INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–103/AWB/2009/11–4–397–400
http://www.fspublishers.org

Full Length Article

Two new Methods for the Estimation of Leaf Area using Digital Photography

ENRIQUE RICO-GARCÍA[1], FABIOLA HERNÁNDEZ-HERNÁNDEZ, GENARO M. SOTO-ZARAZÚA AND GILBERTO HERRERA-RUIZ
Deparment of Biosystems, School of Engineering, Queretaro State University, C.U. Cerro de las Campanas, Querétaro, México
[1]Corresponding author's e-mail: ricog@uaq.mx; garciarico@yahoo.com

ABSTRACT

The aim of this study was to investigate the accuracy of two new leaf area determination methods using digital photographs processed in Matlab and Computer Aided Design (CAD) softwares. A standard method (LICOR LI-3000C & LICOR LI-3050C) was also used for comparing these results with actual measured areas. A group of four rectangles and three circles with known area were used to evaluate Matlab, CAD photograph processing and the standard method. Matlab and CAD photograph processings were also evaluated with a group of 20 leaves, ten for tomato and ten for corn. The results showed coefficients of correlation of above 99% for the standard method and two new methods. Furthermore, no significant difference ($p < 0.05$) was found between actual and estimated data. While measuring leaves they should be kept as flat as possible to avoid area measurement errors due to curved leaves. It was concluded that both methods have good agreement with actual data and are easily applicable.

Key Words: Digital photography; Leaf area estimation; Matlab; Computer aided design

INTRODUCTION

Leaf area is an important agronomical parameter as it is related to plant growth, photosynthetic capacity and many times it is used to asses the effect of different plant treatments (Ali & Anjum, 2004). In literature two categories of non-destructive methods are often reported: regression analysis and optical techniques.

Most of regression analysis studies are based on linear measurements of leaf length and/or width, which are made and correlated with actual leaf area measurements. McKee (1964) devised a method for calculating leaf area of corn. A coefficient (measured area divided by the product of length × width) was calculated for eight different varieties of hybrid corn. No significant difference in leaf area coefficient due to plant population was found nor for variety. Leaf area = 0.73 (length × width) was the equation developed in this study. The coefficient of correlation found between measured and calculated areas was 0.9985. Most coefficients of correlation reported in such studies are above 0.95 (Cristofori *et al.*, 2007; Peksen, 2007; Serdar & Demirsoy, 2007), which indicates that these methods are quite precise. Inclusive Lu *et al*. (2004) reported coefficients of correlation for non-destructive models in taro leaf area estimation above 0.99. An advantage of such developed equations is that they can be easily included in plant growth models. Klaring *et al.* (2007) used an allometric relationship to calculate the leaf area of greenhouse cucumber in order to calculate the net photosynthesis rate. Although these relationships are very useful, the only concern about regression analysis is that it is necessary to develop a different equation for each type of plant.

The optical technique in studying some characteristics of plants is increasing. One of the first microcomputer leaf area measurement systems was developed by Clarke and McCaig (1985) the system used an optical lens interfaced with a microcomputer. The software developed was able to measured different scales of green allowing the differentiation of chlorotic areas in leaves. Sadrnia *et al.* (2007) used 2D images to determine misshapen fruits of long type watermelon; they also found that the weight of normal watermelons can be determined by image analysis with an error of 2.42%. Rashidi and Gholami (2008) found that the volume of kiwifruit can be determined using image processing methods with no significance differences comparing with the water displacement method. Rico-García *et al*. (2009) used digital photography to calculate the leaf area of a hydroponic lettuce plantation.

In this study two new area determination methods using digital photography with Matlab and CAD softwares for processing and a standard method were evaluated comparing their results with real measured areas.

