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# Simple and accurate allometric model for leaf area estimation in *Vitis vinifera* L. genotypes

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## Abstract

The aim of the present experiment was to evaluate the currently used allometric models for *Vitis vinifera* L., as well as to develop a simple and accurate model using linear measurements [leaf length (L) and leaf width (W)], for estimating the individual leaf area (LA) of nine grapevine genotypes. For model construction, a total of 1,630 leaves coming from eight genotypes in 2010 was sampled during different leaf developmental stages and encompassed the full spectrum of leaf sizes. The model with single measurement of L could be considered an interesting option because it requires measurement of only one variable, but at the expense of accuracy. To find a model to estimate individual LA accurately for grapevine plants of all genotypes, both measurements of L and W should be involved. The proposed linear model [LA = –0.465 + 0.914 (L × W)] was adopted for its accuracy: the highest coefficient of determination (> 0.98), the smallest mean square error, the smallest prediction sum of squares, and the reasonably close prediction sum of squares value to error sum of squares. To validate the LW model, an independent data set of 200 leaves coming from another genotype in 2011 was used. Correlation coefficients showed that there was a highly reliable relationships between predicted leaf area and the observed leaf area, giving an overestimation of 0.8% in the prediction.

*Additional key words*: estimation model; linear regression; nondestructive method.

## Introduction

Leaf area (LA), a measure, which is seemingly so simple and fundamental, is really the backbone that provides the framework for further research in areas such as plant pathology, agronomy, and plant physiology. These and many other disciplines including horticulture rely on the measurement of LA for their research, since LA strongly influences crop growth, developmental rate, and productivity (Lizaso *et al.* 2003, Rouphael *et al.* 2004, Rouphael and Colla 2005). LA can be measured by direct or indirect methods (Marshall 1968). Many methods were proposed for direct LA measurement, based on the collection of the leaves and on the subsequent measurements of their area by using specific instruments (*e.g.* planimeter and electronic leaf area meter) or by acquiring and processing leaf images (Giuffrida *et al.* 2011). These methods are all labor and time-consuming, especially for species having small leaves or leaves subjected to rapid withering and curling; they could also affect the accuracy of measurement (Confalonieri *et al.* 2013). In addition, direct LA measurement requires excision of leaves, and it is therefore not possible to make successive measurements of the same leaf. Plant canopy is also damaged, which might affect the other measurements by nonnegligible uncertainty (Rouphael *et al.* 2010a). As a consequence, several authors have pointed out the need for simple, accurate, rapid, inexpensive, and nondestructive method for estimating LA (Salerno *et al.* 2005, Tsialtas and Maslaris 2005, Rivera *et al.* 2007, Fallovo *et al.* 2008, Fascella *et al.* 2009, Rouphael *et al.* 2010b) in various agronomical and physiological experiments, where destructive LA measurement is not desirable, in particular in the case of measurements carried out on genetically segregated populations (De Swart *et al.* 2004) and on rare plants (Misle *et al.* 2013).

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*Abbreviations*: GLM – general linear model; L – leaf midvein length; LA – individual leaf area; LW – product leaf length and width; L:W – length to width ratio or leaf shape; MSE – mean square error; MSPR – mean squared prediction error; OLA – observed leaf area; PLA – predicted leaf area; PRESS – prediction sum of squares; $r^2$ – coefficient of determination; SE – standard errors; SSE – error sum of squares; T – tolerance values; VIF – variance inflation factor; W – maximum leaf width.

A simple approach for LA estimation is to develop ratios and regression estimators by using easily measured leaf parameters, such as leaf length (L) and leaf width (W) (Gao *et al.* 2012). Various models and regression equations have been proposed for several fruit trees, such as avocado (Uzun and Çelik 1999), 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), and citrus (Mazzini *et al.* 2010). In many experiments, the accuracy of the LA estimation model has not been carefully examined, since small or minor violations of the underlying assumptions can invalidate the interferences drawn from the analysis (Pompelli *et al.* 2012). Several models for individual LA estimation have been proposed for grapevine (Manivel and Weaver 1974, Sepúlveda and Kliewer 1983, Elsner and Jubb 1988, Schultz 1992, Montero *et al.* 2000, Williams and Martinson 2003, Tsialtas *et al.* 2008; Table 1). However, these models have been just developed for a specific genotype (*e.g.* only one or two genotypes) and consequently no information is available whether these models could be adapted to other grapevine genotypes without recalibration (Antunes *et al.* 2008). Especially, a leaf shape (L:W ratio) may vary among different genetic materials (Stoppani *et al.* 2003). Moreover, most of these models were selected and based on the highest coefficient of determination ($r^2$) between observed and predicted values and the lowest mean square deviation (MSD). However, it is inappropriate procedure, because high correlations do not indicate that the predicted values agree with the observed ones (Bland and Altman 1986). Based on these considerations, the examination of residual plots is a simple and effective method for detecting model deficiencies in regression analysis (Antunes *et al.* 2008, Pompelli *et al.* 2012). Therefore, the aims of the current paper were: (*1*) to evaluate the currently used models, (*2*) to create a statistical model, based on fast linear measurements (*e.g.* L and W), for grapevine that would fit the effect of leaf size and shape between genotypes, and (*3*) to validate the robustness (*e.g.* unbiased) of the selected model with an independent data set coming from other genotype.

