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Response of Soybean Photosynthesis and Chloroplast Membrane Function to Canopy Development and Mutual Shading

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ABSTRACT

The effect of natural shading on photosynthetic capacity and chloroplast thylakoid membrane function was examined in soybean (*Glycine max.* cv Young) under field conditions using a randomized complete block design. Seedlings were thinned to 15 plants per square meter at 20 days after planting. Leaves destined to function in the shaded regions of the canopy were tagged during early expansion at 40 days after planting. To investigate the response of shaded leaves to an increase in available light, plants were removed from certain plots at 29 or 37 days after tagging to reduce the population from 15 to three plants per square meter and alter the irradiance and spectral quality of light. During the transition from a sun to a shade environment, maximum photosynthesis and chloroplast electron transport of control leaves decreased by two- to threefold over a period of 40 days followed by rapid senescence and abscission. Senescence and abscission of tagged leaves were delayed by more than 4 weeks in plots where plant populations were reduced to three plants per square meter. Maximum photosynthesis and chloroplast electron transport activity were stabilized or elevated in response to increased light when plant populations were reduced from 15 to three plants per square meter. Several chloroplast thylakoid membrane components were affected by light environment. Cytochrome *f* and coupling factor protein decreased by 40% and 80%, respectively, as control leaves became shaded and then increased when shaded leaves acclimated to high light. The concentrations of photosystem I (PSI) and photosystem II (PSII) reaction centers were not affected by light environment or leaf age in field grown plants, resulting in a constant PSII/PSI ratio of 1.6 ± 0.3. Analysis of the chlorophyll-protein composition revealed a shift in chlorophyll from PSI to PSII as leaves became shaded and a reversal of this process when shaded leaves were provided with increased light. These results were in contrast to those of soybeans grown in a growth chamber where the PSII/PSI ratio as well as cytochrome *f* and coupling factor protein levels were dependent on growth irradiance. To summarize, light environment regulated both the photosynthetic characteristics and the timing of senescence in soybean leaves grown under field conditions.

Both light quantity and light quality regulate the photosynthetic properties of higher plants by controlling the activity and the composition of the photosynthetic apparatus (2, 5, 29). Thus, light environment plays a critical role during leaf expansion in determining the photosynthetic properties of the mature leaf. More recently, controlled environment studies with a number of species have shown that fully expanded leaves retain the capacity to 'fine-tune' photosynthesis in response to changes in growth irradiance (8, 12, 14, 15, 33). This study was conducted to determine whether light acclimation is a significant factor under field conditions in leaves that become shaded during canopy development. The first objective was to identify changes in photosynthetic activity and chloroplast membrane composition in fully expanded soybean leaves during the transition from a sun to a shade environment as the upper canopy developed. The second objective was to identify changes in photosynthesis of a shaded leaf during acclimation to an increase in available light induced by removal of adjacent plants.

MATERIALS AND METHODS

Plant Growth and Harvesting

Soybean, *Glycine max* cv Young was planted on June 1, 1989 in a randomized complete block with four replications. Plots consisted of six rows with a row length of 6 m and a row spacing of 1 m. Seedlings were thinned to 15 plants m^{-2} at 20 d after planting. Leaves (5th or 6th trifoliolate nodes) in early expansion at 40 d after planting were tagged. Control plots were maintained at a plant population of 15 plants m^{-2}. Plants were removed at either 29 or 37 DAT[2] to produce treatments of three plants m^{-2} and are referred to as the 15 → 3 plants m^{-2} plots in each figure. On each harvest date, tagged leaves were selected at random from each plot.

For growth chamber studies, soybeans (cv Young) were grown in 6-inch pots of soil (eight seedlings/pot) with 16 h illumination/d and a day/night temperature of 26/22°C, respectively. Illumination conditions have been described in

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[2] Abbreviations: DAT, days after tagging; A_{max}, maximum leaf photosynthetic activity measured under light and CO_2 saturated conditions; DCIP, dichorophenolindophenol; P-700, reaction center of PSI; CF_1, chloroplast coupling factor; LHC, light harvesting complex.

detail elsewhere (15). Initially, seedlings were grown for 11 d under a light intensity of 400 μmol photons m^{-2} s^{-1} to produce plants with fully expanded unifoliolate leaves and the first trifoliolate leaf in the early stage of expansion. Half of the plants were then transferred to an irradiance of 40 μmol photons m^{-2} s^{-1} and the development of the first trifoliolate leaf continued under either high or low light intensity for an additional 8 d before harvest. Development of the first trifoliolate leaf was slow under the low light conditions and the resulting leaves were smaller and thinner than for leaves developed at higher irradiance.

Light Measurements

PPFD was measured with a 1-m line quantum sensor (model LI-191B; Li-Cor, Inc.) and a LI-1000 data logger. The sensor was placed at ground level, perpendicular to the row, with the center intersecting the row. Light penetration was determined as the radiant energy reaching the plant base divided by a paired measurement taken above the plant canopy and expressed as a percentage. Three paired measurements (above and below the canopy) were made within each plot on each measurement date.

