CHARACTERIZATION OF LEAVES GEOMETRY IN *ASCLEPIAS SYRIACA* L. SPECIES

ȘTEF, R. – CARABEȚ, A. – MANEA, D. – SALA, F.*

University of Life Sciences "King Mihai I" from Timisoara
Calea Aradului, 119, 300645, Timisoara, Romania
(phone: +40-256-277-009; fax: +40-256-200-296)

**Corresponding author*
e-mail: florin_sala@usab-tm.ro; phone: +40-256-277-007; fax: +40-256-200-296

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Abstract. The aim of the study was to analyze the *Asclepias syriaca* L. leaves, in order to describe the leaves geometry based on some leaf parameters, and ratios calculated. The leaf samples were taken from mature plants, from the area of Parța locality, Timis County, Romania. 100 leaves from different size categories were randomly analyzed. The scanned leaf area (SLA) was considered as a reference, and the correction factor (CF) was determined for the measured leaf area (MLA). The CF = 0.74 value was confirmed by the mean error (ME) values calculated as the difference between MLA and SLA, and by the RMSEP parameter. The fit between MLA and SLA was described by a linear equation, in conditions of $R^2 = 0.992$, $p < 0.001$. Different correlation levels were found between the foliar parameters and the calculated ratios, under statistical safety conditions ($p < 0.001$). High variability was recorded in the case of leaf area ($CV_{MLA} = 0.360$, $CV_{SLA} = 0.350$), and low variability was recorded in the case of leaf length ($CV_L = 0.150$). The regression analysis conducted to obtain models which described the SLA variation in relation to foliar parameters ($p < 0.001$).
Keywords: *common milkweed, correction factor, invasive alien species, leaf area, modeling*

Introduction

Asclepias syriaca L. is a herbaceous, perennial plant from the Family *Asclepiadaceae (Apocynaceae)*. The species is native to North America, where it occupies large areas, in the ecoregions of southern Canada, and the United States. In its subspontaneous or acclimatized form, it is found in different areas in Europe and Asia Minor (Follak et al., 2021). It is sometimes cultivated as an ornamental plant (Gaertner, 1979).

Since 2017, the species has been included in the list of invasive species and is of interest from this perspective for the European Union (Follak et al., 2021).

As it requires pollinating insects *Asclepias syriaca* depends a lot on habitat conditions (Howard, 2018). *A. syriaca* has been identified in various habitats, in which the primary conditions for perpetuation are found, such as meadows, marshy areas, forest areas, deforested meadows (Lukens et al., 2020).

Depending on the area conditions, isolated plants were identified (initially), in the form of clumps, patches in some meadows, in the form of strips (fences) on the sides of roads, railways, waste storage areas, and areas with uncultivated soils or minimally processed, or in different open habitats (Follak et al., 2018; Szilassi et al., 2021).

Numerous studies have addressed the species *Asclepias syriaca* L. in relation to morphological characteristics, reproduction, distribution and spatiotemporal variability in different European countries, and also in relation to variable habitat types and conditions (Sárkány et al., 2008; Kovács and Pálfalvi, 2012; Pauková et al., 2013; Bartha et al., 2015; Rutkowski et al., 2015; Kaplan et al., 2017; Boršić et al., 2018; Dvirna, 2018; Follak et al., 2018; Dubovik et al., 2021; Zahariev et al., 2021).

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The species *Asclepias syriaca* L. was identified in a variable number of specimens and areas occupied by different agricultural crops and studies and research were carried out regarding the appearance, growth and development of plants in such conditions as well as the behavior of some control measures (Cramer and Burnside, 1982; Hartzler, 2010; Pleasants and Oberhauser, 2013; Shuvar et al., 2021).

At the same time, *A. syriaca* was studied in relation to different ecosystems in order to conserve some insect species (e.g. *Moharch butterfly*) (Pleasants and Oberhauser, 2013; Pleasants, 2017; Pleasants et al., 2017; Pocious et al., 2017).

Also, some studies have evaluated *A. syriaca* in the context of the problems of urban ecosystems, where various other species are considered as evaluation elements of the level of sustainability of these ecosystems (Datcu et al., 2017; MacIvor et al., 2017; Johnston et al., 2019).

Some studies have evaluated morphological, physiological or reproductive aspects of *Asclepias syriaca* regarding seed viability and germination (Kephart et al., 1988; Morgan and Schoen, 1997; Dvirna, 2018).

The present study analyzed and described the leaves geometry of the *Asclepias syriaca* L. species, with the aim to determined the correction factor (CF), very useful for the leaf area calculating based on the leaf parameters (length, width). The obtained results present the importance and practical utility in studies of the leaf area, in the characterization of the *A. siryaca* leaves geometry by non-destructive methods, in the evaluation of the plant growth dynamics, in the promotion of some indicators for the characterization of this species (clones, varieties), in relation to different influencing factors.

