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Failure of corn leaves to acclimate to low irradiance. Role of protochlorophyllide reductase in regulating levels of five chlorophyll-binding proteins

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Primary leaves of corn (*Zea mays*) fail to acclimate successfully to growth irradiances less than or equal to 10 μmol photons per m^2/s. In the range 100–500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, acclimation to low irradiance involves enhanced grana formation and the preferential accumulation of LHC II (the light-harvesting chlorophyll (Chl) *a*/*b* antenna of Photosystem II) relative to the four other major chlorophyll-binding proteins (CPI and LHC I of Photosystem I and CPa-1 and CPa-2 of Photosystem II core). However, at lower irradiances, all chlorophyll-protein complexes are less abundant, especially the Chl *a*/*b* complexes LHC I and LHC II. At 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, LHC I and LHC II apoproteins are below the limit of detection, grana are absent and the apoproteins of the three Chl *a* complexes (CP1, CPa-1 and CPa-2) and Chl *a* itself are only 10–25% as abundant as at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In contrast, protochlorophyllide reductase and prolamellar bodies increase markedly in abundance with decreasing irradiance. The reduced synthesis of Chl at low irradiance is due primarily to a decline in the rate at which light drives each protochlorophyllide reductase molecule.

Introduction

Photosynthetic organisms are able to acclimate to changes in light intensity experienced during growth [1–3]. This is achieved in part by regulating the number of light-harvesting pigments per reaction center. The number increases at low irradiance to enhance photon capture and declines at high irradiance to reduce the probability of photooxidative damage. LHC II is the major light-harvesting Chl *a*/*b*-protein complex of green plants and is associated mainly with Photosystem II (PS II) [4]; it binds about 30% of total Chl *a* and about 80% of total Chl *b*, with a Chl *a*/Chl *b* ratio of about 1. LHC I, the light-harvesting complex of PS I, binds about 25% of total Chl *a* and 15% of total Chl *b*, with a Chl *a*/Chl *b* ratio of about 3.5 [5]. The reaction centers of PS I and PS II contain only Chl *a*-protein complexes: the P-700 Chl *a* protein of PS I and CPa-1 and CPa-2 of PS II [6]. The Chl *a*/Chl *b* ratio of thylakoids is therefore a crude but convenient indication of the ratio of light-harvesting pigments to reaction centers and has been widely used in studies on the acclimation of green plants to changes in growth irradiance [7–9].

The photosynthetic photon flux density of full sunlight is about 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the

Abbreviations: PS, Photosystem; Chl, chlorophyll; LHC, light-harvesting chlorophyll *a*/*b* complex.

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400–700 nm waveband. Recent studies have shown that green plants are able to decrease their Chl *a*/Chl *b* ratio in response to flux densities below 1% of full sunlight [10,11]. However, in many green plants, especially the angiosperms, Chl synthesis is rendered light-dependent by protochlorophyllide reductase [12]. This enzyme catalyzes the NADPH-linked reduction of protochlorophyllide to Chlide. Each enzyme-bound protochlorophyllide molecule must be excited before products can be formed. Thus, at low irradiance, Chl synthesis should be limited by the rate of excitation of protochlorophyllide. Moreover, Chl *b* synthesis should be more adversely affected than Chl *a* synthesis, because Chl *b* is synthesized from Chl *a* and a threshold level of free Chl *a* appears to be required before Chl *b* synthesis can occur [13]. Thus, at flux densities that are limiting for protochlorophyllide reduction, we would expect preferential accumulation of reaction center Chl *a*-proteins compared with light-harvesting Chl *a*/*b* proteins, that is, a failure of acclimation.

We have shown by radioimmune assay that pea leaves grown under white fluorescent light of very low irradiance contain no LHC II [14,15]. Accumulation of the protein requires at least 5 μmol photons per m^2/s, with maximal accumulation per leaf occurring at about 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This result provides clear evidence for failure of the acclimation process at low irradiance but the study did not compare LHC II levels with those of other major Chl-binding proteins. It was therefore unclear whether the same response occurs with LHC I and the Chl *a*-proteins. This situation has now been rectified through preparation of antibodies against apoprotein of LHC I, CPI, CPa-1 and CPa-2. We report here on the acclimation of primary leaves of corn (*Zea mays*) to flux densities in the 0–500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ range. We chose corn rather than pea for this more detailed study because primary leaf expansion is less dependent on irradiance in corn and so it is possible to study chloroplast development against a more uniform background of leaf development.

A key question concerns protochlorophyllide reduction. Does this reaction limit acclimation at low irradiances and, if so, which is the more important factor: the activity of protochlorophyllide reductase or its abundance? Both the reductase and its mRNA are abundant in dark-grown leaves but are unstable in the light. The mRNA is destroyed by a phytochrome-mediated mechanism [16,17] and the enzyme is subject to breakdown following the light-induced consumption of its protective substrates [18,19]. Illumination will promote chlorophyllide synthesis by exciting the protochlorophyllide-enzyme-NADPH ternary complexes and will inhibit chlorophyllide synthesis by increasing destruction of protochlorophyllide reductase and its mRNA. As it is not clear which of these opposing effects will be dominant at any given flux density, we report here the irradiance dependence of both phototransformation and protochlorophyllide reductase accumulation and relate them to the limits of the acclimation process.

