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NON-DESTRUCTIVE ALLOMETRIC METHOD FOR ESTIMATION OF LEAF AREA IN COMMON BEECH (*FAGUS SYLVATICA* L.)

Svetoslav Anev[1*], Petya Dimitrova-Mateva[2], Sabah Lamlom[3], Ganka Chaneva[4], and Nikolina Tzvetkova[1]

[1]Department of Dendrology, Faculty of Forestry, University of Forestry, 10 Kliment Ohridski blvd., 1797 Sofia, Bulgaria
[*]E-mail: svetoslav.anev@gmail.com
[2]South-West State Forest Company, Ministry of Agriculture, 1 Antim I Str., 1000 Sofia, Bulgaria
[3]Faculty of Natural Resources and Environmental Sciences, University of Omar Al-Mukhtar, Al Baida, Libya
[4]Faculty of Biology, Sofia University 'St. Kliment Ohridski', 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

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Abstract

A model for leaf area (L_A) determination in Common beech (*Fagus sylvatica* L.) was developed by using leaves from two different elevation sites. The leaf mass (L_M), lamina width (L_W), lamina length (L_L), and L_A were all measured after excision of leaves from the plants in an open source software ImageJ. Best subsets regression analysis for relations between L_A and different combinations of L_W and L_L was performed. The proposed best-fit L_A-estimation model is: $L_A = 2.40 \cdot L_L + 6.65 \cdot L_W - 23.77$. The established highly significant relation ($R^2 = 0.98$, P-value < 0.001, d.f. = 177) between L_A and leaf width and length allows this mathematical model to be used for simple non-destructive field calculation of L_A in Common beech, which can thus be tremendously time saving without compromising on accuracy.

Key words: allometry, best-fit model, field measurements.

Introduction

Leaf area index is a significant variable in monitoring and modeling forest condition and growth and consequently it is essential for foresters and environmental scientists to determine leaf area regularly and precisely (Coops et al. 2004). The leaf area (L_A) estimation in field experiments is a long and complicated task. There are several direct (Shaban and Vassev 1983, Daughtry 1990, Bartelink 1997, Ferment et al. 2001) and indirect (Welles 1990, Chen 1996, Chen et al. 1997) methods for determination of L_A in the field. The majority of the direct methods, involving physical removal of leaves or other structures from the plants, are called destructive or laboratory methods (Jonckheere et al. 2004). After collection of leaves, L_A can be calculated using planimetric techniques (Daughtry 1990). There are different planimeters on the market for this purpose. The scanning ones (e.g. LI-3000C Area Meter from Li-Cor, Lincoln, USA) utilize an electronic method of rectangular approximation. The portable scanning planimeters (e.g. CI-203 Handheld Laser Leaf Area Meter from CID Bio-Science, Inc., Camas, USA and LI-3100C Portable Area Meter from Li-Cor, Lincoln, USA) are appropriate merely for small plants with few leaves but require the removal of leaves from the plant. All of these are expensive and also use particular software for data preparation.

ImageJ is an open source JAVA application distributed by the National Institutes of Health (NIH, Bethesda, MD, USA), which can find out leaf area by scanned or photographed leaf. The very 'friendly' graphical user interface assists users to manage and edit plant images. The online material as handbooks (Broeke et al. 2015), wikis (Rasband 2016), and plugins leads users through a variety of functions, providing hints about potential new applications. Fiji is one of the most current image processing distribution of ImageJ, bundling many plugins, which help scientific image analysis (Schindelin et al. 2012).

Regardless of the different non-destructive techniques used to estimate leaf area (Lu et al. 2004), the most widespread approach is to develop ratios and regression estimators by using simply measured leaf parameters such as length (L_L) and width (L_W). Such relations can provide researchers with many advantages in physiological experiments (Rouphael et al. 2007). Furthermore, the equations allow researchers to perform L_A measurements on the same plants throughout the plant growth period and may give rise to decreased experimental variability (Serdar and Demirsoy 2006). Various combinations of measurements and various equations relating L_L and L_W to leaf area have been used largely in some horticultural plants (Shaban and Vassev 1983, Stewart and Dwyer 1999, Schwarz and Kläring 2001, Chanda and Singh 2002, Blanco and Folegatti 2003, Tsialtas and Maslaris 2005, Rouphael et al. 2006, Rouphael et al. 2007) and considerably less often in trees (Demirsoy et al. 2004, Serdar and Demirsoy 2006).

