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Responses of Two CAM Species to Different Irradiances during Growth and Susceptibility to Photoinhibition by High Light[1]

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ABSTRACT

Two CAM species, *Kalanchoë daigremontiana* Hamet et Perrier and *Hoya carnosa* (L.) R. Br., were grown under a range of five photon flux area densitites (PFD) and then characterized. Significant acclimation to shade was indicated by progressive decreases in leaf thickness, rates of respiratory O_2 uptake, light compensation point, maximum rates of photosynthetic O_2 evolution, nocturnal acid accumulation, and $\delta^{13}C$ values, and increases in chlorophyll concentration and absolute levels of room temperature (25°C) and 77K fluorescence. Quantum yields (as measured by O_2 exchange) and the ratio of variable 77K fluorescence over the maximum yield (F_v/F_m) were relatively constant across the treatments. The only significant deviation from the above characteristics was in *H. carnosa* grown under full glasshouse PFD, where it apparently experienced photoinhibition. Following a photoinhibitory treatment, *K. daigremontiana* exhibited increases in the light compensation point and progressively greater reductions in the quantum yield, maximum photosynthetic rate, F_v/F_m, and the variable component of room temperature fluorescence with increasing shade during growth. Thus although Crassulacean acid metabolism plants can adjust to shaded conditions, they are susceptible to photoinhibition when exposed to higher PFD than that experienced during growth.

There have been numerous studies of the responses of C_3 species to sun and shade (4), yet little characterization of CAM plants grown under different PFD.[3] Many horticultural CAM plants are grown indoors in dim light, and many other CAM plants grow naturally in deep shade as rainforest epiphytes (3, 13, 16, 25), but little is known of the effects of shade on CAM. Our lack of understanding of these responses is due, in large part, to the complicated gas exchange characteristics exhibited by plants possessing CAM (19). Since CO_2 uptake occurs primarily during the dark it is difficult, if not impossible, to generate typical light response curves for CAM plants using conventional methods of CO_2 exchange analysis. Previous studies of the influences of light on CAM have relied on an integrated measure of nocturnal CO_2 uptake or acid accumulation following exposure to a given PFD for a given daylength (10, 11, 14, 17, 18, 23), and in only one instance was any attempt made to actually grow the plants (for 3 weeks) at different PFD (14). With the advent of the leaf disc O_2 electrode (6), however, it is now possible to measure the light response characteristics of photosynthesis in CAM plants directly and quickly (1, 24).

There has likewise been little consideration of photoinhibition in CAM plants. Although it has been hypothesized that the biochemistry of CAM may provide some degree of protection against photoinhibition through the internal generation and recycling of CO_2 (20), CAM plants transferred from shade to full sunlight experienced photoinhibition (24), as did *Opuntia basilaris* growing in full sunlight (2).

The objectives of this study were therefore 2-fold. First, to ascertain the responses of two CAM species to growth under a range of PFD conditions through the characterization of a number of properties in these plants. Second, to determine the degree to which these plants are susceptible to photoinhibition by high light.

MATERIALS AND METHODS

Kalanchoë daigremontiana and *Hoya carnosa* were propagated from plantlets and cuttings, respectively, and grown as described in Adams *et al.* (1). Growth under reduced PFD was achieved with layers of shade cloth which transmitted approximately 50, 30, 15, and 5% of the daily peak PFD in the glasshouse (approximately 2000 and 1500 μmol quanta m^{-2} s^{-1}, on a clear day, in the summer and winter, respectively). All plants received water daily, and a nutrient solution (one-half strength Hoagland) 3 times a week. Therefore, none of the plants are likely to have experienced water stress, particularly since stomata presumably remained closed throughout much of the day. No attempts were made to provide for uniform leaf temperatures between plants grown under different PFD; however, the air in the glasshouse was well circulated which should have minimized such differences.

