ESTIMATION OF LEAF AREA OF *Tithonia diversifolia* USING ALLOMETRIC EQUATIONS†

[ESTIMACIÓN DEL ÁREA FOLIAR DE *Tithonia diversifolia* UTILIZANDO ECUACIONES ALOMÉTRICAS]

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SUMMARY

This study evaluated different models and proposed a reliable and accurate model using non-destructive measurements of leaf length (L) and/or width (W) for estimating the leaf area of *Tithonia diversifolia* (TD). Rapid, reliable and objective estimation of the leaf area is essential for numerous studies in agronomy and plant physiology, however, it is done usually by the availability of sophisticated and expensive electronic integrate methods. Allometric equations were developed by measuring W, L and LA of 92 leaves of TD, by a linear regression analysis. The current leaf area was measured using *ImageJ* software. With the observed and estimated information, a Pearson correlation analysis was performed. Pearson correlation coefficients oscillated between 0.93-0.94 for the best mathematical models. Equations which used as variable the product L*W presented strong relationships with the observed leaf area, manifested in high determination coefficients ($R^2 = 0.89$). Therefore, the use of the product of L*W as the independent variable was found to be accurate to predict the TD leaf area. In conclusion, a lineal model was developed to predict the leaf area for the *T. diversifolia* (y=0.755+0.438(W*L).
Keywords: Leaf; phenology; physiology plant; forage.

RESUMEN

Este estudio evaluó diferentes modelos y propuso un modelo confiable y preciso que utiliza mediciones no destructivas de la longitud de la hoja (L) y / o el ancho (W) para estimar el área de la hoja de *Tithonia diversifolia* (TD). La estimación rápida, confiable y objetiva del área foliar es esencial para numerosos estudios en agronomía y fisiología de plantas, sin embargo, generalmente se realiza mediante la disponibilidad de métodos de integración electrónica sofisticados y costosos. Las ecuaciones alométricas se desarrollaron midiendo W, L y LA de 92 hojas de TD, mediante un análisis de regresión lineal. El área foliar actual se midió usando el software ImageJ. Con la información observada y estimada se realizó un análisis de correlación de Pearson. Los coeficientes de correlación de Pearson oscilaron entre 0.93 y 0.94 para los mejores modelos matemáticos. Las ecuaciones que utilizaron como variable el producto L * W presentaron fuertes relaciones con el área foliar observada, manifestadas en altos coeficientes de determinación (R^2 = 0,89). Por lo tanto, se encontró que el uso del producto de L * W como la variable independiente era preciso para predecir el área foliar de TD. En conclusión, se desarrolló un modelo lineal para predecir el área foliar de la *T. diversifolia* (y = 0.755 + 0.438 (W * L).
Palabras clave: Lámina foliar; fenología; fisiología vegetal; forraje.

INTRODUCTION

A scale population shows there is a statistical relationship between measures of an individual (Gould, 1966). The proportions between linear measurements of plants and measures of area or volume, obey a rule that is the same for all individuals living in the same conditions; this relationship is derived from the ontogenetic development of individuals, which is the same for all except the variability associated with the personal history of each. This is the basic principle of allometry that predicts a measure of an attribute based on another measure (Picard, Saint-André & Henry, 2012).

Thus, an allometric equation is a formula that formalizes this relationship quantitatively. There is a more restrictive definition of allometry consisting of a relationship of proportionality between the relative increases of measures (Huxley, 1924; Gayon, 2000).

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Estimating the leaf area of plants entails many benefits, such as prediction of the productive potential and the achievement of optimal management agricultural practices as irrigation, fertilization and soil use (Karatassiou *et al.,*2015). Also, the leaf area describes the magnitude of photosynthetic activity of plant species and constitutes an important indicator describing the growth capacity (Dheebakaran and Jagannathan 2009; Caliskanetal. 2010).

