BRIEF COMMUNICATION

Leaf area estimation of sunflower leaves from simple linear measurements

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Abstract

Simple, accurate, and non-destructive methods for determining leaf area (LA) of plants are important for many experimental comparisons. Determining the individual LA of sunflower (*Helianthus annuus* L.) involves measurements of leaf parameters such as length (L) and width (W), or some combinations of these parameters. Two field experiments were carried out during 2003 and 2004 to compare predictive equations of sunflower LAs using simple linear measurements. Regression analyses of LA *vs*. L and W revealed several equations that could be used for estimating the area of individual sunflower leaves. A linear equation having W^2 as the independent variable provided the most accurate estimate (r^2 = 0.98, MSE = 985) of sunflower LA. Validation of the equation having W^2 of leaves measured in the 2004 experiment showed that the correlation between calculated and measured areas was very high.

Additional key words: *Helianthus annuus* L.; leaf parameters; non-destructive methods.

Leaf area (LA) is associated with many agronomic and physiological processes including growth, photosynthesis, transpiration, photon interception, and energy balance (Goudriaan and Van Laar 1994). The equation which can predict LA without causing harm to the plant can provide researchers with many advantages in physiological experiments. Moreover, the equations enable researchers to measure LA on the same plants during the plant growth period and may reduce variability in experiments (Serdar and Demirsoy 2006).

Despite various techniques used to estimate leaf area (Lu *et al*. 2004), the most common approach is to develop ratios and regression estimators by using easily measured leaf parameters such as length (L) and width (W; Květ and Marshall 1971). This method usually saves time and is non-destructive. Various combinations of measurements and various equations relating leaf L and W to leaf area have been used in, for example, tomato (Schwarz and Kläring 2001), cucumber (Robbins and Pharr 1987), pepper (De Swart *et al*. 2004), zucchini (Rouphael *et al*. 2006), maize (Stewart and Dwyer 1999), sugar beet (Tsialtas and Maslaris 2005), taro (Lu *et al*. 2004), grape (Montero *et al*. 2000), and chestnut (Serdar and Demirsoy 2006).

We needed a good equation for non-destructive LA estimation for use in physiological and agronomical studies on the vegetative growth phase of sunflower. Therefore, the aims of this study were (*1*) to compare existing predictive LA equations for sunflower leaves using non-destructive measurements; and (*2*) to assess the accuracy of the optimum equation selected using an independent dataset.

Sunflower (*Helianthus annuus* L., cv. Melody) leaves used for all measurements and estimations were from two field experiments conducted during 2003 (May–September) and 2004 (May–October) at Tal Amara Research Station in the Bekaa Valley of Lebanon (33°51'44"N, 35°59'32"E, 905 m a.s.l.). The plants were grown in a completely randomized block design with 4 replications. The size of each plot was 3×10 m. Row spacing (four rows) was 0.80 m and the distance between plants in the row was 0.20 m providing a sowing density of 6 plants per m^2. In both experiments, fertilizer was applied before seeding at a rate of 300 kg ha^{-1} of NPK (15-15-15). Additionally, 150 kg ha^{-1} of ammonium nitrate (34N) and 50 kg ha^{-1} of potassium nitrate (13N, 44K) were applied 40 and 60 d after sowing, respectively. Irrigation was scheduled twice weekly and applied with overhead sprinklers to ensure that water was non-limiting. Weeds were controlled by hand as needed and there was no problem related to diseases and insects.

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In both experiments, leaf measurements (eight samples per growing season) were taken every 10 d, starting 35 d after sowing and continued until final harvest. On each sampling date, the plants (four in 2003 and three in 2004) were removed and all leaves were detached. Immediately after cutting, the leaves were placed in plastic bags and cooled on ice for transport to the laboratory. Leaf L was measured from lamina tip to the point of intersection of the lamina and the petiole, along the midrib of the lamina, while leaf W was measured from end-to-end between the widest lobes of the lamina perpendicularly to the lamina mid-rib. Values of L and W were recorded to the nearest 0.1 cm. In addition, LA of each leaf was measured using an area meter (*LI-3100*; *LICOR*, Lincoln, NE, USA) calibrated to 0.01 cm^2.

The first dataset (2003) was used to calibrate the equations, the second (2004) dataset was used for validation. The relationship between LA as a dependent variable and L, W, L^2, W^2, or LW as independent variables was determined using regression analysis on data from single leaves from the calibration set (2003). Mean Square Error (MSE) and the values of the coefficients (*b*) and constants (*a*) were also reported (Table 1) and the final equation was selected based on the combination of the highest coefficient of determination (r^2) and the lowest MSE. For the validation experiment (2004), we used the calculated and measured LA dataset, to which we fitted a linear equation. The slope and intercept 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, CA, USA).

