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Regulation of Photosynthetic Induction State by the Magnitude and Duration of Low Light Exposure[1]

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This study was undertaken to examine the dependence of the regulatory enzymes of photosynthetic induction on photon flux density (PFD) exposure in soybean (*Glycine max* L.). The induction state varies as a function of both the magnitude and duration of the PFD levels experienced prior to an increase in PFD. The photosynthetic induction state results from the combined activity of separate processes that each in turn depend on prior PFD environment in different ways. Direct measurement of enzyme activities coupled with determination of in situ metabolite pool sizes indicated that the fast-induction component was associated with the activation state of stromal fructose-1,6-bisphosphatase (FBPase, EC 3.1.3.11) and showed rapid deactivation in the dark and at low PFD. The fast-induction component was activated at low PFD levels, around 70 μmol photons m^{-2} s^{-1}. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 2.7.1.19) deactivated very slowly in the dark and required higher PFD for activation. Both enzymes saturated at lower PFD than did photosynthesis, around 400 μmol photons m^{-2} s^{-1}. Ribulose-5-phosphate kinase (EC 2.7.1.19) appeared never to be limiting to photosynthesis, and saturated at much lower PFD than either FBPase or Rubisco. Determination of photosynthetic metabolite pool sizes from leaves at different positions within a soybean canopy showed a limitation to carbon uptake at the stromal FBPase and possibly the sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37) in shade leaves upon initial illumination at saturating PFD levels.

Recent studies have explored limitations to optimal photosynthesis under rapidly varying light regimes common to canopy environments (Woodrow and Mott, 1989; Pearcy, 1990; Jackson et al., 1991). These studies have provided insight into the dynamic limitations to photosynthetic rate in crop canopies under natural light regimes. Photosynthesis is dependent on the incoming PFD not only for energy to drive carbon assimilation but also for activation of key enzymes of the CRC. The activation levels match the capacity of these metabolic steps to the overall rate of assimilation as determined by external environmental conditions. However, under transient light conditions, this light-activation requirement can temporarily inhibit CO_2 assimilation rate by limiting metabolic flux at a particular site in the CRC. This limitation of photosynthesis upon going from low to high PFD levels results in a lag in the rise of photosynthesis to the maximum light-saturated rate, termed the induction phase of photosynthesis.

The induction phase of photosynthesis has been found to be dependent on three separate processes that operate on different time scales (Pearcy, 1990). A fast phase that activates and deactivates rapidly as PFD increases and decreases is associated with limitations in RuBP regeneration (Sassenrath-Cole and Pearcy, 1992; Sassenrath-Cole et al., 1994). Limitation of enzymes in this part of the CRC by the light activation state is most evident after relatively short low-light periods, when the rate of increase in photosynthesis is limited in the first 1 to 2 min of reillumination. The slow phase of induction is dependent on Rubisco activation, which requires longer illumination at high PFD and exhibits a slower deactivation upon return to low PFD than the RuBP-regenerating enzymes (Kirschbaum and Pearcy, 1988a; Woodrow and Mott, 1989; Sassenrath-Cole and Pearcy, 1992). Rubisco activation appears to be a two-step process involving the initial activation of activase and the subsequent activation of Rubisco (Lan et al., 1992). Rubisco limitations are most evident during the period from 1 to 10 min after an increase in PFD (Seemann et al., 1988; Woodrow and Mott, 1989). Changes in stomatal conductance also contribute to the slow phase of photosynthetic induction. Stomatal limitations can occur at any time during induction, but increases in stomatal conductance are the sole cause of increases in assimilation rate after 10 min of saturating PFD when the enzymes are already fully activated. These three components of the induction state combine to determine the assimilation rate achieved at any given time relative to the steady-state, light-saturated rate.

The heterogeneous nature of a crop canopy results in a wide range of light conditions experienced by leaves at various positions within the canopy (Pearcy et al., 1990). Leaves at the top of the canopy can experience direct sun for most of the day, with changes in solar angle, cloud cover, and leaf angle altering the PFD levels incident on the leaf surfaces. Leaves immediately below this layer experience frequent interruptions in maximal incident PFD due to shading by

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Abbreviations: CRC, carbon reduction cycle; FBPase, fructose-1,6-bisphosphatase (EC 3.1.3.11); IS, induction state; PFD, photon flux density; P-glycerate, 3-phosphoglycerate; Ru5P, ribulose-5-phosphate; Ru5P kinase, ribulose-5-phosphate kinase, (EC 2.7.1.19); RuBP, ribulose-1,5-bisphosphate; Sedo-7-P, sedoheptulose-7-phosphate; Sedo-1,7-P_2, sedoheptulose-1,7-bisphosphate; SBPase, sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37); $t_{1/2}$, half-time.

higher leaves, changing from full sun to shade and back as many as 2000 times per day (Pearcy et al., 1990). Leaves lower in the canopy experience mostly shade, with brief periods of higher PFD. The induction state of photosynthesis for a particular leaf will vary throughout the day as the light environment experienced by that leaf changes. Therefore, the potential contribution of each leaf to the daily carbon uptake of the plant will depend in part on the temporal pattern of PFD incident on the leaf surface. The total carbon assimilation by an individual leaf will be reduced from its maximum potential due to loss of induction.

The objective of this study was to determine the dynamic limitations to CO_2 assimilation in leaves at different positions within a soybean (*Glycine max* L.) crop canopy, particularly the rapidly developing limitations arising after a change in PFD. Measurements of photosynthetic activities and metabolite pool sizes in field-grown plants were made to determine limitations under field conditions. Photosynthetic limitations were explored in more depth with growth-chamber-grown plants by determining the response of enzyme activities and metabolite pool sizes to transient PFD.

MATERIALS AND METHODS

Growth Conditions

Soybeans (*Glycine max* L. cv Williams) were planted in 75-cm rows in a 20- × 30-m plot on May 21, 1990, and June 7, 1991, in Davis, CA. The plot was irrigated as needed and fertilized with a commercial N:P:K (20:10:10) fertilizer at planting and 3 weeks later as previously described (Pearcy and Seemann, 1990). Measurements were begun after canopy closure.

