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Changes in the photosynthetic apparatus in fruit trees during stressful periods

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ABSTRACT The amount of solar radiation absorbed by the leaf is a function of the photosynthetic pigments it contains. Chlorophyll provides an indirect estimate of nutritional status because most of the nitrogen in leaves is incorporated into chlorophyll. The measurements were carried out with three types of leaves (sun, partial shade and shade) for Santa Maria (pear) variety (Tirana region, Albania) 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 in the same period.

KEY WORDS Reflection spectra; Chl(a+b); R550; PRI; Brightness-Y.

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INTRODUCTION

In nature, high light conditions are those exposed to full sunlight such as the leaves of trees and plants grown in the sun, while low light conditions are those of the leaves of trees and plants grown in the shade. 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. Both types show considerable variation in the frequency or density of their thylakoids, the level of light-harvesting protein-pigment complexes, and chloroplast properties such as the rate of electron transport and the rate of photosynthetic CO_2 fixation (Lichtenthaler et al., 1982 a, b; Meier & Lichtenthaler, 1981).

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 (Malkin et al., 1981). 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". Also, the CO_2 fixation rate of their photosynthesis has intermediate values with those of sun and shade leaves (Babani & Lichtenthaler, 1996; Balota et al., 1998).

The aim of this work is to evaluate the functioning of the photosynthetic apparatus in fruit trees (pears) in Tirana region (Albania), in the presence of environmental conditions (solar radiation, temperature, humidity) to which they are exposed.

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 one area in the presence of moisture (under water) and another in absence of water (above water) in September. Both 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 9 mm 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 (1987) and Lichtenthaler & Buschmann (2001). The represented values are the means of six determinations from six leaves.

Reflectance spectra

Leaf reflectance (R) was recorded from upper side of the leaf in a spectral range from 400 nm to 800 nm with a spectral resolution of 2 nm with a spectrophotometer equipped with an integrating sphere attachment (Gitelson et al., 2003; Bushman et al., 2012). 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 centimeters. The measurement of spectral reflectance is a nondestructive and a rapid method (Gamon et al., 1997).

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 1 mm. 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

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

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, period September. Mean values of 6 determinations per leaf-type.

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/ 20 mm.

RESULTS

Photosynthetic pigments

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).

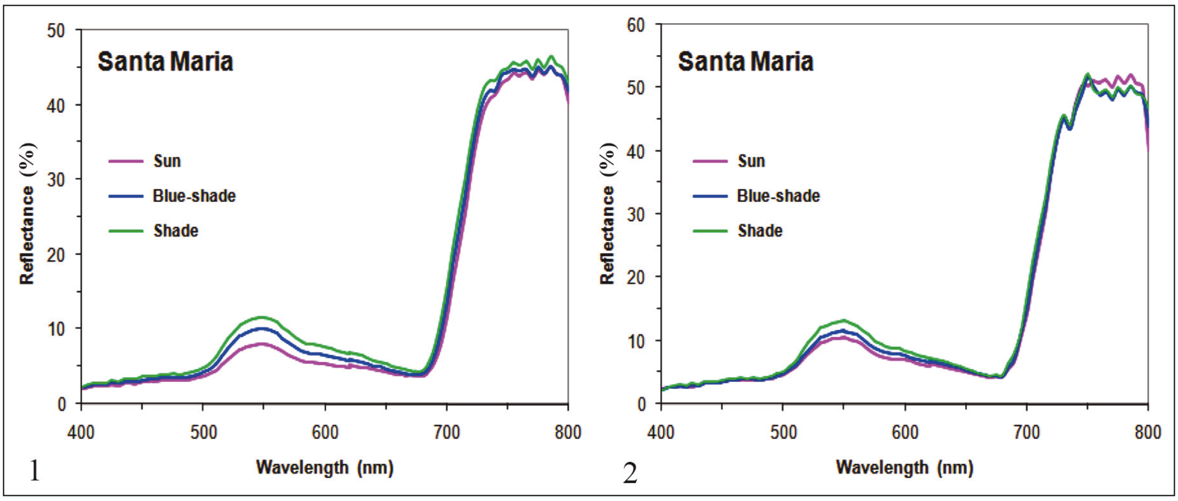
Reflection spectra

Reflection spectra of the three types of leaves exhibited a higher reflectance in the green-to-orange range of the spectrum at wavelengths 500 nm to 650 nm and mainly in the near infrared from 680 nm to 740 nm on both pear varieties. In addition, reflection spectra exhibited a low reflectance from 400 nm to 500 nm in blue part of visible spectra and near 680 nm in red part of visible spectra (Figs. 1, 2). The observed variations correspond to the absorption region of the in-vivo chlorophyll bands. The reflection spectra exhibit the highest values in the green-orange range of the spectrum of shade leaves compared to two other leaf types for variety. 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.

