# LEAF AREA DETERMINATION FOR *ALNUS GLUTIONOSA* (L.) GAERTN USING NONDESTRUCTIVE METHODS

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***Abstract:*** *This study evaluated the determination method of leaf area for common alder, Alnus glutinosa (L.) Gaertn, through nondestructive methods, based on leaves morphometric parameters (L, W) and the correction factor (CF). CF optimum value (CF = 0.73) has been found through successive calculations, with the differences between measured leaf area (MLA) and scanned leaf area (SLA) considered as reference and where minimal error (ME) appeared. The fitting between MLA (for CF = 0.73) and SLA (considered as reference) can be described by the linear equation (2),* $R^2=0.986$, $p<<0.001$. *The interdependency between MLA and L was described by relation (4), with* $R^2= 0.984$, $p<<0.001$, *and between MLA and W by relation (5), de* $R^2=0.988$, $p<<0.001$. *Regression analysis facilitated the development of a model, relation (6), for the MLA estimation only on the leaf parameters L and W, basis, with* $R^2=0.976$, $p<<0.001$.

***Key words**: Alnus glutinosa, correction factor, leaf area, leaf parameters, model, RMSE*

## INTRODUCTION

Black alder (*Alnus glutinosa* (L.) Gaertn), commonly known as black or common alder, is naturally widespread across all Europe, from mid-Scandinavia to the Mediterranean countries [5]. Alder communities represent the main natural vegetation in many wet environments [16].

This species is part of 91E0* habitat - Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*) [18]. It prefers the soils with high organic carbon contents [12], [35]. After afforestation with common alder, an increased accumulation of organic matter and acidification was observed [39], [6], [17].

Alnus species contain various types of secondary metabolites as phenols, flavonoids, terpenoids, steroids, tanins and diarylheptanoids [34]. It has wound healing, anti-inflammatory [34] and antioxidant properties [23], [25].

For the study of foliar surface, nondestructive methods were developed, based on leaf morphometric parameters (L, W) and correction coefficients or surface constant [33], [4], based on models [22], IT applications in Processing with Java cod lines [14]. It is also a known fact that if only a plant species is of interest for a study, for example in genetically segregating populations, nondestructive measurements is extremely useful [31].

Present study determined the optimal value of the correction factor (CF), and based on it and the foliar parameters (L, W), the leaf area was determined in black alder *Alnus glutinosa* (L.) Gaertn.

## MATERIALS AND METHODS

This study evaluated the possibility of leaf area determination for common alder, *Alnus glutinosa* (L.) Gaertn., through nondestructive methods, based on leaves morphometric parameters (L, W), and the correction factor (CF).

The biological material for this investigation was represented by *Alnus glutinosa* (L.) Gaertn., leaves, 100 samples, with different sizes, which were collected randomly. Leaves morphometric parameters (L, W) were determined using a ruler, with a ± 0.5 mm precision. The leaves were then scanned in a 1:1 size ratio. For each leaf, scanned leaf area (SLA) was determined through software analysis [19].

For the determination of the correction factor (CF) specific for common alder leaves, the theoretical variation interval between (0,1) was used, and through tests with 0.1 variation units pas, the value series of measured leaf area (MLA) was determined, using relation (1).

$$MLA = L \times W \times CF \quad (1)$$

where: MLA – measured leaf area; L – leaf length, W – leaf width, CF – correction factor.

For the determination of CF optimal value, differences between MLA and SLA (considered as reference) were calculated, and the optimum CF value was considered in conditions of minimum mean errors (MME).

Experimental data were analyzed using ANOVA test, regression analysis, correlation analysis and for data safety, $R^2$, p and RMSE parameters were determined.

## RESEARCH RESULTS

Leaves morphometric parameters (L, W) were obtained through measuring. Leaf scanned images (in a 1:1 size ratio) were imagistically analyzed (ImageJ software) for the leaf area determination (Scanned Leaf Area – SLA) with a high precision and statistical safety conditions. L, W and SLA parameters values obtained are presented in table 1, selective dates from the 100 studied probes.

Experimental data safety and the presentation of variation in the experimental data set were evaluated using ANOVA single factor set, table 2. The obtained values confirm the variance but also the statistical safety degree of the analyzed experimental data set, $Fcrit << F$, $p << 0.01$, for $Alpha = 0.001$.

Nondestructive methods of determination for foliar surface are useful for vegetation dynamics studies, for lamina growth and development investigations on young plants, when some leaves detaches can affect growth and development of the plant, but also for rare or endangered species studies or in other particular studies when the destruction of the leaves is not allowed.

Based on the model proposed by Sala et al. (2015) [33] correction coefficient for black alder leaves was determined, with the purpose of foliar surface determination using L and W parameters, but also correction coefficient, relation (1).