Light quality was measured as spectral scans utilizing a Li-Cor, Inc. model LI-1800 spectroradiometer equipped with a remote cosine receptor located at the plant base. The generated values represent the mean of four replications taken at 10-nm intervals.

Thylakoid Membrane Isolation

For each membrane preparation, three to six tagged leaves from field plots or 10 to 20 g of leaves from growth chamber plants were combined and used to prepare thylakoid membranes on each harvest day. Leaf tissue was homogenized with two 10-s bursts of a Brinkman Polytron PT 10–35 in ice cold grind buffer containing 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 0.2% (w/v) BSA, 1.0% (w/v) PVP, 10 mM sodium ascorbate, and 50 mM Tricine-NaOH (pH 7.8). The homogenate was filtered through cheesecloth and a low speed pellet collected by centrifugation at 5000*g* for 5 min at 4°C. The initial low speed pellet was resuspended in grind buffer and layered on top of 0.6/1.5 M sucrose step gradients that consisted of 10 mM NaCl, 5 mM $MgCl_2$, 0.2% (w/v) BSA, and 50 mM Tricine-NaOH (pH 7.8) with the appropriate concentration of sucrose. Thylakoid membranes were separated from intact cells, starch grains, and other debris by centrifugation of the gradients at 10,000*g* for 10 min at 4°C. Thylakoid membranes were removed from the 0.6/1.5 M sucrose interface, diluted with resuspension buffer [0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 0.2% (w/v) BSA, and 50 mM Tricine-NaOH (pH 7.8)] and collected by centrifugation at 5000*g* for 5 min at 4°C. The final pellet was resuspended, stored on ice during electron transport measurements, and then frozen with liquid N_2 before long term storage at −80°C.

Activity Measurements

A_{max} was measured as the steady-state rate of O_2 evolution at 25°C using a Hansatech leaf disc O_2 electrode. O_2 evolution was measured in a closed cuvette in the presence of N_2 gas containing 3% (v/v) CO_2 and 17% (v/v) O_2. The rate for each replication was the mean of two linear increases measured for the same disc.

Uncoupled photosynthetic electron transport was assayed as DCIP reduction at 580 nm (E_{580} = 18.0 mM^{-1} cm^{-1}) with water as the electron donor (21). The assay contained 0.1 M sorbitol, 40 mM Tricine-NaOH (pH 8.0), 1 mM NH_4Cl, 30 μM DCIP, and 2 μg Chl/ml. Saturating actinic light was passed through a red filter (Corning 2–58) and the detector was protected with a blue filter (Corning 4–96).

Chl Determination

Chl content of fresh leaf discs was determined by extraction of pigments with dimethylformamide overnight in the dark at 4°C. The Chl concentration and Chl *a/b* ratio of dimethylformamide extracts of leaf tissue and thylakoid membranes were determined spectrophotometrically (27).

Analysis of Thylakoid Membrane Components

The concentration of PSII reaction centers was determined by measuring the specific binding of [^{14}C]atrazine to the high affinity site on thylakoid membranes (30). Cyt *f* content was determined from reduced (hydroquinone) minus oxidized (potassium ferricyanide) difference spectra using an extinction coefficient of 18 mM^{-1} cm^{-1} (20). The concentration of P-700 was determined from the reversible light-induced P-700 A_{697} change using an extinction coefficient of 64 mM^{-1} cm^{-1} (19). Details have been published elsewhere (14).

Chloroplast coupling factor protein was quantified from 10% polyacrylamide gels using the procedure of Laemmli (23) with lithium dodecylsulfate substituted for SDS to facilitate analysis at low temperature. Thylakoid membranes were solubilized at 4°C and electrophoresis was conducted at 8°C to resolve the α- and β-subunits of CF_1 from other polypeptides. The samples were loaded on a Chl basis (10 μg/lane). Purified CF_1 (2 μg protein) was analyzed in one lane on each gel to provide an internal standard. Gels were stained with Coomassie blue and destained before analysis with an LKB laser densitometer. Peak areas for the α- and β-subunits were combined and used as a relative measure of CF_1.

Chl-protein composition was analyzed by mild SDS-PAGE 'green' gels as described previously (15) using the basic procedure of Anderson *et al.* (1).

