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Net Photosynthesis, Electron Transport Capacity, and Ultrastructure of *Pisum sativum* L. Exposed to Ultraviolet-B Radiation[1]

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ABSTRACT

***Pisum sativum* L. was exposed to ultraviolet-B (UV-B) radiation (280-315 nm) in greenhouse and controlled environment chambers to examine the effect of this radiation on photosynthetic processes. Net photosynthetic rates of intact leaves were reduced by UV-B irradiation. Stable leaf diffusion resistances indicated that the impairment of photosynthesis did not involve the simple limitation of CO_2 diffusion into the leaf. Dark respiration rates were increased by previous exposure to this radiation. Electron transport capacity as indicated by methylviologen reduction was also sensitive to UV-B irradiation. The ability of ascorbate-reduced 2,6-dichlorophenolindophenol to restore much of the electron transport capacity of the UV-B-irradiated plant material suggested that inhibition by this radiation was more closely associated with photosystem II than with photosystem I. Electron micrographs indicated structural damage to chloroplasts as well as other organelles. Plant tissue irradiated for only 15 minutes exhibited dilation of thylakoid membranes of the chloroplast in some cells. Some reduction in Hill reaction activity was also evidenced in these plant materials which had been irradiated for periods as short as 15 minutes.**

Ultraviolet radiation in the 280 to 315 nm waveband, usually denoted as UV-B radiation, is readily absorbed by nucleic acid and protein chromophores (8) and effectively inhibits many plant processes (5), including photosynthesis (2, 22). Ultraviolet radiation of this waveband is of particular interest to photobiologists because this radiation occurs in normal sunlight and would be intensified if the atmospheric ozone layer were reduced (3, 10). Although the action of intense 254 nm UV radiation on photosynthetic processes has been well studied (7), this radiation would not reach the Earth's surface even in the event of severe ozone radiation.

The present study was initiated to evaluate effects of UV-B radiation on photosynthesis and aspects of the electron transport system, and to define morphological changes of chloroplasts in *Pisum sativum* L. plants exposed to UV-B radiation. The wavelength compositions of the treatments were selected to approximate ground level, solar spectral irradiance under normal ozone conditions and under conditions of a sizable ozone reduction. Results of this study tend to support the hypothesis that photosynthetic processes primarily associated with PSII are affected by the levels of UV-B radiation tested in this study.

MATERIALS AND METHODS

P. sativum L. cv. Early Alaska plants were grown under greenhouse conditions with an approximate 14-hr photoperiod and PAR[4] of about 400 μeinsteins$\cdot m^{-2}\cdot sec^{-1}$. Studies of the effects of plant biomass were initiated when the seedlings were 7 days old. In all other experiments, the seedlings were 14 days old upon initiation of treatment.

Spectral irradiance for the controlled environment studies was provided by the lamp filter system previously described (21). The PAR was 800 μeinsteins$\cdot m^{-2}\cdot sec^{-1}$ with a 16-hr photoperiod. Temperatures were programmed to simulate a July day in northern Utah (13 to 37 C). Spectral irradiance for both the control and UV radiation treatments are illustrated in Figure 1.

The biomass study was carried out in a greenhouse with the plants exposed to a UV-B radiation supplement. The FS-40 lamp/filter system previously described provided most of the supplemental UV-B radiation below 315 nm (21). The photoperiod during this study was approximately 14 hr and the daily maximum PAR was about 400 μeinsteins$\cdot m^{-2}\cdot sec^{-1}$. At 0900 daily, five samples for biomass determination were taken at random from the control and UV radiation-treated plants. The plants were excised at ground level, weighed, dried at 72 C for 24 hr, and reweighed. This experiment was repeated three times.

