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ORIGINAL ARTICLE

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Loss of quantum yield in extremely low light

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Abstract It has generally been assumed that the photosynthetic quantum yield of all C_3 plants is essentially the same for all unstressed leaves at the same temperature and CO_2 and O_2 concentrations. However, some recent work by H.C. Timm et al. (2002, Trees 16:47–62) has shown that quantum yield can be reduced for some time after leaves have been exposed to darkness. To investigate under what light conditions quantum yield can be reduced, we carried out a number of experiments on leaves of a partial-shade (unlit greenhouse)-grown *Coleus blumei* Benth. hybrid. We found that after leaves had been exposed to complete darkness, quantum yield was reduced by about 60%. Only very low light levels were needed for quantum yield to be fully restored, with 5 μmol quanta m^{-2} s^{-1} being sufficient for 85% of the quantum yield of fully induced leaves to be achieved. Leaves regained higher quantum yields upon exposure to higher light levels with an estimated time constant of 130 s. It was concluded that the loss of quantum yield would be quantitatively important only for leaves growing in very dense understoreys where maximum light levels might not exceed 5 μmol quanta m^{-2} s^{-1} even in the middle of the day. Most leaves, even in understorey conditions, do, however, experience light levels in excess of 5 μmol quanta m^{-2} s^{-1} over periods where they obtain most of their carbon so that the loss of quantum yield would affect total carbon gain of those leaves only marginally.

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Abbreviations *FBPase* Fructose-1,6-bisphosphatase · *RuBP* Ribulose-1,5-bisphosphate · *Rubisco* RuBP carboxylase/oxygenase

Introduction

Plant leaves in understorey environments, or within dense canopies, often experience widely fluctuating light levels, and photosynthesis is usually not fully induced under those conditions (Chazdon and Pearcy 1986; Pearcy 1990; Küppers and Schneider 1993). Photosynthetic carbon gain under those conditions is usually substantially less than could be achieved if leaves were always fully induced (e.g. Pearcy et al. 1994; Stegemann et al. 1999; Timm et al. 2002).

There are at least three factors that together determine the induction state of leaves in fluctuating light levels. The first two are stomatal conductance and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activation that change over similar, and relatively slow, time courses (Kirschbaum and Pearcy 1988a; Kirschbaum et al. 1988; Seemann et al. 1988; Tinocoojanguren and Pearcy 1993).

A third factor, a relatively fast-inducing component, was originally described by Kirschbaum and Pearcy (1988b) who concluded, based on gas-exchange analyses, that it must be part of the ribulose-1,5-bisphosphate (RuBP) regeneration system. Subsequent biochemical work suggested that it could correspond to the activation of fructose-1,6-bisphosphatase (FBPase; Sassenrath-Cole and Pearcy 1992, 1994), although other mechanisms could not be excluded.

These factors were combined in models of photosynthesis under fluctuating light levels (Kirschbaum et al. 1997; Pearcy et al. 1997) that were based on the earlier model of Gross et al. (1991). These models were

able to reproduce in remarkable detail the experimentally observed key features of photosynthetic responses to rapidly changing light levels (Kirschbaum et al. 1997; Küppers et al. 1997).

However, these models assumed, based on a long-standing assumption about the constancy of quantum yield under unstressed conditions (Walker 1992), that the quantum yield of photosynthesis remained unaffected by the induction state of leaves. That is, these models assumed that photosynthesis in moderate and high light would be affected by the induction state of leaves, but that photosynthesis at very low light levels (the ''quantum-yield region'') would remain the same irrespective of the leaves' induction state.

This assumption was challenged through the work of Stegemann et al. (1999) who conducted extensive measurements on understorey plants in a Costa Rican rainforest and fitted an empirical model to their data. The model gave best fit to their observations when the quantum yield was allowed to vary with the general induction state of leaves.

To further investigate whether the quantum yield could really change with the induction state of leaves, Timm et al. (2002) conducted a series of detailed investigations to show conclusively that the quantum yield of leaves could, indeed, be reduced if leaves were pre-conditioned in complete darkness.

