

Use of the elliptical mathematical formula to estimate the surface area of cladodes in four varieties of *Opuntia ficus-indica*

Martín E. Tiznado-Hernández, Judith Fortiz-Hernández, Ángel J. Ojeda-Contreras
and Armida Rodríguez-Félix*

Coordinación de Tecnología de Alimentos de Origen Vegetal. Centro de Investigación en Alimentación y Desarrollo, A.C. Km 0.6 Carretera a la Victoria. CP 83000. Hermosillo, Sonora. México. Tel: +52-662-289-2400. Fax: +52-662-280-0422

*Corresponding author: e-mail, armida@ciad.mx

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Abstract

Estimation of cladode area in *Opuntia* spp. can be a valuable tool for estimating plant growth, development and productivity for either physiological studies or commercial purposes. The objective was to develop an easy, non destructive and inexpensive protocol to estimate the area of cladodes at different stages of development in four varieties of *Opuntia ficus-indica*. The study was carried out by tagging 300 buds of 'Atlixco' and 'Copena F-1' varieties and 180 of 'Milpa Alta'. Further, not tagged buds of 'Jalpa' variety were sampled only once. Sampling of buds in 'Atlixco' and 'Copena F-1' was done at 10, 17 and 23 days after sprout (DAS), whereas for 'Milpa Alta' the sampling was done only at 10 DAS. In the 'Jalpa' variety, sampling was done randomly. The determination of the real cladode area (RCA) was carried out by computer analysis of the digitalized cladodes circumference drawn on a paper sheet and the estimated cladode area (ECA) was done by measuring the minor and major axes, and using the formula of the ellipse. It was found that cladodes of 'Copena F-1' showed a higher growth from 10 to 17 DAS than from 17 to 23 DAS. In contrast, 'Atlixco' showed a constant growth from 10 to 23 DAS. Regression analysis did not allow concluding whether the cladodes growth phenomena follows a straight or exponential behavior. Scatter plots of RCA and ECA recorded a coefficient of determination of 0.94 and 0.96 for 'Atlixco' and 'Copena F-1' varieties, respectively. However, statistical analysis found differences ($p < 0.05$) between the RCA and ECA. It was concluded that the area estimated in 'Atlixco', 'Copena F-1', 'Milpa Alta' and 'Jalpa' varieties with the ellipse mathematical formula is very close to the real cladode area, although not completely accurate.

Key words: Ellipse figure, days after sprout, cladode area, 'Atlixco', 'Copena F-1', 'Milpa Alta', 'Jalpa'.

Introduction

Accurate knowledge of agricultural products surface area is required as an integral part in heat and mass transfer calculations and in determination of physical properties such as gas permeability, respiration rates and weight per unit of surface area estimations (Sabliov *et al.*, 2002).

The volume and surface area of an agricultural product are critical parameters for many food science applications and research disciplines. For instance, in food technology the determination of surface areas is used to calculate the amount of applied chemical, estimating peeling times, and

determining the microbial concentrations present on products (Sabliov *et al.*, 2002). On the other hand, the estimation of the physical dimensions in agricultural crops had been done for different purposes using different approaches. For instance, volume and surface area in axi-symmetric agricultural products such as eggs, lemons, limes, peaches and tamarillos [*Solanum betaceum* (Cav.)] were calculated using image processing (Wang and Nguang, 2006), crop production was estimated by the relationship with remotely sensed leaf area index (Zhang *et al.*, 2005) and visual and the near infra-red spectral bands of canopy reflectance were used to predict properties of oilseed plant (Behrens *et al.*, 2006).

