SCIENTIFIC NOTE

Validation of a leaf area prediction model proposed for rose

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Leaf area (LA) is a valuable key for evaluating plant growth, therefore accurate, simple, and nondestructive methods for LA determination are important for physiological and agronomic studies. A LA prediction model based on leaf length (L) and width (W) and developed under greenhouse on 14 cultivars of rose (*Rosa* hybr.*) was validated on a different cultivar of *R. hybrida* 'Red France' and on a wild rose species (*Rosa sempervirens* L.) grown under open-field conditions with two light environments: ambient and 50% shade. Comparisons between measured *vs*. calculated LA using the following model: LA (cm^2) = 0.56 + 0.717 LW, showed a high degree of correlation ($R^2 > 0.95$) and provided quantitative evidence of the validity of the LA prediction model. Calculated LA values were very close to the measured values, giving an underestimation of 3.5%, 4.2%, 1.1%, and an overestimation of 1.3% in the prediction for *R. hybrida* ambient light, *R. hybrida* 50% shade, *R. sempervirens* ambient light, *R. sempervirens* 50% shade, respectively. This model can provide accurate estimations of rose LA independently of the genetic materials and the growing conditions and can be adopted in many experimental comparisons without the use of any expensive instruments.

Key words: Leaf length, leaf width, *Rosa* hybr.*, *Rosa sempervirens*, light environments, regression analysis, model validation.

INTRODUCTION

Leaf area (LA) is associated with many agronomic and physiological processes including growth, photosynthesis, transpiration, light interception, and energy balance (Rouphael and Colla, 2004; 2005; Antunes et al., 2008; Kandiannan et al., 2009; Spann and Heerema, 2010; Rouphael et al., 2010a). Leaf area can be measured by destructive or nondestructive measurements. Many methods have been devised to facilitate the measurement of LA. 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. Therefore, a nondestructive method for measuring LA is required by agronomists and physiologists. Accurate, nondestructive 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, nondestructive measurements are of a great value. A modeling approach involving linear relationships between LA and one or more dimensions of the leaf (length and width) is an inexpensive, rapid, reliable, and nondestructive method for measuring LA and would be more advantageous than many of the methods mentioned above (Rouphael et al., 2007; Tsialtas et al., 2008).

Various models relating leaf length and width to area have been developed for fruit trees (Demirsoy et al., 2004; Serdar and Demirsoy, 2006; Cristofori et al., 2007; 2008; Mendoza-de Gyves et al., 2007; 2008; Fallovo et al., 2008) and vegetable crops (Schwarz and Kläring, 2001; De Swart et al., 2004; Salerno et al., 2005; Rouphael et al., 2006; Rivera et al., 2007; Kumar, 2009; Rouphael et al., 2010b), whereas the estimation of LA of ornamental plants by using mathematical relationships between LA and one or more dimensions of the leaf has received very little attention (Fascella et al., 2009; Rouphael et al., 2010c; Giuffrida et al., 2011).

A simple model for rose (*Rosa* hybr.*) coming from 14 rose cultivars grown under greenhouse conditions has already been established (Rouphael et al., 2010c). However, 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), and growing conditions (Rivera et al., 2007) we need a good model of nondestructive LA estimation to use in physiological study of rose independently of genotypes and growing conditions.

The aim of this study was to validate robustness of the model proposed by Rouphael et al. (2010c), for a *R. hybrida*

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'Red France' and a wild rose species (*Rosa sempervirens* L.) in different growing environmental conditions (open-field with two light environments: ambient and 50% shading) from those on which it was developed.

