Universal Library of Biological Sciences | Review Article

Volume 1, Issue 1

Open Access | PP: 19-25

DOI: https://doi.org/10.70315/uloap.ulbsc.2024.0101003

Diffuse Reflection in Leaves is the Main Indicator of Evaluating the Activity of the Photosynthetic Apparatus in Fruit Trees

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Abstract

The study with the application of diffuse reflectance imaging of leaves helps to clarify the role and activity of photosynthetic antennae and to evaluate the activity of the photosynthetic apparatus. The measurements were carried out with three types of leaves (sun, partial shade and shade) for Santa Maria (pear) variety in two areas under and above water. The reflectance values in the 400-800nm wavelength provide the opportunity to determine the parameters that evaluate the activity of the photosynthetic apparatus of fruit trees in two different areas.

Keywords: *Chl (a+b), R550, R750, PRI, Brightness-Y, Thickness, Dominant Wavelength.*

INTRODUCTION

All environmental conditions and various stresses directly or indirectly affect the process of plant photosynthesis. Solar radiation, high temperatures, various pollutions affect the reduction of the activity of the photosynthetic apparatus of the leaves. As a result of light absorption there are two types of chloroplasts: the sun type with high light and the shade type with low light. The concentration of pigments Chl (a+b) in the leaves of the sun appears higher compared to the leaves of the shade type, but high values of the Chl a/b ratio correlate with low values of the x/c ratio. The leaves of trees exposed on the north side, which mainly receive the blue light of the sky, present a content of pigments and their ratios with values intermediate to those of leaves in the sun and in the shade. So these are often called "leaves of the north in the shade". Also, the CO2 fixation rate of their photosynthesis has intermediate values with those of sun and shade leaves [1], [2]. "Semi-shade leaves" are those leaves that receive direct sunlight only for a short period of time during the day and are in the shade for the rest of the day. Their pigment composition and CO_2 fixation rate also have values between those of sun and shade leaves. β -carotene decreases, which leads to increased values of the x/c ratio [11]. β-carotene content and the lowest x/c ratio were found in the northern position in fully developed leaves, in the May-June period. 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.

In September, from the drop in temperatures and the beginning of the rains, a stabilization of the rate of CO_2 fixation is observed in the leaves of the sun, but an increase may also occur in the leaves of the shade before the degradation becomes dominant in autumn [1]. The development of chloroplasts of each type is also controlled by the quality of light where: blue light promotes the formation of sun-type chloroplasts and red light promotes the formation of shade-type chloroplasts. Sun-type chloroplasts of plants exposed to light have smaller sizes of pigment antennae compared to shade-type chloroplasts. Sun-type chloroplasts have many more reactive centers and photosynthetic transport chains per chlorophyll unit and far fewer light-harvesting pigment-protein complexes as well as fewer thylakoids and are smaller in size than shade-type plants or leaves grown in the shade [10]. The concentration of pigments Chl (a+b) in the leaves of the sun appears higher compared to the leaves of the shade type, but high values of the Chl a/b ratio correlate with low values of the x/c ratio. The leaves of trees exposed on the north side, which mainly receive the blue light of the sky, present a content of pigments and their ratios with values intermediate to those of leaves in the sun and in the shade. So these are often called "northern leaves in the shade.

The objective of the work is: evidence of damage/or not, induced by different radiations, in the optical characteristics of the leaves, the diffuse reflectance values as early indicators of stress detection.

MATERIAL AND METHODS

Plants

Measurements were made with leaves selected in three 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 *Pyrus Communis* L pear species in the rose family. The study of the variety was done in area: under water (in the presence of moisture) and above water (in the absence of moisture) in period September. The areas have a Mediterranean climate with average annual temperatures of around 16°C.

Pigment Determination

Leaf pigments were extracted with 100% acetone in the 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 [7], [8]. The represented values are the means of six determinations from six leaves.

Reflectance Values

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 [3], [6]. 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.

