Original article



Allometric models for determining leaf area of 'Fino de Jete' cherimoya grown in greenhouse and in the open field

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Summary

Introduction - Cherimoya is a delicious subtropical fruit crop of which obtaining high yields is hampered by the lack of suitable pollinators in most producing countries. Hand-pollination becomes then necessary as a reliable, though expensive, solution for ensuring consistent yield. Growers target crop load levels depending on the size of tree canopy, hand pollinating more flowers in larger trees. The objective of this work was to select the best equations for modelling the area of mature leaves of plants of the main cultivar of cherimoya, 'Fino de Jete', cultivated in the open field and also under a plastic greenhouse as a first step for reliable estimations of the leaf area index. Materials and methods - A random collection of 100 intact, fully developed adult leaves from shoots arising in the four cardinal directions of five adult plants grown at each cultivation environment was generated. Regression equations were calculated by applying linear, quadratic and power models to the leaf area based on single measurements of the leaves. Then, the strength of the chosen equations was confirmed and the results validated with a new set of leaves. Results and discussion - Growing conditions, open field versus greenhouse, did not have significant effects on the models, allowing us to choose the same equations for both environments. The most suitable equations for estimating the area of adult leaves by nondestructive methods in adult plants of the cherimoya 'Fino de Jete' were

 $\hat{Y}_i = -6.1172 + 0.7416 (LxW)_i$ and

$$\hat{Y}_i = -9.1710 + 4.4273 W_i + 0.9309 W_i^2$$

where L is the length, W the maximum width, and $L \times W$ the product of the length by the maximum width of the leaves. *Conclusion* – These equations allow us to estimate the area of individual mature leaves of the cultivar of cherimoya 'Fino de Jete' with very high accuracy in both cultivation environments, open field and under protected cultivation.

Keywords

Spain, *Annona cherimola*, crop management, biometrics, leaf size measurement, morphological traits, protected cultivation

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Significance of this study

What is already known on this subject?

 Crop load in cherimoya trees can be fixed in a certain extent by growers deciding the number of hand-pollinated flowers taking into consideration the size of the canopy.

What are the new findings?

• Leaf area of cherimoya trees grown in open field as well as in greenhouse can be easily calculated by nondestructive methods using simple regression equations.

What is the expected impact on horticulture?

• This is a first step to estimate leaf area index and photosynthetic capacity of individual cherimoya trees and hence their fruit yield capacity.

Résumé

Modèles allométriques pour déterminer la surface foliaire de cherimoya 'Fino de Jete' cultivés en serre ou en plein champ.

Introduction – Le cherimoya est une espèce d'arbre subtropicale aux fruits délicieux dont il est difficile d'obtenir des rendements élevés en raison du manque de pollinisateurs appropriés dans la plupart des pays producteurs. La fécondation manuelle devient alors une solution nécessaire, fiable bien que coûteuse, permettant d'assurer un rendement stable. Les producteurs ciblent le niveau de charge des vergers en fonction de la taille de la canopée, la pollinisation manuelle étant plus efficace sur les arbres de grande taille. L'objectif de ce travail était de sélectionner les meilleures équations pour modéliser la surface des feuilles matures de cherimoya cultivés en plein champ ou sous serre plastique, comme première étape d'une estimation fiable de l'indice de surface foliaire. Matériel et méthodes - Le principal cultivar de cherimoya en production, 'Fino de Jete', a été utilisé dans cette étude. Nous avons établi une collection aléatoire de 100 feuilles à partir des pousses développées aux quatre points cardinaux de cinq plantes adultes placées dans chacune des deux conditions de culture. Les équations de régression ont été calculées en appliquant les modèles linéaires, quadratiques et d'énergie à la surface foliaire à partir des mesures individuelles sur les feuilles. Ensuite, la robustesse des équations choisies a été confirmée et les résultats validés sur une nouvelle série de feuilles. *Résultats et discussion* – Les conditions de culture, plein champ par rapport à la serre, n'ont pas eu d'effets significatifs sur les modèles, ce qui permet de choisir les mêmes équations pour les deux environnements. Les meilleures équations pour estimer la surface des feuilles adultes par méthode non destructive des plantes adultes de cherimoya 'Fino de Jete' étaient

$$\hat{Y}_i = -6.1172 + 0.7416 (LxW)_i$$
 et
 $\hat{Y}_i = -9.1710 + 4.4273W_i + 0.9309W_i^2$

où *L* est la longueur, *W* la largeur maximale et $L \times W$ le produit de la longueur par la largeur maximale des feuilles. *Conclusion* – Ces équations permettent d'estimer la surface foliaire de feuilles matures individuelles chez le cultivar de cherimoya 'Fino de Jete' avec une très grande précision dans les deux conditions de culture, en plein champ et en culture protégée.

