Estimation of cladode area of *Nopalea cochenillifera* using digital images

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ABSTRACT

Determination of photosynthetic area of a plant, leaf or cladode is a fundamental tool in study of transpiration intensity, specific leaf area and leaf area index. The objective of this study was to evaluate *Nopalea cochenillifera* (L.) Salm-Dyck). cladode area, in a non-destructive way, using digital images and test its relation with the variables: product of length and maximum width and real cladode area through regression models. The design used randomized blocks with three replicates and using the *N. cochenillifera* forage cactus clone, Giant Sweet. To determine the real cladode area of cactus forage, 432 cladodes in different stages of growth were randomly collected (162 primary cladodes, 127 secondary and 143 tertiary cladodes), all free from damage, disease or pest attacks. All cladodes were photographed with a digital camera (Sony Mark, model DSC-P72) generating a sample of 432 1200 x 2500 pixel digital images of *N. cochenillifera* cladodes. Linear, gamma and power regression models were adjusted to test the relation between the digital cladode area and the explanatory variables *real cladode area* and *product of length by width*. Models were evaluated with the following criteria: Coefficient of model determination, Akaike information criterion, sum of squares of residuals and Willmott index. The power model gave the best performance, with explanatory power higher than 99.5%, while the Willmott index exceeded 0.99. Sum of squares of residuals and Akaike information criterion had lower values. The digital cladode area of *N. cochenillifera* can be explained by the linear dimensions of cladodes in, and independent of, branching order. The digital cladode area (DCA) of *N. cochenillifera* can be explained as a function of the power model $\widehat{\mathrm{DCA}} = \mathrm{LW}^{0.985}$considering the product of length by width (LW) with explanatory variable, and by $\widehat{\mathrm{DCA}} = \mathrm{RCA}^{1.002}$considering real cladode area (RCA) with explanatory variable.

Keywords: Image J, Regression models, power model, cactus forage.

INTRODUCTION

Species adapted to arid and semi-arid environments, such as forage cacti (*Nopalea* spp. and *Opuntia* spp.), can contribute to increases in biomass yields in agricultural areas, improving the use efficiency of local natural resources (Diniz *et al.*, 2017). The forage cactus is a multiple usage plant, cultivated in a variety of countries across all vegetated continents. 05.05 05.19 It is favored as alternative in semi-arid regions, due to high potential phytomass production, high energy value, high acceptability by ruminants, high digestibility coefficient, large water reserve and easy propagation (Pereira *et al*., 2018). One of determining factors of biomass production is the absorption and use of solar radiation in the photosynthetic process, in the forage cactus this function is conducted by cladodes (Lucena *et al*., 2018).

The quantity of light captured is linked to the use of CO_2 by a plant, and CO_2 assimilation provides both the carbon for plant growth and for maintenance and activation of all metabolic functions, including nutrient acquisition capacity (Lemaire and Chapman, 1996). The forage cactus has a CAM type photosynthetic metabolism (Crassulasean Acid Metabolism), with a low number of stomata in the cladodes (10 to 30 by mm^2), epidermal cells with thick, dense walls (Jaleel *et al*., 2009), high tolerance of high air temperatures (Nobel and Zutta, 2008). This is accompanied by a complex root system and composed of several root types: structural, lateral, originating in the areolas of the cladodes and roots of rain or absorbent (Snyman, 2006).

All these anatomical and morpho-physiological adaptations acquired evolutionarily by forage cacti have contributed to the high agroecological success of this crop; high water use efficiency having resulted in their adaptation to environmental conditions that involve high atmospheric evaporative demand and reduced soil water content (Hartzell *et al*., 2018; Winter *et al*., 2011). One of the important characteristics that can be used in this context is the leaf area index (LAI), a metric forms an important biophysical parameter in plant ecophysiology (Hasegawa *et al*., 2010).

Determination of the photosynthetic area of plant, leaf or cladode is a fundamental tool in study of transpiration intensity, leaf area rate, specific leaf area and leaf area index (Schmildt *et al*., 2014). For species such as forage cacti, which lack leaves, this is replaced by cladode area estimation. Indirect and non-destructive methods are used, allowing successive evaluations of the same cladode and rapid analysis (Lucena *et al*., 2018).

