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THE EFFECT OF THE SPECTRAL COMPOSITION OF THE LIGHT ON THE CHLOROPHYLL AND CAROTENOID CONTENTS OF BEAN LEAVES (PHASEOLUS VULGARIS L.)

L. SZALAY, E. TOMBÁCZ, E. BÁLINT, I. HORVÁTH, GY. SCHNEIDER

Department of Experimental Physics, Department of Botany and Department of Organic Chemistry, Attila József University, Szeged

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Abstract

Experiments under field conditions with perforated coloured polyethylene foils are reported. The amount of the pigments was found to be increased by covering with foils. The amount of the carotenoids decreased with small light intensity in the region of the absorption of carotenoids. The changes of the relative amounts of chromatographically separated chlorophyll-a, chlorophyll-b, α-carotene, α-carotene-monoepoxide and β-carotene-diepoxide, xanthophyll and xanthophyll-epoxide (with some unidentified pigments) are given for three weeks of the vegetation period.

1. Experiments

The experiments were carried out in August and September 1967 under field conditions with coloured polyethylene foils of 0,1 mm thickness. Earlier investigations showed that except the illumination environmental conditions are only slightly influenced by covering with perforated foils (1). During the experiments, in daytime, the intensity of the illumination varied between 10.000 and 50.000 lux.

The spectral distribution of the illumination under the foils was given from the measured spectral distribution of the foils and from the spectral distribution of an average natural illumination (by averageing sunny, cloudy conditions under different heights of the sun). The distribution of the relative intensity is shown in Fig. 1. Here the maximum intensity without foil is arbitrarily taken as unit. From this figure it is seen that the colourless and the blue foils practically cause but a decrease of the intensity (Fig. 1) by 10 and 50 per cent, respectively, whereas under the red foil the spectral distribution of the illumination differs from that under the other foils. Therefore, the application of the colourless foil gives information about the effect of covering; a com-

parison of the results with blue and colourless foils discloses the effect caused by a 50—70 per cent decrease of the intensity of light. Using the red foil the total intensity of light is reduced to 5—7 per cent and 30 per cent in the spectrum region of the absorption of carotenoids and chlorophylls, respectively. The results with red foils, therefore, inform about the case when practically only the chlorophylls are capable to

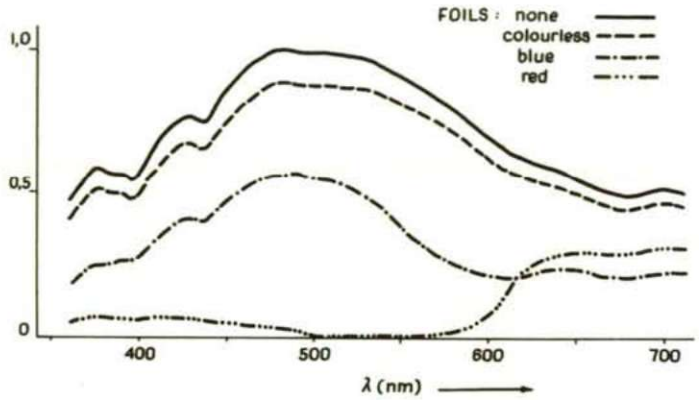


Fig. 1.

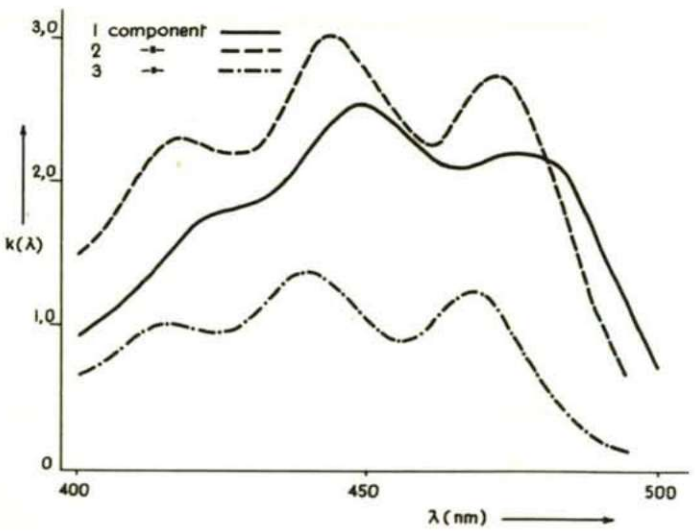


Fig. 2.

