

## PECULIARITIES OF THE LINDEN LEAF AREA IN RELATION TO THE LEAF POSITION ON THE SHOOT

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### Abstract

*The study evaluated the linden leaf area (LA), and found models to describe the leaf area variation in relation to the leaf position on the shoot (Lp) and shoot parameters (CSL, cumulative shoot length). Leaves were studied on annual growth shoots, from the Cenad Forest Protected Area, Timis County, Romania. Leaf area (LA) was determined by scanning (SLA) and calculation based on leaf parameters (L, w) and correction factor (Cf = 0.31, optimal value found), based on relation of type  $MLA = L \times w \times Cf$ . The fit level between MLA and SLA (as reference) was described by a linear equation, and confirmed by  $R^2 = 0.985$  and  $RMSEP = 4.97363$ . The variation of SLA and CSLA (cumulative scan leaf area) in relation to the position of the leaf on the shoot (Lp) and cumulative shoots length (CSL) was evaluated by regression analysis and described by equations, 3D models and in the form of isoquants, under conditions of statistical safety ( $R^2 = 0.960$ ,  $R^2 = 0.999$ ,  $p < 0.001$ ). Some leaves have been found as deviations of SLA from the theoretical model and can be considered as foliar indicators (FI) for growth conditions during the shoot vegetation period.*

**Key words:** annual shoot; correction factor; foliar indicators; leaf area; linden; model.

### INTRODUCTION

The typology of shoots and leaves, respectively the development of the associated physiological processes, varies primarily in relation to the plant species (Jing et al., 2016; Gleason et al., 2018; Rawat et al., 2021).

Elements of characterization of shoots and leaves in different plant species have been studied in relation to their genetic determination (Byrne, 2006; Busch et al., 2011); with the vertical gradient of nutrients in the leaves (eg. N) and the growth of shoots (Weih & Rönnerberg-Wästjung, 2007); with the functionality of arboreal plants under the influence of nutrients (Zhang et al., 2018); with complexity of shoot structure, and leaf dimension (Watanabe, 2015); with increasing shoots and biomass production (Zavistanovicz et al., 2021); with some vegetative and generative processes (Saouab & Amraoui, 2020); with the allocation of biomass and its structure in relation to light (Liu & Su, 2016); with leaf thermoregulation elements and shoot architecture (Bridge et al., 2013); with the vigor of growing shoots in relation to ecological disturbances (Florence, 1996; Au et al., 2019).

Specific aspects of shoots and leaves have been studied in species of economic interest, such as fruit trees, fruit shrubs, vines, or ornamental plants.

Thus, the articulation of shoots and leaves in fruit trees was studied based on allometric relationships and specific patterns, in relation to different elements of influence (Baïram et al., 2017; Wang et al., 2020).

In the case of vines, studies have been conducted in relation to plant nutrition (Sala & Blidariu, 2012) and in relation to the adoption of genotypes and cultivation technologies (Dobrei et al., 2009, 2016).

The development of shoots has been studied in some ornamental plants (eg. rose) in relation to specific maintenance work by cutting (Erwin et al., 1997). The influence of the architecture and status of some shoots on the photosynthetic processes and the production of flowering shoots was evaluated (Zhang et al., 2020). Shoots in relation to growth, development and stress tolerance in ornamental plants have also been studied (Toscano et al., 2020).

Leaf traits were taken into account in the comparative analysis of different species (woody, grassy) in relation to influencing

factors (phylogenetic factors, ecological factors) (Gratani, 2014; Li et al., 2015; An et al., 2021).

The leaves of plants, in different species, have been studied in relation to water, water regime and water loss in the leaves (Zhang et al., 2015; Leuschner et al., 2019), in relation to light (Atherton et al., 2017; Kaitaniemi et al., 2018), nutrient content and associated processes (Huang et al., 2015; Jokar et al., 2021), response to stress factors (Al-Namazi & Bonser, 2020; Reimer et al., 2021; Seleiman et al., 2021), and in relation to pathogen attack (Drienovsky et al., 2017).

The present study analyzed biometric elements and parameters, and physiological indices of linden shoots, *Tilia tomentosa* Moench., in order to evaluate the variation of the leaf area in relation to the position of the leaves on the shoot.