Additional soybeans were planted in 4-L pots and grown under controlled growth conditions in growth chambers at 25/17°C day/night with an incident PFD of 450 μmol photons m^{-2} s^{-1} as previously described (Sassenrath-Cole and Pearcy, 1992). The pots were watered twice daily with half-strength Hoagland nutrient solution (7.5 mM NO_3^-). Measurements were made on the youngest fully expanded leaves 25 to 30 d after planting.

Gas-Exchange Measurements

Photosynthetic activity was determined using an open, fast-response measurement system described previously (Kirschbaum and Pearcy, 1988b). The single-sided leaf chamber that allowed measurement of gas exchange through the abaxial leaf surface was designed in such a way as to minimize air volume and maximize air flow to reduce the lag in measurements due to instrumentation (Sassenrath-Cole and Pearcy, 1992). The full-scale response time of the chamber under field conditions was determined to be 4 s; under laboratory conditions it was reduced to 2 s by further reducing the volume of air in the system. The leaf area enclosed within the chamber was 22 cm^2, and the total chamber volume was 4 cm^3. Air was introduced through a slot at one end and flowed out through a similar slot at the other end. These slots were designed to give a uniform air flow over the leaf surface. Leaf temperature was controlled by flowing thermostatted water through water jackets in the chamber lid and base. During field measurements, the temperature of the leaves was maintained similar to leaf temperatures of adjacent leaves within the canopy. Laboratory photosynthetic measurements were made at a constant 25°C.

Fully expanded leaves of field-grown plants were selected at the top of the canopy and from approximately the middle third of the canopy. Those leaves selected in the middle of the canopy appeared young and healthy. These lower leaves commonly experienced a mix of full sun and shade throughout the day. Incident PFD was recorded for 1 h prior to photosynthetic measurements by mounting gallium arsenide phosphide photodiodes directly on the leaf surface (Gutschick et al., 1985; Pearcy et al., 1990). These sensors were connected to a data logger and PFD levels were recorded every 0.5 s. The leaf was then placed into the chamber and the assimilation rate under the prevailing PFD was determined. The canopy was then parted to expose the leaf to full sun and the increase in photosynthetic rate was monitored by logging the data twice per second for 3 min and then every 7 s for the next 20 min.

Photosynthetic activity in young, fully expanded leaves of growth-chamber-grown plants was determined using the fast-response chamber attached to an open measurement system described previously (Kirschbaum and Pearcy, 1988b). Leaves were illuminated using a 150-W quartz-iodide projection lamp filtered through a mirror (OCLI, Santa Rosa, CA) to remove wavelengths outside of the 400- to 700-nm range. PFD levels were varied using neutral density filters. Leaves were allowed to equilibrate after a change in PFD level until a steady-state rate of carbon assimilation was observed.

The limitations during photosynthetic induction were quantified by calculation of an IS for the leaf. The leaves were first exposed to specific subsaturating PFDs and then the assimilation rate was recorded following the PFD increase to saturating levels. The IS 5 and 120 s after the PFD increase (IS_5 and IS_{120}, respectively) were then calculated by expressing these assimilation rates as a percentage of the steady-state, light-saturated assimilation rate measured after induction was complete. The IS_5 and IS_{120} were taken as measures of the limitations imposed by the fast and slow components of induction, respectively.

Metabolite Pool Size Determinations

Leaves of field-grown plants were selected by position in the canopy to be similar to those used for the gas-exchange measurements. A sample was taken of the leaves under ambient illumination using 35-mm-diameter × 2.5-mm-thick copper plates chilled to liquid N_2 temperature. The edge of one of the plates had a slightly raised stainless-steel lip that served to cut the leaf disc. The plates were attached to tongs and sampling was done by hand. The frozen 9-cm^2 leaf disc was held between the copper plates and transferred in the field directly to liquid N_2 as rapidly as possible. Any leaf disc that inadvertently rewarmed during handling was discarded. The leaf that had been sampled was then exposed to full sun and additional leaf samples were taken after 5 s, 1 min, and 20 min in full sun. The trifoliate leaves of soybean were large enough to allow all four samples to be taken from one leaf,

minimizing leaf age and position effects. Following sampling for metabolite pool size determinations, leaf discs of known area were taken from the same leaf to determine leaf Chl and Chl *a*/*b* ratios as described previously (Sassenrath-Cole and Pearcy, 1992). A minimum of 15 leaves from each PFD level and each time point were taken.

Metabolite pool sizes were determined in leaves from growth-chamber-grown plants using a small double-sided, open gas-exchange chamber with plastic film windows incorporated into a freeze-clamp apparatus (Sharkey et al., 1986). Following exposure of leaves to the desired PFD conditions, the leaves were rapidly frozen by clamping between the hammer and anvil that had been chilled to liquid N_2 temperature. The copper heads punched a 9-cm^2 leaf disc directly through the film windows of the gas-exchange chamber and immediately froze the leaf, stopping metabolism.

The frozen leaf samples from field-grown and controlled-environment plants were stored in vials immersed in liquid N_2. Extraction of metabolites was accomplished by grinding individual leaf discs in a mortar and pestle in liquid N_2 with 3.5% (v/v) $HClO_4$. The frozen leaf powder in $HClO_4$ was transferred to a microfuge tube, and after thawing, a 100-μL aliquot was removed for determination of Chl based on pheophytin content (Vernon, 1960). The remaining extract was centrifuged and the supernatant was used for determination of metabolites. The supernatant was neutralized with KOH, decolorized with activated charcoal, and stored in liquid N_2 as described (Sharkey et al., 1986). Metabolite pool sizes were determined spectrophotometrically in pyridine nucleotide-linked enzyme assays using an SLM Aminco model DW-2000 dual-wavelength spectrophotometer at 340/410 nm as described (Sassenrath-Cole and Pearcy, 1992). Leaf discs were analyzed separately and all metabolites were determined for each leaf sample. Data from controlled-environment studies are means of a minimum of five separate leaves + SE. For field studies, leaves from a minimum of 15 different plants were sampled at each of the four time points (low light and 5 s, 1 min, and 20 min in full sun).

