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Effect of Light Intensity during Growth on Photoinhibition of Intact Attached Bean Leaflets

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ABSTRACT

In the study reported here, two different photoinhibitory phenomena were compared within a single plant species. Bean plants were grown in three different light intensities to simulate sun and shade environments. The effects of photoinhibitory treatments on *in vivo* CO_2 assimilation rates and *in vitro* chloroplast electron transport reactions were investigated and the extent to which carbon metabolism served to prevent photoinhibition was characterized. It was shown that the photoinhibition which follows exposure of intact leaflets of low light-grown bean plants to high light intensity in normal air is essentially similar to that which occurs when leaflets of plants grown in full sunlight are illuminated in the absence of CO_2 at low O_2 partial pressures.

In higher plants, two forms of photoinhibition have been reported. In the first, intact attached leaves of shade plants (plants restricted to growth in low light intensities), when exposed to light intensities greatly in excess of light saturation (in normal air), showed a diminished capacity for CO_2-dependent and light-limited CO_2 assimilation (3, 4, 8, 9). This photoinhibition was assumed to be caused by high light intensities exceeding the capacity for chloroplast electron transport and CO_2 assimilation, resulting in inactivation of the photochemical reaction centers (4, 14). In the second case, a similar photoinhibitory phenomenon occurred when intact attached leaves of sun plants (plants adapted to and grown in high light intensities) were exposed to light intensities similar to those in which they were grown but in an atmosphere low in O_2 and lacking in CO_2 (19, 20). In these experiments, photoinhibition was assumed to result because the normal utilization of photochemically generated energy was prevented by the absence of CO_2 (carbon assimilation) and O_2 (photorespiration) (20).

In the experiments with shade plants, chloroplasts could not be isolated from the treated leaves (4), and a more detailed analysis of the photoinhibition phenomenon was not possible. Inasmuch as it was already known that chloroplast thylakoids could be isolated from leaves of *Phaseolus vulgaris* (20) and that plants could be grown at high or low light intensities (17), we examined the two different photoinhibitory treatments within this individual species. Bean plants were grown at high and low light intensities to simulate sun and shade environments, and the effects of the two different photoinhibitory treatments on *in vivo* CO_2 assimilation and chloroplast photochemical activities were assessed. The particular role of CO_2 assimilation in moderating the effect of illumination of intact bean leaflets at excessive or nonsaturating light intensities was studied. These studies demonstrated that both forms of photoinhibition are essentially similar.

MATERIALS AND METHODS

Seeds of *P. vulgaris* L. (Hawkesbury Wonder) were sown in 5-liter plastic pots containing sterilized soil. All plants were established outdoors under full sunlight. When the first trifoliolate leaf had just unfolded, the plants were separated into three light regimes. The plants which remained under full sunlight received light intensities (400–700 nm) in excess of 2,000 $\mu E\ m^{-2}\ s^{-1}$ for extended periods of the day throughout the entire course of this experiment (midsummer, photoperiod 14 h). Sarlonshade netting (Sarlon, Australia, Waterloo, N.S.W.) was used to provide light regimes of 25% (550 $\mu E\ m^{-2}\ s^{-1}$) and 6% (140 $\mu E\ m^{-2}\ s^{-1}$) of full sunlight. Clear, cloudless conditions prevailed over the entire experimental period. Maximum daytime air temperature was 32 C; minimum night temperature was 18 C; and minimum RH was 30%. All plants were watered daily, at 3-h intervals, to prevent any water stress and the soil in each pot was flushed daily in the late afternoon with 1 liter nutrient solution. Nutrient solutions were based on Hewitt's nitrate-type nutrient solution (11) with double strength nitrate content (24 mM NO_3^-).

Gas Exchange Techniques. CO_2 exchange and leaf conductance measurements were made with an open system gas analysis apparatus as previously described (19, 20). In these studies, illumination was provided by a water-cooled, high pressure xenon lamp (Osram XBF 2,500 w). Individual experiments were conducted on the terminal leaflet of the first trifoliolate leaf when the leaf was just fully expanded (usually 10 days from unfolding in leaves of plants grown in full sunlight and 14 days in plants grown in 6% of full sunlight). A different plant was used for each experiment.

Response of CO_2 Assimilation Rate to Low Irradiances (Apparent Quantum Yield). An attached leaflet was exposed to a CO_2 partial pressure of 330 μbar in air at a light intensity of 180 $\mu E\ m^{-2}\ s^{-1}$. After a constant CO_2 assimilation rate was achieved, the light intensity was reduced in three equal steps, and at each step, CO_2 assimilation was measured as previously described (19).

