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Short note

Accurate and rapid technique for leaf area measurement in medlar (*Mespilus germanica* L.)

E. Mendoza-de Gyves*, V. Cristofori*, C. Fallovo*, Y. Rouphael*, C. Bignami**

* *Dipartimento di Produzione Vegetale, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy.*

** *Dipartimento di Scienze Agrarie, Università degli Studi di Modena e Reggio Emilia, Viale Kennedy, 17, 42100 Reggio Emilia, Italy.*

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Abstract: Accurate measurements of leaf area are important for agronomic and physiological studies. Determining the individual leaf area (A) of medlar (*Mespilus germanica* L.) involves measurements of leaf parameters, such as length (L) and width (W), or some combinations of these parameters. A two-year investigation was carried out in 2005 (on seven genotypes) and in 2006 (on one cultivar) underopen-field conditions to test whether a model could be developed to estimate leaf area across genotypes. Regression analyses of A versus Land W revealed several models could be used for estimating the area of individual medlar leaves. A linear model having LW as the independent variable (A = 1.81 + 0.68 LW) provided the most accurate estimate (R^2 = 0.981, MSE = 7) of medlar leaf area. Validation of the model having LW of leaves from another genotype measured in the 2006 experiment showed that the correlation between calculated and measured areas was very high. Using the selected model, agronomists and physiologists can accurately and reliably estimate the leaf area of medlarwithout the use of expensive instruments.

1. Introduction

The leaf area of a crop is a determinant factor in mechanisms such as radiation interception, water and energy exchange, growth and yield potential. Therefore, accurate measurements of leaf area (A) are essential to understand the interaction between crop growth and environment (de Jesus *et al.*, 2001). The total leaf area of the plant can be obtained either by direct or indirect measurements. The direct method consists of removing all leaves from the plant and measuring them. This method is destructive and requires adequate, potentially expensive equipment. Indirect methods, instead, are non-destructive, less expensive, and can provide accurate leaf area estimates (Norman and Campbell, 1989). Indirect methods are useful when suitable equipment is not available or non-destructive measurements are required, for example measurements carried out on plants growing in pots of controlled experiments or when using unique plants, as in genetically segregating populations. One of the most frequently used non-destructive and indirect methods is estimating leaf area from mathematical equations involving linear measurement such as leaf length, or leaf width, or some combination of these variables, which are generally chosen for their simplicity and accuracy (Blanco and Folegatti, 2003). Leaf area has been found to be related to linear leaf dimensions, which are used in many crops to estimate leaf area (Uzun and Celik, 1999; Kandiannan *et al.*, 2002; Lu *et al.*, 2004).

Various combinations of measurements and various models relating length and width to area have been developed for several fruit trees, such as avocado (Uzun and Celik, 1999), pistachio (Ranjbar and Damme, 1999), grape (Montero *et al.*, 2000; Williams and Martinson, 2003), cherry (Demirsoy and Demirsoy, 2003), peach (Demirsoy *et al.*, 2004), chestnut (Serdar and Demirsoy, 2006), *Actinidia deliciosa* (Mendoza-de Gyves *et al.*, 2007) and hazelnut (Cristofori *et al.*, 2007), while information on the estimation of medlar (*Mespilus germanica* L.) leaf areas is still lacking.

The accuracy of the predictions is dependent on the variation of leaf shape between genotypes. Since leaf shape (length:width ratio) may vary among different genetic materials (Stoppani *et al.*, 2003), a good model

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of non-destructive leaf area estimation is needed for the physiological study of medlar plants independently of the genetic materials.

Therefore, the aims of this study were: (1) to develop a model for leaf area prediction from linear measurements of leaf length and width in medlar that was able to accommodate the effect of changes in leaf shape between genotypes and able to be used for medlar plants of all accessions without recalibration and (2) to assess the robustness of the model on an independent set of data from another genotype grown under different environmental conditions.

2. Materials and Methods

Data collection

Eight medlar (*Mespilus germanica* L.) genotypes, including five cultivars (Goccia, Gigante, Comune, Precoce, and Reale) and three local genotypes (Nespolo d'Olanda, Nespolo di Castelraniero, and Selvatico) were used to develop a leaf area prediction model. Wide varieties of fully expanded leaf samples collected on spurs and on the middle part of shoots were used. Leaves varied in size from large to small for each genotype and were selected randomly from different levels of the canopy ranging between 1 and 2 m from the soil, during the summer growing season in 2005 and 2006. The age of the trees was of 15 years for 'Goccia', 'Gigante', 'Comune', 'Precoce', and 'Reale' and varied between 20-25 years for 'Nespolo d'Olanda', 'Nespolo di Castelraniero', and 'Selvatico'.

Model building

A total of 2819 medlar leaves (about 400 leaves per genotype) were measured for leaf area (A), length (L) and width (W) in the model building experiment coming from seven genotypes: Gigante, Comune, Precoce, Reale, Nespolo d'Olanda, Nespolo di Castelraniero and Selvatico under field conditions at the Experimental Farm of Tuscia University, central Italy (lat. 42º25'N, long. 12º08'E) during the 2005 growing season. The trees were spaced 4 m x 2 m giving a plant density of 1250 plants ha^{-1}.

