## NON-DESTRUCTIVE METHOD FOR DETERMINING THE LEAF AREA OF THE ENERGETIC POPLAR

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#### Abstract

Four genotypes of poplar energetic were evaluated, being determined for a random sample of leaves, leaf area (LA), perimeter (P), length (L) and width (W). High values of the correlation coefficients between the series of data determined by these parameters have been identified, which indicated a geometrically well-defined pattern of the leaves. This gave the possibility to determine a calculation formula which, based on the product of leaf length and width, corrected with a specific constant (k) of each genotype, helped to obtain the foliar surface under the conditions of a minimum acceptable error, and safe statistical. The approximation relation of the foliar surface (LA) is  $LA = l \cdot W \cdot k$ . It was chosen opted for such a relationship, to the detriment of other models, because it is defined by a simple expression that can be used efficiently when calculations for a high volume of samples are required. The foliar surface constants k was obtained for each genotype, which made it easier to obtain optimal foliar surfaces based on the dimensional parameters of the leaves. The k values were different for each genotype, and are within the range (0.62, 0.74).

Key words: energetic poplar, leaf area, model, prediction error, surface constant.

#### INTRODUCTION

Leaves are expressive organs of the plants, in relation to biotypes, pedoclimatic conditions, age, being an important element in the description and classification of species, subspecies, varieties (McDowell et al., 2002; Herrera, 2013). At the leaf level, certain plant reactions to environmental conditions are evident, the leaves being analyzed in relation to the degree of tolerance or sensitivity of plants to biotic or abiotic stress factors, diseases or pests (Fernàdez-Martínez et al., 2013; Drienovsky et al., 2017 a).

The leaves are also active photosynthetic organs, with a major role in the capture of solar energy and the conversion into biochemical energy through the photosynthesis process, for application of the foliar fertilizers and treatments, which are influenced by the pedoclimatic conditions, crops technologies and the state of the plant nutrition (Jivan and Sala, 2014; Rawashdeh and Sala, 2015 a, b, 2016; Peguero-Pina et al., 2016). Starting from the foliar surface, for the characterization of

plants, a series of indices are calculated which give information about the relationship of plants, cultures or vegetal cover with the reception of solar energy or productivity, such as: specific leaf area (SLA), leaf area index LAI), leaf area duration (LAD), specific leaf weight (SLW), net assimilation rate (NAR) (Weiss et al., 2004; Shipley, 2006).

Knowing the foliar surface is important in relation to a number of environmental, physiological and technological factors. For the determination of the foliar surface are known destructive methods, which involve the detachment of the leaves (gravimetric methods, planimetric methods etc.) and non-destructive methods, without detachment of the leaves.

Non-destructive methods are usually based on the measurement of dimensional leaf parameters, on calculation methods based on observations on correlations between the foliar surface and the dimensional parameters and are sometimes combined with imaging methods (Jonckheere et al., 2004; Kirk et al., 2009; Behera et al., 2010). Although sometimes approximations, corrections, or other procedures that may be subjective, these methods are often easy, direct, low cost and often sufficiently high precision for a particular purpose. Non-destructive methods have been increasingly interested in various research and studies (Blanco and Folegatti, 2003; Pandey and Singh, 2011; Drienovsky et al., 2017 b; Sala et al., 2017).

Mathematical models for the estimation of foliar surface with leaf dimensional parameters, length (L) or width (W), or their product (L x W), were obtained using regression analysis (Blanco and Folegatti, 2003; Mokhtarpour et al., 2010; Rouphael et al., 2010). Determination of the foliar surface based on the dimensional parameters of the leaves, length and width, by means of some models, was carried out in various studies, with increasing accuracy (Litschmann et al., 2013; Sala et al., 2015; Souto et al., 2017).

Non-destructive methods of foliar surface determination are of interest in extensive studies that use a large amount of experimental samples, where time or human variables resource required raises problems, or when other, more accurate methods generate economic problems involving high costs. Also, such methods are of interest in very young

biological material studies, such as saplings, at where leaf detachment affects their subsequent growth. Moreover, these methods find their place in experiments where the foliar surface determinations are performed repeatedly at different time intervals, and the repeated and important quantity harvesting of the leaves could cause disturbances in the development of the plants.

The present study aimed to determine a leaf area models for four energy poplar clones, starting from the two parameters, the leaf length (L) and width (W) and a surface constant for each biotype (k).