Materials and Methods

Plants. Seeds of *Z. mays* (Pioneer hybrid no. 3780) were sown in pots containing a mixture of sand and plotting fiber, watered thoroughly and placed inside cylindrical cardboard containers covered with two layers of aluminum foil. The cylinders were placed under a bank of cool white fluorescent tubes (Sylvania, photosynthetic photon flux density, 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 400–700 nm) and lids were placed over them. Each lid contained cinemoid neutral density filters supplemented with several layers of cheese cloth to admit the desired photon flux densities. The latter were measured with a Li-Cor 2 quantum sensor and meter. The cylinders and light bank were housed in a growth chamber maintained at 22° C.

Pigment analyses. Total Chl content of primary leaves was assayed on 90% aqueous acetone extracts as described [20]. Chl *a*, Chl *b*, lutein and β-carotene in similar extracts were analyzed and quantified by HPLC, using 20 μl injector loop, guard column, C18 reverse-phase column (Altex, 3 μm pores, 4 mm inner diameter × 25 cm length), Beckman gradient controller, a gradient of 80% aqueous methanol and ethyl acetate [21], and a calibrated detector fitted with a 436 nm filter.

In vivo phototransformation. 8-day-old dark-grown seedlings were harvested in darkness and duplicate sets of five seedlings were illuminated for 2 s–5 min under warm white fluorescent light

of defined flux density. The seedlings were then homogenized in darkness in 5 ml of 90% aqueous acetone, the total volume was made up to 10 ml with 80% aqueous acetone and filtered, and the 600–700 nm spectrum of each sample was recorded on a Hewlett-Packard diode array spectrophotometer. Protochlorophyllide disappearance and chlorophyllide appearance were measured at 628 and 664 nm, respectively. The initial velocity of the phototransformation was calculated from the linear part of the progress curve and from published extinction coefficients for protochlorophyllide [18].

P-700 assay. P-700 was measured by light-induced absorption difference spectra in the dual wavelength mode of an Aminco DW-2a spectrophotometer with a measuring wavelength at 697 nm relative to an isosbestic wavelength at 720 nm. Thylakoids were extracted from leaves in 50 mM Tris-HCl (pH 7.8) containing 10 mM $MgCl_2$ and 0.05% Triton X-100 [22,23]. Absorption-difference spectra were measured in the presence of methyl vilogen and ascorbate. The P-700 content was normalized to the Chl content of the same sample.

Electroblot radioimmune assays. Sets of five primary leaves were frozen in liquid nitrogen, thawed in the presence of 10 ml of buffer A (0.1 M Tricine-NaOH (pH 8.0)/2% SDS/1 mM dithiothreitol), frozen and thawed again, boiled for 2 min and centrifuged at $10000 \times g$ for 10 min. The supernatants were frozen for later analysis of protochlorophyllide reductase and the apoproteins of CP1, LHC I and LHC II. For measurement of CPa-1 and CPa-2 apoproteins in leaves, the sensitivity of the procedure was increased by isolating thylakoids and analyzing them by SDS-polyacrylamide gel electrophoresis and electroblot radioimmune assay. Ten fresh leaves were homogenized in 0.3 M sucrose, 25 mM Hepes-NaOH (pH 7.6), 1 mM EDTA, 5 mM sodium ascorbate, filtered through one layer of Miracloth (Calbiochem) and centrifuged at $40000 \times g$ for 10 min. The pellet was resuspended in 50 mM Tricine-NaOH (pH 8.0), 2.5 mM $MgCl_2$, 200 μM phenylmethylsulfonylfluoride, and incubated on ice with 100 μg/ml deoxyribonuclease I for 30 min. The membranes were recovered by centrifugation at $40000 \times g$ for 10 min, resuspended in buffer A (2 ml) and heated to 40 °C prior to SDS-polyacrylamide gel electrophoresis and electroblot radioimmune assay. Nuclease treatment greatly reduced non-specific interactions between histones and antibodies.

Samples (usually 30 μl) were subjected to SDS-polyacrylamide gel electrophoresis [24] and analyzed for specific proteins by electroblot radioimmune assay [14,25], using nitrocellulose sheets together with polyclonal antibodies and ^{125}I-protein A. Detection was by autoradiography at −80 °C with a Dupont Hi-Plus intensifying screen. In the case of CPa-1, which was detected with a mouse monoclonal antibody, the primary antibody was reacted with a rabbit anti-mouse antibody prior to addition of ^{125}I-protein A. All the polyclonal antibodies have been characterized: anti-CP1 and anti-LHC I [26], anti-protochlorophyllide reductase [27], anti-CPa-2 [28] and anti-LHC II [14]. The sample with the highest content of the protein of interest was loaded in a dilution series to allow the construction of a standard curve from microdensitometry of the autoradiogram. A slightly curvilinear relation was obtained between volume of sample loaded and area under the microdensitometer peak but this was converted into a linear relationship for all antibody–antigen combinations when a correction was applied for autoradiographic band broadening at higher loadings.

Electron microscopy. The leaf samples were fixed at room temperature in 5% glutaraldehyde buffered to pH 7.4 with 80 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) and stored at 4 °C. Illumination during fixation was at the growth irradiance, and the dark sample was fixed in total darkness. All samples were washed in buffer, post fixed in 2% buffered osmium for 2 h, washed, dehydrated in the cold, and embedded in an epoxy resin. Sections were stained with uranyl acetate and basic lead citrate solutions prior to examination at 80 kV in a transmission electron microscope.

