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The Effect of Solar Radiation on the Structural and Functional Changes of the Photosynthetic Apparatus in Plants

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Abstract

The impact of solar radiation as well as other factors on plants can be analyzed by changes in the emitted fluorescence of leaves, diffuse reflectance and their color. Diffuse reflectance values allow the determination of parameters that evaluate the activity of the photosynthetic apparatus. These spectra allow determination of leaf color with x and y coordinates brightness, luminance as well as "dominant" wavelength. The emission and reflection spectra, as well as the values of certain ratios, show the characteristics and differences between the analyzed leaves, demonstrating structural and functional changes of the photosynthetic apparatus as a result of adaptation to the environment. All parameters make it possible to distinguish between different varieties of pear and to evaluate the influence of growing conditions on the photosynthetic apparatus.

Keywords: Reflection spectra; Chl a, Chl b, Brightness-Y, PRI.

Introduction

The main photoreceptor in the chloroplasts of higher plants is his chlorophyll. Molecules that have the ability to absorb light are called photosynthetic pigments. The color of the pigment is determined by the wavelength of the reflected light. Some of the pigments manage to absorb all wavelengths of light, while others manage to absorb only some wavelengths and reflect those wavelengths that they do not absorb. The green pigment, (chlorophyll) which is prevalent in all photosynthetic cells, absorbs all wavelengths of visible light except green. In plants the pigment that can directly convert light energy into chemical energy in plants is chlorophyll a. In higher plants, in addition to chlorophyll a, there are also chlorophyll b and carotenoids. Carotenoids are red, orange or yellow pigments. Chlorophyll b and carotenoids absorb light at different wavelengths compared to what chlorophyll a absorbs.

This adaptation response to high irradiance refers to grow and development of the entire plant, to leaves, cells, and particularly to the structure, thylakoid arrangement, and photosynthetic function of chloroplasts, ′(Babani F & Lichtenthaler HK, 1996; Anderson J.M, Chow W.S, Park Y.-I, 1995)′. Consecpuence of this a leaf developed under full sunlight (sun leaf) has a higher photosynthetic activity when receiving high irradiance as compared to a leaf that developed in the shade (shade leaf). Plant adaptations to different exposed light environment during their growth affect development of the entire plant and particularly to chloroplasts and their structure, thylakoid arrangement as well as the relative amounts of the photosynthetic pigments, the chlorophylls and carotenoids. As a result, reflectance signals of leaves contain the essential information used for monitoring of plants, from contact measurements of leaves to remote sensing of land vegetation, ′(Babani F& Lichtenthaler HK, 1996; Evert R.F, Russin W.A, Bosabalidis A.M, 1996)′. In this study we present reflectance spectra of single leaves, acquired in a sequence of four spectral bands from the visible to the near infra-red which are of basic interest for leaf evaluation: blue (440 nm), green (550 nm), red (680 nm), and near infra-red (800 nm). During the hot and dry summer season, plants are under the effect of various stresses such as high lighting, high temperature and lack of water. Transmission and reflection spectroscopy are used for the non-destructive assessment of chlorophyll in leaves. Reflectance in the spectral zone around 700nm is the best indicator of chlorophyll and for this the ratios R750/R700 and (R750-R705)/(R750+R705) are used. From these investigations, new vegetation indices were developed using reflectance that correspond to wavelengths with maximal and minimal sensitivity to variation in chlorophyll content. Maximal sensitivity of reflectance to chlorophyll content was found near 550 nm and 700 nm. Two new indices were established on the basis of these two reflectance signatures, which are directly proportional to the chlorophyll content, ′(Lichtenthaler, HK., Wenzel, O., Buschmann, C., Gitelson, A.A. 1998)′. Although a leaf contains of cuticle and the palisade mesophyll cells and appears optically complicated. The relationship between reflectance and leaf chlorophyll content is non-linear, ′(Gitelson, A.A., Merzlyak, M.N., Chivkunova, O.B. 2001)′. The thicker sun leaves and needles in all tree species were characterized by a lower specific leaf area, lower water content, higher total chlorophyll (Chl) a+b and total carotenoid (Cars) content per leaf area unit, as well as higher values for the ratio Chl a/b compared to the much thinner shade leaves and needles that possess a higher Chl a+b and Cars content on a dry matter basis and higher values for the weight ratio Chls/Cars, ′(Lichtenthaler HK., Ac A, Marek MV., Kalina J., Urban O. 2007)′.