Determination of Enzyme Activities

Leaves for determination of in situ enzyme activation state were illuminated in a rapid freeze-quench apparatus as described above. Leaves were illuminated at various PFD levels for a period of time sufficient to achieve steady-state enzyme activities. The leaf disc was then isolated from the leaf by rapidly clamping between a hammer and anvil chilled to liquid N_2 temperatures. The 9-cm^2 leaf disc was divided into three sections that were placed in vials and stored in liquid N_2.

Determination of initial enzyme activity was begun by grinding the frozen leaf sections in liquid N_2 in a mortar and pestle. The frozen leaf powder was scraped into 1 mL of extraction buffer consisting of 100 mM Tricine (pH 8.1), 10 mM $MgCl_2$, 1 mM EDTA, and 15 mM 2-mercaptoethanol. Extraction buffer for determination of FBPase activity also contained 1 mM Fru-1,6-P_2. After suspension of the frozen leaf powder in extraction buffer, a 0.1-mL aliquot was removed for determination of Chl as described previously (Sassenrath-Cole and Pearcy, 1992). The remaining extract was centrifuged for 5 s. An aliquot of the supernatant was then added to the assay buffer to begin the assay reaction. FBPase and Ru5P kinase were assayed spectrophotometrically using pyridine nucleotide-linked enzyme assays as described (Sassenrath-Cole et al., 1994). An SLM Aminco model DW-2000 dual-wavelength spectrophotometer at 340/410 nm was used. Changes in pyridine nucleotide levels were calculated using a molar extinction coefficient at 340 nm of 6.27 M^{-1} cm^{-1}. FBPase activity was determined in 1 mL of assay buffer in a final volume of 100 mM Tricine (pH 8.1), 20 mM $MgCl_2$, 1 mM EDTA, 0.3 mM NADP, 0.6 mM Fru-1,6-P_2, 0.6 units of Glc-6-P dehydrogenase, and 1.2 units of phosphoglucoisomerase, to which was added 0.1 mL of leaf extract. The Ru5P kinase assay buffer consisted of 100 mM Tricine (pH 8.1), 20 mM $MgCl_2$, 1 mM EDTA, 0.2 mM NADH, 2.0 mM Rib-5-P, 1.0 mM PEP, 1.0 mM ATP, 20 units of pyruvate kinase, 30 units of lactate dehydrogenase, and 2.5 units of phosphoriboisomerase, to which 25 μL of leaf extract was added. Rubisco activity was determined by the rate of incorporation of $^{14}CO_2$ into P-glycerate during a 30-s assay using 50 μL of leaf extract (Brooks and Portis, 1988).

RESULTS

Dependence of IS on Prior Light Exposure

Determination of the dependence of photosynthetic induction on the prior PFD was measured by following the increase in photosynthetic activity upon illumination at saturating PFD after exposure to subsaturating PFD (Fig. 1). Prior illumination at subsaturating PFD reduced the time required for induction relative to that observed for leaves held in darkness. Increased PFD during the prior illumination period resulted in more rapid induction of photosynthesis at saturating PFD. Similarly, higher subsaturating PFD levels maintained a higher IS_5 and IS_{120} (Fig. 2). After only 5 min of 0 or 35 μmol photons m^{-2} s^{-1}, IS_5 had decayed to only 20 to 25% of maximal. The decay appeared to be biphasic, with an initial rapid phase followed by a much slower phase. Following a transition from 1500 to 220 μmol photons m^{-2} s^{-1}, IS_5 was maintained at high levels, decaying to only 80% of maximal after 10 min of low PFD. The decrease in IS_5 was only slightly greater at 150 μmol photons m^{-2} s^{-1} than at 220 μmol photons m^{-2} s^{-1}. Thus, both the rate of decay and the final equilibrium value of IS_5 depended on the PFD level and the duration of exposure. As has been observed before (Sassenrath-Cole and Pearcy, 1992), the changes in IS_{120} were much slower with a $t_{1/2}$ of approximately 24 min in darkness or at 35 μmol photons m^{-2} s^{-1}. Preillumination at PFD levels greater than 70 μmol photons m^{-2} s^{-1} resulted in a decline in IS_{120} of no more than 10% after 10 min. The slow component declined similarly at both 35 μmol photons m^{-2} s^{-1} and in darkness but was still 80% of maximal after 10 min at low PFD or in darkness.

Time Course of Light-Dependent Activation and Deactivation of Photosynthetic Enzymes

To determine the contribution of enzymatic activity to the observed delay in activation of photosynthesis upon exposure to saturating PFD, the time course of light activation and

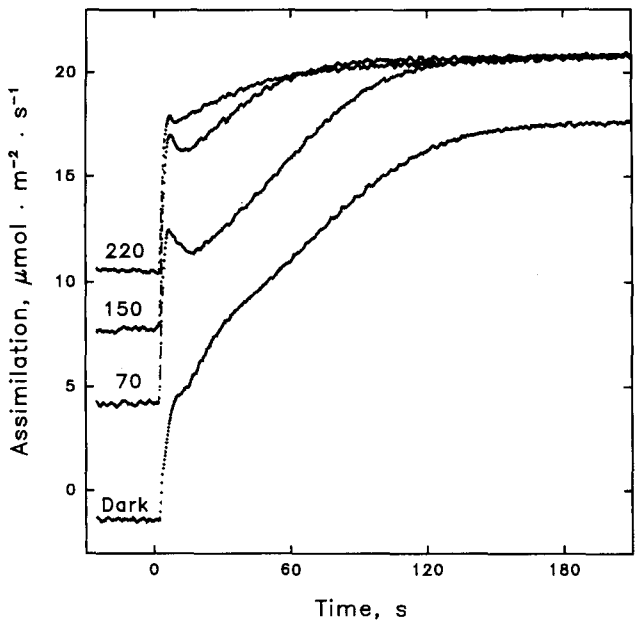

Figure 1. Activation of photosynthesis at saturating PFD following exposure to subsaturating PFD. Activation of photosynthesis at saturating PFD (1500 μmol photons m^{-2} s^{-1}) following exposure to subsaturating PFD (220, 150, or 70 μmol photons m^{-2} s^{-1}) or darkness was determined in intact soybean leaflets. The net carbon assimilation rate at ambient O_2 and CO_2 was measured in a single-sided leaf chamber in an open gas-exchange system. The photosynthetic rate was measured continuously at a sampling rate of four data points s^{-1}. Leaves were preilluminated at a given subsaturating level of illumination or in darkness until a steady-state rate of photosynthesis was observed. The PFD was then increased to saturating and the rate of carbon fixation was determined.

