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Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*

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Key words: Rubisco; Photosynthetic capacity; Quantum yield; ATP synthase; Carotenoid; (*P. sativum*)

Pea plants (*Pisum sativum* L., cv. Greenfeast) were exposed to supplementary UV-B light (up to 8 days) starting on the 17th day after sowing. The effects of this exposure on photosynthesis and the content and activities of some chloroplast components of the mature leaves of these plants were studied. (i) The total chorophyll content of pea leaves was approximately 40% of that in the control leaves on the 8th day of UV-B exposure. Chlorophyll *a* levels decreased to a greater extent than the content of chlorophyll *b*. The decrease in carotenoids paralleled the decrease in chlorophyll *b*. (ii) On a chlorophyll basis, the contents of Photosystem I and cytochrome *f* were stable, whereas Photosystem II, ATP hydrolysis by the ATP synthase and the maximum ribulose-1,5-bisphosphate carboxylase (Rubisco) activity decreased by 55, 47 and 80%, respectively, when compared with the controls at the end of the 8-day illumination period. (iii) On a leaf-area basis, Photosystem I and cytochrome *f* content decreased by 58%, Photosystem II by 80%, ATP hydrolysis by 80%, and Rubisco activity by 90%, when compared with the controls. The in vivo activation of Rubisco was markedly increased in UV-B-treated pea leaves. The underlying mechanisms for these results are discussed.

Introduction

Solar radiation is essential for all plant life. Light is not only the driving force of photosynthesis but it also triggers and regulates many morphogenic responses. Excessive photon flux density, however, is potentially harmful, if not lethal, for plants. Deleterious effects to photosynthesis may be caused by excess visible light (photoinhibition; Ref 1) and ultraviolet (UV) radiation [2,3]. Global ultraviolet irradiance on the earth's surface fluctuates with changes in solar altitude, cloud cover, atmospheric turbidity and stratospheric ozone concentration. Little UV radiation below 290 nm is detected at the earth's surface due to the absorption of solar radiation in the 200–290 nm region by stratospheric ozone. Whereas UV-C radiation (< 280 nm; e.g., the mercury lamp with mostly 254 nm emission) causes damage to photosynthesis, primarily to the water-splitting apparatus of Photosystem II (PS II), and by the destruction of plastoquinone, it is not relevant in the natural environment [4]. However, UV-B radiation (280–320 nm) is readily absorbed by nucleic acids, proteins, pigments and lipids to a lesser extent [2]. UV-B primarily inhibits photosynthesis, as well as damaging many other plant processes [2,3].

The effects of UV-B radiation have been studied on plant communities, individual plants, leaves, isolated chloroplasts or thylakoid membranes using a plethora of UV-B radiation procedures ranging from UV-B radiation only, to various light treatments supplemented with UV-B [2,3,5]. The present study was initiated to evaluate the effects of moderate levels of supplementary UV-B radiation on photosynthesis. The composition and function of chloroplasts from fully expanded mature leaves of peas were compared following exposure of one-half of the pea plants to additional UV-B radiation from the 17th day after planting. Peas were shown

Abbreviations: ATPase, ATP hydrolytic activity of the ATP synthase/hydrolase; Bicine, *N,N*-bis(2-hydroxyethyl)glycine; Car, carotenoids; CF_1, the catalytic component of the chloroplast ATP synthase/hydrolase; Chl, chlorophyll; C_3, plants fixing carbon by the tricarboxylic acid pathway; D_1, the herbicide-binding polypeptide of Photosystem II; F_m, maximum chlorophyll fluorescence; F_o, initial chlorophyll fluorescence when Photosystem II reaction centres are open; F_v, variable chlorophyll fluorescence ($F_m - F_o$); LHCII, the light-harvesting components associated with Photosystem II; PAR, photosynthetically active radiation; PS I, Photosystem I; PS II, Photosystem II; P700, the reaction centre chlorophyll *a* of Photosystem I; Rubisco, ribulose-1,5-bisphosphate carboxylase; RuP_2, ribulose 1,5-bisphosphate; UV-B, ultraviolet-B radiation (280–320 nm); UV-C, ultaviolet-C radiation (< 280 nm).

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to be rather sensitive to our lighting conditions in a preliminary experiment which included nine agricultural plants commonly grown in Australia. Our results show that the ribulose-1,5-bisphosphate carboxylase (Rubsico) activity is most affected by supplementary UV-B radiation, and that Photosystem II function and ATP hydrolysis also drastically decline. We are now in a position to probe the molecular mechanisms of UV-B inactivation of photosynthesis.

Materials and Methods

Growth and treatment of plants

Pea (*Pisum sativum* L., cv. Greenfeast) seedlings were grown in pots of a vermiculite/perlite mixture in a growth room with a daily regime of 12 h light (7 Philips TL 40W 33RS fluorescent tubes, approx. 150 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (400–700 nm) measured with a Li-Cor 195A quantum photometer, 22° C) and 12 h dark (16°C). On the 17th day after sowing, approximately half of the plants were transferred into another compartment where the fluorescent lamps (4 of Philips TL 40W 33RS and 4 of Philips SL25 Prismatic) supplying equal photosynthetically active radiation of approx. 150 μmol photons $\cdot m^{-2} \cdot s^{-1}$ were supplemented by 3 UV-B lamps (Philips TL 40W/12 UV) during the 12 h photoperiod.