The leaf area (LA) is a variable of easy determination with which we can estimate the growth rate of a crop in a given period of time and photosynthetic capacity (Watson, 1937). Thus, measurements of LA are essential for research in plant physiology, agriculture, dendrology, (Cabezas-Gutiérrez *et al.*, 2009; Broadhead*et al.*, 2003) and generate empirical data useful for estimating biomass; how greater leaf area means the amount of light that can be captured increasing primary production and therefore the production of dry matter. This feature is important in forage plants. Furthermore, to evaluate continuous changes in LA and the subsequent growth, a modeling approach is essential (Bonser *et al.*, 2009)

The study of plant growth requires the measurement of two variables, evaluated at defined intervals such as leaf area (LA) and dry weight (DW) (Rojas and Seminario, 2014). With these two values derived by calculation, the growth rates of the individual plant or population (Gardner *et al.*, 1985) have been estimated.

The plant leaf area is related to the number of leaves and the size of them. Several methods are used to estimate leaf area (Lu *et al*., 2004; Morgado *et* al., 2013).

The efficiency of methods estimating LA is determined by their level of precision, their time requirements, the availability of proper equipment and the experimental goals (Karatassiou *et al.*, 2013). Leaf area estimation methods are generally classified as destructive and non-destructive (Karatassiou *et al*., 2015). Some of them are very simples, ranging from visual estimates, to more sophisticated electronic integrators where leaf area is used (Méndez, 1993). In this sense, a scanner image methodology has been used to determine plant dimensions, such as leaf area, length and width, in order to compare with those recorded by the LI-COR leaf area meter (Femat *et al.*, 2011). On the other hand, a quick and inexpensive method to estimate leaf area can be achieved by using a free program called ImageJ (Rincón *et al.*, 2012); this is software for digital image processing programmed in Java and developed at the National Institutes of Health.

On the other hand, the most common way is to estimate the leaf area by mathematical equations involving linear measurements such as length and width of the leaf and length of the petiole, or some combination of these variables, which have generally shown high precision (Blanco and Folegatti, 2005; Lopes *et al*., 2007). These methods are usually quick and not destructive, and they have shown a high accuracy (Campostrini and Yamanishi, 2001; Blanco and Folegatti, 2003).

In agronomic research, determining the leaf area provides reasoning for physiological studies involving plant growth, light interception, photosynthetic efficiency, and response to evapotranspiration and irrigation fertilizers (Blanco and Folegatti, 2005). It is because the leaf is the main organ in the transpiration process, responsible for gas exchange between the plant and the environment, obtaining accurate measurements of leaf area is essential to the understanding of the interaction between crop growth and its environment (Morgado *et al.,* 2013; Cintra de Jesús *et al*., 2001).

There by, the use of linear measurements of leaves ensures, in around about way, the criticality of this variable as important in studies of plant growth without reaching destruction (Peksen, 2007). Determination of a measurement methodology for leaf area that is low-costing, efficient, fast, and non-destructive, is essential for the expansion of research with different species.

For this, we must first have a simple mathematical function (linear) which related to the calculation in a simple way. Several studies suggest that the mathematical model can be obtained by correlation between the length of the leaf (L), width (W) or length by width (L*W) or some combination of these variables, with the LA, through regression analysis (Demirsoy and Lang, 2010; Küçükönder, *et al.,* 2016). Today, non-destructive methods are based on linear measurements that are fast, easy to measure, and provide good accuracy in growth studies of many plant species (Jerez and Diaz, 2014). It is extremely important, however, that such a model be reliable and accurate (Küçükönder, *et al.,* 2016). The objective was to produce a reliable equation to estimate the leaf area of *Tithonia diversifolia* plants, non-destructively, using linear measurements of the leaves as independent variables.

