Australian Journal of Crop Science AJCS

AJCS 11(12):1534-1538 (2017) ISSN:1835-2707
doi: 10.21475/ajcs.17.11.12.pne662

Non-destructive estimation of leaf area in passion fruit (*Passiflora edulis* L.)

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Abstract

The yellow passion fruit is the most commonly cultivated species of the genus *Passiflora* in commercial plantations in Brazil. Obtaining accurate and easy to perform measurements for determination of leaf area in passion fruit is crucial to understand the interaction between the plant and the environment. The objective of this study was to develop reliable mathematical equations to directly estimate leaf area in passion fruit using linear measurements. Passion fruit leaves may exhibit varying morphology across phenological stages, with lanceolate, bilobed or trilobed leaves. For each morphological pattern, 100 leaves without defects were collected. The real leaf area was measured with a LI-COR 3100 area meter and regression equations were estimated for real leaf area and measurements of length, width, and their corresponding products, using a millimeter ruler. The results showed that determination of leaf area can be easily and accurately performed for passion fruit leaves of varying morphology, using the product of the linear measurements of length and width. The linear equations chosen for determining leaf area through the product of width and length were 0.25 + 0.64x (lanceolate), -4.82 + 0.69x (bilobed) and -0.81 + 0.54x (trilobed).

Key words: indirect method; leaf measurements; leaf morphology; linear models; *Passiflora edulis* L..
Abbreviations: L:_Length; W:_Width; Cwa:_Mesothermal climate; r:_Correlation coefficient; d:_Willmott index; d:_Performace or reliability of the observed data; LAe:_Leaf area estimated; LAr:_Real leaf area; Mlar:_Mean of real areas; β_0:_ linear coefficient; β_1 and β_2:_ slope coefficients; $R^2_{adjus.}$:_ adjusted coefficient of determination.

Introduction

The passion fruit belongs to the genus *Passiflora*, which is the most important genus in the family of Passifloraceae in the domestic fruit market (Ferreira et al., 2016). Among the species of this genus, the passion fruit (*Passiflora edulis* L.) is the most commonly grown for commercial purpose in Brazil (Bernacci et al., 2008). According to Melleti (2011), it is estimated that 95% of Brazilian orchards are planted with passion fruit cultivars, mainly due to high yields, fruit quality, plant vigor and juice yield.

Studies related to growth quantitative analysis on passion fruit species that require non-sophisticated equipment (Morgado et al., 2013) enable the assessment of crop production (Souza et al., 2014) and evaluation of biomass accumulation and its partitioning to different plant parts. The evaluation of leaf expansion is important to understand light interception that influences physiological processes related to respiration, transpiration, photosynthesis, use and mobilization of nutrients flowering, fruiting, and crop yield (Silva et al., 2015; Spann and Heerema, 2010).

Obtaining accurate and easy to perform measurements for determination of leaf area in passion fruit is crucial to understand the effect of the interaction between the crop and the environment on the production processes (Morgado et al., 2013). A number of methods have been developed to estimate leaf area such as direct or destructive methods, comprising the removal of the leaves, and indirect or non-destructive methods, using mathematical models and leaf linear measurements, which facilitates the work in the field (Sachet et al., 2015; Schwab et al., 2014; Tartaglia et al., 2016).

Equational models for determining leaf area have been widely used in fruit crops, standing out the models for peach, banana, fig, guava, olive, citrus and strawberry (Sachet et al., 2015; Silva et al., 2015; Souza et al., 2014; Spann et al., 2010; Zuculoto et al., 2008).

In passion fruit, indirect models to estimate leaf area were developed for eight wild species (Morgado et al., 2013). However, the species *P. edulis* was not included in this study. Therefore, reliable estimating equations are required to describe leaf morphological patterns in passion fruit, since the leaf morphology of commercial species differs from those of wild species.

The development of mathematical models for non-destructive, low cost, accurate, and fast leaf area calculation (Sala et al., 2015) specific for passion fruit is essential, due to the fact that is one of the most economically important species in the family Passifloraceae in Brazil. Therefore, the aim of this study was to develop reliable mathematical equations to indirectly estimate the leaf area in passion fruit using linear measurements and non-destructive procedures.