Table 1. Previous models developed for estimating individual leaf area (LA) of grapevine using simple linear measurements. $r^2$ – coefficient of determination. SEE – standard error of estimate; MSD – mean square deviation.

| Model No. | Form of model tested | Genotypes | Independent variable used | Validation experiment | Criteria for model selection | References |
|---|---|---|---|---|---|---|
| 1 | $LA = 1.162\ L^2 - 0.802\ L + 1.051$ | Grenache | L | No | $r^2$ | Manivel and Weaver (1974) |
| 2 | $LA = 0.644\ W^2 + 0.469\ W + 0.109$ | Grenache | W | No | $r^2$ | Manivel and Weaver (1974) |
| 3 | $LA = 0.69\ (L \times W) + 3.17$ | Chardonnay | L × W | No | $r^2$, SEE | Sepúlveda and Kliewer (1983) |
| 4 | $LA = 0.68\ (L \times W) + 2.49$ | Chenin blanc | L × W | No | $r^2$, SEE | Sepúlveda and Kliewer (1983) |
| 5 | $LA = -3.01 + 0.85\ (L \times W)$ | Concord | L × W | No | $r^2$, SEE | Elsner and Jubb (1988) |
| 6 | $LA = -1.41 + 0.527\ W^2 + 0.254\ L^2$ | Concord | L and W | No | $r^2$, SEE | Elsner and Jubb (1988) |
| 7 | $LA = 1.18 \times (L - 2.6) \times (L + 8.75)$ | White Riesling | L | Yes | $r^2$ | Schultz (1992) |
| 8 | $LA = 0.587\ (L \times W)$ | Cencibel | L × W | No | $r^2$ | Montero *et al.* (2000) |
| 9 | $LA = 0.647\ L^{1.956}$ | Cencibel | L | No | $r^2$ | Montero *et al.* (2000) |
| 10 | $LA = 0.637\ W^{1.995}$ | Niagara | W | No | $r^2$, SEE | Williams and Martinson (2003) |
| 11 | $LA = 0.672\ W^{1.963}$ | DeChaunac | W | No | $r^2$, SEE | Williams and Martinson (2003) |
| 12 | $LA = 18.379\ L - 151.41$ | Cabernet-Sauvignon | L | No | $r^2$, MSD | Tsialtas *et al.* (2008) |

## Materials and methods

**Field experiments**: The experiment was carried out during two consecutive growing seasons 2010 and 2011, in a five-year-old, commercial vineyard located at Rutigliano (Bari), Southern Italy (41°10'N, 17°00'E, 250 m a.s.l.). The climate is of the Mediterranean type, with hot and dry summers and mild winters, having an average annual rainfall of 500–600 mm, with about 350–400 mm falling during the autumn and winter months. The soil was a sandy-clay loam soil with the following characteristics: bulk density of 1.1 g $cm^{-3}$, pH 7.1, 2.1% of organic matter, 246 mg(exchangeable K) $kg^{-1}$, with a textural analysis of 49% sand, 17% silt, and 34% clay. The vines were spaced 2.5 m between rows and 2.5 m along rows, summing up about 1,600 plants per hectare. The training system was "tendone", consisting of a continuous overhead canopy under which the bunches are disposed (Rana *et al.* 2004). Standard cultural practices in the region were applied during the two growing seasons.

**Data collection**: Nine grapevine (*Vitis vinifera* L.) genotypes were used to develop the LA prediction model. Wide varieties of fully expanded (> 3 cm) leaves were used. The leaves encompassed the broadest range as possible. The minimum LA sampled was 10.2 $cm^2$ and maximum was 540.0 $cm^2$ (Table 2). In both experiments, the leaves were randomly sampled from different parts of the grapevines,

Table 2. Mean, minimum (min), maximum (max) values for leaf length (L), width (W), leaf area (LA), length:width (L:W) ratio, variation inflation factor (VIF), and tolerance values (T) of grapevine (*Vitis vinifera* L.) genotypes. SE – standard error; $r^2$ – coefficient of determination of the linear regression between W and L.