## MATERIALS AND METHODS

The biological material was annual linden shoots, *Tilia tomentosa* Moench. The shoots samples were taken in July (2021), in four repetitions, from the area of the Cenad Forest Protected Area, Timis County, Romania.

The parameters studied were represented by the length of the internodes, on the basis of which the cumulative shoots length (CSL) was calculated by summing the length of the internodes, in chronological order on the shoot. At the level of the leaves, the dimensions (length - L and width - w) were determined on the basis of which the measured leaf area (MLA) was found. To accurately measured leaf area (MLA) it was necessary to find a correction factor (Cf). To find out Cf, a working model proposed by Sala et al. (2015), which ensures obtaining Cf with high precision, was used. Measured leaf area (MLA) was determined based on the relation (1), which took into account the dimensional parameters of the leaves (L and w) and the correction factor (Cf).

$$MLA = L \times w \times Cf \quad (1)$$

In order to verify the accuracy with which the MLA was found, the scanned leaf area (SLA), which was considered the reference leaf area,

was determined in parallel for each leaf. The calculation accuracy of MLA, in relation to a set of values of the correction factor (Cf), was evaluated based on the error calculated between MLA and SLA. The optimal value for Cf was considered to be in the case of minimum error value (ME) between MLA and SLA. To confirm the Cf, the RMSEP safety parameter, equation (2) was used.

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

The cumulative scanned area (CSLA) was calculated by summing the leaf area of each leaf, in successive order on the shoot.

The analysis and data processing was done to evaluate the statistical safety of the data set obtained, but also to evaluate the presence of the variance in the data set (ANOVA test).

Linear regression analysis was used to assess the degree of fit between MLA and SLA, and the level of statistical safety was assessed based on R<sup>2</sup> and the parameter p (<95%). Multiple regression analysis was used to evaluate the variation of SLA and CSLA in relation to leaf position on shoot (Lp) and cumulative shoot length (CSL), as direct action, and as interaction.

PCA (correlation) was used to evaluate the distribution of leaf samples, L1 to L15, according to the association with the parameters considered (CSL, CSLA, SLA, MLA). Cluster analysis and SDI were used to assess the degree of similarity and association of the leaf sample (L1 to L15) in relation to the values recorded for the parameters considered. The Cophenetic coefficient was used to evaluate statistical safety.

PAST software (Hammer et al., 2001), and Wolfram Alpha (2020) software were used for analysis, data processing and generation of 3D models and in the form of isoquants, and some calculations were made in EXCEL (module of mathematical and statistical calculations).

## RESULTS AND DISCUSSIONS

Based on the length dimensions of each internodes the cumulative values (CSL) were calculated on the total length of the linden

shoots. The determined values were between 6.3 cm specific for L1, and 129.5 cm, as the sum of the individual values, L1 to L15. The values for the scanned leaf area (SLA) were between 42.449 cm<sup>2</sup> for the L1 leaf and 5.053 cm<sup>2</sup> for the L15 leaf, with the maximum value recorded for the L5 leaf. The cumulative foliar surface registered increasing values from 42.449 cm<sup>2</sup>, value afferent to the leaf L1 up to 1275.333 cm<sup>2</sup>, with a variable rhythm of ridges, in relation to the contribution of each leaf, in their order on the shoot length (L1 to L15).

The measured leaf area obtained based on the dimensional parameters of the leaves (L and w) was calculated using the value of the correction factor (Cf), based on a relation of the type of equation (1).

The correction factor was found according to the model proposed by Sala et al. (2015). The values recorded for the determined parameters are presented in Table 1. The ANOVA single factor test, in conditions of Alpha = 0.001, confirmed the safety of the recorded data and

the presence of the variance in the data set (Table 2).

Table 1. Values of the parameters determined at linden shoot

Leaf sample	CSL	SLA	CSLA	MLA
L1	6.3	42.449	42.449	40.622
L2	13.5	78.947	121.396	77.860
L3	23	97.984	219.38	93.828
L4	31.7	125.061	344.441	119.090
L5	41	133.225	477.666	129.642
L6	49.5	130.825	608.491	126.341
L7	58.7	117.910	726.401	117.050
L8	68	107.611	834.012	111.191
L9	78.35	108.251	942.263	114.278
L10	89.85	109.006	1051.269	115.472
L11	101.25	87.871	1139.14	96.565
L12	111.35	78.840	1217.98	89.241
L13	120.25	36.108	1254.088	38.363
L14	125.55	16.192	1270.28	16.508
L15	129.05	5.053	1275.333	4.957