dark deactivation of the stromal enzymes was measured. High light activation was measured by isolating leaf discs during increasing duration of exposure to saturating PFD following preillumination at low PFD. Rubisco and FBPase activities showed similar time courses, reaching maximal activity after 10 min at saturating PFD (Fig. 3). In contrast, Ru5P kinase exhibited transient activity during the early activation and reached a maximal rate after only 2 min of saturating PFD.

The decline in enzyme activity was determined by following the enzymatic activation states with increasing low PFD exposure after preillumination at saturating PFD (Fig. 3). After only 5 s at low PFD, the activity of FBPase increased significantly above the maximal rate observed in leaves at saturating PFD. This increased activity continued for more than 30 s after the light was lowered, after which time the FBPase decayed rapidly to the level measured in low light. After 5 min at low PFD, the FBPase activity was insufficient to support the maximal light-saturated rate of photosynthesis. Rubisco activity declined much more slowly, retaining half-maximal activity even after 20 min at low PFD. Ru5P kinase displayed highly repeatable fluctuations in the activity upon exposure to low PFD, but nonetheless deactivated slowly. After 20 min of low PFD, Ru5P kinase activity was still severalfold greater than that needed to support even the maximal photosynthetic rates at saturating PFD levels.

Light Dependence of Photosynthetic CRC Enzymes

The light dependence of Rubisco, FBPase, and Ru5P kinase were determined in whole-leaf extracts from leaves isolated at various PFD levels after measurement of net photosynthesis (Fig. 4). These enzymes were assayed because they represent likely controlling points of regulation of photosynthetic activity (Heldt et al., 1981). Although SBPase activity was not determined separately, the similarity to FBPase in activation mechanism and midpoint potential indicate that this enzyme most likely follows activation kinetics similar to those of FBPase (Sassenrath et al., 1990; Ort and Oxborough, 1992). The data are shown in such a way as to allow direct comparison of the observed photosynthetic rate and the enzymatic activity necessary to support that level of photosynthetic activity.

Photosynthesis increased with increasing PFD levels in the

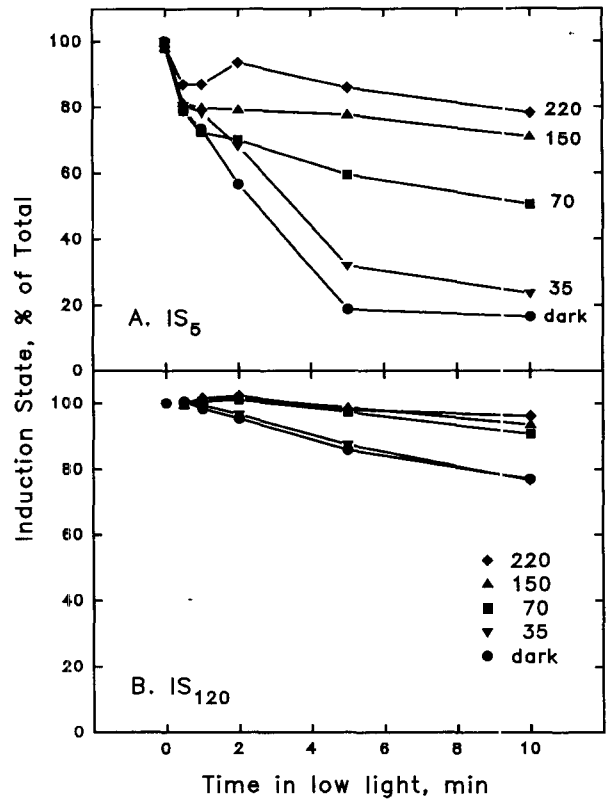

Figure 2. Dependence of photosynthetic induction state on PFD pre-exposure. A measure of the fast and slow components of induction following exposure to low PFD was determined as the photosynthetic rate at 5 s (A, IS_5) and 120 s (B, IS_{120}) during reactivation at saturating PFD (1500 μmol photons m^{-2} s^{-1}). Reactivation of photosynthesis was determined in intact soybean leaflets as described in Figure 1 after 0.5, 1, 2, 5, or 10 min of illumination at low PFD (♦, 220; ▲, 150; ■, 70; or ▼, 35 μmol photons m^{-2} s^{-1}) or darkness (●). Induction state was calculated as the assimilation rate measured after 5 and 120 s of reillumination relative to that measured in fully light-activated leaves prior to low-light exposure.