| Genotype | L [cm] | | | W [cm] | | | LA [cm$^2$] | | | L:W ± SE | $r^2$ | VIF | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | | | | |
| Big Perlon | 11.3 | 4.2 | 25.0 | 14.2 | 5.5 | 28.0 | 154.8 | 20.0 | 540.0 | 0.80 ± 0.013 | 0.915 | 6.14 | 0.16 |
| Black Magic | 9.8 | 3.6 | 23.0 | 12.7 | 4.2 | 23.0 | 134.5 | 16.8 | 424.1 | 0.77 ± 0.012 | 0.896 | 5.07 | 0.19 |
| Crimson | 9.4 | 3.1 | 18.5 | 12.7 | 3.9 | 22.2 | 116.5 | 10.2 | 336.3 | 0.73 ± 0.016 | 0.876 | 4.29 | 0.23 |
| Italia | 10.1 | 4.7 | 17.4 | 13.4 | 6.2 | 24.4 | 128.4 | 26.5 | 379.6 | 0.75 ± 0.017 | 0.859 | 3.81 | 0.26 |
| Michele Palieri | 11.8 | 5.5 | 19.5 | 15.3 | 8.2 | 24.2 | 177.4 | 39.8 | 461.0 | 0.77 ± 0.014 | 0.902 | 5.36 | 0.18 |
| Red Globe | 11.5 | 4.6 | 21.5 | 14.8 | 6.0 | 26.6 | 164.7 | 24.6 | 441.1 | 0.78 ± 0.016 | 0.904 | 5.47 | 0.18 |
| Sugraone | 10.5 | 4.1 | 17.4 | 13.2 | 6.0 | 20.6 | 126.8 | 23.2 | 281.1 | 0.80 ± 0.015 | 0.854 | 3.69 | 0.27 |
| Victoria | 11.7 | 3.4 | 21.2 | 16.2 | 4.5 | 28.0 | 204.6 | 12.8 | 480.6 | 0.72 ± 0.013 | 0.901 | 5.31 | 0.18 |
| Vitroblack | 10.1 | 4.9 | 16.5 | 13.2 | 6.8 | 22.5 | 125.1 | 25.6 | 308.8 | 0.76 ± 0.018 | 0.901 | 5.31 | 0.18 |

during different phenological stages (*e.g.* anthesis, flowering, veraison, and repining). In total, 1,630 healthy leaves (about 200 leaves per genotype) were measured for LA, L, and W in the calibration experiment (*e.g.* model building) coming from eight genotypes: 'Big Perlon', 'Black Magic', 'Crimson', 'Michele Palieri', 'Red Globe', 'Sugraone', 'Victoria', and 'Vitroblack'. These genotypes were selected as a representative sampling of many grapevines cultivated in the Mediterranean region (Spain, Italy, Tunisia, Algeria, and Morocco). For model validation, around 200 leaves of the genotype 'Italia' were used to determine LA, L, and W. Immediately after cutting, leaves were sealed in plastic bags and transported to the laboratory. The maximum leaf L (from lamina tip to the point of the petiole intersection to the midrib), and leaf W (the widest linear length perpendicular to the midrib) were measured by a ruler (Fig. 1). Values of L [cm] and W [cm] were rounded to the nearest 0.1 cm. The area of each leaf (LA) was measured using an area meter (*LI-3100*, *LICOR*, Lincoln, NE, USA) calibrated to 0.01 cm$^2$.

**Methodology and statistical analysis**: Before model calibration, twelve individual LA estimation models proposed previously for grapevine (Table 1) were evaluated for LA prediction with the nine genotypes used in the current experiment.

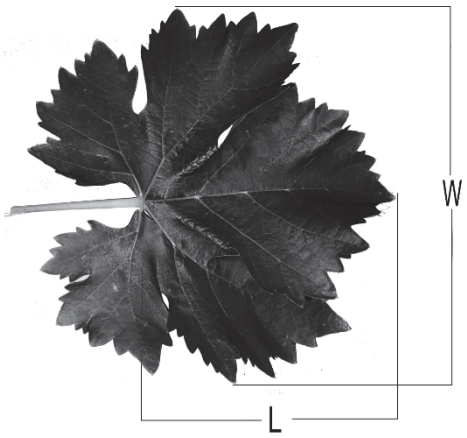


Fig. 1. Grapevine (*Vitis vinifera* L.) leaf showing the position of leaf length (L) and width (W) measurements.

