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Photoinhibition of Intact Attached Leaves of C_3 Plants Illuminated in the Absence of Both Carbon Dioxide and of Photorespiration

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ABSTRACT

When leaflets of bean and leaves of other species of C_3 plants are illuminated in the absence of CO_2 and at low O_2 partial pressure, the capacity for CO_2 assimilation at saturating light and its efficiency at low light intensities are inhibited. This photoinhibition is dependent on leaflet age and period of illumination. In young leaflets and following short exposure to these photoinhibitory conditions, some recovery of CO_2 assimilation capacity is observed immediately after treatment. Following substantial (70 to 80%) photoinhibition of CO_2 assimilation, recovery in fully expanded leaflets is observed only after 48 hours in normal air. The photoinhibition is largely prevented by providing CO_2 at partial pressures equivalent to the CO_2 compensation point, or by >210 millibars O_2 which permits internal CO_2 production by photorespiration. If leaflets are illuminated in 60 microbars CO_2 and 210 millibars O_2 (the CO_2 compensation point in air), no photoinhibition is observed. Electron transport processes and fluorescence emission associated with photosystem II are inhibited in chloroplast thylakoids isolated from leaflets after illumination in zero CO_2 and 10 millibars O_2. These studies support the hypothesis that CO_2 recycling through photorespiration is one means of effectively dissipating excess photochemical energy when CO_2 supply to illuminated leaves is limited.

Excess light energy can have deleterious effects on photosynthetic organisms. This phenomenon has been termed photoinhibition (13). Algae exposed to light intensity greatly in excess of that required to saturate photosynthesis show marked reduction in photosynthetic capacity (13, 16, 21, 22). Shade plants exposed to high light intensities show reduction in rates of light-saturated and light-limited CO_2 assimilation (2, 3). Chloroplast membrane fragments (11, 12, 20) and intact isolated chloroplasts (14) suffer photoinhibition when illuminated at high light intensities or in the absence of an electron acceptor. In the above experiments it has been proposed that the light energy absorbed exceeds the capacity for electron transport and CO_2 fixation so that a large part of the excitation energy is not dissipated in an orderly fashion and the excess excitation energy may result in the inactivation of the photochemical reaction centers (2, 13).

Recently, we reported a similar photoinhibition phenomenon in intact bean leaflets following exposure to normal light intensities (19), under conditions in which the metabolism of the integrated photosynthetic carbon reduction cycle and the photorespiratory carbon oxidation cycle (1) is largely prevented. When leaflets were illuminated under conditions which prevent photorespiration and CO_2 cycling (3 h zero CO_2, 10 mbar O_2, irradiance 2,000 $\mu E/m^2 \cdot s$), substantial reduction in the light-saturated and the light-limited CO_2 assimilation rate was observed (19). Cornic (6, 7) noted a similar decline in light-saturated CO_2 assimilation with *Sinapis* leaf fragments illuminated in a low CO_2 and O_2 atmosphere. In both sets of experiments photoinhibition was markedly dependent upon the length of exposure and the CO_2 and O_2 partial pressures throughout the photoinhibitory treatment.

Here, we report experiments with developing and with fully expanded bean leaflets and with expanded leaves of other C_3 species. These results demonstrate that the reduction in both the light-saturated and the light-limited rate of CO_2 assimilation following exposure to a photoinhibitory treatment is a general response. We also describe some aspects of the short and long term recovery from this photoinhibition. Some properties of electron transport in chloroplast thylakoids were measured after photoinhibition which show that this phenomenon is associated with substantial damage to PSII and lesser damage to PSI.

MATERIALS AND METHODS

Plant Material. Plants were grown from seed in a naturally lit and temperature-controlled glasshouse during the winter and spring months in 15-cm pots of soil. They were well watered and fertilized daily with nutrient solution (24 mM NO_3^-, 4 mM K^+, 4 mM Ca^{2+}, 1.5 mM Mg^{2+}, 13.33 mM Na^+, 1.5 mM SO_4^{2-}, and 1.33 mM PO_4^{3-}). Irradiation during growth regularly reached quantum fluxes (400–700 mm) of 1,800 $\mu E/m^2 \cdot s$. Plants that developed during periods of extended cloudy days were not used. The day/night temperature regime in the glasshouse was approximately 30/18 C and the RH was between 60 and 70%.

Gas Exchange Techniques. CO_2 exchange and leaf conductance measurements were made with an open system gas analysis apparatus, utilizing an IR CO_2 analyzer (model 865 Beckman Instruments, Fullerton, Calif.) and a humidity sensor (model HM-111, Weather Measure Corp., Sacramento, Calif.). The apparatus used was as previously described (19) except for the gas-mixing system. A high flow rate (10 liters/min) was required to permit measurements with large leaves (130 cm^2). To achieve this a precision micro metering valve (SS-22R52 Whitey Company, Oakland, Calif.) was used to bleed 5% CO_2 in N_2 into a CO_2-free gas stream. By using a differential manometer and an electronic sensing device it was possible to maintain a constant pressure difference between the 5% CO_2 and the CO_2-free gas stream. The IR CO_2 analyzer (in the absolute mode) was used to monitor the CO_2 partial pressure.

