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Original article

# A simple and accurate allometric model to predict single leaf area of twenty-one European apricot cultivars

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## Summary

**Research in fruit tree physiology and breeding often requires accurate and non-destructive methods for estimating leaf area (LA). The development of unbiased allometric model from linear measurements [leaf length (L) and/or width (W)] to predict individual LA of apricot irrespective of cultivars is still lacking. The models were built using LA, L, and W data measured in 3,040 leaves collected on trees of nineteen apricot cultivars (calibration experiment). Model(s) were validated on 520 apricot leaves collected from the trees of two additional cultivars (validation experiment). LA prediction models based only on L measurements (L or $L^2$) were not suitable for estimating LA of apricot. A significant improvement in LA prediction was observed when the model including $W^2$ as an independent variable was adopted. However, the coefficients of one dimension LA model ($W^2$) were affected by leaf shape (L:W ratio) and consequently were excluded. To develop an accurate LA model for apricot, independent of leaf shape groups, the product L × W was used as an independent variable. The linear model LA = 1.193 + 0.668 (L × W) exhibited the highest $R^2$, the smallest mean square error (MSE) and predicted residual error sum of squares (PRESS). In the model validation, correlation coefficients showed that there was a highly reliable relationship between the predicted and the observed LA values, giving an underestimation of 2.9% in the prediction. The LA model using LW as independent variable can be successfully adopted in research on apricot, since it provides an accurate, simple and non-destructive estimation of LA across apricot cultivars without the use of any expensive device.**

**Keywords**
estimation model, leaf dimensions, leaf shape, model validation, non-destructive measurement, *Prunus armeniaca* L.

## Significance of this study

*What is already known on this subject?*

- Accurate and non-destructive methods to determine individual leaf areas of fruit and nut trees are a useful tool in physiological and agronomic research.

*What are the new findings?*

- Single leaf area of different apricot cultivars can be easily and accurately predicted by developing non-destructive allometric models from linear measurements such as leaf length and width.

*What is the expected impact on horticulture?*

- The proposed model [LA = 1.193 + 0.668 (L × W)] could be adopted for physiological experiments in which destructive leaf sampling is not possible without the use of expensive devices such as planimeter, plant canopy analyzers and hand scanners.

## Introduction

Over the past ten years, apricot (*Prunus armeniaca* L.) cultivation in the main European producer countries, such as Italy, France and Spain, was characterized by a significant turnover rate of the cultivated varieties. In fact, local varieties were often suddenly replaced, partly or totally, by new cultivars that were preferred for their production and fruit quality (i.e., better sensory attributes and higher phytochemical contents) (Piagnani et al., 2013). The introduction of new cultivars needs to be investigated to allow them to perform well under different environmental conditions (Bassi and Audergon, 2006) and to define specific training systems and orchard management practices (Neri et al., 2010).

Research in fruit trees biology often requires accurate and non-destructive methods for estimating leaf area (LA) (Spann and Heerema, 2010). Indeed, in fruit trees, LA affects several physiological parameters such as net photosynthesis, water relations, transpiration and source-sink relationships (Flore, 1994). These physiological aspects are known to potentially affect plant growth and consequently productivity (Pérez-Pastor et al., 2014).

Leaf area can be measured by destructive (i.e., electronic leaf area meter and planimeter) or non-destructive methods (i.e., plant canopy analyzer, ceptometers and hand scanners) (Rouphael et al., 2010; Giuffrida et al., 2011; Basile et al., 2014). Even though these instruments were demonstrated to be effective for LA estimation, they are still expensive and require the use of complex devices for basic and simple horticultural studies (Pompelli et al., 2012). In addition, direct leaf area measurements require leaf removal, and, therefore they do not allow repeated measurements. Therefore, there is an urgent need in horticultural experiments to adopt simple, inexpensive and nondestructive allometry models for estimating LA using easily measured leaf parameters such as length (L) and width (W) (Rouphael et al., 2006, 2007; Rivera et al., 2007; Zhang and Liu, 2010).

The development of allometric models from linear measurements to predict individual LA has been shown to be a powerful tool in studying plant growth and development for several fruit and nut trees such as cherry (Demirsoy and Demirsoy, 2003), peach (Demirsoy et al., 2004), kiwifruit (Mendoza-de Gyves et al., 2007), medlar (Mendoza-de Gyves et al., 2008), persimmon (Cristofori et al., 2008), citrus (Mazzini et al., 2010), apple (Kishore et al., 2012), common fig (Giaccone et al., 2017), chestnut (Serdar and Demirsoy, 2006), hazelnut (Cristofori et al., 2007) and walnut (Keramatlou et al., 2015). Nevertheless, no published data are available about LA estimation of an important fruit tree species such as apricot, that is considered to be one of the most appreciated temperate tree fruits by consumers (Lo Bianco et al., 2010). Moreover, most of the allometric models previously developed for other fruit trees and vines were calibrated on a limited number of cultivars (Zenginbal et al., 2007; Ghoreishi et al., 2012), and consequently a model recalibration is needed when other varieties are used. Indeed, it is known that leaf shape (L:W ratio) may significantly vary among different cultivars of the same species (Cristofori et al., 2007). In addition, some of these models were selected based on inappropriate statistical procedure (i.e., exclusively the highest coefficient of determination and lowest standard error of estimate), without the examination of residuals, that represent a powerful tool to detect model deficiencies (e.g., biased) in regression analysis (Antunes et al., 2008; Pompelli et al., 2012).

