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Nonsteady-State Photosynthesis following an Increase in Photon Flux Density (PFD)[1]

Effects of Magnitude and Duration of Initial PFD

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ABSTRACT

The response of photosynthesis to an increase in photon flux density (PFD) from low to higher PFD was investigated using spinach (*Spinacia oleracea* L.). The time-course for this response was qualitatively similar to that observed for a dark-to-high-PFD transition, showing an initial, rapid increase in photosynthesis over the first minute or so, followed by a slower increase lasting 5 to 10 minutes. This slow increase was approximately exponential and could be linearized using a semilogarithmic plot. The relaxation time (τ) for this slow phase was found to be a function of the starting PFD value. At starting PFD values below approximately 135 micromoles per square meter per second (including darkness), τ for the slow phase was approximately twice that observed for starting PFD values above 135 micromoles per square meter per second. This indicates a slower approach to steady state for leaves starting at PFD values below this threshold and a greater loss of potential photosynthesis. τ was relatively insensitive to starting PFD values below or above this transition value. The contribution of the slow phase to the total increase in photosynthesis following a low-to-high-PFD transition increased approximately exponentially with time at the lower PFD. The τ for the increase in the contribution of slow phase was determined to be 10.1 minutes. The implications of these data for activation and deactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase and for the functioning of the leaf in a fluctuating light environment are discussed.

Plants growing in both natural and agricultural systems are often exposed to conditions of changing PFD.[2] The rate of photosynthetic carbon assimilation also changes under these conditions, and if the period between fluctuations in PFD is short, then the photosynthetic rate may seldom approach a steady state. This situation has been shown to arise for plants growing beneath dense canopies where photosynthesis during sunflecks may account for more than 40% of the total carbon gain (1, 8). It is likely, therefore, that under such circumstances carbon assimilation will be affected not only by the period of PFD fluctuations, but also by the response times of the biochemical processes that limit the rate of nonsteady-state photosynthesis (3, 13).

The approach of photosynthesis to a new steady-state rate following an increase in PFD appears to reflect at least three basic phases. The first phase responds relatively rapidly (within the first minute or so) to the change in conditions and is thought to involve the autocatalytic buildup of photosynthetic carbon reduction cycle intermediates (6). There is good evidence that the second, slower phase involves an increase in the activity of Rubisco, a process that requires at least 10 to 15 min for completion (11, 13). The slowest of the three phases has been attributed to the change in c_i effected by slowly relaxing stomatal conductance (5). Under some conditions, however, changes in stomatal conductance do not substantially affect the time course of photosynthesis following an increase in PFD (9, 13). When the average period of sunflecks is several minutes, the most important determinant of the total nonsteady-state carbon gain will likely be the rate at which Rubisco activity increases.

The evidence that changes in Rubisco activity are important in determining the time course of nonsteady-state photosynthesis following an increase in PFD comes from two types of experiments. In the first, plants that had been held in darkness for an hour or so were exposed to a sudden increase in PFD, and leaf samples were taken at different times after this treatment. Measurements on these leaf samples showed that an increase in the proportion of Rubisco in the catalytically active form occurred in parallel with the slow phase of the assimilation-*versus*-time time course (11, 13). The second type of experiment involved modifying the amount of active Rubisco present at the onset of illumination by varying the length of the preceding dark period. Woodrow and Mott (13) showed that the increase in the assimilation rate attributable to the second phase was directly proportional to the amount of inactive Rubisco present at the onset of illumination. Taken together, these experiments indicate that Rubisco is almost certainly the primary determinant of the rate of photosynthesis during the second phase.

In this study, we examined the second phase of nonsteady-

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[2] Abbreviations: PFD, photon flux density; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; c_i, intercellular CO_2 concentration; τ, relaxation time; A, photosynthesis.

