Non-destructive Method for Estimating the Leaf Area of Pear cv. ‘Triunfo’

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Abstract
The present study had as objective to determine mathematical equations to estimate the leaf area of pear cv. ‘Triunfo’ using linear dimensions of the leaves. For that, 300 healthy leaves of different sizes from each quadrant of plants from the small farm of Boa Vista located in the city of Montanha, at the northern side of the State of Espírito Santo, Brazil were used. The length (L) along the main vein was measured, along with the maximum width (W) of the leaf blade and observed leaf area (OLA), in addition to the product of the length and width (LW) of each leaf. From these measurements models of linear equations of first degree, quadratic and power were adjusted and their respective \( R^2 \), using OLA as dependent variable and L, W and LW as independent variable. Based on the proposed equations, the data were validated obtaining the estimated leaf area (ELA). The mean of the ELA and OLA were compared by Student \( t \) test 5% probability. The mean error (E), the mean absolute error (MAE) and the root mean squared error (RMSE) was also used as validation criterion. The best equation model was defined based on the non-significant values from the comparison of means of ELA and OLA, E, MAE and RMSE values closer to zero and highest \( R^2 \). The leaf area of pear cv. ‘Triunfo’ can be estimated by the equation ELA = -0.432338 + 0.712862(LW) non-destructively and with a high degree of precision.

Keywords: Pyrus communis L., linear dimensions, mathematical equations

1. Introduction
The pear tree (Pyrus communis L.) belongs to the family of the Rosaceae, being the cv. ‘Triunfo’ a hybrid with characteristics of fast growth, high productivity, vigorous plants, large fruits of green color, oblong shape and punctuation in the shell, firm flesh and with sweet acidulated taste (Nakasu et al., 2007).

The determination of leaf area is a fundamental character in studies involving plant development, light interception, photosynthesis efficiency, evapotranspiration, fertilizer and irrigation related responses, being directly related to the yield and quality of the plant (Blanco & Folegatti, 2005).

The measurement of the leaf area can be done directly or indirectly. Although the direct methods are more efficient, it needs a lot of time, require complex and expensive equipment and are in most cases destructive, preventing successive measurements which often makes their use impracticable (Jonckheere et al., 2004; Pompelli et al., 2012). Indirect methods are based on easy methods to obtain measurements allowing constant measurements of the leaf area, for example during the entire plant development period (Tsialtas & Maslaris, 2005).

One of the most commonly used indirect techniques is based on linear measures of the leaves and the correlation with the leaf area of the plants, generating mathematical models that allow the estimation of the leaf area in a fast and non-destructive way. However this method requires adequate calibration and parameterization to be used. (Peksen, 2007; Costa, Pôças, & Cunha, 2016).
Several authors have proposed models of equations to estimate the leaf area of several plant species as *Helianthus annuus* L. (Rouphael, Colla, Fanasca, & Karam, 2007), *Coffea arabica* (Antunes, Pompelli, Carretero, & Damatta, 2008), *Coffea canephora* (Antunes et al., 2008; Schmildt, Amaral, Santos, & Schmildt, 2015; Espindula et al., 2018), *Jatropha curcas* (Pompelli et al., 2012), *Passiflora* spp. (Morgado, Bruckner, Rosado, Assunção, & Santos, 2013), *Vitis vinifera* L. (Buttaro, Rouphael, Rivera, Colla, & Gonnella, 2015), *Rosa hybrida* L. Costa et al., 2016) and *Prunus armeniaca* L. (Cirillo et al., 2017).

However, studies involving *Pyrus communis* L. are scarce, thus it is fundamental to develop methods to determine the leaf area, using a method simple, non-destructive and easy to measure. Thus, the objective of this study was to establish a mathematical model to estimate the leaf area of pear cv. ‘Triunfo’ using linear dimensions of leaf length and width.

2. Methods

For the accomplishment of the present study, leaves of pear cv. ‘Triunfo’ collected at the Boa Vista small farm located in the city of Montanha, North of Espírito Santo State, Brazil, with geographical coordinates of 18°07′48″ South latitude and 40°13′08″ East longitude.