The earlier work by Kirschbaum and Pearcy (1988b) had already shown that induction loss of leaves differed between leaves exposed to light levels between 0 and 10 μmol quanta m^{-2} s^{-1}. However, quantum yield was not specifically investigated in that work. The question, therefore, arose whether the loss of quantum yield observed by Timm et al. (2002) was equally restricted to pre-conditioning in darkness.

A series of experiments was therefore conducted to assess how quantum yield was affected by pre-conditioning light levels, and over what time course quantum yield could recover to the levels observed for leaves with higher induction state.

Materials and methods

Plant material

The experiment used a *Coleus blumei* Benth. hybrid grown from a cutting (see Timm et al. 2002). The plant had been cultivated in a glasshouse where it was fertilised regularly and watered daily to field capacity. Gas exchange was measured when the plant was approximately 6 months old. In the glasshouse, the plant received about 70% of natural sunlight.

Gas-exchange system

Gas exchange was measured with a field-portable CO_2/H_2O porometer (model 6400; Li-Cor). The double-sided leaf chamber used here consisted of an integrated infrared gas analyser and could seal a leaf area of 6 cm^2. Flow rate through the chamber was maintained at approximately 200 mmol min^{-1}. CO_2 concentrations in the air were adjusted to 380 μmol mol^{-1} with a built-in CO_2 mixer. Leaf temperature was maintained between 24°C and 25°C during the experiment. Relative humidity was not controlled and varied between 30–60% with natural changes in the absolute humidity of the air.

The leaf was illuminated with artificial light, produced by a built-in controllable LED light source. Data were generally recorded every 1 to 2 s which gave an adequate temporal resolution for the given flow rate and chamber size.

All leaves used for measurements were fully grown and had no apparent damage due to herbivory, disease or physical damage. Overnight between measurement days, the plant was kept in the laboratory in an exsiccator at 95–100% relative humidity in order to minimise disturbance and to prevent responses to changing external conditions. The same leaves were generally used for three or four measurements on the same day, but different leaves were used on different days.

Measurement protocol

Leaves were generally pre-conditioned at 800 μmol quanta m^{-2} s^{-1} for at least 1 h in order to ensure that photosynthesis was fully induced. Higher light levels were avoided in order to prevent photoinhibition. Different experiments were then done to investigate whether photosynthetic rates remained constant during step-wise reductions in light level, and to determine the light dependence of quantum yield and the time course of regaining full quantum yield.

For obtaining the photosynthetic pattern upon step-wise decreases in incident light, levels were decreased from 800 to 30, 10, 5 and then 0 μmol quanta m^{-2} s^{-1}. Each light level was maintained for 15 min, with photosynthetic rates being recorded continuously.

To determine the light and time dependence of quantum yield, initially fully induced leaves were pre-conditioned at different set light levels from 0 to 20 μmol quanta m^{-2} s^{-1} for 30 min. Past work on various species has indicated that leaves are likely to be 95–99% deactivated after 30 min in low-light conditions (Kirschbaum and Pearcy 1988b; Stegemann et al. 1999). Therefore, light levels were set to values between 10 and 30 μmol quanta m^{-2} s^{-1} for either 60 or 120 s before being changed to a different light level within that range for the calculation of quantum yields. A total of 20 independent quantum-yield determinations were carried out at 11 different light levels. Further details of the measurement sequence are given in Table 1.

The data analysis was difficult because the time course of induction was as short as the time typically needed to reliably measure a leaf's quantum yield. An example is shown in Fig. 1. Adjustments of the RuBP and triose phosphate pools and increasing induction of Rubisco and the 'fast phase' of induction all

Table 1 Details of measurement protocol for the determination of quantum yields of *Coleus blumei* xzleaves after different pre-conditioning light levels. Light levels and length of time over which leaves were kept at those respective light levels are given

Light level (μmol quanta m^{-2} s^{-1})	Time (s)	Comment
800	3,600	Achieve full induction
0, 2, 3, 4, 5, 6, 7, 10.7, 11, 15 or 20	1,800	Pre-conditioning to different light levels between 0 and 20 μmol quanta m^{-2} s^{-1}
10 or 20	60 or 120	Sequence for determining quantum yield
30	60 or 120	
10 or 20	60 or 120	
30	60 or 120	
More repeats of the sequence until rates stabilised		