Results

Time-course of chlorophyll synthesis in primary leaves of maize

Corn seedlings were grown under continuous warm white fluorescent light for up to 10 days. Fig. 1 shows the time course of Chl accumulation

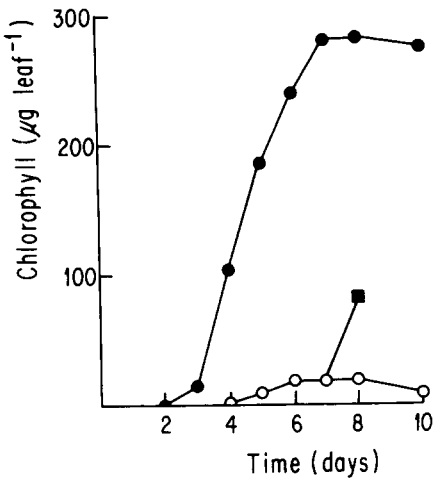

Fig. 1. Time-course of Chl biosynthesis in primary leaves of *Z. mays* grown under white fluorescence light of the indicated photosynthetic photon flux densities. Closed circles: 500 μmol $\cdot m^{-2} \cdot s^{-1}$. Open circles: 1 μmol $\cdot m^{-2} \cdot s^{-1}$. Close square: transferred at 7 days from 1 to 500 μmol $\cdot m^{-2} \cdot s^{-1}$.

in primary leaves exposed to 1 or 500 μmol photons per m^2/s. Chl accumulation began at 3–4 days, when the primary leaves emerged from the soil, was most rapid after 4–5 days, and reached a maximum after 6–7 days. The maximal rate of Chl synthesis was about 8.7 μg Chl per leaf per day at low irradiance and about 86 μg per leaf per day at high irradiance.

Leaves exposed to low irradiance ceased net Chl accumulation at a very low Chl content (23 μg Chl per leaf compared with 280 μg Chl per leaf at high irradiance). This was not due to loss of the capacity for Chl biosynthesis, because when plants grown under low irradiance for 7 days were transferred to high irradiance, Chl accumulation was rapid (Fig. 1) and occurred throughout the primary leaf. An overall rate of accumulation of about 70 μg Chl per leaf per day was obtained, comparable with the maximal rate obtained for plants exposed to high irradiance from the time of emergence.

Differential accumulation of photosynthetic pigments

Corn plants were grown for 8 days under flux densities of 0, 1, 10, 100 or 500 μmol $\cdot m^{-2} \cdot s^{-1}$. The pigments of the primary leaves were extracted into acetone and assayed by HPLC for Chl *a*, Chl *b*, lutein and β-carotene. Fig. 2 shows that the concentrations per leaf of all four pigments de-

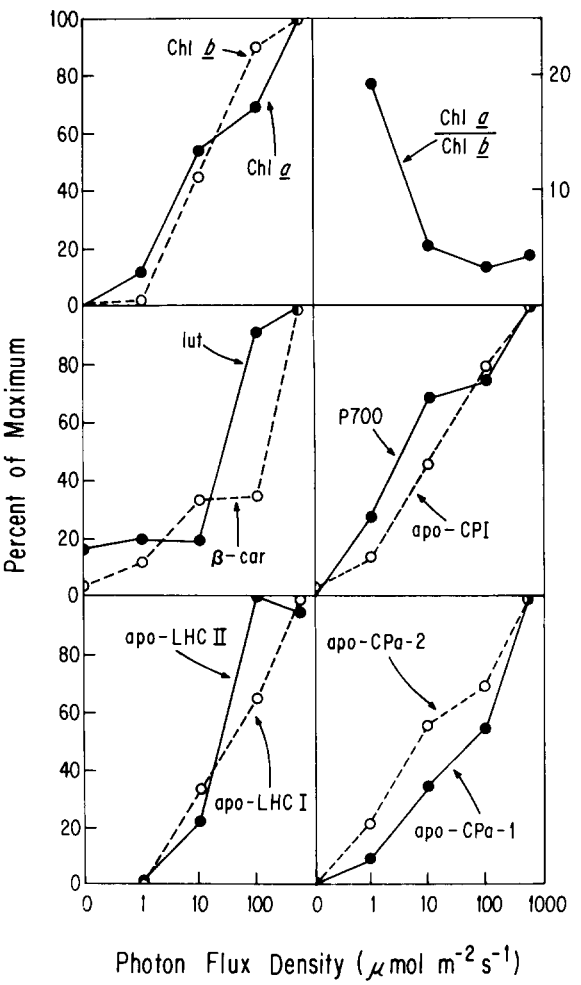

Fig. 2. Concentrations of specific pigments and proteins in primary leaves of 8-day-old corn plants. Parameters are measured per leaf and are expressed as a percentage of maximum value obtained at 0, 1, 10, 100 or 500 μmol $\cdot m^{-2} \cdot s^{-1}$.

creased with decreasing flux density, but with different profiles. Dark-grown leaves contained lutein and β-carotene but no detectable Chl *a* or *b*. Chl *b* was barely detectable in leaves exposed to 1 μmol $\cdot m^{-2} \cdot s^{-1}$ (2.5% of maximum), giving a very high Chl *a*/Chl *b* ratio (19 : 1). The ratio was also somewhat elevated (4.8 : 1) in plants grown at 10 μmol $\cdot m^{-2} \cdot s^{-1}$ but it reached a minimum (3.7 : 1) at 100 μmol $\cdot m^{-2} \cdot s^{-1}$ and then increased significantly (to 4.3 : 1) at 500 μmol $\cdot m^{-2} \cdot s^{-1}$. Since a low Chl *a*/Chl *b* ratio is regarded as being typical of green plants acclimating successfully to low irradiance, these results establish that

corn fails to acclimate below about 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Differential accumulation of chlorophyll-binding proteins