Experiments were carried out during the photoperiod corresponding to that of growth conditions. Intact attached terminal leaflets of the first trifoliate leaf of *Phaseolus vulgaris* L. (Hawkesbury Wonder) were used 25 to 30 days after sowing (average leaflet area was 90–120 cm^2). Experiments with other C_3 species were conducted with large mature leaves.

Response of CO_2 Assimilation Rate to Low Irradiance (Apparent Quantum Yield). An attached leaf was exposed to a CO_2 partial pressure of 330 μbars in air at an irradiance of 160 $\mu E/m^2 \cdot s$. After a constant CO_2 assimilation rate had been achieved the irradiance was reduced in four equal steps and the CO_2 assimilation was determined at least 5 min after each change. Leaf temperature was maintained at 30 ± 0.5 C and leaf to air vapor pressure difference between 12 and 20 mbars. Gas flow rate through the leaf chamber was 4 liters/min. The response of CO_2 assimilation to CO_2 partial pressure was then measured on this same leaf.

Response of CO_2 Assimilation Rate to CO_2 Partial Pressure. After measurement of the low irradiance response the irradiance was increased to 2,000 $\mu E/m^2 \cdot s$. CO_2 partial pressure was maintained at 330 μbars and leaf temperature was held at 30 ± 0.5 C. After a constant CO_2 assimilation rate was achieved the CO_2 partial pressure was reduced in three steps, and after each step CO_2 assimilation was allowed to attain a steady-state. Gas flow rate through the leaf chamber was maintained at 8 liters/min and the ambient vapor pressure difference was from 12 to 16 mbars. The leaf was then exposed to a photoinhibitory treatment following which the light response (quantum yield) and CO_2 response curves were again measured in the same sequence as described above. The results of these experiments are expressed as a function of C_i[1]. C_i is calculated as:

$$C_i = C_a - 1.6\frac{AP}{G}$$

where C_i and C_a are the intercellular and ambient CO_2 partial pressures respectively, A is the assimilation rate, P is the atmospheric pressure, and G is the leaf conductance to H_2O.

Photoinhibitory Treatments. Photoinhibitory treatments were applied by replacing the air with CO_2-free N_2 containing the desired partial pressures of O_2 and CO_2 and illuminating leaves at 2000 $\mu E/m^2 \cdot s$. The temperature of leaflets or leaves was maintained at 30 C, the gas flow rate at 8 liters/min, and vapor pressure difference at 12 to 16 mbars. The standard photoinhibitory treatment used in these experiments was a 3-h exposure to CO_2-free N_2 containing 10 mbars O_2. Further details of different treatments are given for each experiment.

Studies with Chloroplast Thylakoids. PSI and PSII activities were measured in chloroplast thylakoids isolated from one of the subterminal bean leaflets before exposure of the terminal leaflet to photoinhibitory treatments. Chloroplast thylakoids were also prepared from the treated leaflet immediately after exposure to photoinhibitory conditions. The midvein was removed and leaflets were cut into strips (2–3 mm wide) and blended in a Sorvall Omni-Mixer for 10 s (25% line voltage). The extraction buffer (50 ml) contained 0.33 M sorbitol, 30 mM Hepes (pH 7.8), 1 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT, 0.5% BSA, and 2% PVP, mol wt 10,000. Cell debris was removed by filtration through two layers of Miracloth and the filtrate was then centrifuged for 5 min at 1,000*g*. The pellet was resuspended in 10 ml of resuspension medium (0.3 M sorbitol, 10 mM KH_2PO_4, 1 mM $MgCl_2$) and recentrifuged for 5 min at 1,000*g*. Chloroplasts and chloroplast fragments were resuspended in 2 ml of 10 mM phosphate buffer (pH 7.8) with 1 mM $MgCl_2$. All steps were carried out at 4 C.

The Hill reaction, in the resuspended thylakoids, was measured as O_2 evolution with ferricyanide as an electron acceptor. The assay contained 20 mM Tris-HCl (pH 7.8), 20 mM NaCl, 0.5 mM $K_3Fe(CN)_6$, 5 mM methylamine-HCl, and thylakoids (18–25 μg Chl) in a total of 3 ml. Ferricyanide-dependent O_2 evolution was abolished by 3.3 μM DCMU, inhibited 60% by 1 μM DBMIB. Rates of O_2 evolution using dimethylbenzoquinone were identical to those obtained with ferricyanide.

PSI activity was measured by the methylviologen-Mehler reaction using ascorbate-DCPIP as electron donor and measuring O_2 uptake with a Rank O_2 electrode. The assay contained 20 mM Tris-HCl (pH 7.8), 33 μM DCPIP, 3.3 mM Na-ascorbate, 3.3 μM DCMU, 5 mM methylamine-HCl, and 33 μM methylviologen with chloroplasts (18–25 μg Chl) in 3 ml. No catalase activity was detected in the preparations used in these experiments. The assay was insensitive to DBMIB and cyanide.

