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Some effects of enhanced UV-B irradiation on the growth and composition of plants

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Abstract. Barley (*Hordeum vulgare*), corn (*Zea mays*), bean (*Phaseolus vulgaris*), and radish (*Raphanus sativus*) seedlings were continuously irradiated under a lighting device for 5–10 d at an increased ultraviolet (UV)-B fluence rate. In their growth parameters, composition, and leaf surface, these four species responded differently to the increased UV-B exposure. Bean seedlings suffered the most serious effects, radish and barley less, and corn was hardly influenced at all. In all plant species, the fresh weight, the leaf area, the amounts of chlorophylls, carotenoids and the galactolipids of the chloroplasts were reduced. The lipid content of the corn and bean seedlings also diminished. But all the irradiated plants showed a rise in their protein content compared to the control plants. The content of flavonoids increased in barley and radish seedlings by about 50%. The effects on growth parameters and composition were more extensive with increasing UV-B fluence rates, at least as shown in the case of barley seedlings. The fresh weights fell proportionally with the chlorophylls and carotenoids. In contrast, the flavonoid content of barley leaves rose parallel to the increasing UV-B fluence rates and reached 180% of the value in the control plants with the highest UV-B fluence rate. Scorching appeared regularly in the form of bronze leaf discoloration at the highest UV-B fluence rates. Scanning electron micrographs of the leaf surface of UV-B irradiated plants showed deformed epidermal structures.

Key words: Epidermis (UV-B effects) – Growth (UV-B effects) – *Hordeum* – Leaf composition – Ultraviolet (UV-B).

Abbreviations: MGDG = monogalactosyldiglyceride; DGDG = digalactosyldiglyceride; SL = sulfoquinovosyldiglyceride; PG = phosphatidylglycerol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PI = phosphatidylinositol; LA = leaf area; FW = fresh weight; DW = dry weight; SEM = scanning electron microscopy; C = total carotenoids; Chl = total chlorophyll

Introduction

Recent research has established that chlorofluoromethanes and other gases cause a reduction of the ozone layer. Measurements by several groups predicted a reduction of between 7.5% (NAS 1976) and more recently 16% (NAS 1979). As a consequence, a displacement of the spectrum to shorter wavelengths and increased energy fluence rates in the UV-B waveband (280–320 nm) is to be expected.

The biological effects of UV-B on lower and higher plants are well reviewed by Caldwell (1971) and by Klein (1978). So far, reductions in total weight and the leaf area of plants have been regularly observed under UV-B stress (Krizek 1975; Sisson and Caldwell 1976, 1977; Van and Garrard 1976; Van et al. 1976; Basiouny et al. 1978; Tevini et al. 1979; Esser 1979; Teramura 1980; Teramura et al. 1980).

The purpose of the present study was to evaluate the sensitivity of some higher plants (barley, bean, radish, and indian corn) to enhanced UV-B irradiation by measuring the effects on growth and composition of the irradiated plants. With barley seedlings the effect of increasing UV-B fluence rates was studied. Assuming that the leaf surface might also be altered by UV-B irradiation, stereoscan studies were carried out as well.

Material and methods

Irradiation conditions. In the experiments a lighting device built by ourselves and equipped with 3 UV-B tubes (Philips TL 40/12) combined with 4 white light tubes (Philips TL 40/29) was used. The UV tubes could be regulated by a dimmer to give different UV-B fluence rates without causing spectral displacement. A large proportion of the UV-B range was filtered out by a glass pane which covered the control compartment. The spectra of the lighting device without and with a glass filter (control) are shown in Figs. 1 and 2. All spectra were measured by a spectroradiometer type EG & G 550; in the UV region with 2 nm intervals, in the PAR region (PAR = photosynthetic active radiation 400–700 nm) with 10 nm steps (Figs. 1 and 2).

