Europ.J.Hort.Sci., **73** (5). S. 216–221, 2008, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Non-Destructive, Analogue Model for Leaf Area Estimation in Persimmon (*Diospyros kaki* L.f.) Based on Leaf Length and Width Measurement

V. Cristofori[1)], C. Fallovo[1)], E. Mendoza-de Gyves[1)], C.M. Rivera[1)], C. Bignami[2)] and Y. Rouphael[3)]
([1)]Dipartimento di Produzione Vegetale, Università della Tuscia, Viterbo, Italy, [2)]Dipartimento di Scienze Agrarie e degli Alimenti, Università degli Studi di Modena e Reggio Emilia, Reggio Emilia, Italy and [3)]Department of Crop Production, Faculty of Agricultural and Veterinary Sciences, Lebanese University, Dekwaneh-Al Maten, Lebanon)

Summary

Non-destructive methods to determine leaf areas are a useful tool in plant physiology and agronomy research. Determining the leaf area in persimmon (*Diospyros kaki* L.f.) involved measurements of leaf length and width. A two-year investigation was carried out during 2006 (on twelve genotypes) to develop a model and in 2007 (on one cultivar) to validate this model and to estimate the leaf area across persimmon genotypes and environments in Italy. Regression analyses of leaf area versus leaf length and width revealed several models for estimating the leaf area in persimmon. A linear model having leaf length × width as the independent variable (LA = 3.83 + 0.69L×W) provided the most accurate estimate (R^2=0.98, MSE=14, PRESS=32843) of leaf area in persimmon in 2006. Validation of this model in 2007 on one other genotype gave a close correlation and a high R^2 indicating that leaf area may generally be accurately determined from leaf length and width measurements in persimmon.

Key words. *Diospyros kaki* L.f. – leaf area – leaf length and width – non-destructive determination.

Introduction

Leaf area is associated with many agronomic and physiological processes, including growth analysis, photosynthesis, transpiration, light interception and energy balance (De Swart et al. 2004). Plant physiologists and agronomists have demonstrated the importance of this parameter in estimating crop growth, development rate, yield potential, radiation use efficiency, and water and nutrient use (Williams 1987; Olivera and Santos 1995; Bhatt and Chanda 2003; Williams and Martison 2003).

Measuring the surface area of a large number of leaves can be both time-consuming and labour costly. Many methods have been devised to facilitate the measurement of leaf area. However, these methods, including those of tracing, blueprinting, photographing, or using a conventional planimeter, require the excision of leaves from the plants. It is therefore not possible to make successive measurements of the same leaf. Plant canopy is also damaged, which might cause problems to other measurements or experiments. Leaf area can be also measured quickly, accurately, and non-destructively using a portable scanning planimeter (Daughtry 1990), but it is only suitable for small plants with few leaves (Nyakwende et al. 1997) and not feasible for large leaves. An alternative method to measure leaf area is to use image analysis with image measurement and analysis software. The capture of an image by a digital camera is rapid, and the analysis using proper software is accurate (Bignami and Rossini 1996), but the processing is time consuming, and the facility are generally expensive and is not suitable for non-flat leaves measurement, because pictures taken not exactly perpendicular can cause erroneous leaf area measurements. Therefore, an inexpensive, rapid, reliable, and non-destructive method for measuring leaf area is required by agronomists. Accurate, non-destructive measurements permit repeated sampling of the same leaves over time and exclude biological variation in destructive methods (De Swart et al. 2004). Especially when using unique plants, for example in genetically segregating populations, non-destructive measurements are of a great value. If the mathematical relationships between leaf area and one or more dimensions of the leaf (length and width) could be clarified, a method using just these models to estimate leaf area would be more advantageous than many of the methods mentioned above (Villegas et al. 1981; Beerling and Fry 1990). Various combinations of measurements and various models relating length and width to area have been developed for several fruit trees such as grape (Montero et al. 2000; Williams and Martinson 2003), avocado (Uzun and Celik 1999), pistachio (Ranjbar and Damme 1999), cherry (Demirsoy and Demirsoy 2003), peach (Demirsoy et al. 2004), chestnut (Serdar and Demirsoy 2006), apple (Palmer 1987), kiwifruit (Mendoza de Gyves et al. 2007),

and hazelnut (Cristofori et al. 2007), while information on the estimation of persimmon (*Diospyros kaki* L.f.) 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 proveniences (Stoppani et al. 2003), we needed a reliable model of non-destructive leaf area estimation to use in a physiological study of persimmon leaves independently of the genetic material.