Net photosynthetic rates were determined with a Siemens Corp. gas exchange system and IR analysis (13). Photosynthetic resistances were measured and calculated by a modification of the original technique of Gaastra (6). A multiplier (1.59) which relates the diffusion coefficients of CO_2 and H_2O (16) was used to calculate CO_2 diffusive resistances from the H_2O diffusive resistances. In this study, the CO_2 mesophyll resistance term originally defined by Gaastra was replaced by the residual resistance term, since this includes all diffusive and metabolic components of the total CO_2 resistance apart from stomatal and boundary layer resistances (9).

Photosynthetic rates were determined in the Siemens cuvette with and without supplementary UV-B irradiance. The UV-B radiation-enhanced treatment was accomplished by replacing the normal Siemens cuvette cover with one layer of Kodacel TA-401 plastic film. Four Sylvania 300-w lamps provided irradiance above 315 nm and two Westinghouse FS-20 fluorescent "sun lamps" provided UV radiation below 315 nm. PAR was ad-

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[4] Abbreviations: PAR: photosynthetically active radiation (400-700 nm); DCPIP: 2,6-dichlorophenolindophenol.

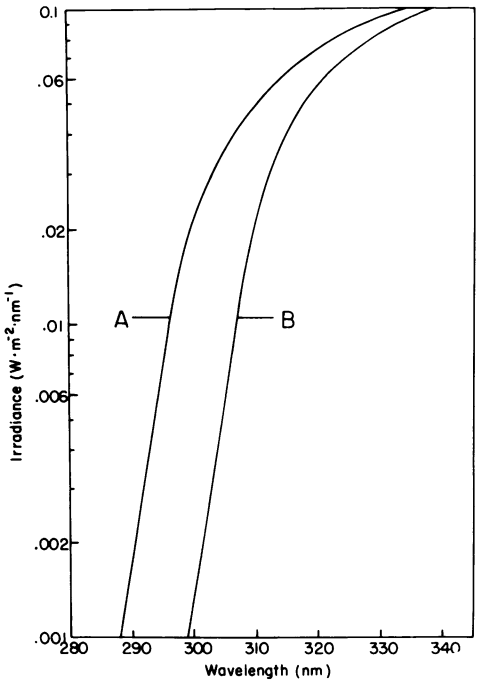

FIG. 1. Spectral irradiance within a controlled environment chamber with a 6000-w xenon arc adjusted to 800 μeinstein·m^{-2}·sec^{-1} (400–700 nm) filtered with (A) Schott Co. WG-320 1-mm glass filters plus two Westinghouse FS-40 "sun lamps" each filtered with one layer of Kodacel TA-401 (5 mil) plastic film (UV-B treatment) and (B) Schott Co. WG-320 2-mm glass filters plus two Westinghouse FS-40 "sun lamps" each filtered by one layer of Mylar type A (10 mil) plastic film (control) (21).

justed to 800 μeinsteins·m^{-2}·sec^{-1} inside the cuvette and continuously monitored by a Lambda Co. model LI-190 SR quantum sensor (4). A constant temperature of 27 C was maintained with a relative humidity of 45% (± 4%).

Chloroplasts were isolated according to the procedures of Jagendorf and Avron (11) with modifications as described by Miles *et al.* (18). Twelve to 15 g of whole plant tissue were homogenized for 15 sec at full speed in a cold Waring Blendor with 75 ml of ice-cold buffer consisting of 20 mM Tricine (pH 8), 10 mM NaCl, 0.8 M sucrose, and 0.1% defatted BSA (Sigma Chemical Co.). The resulting homogenate was strained through single layers of Miracloth and cheesecloth and centrifuged at 1,500 *g* for 7 min at 4 C. The resulting pellet was resuspended with fresh isolation medium and diluted to a concentration of 1 mg Chl/ml (1).