The failure of corn primary leaves to acclimate to very low flux densities was also examined at the protein level. The three major Chl *a*-binding proteins (CP1, CPa-1 and CPa-2) and the two major Chl *a/b*-binding proteins (LHC I and LHC II) were assayed as apoproteins by electroblot radioimmune assay of leaf extracts. Fig. 3 shows typical autoradiograms obtained for each antibody-antigen complex examined in this study. In addition, CP1 was assayed by redox difference spectrophotometry as P-700. Multiple protein species were detected for LHC I and LHC II, corresponding to the several electrophoretically distinct members of these protein families. The two forms of apo-CP1 [29] were not separated sufficiently under these conditions of electrophoresis to be distinguished by autoradiography. CPa-2 and protochlorophyllide reductase gave single bands, as expected. All of the above proteins were detected with monospecific polyclonal antibodies. Apo-CPa-1 was detected with a monoclonal antibody. As we show elsewhere (Sieburth, L.E., Lewis, A. and Bennett, J., unpublished results), the multiple bands detected with this antibody (Fig. 3) were due to incomplete denaturation of apo-CPa-1, not to cross-reactivity with other proteins such as apo-CPa-2. Similar electrophoretic behavior has been reported for tobacco CPa-1 [30].

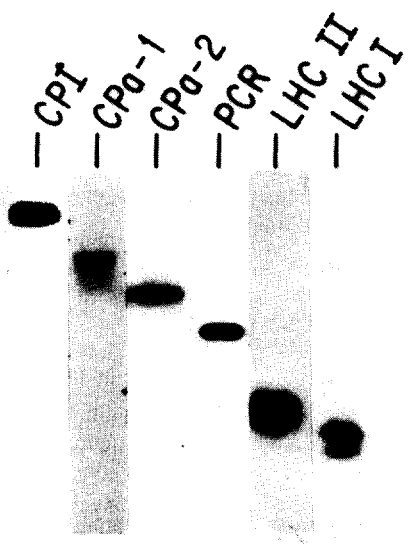

Fig. 3. Electroblot radioimmune assays of six chloroplast proteins in protein extracts of corn leaves. Autoradiogram shows location of antigens as revealed by successive binding of primary antibody, secondary antibody (CPa-1 only) and ^{125}I-protein A. PCR, protochlorophyllide reductase.

The concentrations of the five major Chl-binding proteins at the various flux densities are reported in Fig. 2. In general, they decreased with decreasing flux density. However, three features of the individual patterns are noteworthy: (i) betwen 500 and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, apo-LHC II increased slightly in concentration (by about 4%), while the other Chl-binding proteins decreased in concentration, (ii) like Chl *b*, both apo-LHC I and apo-LHC II were virtually absent from leaves grown at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (less than 2% of maximum, which is our limit of detection), and (iii) apo-CP1 was the only Chl-binding protein detected in dark-grown corn leaves (3% of maximum). No P-700 was detected in dark-grown leaves. In general, the accumulation of Chl *a/b*-binding proteins ran parallel to that of Chl *b* and the accumulation of Chl *a*-binding proteins ran parallel to that of Chl *a*.

Rate of protochlorophyllide phototransformation

The step that is most likely to limit the rate of Chl biosynthesis at low irradiance is the phototransformation of protochlorophyllide to chlorophyllide, catalyzed by protochlorophyllide reductase [12]. To determine the effect of irradiance on the rate of phototransformation in vivo, we exposed 8-day-old dark-grown leaves to flux densities of 1–500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for up to 5 min, extracted leaf pigments with acetone, and assayed protochlorophyllide and chlorophyllide spectrophotometrically. The rate of conversion has been shown to be first-order with respect to protochlorophyllide concentration [31], but the time courses were sufficiently close to linear over the first 30–40% of the progress curves that we were able in most cases to calculate an initial velocity (V_0) of phototransformation. Fig. 4 shows V_0 determined at 0, 1, 10, 100 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. (At 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, transformation was too rapid for us to measure V_0 accurately by this

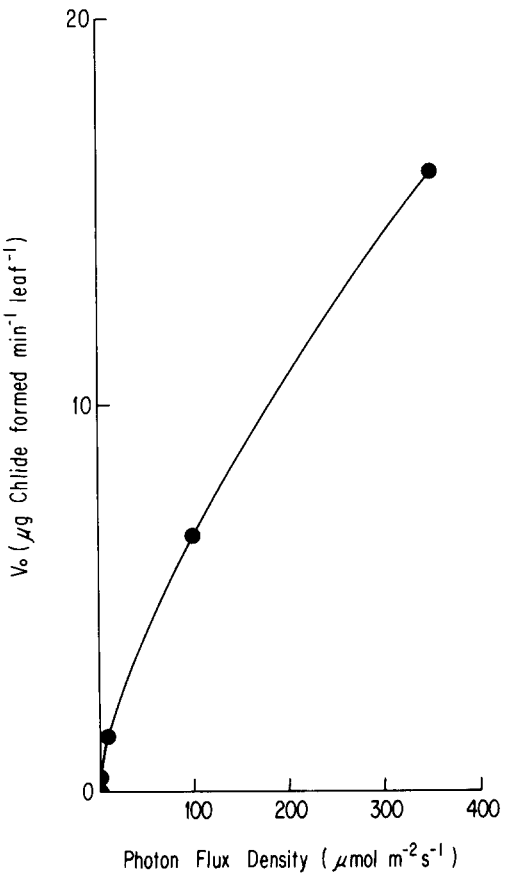

Fig. 4. Initial velocity (V_0) of protochlorophyllide phototransformation in vivo in dark-grown corn seedlings as a function of irradiance.

method.) V_0 increased by (3–4)-fold for every 10-fold increase in flux density, without reaching saturation. By extrapolation, we estimate that phototransformation was at least 50-times faster at 500 μmol · m^{-2} · s^{-1} than at 1 μmol · m^{-2} · s^{-1}. Thus, at low irradiance, the photon requirement for protochlorophyllide reductase function must be a major factor in limiting Chl synthesis in corn.