Ordinarily, correction coefficient has subunit values and for the optimum value determination of correction factor (CF) for black alder laves, the variation interval was limited between 0.68 and 0.78.

Using comparative analysis for the MLA calculated values in relation to SLA (considered as reference) for each leaf, minimum mean error (MME) was noticed at CF = 0.73, table 3. Graphical distribution of MME values (mean of minimum errors) can be observed in figure 1.

Statistical safety parameter RMSE confirmed the same values, CF = 0.73, where it had the minimal value (RMSE=2.25644), when it was compared with other values of CF interval studied, table 3.

**Table 1.**

**Data series containing foliar parameters and leaf area for black alder samples, *Alnus glutinosa* (L.) Gaertn.**

| Sample | L | W | SLA |
|---|---|---|---|
| **1** | 3.6 | 2.65 | 6.68 |
| **2** | 4 | 2.95 | 8.23 |
| **3** | 4.1 | 3.85 | 12.09 |
| **4** | 4.3 | 3.85 | 12.12 |
| **5** | 4.35 | 4.1 | 13.11 |
| **6** | 4.5 | 4.25 | 14.70 |
| **7** | 4.7 | 4.4 | 15.56 |
| **8** | 4.75 | 4.65 | 16.06 |
| **9** | 4.9 | 4.75 | 17.51 |
| **10** | 5.35 | 5 | 20.43 |
| **…** | … | … | … |
| **90** | 8.4 | 7.85 | 47.69 |
| **91** | 8.7 | 8.15 | 50.58 |
| **92** | 9.1 | 8 | 50.98 |
| **93** | 9.2 | 8.1 | 51.58 |
| **94** | 9.55 | 8.55 | 58.75 |
| **95** | 9.85 | 8.4 | 60.28 |
| **96** | 10.05 | 9.8 | 72.78 |
| **97** | 10.75 | 10.35 | 77.56 |
| **98** | 10.8 | 11.05 | 85.85 |
| **99** | 10.85 | 10.5 | 86.21 |
| **100** | 11.1 | 11.15 | 91.70 |
| **SE** | ±0.18 | ±0.17 | ±1.82 |

**Table 2.**

**ANOVA single factor values**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| **Between Groups** | 68036.06 | 3 | 22678.69 | 125.7793 | 5.45E-58 | 5.52274 |
| **Within Groups** | 75007.02 | 416 | 180.3053 | | | |
| **Total** | 143043.1 | 419 | | | | |

Alpha = 0.001

**Table 3.**

**MLA values depending on CF for the optimum MLA determination for black alder**

| CF | SLA | MLA | MME | RMSE |
|---|---|---|---|---|
| **0.68** | 31.72 | 29.70 | -2.02 | 3.11736 |
| **0.69** | | 30.14 | -1.58 | 2.79036 |
| **0.70** | | 30.58 | -1.14 | 2.52410 |
| **0.71** | | 31.02 | -0.70 | 2.33942 |
| **0.72** | | 31.45 | -0.27 | 2.28626 |
| **0.73** | | 31.89 | 0.17 | 2.25644 |
| **0.74** | | 32.33 | 0.61 | 2.42473 |
| **0.75** | | 32.76 | 1.04 | 2.65489 |
| **0.76** | | 33.20 | 1.48 | 2.95540 |
| **0.77** | | 33.64 | 1.92 | 3.30714 |
| **0.78** | | 34.07 | 2.35 | 3.69551 |

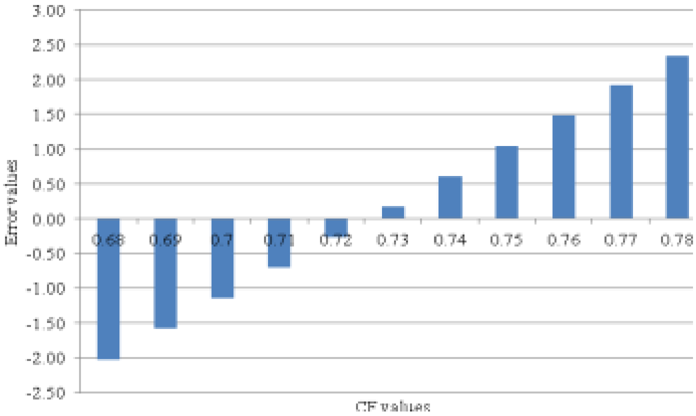


**Figure 1. Graphical distribution of the errors for MLA depending on SLA, based on CF values for black alder**

The fitting between MLA (for CF = 0.73) and SLA (considered as reference) can be described by the linear equation (2), in statistical safety conditions ($R^2$=0.986, p<<0.001), with a graphical distribution in figure 2.

$$MLA = 1.0112 \cdot x = 0.1902 \quad (2)$$

where: x – SLA (scanned leaf area)