The aim of this study was to develop a reliable equation that will allow the non-destructive estimation of Common beech (*Fagus sylvatica* L.) leaves' area through linear measurements.

Materials and Methods

Site description and plant material

Common beech leaves were collected from two sample plots with different elevation in the West Stara Planina mountain region (Table 1).

A total of 178 leaves (89 per sample plot) from two levels of tree crowns were randomly selected and analyzed. They

Table 1. Sites descriptions and plant material.

Site characteristics	Petrohan	Shirine
Altitude, m a.s.l.	1425	600
Latitude	43° 07' 27.2"	43° 10' 34.5"
Longitude	23° 07' 27.6"	23° 09' 28.1"
Aspect	E	E
Slope, °	17	22
Mean trees age, years	20	18
Diameter of breast height (DBH), cm	2.8	4.4
Mean tree height, m	6.2	6.4

were collected during May-August in 2015 and 2016. After leaf detachment, L_M was measured with a portable electronic scale (precision: 0.01 g), and leaves were scanned with portable scanner (Canon Lide 110, Canon Inc., Japan) at 600 dpi resolution in PNG colour mode format.

Leaf allometry and area measurements

Leaf Area Index (LAI) was defined such as the total one-sided area of leaf tissue per unit ground surface area (Watson 1947). According to this definition, LAI is a dimensionless quantity characterizing the canopy of an ecosystem. Leaf allometry and area measurements were performed with open source software ImageJ v.1.51 64-bit (NIH, Bethesda, MD, USA). The scanning scale was set as proportion of dpi/(px/cm), which is 1dpi = 0.393701 px/cm or 600 dpi = 236.2206 px/cm. A rectangular selection tool was used for L_L and L_W measurement and a polygon selection tool was used for L_A determination (Fig. 1).

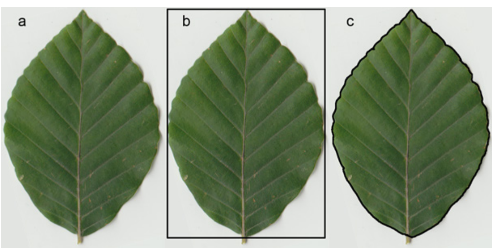

Fig. 1. Leaf allometry processing: a) original image; b) rectangular selection tool for measuring of L_L and L_W; c) polygon selection tool for measuring of L_A.

'Area' and 'bounding rectangle' options were marked in set measurements dialog window. All results were exported in csv format for data preparation and analysis.

Data analysis

Normality test (Shapiro-Wilk) was used for evaluation of the normal distribution of data within each group. Two-tailed Student's t-test was used to detect differences between means of allometric scaling of leaves from Petrohan and Shirine sites. The relationship between L_A as a dependent variable and (1) L_L, (2) L_W, (3) $L_R = L_L \cdot L_W$ or (4) L_M, as independent variables, was determined using linear regression analysis, and two-factorial relationship between L_A as a dependent variable and combination of (5) L_L, L_W and (6) L_L, L_W, L_M as independent variable was determined using multiple linear regression. According to Anev et al. (2013) the six regressions were assessed by values of: coefficient of determination (R^2), adjusted coefficient of determination (AdjR^2), mean absolute error (MAE), root-mean-square error (RMSE) (Zar 2010, Bolboaca and Jäntschi 2013), coefficient of aggregation (W), Akaike information criterion (AIC) and Bayesian information criterion (BIC) (Burnham and Anderson 2004) between measured L_A and modeled with each models L_A. For validation of the models, we used the calculated and measured L_A dataset, to which we fitted a linear equation. The slope and intercept were tested with Z-test (Clogg et al. 1995) to see if they were significantly different from the slope and intercept of the 1:1 correspondence line. All statistical calculations were performed with MS Excel (Microsoft Corp., Redmond, WA) and specially created visual basic user defined functions.

Results

Normality test (Shapiro-Wilk) of all series indicates that the data matches the

Table 2. Leaf dimensions.

Leaf dimension	Petrohan	Shirine
Width (L_W), cm	5.02 (0.10) a	4.94 (0.06) a
Length (L_L), cm	7.15 (0.15) a	7.44 (0.10) a
Rectangle (L_R), cm^2	36.98 (1.44) a	37.10 (0.86) a
Area (L_A), cm^2	27.64 (1.01) a	26.05 (0.59) a
Mass (L_M), g	0.259 (0.011) a	0.309 (0.012) b

Note: Values shown are means (Standard errors of means are shown parenthetically). The same letters on each row indicate that there is no statistically significant difference (Two-tilted t-test, *P*-value < 0.05) between means in both sample plots.

pattern expected if the data was drawn from a population with a normal distribution. Except of L_M, there is no statistically significant difference between allometric scales of leaves from both sample plots (Table 2).