Response of CO_2 Assimilation Rate to CO_2 Partial Pressure. After measurement of the apparent quantum yield, the light intensity was increased whereas CO_2 partial pressure and leaflet temperature were held constant. Measurements of the CO_2-dependent CO_2 assimilation rates were made at a light intensity of 2,000 $\mu E\ m^{-2}\ s^{-1}$ for the leaflets of plants grown in full sunlight, 1,000 $\mu E\ m^{-2}\ s^{-1}$ for plants grown in 25% of full sunlight and 500 $\mu E\ m^{-2}\ s^{-1}$ for plants grown in 6% of full sunlight. Response of CO_2 assimilation to changes in C_i[2] was measured as previously described (20). The leaflet was then exposed to a photoinhibitory treatment after which the light response (apparent quantum yield),

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[2] Abbreviations: C_i: intercellular CO_2 partial pressure; MV: methylviologen; ASC: Na-isoascorbate; DCIP: 2,6-dichlorophenol indophenol.

and CO_2 response curves were again measured. Comparisons of the effects of photoinhibitory treatments on the CO_2-dependent CO_2 assimilation rates of treated leaflets were made at a C_i of 120 μbar as previously discussed (20).

Photoinhibitory Treatments. Photoinhibitory treatments were applied by illuminating the leaflets at 2,000 μE m^{-2} s^{-1} for 3 h in air or in N_2-containing the desired partial pressures of O_2 and CO_2. The standard photoinhibitory treatment was a 3-h illumination in CO_2-free N_2 containing an O_2 partial pressure of 10 mbar. Further details of different treatments are given for each experiment.

Chloroplast Fragment Isolation and Assays. Chloroplasts were isolated from lateral leaflets (controls) and from photoinhibited leaflets by homogenizing at 2 C for 12 s in a Sorvall Omni-Mixer in 50 ml medium containing 0.05 M Sørensen's phosphate buffer (pH 7.5), 50 mM NaCl, 1 mM EDTA and 0.5% (w/v) BSA. The homogenate was filtered through four crosslayers of Miracloth and the filtrate was centrifuged for 5 min at 1000*g*. The chloroplasts were washed twice (1000*g* for 10 min) in the grinding medium without EDTA and 0.05% (w/v) BSA and finally suspended in the same buffer. Chl was determined according to Arnon (1).

All electron transport reactions were measured polarographically in a Clark electrode at 30 C. The final Chl concentration was 10–30 μg ml^{-1}.

Electron transport activity (PSII and PSI) was assayed either with ferricyanide as electron acceptor, measuring O_2 evolution in a 3-ml reaction mixture containing 50 mM Hepes (pH 7.6), 1 mM $MgCl_2$, 1 mM EDTA, 1.5 mM *K*-ferricyanide and chloroplasts, or using MV as electron acceptor and monitoring O_2 consumption in 3 ml medium consisting of 30 mM PPi buffer (pH 8.0), 10 mM $MgCl_2$, 0.1 mM MV and chloroplasts. Both reactions were completely inhibited by DCMU.

PSI activity was assayed with ascorbate/DCIP as electron donor and MV as electron acceptor by following O_2 consumption. The assay mixture contained 30 mM PPi buffer (pH 8.0), 10 mM $MgCl_2$, 1 mM ascorbate, 0.1 mM DCIP, 0.1 mM MV, and chloroplasts (DCMU was not added in these assays). 2.5 mM NH_4Cl was used as an uncoupler.

Actinic light (1,818 μE m^{-2} s^{-1} maximum intensity) was supplied from a 150 w quartz iodine lamp and adjusted to the desired intensity with Balzer's neutral density filters.

Ribulose bis-P carboxylase activity was assayed according to the method of Lorimer *et al.* (16).

Electron Microscopy. Leaf tissue was fixed with 5% glutaraldehyde in 50 mM Sørensen's phosphate buffer (pH 7.5). After washing in the same buffer (without glutaraldehyde), the tissue was refixed in OsO_4, dehydrated in a graded series of acetone, and embedded in Spurr's low viscosity embedding medium. Thin sections were cut on a Reichert ultramicrotome UM2, stained with saturated uranyl acetate and Reynold's lead citrate, and examined in a Hitachi H500 electron microscope.

RESULTS

Relationship between Photosynthetic Capacity and Light Intensity during Growth. A number of the features of leaves of bean plants grown in the three light regimes are listed in Table I. These data have all been expressed on a leaf area basis to enable direct comparison between treatments. The leaf Chl content (per unit leaf area) remained similar between plants grown in the three light regimes. In contrast to previous studies with sun species grown at high or low light intensities (2, 5), the Chl *a/b* ratio remained similar in leaves of bean plants grown in the three light regimes. The leaf fresh and dry weight (g m^{-2}) was highest in plants grown in full sunlight and least in plants grown in 6% of full sunlight. The ribulose bis-P carboxylase activities were higher in leaflets of plants grown in the higher light regimes. Similarly, the CO_2 assimilation rates of intact leaflets of plants grown in full sunlight, when measured at high light intensities, were considerably greater than those of shaded plants (Table I, Fig. 1A). The light intensity required to saturate CO_2 assimilation was also dependent upon the light regime at which the leaflets developed. In leaflets of plants grown in full sunlight, CO_2 assimilation remained unsaturated at 2,000 μE m^{-2} s^{-1} whereas CO_2 assimilation in leaflets of plants that developed in 25% or 6% of full sunlight was saturated at 1,000 μE m^{-2} s^{-1} and 500 μE m^{-2}, respectively (Fig. 1A).