For model construction, the dependent variable LA was regressed on the independent variables L, W, $L^2$, $W^2$, and the product LW. The relationships were evaluated by fitting regression models with the linear regression procedure of *SPSS* (*SPSS Inc*., Chicago, IL, USA) and the stepwise elimination option, as reported by Jiménez and Díaz (2003a). The internal validity of the models was tested by the coefficient of determination ($r^2$), mean square error (MSE), error sum of squares (SSE), predicted residual error sum of squares (PRESS). Residual plots were used to evaluate whether the data points in the residual plot were scattered within a constant width horizontal band centered around zero for an adequate regression model (Weisberg 1985). The final model was selected based on the combination of the highest $r^2$, the lowest MSE, the lowest PRESS, and when the PRESS values were reasonably close to SSE. These criteria allowed us to evaluate the occurrence of bias and the precision and accuracy of the models (Walther and Moore 2005). Individualized models for each genotype were built. In addition, *Shapiro-Wilk* statistic test result revealed that data pooled from all genotypes showed normal distribution. For this reason, data were pooled and a single relationship was calculated to develop LA prediction model for *V. vinifera.* Moreover, using two measurements (L and W) introduced potential problems of collinearity, resulting in poor precision in the estimates of the corresponding regression coefficients. For detecting collinearity, the variance inflation factor VIF = $1/(1 - r^2)$ (Marquardt 1970) and the tolerance values T = 1/VIF (Gill 1986) were calculated, and the following constraint was taken into consideration: 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 (Cristofori *et al.* 2007, Fallovo *et al.* 2008).

In addition to validate the developed model and to assess the robustness, a validation experiment was

conducted on leaf samples of 'Italia' genotype in 2011 growing season. Two hundred leaves of 'Italia' genotype were used to determine LA and leaf L and W by the previously described procedures. This genotype was selected as the most representative grapevine genotype cultivated in Italy.

Two techniques reported by Jiménez and Díaz (2003a), and Jiménez and Díaz (2003b) were used to validate the models: (*1*) the validation data set was used to produce a validation model by re-estimating the model parameters using the stepwise regression option approach to develop the estimation model and the models were compared for consistency; (*2*) regression parameter estimates from the estimation models were used to predict outcomes for observations in the validation data set and 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). In order to compare the predicted LA (PLA) to the observed LA (OLA) for the genotype 'Italia', graphical procedures (Bland and Altman 1986) were used. Plots of values for the PLA against the OLA are presented (Fig. 2). GLM (general linear model) procedure of *SPSS* was used to evaluate the linear relationship for OLA and PLA. Values for PLA were subtracted from OLA for the genotype 'Italia' and differences were plotted against the OLA for each of them. 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 (Fig. 2). Normality (Gaussian distribution) test was carried out to obtain a *Shapiro-Wilk* statistic using examines procedure of *SPSS* (Marini 2001).

## Results and discussion

**Leaf data analysis and collinearity test**: Leaf area of the grapevine leaves ranged between 10.2 to 540 $cm^2$, L from 3.1 to 25 cm, and W from 4.2 to 28 cm (Table 2). Across all genotypes, 'Victoria' had the highest average LA (204 $cm^2$), whereas, the genotype 'Crimson' had the smallest average LA (116 $cm^2$). One of the leaf shape traits was also the L:W ratio. In the current experiment, the L:W ratio of the *V. vinifera* genotypes ranged between 0.72 and 0.80, with the widest leaves recorded for 'Victoria', whereas genotypes 'Big Perlon' and 'Sugraone' had the narrow leaves (Table 2). Moreover, for detecting collinearity, the VIF and the T values of grapevine genotypes were analyzed. Across all genotypes, the VIF ranged from 3.69 to 6.14, whereas the T values ranged from 0.16 to 0.27 (Table 2), indicating that the collinearity between the two measurements (*e.g.* L and W) can be considered negligible (Gill 1986), since VIF was lower than 10 and T higher than 0.10 and consequently both L and W could be included in the calibration model (*e.g.* model construction).

**Model construction**: Separate regression models that estimate LA, from L, W, and the product LW were not significantly different between the eight genotypes for the linear LW model that we developed in our experiment (data not shown). The L, W, and LA data of these genotypes were pooled and single regression models were fit to the combined data (Table 3). Regression analysis demonstrated that there were significant relationships between LA, and L, W, LW, the square of length ($L^2$), and the square of width ($W^2$). Analysis of model deviation showed that among the twelve models of individual LA estimation previously proposed for grapevine, two models: (5) $LA = -3.01 + 0.85\ (L \times W)$ (Elsner and Jubb 1988) and (12) $LA = 18.379\ L - 151.41$ (Tsialtas *et al.* 2008), reported high precision, but they were biased, which leads to a significant underestimation of individual LA. For instance, the model developed by Elsner and Jubb (1988) underestimates in average the LA in about 9%, whereas the model developed by Tsialtas *et al.* (2008), underestimates in average the LA in 18%. However, when the accuracy is not a matter, the model proposed by Tsialtas

