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Gas Exchange Analysis of the Fast Phase of Photosynthetic Induction in *Alocasia macrorrhiza*[1]

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ABSTRACT

When leaves of *Alocasia macrorrhiza* that had been preconditioned in 10 micromoles photons per square meter per second for at least 2 hours were suddenly exposed to 500 micromoles photons per square meter per second, there was an almost instantaneous increase in assimilation rate. After this initial increase, there was a secondary increase over the next minute. This secondary increase was more pronounced in high CO_2 (1400 microbars), where assimilation rate was assumed to be limited by the rate of regeneration of ribulose 1,5-bisphosphate (RuBP). It was absent in low CO_2 (75 microbars), where RuBP carboxylase/oxygenase (Rubisco) was assumed to be limiting. It was therefore concluded that it represented an increase in the capacity to regenerate RuBP. This fast-inducing component not only gained full induction rapidly, but also lost it rapidly in low photon flux density (PFD) with a half time of 150 to 200 seconds. It was concluded that in environments with fluctuating PFD, this fast-inducing component is an important factor in determining a leaf's potential for photosynthetic carbon gain. It is especially important during brief periods (<30 seconds) of high PFD that follow moderately long periods (1 to 10 minutes) of low PFD.

The light environment in forest understories typically consists of long periods of low PFD,[3] separated by periods when short lightflecks lasting from less than a second to several minutes are frequent (1, 3, 14, 15). For the first one or several of these lightflecks, photosynthetic carbon gain by a leaf is likely to be limited by a low photosynthetic induction state. This limitation, however, is gradually removed during subsequent lightflecks (4).

It has been shown previously (4, 10) that increases in both stomatal and biochemical factors are jointly responsible for induction. At least part of the biochemical limitation may correspond to Rubisco activation which was found to increase to a similar extent and with a similar time constant (18) as the biochemical limitation inferred from gas exchange measurements (10). These observed responses were relatively slow, however, occurring over many minutes. Much other work on induction, on the other hand, has reported responses that are completed in shorter times (6, 16, 20). This indicates that there might be more than one factor involved in induction.

In previous work, Chazdon and Pearcy (4, 5) and Kirschbaum and Pearcy (10) used gas exchange techniques to investigate the slow phase of induction from about 1 to 45 min. These studies indicated that there might also be a fast phase of induction that is complete within the first minute after an increase in PFD. This fast induction phase is difficult to analyze with gas exchange techniques because instrument response times are typically so slow as to obscure the underlying plant response. In the present study, measurements of the induction response were made in a gas-exchange system modified to resolve very fast responses. This investigation of the fast induction phase extends our work done previously on the dynamics of photosynthesis of the Australian tropical understory plant, *Alocasia macrorrhiza* (4, 5, 10, 18).

MATERIALS AND METHODS

Plant Material. In all experiments, approximately 1-year-old plants of *Alocasia macrorrhiza* (L.) G. Don, grown from seed collected in a tropical rain forest near Atherton, Queensland, Australia, were used. The plants were grown in 4-L pots in a 50:50 mixture of pumice rock and potting soil, fertilized with half-strength Hoagland solution twice a week, and watered daily. All plants were grown under shade cloth in a glasshouse that reduced natural lighting to 30 μmol photons m^{-2} s^{-1} at maximum.

Gas Exchange. Gas exchange measurements were done on young, fully expanded leaves in an open gas exchange system as described previously (10), but with the following modifications. The depletion of CO_2 by the leaf was measured with a LI-COR 6250 infra-red gas analyzer. The leaf was enclosed in a flow-through chamber with a very small volume (4 ml), and gas exchange was measured only on the lower surface of the hypostomatous leaves of *A. macrorrhiza*. With this configuration, a high flow rate, and the LI-COR analyzer, the system response to a step change in CO_2 injection of a low flow of approximately 1% v/v CO_2 into the chamber was complete in 2 to 3 s (responses shown in Fig. 1 and 2). With further modifications, the response time of the system to a step change in CO_2 injection was further reduced to 1 to 2 s. This configuration was used for later work that is presented in Figures 3 to 5.

The time course of induction was measured by first conditioning the leaf to a background light of 10 μmol photons m^{-2} s^{-1} (low PFD), supplied by an incandescent light bulb, and then increasing the PFD to approximately 500 μmol photons m^{-2} s^{-1} (high PFD), supplied by a 2.5 kW water-cooled xenon-arc lamp. This high PFD was more than saturating for steady-state photosynthesis.