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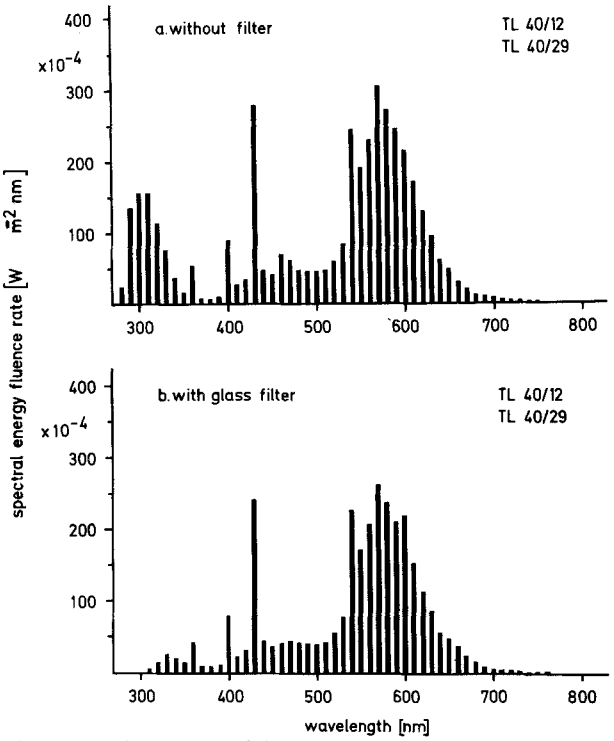

Fig. 1. Complete spectra of the lighting device equipped with UV-B tubes (Philips TL 40/12) and white light tubes (Philips TL 40/29). The fluence rates were measured every 10 nm.
a without filter
b with glass filter (control)

Hordeum vulgare cv. Breuns Villa, *Zea mays, Phaseolus vulgaris* cv. Favorit, *Raphanus sativus* cv. Saxa Treib were grown in soil and continuously irradiated for 5 to 10 days at the following irradiation levels: low UV-B fluence rate = 0.60 W m^{-2} (280–320 nm), medium fluence rate = 1.16 W m^{-2}, high fluence rate = 2.30 W m^{-2}.

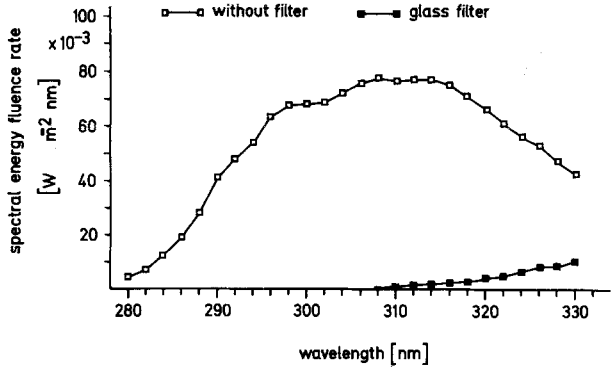

Fig. 2. UV-B spectra of the lighting device with (control) and without glass filter measured in 2 nm intervals

The distance between the tubes and the plants was 50 cm, the experimental chamber was kept at a constant temperature of 20° C ± 1° C.

Quantitative analyses. 10–30 plants were cut 1 cm above the soil and the resulting plant material was used to determine the growth parameters fresh weight, dry weight, and leaf area.

The soluble proteins were determined with the folin-ciocalteus reagent according to Lowry et al. (1951) and also according to the Kjeldahl method, which gave similar results, thus showing that no interference with flavonoids had taken place.

Plant lipids were extracted in ethanol-chloroform (2:1; v/v) and determined gravimetrically. Glycerolipids were separated two-dimensionally by thin-layer chromatography on silica gel (Merck 5721). The solvent for the first dimension consisted of chloroform, methanol, acetic acid, and water (85:25:15:3; v/v). For the second dimension, acetone and acetic acid (80:25; v/v) were used.

The total amount of chlorophyll was calculated according to Arnon (1949). The amounts of chlorophyll a and chlorophyll b were estimated according to Ziegler and Egle (1965).

