Original article

A simple and non-destructive model for individual leaf area estimation in citrus

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A simple and non-destructive model for individual leaf area estimation in citrus.

Abstract — Introduction. Leaf area is often related to plant growth, development, physiology and yield. Many non-destructive models have been proposed for leaf area estimation of several plant genotypes, demonstrating that leaf length, leaf width and leaf area are closely correlated. Thus, the objective of our study was to develop a reliable model for leaf area estimation from linear measurements of leaf dimensions for citrus genotypes. **Materials and methods**. Leaves of citrus genotypes were harvested, and their dimensions (length, width and area) were measured. Values of leaf area were regressed against length, width, the square of length, the square of width and the product (length × width). The most accurate equations, either linear or second-order polynomial, were regressed again with a new data set; then the most reliable equation was defined. **Results and discussion**. The first analysis showed that the variables length, width and the square of length gave better results in second-order polynomial equations, while the linear equations were more suitable and accurate when the width and the product (length × width) were used. When these equations were regressed with the new data set, the coefficient of determination (R^2) and the agreement index 'd' were higher for the one that used the variable product (length × width), while the Mean Absolute Percentage Error was lower. **Conclusion**. The product of the simple leaf dimensions (length × width) can provide a reliable and simple non-destructive model for leaf area estimation across citrus genotypes.

Brazil / *Citrus* sp. / linear models / leaves / dimensions / length / width

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Un modèle simple et non destructif pour estimer la surface individuelle des feuilles d'agrumes.

Résumé — Introduction. La surface foliaire est souvent liée à la croissance, le développement, la physiologie et le rendement des plantes. Beaucoup de modèles non destructifs ont été proposés pour estimer la surface foliaire de plusieurs génotypes végétaux ; ils ont montré que la longueur et la largeur des feuilles, de même que la surface foliaire, étaient étroitement corrélées. L'objectif de notre étude a donc été de développer un modèle fiable pour estimer la surface foliaire à partir de mesures linéaires des dimensions de feuilles pour les génotypes d'agrumes. **Matériel et méthodes**. Les feuilles de certains génotypes d'agrumes ont été récoltées et leurs dimensions (longueur, largeur et surface) ont été mesurées. La surface foliaire a été corrélée à la longueur, la largeur, le carré de la longueur, le carré de la largeur et le produit (longueur × largeur). Les équations les plus précises, qu'elles soient linéaires ou polynomiales de second ordre, ont été testées à nouveau avec un nouvel ensemble de données, puis l'équation la plus fiable a été définie. **Résultats et discussion**. La première analyse a montré que les variables longueur, largeur et carré de la longueur donnaient de meilleurs résultats dans les équations polynomiales de second ordre, tandis que les équations linéaires étaient plus appropriées et précises lorsque la largeur et le produit (longueur × largeur) étaient utilisés. Lorsque ces équations ont été utilisées avec le nouvel ensemble de données, le coefficient de détermination (R^2) et l'indice « d » ont été les plus élevés dans le cas de l'équation utilisant le produit (longueur × largeur) comme variable, tandis que le pourcentage moyen d'erreur absolue était alors le plus faible. **Conclusion**. Le produit (longueur × largeur) des simples dimensions de la feuille peut fournir un modèle non-destructif fiable et facile pour estimer la surface foliaire des génotypes d'agrumes.

Brésil / *Citrus* sp. / modèle linéaire / feuille / dimension / longueur / largeur

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1. Introduction

Knowing the leaf area of a specified plant species is important to understand its growth, development and physiology [1]. Leaves of the same canopy often show different rates of photosynthesis and transpiration. Therefore, estimations of leaf area linked to physiological characteristics are essential to define the overall contribution of each canopy portion to fruit quality and yield [2–4].

Generally, leaf area is determined through processes that are time-consuming and destructive [5], which often require the actual area of each leaf. Leaf area estimation may be improved by using multiple regressions with leaf length and width, dimensions that are closely related to the leaf area. Furthermore, a representative leaf sample, consisting of leaves of different sizes, should be used in order to increase the reliability of the model [6, 7].