Materials and methods

For the characterization of the geometry of the *Asclepias syriaca* L. species leaves, plant samples from the area of Parța locality, Timis County, Romania, were studied.

Leaf sampling

Approximately 200 leaf samples of varying sizes were taken from mature plants, in the flowering stage. The leaves were taken from the basal, middle and apical position of the plants (leaves of variable size and age), from about 50 plants, located at distances between 2 - 5 m. From the basic biological material, 100 leaf samples were randomly analyzed, from different size categories (*Fig. 1*). The simple leaves are opposite, sometimes rounded, broadly ovate lanceolate, with entire edges.

Analyzed elements of leaf geometry

The length and the width of the leaves (L, w) were measured, with a precision of 0.5 mm. The leaves were scanned in a 1:1 ratio (HP CM2320 MFP). The images of the leaves were analyzed with ImageJ (Rasband, 1997) to find the scanned leaf area (SLA) considered as the reference, as a result of the high precision of the determination.

From the analysis of the scanned images for the series of 100 leaves taken in the study, the perimeter of the leaves (Per) was also determined. In practical field studies, in situ, the determination of the leaf area in dynamics, in relation to physiological processes of the plants or to different influencing factors, requires non-destructive methods.

Such a method that leads to the measured leaf area (MLA) is based on dimensional parameters of the leaves (length – L, width – w) and a correction factor – CF (*Eq. 1*).

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$$MLA = L \times w \times CF \quad \text{(Eq.1)}$$

where: L – leaf length; w – leaf width; CF – correction factor.

(a) (b) (c)

Figure 1. *Different size categories of the leaf samples studied, Asclepias syriaca L. species; (a) small size leaf; (b) average size leaf; (c) large size leaf*

In order to achieve high accuracy, it is very important to use a correction factor with a specific value for the considered species. To find out the CF value in the case of the species *Asclepias syriaca* L., taken in the study, the model proposed by Sala et al. (2015) was used.

To characterize the geometry of the leaves, various ratios between leaf parameters were additionally calculated, such as: L/w, Per/L, Per/w, SLA/Per.

Statistical analysis of the data

For the processing and interpretation of the data series obtained, for each leaf parameter, index, and ratio, appropriate mathematical and statistical methods and tools were used (Descriptive Statistics, Correlation Analysis, Regression Analysis).

Appropriate statistical safety parameters were used in relation to each element and statistical analysis performed (p, r, R^2). The RMSEP parameter (*Eq. 2*), was additionally used to certify the accuracy in determining the correction factor (CF), in relation to the MLA.

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

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For data analysis and processing, as well as for the generation of graphs, appropriate software was used, PAST (Hammer et al., 2001), Wolfram Alpha (2020), and JASP (2022).

Results and discussions

Based on the determinations made on the 100 leaf samples, values of the parameters taken into account in order to characterize the geometry of the leaves of the *Asclepias syriaca* L. species were obtained. In the case of leaf length (L), values between 9.00 and 20.80 ± 0.25 cm were recorded, and in the case of leaf width (w), values between 2.80 and 9.50 ± 0.15 cm were recorded. The perimeter of the leaves (P) recorded values between 20.90 and 51.58 ± 0.65 cm. For the scanned leaf area (SLA), values between 18.53 and 143.08 ± 2.81 cm^2 were recorded, and in the case of the measured leaf area (MLA), values between 18.65 - 146.22 ± 2.90 cm^2 were recorded (*Table 1*).

Table 1. *Descriptive statistics for parameters studied in the leaves of Asclepias syriaca L.*

Statistical parameter	L	w	Per	SLA	MLA	L/w	Per/L	Per/w	SLA/Per
	(cm)			(cm^2)		(ratio)			
Valid	100	100	100	100	100	100	100	100	100
Missing	0	0	0	0	0	0	0	0	0
Minimum	9.00	2.80	20.90	18.53	18.65	2.065	2.269	5.147	0.886
Maximum	20.80	9.50	51.58	143.08	146.22	3.307	2.522	7.502	2.774
Median	16.30	6.80	40.18	84.79	81.27	2.481	2.428	6.006	2.072
Mean	16.19	6.60	39.30	81.33	81.63	2.506	2.423	6.058	2.003
Std. error of mean	0.25	0.15	0.65	2.81	2.90	0.026	0.005	0.050	0.041
Coefficient of variation	0.15	0.23	0.17	0.35	0.36	0.105	0.022	0.083	0.202
Variance	5.99	2.26	42.79	788.34	839.94	0.069	0.003	0.251	0.164
Shapiro-Wilk	0.98	0.99	0.98	0.99	0.99	0.948	0.980	0.952	0.978
P-value of Shapiro-Wilk	0.12	0.35	0.17	0.45	0.43	< .001	0.133	0.001	0.095
25th percentile	14.81	5.64	35.49	62.82	62.87	2.29	2.39	5.67	1.76
50th percentile	16.30	6.80	40.18	84.79	81.27	2.48	2.43	6.01	2.07
75th percentile	17.63	7.60	42.90	94.73	96.68	2.65	2.46	6.33	2.26