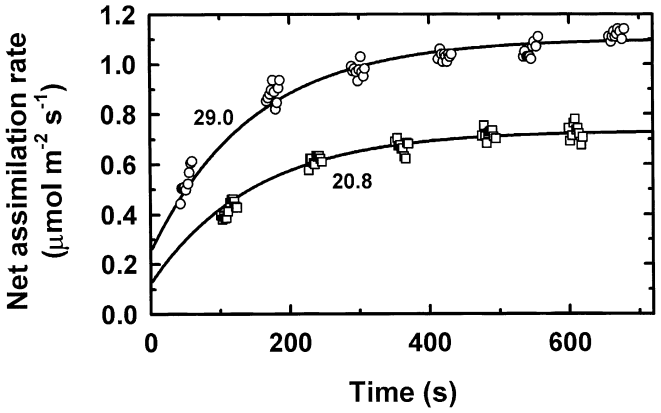

Fig. 1 Net assimilation rate of *Coleus blumei* leaves at different times after increasing the light from complete darkness to either 20.8 or 29.0 μmol quanta m^{-2} s^{-1} as indicated. The light was switched between the two indicated levels for 60-s periods. Only values at the end of each 60-s period are shown. The curves are described by Eq. 1

occurred with similar time constants and added to the increasing carbon gain in the first few minutes after increasing the light level. The glycolate pool must have also been building up and leading to a slight reduction in net carbon gain over the same time period (Kirschbaum et al. 1997).

This resulted in sometimes slow and protracted utilisation of photosynthetic intermediates (see Fig. 10b in Timm et al. 2002 for representative gas-exchange traces). The low gas-exchange rates further added an element of experimental error.

Data for the first 30–40 s after changing the light level were, therefore, generally omitted because they were determined by a combination of plant and system adjustments to the changed light levels. Only subsequent data had no residual system-response included within them and represented almost completely the photosynthetic rate under the current light levels.

The data were analysed by describing assimilation rate, A, as:

$$A = I\{\alpha_0 + (\alpha_f - \alpha_0)[1 - \exp(-t/\tau_i)]\} - R_d \quad (1)$$

where I is incident light, α_0 the incident quantum yield immediately after pre-conditioning at various low light levels (including complete darkness), α_f the incident quantum yield of a fully induced leaf, t the time since increasing the light level, τ_i the time constant for increasing induction and R_d is day respiration (Brooks and Farquhar 1985), which was assumed here to remain constant throughout the period of measurement.

For each measurement sequence, I and t were then given as input variables and A as the measurement variable. The parameters α_0, α_f, τ_i and R_d could then be fitted independently to each measurement sequence with the Solver in Microsoft Excel using a quasi-Newton method. Individual observations could sometimes be described equally well with different combinations of parameters that were believed to reflect experimental errors or differential adjustments of pools of intermediates rather than true differences in quantum yield. After one initial round of fitting parameters, it was then decided to restrict data fitting for all measurement sequences to the use of the same value of $\tau_i = 130$ s to reduce the freedom of parameter fitting. This provided less biased estimates of quantum yield as a function of pre-conditioning light level than if each data set had been fitted without this constraint. A total of 20 quantum-yield determinations were carried out.

To assess the quantitative significance of including variable quantum yield, the model of Kirschbaum et al. (1997) was used. That model is based on the widely accepted Farquhar model of photosynthesis (Farquhar et al. 1980; Farquhar and von Caemmerer 1982), but has been transformed into a dynamic model by explicitly including pools of photosynthetic intermediates and time-dependent changes in the activation state of Rubisco and a step in the regeneration of RuBP. Tests of the model have shown that it can successfully reproduce the dynamic patterns of photosynthesis during rapidly changing light levels (Kirschbaum et al. 1997).