MATERIALS AND METHODS

At first, they were selected at random and collected 93 leaves from the bottom half of bushes *Tithonia diversifolia*. Leaves were harvested, at pre-flowering or early flowering stage in February 2013 in a TD field 60 days old, from different plant accessions, which were grown in the experimental farm at Universidad Nacional de Colombia, Palmira (24°C, 1.000 masl, annual precipitation 1020 mm and relative humidity 72%). It belongs to the warm-moderate climate zone, according to the classification Holdridge, Dry Forest Tropical (BS-T) (Rodríguez, 1999). The soils belong to the order vertisols (Fine clay ustic epiaquert isohyperthermic 1%) (Acosta *et al*., 1997)

These leaves were measured in two perpendicular axes; the measures were registered in a field format and subsequently transcribed into a spreadsheet Excel. The leaves were preserved between piece of papers (paper-Bon of 90 g) and pressed to prevent spoilage. Subsequently, the leaf blades were scanned on a scanner (LaserJet Pro1415fn).

To calibrate the image, we enter a known standard value of a reference mark drawn on the computer screen, using for it the mouse on the mark, which was previously drawn (line of 2 cm) on piece of paper. Once the image is generated (area of the leaves), it is processed to measure the silhouette with ImageJ software applications (Version 1.45) (Rasband, 2007) Thus, the image area equivalent to the leaf area of each leaf was obtained. Having done this exercise, we proceed to manipulate the commands sequentially:

Analyze> Set Scale> Known distance: 2, Unit of length: cm, Global scale.

To process images, commands are used:

Process> Binary> Make binary.

For the measurement of leaf area, this was delineated with the mouse, discarding petioles with the "Wand" tool, and Analyze> Measure commands are used.

Statistical Analysis

Following Rincón *et al.* (2012) value measured leaf area (LA), as measured by *ImageJ* software, is the dependent variable. This was used to estimate the leaf area by operations between real measurements of length (L) and width (W) of the leaves, to generate Allometric equations. Correlation and regression tests were performed.

Leaf morphology varies among species and may be from oblong-oval to trilobate. Thus, modifying the procedure Morgado *et al.* (2013) for species with more than one per lobe, were measured length of the midrib (L) and the largest width between the distal end of the outer lobes (W), as illustrated in Figure 1. These measurements were made up using ruler with accuracy of 0.1 cm.

With these measures, the leaf area is estimated by simple linear regression analysis. This estimate was done, following previous researches as Rojas and Seminario (2014); Rincón *et al.* (2012); Jerez *et al.* *(*2014); Blanco and Folegatti (2003); Morgado *et al.* (2013); Astegiano *et al*. (2001); Casierra-Posada *et al*. (2011); Cabezas-Gutiérrez, *et al.* (2009); Seminario *et al*. (2001); Bhatt and Chanda (2003). Our study was based on the following assumptions: The linear relationship between two variables is given by the equation:

$$y = a + bx$$

Where "y" is the dependent variable and "x" it is the independent variable. That is, in this case, the notation becomes:

$$LA = a + bx$$

Where LA is the leaf area, "x" is any linear measurements, your product, or some combination; a and b are the parameters, intercept and slope, respectively. Thus, relationship between measurements L and W of the leaves (independent variables) and LA (dependent variable) to find a Pearson correlation coefficient (r) significant, suggesting the highest relationship between variables were calculated:

$$r = \frac{s_{XY}}{S_X S_Y}$$

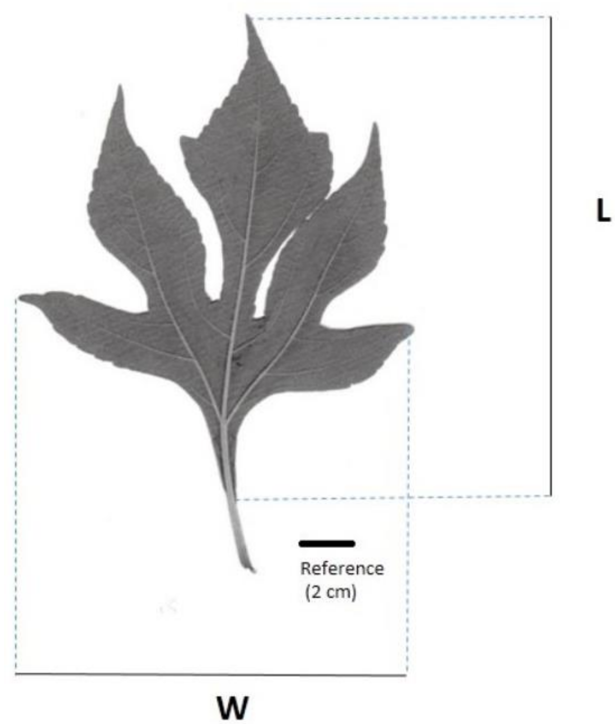

Figure 1. Scheme representing how linear measurements were made length and width of leaves at *Tithonia diversifolia*.

This analysis is valid assuming that the distribution of two continuous variables approaches the normal and were taken randomly from plants. Thus, the best correlation between two variables is one in which the "r" is close to +1 or -1. At the same time, by making a linear regression between the two variables, a mathematical model, whose coefficient of determination measures the proportion of total variability of the dependent variable relative to its mean that is explained by the model. That is, the allometric equation steeper will be the best predictor of LA of each leaf. This is confirmed by a coefficient of determination (R^2) high:

$$R^2 = \frac{S^2{}_{XY}}{S^2{}_X S^2{}_Y}$$

In summary, the analysis steps were:

- Calculate descriptive measures of current area and length and width dimensions, and their combinations in *Tithonia diversifolia* leaves.
- Calculate the correlation coefficient of Pearson between the current area and length and width dimensions, and their combinations in *Tithonia diversifolia* leaves.
- Explore different models of linear regression in order to identify the one that best estimates and predicts leaf area (LA) for plants of *Tithonia Diversifolia*.
- Compare models through goodness-of-fit measures: Mean Squared Error (MSE), Akaike Information Criterion AIC, and Bayesian Information Criterion (BIC).

RESULTS

Linear measurements and its derivatives have important variation. This is understandable in the case of a collection of genetic material from different sources. The variability is also reflected in the dispersion of the values expressed in the averages (x), top and bottom of the parametric data, as presented in Table 1.

Table 1. Descriptive statistics of actual area and length and width dimensions,and their combinations in *Tithonia diversifolia* leaves.

Variables	x (cm)	SD (cm)	Lower Limit (cm)	Upper Limit (cm)
	67.99	32.40	16.20	176.70
L*W	153.37	69.54	32.40	401.70
W	11.28	3.28	4.50	19.50
L	12.95	2.49	7.20	20.60
Ln (L/W)	0.17	0.17	-0.23	0.76
Ln (L-W)	0.41	0.76	-2.30	1.93
Ln (LA)	4.09	0.52	2.79	5.17

L: length, W: width, LA: leaf area

In Model 1, we worked with a level-level model. This equation can be interpreted as per centimeter increases in width, leaf area increased by about 4 units, if the length remains constant. Furthermore, per centimeter to increase the length, leaf area increased by approximately 7 units provided that the width remains constant (Table 2).

In Model 2, the independent variable is the difference (L-W). He worked with a log-log model. With this model, we can interpret that when the difference between the length and width increases by 1%, leaf area is reduced by 0.31% (Table 2).

In Model 3 is the independent variable ratio (L/W). He worked with a log-log model. With this model, we can interpret that when the ratio between length and width increases by 1%, leaf area is reduced by 1.99% (Table 2).

In model 4 the independent variable is product (L*W). We worked with a level-level model. With this equation we can interpret it as when the product of the length and width increases by 1%, leaf area is increased by 0.438% (Table 2).

The the determining coefficient suggest that models 1 and 4 were the ones that best predict leaf area, however MSE and BIC allows you to select one of the two between these two statistics.

Figure 2 shows the results of the test of the methodology performance for estimating LA, where we can see that estimated values of LA were very close to the measured values. The coefficient R^2 indicated that the estimations had a precision of 89%.