Fluorescence emission spectra was recorded at 77 K after freezing chloroplast fragments in 63% glycerol containing 50 mM Hepes, 100 mM sorbitol, 20 mM NaCl, and 4 mM $MgCl_2$. The Chl concentration was approximately 2 μg/ml and the fluorescence was recorded with a spectrophotometer incorporating automatic correction for photomultiplier and monochromator responses, and variation in output of the light source as described previously (4, 5).

Chl concentrations were calculated from spectra of aqueous acetone extracts. Samples of the same area were taken from the bean leaflet subjected to the photoinhibitory treatment and were compared with samples taken from the two lateral leaflets that were not treated. In other C_3 species samples were taken from the photoinhibited leaf and compared with samples taken from a leaf of a similar age on the same plant. Fresh tissues were extracted with 80% (v/v) acetone/H_2O in a glass homogenizer. One volume of chloroplast suspension was extracted in 4 volumes of acetone. Spectra were recorded with a Varian 465 spectrophotometer.

RESULTS

Photoinhibition in Bean Leaflets Illuminated in CO_2-free N_2 Containing 10 mbars O_2. The CO_2 assimilation rate of the terminal leaflet of first trifoliate leaves of bean was measured at intervals from 5 days after unfolding (rapid leaf expansion) through to 50 days from unfolding (early senescence). The CO_2 assimilation rate was at a maximum (16–21 $\mu mol/m^2 \cdot s$ at 200 μbar intercellular CO_2) in leaflets 10 to 14 days after unfolding, as leaves reached full expansion. It declined to 11 and to 2.5 $\mu mol/m^2 \cdot s$ at 30 and 50 days after unfolding. Unless otherwise stated, experiments with beans were carried out with leaflets which had just reached full expansion.

The effect of a standard photoinhibitory treatment on the CO_2 assimilation rate of a recently fully expanded leaflet (10 days after unfolding) is shown in Figure 1. The light-saturated CO_2 assimilation rate was markedly reduced following treatment as observed previously (19). Figure 1A shows the response of CO_2 assimilation rate to different intercellular CO_2 partial pressures before and after the standard photoinhibitory treatment. In all experiments the response of CO_2 assimilation rate to increasing intercellular CO_2 partial pressure (C_i) was linear to above 120 μbars; this partial pressure of CO_2 was thus chosen as a basis for comparing rates of light-saturated CO_2 assimilation within and between experiments. Reduction of the light-saturated CO_2 assimilation rate, at an intercellular CO_2 partial pressure of 120 μbars, in the present experiment (Fig. 1A) was 66%.

The light-limited CO_2 assimilation rate was also markedly reduced after the photoinhibitory treatment (Fig. 1B). The apparent quantum yield (*A* was not measured in these experiments) can be calculated from the linear response of CO_2 assimilation to low irradiance and used to compare the efficiency of the light-harvesting photosynthetic system both within and between experiments. In this experiment (Fig. 1B) photoinhibition reduced the

[1] Abbreviations: C_i: intercellular CO_2 partial pressure; DBMIB: 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

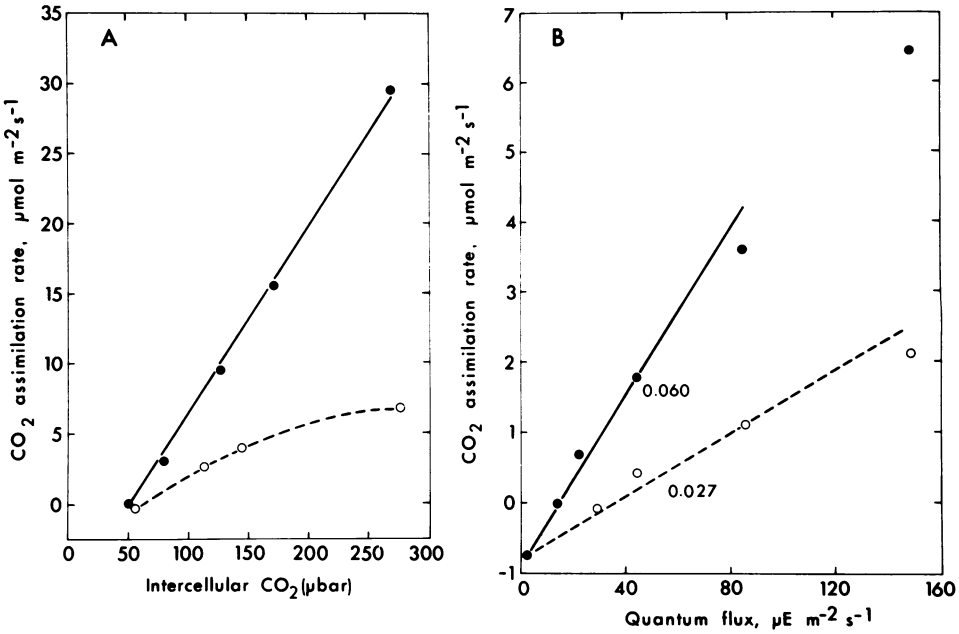

FIG. 1. Effect of standard photoinhibitory treatment (3-h exposure to CO_2-free N_2 containing 10 mbars O_2, irradiance 2,000 $\mu E/m^2 \cdot s$) on (A) light-saturated CO_2 assimilation rate at different intercellular CO_2 partial pressures; and (B) apparent quantum yield. Apparent quantum yield (mol CO_2 assimilated per incident E) is shown on the curve. (●): Before treatment; (○): after treatment.

