Photosynthetic Induction State of Leaves in a Soybean Canopy in Relation to Light Regulation of Ribulose-1,5-Bisphosphate Carboxylase and Stomatal Conductance¹

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ABSTRACT

Photosynthetic induction state, stomatal conductance and light regulation of ribulose-1,5-bisphosphate carboxylase (rubisco) were examined for leaves in a mature, closed soybean (Glycine max) canopy (leaf area index approximately 5) with the objective to determine the extent to which these factors may be limiting the capacity to respond to light transients during sunflecks. When sampled along a vertical gradient, leaves near the bottom of the canopy had lower rubisco contents and chlorophyll a/b ratios as compared with upper leaves. Leaves sampled at midcanopy showed a wide variation in photosynthetic induction state (ratio of the photosynthetic rate achieved after 1 minute exposure to high light to the steady-state assimilation rate achieved after 20 minutes exposure). Both photosynthetic induction state and the initial rubisco activity varied in parallel with stomatal conductance. By contrast there was no correlation between total rubisco activity and stomatal conductance. The results indicate that induction state, as determined by the light regulation of both rubisco activity and stomatal conductance, is an important limitation to the ability of leaves in a soybean canopy to respond to light transients that occur during sunflecks.

The light environment within crop canopies is characterized by fluctuations due to sunflecks, with frequencies ranging from 0.1 to 30 Hz (5, 15). Within soybean canopies, leaves may receive more than 1000 sunflecks per d with a median duration of less than 10 s (20). For leaves within the canopy, sunflecks contribute 60 to 80% of the available PFD². Despite the highly dynamic nature of the light environment, nearly all studies of the photosynthetic performance of crops have examined only steady-state conditions. Models linking canopy structure to leaf photosynthesis rely on sunlit leaf area as a parameter but have been inherently steady-state solutions (7, 14). Studies of forest understory plants have demonstrated that the controls on photosynthesis during transient light changes are quite different than those under steady-state conditions (17, 19). After long periods in low light a photosynthetic induction requirement can limit the capacity of leaves to respond to sunflecks (3). However, during short sunflecks, postillumination CO₂ fixation can contribute substantially to assimilation (4).

The objective of this study was to examine the extent to which the induction requirement of CO₂ assimilation limited the capacity of leaves within a soybean canopy to respond to transient light increases. Since the induction response is primarily determined by light modulation of rubisco activity and the light-mediated increase in stomatal conductance (10, 23), variations in these parameters within the canopy were examined.

MATERIALS AND METHODS

Plant Material

Plots of soybeans (Glycine max var Williams) were planted in rows spaced 75 cm apart and oriented east-west. Three 5 by 20 m plots were planted on May 15, June 15, and July 2, 1988. The plots were irrigated two to three times weekly and were fertilized with a commercial N:P:K (20:10:10) fertilizer at the time of planting and 3 weeks later for a total amount of 200 kg ha⁻¹. Measurements were started after the canopies were fully developed to a height of 1 to 1.2 m and plants were in the flowering stage.

Photosynthesis Measurements

A and gₛ were measured on leaves at about midpoint in the canopy with a transportable, open-system gas exchange apparatus that has been described previously (16, 21). A single attached leaflet was enclosed in a temperature controlled, nickel-plated aluminum chamber with a gas window in the top. Fans within the chamber kept the air well mixed and gave a high boundary layer conductance for water vapor of 2 mol m⁻² s⁻¹. Partial pressures of CO₂ and water vapor in the airstreams were measured with a Binos infrared CO₂ analyzer and Vaisala Humicap thin-film capacitance humidity sensors, respectively. Gas mixtures were obtained by mixing 5% CO₂ in air with CO₂-free air with Tylan mass flow controllers. Humidity of the air was controlled by first saturating with

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² Abbreviations: PFD, photon flux density; rubisco, ribulose-1,5-bisphosphate carboxylase oxygenase; CA1P, 2-carboxyarabinitol-1-phosphate; A, photosynthetic CO₂ assimilation rate; gₛ, stomatal conductance.
water vapor and then condensing to a known dew point temperature in a thermostatted condenser column. Signals from the transducers were logged with a Hewlett-Packard portable computer and data acquisition system. All calculations of gas exchange rates were based on the equations given by von Caemmerer and Farquhar (26).