Mots-clés

Espagne, *Annona cherimola*, conduite des cultures, biométrie, mesure de la taille des feuilles, caractères morphologiques, culture sous abri

Introduction

Cherimoya (Annona cherimola Mill.) is a subtropical fruit crop of controversial origin (Popenoe, 1921; Pozorski and Pozorski, 1997; Bonavia et al., 2004), that belongs to the basal family of the Annonaceae. Spain is the world leader producer of this delicious fruit. Cherimoya acreage occupies 3,200 ha in Spain (Cuevas and Hueso, 2014), most of them grown in the open field, although a small area is under protected cultivation in plastic greenhouses. Other important cherimoya producers are Chile and Ecuador (Cuevas and Hueso, 2014). Cherimoya trees are semi-deciduous, losing their leaves once winter has passed. Blooming starts in late spring after the sub-petiolar buds of 1-year-old shoots get free from the restriction imposed by the presence of the old leaves. In most producing countries, Spain included, cherimoya flowers are hand-pollinated in order to guarantee a regular harvest of large, high quality fruit (González et al., 2006). Therefore, the level of yield can be fixed in great extent by the growers, who decide how many flowers hand-pollinate. Optimal crop load level is under discussion, although depends on the management of the crop and the volume of the canopy (González and Cuevas, 2008). On the other hand, the removal of the leaves by hand or chemically brings forward bud break and bloom, advancing cherimoya harvest (González et al., 2013). Nonetheless, too early defoliation causes a clear diminution of the percentage of buds sprouting probably because the removal of still-functional leaves limits carbohydrate production and their storage.

The leaves undertake primordial functions in a plant life such as light interception and gas exchange (Spann and Heerema, 2010). The accomplishment of these functions partially depends on their size, making important to follow the growth of the leaves along the time (Peksen, 2007). Hence, the measurements of the leaf area should be preferably achieved by non-destructive procedures either by portable planimeters or by estimations applying solid models based on variables easy to measure repeatedly on the plant (Demirsoy, 2009). Soto et al. (2002), who studied the occurrence and development of fungal damages in cherimoya leaves, reported several drawbacks of measuring leaf area by destructive methods. Statistical modelling of leaf size based on simple measurements such as leaf length and width has been investigated in various tropical and subtropical fruit crops, for example medlar (Mespilus germanica L.) (Mendoza-de Gyves et al., 2008), persimmon (Diospyros kaki L.f.) (Cristofori et al., 2008), citrus spp. (Mazzini et al., 2010), mango (Mangifera indica L.) (Lima et al., 2012), passion fruit (Passiflora spp.) (Morgado et al., 2013) and pineapple (Ananas comosus (L.) Merr.) (Francisco et al., 2014). This is the first study of this kind carried out on 'Fino de Jete' or any other cultivars of cherimoya.

The objectives of this work are to compare of the size of the leaves of cherimoya 'Fino de Jete' formed in two different environments (open field and greenhouse), calibrate different equations built for the estimation of leaf area applying linear, quadratic and power models, confirm the validity of the chosen equations, and finally validate the results with a new set of leaves.

Materials and methods

Plant material

This experiment was carried out with leaves of 'Fino de Jete', the most important cultivar of cherimoya in the world, collected in June 2014 from adult plants grown in two contrasting environments (open field and greenhouse) in order to extend the validity of the determinations for both cultivation systems. Trees cultivated at the open field were located at the Experimental Station of Cajamar Foundation in El Ejido (Almería) (latitude 36°48'N, longitude 2°43'W, altitude 155 m a.s.l.). The trees, 40-year-old, were grafted on seedlings, trained in an open vase shape and 8×6 m spaced. Trees were drip irrigated with 7,600 m³ ha⁻¹ year⁻¹. Fertilizers were applied through the irrigation system at a seasonal rate of 150-80-100 kg ha-1 of N, P_2O_5 and K_2O , respectively. The plants were maintained free of pests and diseases by means of conventional practices included in integrated pest management guidelines, involving chemical, biological and cultural methods especially for the control of the insidious fruit fly (Ceratitis capitata).