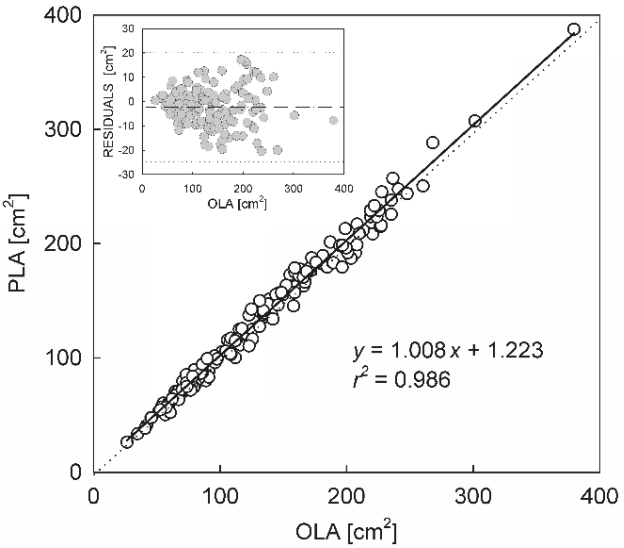


Fig. 2. Plot of predicted leaf area (PLA) using LW model ($LA = -0.465 + 0.914\ L \times W$) *vs.* observed values of single leaf areas (OLA) for 'Italia' genotype (validation experiment). *Solid line* represents linear regression lines of LW model. *Dotted lines* represent the 1:1 relationship between the predicted and observed values. The analysis of dispersion pattern of residuals for LW model is shown in the inset. 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 lie between the limits of agreement.

Table 3. Fitted constant (*a*) and coefficient (*b*) of the models used to estimate the individual grapevine leaf area (LA in $cm^2$) from leaf length (L) and leaf width (W) measurements. Coefficient of determination ($r^2$), mean square errors (MSE), predicted residual error sum of squares (PRESS), and error sum of squares (SSE) of the various models are also given. L and W were in cm. All data were derived from the model construction (calibration experiment, sampled in 2010; 1,630 leaves).

| Model No. | Form of model tested | Fitted coefficient and constant $a$ [$cm^2$] | $b$ | $r^2$ | MSE [$cm^2$] | PRESS [$cm^2$] | SSE [$cm^2$] |
|---|---|---|---|---|---|---|---|
| 1 | $LA = a + b\ L$ | –88.599 ± 2.455 | 20.758 ± 0.195 | 0.884 | 1,254.2 | 2.047.542 | 2.041.810 |
| 2 | $LA = a + b\ W$ | –112.92 ± 4.09 | 20.075 ± 0.292 | 0.754 | 2,547.9 | 4.157.678 | 4.147.960 |
| 3 | $LA = a + b\ (L \times W)$ | –0.465 ± 0.718 | 0.914 ± 0.004 | 0.986 | 240.2 | 392.825 | 391.094 |
| 4 | $LA = a + b\ L^2$ | 38.57 ± 1.576 | 0.738 ± 0.008 | 0.865 | 1,547.0 | 2.528.175 | 2.518.510 |
| 5 | $LA = a + b\ W^2$ | 38.57 ± 1.576 | 0.738 ± 0.008 | 0.765 | 1,547.0 | 2.528.175 | 2.518.510 |

*et al.* (2008) could be adopted, because of its simplicity and convenience, as it only involves one variable (leaf L). As stated by Robbins and Pharr (1987), model selection requires a balance between predictive qualities of the model (*e.g.* $r^2$) and the economy of including the least number of variables necessary to predict LA (*e.g.* L or W). Overall, the models (1 to 12) proposed by the other authors (Table 1) are simple with relatively high $r^2$. However, these models were developed for a reduced number of genotypes and only in a few cases a validation experiment was carried out. It is well established that model calibration based on a high number of genotypes is very important, since a leaf shape (L:W ratio) may vary among different genetic materials (Stoppani *et al.* 2003, Rouphael *et al.* 2010a). Moreover, the amplitude of leaves used in our experiment were 10–540 $cm^2$ compared to 10–350 $cm^2$, 10–360 $cm^2$, and 150–180 $cm^2$ proposed by Montero *et al.* (2000), Williams and Martinson (2003), and Tsialtas *et al.* (2008), respectively; these differences might be responsible for the lower accuracy of the former models. Finally, the previous models (1–12) were selected based on the highest $r^2$ between observed and predicted values and the lowest mean square deviation (MSD), but this is inappropriate procedure, because high correlations do not indicate that the predicted values agree with the observed ones (Bland and Altman 1986), and consequently the examination of residual plots is required to detect model deficiency in regression analysis.

