

LEAF AREA DETERMINATION IN *Populus alba* L. BY NON-DESTRUCTIVE METHOD BASED ON LEAF PARAMETERS

Simona ROȘU, Florin SALA

Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania"
from Timisoara, Timisoara, 300645, Romania

¹*Soil Science and Plant Nutrition*

*E-mail address: florin_sala@usab-tm.ro

Abstract. The study aimed to determine the leaf area of poplar by non-destructive methods, based on foliar parameters. The biological material was represented by the *Populus alba* L. species, from the Protected Area Cenad Forest. Leaves were taken, randomly from the crown of trees, from mature branches. The values of the parameters leaf length (L) and leaf width (w) were obtained by measuring with a ruler, with an accuracy of ± 0.5 mm. The leaves were scanned in a 1: 1 ratio. From the imaging analysis of the images, the values of the scanned leaf area (SLA) and the leaf perimeter (Per) were obtained with a high precision. To determine the values of the measured leaf area (MLA), as a non-destructive method, a relation of type $MLA = L \cdot w \cdot CF$ was used, where CF is the correction factor and its value influences the accuracy in finding the MLA values. The ANOVA single factor test confirmed the data safety and the presence of variance in the experimental data set. The value $CF = 0.66$, at which the minimum value of the mean error (MEM) between SLA and MLA was obtained, was considered as the optimal value of the correction factor (CF). According to the optimal CF value, the measured leaf area (MLA) values were determined. A high degree of matching was found between MLA and SLA values, and the relationship was described by a linear equation, statistically safe, according to $R^2=0.963$, $p<0.001$. The correlation analysis highlighted the existence of very strong correlations between MLA and SLA ($r = 0.981$); between MLA and foliar parameters L ($r = 0.919$), w ($r = 0.954$), Per ($r = 0.961$); between SLA and Per ($r = 0.952$); between SLA and w ($r = 0.951$); between Per and w ($r = 0.935$). Strong correlations were recorded between SLA and L ($r = 0.888$) and between Per and L ($r = 0.882$), and moderate correlation was recorded between L and w ($r = 0.771$). The regression analysis facilitated the evaluation of the variation between SLA, respectively MLA and studied leaf parameters. From the analysis of the values of the coefficients of the regression functions, it was appreciated that the width of the leaves (w) had a stronger influence in the description of the leaf area, compared to L and Per.

Keywords: foliar parameters, leaf area, model, *Populus alba*

INTRODUCTION

The leaves of plants are organs of major importance in relation to the reception of solar energy, and the photosynthetic and metabolic processes of plants (NIINEMETS, 2010; BURGESS et al., 2017). The leaves are characterized by a series of anatomical and morphological elements, in relation to the plant species, genotype, variety etc. (HE et al., 2018; LIU et al., 2019). Also, at the foliar level of the plants there are a series of dimensional and functional variations in relation to the positioning of the leaves in the architecture of the plants, as well as depending on the vegetation conditions, plant nutrition, stress factors, etc. (WATANABE et al., 2005; ENGLAND and ATTIWILL, 2006; DATCU et al., 2017).

Studies at the level of leaves were carried out in relation to the characterization of some plant genotypes (SALA et al., 2017; CÂNDEA-CRĂCIUN et al., 2018), in relation to plant nutrition (RAWASHDEH and SALA, 2013, 2014), with the tolerance to stress factors (CRAMER et al., 2011; KOSOVÁ et al., 2018), with the photosynthetic regime of plants (TERASHIMA et al., 2011), with the characterization of some natural or urban areas (DATCU et al., 2017).

Non-destructive methods in foliar study are very useful, due to the speed of determination and high work accuracy, which has advantages in large numbers of samples (ALI

et al., 2017; DRIENOVSKY et al., 2017a,b; CARVALHO et al., 2018; FARAGÓ et al., 2018; SAVVIDES and FOTOPOULOS, 2018).

The present study evaluated the leaf area of the species *Populus alba* L., by non-destructive method, based on foliar parameters.