Greenhouse plants were kept under high-density $(20 \times 10$ threads cm⁻²) polyethylene mesh in the Experimental Station of the Foundation UAL-ANECOOP sited in Paraje Los Goterones, Retamar (Almería) (latitude 36°86'N, longitude 2°28'W, altitude 88 m a.s.l.). These plants were 4-year-old at the moment the sampling was performed, grafted also on cherimoya seedlings, trained following a horizontal trellis with four wires and 2.5 m tall and spaced 3.5×2.5 m (between rows and within the row, respectively). These plants were also drip irrigated and fertilized following same guide-lines as those trees at the open field. The pressure of pests was lower in the greenhouse.

During the sampling period, the mean temperatures in the open field and in the greenhouse were 19.7 and 21.0°C, the relative humidity 62.3 and 69.9%, and the photosynthetically active radiation 500.7 and 309.8 μ mol m⁻² s⁻¹, respectively. From bud sprout to sampling, the mean temperatures in the open field and in the greenhouse were 20.4 and 20.1°C, the relative humidity 63.9 and 82.8%, and the photosynthetically active radiation 578.3 and 309.9 μ mol m⁻² s⁻¹, respectively.



Data analysis

In each environment, 100 adult fully developed leaves from five plants (20 per plant; 5 per cardinal point) were collected taking good care to sample leaves of very different size in order to obtain a large range of values to calibrate the models and extent their domain of validity. The experiment followed a randomized block design where each plant acted as block (Schmildt *et al.*, 2014). The experiments of the two environments were analyzed together (Steel *et al.*, 1997). The statistical model applied was:

$$Y_{ikj} = m + b_j + A_i + B_k + (AB)_{ik} + e_{ikj}$$

where Y_{ikj} is the observation on the jth repetition of the combination of the ith level of factor A (environment) with the kth level of the factor *B* (cardinal point); *m* is the general mean; b_j is the effect of the jth block (J blocks = 5); A_i is the ith level of the factor A (I levels = 2 environments); B_k is the kth level of the factor B (K levels = 4 cardinal points); $(AB)_{ik}$ is the interaction between environments and cardinal points; and e_{ikj} is the experimental error (Schmildt *et al.*, 2014).

Once the leaves were sampled, they were put correctly identified in plastic bags and rapidly taken to the lab where they were inspected to confirm their mature and undamaged condition (color of the leaf, texture, loss of the velvety appearance and unfolding characteristics of young leaves). On them, we measured maximum length from the base to the tip (L) not considering the petiole, maximum width (W), and leaf area (LA). W and L were measured to the nearest mm using a ruler, while LA was measured using an area meter (Delta-T Devices Ltd.®, Burwell, Cambridge, England). After the measurements, we built regression equations with L, W and their product (L×W) as independent variables, and LA as the dependent one, applying linear

 $(\widehat{\mathbf{Y}}_{i} = \widehat{\boldsymbol{\beta}}_{0} + \widehat{\boldsymbol{\beta}}_{1}\mathbf{x}_{i}),$

quadratic

$$\left(\widehat{Y}_{i} = \widehat{\beta}_{0} + \widehat{\beta}_{1}x_{i} + \widehat{\beta}_{2}x_{i}^{2} \right)$$

and power

$$\left(\bar{Y}_i=\bar{\beta}_0 x_i^{\bar{\beta}_1}\right)$$

models as recommended by Zhang and Liu (2010). The parameters β_0 and β_1 were estimated by using the method of least square, with previous linearization of the power model by a logarithm to the base 10 (log) transformation of both dependent and independent variables: $\log(Y) = Log(\beta_0) + \beta_1 log(X)$. Applying this procedure, nine equations and their respective coefficients of variation were defined.