The use of digital images to measure the leaf area is an alternative to the methods mentioned, because it requires neither the removal of study leaves nor the use of laboratory-based equipment. This makes it a very simple method, and one that is both not harmful to the plant and low cost. Computational advances in recent decades have led to an upsurge of studies of the optical estimation of leaf area. These have included: Lucena *et al.* (2018), *Urochloa mosambicensis* leaf area; Reis *et al.* (2016), *Opuntia fícus-indica* cladode area; Jadoski *et al.* (2012) and Zeist *et al.* (2014) evaluating potato and strawberry leaf areas, respectively. Although there exists information (Silva *et al*., 2014) on the relation between *N. cochenillifera* cladodes and the agronomic characteristics of the species, no studies were found in the literature that estimate cladode area of this species using digital images. Thus, the study of indirect methods to determine cladode area assumes great importance, since this index provides an understanding of plant responses to the environment and allows management strategies to be defined. Accordingly, the objective of this study was to use digital images to non-destructively evaluate *N. cochenilifera* cladode area, and use regression models to test its relation with the variables: product of the length and maximum width of cladode and real cladode area.

MATERIAL AND METHODS

Study area

Research was carried out under field conditions from March 2016 to October 2018, at the Federal Rural University of Pernambuco (Serra Talhada County Campus), located at 07° 57' 01" S, 38° 17' 53" E, at an elevation of 523 m.a.s.l. According to the Köppen system, regional climate is BSwh: semi-arid, hot and dry climate. Annual mean rainfall, temperature and relative air humidity are 632.2 mm, 26ºC and 60%, respectively (Leite *et al.*, 2019).

Experimental conditions
Soils characteristics

The soil used is Typical Haplic Cambisol Ta Eutrophic, and was collected from the first 20 cm. Samples of collected soil had the following attributes: pH (water) = 6.80; P (extractor Mehlich I) = 40 mg dm^{-3}; K^+ = 0.45; Ca^{2+} = 5.50; Mg^{2+} = 1.60; Al^{3+} = 0.0 cmolc dm^{-3}, respectively. Collected soil was subsequently given organic fertilization with 40 t ha^{-1} of bovine manure. The area was weeded and prepared in March 2016, after the forage cactus seed cladodes the clone forage cactus Giant Sweet (*Nopalea cochenillifera* (L.) Salm-Dyck) were planted with 50% of the length of cladode buried in the soil.

Experimental design

A randomized block design was used with three replicates. The experimental unit, with an area of 8.4 m^2 (4.2 m x 2.0 m), was composed of three rows of cacti, in the row spacing of 1.40 (85,714 plants ha^{-1}). Removal of invasive plants occurred every two months. Cultivation was conducted under dry conditions throughout the crop cycle. The evaluations were performed at 550 days after planting (DAP).

Statistical analysis

To determine the real cladode area for this study plants, 432 cladodes in different stages of growth, were randomly collected, 162 primary cladodes, 127 secondary and 143 tertiary cladodes, free of damage, disease or pest attacks, using methodologies already established in the literature (Lucena *et al.*, 2018; Schmildt *et al.*, 2014; Leite *et al.*, 2017).

Study cladodes were numbered from 1 to 432, and length (L, in cm) and width (W, in cm) of each measured using digital calipers, using the region of greatest width and length of each cladode. Length and width were multiplied determining the product (LW) in cm^2. All cladode outlines were delineated graphite on millimeter graph paper according to methodology described by Leite *et al.* (2017). Using scissors, the area of the millimeter graph paper was then cut-out and weighed on electronic balance. From the same paper a 10 x 10 cm square (equivalent to 100 cm^2) was cut out and weighed. The resulting mass (0.630 g), allowed the proportional real cladode area (RCA) to be calculated.

Each cladode was photographed with a digital camera (Sony model DSC-P200) establishing a standard distance above each cladode (45 cm) for all images, making the sample of 432 digital images of cladode of *N. cochenillifera* with dimension 1200 x 2500 pixels (Figure 1a). All images underwent digital processing using Image J software, to transform images to a black and white scale (Figure 1b). After transformation, the extent of the black area was calculated using Image J software (Lucena *et al.*, 2018; Jadoski *et al.*, 2012; Zeist *et al.,* 2014). This process was performed for each of the 432 images in question. To obtain the correction factor of pixels² in cm², a digital photo of 10 x 10 cm was captured. Next, the area of this square was calculated in pixels² according to the methodology described by Lucena *et al.* (2018), and a pixel² value obtained (0.000093 cm²).