energy uptake. A comparison of the results with red and blue foils shows the case when both carotenoids and chlorophylls obtain light energy. (Namely, the spectral distribution of the intensity of light in

the region of the absorption of chlorophylls is practically the same under the blue and the red filters, whereas the blue filter has a considerable transmission in the region of the absorption of carotenoids.)

The leaves were harvested weekly in the same period of the day. 14 g fresh material was homogenized and extracted with petrolether. The components of the pigment system were chromatographically separated with sugar column in the well known manner. The five obtained components (two chlorophylls and three carotenoids) were transferred into ether and the weights were determined from the optical density of the solution. The experiments were done under light protection and at a temperature of 5°C. The criterion of the completeness of the separation of the chlorophylls was to have a ratio of less than 1,33 and 2,82 of the heights of the blue (Soret) and the red maxima for chlorophyll-a and -b, respectively. The absorption spectra of the yellow pigments are shown in Fig. 2 (Fig. 2). The well known chlorophyll spectra are not shown.

2. Results

2.1. The total amount of pigments

Table 1 shows the total amount of the chlorophylls and carotenoids through three weeks in the same units (Table 1). An analysis of the data in this table leads to the following conclusions:

Table 1

Foil	1. week		2. week		3. week	
	Chl.	Car.	Chl.	Car.	Chl.	Car.
none	100	100	90	80	59	83
colourless	98	111	132	103	65	102
blue	104	115	130	112	76	104
red	120	121	122	99	69	86

2.1.1. The amount of pigments is increased by covering.

2.1.2. The amount of pigments is practically not influenced by a 50—60 % decrease of the intensity of illumination.

2.1.3. The amount of the carotenoids compared to that of the chlorophylls decreases if the light intensity is small in the region of the absorption of carotinoids.

2.2. The contribution of the components to the total amount of the pigments

2.2.1. Table 2 exhibits the amount of chlorophyll-a and -b in relative units for the 1—3 weeks with different foils. This table shows that a covering increases the amounts of both chlorophylls (Table 2). The

increase is developed by the 2. and 3. week and especially applies to chlorophyll-b with about 50 per cent. A halving of the illumination does not cause any influence. If only the chlorophylls get light their amount increases in the first week but no increase is found in the further weeks. On the contrary, the amount of chlorophyll-b is considerably reduced by the end of the third week.

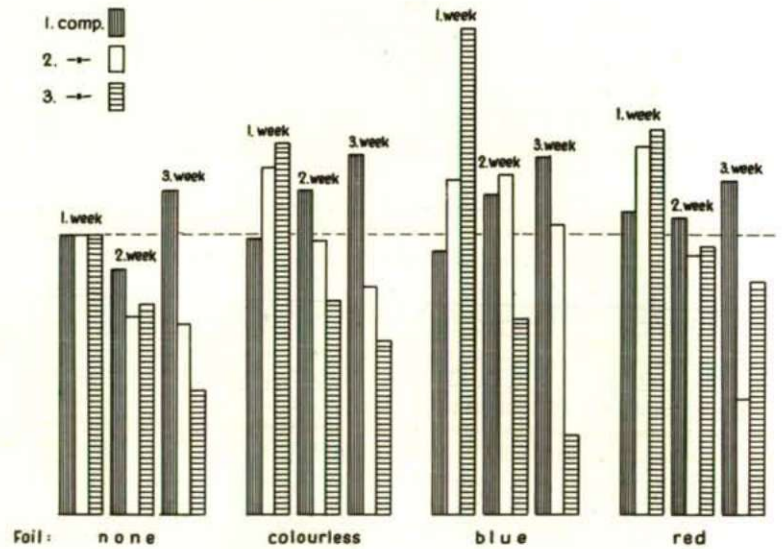
Table 2

Foil	1. week		2. week		3. week	
	Chl-a	Chl-b	Chl-a	Chl-b	Chl-a	Chl-b
none	100	100	88	91	119	102
colourless	98	102	128	128	135	149
blue	105	119	129	127	150	141
red	120	145	120	126	136	121

As for the ratios of the amounts of chlorophyll-a and -b no definite change was found. There is a tendency of increasing of the ratio with the time. A covering and a decrease of the intensity of the illumination by 50—60 per cent show an increased contribution of chlorophyll-b to the ratio.

