Plant Physiol. (1987) 85, 1000–1007
0032-0889/87/85/1000/08/$01.00/0

Reduction State of Q and Nonradiative Energy Dissipation during Photosynthesis in Leaves of a Crassulacean Acid Metabolism Plant, *Kalanchoë daigremontiana* Hamet et Perr.[1]

Received for publication April 13, 1987 and in revised form August 12, 1987

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ABSTRACT

Fluorescence was measured in leaves of the CAM plant *Kalanchoë daigremontiana* using a pulse modulation technique at room temperature. During a 12-h light period at 500 micromole photons per square meter per second (400–700 nanometers) in air containing 350 microbar CO_2, the component of fluorescence quenching related to the reduction state of Q, the primary electron transport acceptor of PSII, remained fairly constant and showed that only 20% of Q were in the reduced form. The reduction state was slightly increased at the onset and at the end of the light period. By contrast, the nonphotochemical component of fluorescence quenching which is a measure of the fraction of nonradiative deexcitation underwent marked diurnal changes. Nonradiative energy conversion was low during the phase of most active malic acid decarboxylation in the middle of the light period when uptake of atmospheric CO_2 was negligible, and when internal CO_2 partial pressures were higher than in air; this allowed for high rates of CO_2 reduction in the chloroplasts. Nonradiative energy conversion was high during the early and the late light period when atmospheric CO_2 was taken up and internal CO_2 partial pressures were below air level. Manipulation of the internal CO_2 partial pressure during the late light period by increasing or decreasing the external CO_2 partial pressure to 1710 and 105 microbar, respectively, led to changes in the magnitude of energy dependent fluorescence quenching which were consistent with the relationship between nonradiative energy dissipation and internal CO_2 partial pressure observed during the diurnal cycle. Again, the reduction state of Q was hardly affected by these treatments. Thus, changes in electron transport rate during the diurnal CAM cycle at a given photon flux density lead primarily to alterations in the rate of nonradiative energy dissipation, with the reduction state of Q being maintained at a relatively low and constant level. Conditions are described under which nonphotochemical dissipation of excitation energy reaches a maximum value and the reduction state of Q is increased.

When leaves are illuminated, the excitation energy of Chl can be dissipated by photosynthesis, as heat or, to a very small extent, as fluorescence. Simultaneous measurements of fluorescence yield and of gas exchange provide information about the partitioning of excitation energy between photochemical and nonphotochemical processes (11, 21). Fluorescence emitted at room temperature emanates predominantly from Chl *a* of PSII and depends on the redox state of the primary electron acceptor of PSII, Q.[2] If, for example, a large percentage of Q is kept oxidized by electron flow to sustain reduction of CO_2, photochemical quenching, q_P, will be high and fluorescence will be low. Nonphotochemical quenching processes, designated q_E, relate, first, to the establishment of a proton gradient across the thylakoid membrane and, thereby, to ATP consumption in photosynthesis (12, 13). Low rates of CO_2 reduction and, hence, ATP consumption will increase Δ pH and thus decrease fluorescence by increasing the proportion of excitation energy lost as heat. q_E may also include a component related to the phosphorylation of the light harvesting complex of PSII (8) and a slowly relaxing type of quenching (4) reflecting a process which represents a major pathway for heat dissipation of excess excitation energy and probably involves operation of the xanthophyll cycle (5). Photochemical quenching, q_P, and nonphotochemical quenching, q_E, are interrelated and linked via photosynthetic electron transport to CO_2 reduction in the chloroplasts.

In a previous study, the light scattering method and the slow decay of Chl *a* fluorescence upon illumination were used as indicators of the energy state in intact leaves of the CAM plant *Kalanchoë pinnata* (10). Although both parameters are related to q_P and q_E, the above measurements did not allow one to distinguish between these two variables. Furthermore, the data on *K. pinnata* were obtained during dark/light transients, which had to be imposed at various times of the light period. In the study presented here, we have used a recently developed pulse modulation fluorescence technique (15) to follow directly changes in q_P and q_E throughout the diurnal cycle of a CAM plant. The method caused little perturbation of the day/night regime. Objectives of this study were (a) to investigate, at least qualitatively, changes in the rate of electron transport and, hence, CO_2 reduction rates in the chloroplasts during a diurnal CAM cycle, and (b) to examine the partitioning of excitation energy between photochemical and nonradiative dissipation throughout such a cycle. Demand for ATP and reducing power changes considerably during the various phases of CAM in the light because of variations in the proportion of CO_2 uptake via PEP and RuBP carboxylase and because of large increases in intercellular CO_2 partial pressure during the period of malic acid decarboxylation (17). The results presented here show that ex-

[1] Supported by the Deutsche Forschungsgemeinschaft.

[2] Abbreviations: Q, primary electron acceptor of photosystem II; A, net CO_2 assimilation rate; F_O, instantaneous fluorescence emission; F_V, variable fluorescence emission; F_M, maximum fluorescence emission; g, leaf conductance to water vapor transfer; p_a, ambient CO_2 partial pressure; p_i, intercellular CO_2 partial pressure; PEP, phosphoenolpyruvate; PFD, photon flux density; q_P, photochemical component of fluorescence quenching; q_E, nonphotochemical component of fluorescence quenching; RuBP, ribulose bisphosphate.

pected diurnal changes of CO_2 reduction rate in the chloroplasts of intact leaves of *K. daigremontiana* are confirmed by fluorescence measurements and that they lead to characteristic alterations in the rate of nonradiative energy dissipation, while the reduction state of Q tends to be maintained at a relatively low and constant level.