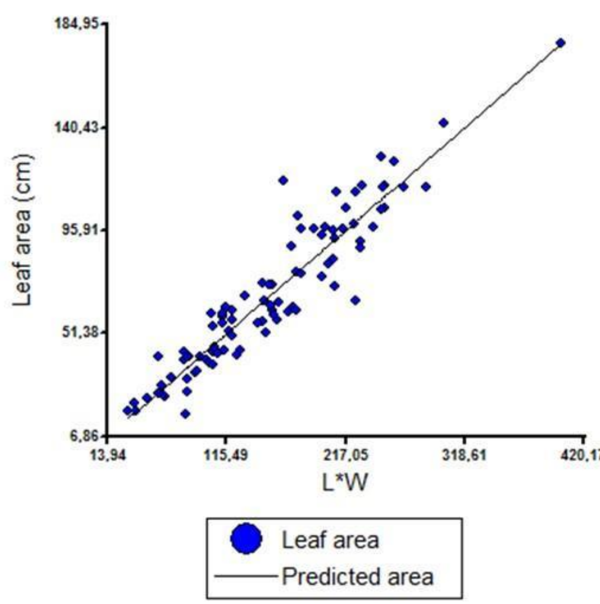

Figure 2. Estimated and measured leaf area (LA) for plants of *Tithonia diversifolia* (R^2 0.89).

In practical terms, the MSE is the sum of the variance and squared deviation estimator (Table 2). In the statistical modeling, the MSE is used to determine the extent to which the model does not fit the information, or if removing certain terms can simplify the model in charitable ways. The MSE provides a way to choose the best estimator: a minimum MSE often, but not always, indicates a minimum variation, and therefore indicates a good estimator. In turn, given two models, estimating the model with the lowest value of BIC is the preferred.

The statistics suggest that model 4 is the allometric equation that best predicts the relationship between variables. Thus, the correlation between the product (L*W) and AF indicated that there is a high association between these variables (R = 0.941). The slope of the model and the coefficient of determination (R^2) of 0.885 suggests that the dependent variable is highly related to the product L*W. This means that the leaf area is 88% explained by the product of the length by the width of the leaf blade. The remainder was due to other unknown factors. The Shapiro-Wilks test modified (p = 0.933) indicate that the sample conforms to the normal distributional assumption

DISCUSSION

From the above it follows that the best estimate of leaf area of *Tithonia diversifolia* obtained by the product of W * L (as independent variable in the Model 4). Several studies have been confirmed that a linear model having L×W as the independent variable provided the most accurate estimate (Rouphael, *et al.,* 2010; Karatassiou *et al.,* 2015).

Although the shape of the leaves of some species is not regular so it is difficult to measure (Campostrini and Yamanishi, 2001), the relationship between measures of L, W and LA follows the same trend. This is a case of isometrics in which the variables grow at the same rate, maintaining a constant proportional size (absolute size but different) (Shingleton, 2010).

Previous studies have shown the adjustment measures throughout and its products as a predictor of leaf area in these type of irregular leaf blades. As the case of *T. diversifolia*, other species such as *Passiflora* sp., and *Ficus carica* L. leaves rough edges have been generated with high setting allometric equations (R^2 0.99). Although the observed and calculated data for these types of leaves have a greater dispersion as shown in lobed limbo leaves, which can lead to errors in determining the width of the leaf, for regularly shaped leaves (Casierra-Posada *et al*., 2008). In the same sense, a strong correlation between the leaf area and the parameters of leaf width and length were effective in the tomato LA estimation (P < 0.01), and thus estimation could be done with these parameters using data transformed into a linear form; other studies on the development of leaf area prediction models also reported similar results (Küçükönder, *et al.,* 2016). High correlation between leaf area and leaf geometry expressed in terms of length and width, in various crop plants have been reported: Küçükönder, *et al*. (2016) in tomato; Karatassiou *et al*. (2015) in *Trifolium pratensis* L., *Cercissi liguastrum* L., *Anthemis arvensis* L.; Rojas and Seminario (2014) in *Valeriana pilosa*; Rincón *et al*. (2012); Jerez, *et al*. (2014) in *Solanum tuberosum. L*.; Blanco and Folegatti (2003) in *Cucumis sativus*; Astegiano *et al*. (2001) in *Lycopersicon esculentum* Mill.; Casierra-Posada, *et al*. (2011) in *Passiflora mollissima* and *Ficus carica L*.; Lu *et al*. (2004) in *Colocasia esculenta* and Warnock *et al.* (2006) in *Phaseolus vulgaris*.