## MATERIALS AND METHODS

## **Obtaining leaf samples**

The biological material was represented by four energy poplar genotypes, denominated Clone 2, ..., Clone 5, typical leaves within each genotype being shown in Figure 1. Random harvested material was represented by a total of 200 leaves, equally distributed from the four poplar genotypes studied (n = 50 leaves).

The leaf form is deltoid to clone C2 and C4, deltoid-cordiform to clone C3, and cordiform to clone C5.

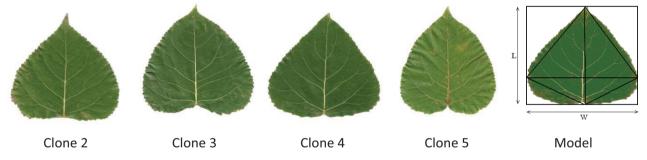


Figure 1. Representative leaves for poplar genotypes studied

# Geographical location of experience and pedoclimatic conditions

The experience was located in the area of Giulvaz, Timiş County, Romania GPS location: 45.53 N. 20.95 E.

The soil in the experimental field is chernozem, moderately strong gley with pH = 7.17, humus content H = 3.43%. Planting distance of trees is  $3 \times 2m$ . The age of the plantation is four years.

# Evaluated parameters and their characteristics

Individual leaf lengths (L) and their width (W) were individually measured with a precision of

± 0.5 mm. Using the Image J software (Rasband, 1997), based on scanned images in a 1: 1 ratio, the surface of each leaf (SLA) or perimeter (PER) was determined. Based on a high resolution imaging method, SLA values were considered as close to reality, with errors being negligible.

The parameters analyzed were variable in size: the length of a minimum of 5.7 cm and a maximum of 20.3 cm and the width of a minimum of 5.5 cm and a maximum of 20.6 cm. The average length was 11.7 cm and the average width was 12.08 cm. Standard

deviations showed high values compared to averages, namely 2.67 for length and 2.81 for width respectively. Hence, there is a relative heterogeneity of the series, indicated by the high values of the coefficients of variation defined as the ratio of the standard deviation to the mean: 0.22 and 0.23, respectively.

The series of leaf perimeters strongly correlated with their surface, r = 0.96, significant with p <0.001. The leaf perimeter strongly correlated with the corresponding leaf width (W) with the leaf length (L), in both cases the correlation coefficient was high, i.e. approximately r = 0.96, significantly with p <0.001. The strong correlation between the L and W series, with r = 0.93, significant with p <0.001, indicated a well-defined pattern of leaves. Thus, the long-legged leaves keep the proportions in the case of the width and similarly with the low length also have the same low width. Moreover, the ratio between leaf length and width indicated values close to the unit averaging 0.97.

This ratio is not highly variability, with a standard deviation of 0.08 and a variation coefficient of about 0.082. The length and width were therefore approximately equal, observing in the testing of the difference between the averages that at = 1.39 was obtained, being in the null hypothesis acceptance region.

### Mathematical model

The geometric shape is well contoured and the leaves preserve the proportions and implicitly the elements of geometric sense. The contour can be approximated by a quadrilateral having the perpendicular diagonals (L, respectively W), the diagonal corresponding to the length being the axis of symmetry.

Such a quadrilateral has the surface of the relationship  $A = 0.5 \cdot L \cdot W$ . Moreover, the correlation between the surface series and the diagonal product  $L \cdot W$  is very strong, with r = 0.98, significant at p < 0.001.

However, due to the presence of irregular contour elements or of another geometric nature, it was necessary to correct the relation of calculus of the area described by the previous formula.

Thus, the surface of each leaf was roughly calculated (LA) using a relationship (1):

$$LA = L \cdot W \cdot k \tag{1}$$

where: k represents a constant specific to each genotype.

This is the proposed method for the approximate calculation of the foliar surface. The values of k were statistically determined by tracking prediction errors ( $\varepsilon_i$ ), representing the differences between the calculated area values (LA) and those measured by scan (SLA), then reported to SLA, the relation (2).

$$\varepsilon_{i} = \left| \frac{LA_{i} - SLA_{i}}{SLA_{i}} \right| \tag{2}$$

During the study for the values  $\epsilon_i$ , the term "prediction errors" was briefly used. Determination of k constants was done in the sense that their values led to minimum predictive errors. Corresponding to the "i",  $i=1,\ldots,n$  index, the values of the parameters evaluated for each of the "n" leaves was evaluated.