MATERIALS AND METHODS

Plant Material. *Kalanchoë daigremontiana* was cultivated in 5 L plastic pots filled with garden soil. Plants were watered daily and received Hewitt's type nutrient solution containing 24 mM NO_3^- once per week (20). They were kept in a glasshouse at 30/15°C (day/night). Osram Power Star metal halide lamps (HQI-T 2000 W/D) provided continuous illumination for 12 h (from 8 AM–8 PM). Experiments commenced when the plants were 3 to 4 months old and had about 12 leaf pairs. Experiments were with fully developed leaves which had been exposed to an average PFD of 400 to 500 μmol m^{-2} s^{-1} during growth. Primary leaves of the C_3 plant *Spinacia oleracea* L. (Yates Hybrid 102) which had developed at a PFD of approximately 600 μmol m^{-2} s^{-1} were used for comparative purposes.

Gas Exchange and Fluorescence Techniques. CO_2 assimilation rate and fluorescence were measured in attached leaves using an open gas exchange system as described previously (18). Leaves were clamped between a double-sided glass and aluminum chamber. The illuminated area was 13.5 cm^2. Fluorescence was measured with a pulse amplitude modulation fluorometer (model PAM 101 Chlorophyll Fluorometer; H. Walz, Effeltrich, West Germany) (3, 15). The tip of the fiber optics was touching the glassplate of the leaf chamber. The distance between tip and the upper leaf surface was 1.5 cm. Excitation of fluorescence through the fiber optics was at an angle of 45°. The experimental routine with the CAM plant was as follows (Fig. 1): at the end of the 12 h dark period (07.58 h), fluorescence was excited with a measuring beam of weak light from a pulsed light-emitting diode to obtain F_O, which designates the fluorescence level when all reaction centers of PSII are open. The frequency of the pulsed measuring light was 1.6 kHz. The integrated PFD was 0.02 μmol m^{-2} s^{-1}. Fluorescence immediately attained a constant level not followed by a further rise indicating that the pulsed measuring light was sufficiently low. During a 1 s pulse of saturating light (above 3000 μmol m^{-2} s^{-1}) to transiently close all reaction centers and completely reduce Q, maximum fluorescence, F_M, was obtained. Response curves of fluorescence *versus* light intensity had shown that the PFD during 1 s pulses was saturating. Variable fluorescence, F_V, equals F_M minus F_O. During the standard 12 h light period (8 AM–8 PM), the leaf inside the gas exchange chamber was illuminated with an Osram Power Star HQI-R 250 W/NDL lamp. PFD incident on the upper leaf surface was either 500 or 1100 μmol m^{-2} s^{-1} (PAR in Fig. 1). At various times throughout the light period, the modulated measuring beam of weak light was turned on to measure steady state fluorescence emission which was composed of a variable component, F_V', plus the actual minimum fluorescence, F_O (Fig. 1). During the saturation pulse, fluorescence emission was usually increased to a new level, composed of a variable component, F_V'', and of F_O. F_O was determined by darkening the leaf for 2 to 5 min (PAR off). This procedure was repeated at frequent intervals. Examples are given for a time point shortly after onset of the light period (08.18 h) when fluorescence quenching was high (Fig. 1B) and for 12.00 h when fluorescence quenching was low (Fig. 1C). The term $1\text{-}q_P$ ($=F_V'/F_V''$) is used as a measure of the reduction state of the acceptor Q of PSII. For the sake of simplicity, we have not considered a possible nonlinearity between $1\text{-}q_P$ and the reduction level of Q caused by energy transfer between PSII units (9). qE is 1 minus F_V''/F_V.

The estimation of the level of fluorescence at open traps (F_O) during the light period was somewhat problematic during periods of pronounced quenching of fluorescence. Upon darkening of the leaves, fluorescence decreased rapidly and immediately increased thereafter (Fig. 1B). In that case, the lowest value of fluorescence was occasionally slightly lower than F_O measured in the dark-adapted leaf, *i.e.* at the end of the standard 12 h dark period. This was particularly so in leaves of another CAM species, *Kalanchoë pinnata* (Lam.) Pers., which otherwise exhibited responses very similar to those shown for *K. daigremontiana* in this paper. We present two values of $1\text{-}q_P$, one calculated from the lowest value of F_O measured during a 5 min dark phase

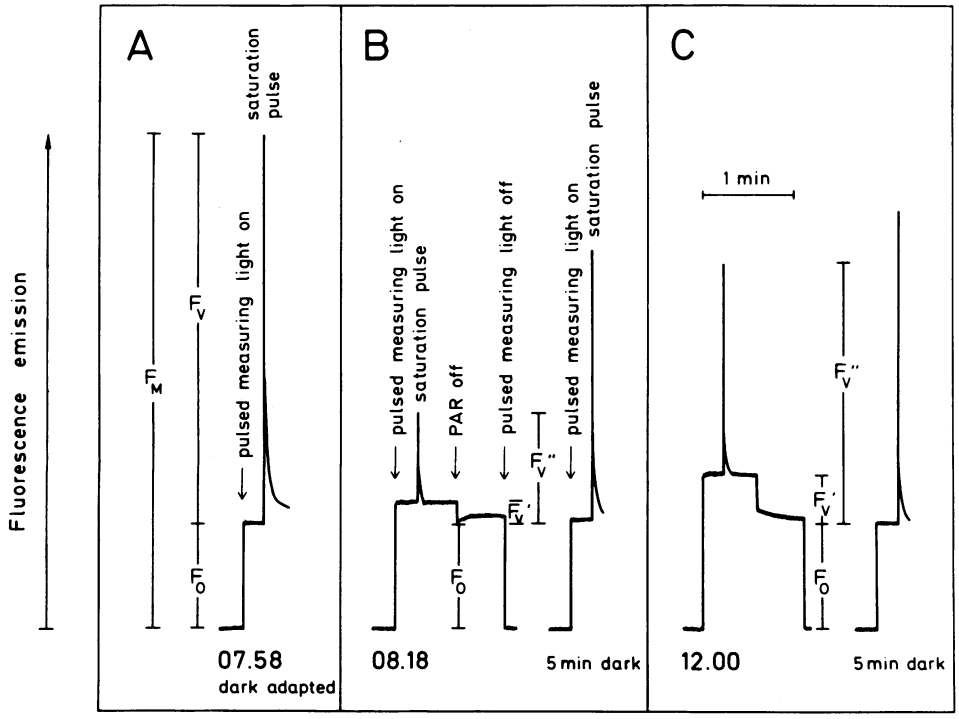

FIG. 1. Fluorescence signals and their terminology. Examples are given for a dark-adapted leaf of *K. daigremontiana* at the end of the night (A) and for the same leaf at two points during the light period (B and C) (see "Materials and Methods" for further details). Examples refer to the experiment of Figure 3. The light period was from 08.00 to 20.00 h.