The model was modified by treating the 'initial slope of triose phosphate production as a function of light', α_{Vj}, not as a constant. Instead, it was calculated as a variable with an equilibrium value for a given light level that could be reached asymptotically with time from an earlier to these new equilibrium values. An equilibrium quantum yield, α_{eq}, for a given light level was thus calculated as:

$$\alpha_{eq} = \alpha_f - (\alpha_f - \alpha_0)\exp(-I/k_i) \quad (2)$$

where I is the light level and k_i a constant that describes the light dependence of the incident quantum yield. The actual incident quantum yield, α_{Vj}, then dynamically changed towards the equilibrium value for the incident quantum yield, with time constants that were different for increases and decreases in quantum yield so that:

$$d(\alpha_{vj})/dt = (\alpha_{eq} - \alpha_{vj})/\tau_i \quad \text{if } \alpha_{eq} > \alpha_{vj} \quad (3)$$

$$d(\alpha_{vj})/dt = (\alpha_{eq} - \alpha_{vj})/\tau_d \quad \text{if } \alpha_{eq} < \alpha_{vj} \quad (4)$$

where τ_i was the time constant for increase in quantum yield and τ_d the equivalent time constant for decrease in quantum yield. This basic modelling approach had been used previously for describing the dynamic changes in other aspects of photosynthetic induction (Gross et al. 1991; Kirschbaum et al. 1997).

Results

Experimental observations

The photosynthetic light response of a typical leaf of the plant is shown in Fig. 2. Steady-state photosynthetic measurements indicated that photosynthetic capacity was about 9 μmol m^{-2} s^{-1} at 800 μmol quanta m^{-2} s^{-1} which appeared to be sufficient to saturate photosynthesis. This light level was therefore used subsequently for pre-conditioning leaves to achieve full induction while avoiding photoinhibition.

As a first step, a leaf that had been pre-conditioned at 800 μmol quanta m^{-2} s^{-1} was then exposed to progres-

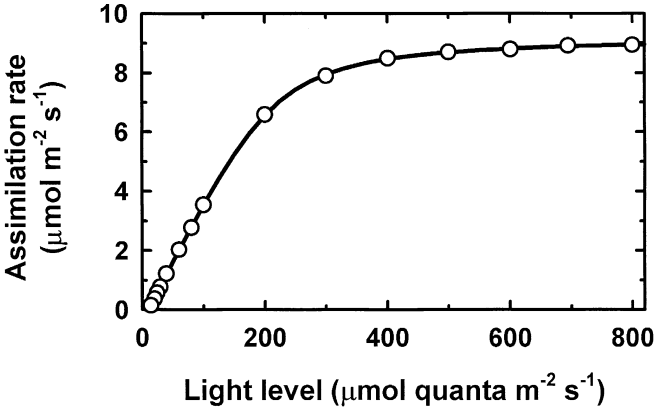

Fig. 2 Steady-state net assimilation rate at different light levels in a fully-induced leaf of the experimental *C. blumei* plant

sively lowered light levels to ascertain to what extent quantum yield and net assimilation rate remained constant under conditions when other processes associated with photosynthetic induction would be deactivated (Fig. 3).

When the light level was first lowered from 800 to 29.2 μmol quanta m^{-2} s^{-1}, assimilation rate decreased to nearly 0 before settling at a higher rate of about 1.2 μmol m^{-2} s^{-1} after about 200 s. This initial decrease (post-illumination burst) was due to the delayed release of CO_2 from photorespiration (Vines et al. 1982). Thereafter, the rate remained fairly constant at that light level. Irregular apparent jumps in assimilation rates at constant light levels were believed to be caused by noise in the analyser rather than being a leaf response.

The pattern was similar for the next decrease in light down to 9.3 μmol quanta m^{-2} s^{-1}, but with a much less pronounced post-illumination burst. Upon the further decrease to 5.1 μmol quanta m^{-2} s^{-1}, a post-illumination burst was not noticeable at all, and upon stepping down further to 0 μmol quanta m^{-2} s^{-1}, there was instead a prolonged period of post-illumination CO_2 fixation, presumably supported by pools of photosynthetic intermediates that had accumulated during the preceding period of slightly higher illumination (Sharkey et al. 1986).

In all these cases, assimilation rates remained steady over the period of constant low illumination, even though it is known from previous work that stomatal conductance, Rubisco activation and the factor in the fast phase of induction must have been reduced over this period in low light (e.g. Kirschbaum and Pearcy 1988a, 1988b). These measurements showed that the quantum yield was not reduced if a leaf was kept in constant moderately low light after a previous period in higher light.

