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Estimation of Leaf Area in Coneflower (*Echinacea purpurea* L.) Using Independent Variables

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Abstract

Leaf area information is required in various horticultural and physiological studies and it will be more useful if done via non-destructive methods. The objective of this study was to establish equations to estimate leaf area (LA) using length (L), width (W), fresh weight (FW), dry weight (DW), length × length (L^2), width × width (W^2), length × width (L×W), length + width (L+W), fresh weight × fresh weight (FW^2) and dry weight × dry weight (DW^2) of coneflower (*Echinacea purpurea* L.) leaves as a medicinal and landscape plant. An open field experiment was carried out to study relationship between leaf dimension and weight with leaf area of this plant. Observed leaf area was obtained by an automatic measuring device and leaf dimensions were measured by a ruler. A linear model employing L × W as an independent variables [LA = 0.575 (L × W) - 0.934] resulted in the most accurate estimation (R^2 = 0.874, RMSE = 2.33) of coneflower leaf area. Validation of the regression model showed that the correlation between measured and simulated values by using this equation was quite acceptable.

Keywords: Leaf length, Leaf width, Linear model, Non-destructive methods.

INTRODUCTION

Green leaves have a key role in plant growth and development. Leaves receive the photosynthetically active radiation (PAR) to produce biomass (Demarty *et al*., 2007). Moreover, they are the main path for transpiration and carbon intake, and are a key variable to study plants response to fertilizer, irrigation, pruning and other physiological functions (Smith and Kliewer, 1984). An accurate leaf area measurement plays a key role in understanding crop growth and its environment (Kumar, 2009). Leaf area measurements, especially under field conditions, are often destructive and time consuming (Tsialtas and Maslaris, 2005). Furthermore, it is not possible to make successive measurement of the same leaf, and plant canopy would be damaged and cause errors in other measurements of the experiment (Tsialtas and Maslaris, 2005).

A large number of methods, either destructive or not, have been developed to measure leaf area. The leaf area can be determined by using some expensive instruments and developed prediction models (Robbins and Pharr, 1987). Recently, new instruments, tools and machines such as hand scanners and laser optic apparatuses have been developed for leaf area measurements that are very expensive and complex devices for both basic and simple studies. Despite various methods 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 and width (Kvet and Marshall, 1971). These methods usually save time and are non-destructive. Non-destructive methods allow measurements to be repeated during the plant growth period, and reduce the variability associated with destructive sampling procedures (Nesmith, 1992). The non-destructive methods based on linear measurements are fast and easy to be executed and resulted in good precision and high accuracy as demonstrated for several crops like lettuce (Guo and Sun, 2001), cucumbers (Cho *et al*., 2007), sunflower (Rouphael *et al.*, 2007), faba bean (Peksen, 2007), small fruits (Fallovo *et al.*, 2008), and rose (Rouphael *et al*., 2010).

Echinacea (*Echinaceae purpurea* L.) is a small genus of the Asteraceae family used as an ornamental and medicinal herb (Hobbs, 1994). This species is strongly used as a landscape plant which is resistant to wind and salt stress. Echinacea is much valued as a cut flower. Medicinal preparations from different plant parts such as flowers and leaves of this species are used worldwide for their healing properties. The dried root is used in modern herbal medicines, skin creams, and shampoos (Carter *et al.*, 2007). The main aim of this study was to find the best model and allometric correlation basis to estimate leaf area of this plant.

MATERIALS AND METHODS

Plant preparation

Coneflower plants were grown under field conditions in College of Agriculture of Birjand University from April to end of the growing season of 2010. Irrigation and fertilization were performed based on local practices. After they flowered and in late June, 50 plants were chosen and one fully-expanded leaf sample was prepared from each plant. Each sample (one leaf) was separately taken into plastic bags and transported to the laboratory for destructive measurement of leaf area using leaf area meter (Delta T-Devices Ltd., Burwell, and Cambridge, England). Consequently, leaf fresh weight, length and width of each sample were measured. The maximum length and width of all leaves were measured by a ruler. Width was evaluated from the widest area to the nearest 1 mm, and length was calculated from the top to the end of the blade without petiole to the nearest 1 mm. Then, samples were taken into oven under 80°C for 24 h and dry weight of each was measured. The fresh and dry weights of leaves were measured to the nearest 0.001 g. Mean, maximum and minimum of all samples were calculated.