Specifically, the value of k has been determined, which achieves the minimum of a regression function denoted  $\bar{\epsilon}(k)$ , determined

for the coordinate points  $^{\left(k,\overline{\epsilon}^{*}\left(k\right)\right)}$  where:

$$\overline{\epsilon}^{*}(k) = \frac{\sum_{i=1}^{n} \epsilon_{i}}{n} = \frac{\sum_{i=1}^{n} \left| \frac{L_{i} \cdot W_{i} \cdot k - SLA_{i}}{SLA_{i}} \right|}{n}$$
(3)

Practically  $\bar{\epsilon}^*(k)$  showed the evolution of average prediction errors of the calculation model (1) compared to the measured SLA surfaces, depending on the k values and  $\bar{\epsilon}(k)$ 

the theoretical path followed by the  $(k, \overline{\epsilon}^*(k))$  coordinate points.

This route was determined using the regression paradigm as a functional model using the Past 3 application (Hammer et al., 2001). The value of k was determined analytically, being in fact the abscissa of the minimal point of the  $\bar{\epsilon}(k)$  parabola. The calculation reasoning was repeated for each poplar genotype. Knowing the length L and the width W of a leaf, once determined the value of k, it could lead to an approximation of the foliar surface for the genotype in question, using a simple geometric calculation, described by relation (1).

As an observation, we mention that a method

of optimizing the value of k was chosen, in which the differences between two values, one calculated and one measured values were measured, relative to the measured value, and not a method in which only the direct differences between sizes.

The varied leaf size due to different periods of vegetation, leaf location, or genotypes could lead to a high degree of subjectivity when assessing only the direct difference between surfaces obtained by two methods. Reporting the direct difference between two sizes to the most accurate one in this case has become a much more objective indicator.

The same aspects led to the idea of the inefficiency of using a  $LA = \phi(L,W)$  regression model as an approximate method of calculating the foliar surface. Although such a regression model would have been easily determined using specific software applications, because regression theory uses the least squares method of direct differences between the values of two sets of data, it could occur in multiple situations with a subjective character.

Another reason for choosing the approximate foliar model (1) is precisely its simplicity. Even though the literature suggests some approximation models with sometimes slightly higher precision, however, often the proposed expressions have a wider form.

Using an extended computation formula would not raise problems for the foliar surface assessment in some small plants where there is no need or large sample volumes. However, in the case of poplar, the formation of an objective image on the leaf image implies, in accordance with the principles of statistics, the evaluation of a sample with a high volume of elements. This could make the evaluation process difficult by another process with a higher difficulty.

## Model performance evaluation

The evaluation of the level of precision provided by the computational model (1) of the foliar surface was performed by following four indicators, for all n = 200 cumulated samples:

- the prediction error size,

$$\overline{\epsilon}^* = \left(\sum_{i=1}^{n} \left| \frac{LA_i - SLA_i}{SLA_i} \right| \right) / n$$

previously indicated, which, in the case of a high performance model, tends to zero;

- the level of association of the measured

results with the calculated ones, following the correlation and slope coefficient (m) for the regression line SLA = m · LA; Nash-Sutclife efficiency index values (E), respective Willmot's index (d):

$$E = 1 - \frac{\sum_{i=1}^{n} (SLA_i - LA_i)^2}{\sum_{i=1}^{n} (SLA_i - \overline{SLA})^2}$$

$$d = 1 - \frac{\sum_{i=1}^{n} (LA_i - SLA_i)^2}{\sum_{i=1}^{n} \left( \left| LA_i - \overline{SLA} \right| + \left| SLA_i - \overline{SLA} \right| \right)^2}$$

values that point toward to 1, for a high performance model.

Methods of testing and validating the performance of some computational models have been commonly used in specialized studies (Willmott et al., 2011; Silva et al., 2018).

## RESULTS AND DISCUSSIONS

Regarding the first poplar genotype (Clone C2), the data were grouped into a table, as Table 1 form. For limitations of the exposure area, from the values for n = 50 evaluated leaves, only the more representative data were presented, and some were exposed by rounding.

For k values, from k=0.57 incremented with a constant step of 0.01, to k=0.67, the surface values were calculated using the relation  $LA=L\cdot W\cdot k$ , respectively the prediction errors  $\epsilon_i.$ 

$$\bar{\epsilon}^*(k) = \frac{\sum_{i=1}^n \epsilon_i}{n}$$

The mean prediction error values are shown in Table 2.