Kalanchoë daigremontiana was characterized primarily in January, and *H. carnosa* in November. All plants were at least 4 months old, and the leaves had developed totally under the indicated light conditions. Only fully developed leaves (in the case of *K. daigremontiana*, only leaves of the 4th to 7th rank from the apex) were used in these experiments. In preliminary trials it was also found that the orientation of the leaves with respect to the light environment was very critical in obtaining consistent results, particularly in high PFD grown plants. Thus, only the south half of east-west pointing *K. daigremontiana* leaves grown under high PFD (the north half was exposed to more direct solar radiation due to their invaginated structure) were used in the experiments described here.

Light response curves of O_2 exchange were determined as described by Adams *et al.* (1) using a leaf disc O_2 electrode (6). Room temperature Chl *a* fluorescence (above 740 nm; primarily

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[3] Abbreviations: PFD, Photon flux area density; F_o, F_m, F_v—instantaneous, maximum, and variable Chl fluorescence at 77K; *P*, *T*—initial peak and terminal steady state level of room temperature (25°C) Chl *a* fluorescence.

PSII) was ascertained prior to the measurement of the light response curves using a Hansatech light source and fluorescence detector (Hansatech Ltd, King's Lyn, UK) based on a light emitting diode producing 300 μmol quanta m^{-2} s^{-1} red light (peak 660 nm) and fluorescence detection by a photodiode (7) following a dark adaptation period of at least 10 min. Chl fluorescence at 77K (690 nm; primarily PSII) and Chl concentrations were determined as described in Adams *et al.* (2). Nocturnal acid accumulation was estimated by sampling leaf tissues near dusk and dawn, extracting the acids in boiling water, and titrating the cooled extractions to pH 7.0 using 0.02 N NaOH. Samples for $\delta^{13}C$ analysis were prepared and analyzed as in Farquhar and Richards (9).

Photoinhibitory treatments consisted of exposure of leaves in air to 1750 μmol quanta m^{-2} s^{-1} (from a water-filtered xenon arc lamp) in a temperature controlled cuvette (leaf temperature of 27°C). As the characteristics of room temperature fluorescence changed during the day with changes in incident PFD, all determinations of fluorescence and light response curves were made in the morning. Thus, photoinhibitory treatments were given midday and the above parameters measured the following morning.

RESULTS

Characteristics of CAM Plants Grown under Different PFD. Leaves of the more shade grown plants of both species were thinner, as evidenced by lower values of fresh weight per leaf area (Tables I and II). Chl concentrations were elevated with reduced PFD during growth (Tables I and II). Measurements of the ratio of variable (maximum-instantaneous fluorescence;$F_m - F_o$) over maximum fluorescence at 77K (F_v/F_m) yielded similar results for both species under all growth conditions except *Hoya carnosa* at full glasshouse PFD, in which F_v/F_m was reduced (Tables I and II). This consistency in the value F_v/F_m was maintained across the range of growth PFD despite increases in the absolute levels of both instantaneous and maximum 77K fluorescence with decreased PFD during growth (Fig. 1). The absolute levels of room temperature fluorescence, the initial peak (P) and terminal steady state level (T), were likewise elevated with decreased PFD during growth (Fig. 1).

Quantum yields were near 0.10 mol O_2 mol^{-1} quanta except in *K. daigremontiana* at 85 and 95% shade and in *H. carnosa* grown under full glasshosue PFD (Tables I and II). Other aspects of the light response curves of O_2 exchange appeared to be dependent on the PFD during growth, with the following general trends being observed (Tables I and II): higher rates of respiratory O_2 uptake, higher light compensation points, and higher levels of photosynthetic capacity (higher rates of photosynthetic O_2 evolution near light saturation) with increased PFD during growth. This was true in all cases except in *H. carnosa* grown at the highest PFD in which reduced maximum rates of photosynthesis concomitant with reduced quantum yields were observed. Thus, the light response curves of *H. carnosa* grown at high PFD did not show the acclimation of photosynthesis found in *K. daigremontiana* (Fig. 2).

An integrated measure of nightly CO_2 uptake, nocturnal acid accumulation, exhibited characteristics similar to those of photosynthetic capacity, with higher levels of acid accumulation in plants grown under higher PFD (Tables I and II). Once again, however, *H. carnosa* grown at the highest PFD deviated from this trend with a reduced level of nocturnal acid accumulation relative to that of plants grown under lower PFD. The $\delta^{13}C$ values of *K. daigremontiana* leaves also varied with PFD during growth, becoming more negative with reduced PFD (Table I).