Leaves were then pre-conditioned at various very low light levels, including darkness, with quantum yields determined upon subsequent exposure to light levels between 10 and 30 μmol quanta m^{-2} s^{-1}. Calculated incident quantum yields after different pre-conditioning light levels are shown in Fig. 4.

Quantum yields determined after pre-conditioning leaves in complete darkness were lower than for leaves pre-conditioned at 5 μmol quanta m^{-2} s^{-1} or more. Quantum yields of six determinations of leaves pre-conditioned in darkness were 0.019 compared to 0.048 for fully induced leaves as deduced from the curve fitted to the data in Fig. 4. An incident quantum yield of 0.048 is in the range of values usually reported for C_3 plants at 25°C (Ehleringer and Björkman 1977; Björkman 1981). The fitted curve in Fig. 4 indicates that a light level of 5 μmol quanta m^{-2} s^{-1} was sufficient for 85% of maximal quantum yield to be maintained.

Modelling results

To give an indication of the quantitative significance of the loss of quantum yield in extremely low light, the model of Kirschbaum et al. (1997) was extended by explicitly including the effect of exposure to darkness or very low light on the quantum yield as expressed in Eqs. 2, 3 and 4. Photosynthesis with constant and variable quantum yield after exposure to darkness is shown in Fig. 5 and the loss in potential carbon gain due to reduced quantum yield after pre-conditioning in darkness in Fig. 6.

With quantum yield held constant, assimilation rate increased sharply upon increasing light levels to 1 or 20 μmol quanta m^{-2} s^{-1} and reached peak rates after about 15 and 25 s, respectively (Fig. 5). This time was required for pools of photosynthetic intermediates to build up to support maximal rates of photosynthesis. Net assimilation rates then decreased to lower values again as the glycolate pool slowly built up and caused an increasing loss of CO_2.

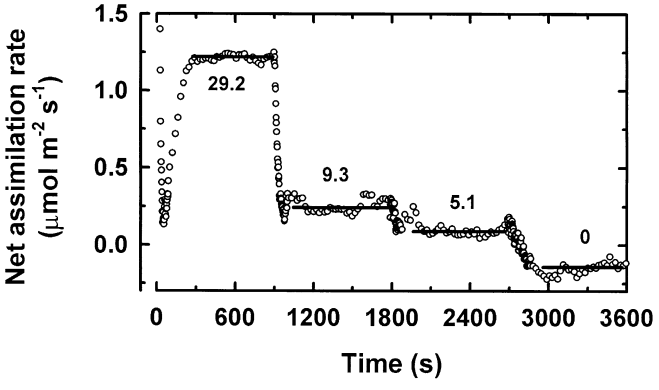

Fig. 3 Net assimilation rate over 1 h after a *C. blumei* leaf pre-conditioned to 800 μmol quanta m^{-2} s^{-1} was exposed to decreasing light levels in four steps, as shown in μmol quanta m^{-2} s^{-1}. *Horizontal lines* fitted by eye give average assimilation rates at respective light levels after the post-illumination burst following a change in light level

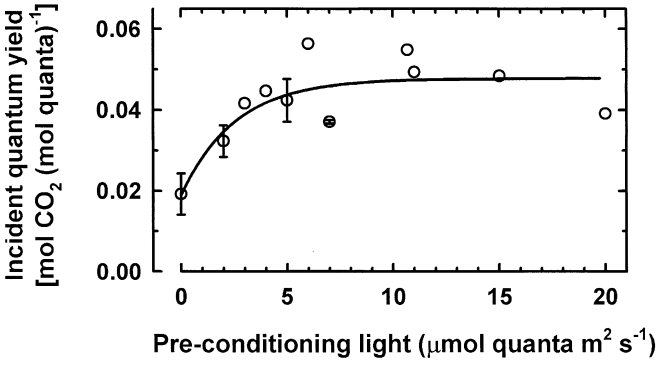

Fig. 4 Dependence of initial incident quantum yield (α_0) on pre-conditioning light level. At pre-conditioning light levels with more than one determination, data are shown as means ± SE. The curve is described by the equation: $\alpha_0 = 0.048 - 0.029 \exp(-I_p/2.6)$, where α_0 is the quantum yield for different pre-conditioning light levels and I_p represents those pre-conditioning light levels ($n = 20$)

With variable quantum yield, assimilation rates increased more slowly after increasing light. The time constant of 130 s for increasing the quantum yield was similar to the time needed for the build-up of the glycolate pool. At a light level of 20 μmol quanta m^{-2} s^{-1}, assimilation rates with variable quantum yield therefore remained below that simulated with constant quantum yield for about 200 s before the effect of reduced quantum yield disappeared (Fig. 5, lower panel).