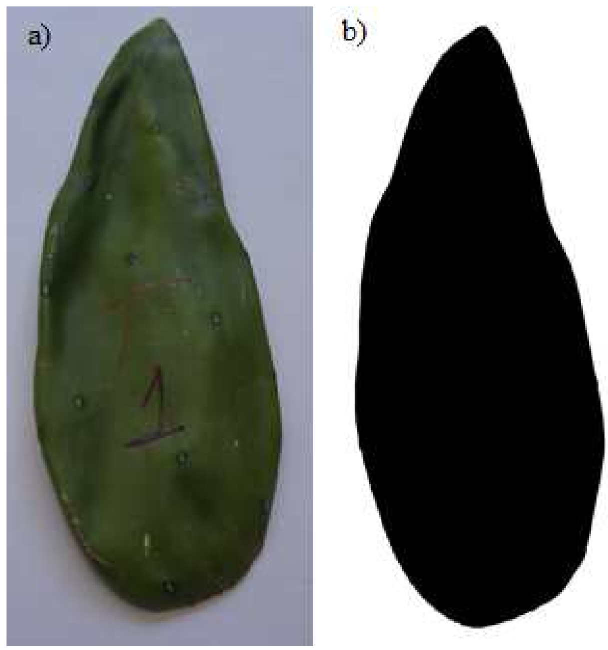

Figure1. Cladode color digital image (a), and cladode black and white digital image (b).

Analysis for the fittest model to predict digital cladode area (DCA) as a function of the real cladode area (RCA), and of the product between L and W (LW), regression studies were performed using linear, gamma and power models (Leite *et al.,* 2017; Lucena *et al.*, 2018) (Table 1). The linear and power models have a normal distribution, assuming that the response variable has values in range (-∞, ∞), while the gamma model assumes that the response variable have values in the range (0; ∞) (Table 1).

Table 1. Regression models for the digital cladode area (DCA) in relation to the explanatory variables: real cladode area (RCA) and product length by width (LW) for *Nopalea cochenillifera* cladodes.

Models	Explanatory variables	
	RCA	LW
Normal	$DCA_i = \beta_0 + \beta_1 RCA_i + \varepsilon_i$	$DCA_i = \beta_0 + \beta_1 LW_i + \varepsilon_i$
Gamma	$DCA_i = \beta_0 + \beta_1 RCA_i + \varepsilon_i$	$DCA_i = \beta_0 + \beta_1 LW_i + \varepsilon_i$

Power	DCA$_i$ = $\beta_0 RCA_i^{\beta_1} \varepsilon_i$	DCA$_i$ = $\beta_0 LW_i^{\beta_1} \varepsilon_i$

Where, DCA$_i$ is the i-th digital cladode area for *N. cochenillifera*; RCA$_i$ is the i-th real cladode area for *N. cochenillifera*; LW$_i$ is the product between length and width of the i-th cladode and ε_i is the i-th error interrelated the digital cladode area, which ε_i exhibited normal distribution of mean 0 and variance constant $\sigma^2 > 0$ to the linear and power model, and gamma distribution of parameters α and β for the gamma models, and β_0 and β_1 are parameters related to the model.

The following criteria were evaluated for the models: Coefficient of determination of the model (R^2), Akaike's Information Criterion (AIC) defined by Akaike (1974), Sum of Square of Residuals (SSR) and the Willmott index (d) defined by Willmott (1981), Table 2.

Table 2. Criteria of model adequacy

Criteria of model adequacy			
R²	**AIC**	**SSR**	**d**
$1-\frac{\sum_{i=1}^{n}(Y_i-\widehat{Y}_i)^2}{\sum_{i=1}^{n}(Y_i-\overline{Y})^2}$	$-2\ln L(x\backslash\widehat{\theta})+2p$	$\sum_{i=1}^{n}(Y_i-\widehat{Y}_i)^2$	$1-\frac{\sum_{i=1}^{n}(\widehat{Y}_i-Y_i)^2}{\sum_{i=1}^{n}(\lvert\widehat{Y}_i-\overline{Y}\rvert+\lvert Y_i-\overline{Y}\rvert)^2}$

Where, $L(x\backslash\hat{\theta})$ is the maximum likelihood function, defined as the product of density function and p is the number of model parameters; $\widehat{Y}_i$ the values of the i-th digital cladode area after model adjustment; $\overline{Y}$ is the mean of the values of the digital cladode area.