The development of mathematical models and equations for predicting total or individual leaf-area has been shown to be very useful in studying plant growth, development and bioproductivity (Uzun and Celik, 1999; Peper *et al.*, 2001). Indeed, it was reported to be useful for estimating plant growth in cucumber (Cho *et al.*, 2007), common bean (Bhatt and Chanda, 2003), faba bean (Peksen, 2007), cottonwood (Farid *et al.*, 2008), onions (Gamiely *et al.*, 1991), and leaves in different horticultural plants (Uzun and Celik, 1999). Besides of the above mentioned, mathematical equations had been used to predict the role of some weather parameters on crop performance (Mouhamed *et al.*, 2006; Austin *et al.*, 2002; Garcia de Cortázar and Nobel, 1990), disease-forecasting (Harikrishnan and del Río, 2008), farming techniques (Board, 2002; Garcia de Cortázar and Nobel, 1991), and packaging systems (Fonseca *et al.*, 2002).

Native of the semi-arid regions of South and Central America, nopal (*Opuntia* spp.) is a branched cactus that grows up to 2 m. Certain species of *Opuntia* have flattened stem segments, the cladodes, which are eaten when young and tender (in this stage of development they are called “nopalitos” in Spanish) in several regions of Mexico (Nerd *et al.*, 1997). The largest diversity of edible *Opuntia* species in Mexico can be found in the semiarid and arid zones mainly located in the north and central parts of the country (Rodríguez-Felix, 2002).

In Mexico during 2006, it was calculated that 66,732.7 ha were cultivated with nopal for forage, for “nopalitos” and cactus pear fruit production (SAGARPA, 2006). It was also reported that “nopalitos” were grown in 11,175.6 ha, reaching an average yield of 61.06 t ha⁻¹, being the most important vegetable produced in arid zones. Furthermore, “nopalitos” for feeding animals were produced in 4,794.25 ha with an average yield of 32.51 t ha⁻¹ (SAGARPA, 2006).

There are several studies available on the “nopalitos”, including nutritional characteristics (Stintzing *et al.*, 2001; Stintzing and Carle, 2005; Feugang *et al.*, 2006), tissue composition (Rodríguez-Felix and Cantwell, 1988; Hegwood, 1990; Betancourt-Domínguez *et al.*, 2006), identification and quantification of nutraceutical compounds (Dok-Go *et al.*, 2003; Lee *et al.*, 2002; Jaramillo-Flores *et al.*, 2003), and post harvest physiology (Cantwell *et al.*, 1992; Rodríguez-Felix and Villegas-Ochoa, 1997; Nerd *et al.*, 1997; Guevara *et al.*, 2001; Guevara *et al.*, 2003; Guevara-Arauz *et al.*, 2006; Rodríguez-Felix *et al.*, 2007).

There is only the study of Flores *et al.* (1996) to the best of our knowledge, in which the area of cladodes was statistically estimated. In this work, the area of cladodes was estimated by using the formula to calculate the area of a perfect circle (πr^2). They found that the method used was an accurate predictor for the variables studied. However, the authors did not recommend the indiscriminate use of the regression model obtained due to the large variability in polymorphism or cladodes' shape of different *Opuntia* species.

Based on the pointed out above, the objective of the present contribution was to estimate the area of the cladodes from plants of four varieties of *Opuntia ficus-indica* through the ellipse mathematical formula by measuring their long and short diameters.

Material and methods

Plant material

The experiment was conducted during the months of June and July, 2007, in an area of seven hectares, located at 29°11'27.53" N, 110°51'17.13" W, besides the road to 'San Miguel de Horcasitas' town, 20 Km far from Hermosillo, Sonora, Mexico. The commercial plantation in which the experiment was carried out was 2.5-years old. Before starting the plantation, the site was fertilized with 140 ton of manure per hectare. During the study, the plantation was drip irrigated every week during 24 hours with a speed of 2 liters per hour. The temperature and relative humidity in the commercial plantation during the period of growth of cladodes were recorded in a data logger model HOBO-XT (Onset Computer Corp., PO Box 3450, Pocasset, MA 02559-3450, USA).

