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Accuracy of linear methods to estimate the leaf area of genotypes of conilon coffee

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This study aimed to establish models of equations to estimate leaf area in genotypes of *Coffea canephora* Pierre ex Froehner var. Conilon using linear measurements of mature leaves as input, and compare its accuracy with other methods that have been used for *Coffee* sp. Linear measurement of leaves of13 genotypes of *Coffea canephora* Pierre ex Froehner, cultivated in field following a randomized blocks design, were evaluated to identify best option for input in linear models, to generate equation models and to validate the method to estimate the leaf area. Differences in the leaf area of the genotypes were detected indicating the possibility to classify the genotypes into two distinct groups for this characteristic.The use of the product of the two linear dimensions, maximum length and maximum width increased the accuracy of the used linear models to estimate the leaf area of conilon coffee. Between the generated equation models with the data, the linear model $\widehat{LA}$=0.6533*LW is adequate to estimate the leaf area of the genotypes CV-04, CV-08, CV-10 and CV-11; and the linear model $\widehat{LA}$=0.6587*LW for the genotypes CV-01, CV-02, CV-03, CV-05, CV-06, CV-07, CV-09, CV-12 and CV-13, respectively. Those equations were validated by reevaluation and compared with other methods, to estimate the leaf area accurately.

Key words: *Coffeacanephora*, morphology, canopy, leaves.

INTRODUCTION

Coffee is one of the most valuable commodities traded in the world and Brazil is the largest producer, exporting both arabica coffee (*Coffea arabica* L.) and conilon coffee (*C. canephora* Pierre ex Froehner). The cultivation of coffee has great economic and social importance in Brazil, mainly in the state of Espírito Santo, which is the largest producer of conilon coffee, accounting for approximately 75% of the national production of this species of coffee (Covre et al., 2013; Martins et al., 2013, 2015).

The estimation of leaf area is important in evaluating

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the plant growth, being a parameter widely used in agronomic and physiological studies, because of its high correlation with the light interception and photosynthetic capacity of the plants (Severino et al., 2004). Destructive methods, such as the use of scanners and area integrators, have been widely used to determine the leaf area directly (Flumignan et al., 2008). However, it is not always possible to use a destructive method, which requires removing leaves from the plants, especially in studies that evaluate the plant growth in longer periods of time (Adami et al., 2008).

The use of non-destructivemethods to estimate the leaf area based onthe linear dimensions of the leaves have been successfully used in *Coffea* spp. (Schmildt et al., 2014; Tavares-Júnior et al., 2002; Barros et al., 1973; Partelli et al., 2006). These methodologies have the great advantage of being able to be used in leaves still attached to the plant, allowing continuous evaluation of the plant growth (Fideles Filho et al., 2010).

Considering the great genetic variability that exists in *C. canephora* Pierre ex Froehner (Fonseca et al., 2007; Martins et al., 2013, 2015; Rodrigues et al., 2013) the revalidation of these non-destructive methods to estimate the leaf area becomes necessary from time to time. With the availability of new improved genotypes by the breeding programs, through the development of new cultivars, the pool of genotypes being used changes and it is possible that some genotypes express different morphological characteristics; consequently, altering the level of accuracy of the methods to estimate the leaf area validated for previous genotypes.

This study aimed to establish models of equations to estimate the leaf area in improved genotypes of *C. canephora* Pierre ex Froehner var. Conilon using linear measurements of mature leaves as input, and compare its accuracy with other methods that have been used for *Coffee* sp.

MATERIALS AND METHODS

Experimental design

The experiment was developed sampling leaves from production field of conilon coffee, located in the experimental area of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), in the municipality of Alegre, southern Espírito Santo State, in Southeast region of Brazil. The region presents altitude of 130 m, with geographical coordinates of 41°29'25.6180"W and 20°45'10.7030"S. The climate is characterized as hot and humid in summer, and dry in the winter, with an average annual rainfall of 1,200 mm and annual mean temperature of 23°C. The soil is classified as a red-yellow latosol.

The experimental fieldwas formed with the13 genotypes that compose the cultivar "VitóriaIncaper 8142" of conilon coffee, the plants were propagated asexually and cultivated in lines, forming a plantation with population of 14,000ortrotopic steams per hectare. In the moment of evaluation, the plants were 6 years old, presenting four completed reproductive cycles. The pruning followed the traditional recommendation, according to the recommendation by Fonseca et al. (2007). Irrigation was done only in periods of adverse conditions, in supplemental character. The correction and fertilization of the soil were based on analyzes of soil, as recommended by Prezotti et al. (2007) and Lani et al. (2007), respectively.