The rates of all measured chloroplast electron transport reactions were very much enhanced when plants were grown under full sunlight as compared to 25 and 6% of full sunlight (Table I). In Figure 1B, electron transport activities (with K-ferricyanide as electron acceptor) of chloroplasts isolated from bean leaflets grown under the three different light regimes are compared as a function of light intensity. The photochemical activity of chloroplasts from bean leaflets grown in full sunlight required considerably higher

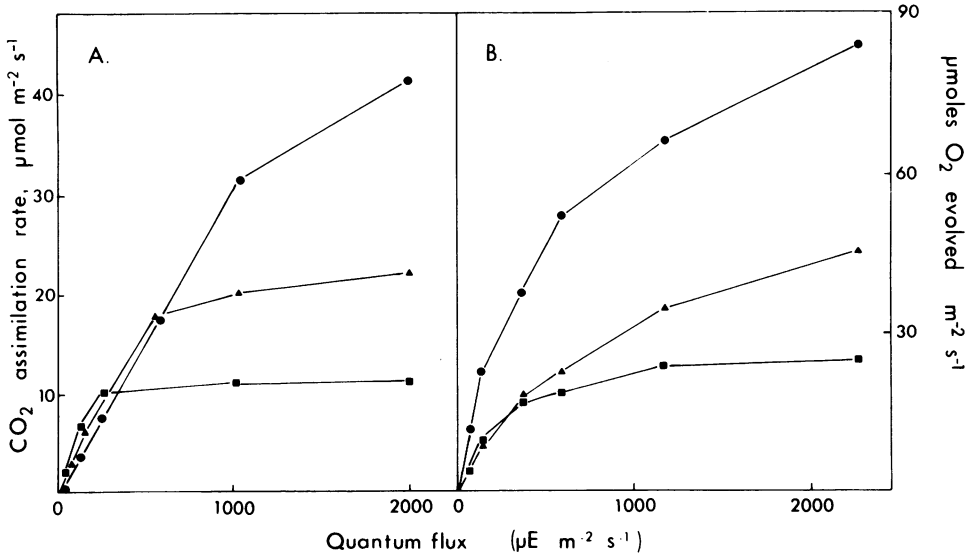

FIG. 1. Effect of light intensity during growth of *P. vulgaris* on: (A) CO_2 assimilation rates of intact attached leaflets and (B) electron transport to $K_3Fe(CN)_6$ of isolated chloroplasts as a function of light intensity. Plants grown at full sunlight (●); 25% of full sunlight (▲); 6% of full sunlight (■).