2.2.2. Carotenoids

Fig. 3 shows the variation of the amount of carotinoids in relative units of the amounts of the first week in all the three components (Fig. 3.) An analysis of the data leads to the following conclusions:



2.2.2.1. The amount of the components increases if the plant is protected with covering by colourless foil. The amount of the 1. component increases with the time as well, whereas the amounts of the 2. and 3. component decrease with time.
2.2.2.2. The decrease of the intensity of illumination by 50—60 per cent does not influence the amount of the 1. component, however, the amount of the 2. component accumulates with the time and the amount of the 3. component highly decreases after a sudden increase in the beginning.
2.2.2.3. If practically only the chlorophylls can access light the amount of the 1. and 2. component highly increases in the first period of time after which a decrease follows. The behaviour of the 3. component is reverse.

2.3. Analysis of the carotenoids

The three components of carotenoids obtained after a single separation with sugar column were identified in the following way.

Component 1. This component is found right below the bands of chlorophyll-a and chlorophyll-b in the sugar column and the maxima of the absorption spectrum of this components are in three different solvents practically at the same wavelengths where the bands of α-carotene are found (see Table 3). Therefore, component 1. is identified as α-carotene.

Table 3

Compound	Solvent	λ_{max} in nm	
		measured	literature/4/
-carotene	hexane	476 ; 449	478 ; 448
	benzene	490 ; 462	492 ; 461
	chloroform	487 ; 460	485 ; 454
-carotene-monoepoxide	hexane	472,2 ; 443,5	478 ; 447
	benzene	487 ; 455	492 ; 460
	chloroform	483 ; 452,5	492 ; 459
-carotene-diepoxide	hexane		470 ; 443
	benzene		485 ; 456
	chloroform		484 ; 456
xanthophyll-epoxide	hexane	465 ; 445	471 ; 442
	benzene	482,5 ; 452	482 ; 453
	chloroform	477,5 ; 449	- -

Component 2. This component seemed to be a mixture of β-carotene-monoepoxide and β-carotene-diepoxide, because it showed a positive hidrochloric acid reaction (3) and the measured maxima of the absorption spectrum in three solvents fitted well into this picture (see Table 3).

Component 3. This component was a mixture of several compounds. The strongly positive hidrochloric reaction and the spectral data mainly show the presence of xanthophyll and xanthophyll-epoxide but the contribution of some other components is not excluded (Table 3.)

This analysis is in accordance with the results of KARRER and coworkers (1948) who reported the presence of α- and β-carotene, xanthophyll and xanthophyll-epoxide in the pigment system of several plants.

In order to draw more detailed conclusions on the physiological role of carotinoids experiments are planned under controlled conditions in phytotrone. With results of better reproducibility the theory of CHOLNOKY and coworkers (1955) could be applied to the changes in the pigment system during the vegetation period. According to this theory primarily carotinoids with ß-ionen rings (ß-carotene and zeaxanthene: 3,3'-dioxy-ß-carotene) are formed in the leaf. On oxygen uptake these will be transformed to epoxides. The epoxides may either be reversibly transformed back to the original compounds with ionen-rings or be transformed to α-isomeres (α-carotene and xantophyll or xantophyll-epoxide).

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Address of the authors:

Prof. Dr. L. Szalay
Dr. Erzsébet Tombácz
Dr. E. Bálint
Department of Experimental Physics
Prof. Dr. I. Horváth
Department of Botany

Dr. Gy. Schneider
Department of Organic Chemistry,
A. J. University, Szeged, Hungary