$) and kurtosis ($-0.88 \leq \text{kurtosis} \leq 0.73$) were observed, wherein normality of data ($P > 0.05$) was verified in all cases using the Kolmogorov-Smirnov test.

Collecting leaves of different sizes is required to generate models with large possibilities of use. In this sense, leaves with great amplitude were used for each measured variable to generate models ($2.80 \text{ cm} \leq \text{length} \leq 16.90 \text{ cm}$, $3.40 \text{ cm} \leq \text{width} \leq 22.80 \text{ cm}$, $9.52 \text{ cm}^2 \leq \text{length} \times \text{width} \leq 385.32 \text{ cm}^2$, and $7.57 \text{ cm}^2 \leq \text{real leaf area} \leq 296.60 \text{ cm}^2$) (Table 1). Leaves with wide amplitude were also used for the validation of the models in 2015/2016 and 2016/2017 growing season ($2.40 \text{ cm} \leq \text{length} \leq 14.80 \text{ cm}$, $2.70 \text{ cm} \leq \text{width} \leq 21.00 \text{ cm}$, $6.48 \text{ cm}^2 \leq \text{length} \times \text{width} \leq 310.80 \text{ cm}^2$, and $5.79 \text{ cm}^2 \leq \text{real leaf area} \leq 240.99 \text{ cm}^2$). Regarding to variability, greater coefficient of variation (CV) scores were observed for length×width product and

real leaf area ($30.46\% \leq CV \leq 49.53\%$) compared to that observed for length and width ($15.82\% \leq CV \leq 27.47\%$), both for leaves used for generation as for leaves used in the validation. Similarly, TOEBE et al. (2012) obtained higher CV scores for length×width product and leaf area in relation to the length and width of snap bean leaves. In jack bean, CARGNELUTTI FILHO et al. (2015) also found greater variability for the real leaf area (CV=49.84%) in relation to leaf width (CV=29.84%).

The proper adjustment of the data to the normal distribution and the high amplitude of leaf size (Table 1) contributed to generate reliable models with wide application. Moreover, the number of leaves used for generate models (n=400 leaves) was higher than that used by ROUPHAEL et al. (2006) in squash ‘Afrodite’ (n=329 leaves) and used by GRECCO et al. (2011) in squash ‘Japonesa’ (n=20 leaves). This number of leaves also exceeds n=200 leaves, which is indicated in sample sizing studies to generate mathematical models in coffee (ANTUNES et al., 2008) and jack bean (CARGNELUTTI FILHO et al., 2015). Likewise, it is close to n=415 leaves, indicated for jatropha (POMPELLI et al., 2012).

Linear associations between length and width and, length×width product and real leaf area were found in data utilized in the model’s generation and validation (Figure 2). For the other associations, nonlinear patterns were visually identified and, therefore, models of different types were generated and validated. Considering leaf length as an explanatory variable for the prediction of real leaf area (LA), the power model ($LA=1.0196L^{2.0432}$, $R^2=0.9723$) presented the best adjustment, followed by the quadratic model ($LA=1.0751L^2+0.5383L$, $R^2=0.9613$) (Figure 3a). When the explanatory variable was leaf width, the power model ($LA=0.5966W^{1.9706}$, $R^2=0.9919$) also provided the best adjustment, followed by the quadratic model ($LA=0.5482W^2+0.0680W$, $R^2=0.9867$) (Figure 3b). In the cases where the models have been generated considering length×width as the explanatory variable, similarity of prediction of the three model types (Figure 3c) was found, being that the power ($LA=0.7393LW^{1.0135}$, $R^2=0.9925$), quadratic ($LA=0.00005LW^2+0.8003LW$, $R^2=0.9871$), and linear model ($LA=0.7918LW$, $R^2=0.9871$) presented high reliability.