Determination of the induction state of leaves under their natural light regimens within the canopies was done with a LI-6000 photosynthesis system in a closed-system mode. A ventilated, 1-L chamber which enclosed 14 cm² of leaf area from a single leaflet was used for the measurements. Three successive measurements, each consisting of the time required for the CO₂ partial pressure to decrease by 1 μbar bar⁻¹, were taken. During these measurements, flow through the desiccant was adjusted so that the humidity in the chamber typically increased only gradually. The calculated values for A, gs, and intercellular CO₂ pressure were stored in the system memory and later transferred to a personal computer for further analysis. All measurements were made on cloudless days.

The relative induction state of photosynthesis and stomatal conductance were determined by first measuring A and gs, under the prevailing nature light within the canopy. The canopy was then parted to expose the leaf to full sunlight and the measurements were repeated 1 min later (A₁, gs₁). The chamber remained on the leaf during this short period between measurements, but a valve was opened so that ambient air was drawn in. Leaf temperatures typically rose 2 to 3°C. After the second measurement the chamber was removed but the canopy remained parted. A third measurement was made 20 min after parting of the canopy, and yielded the fully induced assimilation rate and corresponding gs values (A₂₀, gs₂₀). Time-course measurements made with a controlled temperature cuvette and the open system gas exchange apparatus revealed that a 20 min exposure to high light was adequate for full induction of A. The relative induction state was calculated as 100 × A₁/A₂₀. A similar ratio for gs was calculated to express the comparable change in stomatal conductance during induction.

Rubisco and Measurements

Leaves were sampled for measurement of rubisco initial and total activities, carbamylation percentage, and content using freeze-clamp tongs. These tongs consisted of 35 mm diameter × 30 mm long copper rods that were chilled in liquid nitrogen prior to clamping onto the leaf via scissor-type handles that aligned the ends of the rods. The end of one rod had a small ridge across it and was surrounded by a stainless steel rim so that two frozen half-discs of leaf resulted. One-half disc was used for the assays and was stored in liquid N₂ prior to assay, which was completed within 1 week of sampling.

Measurement of Rubisco activities, carbamylation, and content were made using procedures described by Kobza and Seemann (12). The activation state (carbamylation percentage) of rubisco was calculated as the substrate-saturated initial molar activity, measured following rapid extraction into CO₂-free buffer, divided by the CO₂-Mg⁺⁺ activated molar activity. The Rubisco content was measured using the [¹⁴C]CAIP binding assay as described by Kobza and Seemann (12). Total molar activity was calculated as the measured activity divided by the molar concentration of rubisco on an equal leaf area basis. Total molar activity is dependent on the concentration of tight binding inhibitors such as CAIP in the leaf and changes in this value serve as an indicator of this mode of rubisco regulation. Conversely, measured carbamylation percentage is not strictly dependent on the concentration of these inhibitors (see ref 24), and therefore reflects regulation as a result of changes in the carbamylation state of the enzyme. Chl contents were determined according to the procedures of Arnon (1).

RESULTS

Spatial Variation in Photosynthetic Properties

Measurements of leaves selected at various heights in the canopy showed that gs increased with height above the ground (Fig. 1). This increase corresponds roughly to the increase in average PFD measured in the same canopy (20). The value of gs for individual leaves at any given height varied substantially, however. This variation occurred principally because of the high point to point variability of light within the canopy. Consequently, some leaves were or had been within sunflakes just prior to the measurements, and therefore had increased gs, whereas others had been in only shade light.

Coupled with the decrease in light penetration in the canopy were decreases in rubisco content, Chl a/b ratio and the ratio of rubisco protein to Chl (Fig. 2). The changes in these parameters with height are consistent with a sun-shade differentiation of leaves within the canopy. In contrast to rubisco content, Chl content remained relatively constant with leaf height within the canopy.

![Figure 1. Relationship between gs and height above ground for measurements made at the prevailing PFD during midday in a soybean canopy.](image-url)
Transient Responses to Shade Periods

The time course of A and gs were markedly dependent on the length of the previous low light period (Fig. 3). In Figure 3A, the leaf had received approximately 3 h of shade light (<25 μmol photons m⁻² s⁻¹) in the morning prior to the increase in PFD to 1200 μmol photons m⁻² s⁻¹. A increased gradually over the next 20 min to a steady-state while gs required nearly 40 min to approach a maximum steady-state value. Thus, the leaf exhibited the classic photosynthetic induction response. In Figure 3, B to F, leaves were initially brought to full induction and then shaded before again increasing the PFD. Assimilation rate decreased immediately upon shading; most of the lag in the response is due to the relatively slow response of the gas exchange system rather than the response of the leaf per se (gs also decreased but more gradually than assimilation). Nevertheless, after 16 min in low light, gs approached values observed in Figure 3A where the leaf had not seen high PFD in the previous 13.5 h.