L – leaf length; w – leaf width; Per – perimeter; SLA – scanned leaf area; MLA – measured leaf area; L/w – leaf length/width ratio; Per/L – perimeter/leaf length ratio; Per/w – perimeter/leaf width ratio; SLA/Per – scanned leaf area/perimeter ratio

Values of the ratio between different biometric elements of the leaves (L/w, Per/L, Per/w, SLA/Per) were also calculated, and the obtained data are presented in *Table 1*. The graphic distribution, in the form of a box-plot, of the data series for the foliar parameters studied in the leaves of the species *Asclepias syriaca* L. are shown in *Figure 2*.

For the determination of the leaf area, the non-destructive methods, based on the dimensional parameters of the leaves (L, w), are of interest because they allow the calculation of the leaf surface in situ with a sufficiently good precision, without expensive equipment, and in conditions where the leaves are not detached from the plants, thus being able to be monitored evolutionarily, during the vegetation period.

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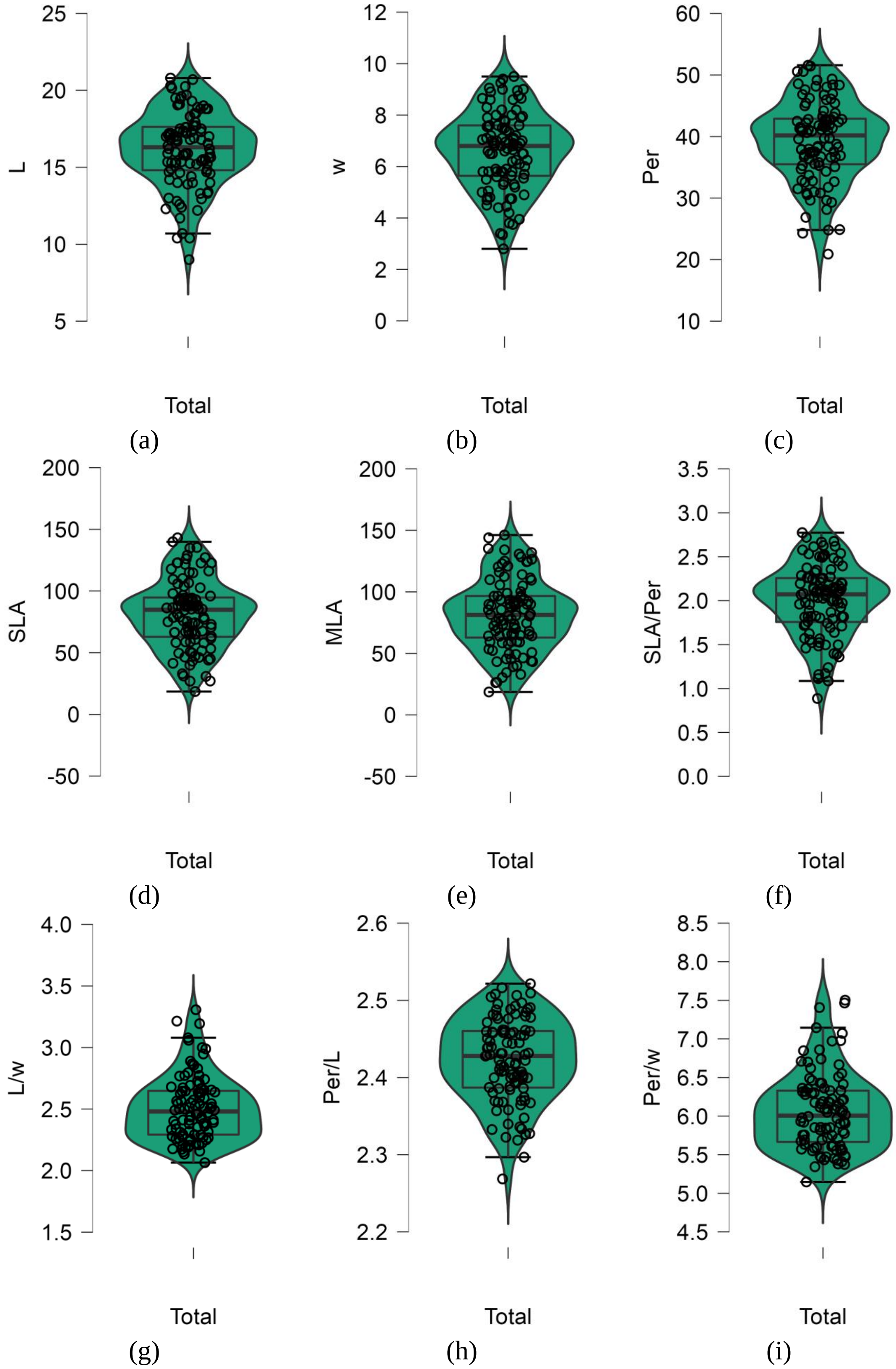