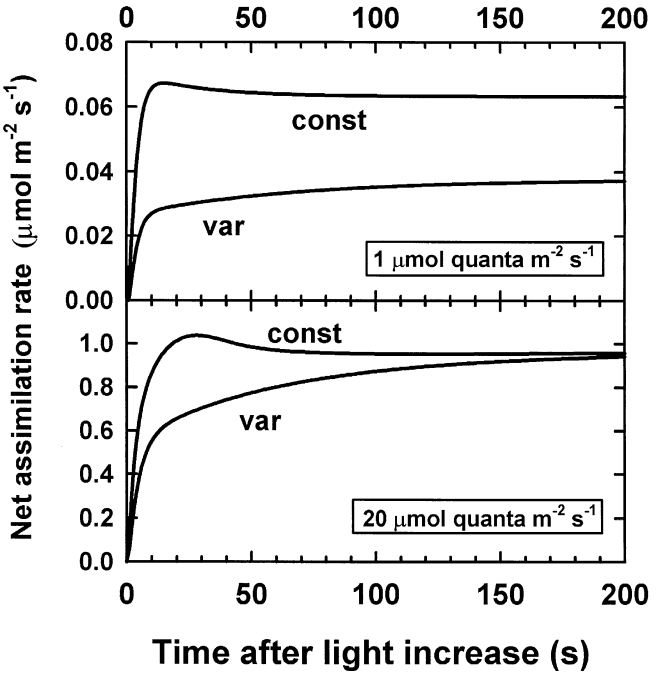

Fig. 5 Modelled net assimilation rate after increasing the light from complete darkness to either 1 μmol quanta m^{-2} s^{-1} (*upper panel*) or 20 μmol quanta m^{-2} s^{-1} (*lower panel*). Simulations are shown either with quantum yield kept constant (*const*) or variable (*var*) based on the experimental evidence shown above

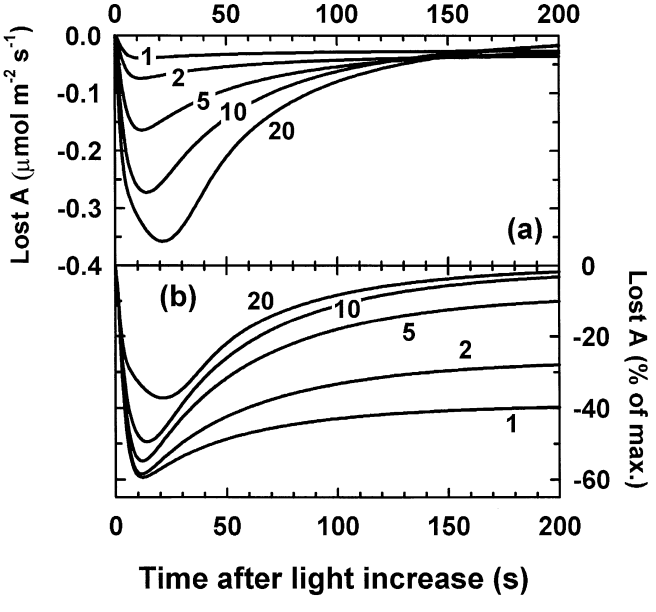

Fig. 6a, b Lost assimilation rate (*A*) due to reduced quantum yield in *C. blumei* leaves at different light levels (1, 2, 5, 10, 20 μmol quanta m^{-2} s^{-1}) after pre-conditioning in darkness. Carbon gain is compared between simulations with constant and variable quantum yield as observed. Data are expressed either as absolute losses (**a**), or relative to the net assimilation rate reached after 200 s at the respective light levels and with constant quantum yield (**b**)

For a light level of 1 μmol quanta m^{-2} s^{-1}, simulations with variable quantum yield continued to remain lower than those with constant quantum yield because quantum yield could not become fully induced at this low light level (see Fig. 4). Thus, plants were not able to fully utilise the available light at these very low light levels.