MATERIALS AND METHODS

A set of seven regular figures, four rectangles (R) and three circles (C) were used to determine the accuracy of the

To cite this paper: Rico-García, E., F. Hernández-Hernández, G.M. Soto-Zarazúa and G. Herrera-Ruiz, 2009. Two new methods for the estimation of leaf area using digital photography. *Int. J. Agric. Biol.*, 11: 397–400

two new methods used to calculate the leaf area. These figures were made of paper, which were then painted black. Finally their actual dimensions were determined with a digital vernier in order to record each figure real area. Also the standard method and the two new methods were studied measuring ten leaves of tomato and ten leaves of corn. For this last part of the study the standard method was used as reference. The digital camera used for the study was a Canon Power Shot A550.

Standard method. In order to compare the two new methods with a standard method the leaf portable area meter (LICOR LI-3000C, Lincoln, Nebraska) and the transparent belt conveyer accessory (LICOR LI-3050C, Lincoln, Nebraska) were used to measure the figures area. The area of figures was calculated from a mean of three measurements as suggested in the LICOR user's manual.

Technique base using matlab processing. The Matlab processing is a semi-automatic method to calculate the leaf area and for more users this will be an easy way to calculate leaf area. The code was written in Matlab version 6.0.0.88. This code will work in any higher version of the program. The following is an explanation of the method.

Photograph acquisition. It is necessary to lay down the leaves on a white background to ensure that the photograph will have only two colors with high contrast. It is also mandatory that no other objects appear in the photograph, because they may be mistakenly included as part of leaf area. Finally, all photographs must be taken from the same position and height to ensure the scale factor will be the same for all photographs (Fig. 1a). In this study the height was kept constant at 40 cm from the lens of the camera to the object being photographed.

Photograph processing. Photographs will be processed by Matlab code in two colors (Fig. 1b). The essence of the Matlab program is to convert pixels to an equivalent real area. An explanation follows each code line (Table I). Once all photographs have been saved into a computer it is necessary to arrange the photograph in sets of nine. The program will analyze at a time just one set. For the photographs in one set their names must be labeled "photo1, photo2, photo3" to "photo9". In analyzing one set of photographs, the Matlab code and the photographs of the set must be in the same folder in order to allow the program to process the images.

Some changes in the Matlab code are needed to be made each time a set of photographs is analyzed. The index "i" must run from 49 to 57 for sets of nine photographs, for sets less than nine photographs the index "i" must run from 49 plus the number of photographs minus one. The scale factor must be calibrated with a figure whose area is already known. The corrected factor *F*, code line 13 (Table I), can be calculated with Eq. (1). Once the factor has been set the camera must remain still until the last photograph is taken.

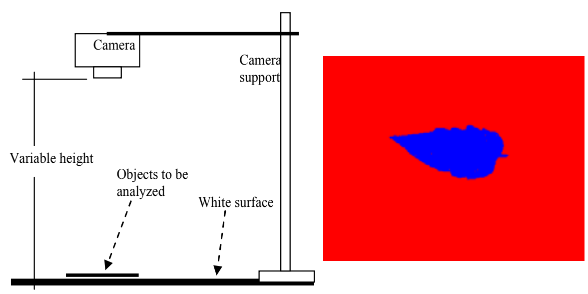
$$F = \frac{\text{(Object known area) (F value writen in the code)}}{\text{Object area value before calibration}} \quad (1)$$

Table I. Matlab code

Code line	Matlab code
1	clear; % Remove items from the workspace
2	j=0; % Sets the counter "j" equals to zero
3	for i=49:57 % The index "i" runs from 49 to 57 for sets of nine photos
4	X = [112 104 111 116 111 i]; % Photo name in numeric code
5	Y = char (X) % Gets the photo mane from the numeric code
6	A = imread (Y,'jpg'); % Reads the photo into A
7	C = (double(A(:, :, 1)) + double(A(:, :, 2)) + double(A(:, :, 3))) / 3; % C is the mean for RGB
8	Cota_color = 90; % Sets the reference color
9	Pre_Area = find(C <= Cota_color); % Takes into account the pixels which value is less than "Cota-color"
10	C(Pre_Area) = 0; % Sets a color for the pixels that are counted
11	%image(uint8(C)) % For calibration process
12	Area = length(Pre_Area); % Gets an area in pixels
13	F = 1.41326e-004; % Must be calibrated each time the program is used
14	Real_area = Area * F; % Gets the real area (cm^2) from the area in pixels
15	j=j+1; % Increases the counter "j" by one
16	Varea(j,:) = Real_area; % Saves the area values in a vector "Varea"
17	end % Ends the cycle witch started in line 3
18	Varea % Displays the vector area in the Matlab Command window