Mathematical models of linear, quadratic, and power types of leaf area estimation by linear dimensions (L, W, or LW) were also generated in other crops such as cucumber (BLANCO & FOLEGATTI, 2003; 2005; CHO et al., 2007), tomato (BLANCO & FOLEGATTI, 2003), hazelnut (CRISTOFORI et al., 2007), fava bean

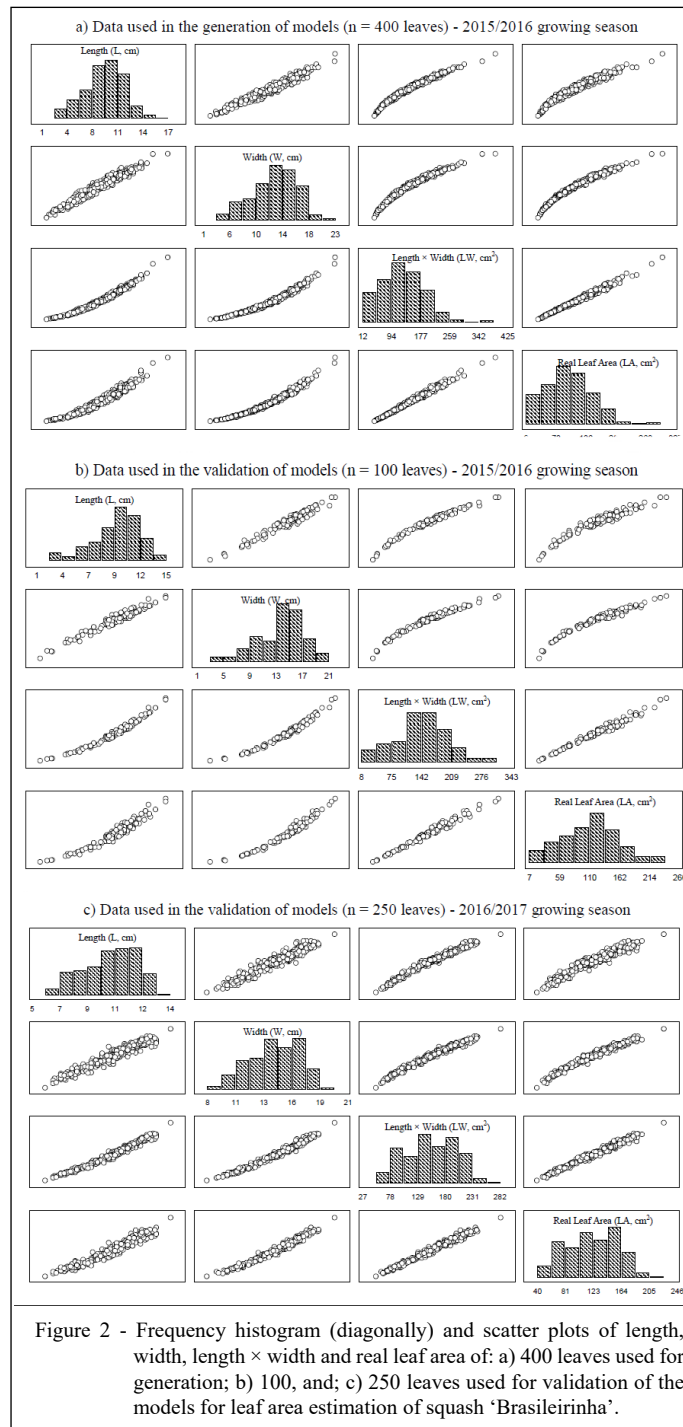
Table 1 - Statistics for the variables: length, width, length×width product and real leaf area of leaves used for generation and for validation of the estimation models for leaf area estimation of squash 'Brasileirinha'.

