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Internal CO_2 Supply during Photosynthesis of Sun and Shade Grown CAM Plants in Relation to Photoinhibition

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WILLIAM W. ADAMS III*[1] AND C. BARRY OSMOND
Plant Environmental Biology Group, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra ACT 2601, Australia

ABSTRACT

Leaves of *Kalanchoë pinnata* were exposed in the dark to air (allowing the fixation of CO_2 into malic acid) or 2% O_2, 0% CO_2 (preventing malic acid accumulation). They were then exposed to bright light in the presence or absence of external CO_2 and light dependent inhibition of photosynthetic properties assessed by changes in 77 K fluorescence from photosystem II (PSII), light response curves and quantum yields of O_2 exchange, rates of electron transport from H_2O through Q_B (secondary electron acceptor from the PSII reaction center) in isolated thylakoids, and numbers of functional PSII centers in intact leaf discs. Sun leaves of *K. pinnata* experienced greater photoinhibition when exposed to high light in the absence of CO_2 if malic acid accumulation had been prevented during the previous dark period. Shade leaves experienced a high degree of photoinhibition when exposed to high light regardless of whether malic acid had been allowed to accumulate in the previous dark period or not. Quantum yields were depressed to a greater degree than was 77 K fluorescence from PSII following photoinhibition.

Leaves of C_3 plants grown in bright light are subject to photoinhibition following illumination at the light intensities experienced during growth in the absence of internal or external sources of CO_2 (23, 24). Photoinhibition has been indicated by impaired quantum yield, impaired light- and CO_2-saturated photosynthesis, both measured *in vivo*, and by impaired PSII electron transport activity in isolated thylakoids. Much the same photoinhibitory responses are shown by shade plants after transfer to bright light in air, and the effects of CO_2 deprivation are additive in this case (25). It has been shown that photorespiration provides a significant internal source of CO_2 which can mitigate photoinhibition in sun grown C_3 plants (10, 26, 27). That is, photorespiration in air makes it unlikely that CO_2 deprivation due to stomatal closure could lead to photoinhibition (14, 22).

Although stomatal closure is unlikely to lead to CO_2 deprivation in C_3 plants, it is well established that stomata of CAM plants close so tightly as to preclude CO_2 exchange in the light during deacidification (16, 19). During this period, high internal CO_2 concentrations are generated by the decarboxylation of malic acid, and this internal CO_2 source is utilized in photosynthesis (9). Many CAM plants, especially stem succulents, respond to soil water deficits by maintaining tightly closed stomata throughout the light period (29) and nocturnal recycling of respiratory CO_2 through malic acid for subsequent photosynthesis. These studies led to the speculation (20, 22) that the internal supply of CO_2 derived from malic acid in CAM plants served to mitigate photoinhibition and maintain the integrity of the photosynthetic apparatus in these plants, in the absence of CO_2 exchange in the light. Although CAM plants grown in the shade are more susceptible to photoinhibition than those grown in bright light (3), the significance of the internal CO_2 source in alleviating high light stress in these plants has not been examined. Preliminary experiments, based on manipulation of dark CO_2 fixation and malic acid pool size, were reported previously (21). This paper describes subsequent experiments in detail, and demonstrates the importance of the internal CO_2 source in preventing photoinhibition of sun-grown CAM plants.

MATERIALS AND METHODS

Kalanchoë pinnata was grown from plantlets under either full glasshouse light (sun-grown plants; peak of approximately 1500 μmol quanta m^{-2} s^{-1} in the winter and 2000 μmol quanta m^{-2} s^{-1} in the summer, on a horizontal plane on a clear day) or under shade cloth (shade-grown plants; irradiance ranged from 15 to 30% of full glasshouse irradiance depending on solar angle and cloud cover). All measurements of light were made with a quantum sensor (Li-Cor 190SB). Glasshouse air temperatures ranged from 14°C at night to as high as 36°C on some summer days. Plants received water daily and nutrients (one-half strength Hewitt's) (30) three times a week. All plants were at least 3 months old and only the terminal leaflet of tripinnate leaves of the third to fifth rank from the apex, in an east-west plane, were used for the experiments described below.

All experiments were carried out with the gas exchange system described in von Caemmerer and Edmondson (7), with the following changes. The leaflet was inserted into a chamber with a volume of 2.5 L and the flow of gases through the chamber was maintained at 2 L min^{-1}, or at 5 L min^{-1} when CO_2 exchange was measured. Light was provided by a metal halide lamp, and different irradiance levels were obtained by changing the height of the lamp and/or inserting neutral density glass filters. Leaf temperature (maintained at 15°C during the dark and 27°C during the light) was monitored with a thermocouple inserted into and parallel with the mesophyll. The vapor pressure deficit was maintained at approximately 8 mbar during the dark and 12 mbar during the light. The CO_2 partial pressure of the air stream prior to entering the chamber was measured with an infrared gas analyzer (model ZAR, Fuji Electric). Calculations of the rates of CO_2 exchange followed von Caemmerer and Farquhar (8).