The carotenoids were separated by high pressure liquid chromatography (HPLC model: Hewlett Packard 1084 B) with a RP-8

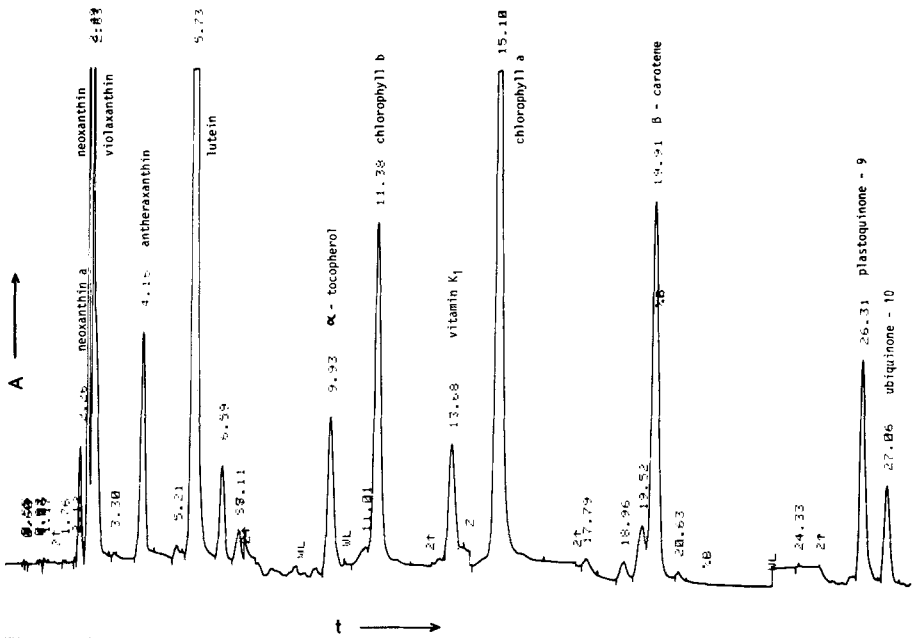

Fig. 3. High pressure liquid chromatographic separation of pigments and quinones of green barley leaves with a reversed phase column (RP 8). Gradient: 85% methanol in water to 95% methanol in water within 20 min

(5 µm, 25 cm, ∅ 4.6 mm) column (Fig. 3). Conditions of chromatography: Rate of flow: 2.5 ml min^{-1}; mobile phase: gradient 85% methanol in water to 95% methanol in water within 20 min; detector: variable wavelength detector (wavelength for chlorophyll a and carotenoids 430 nm, 470 nm for chlorophyll b). The quantitative amounts of the individual pigments were ascertained from calibration curves and summarized for total pigment estimation. Plastid-quinones also separated in the same chromatogram are not expressly mentioned in the text. This new separation method has been developed for routine analysis of photosynthetic pigments and quinones with the advantage to separate them together in one chromatographical run.

The water soluble pigments, including mainly the flavonoids, were extracted in hot water and measured after adding 3 drops of 10% $AlCl_3$-solution (w/v) at 405 nm. The content of the $AlCl_3$-shifted flavonoids was ascertained from a rutin calibration curve.

The data represent average values of three independent experiments with three parallel estimations within one experiment. The standard deviation did not exceed ±10%. The values were statistically checked according to the U-test of Wilcoxon (1945) and Mann and Whitney (1947) and are statistically significant on the 5% level. Values with lower significance are expressly mentioned in the text.

SEM Examination. Leaf discs were fixed for 2 h in darkness in 5% glutaraldehyde buffered by phosphate (pH 7.3) dehydrated in a graded series of acetone and then dried in a critical-point drier using liquid CO_2. The discs were coated with gold in a vacuum evaporator and exmined in a Cambridge Stereoscan Microsope operating at 20 k V.

Results and discussion

Different food plants were exposed directly after sowing for 5 days (radish), 7 days (barley), 8 days (bean), or 10 days (corn) to continuous UV-B irradiation. All four plant species investigated reacted to increased UV-B exposure (Fig. 4).