Material and Methods

Plants. Measurements were made with leaves selected in three types of positions (sun - southern part of the crown, blue shade - northern part and semi-shade/shade - inside a tree crown) for the variety *Santa Maria* (pear) part of a group. of the *Pyrus Communis L* (pear) species and the rose family. The study was conducted in two periods, May and July, in an area with a lack of water, called area 2. The Mediterranean climate prevails with average annual temperatures around 16°C, and low humidity.

Pigment determination. Leaf pigments were extracted with 100% acetone in one circular piece of 9mm in diameter cut from the leaves using a mortar. The pigment extracts were centrifuged for 5 min at 500 X g in glass tubes to obtain the fully transparent extract. The pigment contents, Chl a, Chl b and total carotenoids, were determined spectrophotometrically from acetone extract using the extinction coefficients and equations re-determined by Lichtenthaler, ′(Lichtenthaler, 1987; Lichtenthaler and Buschmann, 2001)′. The represented values are the mean of six determinations from six leaves.

Reflectance spectra. Leaf reflectance (R) was recorded from upper side of the leaf in a spectral range from 400nm to 800nm with a spectral resolution of 2nm with a spectrophotometer equipped with an integrating sphere attachment, ′(Bushmann et al., 2012; Gitelson et al., 2003)′. Leaf reflectance spectra were recorded against barium sulphate as a white reference standard. Leaves were placed on black velvet used as a background which has a reflectance less than 0.5% over the spectral range of measurements. Reflectance (R) was represented as the ratio of the radiation intensities reflected by the leaf sample and the white standard respectively. The leaf spectra were taken in the intercostal fields between the larger leaf veins. These spectra represent an integrated signal over several square centimetres.

Photochemical index (PRI). The photochemical index of diffuse reflectance serves as a photosynthetic indicator of radiation utilization efficiency, ′(Gamon JA, Serrano L, Surfus JS, 1997)′. The values of the photochemical index of diffuse reflectance fluctuate in the range from -1 to 1. The formula for determining the PRI is:

$$PRI = \frac{R531 - R570}{R531 + R570}$$

The photochemical index of diffuse reflectance (PRI) depends on photosynthetic (leaf) pigments, the amount of energy falling from the sun on the surface, the angle of the sun's rays falling on the leaf surface and the water content.

Colorimetry. Evaluation of the visual impression of a leaf sample was assessed by the chromaticity coordinates in the CIE 1931 color space which allow defining quantitative links among wavelengths in the electromagnetic visible spectrum and physiological perceived colors in human color vision [2]. In order to help to assess the visual impression of a sample, the reflectance spectra of the leaf samples were used to define the color as x and y chromaticity coordinates in the CIE 1931 color space, a colorimetric standard widely used in the textile and coating industries, ′(Malacara D, 2002)′. The coordinates x and y, which define a visual color in the CIE 1931 color space chromaticity diagram, were determined using the reflectance data and the color matching functions for daylight illumination (D65). Furthermore, we determined the brightness (values between 0 = dark and 100 = completely bright), the dominant wavelength (the wavelength characteristic for the color of the sample determined by the intersection point with a curved outer boundary line, also called spectrum locus, of the line connecting the achromatic point, i.e., "white" with x = y = 0.33, and the detected color point), and the color saturation (percentage of distance of the color point between the achromatic point and the boundary line: 100% at the spectrum locus, 0% at the achromatic point).

Results

Photosynthetic pigments. The ratios of the photosynthetic pigments, Chl a/b and (a+b)/(x+c), that reflect the light adaptation of the photosynthetic apparatus (Lichtenthaler 2013) show different values in the three leaf types. In sun leaves the mean values of the ratio Chl a/b are higher as compared to blue-shade and shade leaves (Tab. 1). Sun leaves displayed lower values of the ratio (a+b)/(x+c) as compared to two other leaf types (Tab. 1). In the period May-July, the highest values of chlorophyll concentration are presented in the south position. The highest values of Chl a+b and Chl a/b of the variety Santa Maria (pear) are present in the period of May. The reason is related to the optimal influence of solar radiation on the photosynthetic activity of plants.