Electron transport activity was measured by a modified Mehler reaction as described by Miles *et al.* (18). The reaction was followed by measuring O_2 concentration in the reaction chamber with a Yellow Springs model 53 O_2 electrode monitor. The reaction mixture contained 50 mM Tricine (pH 7.8), 30 mM NaCl, 2.5 mM $MgCl_2$, 0.5 mM NaN_3, 0.1 mM methylviologen, 1 mM carbonyl cyanide *m*-chlorophenylhydrazone and chloroplasts (175–200 μg Chl) in a total volume of 5 ml. The reaction chamber was illuminated with a GE DDB 750-w projection bulb filtered with red plastic resulting in 2000 μeinsteins·m^{-2}·sec^{-1} irradiance (570–700 nm). The temperature of the chamber was maintained at 24 C.

As an indication of Hill reaction activity, O_2 evolution was measured in a reaction mixture containing 50 mM Tricine (pH 7.8), 30 mM NaCl, 1 mM $K_3(Fe)CN_6$ and chloroplasts (50 μg Chl) as described above.

Samples for electron microscopy were prepared by punching 1-mm diameter interveinal leaf discs from the youngest mature leaflets. Samples were collected after 0.25, 0.50, 1, 2, 4, and 8 hr, and 2, 4, 8, and 16 days. Leaf discs were fixed in Karnovsky's fixative (12) buffered with cacodylate (pH 7.2), rinsed twice with buffer, and then fixed with 2% osmium tetroxide (pH 7.2) buffered with the same buffer. After three rinses of buffer, discs were dehydrated with ethanol and propylene oxide, infiltrated, and embedded in Spurr's medium (23). Thin sections (50–70 nm) were cut with glass or diamond knives on a Sorvall MT-2 ultra microtome, stained with uranyl acetate (24) and lead citrate (20), and examined with a Zeiss EM-9S-2 or JEM 100B electron microscope operating at 60 kv. A total of 48 sections were examined for each sampling time.

RESULTS

After 5 and 9 days, respectively, fresh and dry weights of the control plants were significantly greater than those of plants exposed to the UV radiation treatment (Fig. 2).

Mean net photosynthetic rates of the UV radiation-treated plants were significantly depressed below the control plant photosynthetic rates after 4 and 5 hr of treatment (Fig. 3). The depressed photosynthetic rates of the plants in the UV radiation treatment were accompanied by increases in residual CO_2 resistance (r_r') (Fig. 3), which also differed statistically for 4 and 5 hr treatment.

The mean dark respiration rate of the UV radiation-treated plants (3.7 mg CO_2·dm^{-2}·hr^{-1}) was significantly ($P < 0.05$) higher than the control plant rate (2.6 mg CO_2·dm^{-2}·hr^{-1}) after 5-hr treatment.

The ability of the photosynthetic apparatus to reduce methylviologen is a measure of the capacity of the electron transport system (17). Following a 20-hr exposure to UV radiation, electron transport (*i.e.* reduction of methylviologen) was significantly reduced by 34% (from 23.1 to 17.9 μmol O_2 uptake·mg Chl^{-1}·hr^{-1}). However, when 1 mM DCPIP reduced by 1 mM ascorbic acid was added as an artificial electron donor to PSI