Photochemical Index (PRI)

The photochemical index of diffuse reflectance serves as a photosynthetic indicator of radiation utilization efficiency [4]. The photochemical reflectance index (PRI), calculated from the reflectance at 531 and 570 nm, is sensitive to the photochemical changes induced by the photoprotective xanthophyll cycle, acting upon light saturation of the chlorophyll antenna [4], [5]. The values of the photochemical index of diffuse reflectance fluctuate in the range from -1 to 1. The PRI values are calculated using the reflectance values at 531 nm and at 570 nm as reference wavelength:

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

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. 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 [9]. With the measured values of diffuse reflectance, it is also possible to calculate color parameters on different leaves by means of a special algorithm in the Excel program such as: brightness-Y, "dominant" wavelength.

Thickness

Measurement of the thickness of the samples (leaves) taken in three positions was accomplished by using a micrometer or Palmer caliper. Micrometers serve to measure the thickness of the object that is clamped between point B of the screw and a stop C attached to the micrometer. The screw is turned by means of a step A that wraps the nut: the step of the screw is 1mm. The number of millimeters with which we have placed the screw on a scale located on the nut and detected by the cap is estimated. We estimate the parts of a millimeter by measuring the parts of a screw lead by a mark removed along a diode conductor and a scale where 30 divisions, we thus estimate the thickness of the leaf placed between two thin glasses, with the proximity of 1/ 20mm.

Statistical analysis

In ANOVA, use the F distribution. Statistical analysis was performed using JMP 7.0 statistical software. All experimental data were processed with the Student's, Turkey-Kramer and Analysis of Variance methods ($P < 0.05$).

RESULTS

Photosynthetic Pigments

The highest value of the chlorophyll content Chl (a+b) is presented by the variety Santa Maria (pear) in the area above water (in the absence of moisture) compared to the area under water (in the presence moisture). It is also observed that the content of chlorophylls Chl (a+b) decreases in variety from sun leaves to blue-shade and shade leaves (Table 1).

Table 1. Content 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 of pear trees. 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– Under water			
Sun	7.022 ±0.067	2.38	4.80
Blue-shade	6.082 ±0.0837	2.25	5.38
Half-shade/shade	4.234 ±0.045	2.12	5.72

Santa Maria– Above water			
Sun	8.461±0.033	2.58	4.97
Blue-shade	6.325±0.033	2.45	5.21
Half-shade/shade	4.834±0.054	2.18	5.51

The ratios of the photosynthetic pigments, Chl a/b and (a+b)/(x+c), reflecting the light adaptation of the photosynthetic apparatus showed different values in the three leaf types. The mean values of the ratio Chl a/b are higher in sun leaves as compared to blue-shade and shade leaves (Table 1). Sun leaves displayed lower values of the ratio (a+b)/(x+c) as compared to two other leaf types (Table 1).

Reflection Values

The values of R700, R750 and R800, for variety Santa Maria are presented in the shaded position. The high values of reflection in the wavelengths 700nm, 750nm and 800nm are explained by the low absorption in the shadow position, in the area above the water. Santa Maria (pears) in the two area under study for three types of analyzed leaves are related to the chlorophyll content being lower in shade leaves and higher in sun leaves (Table 1). In the underwater area, the Santa Maria variety presents the highest value in the shade position and the lowest in the sun position.

Santa Maria (pears) in the two area under study for three types of analyzed leaves are related to the chlorophyll content being lower in shade leaves and higher in sun leaves (Table 1). It is observed that the highest values R550 of variety are presented in the shade position compared to the other two positions (Table 2).

Table 2. Reflectance on sun, blue-shade, shade/half-shade leaves of Santa Maria variety of pear trees. Mean values of 6 determinations per leaf-type.