Among the five models developed in the current experiment (Table 3), the best one was chosen according to the selection criteria described in the Materials and methods (higher $r^2$, lower MSE, lower PRESS, and when the PRESS values were reasonably close to SSE). This study demonstrated that model with a single measurement of W or $W^2$, was less acceptable for estimating the individual LA of grapevine due to the lowest $r^2$, higher MSE, and higher PRESS value. Our results are in contrast to those of Rouphael *et al.* (2006) on zucchini squash, Rouphael *et al.* (2007) on sunflower, Olfati *et al.* (2010) on red cabbage, and Zhang and Liu (2010) on *Bergenia purpurascens*, who observed that W is a suitable variable to estimate the individual LA. An improvement in the accuracy of the model was observed when L was used as independent variable (Table 3). LA prediction models, based on L measurements have already been proposed for different grapevine cultivars, such as 'Grenache' (Manivel and Weaver 1974), 'White Riesling' (Schultz 1992), 'Cencibel' (Montero *et al.* 2000), and 'Cabernet-Sauvignon' (Tsialtas *et al.* 2008). The model with the single measurement of L could be considered an interesting option because it requires measurement of only one variable, thus simplifying measurement procedures (Robbins and Pharr 1987). Moreover, when using the L model, there is obviously no need to measure W, which is not easy to measure, because of the need to consider an imaginary perpendicular line to the leaf L; it causes inaccurate measurements (Antunes *et al.* 2008, Pompelli *et al.* 2012). To find a model to estimate individual LA accurately for *V. vinifera* independently of genotypes, both measurements of L and W should be involved (*e.g.* LW model). We preferred this linear model [$LA = –0.465 + 0.914\ (L \times W)$] for its accuracy: highest $r^2$ (> 0.98), smallest MSE, smallest PRESS, and the reasonably close PRESS value to SSE (Table 3). In the current study, the PRESS value of grapevine was reasonably close to SSE for the LW equation (Table 3), and supports the validity of the fitted regression model and of the MSEs as an indication of the predictive capability of this model (Neter *et al.* 1996). Consequently, involving both dimensions (L and W) was necessary to estimate grapevine LA accurately. This is in agreement with previous studies on cucumber (Blanco and Folegatti 2005), broccoli (Stoppani *et al.* 2003), sweet pepper (de Swart *et al.* 2004), and hazelnut (Cristofori *et al.* 2007). The former authors concluded that models based on the product LW gave a better prediction of LA than models based on either L or W alone.

**Model validation**: For validation of the accuracy and robustness of the LW model, the validation experiment was conducted in 2011 growing season, with an independent data set coming from another genotype ('Italia'). The regression coefficients for LW of the estimation and validation models were not significantly different, and the $r^2$ values were similar for both models (0.91 *vs.* 0.90) (Table 4), indicating the applicability of the proposed LW

model to data beyond those on which the model was based (Neter *et al.* 1996). Moreover, regression parameter estimates from estimation models were used to predict outcomes for observations in the validation data set and then the mean of the squared prediction errors (MSPR) was calculated (Jiménez and Díaz 2003a). If the MSPR is fairly close to the MSE based on the regression fit to the estimation data set, then the MSE for the selected regression model is not seriously biased and gives an appropriate indication of the predictive ability of the model. In the current study, the MSPR from the validation data set for grapevine LA did not differ greatly from the MSE of the estimation data set (Table 4). This implies that the MSE based on the estimation data set is a reasonably valid indicator of the predictive ability of the estimation regression model (Neter *et al.* 1996). In the model validation, correlation coefficients showed that there was a highly reliable relationship between PLA and OLA, giving an overestimation of 0.8% in the prediction (Fig. 2). However, as stated above, correlation is insufficient analysis to explain the relationship between PLA and OLA and plotting the residuals (differences between PLA and OLA) against OLA might be more informative (Bland and Altman 1986, Marini 2001). Plotting differences against the OLA value also allows investigation of possible relationships between a measurement error and the true values. Lack of agreement between estimated 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. 2, a solid line represents the mean of the differences. If the differences are normally distributed, 97% of the differences lie between d ± 3 SD, which is the case in the current study, where a few plots were out of these lines, while the rest of the plots were placed between the lines.

**Conclusions**: The rapid and simple model [LA = –0.465 + 0.914 (L × W)] was developed to predict the leaf area for *V. vinifera.* This model was chosen for its simplicity and accuracy to estimate the individual LA of grapevine irrespective of genotypes, leaf developmental stages, and sizes. The use of this linear model would be an effective tool to predict LA, without the use of any expensive instruments, in various physiological grapevine experiments, where destructive LA measurement is not desirable.

Table 4. Statistics and parameter estimates from a regression model for individual leaf area (LA, $cm^2$) estimation. The estimation model was developed from eight grapevine genotypes sampled in 2010. The validation model was developed from one grapevine genotype ('Italia') sampled in 2011.

| Statistic or parameter estimate | Estimation model | Validation model |
|---|---|---|
| Intercept | –0.465 | 0.143 |
| Standard error of intercept | 0.718 | 0.910 |
| Regression coefficient for L × W | 0.914 | 0.906 |
| Standard error of regression coefficient | 0.004 | 0.008 |
| Prediction sum of squares (PRESS) | 392,825 | - |
| Error sum of squares (SSE) | 391,094 | 9,059.90 |
| Mean squared prediction error (MSPR) | - | 61.50 |
| Mean square error (MSE) | 240.23 | 59.25 |
| Coefficient of multiple determination $r^2$ | 0.9859 | 0.9860 |

## References


Antunes W.C., Pompelli M.F., Carretero D.M., DaMatta F.M.: Allometric models for non-destructive leaf area estimation in coffea (*Coffea arabica* and *Coffea canephora*). – Ann. Appl. Biol. **153**: 33-40, 2008.