Leaves were placed in the chamber in early afternoon and illuminated (sun leaves at 800 μmol quanta m^{-2} s^{-1} and shade leaves at 325 μmol quanta m^{-2} s^{-1}) for 5 to 6 h in air to ensure decarboxylation of any remaining malic acid. This was followed by a 13 h night in either air (allowing fixation of CO_2 into malic acid, thereby providing an internal CO_2 source for photosynthesis

[1] Current address: Lehrstuhl für Botanik II, Mittlerer Dallenbergweg 64, 8700 Würzburg, West Germany.

Table I. *Nocturnal Acid Accumulation and F_v/F_m (Fluorescence from PSII at 77 K) in K. pinnata Leaves in Response to Different Atmospheres in the Dark*

Mean ± SE (*n*).

Growth Conditions	Dark Atmosphere	AM Titratable Acidity	PSII Fluorescence
		μEq g⁻¹ fresh wt	F_v/F_m
Sun	Air	317 ± 8 (11)	0.804 ± 0.006 (12)
	2% O_2	48 ± 3 (13)	0.781 ± 0.012 (16)
Shade	Air	245 ± 12 (4)	0.829 ± 0.008 (8)
	2% O_2	37 ± 0 (3)	0.786 ± 0.004 (4)

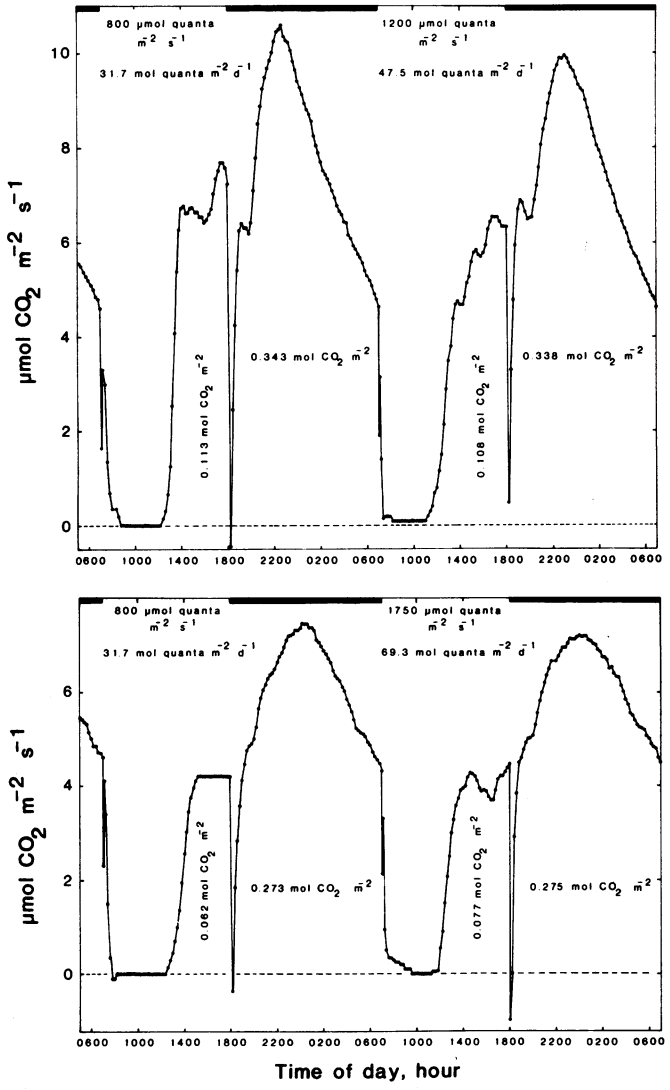

FIG. 1. Diel patterns of CO_2 exchange in air for 2 sun leaves of *K. pinnata* (August), one exposed to 800 μmol quanta m^{-2} s^{-1} for the first 11-h day followed by a day at 1200 μmol quanta m^{-2} s^{-1} (*upper panel*), the other exposed to 800 μmol quanta m^{-2} s^{-1} followed by a day at 1750 μmol quanta m^{-2} s^{-1}. Net CO_2 uptake for each leaf was identical for both exposures.

during the subsequent light period) or in 2% O_2, balance N_2 (preventing the fixation of CO_2 from external sources and suppressing fixation of respiratory CO_2). Following the dark treatment with either air or 2% O_2, the leaves were exposed to light in either air or 2% O_2 and various parameters measured during