In order to eliminate UV radiation below 290 nm, the UV-B lamps were wrapped with cellulose acetate sheets (thickness 0.13 mm). Since the transmission spectrum of cellulose acetate changed after exposure to UV-B (Fig. 1), the sheets were replaced daily during the experiment. At 30 min from the beginning of the photoperiod, the levels of UV-B, measured with an IL1700 Research Radiometer with calibrated photodetector/filters (International Light, Newburyport, U.S.A.), were 50 and 220 mW $\cdot m^{-2} \cdot nm^{-1}$ at 297 nm and 313 nm, respectively. It is obvious that, due to deterioration of cellulose acetate on exposure to UV-B, the spectral irradiance was lower at the end than at the beginning of the photoperiod.

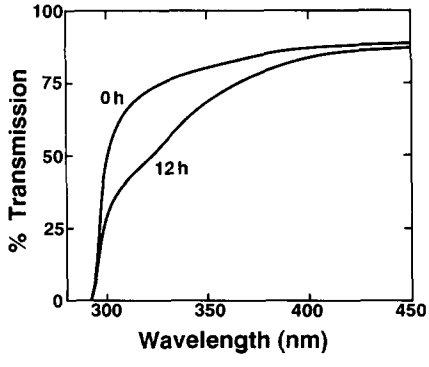

Fig. 1. The transmittance of the cellulose acetate filter used with the UV-B source to diminish light of wavelengths lower than 290 nm. The upper and lower curves show the transmittance for a fresh cellulose acetate sheet and for the same sheet after 12 h of use, respectively.

For 1 week following the transfer of pea plants to a light environment supplemented with UV-B, we monitored the time courses and total extents of a large number of plant and photosynthetic responses. Our photosynthetic measurements were focused on the third pair of leaves from the base of the plant in order to standardize leaf age.

Determination of the chlorophyll and carotenoid content of leaves

Pea leaf discs (2.74 cm^2) were ground in 80% acetone. Chlorophyll (Chl) and carotenoids (Car) were determined according to the simultaneous equations of Porra et al. [6] and Lichtenthaler [7], respectively.

Determination of thylakoid components

The number of functional PS II reaction centres was determined from the O_2 yield per single-turnover xenon flash given to a 2.74 cm^2 leaf disc at 5 Hz in the presence of far-red light [8].

Chloroplasts were isolated as earlier described [9] and frozen at 77 K until used for the determination of thylakoid components. The chlorophyll concentrations of thylakoid stocks were assayed according to Ref. 6.

The number of functional PS I reaction centres was determined by light-induced absorbance changes of the PS I reaction centre chlorophyll *a* (P700) at 703 nm according to Chow and Hope [10].

Cytochrome *f* was assayed [11] from the hydroquinone-reduced minus ferricyanide-oxidized spectra in a Perkin-Elmer 557 double-beam spectrophotometer.

The Mg^{2+}-dependent ATP-hydrolytic activity of thylakoids was assayed in the presence of octyl glucoside as an activator [10,12]. This hydrolytic activity is taken as a measure of the content of CF_1, the coupling factor 1 of chloroplast ATP synthase [13].

Determination of ribulose-1,5-bisphosphate carboxylase

The carboxylase activity and the relative activation of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme were essentially determined as previously described [14]. Leaf discs (2.74 cm^2) were sampled in the middle of the photoperiod and immediately frozen at 77 K. For subsequent assay of Rubisco, each frozen leaf disc was immediately transferred to a mortar and pestle containing liquid N_2 and ground thoroughly to a fine powder. As soon as the liquid N_2 had evaporated, 1 ml of 100 mM *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine, pH 8.1), previously extensively bubbled with pure N_2 to remove CO_2, was added and mixed. An aliquot (25 μl) of the leaf extract in Bicine buffer was mixed with an assay medium (1 ml) containing 100 mM Bicine/KOH (pH 8.1), 20 mM $MgCl_2$, 15 mM $NaH^{14}CO_3$ (10 Bq $\cdot$ $nmol^{-1}$) and 50 μM 6-phosphogluconate. To estimate

the Rubisco activity of leaves in their growth environment, the assay was commenced immediately (within 20 s after mixing the leaf powder with Bicine buffer) by the addition of 0.5 mM ribulose 1,5-bisphosphate (RuP_2). To obtain the maximum activity, the enzyme was allowed to activate at 25°C for 7 min and then assayed by the addition of 0.5 mM RuP_2. In either case, the reaction was stopped after 60 s by injecting 0.1 ml of 12 M formic acid. A blank reaction (assay mixture minus RuP_2) was measured and subtracted from the Rubisco activities obtained. The mixture was dried carefully by gentle heating in a fume hood, mixed with 0.3 ml water and 2.7 ml Beckman Ready Value scintillation fluid, and counted.