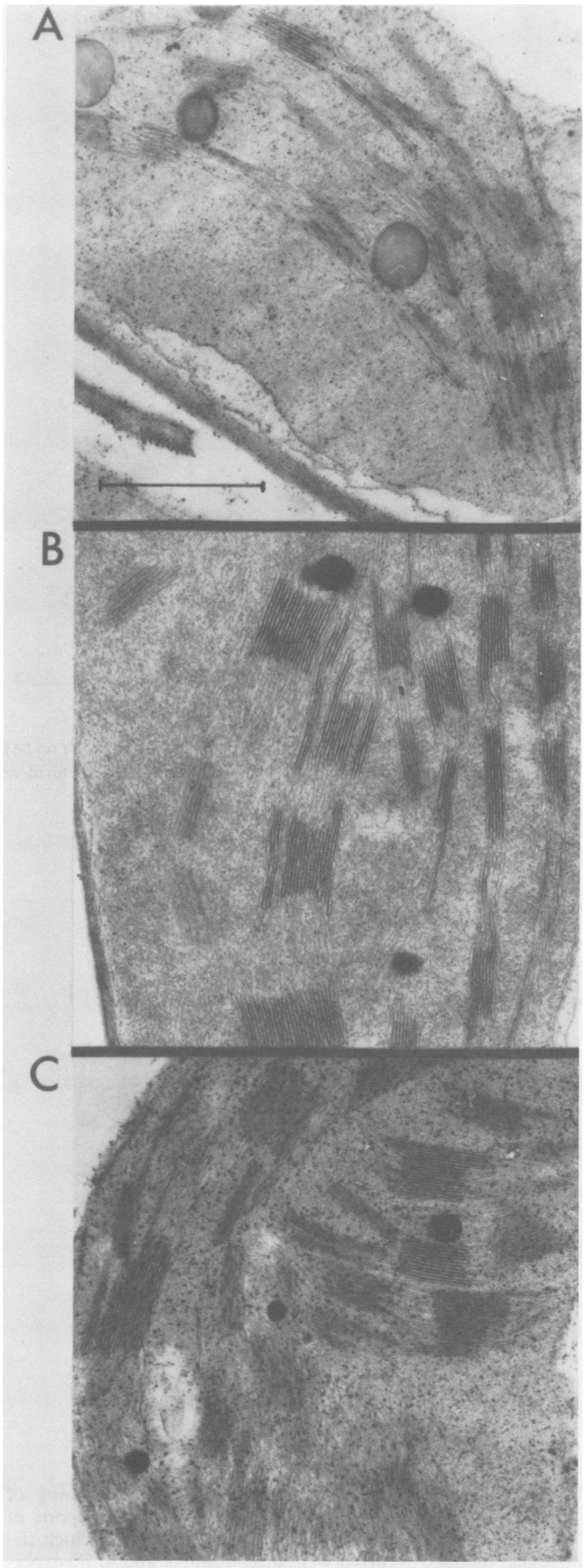

Table I. *The Effect of Light Intensity During Growth on Some Properties of Bean Leaves*

Chlorophyll and leaf area plus weight values are means of 10 determinations. RuBPCase activity was assayed in triplicate. Whole leaf assimilation rates represent one set of experiments, and electron transport activities are mean values of four to six measurements with SE, as in Table II.

		Light Intensity		
		Full Sunlight	25%	6%
Chl content	($g\ m^{-2}$)	0.5	0.5	0.5
Chl *a*/*b* ratio		2.6	2.4	2.8
Leaf area per plant	(m^2)	0.08	0.11	0.10
Leaf fresh weight	($g\ m^{-2}$)	246	198	138
Leaf dry weight	($g\ m^{-2}$)	27	22	16
RuBPCase activity ($\mu mol\ ^{14}CO_2$ fixed $m^{-2}\ s^{-1}$)		137	90	52
Whole leaf CO_2 assimilation rates ($\mu mol\ CO_2$ fixed $m^{-2}\ s^{-1}$)		40	21	10
Electron transport activities (Uncoupled; $\mu mol\ O_2$ evolved or consumed $m^{-2}\ s^{-1}$)				
$H_2O \rightarrow K_3Fe(CN)_6$	(4_e-/O_2)	56	41	25
$H_2O \rightarrow$ MV	(2_e-/O_2)	51	36	24
ASC·DCIP → MV	(2_e-/O_2)	84	59	42

light intensities to approach saturation than did those from plants grown in the lower light intensities. The rates of O_2 evolution of chloroplasts from plants grown in full sunlight were three times those of chloroplasts from plants grown in 6% of full sunlight and twice that of chloroplasts from plants grown in 25% of full sunlight (Fig. 1B). Similar results were obtained earlier (5) with *Atriplex* grown at different light intensities. The work with *Atriplex* showed also that the differences in capacity for electron transport was accompanied by changes in activity of intermediate electron carriers; such measurements were not made in the study reported here.

We examined the chloroplast structure of the upper palisade parenchyma of leaflets from plants grown at the three light intensities. There were no such striking differences in granum size and chloroplast size as has been described for genetically adapted shade plants compared to sun plants (5). However, in chloroplasts from plants grown at full sunlight, rather small grana (approximately six layers/granum) were common whereas rather large grana (12–20 layers/granum) were found in the chloroplasts from the plants that had developed under 25 or 6% of full sunlight (Fig. 2).

Effect of Standard Photoinhibitory Treatment (3 h zero CO_2, 10 mbar O_2, 2,000 $\mu E\ m^{-2}\ s^{-1}$). The effects of the standard photoinhibitory treatment on some photosynthetic properties of recently expanded bean leaflets of plants grown in the three light intensities are shown in Figures 3 and 4. The treatment resulted in substantial reduction of the CO_2-dependent CO_2 assimilation rates at all intercellular CO_2 partial pressures measured (Fig. 3A) with concomitant inhibition of the light-limited CO_2 assimilation capacity (Fig. 3B). Following treatment, there was greater inhibition of the apparent quantum yield of leaflets of plants grown in the lower light intensities than in leaflets of plants grown in full sunlight (Fig. 3B).

FIG. 2. Electron micrographs of thin sections through chloroplasts of bean leaflets grown at: (A) full sunlight, (B) 25% of full sunlight, and (C) 6% of full sunlight. Bar indicates 1 μm.