apparent quantum yield from 0.060 to 0.027 mol CO_2 (einstein of incident $light^{-1}$) (55% reduction). The rate of CO_2 fixation in air remained inhibited at saturating light intensities, as previously shown (19). The dark respiration rate was not affected in any experiment.

Photoinhibition also resulted in a 17% decrease in the Chl content of the leaflet in this experiment. In most experiments where exposure to a photoinhibitory treatment caused marked inhibition of the CO_2 assimilation rate there was a small decline (approximately 10%) in the Chl content of the leaflet; however, in some experiments Chl destruction did not accompany photoinhibition.

Leaflet Age and Photoinhibition in Beans. The inhibition of CO_2 assimilation rate following exposure to a standard photoinhibitory treatment was dependent on leaflet age. Figure 2 shows that the inhibition of the light-saturated CO_2 assimilation rate and reduction in apparent quantum yield was least in a young rapidly expanding bean leaflet (6 days from unfolding) but similar in a recently fully expanded leaflet and a senescing leaflet (50 days from unfolding). Careful attention to leaf age is important in the comparative and recovery experiments described below.

Time and Temperature Dependence of Photoinhibition. The inhibition of both the light-saturated CO_2 assimilation rate (at a C_i of 120 μbars), and the apparent quantum yield, increased to about 70% after 3- to 5-h exposures to CO_2-free N_2 containing 10 mbars O_2 at an irradiance of 2,000 $\mu E/m^2 \cdot s$ (Fig. 3). This time course is quite similar to experiments with leaf fragments treated at much lower light intensities (7). Exposure to the photoinhibitory treatment for 1.5 h or longer resulted in a reduction in the leaflet Chl content.

Bean leaflets were exposed for 3 h to zero CO_2 in N_2 containing 10 mbars O_2 (irradiance 2,000 $\mu E/m^2 \cdot s$) at a leaflet temperature of 15, 18, and 30 C. The light-saturated rate of CO_2 assimilation was inhibited by 73, 70, and 70%, respectively. This indicates that photoinhibition was not affected by treatment temperature within this range.

In a previous communication (19) we noted that very young bean leaflets showed some recovery of CO_2 assimilation rate after initial photoinhibition. In recently fully expanded bean leaflets used in the experiments shown in Figure 3, partial recovery of

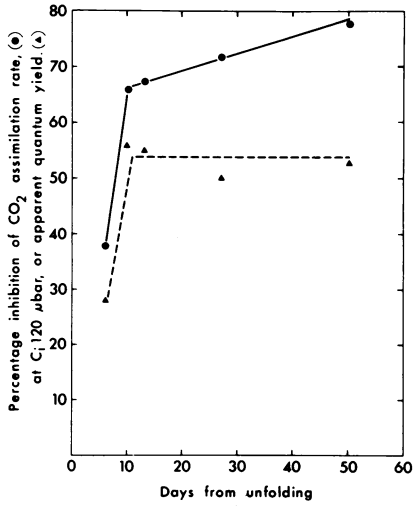

FIG. 2. Effect of leaflet age on inhibition of light-saturated CO_2 assimilation rate (●) and reduction in apparent quantum yield (▲) after exposure to the standard photoinhibitory treatment. Percentage inhibition of the light-saturated CO_2 assimilation rate at a C_i of 120 μbars and reduction in apparent quantum yield were calculated from CO_2 and light response curves of the type shown in Figure 1.

CO_2 assimilation rate was also observed (Fig. 4). The percentage inhibition of CO_2 assimilation rate was greater when measured immediately after the photoinhibition treatment than when measured 1.5 h later, when a steady rate was obtained. This partial recovery was observed only after relatively short photoinhibitory treatments (to 1.5 h); after longer treatments no partial recovery was observed within several hours of treatment. In other experiments we have noted that if low O_2 partial pressures (10 mbars) are used during assay of the CO_2 assimilation rate, partial recovery following photoinhibition is more pronounced. These experiments suggest the recovery process is itself O_2-sensitive. The characteristics and mechanisms of the short term recovery of CO_2 assimilation capacity in photoinhibited leaflets require further investigation.