RESULTS

Light Environment Associated with Canopy Shading

For a soybean leaf destined to function in the lower region of the canopy, the light environment changed dramtically over time. To monitor leaves of the same age throughout the growing season, a population of expanding leaves (5th or 6th trifoliolate) was tagged during early canopy development and analyzed throughout the season. Within 9 DAT, leaf Chl accumulation was complete (Fig. 1). At this time, the fully expanded leaves were functioning in full sun. As the canopy developed, the tagged leaves became shaded. Irradiance in the control plots, measured as PPFD at the plant base, was 824,

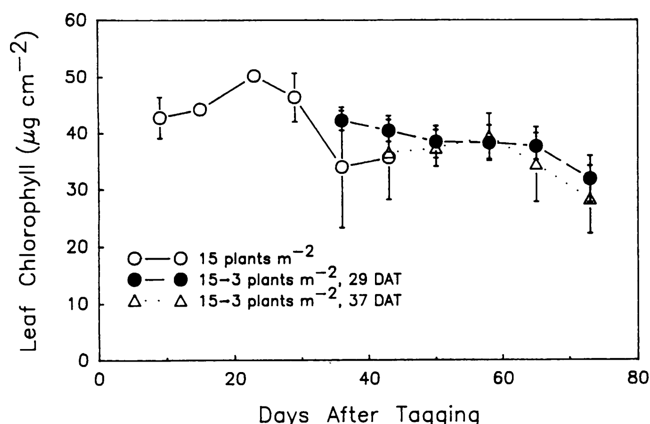

Figure 1. Leaf Chl content. Leaf Chl content was measured in tagged leaves for both control and plant removal treatments throughout the season. Procedures are described in "Materials and Methods." Each point represents the average ± SD of leaves harvested from each of four replicated plots. No tagged leaves remained on the control plants after 55 DAT.

371, and 129 μmol photons m^{-2} s^{-1} at 9, 24, and 37 DAT. These values represented a light penetration of 51, 24, and 7% of available light, respectively. Plant populations were reduced from 15 to three plants m^{-2} in plots at either 29 or 37 DAT to increase the light available to the tagged leaves on the remaining plants. Figure 2 shows the contrasting light environments associated with the plant removal treatments at 44 DAT. The shaded 'control' environment of 15 plants m^{-2} was characterized by a large reduction in total irradiance throughout the visible region and an elevated far-red/red ratio relative to the treatments in which plant populations were reduced from 15 to three plants m^{-2}. Thus, leaves in the shaded regions of the canopy function in an environment of decreased irradiance and altered light quality relative to sun leaves.

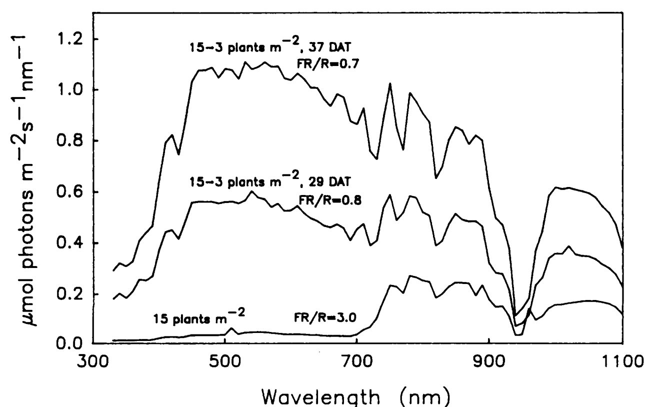

Figure 2. Characterization of the light environment. The spectral scans were acquired at 44 DAT as described in "Materials and Methods." The scans are mean values (n = 4) taken at 10-nm intervals. The far-red/red (FR/R) ratio was calculated from the irradiance level at 730 nm and 640 nm, respectively, to compensate for the Chl induced shift in the phytochrome action spectrum (22).

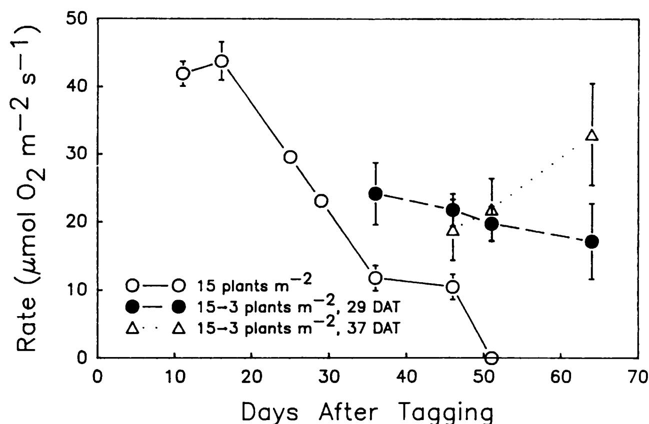

Figure 3. Leaf photosynthesis. Maximum photosynthetic rates were measured as described in "Materials and Methods" using a leaf disc electrode system. Each point represents the average ± SD of one leaf harvested from each of four replicated plots.

Effects of Canopy Shading on Photosynthetic Activities and Leaf Senescence

Both A_{max} (Fig. 3) and electron transport activity of isolated thylakoid membranes (Fig. 4) declined over time as the tagged leaves aged and became shaded. The decline in activity preceded significant Chl loss (Fig. 1). At 35 DAT, Chl loss in shaded control leaves began. The final stages of senescence appeared to be rapid so that the weekly harvests conducted during this study did not provide for a precise kinetic characterization of Chl loss in shaded control leaves. From 35 to 50 DAT, a decrease in leaf Chl and an increase in variability of the data (Fig. 1) were indicators of senescence. Visual inspection of the shaded control leaves revealed that senescence and abscission occurred in a large fraction of the population on a weekly basis during this critical period. No tagged leaves remained on the control plants at 55 DAT.