The loss in carbon gain due to reduced quantum yield is further shown and quantified in Fig. 6, which compares simulations from darkness to varying light levels between simulations with constant or variable quantum yield. The calculations for the light levels of 1 and 20 μmol quanta m^{-2} s^{-1} are based on the data shown in Fig. 5.

For the first 10–20 s after light was increased, assimilation rates remained low because the pools of photosynthetic intermediates had to be built up before maximal rates of photosynthesis for the given conditions could be reached (Sharkey et al. 1986). Such delays were observed with both constant and variable quantum yield (Fig. 5). When photosynthetic intermediates had built up to the levels needed to support photosynthesis, the effect of reduced quantum yield had its greatest effect, with losses of almost 0.4 μmol m^{-2} s^{-1} for leaves exposed to 20 μmol quanta m^{-2} s^{-1} and lower losses for leaves exposed to lower light levels (Fig. 6).

Expressed as a percentage of the maximal photosynthetic carbon gain possible at each light level, these losses in assimilation rates corresponded to peak carbon losses of 40–60% of maximal photosynthetic rates. Percentage losses were greatest for the lowest light levels, and these losses only partly diminished over the 200 s of the simulation because at very low light levels, quantum yields remained permanently reduced (see Figs. 4, 5). At higher light levels, on the other hand, steady-state quantum yields were significantly higher than in darkness so that the assimilation losses mainly reflected the relative slow re-gaining of maximum quantum yields.

Discussion

Previous work had shown that there are at least three distinct processes that are responsible for the loss of photosynthetic induction of leaves in low light, with each having different time courses for induction gain and loss. These are stomatal conductance (Kirschbaum and Pearcy 1988a; Kirschbaum et al. 1988; Tinocoojanguren and Pearcy 1993), activation of Rubisco (Kirschbaum and Pearcy 1988a; Seemann et al. 1988; Tinocoojanguren and Pearcy 1993) and a fast-inducing step in RuBP regeneration (Sharkey et al. 1986; Kirschbaum and Pearcy 1988b; Sassenrath-Cole and Pearcy 1992, 1994; Schulte et al. 2003).

The work presented here builds on the work of Timm et al. (2002) by adding a fourth process: loss of quantum yield after exposure to extremely low light. This factor is characterised by a very low light requirement for complete activation and a fairly fast time constant for re-activation after exposure to higher light levels.

It had been a long-standing assumption that the quantum yield of photosynthesis would be constant unless it was reduced by some kind of stress, especially photoinhibition (Powles 1984; Walker 1992). However, the work of Timm et al. (2002) showed that the quantum yield can be reduced after exposure of leaves to complete darkness.

We confirm here the findings of Timm et al. (2002) and elaborate on them by providing additional information about the light levels above which quantum yield appears to be fully induced and about the time course of increases in quantum yield after increasing light levels. This analysis showed that only very low light levels are needed for quantum yields to reach their maximum values. Assuming that the quantitative results obtained with *Coleus* can be generalised to other species, the loss of quantum yield is probably of limited practical relevance in most understorey environments where light levels, even deep in the understorey, rarely fall below 5 μmol quanta m^{-2} s^{-1}.

Consistent with that, assimilation rates remained essentially constant when light was progressively reduced from saturating levels to levels in the quantum-yield region (Fig. 3). There were initial dynamics associated with delayed CO_2 release from photorespiration, but thereafter, rates remained essentially constant while plants remained at the respective light levels. This indicates that plants, even in understorey environments, probably only rarely experience light conditions that reduced their quantum yield.

Very low light-levels are obviously experienced at the beginning and end of each day, and the reduced quantum yield at those times, especially in the morning, could reduce assimilation rates. While carbon gain would almost certainly be reduced at those times, it would normally constitute only a very slight proportion of daily carbon gain because assimilation rates at those extremely low light levels would contribute only a very small proportion of total daily carbon gain.