MATERIAL AND METHODS

The study aimed to find the value of the correction factor (CF) necessary to determine the leaf area in *Populus alba* L. specie, by non-destructive method, based on foliar parameters.

The biological material was represented by the species *Populus alba* L., from the Protected Area Cenad Forest, Timiș County, Romania. The leaves were taken randomly from the mature branches of the trees, fig. 1.



Figure 1. Leaf of *Populus alba* L.

Measurements of leaf dimensional parameters, length (L) and width (w) were made. The measurement was made with a ruler, with an accuracy of ± 0.5 mm.

The leaves were scanned in a 1:1 ratio. The images were analyzed and the values of the scanned leaf area (SLA) and the perimeter (Per) were obtained (RASBAND, 1997).

The measured leaf area (MLA) was obtained by calculation, according to relation (1), based on the leafs parameters L, w and the correction factor (CF). It is very important to determine the optimal value of the correction factor, as it influences the accuracy of determining the leaf area. To find out the correction factor, was used the model proposed by SALA et al. (2015).

$$MLA = L \cdot w \cdot CF \quad (1)$$

where: MLA – Measured Leaf Area; L – leaf length; w – leaf width; CF – correction factor

The ANOVA test was used for the analysis of the experimental data set, in terms of statistical data safety and the presence of variance. Correlation analysis, regression analysis, and mean of the errors between SLA and MLA were performed (Hammer et al., 2001). The coefficients r, R^2 , and parameters p, F-test and RMSEP (equation (2)), were used for statistical safety of the results.

$$RMSEP = \sqrt{\frac{1}{n} \sum_{j=1}^n (y_j - \hat{y}_j)^2} \tag{2}$$

RESULTS AND DISCUSSIONS

The measurement and imaging analysis of the leaf samples resulted in a data set on biometric parameters length (L) and width (w), scanned leaf area (SLA) and leaf's perimeter (Per). To find the measured leaf area (MLA) based on foliar parameters, the relation (1) was used.

The correction factor determinately influences the value of the leaf area, fact for which it needs to have a correct value in relation to the set of foliar samples (depending to species, genotype, variety, variety).

To find out the correction factor in the case of the studied specie (*Populus alba* L.), the model proposed by Sala et al. (2015) was used, and the values obtained are presented in table 1. It can be observed how the value of the measured leaf area (MLA) varies depending on the values of the correction factor.

Considering as a reference the SLA values, against which the calculated MLA values were reported, the smallest error was recorded under the conditions of using the correction factor (CF) with the value 0.66, at which the average of the minimum error (MEM) was MEM = -0.053 , with the graphical distribution in figure 2.

Table 1

Experimental data in relation to optimal value for the correction factor for *Populus alba* L.

SLA	CF	MLA	ME	RMSEP
22.721	0.61	20.950	-1.770	2.302606
	0.62	21.294	-1.427	2.027366
	0.63	21.637	-1.083	1.782615
	0.64	21.981	-0.740	1.582563
	0.65	22.324	-0.397	1.445884
	0.66	22.668	-0.053	1.391381
	0.67	23.011	0.290	1.428492
	0.68	23.355	0.634	1.550651
	0.69	23.698	0.977	1.740039
	0.70	24.042	1.321	1.977432
	0.71	24.385	1.664	2.247671

The ANOVA test (Alpha = 0.001) highlighted the safety of the experimental data and the presence of variance in the data set, under the conditions $F > F_{crit}$, $p < 0.001$, table 2.