To investigate the time course of induction loss, leaves were kept in high PFD until steady assimilation rates had been achieved. They were then given a period of low PFD, before PFD was increased again. Assimilation rates were recorded just before the PFD was increased and 5 and 90 s after the increase. The increase in the assimilation rate from 0 to 5 s was taken as a measure of the induction state of the fast-inducing component.

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[3] Abbreviations: PFD, photon flux density (of photosynthetically active radiation); RuBP, ribulose-1,5-bisphosphate; Rubisco, RuBP carboxylase/oxygenase (EC 4.1.1.39).

The assimilation rate after 90 s was taken as a measure of the induction state of the slowly inducing component. An exponential curve was fitted to the data, and all data were expressed relative to the extrapolated rates at time 0.

Lightfleck utilization efficiency was determined by first integrating the CO_2 assimilation occurring in response to a lightfleck and then subtracting the assimilation that would have occurred at low light. A comparable value for steady-state assimilation was calculated as the difference in assimilation rates at the lightfleck PFD and at the low light PFD times the lightfleck length. The efficiency was then calculated as the ratio of the total attributable to the lightfleck to the value calculated for the steady state, expressed as a percentage. Details of the procedure are given in Chazdon and Pearcy (5).

RESULTS

When PFD was increased from 10 to 500 μmol photons m^{-2} s^{-1}, there was an initial almost instantaneous increase in assimilation rate, followed by a slower secondary increase that was completed in about 60 s (Fig. 1). This secondary increase was followed by the further and much slower increase over the next 30 to 60 min that has been studied previously (4, 10). The initial increase was completed within 2 to 3 s, with a time course that was similar to the system response to a step change in CO_2 injection into the chamber (inset in Fig. 1). The plant response was therefore likely to have been completed in a much shorter time. The change from 3 to 60 s, however, was much slower than the system response, and as there was no discernible change in stomatal conductance over this interval (data not shown; see Ref. 9), it must have been caused by biochemical changes.

This biochemical change could correspond to a change in either the activation state of Rubisco or the capacity to regenerate RuBP (7). We attempted to distinguish between these possibilities by examining the CO_2 dependence of this fast induction phase. At high partial pressure of CO_2, the rate of RuBP regeneration is likely to be limiting the CO_2 assimilation rate, while the activity of Rubisco even with a fairly low activation state should be high enough to sustain the assimilation rate (7). The converse is true at a low partial pressure of CO_2, where Rubisco is likely to be rate-limiting for CO_2 assimilation, while the smaller required rate of RuBP regeneration should be sustainable even by a RuBP regeneration system that is partly inactivated. Effects due to some activation of RuBP regeneration should then be more apparent at high partial pressure of CO_2 and absent or at least less pronounced at low partial pressure of CO_2. Conversely, if the fast induction phase is due to Rubisco activation, it should be more apparent at low partial pressure of CO_2 and absent or less pronounced at high partial pressure of CO_2.

At high partial pressure of CO_2 (Fig. 2A), assimilation rate increased initially to 2.5 μmol m^{-2} s^{-1}, which was followed by a slower increase over the next 60 to 90 s. The response was therefore similar to that observed at 350 μbar CO_2 (Fig. 1), except for the overall higher assimilation rates and the more prolonged increase. In low CO_2 (Fig. 2B), on the other hand, only the initial almost instantaneous increase was apparent. This pattern is consistent with the hypothesis that the increase in assimilation rate over the first minute was due to an induction of one or several components in the RuBP-regeneration system.

In terms of the utilization of lightflecks, it is important to know not only the rate at which the fast-inducing component can be induced, but also the rate at which induction is lost when an initially fully induced leaf is transferred to low PFD. Induction loss was examined by equilibrating leaves in high PFD and then exposing them to low PFD for varying lengths of time. Figure 3 shows typical responses to a PFD increase in 21% and 2% O_2, the assimilation rate in low PFD was substantially lower if the leaf had been in low PFD for just 40 rather than 300 s (Fig. 3, upper panel). This was primarily due to post-illumination CO_2 release from the glycolate pathway (2), since it was not observed in 2% O_2 (Fig. 3, lower panel) where photorespiration is largely suppressed (7). In *A. macrorrhiza*, the rate of CO_2 release from this pathway decreased with a half-time of about 20 seconds (M. U. F. Kirschbaum, unpublished). For measurements in 21% O_2, there was, therefore, still appreciable CO_2 release after 40 s, which lowered the apparent assimilation rate, but after 300 s it was negligible. In 21% O_2, when PFD was increased after the leaf