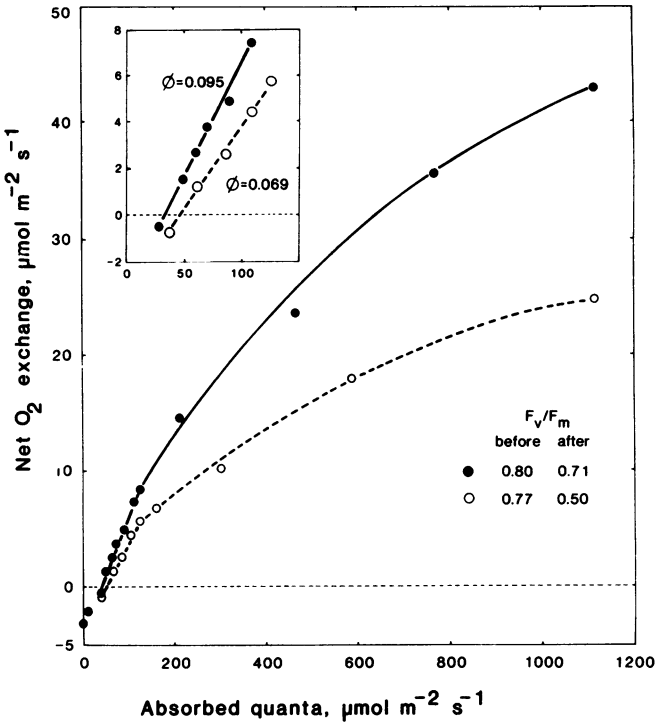

FIG. 2. Selected light response curves of photosynthetic O_2 exchange at 25°C and 5% CO_2 from sun-grown leaves of *K. pinnata* (August). Each curve was obtained at the end of a 4-h exposure to light in 2% O_2, 0% CO_2 at a leaf temperature of 27°C. *Closed circles* = night of air (AM titratable acidity = 307 μEq g^{-1} fresh weight), exposure at 800 μmol quanta m^{-2} s^{-1}; *open circles* = night of 2% O_2, 0% CO_2 (AM titratable acidity = 43 μEq g^{-1} fresh weight), exposure at 1750 μmol quanta m^{-2} s^{-1}.

or at the end of this treatment. Levels of titratable acidity were determined on extracts of leaf punches which were weighed, cut into small pieces, and boiled for 20 to 30 min in water. The extracts were then titrated to an endpoint of pH 7.0 with 0.01 N NaOH.

Fluorescence from PSII (690 nm) from leaf tissues frozen to 77 K for 4 min following a 7-min period of darkness was measured with the system described in Adams (1). Light response curves and quantum yields (ϕ) of O_2 exchange were determined from leaf discs at 25°C and 5% CO_2 as described previously (2, 11).

Uncoupled, light-saturated rates of electron transport through PSII, from H_2O through Q_B[2] to DMQ, were determined polarographically from suspensions of isolated thylakoids maintained at 25°C. Leaf tissues were sliced into small pieces and then ground for 6 s in a chilled medium (approximately 12 ml per 10 cm^2 of tissue) containing 350 mM sorbitol, 150 mM *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulfonic acid (EPPS), 20 mM NaCl, 5 mM $MgCl_2$, and 0.5 mM NaEDTA, at a pH of 8.0 (KOH), to which 1% PVP and 1% BSA were added just prior to use. This suspension was then filtered through 20 μm nylon mesh cloth and the filtrate spun at 2800*g* for 1 min. The supernatant was discarded and the thylakoids resuspended in a small volume of the reaction medium (350 mM sorbitol, 50 mM Hepes, 20 mM NaCl, and 5 mM $MgCl_2$, at pH 6.8, the experimentally determined pH optimum). This suspension, which was kept on ice,

[2] Abbreviations: Q_B, the secondary electron acceptor from the PSII reaction center; DMQ, 2,5-dimethyl-*p*-quinone; F_0, initial, instantaneous level of fluorescence; F_m, maximum level of fluorescence; F_v, variable fluorescence; PFD, photon flux density.

Table II. *Evidence for Different Degrees of Photoinhibition in Sun-Grown Leaves of K. pinnata under Various Conditions in Which Internal and External Sources of CO_2 Have Been Controlled*

Treatment		Titratable Acidity	Quantum Yield	Electron Transport $H_2O \rightarrow DMQ$	PSII Fluorescence at 77 K
Dark	Light[a]				
		μEq g^{-1} fresh wt	*mol O_2 mol^{-1} quanta*	*mmol O_2 mol^{-1} Chl s^{-1}*	*% change in F_v/F_m*
Air	Air	318	0.085	137	−11
2% O_2	Air	49	0.090	105	−15
2% O_2	21% O_2	62	0.074	74	−17
2% O_2	2% O_2	49	0.076	50	−27
21% O_2	2% O_2	54	0.064	47	−25

[a] Treatment was 1750 μmol quanta m^{-2} s^{-1} for 4 h.