The timing of senescence was distinctly different for leaves in plots where plant density was reduced from 15 to three

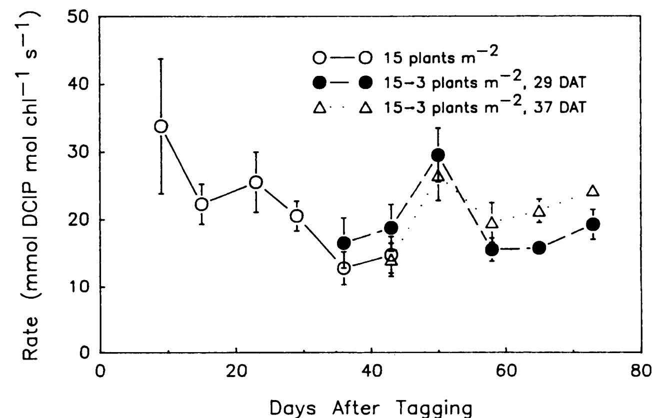

Figure 4. Uncoupled photosynthetic electron transport. Photosynthetic electron transport activity was measured as described in "Materials and Methods." Each point represents the average ± SD activity of four thylakoid membrane preparations, one from each of four replicated plots.

plants m^{-2} at either 29 or 37 DAT. Both leaf photosynthesis (Fig. 3) and chloroplast electron transport activity (Fig. 4) were stabilized or elevated relative to the control rate present at the time removal treatments were imposed. Activities remained high for several weeks beyond the time when abscission of all similar leaves had occurred in control plots. Leaf Chl loss was also delayed by several weeks until the onset of monocarpic senescence (Fig. 1).

Effects on Thylakoid Membrane Components Associated with Electron Transport and Photophosphorylation

In field plants, the concentrations of PSI (Fig. 5A) and PSII (Fig. 5B) reaction centers were not affected significantly by changes in light environments within the canopy or by leaf age. The combination of data from Figure 5, A and B, resulted in a calculated PSII/PSI ratio of 1.6 ± 0.3. In contrast, the level of PSII reaction centers was dependent on light intensity for growth chamber plants grown under constant irradiance (Table I). Plants grown under 400 μmol photons m^{-2} s^{-1}

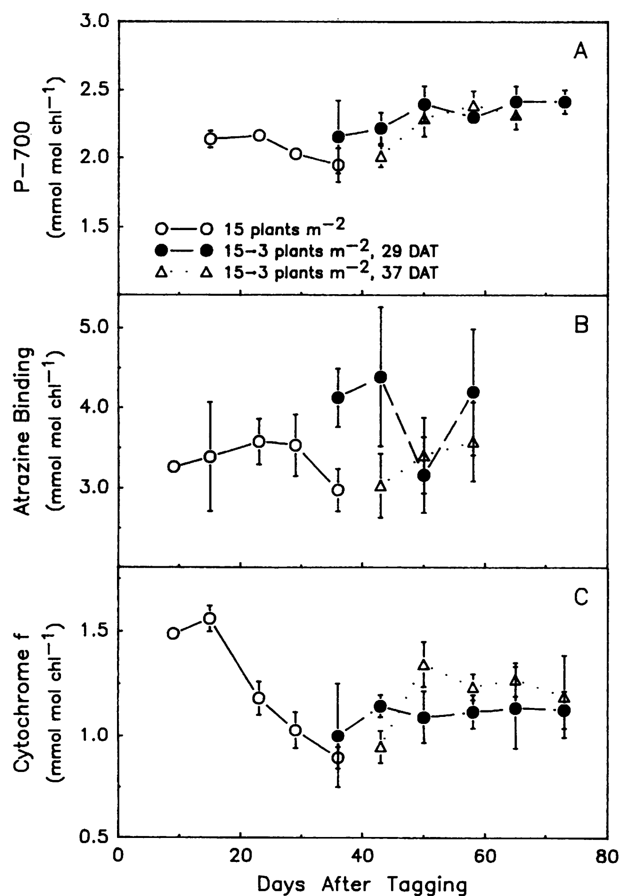

Figure 5. Photosynthetic electron transport components. A, The concentration of P-700 was used as a measure of the number of PSI reaction centers present in the membranes. B, Stoichiometric binding of atrazine to PSII was used as a measure of the number of PSII reaction centers. C, Cyt *f* was measured by reduced minus oxidized difference spectroscopy. Each point represents the average ± SD of four thylakoid membrane preparations, one from each of four replicated plots.