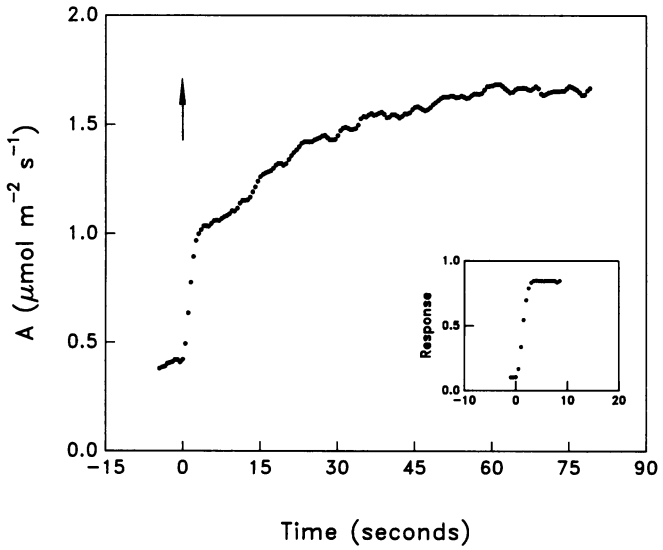

FIG. 1. Time course of assimilation rate (A) for the first 90 s following a step increase in PFD. The arrow in the figure denotes the time when PFD was increased. Points were obtained twice per second. Ambient partial pressure of CO_2 was 350 μbar. The inset in the figure shows the system response to a change in the rate of CO_2 injection into the chamber with the same flow rate and system configuration as was used for the experiment.

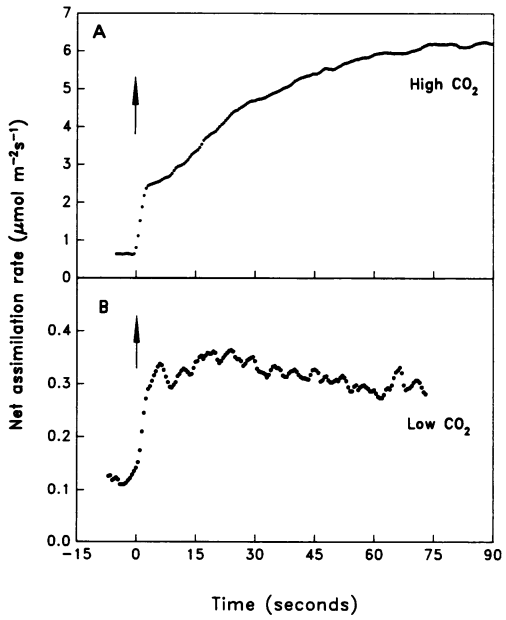

FIG. 2. Time course in assimilation rate (A) following a step increase in PFD with the leaf either in 1400 μbar (in A) or 75 μbar CO_2 (in B). Arrows in the figure denote the time when PFD was increased. Points were obtained twice per second. The oscillations apparent in (B) are largely or entirely an instrumental artifact.

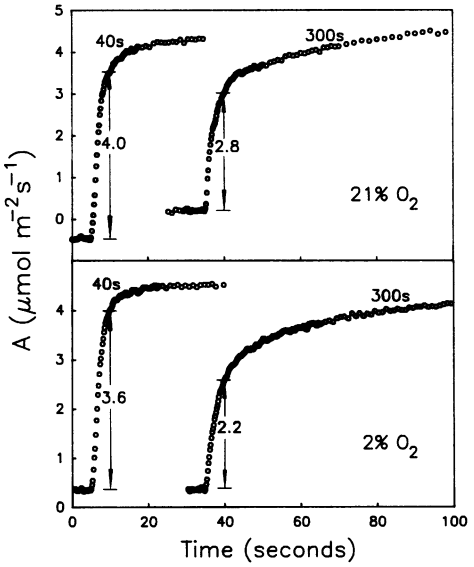

FIG. 3. Assimilation rate (A) following a step increase in PFD in leaves that had been in low PFD for 40 or 300 s, as indicated in the figure. The upper figure was obtained with a leaf in 21% O_2, and the lower figure with a different leaf in 2% O_2. The PFD was increased at 5 and 35 s. Additional numbers in the figure give the increase in assimilation rate over the first 5 s in high PFD. Points were recorded 5 times per second. The system response to a step change in CO_2 injection was completed in 1 to 2 s.

