Original article

A non-destructive, simple and accurate model for estimating the individual leaf area of kiwi (*Actinidia deliciosa*)

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A non-destructive, simple and accurate model for estimating the individual leaf area of kiwi (*Actinidia deliciosa*).

Abstract – **Introduction**. Simple, accurate and non-destructive models determining leaf area of plants are important in many experimental comparisons. Determining the individual leaf area (La) of *A. deliciosa* (A. Chev.) vines involves the measurements of leaf parameters such as leaf length (Ll) and width (Lw), or some combinations of these parameters. **Materials and methods**. A 2-year investigation was carried out in Italy during 2005 (calibration experiments) and 2006 (validation experiment) under open field conditions. It aimed at comparing existing predictive leaf area models for *A. deliciosa* leaves using non-destructive measurements, and assessing the accuracy of the optimum model selected using an independent dataset. **Results and discussion**. Regression analyses of (La) *vs.* (Ll) and (Lw) revealed several models that could be used for estimating the area of individual *A. deliciosa* leaves. A linear model with (Ll × Lw) as the independent variable [La = 0.82 (Ll × Lw) – 0.28] provided the most accurate estimate (R^2 = 0.985, mean standard error = 25) of *A. deliciosa* leaf area. Validation of the model with (Ll) and (Lw) measured on leaves from other orchards grown in different locations showed that the correlation between calculated and measured areas was very high. **Conclusions**. With the model selected, agronomists and physiologists can estimate accurately and reliably the leaf area of *A. deliciosa* plants without the use of expensive instruments, *e.g.*, a leaf area planimeter or digital camera with image measurement software.

Italy / *Actinidia deliciosa* / leaf area / mathematical models

Un modèle non destructif, simple et précis pour évaluer la surface d'une feuille de kiwi (*Actinidia deliciosa*).

Résumé – **Introduction**. Les modèles simples, précis et non destructifs permettant de mesurer la surface de la feuille d'un plant sont importants pour de nombreuses comparaisons expérimentales. Déterminer la surface de la feuille (La) du kiwi [*A. deliciosa* (A. Chev.)] suppose d'utiliser certains paramètres tels que la longueur (Ll) et la largeur (Lw) de la feuille ou certaines combinaisons de ces paramètres. **Matériel et méthodes**. Une recherche de 2 ans a été effectuée en plein champ, en Italie, pendant les années 2005 (expérimentations de calibrage) et 2006 (validation du modèle). Elle a visé à comparer des modèles existants de prédiction de la surface de feuille par mesures non destructives, appliqués à des feuilles de kiwi, et elle a permis de comparer la fiabilité du meilleur modèle sélectionné en utilisant un ensemble de données indépendantes. **Résultats et discussion**. Les analyses de régression du paramètre (La) vis-à-vis de (Ll) et (Lw) ont mis en évidence plusieurs modèles qui pourraient être utilisés pour évaluer la surface de la feuille de kiwi. Un modèle linéaire utilisant le produit (Ll × Lw) comme variable indépendante [La = 0,82 (Ll × Lw) – 0,28] a permis l'évaluation la plus précise (R^2 = 0,985, erreur standard moyenne = 25) de la surface de feuille du kiwi. La validation du modèle à l'aide des paramètres (Ll) et (Lw) mesurés sur des feuilles provenant d'autres vergers développés en divers lieux a montré que la corrélation entre les surfaces calculées et mesurées était très forte. **Conclusions**. Le modèle sélectionné permet aux agronomes et aux physiologistes d'estimer exactement et fiablement la surface de feuille de plants de kiwi sans avoir à utiliser des instruments couteux, par exemple, un planimètre ou un appareil photo numérique couplé avec un logiciel d'interprétation d'image.

Italie / *Actinidia deliciosa* / surface foliaire / modèle mathématique

1. Introduction

Leaf area is associated with many agronomic and physiological processes, including growth analysis, photosynthesis, transpiration, light interception and energy balance [1]. 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 [2–5].