Results and Discussion

Validation of linear models

Table 1 shows the results of the regression analysis with the linear (β_0) and angular (β_1 and β_2) coefficients of the equations, the value of the F-statistic, and the coefficient of determination (R^2). The use of the greatest linear length and width measurements for the different morphological patterns

Table 1. Values of the estimated linear coefficient (β_0), slope coefficients (β_1 and β_2), standard error of the slope coefficients (values in parentheses) calculated values of F (F_{calc}) and the adjusted coefficient of determination ($R^2_{adjus.}$) to adjusted regression models between the real leaf area (dependent variable) and the linear dimensions for length (L) and width (W) of the morphological patterns of passion fruit leaves.

Lanceolate	β_0	β_1	β_2	F_{calc}	$R^2_{adjus.}$
LA = $\beta_0 + \beta_1$ (L)	-13.80	4.86(0.17)**	-	770.34**	0.8859
LA = $\beta_0 + \beta_1$ (W)	-11.87	8.50(0.29)**	-	856.02**	0.8962
LA = $\beta_0 + \beta_1$ (L × W)	0.25	0.64(0.12)**	-	2610.95**	0.9634
LA = $\beta_0 + \beta_1$(L) + β_2 (W)	-12.74	5.22(1.24)**	1.93(0.71)**	459.35**	0.9025
Bilobed	β_0	β_1	β_2	F_{calc}	$R^2_{adjus.}$
LA = $\beta_0 + \beta_1$ (C)	-63.12	13.38(0.55)**	-	584.96**	0.8550
LA = $\beta_0 + \beta_1$ (L)	40.00	10.43(0.40)**	-	664.00**	0.8700
LA = $\beta_0 + \beta_1$ (C × L)	-4.82	0.69 (0.16)**	-	1836.70**	0.9488
LA = $\beta_0 + \beta_1$(C) + β_2 (L)	54.26	5.97(0.92)**	6.25(1.19)**	434.22**	0.8974
Trilobed	β_0	β_1	β_2	F_{calc}	$R^2_{adjus.}$
LA = $\beta_0 + \beta_1$ (C)	-53.65	12.76(0.43)**	-	847.58**	0.8953
LA = $\beta_0 + \beta_1$ (L)	-38.51	9.10 (0.40)**	-	499.28**	0.8342
LA = $\beta_0 + \beta_1$ (C × L)	-0.81	0.54(0.16)**	-	1130.82**	0.9194
LA = $\beta_0 + \beta_1$(C) + β_2 (L)	-53.15	2.89(0.76)**	9.17(1.02)**	489.40**	0.9079

(** p<0.01)