Figure 2. *The graphic distribution of the data series for leaf parameters in the Asclepias syriaca L. species. (a) L – leaf length; (b) w – leaf width; (c) Per – leaf perimeter; (d) SLA – scanned leaf area; (e) MLA – measured leaf area; (f) SLA/Per ratio; (g) L/w ratio; (h) Per/L ratio; (i) Per/w ratio*

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For the purpose of a high precision of calculation of the leaf area, the use of the correction factor (CF) with a specific value is very useful. Based on the model proposed by Sala et al. (2015), the value of the correction factor was determined for the *Asclepias syriaca* L. species. The value of CF = 0.74 was obtained under statistical safety conditions, assessed on the basis of the minimum error (ME) between SLA (considered as reference) and MLA calculated on the basis of the obtained CF value (*Eq. 1*). At the same time, the parameter RMSEP = 2.64540 (*Eq. 2*), resulting from the series of considered values, confirmed the respective value for the correction factor CF = 0.74 (*Table 2; Fig. 3*).

Table 2. *Values for the measured leaf area in relation to correction factor and considered statistical safety parameters*

CF	SLA	MLA	ME	RMSEP
	(cm^2)	(cm^2)	(cm^2)	(cm^2)
0.69		76.12	-5.21	5.83435
0.70		77.22	-4.11	4.81382
0.71		78.32	-3.01	3.87862
0.72		79.43	-1.90	3.10679
0.73		80.53	-0.80	2.66115
0.74	81.33	81.63	0.30	2.64540
0.75		82.74	1.41	3.14688
0.76		83.84	2.51	3.93213
0.77		84.94	3.61	4.87421
0.78		86.05	4.72	5.89846
0.79		87.15	5.82	6.96874

CF – correction factor; SLA – scanned leaf area; MLA – measured leaf area; ME – mean of errors; RMSEP – root mean square error of prediction

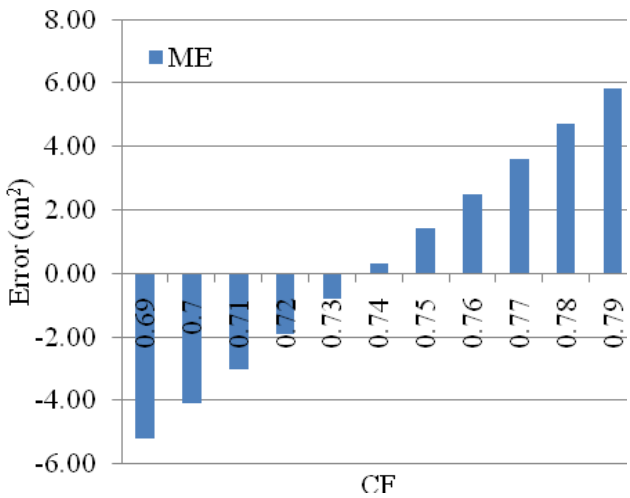

Figure 3. *Minimum error values as the difference between measured leaf area and scanned leaf area related to correction factor values, Asclepias syriaca L. species*

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Additionally, the degree of fit between MLA (calculated by CF = 0.74) and SLA (considered as reference) was analyzed. The obtained linear equation (*Eq. 3*) described with high statistical certainty the match between MLA and SLA ($R^2 = 0.992$, $p < 0.001$). The graphic distributions of MLA values according to SLA, and of the fitting line, are represented in *Figure 4*.

$$MLA = 1.028x - 1.994 \qquad \text{(Eq.3)}$$

where: *x* – SLA values.

To evaluate the relationships between leaf parameters (L, w, Per), scanned leaf area or obtained by measurement (SLA, MLA), as well as the different calculated ratios (L/w, Per/L, Per/w, SLA/Per) in the studied species (*Asclepias syriaca* L.), correlation analysis was used. Different levels of correlation were recorded between the considered elements of leaf geometry, under statistical safety conditions, $^{***}p < 0.001$ (*Table 3*). The closest interdependence relationships between the analyzed parameters are shown in *Figure 5*.