Table 1: *Levels of Chl a+b and total carotenoids (x+c) per leaf area unit as well as the pigment ratios Chl a/b and chlorophylls (a+b) to carotenoids (a+b)/(x+c) between sun, blue-shade, shade/half-shade leaves of Santa Maria variety trees, above water, area 2. Mean values of 6 determinations per leaf-type.*

Leaf-type	**Chl a+b (mg dm^{-2})**	**Chl a/b**	**(a+b)/(x+c)**
***Santa Maria* - May**			
Sun	8.865 ± 0.028	2.94	4.64
Blue-shade	6.092 ± 0.019	2.85	4.637
Shade	5.073 ± 0.015	2.46	4.94
***Santa Maria* -July**			
Sun	7.868 ± 0.078	2.45	5.39
Blue-shade	5.630 ± 0.050	2.38	5.56
Shade	4.339± 0.079	2.21	6.23

Reflectance spectra. Reflectance spectra of the three types of leaves of both pear varieties exhibited a higher reflectance between 500nm and 650nm, in the green-to-orange range of the spectrum, and mainly at

Matilda Mema / Afr.J.Bio.Sc. 6(5) (2024). 4184-4194

wavelengths between 680nm and 740nm in the near infra-red. In addition reflectance spectra exhibited a low reflectance between 400nm and 500nm in blue part of visible spectra and also near 680nm in red part of visible spectra (Figure 1). The observed variations correspond to the absorption region of the in-vivo chlorophyll bands.

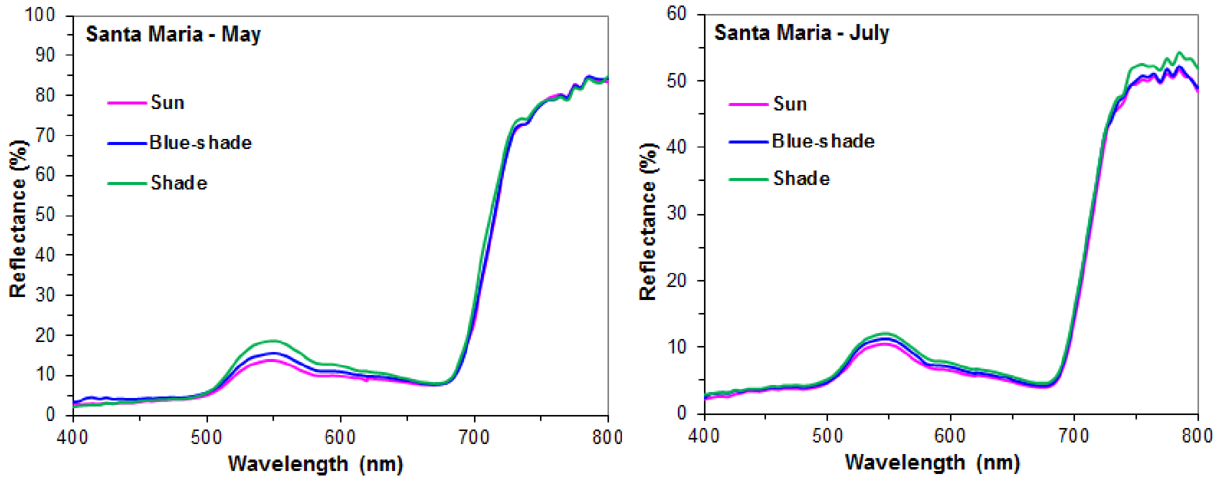

Figure 1: *Reflectance spectra of the sun (south part), blue-shade (north part) and shade/half shade leaves of Santa Maria pear variety on May-July, area 2. Mean of 6 reflectance spectra per leaf-type.*

The reflection spectra for variety exhibit the highest value in the green-orange range of the spectrum of shade leaves compared to two other leaf types. Also, could be observed a blue shift of the "red edge" (inflection point of the rise of signal at wavelengths between 680nm and 740nm) towards shorter wavelengths to the shade leaves. These variations among three types of analysed leaves are related to the chlorophyll content being lower in shade leaves and higher in sun leaves (Table 1)

Table 2: *Levels of reflectance between sun, blue-shade, shade/half-shade leaves of Santa Maria variety trees in periods May- July, area 2. Mean values of 6 determinations per leaf-type.*