The objectives of this study were: (1) to create a simple, accurate and non-destructive LA model based on simple linear measurement (L and/or W) that would include the variability in L:W ratio (i.e., leaf shape) among nineteen apricot cultivars and (2) to validate the selected model(s) using an independent data set coming from two of the most representative cultivars used in Italy and in Europe (Piagnani et al., 2013).

## Materials and methods

### Plant material, growth conditions and data collection

Two experiments were conducted at the Regional Experimental Station IMPROSTA Battipaglia, Southern Italy (40°61'N, 14°99'E, 72 m a.s.l.; calibration experiment) and at the experimental orchard of the University of Naples Federico II, located in Portici (40°46'N, 14°21'E, 70 m a.s.l.; validation experiment).

The leaf area model was developed using the data collected in 2015 on nineteen apricot cultivars including nine Italian apricot varieties ('Boccuccia Liscia', 'Cafona', 'Ceccona', 'Monteruscello', 'Ninfa', 'Prete', 'Stella', 'Taviello', 'Vitillo') and ten French and Belgian cultivars ('Faralia', 'Flavour Cot', 'Lilly Cot', 'Magic Cot', 'Pearl Cot', 'Pink Cot', 'Silver Cot', 'Tardi Cot', 'Tom Cot', 'Wonder Cot') (Figure 1). Model validation was carried out on two different cultivars, 'Pellecchiella' and 'Orange Rubis'. These two cultivars were selected because they are among the most appreciated apricot varieties cultivated in Italy and in other European countries (Piagnani et al., 2013). Trees used in both experiments were mature trees (8–10 years old), grafted onto Myrobalan 29 plum (*P. cerasifera* Ehrh.), trained to an open vase system, and spaced 3 × 4 m (calibration experiment) and 2 × 4.5 m (validation experiment), corresponding to a tree density of 833 and 1,111 plants $ha^{-1}$, respectively. The orchards were drip irrigated and managed according to conventional technical practices.

Model calibration was done using a total of 3,040 randomly collected leaves (160 leaves per cultivar). Leaves were immediately transported to the laboratory to measure their LA, L, and W. Leaf L and W were measured with a ruler and the values were rounded to the nearest 0.1 cm. Leaf L was measured along the midrib from the lamina tip to the point of intersection between the lamina and the petiole, whereas leaf W was measured perpendicularly to the midrib between the widest margins of the lamina. The actual area of each leaf (LA) was individually measured using an area meter (LI-3100; LICOR, Lincoln, NE, USA) calibrated to 0.01 $cm^2$. For model validation, a total of 520 apricot leaves (260 leaves per cultivar) was used to measure LA, L and W as previously described for the calibration experiment.

### Statistical methodology

For model construction (i.e., calibration experiment), the dependent variable (LA) was regressed on the following independent variables L, W, L × W, $L^2$, and $W^2$. The relationships were evaluated by fitting regression models with the linear regression procedure and the stepwise elimination option (Miranda and Royo, 2003).

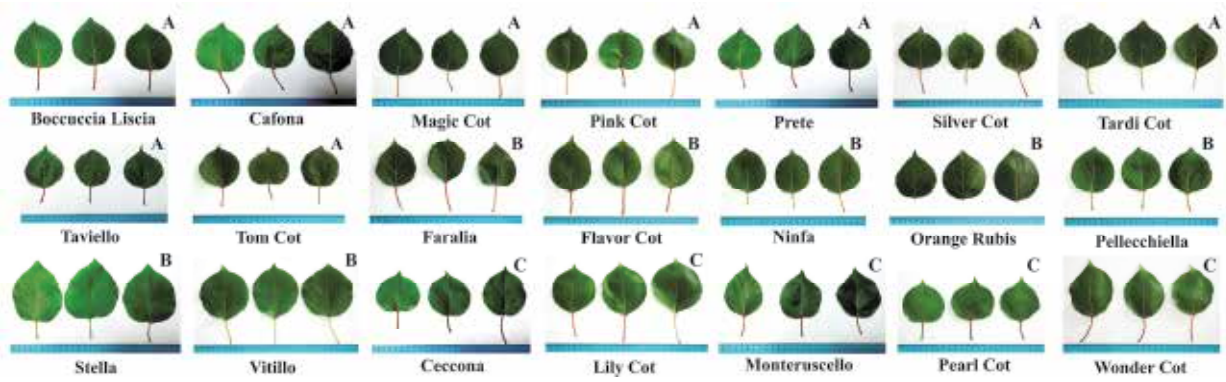



**Figure 1.** Leaf samples of the nineteen apricot cultivars used to calibrate the leaf area estimation models and of the two cultivars used to validate them ('Orange Rubis' and 'Pellecchiella'). The cultivars were separated and categorized into three groups (A, wide; B, intermediate; C, narrow) based on leaf shape expressed as the ratio of leaf length to width (L:W).

We first developed models using data from each apricot cultivar groups. Cultivars were separated and categorized into three groups (wide, intermediate and narrow leaves) based on leaf shape defined as the ratio of leaf length to width (L:W) (Table 1). Then, the internal validity of the models was tested based on the following parameters: coefficient of determination ($R^2$), mean square error (MSE), predicted residual error sum of squares (PRESS), error sum of squares (SSE).