The sample size needed for each regression model was determined as follows:

$$\eta_{eq} = \frac{t_{\alpha/2}^2 RMSE^2}{e^2 \overline{Y}^2}$$

according to suggestions made by Schmildt *et al.* (2014) and based on Cochran (1977), where η_{eq} is the size of the sample for building the model; \overline{Y} is the mean of LA in a sample of 200 leaves; $t_{\alpha/2}$ is the critical value of the t of Student, which area is equal to $\alpha/2$ with (*n*-1) degrees of freedom for *P*=0.05; *e* is the error of the mean assumed to be 3%, and RMSE is the root mean square error calculated as:

$$RMSE = \sqrt{\frac{\sum\limits_{i=1}^{n} \left(\hat{Y}_{i} - Y_{i}\right)^{2}}{n}}$$

Where \hat{Y}_i are the estimated leaf area values and Y_i the observed ones.

On the other hand, as suggested by different authors (Zhang and Liu, 2010; Normand and Lauri, 2012), an analysis of covariance was carried out using environment as covariate to determine if the environment where the plants grew has an effect on the parameters (slope and intercept) of the linear models.

As recommended by Demirsoy (2009), the validation of the different models selected for estimating LA was performed with a new set of leaves. In our case, this was performed three weeks after the first collection (in July 2014) sampling a new set of 50 leaves from open field plants to compare the leaf area measured (Y_i) with the values estimated by the model (\hat{Y}_i). For each model, measured and estimated leaf area values were compared with a linear regression

$$\left(\hat{\mathbf{Y}}_{i}=\hat{\boldsymbol{\beta}}_{0}+\hat{\boldsymbol{\beta}}_{1}\mathbf{Y}_{i}\right).$$

The hypotheses $H_0: \beta_0 = 0$ versus $H_a: \beta_0 \neq 0$ and $H_0: \beta_1 = 1$ versus $H_a: \beta_1 \neq 1$ were verified with the test of Student at $P \leq 0.05$. We also calculated the coefficient of determination (R²), the mean absolute error (MAE), RMSE and the d index of Willmott (Willmott, 1981; Yang *et al.*, 2009; José *et al.*, 2014) (an index of agreement between simulated and actual data, varying between 0 and 1), where MAE and d are:

$$\mathsf{MAE} = \frac{\sum_{i=1}^{n} |\hat{\mathbf{Y}}_{i} - \mathbf{Y}_{i}|}{n}, \quad \mathbf{d} = 1 - \left[\frac{\sum_{i=1}^{n} (\hat{\mathbf{Y}}_{i} - \mathbf{Y}_{i})^{2}}{\sum_{i=1}^{n} \left(\left|\hat{\mathbf{Y}}_{i} - \overline{\mathbf{Y}}\right| + \left|\mathbf{Y}_{i} - \overline{\mathbf{Y}}\right|\right)^{2}}\right]$$

The criteria used for the selection of the model to estimate leaf area were intercept (β_0) to be non-different of zero, the slope (β_1) non-different of one, MAE and RMSE close to zero, and the Willmott's index close to one. Statistical analyses were performed using Microsoft Office Excel (Levine *et al.*, 2012) and Genes software (Cruz, 2013) and R software (R Core Team, 2016).

Results and discussion

The analyses of variance show that neither the cardinal points where the leaves were sampled, nor the growing conditions where the trees grew, or their interaction had significant effects on leaf length, width or their product (Table 1). This can also be visualized in Figure 1, where the relationships between leaf length and width versus leaf area are represented for both environments: open field and greenhouse.

The covariance analysis showed that the environment has no effect on the slope or on the y-intercept of the linear model with $L \times W$ as a predictor (*P* value of t-test=0.332). The same applied for L (*P* value of t-test=0.528) and for W as predictors of leaf area (*P* value of t-test=0.398). Thus, the modelling of the area of the leaves was performed with leaves taken from both growing conditions together.

The number of leaves needed to build the equations for leaf area estimation per genotype varies across the literature from less than 50 (Lu *et al.*, 2004), to more than 1,000 leaves



FIGURE 1. Dispersion of the observed leaf area data as a function of leaf length (A) and width (B) of 200 leaves of 'Fino de Jete' cherimoya. One hundred leaves were sampled from five plants grown in the open field, while another set of 100 leaves proceeded from five plants grown under a plastic greenhouse.