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

Materials and Methods

Data collection

Thirteen persimmon (*Diospyros kaki* L.f.) genotypes collected from four Italian locations [Viterbo, Caprarola and Vetralla (Latium region, central Italy) and Caserta (Campania region, South Italy)] were used to develop a leaf area prediction model. Leaves of different sizes collected on spurs and on the middle part of non bearing shoots were used. Area of the different genotype leaves ranged from 4 to 143 cm^2, length from 2.3 to 18.1 cm and width from 1.5 to 12.5 cm. Leaves were selected randomly from different levels of the tree canopy ranging between 1 m and 2 m from the soil and all around the crown, during the summer growing season in 2006 and 2007. The age of the trees varied between ten and nineteen years.

A total of 2358 persimmon leaves (about 200 leaves per genotype) were measured for leaf area (LA), length (L) and width (W) in the preliminary calibration experiment coming from twelve genotypes: 'Izu', 'Mandarino', 'Jiro', 'Hachiya', 'Atago', 'Dolce', 'Suruga', 'O'Gosho' 'Hana Fuyu', 'Cioccolatino', 'Vaniglia', and 'Call Fuyu' under field conditions. These genotypes were selected as a representative sampling of many Japanese persimmons cultivated in the warm areas of China, Korea, Japan and for some of them, introduced in the Mediterranean region (Spain, Italy, Greece and France) (Bellini and Giordani 2005). The single-trunk trees trained as free vase were spaced 4×5 m giving a plant density of 500'plants'ha^{-1}.

Immediately after cutting, leaves were placed in plastic bags and were transported on ice to the laboratory. Leaf length was measured from lamina tip to the point of intersection of the lamina and the petiole, along the mid-rib of the lamina, while leaf width was measured from end-to-end between the widest lobes of the lamina perpendicular to the lamina mid-rib (Fig. 1). Values of length and width were recorded to the nearest 0.1 cm. The area of each leaf (LA) was measured using laboratory stationary area meter (LI-3100; LICOR, Lincoln, NE, USA) calibrated to 0.01 cm^2 using the silver disc supplied with the unit.

Model building

The dependent variable (LA) was regressed with different independent variables, including L, W, L^2, W^2, $(L + W)^2$ and the product L × W. Mean Square Error (MSE), Predicted Residual Error Sum of Squares (PRESS), Error Sum of Squares (SSE), and the values of the constants (a) and coefficients (b) were also reported, and the final model was selected based on the combination of the highest coefficient of determination (R^2), the lowest MSE, the lowest PRESS, and when the PRESS values are reasonably close to SSE. These statistics were applied to each individual genotype and to combined data points of all genotypes for each model. Moreover, using two measurements (i.e., length and width) introduces potential problems of collinearity, resulting in poor precision in the estimates of the corresponding regression coefficients. For detecting collinearity, the variance inflation factor (VIF) (Marquardt 1970) and the tolerance values (T) (Gill 1986) were calculated.

$$VIF = \frac{1}{(1 - r^2)} \quad (1)$$

$$T = \frac{1}{VIF} \quad (2)$$

where r, is the correlation coefficient. If the VIF value was higher than 10 or if T value was smaller than 0.10, then collinearity may have more than a trivial impact on the estimates of the parameters, and consequently one of them should be excluded from the model.

Model validation

In addition to validate the developed model and to increase practical applicability in different environmental conditions, a validation experiment was conducted in the summer 2007 on leaf samples of cv. 'Kaki Tipo' grown at the Experimental Farm of Tuscia University, central Italy (latitude 42 °25 ′ N, longitude 12 °08 ′ E, altitude 310 m). This genotype was selected as one of the most representative persimmon genotypes cultivated in Italy. The trees were spaced at 5×5 m giving a plant density of 400'plants'ha^{-1}.