The main advantages of reliable models for estimating leaf area are the preservation of the canopy because of the possible utilization of the same leaves through equations [8], the lack of expensive equipment and labor, and the ease and rapidity in getting final results [6, 9]. In fact, the utilization of regression equations for estimating leaf area through simple measurements of leaf dimensions is an inexpensive, easy, rapid, accurate and non-destructive alternative for assessing general plant leaf area in the field [5, 9]. However, any proposed model should be validated with different leaf samples before using it for other experiments [6], as the accuracy of the prediction is dependent on the variation of leaf shape amongst species or cultivars [10].

Many non-destructive models have been recommended for estimating individual leaf area from simple measurements for several tree species [4, 5, 7, 8–15], vegetables [2, 16–18], ornamentals [19–21] and other important crops [22], but a leaf area estimation model for citrus genotypes has not been found. The reported models have shown that there are close relationships among leaf width, leaf length and leaf area [4].

The objective of our study was to develop a reliable model for leaf area estimation from linear measurements of leaf dimensions for citrus genotypes, independent of the genetic material.

2. Materials and methods

Six genotypes of citrus were used to develop leaf area estimation models: Buddha's Hand citron [*Citrus medica* var. *sarcodactylis* (Hoola van Nooten) Swingle], Cipo orange [*Citrus sinensis* (L.) Osbeck], Imperial orange (*C. sinensis*), Jaboticaba orange (*C. sinensis*), Faustrine lemon [*Fortunella*

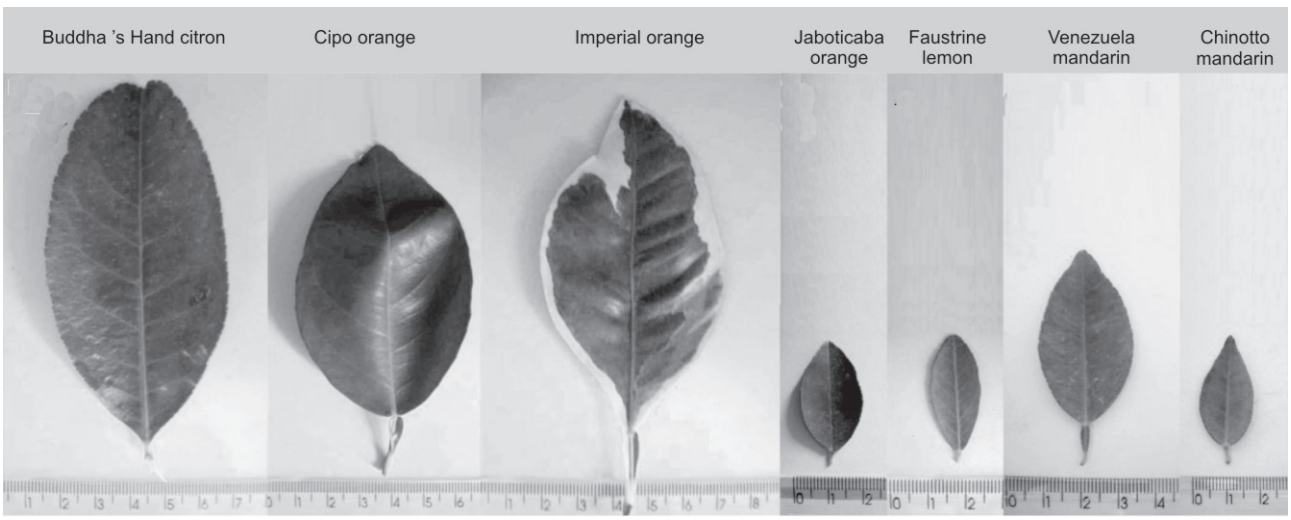

Figure 1.
Leaf visual aspect of seven citrus genotypes used to develop leaf area estimation models in Brazil (Cordeiropolis County, Sao Paulo State).

Table I.
Measured mean, minimum and maximum values of length, width and leaf area for single leaves of citrus genotypes (n = 90 leaves for each genotype, data are mean values ± standard error).