The regression function determined for the data in Table 2 is:  $\bar{\epsilon}(k)=14.57k^2-18.18k+5.728$  and the graphical representation is shown in Figure 2, curve a).

The value of k for which  $\overline{\epsilon}(k)$  achieves a minimum is:

$$k = -\frac{-18.18}{2 \cdot 14.57} = 0.623$$

In conclusion, for the first studied poplar genotype (clone C2), the proposed relationship for the approximate determination of the individual foliar surface is: LA = L · W · 0.62 . The whole approach described above was repeated for all the other leaves harvested from the other three poplar genotypes (clones C3, C4, C5) in a number of n = 50 samples for each genotype. We summed up to only present the table indicating the values  $\overline{\epsilon}^*(k)$ , Table 3.

For clone C3, the expression of the regression function determined for the values in Table 3 is:  $\bar{\epsilon}(k) = 13.43 \, k^2 - 18.78 \, k + 6.605$  and it is graphically represented in Figure 2, curve b). The value of k that leads to the minimum of

$$k = -\frac{-18.78}{2 \cdot 13.43} = 0.699$$

this function is:

Thus, the approximate expression of the foliar

surface for clone 3 is:  $LA = L \cdot W \cdot 0.7$ .

Table 1. Parameter values for leaves harvested from the first poplar genotype (Clone C2)

Leaf code	$L_{i}$	$W_{i}$	$SLA_i$	LA <sub>i</sub> k=0.57	k=0.57	LA k=0.58	ε <sub>i</sub> k=0.58	LA <sub>i</sub> k=0.6	ε <sub>i</sub> k=0.62	LA <sub>i</sub> k=0.67	ε <sub>i</sub> k=0.67
C41_1	14.5	12.6	114.4	104.1	0.0898	106.0	0.0739	 113.3	0.0100	 122.4	0.0698
C41_2	6.2	5.5	19.9	19.4	0.0248	19.8	0.0077	 21.1	0.0606	 22.8	0.1461
C41_9	9.7	9.8	61.0	54.2	0.1117	55.1	0.0961	 58.9	0.0338	 63.7	0.0440
C41_10	9.75	9.15	54.2	50.9	0.0615	51.7	0.0450	 55.3	0.0207	 59.8	0.1030
C42_1	11.8	12.1	96.5	81.0	0.1599	82.5	0.1452	 88.1	0.0863	 95.3	0.0126
C42_2	12.1	10.95	86.8	75.5	0.1301	76.8	0.1148	 82.1	0.0537	 88.8	0.0225
C42_9	14.3	14.25	133.6	116.2	0.1304	118.2	0.1151	 126.3	0.0541	 136.5	0.0221
C42_10	9	10.05	60.7	51.6	0.1499	52.5	0.1350	 56.1	0.0753	 60.6	0.0008
C43_1	13.1	13.35	115.1	99.7	0.1337	101.4	0.1185	 108.4	0.0578	 117.2	0.0181
C43_2	11.7	10.7	75.0	71.4	0.0489	72.6	0.0323	 77.6	0.0344	 83.9	0.1178
C43_9	16.5	16.7	184.3	157.1	0.1476	159.8	0.1327	 170.8	0.0729	 184.6	0.0018
C43_10	8.6	9.2	52.1	45.1	0.1348	45.9	0.1197	 49.1	0.0589	 53.0	0.0168
C44_1	17.3	17.4	199.8	171.6	0.1413	174.6	0.1262	 186.6	0.0660	 201.7	0.0093
C44_2	16.9	17.1	188.6	164.7	0.1264	167.6	0.1111	 179.2	0.0498	 193.6	0.0267
C44_9	10.2	9.6	61.4	55.8	0.0910	56.8	0.0751	 60.7	0.0113	 65.6	0.0684
C44_10	9	8.05	41.1	41.3	0.0037	42.0	0.0213	 44.9	0.0917	 48.5	0.1798
C45_1	15.7	15.5	149.4	138.7	0.0713	141.1	0.0550	 150.9	0.0101	 163.0	0.0915
C45_2	15.3	17.15	190.0	149.1	0.2153	151.7	0.2015	 162.2	0.1464	 175.2	0.0776
C45_9	9.6	8.35	45.8	45.7	0.0031	46.5	0.0143	 49.7	0.0842	 53.7	0.1717
C45_10	8.3	7.95	39.1	37.6	0.0375	38.3	0.0206	 40.9	0.0468	 44.2	0.1313