FIGURE 2. Linear equation for the estimation of leaf area of 'Fino de Jete' cherimoya based on the product of leaf length by width (L×W). A: Equation based on 200 leaves sampled from open field and greenhouse grown plants (100 from each growing condition); B: Validation of the model based on a new set of 50 leaves.

	L (cm		W (cm)	LA (cm ²)		
Minimum value		7.50	4.00	25.01		
Maximum value		26.30	17.10	329.27		
Mean		17.48	9.56	125.24		
CV (%)		8.80	11.44	19.39		
SV	DF		Mean squares			
Block	4	5.61 ns	3.05 ns	1340.84 ns		
СР	3	0.40 ns	0.16 ns	118.77 ns		
E	1	1.58 ns	0.02 ns	687.57 ns		
00.5	3	0.75 ns	0.85 ns	432.24 ns		
CPXE	v	••				

TABLE 1. Summary of the analyses of variance of leaf length (L), width (W) and area (LA) of 'Fino de Jete' cherimoya. SV= re of variation, DF = degree of freedom, CV = coefficient of variation, ns = non-significant (P>0.05) by the E-test, CP - cardinal

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(Lima *et al.*, 2012). Our results show that the sample size required for the modelling of LA varied widely depending on the chosen equation from a minimum of 8 leaves for a linear model based on L × W to more than a hundred leaves (102) for a lineal model based on L (Table 2). Sample size of 200 leaves used in this experiment (100 from each environment) resulted therefore endorsed. Silva *et al.* (2004) modelled leaf area of different progenies of *Annona squamosa* using 100 leaves per genotype. A prerequisite to build solid models is to sample leaves of very different dimensions, as we did, in order to represent a wide variety of sizes. Besides finding a solid equation, it is also important to provide the range of confidence of the selected equation.

The linear, quadratic and power models using L×W as predictor showed the highest values of the coefficients of determination among the different models studied (Table 2). Nevertheless, in the selection of a model we must consider not only a good fitting between predicted and observed values, but the performance of the chosen equation validating its appropriateness preferably with a new set of leaves (Pire and Valenzuela, 1995; Fascella *et al.*, 2013), as we did in this work.

The test of validation carried out showed that 67% of the equations were adequate according to the criteria of linear coefficient statistically equal to 0, and angular coefficient equal to 1 (Table 3). Of the validated models, linear and quadratic equations built with L × W as independent variable reached the highest R^2 (Table 2). These equations presented also the lowest mean absolute error (MAE), the lowest root mean square error (RMSE) and the highest value for Willmott d index, indicating that these equations are the most suitable for the estimation of LA in the cultivar of cherimoya 'Fino de Jete'. Thus, considering the facility of the interpretation, we recommend the use of the linear equation

$$\hat{Y}_i = -6.1172 + 0.7416 (LxW)_i$$

(Table 2; Figure 2A). The validation criteria are included in Table 3 and Figure 2B.

Despite the above, the use of linear models based on single one-dimension parameter is frequently preferred (Tsialtas and Maslaris, 2005; Galindo and Clavijo, 2007; Rouphael *et al.*, 2007; Tsialtas *et al.*, 2008). Considering that maximum width provides higher accuracy than length we recommend the quadratic equation

$$\hat{Y}_{i} = -9.1710 + 4.4273 W_{i} + 0.9309 W_{i}^{2}$$

(Table 2; Figure 3A) that showed a satisfactory prediction capacity (Table 3; Figure 3B). Since extrapolation is not possible using regression models (Levine *et al.*, 2012), a prerequisite for useful models is to sample leaves of a wide range of sizes. Therefore, the above selected equation must only be applied to leaves which width is roughly between 4 and 17 cm (Table 1).