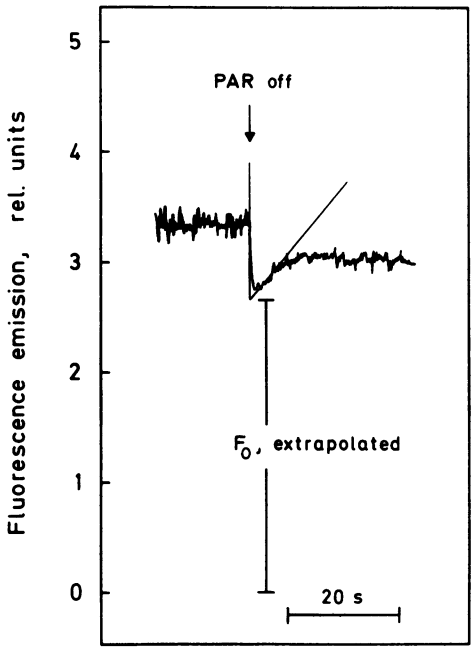

FIG. 2. Example of rapid fluorescence changes upon darkening of *K. daigremontiana* during a period of pronounced quenching during the diurnal cycle (experiment of Fig. 7, measurement at 08.18 h) and the estimation of F_O by extrapolation.

throughout the light period, and a second one, obtained by extrapolation (Fig. 2) and based on the assumption that the true F_O in the light (time zero in Fig. 2) was even lower (1, 4). The latter procedure resulted in values of F_O which were at most 10% lower than the lowest measured value during the respective 5 min dark interruption. There is increasing evidence that strong nonphotochemical fluorescence quenching in the light reduces not only F_M but also F_O which leads to lower fluorescence values immediately after darkening compared to the F_O level after long-term dark adaptation (1, 4).

Data shown are representative of between 2 and 5 experiments with different leaves which exhibited little variation in response.

RESULTS

Figure 3A shows day/night cycles of gas exchange and fluorescence characteristics of a *Kalanchoë* leaf under standard conditions (12 h light, 500 μmol photons m^{-2} s^{-1}, 25°C/12 h dark, 15°C). Adopting the approach used by Osmond (14) to describe the net CO_2 exchange of CAM plants, four typical phases can be distinguished: the period of net CO_2 dark fixation (phase I), followed by a burst in uptake of atmospheric CO_2 during the initial part of the light period (phase II). During the middle of the light period, stomatal conductance is low, uptake of atmospheric CO_2 is negligible, and photosynthesis is based on reduction of CO_2 supplied internally via decarboxylation of malic acid (phase III). Thereafter, atmospheric CO_2 is taken up again for the remaining part of the light period (phase IV). The intercellular CO_2 partial pressure, p_i, increases during phase II and decreases during phase IV. No data are shown for phase III when leaf conductance is low and substantial errors can be introduced in the calculation of p_i, *e.g.* because of the increased contribution of cuticular transpiration to total transpiration, It is nevertheless safe to assume that p_i is above, or at least close to, atmospheric CO_2 partial pressure during phase III.

Figure 3B depicts alterations in the absolute levels of fluorescence emission in the dark and light. F_O remained virtually unchanged during the standard day/night cycle. After onset of the dark period, fluorescence during the saturation pulses reached a maximum level after about 1 h. The ratio F_V/F_M was then close to 0.8 which is typical of healthy, nonphotoinhibited, dark-adapted leaves (2). During the light period, the fluorescence signal composed of F_O and F_V' increased for the first 2 h and gradually declined after the 5th h of light. Similar, but much more pronounced changes were observed in the fluorescence emission during 1 s pulses of saturating light ($F_O + F_V''$). Fluorescence during saturation pulses increased during phase II, attained a plateau during phase III, and decreased during phase IV. Application of a pulse of saturating light, following 5 min of darkness at various times throughout the light period (Fig. 1, B and C) showed that fluorescence increased to a similar level in all cases, which was about 20% lower than F_M obtained after 1 h of darkness. Thus, there is a component of nonphotochemical quenching which relaxes rapidly upon darkening. This component is very small during phase III, when total nonphotochemical fluorescence quenching is only moderate, and increases at the beginning and at the end of the light period. The remaining component of nonphotochemical quenching, which relaxes slowly, has a more uniform magnitude during the light period.

The term $1-q_P$, which was calculated from these fluorescence signals, remained remarkably constant at around 0.2 and showed that the reduction state of Q was approximately 20% throughout the light period (Fig. 3 C). q_E increased strongly at the onset of illumination and then decreased during phase II, remained at a low level during phase III and increased again during phase IV, showing that the nonradiative energy dissipation was highest at the beginning and the end of the light period and lowest in the middle of the light period when photosynthesis rates were presumably highest due to malate decarboxylation and resulting high intercellular CO_2 partial pressures.