Sampling

The involved *Opuntia ficus-indica* varieties were 'Copena F-1', 'Atlixco', 'Jalpa' and 'Milpa Alta'. Cladode buds were tagged in order to know the stage of cladode development in days after sprout (DAS). Eighty cladodes or "nopalitos" randomly chosen from each of 'Copena F-1' and 'Atlixco' varieties were sampled at 10, 17 and 23 days after sprout (DAS). Furthermore, 27 cladodes with 10 DAS were sampled in the case of 'Milpa Alta' variety, and 66 cladodes in that of 'Jalpa' variety. It deserves to be mentioned that for the last two varieties, there were not enough cladodes to carry out the sampling at three different DAS. Sampled cladodes were packaged carefully in plastic boxes and transported immediately to the laboratory with the aim to have cladodes free from physical damage.

Determination of Estimated and Real area of cladodes

The width and length of each cladode were measured. We considered maximum cladode width (W) as the widest point perpendicular to the half part of the cladode, and the length (L) as the distance from one end to the other end along the longest axis of the cladode. We used 80 freshly harvested cladodes for 'Copena F-1' and 'Atlixco' varieties at three different DAS; whereas for 'Milpa Alta' and 'Jalpa' varieties, the measurements were carried out using 27 and 66 cladodes, respectively.

These values were used to estimate the area of the cladode using the formula of the ellipse:

$$X = (W/2)*(L/2)*\pi,$$

where:

X = estimated area; W = width, minor axis; L = length, major axis; and $\pi = 3.141516$.

In order to compute the real area, a line was drawn in a paper following the circumference of each cladode. Then, the resulting figure was digitalized using a scanner and saved; a 10 cm long line was drawn in every sheet to be used as a reference for the calculations by the image analysis software. The estimation of the real area in each cladode was done from the digitalized figure created as mentioned above using the Imaging J software and its Java source code, version 1.41, which is software freely available from the website <http://rsbweb.nih.gov/ij/index.html>.

Statistical analysis

Cladode's width, length, estimated area and real area were considered as variables. Then, we calculated their basis statistica. Normality tests were also performed. Comparisons were performed through paired t test for data normally distributed; whereas the no parametric Wilcoxon Signed-Rank test for difference in medians was used when data sets were not normally distributed. All the statistical analyses were carried out at 5% of significance level with the NCSS software (Hintze, 2004).

Results

The average temperature during the period of study was 38.4°C during the day (from 7:00 to 18:00 hours) with a minimum of 27.9°C and a maximum of 43.9°C. During the night (from 19:00 to 6:00 hours), average temperature was 30.9°C with a minimum of 24.4°C and a maximum of 38.8°C. The average relative humidity (RH) during the day was 27.2% with a minimum of 24.0% and a maximum of 29.6%. During nights, the average RH was 24.9% with a minimum of 23.6% and a maximum of 27.7%.

Cladode surface areas of 'Copena F-1', 'Atlixco', 'Jalpa' and 'Milpa Alta' varieties were measured through the image processing (real area) and geometrical methods (estimated areas). The average and the standard error of the cladode real and estimated areas at different DAS, the percentage of difference (PD) between real and estimated cladode areas, and the results of the statistical comparison between areas are shown in Table 1. It can be observed that both methods were similar in precision as suggested by rather low and similar standard errors related to the average area at the different DAS for all varieties. Exception was found for 'Jalpa' variety because there was only a level of DAS. It deserves to be noted that low standard errors suggests use of cladodes of the same age is an advantage allowing reliable comparisons. In almost all cases, real and estimated areas did not show obvious trends. That is, only at 10 DAS, real area was higher than the estimated one, but this pattern was not appreciated in other DAS.

The PD between approaches was never more than 12% indicating small differences. However, PD values found in this study are higher than the data reported by Sabliov *et al.* (2002), who analyzed axi-symmetric agricultural products such as eggs, lemons, limes and peaches. In this experiment, the surface area and volume of five 1.5'' diameter spheres obtained using image processing and traditional methods were compared against calculated values to assess the accuracy of the used method. It was found that the image processing procedure overestimate by 1.34% the surface area than the calculated value.