The evaluation of the differences in the linear measurements between genotypes were performed sampling leaves of the adult plants from the experimental field. The 13 genotypes of the conilon coffee (CV-1, CV-2, CV-3, CV-4, CV-5, CV-6, CV-7, CV-8, CV-9, CV-10, CV-11, CV-12 and CV-13) were installed in the field following a randomized blocks design, using 5 replications and 8 plants by experimental plot, surround by at least 2 rows of plants as borders.

Sampling and measurements

The sampling was performed between March and April 2013, collecting leaves randomly from the lower, middle and upper sections of the canopy; only mature and healthy leaves were selected. Immediately after collection, the leaves were placed in plastic bags, containing drops of water, and taken to laboratory to determine the real leaf area (LA), using a digital integrator of leaf area (area meter, Li-Cor, model LI 3100, precision: 0.01 cm^2).

To identify the linear measurements of the leaf that has better correlationwith the real leaf area (LA), the maximum length (L), the maximum width (W) and the product of those (LW) were measured in each leaf to be used as potential input variables for the models (Johnson and Wichern, 1998). The maximum length corresponds to the distance between the distal base of the petiole and the terminal end of the sheet, while the maximum width corresponds to the greatest distance perpendicular to the length axis, both measured with rulers and expressed in centimeters (cm).

Grouping genotypes

The data were subjected to variance analysis to study the existence of significant differences between leaf morphology of the genotypes and to identify the possibility of grouping them according to the homogeneity of leaf area, through the Scott-Knott criteria (<0.05).

Linear models to estimate the leaf area

To identify equation models that could be useful to estimate the leaf area from the linear measurements, a new leaf sampling was performed, collecting 30 leaves per experimental plot, in order to increase the accuracy of the estimate parameters for the equations. The models were chosen based on a biological logic, taking into account the influences suffered by the plant, both intrinsic and extrinsic, in its simplicity and ease of use. Using the set of models that were superior in the preliminary studies, models from linear, quadratic, exponential and neperian characteristic were adjusted.

Validation of models

To validate the models, a new sampling was performed from the plants in the experimental field, now using 50 leaves per experimental plot. The linear measurements were evaluated and data used to estimate the leaf area using the models which best fitted for each genotype. The estimated leaf area obtained by this method were compared with the estimates from the methods proposed by Barros et al. (1973), widely used for *C.arabica* L.; by Partelli et al. (2006), recommended for its simplicity and for only requiring one linear measurement; and the real value of leaf area, evaluated with the same area integrator previously described. The results from the linear models, from Barros et al. (1973) and from

Table 1. Means of leaf area of adult plants of 13 genotypes of conilon coffee.

Genotypes	Unitary leaf area (cm^2)
CV-10	73.3824 [a]
CV-08	68.9575 [a]
CV-04	66.9674 [a]
CV-11	66.2605 [a]
CV-07	64.3400 [b]
CV-05	63.7439 [b]
CV-02	63.4819 [b]
CV-03	61.5213 [b]
CV-12	60.7843 [b]
CV-06	60.5755 [b]
CV-09	60.5331 [b]
CV-13	59.0439 [b]
CV-01	54.0392 [b]

Means followed by the same letter do not differ by test of Scott-Knott (p≤0.05).

Partelli et al. (2006) were compared with the real leaf area by the criteria of Bonferroni (p <0.05).

To determine the leaf area by the method of Barros et al. (1973), a circumscribed rectangle to the leaf bladeswere obtained. The adjustment was done by Equation (1), where Y represents the estimated leaf area, and the area of X is restricted to the leaf surface obtained by the product of the larger length and larger width of the sheet, provided that they do not have length less than 2.5 cm.

$$Y = 0.6670 * \mathrm{X} \quad (1)$$

For determination of leaf area by the model proposed by Partelli et al. (2006), the Equation (2), where LA represents leaf area; AGE is the age of the adult plants, in months; LMR is the length of the midrib. All the analyses were done using the statistical software GENES (Cruz, 2013).

$$\mathrm{LA} = (0.3064 * \mathrm{AGE}^{-0{,}0556}) * (\mathrm{LMR}^{2{,}0133}) \quad (2)$$

RESULTS AND DISCUSSION

Difference between genotypes

The variance analyses showed a significant effect of the genotypes over the means of leaf area and two distinct groups of genotypes were formed through the criteria of Scott-Knott (Table 1). The genotypes CV-10, CV-08, CV-04 and CV-11 formed the first group, with values ranging from 66.26 to 73.38 cm^2, having unitary leaf areas higher than the rest of the genotypes of this cultivar. The second group, formed by the remaining clones (CV-07, CV-05, CV-02, CV-03, CV-12, CV-06, CV-09, CV-13 and CV-01), presented means ranging from 54.03 to 64.34 cm^2.