Table 2

ANOVA test, single factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7469.89	3	2489.963	147.2865	2.13E-39	5.794287
Within Groups	1961.048	116	16.90558			
Total	9430.938	119				

Alpha=0.001

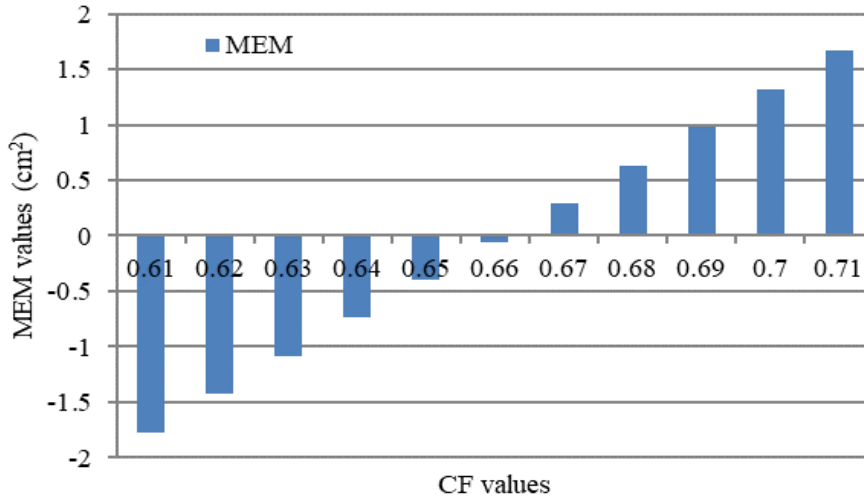


Figure 2. Graphical distribution of MEM values according to CF values, *Populus alba* L.

The correlation analysis showed the existence of very strong correlations between MLA and SLA ($r = 0.981$), between MLA and foliar parameters L ($r = 0.919$), w ($r = 0.954$), and Per ($r = 0.961$), between SLA and Per ($r = 0.952$), between SLA and w ($r = 0.951$), as well as between Per and w ($r = 0.935$), Table 3. Strong correlations were recorded between SLA and L ($r = 0.888$), and between Per and L ($r = 0.882$), and a moderate correlation was recorded between L and w ($r = 0.771$).

Table 3

	L	w	SLA	Per	MLA
L					
w	0.771				
SLA	0.888	0.951			
Per	0.882	0.935	0.952		
MLA	0.919	0.954	0.981	0.961	

The fit analysis between SLA, as reference values, and MLA (values determined on the basis of optimal CF), in the case of leaf samples studied in *Populus alba* L., led to the relationship (3), in statistical safety conditions, $R^2 = 0.963$, $p < 0.001$, and the graphical distribution is shown in fig. 3.

$$MLA = 0.9702x + 0.6251 \tag{3}$$

The regression analysis facilitated the evaluation of the variation between the SLA and the studied leaf parameters, L, w and Per. Equation (4) was obtained, which described the variation of the SLA in relation to the studied parameters, in general conditions of statistical safety, according to $R^2 = 0.981$, $p < 0.001$.

By the analysis of the values of the equation (4) coefficients was found a

differentiated contribution of foliar parameters to the definition of SLA values. The highest contribution was the width of the leaves (w), followed by the length (L) and the perimeter (Per).

$$SLA = 0 - 0.1514L + 4.54865w - 0.08982Per \quad (4)$$

The regression analysis regarding the relationship between MLA and studied leaf parameters, L, w and Per, led to the general relationship (5), in statistical safety conditions, according to $R^2 = 0.982$, $p < 0.001$.

From the analysis of the values of the coefficients of equation (5) it was found the differentiated contribution of the foliar parameters to the definition of the MLA values. The highest contribution was the width of the leaves (w), followed by the length (L) and perimeter (Per), similar as in the case of SLA.