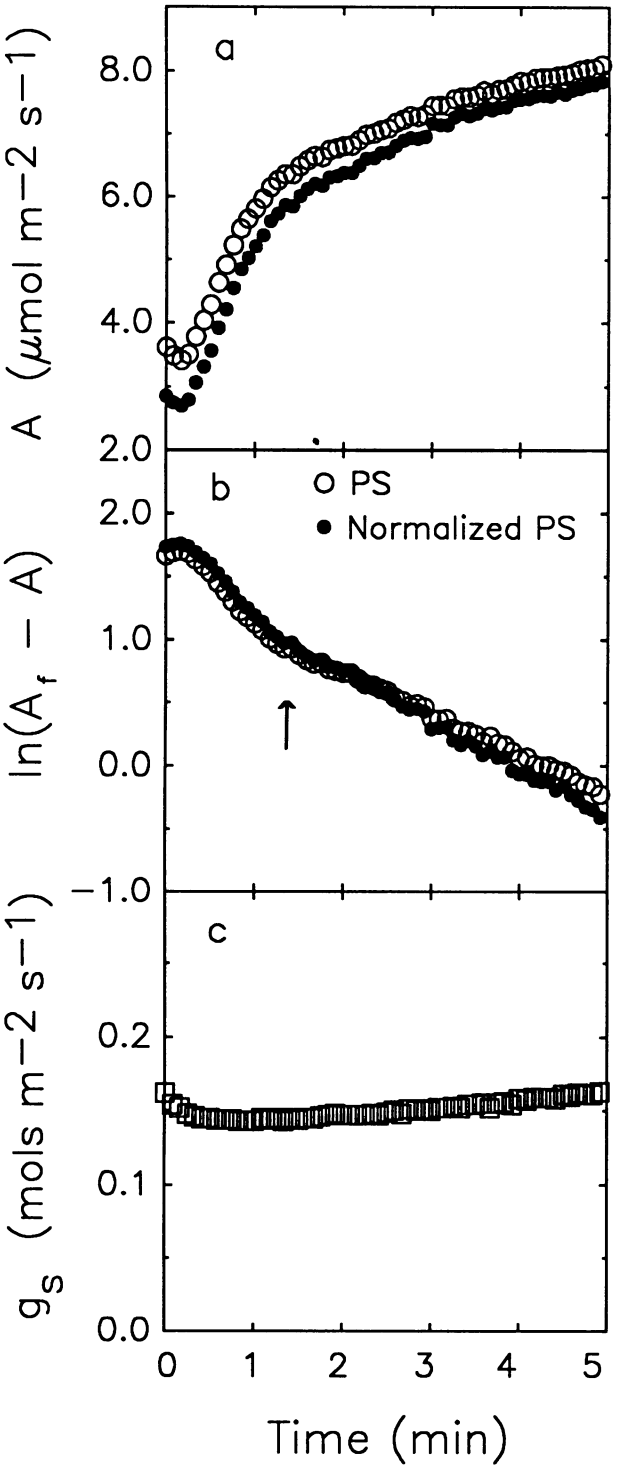

Figure 1. Time courses for (a) photosynthesis, (b) the natural logarithm of the difference between maximum and measured photosynthesis, and (c) stomatal conductance following an increase in PFD from 182 μmol m^{-2} s^{-1} to 690 μmol m^{-2} s^{-1}. Normalized photosynthesis values were adjusted to a c_i of 250 ppm by assuming a linear relationship for A and c_i through the measured points and a compensation point of 50 ppm. The adjustment was made to remove the effects c_i on photosynthesis rate. For the semilogarithmic timecourse ($\ln(A_f - A)$ *versus* time), an initial nonlinear portion of the graph corresponding to one or more initial fast phases of increased photosynthesis (see text introduction) is visible for approximately 1.5 min and is then followed by a linear portion (beginning at the arrow) corresponding to the slower phase of nonsteady-state photosynthesis.

state photosynthesis that occurs after an increase in PFD. The effect of different starting PFD values and different periods of time at the starting PFD were examined using the analysis presented by Woodrow and Mott (13). By identifying factors that affect the relaxation time of this phase, we gain insight into processes involved in the regulation of nonsteady-state photosynthesis.