The higher L_M of leaves from Shirine is probably due to greater water content in these leaves in comparison to leaves from the higher altitude sample plot (Petrohan).

The six models for calculation of L_A by allometric scales of leaves are described in Table 3.

The best-fit is model 5, which includes L_L and L_W as factors and was prepared by multifactorial regression analysis (Fig. 2).

Only model 4, which calculates L_A by L_M as a factor, is insignificant and should not be used for L_A modeled. In model 6, the regression coefficient of L_M is statistically insignificantly different from zero, which is evidence that leaf mass is inconsequential as factor in this model and the calculation of L_A is unnecessarily complicated. Therefore, it will not be included in the model assessments and comparison.

All the indicators, used for assessment of models, confirm the conclusion that model 5 is the best-fit among the assessed ones. However, the slope of it is close, but different (*P*-value = 0.044) from the slope of 1:1 regression (Table 4).

Model 3 also has good fit indicators, but both the intercept and the slope in it are statistically significantly different from the same of 1:1 regression (*P*-value is 0.038 and 0.032 for intercept and slope respectively).

Table 3. Descriptions and statistics of allometric models for determining leaf area (LA).

Model number	Model equation	AdjR^2	ANOVA-F	ANOVA-P
1	$L_A(1) = 5.58 \cdot L_L - 13.88$	0.765	576	0.033
2	$L_A(2) = 9.42 \cdot L_W - 20.04$	0.904	1671	0.020
3	$L_A(3) = 0.69 \cdot L_R + 1.40$	0.962	4535	0.012
4	$L_A(4) = 49.60 \cdot L_M + 12.77$	*0.471*	*159*	*0.063*
5	$\mathbf{L_A(5) = 2.40 \cdot L_L + 6.65 \cdot L_W - 23.77}$	**0.968**	**5350**	**0.011**
6	$L_A(6) = 2.42 \cdot L_L + 6.68 \cdot L_W - \underline{0.53} \cdot L_M - 23.77$	0.968	5354	0.011

Note: The best-fit model is bolded. The insignificant models are presented in italic. The insignificant different from zero (Two-tilted t-test, *P*-value < 0.05) regression coefficients are underlined.