Even in this case, the reflection spectra exhibit the highest values in the green-orange range of the spectrum of shade leaves compared to two other leaf types of both varieties. 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).

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 700 nm, 750 nm and 800 nm are explained by the low absorption in the shadow position, in the area above the water. In the two area under study, higher values of R550 are observed in the sun position (Figs. 3, 4). High values of R550 in the shade posi-



Figures 1, 2. Reflectance spectra of the sun (south part), blue-shade (north part) and shade/half shade leaves of Santa Maria pear variety. Fig. 1: area under water. Fig. 2: area above water.

tion indicate that the leaves of green plants absorb less sunlight and reflect more. In the shade position, the R550 value is higher and the chlorophyll concentration value is lower (Figs. 5, 6).

In the shadow position, the concentration value of Chl (a+b) is low, while the value of R550 is high (Fig. 3). 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. Thickness of the leaves for the variety Santa Maria presents higher values in the area above water, compared to the area under water. Leaves in the sun position in the two area present higher values (Figs. 7, 8).

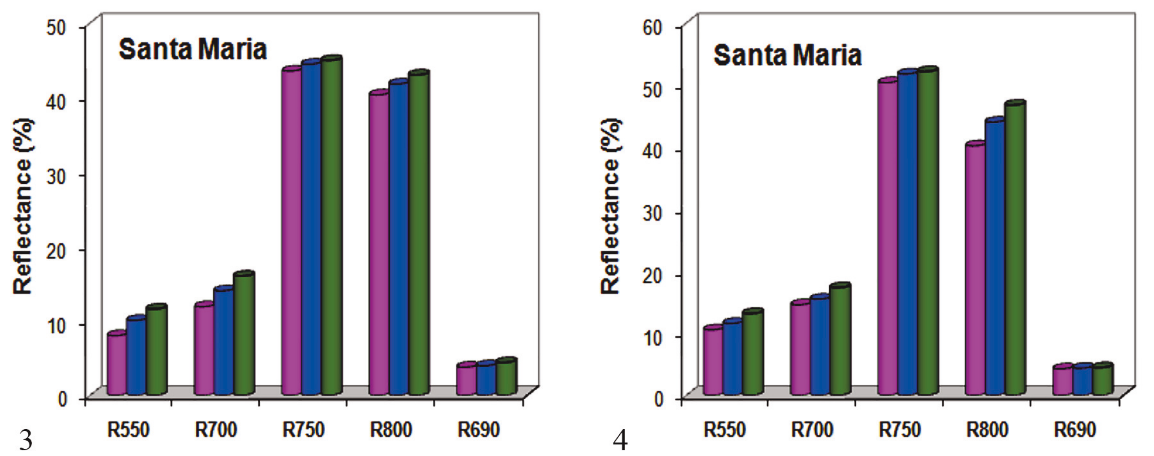
The leaves in the shade are thinner; the concentration of chlorophylls is lower compared to the other two positions. The leaves of the shade are thinner but with higher water content compared to the leaves of the sun and blue-shade.

DISCUSSION

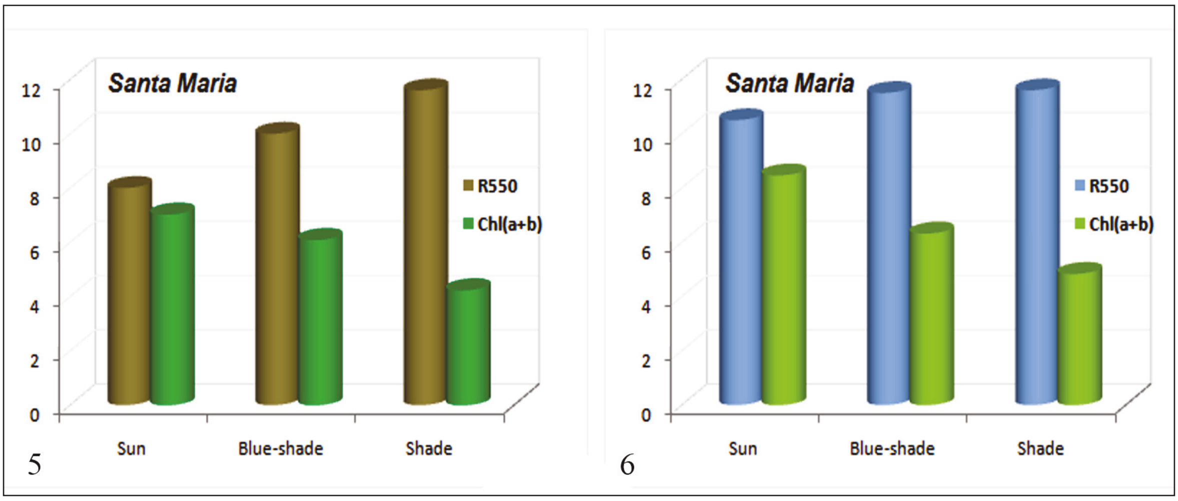
Shade-type chloroplasts, in which there is a greater amount of light-harvesting chlorophyll-protein complexes per chlorophyll unit, have a significantly higher amount of antennae than sun-type chloroplasts, in which a greater number of photosystems and electron transport chains per chlorophyll unit. Since