MATERIAL AND METHODS

Rosa hybrida L. cv. Red France and a wild rose species (*Rosa sempervirens* L.) were grown under open-field conditions at the experimental farm of the Agricultural Research Council, Research Unit for Mediterranean Flower Species of Palermo (38°5' N, 13°30' E, 23 m a.s.l.), Italy, during the 2011 growing season. The local climate was characterized by mild and moderately rainy winters, and hot dry summers. The plants were grown into plastic pots containing 3 L of peat and perlite (1:1, v/v) in single rows with a plant density of 6.7 plants m^{-2}. Fertigation was scheduled daily and applied with drip-irrigation system to ensure that water and nutrients were non-limiting. The nutrient solution had the following composition (mg L^{-1}): 180 N, 50 P, 200 K, 120 Ca, 30 Mg, 1.3 Fe, 0.2 Cu, 0.2 Zn, 0.3 Mn, 0.2 B, and 0.03 Mo. The pH and the electrical conductivity were maintained at 6.0 and 2.0 dS m^{-1}, respectively. Shading treatment of both species was achieved by suspending 1.8 m above the ground level a single layer of black polyethylene net which allowed a reduction of 50% of full sunlight. During the experiment mean max/min (July/December) temperatures 31.2/11.4 °C and 29.1/120 °C were recorded for plants cultivated at ambient light and under 50% shading, respectively. Average daily photosynthetic active radiation (PAR) during the experiment ranged from a minimum of 69.7 µmol m^{-2} s^{-1} in December to a maximum of 1786.5 µmol m^{-2} s^{-1} in July for plants grown at ambient light, and from 35.7 to 969.2 µmol m^{-2} s^{-1} for plants under 50% shading. The experiment was designed as a factorial combination of two rose species (*R. hybrida* L. and *R. sempervirens* L.) and two light environments (ambient light level and 50% shading). The four treatments were arranged in a randomized complete block design with three replicates per treatment. Each replicate consisted of 20 plants (a total of 120 plants per species). Leaves varied in size from large to small for each treatment and were selected randomly from different levels of the canopy and during different growth stages (vegetative stage, first appearance of flowers, and full blooming). A total of 600 whole leaves (about 150 leaves per treatment: *R. hybrida* ambient light level, *R. hybrida* 50% shading, *R. sempervirens* ambient light level, *R. sempervirens* 50% shading) were measured for LA, length (L) and width (W). Immediately after cutting, leaves 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 midrib 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. L (cm), W (cm) and LA (cm^2) of each leaf were measured and recorded using a WinDIAS image analysis system (Delta-T Devices, Cambridge, UK) calibrated to 0.01 cm^2.

The rose LA estimation model produced by Rouphael et al. (2010c) was

$$LA = 0.56 + 0.717\ LW \qquad [1]$$

In the former study, LA estimation rose model was calibrated using 13 cultivars ('Vivaldi', 'Queen Elizabeth', 'Virgo', 'Velvet Star', 'Anna', 'New Dawn', 'Alba', 'Fairy', 'Iceberg', 'White Success', 'Kardinal', 'Rockstar', and 'Grand Gala') grown under greenhouse conditions during 2007 growing season, and the model was also validated using leaves from a different cultivar ('Dallas') during 2008 growing season.

For the validation of the LA prediction model proposed for rose, LA values obtained using the Rouphael et al. (2010c) model was plotted against observed LA measured by the area meter and the accuracy was based on the highest coefficient of determination (r^2) and the lowest mean squared error (MSE). 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).

RESULTS AND DISCUSSION

Leaf shape

Area of *R. hybrida* leaves under ambient light level and 50% shading ranged from 6.0 to 39.5 cm^2, length from 3.3 to 8.1 cm, and width from 2.4 to 6.6 cm, whereas LA of *R. sempervirens* under ambient light level and 50% shade ranged from 1.3 to 9.1 cm^2, length from 1.8 to 6.0 cm, and width from 0.9 to 2.4 cm (Table 1). One leaf-shape parameter is the L:W ratio. In the current study, the leaf shape (L:W ratio) varied between the two species. *Rosa sempervirens* under ambient light level and 50% shading had narrow leaves (L:W = 2.05 and 2.24, respectively), whereas *R. hybrida* under ambient light level and 50% shading had wider leaves (L:W = 1.24 and 1.34, respectively; Table 1). Moreover, leaf shape did not change with leaf size, since L:W ratio was constant with increasing LA, with a similar pattern between growing conditions.