Table 2. ANOVA test, single factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5327627	3	1775876	35.38764	5.81E-13	6.229585
Within Groups	2810276	56	50183.49			
Total	8137902	59				

Alpha=0.001

For the calculation of the measured leaf area (MLA) based on the dimensional parameters of the leaves, the optimal value for the correction factor (Cf) was initially determined. The theoretical value falls within the range (0.1), and the finding of the optimal value was made by calculating a series of CF values, determining the MLA, and finding the minimum error compared to the SLA which was considered the reference value. Thus, the value Cf = 0.31 was found, in the conditions of the working samples, and the values of the average of the calculated errors, as the difference MLA - SLA, are presented in Table 3.

As a statistical safety parameter, RMSEP, relation (2), was determined. The minimum error (ME = 1.04 cm<sup>2</sup>) was recorded in the case of the value of Cf = 0.31, and the value RMSEP = 4.973634, confirmed the value found for the correction factor (Cf).

Table 3. Values calculated for Cf and MLA and statistical safety parameters

Cf	SLA	MLA	ME	RMSEP
0.26	85.02	72.19	-12.84	15.19424
0.27		74.96	-10.06	12.33626
0.28		77.74	-7.28	9.60363
0.29		80.51	-4.51	7.14172
0.30		83.29	-1.73	5.33915
0.31		86.07	1.04	4.97363
0.32		88.84	3.82	6.30043
0.33		91.62	6.60	8.56632
0.34		94.40	9.37	11.2159
0.35		97.17	12.15	14.03349
0.36		99.95	14.93	16.93545

Simple regression analysis was used to find the level of match between MLA, calculated on the basis of L, w and Cf, and the SLA obtained by scanning.

Equation (3) was obtained, which describes the fit between MLA and SLA in conditions of high statistical safety,  $R^2 = 0.985$ ,  $r = 0.993$ ,  $p < 0.001$ . The graphical distribution of MLA vs SLA values and regression line are shown in Figure 1.

$$MLA = 0.9834x + 2.4533 \quad (3)$$

where:  $x$  – SLA ( $cm^2$ )

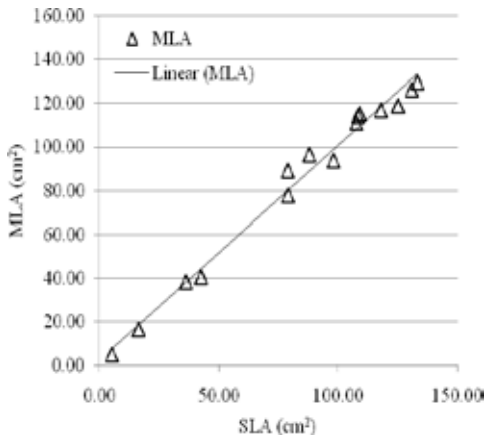


Figure 1. Graphic expression of the fit line, MLA vs SLA, linden leaves

The PCA analysis led to the diagram in Figure 2, in which the distribution of the leaf samples, L1 to L15, was obtained, depending on the association with the considered parameters (CSL, CSLA, SLA, MLA), as biplot.

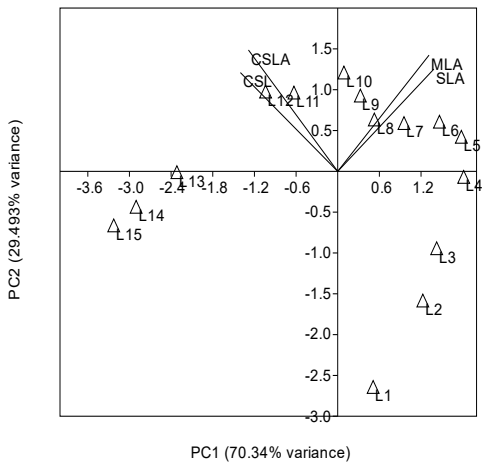


Figure 2. PCA diagram on leaf sample distribution (L1 to L15) in relation to reference parameters, as biplot

It was found the independent positioning of some leaves (positions L1 to L4, and L13 to L15) compared to the parameters taken into account. Leaves in positions L12 and L13 were associated with cumulative shoot length (CSL) and cumulative leaf area (CSLA) parameters. The leaves on positions L5 to L10 were associated with individual leaf surface parameters (SLA, MLA). PC1 explained 70.34% of variance, and PC2 explained 29.493% of variance (Figure 2).