The orchard is composed of 300 plants planted in September of 2014, the fertilization was made using the organic fertilizer Minho Fértil®, phytosanitary treatments and control of invasive plants were done according to need and the whole crop management was made following the recommendations of Nakasu et al. (2007). The plants were spaced 1.5 m between row and 4.0 m between plants, being used Microjet irrigation system with flow rate of 20 L/h, with 1 hour of watering time per day. The weather of the region according to Köppen classification is Tropical Aw (Köppen, 1936).

On April 17, 2018, 300 healthy leaves of different sizes of each plant quadrant were collected as recommended by Oliveira, Silva, Costa, Schmildt, and Vitória (2017). The leaves were packed in plastic bags and transported to the Laboratory of Plant Breeding of the Postgraduate Program in Tropical Agriculture of the Centro Universitário Norte do Espírito Santo (CEUNES/UFES), located in the city of São Mateus.

In the laboratory the leaves were scanned in a HP® Deskjet F4280 model scanner and the images were saved in TIF format with 75 dpi, then the length (L) along the main vein in cm, the maximum length of leaf blade (W) in cm (Figure 1) and observed leaf area (OLA, cm²) were evaluated using the software ImageJ® (Schindelin, Rueden, Hiner, & Eliceiri, 2015). Multiplying the length along the main vein with the largest width of leaf blade was possible to obtain the product of width and length (LW).

![Figure 1. Measures of the length (L) along the main vein and the maximum width (W) of the pear cv. ‘Triunfo’ leaf blade](image)

The data were submitted to descriptive statistics analysis, obtaining the minimum, maximum, mean and the coefficient of variation (CV) values. The equations were estimated from a 250 leaves sample using the first order linear model represented by $\text{ELA} = \beta_0 + \beta_1 x$, quadratic represented by $\text{ELA} = \beta_0 + \beta_1 x + \beta_2 x^2$ and power represented by $\text{ELA} = \beta_0 x^{\beta_1}$, where OLA was the dependent variable in function of L, W and LW as independent variable. Thus, nine equations were obtained to estimate the leaf area of pear cv. ‘Triunfo’ and their respective determination coefficients ($R^2$).

The models validation was made from a sample of 50 leaves collected for this purpose. The parameters L, W, LW and OLA were measured by previously mentioned methodology. The values of the L, W and LW variables
were substituted in the proposed equations for the mathematical modeling, obtaining the estimated leaf area (ELA), in cm². For each model, a first order linear regression and its respective determination coefficient (R²) were adjusted, using ELA values as dependent variable and OLA values as independent variable. The obtained means from ELA and OLA were compared using the Student’s t test at a 5% probability level. For each proposed model the mean error (E) (Equation 1), the mean absolute error (MAE) (Equation 2) and the root mean squared error (RMSE) (Equation 3) were evaluated, using the following equation:

\[
E = \frac{1}{n} \sum_{i=1}^{n} (ELA - OLA)
\]  

\[
MAE = \frac{1}{n} \sum_{i=1}^{n} |ELA - OLA|
\]  

\[
RMSE = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (ELA - OLA)^2}
\]

where, ELA are the values of the estimated leaf area; OLA are the values of observed leaf area and n is the amount of leaves contained in the validation sample being 50 in the present study.

The choice of the best model to estimate the leaf area of pear cv. ‘Triunfo’ was based on the highest values obtained from the coefficient of determination (R²) and the non-significant values from comparing the means of ELA, OLA and E, MAE and RMSE with greater proximity to zero. All the statistical analyzes were performed using the R software (R Core Team, 2018) and scripts from the ExpDes.pt data package version 1.2 (Ferreira, Cavalcanti, & Nogueira, 2018).