Blanco F.F., Folegatti M.V.: Estimation of leaf area for greenhouse cucumber by linear measurements under salinity and grafting. – Sci. Agric. **62**: 305-309, 2005.

Bland J.M., Altman D.G.: Statistical methods for assessing agreement between two methods of clinical measurement. – Lancet **1**: 307-310, 1986.

Confalonieri R., Foi M., Casa R. *et al.*: Development of an app for estimating leaf area index using a smartphone. Trueness and precision determination and comparison with other indirect methods. – Comput. Electron. Agr. **96**: 67-74, 2013.

Cristofori V., Rouphael Y., Mendoza-de Gyves E., Bignami C.: A simple model for estimating leaf area of hazelnut from linear measurements. – Sci. Hortic.-Amsterdam **113**: 221-225, 2007.

Cristofori V., Fallovo C., Mendoza-de Gyves E. *et al.*: 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, 2008.

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

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

de Swart E.A.M., Groenwold R., Kanne H.J. *et al.*: Non-destructive estimation of leaf area for different plant ages and accessions of *Capsicum annuum* L. – J. Hortic. Sci. Biotech. **79**: 764-770, 2004.

Elsner E.A., Jubb Jr. G.L.: Leaf area estimation of Concord grape leaves from simple linear measurements. − Am. J. Enol. Viticult. **39**: 95-97, 1988.

Fallovo C., Cristofori V., Mendoza-de Gyves E. *et al.*: Leaf area estimation model for small fruits from linear measurements. –