Leaf-type	**R550**	**R700**	**R750**	**R800**
Santa Maria - May				
Sun	13.7 ±0.52	23.1	77.8	83.4
Blue-shade	15.5 ±0.63	24.9	78.0	84.2
Shade	18.6 ±0.61	27.8	78.1	84.8
Santa Maria - July				
Sun	10.4 ±0.11	13.8	49.5	48.4
Blue-shade	11.2 ±0.54	14.8	49.9	49.0
Shade	11.9 ±0.83	15.5	52.1	51.9

The highest value of diffuse reflection, R550 Santa Maria variety (pear) presents in the period of May (Table 1). In the two periods: May and July, the highest values are presented in the shadow position compared to the other two positions. The high values of the R750/R550 ratio are presented in the period of May (Table 2).

Table 3: *Levels of reflectance ratios between sun, blue-shade, shade/half-shade leaves of Santa Maria variety trees in periods May- July, area 2. Mean values of 6 determinations per leaf-type.*

Leaf-type	**R750/R800**	**R750/R700**	**R750/R550**	**R800/R550**
***Santa Maria* - May**				
Sun	0.93	3.36	5.66	6.06
Blue-shade	0.92	3.11	5.00	5.42
Shade	0.92	2.80	4.19	4.55
***Santa Maria* - July**				
Sun	1.02	3.58	4.76	4.66
Blue-shade	1.01	3.36	4.46	4.38
Shade	1.00	3.35	4.36	4.34

Photochemical index (PRI). The highest values of the photochemical index (PRI), are presented in July, a period characterized by high temperatures. In the two periods under study, the highest values are presented in the south (sun) position the Santa Maria variety (pear) (Figure 2).

Table 4: *PRI values for Santa Maria (Pear) variety in periods May-July, area 2*

Leaf-type	**PRI**
***Santa Maria* - May**	
Sun	0.041
Blue-shade	0.038
Shade	0.031
***Santa Maria* - July**	
Sun	0.091
Blue-shade	0.079
Shade	0.069

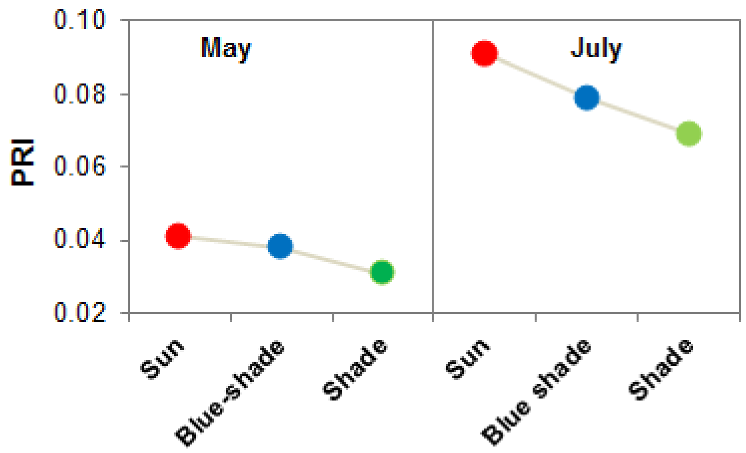

Figure 2: *Presentation of the photochemical index (PRI) values of the leaves in three positions; sun, blue - shade and shade of Santa Maria variety, in periods May-July, area 2*

Colorimetry. In the study, from the diffuse reflectance values, in the three positions, some chromatic parameters can be determined which are: chromatic coordinates x and y, "dominant" wavelength, luminance and Y-brightness based on the algorithm of the CIE 1931 system, for variety Santa Maria (pear), area 2.

The highest values of the "dominant" wavelength are presented for the leaves in the shade position, compared to the leaves in the other two positions, for the May-July period. In the two periods under study, the highest values are presented in May. The "dominant" wavelength depends on the concentration of chlorophylls. The dependence between them is oblique, which means that the leaves in the shade position have a lower concentration of chlorophylls, but a higher "dominant" wavelength value. It is also noticed that the values of luminosity and "dominant" wavelength present lower values in the shade position, compared to the other two positions. The dependence of luminosity and "dominant" wavelength is linear from south to shadow in the two periods under study.