To test the possibility that q_E is sensitive to changes in photosynthesis rates caused by changes in CO_2 supply, *i. e.* in p_i, the intercellular CO_2 partial pressure was varied during phase IV by changing the CO_2 level of the ambient air. During phase IV, malic acid has been consumed and photosynthesis is almost exclusively based on uptake of atmospheric CO_2 (17). When p_a was increased to 1710 μbar between 17 and 19 h, p_i increased up to 750 μbar and A was stimulated by a factor of 2 (Fig. 4A). During this period, q_E continued to remain at the low level typical of phase III and did not increase at about 16 h as was observed in the control treatment (Fig. 3). Upon return to 350 μbar external CO_2, q_E increased sharply. The reduction state of Q was fairly constant throughout the treatment. Values of $1-q_P$, based on the lowest measured level of fluorescence during the dark interruptions, show an apparent transient decrease upon the change in p_a from 1710 to 350 μbar at 19 h. No such change was seen when $1-q_P$ was calculated using extrapolated values of F_O (Fig. 2).

When p_a was decreased to 105 μbar during phase IV (Fig. 5), p_i dropped to below 100 μbar, causing net CO_2 assimilation rate, A, to decrease to about 1 μmol m^{-2} s^{-1}. The decrease in p_a was accompanied by an abrupt increase in q_E. The reduction state of Q was again no higher than 20 to 30%. When the lowest measured level of fluorescence during the dark interruptions was used to calculate $1-q_P$, values transiently decreased during exposure of leaves to 105 μbar CO_2, suggesting that the reduction state of Q became lower in spite of the reduction in CO_2 supply and in spite of a decreased rate of photosynthetic electron flow as indicated by increased nonphotochemical quenching, q_E. If determination of $1-q_P$ was based on extrapolated values of F_O, the decrease in the rate of electron transport was accompanied by a more likely small increase in the reduction state of Q, from 0.175 (lowest value at noon) to 0.3 (highest value during the exposure to 105 μbar CO_2). Upon return to 350 μbar CO_2, $1-q_P$ decreased to 0.23.