Protochlorophyllide reductase content

Although phototransformation in dark-grown leaves was 50-times faster after brief exposure to 500 μmol · m^{-2} · s^{-1} than after brief exposure to 1 μmol · m^{-2} · s^{-1}, the observed rates of Chl synthesis during growth at these two flux densities differed by only 10-fold (Fig. 1). This implied that some other factor was at work to enhance Chl synthesis at low irradiance.

One possible factor was the protochlorophyllide reductase content. It has been shown that light can affect the protochlorophyllide reductase content of leaves in two ways: by phytochrome-mediated promotion of the breakdown of its mRNA [16,17] and by stimulating proteolysis of the enzyme through consumption of protective substrates [18,19]. The enzyme was found, by SDS-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue, to be one of the most abundant membrane-bound proteins in primary leaves of dark-grown maize, but its concentration declined sharply with increasing flux density (data not shown). To assay the protein in leaves grown at high irradiance we used electroblot radioimmune assay (Fig. 5). Plants grown for 8 days at 1 μmol · m^{-2} · s^{-1} contained 13–15 times as much reductase as plants grown at 100–500 μmol · m^{-2} · s^{-1}, and dark-grown plants contained even more of the enzyme. Moreover, the reductase content of leaves grown in darkness or at 1 μmol · m^{-2} · s^{-1} increased between the 4th and 8th day after sowing, establishing that these leaves contained functional mRNA for this pro-

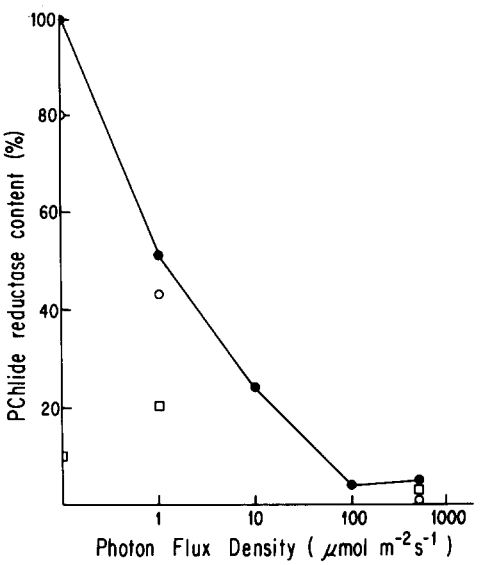

Fig. 5. Protochlorophyllide (PChlide) reductase content of primary leaves of corn as a function of age and growth irradiance. Measured by radioimmune assay and expressed as percentage of 8-day-old dark-grown control. Open squares: 4 days old, open circles: 6 days old, closed circles: 8 days old.