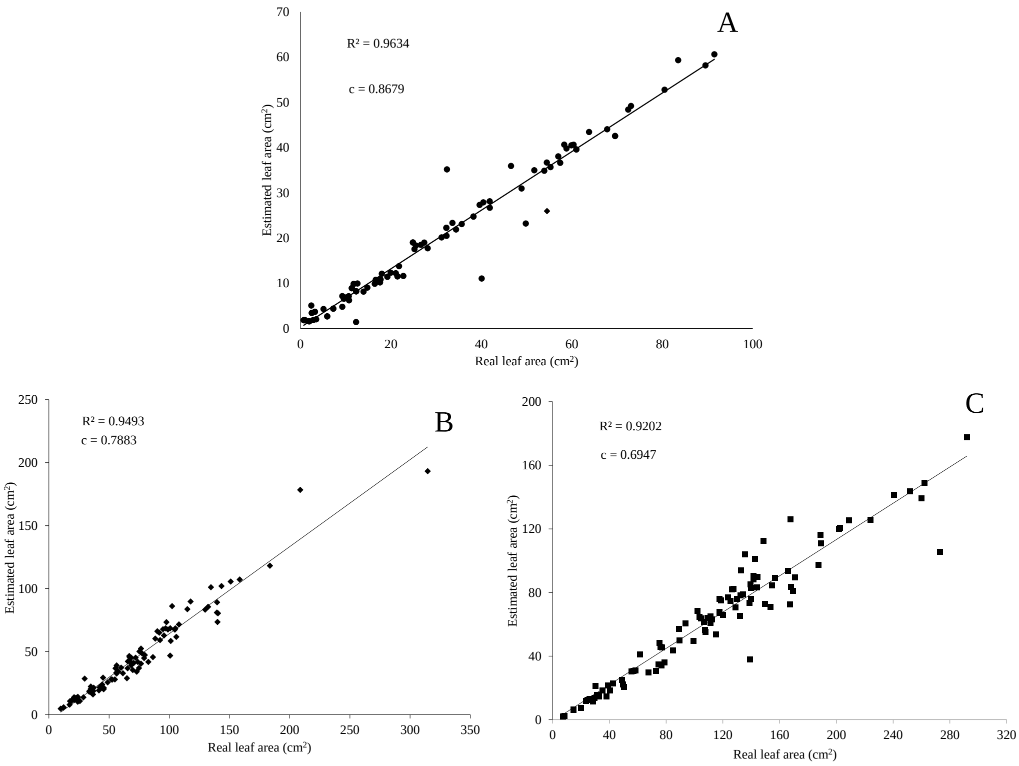

Fig 1. Relationship between real leaf area and estimated leaf area, and their statistical indices: coefficient of determination (R^2) and confidence index regression model (c) for morphological patterns of passion fruit leaves. A: Leaf lanceolate, B: Leaf bilobed and C: Leaf trilobed

Table 2. Pearson's correlation coefficient between actual leaf area and linear dimensions of length, width and the product of length and width of the leaf morphological patterns of passion fruit.

Morphological pattern		Length	Width	Length × Width
Lanceolate		0.9419**	0.9472**	0.9817**
Bilobed	Leaf area	0.9255**	0.9335**	0.9743**
Trilobed		0.9468**	0.9143**	0.9593**

(** p<0.01 – t test).

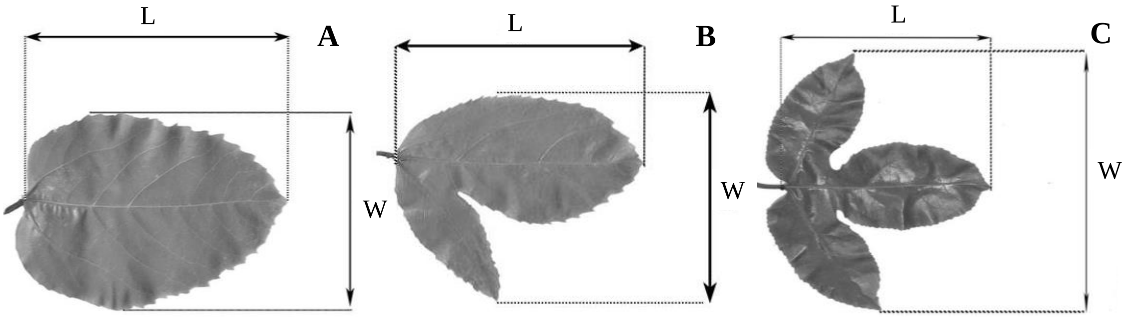

Fig 2. Graphical representation of the length dimensions (L) and width (W) of different leaf morphological patterns observed in passion fruit. A: Leaf lanceolate, B: Leaf bilobed and C: Leaf trilobed.

Table 3. Classification range of the indices of confidence or determination "c" with the respective determinations of performance and interpretation.

Values "c"	Interpretation
>0.85	Excellent
0.76 a 0.85	Very good
0.66 a 0.75	Good
0.61 a 0.65	Average
0.51 a 0.60	Fair
0.41 a 0.50	Poor
<0.40	Very poor

observed in passion fruit leaves afforded a good accuracy in estimating the leaf area.

It was found that for the obtained linear and quadratic models, the relationship between the linear measurements of the length (L) and the width (W), and the product between them (L × W), for determining the leaf area (LA) showed high correlation coefficients (R^2), with values greater than 0.8342. However, according to the high determination coefficients for lanceolate (0.9634), bilobed (0.9488) and trilobed (0.9194) leaves, the model that estimates leaf area using the product between the length and width of leaf was chosen for determining the leaf area in the different morphological patterns of passion fruit leaves (Table 2).