Measuring the surface area of a large number of leaves is often costly and time-consuming. Many methods have been devised to facilitate measurement of the leaf area. However, these methods, including those of tracing, blueprinting, photographing, or using a conventional planimeter, although accurate, require the excision of leaves from the plants. It is therefore not possible to make successive measurements of the same leaves. The plant canopy is also damaged, which might cause problems for other measurements or experiments. Nowadays, it is possible to measure leaf area quickly and non-destructively by using a portable scanning planimeter [6], but it is suitable only for small plants with few leaves and not feasible for large leaves [7]. An alternative method for measuring leaf area is to use a digital camera with image measurement and analysis software. The capture of images by a digital camera is rapid, and the analysis using proper software is accurate, but the equipment is generally expensive and is not suitable for non-flat leaf measurement, because pictures taken not exactly perpendicular can cause erroneous leaf area measurements. Therefore, an inexpensive, rapid, reliable and non-destructive method for measuring individual leaf area is required by agronomists. 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 linear measurements to estimate leaf area would be more advantageous than many of the methods mentioned above [8, 9].

Various combinations of measurements and various models relating length and width to area have been developed for several fruit trees such as avocado [10], cherry [11] and peach [12], while information on the estimation of *A. deliciosa* (A. Chev.) leaf area is still lacking.

We needed to use a good model for leaf area estimation in physiological studies of *A. deliciosa* vines independently of location. Therefore, the aims of our study were, firstly, to develop a model for leaf area prediction from linear measurements of leaf length and width in *A. deliciosa* vines grown in different locations; secondly, to assess the robustness of the optimum model selected using an independent dataset.

2. Materials and methods

The *A. deliciosa* (A. Chev.) vines, cv. Hayward, used for all measurements and estimations, were from four field experiments conducted during the summer growing season (June to September) in 2005 and 2006. In all experiments, leaf measurements were taken every 30 days, starting in June and continuing until September. On each sampling date, leaves were randomly selected from different *A. deliciosa* vines placed in different parts of the orchard. A wide variety of leaf samples was used, varying in size from large to small. The area of the leaves ranged from (40 to 312) cm^2, length from (3.0 to 19.5) cm and width from (2.0 to 20.5) cm.

2.1. Calibration experiments

In the preliminary calibration experiments (2005), a total of 1800 *A. deliciosa* leaves was measured for leaf area (La), length (Ll) and width (Lw). They were collected in three commercial orchards at the rate of 600 leaves per orchard. The orchards were located in Italy:

– in the north, at latitude 44° 23' N, longitude 7° 33' E, altitude 390 m for experiment 1,

– in the centre, at latitude 43° 7' N, longitude 12° 23' E, altitude 400 m for experiment 2,

– in the south, at latitude 40° 50' N, longitude 14° 15' E, altitude 150 m for experiment 3.

All vines were more than 10 years old, trained on T-bar trellises and managed using standard commercial practices. The [male:female] ratio of the vines in the block was [1:5] in experiment 1, [1:6] in experiment 2, and [1:7] in experiment 3. The vines were spaced 5 m × 5 m, 4 m × 4 m, and 3 m × 4.5 m, giving a plant density of 320 female vines·ha^{-1} in experiment 1; 521 female vines·ha^{-1} in experiment 2; and 635 female vines·ha^{-1} in experiment 3. In the current study, the female 'Hayward' leaves were used for the leaf measurements.

Immediately after cutting, leaves were placed in plastic bags and cooled on ice for transport to the laboratory. Leaf length (Ll) was measured from the lamina tip to the point of intersection of the lamina base line and the petiole, along the midvein of the lamina, while leaf width (Lw) was measured from end-to-end between the widest lobes of the lamina, perpendicular to the lamina midvein (*figure 1*). Values of length and width were recorded 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.

The dependent variable (La) was regressed with different independent variables, including (Ll), (Lw), (Ll^2), (Lw^2) and the product (Ll × Lw). The Mean Square Error (MSE) and the values of the regression coefficients (*b*) and regression intercepts (*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 site (experiments 1, 2 and 3) and combined data points of all sites for each model.