Sabliov *et al.* (2002) found that the tape method yielded a surface area 3.20% larger than the calculated value, which further suggests that the image processing method is more accurate than the tape method. Besides of the higher accuracy, the image processing method also showed a lower standard deviation. Indeed, it was recorded 0.32 cm² and 0.64 cm² for image processing and the tape method, respectively. Furthermore, those authors mentioned that the difference between image processing accuracy for different agricultural products may be explained under the basis of their geometry: the closer the product is to axi-symmetric geometry, the better the accuracy of the method. In this way, objects such as eggs, which are close to axy-symmetric, lead to small average standard deviations. In contrast, higher standard deviations were associated with objects such as limes, lemons, and peaches (1.98, 0.88, and 2.34, respectively) because they are less axy-symmetric. For eggs and spheres, the image processing method gave results within 5% lower than the tape method whereas for all other agricultural products there was not a clear trend in the data.

Table 1. Cladode real and estimated areas (mean±standard error) for ‘Atlixco’, ‘Copena F–1’, ‘Milpa Alta’ and ‘Jalpa’ varieties at different days after sprout. For ‘Jalpa’ variety, days after sprout variable was not taken into account.

Varieties	Days After Sprout	Real Area (cm ²)	Estimated Area (cm ²)	Statistical Test ¹	Percentage of Difference
Copena	10	58.41±1.26 ^a	53.23±1.25 ^b	9.55	9.65±6.67
Copena	17	151.84±2.45 ^a	141.46±2.77 ^b	9.80	8.05±4.77
Copena	23	161.85±4.27 ^a	169.48±4.68 ^b	5.66*	5.87±5.01
Atlixco	10	69.20±2.07 ^a	61.23±1.90 ^b	7.55*	11.81±6.03
Atlixco	17	159.61±4.41 ^a	171.24±4.68 ^b	6.00*	10.51±10.19
Atlixco	23	220.34±5.18 ^a	233.92±4.98 ^b	5.40*	9.79±8.74
Jalpa	–	129.76±6.94 ^a	122.55±7.32 ^b	5.07*	10.35±6.65
Milpa Alta	10	36.17±2.20 ^a	35.03±2.41 ^b	2.81*	5.88±5.46

¹ Numbers without asterisk are paired *t* values at $p \leq 0.05$; whereas those with asterisk are *Z* values estimated when the non parametric Wilcoxon Signed–Rank test for difference in medians ($p \leq 0.05$) was performed.

^a Different superscripts within rows are indicating statistical differences ($p \leq 0.05$).

Some products resulted in higher image processing values while others resulted in higher values calculated using the tape method. In agreement with this study, we did not appreciate a clear trend in such a way that sometimes the estimated areas were higher and sometimes lower than the real ones. Reinforcing Sabliov *et al.* (2002) and this study findings, Wang and Nguang (2006) reported that estimated surface area of eggs, lemons, limes and tamarillos [*Solanum betaceum* (Cav.)] did not show a clear trend in the data, however, in all cases, the differences between their image processing values and their values calculated using the tape method were less than 6%. It deserves be noted that in this study, we recorded differences from less than 6 % up to almost 12%, showing a larger bias in the estimation of the cladodes areas by the mathematical approach.

In Figure 1A, changes in the area of the cladodes at different DAS for the ‘Copena F–1’ variety are shown. There were few changes in the area of the cladodes after 17 DAS; whereas from 10 to 17 DAS, it was recorded a large increase in the surface area. The shape of the graph suggest a logarithmic type of growth, however, the coefficients of determination for the exponential regression and the straight linear model were very similar. Indeed, for exponential and straight line fits, the coefficients of determination were 0.7 and 0.65, respectively, in the case of the real areas; whereas for estimated areas, they were 0.73 and 0.7. Therefore, from the data generated in this study, it was not possible to conclude which type of behavior describes the changes in the area of cladodes during the growth for the variety ‘Copena F–1’. In the Figure 1A, it can be clearly seen that estimated and real areas of the cladodes are very similar at the different evaluated DAS. Furthermore, the scatter plot of Figure 1B showed a close linear relationship between the real and estimated area with a coefficient of determination of 0.96, which strongly supports the large similarity between the real and estimated areas of the cladodes (Nagelkerke, 1991). However, statistical analysis showed a significant differences between real and estimated areas ($p < 0.05$). Furthermore, at different DAS, it was found that the ellipses underestimate or overestimate the real area value suggesting a lack of accuracy of the mathematical approach carried out in this study. The large numbers of buds from different plants allowed us to have reliable results from the statistical analysis carried out in the present study showing a statistical difference between real and estimated areas.