Table 3. *Correlation table*

Variable		**L**	**w**	**Per**	**SLA**	**MLA**	**L/w**	**Per/L**	**Per/w**	**SLA/Per**
		(cm)			**(cm²)**		**(ratio)**			
L	Pearson's r	—								
	p-value	—								
w	Pearson's r	0.964	—							
	p-value	< .001	—							
Per	Pearson's r	0.996	0.981	—						
	p-value	< .001	< .001	—						
SLA	Pearson's r	0.978	0.986	0.990	—					
	p-value	< .001	< .001	< .001	—					
MLA	Pearson's r	0.976	0.99	0.986	0.996	—				
	p-value	< .001	< .001	< .001	< .001	—				
L/w	Pearson's r	-0.789	-0.907	-0.830	-0.841	-0.845	—			
	p-value	< .001	< .001	< .001	< .001	< .001	—			
Per/L	Pearson's r	0.717	0.844	0.776	0.802	0.79	-0.931	—		
	p-value	< .001	< .001	< .001	< .001	< .001	< .001	—		
Per/w	Pearson's r	-0.791	-0.907	-0.826	-0.836	-0.845	0.995	-0.894	—	
	p-value	< .001	< .001	< .001	< .001	< .001	< .001	< .001	—	
SLA/Per	Pearson's r	0.975	0.990	0.989	0.990	0.982	-0.887	0.835	-0.882	—
	p-value	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	—

L – leaf length; w – leaf width; Per – perimeter; SLA – scanned leaf area; MLA – measured leaf area; L/w – leaf length/width ratio; Per/L – perimeter/leaf length ratio; Per/w – perimeter/leaf width ratio; SLA/Per – scanned leaf area/perimeter ratio

Based on the calculated coefficient of variation (CV), the variability within each data set for the considered parameters was evaluated. The highest level of variability was recorded in the case of the measured leaf area ($CV_{MLA} = 0.360$) and the scanned leaf area ($CV_{SLA} = 0.350$).

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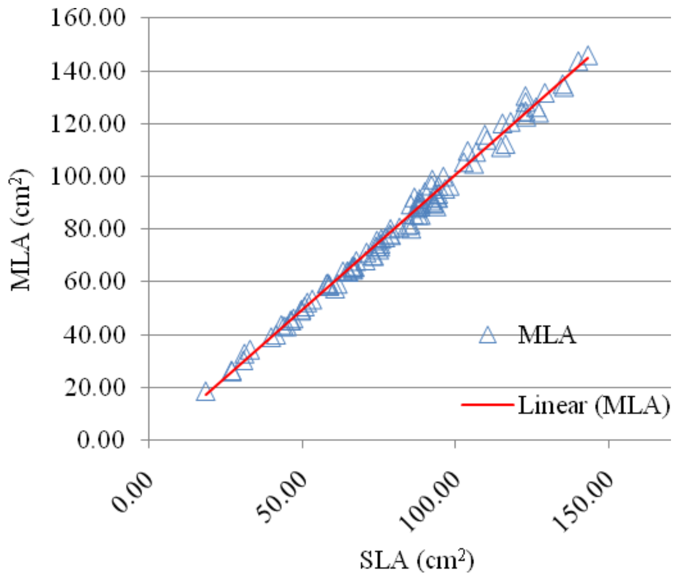

Figure 4. *The graphic distribution of measured leaf area values in relation to scanned leaf area and the fitting line, the Asclepias syriaca L. species*

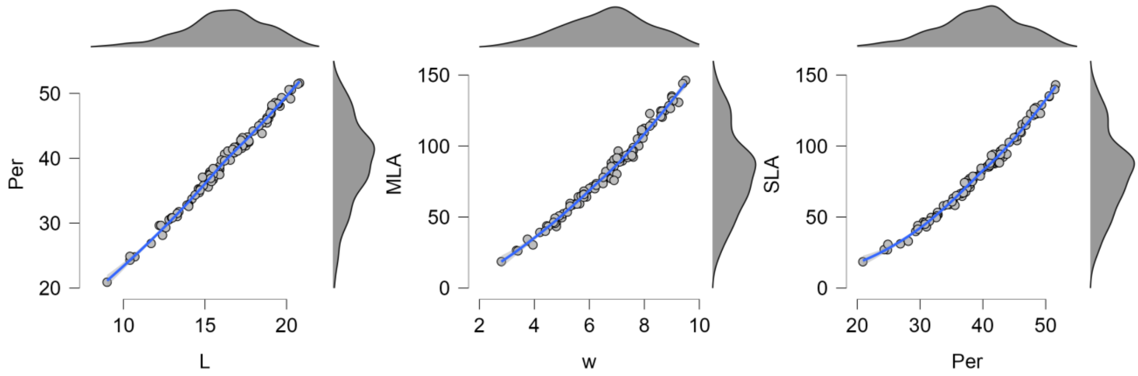

Figure 5. *Examples for the closest interdependence relationships between leaf parameters studied in Asclepias syriaca L. L – leaf length; w – leaf width; Per – perimeter; SLA – scanned leaf area; MLA – measured leaf area*

Low level of variability was recorded in the case of leaf length (CVL = 0.150). The graphic representation of the data series, which expresses the variability in the form of a matrix plot (*Fig. 6*). Regarding the calculated ratios, a low level of variability was recorded in the case of Per/L ($CV_{Per/L}$ = 0.022), and a high value was recorded in the case of SLA/Per ($CV_{SLA/Per}$ = 0.202).