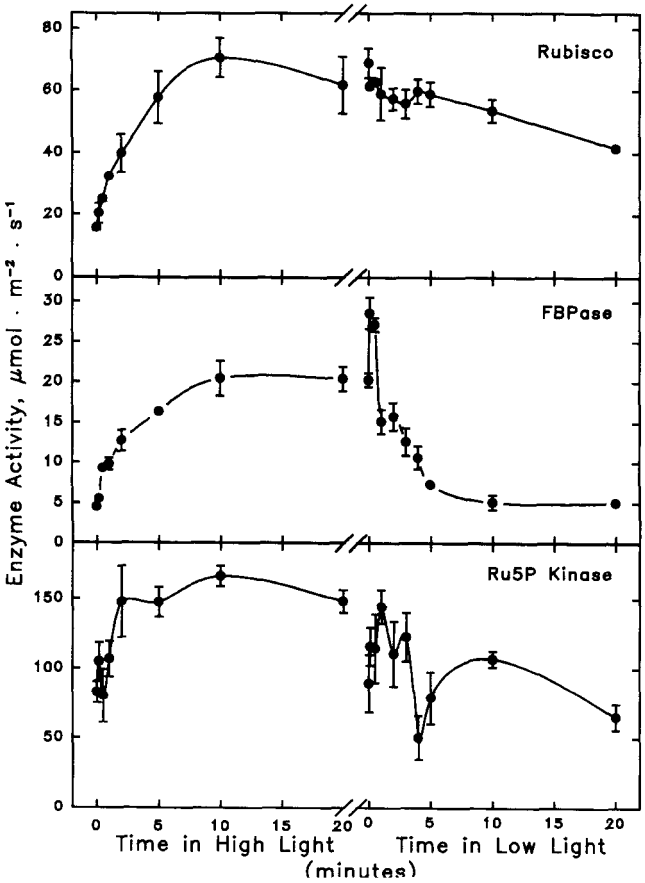

Figure 3. Time course of high-light activation and low-light deactivation of stromal enzymes. High-light activation of stromal enzymes was determined by sampling leaves after increasing times at saturating PFD (1500 μmol photons m^{-2} s^{-1}) following preillumination at low PFD (35 μmol photons m^{-2} s^{-1}). Low-light deactivation of enzymatic activity was followed by first preilluminating leaves at saturating PFD (1500 μmol photons m^{-2} s^{-1}) for a minimum of 20 min until maximal steady-state photosynthetic rates were observed. Leaves were then sampled after increasing durations of low PFD exposure (35 μmol photons m^{-2} s^{-1}). Leaves were sampled in a freeze-quench apparatus that rapidly isolated a leaf punch between two copper heads cooled to liquid N_2 temperatures. Leaf discs were divided into three pieces for separate determination of stromal enzyme activities. FBPase and Ru5P kinase activities were determined spectrophotometrically, and Rubisco activity was determined radiometrically, as described. Reported values are means ± SE of a minimum of three separate leaves.

familiar light response of photosynthesis and began to saturate around 1000 μmol photons m^{-2} s^{-1}. The enzymatic activities also displayed a strong light dependence, increasing rapidly at low PFD levels until they reached a saturating level. Rubisco and FBPase activities exhibited dependencies on PFD similar to that for assimilation. Ru5P kinase activity, however, increased more rapidly at low PFD levels and was maximal near 200 μmol photons m^{-2} s^{-1}. The PFD level at which enzymatic activity was sufficient to support maximal light-saturated rates of photosynthesis is shown by the line in Figure 4. Substrate-saturated Rubisco activity exceeded light-saturated photosynthetic activity at PFD greater than 100 μmol photons m^{-2} s^{-1}. Similarly, FBPase activity was in excess above about 70 μmol photons m^{-2} s^{-1}. Ru5P kinase activity exceeded light-saturated photosynthesis at all PFDs. These enzyme activities are measured at substrate saturation and therefore overestimate the true enzymatic turnover in situ, where substrate limitations are important. Nevertheless, they do indicate differences in the minimum PFD level at which the enzymatic activity is sufficient to support maximal photosynthesis and the relative limitations imposed by each enzyme at low PFD.

Metabolite Pool Size Changes as a Function of Incident PFD

Limitations at a particular enzymatic reaction in a linear series of reactions will be evident as a buildup of substrate and depletion of product. However, because the CRC is a cycle and not a linear sequence, pool sizes can depend on both the preceding and subsequent enzymatic reactions, making exact localization of a limiting step difficult. To determine if there were shifts of enzymatic limitations with incident

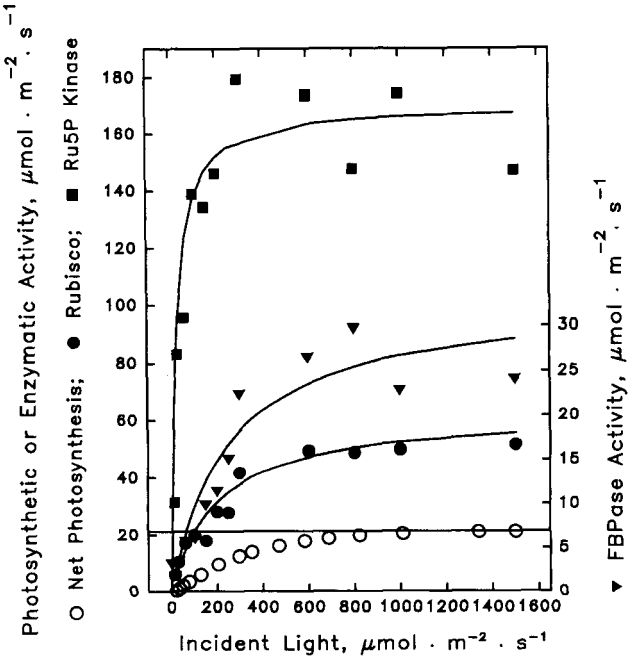

Figure 4. Dependence of enzymatic and photosynthetic activity on incident PFD. Steady-state carbon assimilation rates were measured in young, fully expanded soybean leaflets in an open gas-exchange system as a function of incident PFD. A rapid freeze-quench apparatus incorporated into the gas-exchange system was used to rapidly isolate leaf discs from leaves after steady-state rates of photosynthesis were attained at various incident PFD levels (○). Each individual leaf disc was then separated into three samples and stored in liquid N_2 for determination of FBPase (▲), Ru5P kinase (■), and Rubisco (●) activities as described. Data are plotted in such a way that the photosynthetic activity is plotted directly across from the enzymatic activity necessary to support the observed rate of photosynthesis.

PFD, CRC metabolite pool sizes were determined in leaf samples illuminated at various PFD levels. The results are presented in the sequence of reactions of carbon fixation to facilitate comparisons of substrate and product pools and aid in identification of limitations to carbon fixation (Fig. 5).