An explanation follows code lines

Fig. 1. (a) Camera set up (b) objects treated with Matlab processing



(a) (b)

During calibration only the figure with the known area must be used and named "photo1". The index "i" must be set from 49 to 49 to read only one figure. The code line number 11 (Table I) may be activated to aid the calibration process. To activate this code line the symbol "%", at the beginning of the line, must be erased. It is suggested that this code line may be deactivated after the process of calibration. However, if the code line is not deactivated it will not cause any problem.

Technique based in CAD processing. The CAD processing (AutoCad, 2007) is a manual method to calculate the leaf area. For those users who are not familiar with the use of CAD software, this method may be arduous to calculate the area due to the manual work required to treat the image. The following explains the method.

Photograph acquisition. Once the leaves have been laid down on a white surface it is necessary to draw a reference line whose linear dimension is known. To take the picture it is necessary to view all leaves (or leaf) and the reference line (Fig. 2). The picture must be taken parallel to the surface where the leaves are lying to minimize any error cause by camera rotation.

Table II. Steps to calculated the leaf area in AutoCAD 2007

Step	Description of action	Commands		
		Comm	Start	End
1	The photograph should be pasted in Autocad work space	In ordinary way		
2	A line must be drawn over the reference line of the photograph	Line (L)	Enter	Enter
3	Knowing the line dimension before scaling the photograph. A window will appear where you can read the geometric line features. You can red the length of the line. The scaling factor can be calculated as follow: $SF = \frac{\text{Known dimension of reference line}}{\text{Dimension of the reference line before scaling}}$	List	LC on the line	Enter
4	Scaling the photograph to real scale	Scale (SC)	Enter	Enter
5	Drawing a close line around the leaves. While drawing the line around the leaves and approaching to the starting point "close" should be written to form a closed object	Spline (SPL)	Enter	Close enter Enter
6	Knowing the area of the close object	List	LC on close object	Enter

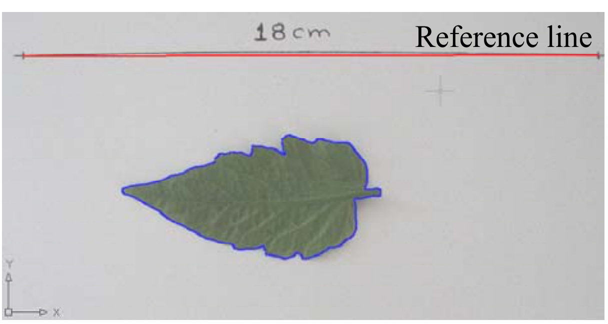
To activate the commands they should be written as they are listed. For some command the short way is shown in brackets
Most of commands have sub-steps, the program will lead the user through sub-steps
LC: Left click

Table III. Comparison of area values for the standard method and the two new methods

Figure	Area (cm^2)					Error (%)		
	Actual	LICOR	CAD	Matlab		LICOR	CAD	Matlab
R 1	137.411	138.100	135.639	137.411		0.50	-1.29	0.00
R 2	91.317	91.900	90.282	91.615		0.64	-1.13	0.32
R 3	64.212	64.400	64.758	63.931		0.29	0.85	-0.44
R 4	45.783	46.100	47.099	46.577		0.69	2.88	1.72
C 1	50.228	50.300	51.158	49.356		0.14	1.85	-1.73
C 2	24.930	25.400	25.219	25.891		1.88	1.16	3.78
C 3	9.954	10.300	10.425	9.905		3.48	4.73	0.48
		R^2			Mean	1.09	1.29	0.45
		0.99998	0.99976	0.99979	SD	1.20	2.14	1.80