The best model was selected based on the combination of the highest $R^2$, the lowest MSE, and PRESS and also when the PRESS values are reasonably close to SSE. Additionally, the significance of the differences between models developed for different apricot groups in the slope and in the intercept was tested with the analysis of covariance (ANCOVA). When no significant differences in the slope and the intercept were observed, data from the three groups were pooled to construct a single allometric model (Zhang and Pan, 2011; Wang and Zhang, 2012).

Taking into account the potential problems of collinearity caused by the use of two dimensional variables (L and W), the variance inflation factor, VIF = $1/(1-R^2)$ (Marquardt, 1970), and the tolerance factor, T = 1/VIF (Gill, 1986) were also calculated. If the VIF value was higher than 10 or if T value was smaller than 0.10, then collinearity is detected, and consequently one of the two leaf measures should be excluded from the model.

The following two techniques described by Miranda and Royo (2003) were used to validate the models:

(1) The Stepwise Regression Option was applied on the validation data for re-estimating model parameters and producing validation models that were compared for consistency;

(2) Outcomes for observations in the validation data set were predicted using regression parameter estimates from the estimation models; then the mean squared prediction error (MSPR) was calculated and compared with the MSE of the regression fit to the model building data set (Neter et al., 1996).

A graphical procedure was used to compare the predicted and the observed LA for the two cultivars 'Orange Rubis' and 'Pellecchiella' (Bland and Altman, 1986). Therefore, LA predicted values were plotted against the observed LA (Figure 2) to evaluate the linear relationship between them. Besides, predicted LA values were subtracted from observed LA and the residuals were plotted against the observed LA. The lack of agreement between observed and estimated values was evaluated calculating the relative bias, estimated by the mean of the differences (d) and the standard deviation (sd) of the differences (Figure 2). Normality test (Gaussian distribution) was conducted to attain a Wilkes-Shapiro W statistic using the examines procedure of SPSS software package (SPSS 13.0 for Windows) (Marini, 2001).

## Results and discussion

In the present study, leaf shape expressed as the ratio of leaf length to width (L:W) varied among the twenty-one cultivars (Figure 1; Table 1). The L:W ratios were separated into three groups. The first group included 'Boccuccia Liscia', 'Cafona', 'Magic Cot', 'Pink Cot', 'Prete', 'Silver Cot', 'Tardi Cot', 'Taviello' and 'Tom Cot'. These cultivars were mostly characterized by wide leaves (L:W ratio between 1.00 and 1.09; Table 1). The second group including 'Ceccona', 'Lily Cot', 'Monteruscello', 'Pearl Cot' and 'Wonder Cot' had narrow leaves (L:W ratio between 1.21 and 1.33), whereas the third group ('Faralia', 'Flavour Cot', 'Ninfa', 'Stella', 'Vitillo', 'Orange Rubis' and 'Pellecchiella') presented intermediate leaf shape (L:W ratio between 1.14 and 1.20) (Table 1).

It is well established that the use the two leaf parameters (L and W) can introduce problems of collinearity (Marquardt, 1970; Gill, 1986). Therefore, as a preliminary step for model calibration, both VIF and T values of the cultivars were calculated and analyzed to check collinearity. Except for 'Magic Cot', all the other cultivars coming from the calibration experiment had a VIF always lower than 10 (from 3.20 to 9.80), and a T value higher than 0.10 (from 0.102 to 0.310) (Table 1). Consequently, our results indicated that the collinearity between L and W was negligible for most of the cultivars, but not all and consequently both leaf measurements could be included to build the model.

Regression analysis of the calibration experiment (3,040 leaves) for each leaf shape group demonstrated a significant ($P<0.001$) relationships between LA and the independent variables L, W, L×W, the square of length ($L^2$) and the square width ($W^2$). In the current study, the linear regression models have been preferred over nonlinear

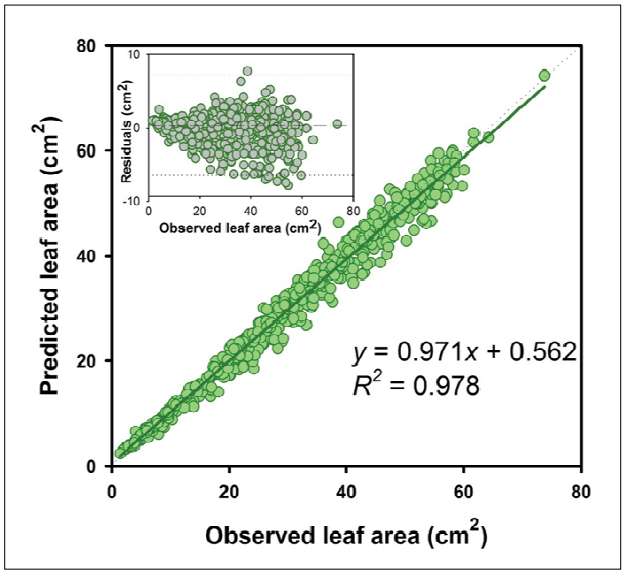



**Figure 2.** Relationship between observed values of single LA and predicted LA using the L×W model [LA = 1.193 + 0.668 (L×W)] for the cultivars 'Orange Rubis' and 'Pellecchiella' (validation experiment). In the main panels, solid lines represent linear regression lines, whereas dotted lines represent the 1:1 relationship between predicted and observed values. The analysis of dispersion pattern of residuals for L×W model is shown in the inset. In the insets, the horizontal dashed lines indicate the mean of the differences, whereas the horizontal dotted lines indicate the limits of agreement, calculated as d ± 3 SD; where d is the mean of the differences, and SD is the standard deviation of the differences. If the differences are normally distributed, 97% of the differences in a population will lie between the limits of agreement.