To validate the model, 210 leaves of cv. 'Kaki Tipo' were used to determine leaf area and leaf width and length by the previously described procedures. Leaf area

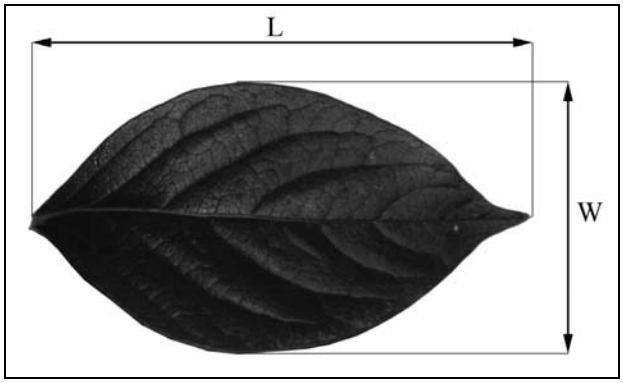


Fig. 1. Persimmon leaf showing the position of leaf length (L) and width (W) measurement.

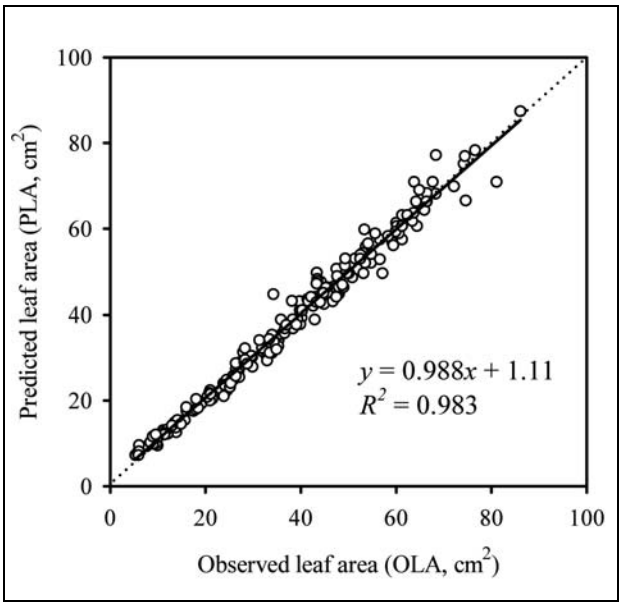


Fig. 2. Plot of predicted leaf area (PLA) using model 3 (LA = 3.83 + 0.69 LW) versus observed values of single leaf areas (OLA) of cv. 'Kaki Tipo', during 2007 (validation experiment). Solid line represents linear regression lines of model 3. Dotted lines represent the 1:1 relationship between the predicted and observed values.

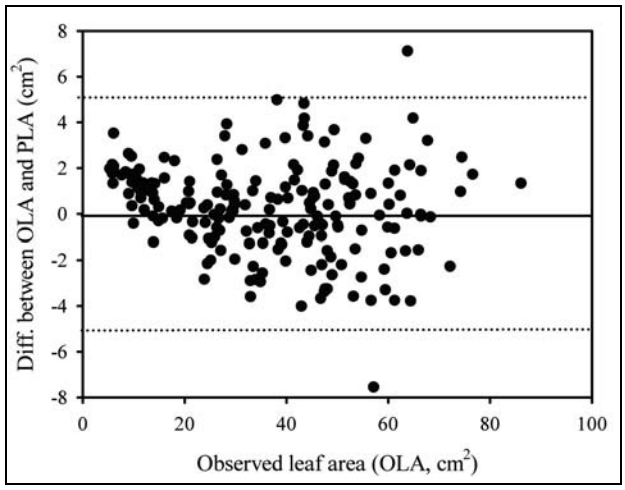


Fig. 3. The difference between predicted leaf areas (PLA) estimated by model 3 from pooled data from twelve persimmon genotypes and observed leaf area (OLA) versus the observed leaf area of 'Kaki Tipo' genotype sampled in 2007 (validation experiment). The solid line is the mean of the differences. The broken lines are 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 be lie between the limits of agreement.

of individual leaves was predicted using the best model from the calibration experiment and was compared with the actual leaf area. Moreover, to compare the predicted leaf area (PLA) to the observed leaf area (OLA) for the genotype 'Kaki Tipo' during 2007 growing season, graphical procedures (Bland and Altman 1986) were used. Scatter plots of values for the PLA against the OLA are presented in Fig. 2. GLM Procedure of SPSS was used to evaluate the linear relationship for OLA and PLA of 'Kaki Tipo'. Values for PLA were substracted from OLA, and differences were plotted against the OLA. Lack of agreement was evaluated by calculating the relative bias, estimated by the mean of the differences (d) and the standard deviation (SD) of the differences. Normality test was carried out to obtain a Wilkes-Shapiro W statistic using examines procedure of SPSS (Marini 2001).