Table 5: *Colorimetric determination according CIE 1931 for the leaf samples: sun, blue-shade, shade/half-shade leaves of Santa Maria variety trees, in periods May- July, area 2. Mean values of 6 determinations per leaf-type.*

Leaf-type	x-coordinate	y-coordinate	Brightness -Y	Dominant wavelength (nm)	Limonsity (%)
Santa Maria - May					
Sun	0.37	0.45	10.5	561.9	46.1
Blue-shade	0.37	0.45	11.8	562.9	50.5
Shade	0.37	0.48	13.7	563.2	53.8
Santa Maria - July					
Sun	0.33	0.44	7.76	552.0	30.9
Blue-shade	0.33	0.44	8.36	552.7	33.4
Shade	0.33	0.44	8.99	555.0	34.9

For three calculated parameters: "dominant" wavelength, luminosity and brightness -Y, in the two periods under study, the highest values are presented in May, south position, for the Santa Maria variety, area 2 (Table 5). The explanation is related to the fact that the Santa Maria variety (pears) in this period presents a higher development of the photosynthetic apparatus than in July.

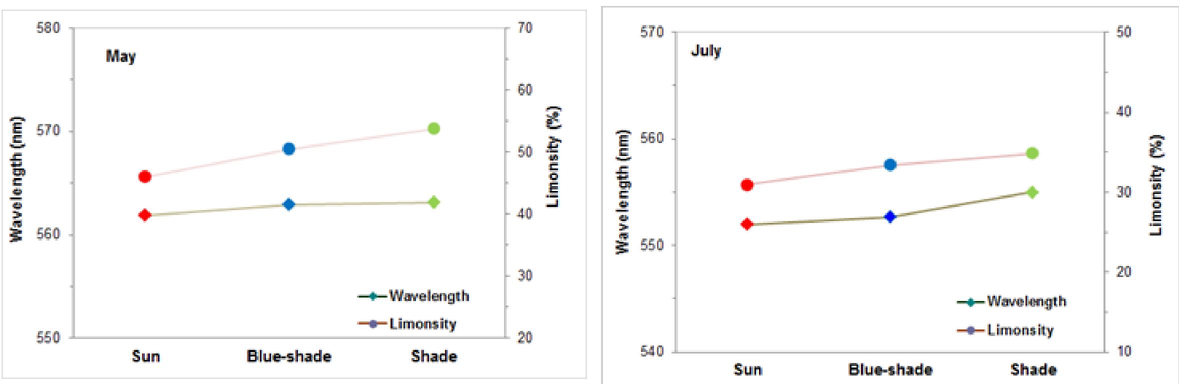

Figure 3: *Presentation of the values of the "dominant" wavelength, luminosity , of the leaves in three positions; sun, blue -shade and shade of Santa Maria variety, in periods May-July, area 2*

The reason is related to the structure of the leaf, which begins to lose its luster in color as a result of the action of environmental stress, such as solar radiation, with very high temperatures.

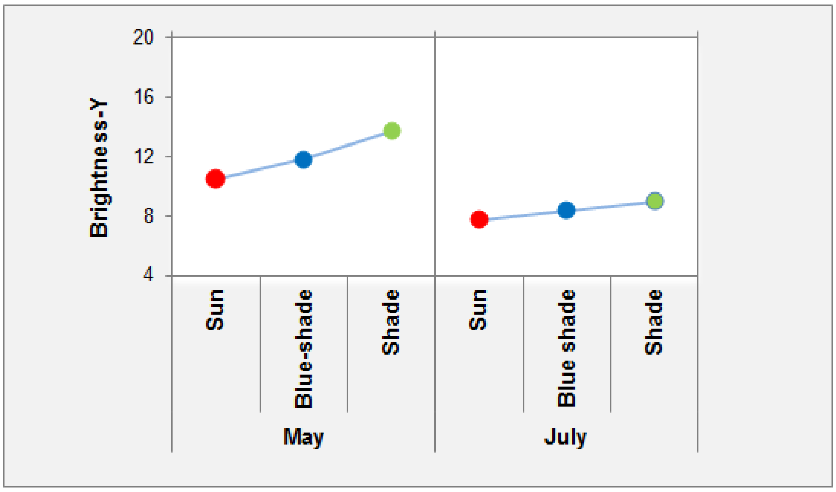

Figure 4: *Presentation of the values of the Brightness-Y, of the leaves in three positions; sun, blue -shade and shade of Santa Maria variety, in periods May-July, area 2*

The influence of solar radiation is also reflected in the change in leaf thickness (Table 6).