Leaf-type	**R550**	**R700**	**R750**	**R800**
Abbas– Under water				
Sun	8.02 ±0.86	11.8	43.4	40.4
Blue-shade	10.1 ±0.63	14.1	44.5	41.8
Half-shade/shade	11.4 ±0.50	15.8	44.8	42.8
Abbas– Above water				
Sun	10.4 ±0.33	14.3	50.2	40.1
Blue-shade	11.4 ±0.50	15.3	51.6	43.8
Half-shade/shade	13.2 ±0.80	17.2	52.2	46.6

In the two area under study, higher values of R550 are observed in the sun position (Fig. 1). High values of R550 in the shade position indicate that the leaves of green plants absorb less sunlight and reflect more.

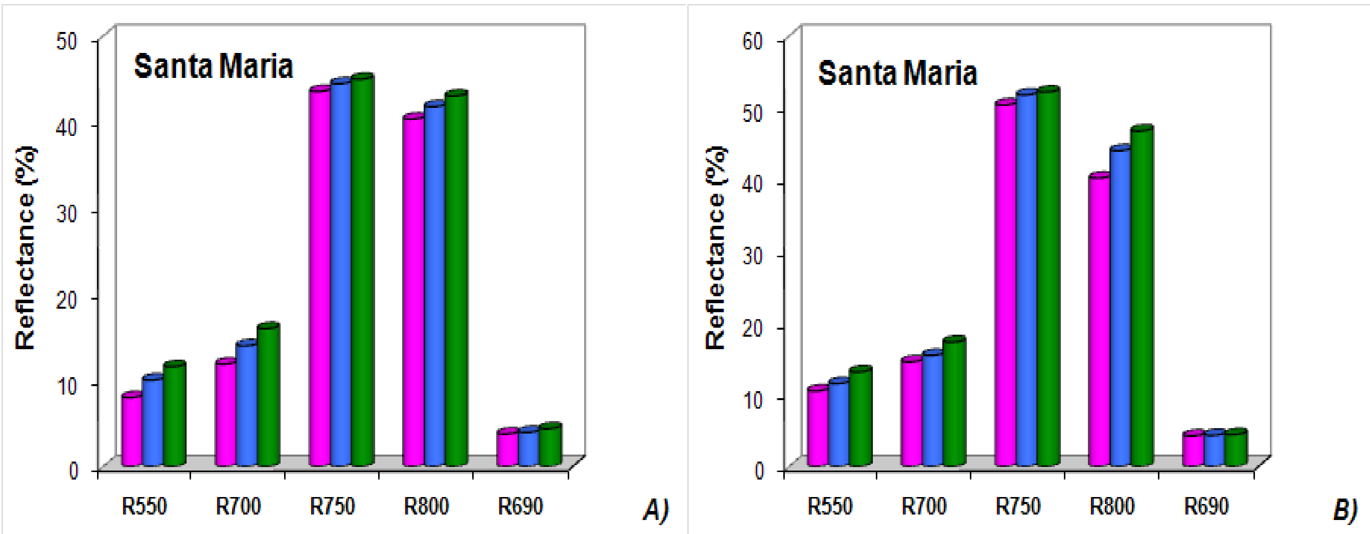

Fig. 1. Values reflectance of the sun (south part), blue-shade (north part) and shade/half shade leaves of Santa Maria pear variety; A) Area under water, B) Area above water.

In the shade position, the R550 value is higher and the chlorophyll concentration value is lower (Fig 2).