As photosynthetic activity increased with increasing PFD, the pool sizes of CRC metabolites increased (Fig. 5). The largest pool was P-glycerate, with substantial amounts also in the Rib/ribulose and Fru-6-P pools, and in Glc-6-P and Glc-1-P. Most metabolites showed very little change in pool size at low PFD, with an increase in pool size at saturating PFD levels. A notable exception was the more than 4-fold increase in the Fru-1,6-P_2 + Sedo-1,7-P_2 pool from 10 to 30 μmol photons m^{-2} s^{-1}. The Fru-1,6-P_2 + Sedo-1,7-P_2 level began to decrease at 50 μmol photons m^{-2} s^{-1} and was very low at 500 μmol photons m^{-2} s^{-1}, before increasing again at saturating PFD levels. The 3-fold increase in triose-P levels at 30 μmol photons m^{-2} s^{-1} most probably reflected the increase in Fru-1,6-P_2 levels due to the near equilibrium of the aldolase reaction in situ. The absence of a commensurate decrease in product pools of Fru-6-P at 30 and 50 μmol photons m^{-2} s^{-1} was most likely due to the regulation of this pool by subsequent enzymatic steps involved in either starch synthesis or RuBP regeneration. The limitation in enzyme activation of FBPase near 30 μmol photons m^{-2} s^{-1}, as seen by the buildup in Fru-1,6-P_2 + Sedo-1,7-P_2 at 30 μmol photons m^{-2} s^{-1} and subsequent decline with increasing PFD, was similar to the PFD level at which FBPase activity was measured to be in excess of that needed to support maximal photosynthetic rates (Fig. 4). Interestingly, although the saturating PFD levels required for full activation of Rubisco and FBPase were not significantly different, no buildup in Rib/ribulose pools was observed in leaves at low PFD. This may indicate that the limitation at FBPase at these low PFD levels was more substantial, and masked any limitation at Rubisco.

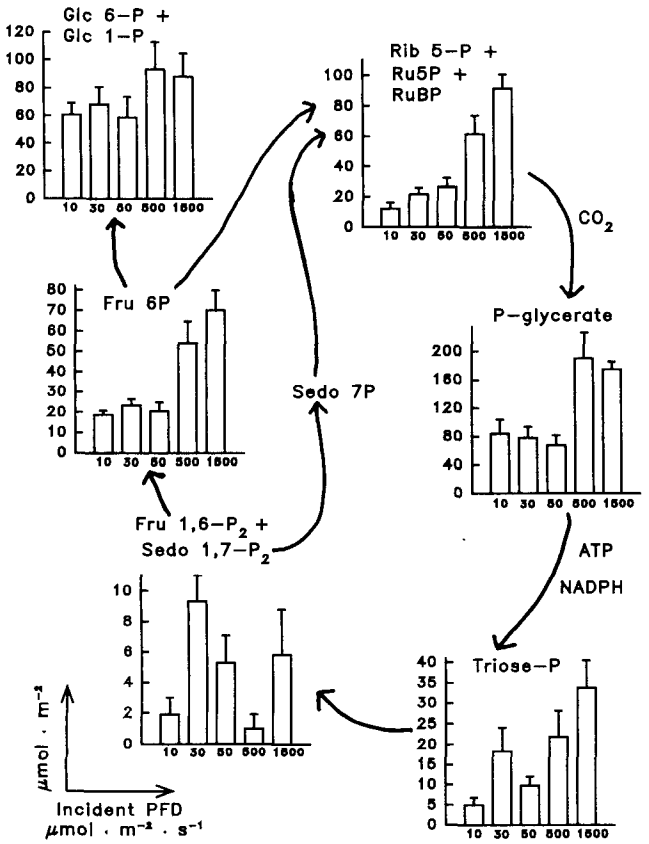

Figure 5. Levels of photosynthetic CRC metabolites as a function of incident PFD. Leaves were exposed to 10, 30, 50, 500, or 1500 μmol photons m^{-2} s^{-1} until steady-state photosynthetic rates were observed. Leaf discs were then isolated by rapidly quenching the leaves to liquid N_2 temperature using the freeze-quench apparatus. Photosynthetic CRC metabolites were determined spectrophotometrically in an $HClO_4$ extraction of the leaf tissue using pyridine nucleotide-linked enzyme assays. Data are presented as the sequence of steps involved in carbon fixation to facilitate identification of rate-limiting steps to carbon uptake. Data are from a minimum of five separate leaves + SE.

Limitations to Carbon Fixation under Field Conditions in a Crop Canopy

Natural and agricultural canopies are highly heterogeneous in nature, being composed of leaves of many different ages and at widely varying positions within the canopy. The position of a leaf within a canopy will determine the PFD environment experienced by the leaf, which will in turn determine its IS. To assess limitations to photosynthesis operative within a natural plant canopy, we artificially defined leaves within the canopy to be either "sun" leaves, which were at or near the top of the canopy and received near-full sun throughout the day, or "shade" leaves, which were lower in the canopy and received intermittent high PFD levels interspersed with low PFD levels of varying durations. These categories refer only to the current environment and do not imply a particular developmental history.

The induction of photosynthesis of sun and shade leaves differed dramatically (data not shown; see also Pearcy and Seemann, 1990). A typical sun leaf received an average PFD of 420 μmol photons m^{-2} s^{-1} for the 1 h preceding measurement of the photosynthetic rates, whereas a typical shade leaf received only 83 μmol photons m^{-2} s^{-1} for the same period. When exposed to full sun after a brief (<1 min) low-PFD period, the sun leaves showed a rapid increase in assimilation rate, indicating essentially no induction limitation. In contrast, a typical shade leaf showed a significant induction requirement for assimilation. In addition, the maximum photosynthetic activity of shade leaves was lower than that observed for the sun leaf (29 versus 37 μmol CO_2 m^{-2} s^{-1}).