**Table 1.** Mean, minimum (min) and maximum (max) values for the leaf length (L), leaf width (W), leaf area, length:width ratio (L:W), variation inflation factor (VIF) and tolerance values (T) of apricot cultivars grouped on leaf shape basis (A, wide; B, intermediate; C, narrow).

| Cultivars | Leaf shape group | Leaf length (cm) | | | Leaf width (cm) | | | Leaf area ($cm^2$) | | | L:W (±SE)[a] | $R^2$[b] | MSE[b] | VIF | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Min. | Max. | Mean | Min. | Max. | Mean | Min. | Max. | | | | | |
| 'Boccuccia Liscia' | A | 6.1 | 2.1 | 8.6 | 5.7 | 1.9 | 8.4 | 25.2 | 2.2 | 45.1 | 1.06 (±0.01) | 0.798 | 0.36 | 4.95 | 0.202 |
| 'Cafona' | A | 7.6 | 3.0 | 11.6 | 7.6 | 3.1 | 10.5 | 41.3 | 5.2 | 75.7 | 1.02 (±0.01) | 0.690 | 0.67 | 3.23 | 0.310 |
| 'Magic Cot' | A | 6.2 | 1.9 | 10.1 | 5.6 | 1.3 | 9.4 | 26.6 | 2.0 | 62.2 | 1.09 (±0.01) | 0.915 | 0.20 | 11.76 | 0.085 |
| 'Pink Cot' | A | 6.3 | 1.6 | 9.2 | 6.1 | 1.2 | 9.4 | 30.5 | 1.7 | 65.0 | 1.05 (±0.01) | 0.838 | 0.47 | 6.17 | 0.162 |
| 'Prete' | A | 6.3 | 2.8 | 9.6 | 6.1 | 3 | 9 | 27.6 | 4.5 | 54.4 | 1.04 (±0.01) | 0.861 | 0.30 | 7.19 | 0.139 |
| 'Silver Cot' | A | 6.2 | 1.4 | 9.2 | 5.9 | 1.5 | 9.2 | 29.1 | 1.7 | 61.9 | 1.07 (±0.01) | 0.874 | 0.37 | 7.94 | 0.126 |
| 'Tardi Cot' | A | 6.4 | 1.6 | 9.2 | 6.5 | 1.6 | 9.8 | 33.5 | 2.7 | 60.1 | 1.00 (±0.01) | 0.810 | 0.69 | 5.26 | 0.190 |
| 'Taviello' | A | 6.4 | 2.6 | 9.0 | 6.2 | 2.9 | 8.5 | 26.6 | 5.4 | 43.7 | 1.04 (±0.01) | 0.692 | 0.46 | 3.25 | 0.308 |
| 'Tom Cot' | A | 5.4 | 2.3 | 8.9 | 5.2 | 1.6 | 8.2 | 22.6 | 2.7 | 47.1 | 1.05 (±0.01) | 0.895 | 0.25 | 9.52 | 0.105 |
| 'Faralia' | B | 7.3 | 2.0 | 12.0 | 6.4 | 1.6 | 10.4 | 37.1 | 2.8 | 83.1 | 1.15 (±0.01) | 0.850 | 0.55 | 6.67 | 0.150 |
| 'Flavour Cot' | B | 6.7 | 2.0 | 9.5 | 5.8 | 1.3 | 8.4 | 30.4 | 3.0 | 60.1 | 1.16 (±0.01) | 0.873 | 0.36 | 7.87 | 0.127 |
| 'Ninfa' | B | 6.8 | 1.9 | 9.5 | 5.7 | 1.4 | 7.8 | 28.5 | 2.5 | 49.3 | 1.18 (±0.001) | 0.898 | 0.24 | 9.80 | 0.102 |
| 'Orange Rubis' | B | 7.1 | 2.2 | 10.5 | 6.0 | 1.5 | 9 | 33.3 | 3.0 | 64.2 | 1.20 (±0.01) | 0.895 | 0.30 | 9.52 | 0.105 |
| 'Pellecchiella' | B | 6.5 | 1.7 | 11.4 | 5.8 | 1.1 | 9.6 | 27.9 | 1.5 | 73.8 | 1.14 (±0.01) | 0.896 | 0.32 | 9.615 | 0.104 |
| 'Stella' | B | 7.7 | 2.3 | 11.5 | 6.5 | 1.4 | 9.7 | 36.5 | 0.9 | 70.1 | 1.19 (±0.01) | 0.808 | 0.63 | 5.21 | 0.192 |
| 'Vitillo' | B | 7.1 | 1.7 | 11.7 | 6.0 | 1.2 | 10 | 32.7 | 2.0 | 80.5 | 1.19 (±0.01) | 0.898 | 0.43 | 9.80 | 0.102 |
| 'Ceccona' | C | 6.8 | 2.6 | 10.0 | 5.7 | 1.8 | 8.5 | 27.0 | 1.7 | 54.6 | 1.21 (±0.01) | 0.823 | 0.33 | 5.65 | 0.177 |
| 'Lily Cot' | C | 7.6 | 3.2 | 10.7 | 5.8 | 2.1 | 8.6 | 31.0 | 4.9 | 61.9 | 1.33 (±0.01) | 0.871 | 0.28 | 7.75 | 0.129 |
| 'Monteruscello' | C | 7.3 | 1.9 | 9.6 | 5.9 | 1.8 | 7.6 | 30.0 | 2.1 | 51.7 | 1.24 (±0.01) | 0.733 | 0.38 | 3.75 | 0.267 |
| 'Pearl Cot' | C | 5.8 | 2.1 | 10.0 | 4.7 | 1.1 | 8 | 19.7 | 1.9 | 50.2 | 1.24 (±0.01) | 0.889 | 0.21 | 9.01 | 0.111 |
| 'Wonder Cot' | C | 7.0 | 2.1 | 11.0 | 5.3 | 2 | 8.4 | 27.0 | 3.7 | 60.4 | 1.33 (±0.01) | 0.888 | 0.25 | 8.93 | 0.112 |