Spann and Heerema (2010) determined the leaf area using non-destructive measurements (linear) in different species of fruit trees (citrus, pear, olive and walnut) and found high R^2 values for the models obtained (ranging from 0.7258 to 0.9340). In fig tree 'Roxo de Valinhos', determination coefficients above 0.6991 were obtained when determining various mathematical models using linear measurements (Souza et al., 2014). In guava (*Psidium guajava*) and peach (*Prunus persica*) leaves, high R^2 values were also observed in the models tested (≥ 0.672 and ≥ 0.7225, respectively), showing that linear measurements can provide highly accurate equations to determine real area rapidly and with low cost (Sachet et al., 2015; Silva et al., 2015). Based on the interpretation criterion of model performance using the statistical indicators proposed by Camargo and Sentelhas (1997) (Table 1), we observed distinct confidence indices among the different morphologies of passion fruit leaves. The equation generated for the lanceolate leaf had the "c" index equal to 0.8679, which is considered "excellent", while the bilobed and trilobed leaves had "c" indices equal to 0.7883 and 0.6947, with "very good" and "good" performances, respectively (Fig 2).

Similarly, Morgado et al. (2013) reported high coefficients of determination in the generated models (≥ 0.772), and the product between the length and the greatest width was chosen to determine directly and easily the leaf area in lanceolate and trilobed leaves of wild Passiflora species. According to the authors, the leaf area is an important parameter in experimental studies of fruit crops because, according to Spann and Heerema (2010), it can influence photosynthesis, transpiration, and leaf expansion, and it affects the growth rate and the quality of production. With the validation of mathematical models using precise and accurate indices, the equations in the study can be used to measure the area of different morphological patterns of passion fruit leaves quickly, accurately and non-destructively, as also observed by Morgado et al. (2013) in obtaining the leaf area of wild Passiflora species.

Validation of equational models through statistical indices has been commonly used in crop species to determine the reliability of the generated equations (Sala et al., 2015). When determining models to calculate the leaf area for fig trees, Souza et al. (2014) found that most of the tested mathematical models showed performance "c" indices considered optimum or good. Similar trends were observed in guava and gladioli, where the confidence indices ranged from 0.636 to 0.9371 (Schwab et al., 2014; Silva et al., 2015), which according to Table 1 are classified with performance ranging from "good" to "excellent".

The Pearson correlation coefficients between observed leaf area and non-linear measurements for the passion fruit are presented in Table 2. Among the measurements, the model considers the product of the length and width with higher correlations, independent of the morphological pattern studied, with values of 0.9817, 0.9743 and 0.9593 for lanceolate, bilobed and trilobed leaves, respectively. The results demonstrate that the increase in the number of lobes in passion fruit leaves provides a reduction in the correlation between the values of leaf area and linear measurements, due to occurrence of major empty spaces between the lobes.

The Pearson correlation coefficients (Table 3) were higher (> 0.9) and positive. According to Partelli et al. (2006), they indicate that the increase in a trait influenced the positive increase in another correlated trait. Buttaro et al. (2015), compared the estimated area using the length or width and found the highest correlation coefficient for the product between the length of the leaf blade and the width in the estimation of leaf area by non-destructive methods in grapevine (*Vitis vinifera* L.).

The leaf area estimation considering the product between the length and the width of the leaf has been recommended for various species. In physic nut (*Jatropha curcas* L.), Pompelli et al. (2012) obtained a model based on the product of the length and width to predict leaf area, highlighting the advantages of the simplicity and speed of the model, allowing evaluation in the field or greenhouse, but with similar precision of estimates obtained by equipment. Antunes et al. (2008) point out that non-destructive models are excellent tools to estimate the leaf area of coffee, applying them in research on the growth and development of plants.

The estimation of leaf area in passion fruit by non-destructive methods at different phenological stages will expand the studies related to severity of disease, pest attack and physiological parameters related to growth without causing destruction of plants taken as experimental plots, resulting in greater accuracy of the results obtained. The determination of leaf area in different morphological patterns (lanceolate, bilobed and trilobed) of passion fruit leaves can be performed simply, accurately and safely using the linear measurements of length and width, and the equation that considered the product between these measurements.

Materials and Methods

Site characterization

The experiment was conducted at the Fruit Breeding Laboratory at the Plant Science Department of the University Federal of Viçosa, Viçosa, Minas Gerais in May 2016. The municipality is georeferenced at coordinates 20º 45'S, 42º 52' W, 648 m altitude, climate Cwa type (mesothermal climate, Koppen), with average annual rainfall of 1.200 mm and average temperature of 19 °C.