$$MLA = 0 + 0.43863L + 4.46049w - 0.2389Per \quad (5)$$

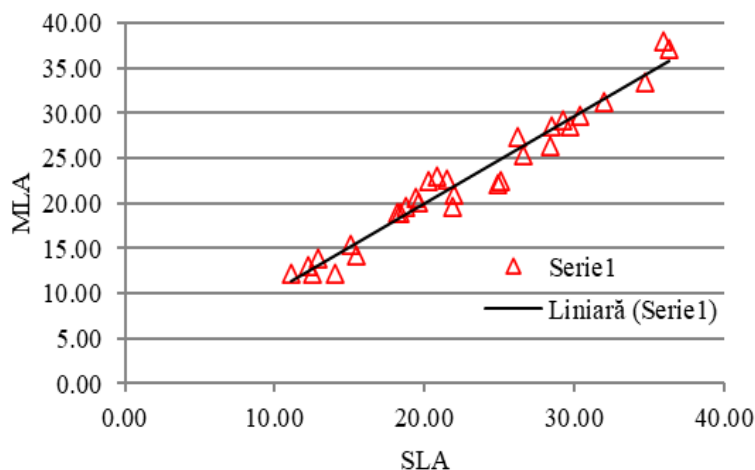


Figure 3. Graphical distribution of MLA values in relation to SLA in the species *Populus alba* L.

Differentiated values of foliar parameters (L, w) when estimating MLA, or in leaf geometry analysis, based on models and equations, were also communicated in other studies (SALA et al., 2015, 2017; CÂNDEA-CRĂCIUN et al., 2018).

Models for estimating the leaf area, based on foliar parameters, were obtained in different plant species, in statistical safety conditions (BLANCO and FOLEGATTI, 2003; GIUFFRIDA et al., 2011; FASCELLA et al., 2013). The fact that more and more models for estimating the leaf area based on dimensional parameters of the leaves are promoted, shows the importance of the non-destructive method of determining the leaf area in various studies and for practice.

The results communicated in the present study are in concordance with the scientific literature in the field, which was consulted and was the basis for documenting the present research.

CONCLUSIONS

The determination of the leaf area in the species *Populus alba* L. was possible based on the leaf parameters and the correction factor found ($CF = 0.66$), in statistically safety conditions. Among the foliar parameters, leaf width (w) had a higher contribution in the precise definition of SLA and MLA, based on equations resulting from regression analysis. The matching relationship between SLA and MLA values was described by a linear equation, in conditions of high statistical safety, which highlights the accuracy of MLA determination.