Following reillumination with high PFD, the A achieved 1 min after the light increase was strongly dependent on the length of the low light period. After only 1 min of shade, the assimilation rate returned to 95% of the maximum steady-state rate within 1 min. However, with longer shade periods the initial rapid increase in A was smaller and depended on the duration of the shade period. After the initial increase, a further gradual increase in A and gs occurred until steady-state rates of A were again reached. These results show that a significant induction requirement developed after only a few minutes in shade light. This induction requirement constrains the ability of the leaves to utilize a sudden increase in PFD, as would occur during sunflecks.

The variation in the induction state of leaves subjected to natural light regimes in the canopy was measured by first determining A and gs for leaves under natural illumination with the LI-COR 6200 photosynthesis system. The canopy was then parted to expose the leaf to full sunlight and A and gs were measured after 1 and 20 min exposure. The measurements at 1 min and 20 min were under essentially identical light conditions and, thus, any differences in assimilation and gs were due to the induction occurring during the exposure to high PFD. The 20 min exposure was deemed long enough to allow achievement of steady-state light saturated assimilation rates (Fig. 3) but it is likely that gs would have increased further with longer exposure. However, this further increase would have had little or no effect on the assimilation rate.

The assimilation rates of leaves prior to parting of the canopy varied substantially since some leaves were either partially or wholly within sunflecks at the time of sampling. There was wide variation in the induction state (ratio of A at 1 min to that at 20 min) of leaves within the canopy, with some having values as low as 10% while others had values as high as 90% (Fig. 4). Induction state measured for assimilation was correlated with a similar induction state for gs. Leaves

Figure 2. Dependence of Chl content (A), rubisco content (B), the ratio of rubisco to Chl contents (C), and Chl a/b ratio (D) on leaf height above ground in a closed soybean canopy.

Figure 3. Time courses of assimilation (C) and stomatal conductance (V) following different periods of shade. A. After 10.5 h of darkness and 3 h of shade light; B to F, leaves were brought to steady-state assimilation rates in full sunlight (1500–1800 μmol photons m⁻² s⁻¹) before shading (20–30 μmol photons m⁻² s⁻¹) for various durations. Leaf temperatures were 25 to 28°C and the leaf-air vapor pressure gradient was 12 to 17 mbar bar⁻¹. The PFD was increased and decreased at the arrows.
that were in sunflecks at the time of the initial measurements had a higher induction state for assimilation at a given induction state for \( g_s \) than leaves that were in the shade. The wide variation in induction state and the relative conductance value was due to the past light history; some leaves that were not in sunflecks at the time of sampling undoubtedly had been just prior to sampling. The induction state and relative conductance should be highest in those leaves that had been exposed to significant periods of sunfleck activity just prior to the measurements.

**Light-Reduction of Rubisco**

A similar experiment was conducted to examine changes in rubisco regulation in the canopy. It was not possible to follow simultaneously the time course of rubisco activation and stomatal conductance in a single leaflet, so a trifoliate was utilized. The trifoliate leaves selected were all shaded (PFD of 15–50 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \)) and in canopy locations where they had almost certainly received few sunflecks in the 0.5 to 1 h prior to the measurements. Stomatal conductance was measured on one leaflet, which was then freeze clamped for later determination of rubisco activity. The canopy was then parted to expose the remaining two leaflets to high PFD (1700–2000 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \)) and 1 min later a second leaflet was freeze clamped. Because of the short time, it was not possible to also measure \( g_s \) on this leaflet. For the third leaflet, however, \( g_s \) was measured just prior to freeze-clamping at 20 min after the canopy was parted.

Leaflets sampled in the shade had low initial rubisco activities and low \( g_s \) (Table I). After 1 min in high light there was a small increase in initial rubisco activity which was followed by a much larger increase after 20 min of high light. Stomatal conductance also increased substantially during the 20 min of high light. The increase in initial rubisco activity was explained primarily by an increase in the extent of CO\(_2\)-Mg\(^{2+}\) carboxylation in vivo. Total activities did not vary much or vary in a consistent fashion with time of light exposure. Therefore, removal of a tight-binding inhibitor such as CA1P cannot account for more than a small part of the observed increase in initial rubisco activities.

Leaves sampled throughout the canopy showed wide variation in initial rubisco activity, percent carboxylation and conductance (Fig. 5). Both initial rubisco activity and percent carboxylation were significantly correlated with conductance (Fig. 5, A and B) whereas total rubisco activity and conductance were not correlated (Fig. 5C).