Measurements of photosynthesis

The quantum yield of O_2 production and the maximum photosynthetic capacity of pea leaf discs (2.74 cm^2) were determined by light-saturation experiments performed with a Hansatech leaf disc O_2-electrode (King's Lynn, U.K.) at 25.0°C and at saturating CO_2 conditions (1% CO_2 from a 1 M carbonate/bicarbonate buffer, pH 9), as previously described [15].

Measurements of Photosystem II fluorescence

Chlorophyll fluorescence from leaves attached to plants was assayed by excitation with blue-green light (Corning 4-72 filter, approx. 500 μmol photons $\cdot m^{-2} \cdot s^{-1}$) passed through an electronic shutter which opened in 1.2 ms. The fluorescence at room temperature was detected at 685 nm using a light-guide, a photodiode and a Gould 1421 digital storage oscilloscope. The fluorescence level corresponding to open PS II reaction centres, F_o, was taken as the fluorescence at 1.5 ms after the start of shutter opening. The variable fluorescence (F_v) was obtained as the difference between F_m and F_o, where F_m is the maximum fluorescence reached within 0.4 s after shutter opening, i.e., when the PS II reaction centres are closed.

Results

Growth and development

Nine different species (barley, beans, cotton, maize, pea, sorghum, soybean, spinach and wheat) were tested in a preliminary experiment (pigment content, photosynthetic capacity and quantum yield) under our supplementary UV-B light regime. Pea, the second most important legume crop of Australia, was the most sensitive, whereas the other species showed less drastic declines in photosynthetic responses.

Commencing on the 17th day from sowing, illumination of half the pea plants was supplemented with UV-B light (12 h daily). Note that the photosynthetically active radiation supplied to the control plants was equal to that supplied to the plants transferred to the supplementary UV-B regime. Measurement of the spectral irradiance from 380 to 720 nm with a spectroradiometer (SR 3000A, Macam Photometrics, Livingston, Scotland) showed that the blue component (380–470 nm) was 18.0% of the total PAR for control plants, and 18.7% for the supplementary UV-B-treated plants, respectively (not shown).

The effects of supplementary UV-B irradiation on pea plants were clearly visible as bronzing of the leaves occurred. The gross physiological effects were further studied by an extensive examination of the underlying chloroplast responses. After the finish of UV-B exposure, some UV-B-treated pea plants were kept for a period of 2 weeks in normal fluorescent light. Recovery of the UV-B-treated plants was seen with fresh shoots emerging.

Pigment content of pea leaves

In all experiments, leaf discs were taken from only the third pair of leaves above the base of the pea plants so that the photosynthetically active radiation (PAR) received by the leaves would have been identical. Also, this means that the tissue was of comparable physio-

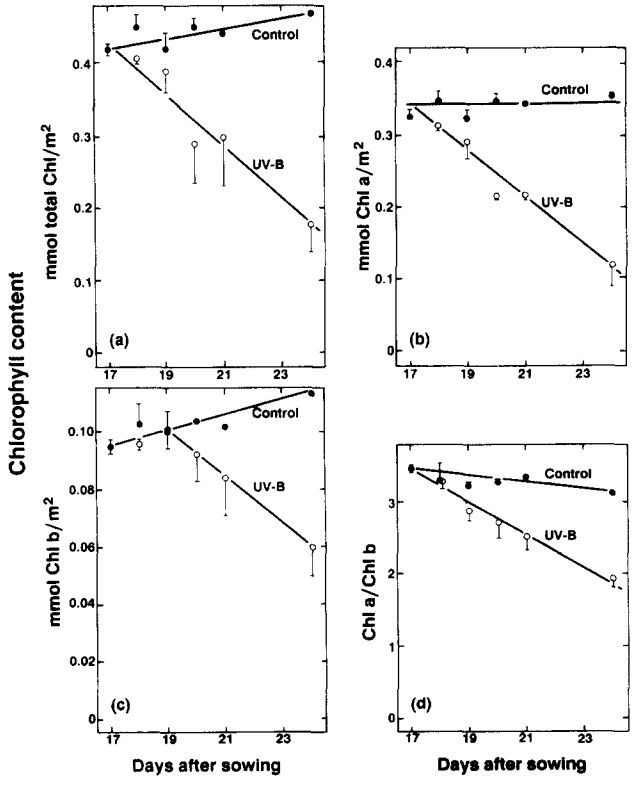

Fig. 2. The content of chlorophyll in pea leaves as a function of the duration of the UV-B treatment which commenced on the 17th day after sowing. (a) The total amount of chlorophyll, (b) the amount of chlorophyll *a*, and (c) the amount of chlorophyll *b* of UV-B-treated (○) and control (●) peas expressed on a leaf area basis. (d) The effect of enhanced UV-B radiation on the chlorophyll *a* to chlorophyll *b* ratios of pea leaves. The standard errors of the determinations are indicated with bars.