Statistics	Length (L, cm)	Width (W, cm)	Length×Width (LW, cm ²)	Real Leaf Area (LA, cm ²)
-----Data used in the generation of models (n=400 leaves) - 2015/2016 growing season-----				
Minimum	2.80	3.40	9.52	7.57
Maximum	16.90	22.80	385.32	296.60
Mean	9.05	12.90	125.05	98.97
Median	9.20	13.30	122.76	96.79
Variance	5.87	12.55	3767.01	2402.75
Standard Deviation	2.42	3.54	61.38	49.02
Coefficient of variation	26.79	27.47	49.08	49.53
Standard Error	0.12	0.18	3.07	2.45
Asymmetry ⁽¹⁾	-0.23 ^{ns}	-0.35*	0.41*	0.36*
Kurtosis ⁽²⁾	-0.20 ^{ns}	-0.31 ^{ns}	0.28 ^{ns}	0.11 ^{ns}
P-value of K-S ⁽³⁾	>0.20	>0.10	>0.20	>0.20
-----Data used in the validation of models (n=100 leaves)- 2015/2016 growing season-----				
Minimum	2.40	2.70	6.48	5.79
Maximum	14.80	21.00	310.80	240.99
Mean	9.66	13.78	141.64	111.95
Median	10.05	14.50	144.63	114.05
Variance	6.22	12.52	3950.14	2528.38
Standard Deviation	2.49	3.54	62.85	50.28
Coefficient of variation	25.82	25.68	44.37	44.92
Standard Error	0.25	0.35	6.29	5.03
Asymmetry ⁽¹⁾	-0.70*	-0.78*	0.07 ^{ns}	0.02 ^{ns}
Kurtosis ⁽²⁾	0.54 ^{ns}	0.73 ^{ns}	0.03 ^{ns}	-0.19 ^{ns}
P-value of K-S ⁽³⁾	>0.10	>0.15	>0.20	>0.20
-----Data used in the validation of models (n=250 leaves) - 2016/2017 growing season-----				
Minimum	6.20	8.20	50.84	38.97
Maximum	14.10	20.10	283.41	226.76
Mean	10.30	14.49	152.98	120.95
Median	10.45	14.70	152.95	121.46
Variance	2.73	5.55	2169.91	1438.59
Standard Deviation	1.65	2.36	46.58	37.93
Coefficient of variation	15.82	16.02	30.46	31.23
Standard Error	0.10	0.15	2.95	2.40
Asymmetry ⁽¹⁾	-0.34*	-0.34*	-0.08 ^{ns}	-0.05 ^{ns}
Kurtosis ⁽²⁾	-0.71*	-0.74*	-0.83*	-0.88*
P-value of K-S ⁽³⁾	>0.15	>0.05	>0.20	>0.15

⁽¹⁾*Asymmetry differs from zero by the t-test at 5% probability level. ^{ns}non-significant. ⁽²⁾*Kurtosis differs from zero by t-test at 5% probability level. ^{ns}non-significant. ⁽³⁾P-value of the normality test of Kolmogorov-Smirnov.