2.2. Validation experiment

To validate the developed model and to increase practical applicability in different locations, a validation experiment (experiment 4) was conducted in the summer (June to September) of 2006 on leaf samples of *A. deliciosa* cv. 'Hayward' grown at the experimental farm of Tuscia University (latitude 42° 25' N, longitude 12° 08' E, altitude 310 m). The [male:female] ratio of vines in the block was then [1:5], and vines were planted at a spacing of 4 m × 5 m, giving a plant density of 400 female vines·ha^{-1}.

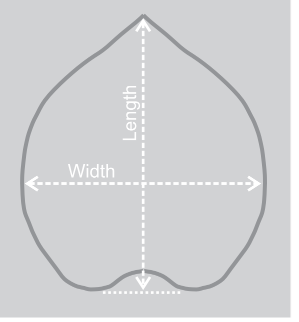

Figure 1.
Diagram of *Actinidia deliciosa* vine leaf showing positions of length and width measurements.

About 900 leaves of 'Hayward', with actual leaf area, width and length, were determined by the previously described procedures. Leaf area of individual leaves was predicted using the best model from the calibration experiments and was compared with the actual leaf area. The regression coefficient and regression intercept of the model were tested to see if they were significantly different from the regression coefficient and regression intercept of the [1:1] correspondence line [13]. Regression analyses were conducted using the SigmaPlot 8.0 package (SigmaPlot, Richmond, California, USA).

3. Results and discussion

3.1. Model calibration

Regression analysis demonstrated strong relationships ($P < 0.001$) between leaf area (La) and leaf width (Lw) and leaf length (Ll), the product (Lw × Ll), the square of leaf width (Lw^2), and the square of leaf length (Ll^2) (*table I*). In accordance with the present study, other research has been carried out to establish reliable relationships between the leaf area and the leaf dimensions of different plant species such as pepper [1], bean [3], grapevine [5], avocado [10], pecan [14], strawberry [15] and zucchini squash [16]. Except for model 1 [La = $a + b$(Ll)],

Table I.
Regression coefficient (*b*) and regression intercept (*a*) values of five models used to estimate the *A. deliciosa* vine leaf area (La) of single leaves from length (Ll) and width (Lw) measurements, from combined data of experiments located in three different orchards of Italy during the 2005 summer growing season (n = 1800 leaves).

Model No.	Form of model tested	Regression coefficient[1] (*a*)	Regression intercept[1] (*b*)	Coefficient of determination R^2	Mean square errors (cm^2)
1	La = *a* + *b* (Ll)	– 82.84 (1.62)	17.25 (0.15)	0.899	259
2	La = *a* + *b* (Lw)	– 65.06 (1.20)	15.51 (0.10)	0.933	159
3	La = *a* + *b* (Ll × Lw)	– 0.28 (0.15)	0.82 (0.002)	0.985	25
4	La = *a* + *b* (Ll^2)	0.01 (0.29)	0.82 (0.006)	0.915	211
5	La = *a* + *b* (Lw^2)	8.60 (0.61)	0.73 (0.004)	0.952	101

[1] Standard errors in parenthesis; (Ll) and (Lw) were in cm.