Validation of the leaf area prediction model

Regression analysis demonstrated strong relationships ($P < 0.001$) between LA and midvein length (L), maximum leaf width (W), product of length and width (LW), square of length (L^2), and square of width (W^2) (data not shown). This is in agreement with previous studies (Cristofori et al., 2007; Mendoza de Gyves et al., 2007; Rouphael et al., 2007) on non-destructive model development for predicting LA using simple linear measurements. Moreover, models with a single measurement of L (L and L^2) were less acceptable for estimating rose LA as

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 *Rosa hybrida* and *Rosa sempervirens* under ambient light level and 50% shading.

		Leaf length			Leaf width			Leaf area					
Species	Light	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	L:W (± SE)	r^2	MSE[†]
		cm						cm^2					
R. hybrida	Ambient	5.3	3.8	8.0	4.2	2.9	6.6	17.7	9.0	39.5	1.24 (0.011)[*]	0.844	0.13
	Shade	5.7	3.3	8.1	4.2	2.4	6.5	18.1	6.0	37.5	1.34 (0.009)	0.894	0.14
R. sempervirens	Ambient	3.0	1.8	4.4	1.4	0.9	2.0	3.1	1.3	5.5	2.05 (0.005)	0.736	0.07
	Shade	3.5	1.9	6.0	1.6	0.9	2.4	1.7	1.7	9.1	2.24 (0.004)	0.782	0.06

[*]Standard error (SE) in parenthesis.
[†]Coefficient of determination (r^2) and mean squared error (MSE in cm^2) of the linear regression between leaf width (W) and leaf length (L).

a result of their lowest coefficient of determination (R^2), higher MSE. An improvement was possible for single LA estimation when W^2 was used as an independent variable. To find a model to predict single LA accurately for rose species in different growing environmental conditions, the product of L × W was used as the independent variable (data not shown). This is in agreement with previous findings (Cristofori et al., 2007; 2008; Fallovo et al., 2008; Mendoza de Gyves et al., 2007; 2008; Rouphael et al., 2010b; 2010c), who reported that the model containing the product L × W gave a better estimation than models based on either L or W alone. Therefore, neither L nor W can be dropped when estimating rose LA.

When rose LA values measured by digital planimeter were plotted against calculated LA, coefficients of determination in all treatments were equal to or greater than 0.95 (Figure 1). The MSE was relatively low in all treatments and ranged from 0.03 to 0.06 cm^2 for *R. sempervirens* under ambient and 50% shade, respectively, while for *R. hybrida* cv. 'Red France' the MSE ranged from 0.42 to 0.62 cm^2. Comparisons between measured *vs*. calculated LA using the Rouphael et al. (2010c) model (Equation [1]), showed a high degree of correlation and provided quantitative evidence of the validity of the area prediction model (Figure 1). The regression lines of the measured *vs*. calculated values were not significantly (P = 0.53 and 0.78 for *R. sempervirens* under ambient light level and 50% shade, respectively; P = 0.65 and 0.54 for *R. hybrida* 'Red France' under ambient light level and 50% shade, respectively) different from the 1:1 correspondence (Figure 1). Moreover, calculated LA values were very close to the measured values, giving an underestimation of 3.5%, 4.2%, 1.1% and an overestimation of 1.3% in the prediction for *R. sempervirens* under ambient light level, *R. sempervirens* 50% shade, *R. hybrida* under ambient light level, *R. hybrida* 50% shade, respectively (Figures 1A, 1B, 1C, 1D, respectively). The close relationship between calculated and measured LA values has been observed earlier on other ornamental species such as *Euphorbia x lomi* (Fascella et al., 2009), bedding plants (Giuffrida et al., 2011) and rose (Rouphael et al., 2010c), when the product L × W was used as independent variable. Besides, the underestimation recorded in the current experiment, especially for *R. sempervirens*, could be attributed to the genetic variation (e.g. smaller leaves).