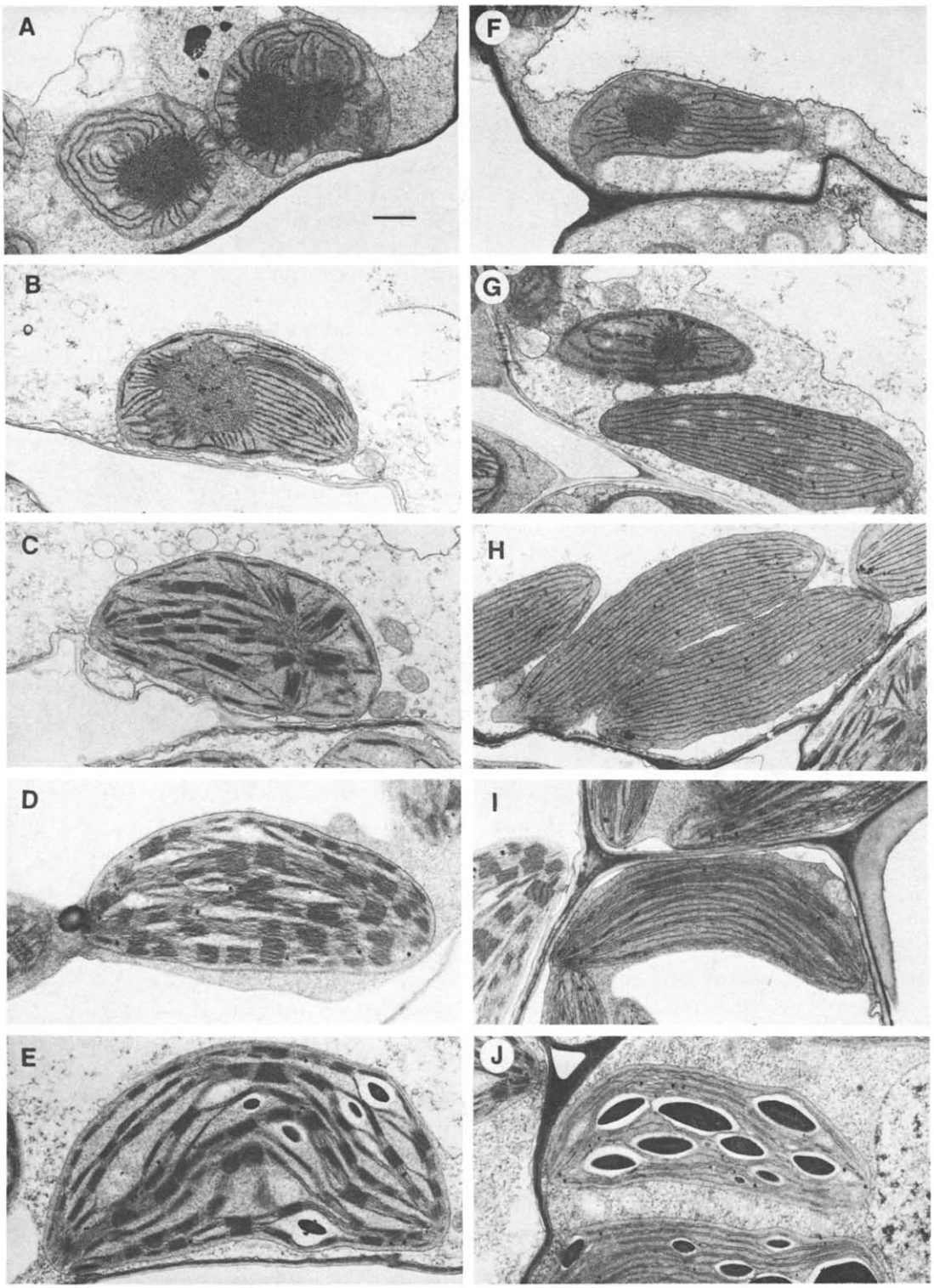

Fig. 6. Mesophyll plastids (A–E) and bundle sheath plastids (F–J) in primary leaves of 8-day-old corn grown in darkness (A, F) or at the following photon flux densities ($\mu mol \cdot m^{-2} \cdot s^{-1}$): 1 (B, G), 10 (C, H), 100 (D, I) and 500 (E, J). The bar corresponds to 1 μm.

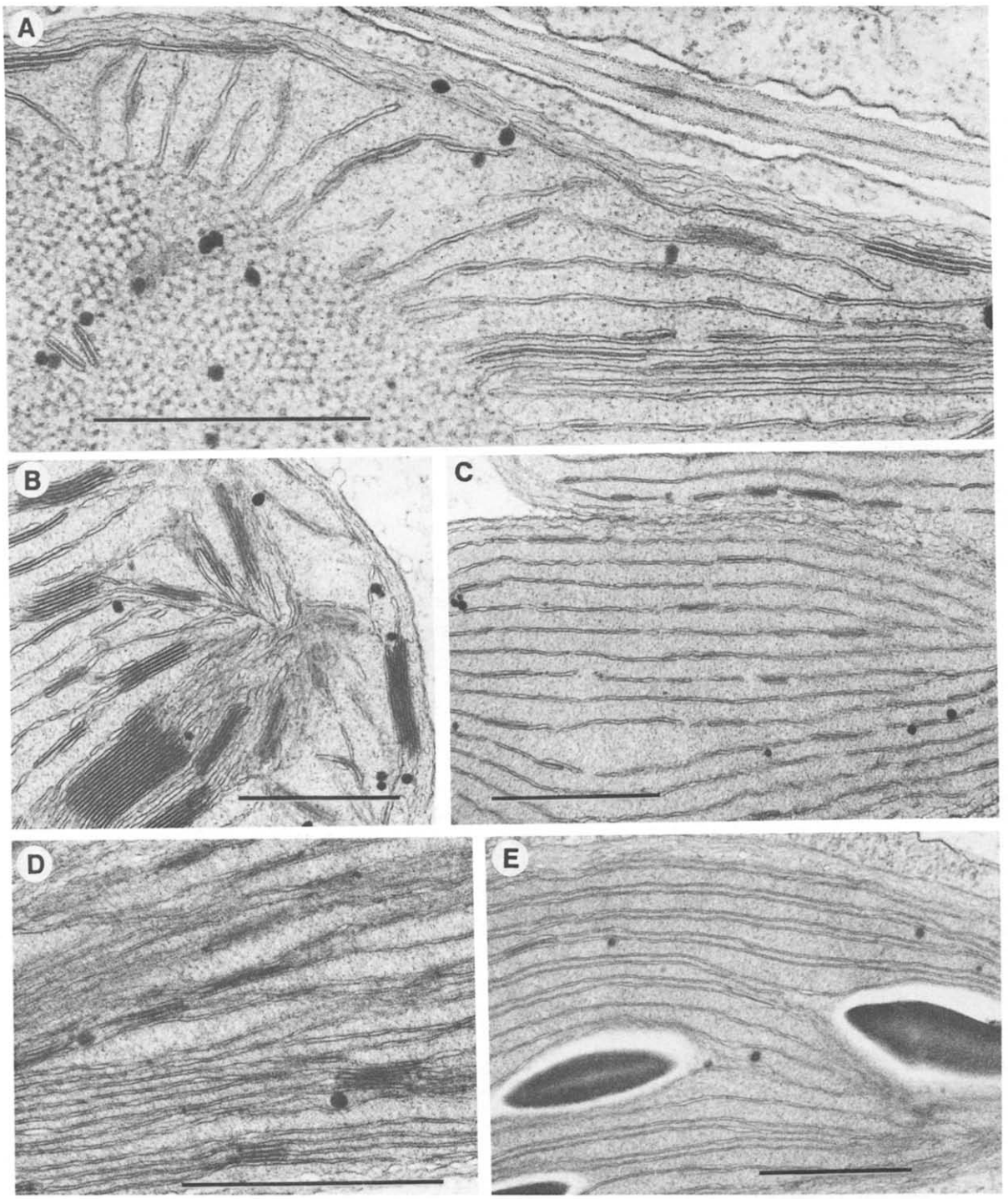

Fig. 7. Details of plastids in 8-day-old corn leaves (grown under the indicated photon flux densities, $\mu mol \cdot m^{-2} \cdot s^{-1}$). A, mesophyll plastid (1). B, mesophyll plastid (10). C, bundle sheath plastid (10). D, bundle sheath plastid (100). E, bundle sheath plastid (500). The bar corresponds to 1 μm.