Table 1. The leaf shape (length: width ratio), mean, minimum (min) and maximum (max) values for the leaf length, leaf width, and leaf area of persimmon genotypes.

	Leaf length (cm)			Leaf width (cm)			Leaf area (cm^2)			L:W (±SE)	R^2	MSE†
Genotypes	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max			
'Izu'	11.1	3.3	18.1	7.4	2.7	11.6	64.7	7.7	133.2	1.51 (0.015) *	0.805	1.42
'Mandarino'	9.3	4.2	17.5	6.5	2.5	10.6	48.3	9.4	119.4	1.42 (0.015)	0.838	1.01
'Jiro'	9.0	2.7	14.6	6.1	2.2	9.3	43.6	4.9	95.5	1.47 (0.014)	0.885	1.37
'Hachiya'	9.5	3.0	18.0	6.4	1.5	12.5	50.9	3.7	162.1	1.49 (0.021)	0.786	2.10
'Atago'	8.6	2.3	12.5	5.7	2.2	9.0	39.5	4.5	80.1	1.51 (0.016)	0.765	1.07
'Dolce'	9.4	3.7	14.9	6.6	2.5	9.8	50.8	7.7	94.5	1.43 (0.012)	0.884	0.81
'Suruga'	9.0	3.3	18.0	6.5	2.7	11.2	49.4	7.6	135.5	1.38 (0.017)	0.909	0.91
'O'Gosho'	8.2	3.0	14.0	5.9	2.4	9.3	39.6	6.7	87.6	1.37 (0.012)	0.906	1.06
'Hana Fuyu'	9.6	5.1	16.0	6.0	3.2	8.6	44.6	15.0	91.0	1.59 (0.023)	0.768	2.15
'Cioccolatino'	9.3	2.5	17.0	6.7	2.5	11.5	50.8	4.5	123.9	1.40 (0.012)	0.862	1.06
'Kaki Tipo'	7.9	2.3	13.2	5.6	1.5	8.5	36.8	5.2	76.6	1.42 (0.018)	0.874	0.96
'Vaniglia'	11.0	3.5	17.7	7.3	3.4	12.0	64.2	12.4	143.3	1.50 (0.016)	0.804	1.79
'Call Fuyu'	7.8	2.8	13.5	5.5	2.1	9.2	35.7	6.4	84.1	1.42 (0.018)	0.810	1.26

*Standard errors in parenthesis.
†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).

Results and Discussion

One of the leaf shape traits is the length:width ratio (L:W). In the current experiment, significant differences ($P<0.05$) in L:W ratio were recorded among genotypes (Table 1). cvs. 'Mandarino', 'Dolce', 'Suruga', 'O'Gosho', 'Cioccolatino', 'Kaki Tipo', and 'Call Fuyu' produced the largest leaves (L:W ratio ranged from 1.37 to 1.43). Moreover, cv. 'Hana Fuyu' had narrow leaves (L:W ratio was 1.59), while cvs. 'Izu', 'Jiro', 'Hachiya', 'Atago', and 'Vaniglia' exhibited an intermediate leaf shape value (L:W ratio ranged from 1.47 to 1.51) (Table 2).

As a preliminary step to model calibration, the degree of collinearity among leaf width and length was analyzed. The VIF ranged from 2.7 to 8.3 and T values ranged from 0.12 to 0.35, depending on the cultivar. In all genotypes, VIF was < 10 and T was > 0.10, showing that the collinearity between length and width can be considered negligible (Gill 1986) and these variables can be both included in the model.