The cluster analysis led to the dendrogram in Figure 3. It was found the formation of two distinct clusters, with several sub-clusters each, which include leaf samples (L1 to L15) depending on the degree of similarity in relation to the parameters considered, in statistical safety conditions (Coph.corr = 0.762).

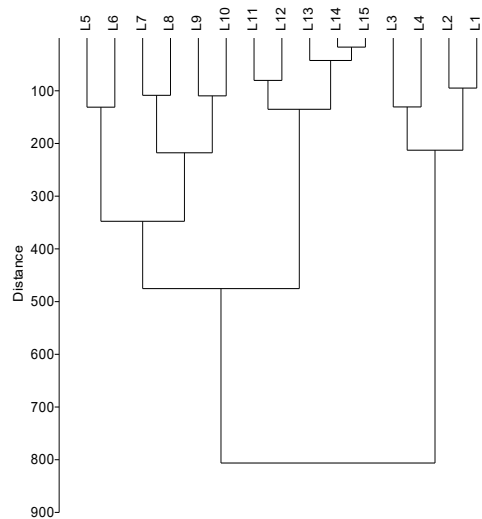


Figure 3. Leaf sample association dendrogram, by degree of similarity, based on Euclidean distances

The obtained SDI values are presented in Table 4. A high degree of similarity was recorded in leaves L14 and L15 (SDI = 17.184), followed by leaves L14 and L13 (SDI = 34.126).

These leaves are grouped in a common cluster in the dendrogram, [L13 (L14, L15)] (Figure 3), and in the PCA diagram they are in an independent position in relation to the parameters considered, as biplot. They are in the apical position on the shoot, with the lowest values for SLA.

Table 4. SDI values for linden leaf sample

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15
L1		94.885	193.65	323.77	454.73	580.89	694.31	799.75	908.08	1017.2	1103.1	1181.8	1217	1234.1	1240.1
L2	94.885		101.53	232.18	365.12	493.56	609.2	716.05	824.75	934.24	1021.7	1101	1139.2	1157.7	1164.3
L3	193.65	101.53		130.72	263.74	392.74	509.2	616.6	725.36	834.92	923.14	1002.7	1042.6	1061.9	1069
L4	323.77	232.18	130.72		134.21	264.81	382.99	491.29	599.89	709.41	798.92	878.89	921.81	942.53	950.51
L5	454.73	365.12	263.74	134.21		131.16	250.15	358.76	467.02	576.36	666.58	746.73	791.75	813.56	822.19
L6	580.89	493.56	392.74	264.81	131.16		119.33	227.97	335.99	445.28	535.72	615.94	662.2	684.8	693.94
L7	694.31	609.2	509.2	382.99	250.15	119.33		108.66	216.99	326.48	416.52	496.71	543.25	566.33	575.83
L8	799.75	716.05	616.6	491.29	358.76	227.97	108.66		108.79	218.4	307.92	388.1	435.44	459.31	469.36
L9	908.08	824.75	725.36	599.89	467.02	335.99	216.99	108.79		109.62	200.04	280.36	331.6	357.57	368.93
L10	1017.2	934.24	834.92	709.41	576.36	445.28	326.48	218.4	109.62		93.035	172.78	230.91	260.09	273.42
L11	1103.1	1021.7	923.14	798.92	666.58	535.72	416.52	307.92	200.04	93.035		80.33	140.15	171.28	185.94
L12	1181.8	1101	1002.7	878.89	746.73	615.94	496.71	388.1	280.36	172.78	80.33		76.142	110.24	127.09
L13	1217	1139.2	1042.6	921.81	791.75	662.2	543.25	435.44	331.6	230.91	140.15	76.142		34.126	51.08
L14	1234.1	1157.7	1061.9	942.53	813.56	684.8	566.33	459.31	357.57	260.09	171.28	110.24	34.126		17.184
L15	1240.1	1164.3	1069	950.51	822.19	693.94	575.83	469.36	368.93	273.42	185.94	127.09	51.08	17.184	

The variation of the SLA in relation to the position of the leaves on the shoot was described by a polynomial equation of degree 3, equation (4), in conditions of statistical safety, according to  $R^2 = 0.960$ ,  $p < 0.001$ .