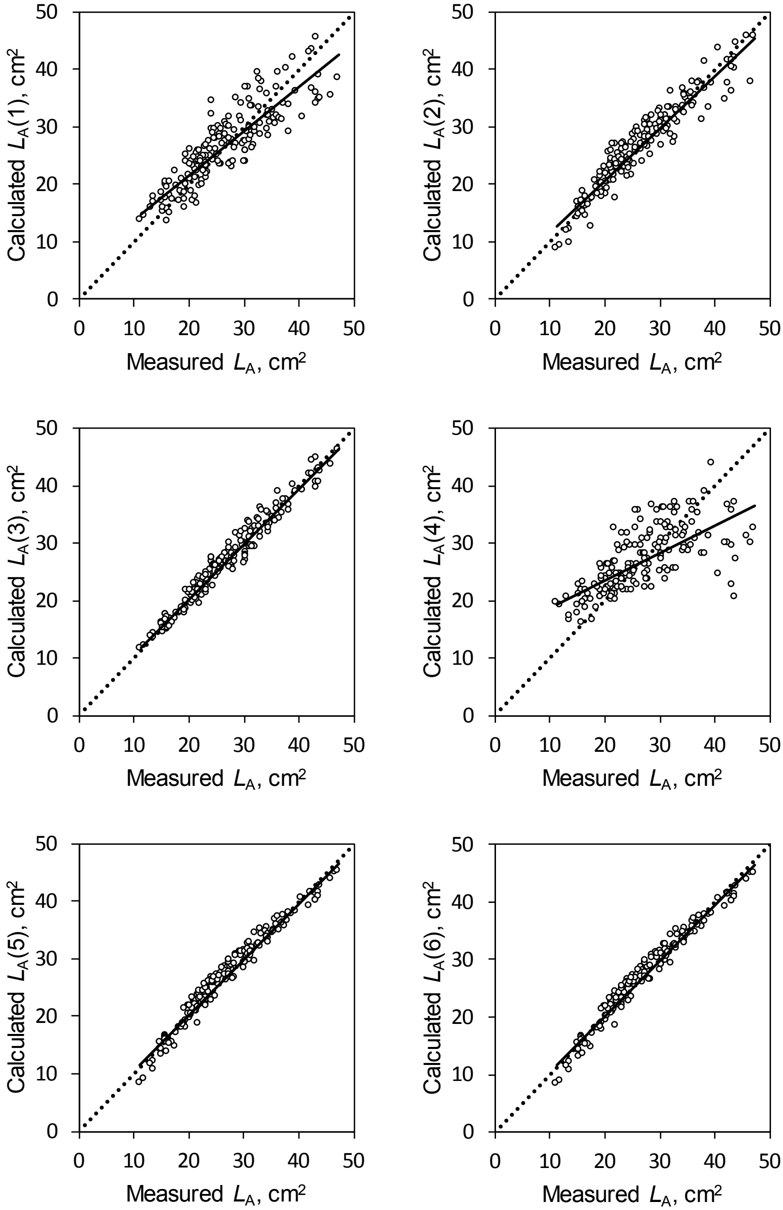

Fig. 2. Regression between measured in ImageJ leaf area (L_A) and calculated by corresponding model leaf area – L_A (N).

Note: *N* is the number (from 1 to 6) of each model, described in Table 3.

Table 4. Descriptions and statistics of allometric models for determining leaf area (LA).

Statistical indices	Model number				
	1	2	3	4	5
Coefficient of determination (R^2)	0.766	0.905	0.963	0.474	**0.968**
Adjusted coefficient of determination (AdjR^2)	0.765	0.904	0.962	0.471	**0.968**
Mean absolute error (MAE)	3.096	1.835	1.238	4.113	**1.176**
Root mean square error (RMSE)	3.781	2.412	1.511	5.668	**1.395**
Akaike information criterion (AIC)	428.0	297.7	142.1	486.7	**114.7**
Bayesian information criterion (BIC)	431.2	300.9	145.2	489.9	**117.9**
Coefficient of aggregation (W)	0.930	0.974	0.990	0.795	**0.992**
Intercept (b_0)	***	**	*	***	**n.s.**
Slope (b_1)	***	**	*	***	*****

Note: In the best-fit model, R^2, AdjR^2 and W must be maximal and close to 1, MAE, RMSE, AIC and BIC must be minimal. For the intercept (b_0) and the slope (b_1) are shown significance of difference (Z-test, P-value <: * 0.05; **0.01; ***0.001) from the same of 1:1 regression line.

Discussion

Leaf area is a valuable variable for the majority of physiological studies, concerning plant growth, light interception, photosynthetic efficiency, evapotranspiration and response to fertilizers and irrigation (Blanco and Folegatti 2003). It is known that (Bréda 2003) allometric relationships are site and species dependent or in some cases – year dependent. By Bidlake and Black (1989), Maguire and Bennett (1996) estimating allometric relationships through destructive sampling is a reliable method to assessing LAI for a given experimental sites but remains year-dependent. The findings of this evaluation of the six non-destructive methods for leaf area determination of Common beech, indicate that closest to the measured areas are those calculated by two-factor model 5 including the length and width of the leaves. As recommended by other authors (Blanco and Folegatti 2003, Peksen 2007, Rouphael et al. 2007, Ravi et al. 2010), model 3, including the product of length and width of the leaves, also has good fit indicators, but both intercept and slope are statistically significantly different from the same of 1:1 regression. This calls into question of its accuracy for leaf area determination in species with highly variable leaf size (L_L and/or L_W). The models which include only one dimension of the leaf (whether width, height or especially mass) are with insufficient precision and will not be recommended for scientific experiments. The unnecessarily complicated calculation of L_A with including of more than two dimensions does not improve the models. The validation of model 5, which is of the type $L_A = a \cdot L_L + b \cdot L_W + c$, showed that

Common beech leaf area could be measured quickly, accurately, and non-destructively by using the simple allometric leaf dimensions.

Conclusions

In this study, a fast and simple model was developed to predict the leaf area of Common beech. The use of this equation would enable researchers to make non-destructive or repeated measurements, which can thus be tremendously time saving without compromising on accuracy.

The approach for constructing and validation of the model could be used for estimation of leaf area in other plant species. However, it has been considered that prediction equation developed for the present region may need to be adjusted (validated) for diverse environmental conditions.

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