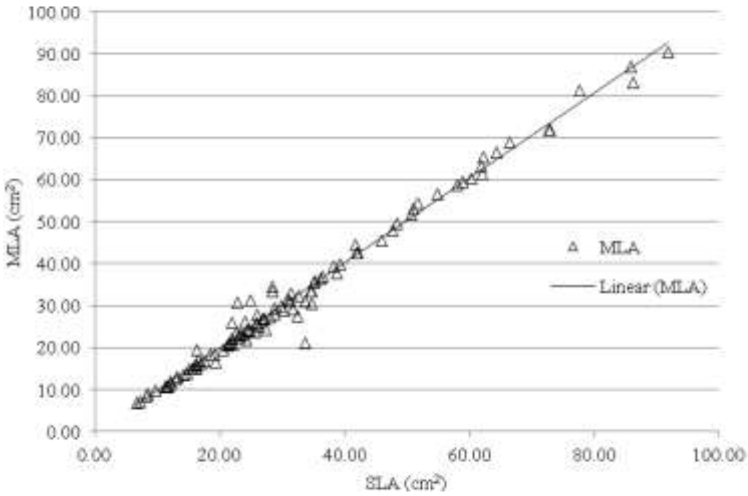


**Figure 2. Graphical distribution of MLA (CF=0.73) depending on SLA for black alder**

The relation between foliar parameters L and W, utilized for MLA determination was explained by a third degree polynomial equation, relation (3), with $R^2$=0.962, p<<0.001, F=860.99. Graphical distribution can be observed in figure 3.

$$W = 0.03021 \cdot x^3 - 0.6283 \cdot x^2 + 5.027 \cdot x - 8.537 \quad (3)$$

where: W – leaf width; x – leaf length (L)

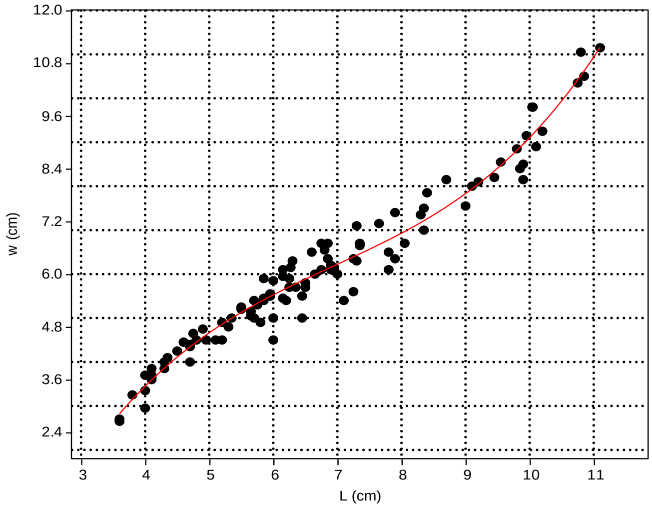


**Figure 3. Graphical distribution of W values depending on L values for black alder leaves**

Considering the fact that MLA was determined based on foliar parameters L and W and the correction constant (KA), the interdependency between MLA and each parameter was analyzed. The interdependency between MLA and L was described by relation (4), with $R^2$= 0.984, p<<0.001, and between MLA and W by relation (5), de $R^2$=0.988, p<<0.001, with graphical distributions in figures 4 and 5.

$$MLA = 0.8493 \cdot L^2 - 2.543 \cdot L + 7.895 \tag{4}$$

$$MLA = 0.5712 \cdot W^2 + 2.624 \cdot W - 6.643 \tag{5}$$

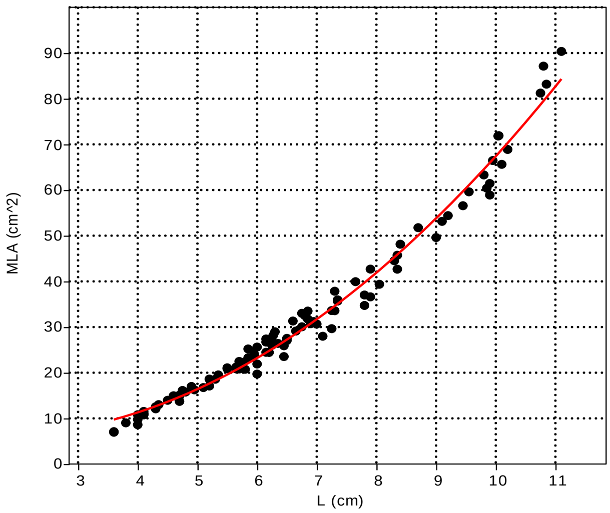


**Figure 4. Graphical distribution of MLA values depending on L values for black alder leaves**

Regression analysis facilitated the development of a model, relation (6), for the MLA estimation only on the leaf parameters L and W, basis, with $R^2$=0.976, p<<0.001.

$$MLA = -31.7074 + 3.3324 \cdot L + 6.85172 \cdot W \tag{6}$$

From the analysis of relation (6) coefficients, it was found that the highest contribution was of W parameter (Coef W= 6.85172) for the determination of MLA when compared to the L parameter contribution (Coef L = 3.3324). Similar results were communicated by previous studies [1], [24].