Figure 2A depicts the changes in the area of the cladodes at different DAS for ‘Atlixco’ variety. The shape of the graph shows almost a straight line from 10 to 23 DAS. However, the correlations of determination for exponential and straight lines were 0.74 and 0.73 respectively; whereas for

estimated areas, the coefficients were 0.79 and 0.78, respectively. Therefore, as in the case of 'Copena F-1' variety, it is not possible to know the behavior of growth in area of the cladodes of 'Atlixco' variety. In Figure 2A, it can be observed that similar values of estimated and real areas of the 'Atlixco' cladodes were recorded during the time of the study. Furthermore, in Figure 2B, it is presented the scatter plot between the real and estimated area of the cladodes with a coefficient of determination of 0.94 indicating the large similarity between the real and estimated area of cladodes. However, likewise 'Copena F-1' variety, statistical analysis showed differences ($p < 0.05$) between real and estimated areas. In addition, it was found that the ellipse over- and underestimate the real area, suggesting a lack of accuracy of the mathematical approach used in this work to estimate the cladode area.

Different growth patterns between 'Copena F-1' (Figure 1A) and 'Atlixco' (Figure 2A) varieties were observed. Although the coefficients of determination for both curves did not allow us concluding whether the behavior of growth is linear or logarithmic in the case of both varieties. Luo and Nobel (1993) concluded that in new daughter cladodes of *Opuntia ficus-indica* growing under controlled conditions, the area expansion of cladodes follows a combination of sigmoidal-linear behavior.

For the case of the 'Milpa Alta' and 'Jalpa' other varieties, the real and estimated areas of the cladodes were very similar; however, they were found to be different ($p < 0.05$) based in the statistical analysis. In both cases, it was recorded an underestimation of the area by the mathematical approach.

In this work, it was recorded an under- and overestimation of the cladode area by using the ellipse mathematical formula. Based in this observation together with the results of the statistical comparison carried out between the estimated and real areas, it is clear that for the varieties of *Opuntia* involved in this study, the estimation of the area by the mathematical formula is not completely accurate. Despite of this findings, the deviation from the real value are very small in such a way that the developed methodology can be used to estimate the cladode area in many practical applications. However, it is important to mention that the model used in this work can not be used for other species of *Opuntia* due to the large polymorphism in the cladodes from different species belonging to this genus (Flores *et al.*, 1996). In agreement with this, there is a work in which the estimation of the cladode area in three varieties of *Opuntia megacantha*, four varieties of *Opuntia ficus-indica* and three varieties of *Opuntia amyclaea*, was made using the formula to calculate the circle area whereas the real cladode area was calculated by electronic detection.

Different models of simple linear regression were tested and were selected based in their coefficient of determination, error mean square and variation coefficient. It was found a coefficient of determination between measured and estimated areas of cladodes from 0.92 to 0.95 (Flores *et al.*, 1996). These values are similar with the data generated in the present study. Thus, taking into account the results of this study, it will be difficult to conclude whether the cladode area can be better estimated by using the formula of a circle or the ellipse.

The protocol developed in this work for estimating the area of the cladode by measuring the longitudinal and equatorial diameters of the cladodes is easy and inexpensive. Also, the protocol developed avoids the need to manipulate these tissues at late stages of development in which spines are fully developed. With two simple measurements, the area can be estimated in many cladodes in a very short period. Leaf surface area determines in large part the amount of carbon gained through photosynthesis and the amount of water lost through transpiration and ultimately the crop yield. In

many crop growth studies leaf area index has been reported to be related with both biological and economical yield (Kathirvelan and Kalaiselvan, 2007).