Bean seedlings were especially sensitive, their fresh weight as well as their leaf area being approximately halved in comparison to the control seedlings. The reduction of fresh weight and leaf area was almost as pronounced in radish seedlings. The monocotyledonous plants, barley, and especially corn showed a much smaller reduction in fresh-weight and leaf area. The dry weight of barley, corn, and radish seedlings was not significantly changed compared to the control plants. Therefore, it is obvious that UV-B irradiated plants suffer water deficiency. The reason for this might be a deformed epidermis, which was regularly observed on SEM pictures of UV-irradiated leaves (Figs. 5 and 6).

In all four plant species exposed to enhanced UV-B irradiation a rise in the content of water soluble proteins was found as compared to the control plants. At the moment it is still unknown whether the biosynthesis of all proteins and their amino acids is stimulated. It is possible that only the synthesis of the aromatic amino acids is enhanced. They are precursors for the biosynthesis of flavonoids.

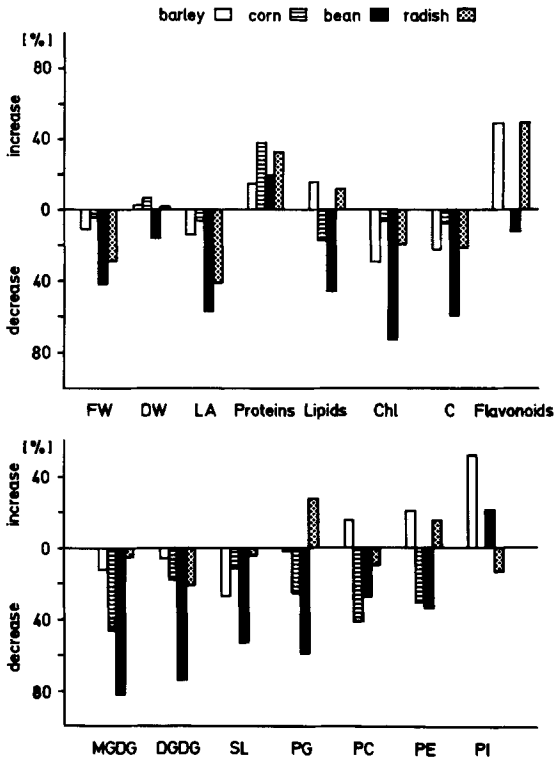

Fig. 4. Effects of enhanced UV-B irradiation on the growth parameters and the content of some leaf compounds in barley, corn, bean and radish seedlings. The columns represent relative increases or decreases compared to the control plants (with glass filter). The absolute amounts are based on 100 plants

The concentration of the flavonoids rose in barley and radish seedlings (Fig. 4). Synthesis of these pigments which are localized in the epidermis and mesophyll has been shown in some cases to be stimulated by ultraviolet radiation (Wellmann 1971). A higher concentration of flavonoids was also always found in alpine plants which were exposed to increased ultraviolet irradiation (Caldwell 1968). It has been shown, therefore, that plants may protect themselves from increased UV irradiation by accumulating substances that absorb the radiation. In greenhouses under normal window glass, plants do not adapt to solar UV-B radiation, therefore, they produce considerably fewer flavonoids and thus are unprotected when suddenly exposed to the UV irradiation present outdoors (Bogenrieder and Klein 1978).

The chlorophyll content was decreased by about 70% in bean seedlings and by about 30% in barley seedlings. The carotenoids were less damaged than the chlorophylls, although carotenoids help to protect the chlorophylls from photodestruction. Therefore, the biosynthetic pathway of chlorophyll might be more influenced by UV-B than that of carotenoids. Furthermore, since the proportion of chlorophyll a to chlorophyll b rises in UV-irradiated plants, it is reasonable to conclude that UV-B radiation stress