[a] Standard errors in parentheses.
[b] Coefficient of determination ($R^2$), mean square errors (MSE in $cm^2$), of the linear regression between leaf L and W.

models because of the calculation ease (Castelan-Estrada et al., 2002). The coefficient of determination ($R^2$) of the five models used in the calibration experiment ranged between 0.848 and 0.976 (Table 2). According to the selection criteria previously described (highest $R^2$; lowest MSE and PRESS; similarity between PRESS and SSE values), LA prediction models based only on L measurements (models 1 and 4) were not acceptable for estimating LA of apricot, because of the relatively low $R^2$ (0.848–0.893) and high MSE (15.5–29.6 cm$^2$) and PRESS (12,424–38,134 cm$^2$) (Table 2). However, a significant improvement in LA prediction was observed when the model including W$^2$ as an independent variable was adopted. Indeed, the model based on W$^2$ (model 5) showed a high accuracy in prediction for the three apricot groups, as suggested by the high $R^2$ (0.951–0.967), the small MSE (6.9–8.7 cm$^2$) and the reasonable similarity between PRESS and SEE values (Table 2). LA prediction model based only on W measurement (W$^2$, model 5) is highly appreciated because of its simplicity and convenience, as it involves the utilization of one variable (W) compared to the L × W model characterized by the double measurements of leaf dimensions (Robbins and Pharr, 1987). Although model 5 exhibited accurate LA estimates for each L:W class, the major interest was to verify whether a single common W$^2$ model could be adopted for apricot irrespective of L:W classes. This was not the case in the current experiment since significant differences between the slopes and intercepts were observed among the three L:W groups (Table 2).

To find a model able to predict single LA accurately for apricot across the L:W ratios (i.e., cultivars), the product L × W was used as an independent variable (model 3; Table 2). In fact this linear model exhibited the highest $R^2$ (0.956–0.976), the smallest MSE (4.3–7.7 cm$^2$) and PRESS (3,490–11,119 cm$^2$) and also a reasonable similarity between PRESS and SEE values, compared to the other models (Table 2). Furthermore, analysis of covariance was performed to test the significance of the differences in the slopes and the intercepts among the three groups of cultivars. Since pairwise differences in slopes and intercepts were not significant ($P > 0.05$), data for the three L:W groups were pooled and a single regression using model 3 [LA = 1.193 + 0.668 (L × W)] was adopted. Consequently, the use of both leaf measures was necessary to predict apricot LA accurately. This is in agreement with previous findings on other fruit and nut trees (Demirsoy et al., 2004; Cristofori et al., 2007, 2008; Mendoza-de Gyves et al., 2007, 2008; Mazzini et al., 2010; Kishore et al., 2012; Keramatlou et al., 2015) as well as on ornamental species (Fascella et al., 2015). All these previous studies highlighted that models containing the product L × W gave a more accurate prediction than models based on single measurement (i.e., either L or W). In addition, the regression coefficient (i.e., shape coefficient) of the L × W model (0.67) obtained in this experiment agreed closely with those reported (0.61–0.82) for other fruit and nut trees such as peach (Demirsoy et al., 2004), hazelnut (Cristofori et al., 2007), persimmon (Cristofori et al., 2008), kiwifruit

**Table 2.** Fitted constant ($a$) and coefficient ($b$) of the five linear models used to estimate the individual apricot leaf area (LA in cm$^2$) from length (L) and width (W) in different leaf shape classes and in the combined models. All data were derived from the model construction (3,040 leaves). Differences in slopes and intercepts between the three leaf shape classes were tested using analysis of covariance.