HortScience **43**: 2263-2267, 2008.
Fascella G., Maggiore P., Zizzo G.V. *et al.*: A simple and low-cost method for leaf area measurement in *Euphorbia* × *lomi* Thai hybrids. – Adv. Hort. Sci. **23**: 57-60, 2009.
Gao M., Heijden G.W.A.M., Vos J. *et al.*: Estimation of leaf area for large scale phenotyping and modeling of rose genotypes. – Sci. Hortic.-Amsterdam **138**: 227-234, 2012.
Gill J.L.: Outliers, and influence in multiple regression. – J. Anim. Breed. Genet. **103**: 161-175, 1986.
Giuffrida F., Rouphael Y., Toscano S. *et al.*: Simple model for nondestructive leaf area estimation in bedding plants. – Photosynthetica **49**: 380-388, 2011.
Jiménez C.M., Díaz J.B.R.: A statistical model to estimate potential yields in peach before bloom. – J. Am. Soc. Hortic. Sci. **128**: 297-301, 2003a.
Jiménez C.M, Díaz J.B.R.: Statistical model estimates potential yields in pear cultivars 'Blanquilla' and 'Conference' before bloom. – J. Amer. Soc. Hort. Sci. **128**: 452-457, 2003b.
Lizaso J.I., Batchelor W.D., Westgate M.E.: A leaf area model to simulate cultivar-specific expansion and senescence of maize leaves. – Field Crop. Res. **80**: 1-17, 2003.
Manivel L., Weaver R.J.: Biometric correlations between leaf area and length measurements of 'Grenache' grape leaves. − HortScience **9**: 27-28, 1974.
Marini R.P.: 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, 2001.
Marquardt D.W.: Generalized inverse, ridge regression and biased linear estimation. – Technometrics **12**: 591-612, 1970.
Marshall J.K.: Methods for leaf area measurement of large and small samples. − Photosynthetica **2**: 41-7, 1968.
Mazzini R.B., Ribeiro R.V., Pio R.M.: A simple and non-destructive model for individual leaf area estimation in citrus. – Fruits **65**: 269-275, 2010.
Mendoza-de Gyves E., Rouphael Y., Cristofori V., Rosana Mira F.: A non-destructive, simple and accurate model for estimating the individual leaf area of kiwi (*Actinidia deliciosa*). – Fruits **62**: 171-176, 2007.
Mendoza-de Gyves E., Cristofori V., Fallovo C. *et al.*: Accurate and rapid technique for leaf area measurement in medlar (*Mespilus germanica* L.). – Adv. Hort. Sci. **22**: 223-226, 2008.
Misle E., Kahlaoui B., Hachicha M., Alvarado P.: Leaf area estimation in muskmelon by allometry. – Photosynthetica **51**: 613-620, 2013.
Montero F.J., de Juan J.A., Cuesta A., Brasa A.: Nondestructive methods to estimate leaf area in *Vitis vinifera* L. −HortScience **35**: 696-698, 2000.
Neter J., Kutner M.H., Nachtshein C.J., Wasserman W.: Applied Linear Regression – Models. 3rd Ed. Pp. 1408. Homewood III, Irwin 1996.
Olfati J.A., Peyvast G.H., Shabani H., Nosratie-Rad Z.: An estimation of individual leaf area in cabbage and broccoli using non-destructive methods. – J. Agr. Sci. Tech. **12**: 627-632, 2010.
Pompelli M.F., Antunes W.C., Ferreira D.T.R.G. *et al.*: Allometric models for non-destructive leaf area estimation of *Jatropha curcas*. – Biomass Bioenerg. **36**: 77-85, 2012.
Rana G., Katerji N., Introna M., Hammami A.: Microclimate and plant water relationship of the "overhead" table grape vineyard managed with three different covering techniques. – Sci. Hortic.-Amsterdam **102**: 105-120, 2004.
Rivera C.M., Rouphael Y., Cardarelli M., Colla G.: A simple and accurate equation for estimating individual leaf area of eggplant from linear measurements. – Eur. J. Hortic. Sci. **72**: 228-230, 2007.
Robbins S.N., Pharr D.M.: Leaf area prediction models for cucumber from linear measurements. – HortScience **22**: 1264-1266, 1987.
Rouphael Y., Colla G.: Radiation and water use efficiencies of greenhouse zucchini squash in relation to different climate parameters. – Eur. J. Agron. **23**: 183-194, 2005.
Rouphael Y., Colla G., Battistelli A. *et al.*: Yield, water requirement, nutrient uptake and fruit quality of zucchini squash grown in soil and closed soilless culture. – J. Hortic. Sci. Biotech. **79**: 423-430, 2004.
Rouphael Y., Colla G., Fanasca S., Karam F.: Leaf area estimation of sunflower leaves from simple linear measurements. – Photosynthetica **45**: 306-308, 2007.
Rouphael Y., Mouneimne A.H., Ismail A. *et al.*: Modeling individual leaf area of rose (*Rosa hybrida* L.) based on leaf length and width measurement. – Photosynthetica **48**: 9-15, 2010a.
Rouphael Y., Mouneimne A.H., Rivera C.M. *et al.*: Allometric models for non-destructive leaf area estimation in grafted and ungrafted watermelon (*Citrillus lanatus* Thunb.). – J. Food Agric. Environ. **8**: 161-165, 2010b.
Rouphael Y., Rivera C.M., Cardarelli M. *et al.*: Leaf area estimation from linear measurements in zucchini plants of different ages. – J. Hortic. Sci. Biotech. **81**: 238-241, 2006.
Salerno A., Rivera C.M., Rouphael Y. *et al.*: Leaf area estimation of radish from simple linear measurements. – Adv. Hort. Sci. **19**: 213-215, 2005.
Schultz H.R.: An empirical model for the simulation of leaf appearance and leaf area development of primary shoots of several grapevine (*Vitis vinifera* L.) canopy-systems. − Sci. Hortic.-Amsterdam **52**: 179-200, 1992.
Sepúlveda G.R., Kliewer W.M.: Estimation of leaf area of two grapevine cultivars (*Vitis vinifera* L.) using laminae linear measurements and fresh weight. − Am. J. Enol. Viticult. **34**: 221-226, 1983.
Stoppani M.I., Wolf R., Francescangeli N., Martí H.R.: A non-destructive and rapid method for estimating leaf area of broccoli. – Adv. Hort. Sci. **17**: 173-175, 2003.
Tsialtas J.T., Maslaris N.: Leaf area estimation in a sugar beet cultivar by linear models. – Photosynthetica **43**: 477-479, 2005.
Tsialtas J.T., Koundouras S., Zioziou E.: Leaf area estimation by simple measurements and evaluation of leaf area prediction models in cabernet-Sauvignon grapevine leaves. – Photosynthetica **46**: 452-456, 2008.
Uzun S., Celik H.: Leaf area prediction models (*Uzcelik-I*) for different horticultural plants. – Turk. J. Agric. Forest. **23**: 645-650, 1999.
Walther B.A., Moore J.L.: The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. – Ecography **28**: 815-829, 2005.
Weisberg S.: Applied Linear Regression, 2nd Ed. Pp. 324. J. Wiley & Sons, Inc., New York 1985.
Williams L., Martinson T.E.: Nondestructive leaf area estimation of 'Niagara' and 'DeChaunac' grapevines. – Sci. Hortic.-Amsterdam **98**: 493-498, 2003.
Zhang L., Liu X.-S.: Non-destructive leaf-area estimation for *Bergenia purpurascens* across timberline ecotone, southeast Tibet. – Ann. Bot. Fenn. **47**: 346-352, 2010.