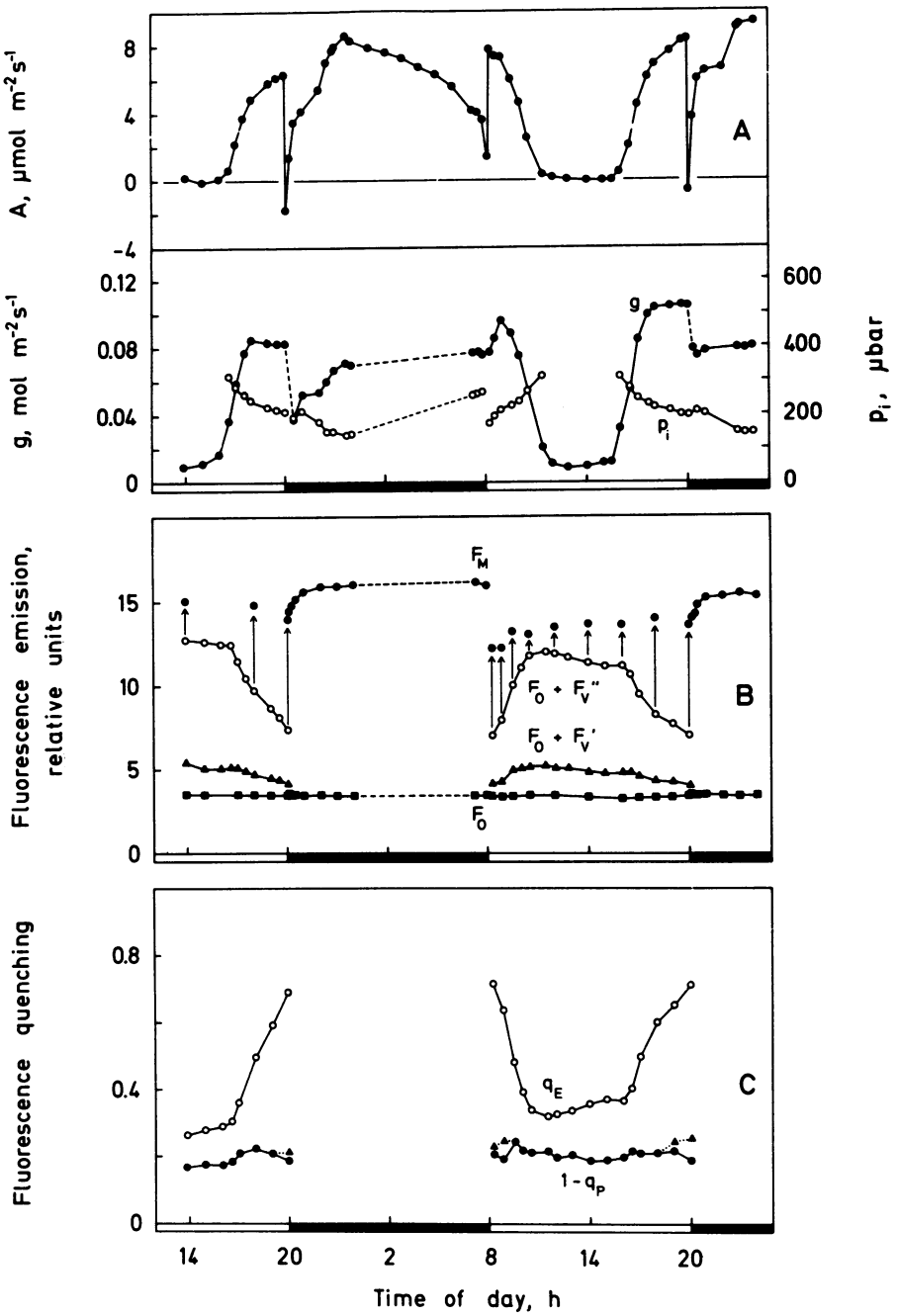

FIG. 3. Day/night changes of A, g, p_i, fluorescence yield (F_M, F_O, $F_O + F_V'$, $F_O + F_V''$), nonphotochemical quenching (qE) and reduction state of Q ($1-q_P$) in *K. daigremontiana*. PFD during the light period was 500 μmol photons m^{-2} s^{-1}. Leaf temperature and leaf-air vapor pressure difference were 25 to 26°C and 16 to 20 mbar bar^{-1} during the light period, and 15°C and 7 mbar bar^{-1} during the dark period. The arrows indicate the increase in fluorescence yield during a saturation pulse of light after 5 min of darkness at various times throughout the diurnal cycle. F_V' designates variable fluorescence during steady state photosynthesis and F_V'' the variable fluorescence during a pulse of saturating light in combination with the PFD of 500 μmol m^{-2} s^{-1}. Values of $1-q_P$ were either based on F_O taken as the lowest measured fluorescence level during dark interruptions (●) or on extrapolated levels of F_O (▲).

Following a night in CO_2-free air to minimize nocturnal CO_2 uptake and acidification (respiratory CO_2 still available) (19), uptake of atmospheric CO_2 during phase II was stimulated, leading to a 2-fold higher carbon gain than during phase II after a night at 350 μbar CO_2 (Fig. 6). Duration of phase III was greatly reduced and uptake of atmospheric CO_2 of phase IV commenced earlier compared to a standard light period (Fig. 3). q_E remained at a very high level of 0.83 for 2 h after onset of the light period, which contrasts with the rapid decline of q_E during corresponding periods of previous experiments. Thus, high values of q_E are clearly related to the duration of phase II and q_E decreases at the transition between phase II and phase III as was seen in the previous experiments. Irrespective of the fashion in which F_O during dark interruptions of the light period was estimated, there was a strong increase in $1-q_P$ at the beginning of the light period, indicating that Q was reduced to 50% initially, *i.e.* much more than seen in the experiments of Figures 3 to 5. Concomitant with the decrease in nonphotochemical quenching at the transition between phases II and III, the reduction state of Q decreased to values around 20% which corresponds to those observed during the standard light period (Fig. 3).

When the PFD during the light period was increased from 500 to 1100 μmol m^{-2} s^{-1}, phase II CO_2 fixation was decreased, the duration of phase III shortened, and that of phase IV prolonged (Fig. 7). The overall level of q_E was increased, yet as in the previous experiments changes in q_E were linked to changes in net CO_2 assimilation rate, A, inasmuch as q_E decreased during phase II to attain a minimum at that time when net CO_2 uptake was at its minimum (yet photosynthetic activity in the chloroplasts at its maximum), and that q_E increased again when net uptake of CO_2 increased in the second half of the light period. At 1100 μmol m^{-2} s^{-1}, the overall level of $1-q_P$ was also increased

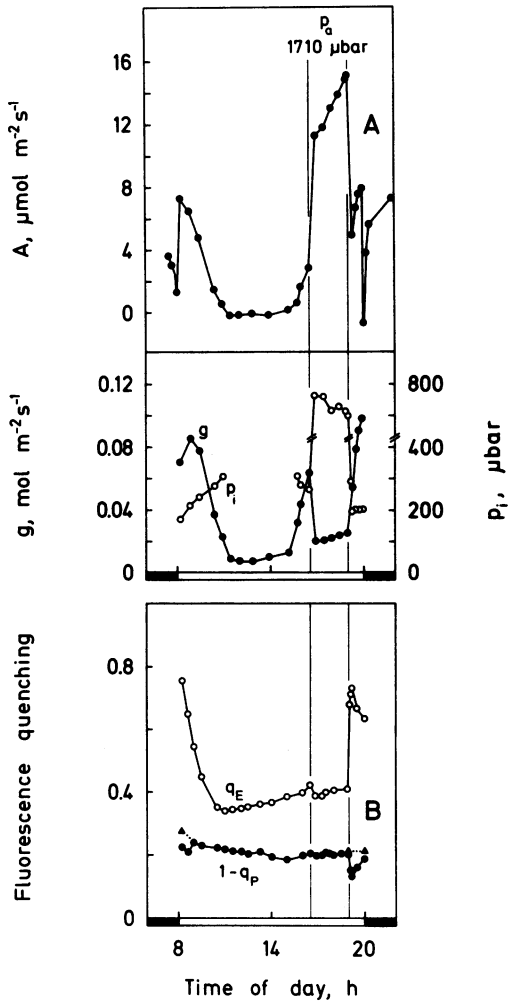

FIG. 4. Response of A, g, p_i, q_E, and reduction state of Q (1-q_P) to an increase in the ambient CO_2 partial pressure, p_a, from 350 to 1710 μbar in *K. daigremontiana* during the second half of a standard 12 h light period. Values of 1-q_P were either based on F_O taken as the lowest measured fluorescence level during dark interruptions (●) or on extrapolated levels of F_O (▲).