Table I. *Comparison of Soybeans Grown under Moderate or Low Irradiance in a Growth Chamber*

Values are the average ± SD of three independent thylakoid membrane preparations from 8 d old first trifoliolate leaves.

	Growth Irradiance	
	400 μmol photons m^{-2} s^{-1}	40 μmol photons m^{-2} s^{-1}
Leaf Chl content (μg cm^{-2})	47 ± 5	18 ± 3
Chl *a*/*b* ratio	4.14 ± 0.14	3.51 ± 0.19
Uncoupled electron transport (mmol DCIP mol Chl^{-1} s^{-1})	26.5 ± 1.5	22.0 ± 0.9
Atrazine binding sites (mmol mol Chl^{-1})	3.9 ± 1.0	1.9 ± 0.2
P-700 (mmol mol Chl^{-1})	1.7 ± 0.1	2.1 ± 0.2
PSII/PSI	2.3 ± 0.5	0.9 ± 0.1
Cyt *f* (mmol mg Chl^{-1})	1.5 ± 0.1	1.0 ± 0.2
Relative CF_1 protein content	1.00	0.61 ± 0.07

contained 3.9 mmol atrazine binding sites/mol Chl, a value similar to that of field grown plants. In contrast, plants grown under an irradiance of 40 μmol photons m^{-2} s^{-1} contained approximately half the number of atrazine binding sites observed under moderate irradiance conditions. Because the P-700 was not affected by irradiance (Table I), the PSII/PSI ratio of moderate irradiance plants was a factor of 2 greater than that of low irradiance plants (Table I).

Cyt *f* was observed to be a dynamic component of the thylakoid membrane. In field grown plants, Cyt *f* decreased by approximately 40% as the tagged leaves became shaded in the 15 plants m^{-2} plots (Fig. 5C). During acclimation of shaded leaves to increased light, Cyt *f* levels increased by 10 to 40% in the tagged leaves of plots where the plant population was reduced from 15 to three plants m^{-2} (Fig. 5C). Plants grown under a constant irradiance of 400 μmol photons m^{-2} s^{-1} in the growth chamber contained approximately 40% more Cyt *f* than plants grown under 40 μmol photons m^{-2} s^{-1} (Table I).

CF_1 was also a dynamic component within the thylakoid membrane. The gel in Figure 6 shows a large decrease in the α- and β-subunits of CF_1 of control plants from 9 to 36 DAT. CF_1 levels increased by 50 DAT in plots where the plant population was reduced at 37 DAT (Fig. 6). Densitometry was used to quantitate CF_1 protein levels in Coomassie stained gels loaded on an equal Chl basis, and the results are presented in Figure 7. CF_1 protein levels decreased by 80% relative to the maximum observed 9 DAT as the leaves became shaded and then increased after the plant removal treatments were imposed. An irradiance effect was also observed in the growth chamber studies. Plants grown under a constant irradiance of 40 μmol photons m^{-2} s^{-1} contained only 60% of the CF_1 protein found in plants grown under 400 μmol photons m^{-2} s^{-1} (Table I).

Effects on Chl Organization

Chl organization was altered in response to canopy development. The response was reflected in the Chl *a*/*b* ratio (Fig. 8). This ratio decreased as control leaves became shaded and

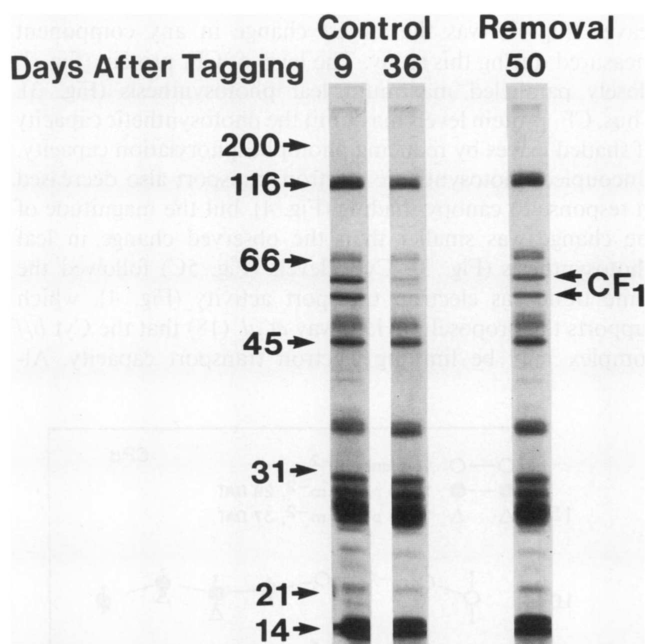

Figure 6. SDS-PAGE of chloroplast thylakoid membranes. Electrophoresis was conducted as described in "Materials and Methods" for CF_1 quantitation. Each lane contains thylakoid membranes equivalent to 10 μg of Chl. Molecular mass markers are given in kD.

then increased when plant populations were reduced. Because all Chl pigment is noncovalently attached to one of several polypeptides within the thylakoid membrane, the change in the Chl *a*/*b* ratio indicated a redistribution of Chl between the Chl-protein complexes.