Model calibration

Regression analysis demonstrated strong relationships ($P<0.001$) between leaf area (LA) and midvein length (L), leaf width (W), the product of length and width (LW), the square of the sum of length and width $(L + W)^2$, the square of length (L^2), and the square of width (W^2) (Table 2) as expected. This is in agreement with previous (Rouphael et al. 2006, 2007; Serdar and Demirsoy 2006; Cristofori et al. 2007; Mendoza de Gyves et al. 2007; Peksen 2007) non-destructive models for predicting leaf area using measurement of leaf length and width. However, suitability of these models varied based on the selection criteria previously described. Except for model 1, all models produced a coefficient of determination (R^2) equal to or greater than 0.90 (Table 2). Based on selection criteria previously described (higher R^2, lower MSE, lower PRESS, and when the PRESS values are reasonably close to SSE), this study demonstrated that models with a sole measurement of leaf length (models 1 and 5, Table 2) were less acceptable for estimating leaf area, due to their lowest coefficient of determination (R^2), higher MSE, and higher PRESS values. An improvement was possible for leaf area estimation when leaf width (model 6), and the additive model (model 4) were used as independent variable (Table 2). To find a model to predict leaf area accurately for plants of all genotypes, the product of leaf length × leaf width was used as independent variable (model 3). We preferred this model ($LA = 3.829 + 0.688LW$) for its accuracy: highest R^2 (0.98), smallest MSE (14), smallest PRESS (32843), and to the

Table 2. Fitted constant (a) and coefficient (b) of the models to estimate the leaf area (LA) from leaf length (L) and leaf width (W) measurements in persimmon.

Model No.	Model tested	Fitted coefficient and constant		R^2†	MSE†	PRESS†	SSE†
		a (±SE)	b(±SE)				
1	$LA = a + bL$	−23.01 (0.63)*	7.58 (0.060)	0.863	82.62	195079	194647
2	$LA = a + bW$	−36.69 (0.57)	13.21 (0.090)	0.918	51.61	121862	121591
3	$LA = a + b(L \times W)$	3.83 (0.16)	0.69 (0.002)	0.985	13.91	32843	32779
4	$LA = a + b(L+W)^2$	4.56 (0.20)	0.16 (0.001)	0.969	20.58	48586	48488
5	$LA = a + bL^2$	10.40 (0.36)	0.39 (0.003)	0.903	79.56	187881	187447
6	$LA = a + bW^2$	4.05 (0.30)	0.99 (0.006)	0.933	43.19	101945	101746

*Standard errors in parenthesis; L and W were in cm.
†Coefficient of determination (R^2), mean square errors (MSE in cm^2), predicted residual error sum of squares (PRESS), and error sum of squares (SSE) of the various models are also given. All data were derived from the calibration Experiment 2006 (n=2358 leaves).

Table 3. Statistics and parameter estimates from regression model for leaf area (LA, cm^2) estimation. The estimation model was developed from twelve persimmon genotypes sampled in 2006. Validation model was developed from one persimmon genotype ('Kaki Tipo') sampled in 2007.

Statistic or parameter estimate	Estimation model	Validation model
Intercept	3.829	3.346
Standard error of intercept	0.163	0.345
Regression coefficient for LW	0.688	0.690
Standard error of regression coefficient	0.002	0.006
Prediction sum of squares (PRESS)	32843	
Error sum of squares (SSE)	32779	1217
Mean square error (MSE)	13.931	5.850
P > F	<0.0001	<0.0001
Coefficient of multiple determination R^2	0.985	0.989

reasonably close PRESS value to SSE (Table 2). PRESS and SSE are measures of how well the fitted values for a subset model can predict the observed values of the response value Y_i. Some evidence of the internal validity of the fitted model is to compare PRESS and SSE (MIRANDA and ROYO 2003). The PRESS value is always larger than SSE, because the regression fit for the i^{th} case, when this case is deleted in fitting, can never be as good as that when the i^{th} case is included. In the current study, the PRESS value (32843) was reasonably close to SSE (32779) for the leaf area model 3, and support the validity of the fitted regression model and of the MSEs as an indications of the predictive capability of this model (NETER et al. 1996). Based on the above considerations, both leaf length and width measurements were necessary to estimate persimmon leaf area accurately. The results reported in the current research were in accordance with previous studies on some species of fruit tree such as hazelnut (CRISTOFORI et al. 2007), apple (PALMER 1977), and kiwifruit (MENDOZA DE GYVES et al. 2007), where the leaf area estimation models were developed using the multiplicative equation (length × width).

The shape coefficient (regression coefficient 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 (0.69) agreed closely with those calculated for other crops. Values of 0.68 have been reported for sunflower (ROUPHAEL et al. 2007), 0.63 for broccoli (STOPPANI et al. 2003), 0.62 for blueberries (NESMITH 1991), 0.63 for zucchini squash (ROUPHAEL et al. 2006), 0.74 for hazelnut (CRISTOFORI et al. 2007), and 0.59 for *Vitis vinifera* L. (MONTERO et al. 2000).