This discrimination between groups of genotypes which are more homogeneous inside the groups is necessary because the genotypes of this cultivar present phenotypic dissimilarity, showing differences regarding the growth, morphology, leafiness and canopy structure (Contarato et al., 2010; Martins et al., 2013; Covre et al., 2013). Therefore, grouping them regarding the differences in leaf area may increase the efficiency of the models proposed to study their leaf area.

Selection of measurements for input

The coefficients of correlation between the real leaf area and the linear measurements of the leaves were high and positive for the single linear measurements of the limbo (Land W) in most genotypes. However, some low correlations were observed in specific cases, like for the length in the genotype CV-10 and for the width in the genotype CV-12 (Table 2), making it impractical to define equations to estimate the leaf area based on only one of the linear measurements(L or W).

Considering the product of the maximum length and maximum width (LW), strong correlations were observed, being above 0.93 for all 13 genotypes, showing the potential of combining those two linear measurements to estimate the leaf area. These results corroborate the findings of Tavares-Júnior et al. (2002), working on genotypes of *C. arabica* L., showed the satisfactory correlation between the products of linear measurements of dimensions of the limbo with the leaf area. The results obtained by Barros et al. (1973) and Rey and Alvarez (1991), on genotypes of *C. arabica* L., showed viability of estimating the leaf area of coffee plantsbased on the product of the measures of length and width, presenting equations that are widely used for this species.

Selection of linear model and establishment of equations

Based on the best correlation values presented by the

Table 2. Coefficients of correlation between the real leaf area and maximum length (L), maximum width (W) and the product of the linear dimensions (LW) of leaves from the genotypes of conilon coffee.

Genotypes	L	W	LW
CV-01	0.8373	0.8431	0.9659
CV-02	0.8081	0.8421	0.9574
CV-03	0.8164	0.8480	0.9624
CV-04	0.8607	0.8832	0.9802
CV-05	0.8582	0.8639	0.9669
CV-06	0.8331	0.8633	0.9684
CV-07	0.8397	0.8784	0.9707
CV-08	0.8819	0.9136	0.9804
CV-09	0.8414	0.8990	0.9843
CV-10	0.6587	0.7992	0.9652
CV-11	0.8594	0.8815	0.9805
CV-12	0.8630	0.5505	0.9840
CV-13	0.8096	0.8404	0.9356

Pearson correlation analysis.

Table 3. Equation models to estimate the leaf area (LA) as a function of the product between the maximum length and maximum width (LW) of leaves of genotypes of conilon coffee from different groups.

Model	Equation	R^2
	- Group 1 -	
	(CV-04, CV-08, CV-10, CV-11)	
Linear	$\widehat{LA} = 0.1258^{ns} + 0.6533^{*}LW$	97.66^{*}
Quadratic	$\widehat{LA} = -2.458^{*} + 0.70478^{*}LW - 0.0003^{*}(LW)^2$	97.69^{*}
Exponential	$\widehat{LA} = 22.642 \times (1.0101^{LW})$	92.37^{*}
Ln-Ln	$\widehat{LA} = -0.46581 + 1.008824 \ Ln(LW)$	98.13^{*}
	- Group 2 -	
	(CV-01, CV-02, CV-03, CV-05, CV-06, CV-07, CV-09, CV-12, CV-13)	
Linear	$\widehat{LA} = 0.1236^{ns} + 0.6587^{*}LW$	96.71^{*}
Quadratic	$\widehat{LA} = -2.8775^{*} + 0.7246^{*}LW - 0.0004^{*} (LW)^2$	96.73^{*}
Exponential	$\widehat{LA} = 21.3178 \times (1.0111^{LW})$	93.12^{*}
Ln-Ln	$\widehat{LA} = -0.46281 + 1.010323 \ Ln (LW)$	93.12^{*}