Table 2. The mean values of predictive errors given by the foliar plot values calculated as compared to the ones measured for the first clone genotype (Clone C2) for various k values

k	$\left(\sum_{i=1}^n \epsilon_i\right)\!/n$	k	$\left(\sum_{i=1}^n \epsilon_i\right)\!/n$	k	$\left(\sum_{i=l}^n \epsilon_i\right)\!/n$
0.57	0.100194	0.61	0.060109	0.65	0.069166
0.58	0.087397	0.62	0.058257	0.66	0.078162
0.59	0.076379	0.63	0.059625	0.67	0.089491
0.60	0.066715	0.64	0.063684		

Table 3. Average predictive error values, based on the values of foliar surfaces calculated in comparison to the measured values, for poplar genotypes - Clones C3, C4 and C5 for different k values

Cl	one C3		Clone C4	Clone C5		
k	$\overline{\epsilon} * (k) = \left(\sum_{i=1}^{n} \epsilon_{i}\right) / n$	k	$\bar{\epsilon}*(k) = \left(\sum_{i=1}^{n} \epsilon_{i}\right)/n$	k	$\bar{\epsilon}*(k) = \left(\sum_{i=1}^{n} \epsilon_{i}\right)/n$	
0.65	0.074324	0.63	0.083959	0.68	0.075765	
0.66	0.06426	0.64	0.07207	0.69	0.065467	
0.67	0.054804	0.65	0.063072	0.7	0.057469	
0.68	0.047896	0.66	0.056749	0.71	0.05115	
0.69	0.044616	0.67	0.052148	0.72	0.048317	
0.70	0.042617	0.68	0.050144	0.73	0.046453	
0.71	0.044166	0.69	0.050866	0.74	0.047141	
0.72	0.048667	0.7	0.05358	0.75	0.050976	
0.73	0.056127	0.71	0.059956	0.76	0.056439	
0.74	0.066673	0.72	0.068376	0.77	0.063532	
0.75	0.077844	0.73	0.077547	0.78	0.072977	

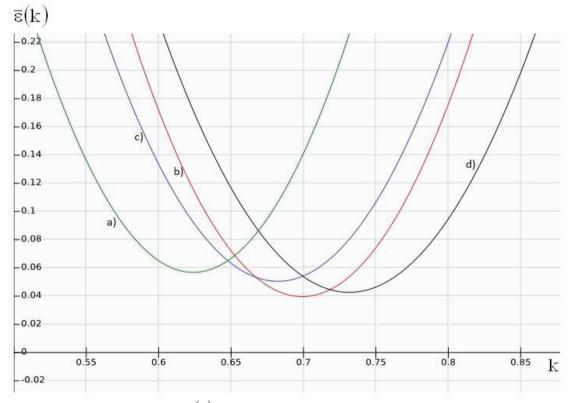


Figure 2. Theoretical  $\overline{\epsilon}(k)$  course of average prediction errors based on k values: a) Clone C2; b) Clone C3; c) Clone C4; d) Clone C5

The regression function for the clone 4 data, with the graphical distribution in Figure 2, curve c), has the expression:

$$\bar{\epsilon}(k) = 12.26k^2 - 16.73k + 5.758$$

and the value k which gives the minimum of

$$k = -\frac{-16.73}{2 \cdot 12.26} = 0.682$$

this function is:

The approximate expression of the foliar surface is:  $LA = L \cdot W \cdot 0.68$ . Similarly, for the fourth poplar genotype (clone C5), the regression function with graphical distribution in Figure 2, curve d) is:

 $\overline{\epsilon}(k) = 11.12k^2 - 16.27k + 5.994$ , and k value, which gives the minimum of this function is:

$$k = -\frac{-16.27}{2 \cdot 11.12} = 0.731$$

The approximate calculation of a leaf surface for this genotype can be made using the expression:  $LA = L \cdot W \cdot 0.73$ .

The graphical representation in Figure 2 was done using the Foo Plot application.

**Model validation**. Using the four computational relationships and aggregating in a single series all four series of values of  $^{\epsilon_i}$  differences expressed as a percentage, the data in Table 4 was obtained, where some parameters of the cumulative series are presented.

It is worth noting that about 75% of  $^{\epsilon_i}$  differences do not exceed 6.9% of the real leaf area, 50% do not exceed 3.83% and about 25% do not exceed 1.98%, if the proposed method of calculation is used.