The regression analysis was used to evaluate the interdependence relationship of some parameters at the level of the leaves of the species *Asclepias syriaca* L. For the safety and high precision of the calculations, the values of the coefficients of the obtained equations had up to 16 decimal.

The variation of SLA in relation to L and w, as a direct relationship and interaction of the two-dimensional leaves parameters, was described by *Equation 4*, under statistical safety conditions (R^2 = 0.999, p < 0.001). The graphic distribution of SLA in

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relation to L and w is presented as a 3D model in *Figure 7a*, and in the form of isoquants in *Figure 7b*.

$$SLA = ax^2 + by^2 + cx + dy + exy + f \tag{Eq.4}$$

where: SLA – scanned leaf area; *x* – leaf length (L); *y* – leaf width (w); a, b, c, d, e, f – coefficients of *Equation 4;* a = -0.50516384; b = -2.76674045; c = 1.37749581; d = -3.01243677; e = 3.09035970; f = 0.

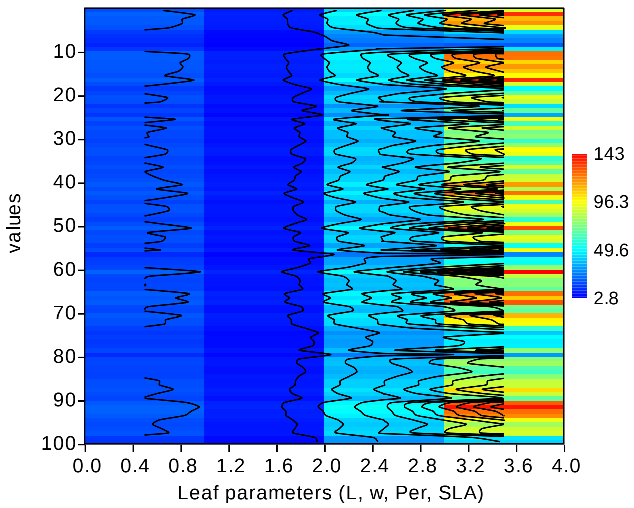

Figure 6. *The graphic representation in matrix plot format of the data series for leaf parameters in the Asclepias syriaca L. species; high variability for SLA, right side. L – leaf length; w – leaf width; Per – perimeter; SLA – scanned leaf area; MLA – measured leaf area*

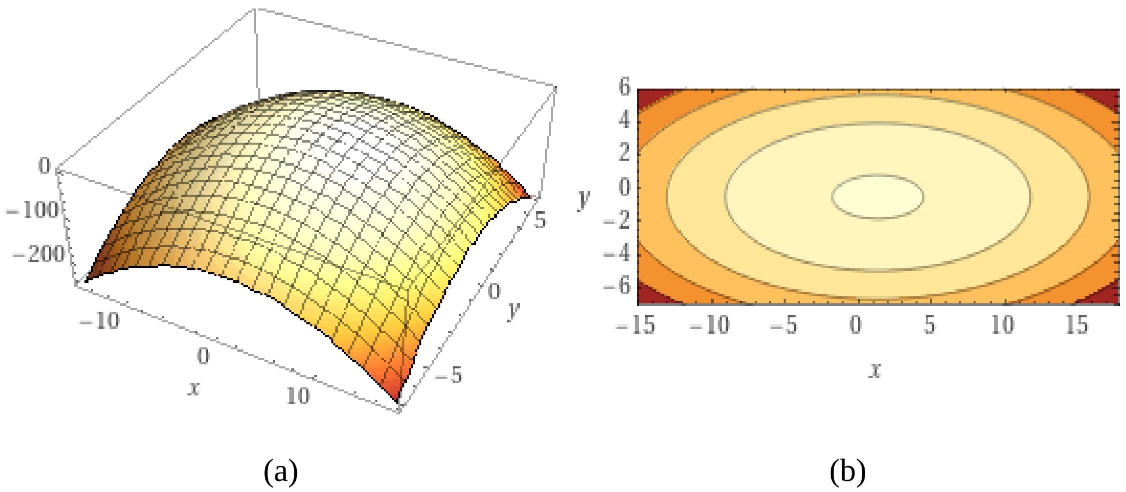

Figure 7. *Scanned leaf area variation in relation to leaf length (x-axis) and leaf width (y-axis) in the Asclepias syriaca L. species; (a) 3D model, (b) model in the form of isoquants*