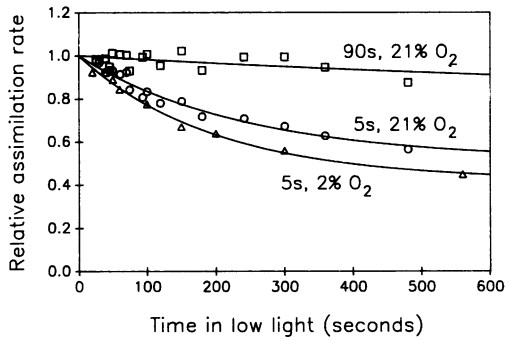

FIG. 4. Time course of induction loss of the fast-inducing (○) and the slowly inducing component (□) in 21% O_2, and of the fast-inducing component in 2% O_2 (△). The increase in assimilation rate over the first 5 s after an increase in PFD was taken as a measure of the induction state of the fast-inducing component. The assimilation rate 90 s after the increase in PFD was taken as a measure of the induction state of the slowly inducing component. Data at 2% and 21% O_2 had been obtained with different leaves. Further details are given in "Materials and Methods".

had been in low PFD for 40 s assimilation rate increased by 4.0 μmol m^{-2} s^{-1} within 5 s, and steady-state rates were reached within about 20 s. After 300 s in low PFD, on the other hand, assimilation rate increased by only 2.8 μmol m^{-2} s^{-1} over the first 5 s after the increase in PFD, and attainment of steady-state rates required more than 40 s. In 2% O_2, the increase in assimilation rate over the first 5 s after the increase in PFD decreased similarly as in 21% O_2 with length of the low-PFD period.

Data obtained with different times in low PFD are presented in Figure 4. It shows the rate of induction loss, measured as the relative increase in the assimilation rate over the first 5 s following an increase in PFD after the leaf had been in low PFD for different lengths of time. Induction of the fast-inducing component was lost in an exponential manner with a half-time between

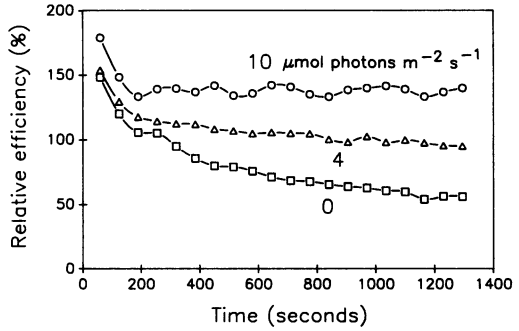

FIG. 5. Time course of utilization efficiency of 5-s lightflecks separated by low PFD periods of different PFD. All measurements were obtained on the same leaf on the same day. In each case, the leaf had reached a steady assimilation rate at 500 μmol photons m^{-2} s^{-1} before being given a series of lightflecks. Each lightfleck consisted of 5 s at 500 μmol photons m^{-2} s^{-1} and followed 60 s of low PFD. Different values of PFD were used between lightflecks, as indicated in the figure. The first low-PFD period was given at time 0.

150 and 200 s. Induction was not lost completely, however, but only to a minimum of 40 to 50% of full induction. This is consistent with the responses shown in Figures 1 and 2A, which showed that some residual, fairly high induction state must have been maintained in low PFD to allow the almost instantaneous increase in assimilation rate in an uninduced leaf when PFD was increased (Figs. 1 and 2A).

In 2% O_2, loss of induction of the fast-inducing component was even more pronounced (Fig. 4). Since photorespiration is largely suppressed in 2% O_2, this indicates that the observed induction loss of the fast-inducing component is not due to changes in photorespiratory CO_2 release with different lengths of time in low light. For comparison, Figure 4 also shows the relative assimilation rates obtained 90 s after the increase in light. After 90 s, the fast-inducing component had been fully induced so that the rates at that point were a measure of the induction loss due to inactivation of Rubisco and stomatal closure. It declined only slowly, still being more than 90% of its maximum after 500 s in low PFD, which is consistent with previous work (4).