*Significant and ^{ns}Non significant by the t-test ($p \leq 0.05$).

product of LW, this variable was used as input variable to estimate the leaf area. The same product of linear dimensions were also successfully used to estimate leaf area, with good accuracy and excellent precision, in different agronomic species, such as cotton (Monteiro et al., 2005), soybean (Adami et al., 2008), mango (Lima et al., 2012) and beans (Toebe et al., 2012). Using the product of LW, the models were used to generate equations for each of the two homogeneous groups of genotypes. All the models were significant at 5% of probability by the t-test, also showing determination coefficients above 0.95, which proves the high accuracy of these models in the determination of the leaf area of those genotypes (Table 3).

Although all the models presented significant precision, the linear model showed better expression for estimating the leaf area in both groups of genotypes associated with a biological logic and greater simplicity to determinate the leaf area, being favored between the models studies in this experiment. Considering the linear model, the regression coefficient (β_0) for both groups of genotypes were not significant, therefore, the equationscan be simplified as $\widehat{LA}$=0.6533LWfor the genotypes CV-04, CV-08, CV-10 and CV-11; and $\widehat{LA}$=0.6587LW for the genotypes CV-01, CV-02, CV-03, CV-05, CV-06, CV-07, CV-09, CV-12 and CV-13, respectively.

Table 4. Comparison between the linear models found in this study and other methods to estimate leaf area in coffee plants used to study the leaf area of genotypes of Conilon coffee.

Method	Comparison
- -Group 1 -	
(CV-04, CV-08, CV-10, CV-11)	
Linear model ($\widehat{LA}$=0.6533LW)	67,3850
Real leaf area	67,6850
Y=0.6670*X [(1)]	68,9700
LA= (0.3064*AGE$^{-0,0556}$)*(LMR^{2,0133}) [(2)]	75,7100*
- -Group 2 -	
(CV-01, CV-02, CV-03, CV-05, CV-06, CV-07, CV-09, CV-12, CV-13)	
Linear model ($\widehat{LA}$ =0.6587LW)	58,9813
Real leaf area	58,9074
Y=0.6670*X [(1)]	59,9614
LA=(0,3064*AGE$^{-0,0556}$)*(LMR^{2,0133}) [(2)]	65,4365*

*Statistically different from the method proposed (Bonferroni test, p <0.05).[1]Barros et al. (1973); [2]Partelli et al. (2006).

Validation and comparison between methods

To validate the proposed model and to test it together with other methods that have been used for estimating leaf area in *C.canephora* Pierre ex Froehner, the methods were confronted with the real leaf area by the Bonferroni method, as shown in Table 4. The linear equations proposed for each group were statistically similar to the means of real leaf area obtained in those genotypes, showing the high accuracy of the set of equations to estimate the leaf area for those genotypes. The proposed equations were similar to the non-destructive method proposed by Barros et al. (1973). The set of linear equations for the groups of genotypes are compatible with others equations that have the same purpose, indicating that the method proposed by Barros et al. (1973) may be used to estimate the leaf area of some genotypes of *C. canephora* Pierre ex Froehner, even if it was originally proposed for *C. arabica* L.

Despite being of different species and present certain different characteristics, *C. Canephora* Pierreex Froehner and *C. Arabica* L. Belong to the same genus, andalso possesssome similarities, which explains the good fit of the model proposed by Barros *et al.*(1973) for coniloncoffee, since the method of estimation based on the circumscribed rectangle to the leaf blades also proved efficient for this species (Brinate et al., 2015; Antunes et al., 2008).

There was no resemblance between the means obtained from the set of equations of this study and method described by Partelli et al. (2006) for Conilon coffee. This difference may be related to the higher requirement of standardization of leaves used in this method, andto the use of only one linear measurement of the leaves, which although reducing the costs of evaluation, may be more subjected to variation.

Conclusions

The use of the product of the two linear dimensions, maximum length and maximum width, increases the accuracy of the linear models used to estimate the leaf area of conilon coffee. The linear model $\widehat{LA}$=0.6533*LW

is adequate to estimate the leaf area of the genotypes CV-04, CV-08, CV-10 and CV-11; and the linear model $\widehat{LA}$=0.6587*LW for the genotypes CV-01, CV-02, CV-03,

CV-05, CV-06, CV-07, CV-09, CV-12 and CV-13, respectively.

Conflict of Interest

The authors have not declared any conflict of interest.

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