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

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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 CO₂ 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.

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 of about 400 μEinsteins·m⁻²·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·m⁻²·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·m⁻²·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 Gaasstra (6). A multiplier (1.59) which relates the diffusion coefficients of CO₂ and H₂O (16) was used to calculate CO₂ diffusive resistances from the H₂O diffusive resistances. In this study, the CO₂ mesophyll resistance term originally defined by Gaasstra was replaced by the residual resistance term, since this includes all diffusive and metabolic components of the total CO₂ 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.
sky'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 CO2 resistance (tC) (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 CO2·dm⁻²·hr⁻¹) was significantly (P < 0.05) higher than the control plant rate (2.6 mg CO2·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 O2 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
(18), electron transport of the UV radiation-treated plants was significantly reduced by only 12% (from 58.9 to 52.4 μmol O₂ uptake·mg Chl⁻¹·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 O₂ evolution associated with the Hill reaction after various UV radiation exposure periods. As shown in Figure 4, O₂ evolution was decreased by increasing the exposure time to UV radiation. A similar pattern of decreased O₂ 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 O₂ evolution was observed after 15 min exposure to the UV radiation treatment (Fig. 4). Statistical analysis using a paired t test indicated that O₂ evolution of the UV-irradiated plants was significantly lower than that of the control plant tissue at all sampling times. Although O₂ evolution from the UV irradiated plant tissue always decreased after 0.5 hr exposure, the plant tissue exposed to UV radiation for 1 hr always had a higher mean O₂ 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 O₂·mg Chl⁻¹·hr⁻¹) from the O₂ evolution rate of the control plants was observed after 19 hr. Additional decreases were not recorded after 43 hr of exposure. The depressed O₂ evolution rates of the UV irradiated plants suggest that photosynthetic rate depression 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. Furthermore, 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 tonoplasma 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. Distortion 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 percent 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 (rₑ + rₘ) and the increasing residual resistance (rₑ) 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 O₂ 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.
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, O₂ 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 PSI or some specific step in the electron transport associated with PSI was partially inhibited. This may have resulted from a specific impact of UV radiation on PSI 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 PSI. This corroborates the suggestion of a direct impact on PSI apart from secondary effects due to membrane disruption.

Okada et al. (19) also reported that the 254 nm radiation could inactivate P₇₃₀ 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 PSI, 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 CO₂ 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.

LITERATURE CITED