Citrus genotype	Length (cm)			Width (cm)			Leaf area (cm^2)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Buddha's Hand citron	9.38 ± 0.35	3.32	15.64	4.93 ± 0.18	1.80	8.07	38.67 ± 2.59	4.40	93.14
Cipo orange	8.18 ± 0.31	3.00	14.97	4.64 ± 0.21	0.90	8.35	28.84 ± 1.98	2.00	76.25
Imperial orange	8.79 ± 0.31	3.51	16.32	4.72 ± 0.17	1.53	7.00	30.94 ± 1.86	3.64	76.15
Jaboticaba orange	3.62 ± 0.11	1.85	5.75	2.20 ± 0.08	1.03	4.14	6.10 ± 0.43	1.34	16.54
Faustrine lemon	3.40 ± 0.09	1.55	5.05	1.64 ± 0.04	0.81	2.53	3.96 ± 0.19	1.28	8.11
Venezuela mandarin	4.16 ± 0.14	1.86	7.04	2.30 ± 0.09	0.83	4.07	7.08 ± 0.51	1.09	18.84
Chinotto mandarin	2.88 ± 0.07	1.60	4.30	1.52 ± 0.05	0.80	2.52	3.00 ± 0.15	0.81	6.57

sp. Swingle × *Microcitrus australasica* Swingle × *Citrus aurantifolia* (Christm.) Swingle] and Venezuela mandarin (*Citrus reticulata* Blanco) (*figure 1*). These citrus genotypes are cultivated under field conditions at the germplasm collection of the *Centro APTA Citros Sylvio Moreira, Instituto Agronomico*, located in Cordeiropolis County, Sao Paulo State, Brazil (22°32' S, 47°27' W, altitude of 639 m).

Different sizes of leaves from three trees of each genotype were sampled randomly from different canopy positions, between (1 and 2) m from the soil surface, on the four cardinal points of the tree crown. Sampling was carried out during the 2007-2008 summer growing season and trees were spaced at 7.5 m × 5.5 m, and aged between (18 and 24) years.

Length (L), width (W) and leaf area (LA) were measured in a total of 540 leaves, ninety leaves for each genotype (*table I*). L was measured from the lamina tip to the point of petiole intersection along the midvein, while W was determined by the widest point across the leaf, perpendicular to the lamina midvein. LA was measured with the LI-3100 area meter (LI-Cor, Lincoln, NE, USA), with resolution of 0.01 cm^2. The L and W values were recorded with the help of a scale ruler, as accurate as 0.01 cm.

Ninety leaves of small, medium and large sizes for each genotype (a total of 540 leaves) were used in the preliminary experiment, where the dependent variable LA was regressed against the independent variables L, W, the square of L (L^2), the square of W (W^2) and the product of L × W, using the Microsoft Office Excel program. The utilization of both dimensions may introduce collinearity problems, which results in poor precision in the estimation of the regression coefficients. To detect the existence of collinearity, the variance inflation factor (VIF) [23] and the tolerance values (T) [24] were calculated. The VIF was estimated as {VIF = [1 / (1 – R^2)]}, where R is the correlation coefficient; and the T as [T = (1 / VIF)]. VIF has to be lower than 10 and T greater than 0.10 to indicate that collinearity does not imply a real effect on the estimation through both dimensions. If collinearity does exist, then one of the dimensions of that genotype is excluded from the models.

The regression analysis resulted in both linear and second-order polynomial regression equations for each variable. One equation for each variable [L, W, L^2, W^2 and (L × W)] was selected, either linear or second-order polynomial, based on the best combination of the highest coefficient of determination (R^2) and the data scattering. Once the best fitted regression equation for each variable was selected, leaves from the genotype Chinotto mandarin [*Citrus myrtifolia* (Raf.)] were used to validate the proposed models. It was chosen because it shows the most different leaf shape amongst the genotypes (*figure 1*).

Table II.

Linear and second-order polynomial regression equations, with their correspondent R^2, for the dependent variable leaf area and the independent variables length, width, the square of length, the square of width and the product of (length × width) for single leaves of citrus genotypes ($p < 0.0001$).

Independent variable	Number of leaves	Linear equation	R^2	Second-order polynomial equation	R^2
Length (L)	360	$y = 4.992\,x - 12.900$	0.956	* $y = 0.242\,x^2 + 1.607\,x - 3.982$	0.977
Width (W)	540	$y = 10.030\,x - 14.910$	0.924	* $y = 1.319\,x^2 - 0.491\,x + 0.689$	0.961
Square of length (L^2)	360	$y = 0.352\,x + 0.421$	0.972	* $y = -0.000\,x^2 + 0.432\,x - 0.782$	0.980
Square of width (W^2)	540	* $y = 1.259\,x - 0.072$	0.961	$y = 0.000\,x^2 + 1.253\,x - 0.041$	0.961
Product (L × W)	360	* $y = 0.680\,x - 0.103$	0.997	$y = 0.000\,x^2 + 0.671\,x - 0.038$	0.997

* Indicates the selected equation for each variable.