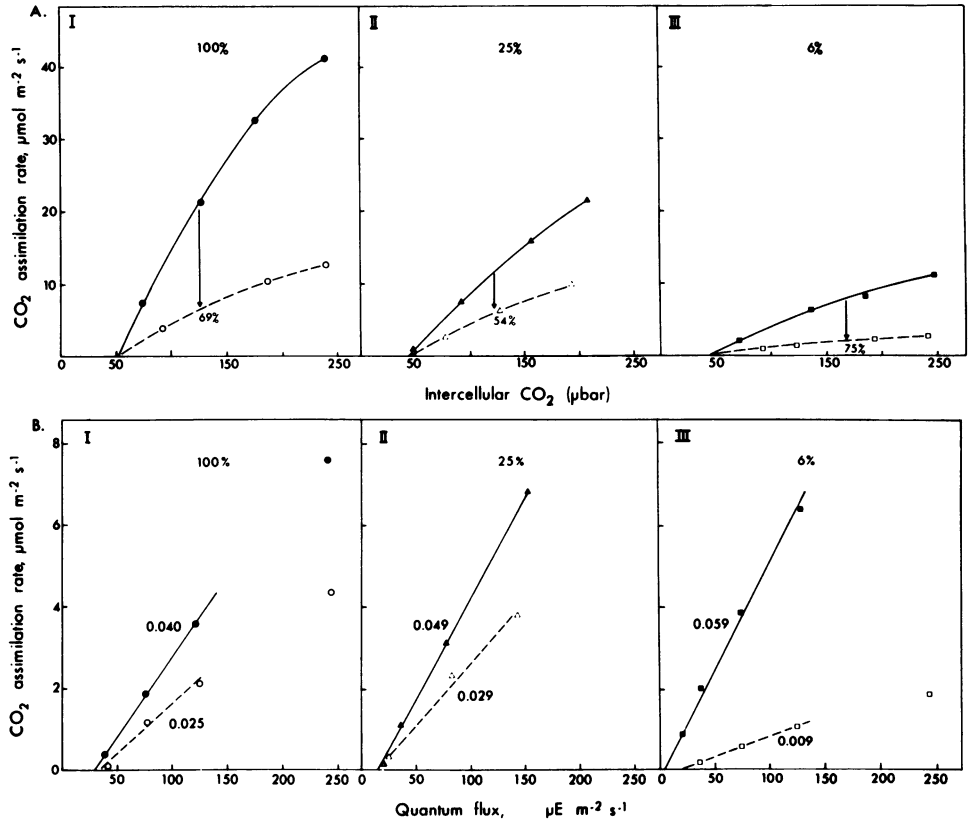

FIG. 3. Effect of the standard photoinhibitory treatment (3-h exposure to CO_2-free N_2 containing 10 mbar O_2, irradiance 2,000 μE m^{-2} s^{-1}) on (A) CO_2-dependent CO_2 assimilation rate at different intercellular CO_2 partial pressures and (B) apparent quantum yield (μmol CO_2 assimilated per incident μE). (●) (▲) (■): controls; (○) (△) (□): photoinhibited.

Table II. *Effect of the Standard Photoinhibitory Treatment on Some Electron Transport Activities in Isolated Bean Chloroplasts*

Rates are expressed as μmol O_2 evolved or consumed m^{-2} s^{-1} and were measured at light saturation and in the presence of 2.5 mM NH_4Cl as uncoupler. They represent mean values of four to six experiments ± SE of mean.

Growth Sunlight Intensity	$H_2O \rightarrow K_3Fe(CN)_6$		Inhibition	$H_2O \rightarrow MV$		Inhibition	ASC·DCIP → MV		Inhibition
	Control	Treated		Control	Treated		Control	Treated	
			%			%			%
Full	55 ± 6	28 ± 4	49	51 ± 5	19 ± 3	63	84 ± 5	45 ± 4	46
25%	41 ± 2	15 ± 1	63	36 ± 1	10 ± 1	72	59 ± 2	31 ± 2	47
6%	25 ± 3	7 ± 1	72	24 ± 3	4	83	42 ± 5	15 ± 1	64

Table III. *Effect of 3-h Exposure to an Irradiance of 2,000 μE m^{-2} s^{-1} at Conditions Approximating the CO_2 Compensation Point (60 μbar CO_2, 210 mbar O_2, 30 C) on Some Electron Transport Activities of Isolated Chloroplasts*

Rates are expressed as μmol O_2 evolved or consumed m^{-2} s^{-1} and were measured at light saturation and in the presence of 2.5 mM NH_4Cl as uncoupler. Rates are means of two or three experiments.