3. Results and Discussion

In Table 1, it is possible to observe the minimum, maximum and average values L, W, LW and OLA characteristics from the 250 leaves used to propose the mathematical models of leaf area estimation and of the 50 leaves that were used to validate the data. All the values used in the validation are presented among the values used to propose the mathematical models, these values are adequate since according to Levine, Berenson, Krehbiel, and Stephan (2012) the measurements should not exceed those used to fit the models.

| Variable | Unit | Minimum | Max  | Average | CV (%) |
|----------|------|---------|------|---------|--------|
| 250 leaves were used for modeling | | | | | |
| L | cm | 2.78 | 12.15 | 8.16 | 23.63 |
| W | cm | 1.62 | 7.95 | 4.92 | 24.36 |
| LW | cm² | 4.62 | 96.61 | 42.17 | 39.19 |
| OLA | cm² | 3.18 | 69.63 | 29.63 | 39.97 |
| 50 leaves for validation | | | | | |
| L | cm | 3.91 | 10.99 | 7.52 | 23.00 |
| W | cm | 2.43 | 7.00 | 4.50 | 23.71 |
| LW | cm² | 9.48 | 69.52 | 35.41 | 42.97 |
| OLA | cm² | 6.12 | 48.73 | 25.01 | 43.53 |

When the coefficient of variation (CV) of the leaves used for modeling was analyzed, it was possible to see that the values were presented in a range between 23.63 and 39.97%, furthermore the CV values for the leaves used in the validation had a variation from 23.00 to 43.53% range (Table 1). According to Pimentel-Gomes (2009), all these values are considered high or very high, attesting high variability of the sampled data. However, it is desired a high CV value in works that seek a mathematical model to estimate the leaf area, since equations that take into account different leaves forms and sizes present better adjustments, besides representing all the stages of vegetative development of the plants (Toebe et al., 2011; Espindula et al., 2018; Pezzini et al., 2018).

The adjusted equations and their respective coefficient of determination (R²) are shown in Table 2. All proposed models had a high correlation between the dependent variable (OLA) and the independent variables (L, W and LW) with R² greater than 0.8671. However it should be noted that for all LW based models as an independent variable R² had a value of 0.989 being superior to the others. Despite that the choice of the best model should not only take into account the highest value of R², since they may be inadequate, provoking a underestimation of the
leaf area (Antunes et al., 2008). In this way, the validation of the data becomes indispensable to choose the best model that estimate the leaf area of pear cv. ‘Triunfo’.

Table 2. Regression models for the estimation leaf area of pear cv. ‘Triunfo’ with the respective coefficients of determination, as a function of length (L), width (W) and the product of length versus width (LW)

| Model   | Equation                                                                 | \( R^2 \) |
|---------|---------------------------------------------------------------------------|-----------|
| Linear  | \( ELA = -17.0379 + 5.7175(L) \)                                         | 0.8671    |
| Linear  | \( ELA = -17.1664 + 9.5104(W) \)                                         | 0.9263    |
| Linear  | \( ELA = -0.432338 + 0.712862(LW) \)                                     | 0.9894    |
| Quadratic | \( ELA = -6.69715 + 2.57007(L) + 0.21825(L)^2 \)                      | 0.8751    |
| Quadratic | \( ELA = -6.6396 + 4.2873(W) + 0.5917(W)^2 \)                          | 0.9353    |
| Quadratic | \( ELA = 0.0330344 + 0.6834573(L) - 0.0003778(LW)^2 \)                 | 0.9895    |
| Power   | \( ELA = 0.6958(L)^{1.7697} \)                                          | 0.8701    |
| Power   | \( ELA = 1.740(W)^{1.7557} \)                                           | 0.9319    |
| Power   | \( ELA = 0.6392(LW)^{1.0246} \)                                         | 0.9896    |

The behavior of the validation equations obtained through the leaves samples and their respective determination coefficient (\( R^2 \)) are represented in Figure 2. It is noted that the highest values of \( R^2 \) were obtained using LW as independent variable being the same equations that presented higher values of \( R^2 \) in the modeling.