A search for the best model to predict leaf area (LA) was conducted with various subsets of the independent variables namely, length (L), length square (L^2), width (W), width square (W^2), length × width (L × W), fresh weight (FW), dry weight (DW), Length + Width (L + W), fresh weight square (FW^2) and dry weight square (DW^2). The best model was selected based on coefficient of determination (R^2), root mean square of error (RMSE), efficiency (E), index of agreement (d), variance inflation factor (VIF) and tolerance value (T).

The relationship between leaf area as a dependent variable and independent variables was determined using regression analysis on data from 50 leaves. Coefficients of determination (R^2) were calculated and the equation that presented the highest R^2 was used in the estimations. Then estimated and measured leaf areas were compared by testing the significance of regression equation and degree of goodness of fit (R^2) between estimated and observed values. The final model was selected based on the combination of the highest R^2 and the lowest root mean square error (RMSE). Root mean square error of estimation was calculated based on Janssen and Heuberger (1995):

$$RMSE = [\sum (Pi - Oi)^2 / N]\ 0.5$$

where P = predicted leaf area, O = measured leaf area, N = number of observation, and i = 1…N.

Comparison between the best two models (higher R^2 and lower MSE) was addressed by calculating the statistic E, i.e., the accuracy of model 1 relative to model 2 (Allen and Raktoe, 1981):

$$E12 = MSE1 / MSE2$$

where MSE1 and MSE2 are the mean square error of the predictions with model 1 and 2, respectively:

$$MSE1 = \Sigma (P1i - Oi)^2$$

$$MSE2 = \Sigma (P2i - Oi)^2$$

The statistic E is dimensionless and varies from 0 to infinity. A value of E between 0 and 1 implies that model 1 is superior to model 2. If E is greater than 1 then model 2 is better.

The index of agreement (d) measures the degree to which the predictions of a model are error free, and is dimensionless (Willmott, 1981). The d values range from 0, for complete disagreement, to 1, for perfect agreement between the observed and predicted values. The index d was calculated as:

$$d = 1 - [\Sigma (Pi - Oi)^2] / \Sigma [(|Pi - \bar{O}|) + (|Oi - \bar{O}|)]^2$$

where $\bar{O}$ is the average of the observed values.

For detecting collinearity, the variance inflation factor (VIF) (Marquardt, 1970) and the tolerance values (T) (Gill, 1986) were calculated:

$$VIF = 1 / 1 - r^2$$

$$T = 1 / VIF$$

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.

RESULTS

Minimum and maximum data for considering independent variables are shown in Table 1.

Table 1. Mean, minimum and maximum values for measured independent variables of coneflower leaves.

Plant parameters	Sample number	Mean± SE	Min.	Max.
Length (cm)	50	10.27 ± 0.65	7.80	13.20
Width (cm)	50	3.29 ± 0.81	2.60	5.80
Fresh weight (g)	50	1.01 ± 0.84	0.49	1.96
Dry weight (g)	50	0.20 ± 0.81	0.08	0.40
$Length^2$ (cm)	50	107.22 ± 0.64	60.84	174.24
$Width^2$ (cm)	50	15.89 ± 0.81	6.76	33.64
Length × Width (cm^2)	50	40.65 ± 1.53	23.40	66.70
Length + Width (cm)	50	14.19 ± 0.82	10.80	17.50
Fresh weight ^{2} (g)	50	1.16 ± 0.13	0.24	3.84
Dry $weight^2$ (g)	50	0.04 ± 0.78	0.01	0.16

Standard deviations (SE), minimum (Min) and maximum (Max), length (L), width (W), fresh weight (FW), dry weight (DW), $length^2$ (L^2), $width^2$ (W^2), length× width (L × W), length + width (L + W), fresh $weight^2$ (FW^2) and dry $weight^2$ (DW^2).

Table 2. Mathematical models for leaf area estimation of coneflower.

Equation no.	Variable	Regression model	R^2	RMSE
1	Length (cm)	LA = 3.420 (L) - 12.673	0.471	4.79
2	Width (cm)	LA = 7.979 (W) - 8.834	0.749	3.30
3	Fresh weight (g)	LA = 15.77 (FW) + 6.499	0.800	2.94
4	Dry weight (g)	LA = 79.33 (DW) + 6.843	0.734	3.40
5	$Length^2$ (cm)	LA = 0.164 (L × L) + 4.803	0.470	4.79
6	$Width^2$ (cm)	LA = 0.951 (W × W) + 7.324	0.733	3.40
7	Length × Width (cm^2)	LA = 0.575 (L × W) - 0.934	0.874	2.33
8	Length + Width (cm)	LA = 3.303 (L + W) - 24.42	0.766	3.19
9	Fresh weight ^{2} (g)	LA = 6.330 (FW^2) + 15.07	0.758	3.23
10	Dry weight ^{2} (g)	LA = 161.1 (DW^2) + 15.38	0.687	3.68

Table 3. Statistics and parameters yielded from regression models for LA estimation to compare models for coneflower.