logical age and the leaves were almost fully expanded, thereby eliminating developmental effects. Fig. 2 shows the effect of different levels of UV-B radiation on the chlorophyll content. Whereas the total chlorophyll content per unit leaf area in the controls was increased by almost 12% in the 8-day test period, the UV-B-treated peas lost more than 55% of their total chlorophyll (Fig. 2a). This means that on the 8th day of exposure to UV-B-enriched light, the chlorophyll content was only 40% of that in the control leaves. It is evident from Fig. 2b that the drop in total chlorophyll was due mainly to the substantial lowering of the Chl *a* from 0.34 to 0.12 mmol · m^{-2}, or by 65%; the level of Chl *a* in the controls remained constant. On the other hand, the Chl *b* levels in control leaves were increased by nearly 20%, probably due to shading of the third leaf pairs during the development of the plants (Fig. 2c). A comparison of Figs. 2b and 2c reveals that, in the UV-B-treated plants, Chl *a* declined to a greater extent than Chl *b*. The latter decreased by approx. 35%, being 52% of the value in the controls on the 8th day of UV-B treatment. Furthermore, no lowering of the Chl *b* was found until the 4th day of treatment. Taken together, these UV-B-treated plants exhibit drastic lowering of the Chl *a*/Chl *b* ratio (Fig. 2d) from about 3.45 to 1.85 (by 45%). This decrease implies that severe stress is imposed on the plants. The lowering of the Chl *a*/Chl *b* ratio in the controls from 3.45 to 3.12 (by less than 10%) can be attributed to the aforementioned shading of the third leaf pair.

The content of carotenoids was also lowered by UV-B radiation. On a unit area basis, approx. 50% of the amount of carotenoids present in healthy leaves remains in leaves exposed to eight 12 h periods of UV-B (Fig. 3a). However, the relative decrease in total chlorophyll was significantly larger (Fig. 3b) than the drop in the total carotenoid content, and increasingly so the longer the period of treatment. The weight ratio Chl/Car decreased from 5.1 to 3.6 (approx. 30%) during the treatment. Furthermore, only Chl *a* decreased more rapidly than the carotenoids (by 35% in Fig. 3c), whereas the Chl *b*/Car ratio was not affected by UV-B exposure (Fig. 3d). Nevertheless, the latter ratio is increased by approx. 15% during the course of the study for both treated and untreated leaves.

Thylakoid membrane components involved in electron transport and photophosphorylation

The oxygen yield (i.e., a measure of the electrons extracted from H_2O) per single-turnover flash is an estimation of the number of PS II reaction centres

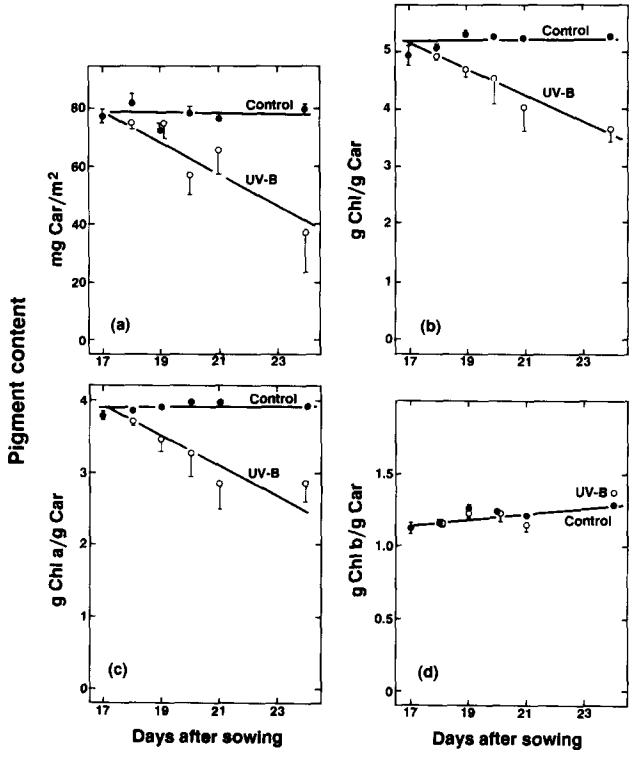

Fig. 3. (a) The amount of carotenoids (expressed as mg per unit leaf area) as a function of the duration of UV-B-treatment, which started on the 17th day from sowing. The w/w ratios of (b) total chlorophyll to carotenoids, (c) chlorophyll *a* to carotenoids, and (d) chlorophyll *b* to carotenoids are also shown as a function of UV-B treatment. (●) denotes control samples and (○) the UV-B-exposed samples. The standard errors of the determinations are indicated with bars.