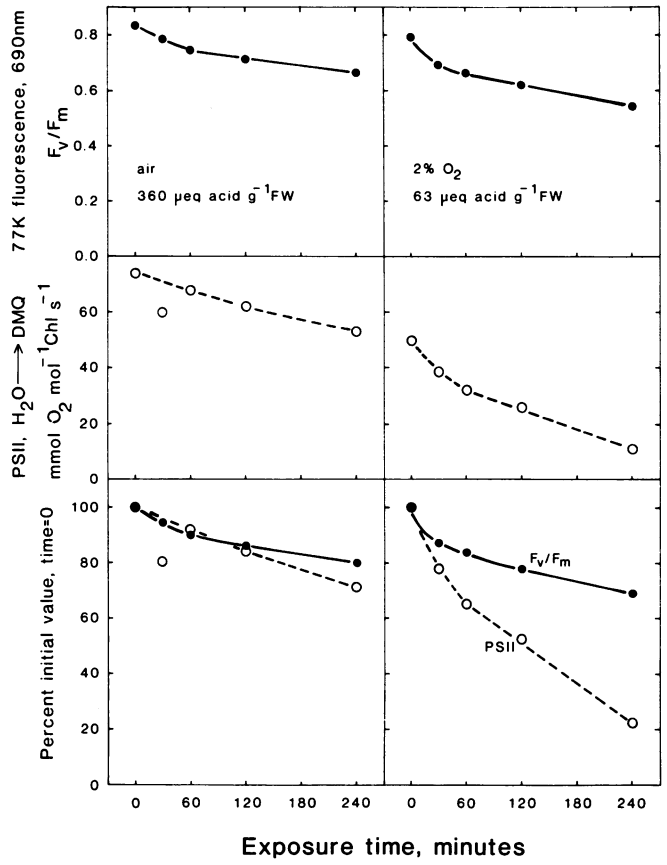

FIG. 3. Time course of changes in F_v/F_m (77 K fluorescence from PSII, *closed circles*) and electron transport through PSII from H_2O to DMQ (*open circles*) in sun-grown leaves of *K. pinnata* (November) during exposure to 1750 μmol quanta m^{-2} s^{-1} in air *left panel* and in 2% O_2 (*right panel*). The leaf illuminated in air had been exposed to air during the previous night allowing accumulation of malic acid, while the leaf illuminated in 2% O_2, 0% CO_2 had been exposed to 2% O_2, 0% CO_2 during the previous night preventing accumulation of malic acid.

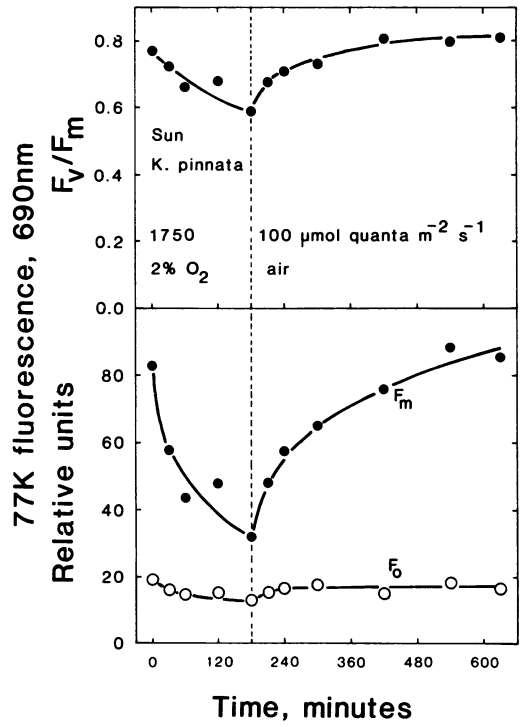

FIG. 4. Time course of changes in fluorescence from PSII at 77 K for a sun-grown leaf of *K. pinnata* (November) during exposure to 1750 μmol quanta m^{-2} s^{-1} in 2% O_2, 0% CO_2 followed by recovery at 100 μmol quanta m^{-2} s^{-1} in air. The leaf had been prevented from accumulating malic acid during the previous 13-h night by exposure to 2% O_2, 0% CO_2.

was then immediately assayed. Each preparation was assayed a minimum of three times and the mean rate determined. The reaction medium included 10 mM NH_4Cl and 1 mM DMQ. The Chl concentration in the reaction suspension was determined according to Arnon (6) following the electron transport assays.