To identify the specific complexes involved, soybean Chl-protein complexes were separated on mild SDS-PAGE 'green' gels. Eight Chl-containing bands were resolved (Fig. 9) and are labeled according to the nomenclature of Anderson *et al.* (1). CP1a and CP1 are PSI Chl-protein complexes. In soybean,

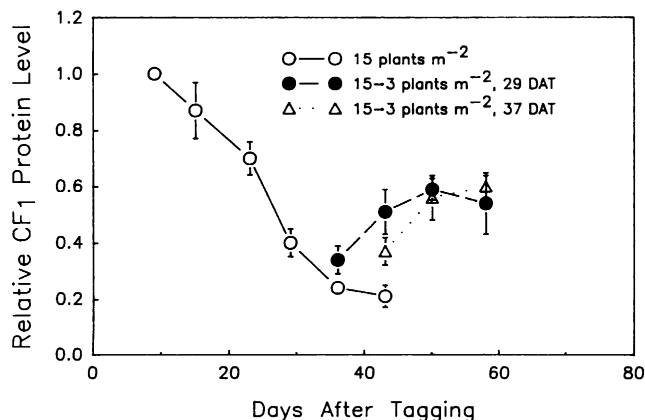

Figure 7. CF_1 protein content of thylakoid membranes. CF_1 protein levels were measured as described in "Materials and Methods." The data were normalized to the CF_1 level in control leaves functioning in full sun before canopy shading. Each point represents the average ± SD of four thylakoid membrane preparations, one from each of four replicated plots.

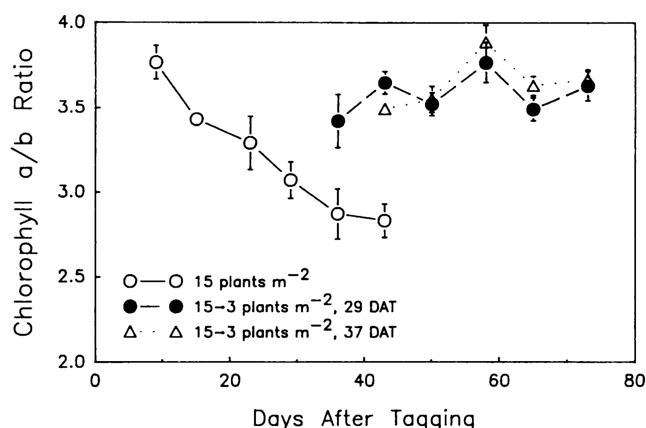

Figure 8. Chl *a*/*b* ratio. Chl *a*/*b* ratio was measured as described in "Materials and Methods." Each point represents the average ± SD of four independent determinations, one from each of four replicated plots.

CP1 migrated as two bands with similar absorbance spectra that showed no evidence of Chl *b* (data not shown). The absorbance spectrum of CP1a contained a small amount of Chl *b* (data not shown), an indication that CP1a consisted of CP1 associated with the LHC-I of PSI. CPa contains mainly PSII Chl *a* complexes, but may contain a variable amount of LHC-I under conditions that dissociate CP1a into CP1 and LHC-I components (26). $LHCP^1$, $LHCP^2$, and $LHCP^3$ are different molecular weight forms of LHC-II, the major light-harvesting Chl-protein complex of PSII. FP is free pigment dissociated from the complexes during the detergent solubilization.

Quantitation was performed by integration of gel scan peak areas. Free pigment levels averaged 7.3 ± 0.8% for the thylakoid membrane preparations analyzed during this study. As the tagged leaves became shaded, CP1a decreased and LHC-II increased in control plants with no effect on CP1 or CPa (Fig. 10). After plant populations were reduced, CP1a increased and there was a trend upward in CP1 while LHC-II decreased (Fig. 10).

DISCUSSION

Two types of senescence have been described for soybean under field conditions: progressive senescence of lower leaves before reproductive growth and monocarpic senescence of the remaining leaves during the pod fill phase of reproductive growth (28). Wells (32) showed that the loss of lower canopy leaves was not closely associated with light interception subsequent to canopy closure, an indication that the shaded leaves do not contribute significantly to whole plant photosynthesis. In this study, light environment was found to be a factor that controls the timing of progressive leaf senescence in soybean. For control leaves destined to function in the shaded regions of the canopy, A_{max} declined (Fig. 3) as the upper canopy developed, and this decline occurred before reduction of leaf Chl (Fig. 1). Beginning approximately 35 DAT, the Chl content of shaded leaves decreased, followed by a period of rapid senescence and abscission. Photosynthetic activity was stabilized or increased relative to shaded controls