Table 6: *Thickness values for Santa Maria (Pear) variety in periods May-July, area 2*

Leaf-type	**Thickness (mm)**
***Santa Maria* - May**	
Sun	0.342 ± 0.013
Blue-shade	0.295 ± 0.013
Half-shade/shade	0.267 ± 0.010
***Santa Maria* - July**	
Sun	0.336 ± 0.025
Blue-shade	0.297 ± 0.022
Half-shade/shade	0.273 ± 0.018

Shade and low light leaves are thinner and have a larger average surface area than sun or high light leaves. The total content of chlorophylls and their carotenoids per unit leaf area is significantly lower than in sun or high light leaves.

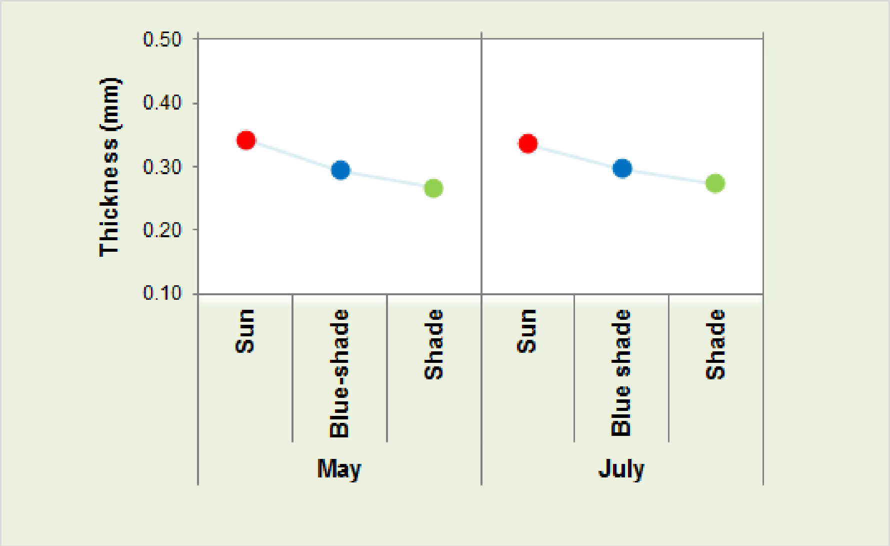

Figure 5: *Presentation of the values of the thickness, of the leaves in three positions; sun, blue -shade and shade of Santa Maria variety, in periods May-July, area 2*

Conclusions

The concentration of pigments Chl (a+b) presents higher values in the leaves in south position compared to the other two positions for periods of May and July. The values of the Chla/Chlb ratio from the south-shadow position increase, while the a+b/x+c ratio decreases from the south-shadow position.

In area over water in the two periods under study, the values of R550 are lower in the south position and higher in the shadow position, while the values of the R750/R550 ratio are higher in the south position compared to the other two positions . Shade leaves absorb less sunlight and reflect more. Low absorption of sunlight leads to lower chlorophyll concentration.

The highest value of the spread reflection at R550, for the variety Santa Maria (pears) is presented in the period of May. The reason for the higher diffuse reflectance values for the variety Santa Maria (pear) is related to the high photosynthetic development of the variety in this period.

The highest values of the R750/R550 ratio, the Santa Maria (pear) variety are present in the period of May.

In the study area the highest value of the photochemical index (PRI), the variety Santa Maria (pear) are presented in the shade position in July.

Santa Maria varieties (pear) in the two periods under study (May and July), present higher values of the "dominant" wavelength and luminance in the shadow position, compared to the other two positions. The two parameters are related to the structure of the leaf itself. The variety Santa Maria (pear) presents a higher value of the "dominant" wavelength in the May period, shade position. While the highest luminosity value in the May period, the shadow position.

Brightness-Y, is also a parameter which is on to the structure of the leaf. In the two periods taken in the study, for the variety Santa Maria (pear), higher values are presented in the shade position compared to the other two positions.

For the Santa Maria (pear) variety, higher thickness values are presented in the leaves of the south position compared to the other two positions. In the two periods, the highest value is observed in May compared to the July period.
May is the period of maximum development of the photosynthetic apparatus which is observed in all the defined parameters.

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