The number of functional PSII centers in intact leaf discs was determined from the oxygen yield induced by single-turnover flashes (approximately 3 μs duration at half peak height). The flashes were supplied by a xenon flash lamp (Stroboslave type 1539-A) at a frequency of 4 Hz. The rate of oxygen evolution (minus dark drift), determined with a leaf disc O_2 electrode, was compared with the flash frequency to give the oxygen yield per flash. The number of O_2 molecules evolved per flash was multiplied by 4 to give the number of functional PSII centers. Background far-red light (approximately 17 μmol quanta m^{-2} s^{-1}, 700–730 nm) was present during flash illumination in order to keep PSI turning over and the plastoquinone pool in an oxidized state.

RESULTS AND DISCUSSION

Leaves of *K. pinnata*, grown in sun or shade, exhibited greatly reduced levels of nocturnal acid accumulation when kept in 2% O_2 in the dark for 13 h (Table I). This result, similar to that obtained by Moyse (17) with N_2 atmospheres, provided a ready means of controlling internal CO_2 supply in the subsequent light phase. However, we found that N_2 atmospheres led to flaccid leaves, for unknown reasons, and this complication could be avoided in 2% O_2. Several other complications also arose from the protocol employed in these experiments. One is that F_v/F_m

Table III. *Photoinhibition in Leaves of Sun-Grown K. pinnata following CO_2 Deprivation in Bright Light*

Leaves were kept in 2% O_2 in dark, then 3 h at 1750 μmol quanta m^{-2} s^{-1} in 2% O_2 followed by recovery at 100 μmol quanta m^{-2} s^{-1} in air (November).

Experiment	PSII Fluorescence at 77 K	Electron Transport $H_2O \rightarrow DMQ$	Functional PSII Centers
	F_v/F_m	*mmol O_2 mol^{-1} Chl s^{-1}*	*mmol mol^{-1} Chl*
No. 1 control	0.83	132	2.34
Photoinhibited	0.58	97	0.52
Recovery 3 h	0.74	141	2.67
Recovery 7 h	0.76	153	2.34
No. 2 control	0.79	170	
Photoinhibited	0.53	118	
Recovery 3 h	0.76	172	

was usually slightly reduced in leaves exposed to 2% O_2 overnight relative to those exposed to air (Table I). Another is that 2% O_2 in the light substantially delayed deacidification in tissues which had been kept in air in the dark. For instance, in one typical experiment the level of titratable acidity dropped from 360 to 81 μEq acid g^{-1} fresh weight during the 4-h exposure to 1750 μmol quanta m^{-2} s^{-1} in air, whereas in 2% O_2 it only decreased from 350 to 309 μEq acid g^{-1} fresh weight. Therefore, controls were usually plants exposed to air in the dark and light and treatments were usually plants kept in 2% O_2 in the dark and light.

Another aspect of the controls used in these experiments which required evaluation at the outset was the intrinsic sensitivity of *K. pinnata* to photoinhibition in bright light. Some CAM plants show light dependent reduction in dark CO_2 fixation capacity when exposed to high PFD for several days (18). Figure 1 shows that, in the relatively short-term experiments employed here, leaves of sun-grown *K. pinnata* did not show any evidence of light dependent reduction in maximum rates of CO_2 uptake in air in the late afternoon. Moreover, increasing the PFD from 800 to 1200 or 1750 μmol quanta m^{-2} s^{-1} did not alter the amount of CO_2 fixed in the light or the dark. That is photosynthetic CO_2 fixation in these plants was light saturated at 800 μmol quanta m^{-2} s^{-1}, but higher PFD did not impair CO_2 uptake when air levels of CO_2 were available.

Comparison of Figures 1 and 2 shows that the CO_2-saturated rate of O_2 exchange is much greater (5–8 times) than the rates of photosynthetic CO_2 uptake achieved in air in the late afternoon. This feature of CAM plants, noted earlier by Spalding *et al.* (28) in comparisons of the rate of deacidification and photosynthetic CO_2 exchange in *Sedum praealtum* and more recently for rates of deacidification and photosynthetic O_2 exchange versus CO_2 exchange in *Opuntia stricta* (1), suggests that the photosynthetic apparatus is optimized for rapid and efficient photosynthesis under the high CO_2 concentrations prevailing during deacidification, rather than for photosynthesis in air.