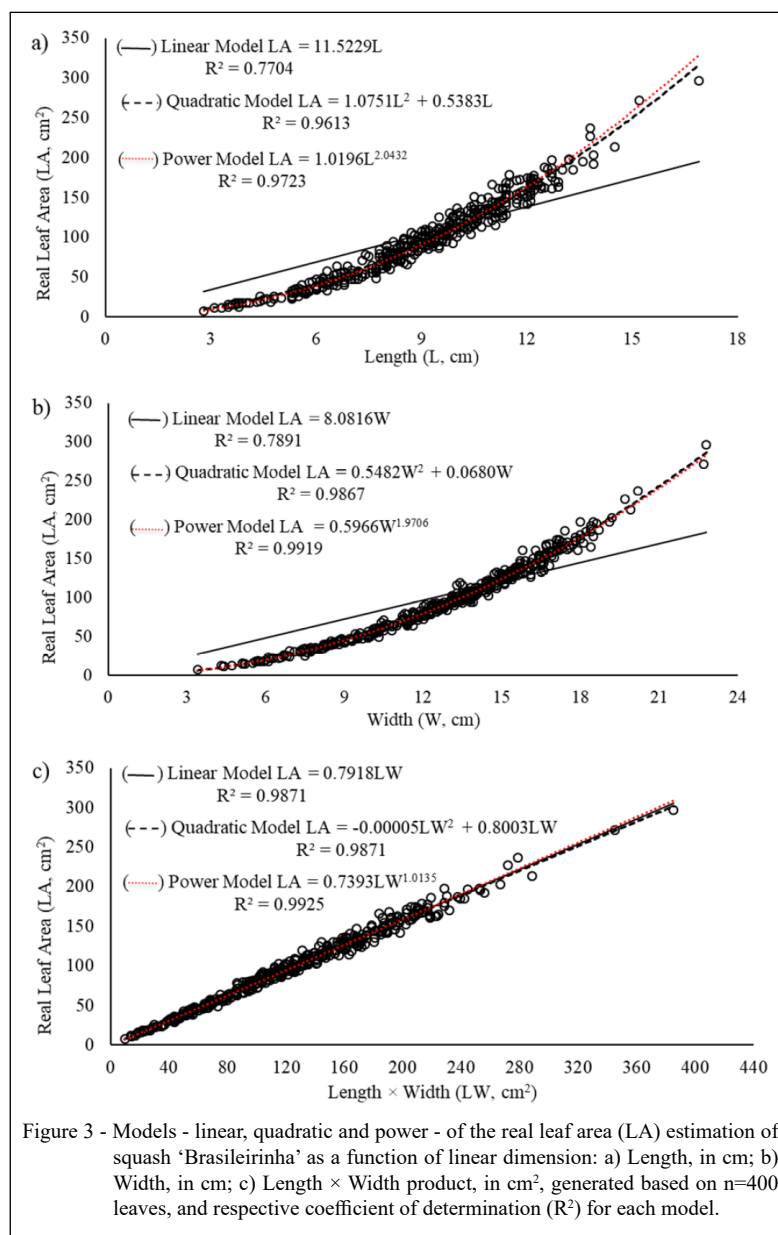
(PEKSEN, 2007), melon (LOPES et al., 2007), kiwi (MENDOZA-DE GYVES et al., 2007), small fruits (FALLOVO et al., 2008), ginger (KANDIANNAN et al., 2009), bedding plants (GIUFFRIDA et al., 2011), squash (*Cucurbita moschata*) 'Japonesa' (GRECCO et al., 2011), snap bean (TOEBE et al., 2012), coffee (ANTUNES et al., 2008), maize (VIEIRA JÚNIOR et al., 2006), soybean (ADAMI et al., 2008), jatropa

(POMPELLI et al., 2012), gladiolus (SCHWAB et al., 2014), jack bean (CARGNELUTTI FILHO et al., 2015), *Vitis vinifera* L. (BUTTARO et al., 2015), *Plumeria rubra* L. (FASCELLA et al., 2015) and apricot cultivars (CIRILLO et al., 2017), with high prediction capacity and reliability, indicating the suitability of the use of indirect and non-destructive methods of leaf area measurement.



Based on the nine generated models (Figures 3a, b, c), there was proper adjustment of power ($0.9723 \leq R^2 \leq 0.9925$) and quadratic ($0.9613 \leq R^2 \leq 0.9871$) models, regardless of the considered linear dimension (L, W, or LW). The linear model presented proper adjustment only in the case

where the independent variable was LW ($R^2=0.9871$). In this study, the linear and quadratic models were generated using the intersection (through the origin), being the most appropriate procedure from a biological point of view (SCHWAB et al., 2014). In the validation phase, six models (quadratic and



power based on length, quadratic based on width, and quadratic, power, and linear based on length×width) exhibited linear coefficients not different from zero in the 2015/16 growing season, indicating that if the leaf area observed is zero, the estimate leaf area will also be close to zero (Table 2). These models also presented slope coefficient no different than one, indicating that increased 1 cm² of observed leaf area results in an increase of approximately 1 cm² in the estimated leaf area. In the 2016/17 growing season, all models exhibited linear coefficients different

from zero and only the power model based on length presented slope coefficient no different than one. These significant deviations are due to the sensitivity of the t-test to the increase in sample size (from 100 to 250 leaves). These six models also presented r and R² closer to one, MAE and RMSE closer to zero and d index closer to one.

Although the power, quadratic and linear models of LA based-on LW have excellent predictive capacity (Figure 3c) and the best precision indicators in the two validation periods (Table 2), was verified

Table 2 - Validation of models based on indicators: linear coefficient (a), slope coefficient (b), Pearson correlation coefficient (r) and determination coefficient (R²), mean absolute error (MAE), root mean square error (RMSE) and d Willmott index (d), calculated based on observed leaf area and estimated leaf area of leaves from squash 'Brasileirinha'.