**DISCUSSION**

Photosynthetic CO\(_2\) assimilation in a canopy is strongly dependent on light penetration. Photosynthetic acclimation in response to the gradient in PFD within the canopy as well as age-dependent changes, results in differences in photosynthetic capacity, specific leaf weight, and rubisco content in leaves at the top as compared to leaves within a canopy (8). The decreasing photosynthetic capacity, rubisco content, and changes in Chl to rubisco ratios with depth in the canopy are similar to results obtained when soybeans were grown at different photon flux densities (6, 22, 25). This sun-shade differentiation limits the capacity of lower leaves to utilize high PFD. However, from this study, it is apparent that photosynthetic induction state of lower canopy leaves poses

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**Table I. Response of Rubisco Activity (Initial and Total) and Stomatal Conductance of Glycine max to a Sudden Increase in PFD Resulting from a Parting of the Canopy**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conductance ( \text{mol m}^{-2} \text{s}^{-1} )</th>
<th>Rubisco Initial Activity ( \text{s}^{-1} )</th>
<th>Total Activity ( \text{s}^{-1} )</th>
<th>Carbamylation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.167 ± 0.028</td>
<td>3.51 ± 0.45</td>
<td>11.56 ± 1.06</td>
<td>31.4 ± 3.9</td>
</tr>
<tr>
<td>1</td>
<td>Not measured</td>
<td>5.34 ± 0.43</td>
<td>10.89 ± 0.90</td>
<td>51.5 ± 5.3</td>
</tr>
<tr>
<td>20</td>
<td>0.690 ± 0.060</td>
<td>10.11 ± 0.46</td>
<td>13.57 ± 0.97</td>
<td>78.3 ± 6.2</td>
</tr>
</tbody>
</table>

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Figure 5. Relationships between initial rubisco activity (A), percent carboxylation of rubisco (B), and total rubisco molar activity (C) and stomatal conductance for leaves within a soybean canopy. The units for rubisco molar activity (initial and total) are mol CO₂ mol rubisco⁻¹ s⁻¹.

an additional limitation on the capacity to utilize high PFD occurring transiently during sunflecks.

After a long period at low PFD, the CO₂ assimilation rate in leaves has an induction phase following a light increase in which photosynthesis only gradually approaches the maximum rate (3, 27). In the shade plant Alocasia macrorrhiza, this induction response is known to be due to both a fast activating component that is important in the first minute (11), and increases in gₛ and rubisco activity that are important over longer times (10). Light modulated increases in rubisco activity are generally complete in 5 to 10 min, whereas gₛ continues to increase for longer periods. Light-modulation of Rubisco involves both an activation caused by carboxylation and an inhibition caused by the tight binding of CA1P (12, 13). Plants differ in the amount to which each regulatory mechanism contributes to the light modulation of rubisco (12); soybean has been shown to be variable between cultivars (2). In our measurements, most of the regulation of rubisco activity could be accounted for by carboxylation whereas metabolism of CA1P appeared to be relatively unimportant.

The induction state of a leaf is determined by the immediate past light environment. The induction state declines when an induced leaf is shaded for longer than a minute or so and can be increased by exposure of a shaded leaf to a series of sunflecks (18). The light environment within a soybean canopy is characterized by periods of rapid sunfleck activity separated by periods of shade (20). The spatial variability is also large so that at any given time, leaves at the same height in the canopy could be expected to be at quite different induction states. This is shown in Figure 4 where leaves sampled at 40 to 70 cm height in the canopy had induction states ranging from 20 to 80% with those leaves in sunflecks at the time of sampling having a higher induction state than leaves sampled in the shade. These data indicate a close relationship between induction state and a relative stomatal opening. While rubisco initial activity could not be directly compared to induction state, the correlation with gₛ (Fig. 4A) indicates that it was also covarying with induction state in the canopy.

The controls on photosynthetic induction state in fluctuating light are not well known. Under steady-state conditions, initial rubisco activities and photosynthetic rate usually exhibit similar light-dependent responses in soybean (22, 25). However, initial rubisco activities in soybean leaves were maintained at higher values in a fluctuating than in a constant light regime where both had the same average PFD (6). This result can be explained by the faster increase than decrease in initial rubisco activities following increases and decreases in PFD, respectively (13, 23). Stomatal conductance can also exhibit a hysteretic response, with a faster opening than closing, especially in response to brief lightflicks (9). As a consequence of these hysteretic responses it is unlikely that induction state, and hence the capacity to utilize sunflecks, can be readily predicted from steady-state measurements. Further experiments on the dynamics of the induction response in transient light will be required to assess their quantitative role in canopy photosynthesis.

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LITERATURE CITED