The variation of the cumulative scanned leaf area (CSLA) was described by a polynomial equation of degree 3, equation (5), also in statistical safety conditions,  $R^2 = 0.999$ ,  $p < 0.001$ .

$$SLA = 0.099x^3 - 4.3783x^2 + 43.024x + 7.7569 \quad (4)$$

$$CSLA = -0.5787x^3 + 10.069x^2 + 66.754x - 42.546 \quad (5)$$

where: SLA – scanned leaf area;  
 CSLA – cumulative scanned leaf area;  
 x – leaf position on the shoot.

Multiple regression analysis was used to evaluate the variation of the SLA according to the position of the leaves on the shoot (Lp) and the cumulative length of the shoot (CSL). Equation (6) was obtained which described the variation of SLA as a function of Lp (x-axis) and CSL (y-axis), under conditions of  $R^2 = 0.990$ ,  $p < 0.001$ ,  $F = 204.2354$ .

The SLA variation according to Lp and CSL is presented in the form of a 3D model in Figure 4, and in the form of isoquants in Figure 5.

$$SLA = ax^2 + by^2 + cx + dy + exy + f \quad (6)$$

where: SLA – Scan leaf area; x – Lp – leaves position (no); y – CSL – Cumulative shoot length (cm); a, b, c, d, e, f – coefficients of the equation (6); a= 39.90880651; b= 0.55981845; c= 71.89687748; d= -4.58511570; e= -9.70627938; f= 0

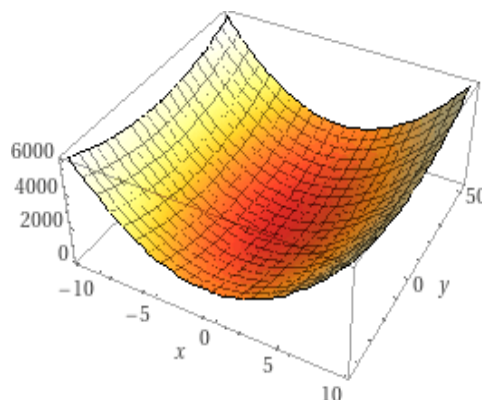


Figure 4. 3D model of SLA variation in relation to Lp (x-axis) and CSL (y-axis) in linden

The CSLA variation depending on the position of the leaves (Lp) and the cumulative length of the shoot (CSL) was described by equation (7) under conditions of  $R^2 = 0.999$ ,  $p < 0.001$ ,  $F = 5855.883$ .

A 3D graphic model was obtained (Figure 6) and in the form of isoquants (Figure 7) which represented graphically the variation of CSLA according to Lp (x-axis) and CSL (y-axis).

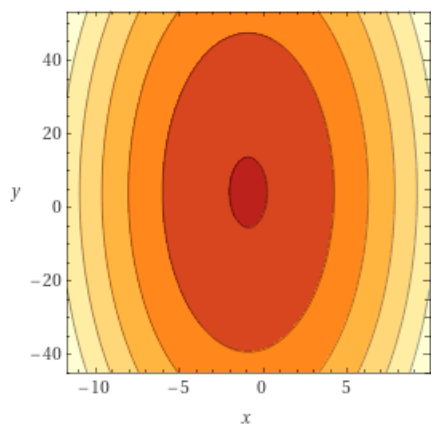


Figure 5. Model in isoquant format of SLA variation in relation to Lp (x-axis) and CSL (y-axis) in linden

$$CSLA = ax^2 + by^2 + cx + dy + exy + f \quad (7)$$

where: CSLA - Cumulative Scan Leaf Area; x - Lp - leaves position (no); y - CSL - Cumulative shoot length (cm); a, b, c, d, e, f - coefficients of the equation (1); a = -12.15325041; b = -0.39940623; c = -237.28979892; d = 42.24825265; e = 4.53261605; f = 0