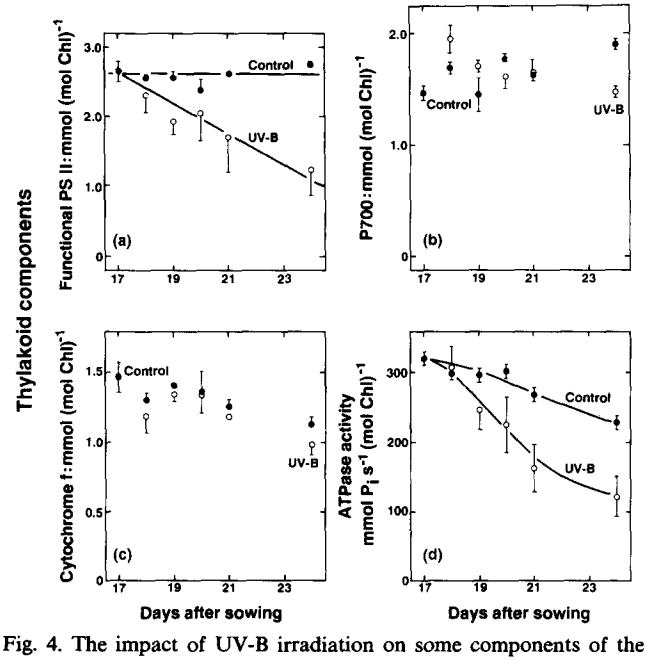

Fig. 4. The impact of UV-B irradiation on some components of the thylakoid membranes. (a) Photosystem II was measured by oxygen evolution induced by single-turnover flashes, (b) Photosystem I as P700 content, (c) cytochrome *f* from the reduced minus oxidized difference spectrum, and (d) ATP synthase as the octyl glucoside-stimulated Mg^{2+}-dependent ATPase activity. UV-B treatment commenced on the 17th day after sowing and the activities of the different components of control (●) and UV-B-exposed (○) pea plant leaves were assayed until the 24th day. The bars indicate the standard errors of the data.

functional in charge separation and electron transport [8]. Fig. 4a shows this quantity as a function of the time of exposure to UV-B: a drastic decrease by 55% was seen over the experimental period. Since the flash yield in Fig. 4a is expressed per mol Chl, the actual decrease per unit leaf area is even more severe, in fact nearly 80% when the results from the 24th day after sowing (Figs. 2a and 4a) are combined.

On the other hand, the number of functional PS I reaction centres (Fig. 4b) and cytochrome *f* (Fig. 4c), expressed on the basis of chlorophyll content, are nearly the same in both UV-B-treated and untreated leaves. This means that per unit leaf area, PS I and cytochrome *f* decreased in parallel with the chlorophyll content by approx. 58% (Fig. 2a). Both controls and UV-B radiation-exposed plants showed a slight decline in cytochrome *f* concentration over time (Fig. 4c).

The ATP hydrolytic activities of octyl glucoside-treated thylakoids were used as a relative measure of the number of functional ATP synthases [10,12]. Fig. 4d shows the ATPase activity as a function of the time of exposure. It is important to note that ATP hydrolysis is expressed on the basis of chlorophyll content. The activity of control leaves decreased by 28% over the test period, whereas UV-B illumination resulted in a 62% lowering of the activity. At the end of the treatment, the ATPase activity of UV-B-exposed thylakoids was about 50% of that in the controls.

Based on unit leaf area (cf. Fig. 2a), the ATP hydrolysis rate on the 8th day of treatment was diminished to a mere 16% of the rate found in leaves before exposure to UV-B. The control leaves, on the other hand, retained more than 80% of its ATPase activity from day 17 through day 24 since the total chlorophyll content was increased in these leaves over the period. Compared with the thylakoids from the control plants, those from UV-B-irradiated plants had an ATPase activity of less than 20% on the last day of exposure.

Rubisco activity

The first enzyme involved in CO_2 assimilation and carbon fixation, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), was examined for its carboxylase activity in leaves from plants treated with UV-B and in leaves from control plants. It is obvious from Fig. 5a (activity is expressed on the basis of Chl content) that the diminution from 75 to 15 mmol CO_2/s per mol Chl, i.e. by 80%, is far greater than the decrease of approx. 29% in the control leaves over the same period of time. On the last day, the UV-B-treated leaves had a carboxylase activity of 28% of the controls.

On a leaf area basis, the decrease of functional Rubisco in UV-B-irradiated and control leaves over the period was 92 and 21%, respectively. The carboxylase activity in UV-B-treated pea plants was down by 90% when compared to control leaves on the last day.

The smaller the remaining carboxylase activity became during the experimental period, the more the enzyme was activated in vivo (Fig. 5b). The relative activation of the carboxylase from the control leaves was constant between the 17th and the 24th days after sowing.

Interestingly, PS II content, ATPase activity and Rubisco carboxylase activity decreased to a comparable extent on a unit leaf area basis: by 80, 80 and 90%, respectively. This is considerably more than the contents of PS I, cytochrome *f*, chlorophyll or carotenoids were decreased, the latter being affected least.