Growth Sunlight Intensity	$H_2O \rightarrow K_3Fe(CN_6)$		Inhibition	$H_2O \rightarrow MV$		Inhibition	ASC·DCIP → MV		Inhibition
	Control	Treated		Control	Treated		Control	Treated	
			%			%			%
Full	56	59	0	46	49	0	85	88	0
25%	46	35	24	34	38	0	59	50	16
6%	20	15	25	16	9	44	39	33	14

In a minority of experiments, exposure of intact leaflets of bean plants grown in full sunlight to the standard photoinhibitory treatment resulted in an unusual physiological response. Within 1 h of commencement of treatment, visible damage to leaflets became evident; the symptoms involved a mild bleaching of pigments. These symptoms did not appear in earlier reports of photoinhibition in C_3 plants (19, 20). Experiments in which the visible leaf damage occurred were discontinued, and in no exper-

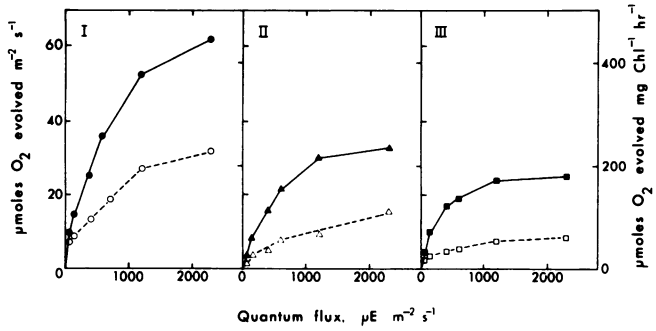

FIG. 4. Effect of standard photoinhibitory treatment on electron transport to $K_3Fe(CN)_6$ of chloroplasts isolated from treated and control leaflets. Plants grown at (I) full sunlight, (II) 25% of full sunlight, (III) 6% of full sunlight. (●) (▲) (■): controls; (○) (△) (□): photoinhibited.

iment reported in this paper, did these visible symptoms or changes in leaf Chl content occur.

The differences in the effect of the standard photoinhibitory treatment on the apparent quantum yield (Fig. 3B) of leaflets from plants grown in the different light environments were also found for the inhibition of electron transport in thylakoids isolated from the treated leaflets. Table II shows that the degree of inhibition of uncoupled electron transport rates, which resulted from the standard photoinhibitory treatment, was directly correlated to the growth conditions of the plant. The average reduction (4 to 6 experiments), for example, of K-ferricyanide-dependent electron transport activity was 72% in chloroplasts isolated from treated leaflets of plants grown in 6% of full sunlight, but only 62 and 50% in chloroplasts from plants grown in 25 and 100% of full sunlight, respectively (Table II). This was the case for the three electron transport partial reactions measured, but the activity of PSI (ASC, DCIP → MV) was inhibited somewhat less than PSII. As DCMU was not added to the PSI assay it is possible that some of the inhibition of PSI activity may be due to resident PSI activity. Where 3.3 μM DCMU was added to the assay mix, considerably less inhibition of PSI activity was measured (data not shown).

In Figure 4, the K-ferricyanide-dependent electron transport rates of chloroplasts from control and treated leaflets (from plants grown at the three light intensities) were compared as functions of light intensity. There was no significant change in the light saturation characteristics between chloroplasts isolated from treated and control leaflets. Electron microscopy of the chloroplasts of control and photoinhibited leaflets revealed no marked structural differences.

Photoinhibition following Treatment at Different CO_2 Partial Pressures (at Ambient O_2 Partial Pressures). It was previously shown (19, 20) that the extent of photoinhibition in intact bean leaflets (plants grown in full sunlight) can be lessened by the presence of O_2 and/or CO_2 during treatment and can be completely prevented by treatment of conditions that approximate the CO_2 compensation point (50–60 μbar CO_2, 210 mbar O_2). In the experiments reported here, the above results were confirmed. However, the extent of photoinhibition was additionally affected by the light intensity at which the plants had developed. When intact leaflets were treated (3 h exposure to a light intensity of 2,000 μE m^{-2} s^{-1}, ambient O_2 partial pressure of 210 mbar) in the absence of external CO_2, inhibition of the CO_2-dependent CO_2 assimilation rates resulted (Fig. 5). The effect of this treatment was greatest in leaflets of plants grown in 6% of full sunlight, less in leaflets that had developed in 25% of full sunlight, and least in leaflets that had developed in full sunlight. In all cases, the extent of photoinhibition could be diminished if external CO_2 was present during the treatment period. In leaflets of plants grown in full sunlight, photoinhibition was completely prevented where an

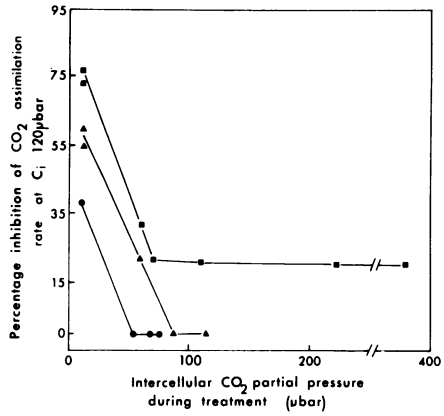

FIG. 5. Effects of intercellular CO_2 partial pressure during a 3-h photoinhibitory treatment (O_2 partial pressure 210 mbar) on reduction of the CO_2-dependent CO_2 assimilation rate. Percentage inhibition of CO_2 assimilation rate, at a C_i of 120 μbar, was calculated from CO_2 response curves of the type shown in Figure 3A. Plants grown at full sunlight (●); 25% of full sunlight (▲); 6% of full sunlight (■).