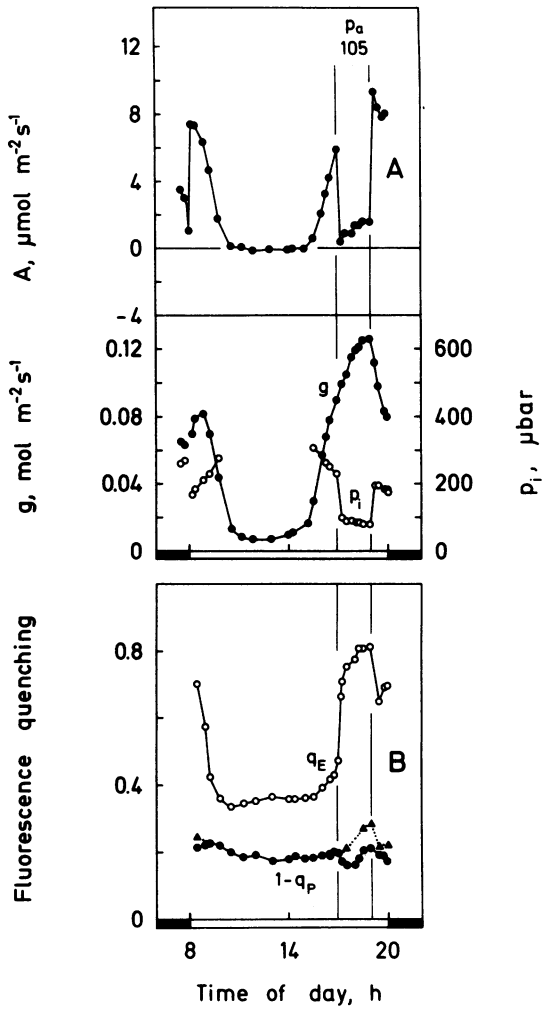

FIG. 5. As in Figure 4, but p_a was decreased from 350 to 105 μbar.

with 1-q_P being higher at the beginning and at the end than in the middle of the light period, particularly when calculations were based on extrapolated values of F_O.

Full CO_2 response curves of photosynthetic CO_2 assimilation via RuBP carboxylase, *e.g.* after completion of malic acid decarboxylation in the second half of a 12 h light period, are difficult to obtain in CAM plants, because CO_2 assimilation rates hardly attain a steady state. To further study the relationship between p_i, net CO_2 assimilation rate, q_E, and 1-q_P, CO_2 response curves were obtained for spinach leaves at two levels of PFD similar to those used in the experiments with *K. daigremontiana* (Fig. 8). At 472 μmol m^{-2} s^{-1}, which corresponds to the PFD during the standard 12-h light period with *K. daigremontiana*, q_E increased abruptly and strongly at p_i values below 200 μbar from 0.4 to 0.75 as net CO_2 assimilation rate decreased linearly with p_i. Above p_i = 200 μbar, A increased from 16 to 23 μmol m^{-2} s^{-1}, whereas q_E remained at 0.3. The reduction state of Q was remarkably constant at 10% over the whole range of p_i values and was only marginally increased at the CO_2 compensation point. At the higher PFD of 940 μmol m^{-2} s^{-1}, the overall values of q_E and 1-q_P were increased. Changes in A were again mainly accompanied by changes in q_E, which markedly increased from 0.5 to almost 0.9 below 400 μbar CO_2 when the decline in CO_2 assimilation rate with p_i became most pronounced. Unlike the situation at 472 μmol m^{-2} s^{-1}, q_E did not remain at its minimum at p_i above 400 μbar CO_2 but rather tended to increase again to about 0.6. Again, 1-q_P only increased at the CO_2 compensation point, but to a greater extent than at 472 μmol photons m^{-2} s^{-1}.

DISCUSSION

Measurements of leaf fluorescence were used to study photosynthetic activity at the chloroplast level, *i.e.* changes in the rate of electron transport, in the CAM plant *K. daigremontiana*. Diurnal changes in leaf gas exchange and in expected rates of CO_2 reduction were accompanied primarily by alterations of the nonphotochemical component of fluorescence quenching and only to a much lesser extent by fluorescence quenching related to the reduction state of Q, the primary electron acceptor of PSII.

When rates of photosynthetic electron flow are high compared to the rate of absorption of excitation energy, Q should be expected to be highly oxidized, and a small portion of excitation energy would be dissipated as heat. On the other hand, when rates of electron transport are insufficient to dissipate the absorbed excitation energy, an increasing proportion of this energy should be dissipated as heat. q_E is mainly a reflection of such nonradiative decay. It underwent marked changes in *K. daigremontiana* in the course of a 12 h light period.

Low values of q_E during the middle of the light period, when uptake of external CO_2 was negligible, are consistent with the view that CO_2 reduction rates within the chloroplasts are maxi-

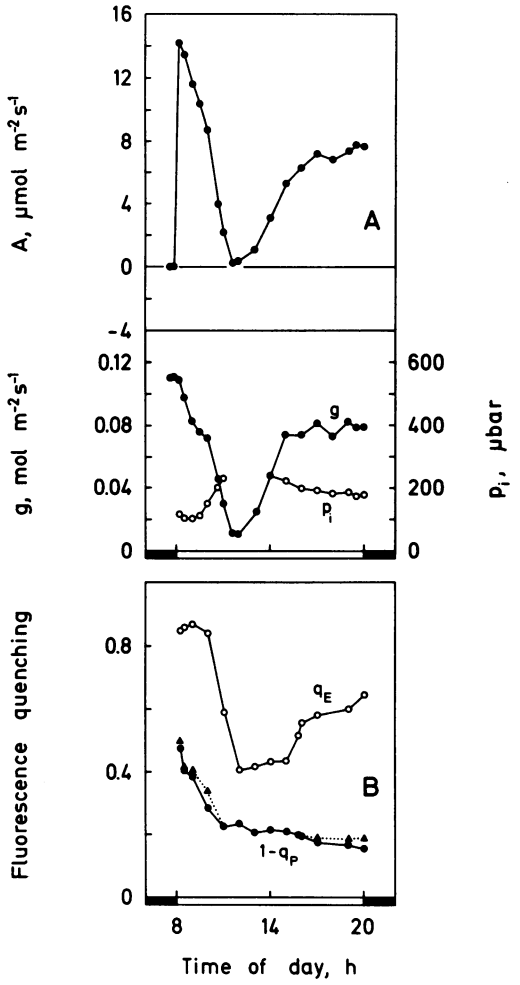

FIG. 6. As light period in Figure 3, but following a 12 h dark period in CO_2 free air.