Figure 2 shows the light response curves for photosynthetic O_2 evolution in *K. pinnata* leaves exposed to 800 μmol quanta m^{-2} s^{-1} for 4 h in 2% O_2 after a night in air (titratable acidity following darkness = 307 μEq g^{-1} fresh weight) and to 1750 μmol quanta m^{-2} s^{-1} for 4 h in 2% O_2 after a night in 2% O_2 (titratable acidity following darkness = 43 μEq g^{-1} fresh weight). Illumination in the absence of an internal CO_2 supply (malic acid), and in the absence of external CO_2, led to a reduction in quantum yield, a reduction in photosynthesis at high PFD, and a reduction in F_v/F_m of PSII fluorescence. These changes suggest that photoinhibition of these sun-grown plants depends on low malic acid content and high PFD.

These indications were confirmed in other experiments with leaves of sun-grown plants (Table II). As an additional measure of the occurrence of photoinhibition, the rate of PSII mediated electron transport from H_2O to DMQ was measured. Compared with leaves which had access to an internal and/or external source of CO_2, leaves exposed to high light in the absence of either experienced greater reductions in quantum yield, light-saturated electron transport, and F_v/F_m fluorescence from PSII. Treatment in air following 2% O_2 in the dark prevented photoinhibition, although the most sensitive parameter, electron transport activity, decreased to some extent. The ability of photorespiration to prevent photoinhibition through the maintenance of C_3 plants at the CO_2 compensation point in 21% O_2 at high light was previously demonstrated (24, 26). However, treatment of *K. pinnata* in 21% O_2 in the light, which would permit photorespiration to proceed, was not very effective, possibly because the flowing gas stream flushed photorespiratory CO_2 from the chamber. Perhaps for the same reason, 21% O_2 in the dark, which would permit respiration to proceed, did not result in a significant increase in malic acid synthesis, although this may also indicate that 2% O_2 did permit respiration to continue. These experiments establish that in the absence of an internal CO_2 supply (malic acid) and external CO_2, this CAM plant shows evidence of photoinhibition in bright light. The results are comparable to those obtained with C_3 plants (24, 26) and demonstrate that in CAM plants, internal refixation of CO_2 from malic acid decarboxylation, like recycling of photorespiratory CO_2 in C_3 plants, mitigates photoinhibition.

In most of these experiments with leaves of sun-grown plants in which photoinhibition was brought about by CO_2 deprivation in bright light, changes in PSII electron transport were greater

Table IV. *Evidence for Photoinhibition of Shade-Grown Leaves of K. pinnata in High Light Regardless of Whether an Internal Source of CO_2 (Malic Acid) Is Present or Not*

Treatment	Titratable Acidity	Quantum Yield	Electron Transport $H_2O \rightarrow DMQ$	PSII Fluorescence at 77 K
	μEq g^{-1} fresh wt	*mol O_2 mol^{-1} quanta*	*mmol O_2 mol^{-1} Chl s^{-1}*	*% change in F_v/F_m*
Air (dark/light[a])				
800 μmol quanta m^{-2} s^{-1}	225	0.094	84	−9
1750 μmol quanta m^{-2} s^{-1}	237	0.047	44	−38
2% O_2 (dark) air (light[a])				
800 μmol quanta m^{-2} s^{-1}	36	0.090	79	−5
1750 μmol quanta m^{-2} s^{-1}	38	0.054	41	−27

[a] Leaves exposed to light for 4 h.