| No. and form of the tested model | Leaf shape (L:W) group | Fitted coefficient and constant | | $R^{2a}$ | MSE[a] (cm$^2$) | PRESS[a] (cm$^2$) | SSE[a] (cm$^2$) |
|---|---|---|---|---|---|---|---|
| | | $a$ (cm$^2$) | $b$ | | | | |
| (1) LA = $a$ + $b$L | 1.00-1.09 | -16.286[b] | 7.184[a] | 0.848 | 26.421 | 38093 | 37993 |
| | 1.14-1.20 | -18.095[a] | 7.179[a] | 0.893 | 27.821 | 22320 | 22201 |
| | 1.21-1.33 | -15.994[b] | 6.211[b] | 0.891 | 15.490 | 12424 | 12361 |
| | Combined | -15.769 | 6.782 | 0.839 | 31.2 | 94813 | 94686 |
| (2) LA = $a$ + $b$W | 1.00-1.09 | -17.565[b] | 7.657[B] | 0.938 | 10.760 | 15532 | 15472 |
| | 1.14-1.20 | -19.904[a] | 8.671[a] | 0.942 | 15.225 | 12242 | 12150 |
| | 1.21-1.33 | -16.838[b] | 7.970[B] | 0.930 | 9.891 | 7943 | 7893 |
| | Combined | -17.84 | 7.98 | 0.922 | 15.066 | 45854 | 45772 |
| (3) LA = $a$ + $b$($L$×W) | 1.00-1.09 | 1.196[a] | 0.674[a] | 0.956 | 7.710 | 11119 | 11087 |
| | 1.14-1.20 | 1.183[a] | 0.673[a] | 0.976 | 6.287 | 5048 | 5017 |
| | 1.21-1.33 | 1.180[a] | 0.669[a] | 0.969 | 4.350 | 3490 | 3471 |
| | Combined | 1.193 | 0.668 | 0.964 | 7.055 | 21465 | 21433 |
| (4) LA = $a$ + $b$L$^2$ | 1.00-1.09 | 3.445[b] | 0.600[a] | 0.848 | 26.445 | 38134 | 38028 |
| | 1.14-1.20 | 4.113[a] | 0.524[b] | 0.886 | 29.652 | 23798 | 23662 |
| | 1.21-1.33 | 3.302[b] | 0.463[c] | 0.884 | 16.454 | 13201 | 13130 |
| | Combined | 4.632 | 0.517 | 0.827 | 33.469 | 101829 | 101679 |
| (5) LA = $a$ + $b$W$^2$ | 1.00-1.09 | 2.620[a] | 0.663[b] | 0.951 | 8.518 | 12298 | 12249 |
| | 1.14-1.20 | 1.851[b] | 0.769[a] | 0.967 | 8.684 | 6971 | 6930 |
| | 1.21-1.33 | 1.688[b] | 0.783[a] | 0.951 | 6.964 | 5586 | 5557 |
| | Combined | 2.582 | 0.709 | 0.937 | 12.155 | 36996 | 36926 |

[a] Coefficient of determination ($R^2$), mean square errors (MSE in cm$^2$), predicted residual error sum of squares (PRESS in cm$^2$), and error sum of squares (SSE in cm$^2$) of the various models are also given. [b] Standard errors in parenthesis; L and W were in cm.
Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P = 0.05$).

(Mendoza-de Gyves et al., 2007), medlar (Mendoza-de Gyves et al., 2008), raspberry, redcurrant, blackberry, gooseberry, highbush and blueberry (Fallovo et al., 2008), citrus (Mazzini et al., 2010), apple (Kishore et al., 2012) and Persian walnut (Keramatlou et al., 2015).

The prediction capacity of the best model (L × W) was validated using an independent data set (520 leaves) coming from two different cultivars, 'Orange Rubis' and 'Pellecchiella'. The regression coefficients (b parameter) for the L × W did not differ between the estimation and validation models (0.668 vs. 0.673) (Table 3). Besides, the coefficient of multiple determination of the estimation and validation models were similar for L × W model (0.964 vs. 0.978), demonstrating the robustness of the proposed model (Table 3). Another important criteria to assess the model predictive capability is using them to estimate each case in the validation data set and then to calculate the MSPR. If the MSPR is close to the MSE of the estimation model, then the MSE of the selected model is not biased and could be adopted for accurate LA estimation. In the present study, the MSPR from the validation data set for the L × W model did not differ greatly from the MSE of the estimation data set (Table 3). This indicates a good predictive ability of this estimation model.

In the model validation, correlation coefficients showed that there was a highly reliable relationship between the predicted and the observed LA values, giving an underestimation of 2.9% in the prediction (Figure 2). Moreover, the correlation coefficients between predicted LA and observed LA for L × W model was 0.978 (Table 3). However, correlation is an insufficient statistical analysis to elucidate the relationship between the predicted LA and the observed LA and plotting the residuals against the observed LA would be more informative (Marini, 2001) as reported in Figure 2. The lack of agreement between the predicted LA and the observed LA can be assessed by calculating the bias (i.e., mean of the differences [d] and the standard deviations [sd] of the differences). As illustrated in Figure 2, the central dashed lines represent the mean of the differences. In the current study, the differences were distributed normally, since 97% of the differences lies within the range d ±3 sd, whereas very few points were outside these dotted lines (Figure 2).

**Table 3.** Statistics and parameter estimates from regression model L×W for individual leaf area (LA, $cm^2$) estimation. The estimation model was developed from nineteen apricot genotypes (3,040 leaves). Validation model was developed from two apricot cultivars 'Orange Rubis' and 'Pellecchiella' (520 leaves).

| Statistic or parameter estimate | Estimation model L × W | Validation model L × W |
|---|---|---|
| Intercept | 1.193 | 1.301 |
| Standard error of intercept | 0.111 | 0.216 |
| Regression coefficient | 0.668 | 0.673 |
| Standard error of regression coefficient | 0.002 | 0.004 |
| Prediction sum of squares (PRESS) | 21465 | – |
| Error sum of squares (SSE) | 21433.00 | 2681.00 |
| Mean squared prediction error (MSPR) | – | 5.28 |
| Mean square error (MSE) | 7.10 | 5.18 |
| Coefficient of multiple determination $R^2$ | 0.964 | 0.978 |

## Conclusions

Very close relationships were found between the observed and the predicted leaf area values by the model having L × W as an independent variable. The model that required two measures per leaf [LA = 1.193 + 0.668 (L × W)] does not only provide more accurate estimations than one-dimensional models, but also was independent of the cultivar (i.e., L:W groups). The results of this study suggest that the L × W model can be successfully adopted in research on apricot, since it allows to estimate accurately, nondestructively and in large quantities the individual LA of several apricot cultivars without the use of expensive devices such as planimeter, plant canopy analyzers and hand scanners.