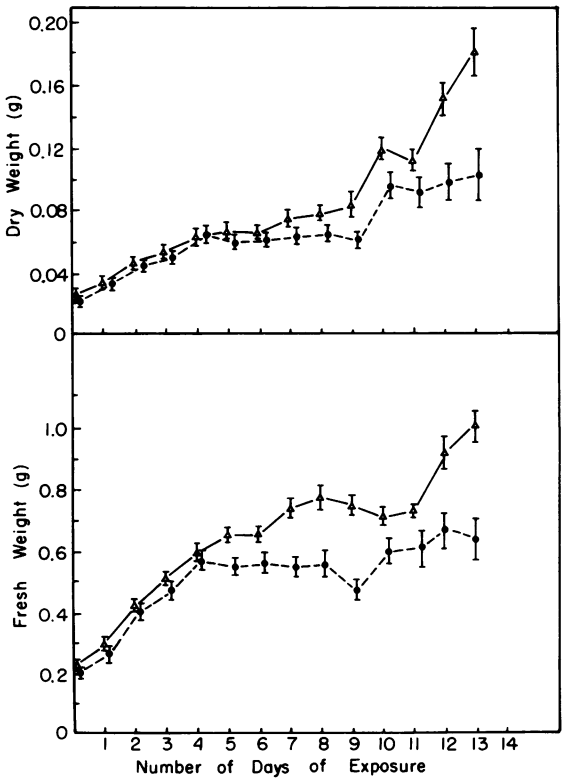

FIG. 2. Dry and fresh weights of *P. sativum* L. for 13 days of UV irradiation (●- - -●) and control treatment (△——△). Vertical bars represent ± 1 SE.

(18), electron transport of the UV radiation-treated plants was significantly reduced by only 12% (from 58.9 to 52.4 μmol O_2 uptake · mg $Chl^{-1} \cdot hr^{-1}$).

The ability of ascorbate-reduced DCPIP to restore much of the electron transport capacity of the UV radiation-treated plant tissue suggested that inhibition by UV-B radiation was more closely associated with PSII than with PSI. We examined the reactions of PSII more carefully by measuring O_2 evolution associated with the Hill reaction after various UV radiation exposure periods. As shown in Figure 4, O_2 evolution was decreased by increasing the exposure time to UV radiation. A similar pattern of decreased O_2 evolution as a function of UV-B radiation exposure was observed over a range of chloroplast concentrations (0.025–0.175 mg Chl). A depression of O_2 evolution was observed after 15 min exposure to the UV radiation treatment (Fig. 4). Statistical analysis using a paired t test indicated that O_2 evolution of the UV-irradiated plants was significantly lower than that of the control plant tissue at all sampling times. Although O_2 evolution from the UV irradiated plant tissue always decreased after 0.5 hr exposure, the plant tissue

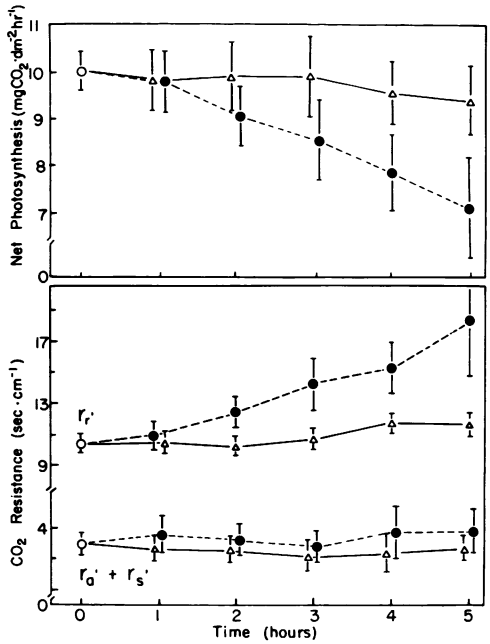

FIG. 3. Net photosynthesis and the associated CO_2 resistances (r_r' = residual resistance; $r_r' + r_s'$ = leaf resistance) of *P. sativum* L. for 5 hr of UV irradiation (●– – –●) and control (△——△) treatment. Photosynthetically active irradiance was 800 μeinsteins · m^{-2} · sec^{-1}. Vertical bars represent ± 1 SD.

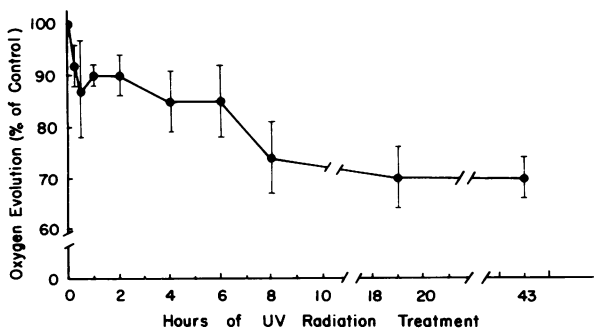

FIG. 4. Oxygen evolution of *P. sativum* L. for 43 hr of UV irradiation treatment expressed as a per cent of the control treatment plant rates. Chloroplast concentration was 0.05 mg chloroplast · ml^{-1}. Vertical bars represent ± 1 SD, 100% O_2 evolution = 96.09 ± 8.04 μmol O_2 · mg $Chl^{-1} \cdot hr^{-1}$.