MATERIALS AND METHODS

Spinacea oleracea L. was grown in a controlled-environment growth chamber with day and night temperatures of 25 and 20°C, respectively. The chamber photoperiod was 10 h, and the PFD during the photoperiod was 350 μmol m^{-2} s^{-1}. The plants were grown hydroponically in aerated, half-strength, modified Hoagland solution. Gas-exchange measurements were taken with a standard single-pass system described previously (7, 13). The rate of CO_2 assimilation was normalized to a c_i of 250 ppm by assuming that the relationship between photosynthesis rate and c_i was linear and passed through the compensation point (13). This procedure compensated for the effect of changes in c_i on photosynthesis. Normalized rates of photosynthesis are indicated by the superscript*.

The first set of experiments compared the effects of low PFD and darkness on the relationship between photosynthesis rate and time. Spinach leaves were illuminated for 1 h at 690 μmol m^{-2} s^{-1} (which was slightly below light saturation and was not photoinhibitory) and were next placed for 45 min in either darkness or low PFD (between 25 and 350 μmol m^{-2} s^{-1}). The leaves were then reilluminated at 690 μmol m^{-2} s^{-1}, and gas-exchange measurements were recorded at intervals of 5 s.

The second set of experiments tested the effect of time at low PFD on the photosynthesis-rate-*versus*-time trajectory. Spinach leaves were equilibrated for 1 h at a PFD of 690 μmol m^{-2} s^{-1}; the PFD was then decreased to 182 μmol m^{-2} s^{-1}. The leaves remained in low PFD for 10, 15, 30, or 60 min, after which the light intensity was returned to 690 μmol m^{-2} s^{-1}. Gas-exchange measurements recorded the response of the leaf to this final increase in PFD.

RESULTS

Increasing the PFD from an initially low value (182 μmol m^{-2} s^{-1}) to a higher one (690 μmol m^{-2} s^{-1}) caused the photosynthesis rate to increase over a period of minutes, eventually approaching a new steady state after approximately 20 min (open circles, Fig. 1a). Stomatal conductance also increased after the adjustment in PFD but changed little during the first 5 min of higher PFD (Fig. 1c). By plotting the logarithm of the difference between maximum and measured (normalized) photosynthesis rates ($\ln(A_f^* - A^*)$) *versus* time

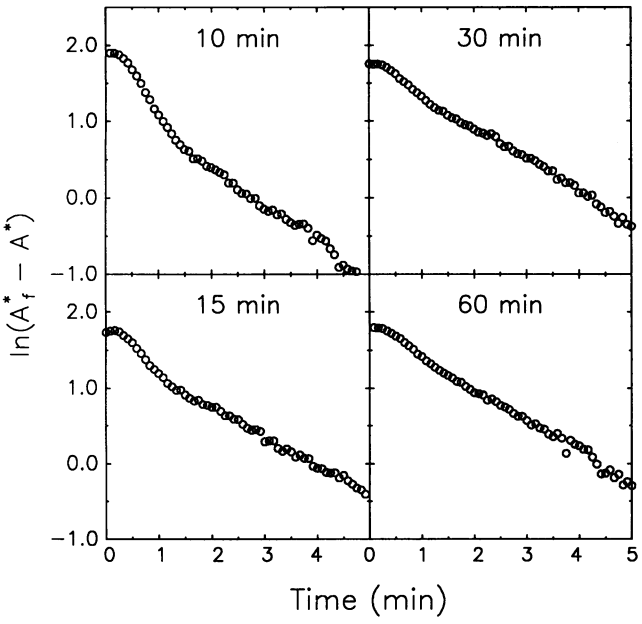

Figure 2. Natural logarithm of the difference between the maximum and the measured (normalized) photosynthesis rates as a function of time. The leaf was equilibrated for 1 h at 690 μmol m^{-2} s^{-1}, illuminated at 182 μmol m^{-2} s^{-1} for 10, 15, 30, or 60 min, and then reilluminated at 690 μmol m^{-2} s^{-1} at time zero. The length of time the leaves were at low PFD had no apparent effect on the slope of the slow phase of nonsteady-state photosynthesis.