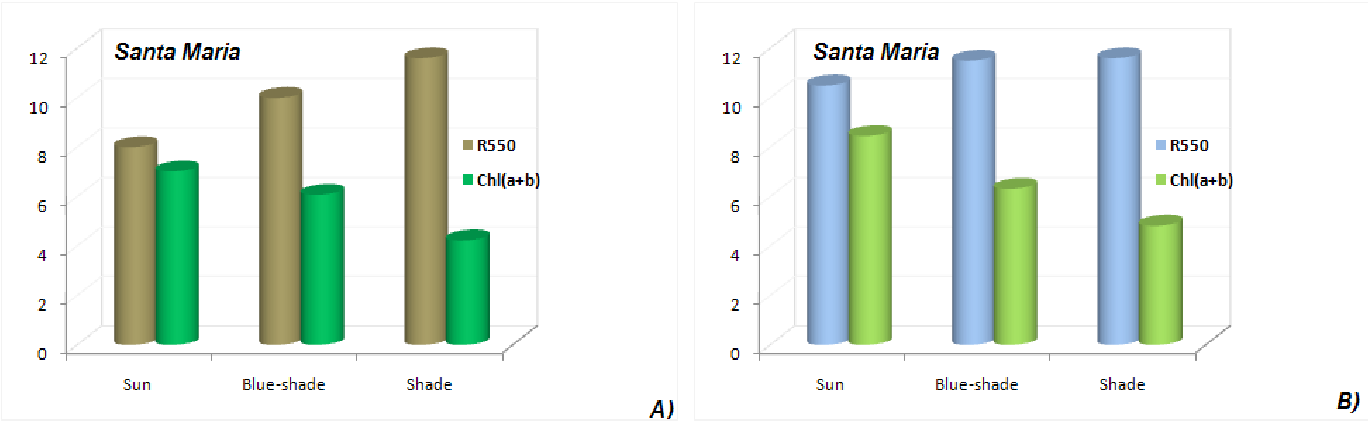

Fig. 2. Values reflectance R550 and content of Chl (a+b) of the sun (south part), blue-shade (north part) and shade/half shade leaves of Santa Maria (pear) variety; A) Area under water, B) Area above water.

In the shadow position, the concentration value of Chl (a+b) is low, while the value of R550 is high (Fig. 2).

In shade leaves, chloroplasts invest heavily in the light absorption apparatus and have larger antennae to absorb as efficiently as possible all the light that reaches them.

Photochemical Index (PRI)

The photochemical reflectance index (PRI) depends on the amount of energy falling from the sun on the leaf surfaces. Photochemical reflectance index, the PRI, has been demonstrated to be related to light use efficiency [12]. The highest value of the photochemical index (PRI) is presented in the area under water for Santa Maria (pear), sun position (Table 3). The influence of solar radiation on the leaves of the sun brings higher values in this position, compared to the other two positions.

Table 3. PRI values for Santa Maria (Pear) variety

Leaf-type	**PRI**
Santa Maria–Under water	
Sun	0.052
Blue-shade	0.044
Half-shade/shade	0.041
Santa Maria–Above water	
Sun	0.049
Blue-shade	0.041
Half-shade/shade	0.038

Santa Maria (pear) variety presents the highest values of the photochemical index (PRI) in the sun position, in the two area under study (Fig. 3).