Quantum yield and photosynthetic capacity

The decline of chloroplast components due to UV-B treatment could lower both the quantum yield of photo-

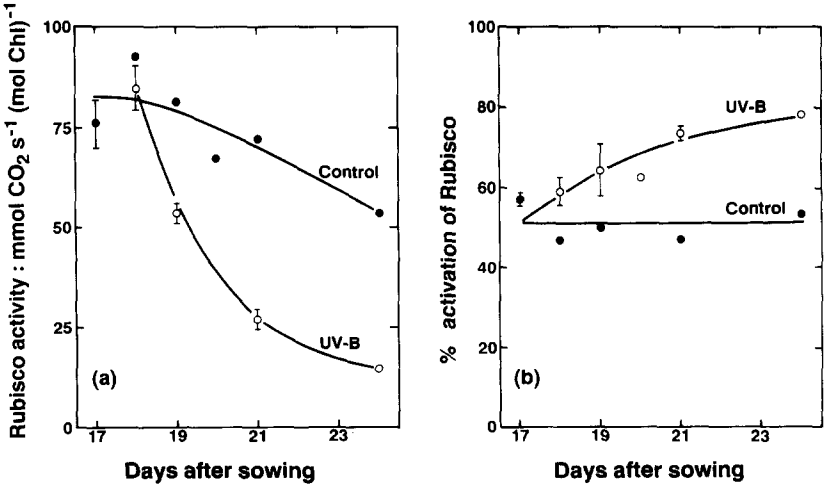

Fig. 5. (a) The ribulose 1,5-bisphosphate carboxylase activity, and (b) the in vivo activation of the Rubisco activity in leaves from control (●) and UV-B-treated (○) pea plants as a function of the duration of the experiment. UV-B treatment started on the 17th day after sowing, and leaves were sampled in the middle of each photoperiod. The bars indicate the standard errors of the experiments.

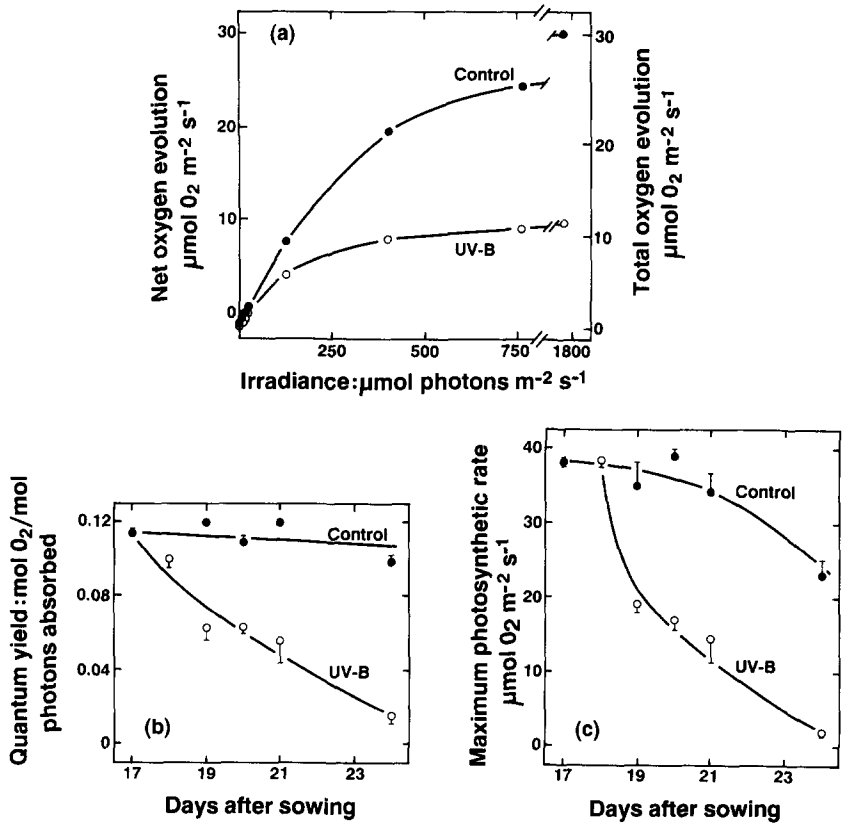

Fig. 6. (a) The rate of oxygen evolution from pea leaf discs as a function of the incident light intensity on the 21st day after sowing or the 5th day of UV-B treatment. Both the total production of O_2 and the net oxygen evolution (total oxygen production minus respiration) are given for control (●) and UV-B-exposed (○) pea leaves. (b) The quantum yields of O_2-evolution, derived from the linear part of the light-saturation curve and corrected for the absorptance of the leaf discs, is shown as a function of the time of exposure to supplementary UV-B radiation (12 h light/12 h dark). Transfer to UV-B radiation was made on the 17th day after sowing. (c) The maximal production of oxygen per unit leaf area, as derived from applying Michaelis-Menten kinetics to the light-saturation of O_2 evolution, is given as a function of time of exposure to UV-B. The bars show the standard errors of the data.

synthesis at limiting light and the maximal capacity for photosynthesis at light- and CO_2-saturation. A comparison of the photosynthetic capacity per unit leaf area between control and UV-B-exposed pea leaves is shown in Fig. 6a. The actual example is from the 21st day after sowing (i.e. on the 5th day of UV-B treatment). The slope of the linear part of this curve at low light intensities is a measure of the maximum quantum yield of photosynthesis in the tested leaves. This quantity is shown in Fig. 6b as a function of the time of exposure