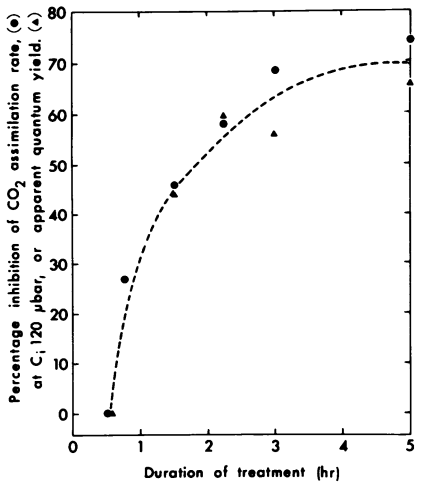

FIG. 3. Effect of length of exposure to standard photoinhibitory treatment on inhibition of the light-saturated CO_2 assimilation rate (●) and reduction in apparent quantum yield (▲). Percentage inhibition was calculated as in Figure 2.

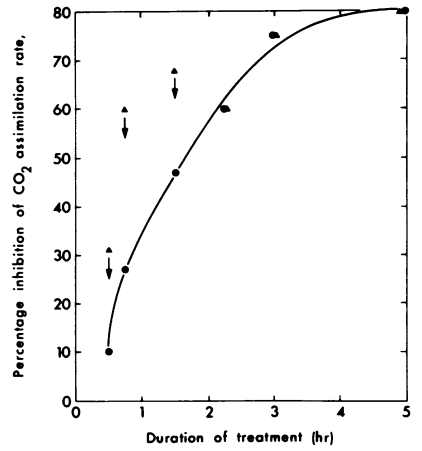

FIG. 4. Short term recovery of light-saturated CO_2 assimilation rate. Rate immediately after a photoinhibitory treatment (▲) and then 1.5 h after treatment (●).

Bean leaflets subjected to a standard 3-h photoinhibitory treatment recovered their former CO_2 assimilation rate over a longer period. A recently fully expanded bean leaflet (10 days after unfolding) showed almost complete recovery of light-saturated CO_2 assimilation rate (Fig. 5A) and apparent quantum yield (Fig. 5B) when returned to the glasshouse for 48 h. A similar potential for recovery of CO_2 assimilation capacity within 24 to 48 h was observed in both young and senescing leaflets exposed to the photoinhibitory treatment. However, with these leaflets it was not possible to separate recovery after treatment from the ontogenetic change in CO_2 assimilation rate over the recovery period. Exposure to a photoinhibitory treatment for 3 h usually caused a decline in the Chl content of the leaflet, but there was no increase in leaflet Chl content in any recovery experiment.

Dependence of Photoinhibition on O_2 and CO_2 Partial Pressures. The inhibition of both light-saturated and light-limited CO_2 assimilation rates in recently fully expanded bean leaflets (illuminated at 2,000 $\mu E/m^2 \cdot s$ for 3 h in 10 mbars O_2) was dependent on the intercellular CO_2 partial pressure maintained throughout the treatment period. Maximum inhibition of the light-saturated CO_2 assimilation rate (Fig. 6A) and the apparent quantum yield (Fig. 6B) occurred when the intercellular CO_2 partial pressure was near zero. In 10 mbars O_2 at an intercellular CO_2 partial pressure of 70 μbars (approximately equal to the CO_2 compensation point in 210 mbars O_2) there was only a small reduction in the light-saturated and light-limited CO_2 assimilation rate following a photoinhibitory treatment. In other experiments with younger leaflets (19) no photoinhibition was observed when CO_2 partial pressure was maintained at 60 to 70 μbars.

The degree of photoinhibition resulting from a 3-h exposure to zero CO_2 and irradiance of 2,000 $\mu E/m^2 \cdot s$ was dependent on the O_2 partial pressure maintained throughout the treatment period. Maximum inhibition of the light-saturated CO_2 assimilation rate occurred when the O_2 partial pressure was lowest (Fig. 7A) and the degree of photoinhibition decreased with increase in the O_2 partial pressure. Even in the presence of atmospheric partial pressures of O_2 (210 mbars) there was reduction of the light-saturated CO_2 assimilation rate after treatment. O_2 was less effective in preventing reduction of the apparent quantum yield (Fig. 7B); about 40% inhibition remained after treatment at zero CO_2 and 210 mbars O_2. Reduction in leaflet Chl content (about 10%) occurred regardless of the O_2 partial pressure used in these experiments.