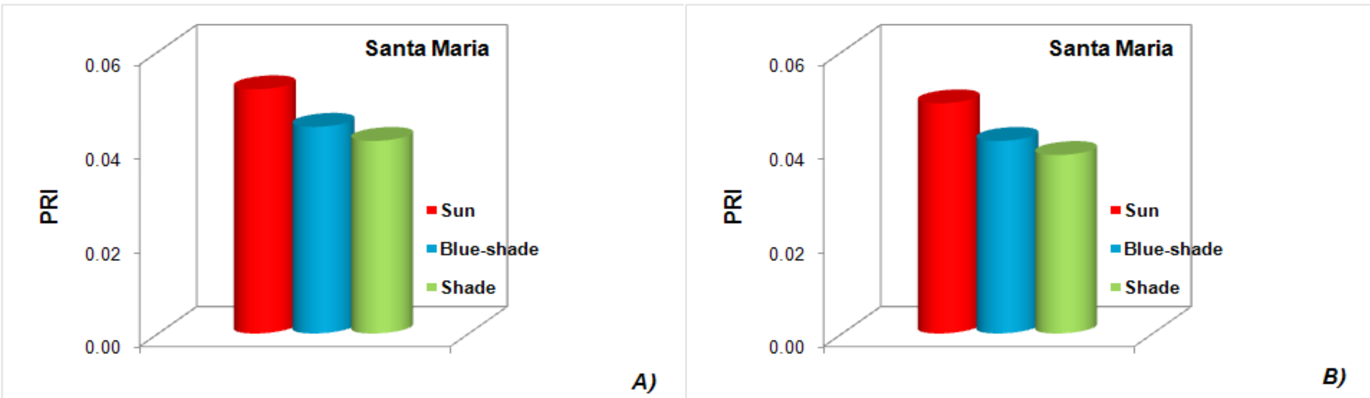

Fig. 3. Photochemical index (PRI) values of the leaves of the sun, blue-shade and shade type leaves; of Santa Maria (pear) variety; A) Area under water, B) Area above water.

From the experimental data it is observed that: the concentration of Chl (a+b) chlorophylls from the sun-shade position decreases and the values of the PRI photochemical index decrease (Fig. 3). The highest value of PRI appears in the sun position, in the area in the presence of moisture. The value decreases in the shade position, in the absence of moisture.

Colorimetric Parameters

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

Table 4. Colorimetric determination according CIE 1931 for the leaf samples: sun, blue-shade, shade/half-shade leaves of Santa Maria variety trees. Mean values of 6 determinations per leaf-type.

Leaf-type	Brightness -Y	Dominant wavelength (nm)
Santa Maria–Under water		
Sun	6.45	557.0
Blue-shade	7.48	557.0
Half-shade/shade	7.78	557.5
Santa Maria–Above water		
Sun	7.96	555.8
Blue-shade	8.94	558.0
Half-shade/shade	9.40	558.0

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 area under-above water in the period September. In the two areas under study, the highest values are presented in above water, position shade.

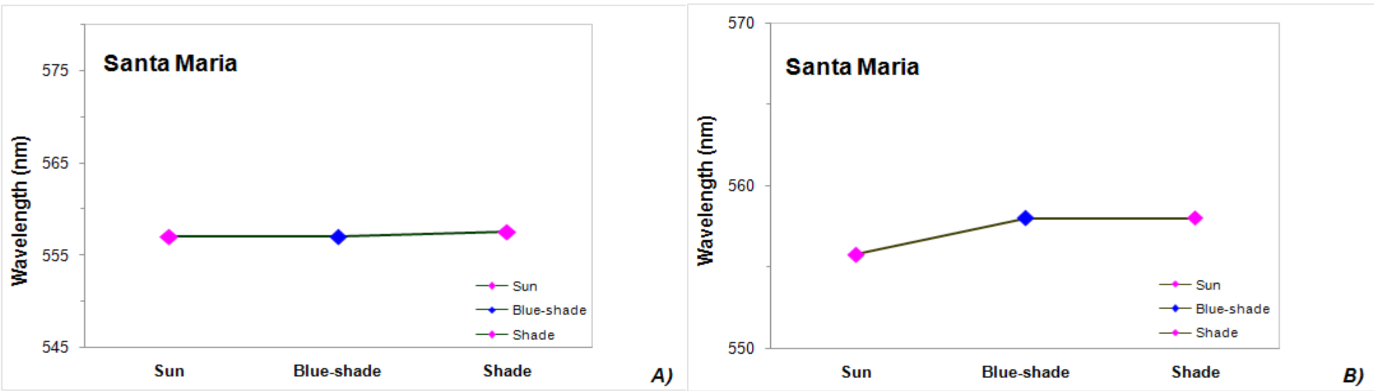

Fig. 4. Values of the "dominant" wavelength, of the sun, blue-shade and shade type leaves; of Santa Maria (pear) variety; A) Area under water, B) Area above water.