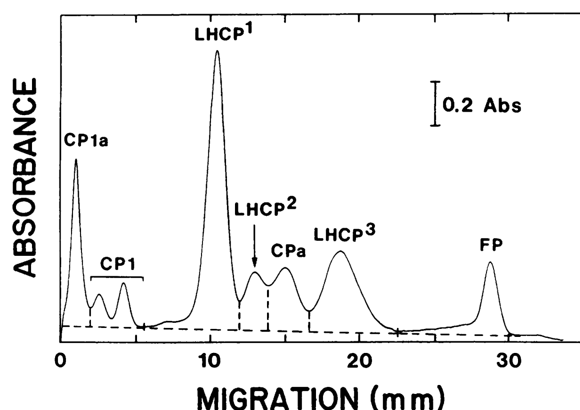

Figure 9. Gel scan of soybean Chl-protein complexes separated by mild SDS-PAGE. To include contributions from both Chl *a* (675 nm) and Chl *b* (650 nm), gels were scanned at 720, 675, and 650 nm. After computer subtraction of the 720 nm baseline, baseline corrected 675 nm and 650 nm scans were added and then divided by two to produce the gel scan shown. Individual Chl-containing bonds are defined in the text. Abs = absorbance

(Fig. 3) and Chl loss was delayed by several weeks (Fig. 1) when plant populations were reduced to increase available light within the canopy. Thus, manipulation of the light environment by alteration of plant density delayed progressive senescence of leaves within the lower canopy.

There is evidence that light can act to delay senescence (3). Although the mechanism for light regulation of senescence is not known, effects on photosynthesis (3) or the action of phytochrome on protein synthesis (3, 6) and hormone levels (3, 31) have been suggested. A role for phytochrome has been clearly demonstrated in mustard cotyledons (4) and barley leaf segments (11), in which dark-induced senescence was delayed by pulses of red light and reversed by far-red pulses. Phytochrome could be involved in the progressive senescence of shaded leaves in a natural canopy. A major difference between field conditions and the classical phytochrome experiment is that shaded leaves within the canopy are exposed to long periods of continuous low irradiance enriched in far-red light (see Fig. 2), not short pulses of red or far-red light. Under such steady state conditions, the rates of Pr synthesis and Pfr breakdown, not the Pr/Pfr photochemical equilibrium, would be the critical factors controlling a phytochrome response (17). Thus, a relationship may exist between the steady state level of phytochrome protein in the leaf and the timing of progressive leaf senescence in the shaded regions of the canopy.

A major objective of this study was to determine the significance of light acclimation in the field during canopy development. The steady state levels of specific thylakoid membrane-proteins are known to change in response to irradiance manipulations under growth chamber conditions. Experiments with barley (14), lettuce (13), mustard (33), pea (9, 16, 24), soybean (Table I), spinach (10), and tomato (12) have shown that levels of CF and Cyt *f* are dependent on growth irradiance. In results reported here, both coupling factor and Cyt *f* levels were affected by canopy shading under field conditions. The fourfold decrease in CF_1 protein in control leaves (Fig. 7) was the largest change in any component measured during this study. The level of CF_1 protein (Fig. 7) closely paralleled maximum leaf photosynthesis (Fig. 3). Thus, CF_1 protein levels may limit the photosynthetic capacity of shaded leaves by reducing photophosphorylation capacity. Uncoupled photosynthetic electron transport also decreased in response to canopy shading (Fig. 4), but the magnitude of the change was smaller than the observed change in leaf photosynthesis (Fig. 3). Cyt *f* levels (Fig. 5C) followed the same trends as electron transport activity (Fig. 4), which supports the proposal of Halloway *et al.* (18) that the Cyt *b/f* complex may be limiting electron transport capacity. Al-