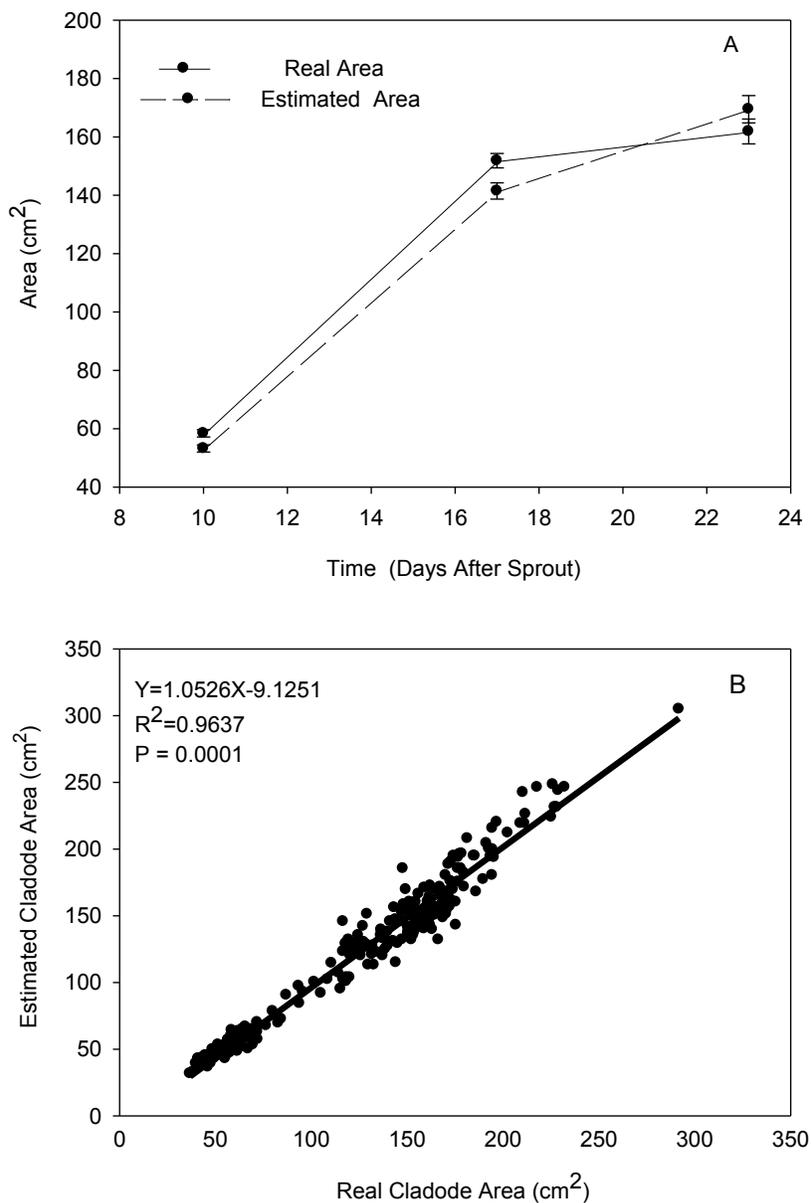


Figure 1. Results of the analysis carried out in the 'Copena F-1' variety. In panel A, it is depicted the changes in cladode areas at different days after sprout. In panel B, it is shown the scatter plot done with data of real cladode area versus estimated cladode area. Vertical lines in panel A correspond to standard errors.