## References


Antunes, W.C., Pompelli, M.F., Carretero, D.M., and DaMatta, F.M. (2008). Allometric models for non-destructive leaf area estimation in coffee (*Coffea arabica* and *Coffea canephora*). Ann. Appl. Biol. *153*, 33–40. https://doi.org/10.1111/j.1744-7348.2008.00235.x.

Basile, B., Giaccone, M., Shahak, Y., Forlani, M., and Cirillo, C. (2014). Regulation of the vegetative growth of kiwifruit vines by photo-selective anti-hail netting. Sci. Hortic. *172*, 300–307. https://doi.org/10.1016/j.scienta.2014.04.011.

Bassi, D., and Audergon, J.M. (2006). Apricot breeding: update and perspectives. Acta Hortic. *701*, 279–294. https://doi.org/10.17660/ActaHortic.2006.701.43.

Bland, J.M., and Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet *1*, 307–310. https://doi.org/10.1016/S0140-6736(86)90837-8.

Castelan-Estrada, M., Vivin, P., and Gaudillère, J.P. (2002). Allometric relationships to estimate seasonal above-ground vegetative and reproductive biomass of *Vitis vinifera* L. Ann. Bot. *89*, 401–408. https://doi.org/10.1093/aob/mcf059.

Cristofori, V., Rouphael, Y., Mendoza-de Gyves, E., and Bignami, C. (2007). A simple model for estimating leaf area of hazelnut from linear measurements. Sci. Hortic. *113*, 221–225. https://doi.org/10.1016/j.scienta.2007.02.006.

Cristofori, V., Fallovo, C., Mendoza-de Gyves, E., Rivera, C.M., Bignami, C., and Rouphael, Y. (2008). Non-destructive, analogue model for leaf area estimation in Persimmon (*Diospyros kaki* L.f.) based on leaf length and width measurement. Eur. J. Hortic. Sci. *73*, 216–221.

Demirsoy, H., and Demirsoy, L. (2003). A validated leaf area prediction model for some cherry cultivars in Turkey. Pak. J. Bot. *35*(3), 361–367.

Demirsoy, H., Demirsoy, L., Uzun, S., and Ersoy, B. (2004). Nondestructive leaf area estimation in peach. Eur. J. Hortic. Sci. *69*, 144–146.

Fallovo, C., Cristofori, V., Mendoza-de Gyves, E., Rivera, C.M., Rea, R., Fanasca, S., Sassine, Y., and Rouphael, Y. (2008). Leaf area estimation model for small fruits from linear measurements. HortScience *43*, 2263–2267.

Fascella, G., Cristofori, V., Rivera, C.M., and Rouphael, Y. (2015). A simple and accurate model for the non-destructive estimation of leaf areas in genotypes of *Plumeria rubra* L. J. Hortic. Sci. & Biotechnol. *90*(3), 267–272. https://doi.org/10.1080/14620316.2015.11513181.

Flore, J.A. (1994). Stone fruit. In Handbook of Environmental Physiology of Fruit Crops, Vol. I, Temperate crops, B. Schaffer, and P.C. Anderson, eds. (Boca Raton, Florida: CRC Press), p. 233–270.

Giaccone, M., Pannico, A., Scognamiglio, P., Rivera, C.M., Cirillo, C., Rouphael, Y., De Pascale, S., and Basile, B. (2017). Regression model for leaf area estimation in *Ficus carica* L. Acta Hortic. (in press).

Gill, J.L. (1986). Outliers, and influence in multiple regression. J. Anim. Breed. Genet. *103*, 161–175. https://doi.org/10.1111/j.1439-0388.1986.tb00079.x.

Giuffrida, F., Rouphael, Y., Toscano, S., Scuderi, D., Romano, D., Rivera, C.M., Colla, G., and Leonardi, C. (2011). Simple model for nondestructive leaf area estimation in bedding plants. Photosynthetica *49*, 380–388. https://doi.org/10.1007/s11099-011-0041-z.

Ghoreishi, M., Hosseini, Y., and Maftoon, M. (2012). Simple models for predicting leaf area of mango (*Mangifera indica* L.). J. Biol. Earth Sci. *2*, 45–53.

Keramatlou, I., Sharifani, M., Sabouri, H., Alizadeh, M., and Kamkar, B. (2015). A simple linear model for leaf area estimation in Persian walnut (*Juglans regia* L.) Sci. Hortic. *184*, 36–39. https://doi.org/10.1016/j.scienta.2014.12.017.

Kishore, D.K., Pramanick, K.K., Verma, J.K., and Singh, R. (2012). Non-destructive estimation of apple (*Malus ×domestica* Borkh.) leaf area. J. Hortic. Sci. Biotechnol. *87*, 388–390. https://doi.org/10.1080/14620316.2012.11512881.

Lo Bianco, R., Farina, V., Indelicato, S.G., Filizzola, F., and Agozzino, P. (2010). Fruit physical, chemical and aromatic attributes of early, intermediate and late apricot cultivars. J. Sci. Food Agric. *90*, 1008–1019. https://doi.org/10.1002/jsfa.3910.