Possible genotype differences using the selected model were analyzed. Regression coefficients of the genotypes were slightly different (data not shown). However, when leaf area estimations using an equation derived for a single cultivar versus the overall model were compared, they were not significantly different. These results suggest that a universal leaf area estimation model for persimmon is plausible, unless other genotypes differ greatly in leaf morphology from those used in this experiment.

Model validation

Parameter estimates and statistics obtained from SPSS outputs are presented for the leaf area estimation and validation model (Table 3). The intercept, the regression coefficient for length × width of the estimation and validation models were not significantly (P=0.20 and P=0.58, respectively) different, and the R^2 values were similar for both models, indicating the applicability of the proposed model 3 to data beyond those on which the model is based (NETER et al. 1996).

Comparisons between observed leaf area (OLA) versus predicted leaf area (PLA) using model 3 (LA = 3.829 + 0.688 L×W) for the validation set derived from 2007 experiment on cv. 'Kaki Tipo', showed a close correlation (r=0.99, P<0.0001), and the PLA values were very close to the OLA values, giving an underestimation of 1.2 % in the prediction. However, correlation is an inappropriate analysis to explain relationship between PLA and OLA, and a plot of the differences between PLA and OLA against OLA may be more informative (BLAND and ALTMAN 1986; MARINI 2001). Plotting differences against OLA value also allow investigation of possible relationships between measurement error and the true values. Lack of agreement between estimation PLA and OLA can be evaluated by calculating the bias, estimated by the mean of the differences (d) and the SD of the differences. In Fig. 3, a solid line represents the mean of the differences. If the differences are normally distributed, 97 % of the differences will lie between d ± 3 SD, which is the case in the current study.

To summarize, we can conclude that the length-width model; can provide more accurate estimations of persimmon leaf area across genotypes and environments than those based on single length or width measurement. Because leaf width and midvein length are dimensions that can be easily measured in the field, greenhouse and pod experiments, use of this equation would enable researchers to make non-destructive measurements or repeated measurements on the same leaves. Such a model can estimate accurately and in large quantities the leaf area of persimmon plants in many experimental comparisons without the use of any expensive instruments, e.g., a leaf area planimeter or digital camera with image measurement software.

References

BEERLING, D.J. and J.C. FRY 1990: A comparison of the accuracy, variability and speed of five different methods fro estimating leaf area. Ann. Bot. **65**, 483–488.

BELLINI, E. and E. GIORDANI 2005: Germplasm and breeding of persimmon in Europe. Acta Hort. **685**, 65–75.

BHATT, M. and S.V. CHANDA 2003: Prediction of leaf area in *Phaseolus vulgaris* by non-destructive method. Bulg. J. Plant Physiol. **29**, 96–100.

BIGNAMI, C. and F. ROSSINI 1996: Image analysis estimation of leaf area index and plant size of young hazelnut plants. J. Hort. Sci. **71**, 113–121.

BLAND, J.M. and D.G. ALTMAN 1986: Statistical methods for assessing agreement between two methods of clinical measurements. Lancet, 1986I, 307–310.

CRISTOFORI, V., Y. ROUPHAEL, E. MENDOZA-DE GYVES and C. BIGNAMI 2007: A simple model for estimating leaf area of hazelnut from linear measurements. Sci. Hort. **113**, 221–225.

DAUGHTRY, C. 1990: Direct measurements of canopy structure. Remote Sens. Rev. **5**, 45–60.

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

DEMIRSOY, H., L. DEMIRSOY, S. UZUN and B. ERSOY 2004: Non-destructive leaf area estimation in peach. Eur. J. Hort. Sci. **69**, 144–146.

DE SWART, E.A.M., R. GROENWOLD, H.J. KANNE, P. STAM, L.F.M. MARCELIS and R.E. VOORRIPS 2004: Non-destructive estimation of leaf area for different plant ages and accessions of *Capsicum annuum* L. J. Hort. Sci. Biotechnol. **79**, 764–770.

GILL, J.L. 1986: Outliers, and influence in multiple regression. J. Anim. Breed. Genet. **103**, 161–175.

MARINI, R.P. 2001: Estimating mean fruit weight value for apple trees: comparison of two sampling methods with the true mean. J. Amer. Soc. Hort. Sci. **126**, 503–510.