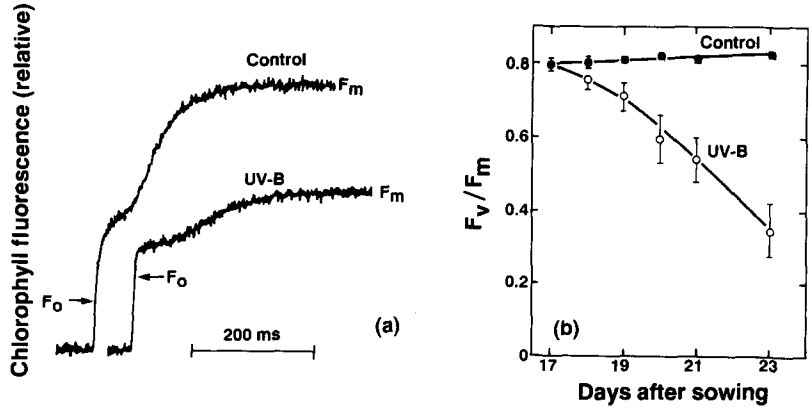

Fig. 7. (a) Typical traces from assaying of the chlorophyll fluorescence of UV-B-exposed and control pea leaves (attached to the plants) on the 20th day after sowing, i.e., on the 4th day of UV-B treatment. F_m denotes the maximal fluorescence, and F_o the fluorescence when the Photosystem reaction centres are open. F_v is defined as $F_m - F_o$. (b) F_v/F_m (a measure of the photochemical efficiency of Photosystem II) is shown as a function of the time of exposure to UV-B-supplemented light, commencing on the 17th day after sowing; (●) controls and (○) for UV-B-treated leaves. The standard errors of our data are shown with bars.

to UV-B radiation. An acute decline by more than 90% over the test period is obtained for UV-B-treated peas.

The total maximal O_2 production in the leaves, can be obtained from the asymptotes of the light-saturation curves which are hyperbolic in appearance (Fig. 6c). The maximal rate of total oxygen evolution is constant in the controls over the first 5 days but then decreased by almost 35% by the end of the treatment. After 2 days of treatment with UV-B light, a severe decrease in maximal O_2-evolution rate is obtained and by the end of the experiment only 5% of the full rate of the controls remained.

To see if a decrease of quantum efficiency of PS II might have contributed to the decrease in overall quantum yield of O_2 evolution in treated leaves, we monitored the efficiency of PS II photochemistry. Note that the same leaves from one pot were measured daily for these fluorescence experiments, whereas the quantum yields were measured by using leaves from many plants located in different pots, so the experiments are not strictly comparable. The efficiency of photochemistry of PS II can be expressed as the ratio between the rate of the photochemical activity and the rate of total dissipation of the energy absorbed. It is given by the ratio between the variable fluorescence (F_v) and the maximal fluorescence (F_m) of the PS II reaction centres, where F_v is defined as the difference $F_m - F_o$, and F_o is the fluorescence of the open PS II reaction centres [16]. The fluorescence of PS II for control and UV-B-treated pea leaves at 20 days from sowing is shown in Fig. 7a. F_v/F_m is depicted in Fig. 7b as a function of the time of UV-B treatment. A decrease of 55% in the F_v/F_m ratio was seen after 7 days, whereas the control leaves were unaffected. The lowering of $F_v/F_m = (1 - F_o/F_m)$ due to UV-B treatment is mainly a result of a decrease in F_m, but some increase in F_o could also be discerned (Fig 7a).

Discussion

The enhanced level of UV-B radiation used, resulted in visible signs of stress 2 days after the transfer to supplementary UV-B, rather than after a few hours as occurred with high UV-B radiation alone [17]. Many studies carried out in growth chambers, greenhouses, or the field, have shown that supplementary UV-B significantly depresses growth and biomass accumulation of UV-B sensitive species [2,3]. However, studies with supplementary UV-B light and high PAR show less deleterious UV-B effects, with field and glasshouse plants being less sensitive to enhanced UV-B-radiation when compared with plants raised in growth rooms with lower irradiance [2,3,18]. Possibly, protective as well as photorepair mechanisms may be operating under high PAR thus reducing the damage due to UV-B radiation.

In this study, substantial reduction in the total chlorophyll and carotenoid content on a leaf area basis (Figs. 2a and 3a) was evident. The reduction in chlorophyll was more marked in Chl *a* than in Chl *b*, resulting in an overall decrease in the Chl *a*/Chl *b* ratio from 3.1 to 1.9 after 7 days of supplementary UV-B-exposure (Fig. 2d). Stress imposed by low dosages of PS II herbicides, induces a marked decline in the Chl a/Chl *b* ratio of plants, but in this case there is a marked increase in chlorophyll per unit leaf area (cf. Ref. 19). Both the total chlorophyll/ carotenoid and Chl *a*/carotenoid weight ratios declined with increasing supplementary UV-B, while the Chl *b*/carotenoid ratio remained rather constant (Fig. 3d). The latter constancy is probably due to the xanthophylls and chlorophyll *b* being present together as major constituents of LHCII, which comprises over 50% of the total chlorophyll. The decline in chlorophyll content may reflect either a decline in pigment synthesis, or an increase in pigment degradation, or both. High UV-B irradiance with low PAR produced significant reductions in pigment content in peas [20], soybean [21], bean, barley and corn [22].