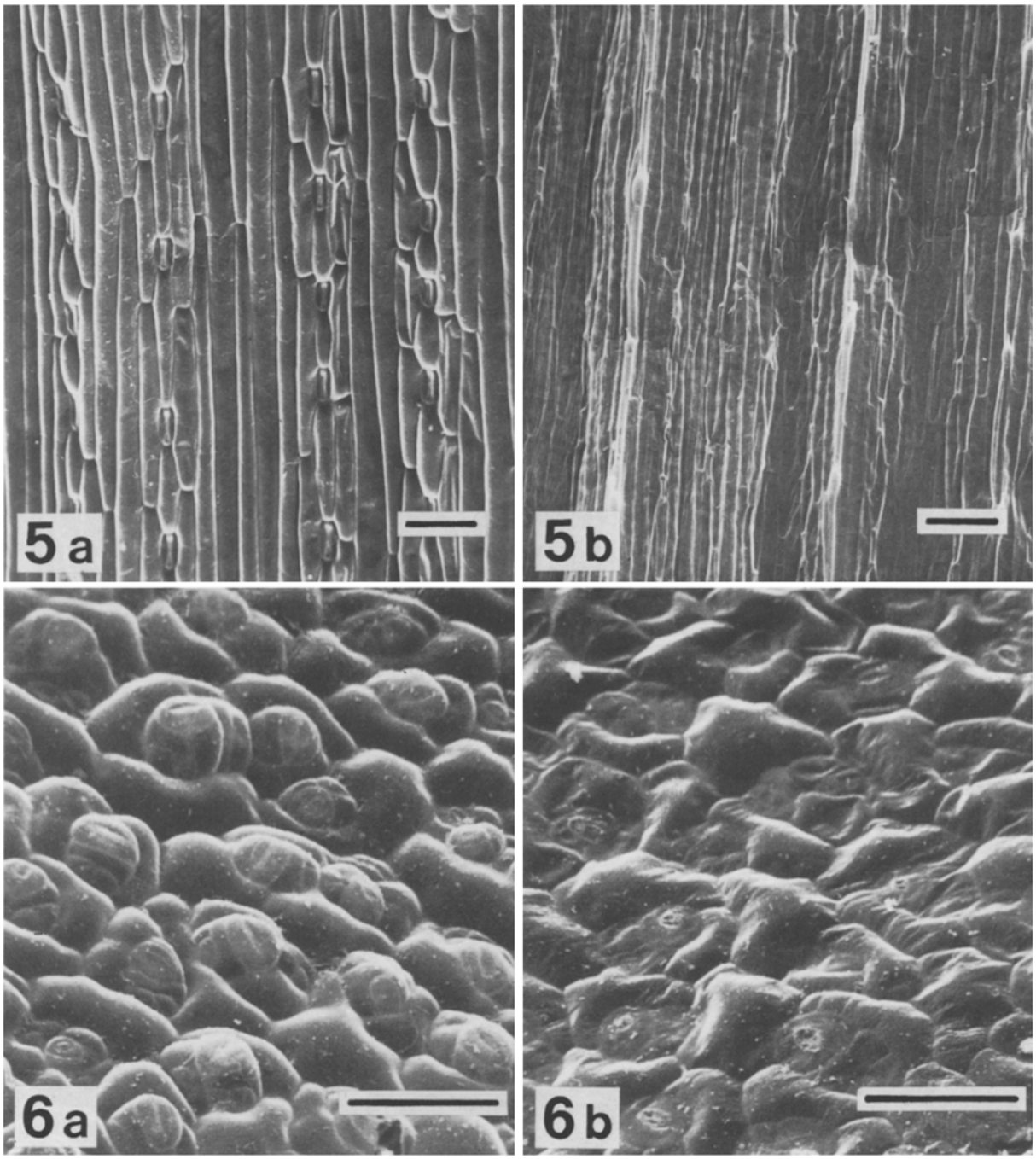

Fig. 5–6. SEM pictures of the epidermal surfaces of barley and radish leaves

Fig. 5a, b. Upper surface of a barley leaf **a** control **b** UV-B irradiated (all 110×, bar = 100 μm)

Fig. 6a, b. Leaf surface of radish **a** control **b** UV-B irradiated (all 230×, bar = 100 μm)

inhibits the biosynthesis of chlorophyll b more than chlorophyll a (Table 1).