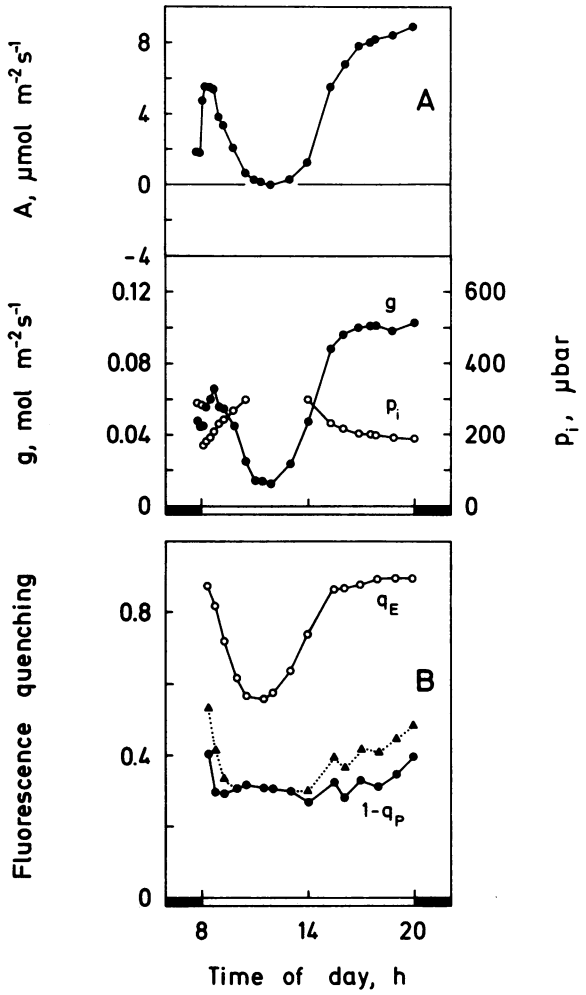

FIG. 7. As light period in Figure 3, but PFD was 1100 μmol photons m^{-2} s^{-1}.

mal during this time because malic acid decarboxylation provides high, saturating intercellular CO_2 partial pressures for photosynthesis. In *K. pinnata*, ATP/ADP ratios have indeed been shown to be lowest during this part of the diurnal cycle (10). p_i values are much lower at the beginning and the end of the light period, and although atmospheric CO_2 is taken up, the high values of q_E indicate decreased rates of ATP consumption and therefore lower CO_2 reduction rates than at noon. This interpretation is supported by changes in q_E induced by changes in CO_2 uptake rates during phase IV (malic acid pool depleted) resulting from either increasing or decreasing p_a and hence p_i (Figs. 4 and 5).

Fluorescence is a function of total electron transport, which apart from CO_2 reduction sustains other processes such as photorespiration. CO_2 response curves with spinach show that, at a PFD similar to that of the standard light period, q_E stays constant between p_i values of 240 and 1500 μbar suggesting that electron transport rate related to the sum of CO_2 reduction and photorespiration remains largely unchanged. It is only at p_i values below 200 μbar that the partitioning of absorbed light energy into heat is markedly increased (as indicated by the increase in q_E) showing that the combined action of CO_2 reduction and photorespiration dissipates less excitation energy at these low internal CO_2 partial pressures. Indeed, electron transport rates calculated after Farquhar and von Caemmerer (7) (based on a CO_2 compensation point in the absence of day respiration of 33 μbar and a day respiration of 0.5 μmol m^{-2} s^{-1}) remained constant around 118 μmol m^{-2} s^{-1} at p_i values above 240 μbar, and decreased by about 30% between 240 and 80 μbar CO_2. The critical p_i below which electron transport rate apparently declines is shifted upwards with increasing PFD and is found to be at about 400 μbar at 940 μmol m^{-2} s^{-1}. Thus, although changes in q_E are at least a qualitative measure of CO_2 reduction rate at subatmospheric internal CO_2 partial pressures, changes in CO_2 reduction rate at higher CO_2 pressures are no longer indicated by q_E, because increased electron transport rates due to increased rates of CO_2 reduction may be balanced by decreased rates of photorespiration and vice versa. At the PFD of 940 μmol m^{-2} s^{-1}, calculated electron transport rates were around 160 μmol m^{-2} s^{-1} between p_i of 1420 and 400 μbar, and decreased to 80 μmol m^{-2} s^{-1} between 400 and 80 μbar CO_2.

There was a component of q_E which rapidly relaxed upon darkness (Fig. 3B). This nonphotochemical quenching process is probably related to Δ pH across the thylakoid membranes and was shown to relax within 10 to 30 s in *Chlorella* cells and spinach chloroplasts (12). This rapidly relaxing component of q_E is very small in the middle of the light period when photosynthetic activity is maximal in the chloroplasts, *i.e.* when Δ pH is expected to be smallest due to high ATP demand (10). The rapidly relaxing component of q_E is responsible for the strong increase in total q_E at the beginning and at the end of the light period, when Δ pH is expected to increase because rates of electron flow fall behind the rate of absorption of excitation energy.