Metabolite pool sizes of photosynthetic CRC intermediates were determined in shade leaves at ambient light and after 5 s, 1 min, and 20 min of illumination at full sun, and for sun leaves at full sun (Fig. 6). Within 5 s of exposure of shade leaves to full sun, the P-glycerate levels dropped more than 4-fold. With increasing exposure to full sun, P-glycerate pool sizes then increased slowly but never reached the level observed in the sun leaves. Commensurate with the drop in P-glycerate levels was an increase in the pools of Fru-1,6-P_2 +

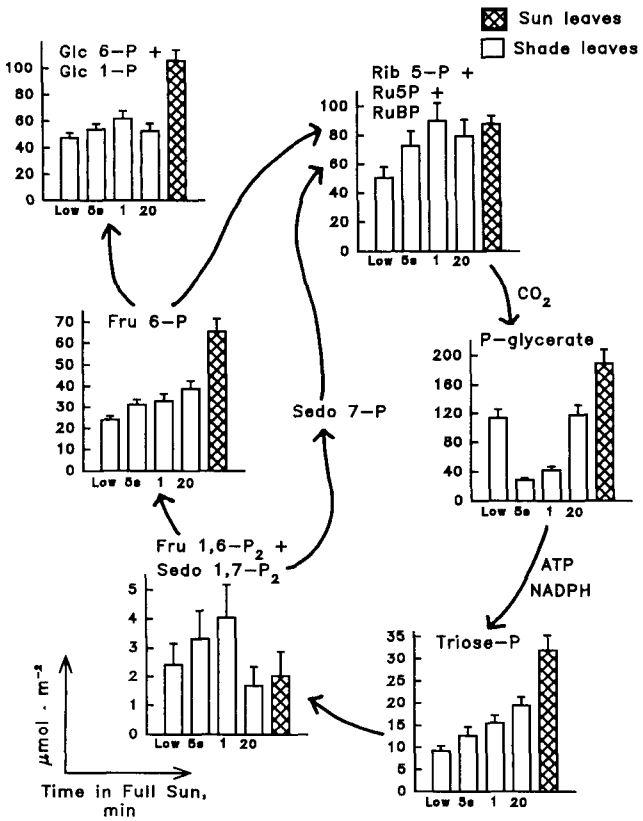

Figure 6. Photosynthetic CRC metabolite pool sizes in sun and shade leaves under field conditions. Metabolite pool sizes of photosynthetic CRC intermediates were determined in sun (▩) and shade (□) leaves from field-grown soybeans. Copper plates attached to tongs were immersed in liquid N_2 and used to rapidly isolate leaf discs from leaves after specific light exposure. The frozen leaf discs were quickly transferred to liquid N_2 and brought to the lab for spectrophotometric determination of metabolite pool sizes from $HClO_4$ extracts of leaf tissue. Sun leaves were chosen from the top canopy layer and exposed to full sun prior to isolation of leaf discs. Shade leaves were from lower canopy layers and were sampled at ambient PFD levels (Low). The canopy above the leaf was then parted and the leaves were again sampled after 5 s, 1 min, and 20 min of illumination at full sun. The trifoliate leaves of soybean were large enough to allow all four samples to be taken from one leaf, circumventing differences in leaf age and position within the canopy. A minimum of 15 leaves from each canopy layer and each time point were sampled and analyzed separately.

Sedo-1,7-P_2 and Rib/ribulose, which became maximal after 1 min of illumination at full sun, and then decreased with additional time in full sun. A small increase in triose-P, Fru-6-P, and Glc-6-P + Glc-1-P pools also occurred. The buildup in the Fru-1,6-P_2 + Sedo-1,7-P_2 pool after 1 min of illumination at full sun was significantly greater than that of the control plants (95% confidence level), indicating an initial limitation in flux through these pools following the transition from low to high PFD. The drop in the Fru-1,6-P_2 + Sedo-1,7-P_2 pool to near full-sun levels after 20 min of illumination at full sun indicated a removal of this limitation with longer illumination times.

DISCUSSION

Leaves within natural plant canopies experience highly variable light environments over the course of a day due to changes in leaf and solar angle, cloud cover, and overshadowing canopy cover. Photosynthesis is dependent on incoming light both for energy and for light-dependent activation of regulatory enzymes of assimilation. Declines in PFD levels within a canopy result in immediate decreases in net carbon uptake. Prolonged shading of a leaf may further limit total carbon uptake because inactivation of photosynthetic CRC enzymes limits assimilation when the light again increases. Light-regulatory mechanisms of photosynthesis serve to modulate carbon fixation to external environmental conditions, but can also hamper maximal carbon uptake during sunflecks because of the only partially activated photosynthetic enzymes. This loss of potential fixed carbon is readily apparent as the lag in attainment of maximal photosynthetic rate upon illumination at saturating PFD.

Stromal FBPase and SBPase belong to a group of enzymes that require reduction by the thioredoxin system for activation (Buchanan, 1980), specifically thioredoxin *f*. The thioredoxin system receives reducing equivalents from PSII via Fd-thioredoxin reductase, and subsequently reduces thiol groups on the targeted enzymes, inducing conformational changes to a more active form. This reductive activation is one mechanism through which the activities of the biochemical reactions in the CRC are coordinated with electron transport activity and energy flux (Harbinson et al., 1990). The CF_1CF_0-ATP synthase (coupling factor) is also reductively activated via the thioredoxin system and has a redox potential that is nearly equipotential with thioredoxin *f* (Ort and Oxborough, 1992, and refs. therein). In field-grown sunflower, the coupling factor is fully activated at PFD levels below 20 µmol photons m^{-2} s^{-1} (Kramer et al., 1990), suggesting that very low PFD levels are sufficient to reduce the thioredoxin system. Determination of enzymatic activities (Fig. 4) and in situ pool sizes of photosynthetic carbon intermediates (Fig. 5) indicate that FBPase activity is probably more limiting to photosynthesis than other steps in the CRC below about 70 µmol photons m^{-2} s^{-1}. An apparent lessening of the limitation at FBPase at PFDs above 70 µmol photons m^{-2} s^{-1} is consistent with the observed light-dependent thioredoxin activation, since the midpoint potential of this enzyme is slightly more reducing than that of thioredoxin *f* (Ort and Oxborough, 1992). The maintenance of high IS_5 at PFDs greater than 70 µmol photons m^{-2} s^{-1} (Fig. 2) is also consistent with a lesser FBPase limitation at higher PFD.