Plant parameters	Equation no.	SD	MSE	df	VIF	T
Length (cm)	1	4.61	22.91	0.99	1.89	0.53
Width (cm)	2	5.82	10.86	0.95	3.98	0.25
Fresh weight (g)	3	6.01	8.67	0.87	5.00	0.20
Dry weight (g)	4	0.81	11.54	0.95	3.76	0.27
$Length^2$ (cm)	5	4.59	22.98	0.98	1.89	0.53
$Width^2$ (cm)	6	5.75	11.55	-2.45	3.74	0.27
Length × Width (cm^2)	7	10.92	5.44	0.99	7.93	0.13
Length + Width (cm)	8	5.88	10.15	0.99	4.27	0.23
Fresh weight ^{2} (g)	9	0.92	10.46	0.95	4.13	0.24
Dry $weight^2$ (g)	10	5.57	13.57	0.53	3.19	0.31

f Mean square errors (MSE), index of agreement (d), variance inflation factor (VIF) and tolerance value (T).

Table 4. Calculation of statistic E to find the best equation.

Equations	MSE	E12
Equation 3	8.67	(MSE7 / MSE8) = 1.59
Equation 7	5.44	(MSE8 / MSE7) = 0.63

The results indicated that among tested equations, the seventh equations considering leaf length × leaf width (LA = 0.575 (L × W) - 0.934) showed the highest R^2 (0.874) and the lowest RMSE (2.33) is good means for non-destructive measurement of leaf area compared to others, although third equation considering leaf fresh weight (LA = 15.77 (Fresh W.) + 6.499) indicated $R^2 = 0.800$ and RMSE = 2.94 that is near to equation seven (Table 2 and Fig. 1). Regarding other independent variables, width and fresh weight related equations were better and showed higher R^2 and lower RMSE than length and dry weight related ones, respectively (Table 2 and Fig. 1).

The lowest MSE (5.44) were found in seventh equation that followed by third, eighth, ninth, second, fourth, sixth, tenth and first equations and the highest rate was obtained in fifth equation (Table 3). The highest index of agreement was resulted from first, seventh and eighth equations (Table 3). The variance inflation factor (VIF) and the tolerance values (T) indicated the values lower than 10 and higher than 0.10, respectively (Table 3). For leaf length and width, the VIF and T values were (1.89, 3.98) and (0.53, 0.25), respectively. Statistic E was used to compare these equations and models, and results indicated that model 7 was better than model 3 (Table 4).