Susceptibility to Photoinhibition in CAM Plants. The time-course of the effects of the photoinhibitory treatment on *K. daigremontiana* grown under different PFD were followed with 77K fluorescence (Fig. 3). These data show that photoinhibition occurred more rapidly in plants grown in deep shade. The extent of photoinhibition after a standard 4 h exposure to bright light was indicated by changes in F_v/F_m (77K fluorescence) and P-T (an indicator of the variable component of room temperature fluorescence) (Fig. 4). The changes in P-T were due solely to reductions in P, as the level of T was uanffected by the treatment. There were likewise significant changes in the light response curves of *K. daigremontiana* following photoinhibition, with increases in the light compensation point and decreases in the apparent quantum yield across the range of growth conditions (Fig. 5). The maximum rates of photosynthesis were also reduced by more than 50% in the plants grown at 85 and 95% shade, and by approximately 25% in the plants grown at 50 and 70% shade (data not shown; see Table I for control values).

The changes in all of these parameters indicate that the extent of photoinhibition was progressively greater in plants grown

Table I. *Some Characteristics of K. daigremontiana Grown at Different PFD*

Mean ± SE (n).

	Percent Shade during Growth				
	0	50	70	85	95
g Fresh wt cm^{-2}	0.181 ± 0.013 (4)	0.122 ± 0.003 (4)	0.119 ± 0.006 (4)	0.108 ± 0.001 (4)	0.094 ± 0.004 (4)
Chl concentration					
mg Chl m^{-2}	448	517	655	707	742
μg Chl g^{-1} fresh wt	182	288	440	475	519
77K fluorescence					
F_v/F_m	0.81 ± 0.00 (24)	0.81 ± 0.00 (3)	0.81 (2)	0.81 ± 0.01 (6)	0.81 ± 0.01 (4)
Apparent quantum yield					
mol O_2 mol^{-1} quanta	0.098 ± 0.005 (8)	0.099 ± 0.004 (3)	0.100 (2)	0.084 ± 0.005 (4)	0.088 (2)
Dark respiration rate					
μmol O_2 m^{-2} s^{-1}	−4.0 ± 0.2 (8)	−4.0 ± 0.5 (3)	−3.2 (2)	−1.4 ± 0.3 (4)	−0.7 (2)
Light compensation point					
μmol quanta m^{-2} s^{-1}	30.4 ± 3.2 (8)	27.7 ± 2.3 (3)	22.0 (2)	9.5 ± 1.5 (4)	3.5 (2)
Max photosynthetic rate[a]					
μmol O_2 m^{-2} s^{-1}	28.4 ± 2.2 (8)	33.1 ± 5.9 (3)	27.9 (2)	20.9 ± 2.2 (4)	13.4 (2)
Nocturnal acid accumulation					
μeq acid g^{-1} fresh wt	305 (2)	288 (2)	168 (2)	158 (2)	104 (2)
$\delta^{13}C$	−19.1 ± 0.2 (3)	−19.7 ± 0.5 (3)	−19.8 ± 0.2 (3)	−22.2 ± 0.1 (3)	−24.2 ± 0.2 (3)

[a] Near light saturation.

Table II. *Some Characteristics of H. carnosa Grown at Different PFD*
Mean ± SE (*n*). ND, not determined.