RESULTS AND DISCUSSION

Table 3 shows a large variance in the size of the analysed *N. cochenillifera* cladodes, independent of cladode order. Highest mean real cladode area occurred with primary cladodes (321.43 ± 106.69 cm²), while tertiary cladodes had the lowest (140.15 ± 68.83 cm²). Primary cladodes had a mean digital area of 324.29 ± 108.16 cm², for secondaries the mean was 302.04 ± 131.45 cm² and 142.72 ± 68.65 cm² for tertiaries. Product of length by width showed that the highest mean occurred for the primary cladodes (333.27 ± 128.39 cm²), and the lowest was for tertiary cladodes (155.22 ± 76.92 cm²).

For *N. cochenillifera* Guimarães *et al.* (2018) reported a mean cladode area of 299.66 cm² at 930 DAP. Silva *et al.* (2010) reporting on the morphology and productive characteristics of 50 clones of forage cactus at 720 DAP found the mean product of length by width to be 463.65 cm² for primary cladodes, 242 cm² for secondary and 151.68 cm² for tertiary. Silva *et al.* (2015) found primary, secondary and tertiary 745 DAP Clone Giant Sweet cladodes to have mean areas of 243.11, 229.71 and 23.63 cm², respectively. A mean of 73.73 cm² for 210 DAP clone Giant Sweet *N. cochenillifera* cladodes was reported by Gomes *et al.* (2016), while Reis *et al.* (2016) gave 586.71 cm² as the mean cladode area for *Opuntia fícus-indica* and 722.01 cm² as the mean product of length by width.

Table 3. Descriptive analysis of real area (RCA), digital (DCA) and product of length by width (LW) for *Nopalea cochenillifera* cladodes.

	Min.	**Mean**	**Standard deviation**	**Max**
		Real Cladode Area (RCA)		
Primary cladode	78.93	321.49	106.69	714.08
Secondary cladode	59.86	301.43	132.23	745.60
Tertiary cladode	17.14	140.15	68.83	396.95
Independent of order	17.14	236.82	125.33	745.60
		Digital Cladode Area (DCA)		
Primary cladode	80.15	324.29	108.16	729.92
Secondary cladode	60.00	302.04	131.45	744.21
Tertiary cladode	20.05	142.72	68.65	400.00
Independent of order	20.05	238.71	125.40	744.21
		Product of length by width (LW)		
Primary cladode	91.84	333.37	128.39	828.75
Secondary cladode	59.00	323.08	142.84	820.00
Tertiary cladode	20.06	155.22	76.92	445.56
Independent of order	20.06	260.13	139.98	828.75

As shown in Table 4, for primary cladodes a positive correlation exists between DCA and RCA ($r = 0.997$; $p<0.0001$) and DCA and LW ($r = 0.954$; $p<0.0001$). For secondary cladodes DCA correlates positively with RCA ($r = 0.995$; $p<0.0001$), and LW ($r = 0.979$; $p<0.0001$). A positive correlation in tertiary cladodes between DCA and RCA ($r = 0.999$; $p<0.0001$) and LW ($r = 0.96$; $p<0.0001$), is also present. Independent of cladode order a positive correlation between RCA and product of length by width has reported by Lucena *et al.* (2018) in *Opuntia stricta* ($r = 0.90$) and by Reis *et al.* (2016) for *Opuntia ficus-indica* ($r = 0.95$).

Table 4. Correlation matrix between real cladode area (RCA), digital cladode area (DCA) and product of length by width (LW) by order and independent of order of *Nopalea cochenillifera* cladodes.