There are, however, plants that are growing in deep shade where they may be overshadowed by several layers of competing plants. Such plants live a precarious existence, with their very limited carbon gain needed to sustain their own metabolism and support the eventual regrowth of new leaves and other plant organs. It is the persistence of such plants that would be made even more difficult through the loss of quantum yield described here. Such plants would rely for most of their carbon gain on light levels of less than 10 μmol quanta m^{-2} s^{-1} and for a substantial proportion of their carbon gain on the even lower light levels that are low enough to reduce their quantum yield. If their quantum yield is further diminished then their on-going survival becomes even more difficult.

It would be interesting to follow the present work by further comparative ecophysiological studies on species from different light habitats, or on plants that have experimentally been grown under different light conditions. Such studies could ascertain whether different plants have different thresholds or time constants for change in their quantum yield similar to the differences that have been observed between plants in their light-fleck-use efficiency (Küppers et al. 1996; Ögren and Sundin 1996).

Significant differences in acclimation potential of photosynthetic induction dynamics have been observed in saplings of different successional positions (e.g. ranging from shade tolerant or even extremely shade tolerant to intolerant) when they were grown in deep shade under identical glasshouse conditions (Schneider et al. 1993; Paliwal et al. 1994; Küppers et al. 1996). If there are species-specific differences in their quantum-yield and induction characteristics under extremely low light, it could have important effects on their survival potential at the limits of light tolerance.

The possible mechanistic cause of the observed quantum-yield loss is not clear. Fluorescence measurements indicate that the photochemical reactions of photosynthesis have no induction requirement but, unless damaged by photo-oxidative stress, are able to always function at optimal capacity (Walker 1992). Laisk et al. (1992), for example, observed that bursts of oxygen evolution continued upon re-illumination after dark phases of different lengths, while the ability for immediate CO_2 fixation at maximal rates was lost. The photosynthetic enzymes that are responsible for other aspects of photosynthetic induction change their activation state with light level to exert different levels of control over photosynthesis (Seemann et al. 1988; Sassenrath-Cole and Pearcy 1994; Raines 2003) but it is not clear to what extent they may become so completely de-activated in the dark to not even be able to support the low assimilation rates possible in the quantum-yield region.

The process most likely to be responsible for the loss of quantum yield might be the build-up of the trans-thylakoid proton gradient that is the driving force for ATP generation (Rappaport and Lavergne 2001; Kramer et al. 2003). Maintenance of the gradient is hindered by a small rate of on-going leakage of protons across the membrane without producing ATP. That is not believed to constitute a major loss under normal photosynthetic conditions (Walker 1992), but may attain greater significance under the extreme low-light conditions studied here.

Stomatal conductance was recorded during the measurement sequence, but changes were too slow to have appreciably contributed to the change in observed photosynthetic rates after changing light levels. That was consistent with past work that has shown stomatal responses to changing light levels to typically occur over

15 min or longer (Kirschbaum et al. 1988). Changes between light levels in the quantum-yield region were also too small to trigger large changes in stomatal conductance.

Recognition of the potential loss of quantum yield described here is important for quantum-yield determinations where it is important to avoid measuring leaves immediately after transferring them from darkness. It is also important for research in that the important difference between pre-conditioning of leaves in low light compared to complete darkness needs to be recognised. Kirschbaum and Pearcy (1988b) showed that there were differences in induction loss between leaves exposed to low light levels of 10, 4 or 0 μmol quanta m^{-2} s^{-1} in that there were more substantial induction losses at light levels less than 10 μmol quanta m^{-2} s^{-1}. Even for apparently identical photosynthetic induction states, such as determined via the procedure and mathematical expression given by Stegemann et al. (1999), there could be different induction states of the various processes that together account for photosynthetic induction for leaves pre-conditioned in darkness compared to low-light levels.

The measurements done as part of the present work indicate that the quantum-yield component of induction saturates at the very low light level of a few μmol quanta m^{-2} s^{-1}, whereas the other 'fast-inducing' component in induction that also affects RuBP regeneration does not saturate at such low light levels (Kirschbaum and Pearcy 1988b). Hence, quite different patterns of induction loss would be generated by leaves incubated in darkness rather than at low light levels. This is not to say that one pattern is more meaningful than another, but it is important to know that there are different patterns, and that an experimental protocol must be chosen to be consistent with the intent of any experiment.

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