exposed to UV radiation for 1 hr always had a higher mean O_2 evolution rate than did those exposed for only 0.5 hr (Fig. 4). A maximum decrease of 30% (from 86.1 to 60.1 μmol O_2 · mg $Chl^{-1} \cdot hr^{-1}$) from the O_2 evolution rate of the control plants was observed after 19 hr. Additional decreases were not recorded after 43 hr of exposure. The depressed O_2 evolution rates of the UV irradiated plants suggest that photosynthetic rate repression resulted at least partially from PSII inhibition.

Mantai (14) and Mantai *et al.* (15) exposed spinach chloroplasts to 254 nm UV radiation and similarly observed an inhibition of PSII activity. They suggested this may have resulted when chloroplast lamellar membranes incurred structural damage, thereby impairing PSII and its associated reactions. Futhermore, they concluded that plastoquinone destruction was not the major cause of UV inhibition of photosynthesis (15). To determine if the structural integrity of chloroplast membranes had been altered in plants of this experiment, an ultrastructural analysis of UV irradiated and control plant tissue was undertaken. Figure 5A is a representative electron micrograph of the ultrastructure of freshly harvested leaflets of control plants. Each mesophyll cell was characterized by a large central vacuole within a peripheral layer of cytoplasm that was appressed to the cell wall. Chloroplasts, usually lens-shaped, had an average length of 3 μm. Each chloroplast was surrounded by a double membrane, had well developed granal and stromal lamellae, and one or more starch grains. A few, very small osmiophilic globules were also observed. Mitochondria had well developed cristae. The ER, plasmalemma, and tonoplast were regular in outline. Observed nuclei had well defined nuclear membranes.

An electron micrograph of mesophyll cells from pea plants exposed to the UV radiation for 0.25 hr is shown in Figure 5B. Dilation of the thylakoid membranes in the chloroplast is indicated by the double arrow. Although this was not apparent in all chloroplasts, it did occur in some chloroplasts after this short irradiation period. Table I summarizes the sequence of ultrastructural changes observed in chloroplasts and other cell organelles following various periods of UV irradiation. The per cent of cells exhibiting damage to organelles ranged from 0.05% for the control plant tissue to 26.2% for leaves exposed to UV radiation for 16 days. During the 1st day of UV radiation treatment, the outer double membrane of the chloroplast in some of the cells was disrupted and, in the more severely damaged cells, the chloroplast membrane was completely disrupted with vesicles being formed in the stroma from the thylakoids. Swollen cisternae were also observed in the ER. These cisternae developed into vesicles after the 2nd day of UV radiation treatment. Small vesicles were also produced in the plasmalemma and tonoplast during this period of time. These vesicles increased in size until they became disrupted after 4 to 8 days of UV irradiation. The mitochondria appeared normal until after a 2-day exposure period, when the mitochondria appeared to possess fewer cristae. This observation was, however, applicable only to cells having damaged chloroplasts.

DISCUSSION

The decreased photosynthetic rates of the UV irradiated plants following 4 hr of exposure were not the result of reduced stomatal aperture (Fig. 3). Instead, the stable leaf resistances ($r_a' + r_s'$) and the increasing residual resistance (r_r') indicated that processes other than stomatal closure were involved in the depressed photosynthetic rates.

Results of this study indicate that an inhibition of PSII activity (Fig. 3) and structural damage to chloroplasts were contributing factors in the depressed photosynthetic rates of the UV irradiated plants.