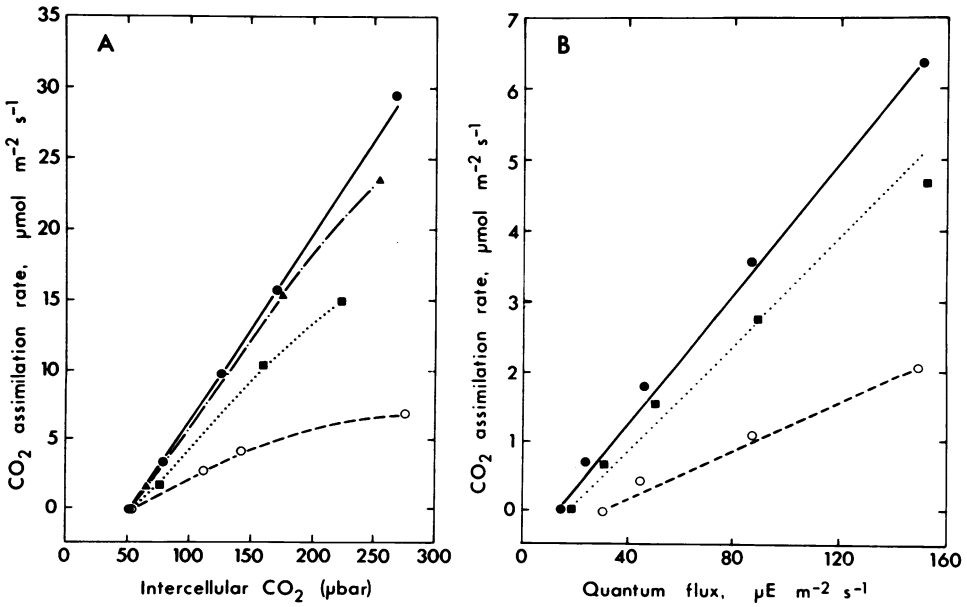

FIG. 5. Long term recovery of CO_2 assimilation rate after a standard photoinhibitory treatment (A) recovery of light-saturated rate and (B) recovery of apparent quantum yield. (●): Before treatment; (○) after treatment; (■): 24 h after treatment; (▲): 48 h after treatment.

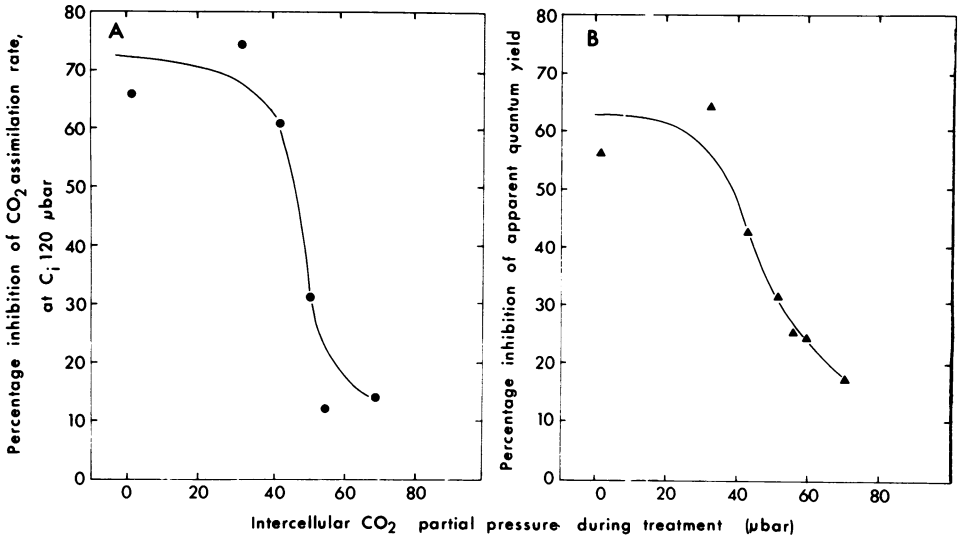

FIG. 6. Effect of intercellular CO_2 partial pressure during a 3-h photoinhibitory treatment (O_2 partial pressure 10 mbars) on (A) inhibition of light-saturated CO_2 assimilation rate; and (B) reduction in apparent quantum yield. Percentage inhibition was calculated as in Figure 2.

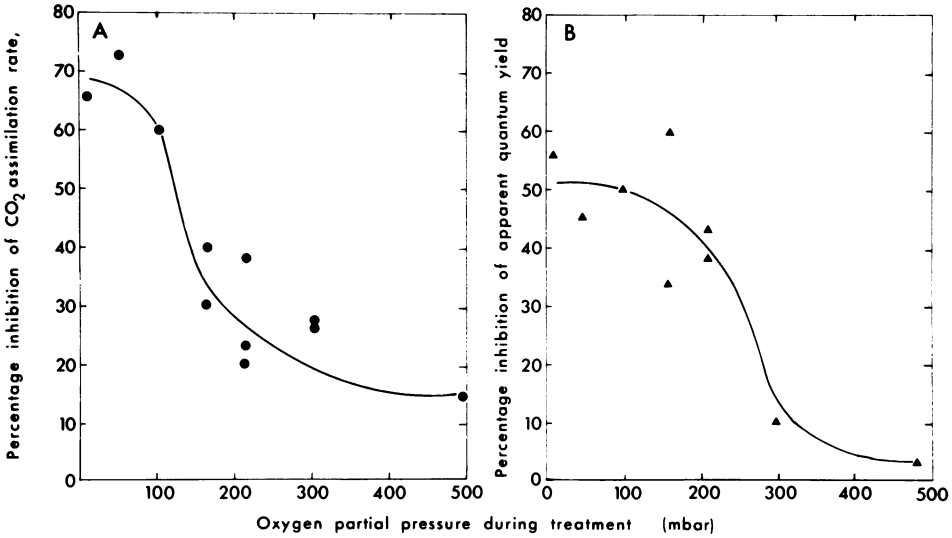

FIG. 7. Effect of O_2 partial pressure during a 3-h photoinhibitory treatment (zero CO_2) on (A) inhibition of light-saturated CO_2 assimilation rate; and (B) reduction in apparent quantum yield. Percentage inhibition was calculated as in Figure 2.