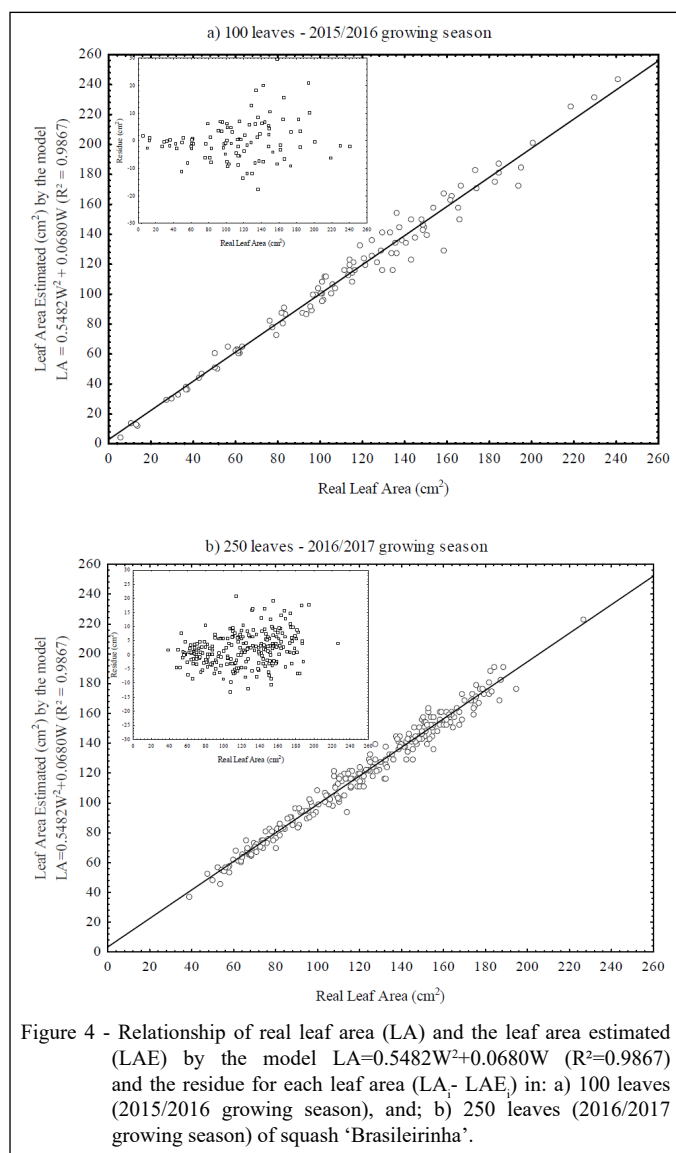
Model Type	Independent variable	a ⁽¹⁾	b ⁽²⁾	r ⁽³⁾	R ²	MAE	RMSE	d
-----Validation of models (n = 100 leaves) - 2015/2016 growing season-----								
1) Linear	Length	49.850*	0.549*	0.960*	0.922	19.367	24.059	0.906
2) Quadratic	Length	5.029 ^{ns}	0.956 ^{ns}	0.972*	0.946	9.431	11.694	0.986
3) Power	Length	0.995 ^{ns}	0.994 ^{ns}	0.972*	0.945	9.737	12.049	0.986
4) Linear	Width	49.545*	0.552*	0.970*	0.943	18.744	23.544	0.910
5) Quadratic	Width	2.958 ^{ns}	0.972 ^{ns}	0.989*	0.978	5.303	7.425	0.994
6) Power	Width	3.806*	0.961*	0.989*	0.978	5.274	7.475	0.994
7) Linear	Length×Width	2.593 ^{ns}	0.978 ^{ns}	0.988*	0.978	5.721	7.504	0.994
8) Quadratic	Length×Width	3.358 ^{ns}	0.972 ^{ns}	0.988*	0.978	5.715	7.482	0.994
9) Power	Length×Width	1.439 ^{ns}	0.988 ^{ns}	0.988*	0.978	5.709	7.550	0.994
LA = 6.7940 + 0.8259LW (Grecco et al. 2011)	Length×Width	9.499*	1.020 ^{ns}	0.988*	0.978	12.187	14.222	0.981
LA = 4.77 + 0.61W ² (Rouphael et al. 2006)	Width	7.598*	1.077*	0.989*	0.978	16.521	18.600	0.969
-----Validation of models (n = 250 leaves) - 2016/2017 growing season-----								
1) Linear	Length	59.963*	0.485*	0.966*	0.935	17.091	20.232	0.872
2) Quadratic	Length	9.264*	0.936*	0.969*	0.939	7.470	9.498	0.984
3) Power	Length	4.531*	0.978 ^{ns}	0.968*	0.939	7.474	9.709	0.984
4) Linear	Width	57.289*	0.494*	0.984*	0.970	16.706	19.848	0.878
5) Quadratic	Width	3.452*	0.955*	0.988*	0.977	4.704	6.092	0.993
6) Power	Width	4.523*	0.943*	0.988*	0.977	4.868	6.314	0.993
7) Linear	Length×Width	4.797*	0.961*	0.989*	0.978	4.358	5.646	0.994
8) Quadratic	Length×Width	5.741*	0.954*	0.989*	0.978	4.393	5.687	0.994
9) Power	Length×Width	3.378*	0.973*	0.989*	0.978	4.336	5.613	0.994
LA = 6.7940 + 0.8259LW (Grecco et al. 2011)	Length×Width	11.797*	1.003 ^{ns}	0.989*	0.978	12.309	13.468	0.970
LA = 4.77 + 0.61W ² (Rouphael et al. 2006)	Width	8.075*	1.058*	0.988*	0.977	15.250	16.569	0.957