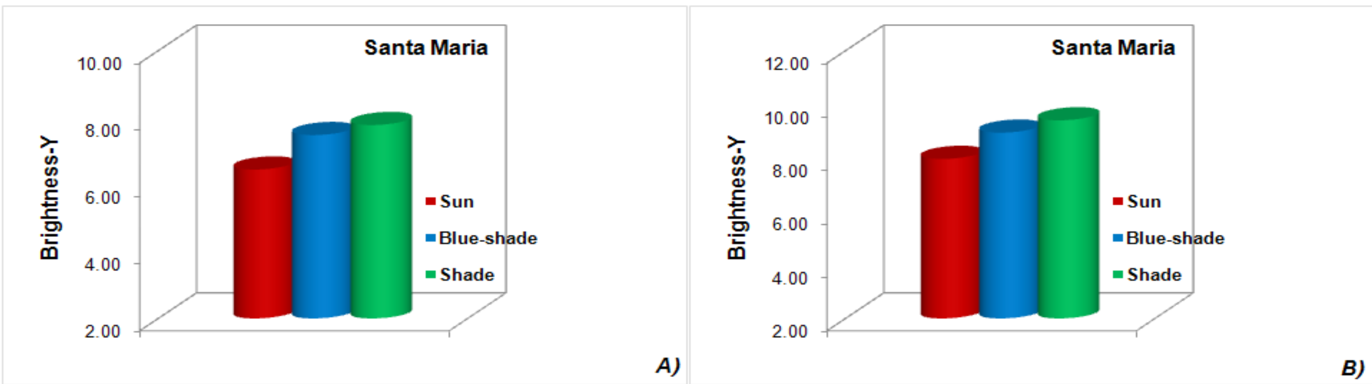

Fig. 5. Brightness-Y values of the leaves of the sun, blue-shade and shade type leaves; of Santa Maria (pear) variety; **A)** Area under water, **B)** Area above water.

The "dominant" wavelength depends on the concentration of chlorophylls. In the two areas under study, the brightness values are higher in the shadow position (Fig. 4). The gloss values are higher in the shadow position in the two areas under study. The shine in the leaves of the shade is related to the low content of chlorophylls and the high content of water in them (Fig. 4).

Thickness

Thickness of the leaves for the variety Santa Maria presents higher values in the area above water, compared to the area under water (Table 5). Leaves in the sun position in the two area present higher values (Fig. 6).

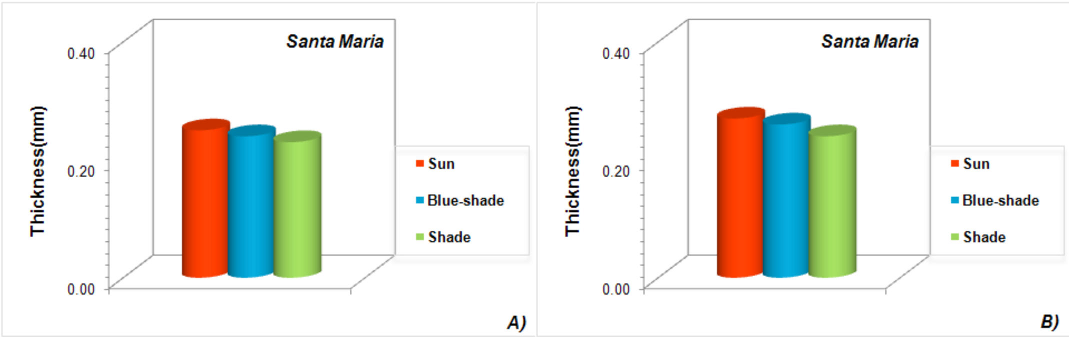

Fig. 6. Thickness values of the leaves of the sun, blue-shade and shade type leaves; of Santa Maria (pear) variety; **A)** Area under water, **B)** Area above water.