Leaf-type	R550	R700	R750	R800
Santa Maria - under water				
Sun	8.00 ± 0.86	11.9	43.5	40.3
Blue-shade	10.0 ± 0.63	14.0	44.4	41.7
Half-shade/shade	11.6 ± 0.50	15.9	44.9	42.9
Santa Maria - under water				
Sun	10.5 ± 0.33	14.5	50.3	40.2
Blue-shade	11.5 ± 0.50	15.5	51.7	43.9
Half-shade/shade	13.1 ± 0.80	17.3	52.1	46.7

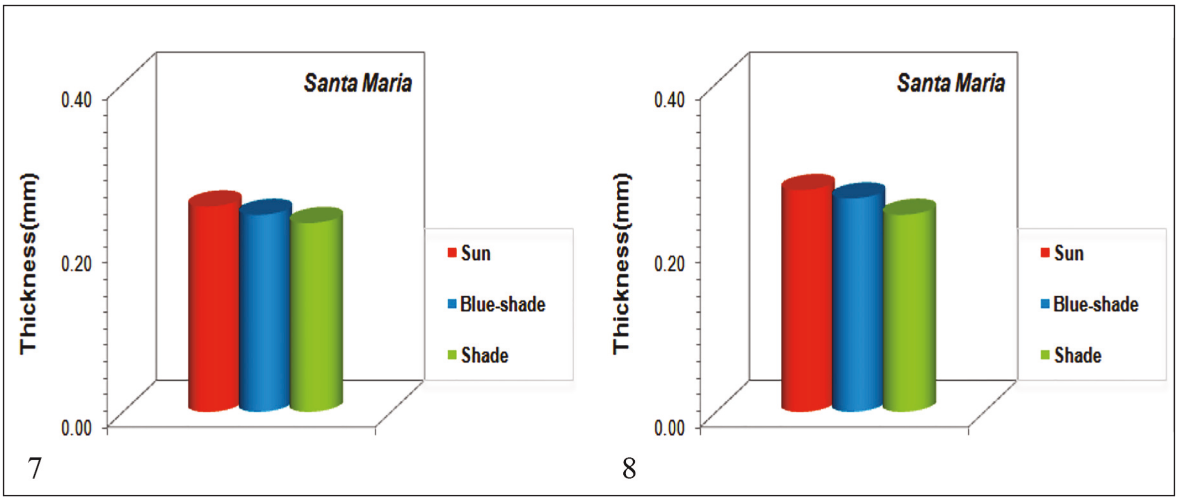
Table 2. Reflectance on sun, blue-shade, shade/half-shade leaves of Santa Maria variety of pear trees. Mean values of 6 determinations per leaf-type.



Figures 3, 4. 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. Fig. 3: area under water. Fig. 3: area above water.



Figures 5, 6. 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. Fig. 5: area under water. Fig. 6: area above water.



Figures 7, 8. Thickness values of the leaves of the sun, blue-shade and shade type leaves of Santa Maria (pear) variety. Fig. 7: area under water. Fig. 8: area above water.

the level of light-harvesting chlorophyll-xanthophyll-protein complexes is quite low in sun-type chloroplasts in leaves grown in the sun and under high light conditions, the concentration of their pigments is quite different from that of chloroplasts of the leaves grown in shade and under low light conditions.

The concentration of pigments is higher in the south position compared to the shade position (Lichtenthaler et al., 1982 a, b). Shade and low light leaves are thinner and have a larger average surface area than sun or high light leaves. In fact, the total content of chlorophylls and their carotenoids per unit leaf area is significantly lower than in sun or high light leaves (Lichtenthaler et al., 1981). The results of the comparative study of plant leaves show that leaf reflectance signals are significantly and fundamentally influenced by three basic factors: (a) leaf pigment (content and types), (b) leaf tissue structure, and (c) leaf surface properties. It has been shown that hairs and waxes on the leaf surface can protect plant leaves against photo inhibition of the photosynthetic apparatus.

CONCLUSIONS

From this study, it was possible to notice the changes in the leaves of the fruit trees in three positions: south, north and shade. The change is observed by the phenomenon of diffuse reflection.

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 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.

The reflectance spectra, as well as the 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. Direct action of solar radiation on the leaf also affects its thickness.

The study carried out in the framework of the topic is a contribution regarding the effect of solar radiation (direct and diffused), on the activity of the photosynthetic apparatus of the analyzed fruit trees.

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