With the exception of three outlier values, calculated using the interquartile range  $(Q_1 - 1.5 \text{ IQR}, Q_3 + 1.5 \text{ IQR})$  multiplication, the maximum difference was 14.2%.

Approximately 25% of the  $^{\varepsilon_i}$  difference values were between 6.9% and 14.2%.

These aspects can also be noted in the box and jitter plot of Figure 3.

Table 4. Statistical indicators for the cumulative series (n = 200) of prediction errors expressed per cent, given by the values of the foliar surfaces calculated as compared to the measured ones

n (volume)	200	Max	14.2%		
First quartile (Q <sub>1</sub> )	1.98%	Interquartile range (IQR)	4.91%		
Median	3.83%	Q <sub>1</sub> -1,5 IQR	-5.38%		
Third quartile (Q <sub>3</sub> )	6.90%	Q <sub>3</sub> +1.5 IQR	14.28%		
Min	0.1%	Outliers	14.6%, 14.8%, 67.4%		

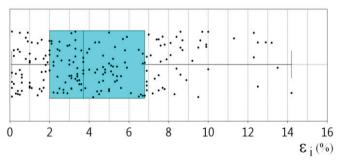


Figure 3. Box and jitter plot of distribution of predictive error series values between measured and calculated foliar surface area values, based on their size (min = 0.1%, max = 14.2%, n = 200)

The values obtained are similar to those reported in the literature. Thus, the length and width of the leaves, as independent factors, were used in the construction of P. kurroa leaf surface approximation models. In this sense, the relationship of form y = 0.333 + 0.603 LW was proposed (Kumar and Sharma, 2013), that indicated a high level of association between measured and calculated values. For the

estimation of the leaf area of *Combretum leprosum* Mart. a mathematical relationship based on L and W has been proposed, through a similar expression as a form A=0.7103 L W (Candido et al., 2013). For the leaves of *Rosa sempervirens* a relationship was proposed, of the form: LA=0.717 L W+0.56 (Fascella et al., 2013). To test the association between the series of measured and calculated values,

aggregating all 200 data and using the Past 3/Linear/Bivariate application with zero intercept, the relationship was obtained:  $y = 1.0234 \times (Figure 4)$ , so the slope value was m = 1.0234. The standard error for the coefficient obtained was 0.0041 and the 95% confidence interval for m was (1.014; 1.032). The value of the correlation coefficient was r = 0.99 with p < 0.001.

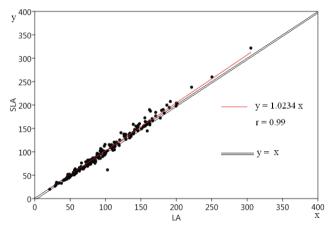
The prediction error, which had a low value  $\bar{\epsilon}^* = 0.0493$ , and the Nash-Sutcliffe (E), Willmot's (d) index: E = 0.97, d = 0.99 close to 1, showed a high degree of confidence in the calculations obtained, using the model given by equation (1).

Similar values describing the degree of precision of foliar surface estimation models

were indicated for tomato and cucumber leaves, the relationship between the estimated area of the measured one being given by the expression: y = 1.017 x, and having a high level of correlation coefficient of  $R^2 = 0.98$  (Blanco, 2003).

For the testing and validation of the proposed model for estimating the surface of marigold pots, a relationship of a type y = 0.948 x + 0.49 with  $R^2 = 0.987$ , was indicated (Giuffrida et al., 2011).

In the proposed model for *Rosa sempervirens* surface approximation, the relationship between predicted and measured values was  $y = 0.965 x + 0.089 \text{ with } R^2 = 0.953 \text{ (Fascella et al., 2013)}.$ 



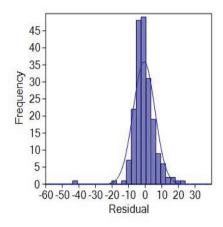


Figure 4. Testing the association between the series of measured values of foliar surfaces (SLA) and the calculated (LA) for the four poplar species, respectively the residual histogram (cm<sup>2</sup>)

## **CONCLUSIONS**

The foliar surface constants k was obtained for each genotype, which made it easier to obtain optimal foliar surfaces based on the dimensional parameters of the leaves.

The surface of each leaf at the four poplar genotypes studied was approximated using the relationship:  $LA = L \cdot W \cdot k$ .

It was chosen opted for such a relationship, to the detriment of other models, because it is defined by a simple expression that can be used efficiently when calculations for a high volume of samples are required.

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