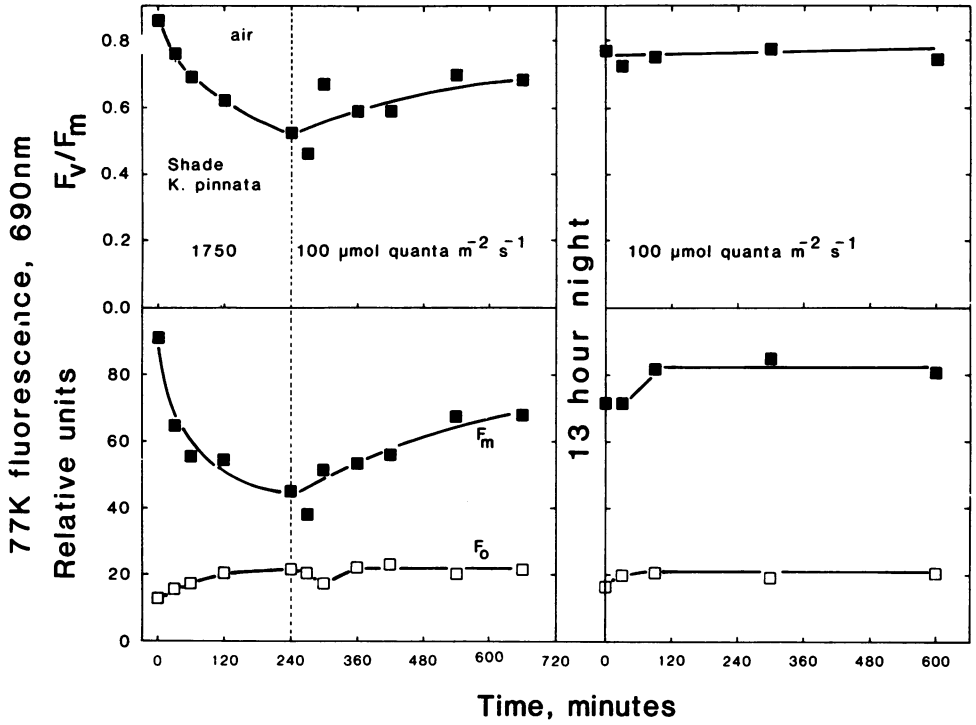

FIG. 5. Time course of changes in fluorescence from PSII at 77 K for a shade-grown leaf of *K. pinnata* (November) during exposure to 1750 μmol quanta m^{-2} s^{-1} in air followed by recovery at 100 μmol quanta m^{-2} s^{-1} in air. The leaf had been allowed to accumulate malic acid during the previous night by exposure to air.

Table V. *Photoinhibition of Leaves of Shade-Grown K. pinnata Exposed to Bright Light in Air*

Leaves were kept in air in the dark, then 4 h at 1750 μmol quanta m^{-2} s^{-1} in air, followed by recovery at 100 μmol quanta m^{-2} s^{-1} in air (November).

Experiment	PSII Fluorescence at 77 K	Electron Transport $H_2O \rightarrow DMQ$	Functional PSII Centers
	F_v/F_m	*mmol O_2 mol^{-1} Chl s^{-1}*	*mmol mol^{-1} Chl*
No. 1 control	0.84	128	1.84
Photoinhibited	0.48	51	0.87
Recovery 2 h	0.71	98	2.02
Recovery 4 h	0.73	118	2.45
Recovery 6 h	0.72	123	1.81
No. 2 control	0.80	143	
Photoinhibited	0.45	62	
Recovery 1.5 h	0.58	96	
Recovery 3 h	0.63	124	
Recovery 6 h	0.75	134	

than changes in fluorescence. Figure 3 compares the time course of the former two parameters in control and photoinhibitory treatments, and shows that inhibition of PSII electron transport exceeded the changes in fluorescence in the absence of CO_2. Further time courses of changes in 77 K fluorescence and electron transport during photoinhibition in leaves of sun-grown plants deprived of CO_2 are shown in Figure 4 and Table III. The decrease in F_v/F_m during the exposure to high light in the presence of 2% O_2 was due to a massive quenching of F_m and a smaller, but significant, quenching of F_0. This quenching was rapidly reversible upon transfer of the leaf to low light and air, and F_v/F_m recovered within 4 to 5 h (Fig. 4).

These changes in fluorescence are consistent with those believed to indicate the operation of a mechanism whereby energy in excess of that which can be utilized for photosynthesis and photorespiration is diverted from the reaction centers through

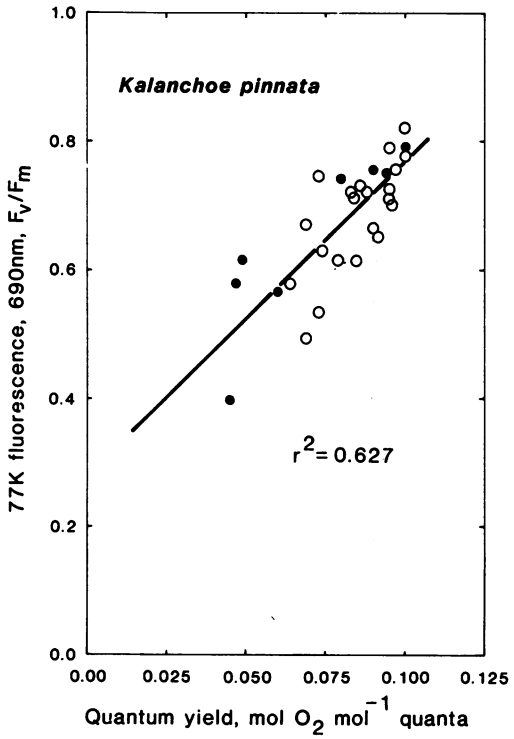

FIG. 6. Relationship between the quantum yields of O_2 exchange and F_v/F_m (fluorescence from PSII at 77 K) in sun-grown (*open circles*) and shade-grown (*closed circles*) leaves of *K. pinnata* subjected to various degrees of photoinhibition.

nonradiative dissipation, thus protecting them from photoinhibitory damage (12, 13, 15). According to this model an increase in F_0 is indicative of damage to PSII. However, these fluorescence changes were regularly accompanied by a decrease in PSII electron transport activity, and a decrease in functional PSII reaction centers as measured by flash yield, both of which were reversible within 3 h (Table III). It is possible that an increase in F_0 did

occur in these experiments but that it was masked by concurrent quenching. Alternatively, it is possible that in spite of the photoprotective dissipation of excitation indicated by the fluorescence changes, lesions occurred elsewhere in the photosynthetic apparatus which were responsible for impaired PSII electron transport and PSII reaction center function.