Table 5. Thickness values for Santa Maria (pear) variety

Leaf-type	**Thickness (mm)**
Santa Maria – Under water	
Sun	0.254± 0.010
Blue-shade	0.245 ± 0.009
Half-shade/shade	0.233 ± 0.012
Santa Maria–Above water	
Sun	0.275 ± 0.006
Blue-shade	0.262 ± 0.007
Half-shade/shade	0.247 ± 0.006

The leaves in the shade are thinner; the concentration of chlorophylls is lower compared to the other two positions. The leaves of the shade are thinner but with higher water content compared to the leaves of the sun and blue-shade.

Statistical Analysis

Based on the unit variable analysis and Student's and Kramer's criterion, for the parameter Chl(a+b) for the leaves of the variety Santa Maria (pear), area under and above water with Student's and Kramer's criterion, statistical differences are observed in period between positions (Fig. 7).

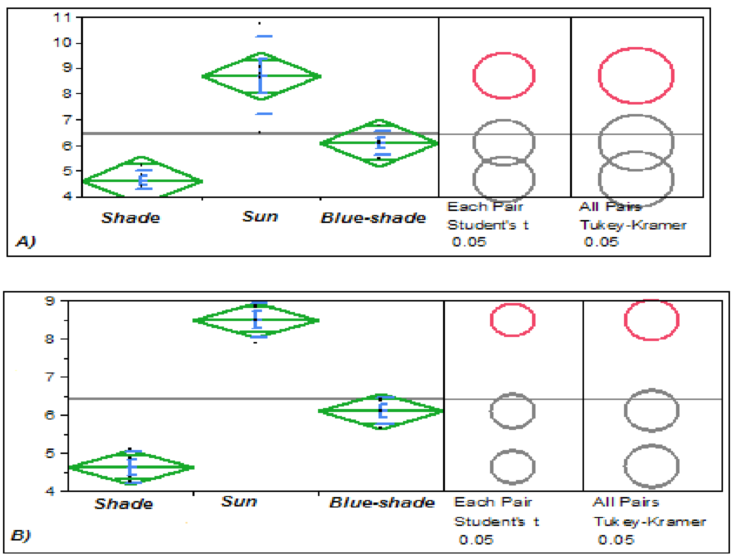

Fig. 7. Unidirectional analysis of the variety Santa Maria (pear) variety; **A)** Area under water, **B)** Area above water.

CONCLUSIONS

With the phenomenon of diffuse reflection, it was possible to determine the reflection values, photochemical index and color by means of chromatic parameters.

The pigment content Chl (a+b) represents the highest values on the sun leaves (sun position) and the lowest values on half-shade/shade leaves (inside a tree crown). The decrease in the concentration of chlorophylls from the position of the sun to the shade shows that the photosynthetic apparatus becomes more and more unstable and the sensitivity to stresses increases. Slow reduction of Chl (a+b) level and Chl (a+b)/(x+c) ratio values during September occurs especially in sun-exposed leaves that are continuously exposed to combined stresses such as high lighting, lack of water and high temperature.

Reflection values (R550, R750, R800) show the characteristics and differences between the analyzed leaves demonstrating structural changes in the photosynthetic apparatus as a result of adaptation to the environment. PRI as a parameter is related to the action of solar radiation on leaves. Direct action of solar radiation on the leaf also affects its thickness.

The study is important and aims to present valuable recommendations for evaluating and increasing the efficiency of the photosynthetic conversion of solar radiation in fruit trees (pears) for increasing productivity or creating new cultivars.

Acknowledgments

Spectrophotometry of reflection in leaves, are methods cultivated for a long time in the laboratories of the Faculty of Natural Sciences (Department of Physics, University of Tirana), creating the basic basis for the realization of this study.

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Citation: Matilda Mema, Merita Rumano, et al., “Diffuse Reflection in Leaves is the Main Indicator of Evaluating the Activity of the Photosynthetic Apparatus in Fruit Trees”, Universal Library of Biological Sciences, 2024; 1(1): 19-25. DOI: https://doi.org/10.70315/uloap.ulbsc.2024.0101003.