Marini, R.P. (2001). Estimating mean fruit weight and mean fruit value for apple trees: comparison of two sampling methods with the true mean. J. Am. Soc. Hortic. Sci. *126*, 503–510.

Marquardt, D.W. (1970). Generalized inverse, ridge regression and biased linear estimation. Technometrics *12*, 591–612. https://doi.org/10.2307/1267205.

Mazzini, R.B., Ribeiro, R.V., and Pio, R.M. (2010). A simple and non-destructive model for individual leaf area estimation in citrus. Fruits *65*, 269–275. https://doi.org/10.1051/fruits/2010022.

Mendoza-de Gyves, E., Rouphael, Y., Cristofori, V., and Rosana Mira, F. (2007). A non-destructive, simple and accurate model for estimating the individual leaf area of kiwi (*Actinidia deliciosa*). Fruits *62*, 171–176. https://doi.org/10.1051/fruits:2007012.

Mendoza-de Gyves, E., Cristofori, V., Fallovo, C., Rouphael, Y., and Bignami, C. (2008). Accurate and rapid technique for leaf area measurement in medlar (*Mespilus germanic*a L.). Adv. Hortic. Sci. *22*(3), 223–226.

Miranda, C., and Royo, J.B. (2003). A statistical model to estimate potential yields in peach before bloom. J. Am. Soc. Hortic. Sci. *128*, 297–301.

Neri, D., Morini, F., Massetani, F., and Pirazzini, P. (2010). Pruning: How to manage shoot growth. Acta Hortic. *862*, 355–364. https://doi.org/10.17660/ActaHortic.2010.862.55.

Neter, J., Kutner, M.H., Nachtshein, C.J., and Wasserman, W. (1996). Applied Linear Regression – Models. 3rd ed. (Irwin: Homewood III).

Pérez-Pastor, A., Ruiz-Sánchez, M.C., and Domingo, R. (2014). Effects of timing and intensity of deficit irrigation on vegetative and fruit growth of apricot trees. Agric. Water Manag. *134*, 110–118. https://doi.org/10.1016/j.agwat.2013.12.007.

Piagnani, M.C., Castellari, L., Sgarbi, P., and Bassi, D. (2013). Fruit quality evaluation of diverse apricot cultivars. Asp. Appl. Biol. *119*, 139–144.

Pompelli, M.F., Antunes, W.C., Ferreira, D.T.R.G., Cavalcante, P.G.S., Wanderley-Filho, H.C.L., and Endresc, L. (2012). Allometric models for non-destructive leaf area estimation of *Jatropha curcas*. Biomass Bioenergy *36*, 77–85. https://doi.org/10.1016/j.biombioe.2011.10.010.

Rivera, C.M., Rouphael, Y., Cardarelli, M., and Colla, G. (2007). A simple and accurate equation for estimating individual leaf area of eggplant from linear measurements. Eur. J. Hortic. Sci. *70*, 228–230.

Robbins, S.N., and Pharr, D.M. (1987). Leaf area prediction models for cucumber from linear measurements. HortScience *22*, 1264–1266.

Rouphael, Y., Rivera, C.M., Cardarelli, M., Fanasca, S., and Colla, G. (2006). Leaf area estimation from linear measurements in zucchini plants of different ages. J. Hortic. Sci. Biotechnol. *81*, 238–241. https://doi.org/10.1080/14620316.2006.11512056.

Rouphael, Y., Colla, G., Fanasca, S., and Karam, F. (2007). Leaf area estimation of sunflower leaves from simple linear measurements. Photosynthetica *45*, 306–308. https://doi.org/10.1007/s11099-007-0051-z.

Rouphael, Y., Mouneimne, A.H., Ismail, A., Mendoza-de Gyves, E., Rivera, C.M., and Colla, G. (2010). Modeling individual leaf area of rose (*Rosa hybrida* L.) based on leaf length and width measurement. Photosynthetica *48*, 9–15. https://doi.org/10.1007/s11099-010-0003-x.

Serdar, U., and Demirsoy, H. (2006). Non-destructive leaf area estimation in chestnut. Sci. Hortic. *108*, 227–230. https://doi.org/10.1016/j.scienta.2006.01.025.

Spann, T., and Heerema, R. (2010). A simple method for non-destructive estimation of total shoot leaf area in tree fruit crops. Sci. Hortic. *125*(3), 528–533. https://doi.org/10.1016/j.scienta.2010.04.033.

Wang, Z., and Zhang, L. (2012). Leaf shape alters the coefficients of leaf area estimation models for *Saussurea stoliczkai* in central Tibet. Photosynthetica *50*(3), 337–342. https://doi.org/10.1007/s11099-012-0039-1.

Zenginbal, H., Uzun, S., Ozcan, M., and Demir, T. (2007). Leaf area prediction model for some kiwifruit (*Actinidia chinensis* Planch.) cultivars. J. Plant Sci. *2*, 470–474. https://doi.org/10.3923/jps.2007.470.474.

Zhang, L., and Liu, X.S. (2010). Non-destructive leaf-area estimation for *Bergenia purpurascens* across timberline ecotone, southeast Tibet. Ann. Bot. Fennici *47*, 346–352. https://doi.org/10.5735/085.047.0504.

Zhang, L., and Pan, L. (2011). Allometric models for leaf area estimation across different leaf age groups of evergreen broadleaved trees in a subtropical forest. Photosynthetica *49*(2), 219–226. https://doi.org/10.1007/s11099-011-0027-x.

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