Some of these experiments were repeated with leaves of shade-grown *K. pinnata*. Table IV shows that neither external nor internal sources of CO_2 were effective in protecting against photoinhibition when leaves were exposed to 1750 μmol quanta m^{-2} s^{-1}. These results are similar to those obtained previously with shade-grown *K. daigremontiana* (3). In the experiments described in Table IV, unlike those in Table II, there was good proportional agreement between the changes in quantum yield, electron transport, and fluorescence. However, the time course of changes in fluorescence exhibit quite a different pattern of response to that shown in Figure 4. In the leaves of shade-grown *K. pinnata* photoinhibited in air, the decrease in F_v/F_m was due to an increase in F_0 as well as a decrease in F_m (Fig. 5). Recovery was slower, incomplete, and primarily due to an increase in F_m. Even after 24 h, F_0 remained higher than the initial control value. This pattern of photoinhibition was associated with much the same reversible reductions in PSII electron transport activity and functional PSII reaction centers as observed previously (*cf.* Table V and Table III). In these experiments it is presumed that, in spite of the potentially photoprotective processes indicated by the decrease in F_m (due to an increase in nonradiative dissipation of excess energy), the increase in F_0 indicates photoinhibitory damage, and it was not surprising to find this associated with impaired PSII electron transport and flash yield.

The possibility of photoinhibitory damage at sites in addition to the PSII reaction center is indicated by plotting the relationship between F_v/F_m and quantum yield measured in leaves of sun- and shade-grown plants which had been subjected to different treatments resulting in various degrees of photoinhibition. The regresssion line (Fig. 6), which was very similar to that observed previously in *K. daigremontiana* photoinhibited in air (3), does not pass through the origin. This implies that the greater change in quantum yield than in fluorescence could involve factors other than the balance of excitation dissipation in the reaction centers, as interpreted by current models of *in vivo* fluorescence. The more perfect correlations obtained by Demmig and Björkman (12) with C_3 plants were based on quantum yield and fluorescence measurements after periods of recovery in air at low PFD. Their period of recovery may have allowed repair of additional sites of damage other than the PSII reaction center, perhaps between the water-splitting apparatus and Q_B (see Fig. 3, and compare rates of recovery in Tables III and V), or differences between C_3 and CAM plants may explain the difference between these correlations. However, the relationships between the two different responses behind the change in F_v/F_m and the similarity of changes in PSII function indicated *in vivo* and *in vitro* in the data sets for sun-grown and shade-grown leaves cannot, at present, be explained.

In conclusion it seems clear that the internal source of CO_2 provided by the nocturnal fixation of CO_2 into malic acid can provide photosynthetic tissues of CAM plants adequate protection against photoinhibition during deacidification when stomata are closed. If no internal source of CO_2 is available then photoinhibition ensues, just as it does in CO_2 deprived C_3 plants. Despite the apparent protection against photoinhibition afforded by the internal supply of CO_2 in these short-term experiments with sun-grown leaves of *K. pinnata*, it has recently become clear that CAM plants growing in full sunlight under natural conditions may experience photoinhibition, often to a severe degree (1, 4, 5). Some degree of CAM-idling (29) probably occurs in many CAM plants under natural conditions, and it is likely that the CO_2 supplied in this manner is insufficient to prevent photoinhibition in conditions of full sunlight. Afterall, deacidification is usually complete in about 4 h and if stomata remain closed, photorespiratory CO_2 cycling is likely to be the only other source of CO_2 available. This may mitigate photoinhibition in C_3 plants for several hours (26) but its long-term efficacy has not been demonstrated. It should be noted that the photoinhibited CAM species *O. basilaris*, growing in Death Valley, exhibited only 10% of the nocturnal acidification observed in *K. pinnata* (4). In *O. basilaris* the internal CO_2 pool is clearly of limited significance, and processes involved in the nonradiative dissipation of excitation may be more important. Our experiments also indicate that, in shade-grown CAM plants, the internal supply of CO_2 probably does not provide significant protection against photoinhibition, should these plants experience bright light. Like shade-grown C_3 plants, they will be photoinhibited when exposed to high light even in the presence of adequate internal or external CO_2 sources.

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