Other studies have been demonstrated that the power models based on leaf L or W have been considered the most adequate for estimating LA, for example, in perennial crops, such as black pepper, grapevine, dwarf coconut tree (Pompellia *et al.*, 2012) and *Carya illinoinensis* (Torri *et al.,* 2009.

Table 2. Equations and statistical estimators derived from the analysis of correlation between the actual area and length and width dimensions, and their combinations in *Tithonia diversifolia* leaves

Model	Variable	Equation	R	R^2	MSE	AIC	BIC
1	W, L	y=-69.77+4.07*W+7.09*L	0.932	0.869	151.97	728.69	738.82
2	Ln (L-W)	ln y =4.19-0.31*ln (L- W)	0.453	0.205	0.22	121.02	128.59
3	Ln (L/W)	ln y =4.43-1.99*ln (L/ W)	0.657	0.431	0.16	92.60	100.16
4	W*L	y=0.755+0.438(W*L)	0.941	0.885	126.15	714.45	722.04

L: length, W: width

In addition, when using the W in power model, there is no need to measure L, facilitating the work on the field; this is particularly relevant in modeling of *T. diversifolia* LA, because W is solely measured using an imaginary perpendicular line to the leaf L, which, reduce the difficulties derived of measurements of irregular forms of the leafs, both which can cause inaccurate measurements (Pompellia *et al.,* 2012).In agree with Pompelli *et al*. (2012), these aspects must be judiciously considered when measuring W to obtain reliable LA estimations using the LW model. These issues were also discussed by Torri *et al*. (2009) in relation to leaf area estimation of *Carya illinoinensis* leafs. On the other hand, Williams and Martinson (2003) reported that single variable models could avoid issues of collinearity between leaf W and L. In the same sense to Pompelli *et al*. (2012), when we use LW as variable, we took into consideration that it is one variable the product of both dimensions.

Although the measurements require more time for determining the leaf area by taking data on length and width, these are the parameters commonly used, due to the high precision results and low error in predictions (Blanco and Folegatti, 2005). Although the measurements require more time for determining the leaf area by collecting data on length and width, these are the parameters commonly used, due to the high precision results and low error in predictions (Blanco and Folegatti, 2005)

CONCLUSIONS

In our study, very close relationships between the actual leaf area and the predicted leaf area by the model were found. A rapid and simple model was developed to predict the leaf area for the *T. diversifolia* (y=0.755+0.438(W*L). This model was chosen for its simplicity and capacity to produce results with the same level of accuracy as other more complex estimation models or expensive equipment. The equations relating the leaf area with the L, W and LxW for all crops demand less effort and time in the measurement at field because just two dimensions could be measured. Dimensions of the leaves can be easily measured in the field. Use of this equation would enable researchers to make non-destructive or repeated measurements of the same leaves. Thus, use this non-destructive method to quickly obtain the leaf area of a crop and its use in both field work fieldwork and laboratory. Linear equations obtained for pre-flowering allowed having a quick, reliable and easy estimation of leaf area. However, you may have required equations for other stages of the phenological cycle when the need for information requires greater precision. Moreover, it is suggested even within the same cultivar, try the best estimate of leaf area by separate leaves of different sizes. This then entails taking equations phenological stage and size of the leaves.

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