(Fig. 1b), at least two phases of the increase in photosynthesis were resolved: one or more rapid phases lasting approximately 1 min and a slower phase lasting at least 5 min. This second phase is approximately exponential and appears as the linear portion of the semilog plot beginning 1 to 1.5 min (marked by the arrow in Fig. 1b) after the increase in PFD. The negative slope of this linear portion of the curve equals the first order rate constant for the exponential process governing this phase. The reciprocal of this value is the relaxation time (τ) for the phase (13). For most leaves this value was approximately 2 min; it was repeatable for a given leaf but varied somewhat among leaves.

The semilogarithmic plot can also be made using assimilation rates that have been normalized to a constant c_i (triangles, Fig. 1b) (see "Materials and Methods"), thus removing the effect of changes in c_i. This correction did not greatly affect the slope of the linear portion of the semilogarithmic plot in the present experiments (Fig. 1b), but may be very important in determining τ at low ambient CO_2 concentrations or for leaves with a relatively low stomatal conductance. It should be emphasized, however, that normalization of assimilation rates to a constant c_i generally extends the linear portion of the semilogarithmic plot, thus improving the accuracy of the calculation of τ.

The value of τ for the slow phase appeared to be independent of the length of time that a leaf was illuminated at 182 μmol m^{-2} s^{-1} before increasing the PFD to 690 μmol m^{-2} s^{-1} (Fig. 2). For these experiments leaves were allowed to reach a steady state at a PFD of 690 μmol m^{-2} s^{-1} and were then exposed to a PFD of 182 μmol m^{-2} s^{-1} for 10, 15, 30, or 60 min, after which the PFD was returned to 690 μmol m^{-2} s^{-1}.

In contrast, the contribution of the slow phase to the total increase in photosynthesis increased with time at the lower PFD. This was quantified by extrapolating the linear portion of the semilogarithmic plots (Fig. 2) to the ordinate axis. The inverse logarithm of this intercept is A_f^*—A_i, where A_f^* is the final, normalized, steady-state rate of photosynthesis and A_i is the rate of photosynthesis that would have occurred had there been no slow phase. In other words, the intercept gives the change in photosynthesis that is attributable to the slow phase (13). This value was divided by the total increase in photosynthesis to give the relative contribution of the slow phase to the total increase in photosynthesis. The results of several experiments similar to the one shown in Figure 2 were combined to define the relationship between the contribution of the slow phase to the total increase in photosynthesis and the time at low PFD. The percent increase in photosynthesis