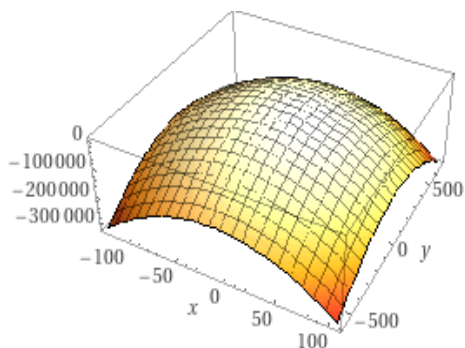


Figure 6. 3D model of CSLA variation in relation to Lp (x-axis) and CSL (y-axis) in linden

The value determined for the correction factor ( $C_f = 0.31$ ) facilitated the obtaining with high precision of the measured leaf area (MLA), in relation to the SLA. Linear equation (3) described the fitting between MLA and SLA in statistical safety conditions, which confirmed the accuracy of the work. The validation of leaf area values, or of associated leaf area indices, determined by different methods, models and techniques, has been communicated in many studies, by means of specific coefficients such as  $R^2$ , RMSEP, coefficient of variation (CV), or other appropriate statistical parameters (Kumar

& Sharma, 2013; De Carvalho et al., 2017; Yang et al., 2022).

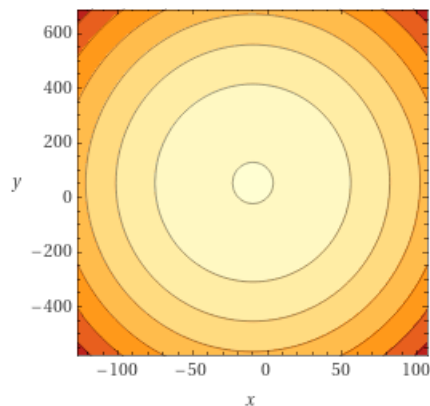


Figure 7. Model in isoquant format of variation of CSLA in relation to Lp (x-axis) and CSL (y-axis) in linden

The variation of SLA and CSLA was described in relation to the position of the leaves on the shoot (Lp) and the cumulative length of the shoot (CSL) by equations obtained on the basis of regression analysis, in conditions of statistical safety. The information obtained is useful for evaluating the LA variation in relation to the length of the shoots and the position of the leaves, and present importance through the models obtained. Based on leaf area, some studies evaluated cumulative leaf area index and leaf area index profile, in relation to biomass, biomass carbon and growth rate of some plant species (eg. pine) determined by LiDAR techniques (Beets et al., 2011). The study authors reported statistical safety values of the statistical parameters used to confirm data validation, and appreciated that LiDAR regression equations could be improved by integrating additional location and crops data.

Some studies have used models to describe the branch architecture and leaves based on allometric relationships, in order to predict the leaf area at different genotypes of fruit trees (eg. apple) in statistical safety conditions (Baïram et al., 2017). The authors reported that refining the models to increase predictive safety was made possible by focusing more on the skeletal structure of the tree crown (shoots length, spurs).

Structural equation models were used, in terms of statistical certainty ( $p < 0.0001$ ), in studies to evaluate traits associated with "hydraulics,

biomechanics and the spectrum of leaf economy" considered as factors with variable and independent contribution to growth and branch thickening (Gleason et al., 2018).

In the present study, from the detailed analysis of the graphical distribution, as well as from the obtained equations, it was found the deviation of the values of the leaves L7 and L8 in relation to the theoretical model, given by the function (4), fact that can be associated with vegetation conditions and the growth of those leaves. Such morphological elements can be considered as indicators for the expression of stress conditions during the vegetation period of the plants. The approach used in this study can be adapted to the study of species of high economic interest and can provide information for adapting cutting technologies, maintenance works in order to ensure a balance between vegetative growth / fruiting.

## CONCLUSIONS

The present study facilitated the finding of the correction factor (Cf) and the determination of the leaf area by measurement of linden leaves in high statistical safety conditions, confirmed by the value of the coefficient  $R^2$  of the fit equation between MLA and SLA, and by the RMSEP statistical parameter.

It was possible to obtain models describing the variation of the cumulative leaf area (CSLA) in relation to the position of the leaves on the shoot and cumulative shoot length (CSL).

The obtained equations and the graphical models highlighted the positioning of some leaves as a deviation from the theoretical model of variation of LA, which leads to the possibility of considering such cases as indicators of plant growth and development in relation to certain environmental factors and conditions of influence.

The approach models can be adapted to species of economic interest (fruit trees, ornamental species) in relation to maintenance works for vegetative growth, fruiting or floral elements and indicators of ornamental quality.

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