Table III.

Coefficient of determination (R^2), agreement index (d) and Mean Absolute Percentage Error (MAPE) for each model used to estimate leaf area in Chinotto mandarin (n = 90 leaves).

Independent variable	Chosen equation (from *table 2*)	R^2	d	MAPE (%)
Length (L)	$y = 0.242\,x^2 + 1.607\,x - 3.982$	0.926	0.953	32.24
Width (W)	$y = 1.319\,x^2 - 0.491\,x + 0.689$	0.952	0.979	11.48
Square of length (L^2)	$y = -0.000\,x^2 + 0.432\,x - 0.782$	0.928	0.977	14.59
Square of width (W^2)	$y = 1.259\,x - 0.072$	0.954	0.980	9.82
Product (L × W)	$y = 0.680\,x - 0.103$	0.982	0.995	4.99

Model validation is necessary in order to be applicable to other citrus genotypes and in different research areas. Leaf area of individual leaves was therefore estimated with each equation for 90 samples of that genotype (*table I*), and then regressed against the actual (measured) values. The index '*d*' [25] indicates the accuracy degree between the estimated and the actual values. The Mean Absolute Percentage Error (MAPE) was estimated as MAPE = $\Sigma\,(E_n - O_n) \times (100 / O_n)$, where Σ represents the total number of measured leaves, E_n is the estimated n-value and O_n is the measured n-value. The best combination here is the highest agreement index '*d*' and R^2, with the lowest MAPE.

3. Results and discussion

Two citrus genotypes showed collinearity between L and W: Buddha's Hand citron (VIF = 16.949 and T = 0.059) and Cipo orange (VIF = 12.987 and T = 0.077). For the others, VIF was lower than 10 (from 7.246 to 9.091), and T was greater than 0.10 (from 0.110 to 0.138), indicating that collinearity between the two dimensions may be negligible. As one of the dimensions has to be excluded when there is collinearity [23, 24], only the W was included in the models for both Buddha's Hand citron and Cipo orange, based on the highest R^2 obtained on a regression analysis performed individually for each genotype.

All tested dimensions [L, W, L^2, W^2 and (L × W)] were significantly ($p < 0.0001$) correlated with LA (*table II*). For all relationships, linear and second-order polynomial equations were obtained for LA estimation. Based on the selection criteria described (highest R^2, statistical significance and data scattering), the analysis demonstrated that the variables L, W and L^2 gave better results in second-order polynomial equations, although all of them, including the linear

ones, had R^2 equal to or higher than 0.924. The linear regressions for the variables W^2 and (L × W) showed equal R^2 to the second-order polynomial equations and, therefore, were more suitable for those variables. This previous analysis demonstrated that the second-order polynomial equations with L, W or L^2, and the linear equations with W^2 or (L × W), were more acceptable for estimating LA than the other ones (*table II*), as found on linear models for zucchini plants [17]. However, the utilization of the variable (L × W) is more laborious due to double sampling of dimensions. The utilization of the independent variables W or W^2 resulted in lower R^2 than L or L^2, as was also observed for eggplant [18]. However, as found in this study, research with hazelnut [5], pecan [7] and kiwi [8] also showed that the most accurate regression was the linear regression of LA against (L × W).

Once the best fitted regression equation was chosen for each variable, models were tested for the Chinotto mandarin genotype, and the coefficient of determination (R^2), the agreement index 'd' and the Mean Absolute Percentage Error (MAPE) were calculated for leaf area estimation (*table III*). The R^2 ranged from 0.926 to 0.982 for all variables, while the agreement index 'd' ranged from 0.953 to 0.995. The MAPE showed a greater variation amongst the variables, from 4.99% to 32.24%.