The glycerolipids represent important components of membranes and fulfill mostly structural, but also functional, purposes. Thus, the galactolipids monogalactosyldiglyceride (MGDG) and digalactosyldiglyceride (DGDG) as well as the phospholipid phosphatidylglycerol (PG) are main components of the chloroplast. Phosphatidylethanol-amine (PE) and the greater part of phosphatidylcholine (PC) are to be found in mitochondria and in plasma membranes. Judging from the change in concentration of the indi-

Table 1. Chlorophyll a/b and MGDG/DGDG ratios in leaves of different plants irradiated with a low UV-B fluence rate (0.6 W m^{-2})

Plant	Chl a/Chl b		MGDG/DGDG	
	Control	UV-B enhanced	Control	UV-B enhanced
Bean	2.71	2.87	2.52	1.75
Barley	2.49	2.65	2.05	1.91
Radish	2.86[a]	2.89[a]	2.82[a]	2.60[a]
Corn	3.09	3.37	1.72	1.34

[a] not significant

vidual lipids as a result of irradiation by UV-B it appears logical to conclude that the various membrane systems are influenced to different degrees.

The concentration of the galactolipids MGDG and DGDG as well as of the sulpholipid was diminished throughout. It is especially notable that the corn seedlings showed a reduction of almost 50% in their MGDG content, although the influence of the UV-B irradiation on the chlorophyll concentration was only very slight. In this case structural and functional lipids seem to react quite differently, while chlorophyll and galactolipid formation were both inhibited to the same degree in bean seedlings.

In all seedlings irradiated with enhanced UV-B the MGDG to DGDG ratio was shifted in favor of DGDG (Table 1). Similar changes have been frequently observed in yellowing tissues and may indicate the start of senescence (Tevini 1976).

The concentration of the phospholipids varied greatly. In corn and bean seedlings a reduction was found in the content of PC and PE up to 40%, whereas in barley seedlings there was even a rise in the amount of PC, PE, phosphatidylinositol (PI) between 16% and 50% in comparison to the control plants (Fig. 4). It is not yet known whether the number of mitochondria is related to these various changes in the amounts of the phospholipids.

In the second part of the experiments, the dependence of effects on the fluence of the UV-B irradiation was ascertained for barley. Seedlings were exposed to 3 fluence rates for 7 days.

Increasing damage in the external appearance of the plants could be very clearly correlated with rising UV-B fluence rates. After exposure to the highest fluence rate, the plants exhibited stunted, crooked, and irregular growth. There was a considerable leaf bronzing. The plants exposed to the medium fluence rate were less affected, grew straighter and reached more than half the height of the control plants. After exposure to the low UV-B fluence rate, the irradiated barley seedlings were only slightly smaller than the controls and showed hardly any signs of scorching. The extent of decrease in fresh weight, dry weight,