tein. Thus, a flux density of 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ does not induce total destruction of this mRNA, even though, as we show elsewhere (Sutton, A., Sieburth, L.E. and Bennett, J., unpublished results), it is adequate to induce high levels of the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, another phytochrome-mediated response in corn [32,33].

Prolamellar bodies and grana

In dark-grown plants both protochlorophyllide and its reductase accumulate in the prolamellar bodies [34–36]. These structures tend to disintegrate rapidly when dark-grown plants are illuminated, disintegration being correlated with esterification of chlorophyllide rather than with phototransformation itself [37]. Since plants grown at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ contain about 50% as much protochlorophyllide reductase as dark-grown plants, they might also contain a significant population of prolamellar bodies.

We used transmission electron microscopy to test this prediction. Fig. 6 shows the overall ultrastructural appearance of mesophyll and bundle sheath plastids in 8-day-old corn leaves grown in darkness or at 1, 10, 100 and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plastids increase in size significantly over this range of irradiance. Prolamellar bodies are indeed prominent in plants grown in darkness or at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The prolamellar bodies of dark-grown plants tend to be larger and more regular in their *para*crystalline organization and more likely to be seen in any given plastid section than prolamellar bodies of plants grown at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This result is in agreement with the higher protochlorophyllide reductase content of dark-grown plants (Fig. 5). There is also a significant level of reductase in leaves grown at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but we have not been able to locate a single, well-defined prolamellar body in sections of these leaves. However, poorly defined remnants of such bodies are found in mesophyll plastids at this irradiance (Figs. 6C and 7B). Membranes lie along radii from the prolamellar body remnants much as they do from the well-defined prolamellar bodies found in plants grown at lower irradiance or in darkness, and the thylakoids are found parallel to the long axis of the plastid at distances from the prolamellar body remnants. There are no detectable remnants of prolamellar bodies in plastids grown at the higher levels of 100–500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the thylakoids are organized to run roughly parallel to the long axis of the plastid. Thus, a close correlation exists at all irradiances between the biochemical data (protochlorophyllide reductase content) and ultrastructure (occurrence of prolamellar bodies).

Close agreement between biochemistry and ultrastructure is also seen for LHC II content and the occurrence of grana in mesophyll plastids. Like LHC II (Fig. 2), grana are most conspicuous in leaves grown at 100 $\mu mol \cdot m^{-1} \cdot s^{-1}$ (Fig. 6D). Grana are also abundant at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ but contain fewer thylakoids per granum and more prominent stromal lamellae. A reduced content of grana is seen at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$, when LHC II content is 22% of maximum. At 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, very little Chl *b* and essentially no LHC II are found; grana are also absent but membrane appression is observed on a limited scale (Fig. 7A). In contrast, membrane appression is not a conspicuous feature of bundle sheath plastids at any irradiance (Figs. 6 and 7C–E) but thylakoids become more abundant with increasing flux density and show incipient grana formation at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 7D); finally, a distinctive form of thylakoid membrane pairing without appression is observed at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in bundle sheath chloroplasts (Fig. 7E).

Starch accumulated in the plastids of both cell types at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 6). It has been reported that 95% of the activity of the starch synthesizing enzymes is found in bundle sheath plastids and only 5% in mesophyll cell plastids [38]. Our results suggest that this distribution may underestimate the contribution of mesophyll plastids to starch synthesis.

Discussion

Differential accumulation of Chl-protein complexes at different irradiances

Corn plants are able to acclimate successfully to different flux densities within the range of 50–1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [39]. In the present study, corn plants were grown at 1, 10, 100 and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to determine the lower limits for acclimation. After 8 days of growth, leaves

were found to differ in Chl *a* content by as much as 10-fold. Similar differences were found by radioimmune assay for CP1 of PS I core and CPa-1 and CPa-2 of PS II core. The CP1/CPa-1 ratio was essentially invariant with respect to irradiance, as reported previously for the PS I/PS II ratio of pea plants exposed to a narrower range of irradiances [9–11].

An unexpected feature of leaves exposed to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was their high LHC I content. This result is difficult to understand if LHC I is regarded simply as a light-harvesting complex, which should be of more use at low light than at high light. However, Haworth et al. [40] have suggested that LHC I may also have a role in energy dissipation when PS I traps are closed. As such, a high concentration of LHC I would not be out of place at high light.

The LHC I/CP1 and LHC II/CPa-1 ratios were maximal at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Below this flux density the strategy of maximizing light-harvesting per reaction center in response to declining irradiance began to fail, until, at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, LHC I and LHC II were undetectable by our sensitive radioimmune assays. Thus, corn LHC I and LHC II resemble pea LHC II [14] in the minimal flux density required for accumulation.

Leong and Anderson [10,11] studied the development of pea leaves as a function of irradiance, but did not report a failure of the acclimation process. They used a different white light source (a combination of fluorescent and tungsten) and did not use a white light flux density below 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. They did use red light of very low flux density but, since red light is the most effective waveband for promoting Chl biosynthesis [41], it is equivalent to using a white light source 5–10 times more intense.