Contrary to what was found in the preliminary experiment, the variables W and W^2 showed better results than L and the correspondent L^2, with higher R^2 and agreement index 'd', and much lower MAPE. The single dimension L showed the lowest values for R^2 and agreement index 'd', together with the highest MAPE. The equation with (L × W) conferred, once more, the highest R^2, which is in accordance with the highest agreement index '*d*' and the lowest MAPE. Therefore, it is the most suitable model to estimate individual leaf area of citrus (*figure 2*), as it is for other fruit crops, such as watermelon [10], persimmon [12] and medlar [14], and for some ornamental crops, such as roses [21].

The majority of the leaves produced by all genotypes is elliptic-shaped, with the exception of Chinotto mandarin's, which are ovate (*figure 1*). However, it is also possible to find leaves with different shapes. For example, Buddha's Hand citron produces few oval leaves, while Cipo orange and Venezuela mandarin have some ovate leaves. Imperial orange and Faustrine lemon show some obovate leaves. Although intraspecific variation in size and shapes within the same genotype was considered to build the models, the results show that the R^2 and the agreement index '*d*' were high for all variables, while the MAPE was low for all of them, with the exception of the L dimension. Therefore, the models, especially the one with (L × W), are also applicable to other citrus genotypes, unless their leaf morphology differs considerably from those used in this study.

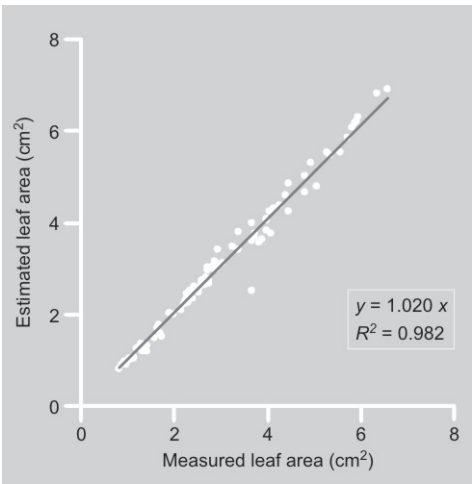

Figure 2.
Measured leaf area (LA) against estimated LA [LA = 0.680 (L × W) – 0.103] of single leaves of Chinotto mandarin (n = 90 leaves).

4. Conclusion

The product of the simple leaf dimensions (length × width) can provide a reliable and simple non-destructive model for leaf area estimation across citrus genotypes.

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Un modelo simple y no destructivo para estimar la superficie individual de las hojas de cítricos.

Resumen — Introducción. La superficie foliar a menudo está ligada al crecimiento, al desarrollo, a la fisiología, así como al rendimiento de las plantas. Ya se propusieron sendos modelos no destructivos para estimar la superficie foliar de varios genotipos vegetales. Éstos mostraron que la longitud y la anchura de las hojas, así como la superficie foliar, estaban estrechamente relacionadas. Por ello, el objetivo de nuestro estudio fue el desarrollo de un modelo fiable para estimar la superficie foliar a partir de medidas lineares de las dimensiones de las hojas para los genotipos de cítricos. **Material y métodos**. Se cosecharon las hojas de ciertos genotipos de cítricos, y se midieron sus dimensiones (longitud, anchura y superficie). La superficie foliar se relacionó en cuanto a la longitud, a la anchura, al cuadrado de la longitud, el cuadrado de la anchura, y al producto (longitud por anchura). Las ecuaciones más precisas, que fueran lineares o polinómicas de segundo orden, se sometieron nuevamente a prueba con un nuevo conjunto de datos. Por último, se definió la ecuación más fiable. **Resultados y discusión**. El primer análisis mostró que las variables de longitud, anchura y cuadrado de la longitud ofrecían mejores resultados en las ecuaciones polinómicas de segundo orden. Sin embargo las ecuaciones lineares eran más apropiadas y precisas cuando se empleaba la anchura y el producto (longitud por anchura). Cuando dichas ecuaciones se emplearon con el nuevo conjunto de datos, tanto el coeficiente de determinación (R^2) como el índice «d» fueron más elevados, en el caso de la ecuación que empleaba el producto (longitud por anchura) como variable. Sin embargo entonces el porcentaje medio de error absoluto era el más débil. **Conclusión**. El producto (longitud por anchura) de las simples dimensiones de la hoja puede proporcionar un modelo no destructivo, fiable y fácil para la estimación de la superficie foliar de los genotipos de cítricos.

Brasil / *Citrus* sp. / modelos lineales / hojas / dimensión / largura / anchura