Primary cladode				**Secondary cladode**			
	RCA	DCA	LW		RCA	DCA	LW
RCA	1.000	---	---	RCA	1.000	---	---
DCA	0.997	1.000	---	DCA	0.995	1.000	---
LW	0.951	0.954	1.000	LW	0.980	0.979	1.000
Tertiary cladode				**Independent of order**			
	RCA	DCA	LW		RCA	DCA	LW
RCA	1.000	---	---	RCA	1.00	--	--
DCA	0.999	1.000	---	DCA	0.998	1.00	--
LW	0.960	0.960	1.000	LW	0.974	0.973	1.00

Analyzing the relation of the digital cladode area as a function of the product of length by width showed that for primary cladodes the power model was the most efficient in explaining this relationship, as it had the highest coefficients of model determination (R^2 = 99.97%), the greatest Willmott index (d = 0.978), and the smallest values of sum of squares of residuals (SSR = 163,450.50) and Akaike's Information Criterion (AIC = 315.09) when compared to the linear and Gamma models (Table 5). The same is true for secondary cladodes (R^2 = 99.94%; SSR = 89012.24; AIC = 349.19 and d = 0.991), tertiary (R^2 = 99.77%; SSR = 53630.44; AIC = 932.78 and d = 0.983) and independent of order (R^2 = 99.91%; SSR = 340303.10; AIC = 342.58 and d = 0.987) when compared to linear and Gamma models (Table 5). Considering the four criteria evaluated, the power model showed the highest values of coefficient for model determination and Willmott index and the smallest values of sum of squares of residuals and Akaike's Information Criterion, thus indicating is as the most appropriate means of explaining the relation between DCA and LW.

Table 5. Estimates of parameters and adequacy criteria for the models of digital cladode area (DCA) of *Nopalea cochenillifera* taking into account the product of length by width (LW) as the explanatory variable.

Models	**Equation of digital cladode area**	**Criteria of model adequacy**			
		R^2	**SSR**	**AIC**	**d**
	Primary Cladode				
Linear	$\widehat{DCA} = 0.889LW$	98.66	166769.70	1589.49	0.976
Power	$\widehat{DCA} = LW^{0.983}$	99.97	163450.50	315.09	0.978
Gamma	$\widehat{DCA} = 0.916LW$	90.42	180357.30	1474.20	0.975
	Secondary Cladode				
Linear	$\widehat{DCA} = 0.929LW$	99.35	89080.69	1196.66	0.989
Power	$\widehat{DCA} = LW^{0.989}$	99.94	89012.24	349.19	0.991
Gamma	$\widehat{DCA} = 0.954LW$	95.46	98744.77	1320.50	0.988
	Tertiary Cladode				
Linear	$\widehat{DCA} = 0.904LW$	98.47	54428.20	1259.49	0.979
Power	$\widehat{DCA} = LW^{0.982}$	99.77	53630.44	932.78	0.983
Gamma	$\widehat{DCA} = 0.963LW$	90.65	69248.09	1447.40	0.976
	Independent cladode order				
Linear	$\widehat{DCA} = 0.908LW$	98.90	346409.30	4118.73	0.987
Power	$\widehat{DCA} = LW^{0.985}$	99.91	340303.10	342.58	0.987
Gamma	$\widehat{DCA} = 0.943LW$	94.61	392629.80	4440.80	0.987

R^2 (coefficients of determination); SSR (sum squared of residuals); AIC (Akaike information criteria); d (Willmott index).

Lucena *et al.* (2018), evaluating *Urochloa mosambicensis* found the power model to be the most appropriate when explaining the relation of digital leaf area as a function of the product of length by width with explanatory power (R^2) of 99.99%. Reis *et al*. (2016), reported that the linear model (explanatory power, 91%) was best explained the digital cladode area of *Opuntia fícus-indica* as a function of the product of length by width with explanatory variable. Jadoski *et al.* (2012) evaluating digital leaf area of potato as a function of the product length by width considered the best model to explain the relation to be a cubic polynomial (explanatory power, 94%). Silva *et al.* (2014) studying *Nopalea* sp., Hernández *et al.* (2010) for *Opuntia fícus-indica* and Zeist *et al.* (2014) with strawberry all found that the linear model best explained digital area as a function of the product of length by width with precisions (R^2) of 98.47%, 96.37% and 96.75%, respectively. When testing the relation of *Opuntia stricta* cladode area to the product of length by width Lucena *et al.* (2018) reported the linear model had an explanatory power of 96.66%.