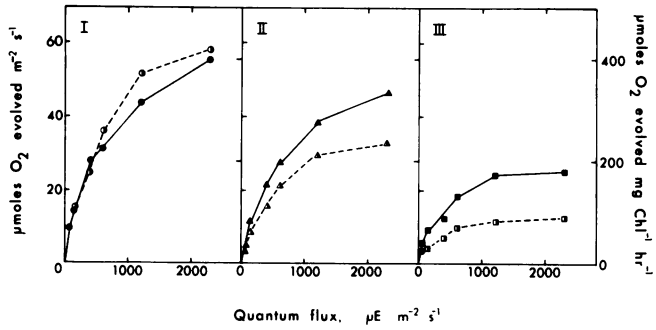

FIG. 6. Effect of treatment at conditions approximating the CO_2 compensation point (60 μbar CO_2; 210 mbar O_2) on electron transport to $K_3Fe(CN)_6$ of chloroplasts isolated from treated and control leaflets. Plants grown at (I) full sunlight, (II) 25% of full sunlight, (III) 6% of full sunlight. (●) (▲) (■): controls; (◑) (◭) (◧): photoinhibited.

intercellular CO_2 partial pressure of 40 μbar or greater was maintained during the treatment period. A higher intercellular CO_2 partial pressure (80 μbar or greater) completely prevented photoinhibition in treated leaflets of plants grown in 25% of full sunlight. In treated leaflets of plants grown in 6% of full sunlight, photoinhibition was markedly reduced over the same range of CO_2 partial pressures but could not be prevented by an increased CO_2 partial pressure, and some photoinhibition still occurred following treatment at CO_2 partial pressures considerably in excess of ambient (Fig. 5). The characteristics of this photoinhibition in leaves of low-light-grown bean plants are under further study.

Electron transport activities of chloroplasts isolated from treated leaflets reflected similar trends to the *in vivo* results. In all measured electron transport reactions, inhibition was significantly alleviated or even abolished when treatment was performed at CO_2 partial pressures approximating the CO_2 compensation point (60 μbar CO_2, 210 mbar O_2). Exposure of leaflets of plants grown in full sunlight to conditions approximating the CO_2 compensation point resulted in no inhibition of electron transport reactions in chloroplasts isolated from the treated leaflets. However, this same treatment resulted in some inhibition of electron transport reactions in thylakoids from leaflets that had developed in 25 or 6% of full sunlight (Table III). Photoinhibitory treatments at conditions that approximate the CO_2 compensation point did not significantly change light saturation characteristics of chloroplast electron transport activity, (Fig. 6), where K-ferricyanide-dependent elec-

tron transport was measured as a function of light intensity. Control and photoinhibited leaves were examined by electron microscopy. There were no changes in chloroplast structure and organization as examined by electron microscopy after treatment of the leaflets under conditions approximating the compensation point.

Dependence of Photoinhibition on the O_2 Partial Pressure during Treatment. The extent of photoinhibition following exposure of intact bean leaflets to a 3-h illumination (2,000 μE m^{-2} s^{-1}) in the absence of CO_2 was influenced by the O_2 partial pressure maintained during treatments and by the light intensity at which the plants had developed. As previously discussed, exposure to the standard photoinhibitory treatment resulted in inhibition of the CO_2-dependent CO_2 assimilation rates (with no apparent trend between plants grown in the three light intensities). Treatment at an increased O_2 partial pressure (210 mbar, zero external CO_2) resulted in less photoinhibition in leaflets of plants grown in the highest light intensities (Fig. 7A). Similar results were obtained for the effects of these treatments on the light-limited CO_2 assimilation rates of the treated leaflets (Fig. 7B).

DISCUSSION

It has been shown in earlier work (5, 9, 10) and the experiments reported here (Table I) that sun plants when grown in high or low light regimes have different and characteristic photosynthetic features. Leaves of bean plants grown in low light intensities displayed much lower fresh weights per unit area (Table I). However, the Chl content per unit leaf area was not different from that of plants grown in high light intensities. The leaves of low light grown bean plants had lower rates of photosynthetic electron transport and CO_2 assimilation than leaves of plants grown in full sunlight (Table I). In this sense, the photosynthetic parameters were adapted to perform adequately in low light environments. Conversely, in plants grown in full sunlight, the higher electron transport and CO_2 assimilation capacities were adapted to function at higher light intensities (Table I, Fig. 1). Thus, bean plants grown in high or low light intensities showed properties similar to those described for sun and shade plants (3, 9). This provided us with a model system to compare directly a range of phenomena previously observed in photoinhibition studies on a variety of organisms and under various experimental conditions.