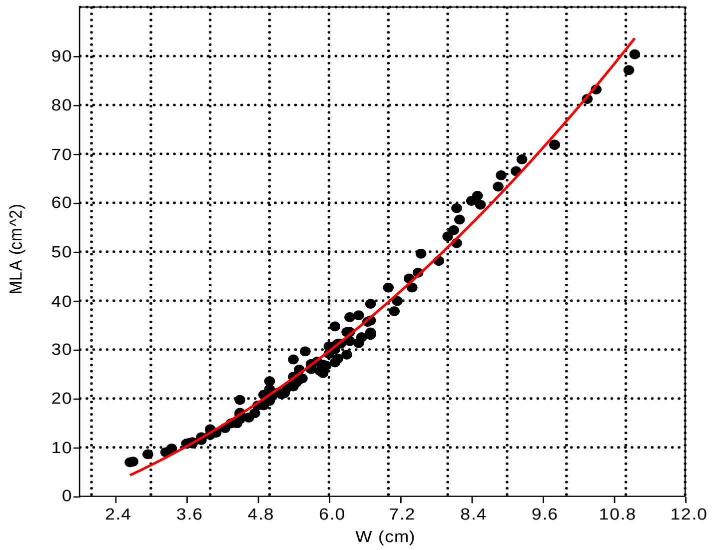


**Figure 5. Graphical distribution of MLA depending on W values for black alder leaves**

Comparative analysis of five models for the determination of leaf area (MLA) for black alder based on foliar parameters and correction factor (CF), relation (1), on SLA, relation (2), on each foliar parameter, relation (4) for L, and relation (5) for W and foliar parameters, respectively, relation (6) conducted to synthetic data from table 5.

The best determination of MLA is as a function of SLA, equation (2), when the safety parameter RMSE and SEP had the lowest values (RMSE=0.860584, SEP=2.786089), but this requires a known SLA and in this conditions is not necessary to determine MLA.

When SLA is not known, then the other 4 models can be considered. Between them, MLA determination based on L and W foliar parameters and correction factor (CF), equation (1) presented the highest statistical safety based on RMSE and SEP parameters. In a decreasing order of statistically safety the model with W, equation (5), next the model with L, equation (4) and the last was the model given by both foliar parameters (L, W), but without correction coefficient, equation (6).

Leaf lamina was studied in relation with the presence and the distribution of some pathogens [36], [15], but also in relation with the aerosols influence on forest trees [38], biomonitoring of some mineral elements in urban habitat [32]. In addition, leaf area was studied on different species from grassland communities [37].

**Table 5.**

**Synthetic data regarding prediction/determination safety for MLA**

| | MLA = f(var) | Equation | RMSE | SEP |
|---|---|---|---|---|
| **MLA** | L×W×CF | (1) | 2.25644 | 7.17417 |
| **MLA=f(SLA)** | SLA | (2) | 0.860584 | 2.786089 |
| **MLA=f(L)** | L | (4) | 3.619897 | 11.32349 |
| **MLA=f(W)** | W | (5) | 2.730326 | 8.561764 |
| **MLA=f(L,W)** | L,W | (6) | 3.765082 | 11.80678 |

In different plant species, especially and of greater importance to those cultivated, the leaves have been studied in relation to the application of foliar fertilizers and the variation of ecophysiological parameters [26], [2], [8], relative to the elements of productivity, gravimetric parameters, production and quality [13], [21], [27], [28], [29], [3], [10], in relation to biomass parameters and biomass production [20], [7], [9].

Physiological studies on leaf area are of interest due to the fact that they can give information about photosynthetic efficiency, plant growth, light interception, transpiration and responses to stress [11], [30].

The results communicated in this study are in agreement with other studies and complement optimized values for the necessary parameters in determining the leaf surface in plants of interest, by non-destructive methods.

## CONCLUSIONS

The image analysis and the comparative mathematical and statistical analysis between the MLA and SLA values (considered as reference) led to obtaining the optimum value of the correction factor (CF) and to determining the leaf area at *Alnus glutinosa* (L.) Gaertn.

Comparative analysis of these five models used for the leaf area (MLA) determination in the black alder case, based on foliar parameters and correction factor (CF), or based on SLA, foliar parameters considered simultaneously (L, W), and separately for L, and respectively for W evidenced that under conditions in which it is not possible to determine the SLA, the safest method is based on the parameters L, W and the correction factor CF, which for the black alder was optimally found at CF = 0.73.

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