Immediately after cutting, leaves were placed in plastic bags and were transported on ice to the laboratory. Leaf L was measured from lamina tip to the point of intersection of the lamina and the petiole, along the midrib of the lamina, while leaf W was measured from end-to-end between the widest lobes of the lamina perpendicular to the lamina mid-rib. Values of L and W were recorded to the nearest 0.1 cm. The area of each leaf (A) was measured using an area meter (LI-3100; LICOR, Lincoln, NE, USA) calibrated to 0.01 cm^2.

The dependent variable (A) was regressed with different independent variables, including L, W, L^2, W^2, and the product L x W. Mean Square Error (MSE) and the values of the coefficients (b) and constants (a) were also reported, and the final model was selected based on the combination of the highest coefficient of determination (R^2) and the lowest MSE. These statistics were applied to each individual genotype and to combined data points of all genotypes for each model.

Model validation

To validate the developed model and to increase practical applicability in different environmental conditions, a validation experiment was conducted in the summer 2006 on leaf samples of 'Goccia' grown at the Experimental Farm of Lago di Vico, central Italy. This genotype was selected as one of the most representative medlar genotype cultivated in Italy. The trees were spaced at 4 m x 4 m giving a plant density of 625 plants ha^{-1}.

To validate the model, on about 400 leaves of 'Goccia', the actual leaf area and leaf width and length were determined by the previously described procedures. Leaf area of individual leaves was predicted using the best model from the calibration experiment (model building) and was compared with the actual leaf area. The slope and intercept of the model were tested to see if they were significantly different from the slope and intercept of the 1:1 correspondence line (Dent and Blackie, 1979). Regression analyses were conducted using the SigmaPlot 8.0 package (SigmaPlot, Richmond, California, USA).

3. Results and Discussion

One of leaf shape traits is the length:width ratio (L:W). In the current experiment, significant differences ($P < 0.05$) were recorded on L:W ratio among genotypes (Table 1). 'Gigante'and 'Comune'produced the largest leaves (L:W ratio ranged from 2.12 to 2.14); 'Nespolo di Castelraniero', 'Selvatico' and 'Precoce' had narrow leaves (L:W ratio ranged from 2.57 to 2.60), while 'Reale', 'Nespolo d'Olanda' and 'Goccia' exhibited an intermediate leaf shape value (L:W ratio ranged from 2.21 to 2.47) (Table 1).

Table 1 - Leaf shape (length: width ratio) values for individual medlar genotypes

Genotypes	L:W	R^2 (z)	MSE (z)
Gigante	2.12 (0.016) (y)	0.800	0.96
Comune	2.14 (0.012)	0.750	0.92
Precoce	2.60 (0.016)	0.720	1.10
Reale	2.21 (0.013)	0.744	1.09
Nespolo d'Olanda	2.38 (0.014)	0.805	0.92
Nespolo di Castelraniero	2.57 (0.017)	0.789	0.96
Selvatico	2.56 (0.016)	0.807	0.81
Goccia	2.47 (0.017)	0.867	0.93

(z) Coefficient of determination (R^2) and mean square errors (MSE, in cm^2) of the linear regression between leaf width (W) and leaf length (L).

(y) Standar errors in parentheses.

Model calibration

Leaf W, leaf L and functions of these dimensions were significantly ($P < 0.001$) correlated with A (Table 2). When A was regressed with L or W alone (Models 1 and 2), a curvilinear relationship was obtained. A linear relationship was found between A and L x W, and between A and L^2 or W^2 (Models 3, 4 and 5). Except for Model 1, all models produced a coefficient of determination (R^2) equal to or greater than 0.91 (Table 2). Based on the selection criteria previously described (higher R^2 and lower MSE), this study demonstrated that models with a single measurement of L (Models 1 and 4, Table 2) were less acceptable for estimating leaf area, due to their lowest coefficient of determination and higher MSE values. An improvement was possible for single LA estimation when W^2 was used as independent variable (Model 5). To find a model to predict single leaf area accurately for plants of all genotypes, the product of L x W was used as independent variable (Model 3). We preferred this linear model for its accuracy (smallest MSE and the highest R^2, Table 2). Therefore, both L and W measurements were necessary to estimate medlar leaf area accurately. The shape coefficient (slope of Model 3) can be described by a shape between an ellipse (0.78) and a triangle (0.5) of the same length and maximum width. Our shape coefficient (0.68) agreed closely with those calculated for other fruit trees. Values of 0.59 have been reported for *Vitis vinifera* L. (Montero *et al*., 2000) and 0.74 for hazelnut (Cristofori *et al*., 2007). Moreover, results of the present study were in accordance with previous studies on establishing reliable equations for predicting leaf area through measuring leaf dimensions. Leaf area estimation models in some species of fruit trees such as cherry (Demirsoy and Demirsoy, 2003), peach (Demirsoy *et al*., 2004) and pistachio (Ranjbar and Damme, 1999) were developed using leaf length and width as performed in our study. These models can also easily be used in physiological and agronomical studies.