Evaluating the relationship between digital and real areas of primary cladodes, it was observed that the evaluated models performed in very similar manners, as all had explanatory power greater than 99%, a Willmott index higher to 0.99 and sum of square of residuals exceeding 11,600 (Table 6). However, the power model best explained this relationship, because it had the highest R^2 and d index, and smaller SSR and AIC values when compared to the linear and Gamma models.

The relation of the digital and real areas of secondary cladodes is best explained by power regression model, because of the four criteria analyzed, it showed the best performance in three (R^2 = 99.83%; SSR = 7045.43 and AIC = 520.28), when compared to the linear and Gamma models. The Wilmott index did not differ between models (Table 6). For tertiary cladodes the power model most appropriately explained the relation between the digital and real areas, having greater explanatory power (R^2 = 99.75%), and smaller SSR and AIC when compared to the linear and Gamma models. When analysed independent of cladode order, the best relation between the digital and real areas was given when using the power model, which had the best performance in three criteria of the four analyzed (larger R^2 = 99.89%, smaller SSR = 20586.61 and AIC = 1814.37) when compared to the linear and Gamma models.

Table 6. Estimates of parameters and criteria of adequacy of the models for digital cladode area (DCA) of *Nopalea cochenillifera,* with real cladode area (RCA) as the explanatory variable.

Models	Equation of digital cladode area	Criteria of model adequacy			
		R^2	SSR	AIC	d
		Primary Cladode			
Linear	$\widehat{DCA} = 1.01RCA$	99.32	11630.30	1156.09	0.998
Power	$\widehat{DCA} = ARC^{1.002}$	99.69	11619.25	735.78	0.999
Gamma	$\widehat{DCA} = 0.88RCA$	99.38	11635.48	1057.10	0.998
		Secondary Cladode			
Linear	$\widehat{DCA} = 1.001RCA$	99.35	7352.52	870.91	0.999
Power	$\widehat{DCA} = ARC^{1.001}$	99.83	7045.43	520.28	0.999
Gamma	$\widehat{DCA} = 1.004RCA$	99.67	7128.39	902.37	0.999
		Tertiary Cladode			
Linear	$\widehat{DCA} = 1.011RCA$	99.36	1439.70	740.05	0.999
Power	$\widehat{DCA} = ARC^{1.003}$	99.75	1206.72	577.87	0.999
Gamma	$\widehat{DCA} = 1.019RCA$	99.75	1652.27	805.58	0.999
		Independent cladode order			
Linear	$\widehat{DCA} = 1.006RCA$	99.69	20681.98	2901.20	0.999
Power	$\widehat{DCA} = ARC^{1.002}$	99.89	20586.61	1814.37	0.999
Gamma	$\widehat{DCA} = 1.012RCA$	99.68	21702.18	2782.40	0.999

R^2 (coefficients of determination); SSR (sum squared of residuals); AIC (Akaike information criteria); d (Willmott index).

Lucena *et al.* (2018), found that the power model was appropriately explained the relation of digital and real leaf area of *Urochloa mosambicensis* with an explanatory power of 99.98%, SSR= 161,451.8, AIC= -203.08 and index d=0.9999. Peksen (2007) for *Vicia faba* L., and Godoy *et al.* (2007) for the orange, Adami *et al.* (2008) for soybean leaflets and Ferreira *et al.* (2017) for leaves of a variety of species all found the linear model provided the best explanation for the relation between the digital and real area with explanatory power of 97.7, 99.99, 99.7 and 99%, respectively.

CONCLUSIONS

Digital cladode area of *Nopalea cochenillifera* Giant Sweet clone can be explained by linear dimensions of cladodes by order and independent of cladode order, notably by product of cladode length by width. Cladode length and width can be easily measured in the field, periodically, without the need to destroy the plant.

Digital cladode area (DCA) of *Nopalea cochenillifera* Giant Sweet clone can be estimated, with high level of accuracy, as a function of power models $\widehat{\mathrm{DCA}} = \mathrm{LW}^{0,985}$ taking into account the product of length by width (LW) as explanatory variable, and by $\widehat{\mathrm{DCA}} = \mathrm{RCA}^{1,002}$ taking into account the real cladode area (RCA) as the explanatory variable. Cladode digital images can easily be obtained with a photographic or smartphones camera.

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