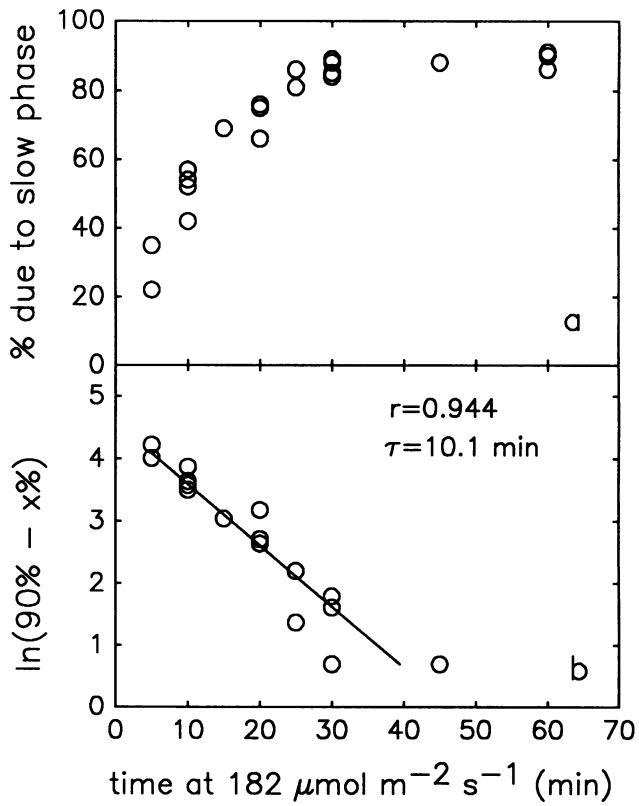

Figure 3. (a) The percentage of the total increase in photosynthesis rate that was due to the slow phase *versus* time at the lower PFD. (b) The natural logarithm of the maximum percentage contributed by the slow phase (90%, from panel a) minus the observed percentage contributed by the slow phase *versus* time at the lower PFD. Semilogarithmic plots (*e.g.* Fig. 2) were analyzed by taking the inverse logarithm of the *y*-intercept of the extrapolated linear portion of the curve (A_f − A_i) and dividing by the total increase in photosynthesis; this provides a quantitative estimate of the relative contribution of the slow phase to total photosynthesis. Leaves were treated as in Figure 2, but with a broader range of times at 182 μmol m^{-2} s^{-1}. The negative reciprocal of the slope of the lower graph is the relaxation time for the increase in the contribution of the slow phase to the total increase in assimilation.

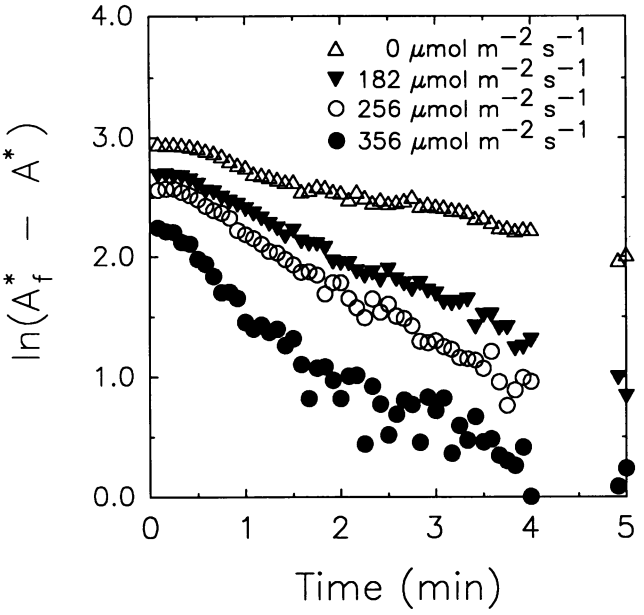

Figure 4. Natural logarithm of the difference between the maximum and the measured (normalized) photosynthesis rate as a function of time for different PFD values. The leaf was equilibrated for 1 h at a PFD of 690 μmol m^{-2} s^{-1}, placed in either darkness or low PFD for 45 min as noted in the figure, and then returned at time zero to 690 μmol m^{-2} s^{-1}. The slope of the slow phase (and hence τ) differed between the dark (0 μmol m^{-2} s^{-1}) and low-PFD time courses (182, 256, and 356 μmol m^{-2} s^{-1}), but did not differ appreciably among the latter.

attributable to the slow phase increased in an approximately exponential manner with increasing time at 182 μmol m^{-2} s^{-1} (Fig. 3a). The relaxation time for the relative contribution of the slow phase to the overall increase in photosynthesis was calculated from this exponential curve to be 10.1 min. (Fig. 3b, $r^2 = 0.89$, slope significantly different from zero at $P < 0.001$).