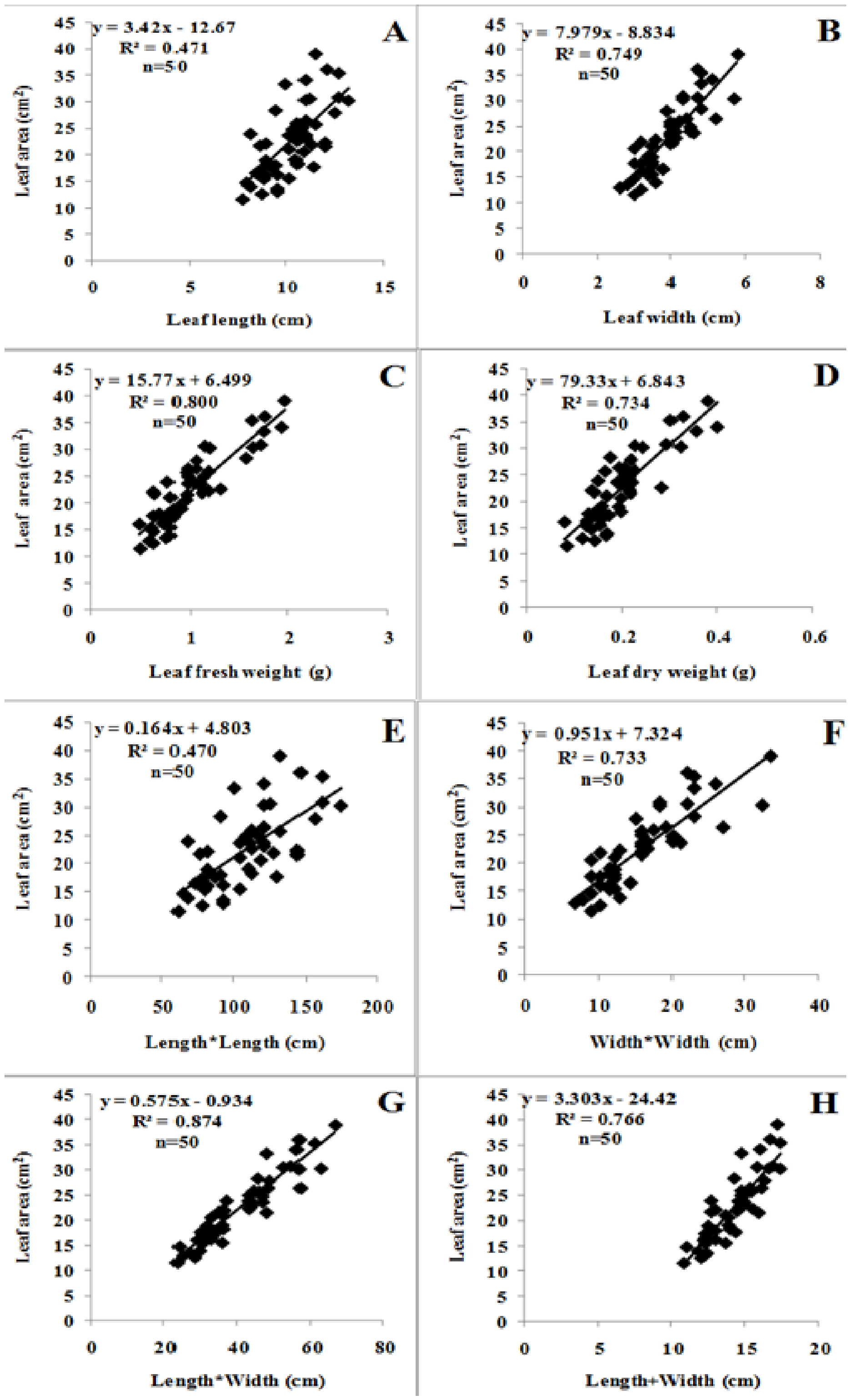

Fig. 1. Plot of predicted leaf area, estimated by model vs. the observed leaf area using independent variables (A to J).

DISCUSSION

Leaf area is one of the important growth parameters and one must record it for effective monitoring of the growth and development of plant in the experiment. Lack of accurate model is a limitation for calculating LA. Non-destructive method of the estimation of LA has several advantages without compromising on accuracy (Peksen, 2007; Kandiannan *et al.*, 2009). Various mathematical models for indirect estimation of leaf area of different plant species have been presented (Guo and Sun, 2001; Cristofori *et al.*, 2008; Fallovo *et al.*, 2008; Spann and Heerema, 2010). Although no model has been developed to predict coneflower leaf area, the present study results were in agreement with some of the previously mentioned investigations on non-destructive model development for predicting leaf area using simple linear leaf measurements. Many studies have been carried out to estimate leaf area by measuring leaf dimensions. In general, the combination of leaf length (L) and maximum width (W) has been used as the parameters of leaf area models (Peksen, 2007). Similar results were also reported in other horticultural crops like *Vitis vinifera* L. (Montero *et al.*, 2000), broccoli (Stoppaniet *et al.*, 2003), pepper (De Swart *et al.*, 2004) and zucchini squash (Rouphael *et al.*, 2006). In this study, very close relationships were found between actual leaf area and predicted leaf area using the proposed model. Results showed that coneflower leaf area could be monitored quickly, accurately, and non-destructively by using the leaf length × leaf width models.

CONCLUSION

In conclusion, coneflower plant is used worldwide for medicinal and landscape purposes and so it is necessary to study the plant responses to different environmental conditions. Thus, non-destructive estimation of leaf area as a main path for those studies can be used as a useful means. We can conclude that the length–width model (i.e. Model 7) can provide accurate estimations of coneflower leaf area. With this model, agronomists and physiologists can estimate the leaf area of coneflower plants accurately and in large quantities.

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