	Percent Shade during Growth				
	0	50	70	85	95
g Fresh wt cm^{-2}	0.154 ± 0.001 (4)	0.088 ± 0.001 (4)	0.089 ± 0.002 (4)	0.060 ± 0.002 (4)	0.053 ± 0.001 (4)
Chl concentration					
mg Chl m^{-2}	276	586	586	724	638
μg Chl g^{-1} fresh wt	138	683	552	888	921
77K fluorescence					
F_v/F_m	0.76 ± 0.02 (11)	0.80 (2)	0.82 ± 0.00 (4)	0.82 ± 0.00 (4)	0.82 ± 0.01 (4)
Quantum yield					
mol O_2 mol^{-1} quanta	0.083 ± 0.001 (4)	0.098	0.099	0.099	0.092
Dark respiration rate					
μmol O_2 m^{-2} s^{-1}	−2.5 ± 0.6 (4)	−1.6	−1.3	−1.5	−1.1
Light compensation point					
μmol quanta m^{-2} s^{-1}	18.5 ± 3.2 (4)	17	13	5	5
Max photosynthetic rate[a]					
μmol O_2 m^{-2} s^{-1}	11.0 ± 0.1 (4)	23	18	16.5	14.5
Nocturnal acid accumulation					
μeq acid g^{-1} fresh wt	46 (2)	96 (2)	ND	76 (2)	58 (2)
$\delta^{13}C$	−20.1 ± 0.1 (3)	−21.8 ± 0.2 (3)	−21.6 ± 0.1 (3)	−22.2 ± 0.2 (3)	−21.3 ± 0.1 (3)

[a] Near light saturation.

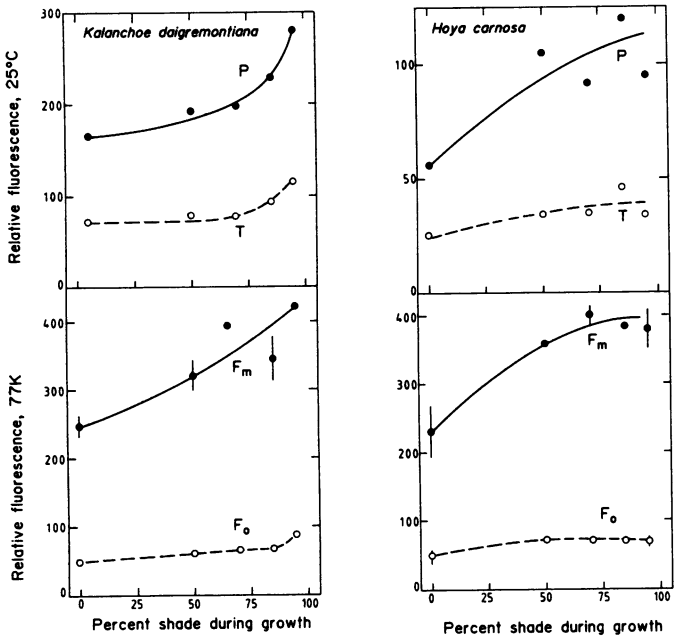

FIG. 1. Changes in the initial peak (*P*) and terminal steady state (*T*) values of room temperature Chl *a* fluorescence (upper panels), and the instantaneous (F_o) and maximum (F_m) levels of 77K fluorescence (lower panels) with changes in PFD during growth in *K. daigremontiana* and *H. carnosa*. Standard error bars indicated; *n* ranged from 2 to 10.

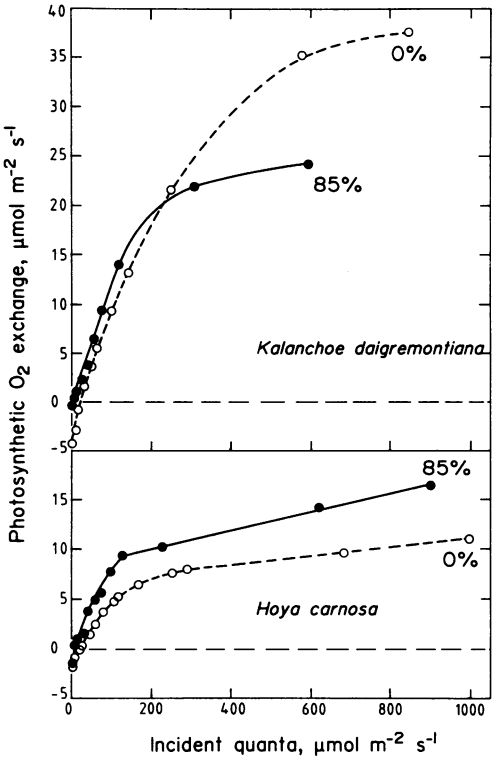

FIG. 2. Comparison of the light response curves of *K. daigremontiana* and *H. carnosa* grown at high light (0% shade) and at low light (85% shade).

under lower PFD. The reductions in F_v/F_m (Fig. 4) and apparent quantum yield (Fig. 5) following photoinhibition were strikingly similar. A plot of one against the other (Fig. 6) revealed that the two were well correlated.