⁽¹⁾Linear coefficient differs from zero, according to the t test at P<0.05. ^{ns}non-significant. ⁽²⁾Slope coefficient differs from one, according to the t test at p<0.05. ^{ns}non-significant. ⁽³⁾Pearson correlation coefficient differs from zero, according to the t test at p<0.05. ^{ns}non-significant.

collinearity between L and W. In this sense, the VIF was 22.01 and the tolerance 0.045 between L and W, indicating the existence of serious collinearity problems (CRISTOFORI et al., 2007; FALLOVO et al., 2008; TOEBE & CARGNELUTTI FILHO, 2013; BUTTARO et al., 2015). Therefore, models that consider LW are not recommended to estimate the leaf area of squash 'Brasileirinha'. Among the models that considered only one linear dimension (length or width), superior adjustment was found in the validation of the quadratic model in function of width (LA=0.5482W²+0.0680W). In this model the real and estimated leaf area showed a linear relationship and a well distributed residue, without trends biased in small and large leaves (Figures 4a, b). Thus, considering the

proper adjustment of the model, the measurement simplicity of only one dimension (width) and absence of collinearity, this model is recommended to estimate the leaf area of squash 'Brasileirinha' (Figure 3b, Table 2).

If the researcher only has information of length, the power model (LA=1.0196L^{2.0432}) can be used with proper adjustment and validation criteria compliance (Figure 3a, Table 2). However, in this case the quality indicators are slightly lower than those found for the quadratic model as a function of width. The model's generated for squash 'Japonesa' by GRECCO et al. (2011) based on length×width of the leaves and for squash 'Afrodite' by ROUPHAEL et al. (2006) based on the leaf width had similar patterns among



themselves and were slightly lower than those described for the models recommended in this study. However, considering the problems previously reported on collinearity between L and W, it is not recommended to use the model proposed by GRECCO et al. (2011) to estimate leaf area of squash 'Brasileirinha'.

CONCLUSION

There is collinearity between L and W and, therefore, models using the LW product are not recommended. The model $LA=0.5482W^2+0.0680W$ ($R^2=0.9867$) is adequate for leaf area estimation of squash 'Brasileirinha'.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results

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AUTHORS' CONTRIBUTIONS

All authors conceived and designed experiments. RRS, ACM, PJM, AS and ACC performed the experiments and data collection. MT supervised and coordinated the experiments, performed the image processing, statistical analyses and prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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