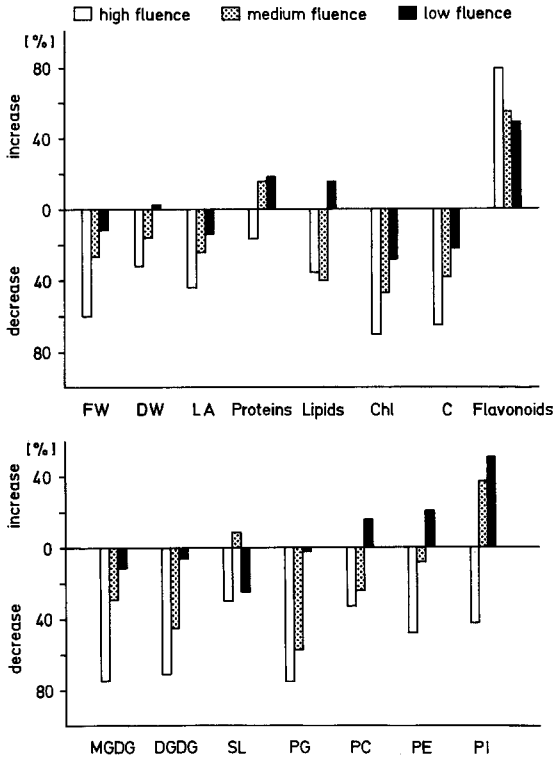

Fig. 7. Effects of low (0.60 W m^{-2}), medium (1.16 W m^{-2}) and high (2.30 W m^{-2}) UV-B fluences (irradiation time 7 days) on the growth parameters and the content of substances contained in barley seedlings expressed as relative increases or decreases compared to the control plants (with glass filter). The absolute amounts are based on 100 plants

and leaf area depended directly on the UV-B fluence rate (Fig. 7). The reduction in the fresh weight at the high fluence rate was 60%, at the medium fluence rate 27%, and at the low fluence rate only 11%. Similar values were obtained for the leaf area. The reduction in the dry weight was much less severe. The negative effects of UV-B on the pigment content of the plants were especially pronounced. The high UV-fluence rate led to a reduction in the chlorophylls of 70% and in the carotenoids of 65%, the low fluence rate to a reduction of 29% and 22%, respectively, compared to the control plants. Much as in the case of the photosynthetic pigments, an especially steep drop in the content of the glycerolipids MGDG, DGDG, and PG was found with a decrease of about 75% at high UV-B fluence. The low fluence promoted the formation of the other phospholipids, while high fluences, on the other hand, inhibited this accumulation (decrease of almost 50%) (Fig. 7). No clear correlation can be drawn between the UV-B fluence and the increase or decrease in the content of proteins and lipids. A rise in the protein content could be seen at low and medium UV-B fluence rates, while the total lipid content only increased at the low UV-B fluence rate (Fig. 7). It seems that retardation or inhi-

bition of biosynthetic pathways by high UV-B fluence rates is not a general rule, since the amount of flavonoids actually increases with UV-radiation (Fig. 7). This result fully confirms earlier findings in cell-suspension cultures of *Petroselinum*, in which the flavonoid synthesis also depended on the UV-fluence (Wellmann 1975). The transmission of the epidermis may be an important factor to consider among the effects of UV-B on cellular and metabolic processes. Investigations of about 25 species with different epidermal properties showed a decrease of transmission with increasing UV fluences (Robberecht and Caldwell 1978). The flavonoids and phenolics may be partly responsible for this tendency, as it is indicated by flavonoid accumulation in the leaves. In our case, the destruction of the epidermal layer with increasing UV-B fluence, as shown by the scanning electron microscope (SEM)-pictures, makes it unlikely that all flavonoids are synthezised and accumulated in the epidermis.

The results obtained for the flavonoids as well as for the fresh weight and leaf area are in agreement with recently published results on other plants under different cultivation conditions (e.g., Brandle et al. 1977; Vu et al. 1978; Lindoo and Caldwell 1978; Semeniuk and Stewart 1979; Teramura 1980; Teramura et al. 1980). With respect to the protein content, a stimulation under the low UV-B fluence rate is in accordance with the results obtained in field experiments (Esser 1980) and the results with alpine plants (Pirschle 1941). However, we found in barley seedlings a reduction of the protein content after irradiation at the high UV-B fluence rate. Similar results have been reported for some other plants, although the irradiation conditions were not identical (Andersen and Kasperbauer 1973; Basiouny et al. 1978).

The results presented here clearly show, that UV-B irradiation, depending on its fluence, causes changes in the growth, composition, and leaf surface of plants. The plant species investigated here vary, however, in their response to UV-B irradiation. Bean, barley, and radish plants are very susceptible to the effects of continuous UV-B irradiation, corn, on the other hand, appeared resistant.

A consideration of our results with respect to the ozone problem does not appear useful at the moment, until any information on the fluence effect relationships is available for the various responses to the relevant UV-B wavebands.

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