It has previously been shown (19, 20), and confirmed here, that intact leaflets from plants grown in moderate-to-high light intensities suffer photoinhibition when illuminated (3 h) in the absence of CO_2 and at low O_2 partial pressures (Fig. 3). This treatment largely prevents carbon metabolism and therefore removes the major sink for photochemically generated energy ($NADPH_2$ and ATP). As previously observed (20), the capacity for PSII electron transport is substantially depressed following this photoinhibitory treatment (Table II). The photoinhibition observed in these *in vivo* experiments with bean leaflets illuminated in the absence of CO_2 and "photorespiration" bears similarities to previous *in vitro* studies of photoinhibition observed with leaf fragments (6) and broken chloroplasts (23) illuminated in the absence of an electron acceptor.

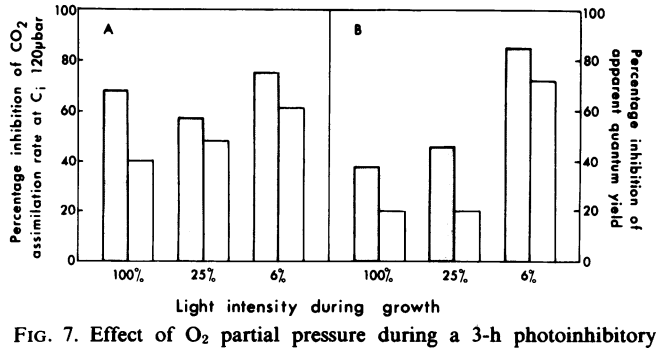

FIG. 7. Effect of O_2 partial pressure during a 3-h photoinhibitory treatment (zero external CO_2) on (A) inhibition of the CO_2-dependent CO_2 assimilation rate, and (B) reduction in apparent quantum yield. *Dense shading:* treatment at zero CO_2 10 mbar O_2; unshaded: treatment at zero CO_2 210 mbar O_2. Percentage inhibition of the CO_2-dependent CO_2 assimilation rate was calculated as in Figure 5. Percentage inhibition of the apparent quantum yield was calculated from light response curves of the type shown in Figure 3A.

In leaflets of plants grown in full sunlight or 25% of full sunlight, photoinhibition was completely prevented by the presence of greater than 40 μbar and 80 μbar intercellular CO_2 partial pressures, respectively, throughout the treatment period (Fig. 5). It was suggested that the presence of CO_2 allows carbon assimilation throughout the treatment period, thereby providing a sink for photochemically generated energy and ensuring the continued ability of chloroplasts to transduce light energy (20). Similarly, an increased O_2 partial pressure throughout the treatment reduced photoinhibition in all experiments (Fig. 7). The presence of O_2 allows CO_2 production in photorespiration and the consumption of photochemically generated energy via the integrated carbon reduction and photorespiratory cycles (19). Additional energy consumption via O_2 uptake in a Mehler-type reaction may also occur under these conditions (7, 21).

The present experiments show, that photoinhibition suffered by plants grown in 6% of full sunlight was not prevented by an increased CO_2 partial pressure during the treatment at an illumination of 2,000 μE m^{-2} s^{-1} (Fig. 5). Evidently the increased light harvesting capacity of these leaflets, relative to their electron transport and CO_2 assimilation capacities (Table I), is such that when illuminated at high light intensities, even at a saturating CO_2 partial pressure, photoinhibition results. Photoinhibition presumably occurs because the capacity for light trapping exceeds the capacity for electron transport and CO_2 assimilation (20) and other means of dissipating light energy (22). In this sense, the photoinhibition observed in these experiments with leaflets of bean plants grown in 6% of full sunlight and exposed to light intensity of 2,000 μE m^{-2} s^{-1} bears similarities to reports of photoinhibition with intact leaves of shade plants (4, 9), algal cells (13, 18), isolated chloroplast thylakoids (12, 14), and intact isolated chloroplasts (15), illuminated at excess light intensities. Photoinhibition under these conditions may result from an over energization of photosynthetic reaction centers as a consequence of excess light (4, 14).

In summary, the present experiments show that the consequences of photoinhibition in leaves of plants grown in high light intensities when they cannot utilize photochemically generated energy because CO_2 is limiting, and in leaves of low light-grown plants when illuminated at high light intensities, are essentially the same. In both cases, photoinhibition involves concomitant reductions in the CO_2-dependent rates of CO_2 assimilation, apparent quantum yields, and chloroplast electron transport reactions. Electron flow through PSII (Table II) seems to be primarily affected.

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