Since it is in environments with variable PFD that changes in induction have the greatest effect on leaves' photosynthetic performance, it is of particular interest how lightfleck utilization efficiency is affected by induction. Fully induced leaves that were given a single 60-s low-PFD period were able to utilize 5-s lightflecks 1.5 to 1.8 times as efficiently as the same photon flux in the steady state (Fig. 5). However, induction was lost rapidly over the first three minutes, and subsequent lightflecks were utilized less efficiently. Efficiency decreased especially strongly when the leaf was in the dark between lightflecks, while it decreased only slowly if the leaf received 10 μmol photons m^{-2} s^{-1} between lightflecks and was above steady-state efficiencies even after 1300 s (Fig. 5).

DISCUSSION

The data presented here indicate that there is also a fast-inducing component of assimilation that is saturated within 1 min of an increase in PFD in addition to the slower-inducing components previously reported for *A. macrorrhiza* (4, 10, 18). In the absence of studies of the time courses of enzyme activation and metabolite pool sizes during the first minute or so of induction, it is difficult to be specific about the biochemical nature of the fast-inducing component. A triose-phosphate utilization limitation to assimilation (19) can probably be ruled out on the grounds that assimilation during the first 60 s responded to increased CO_2 partial pressure (cf. Figs. 1 and 2A). It should

have been insensitive to CO_2 partial pressure if a triose-phosphate limitation were operational (8, 19). A role for Rubisco activation also appears to be ruled out by the CO_2-dependence of this fast induction, which is consistent with an induction of RuBP regeneration but not with Rubisco activation. Several of the enzymes in the Calvin cycle are known to be light activated (17). These enzymes are generally activated within about 1 min after a transfer to high PFD (11–13) and could therefore be responsible for an increased capacity for RuBP regeneration. An autocatalytic buildup of metabolites, which has been well documented in isolated chloroplast systems (6), could also be involved, although its importance *in vivo* has been questioned (20). Finally, the possibility of a limitation in ATP synthesis in this time scale cannot be discounted.

A particular problem in comparing the present results with previous biochemical work is that, in virtually all biochemical studies of induction, plant material was transferred from darkness to high PFD, which may differ significantly from the more ecologically relevant change from low to high PFD. As shown in Figure 5, there is a significant difference in the rate and extent of induction loss between responses in the dark and in low light.

In previous experiments, it was shown that the slow phase of induction was due to light activation of Rubisco and stomatal opening (10, 18). The results reported here show that a fast-activating component is also important in the induction response. The complexity of these various factors precludes generalized statements about the relative importance of one or the other in induction. The relative importance may differ from leaf to leaf due to variations in the minimum conductance in low PFD (9), and it may shift with preconditioning, such as the previous length and PFD of the low-light period or ambient humidity. After an increase in PFD, differences in stomatal conductance, for example, will primarily affect the balance of importance between stomatal and biochemical factors (10). But by also influencing the intercellular partial pressure of CO_2, stomatal conductance also has an effect on the required rate of RuBP regeneration (7, 8). This in turn would affect the relative importance of the induction requirements of Rubisco and RuBP regeneration.

Previous research (5, 15) has shown that the timing and duration of lightflecks is an important determinant of the efficiency with which they are utilized for photosynthesis. The results here show that, additionally, the PFD during the low-PFD periods can have a significant effect on the efficiency of lightfleck utilization. In forest understories, the PFD during periods of diffuse photon flux is typically between 5 and 15 μmol photons m^{-2} s^{-1} so that the fast induction and induction loss effects reported here would be of importance. Thus, in order to gain a full understanding of the controls on lightfleck utilization in forest understories, the PFD and duration of the periods of low diffuse light must be known as well as the properties of the lightflecks.

It can be said in general that the importance of the fast-inducing component would be greatest for short lightflecks of less than 30 s that are too short to lead to significant activation and after low light periods from 1 to 10 min, which are long enough to lead to significant loss of induction of the fast-inducing component but too short for the quantitatively more important induction loss due to stomatal closure and deactivation of Rubisco (4, 10, 18). In general, the quantitative importance of this component can only be assessed within a given set of changes in PFD and other environmental conditions and leaf parameters.

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