To determine the effect of starting PFD on the τ of the slow exponential increase in photosynthesis following an increase in PFD, leaves were held for 1 hr at 690 μmol m^{-2} s^{-1}, placed in either darkness or low PFD (182, 256, or 350 μmol m^{-2} s^{-1}), and then reilluminated at 690 μmol m^{-2} s^{-1} (Fig. 4). For the leaf used in Figure 4, τ for the dark-to-high PFD treatment was about 6 min and τ for each low-to-high-PFD treatment was about 2.5 min. These values were consistent and repeatable for that leaf, and the difference between the values was statistically significant at $P < 0.001$. For other leaves, particularly those from different age classes, τ for low-to-high PFD treatments was between 1.5 and 2.5 min, and τ for dark-to-high PFD treatments was between 3.5 and 7 min. For each individual leaf, however, the τ value for a dark-to-high PFD treatment was at least twice that for a low-to-high PFD treatment. Moreover, there were no large differences in τ values among treatments with starting PFD values of 182 μmol m^{-2} s^{-1} or above, but the amount of scatter in the slow phase of the curves for these higher starting PFD values made it difficult to accurately determine τ for these treatments.

Because the τ of the slow phase differed between dark and low-PFD treatments, we performed further experiments with lower starting PFD values to determine the approximate PFD at which the change in τ occurred. To obtain consistent τ values among leaves, we used leaves from the same age-class. The effect of initial PFD values of 25 to 135 μmol m^{-2} s^{-1} on the relaxation time of the slow phase following an increase in PFD to 690 μmol m^{-2} s^{-1} was tested. These data indicate a relatively abrupt change in τ at a starting intensity of approximately 135 μmol m^{-2} s^{-1} (Fig. 5).

DISCUSSION

In the spinach leaves used in this study the time-course for photosynthesis following an increase in PFD from a low to a higher value was qualitatively similar to that for an increase in PFD from dark to a high value. In both cases photosynthesis showed an initial rapid increase over the first minute or so, followed by a slower increase over the next several minutes. This slower increase was approximately exponential and could be resolved using a semilogarithmic plot. In addition, the contribution of this slow increase to the total increase in photosynthesis following the increase in PFD was dependent on the length of time spent at the lower PFD or in darkness.

The qualitative similarities between the slow phase following an increase from low to higher PFD and the slow phase following an increase from darkness to high PFD suggest that they are controlled by the same factor. The slow exponential increase in photosynthesis following a dark-to-high-PFD transition has been shown to be caused by an increase in the

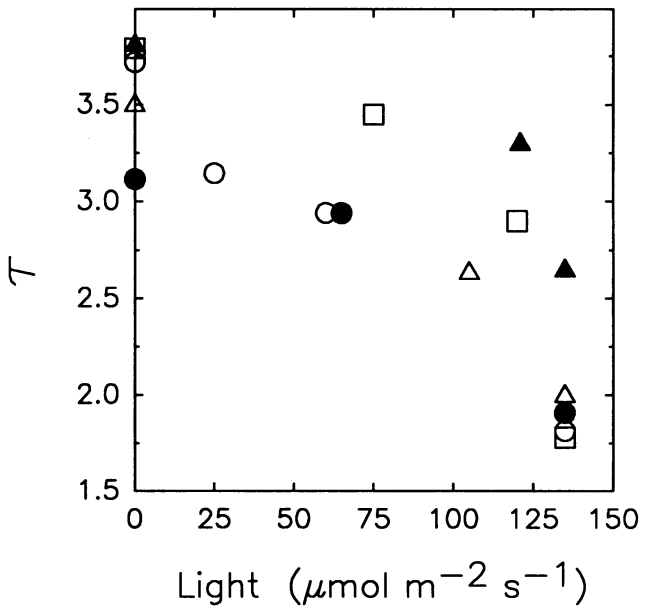

Figure 5. The τ values for the slow phase as a function of PFD. τ values were calculated from natural logarithm time courses similar to those shown in Figure 4. The procedure was the same as for the experiment documented in Figure 4, except the low-PFD levels tested were between 25 and 135 μmol m^{-2} s^{-1}. The linear portion of each timecourse was regressed to determine τ; a relatively large change in τ was detectable at 135 μmol m^{-2} s^{-1}. Each symbol represents the results within a given leaf tested at various PFD values.

activity of Rubisco (13). Because of the similarities discussed above, we suggest this enzyme also controlled the slow exponential increase in photosynthesis observed in this study following an increase from a low to higher PFD.