One interpretation of the data in Figure 7 is that high levels of O_2 permit internal CO_2 production via photorespiration. However, the actual intercellular CO_2 partial pressure maintained is dependent upon the CO_2 and O_2 partial pressures and the stomatal conductance. In the experiments shown in Figure 7, high stomatal conductances, high flow rates, and zero external CO_2 ensured that the intercellular CO_2 partial pressure did not rise above 6 μbars CO_2.

There are discrepancies between the data reported here and those reported earlier (7, 19) which may be ascribed to differences in technique and leaf age. In the younger bean leaflets used previously (leaflet area 35 cm^2), the time course of photoinhibition and the O_2 requirement for prevention of photoinhibition differ (19). Younger leaflets are clearly less sensitive to photoinhibition (Fig. 2) and show more rapid recovery. We suspect that such processes in younger leaves, and the very different techniques used by Cornic (7) may be responsible for the above discrepancies.

In another series of experiments the interactions of O_2 and CO_2 partial pressures were examined. Leaflets were provided with 210 mbars O_2 (normal atmospheric partial pressure) and with different partial pressures of CO_2 during the 3-h photoinhibitory period. When leaflets were illuminated at 2,000 μE/m^2·s for 3 h in the presence of 210 mbars O_2 and an intercellular CO_2 partial pressure of 45 μbars or greater, no inhibition of the light-saturated CO_2 assimilation rate or of the apparent quantum yield was observed. These treatments correspond to the internal CO_2 and O_2 partial pressures prevailing at the compensation point in C_3 plants. If leaflets were treated at intercellular CO_2 partial pressures below that of the compensation point photoinhibition resulted (data not shown). These data show that illumination of leaves under conditions equivalent to the normal intercellular CO_2 and O_2 partial pressures at the CO_2 compensation point provides complete protection against photoinhibition.

Changes in Photochemical Properties of Chloroplast Thylakoids Isolated from Bean Leaflets following Photoinhibition. The inhibition of CO_2 assimilation rate and the apparent quantum yield is associated with substantial changes in photochemical properties of the chloroplast thylakoids. In thylakoids from bean leaflets the Hill reaction was consistently reduced by about 60% following a 3-h photoinhibitory treatment, but was practically unchanged if leaflets were maintained at conditions that approximate the CO_2 compensation point during illumination (Table I). The Mehler

Table I. *Capacity for Electron Transport in Thylakoids Isolated from Chloroplasts of Bean Leaflets Before and After Photoinhibition*

Treatment	Hill Reaction		Mehler Reaction	
	Before	After	Before	After
	μmol O_2 evolved/mg Chl·min		μmol O_2 absorbed/mg Chl·min	
3-h zero CO_2; 10 mbar O_2	2.2	0.6 (72%)	4.0	2.4
2000 μE/m²·s	1.7	0.7 (60%)	3.6	3.1
3-h 70 μbar CO_2; 210 mbar O_2	1.7	1.7	3.6	3.6
2000 μE/m²·s	1.1	1.0	1.6	1.4

reaction in the same thylakoids was less consistently reduced by the photoinhibitory treatment. The rates of electron transport obtained in these chloroplast thylakoid preparations were substantially lower than the light-saturated rates of CO_2 fixation in intact leaflets in air (3.6 μmol/mg Chl·min). Further experiments are in progress to evaluate the changes in electron transport capacity in these preparations.

Clear evidence of substantial changes in the photochemical processes associated with PSII, following these photoinhibitory treatments, was obtained from fluorescence emission spectra of thylakoids at liquid N_2 temperature. Following a 3-h illumination at 2,000 μE/m²·s in zero CO_2 at 10 mbars O_2 the fluorescence emission at 683 nm, characteristic of PSII, is depressed by about 60% when spectra were normalized in terms of PSI fluorescence at 735 nm (Fig. 8A). If leaflets were maintained at conditions that approximate the CO_2 compensation point (210 mbars O_2 and 60 μbars CO_2) while illuminated, no changes in the relative intensity of PSII and PSI fluorescence (735 nm) were observed (Fig. 8B).