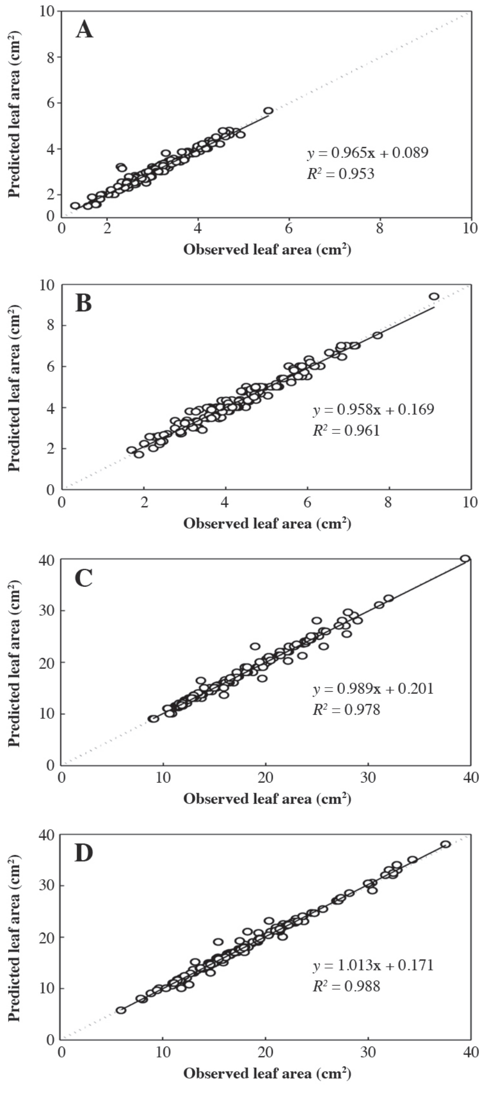

Figure 1. Relationship between measured *vs*. calculated leaf area using the model (Equation [1]) for *Rosa sempervirens* ambient light (A), *R. sempervirens* 50% shade (B), *R. hybrida* 'Red France' ambient light (C), *R. hybrida* 'Red France' 50% shade (D). Solid lines represent linear regression lines of the model. Dotted lines represent the 1:1 relationship between measured and calculated values.

Validation of LA models is an important step for determining usefulness and accuracy of LA estimation methods. Several researchers have validated LA prediction models for other species, such as grapevine (*Vitis vinifera* L.; Tsialtas et al., 2008), avocado (*Persea americana* Mill.; Celik and Uzun, 2002), chestnut (*Castanea dentata* L.; Serdar and Demirsoy, 2006), peach (*Prunus persica* L.; Demirsoy et al., 2004), kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson; Mendoza-de Gyves et al., 2007), medlar (*Mespilus germanica* L.; Mendoza-de Gyves et al., 2008), persimmon (*Diospyros kaki* L.f.; Cristofori et al., 2008), hazelnut (*Corylus avellana* L.; Cristofori et al., 2007), small fruits (Fallovo et al., 2008), zucchini squash (*Cucurbita pepo* L.; Rouphael et al., 2006), sunflower (*Helianthus annuus* L.; Rouphael et al., 2007), and eggplant (*Solanum melongena* L.; Rivera et al., 2007). Our results indicated that the proposed model could predict rose LA accurately independently from species (*R. hybrida* and R. *sempervirens*) and growing conditions (ambient light level *vs*. 50% shade).

CONCLUSIONS

To summarize, the length-width model proposed by Rouphael et al. (2010c) can provide accurate estimations of rose leaf area regardless of the genetic materials and the growing conditions. Because leaf width and midvein length are dimensions that can be easily measured in the field, greenhouse and pod experiments, use of this model would enable researchers to make non-destructive measurements or repeated measurements on the same leaves. Such model can estimate accurately and in large amounts the leaf area of rose plants in many experimental comparisons without the use of any expensive instruments, e.g., a leaf area meter or digital camera with image measurement software.

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