MARQUARDT, D.W. 1970: Generalized inverse, ridge regression and biased linear estimation. Technometrics **12**, 591–612.

MENDOZA-DE GYVES, E., Y. ROUPHAEL, V. CRISTOFORI and F. ROSANA MIRA 2007: A non-destructive, simple and accurate model for estimating the individual leaf area of kiwi (*Actinidia deliciosa*). Fruits **62**, 171–176.

MIRANDA, C. and J.B. ROYO 2003: A statistical model to estimate potential yields in peach before bloom. J. Amer. Soc. Hort. Sci. **128**, 297–301.

MONTERO, F.J., J.A. DE JUAN, A. CUESTA and A. BRASA 2000: Non-destructive methods to estimate leaf area in *Vitis vinifera* L. HortScience **35**, 696–698.

NESMITH, D.S. 1991: Nondestructive leaf area estimation of rabbiteye blueberries. HortScience **26**, 1332.

NETER, J., M.H. KUTNER, C.J. NACHTSHEIN and W. WASSERMAN 1996: Applied Linear Regression Models. 3rd ed. Homewood III, Irwin.

Nyakwende, E., C.J. Paull and J.G. Atherton 1997: Non-destructive determination of leaf area in tomato plants using image processing. J. Hort. Sci. **72**, 225–262.
Olivera, M. and M. Santos 1995: A semi-empirical method to estimate canopy leaf area of vineyards. Am. J. Enol. Viticult. **46**, 389–391.
Palmer, J.W. 1987: The measurement of leaf area in apple trees. J. Hort. Sci. **62**, 5–10.
Peksen, E. 2007: Non-destructive leaf area estimation model for faba bean (*Vicia faba* L.). Sci. Hort. **113**, 322–328.
Ranjbar, A. and P. Damme 1999: Estimation of leaf area by non-destructive methods in three Iranian pistachio species (*Pistacia mutica* subsp. Cabulica, *Pistacia khinjuk* subsp. Oblonda and *Pistacia khinjuk* subsp. Populifolia). Mededelingen-Faculteit Landbouwkundige en Toegepaste Biologische Wtenschappen Universiteit Gent, Belgium.
Rouphael, Y., C.M. Rivera, M. Cardarelli, S. Fanasca and G. Colla 2006: Leaf area estimation from linear measurements in zucchini plants of different ages. J. Hort. Sci. Biotechnol. **81**, 238–241.
Rouphael, Y., G. Colla, S. Fanasca and F. Karam 2007: Leaf area estimation of sunflower leaves from simple linear measurements. Photosynthetica **45**, 306–308.
Serdar, U. and H. Demirsoy 2006: Non-destructive leaf area estimation in chestnut. Sci. Hort. **108**, 227–230.
Stoppani, M.I., R. Wolf, N. Francescangeli and H.R. Martí 2003: A nondestructive and rapid method for estimating leaf area of broccoli. Adv. in Hort. Sci. **17**, 173–175.
Uzun, S. and H. Celik 1999: Leaf area prediction models for different horticultural plants. Tr. J. Agric. For. **23**, 645–650.
Villegas, C.D., A.T. Bautista and F.R. Cotejo Jr 1981: Accurate and rapid techniques for leaf area measurement in cassava and sweet potato. Radix **3**, 10.
Williams, L.E. 1987: Growth of 'Thompson seedless' grapevines. I. Leaf area development and dry weight distribution. J. Am. Soc. Hort. Sci. **112**, 325–330.
Williams, L. and T.E. Martinson 2003: Nondestructive leaf area estimation of 'Niagara' and 'DeChaunac' grapevines. Sci. Hort. **98**, 493–498.

Received April 22, 2008 / Accepted July 22, 2008

Addresses of authors: Valerio Cristofori (corresponding author), Carlo Fallovo, Emilio Mendoza-de Gyves, Carlos Mario Rivera, Dipartimento di Produzione Vegetale, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy, Cristina Bignami, Dipartimento di Scienze Agrarie e degli Alimenti, Università degli Studi di Modena e Reggio Emilia, Via Amendola 2, pad. Besta, 42100 Reggio Emilia, Italy, and Youssef Rouphael, Department of Crop Production, Faculty of Agricultural and Veterinary Sciences, Lebanese University, Dekwaneh- Al Maten, Lebanon. e-mail: valerio75@unitus.it.