Although the response of leaves to an increase from low to high PFD was qualitatively similar to that observed for increases from dark to high PFD, there were important quantitative differences between the two. Leaves held at 182 μmol m^{-2} s^{-1} before illumination at 690 μmol m^{-2} s^{-1} showed a consistently smaller relaxation time (τ) for the slow phase than leaves held in darkness (Fig. 4), indicating that Rubisco activated more slowly for leaves held in darkness. The relationship between the starting PFD and the τ for the slow phase appeared roughly sigmoidal (Fig. 5); τ changed very little as starting PFD was increased from dark to 135 μmol m^{-2} s^{-1}, but decreased substantially near 135 μmol m^{-2} s^{-1}. The value for τ appeared relatively constant for starting PFD values above 182 μmol m^{-2} s^{-1} (Fig. 4). This variation in τ suggests differences in the process(s) controlling Rubisco activation, and hypotheses concerning these differences are discussed below.

Another quantitative difference between leaves exposed to either darkness or low PFD before an increase in PFD was the relationship between the time at the starting PFD and the magnitude of the ordinate intercept of the semilogarithmic plot (indicating the proportion of the overall change in assimilation rate attributable to the slow phase). When leaves were held at 182 μmol m^{-2} s^{-1} for increasing time periods before returning the PFD to 690 μmol m^{-2} s^{-1}, the contribution of the slow phase to the total increase in photosynthesis increased with a τ of 10.1 min (Fig. 3b). Using the analysis presented by Woodrow and Mott (13), these data indicate that Rubisco activity declined with τ of 10.1 min following a decrease in PFD from 690 to 182 μmol m^{-2} s^{-1}. This value is less than half that observed for the decline in Rubisco activity following a decrease in PFD from 690 μmol m^{-2} s^{-1} to darkness (13).

These differences in the rate of change of Rubisco activity have important ecological implications. The existence of a threshold intensity above which plants can rapidly activate Rubisco may significantly decrease the total amount of photosynthesis that is "lost" during sunflecks because of the slow increase in Rubisco activity. Woodrow and Mott (13) showed that the amount of CO_2 assimilation that is forgone (F) due to the slow increase in Rubisco activity is given by

$$F = \tau(A_f - A_i)$$

where τ is the relaxation time for the slow phase, A_f is the final, steady-state rate of CO_2 assimilation, and A_i is the rate of CO_2 assimilation that would have occurred had Rubisco not undergone an increase in activity. Thus, if the PFD between sunflecks is greater than 135 μmol m^{-2} s^{-1}, then τ will be lower than it would be if the PFD between sunflecks was less than 135 μmol m^{-2} s^{-1} and F will also be lower. On the other hand, the decline in Rubisco activity following a decrease in PFD was more rapid for leaves held in low PFD than for leaves held in darkness. Therefore, the value of A_i will be higher (tending to decrease F) for a leaf held in darkness than for a leaf held in low PFD if the period of time is relatively short. Thus, the amount of forgone assimilation will be a function of τ and A_i, which will vary in a complicated manner with the period of time at the lower PFD and the value of the lower PFD.