Although O_2 evolution associated with electron transport activity was depressed and ultrastructural damage was discernible in the mesophyll cells after 0.25 hr of UV irradiation, variability

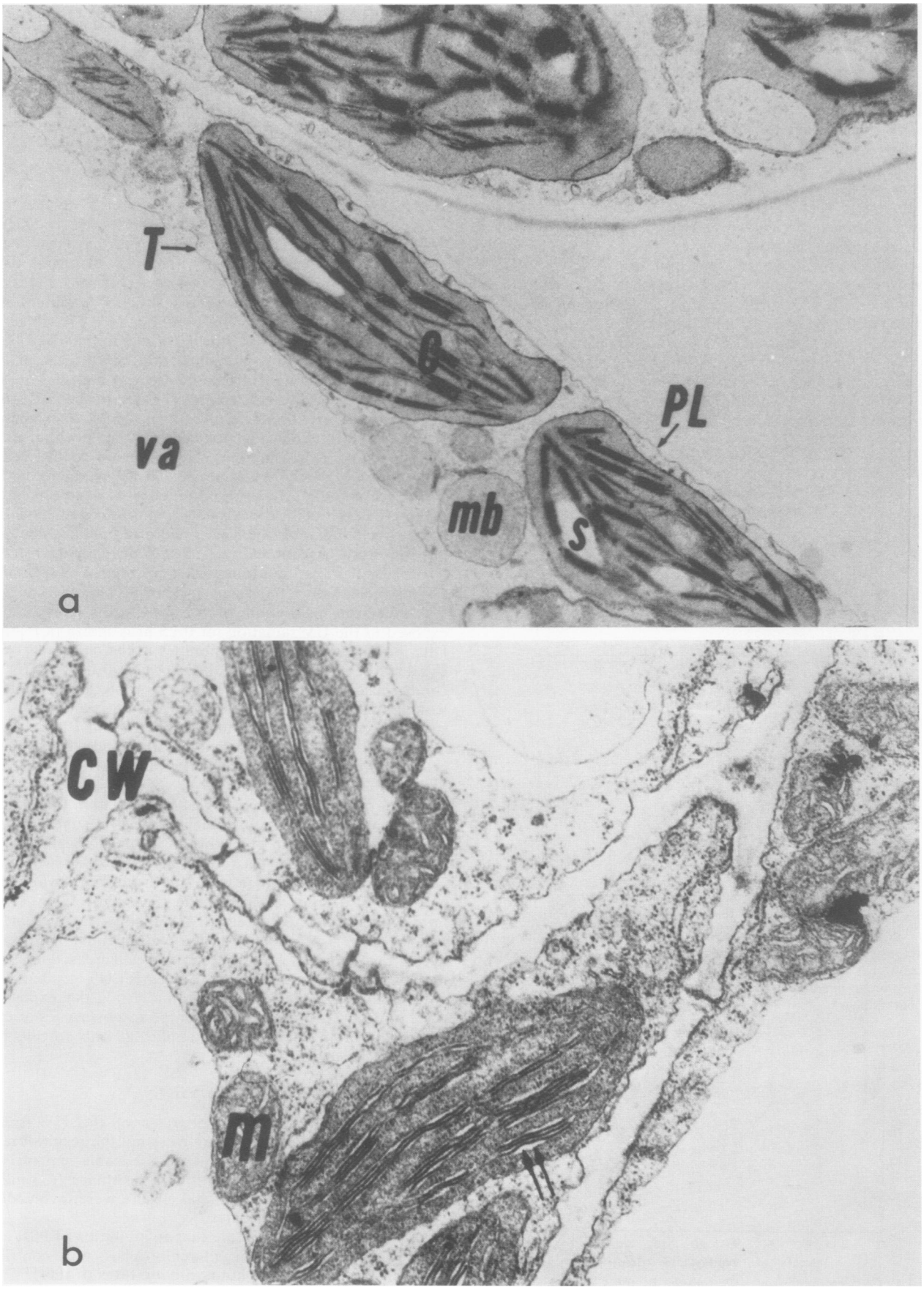

FIG. 5. Electron photomicrograph of a portion of mesophyll cells from *P. sativum* L. a: Normal cells showing typical arrangement of the organelles. × 18,000. b: Cells from a plant exposed to 0.25 hr of UV-B radiation. × 14,500. Note the dilation of the thylakoid membranes of the chloroplast (double arrow). C: chloroplast; CW: cell wall; mb: microbody; m: mitochondria; PL: plasmalemma; S: starch grain; T; tonoplast; va: vacuole.

Table I. Sequence Of Ultrastructural Events Occurring In Mesophyll Cells Of *Pisum sativum* L. Following UV-B Irradiation

UV-B Radiation	Percent cells showing injury	Nucleus	Chloroplasts	Mitochondria	Endoplasmic Reticulum	Plasmalemma	Tonoplast
HR	% S_d						
0	0.05 ± 0.02	Normal	Normal	Normal	Normal	Normal	Normal
¼	0.5 ± 0.48	Slight Dilation of Nuclear Membrane	Slight Swelling Thylakoid Dilation Outer Membrane Broken	Normal	Swollen Cisternae	Vesiculation	Vesiculation
½	0.9 ± 0.53	"	"	"	"	"	"
1	1.3 ± 0.67	"	"	"	"	"	"
2	2.4 ± 1.40	"	"	"	"	"	"
4	3.7 ± 1.66	"	"	"	"	"	"
8	6.9 ± 2.46	"	"	"	"	"	"
DAY							
2	13.3 ± 1.34	"	Vesiculation In Stroma	Fewer Cristae	Vesiculation	"	"
4	17.1 ± 2.01	"	"	"	"	"	"
8	19.6 ± 2.39	"	"	"	"	Disrupted	Disrupted
16	26.2 ± 2.60	"	"	"	"	"	"

among replicate samples obscured statistical differences in photosynthetic rates until the plants had been exposed to UV radiation for 4 hr. It seems that reduction of photosynthetic activity roughly paralleled the decrease in electron transport capacity.