Under natural conditions, flux densities below 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ are encountered at dawn and dusk. Our data indicate that at these times Chl biosynthesis will be severely limited, and plants will accumulate mainly PS I and PS II cores. We assume that the cores become surrounded later by LHC I and LHC II, when higher irradiances allow synthesis of sufficient Chl *a* and Chl *b* to stabilize the peripheral antenna proteins. A precedent for such a two-stage assembly of photosystems in higher plants is seen for growth under light-dark cycles (e.g., 2 min of white light every 100–120 min). The PS I and PS II reaction centers formed during exposure to light-dark cycles become surrounded by light-harvesting complexes when plants are transferred to continuous illumination [42–44]. Under intermittent illumination, the apoproteins of LHC I and LHC II are synthesized but are unstable in the absence of adequate levels of Chl *a* and Chl *b* (Refs. 45 and 46; and see also Williams, R.S. unpublished results).

Intermittent light [42–44] and very low light (Fig. 2) both inhibit Chl *b* synthesis more than Chl *a* synthesis. It would appear that the threshold level of free Chl *a* required for Chl *b* synthesis [13] cannot accumulate at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Part of the reason must lie in the slow rate of Chl *a* synthesis at such a low flux density (see next section), but part may also lie with the tendency of the three Chl *a*-binding proteins to bind Chl *a* as rapidly as it is made. Fig. 2 shows clearly that formation of CP1, CPa-1 and CPa-2 is severely limited by irradiance. We are accustomed to thinking of the levels of LHC I and LHC II being determined in part by the stabilizing effect of bound Chl *a* and Chl *b* [4,14,45,46]; perhaps the 10-fold change in CP1, CPa-1 and CPa-2 levels shown in Fig. 2 reflects a similar stabilizing effect on these proteins by Chl *a* alone.

Activity and abundance of protochlorophyllide reductase

We examined protochlorophyllide phototransformation in dark-grown corn leaves to estimate the rate of protochlorophyllide reductase at different flux densities. The initial rate (V_0) was about 50-times faster at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a ratio that was more than adequate to account for the 10 : 1 ratio in the maximal rates of Chl synthesis observed during leaf development at these irradiances. Plants grown at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ appeared to phototransform protochlorophyllide more efficiently at very low irradiance than high light plants did at high irradiance, for at least two reasons. First, electroblot radioimmune assays established that leaves grown at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ accumulated about 15-fold more protochlorophyllide reductase than high-light leaves. Since reductase-bound protochlorophyllide

is the only form of protochlorophyllide that is immediately phototransformable [12,18], a higher enzyme concentration should give rise to a higher steady-state level of active protochlorophyllide and thus a higher rate of phototransformation. Second, electron microscopy revealed that these leaves also contained numerous prolamellar bodies, which are the site of accumulation of both protochlorophyllide and its reductase [34–36]. Prolamellar bodies presumably facilitate enzyme–substrate interaction and may further enhance the phototransformation rate.

Santel and Apel [18] have drawn attention to the very marked decline in protochlorophyllide reductase concentration which occurs when dark-grown plants are illuminated. They have suggested that the decline must seriously impair the ability of the enzyme to contribute to Chl synthesis during greening. However, as Griffiths, Kloppstech and their coworkers have pointed out [27,47], protochlorophyllide reductase does not disappear completely from illuminated leaves and the activity that remains is more than adequate to support the observed level of Chl synthesis.

Our data show that the argument of Griffiths is also applicable to maize. If the amount of protochlorophyllide reductase found in 8-day-old dark-grown maize leaves were present also in leaves grown at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the maximum potential rate of phototransformation would exceed 16 μg protochlorophyllide per min per leaf (Fig. 4), or 23 mg protochlorophyllide per leaf per day. This rate is more than 200-fold greater than the maximum rate of Chl synthesis observed in leaves grown at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (86 μg Chl per leaf per day, Fig. 1). Such a rate of Chl synthesis is probably obtainable only in leaves containing a pool of protochlorophyllide and then for no more than about 5 s. Once the pool of protochlorophyllide is consumed, reductase destruction would begin [18,19] and would probably not cease until the rate of protochlorophyllide reduction matched the rate of protochlorophyllide synthesis. This is undoubtedly the reason why the protochlorophyllide reductase content of high-light leaves 5–6 days after sowing (when Chl synthesis is maximal) is about 2% of the reductase content of 8-day-old dark-grown plants (Fig. 5); a higher level of enzyme would be both superfluous and unstable. The argument suggests that the rate-limiting step in Chl synthesis at high light is protochlorophyllide synthesis, which is in turn limited by δ-aminolevulinate synthesis [48,49].

The situation is different at very low irradiance. As mentioned above, a flux density of 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is adequate to stimulate the phytochrome-mediated accumulation of Rubisco small subunits in maize primary leaves. This irradiance should also activate phytochrome-mediated destruction of reductase mRNA [16,17]. However, the fact that the protochlorophyllide reductase content of leaves exposed to 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increases with time (Fig. 5) shows that at least some mRNA remains. It implies, moreover, that there is sufficient protochlorophyllide and NADPH to stabilize the new reductase molecules. At this point, irradiance itself must be limiting, in agreement with the 50-fold decline in phototransformation rate seen when dark-grown leaves are illuminated at 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ rather than 500 $\mu mol \cdot m^{-1} \cdot s^{-1}$ (Fig. 4).

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