Conclusion

Based on the above results, we may affirm that the area of individual adult leaves in bearing plants of cherimoya (*Annona cherimola* Mill. 'Fino de Jete') can be accurately modelled by non-destructive methods measuring the length and width of leaves of plants grown in both cropping environments, open field and greenhouse, sampled from any of the four cardinal points. The leaf area of the cultivar of cherimoya 'Fino de Jete' is best determined by the product of their length by width as independent variables:

Models	Equations	R ²	n _{eq}
	L (cm)		
Linear	$\hat{Y}_{j} = -151.1050 + 15.8175L_{j}$	0.910	102
Quadratic	$\hat{Y}_{i}=66.4139\!-\!10.6761L_{i}+0.7663L_{i}^{^{2}}$	0.960	45
Power	$\hat{Y} = 0.1854 L_i^{^{2,2499}}$	0.951	55
	W (cm)		
Linear	$\hat{Y}_{j} = -93.9887 {+} 22.9333 W_{j}$	0.966	38
Quadratic	$\hat{Y}_{j} = -9.1710 + 4.4273 \text{W}_{j} + 0.9309 \text{W}_{j}^{2}$	0.984	18
Power	$\hat{Y}_i = 2.0190 \ W_i^{_{18015}}$	0.981	18
	L × W (cm ²)		
Linear	$\hat{Y}_{j} = -6.1172 \! + \! 0.7416 \left(\text{LxW} \right)_{j}$	0.993	8
Quadratic	$\hat{Y}_{j} = 0.4273 + 0.6625(LxW)_{j} + 0.0002(LxW)_{j}^{2}$	0.994	8
Power	$\hat{Y}_i = 0.6054 (LxW)_i^{_{1.0287}}$	0.995	8

TABLE 2. Leaf area (\hat{Y}) equations, coefficients of determination (R^2), and required sample size (n_{eq}) for linear, quadratic, and power models based on leaf length (L), width (W), and product of length by width (L×W), derived from observed, measured, area of 200 leaves (100 from open field and 100 from greenhouse plants) of 'Fino de Jete' cherimoya.

 $\hat{Y}_i = -6.1172 + 0.7416 (LxW)_i$

The estimation of leaf area by equations using only leaf length or leaf width is also possible with a small loss of precision. For the assessment of the leaf area of cherimoya 'Fino de Jete', using only one parameter, we recommend the following equation:

$$\hat{Y}_i = -9.1710 + 4.4273 W_i + 0.9309 W_i^2$$

given its high accuracy and easiness of measurement.

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FIGURE 3. Quadratic equation for the estimation of leaf area of 'Fino de Jete' cherimoya plants based on its leaf width (W). A: Equation based on 200 leaves sampled from open field and greenhouse grown plants (100 from each growing condition); B: Validation of the model based on a new set of 50 leaves.

TABLE 3. Validation of the leaf area model. Linear ($\hat{\beta}_0$), angular ($\hat{\beta}_1$), and determination of coefficients (R²) obtained by regression between estimated values of leaf area (as dependent variable) and observed values (independent variable), mean square errors (MSE), root mean square errors (RMSE) and Willmott index (d) calculated on a new set of 50 leaves of 'Fino de Jete' cherimoya.

Models	Â ₀ (1)	$\hat{\boldsymbol{\beta}}_{1}^{(2)}$	R ²	MSE	RMSE	d		
		L (cm)						
Linear	6.2607 ns	0.9654 ns	0.933	10.8186	13.0636	0.9822		
Quadratic	0.4484 ns	0.9802 ns	0.976	6.2446	7.9016	0.9935		
Power	8.4519 **	0.9117 **	0.973	7.2089	9.0534	0.9909		
		W (cm)						
Linear	6.5577 *	0.9785 ns	0.981	6.1868	7.8676	0.9936		
Quadratic	3.1610 ns	0.9827 ns	0.986	4.9292	5.8601	0.9964		
Power	3.2655 ns	0.9789 ns	0.986	4.9334	5.8944	0.9964		
		L × W (cm ²)						
Linear	-0.2855 ns	1.0048 ns	0.996	2.4638	3.0967	0.9990		
Quadratic	-0.1584 ns	1.0015 ns	0.996	2.2839	3.0333	0.9991		
Power	2.2990 ns	0.9802*	0.996	2.4011	3.1017	0.9990		

(1) *, ** Linear coefficient different to zero by t-test at 5% and 1% probability. Ns: non-significant.

⁽²⁾ *, ** Slope different to one by t-test at 5% and 1% probability. Ns: non-significant.



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