Error = [$\text{Area}_{(\text{LICOR, CAD, Matlab})}$ – Actual Area] / Actual area × 100
The Matlab program was calibrated with the figure R 1. That is way the error is zero

Fig. 2. Objects for CAD processing
The red line is the one drawn over the reference line to scale the photograph. The blue line is the one drawn oround the leaft allowing the calculation of the area (see PDF version for colors)



Photograph processing. Once the photograph has been taken and saved in to a computer the photograph must be pasted into the work space of the CAD software. The reference line will serve to scale the photograph to a real scale. Once the photograph has been sized to its real scale a continuous line must be drew around the leaves to form a closed object (Fig. 2). Finally, the area of the object must be calculated. In Table II the steps to process the photograph are listed. In this work the area of the figures was calculated from a mean of three measurements.

RESULTS AND DISCUSSION

In calculating the area of the regular figures, the standard method and the two new methods had coefficients of correlation above 0.99 (Table III). In fact the areas determined were found not to be significantly different ($p < 0.05$) from the measured areas (Fig. 3). Mean of the error was 1.09, 1.29 and 0.45% and the standard deviation of the error was 1.20, 2.14 and 1.80%, for LICOR, CAD and Matlab methods, respectively. While in measuring real crop leaves the error incremented (Table IV). For leaf area in tomato, the mean of the error was 0.14 and -1.89%, the standard deviation was 1.58 and 1.38% for CAD and Matlab, respectively. For leaf area in corn, the mean of the error was -3.15 and -3.96%, the standard deviation was 4.37 and 3.14% for CAD and Matlab, respectively. The latest results for corn showed an increment in the error. This was due to the curved shape of corn leaves in width direction that made appear the width shorter that it is while taking the photograph. The error found in measuring the tomato leaves was similar to that found for the regular figures as these leaves could be kept almost flat. For the time of analysis, the standard Matlab methods took about a minute to get the area of one leaf of tomato, while the CAD method will take about 5−10 min per leaf of tomato depending on the user's ability to use the CAD software.

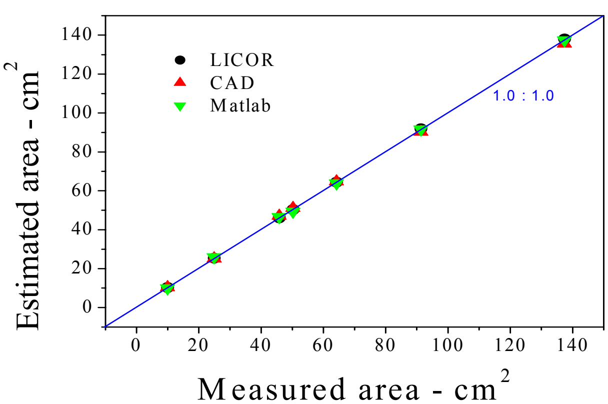
Clarke and McCaig (1985) reported a microcomputer leaf area measurement system able to measure different green scales, which allowed the system to measured chlorotic parts on leaves. The Matlab system presented in this work is not able to measure scales of green in the same photograph. However, changing the value of Cota_color, in Matlab code line 8 (Table I), will allow taking into account different scales of green. The greater the value, the lower the scale of green the program will be able to measure and viceversa. For the CAD method, as it is manual the user can differ from parts in leaves and make any area measurements. Thus the reported methods have many advantages over some orthodox methods of leaf area determination (McKee, 1964; Klaring *et al.*, 2007).