The remaining component of q_E was characterized by relatively slow relaxation kinetics and may be connected to the

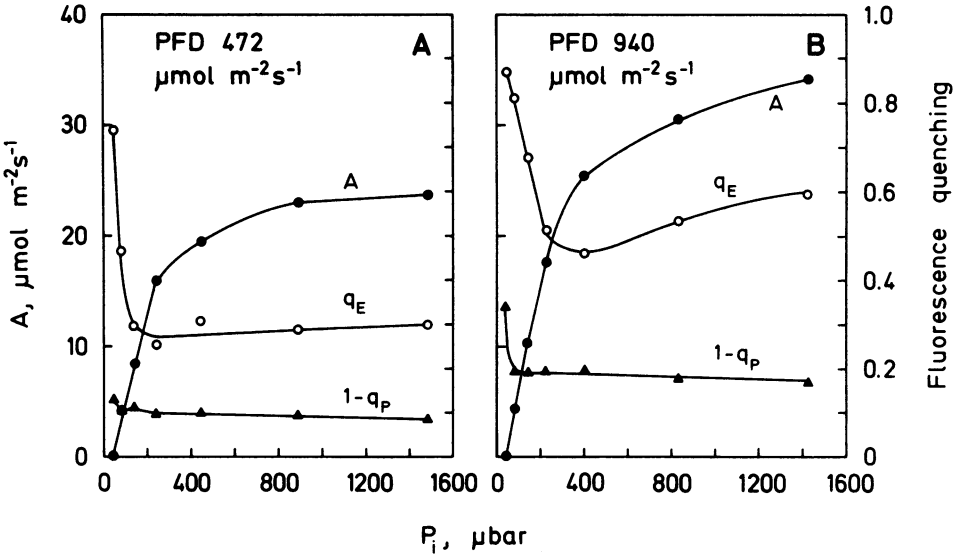

FIG. 8. CO_2 response curves of A, q_E, and the reduction state of Q ($1-q_P$) in spinach leaves at two levels of PFD. Leaf temperature was 22°C and leaf-air vapor pressure difference 10 mbar bar^{-1}. Measurements were taken 15 to 30 min after changes in the ambient CO_2 partial pressure.

production of zeaxanthin in the xanthophyll cycle possibly acting as a quencher of excess excitation energy in the antenna bed (5). Furthermore, a portion of the slowly relaxing component of q_E may be related to phosphorylation of the light harvesting complex of PSII, a process which is thought to divert light energy from PSII to PSI (8). Both the rapidly relaxing, Δ pH dependent component of q_E as well as the portion of the slowly relaxing component which is not related to energy transfer to PSI reflect nonradiative decay processes. The Δ pH dependent mechanism seems sensitive to small fluctuations in demand for ATP and reducing equivalents. The quenching mechanism with the slower relaxation kinetics has been shown to be predominant when excitation energy becomes greatly excessive (4), *e.g.* at very high levels of PFD.

In contrast to q_E, the reduction state of Q (= $1-q_P$) does not seem to be a good indicator of the changes in the rate of electron transport as $1-q_P$ stays low and constant during a standard day. Whenever rates of electron transport decrease, as indicated by increased nonradiative decay, $1-q_P$ increases only marginally. This suggests that an altered balance between the absorption of excitation energy and photochemistry leads to an altered partitioning of excitation energy in favor of nonradiative decay, thereby counteracting an increased reduction of Q.

Calculated values of $1-q_P$ are very sensitive to changes in the level of F_O. Therefore, values of $1-q_P$ were based on two different levels of F_O. When nonphotochemical quenching was strong, fluorescence upon darkening showed a complex behavior as it first decreased and then increased. When $1-q_P$ was calculated using a value of F_O which was extrapolated back to an even lower putative level than the lowest measured value of F_O following darkening during the light period, the reduction state of Q ($1-q_P$) had slightly higher values during phases II and IV when nonphotochemical fluorescence quenching was pronounced, than during phase III. Yet irrespective of the way in which F_O was estimated, the reduction state of Q tended always to be remarkably low.

Substantial increases in $1-q_P$ were observed in only those instances in which q_E was very high indicating that the capacity of the processes underlying q_E to dissipate excitation energy was exceeded. This was the case, for example, when the balance between absorbed excitation energy and photochemistry was altered by increasing the PFD from 500 to 1100 μmol m^{-2} s^{-1}. Under these conditions, increases in $1-q_P$ were observed at the beginning and at the end of the light period. Even so, the capacity to maintain a low reduction state of Q seemed remarkably high, because $1-q_P$ did not exceed 50% at 1100 μmol m^{-2} s^{-1}, *i.e.* at more than twice the PFD than during growth.

A relatively high reduction state of Q of 50% was obtained at the beginning of a light period at 500 μmol photons m^{-2} s^{-1} following a night in CO_2-free air. Under these conditions, uptake of atmospheric CO_2 during phase II was greatly stimulated, because CO_2 dark fixation processes involving PEP carboxylase, which had been held in check during the previous dark period, were presumably highly active during the early light period and competed with RuBP carboxylase for CO_2. Since the biochemistry of CO_2 dark fixation is not linked to photosynthetic electron transport, phase II is characterized by a transient large surplus of excitation energy. The observed increase in nonradiative decay was apparently no longer sufficient to maintain a reduction state of 20 to 25% which existed during the second half the light period and which was typical throughout a standard diurnal cycle at 500 μmol m^{-2} s^{-1}.

The conclusion that the reduction state of Q is much less sensitive to alterations in the balance of excitation energy and photochemistry than nonradiative decay processes (see also Ref. 16) is supported by studies of CO_2 response curves in spinach (Fig. 8). $1-q_P$ stayed low and constant at nearly all CO_2 partial pressures used. Only at the CO_2 compensation point at 940 μmol photons m^{-2} s^{-1}, when q_E was close to its maximum possible value, was there an increase in $1-q_P$ from 0.2 to 0.35, whereas calculated rates of electron transport already started to decrease at p_i values lower than 400 μbar. Maintenance of a low reduction state of Q can be considered advantageous as it decreases the probability of the creation of potentially harmful oxygen radicals (6). It is conceivable that at higher light intensities than those used in the present study, which greatly exceed the growth light intensity, q_E would be saturated in *K. daigremontiana* throughout the day and in spinach over the whole range of p_i values. Only under these conditions are changes in electron transport rate expected to be fully reflected by the reduction state of Q.

Acknowledgments—We thank K. J. Dietz and U. Heber for critically reading the manuscript.

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