Supplementary UV-B radiation markedly reduced the content of functional PS II reaction centres, while functional PS I reaction centres on a chlorophyll basis were unaffected (Figs. 4a and 4b). The lack of modulation of P700 on a chlorophyll basis is also observed under other normal and stressed environmental conditions. For example, peas grown under a wide range of growth irradiances have constant Chl/P700 ratios of about 600 [23], as have many other sun or shade plants [24], or plants exposed to high salinity [15]. Not only is the PS I complex relatively stable under most environmental conditions, but plants are generally able to maintain the same amount of P700 per total chlorophyll content. In contrast, the PS II complex is highly vulnerable to many environmental stresses, including UV-C alone (cf. Ref. 4) and UV-B alone [4,25]. The oxygen yield per single turnover flash, a measure of the number of functional PS II reaction centres [8], shows a dramatic decline of about 50% on a chlorophyll basis (Fig. 4a) and 80% on a leaf area basis after 7 days of exposure to enhanced UV-B. Over the same period of UV-B radiation, the PS II/PS I reaction centre stoichiometry declines from the normal value of 1.8 to 0.82.

The content of cytochrome *f*, an indicator of the content of the cytochrome *b*/*f* complex, on a chlorophyll basis is unaffected by supplementary UV-B radiation (Fig. 4c). This means that the cytochrome *f*/700 ratios remain constant with increasing supplementary UV-B exposure, in contrast to the PS II/PS I reaction centre ratios. On a leaf area basis, PS I, cytochrome *f* and total chlorophyll decline in parallel.

The number of functional chloroplast ATP synthases per unit chlorophyll declines significantly during UV-B radiation exposure (Fig. 4d), indicating for the first time

a substantial decrease in photophosphorylation capacity.

After the second day of UV-B exposure, there was a rapid decline in Rubisco activity on a leaf area basis, falling to only 10% of that of the controls by the end of the treatment. The smaller the remaining carboxylase activity, the more the enzyme was activated in vivo (Fig. 5b). Vu et al. [20,26] showed that the Rubisco carboxylase activity declined with increasing exposure of both peas and soybean leaves to UV-B; on a fresh weight basis, there was also a qualitative decrease in Rubisco content [20].

Given that supplementary UV-B caused a reduction in the content of PS II complex and of ATP hydrolysis by the ATP synthase, a decline in both electron transport and photophosphorylation would decrease both photosynthetic capacity and quantum yield. Comparison of the overall photosynthetic capacity at saturating light, showed that this was indeed the case (Fig. 6c). The decline in photosynthetic capacity approximately mimicked the exponential declines for Rubisco (Fig. 5a) and ATP hydrolytic activities (Fig. 4d).

At limiting light, the mean quantum yield value of control peas was 0.114 ± 0.002 mol O_2 per mol photons absorbed, within the range of the quantum yields reported for 37 different C_3 species [16]. In contrast, following UV-B radiation exposure, there was a drastic non-linear decline in quantum yields. A lowering in the F_v/F_m ratio, which represents the efficiency of PS II photochemistry [16], was also evident (Fig. 7). The diminishing efficiency of PS II photochemistry is substantially responsible for the diminution in the overall photosynthetic quantum yield of UV-B-irradiated leaves. Furthermore, the decline in the F_v/F_m ratio was closely paralleled by that in the concentration of functional PS II complexes (Fig. 4a), suggesting that the loss of PS II photochemical efficiency could have arisen because non-functional PS II complexes (damaged by UV-B) absorbed, but did not utilize, PAR.

We conclude that supplementary UV-B radiation with low PAR is detrimental to mature pea leaves causing a plethora of changes to the photosynthetic apparatus. These changes, occurring progressively over the entire period of UV-B treatment, appear to be responses to the cumulative UV-B dosage. Indeed, Sisson and Caldwell [27] have shown that nearly any level of UV-B radiation caused cumulative suppression of leaf photosynthesis in *Rumex patientia*, such that short periods of high UV-B irradiance were equivalent to long periods of low irradiance. In this study, the progressive suppression of photosynthesis by UV-B radiation resulted from differential declines in pigment and protein contents and enzyme activities.

Since UV-B radiation denatures proteins, and also damages nucleic acids, reductions in the amounts of stroma and thylakoid membrane proteins would be expected. Rubisco, located in the stroma accounts for about 50% of the total soluble proteins. The D1-protein of the core PS II complex is the most rapidly turned-over thylakoid protein, at a rate 80–100-times greater than that of any other protein [28]. Thus, if rates of protein synthesis were to be severely impaired by UV-B radiation, one would expect that Rubisco (by virtue of its high concentration) and the D1 protein of the PS II complex (by virtue of its rapid turnover) would be adversely affected by UV-B radiation leading to the inhibition of both the CO_2-fixing and the energy-transducing processes of photosynthesis.

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