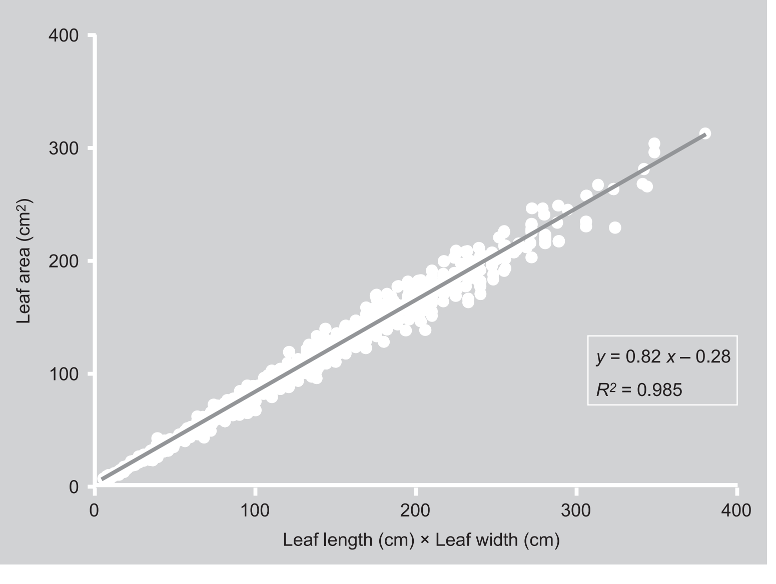

Figure 2.
Relation between leaf area (La) and [leaf length (Ll) × leaf width (Lw)] of single leaves of *Actinidia deliciosa* vines measured in three calibration experiments in 2005 (combined data of the three experiments). The equation for the regression line is [La = 0.82 (Ll × Lw) – 0.28].

all models produced a coefficient of determination (R^2) equal to or greater than 0.91 (*table I*). Based on previously described selection criteria (higher R^2 and lower MSE), this study demonstrated that models with a single measurement of Ll (models 1 and 4, *table I*) were less acceptable for estimating leaf area than the other ones, due to their lower coefficient of determination and higher MSE values. An improvement was possible for single leaf area estimation when Lw^2 was used as an independent variable (model 5). To find a model to predict single leaf area accurately for plants, the product of [Ll × Lw] was used as an independent variable (model 3). We preferred this linear model due its accuracy (the smallest MSE and the highest R^2, *table I*; *figure 2*). Therefore, both (Ll) and (Lw) measurements were necessary to estimate *A. deliciosa* leaf area accurately.

The effect of location on the regression coefficient using the selected model (model 3) was analysed. Regression coefficients of the *A. deliciosa* cultivar from experiment 1 (R^2 = 0.82), experiment 2 (R^2 = 0.80), and experiment 3 (R^2 = 0.83) were not significantly different. Moreover, when leaf area estimations using an equation derived for a single experiment *vs.* the overall model were compared, they were not significantly different. Therefore, the model: [La = 0.82 (Ll × Lw) – 0.28] can provide accurate estimations of *A. deliciosa* leaf area across different locations. Our regression coefficient (R^2 = 0.82) agreed closely with those calculated for other crops. Values of R^2 = 0.80 have been reported for grapevine [5], R^2 = 0.89 for cucumber [17] and R^2 = 0.86 for melon [18].

3.2. Model validation

Comparisons between measured *vs.* calculated leaf area using model 3 [La = 0.82 (Ll × Lw) – 0.28] for the validation set derived from the 2006 experiment on *A. deliciosa* cv. 'Hayward' showed a high degree of correlation and provided quantitative evidence of the validity of the area estimation model (*figure 3*). The regression lines of the measured *vs.* calculated values were not significantly (P = 0.59) different from the [1:1] correspondence. The 95% confidence intervals were 0.983 to 0.999 for the regression coefficient (a) and 0.176 to 2.115 for the regression intercept (b). Moreover, the calculated values of leaf area were very close to the measured values, giving an underestimation of 0.9% in the prediction. The high-accuracy prediction of individual leaf area observed in the current study has been reported previously on zucchini squash [16] grown under different environmental conditions.

4. Conclusions

The length-width model (*i.e.,* model 3) can provide the most accurate estimations of *A. deliciosa* leaf area across different locations. With this model, agronomists and physiologists can estimate accurately and in large quantities the leaf area of *A. deliciosa* plants without the use of any expensive instruments, *e.g.,* a leaf area planimeter or digital camera with image measurement software.