Table IV. Comparison of area values between the standard method and the two new methods

LICOR	Tomato				Corn				
	Area (cm^2)		Error (%)			Area (cm^2)		Error (%)	
	CAD	Matlab	CAD	Matlab	LICOR	CAD	Matlab	CAD	Matlab
20.40	19.921	20.089	-2.35	-1.52	102.00	103.462	102.495	1.43	0.49
18.40	18.360	18.495	-0.22	0.51	154.30	160.773	155.072	4.19	0.50
21.10	21.069	20.954	-0.15	-0.69	173.30	170.654	158.463	-1.53	-8.56
20.60	20.488	19.988	-0.54	-2.97	66.10	65.168	64.451	-1.41	-2.49
14.00	14.101	13.570	0.72	-3.07	122.20	118.834	118.893	-2.75	-2.71
24.70	24.883	24.174	0.74	-2.13	182.70	178.178	175.540	-2.47	-3.92
23.60	23.830	22.587	0.97	-4.29	105.50	100.803	102.580	-4.45	-2.77
19.70	19.277	19.417	-2.15	-1.43	43.20	39.909	40.479	-7.62	-6.30
19.90	18.190	17.740	1.62	-0.90	50.10	44.604	46.728	-10.97	-6.73
19.30	17.776	16.888	2.75	-2.38	144.70	136.065	134.430	-5.97	-7.10
Mean			0.14	-1.89	Mean			-3.15	-3.96
SD			1.58	1.38	SD			4.37	3.14

Error = $[Area_{(CAD, Matlab)} - Area_{(LICOR)}] / Area_{(LICOR)} \times 100$

Fig. 3. Measured areas and estimated areas using LICOR, CAD and Matlab with the line of equality (1.0: 1.0)



CONCLUSION

Two new methods for leaf area estimation using digital photographs have been evaluated finding a positive correlation and good agreement with actual data. These methods can be used to calculate the area of any flat objects without the need for expensive leaf area meters. While measuring leaves they should be kept as flat as possible to avoid area measurement errors due to curved leaves.

Acknowledgement. This research was supported by FIFI 2009 de la Facultad de Ingeniería (UAQ). Authors acknowledge to Veronica Aguayo and Silvia C. Stroet.

REFERENCES

Ali, H. and M.A. Anjum, 2004. Aerial growth and dry matter production of potatoe (*Solanum tiberosum* L.) cv. Desiree in relation to phosphorus application. *Int. J. Agric. Biol.,* 6: 458–461

Clarke, J.M. and T.N. McCaig, 1985. A versatile, inexpensive microcomputer-based leaf area measurement system. *Agron. J.,* 77: 966–968

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

Klaring, H.P., C. Hauschild, A. Heiβner and B. Bar-Yosef, 2007. Model-based control of CO_2 concentration in greenhouses at ambient levels increases cucumber yield. *Agro. Forest. Meteorol.,* 143: 208–216

Lu, H.Y., C.T. Lu, M.L. Wei and L.F. Chan, 2004. Comparison of different models for nondestuctive leaf area estimation in taro. *Agron. J.,* 96: 448–453

McKee, G.W., 1964. A coefficient for computing leaf area in hybrid corn. *Agron. J.,* 56: 240–241

Peksen, E., 2007. Non-destructive leaf area estimation model for faba bean (*Vicia faba* L.) *Sci. Hortic.,* 113: 322–328

Rashidi, M. and M. Gholami, 2008. Determination of kiwifruit volume using ellipsoid approximation and image-processing methods. *Int. J. Agric. Biol.,* 10: 375–380

Rico-García, E., V.E. Casanova-Villareal, A. Mercado-Luna, G.M. Soto-Zarazúa, R.G. Guevara-González, G. Herrera-Ruiz and R.V. Velázquez-Ocampo, 2009. Nitrate content on summer lettuce production using fish culture water. *Tren. Agric. Econ.,* 2: 1–9

Sadrnia, H., A. Rajabipour, A. Jafary, A. Javadi and Y. Mostofi, 2007. Classification and analysis of fruit shapes in long type watermelon using image processing. *Int. J. Agric. Biol.,* 9: 68–70

Serdar, Ü. and H. Demirsoy, 2007. Non-destructive leaf area estimation in chestnut. *Sci. Hortic.,* 108: 227–230

(Received 03 April 2009; Accepted 17 April 2009)