Table 1. Fitted coefficient (*b*) and constant (*a*) values of the equations used to estimate the sunflower leaf area (LA) of single leaves from length (L) and width (W) measurements [cm]. *Standard errors in parenthesis. Coefficient of determination (r^2) and mean square errors, MSE [cm^2] of the various equations are also given. All data were derived from the preliminary calibration. Experiment 2003 (n = 526 leaves).

Equation No.	Form of equation tested	Fitted coefficient and constant *a*	*b*	r^2	MSE
1	LA = $a + b$L	–255.61 (11.92)*	27.45 (0.50)	0.870	5964
2	LA = $a + b$W	–202.26 (7.89)	25.93 (0.33)	0.928	3276
3	LA = $a + b$LW	–12.73 (4.36)	0.68 (0.006)	0.960	1863
4	LA = $a + b$L^2	–22.05 (6.57)	0.68 (0.01)	0.913	4168
5	LA = $a + b$W^2	6.72 (3.31)	0.65 (0.005)	0.978	985

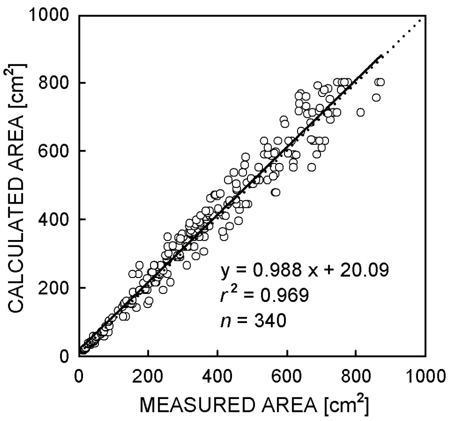

Fig. 1. Measured *vs.* calculated values of single leaf areas [cm^2] of sunflower in Experiment 2 (2004) using Eq. 5 [LA = 6.720 + 0.6494 W^2, where LA is individual leaf area and W^2 is the leaf-width squared]. Solid line represents linear regression line of Eq. 5. *Dotted line* represents the 1 : 1 relationship between the measured and calculated values.

A total of 526 sunflower leaves were measured for LA, L, and W in the preliminary calibration experiment (2003), and 340 leaves were measured for the validation experiment (2004), respectively. Leaf W, leaf L, and functions of these dimensions were significantly ($p<0.0001$) correlated with LA (Table 1). When LA was regressed with L or W alone (equations 1 and 2), a curvilinear relationship was obtained. A linear relationship was found between LA and L×W, and between LA and L^2 or W^2 (equations 3, 4 and 5, respectively). Except for Eq. 1, all equations produced a coefficient of determination (r^2) equal to or greater than 0.91 (Table 1). Based on selection criteria previously described (higher r^2 and lower MSE), this study demonstrated that equations with a single measurement of L (Eqs. 1 and 4, Table 1) were less acceptable for estimating LA, due to their lowest r^2 and higher MSE values. Eq. 3 (Table 1) that required two measurements per leaf could estimate LAs of open field sunflower accurately, but doubled the time required for leaf measurement. Eq. 3 showed a negative intercept, meaning that the shape coefficient was larger for larger leaves (Table 1). The shape coefficient (slope of Eq. 3) can be described by a shape between an ellipse (0.78) and a triangle (0.5) of the same length and maximum width. Our shape coefficient (0.68) agreed closely with those calculated for other crops. Values of 0.69 have been reported for pepper (De Swart *et al*. 2004), 0.63 for zucchini (Rouphael *et al*. 2006), 0.59 for *Vitis vinifera* L. (Montero *et al*. 2000), 0.62 for soybean (Wiersma and Bailey 1975), and 0.73 for corn (McKee 1964). Except for Eq. 2, which had a large negative intercept and was not further considered, the linear Eq. 5 having W as independent variable exhibited the highest accuracy and precision (one of the smallest MSE values and one of the highest r^2 values) in estimating individual sunflower LAs (Table 1). We preferred this linear equation because of its simplicity and convenience, as it only involves one variable. As stated by Robbins and Pharr (1987), model selection requires a balance between predictive qualities of the model and the economy of including the least number of variables necessary to predict LA.

Comparison between measured *vs*. calculated LAs using Eq. 5 for the 2004 experiment showed a high degree of correlation and provided quantitative evidence for this method of LA estimation (Fig. 1). The linear regression for the relationship between measured and calculated values was not significantly different from the 1 : 1 line. Moreover, the calculated values of LA were very close to the measured values, giving an underestimation of 1.2 % in the prediction.

We conclude that the sunflower LA can be accurately estimated when W^2 is used as an independent variable (*i.e*. Eq. 5). With this equation, agronomists and physiologists can estimate accurately and in large quantities the LA of sunflower plants without the use of any expensive instruments, *e.g*. leaf area planimeter or digital camera.

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