Biological material

To determine the linear equations of passion fruit leaves, three leaf morphological patterns observed for this species (lanceolate, bilobed and trilobed) were collected from plants grown in a greenhouse, belong to three commercial varieties already established in the fruit production market (BRS Sol do Cerrado, BRS Gigante Amarelo and FB-200 Yellow Master). Leaves were collected with the greatest variability of size. For each leaf morphological pattern, 100 units were collected without any damage or defects caused by disease, pests or climatic conditions.

Estimation of leaf area

Leaves of each morphological pattern were placed in paper bags and taken to the laboratory, and the real leaf area was measured with a LI-COR 3100 area meter. For each leaf morphology pattern (lanceolate, bilobed and thilobed), the measurements were performed with a millimeter ruler as shown in Figure 1 as follows: the length of the midrib (L) and the largest width of the leaf (W) were measured on lanceolate leaves; the length of the central width (L) and the largest width between the distal end of the lobe to the distal end side without the lobe (W) were measured on bilobed leaves; and the major length of the central width (L) and the largest width between the distal end of the outer lobes (W) were measured on trilobed leaves.

Regression models, validation and statistical analysis

For each leaf morphological pattern, linear models were estimated for the relationship between the real area of each leaf and the linear dimensions of measurements between the length (L) and width (W) and the product of length and width, using the mathematical models: $LA = \beta_0 + \beta_1(L)$; $LA = \beta_0 + \beta_1(W)$; $LA = \beta_0 + \beta_1(L \times W)$ and $LA = \beta_0 + \beta_1(L) + \beta_2(W)$, where LA is the leaf area and represents the dependent variable; β_0, β_1 and β_2 are the parameters to be estimated; 'L' is the independent variable relative to the length of the leaf; and "W" the independent variable referring the width of leaf. To statistically evaluate the performance of mathematical models obtained in different morphological patterns, 100 leaves of each pattern were used to correlate the real leaf area with the estimated leaf area using the linear measurements, based on statistical indicators proposed by Camargo and Sentelhas (1997), defined as follows: the correlation coefficient "r" - determining the accuracy; the Willmott index "d" - that determines the exactness, and the performance or reliability "d" of the observed data. The correlation coefficient "r" allows the quantification of the degree of association between two variables involved in the analysis (real leaf area and estimated leaf area), which indicates the accuracy of the model tested. The concordance index "d" determines the degree of exactness between the variables involved, since it is related to the differences between estimated and observed values, and ranges from 0 (no concordance) to 1 (perfect concordance), according to the methodology proposed by Willmott et al. (1985):

$$d = 1 - \left[\Sigma\left(LAe - LAr\right)^2 / \Sigma\left(|LAe - Mlar|\right) + |LAr - Mlar|\right)^2$$

Where; LAe = Values of leaf area estimated by the developed equation;
LAr = Values observed in the real leaf area;
Mlar = Mean of real leaf areas.

Then, the confidence index or determination "c", which indicates the performance of the models, as proposed for Camargo and Sentelhas (1997), was calculated by the product of the correlation coefficient "r" and the concordance index "d". Table 3 shows the criteria were used to analyze and interpret the performance of the models using the confidence index. The regression and correlation analyses between the real leaf area and linear measurements were performed using the statistical program GENES (Cruz, 2013).

Conclusion

The determination of leaf area in the different morphological patterns (lanceolate, bilobed and trilobed) of passion fruit leaves can be carried out in a simple, precise and safe way using linear measurements between length and width. The equation that considered the product between leaf length and leaf width is the most reliable and accurate to be used as independent variable in leaf area estimation of the different morphological stages of the passion fruit leaf. The linear equations chosen for determining leaf area through the product of width and length were 0.25 + 0.64x (lanceolate), -4.82 + 0.69x (bilobed) and -0.81 + 0.54x (trilobed).

Acknowledgments

The authors would like to thank the Brazilian National Council for Scientific and Technological Development

(CNPq), the Coordination for the Improvement of Higher Level Personnel (CAPES) and Minas Gerais State Research Assistance Foundation (FAPEMIG – CAG – APQ-01655-14) for the grants and the financial support.

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