The variation of SLA in relation to L and Per, as a direct and interaction contribution to the leaf samples studied, the *Asclepias syriaca* L. species, was described by *Equation 5* under conditions of $R^2 = 0.999$, $p < 0.001$. The graphic representation of

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SLA distribution in relation to L and Per is shown in the form of a 3D model in *Figure 8a* and in the form of isoquants in *Figure 8b*.

$$SLA = ax^2 + by^2 + cx + dy + exy + f \quad \text{(Eq.5)}$$

where: SLA – scanned leaf area; *x* – leaf length (L); *y* – leaf perimeter (Per); a, b, c, d, e, f – coefficients of *Equation 5;* a = -4.47643302; b = -0.63496765; c = -0.60301073; d = 0.18876967; e = 3.51370358; f = 0.

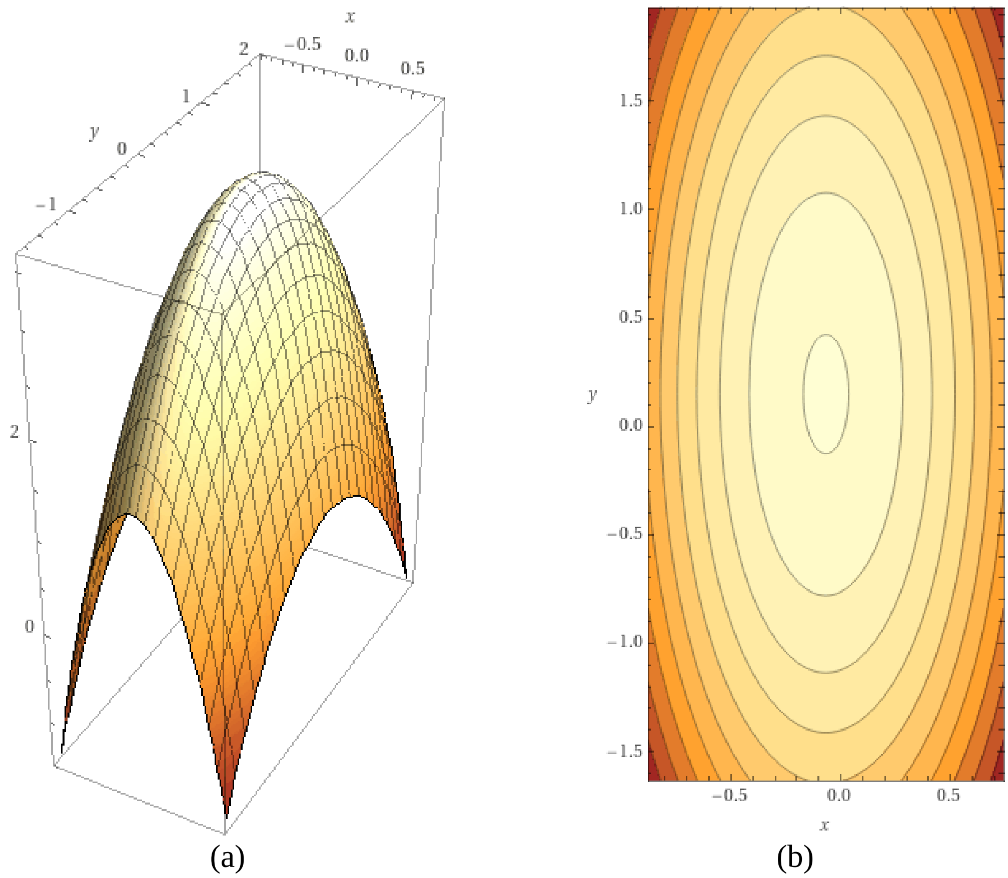

Figure 8. *Scanned leaf area variation in relation to leaf length (x-axis) and perimeter (y-axis) in the Asclepias syriaca L. Species; (a) 3D model, (b) model in the form of isoquants*

The variation of SLA in relation to w and Per, as a direct contribution and interaction of the two leaf parameters, in the leaf samples studied, the *Asclepias syriaca* L. species, was described by *Equation 6* under conditions of $R^2 = 0.999$, $p < 0.001$. The graphic representation of SLA variation in relation to w and Per is shown in the form of a 3D model in *Figure 9a* and in the form of isoquants in *Figure 9b*.

$$SLA = ax^2 + by^2 + cx + dy + exy + f \quad \text{(Eq.6)}$$

where: SLA – scanned leaf area; *x* – leaf width (w); *y* – leaf perimeter (Per); a, b, c, d, e, f – coefficients of *Equation 6*; a = -2.69169792; b = -0.04919706; c = 0.60868238; d = -0.08847775; e = 1.04908288; f = 0.

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The leaves of *A. syriaca* was described as opposite, long, the leaf blade ovate or elliptical, with variable sizes, 10-26 cm long, respectively 4-18 cm wide, in the case of mature leaves (Gudžinskas et al., 2019).