If PFD-dependent increases in Rubisco activity are reflected by the slower, exponential phase of the photosynthesis-*versus*-time curve (see introduction), then there are at least two basic mechanisms that are consistent with the data presented in this study and those of Woodrow and Mott (13). First, increases in Rubisco activity may involve a two-stage, sequential process in which both stages proceed relatively slowly (*i.e.* over several minutes):

$$E \overset{\text{slow}}{\rightleftharpoons} E' \overset{\text{slow}}{\rightleftharpoons} E_a'$$

where E and E' are catalytically inactive forms of Rubisco, and E_a' is catalytically active. Our data are consistent with both equilibria being directly or indirectly PFD-dependent. Moreover, at low PFD values (around 135 μmol m^{-2} s^{-1} in our experiments) both equilibria favor E', whereas at higher PFD values the overall equilibrium favors E_a'. Thus, in the current experiments, the relaxation time for the slow phase after a 182-to-690 μmol m^{-2} s^{-1} transition (Fig. 4) primarily reflects E' conversion to E_a'. During a dark-to-690 μmol m^{-2} s^{-1} transition, however, the slow phase relaxation time reflects E to E_a' conversion via the two slow steps.

The second possible mechanism involves an activator (*Act*), such as Rubisco activase (10), which itself is slowly activated (to *Act'*):

$$Act \underset{\text{slow}}{\rightleftharpoons} Act'$$

$$E \overset{Act'}{\underset{\text{slow}}{\rightleftharpoons}} E_a$$

where E and E_a are the catalytically inactive and active forms of Rubisco, respectively. Like the first mechanism, our data are consistent with both equilibria being either directly or indirectly PFD-dependent. In this case, however, the production of *Act'* and E are favored at low PFD values (around 135 μmol m^{-2} s^{-1} in our experiments), and the rate of E_a production depends upon the concentration of *Act'*. Thus, the slow phase during a dark-to-690 μmol m^{-2} s^{-1} transition reflects both slow steps, whereas during the 182-to-690 μmol m^{-2} s^{-1} transition only the E to E_a conversion is reflected in the slow phase.

When leaves undergoing steady-state photosynthesis at a PFD of 690 μmol m^{-2} s^{-1} were exposed to a period of low PFD and then reilluminated at 690 μmol m^{-2} s^{-1}, it was found that the relaxation time for the slow phase was constant when the lower PFD was above about 135 μmol m^{-2} s^{-1} (Fig. 4). According to the above mechanisms, these data indicate that the $E \rightleftharpoons E'$ (first mechanism) or the $Act \rightleftharpoons Act'$ (second mechanism) reactions are PFD-dependent and proceed almost entirely to the right at PFDs above 135 μmol m^{-2} s^{-1}. Moreover, the sigmoid relationship between the relaxation time of the slow phase and lower PFD (Fig. 5) indicates that the two equilibria are not displaced in a manner that is proportional to PFD. One possibility is that the first slow step (*i.e.* $Act \rightleftharpoons Act'$ or $E \rightleftharpoons E'$) is affected by stromal pH. Supporting this view is the observation that the largest change in stromal pH occurs at a PFD that is several times less than that required for maximum rates of photosynthesis (4). Furthermore, if one takes into account the buffer capacity of the

stroma, then one would expect a sigmoid response of the equilibria involving one or more protonation reactions. It is also feasible that the slow equilibria are affected by changes in the concentrations of divalent cations, ATP/ADP, or by changes in the activity of Rubisco activase (2). At this stage, however, it is not possible to attribute a mechanism to the variation in relaxation time for the slow phase with PFD as we do not have a clear understanding of the principle mechanism underlying the PFD-dependent increase in activity of Rubisco (12), especially the mechanism and role of Rubisco activase (10).

In summary, the degree to which Rubisco activation limits the approach of photosynthesis to a new steady state following an increase in PFD is not constant. It varies among leaves and with the period and intensity of the preceding low-PFD exposure. Because nonsteady-state photosynthesis can be important to the total daily carbon gain of many understory plants, these differences may have a role in determining the success of a plant in an environment with fluctuating PFD. Our data should lead to a better understanding of both the processes controlling Rubisco activation and the carbon balance of leaves that experience unpredictable changes in PFD.

ACKNOWLEDGMENT

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