Models that take into account individualized linear dimensions are easier to measure, making work in practice simpler to execute. However, for Espindula et al. (2018) equations that considerer a single linear measure of leaves are less efficient, being used only on certain occasions, in this way, a model to estimate leaf area using combined linear measures such as the interrelation between length and width, presented a higher degree of precision and are more desirable conforming with the results found in the present study.
It was found that the OLA and ELA values did not differ significantly by the Student t test ($p < 0.05$) for all the equations (Table 3), however the first-order linear equation that uses LW as an independent variable showed higher values attesting greater similarity between OLA and ELA. This equation was also shown more precise when we consider the validation criterios of E, MAE and RMSE, where values must be closer to zero (Table 3). Lower values for these criterios were also observed by Schmildt et al. (2015) in a modeling study of the linear
equation of first degree based on LW for estimating leaf area of a variety of *Coffea canephora* concluding that this is the best model for such a species.

All equations based on only one linear measure (L and W) presented lower values of $R^2$ and higher values of E, MAE and RMSE. These results demonstrate that these equations were less precise to estimate of leaf area. Similar results were found by Rouphael et al. (2007) and studying leaf area models of *Helianthus annuus* L., observed lower values of RMSE and greater value of $R^2$ of the equation that relates L and W compared to the others, indicating that equations that use these features together have a better fit.

Table 3. Observed leaf area (OLA) and estimated leaf area (ELA) of linear equations of first degree, quadratic and power for the independent variable length (L), width (W) and product of length with width (LW), besides the value of $p$, the mean error (E), the mean absolute error (MAE) and the root mean squared error (RMSE) of pear cv. ‘Triunfo’ leaves used for the validation

| Model     | Variable | OLA (cm²) | ELA (cm²) | $p^*$ value | E     | MAE    | RMSE    |
|-----------|----------|-----------|-----------|-------------|-------|--------|---------|
| Linear    | L        | 25.95     | 0.651     | 0.963       | 2.746 | 3.471  |
| Linear    | W        | 25.63     | 0.770     | 0.628       | 2.632 | 3.298  |
| Linear    | LW       | 24.81     | 0.924     | -0.211      | 0.718 | 0.976  |
| Quadratic | L        | 25.61     | 0.777     | 0.610       | 2.632 | 3.301  |
| Quadratic | W        | 25.29     | 0.8945    | 0.286       | 2.461 | 3.076  |
| Quadratic | LW       | 23.67     | 0.521     | -1.370      | 1.452 | 1.882  |
| Power     | L        | 25.60     | 0.781     | 0.598       | 2.679 | 3.323  |
| Power     | W        | 25.30     | 0.891     | 0.292       | 2.477 | 3.073  |
| Power     | LW       | 24.76     | 0.908     | -0.255      | 0.736 | 0.988  |

*Note. P values greater than 0.05 indicate that the observed leaf area (OLA) and estimated leaf area (ELA) did not differ by Student t test.*

Thus, considering the validation criteria defined above, as well as the higher coefficient of determination ($R^2$) of the equation, the linear first-degree model using the product of length along the main vein with the largest width of the leaf limb (LW) as independent variable presents a higher degree of precision. Foliar area estimation models based on more than one measure are more notorious, according to those proposed for several species as *Jatropha curcas* (Pompelli et al., 2012), *Passiflora* spp. (Morgado et al., 2013), *Vitis vinifera* L. (Buttaro et al., 2015), *Coffea canephora* (Schmildt et al., 2015) and *Prunus armeniaca* L. (Cirillo et al., 2017), concluding that the best adjustments were found relating L and W in comparison with models that take into account only one dimension.

Therefore, it is recommended to use equation ELA = -0.432338 + 0.712862(LW) to estimate the leaf area of pear tree cv. ‘Triunfo’. It should be emphasized as described by Schmildt et al. (2015), for the determination of models that estimate the leaf area is necessary the destruction of the leaves, however after establishing the equation the leaf area can be estimated by obtaining the length along the main vein and the largest width of the leaf blade, with simple equipment such as ruler and or tape measure, non-destructively.

4. Conclusions

The leaf area of pear cv. ‘Triunfo’ can be estimated by the length (L) ratio along the main vein and width (W) maximum of the leaf limb, through the following first degree equation ELA = -0.432338 + 0.712862(LW), non-destructively and with a high degree of precision.

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