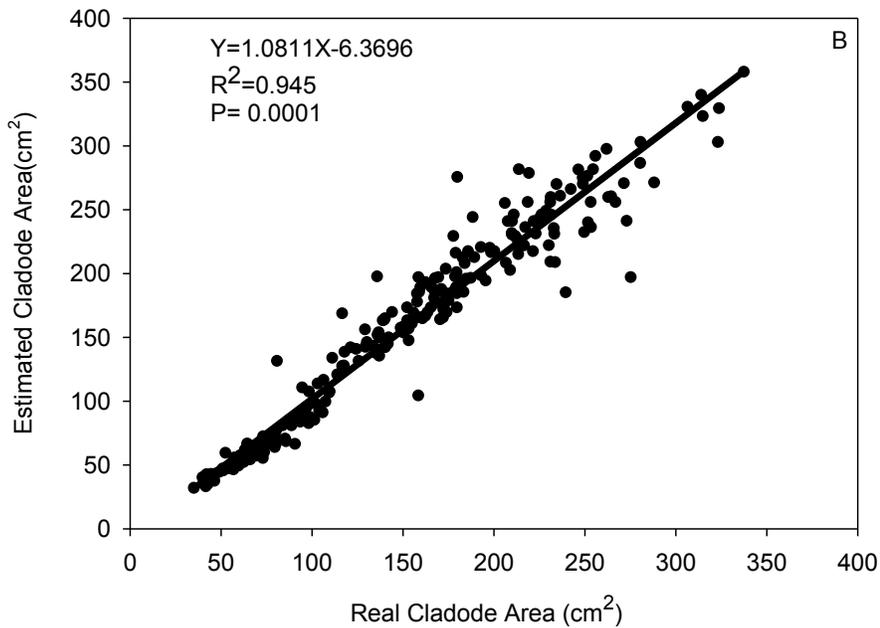
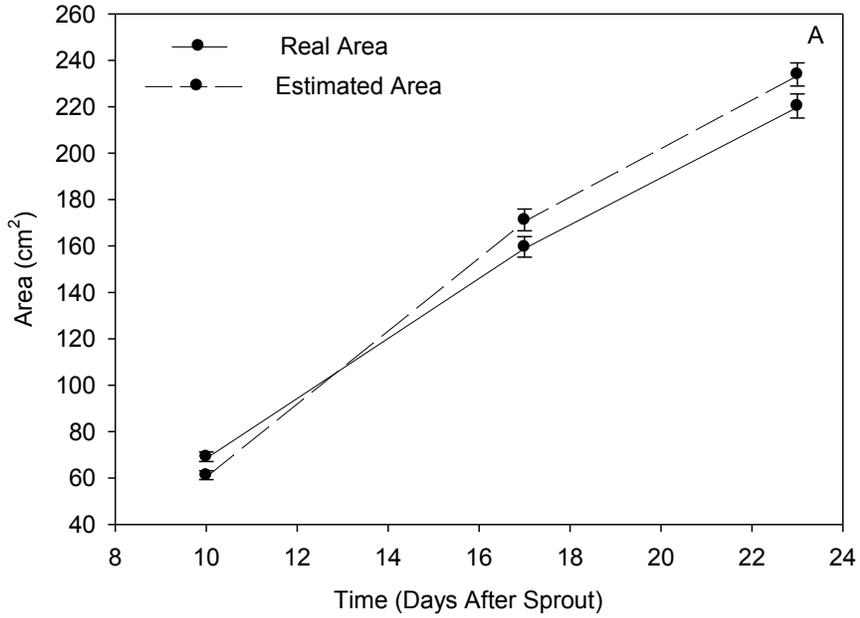


Figure 2. Results of the analysis carried out in the ‘Atlixco’ variety. In panel A, it is depicted the changes in cladode areas at different days after sprout. In panel B, it is shown the scatter plot done using the data of real cladode area versus estimated cladode area. Vertical lines in Panel A correspond to standard errors.

Conclusions

In this work, a rapid, inexpensive and simple method for estimating cladode area in four varieties of *Opuntia ficus-indica*, using the elliptical mathematical formula was developed. Although the procedure is not completely accurate to estimate cladode area, it is enough for many practical purposes.

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