Possible genotype differences using the selected model were analyzed. Slopes of the genotypes were slightly different (Table 3). However, when leaf area estimations using an equation derived for a single cultivar vs. the overall model were compared. They were not significantly different. These results suggest that a "universal" leaf area estimation model for medlar is plausible, unless other genotypes differ greatly in leaf morphology from those used in this experiment.

Model validation

Comparisons between measured vs. calculated leaf area using Model 3 (A = 1.81 + 0.68 LW) for the validation set derived from the 2006 experiment on 'Goccia' showed a high degree of correlation and provided quantitative evidence of the validity of the area estimation model (Fig. 1). The regression line of the measured vs. calculated values were not significantly (P = 0.68) different from the 1:1 correspondence. Moreover, the calculated values of A were very close to the measured values, giving an underestimation of 1.6% in the prediction.

Table 2 - Fitted coefficient (b) and constant (a) values of the models used to estimate the medlar leaf area (A) of single leaves from length (L) and width (W) measurements (combined data for seven medlar genotypes)

Model no.	Form of model tested	Fitted coefficient and constant		$R^{2\,(z)}$	MSE (z)
		a	b		
1	A= a + bL	-29.71 (0.49) (y)	6.28 (0.05)	0.899	41
2	A= a + bW	-23.07 (0.41)	13.27 (0.09)	0.915	35
3	A= a + bLW	1.81 (0.10)	0.68 (0.002)	0.981	7
4	A= a + bL^2	2.02 (0.24)	0.28 (0.002)	0.919	33
5	A= a + bW^2	5.36 (0.21)	1.39 (0.009)	0.930	29

(z) Coefficient of determination (R^2) and mean square errors (MSE, in cm^2) of the various models are also given. All data were derived from the calibration Experiment 2005 (n= 2819 leaves).
(y) Standar errors in parentheses, L and W were in cm.

Table 3 - Fitted coefficient (b) and constant (a) values for individual medlar genotypes using the model A= a + bLW, where A is the leaf area of single leaves, and LW is the product of length and width measurements

Genotypes	Fitted coefficient and constant		$R^{2\,(z)}$	MSE (z)
	a	b		
Gigante	4.20 (0.73)	0.66 (0.008)	0.971	8
Comune	3.02 (0.43)	0.65 (0.007)	0.962	7
Precoce	2.36 (0.45)	0.67 (0.009)	0.976	6
Reale	2.41 (0.46)	0.68 (0.011)	0.969	4
Nespolo d'Olanda	1.85 (0.26)	0.68 (0.008)	0.970	3
Nespolo di Castelraniero	1.60 (0.22)	0.69 (0.008)	0.967	2
Selvatico	2.73 (0.28)	0.61 (0.012)	0.952	2

(z) Coefficient of determination (R^2) and mean square errors (MSE, in cm^2). All data were derived from the calibration Experiment 2005.
(y) Standar errors in parentheses, Land W were in cm.

To summarize, we can conclude that the length-width model (i.e. Model 3) can provide accurate estimations of medlar leaf area across genotypes and environments. With this model, agronomists and physiologists can estimate, accurately and in large quantities, the leaf area of medlar plants.

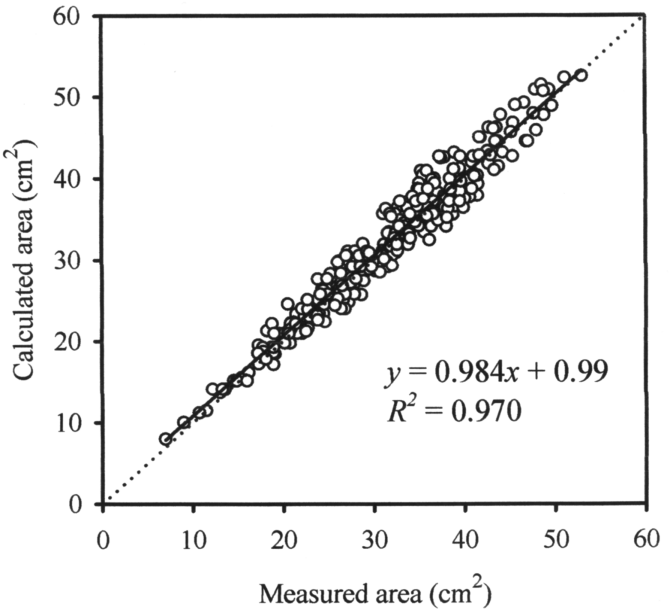

Fig. 1 - Measured vs. calculated values of single leaf area of 'Goccia' during 2006 (validation experiment) using Model 3 [A= 1.81 + 0.68 LW], where A is individual leaf area (cm^2) and LW is the product of leaf length (cm) x leaf width (cm). Solid line: linear regression line of Model 3; dotted line: the 1:1 relationship between the measured and calculated values.

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