Results of this study agree with the suggestion of Mantai (14) and Mantai *et al.* (15) that structural damage to the chloroplast lamellae results from exposure to UV radiation and coincides with a decrease in PSII activity. However, physical disruption of the chloroplast membranes may not be the only factor causing disruption of PSII activity. Following 20 hr of UV-B irradiation, electron transport (*i.e.* reduction of methylviologen) was reduced by 34% compared to control plant tissue. In the reaction vessel, O_2 evolution was restored to within 12% of control plant rates after an artificial electron donor to PSI was introduced. This restoration of most of the activity indicates that possibly either PSII or some specific step in the electron transport associated with PSII was partially inhibited. This may have resulted from a specific impact of UV radiation on PSII apart from the indirect effects of chloroplast membrane disruption. Recent studies by Okada *et al.* (19) suggest that 254 nm UV radiation can directly inhibit primary photochemistry at the reaction center Chl of PSII. This corroborates the suggestion of a direct impact on PSII apart from secondary effects due to membrane disruption.

Okada *et al.* (19) also reported that the 254 nm radiation could inactivate P_{700} PSI but to a much smaller degree than PSII. This also seems consistent with our experiments. Although most of the inhibition of electron transport capacity was associated with PSII, electron transport activity was still reduced by 12% when DCPIP with ascorbic acid was added to the reaction vessel as an electron donor to PSI.

This study is consistent with the hypothesis that the short term effects of UV-B radiation on net photosynthesis do not involve increased stomatal diffusion resistance to CO_2 but directly affect the photochemical apparatus. Some of the damage may be an indirect effect resulting from chloroplast membrane disruption. However, direct effects on PSII, and to a much lesser extent, on PSI, may also be involved.

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