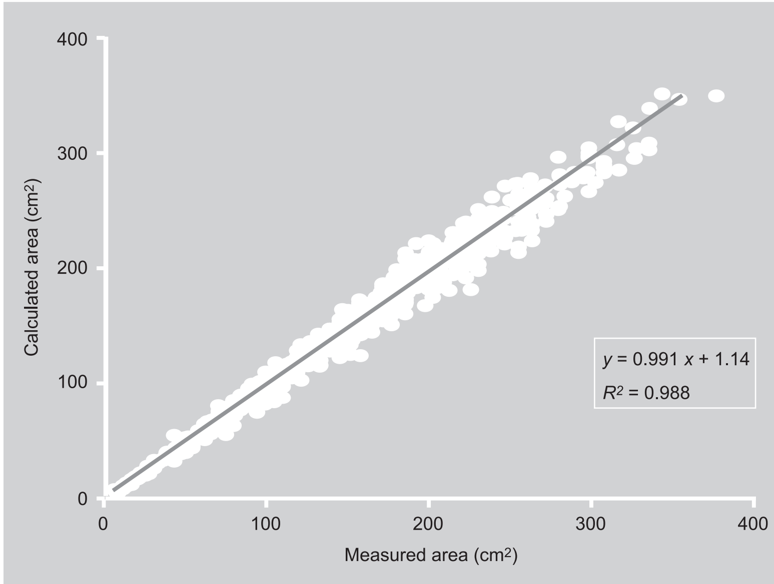

Figure 3.
Measured *vs*. calculated values of single leaf areas of *Actinidia deliciosa* cv. 'Hayward', during a validation experiment in 2006, using the model [La = 0.82 (Ll × Lw) – 0.28], where (La) is individual leaf area (cm^2) and (Ll × Lw) is the product of [leaf length (cm) × leaf width (cm)]. Solid line represents linear regression line of the model. Dotted line represents the 1:1 relationship between the measured and calculated values.

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[18] Panta G.R., NeSmith D.S., A model for estimating area of muskmelon leaves, HortScience 30 (1995) 624–625.Un modelo no destructivo, simple y preciso para evaluar la superficie de una hoja de kiwi (*Actinidia deliciosa*).

Resumen – Introducción. Los modelos simples, precisos y no destructivos, que permitan medir la superficie de la hoja de un plantón, son importantes para una gran cantidad de comparaciones experimentales. Determinar la superficie de la hoja (La) del kiwi [*A. deliciosa* (A. Chev.)] supone el uso de ciertos parámetros tales como la longitud (Ll) y el ancho (Lw) de la hoja o ciertas combinaciones de estos parámetros. **Material y métodos**. Un experimento de 2º años se llevó a cabo en pleno campo, en Italia, durante los años 2005 (experimentos del calibre) y en 2006 (validación del modelo). Éste tuvo como objetivo el comparar modelos existentes de predicción de la superficie de hoja mediante medidas no destructivas, aplicadas a hojas de kiwi; y permitió comparar la fiabilidad del mejor modelo seleccionado gracias al uso de un conjunto de datos independientes. **Resultados y discusión**. Los análisis de regresión del parámetro (La) con respecto a (Ll) y (Lw) pusieron de manifiesto varios modelos que podrían utilizarse para evaluar la superficie de la hoja de kiwi. Un modelo linear usando el producto (Ll × Lw) en tanto que variable independiente [La = 0,82 (Ll × Lw) – 0,28] permitió la evaluación más precisa (R^2 = 0,985, error estándar medio = 25) a validación del modelo con la ayuda de parámetros (Ll) y (Lw) medidos en hojas procedentes de otros vergeles desarrollados en diversos lugares, mostró que la correlación entre las superficies calculadas y medidas era fuerte. **Conclusión**. El modelo seleccionado permite tanto a los agrónomos como a los fisiologistas estimar exactamente y fiablemente la superficie de la hoja de plantones de kiwi, sin tener que recurrir al uso de instrumentos costosos, como, por ejemplo, un planímetro o una cámara digital junto con el software de interpretación de la imagen.

Italia / *Actinidia deliciosa* / superficie foliar / modelos matemáticos