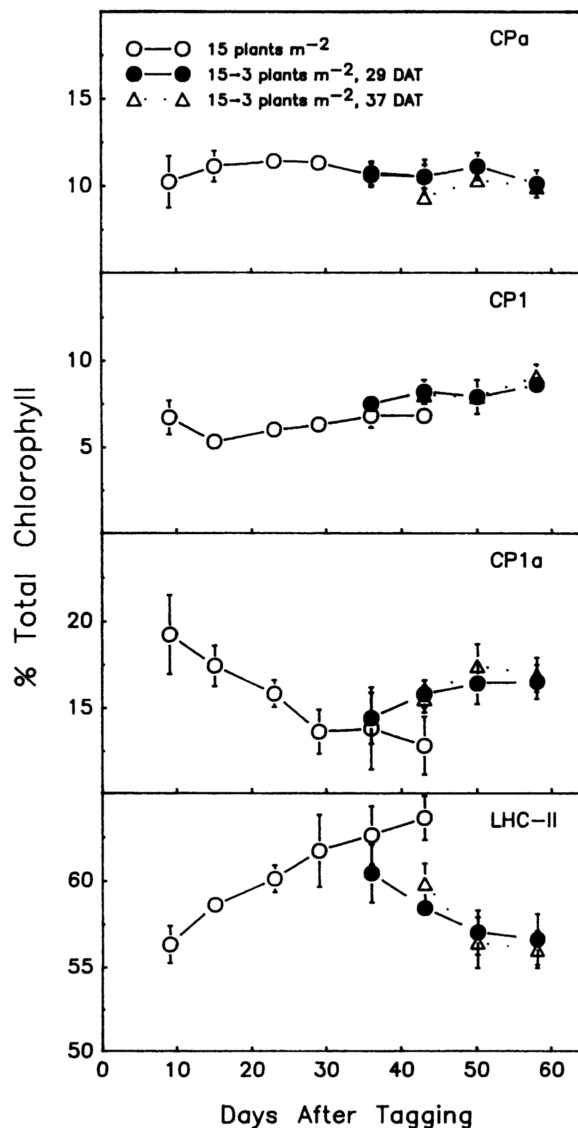

Figure 10. Distribution of Chl between Chl-protein complexes. Computer integration of peak areas was conducted to determine the percentage of total Chl associated with the CP1a, CP1, CPa, and LHC-II Chl-protein complexes. LHC-II represents the summation of areas for $LHCP^1$, $LHCP^2$, and $LHCP^3$. The solid and dashed lines shown in Figure 9 define the areas assigned to each complex. Each point represents the average ± SD of four thylakoid membrane preparations, one from each of four replicated plots.

though the values reported here are on a Chl basis, the interpretation would be similar on a leaf area basis because changes in photosynthetic activity, CF_1, and Cyt *f* preceded the loss of leaf Chl.

Light effects on the PSII/PSI ratio were different for growth chamber and field grown plants. The concentrations of PSI (Fig. 5A) and PSII (Fig. 5B) reaction centers were not significantly different over the wide range of light environments imposed by canopy development in the field, resulting in a constant PSII/PSI ratio of 1.6 ± 0.3. In contrast, a number of controlled environment studies have shown that the PSII/PSI ratio is dependent on irradiance (9, 10, 14, 16, 24, 33, and Table I) although the physiological significance has been questioned because the effects on PSII/PSI ratio are small for certain species (24). Both the field data (Fig. 5A) and the irradiance studies (9, 10, 14, 16, 24, 33, and Table I) confirm that light environment does not significantly affect the number of PSI reaction centers. The conflict concerns the concentration of PSII reaction centers. The level of PSII centers was found to be dependent on growth irradiance for a number of species (9, 10, 14, 16, 24, 33) including soybean (Table I). Yet canopy shading did not affect the number of PSII reaction centers (Fig. 5B). Light quality effects may explain the differences between irradiance studies and canopy shading. The higher far-red/red ratio associated with shade environments within the canopy (Fig. 2) will preferentially excite PSI. Plants grown under light that favors PSI have been shown to contain more PSII reaction centers to compensate for the illumination conditions (7). Therefore, during canopy development, the reduction in PSII reaction centers predicted for a low irradiance environment may be offset by light quality conditions that increase the number of PSII centers. The result of these two factors would be a PSII/PSI ratio that appears to be unaffected by light.

Although the PSII/PSI ratio was constant under field conditions, the distribution of Chl between the two reaction centers was found to be regulated by light environment. This was reflected in both the Chl *a/b* ratio (Fig. 8) and the Chl-protein composition (Fig. 10). As control leaves became shaded, a decreased Chl *a/b* ratio was associated with a decrease in the CP1a complex of PSI and an increase in the LHC-II of PSII. Because the number of P-700s did not change (Fig. 5A), the decrease in CP1a was probably the result of a reduction in the LHC-I that contributes Chl to the CP1a complex. The net effect of a decrease in LHC-I accompanied by an increase in LHC-II is to shift Chl from PSI to PSII in response to shade. Following a reduction in plant density, the increased available light caused a shift in Chl from PSII to PSI, effectively reversing the shade response. The results suggest that light environment within the canopy controls the distribution of Chl between PSI and PSII through the synthesis and breakdown of LHC-I and LHC-II. The kinetics of the response were slow (days), an indication that light acclimation of this type is a response to long term changes in light environment.

A comparison of canopy shading and irradiance studies revealed significant differences in light environment effects on Chl organization. The Chl *a/b* ratio is affected by both canopy shading (Fig. 8) and irradiance manipulations (15, 25). Generally, the Chl *a/b* ratio is higher for leaves exposed to full sun or high irradiance growth chamber conditions relative to shade or low irradiance acclimated leaves. However, the associated changes in Chl-protein composition are different for the two light environments. As discussed above, the combination of irradiance and light quality changes within plant canopies affects the distribution of Chl between PSI and PSII by altering LHC-I and LHC-II levels. In contrast, irradiance alone affects the distribution of Chl within PSII by altering the levels of CPa and LHC-II without changing the relative distribution of Chl between PSII and PSI (15, 25). Overall, these results support the concept that chloroplast thylakoids are a dynamic membrane system that responds to light environment.

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LITERATURE CITED

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