The light-dependent activation and dark deactivation of the CRC enzymes following PFD changes leads to transiently different limitations than those ultimately controlling under steady-state conditions. Although Ru5P kinase activity exhibited repeatable oscillations (Fig. 3), at no time did this enzyme appear to be limiting to CO_2 assimilation. Indeed, the maximal activity was 10-fold greater than that needed to support light-saturated photosynthesis. Following an overnight dark period, Rubisco activated with a $t_{1/2}$ of 5 min and deactivated very slowly in low light or dark, with a $t_{1/2}$ of 24 min (Fig. 3). The FBPase showed a similar $t_{1/2}$ for activation. Obser-

vations from other studies have found similarly long activation times for FBPase following overnight darkness but more rapid light activation following shorter periods of darkness (Leegood and Walker, 1982; Stitt and Grosse, 1988). FBPase deactivated much more rapidly in low light than Rubisco, with a $t_{1/2}$ on the order of 2.5 min. These $t_{1/2}$ values for deactivation of carbon-metabolizing enzymes are similar to the decay of the slow- and fast-induction components, respectively (Fig. 2; Sassenrath-Cole and Pearcy, 1992).

The substantial increase in FBPase activity immediately following a PFD decrease was rather surprising. Activation of FBPase occurs via reduction by thioredoxin *f* (Scheibe, 1991), which lowers the K_m for the substrate (Mg-Fru-1,6-P_2 complex) to the biological range (Baier and Latzko, 1975; Portis and Heldt, 1976). Given its negative midpoint potential (Ort and Oxborough, 1992), FBPase could be expected to rapidly deactivate upon transfer to light-limiting conditions. The initial burst in activation state upon transfer to low PFD may reflect the same increase in chloroplast redox state observed by Groom et al. (1993), who suggested that the initial increase in chloroplast redox state upon transfer to low PFD may result from a large reductant pool in the chloroplast. The negative redox potential of the FBPase may allow an increase in activity for a brief period after transfer to low light (<30 s), but the large reduction energy level necessary to maintain the FBPase fully active is soon not available, leading to a rapid decay of FBPase activity to the equilibrium level set by the low PFD level. The decrease in carbon uptake due to limitations in RuBP regeneration (Sassenrath-Cole and Pearcy, 1992) thus appears to result from a loss of FBPase activity.

The slow-induction component of photosynthesis is due to the requirement for stomatal opening and for light activation of Rubisco (Kirschbaum and Pearcy, 1988a; Woodrow and Mott, 1989). Although the regulatory mechanisms for Rubisco and FBPase are different, they exhibit a similar PFD dependence. Rubisco is activated via carbamylation and by removal of bound sugar phosphate inhibitors, which depends on another light-activated enzyme, Rubisco activase (Portis, 1992). As with the thioredoxin-mediated activation of FBPase, the activation of Rubisco has also been shown to be dependent on PSI electron transport (Campbell and Ogren, 1990). Mott and co-workers (Jackson et al., 1990; Woodrow and Mott, 1992) have explored the dual light-activation steps for Rubisco, showing that they have different light dependencies and rates of activation. A prominent feature of the light regulation of Rubisco is the very slow loss of activity upon transfer to shade. This causes the decline in IS_{120} to be much slower than the decline in IS_5 (Fig. 1).

The inherent regulatory mechanisms of photosynthesis that act to coordinate the capacities of photosynthetic biochemistry with the available PFD can also result in distinct limitations to optimal canopy assimilation by virtue of their time-dependent requirement for full activation. Our data indicate that the initial limitation to photosynthesis in lower canopy leaves upon illumination at saturating PFD is at least partially due to FBPase/SBPase (Fig. 6), as seen by the buildup in substrate pool after 1 min of full sun. Additional limitations due to down regulation of Rubisco also contribute to limitations in canopy photosynthesis (Pearcy and Seemann, 1990).

Although FBPase activity is competent to support maximal light-saturated photosynthesis at fairly low PFD levels, the light levels within a canopy can fall below that necessary to maintain adequate carbon flux through FBPase if the PFD increases again. Pearcy et al. (1990) showed that leaves in the upper half of a soybean canopy experienced mean PFD levels in excess of 100 μmol photons m^{-2} s^{-1}. For leaves lower in the canopy, mean PFD levels fell below that necessary to maintain a minimum required FBPase activity. Even for the upper canopy leaves, the rapid rate of FBPase deactivation (Fig. 3) would result in inhibition at lower PFD levels. Additional evidence suggests that FBPase deactivation is relatively more sensitive to rapidly varying PFD regimes than is Rubisco, as seen by a greater decline in enzymatic activity under flashing light (Sassenrath-Cole et al., 1994).

Rubisco activation requires higher PFD levels (Fig. 4; Woodrow and Mott, 1992), but the hysteretic nature of activation, with a much slower deactivation than activation, results in the maintenance of Rubisco activity for a longer time after return to low PFD. In this way, an initial cluster of sunflecks "readies" the photosynthetic CRC for subsequent sunflecks (Pearcy, 1990). Although this may reduce the loss of carbon due to deactivation of Rubisco, Rubisco activation state nonetheless can present a limitation to carbon assimilation in a soybean canopy. The loss in induction state observed previously for leaves in soybean canopies (Pearcy and Seemann, 1990) most likely included effects of down regulation of both FBPase/SBPase and Rubisco. The relative contribution of each site to limiting carbon uptake depends on the PFD exposure history of individual leaves. Relatively high PFD levels are required to maintain Rubisco in an active state, but the slow down regulation maintains activities at reasonably high rates even after fairly lengthy low-PFD periods. In contrast, FBPase deactivates rapidly upon shading, but activity is regained at low PFD levels.

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