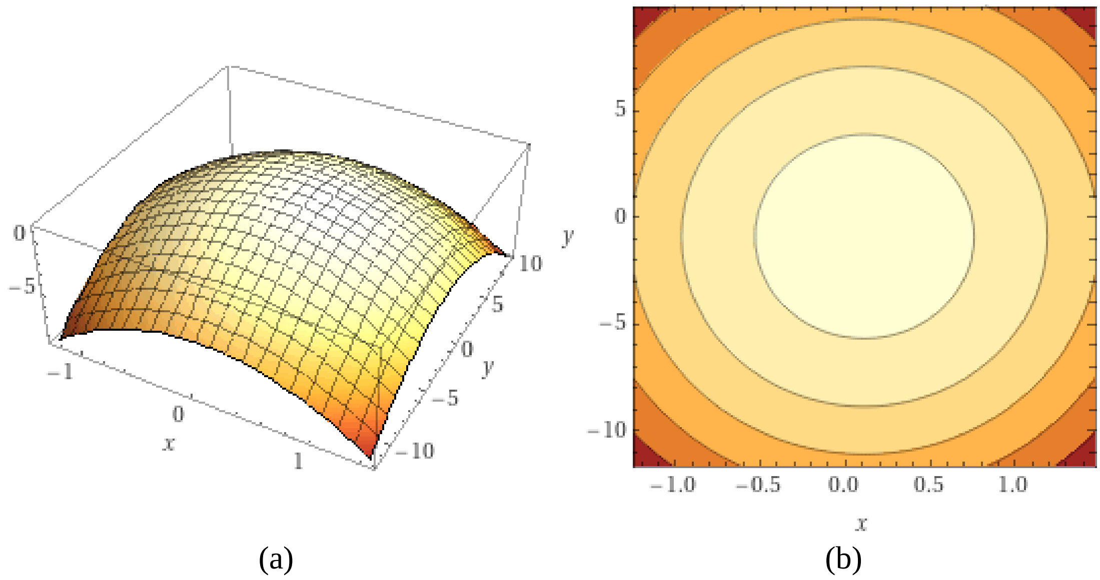

Figure 9. *Scanned leaf area variation in relation to leaf width (x-axis) and perimeter (y-axis) in the Asclepias syriaca L. species; (a) 3D model, (b) model in the form of isoquants*

Some studies have addressed phylogenetic aspects in relation to the function and certain traits with a functional role of the leaf surface in *Asclepias* (Agrawal et al., 2009, 2015). Leaf shape and size were studied for the purpose of comparative analyzes with other related species (Gudžinskas et al., 2019).

The leaves of *A. syriaca* were studied in relation to the photosynthetic processes determined by leaf injury and the recovery period (Delaney et al., 2008), in relation to the profile of some bioactive compounds with a defense role (Agrawal, 2005; Sánchez-Gutiérrez et al., 2020; Follak et al., 2021). The non-destructive methods for plants leaves studies, some as an application on mobile devices, are of interest for leaf area determination, for the leaf surface level affected by stress factors, in the evaluation of some physiological processes, the evaluation of the plants relationship with ecological factors etc. (Schrader et al., 2017; Suárez et al., 2022).

From another perspective, the antioxidant capacity of some extracts of *A. syriaca* (based on water and methanol) and the influence of these extracts on seed germination, growth and development of corn, soybean and sunflower seedlings were tested (Popov et al., 2021).

In the context of the current interest in *A. syriaca*, for the study of the species from individuals to plant communities, in relation to habitat conditions and influencing factors, with vegetation dynamics, or other objectives, the value of the correction factor (CF = 0.74) determined by the present study can be useful for high precision in finding the leaf surface based on the dimensional parameters (L, w). Also, other aspects of the geometry of the leaves described by the present study can be useful in evaluating the status of the plants under normal growth conditions, in relation to the habitat conditions, or in response to different stress factors.

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Conclusions

The study facilitated the description of the leaf geometry of the *Asclepias syriaca* L. species, based on some leaf parameters (L, w, Per), the scanned and measured leaf area (SLA, MLA), as well as some calculated ratios (L/w, Per/L, Per/w, SLA/Per).

For high precision in the MLA calculation, the value of the specific correction factor (CF) was determined for the typology of *A. syriaca* leaves, in the amount of CF = 0.74. The statistical certainty was given by the value of the minimum error (ME), as the difference between MLA and SLA, and by the RMSEP parameter, values related to some calculations for series of CF values.

The regression analysis facilitated the obtaining of equation-type models, which described the variation of SLA in relation to foliar parameters in conditions of statistical safety ($p < 0.001$).

The obtained values can be used for different studies on *A. syriaca* in relation to habitat conditions, genotypes, and stress factors.

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