REFERENCES

- ALI, M.M., AL-ANI, A., EAMUS, D., TAN, D.K.Y., 2017 - Leaf nitrogen determination using nondestructive techniques - A review. *Journal of Plant Nutrition*, 40(7): 928-953.
- BLANCO, F.F., FOLEGATTI, M.V., 2003 - A new method for estimating the leaf area index of cucumber and tomato plants. *Horticultura Brasileira*, 21(4): 666- 669.
- BURGESS, A.J., RETKUTE, R., HERMAN, T., MURCHIE, E.H., 2017 - Exploring relationships between canopy architecture, light distribution, and photosynthesis in contrasting rice genotypes using 3D canopy reconstruction. *Frontiers in Plant Science*, 8: 734.
- CARVALHO, L.B., ALVES, E.A., BIANCO, S., 2018 - Non-destructive model to predict *Commelina diffusa* leaf area. *Planta Daninha*, 35: e017167226.
- CÂNDEA-CRĂCIUN, V.-C., RUJESCU, C., CAMEN, D., MANEA, D., NICOLIN, A.-L., & SALA, F., 2018 - Non-destructive method for determining the leaf area of the energetic poplar. *AgroLife Scientific Journal*, 7(2): 22-30.
- CRAMER, G.R., URANO, K., DELROT, S., PEZZOTTI, M., SHINOZAKI, K., 2011 - Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biology*, 11: 163.
- DATCU, A.-D., SALA, F., IANOVICI, N., 2017 - Studies regarding some morphometric and biomass allocation parameters in the urban habitat on *Plantago major*. *Research Journal of Agricultural Science*, 49(4): 96-102.
- DRIENOVSKY, R., NICOLIN, A.L., RUJESCU, C., SALA, F., 2017a - Scan LeafArea – A software application used in the determination of the foliar surface of plants. *Research Journal of Agricultural Sciences*, 49(4): 215-224.
- DRIENOVSKY, R., NICOLIN, A.L., RUJESCU, C., SALA, F., 2017b - Scan Sick & Healthy Leaf – A software application for the determination of the degree of the leaves attack. *Research Journal of Agricultural Sciences*, 49(4): 225-233.
- ENGLAND, J.R., ATTIWILL, P.M., 2006 - Changes in leaf morphology and anatomy with tree age and height in the broadleaved evergreen species, *Eucalyptus regnans* F. Muell. *Trees*, 20: 79.
- FARAGÓ, D., SASS, L., VALKAI, I., ANDRÁSI, N., SZABADOS, L., 2018 - PlantSize offers an affordable, non-destructive method to measure plant size and color *in Vitro*. *Frontiers in Plant Science*, 9: 219.
- FASCELLA, G., DARWICH, S., ROUPHAEL, Y., 2013 - Validation of a leaf area prediction model proposed for rose. *Chilean Journal of Agricultural Research*, 73(1): 73-76.
- GIUFFRIDA, F., ROUPHAEL, Y., TOSCANO, S., SCUDERI, D., ROMANO, D., RIVERA, C.M., COLLA, G., LEONARDI, C., 2011 - A simple model for nondestructive leaf area estimation in bedding plants. *Photosynthetica*, 49(3): 380-388
- HAMMER, Ø., HARPER, D.A.T., RYAN, P.D., 2001 - PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1): 1-9.
- HE, N., LIU, C., TIAN, M., LI, M., YANG, H., YU, G., GUO, D., SMITH, M.D., YU, Q., HOU, J., 2018 - Variation in leaf anatomical traits from tropical to cold-temperate forests and linkage to ecosystem functions. *Functional Ecology*, 32: 10-19.
- KOSOVÁ, K., VITÁMVÁS, P., URBAN, M.O., PRAŠIL, I.T., RENAUT, J., 2018 - Plant abiotic stress proteomics: the major factors determining alterations in cellular proteome. *Frontiers in Plant Science*, 9: 122.
- LIU, C., LI, Y., XU, L., CHEN, Z., HE, N., 2019 - Variation in leaf morphological, stomatal, and anatomical

Research Journal of Agricultural Science, 52 (3), 2020

- traits and their relationships in temperate and subtropical forests. *Scientific Reports*, 9: 5803.
- NIINEMETS, Ü., 2010 - A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecological Research*, 25: 693-714.
- RAWASHDEH, H.M., SALA, F., 2013 - The effect of foliar application of iron and boron on early growth parameters of wheat (*Triticum aestivum* L.). *Research Journal of Agricultural Science*, 3(1): 21-26.
- RAWASHDEH, H.M., SALA, F., 2014 - Foliar application of boron on some yield components and grain yield of wheat. *Academic Research Journal of Agricultural Science and Research*, 2(7): 97-101.
- SALA, F., ARSENE, G.-G., IORDĂNESCU, O., BOLDEA, M., 2015 - Leaf area constant model in optimizing foliar area measurement in plants: A case study in apple tree. *Scientia Horticulturae*, 193: 218-224.
- SALA, F., IORDĂNESCU, O., DOBREI, A., 2017 - Fractal analysis as a tool for pomology studies: Case study in apple. *AgroLife Scientific Journal*, 6(1): 224-233.
- SAVVIDES, A.M., FOTOPOULOS, V., 2018 - Two inexpensive and non-destructive techniques to correct for smaller-than-gasket leaf area in gas exchange measurements. *Frontiers in Plant Science*, 9: 548.
- TERASHIMA, I., HANBA, Y.T., THOLEN, D., NIINEMETS, Ü., 2011 - Leaf functional anatomy in relation to photosynthesis. *Plant Physiology*, 155: 108-116.
- WATANABE, T., HANAN, J.S., ROOM, P.M., HASEGAWA, T., NAKAGAWA, H., TAKAHASHI, W., 2005 - Rice morphogenesis and plant architecture: measurement, specification and the reconstruction of structural development by 3D architectural modelling. *Annals of Botany*, 95(7): 1131-1143.