Photoinhibition in Other C_3 Species. Intact leaves of all C_3 species examined (herbs, shrubs, and trees) show photoinhibition following illumination at 2000 μE/m²·s in CO_2-free N_2 containing 10 mbars O_2. The light-saturated CO_2 assimilation rate and the apparent quantum yield were reduced for three C_3 species (*Gossypium hirsutum, Spinacia oleracea, Helianthus annuus*), as detailed for bean leaflets above (data not shown). Reduction in the light-saturated CO_2 assimilation rate also occurred in intact leaves of *Vigna unguiculata, Gingko biloba, Eucalyptus camaldulensis,* and *Eucalyptus pauciflora* following exposure to a photoinhibitory treatment (data not shown). Photoinhibition was totally prevented if leaves of *V. unguiculata* were illuminated at conditions that approximate the CO_2 compensation point.

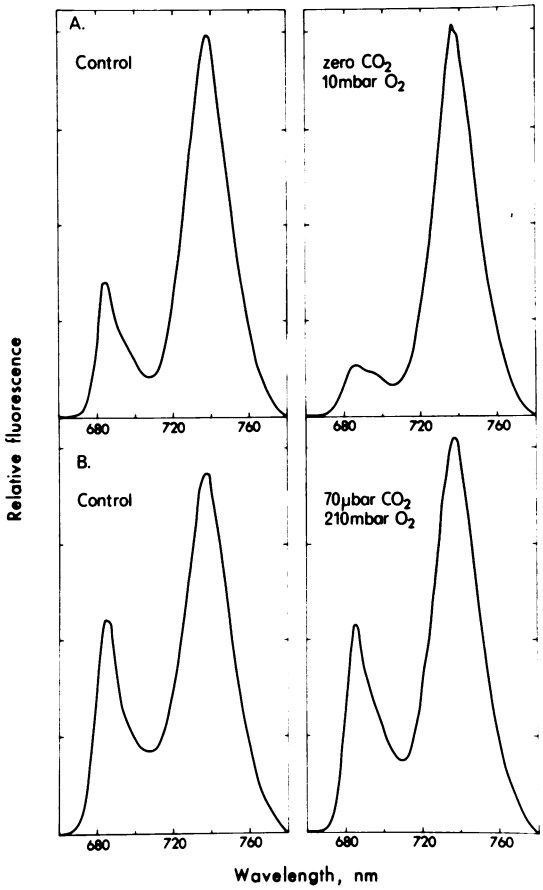

FIG. 8. Fluorescence emission spectra (at 77 K) of thylakoids isolated from bean leaflets exposed to the standard photoinhibitory treatment and after treatment at conditions approximating the CO_2 compensation point.

DISCUSSION

These experiments show that when the supply of external CO_2 to illuminated leaves is removed, and when photorespiration (a major internal source of CO_2 in the light) is prevented, intact leaves of C_3 plants are susceptible to photoinhibition. This photoinhibition is manifest as substantial reduction in the capacity for light-saturated CO_2 assimilation and apparent quantum yield, as noted previously (19). The reduced apparent quantum yield in intact leaflets is reflected in a reduction of PSII activity of chloroplast thylakoids isolated from treated leaflets (Table I and Fig. 8). Further experiments are required to determine if the changes in photochemical properties are due to primary damage to the reaction centers, interference with the transfer of excitation energy to the reaction centers, or to inhibition of specific steps in electron transport. Such primary changes in the properties of thylakoid membranes may affect the coupling of photophosphorylation and/or the capacity of chloroplasts to maintain enzymes such as ribulose bisP carboxylase in a fully active state (10). One or all of these processes would result in CO_2 assimilation remaining depressed following photoinhibition. The mechanism of the photoinhibition evident in these experiments is unknown.

The inhibition of CO_2 assimilation following illumination in zero CO_2 at low partial pressures of O_2 is a general phenomenon in C_3 plants. Photoinhibition can be largely prevented by increased partial pressures of CO_2 and/or O_2 (Figs. 6 and 7) and is prevented if the leaf is illuminated at conditions that approximate the CO_2 compensation point. Evidently, carbon flux through the integrated carbon reduction and oxidation cycles, at the CO_2 compensation point, allows adequate dissipation of photochemically generated energy which might otherwise result in photoinhibition. The potential contributions of additional CO_2 evolving reactions in the photorespiratory carbon oxidation cycle (8) or continued CO_2 production in the light via the tricarboxylic acid cycle, remain to be assessed. The possibility of additional energy dissipation in O_2 uptake by a Mehler-type reaction cannot be excluded (9). However, if this reaction occurs *in vivo*, and is coupled to ATP synthesis, a sufficiently active sink for the ATP generated (in the absence of CO_2 assimilation) is not immediately evident.

Speculations on the importance of these and other CO_2-recycling processes in photosynthetic metabolism under stress conditions have been developed elsewhere (17, 18). The prevailing partial pressure of O_2 in the atmosphere, and the oxygenase activity of ribulose bisP carboxylase make it inevitable that in C_3 plants, photorespiration provides protection against photoinhibition in most terrestrial habitats when external CO_2 supply is limited by stomatal closure in the light. This is not true of freezing tolerant C_3 species in which carbon turnover is limited by very low temperatures. It is not surprising that substantial photoinhibition is observed following illumination of these leaves in the vicinity of zero C (15).

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