DISCUSSION

Our experiments describe, for the first time, the effects of long-term growth and development under prescribed light conditions on the photosynthetic properties of CAM plants. These data serve to extend earlier studies on the effects of light intensity during growth on photosynthetic properties of C_3 and C_4 plants (4) to CAM plants, and are relevant to field studies of CAM species which grow in naturally shaded habitats (24). They can also be related to earlier investigations of the effects of short-term changes in light regime on some aspects of the expression of CAM. For example, Kaplan *et al.* (10) found that respiration of *K. daigremontiana* responded to light intensity within 3 d, and our plants grown in deep shade had the lowest respiration rates (Tables I and II). The extent of nocturnal acidification in CAM plants is dependent on the total PFD received by the tissues in the previous day (10, 11, 17, 18, 23) and in our experiments it similarly decreased with shading during growth

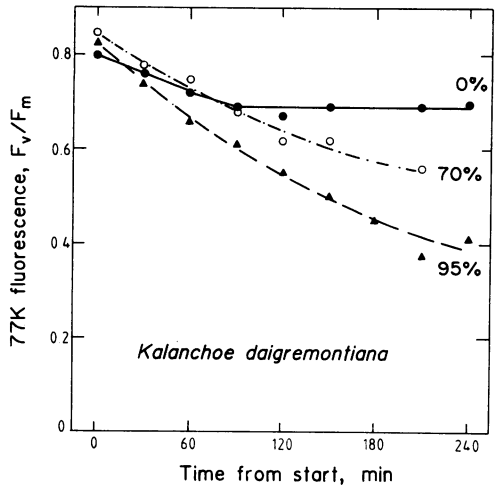

FIG. 3. Timecourse of changes in F_v/F_m (77K fluorescence) during a photoinhibitory treatment (1750 μmol quanta m^{-2} s^{-1} in air, leaf temperature of 27°C) in *K. daigremontiana* which received different PFD during growth (0, 70, and 95% shade).

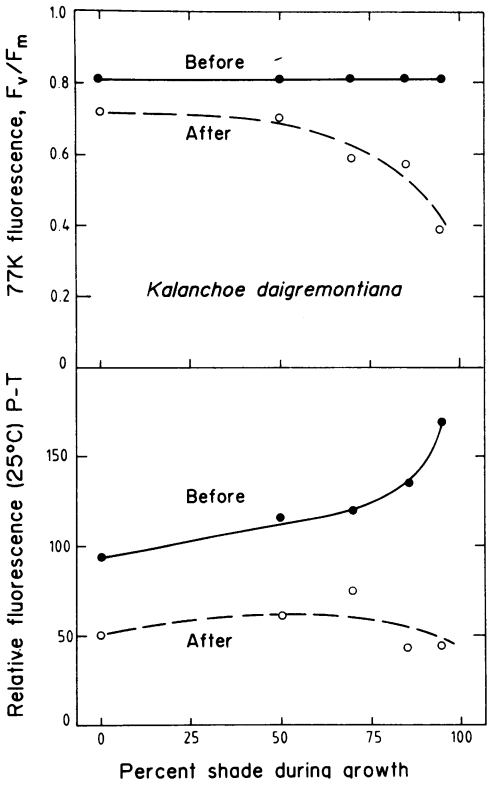

FIG. 4. Changes in F_v/F_m (77K fluorescence) and *P-T* (room temperature Chl *a* fluorescence) in *K. daigremontiana* grown at different PFD following a 4 h photoinhibitory treatment. Control data taken from Figure 1.

(Tables I and II). However, reduced acidification in CAM plants grown under reduced PFD may also be due to changes in intrinsic factors as well, such as photosynthetic capacity (Tables I and II). The low level of acidification exhibited by *H. carnosa* grown at the highest PFD seems to be related to photoinhibition as discussed below.

There are at least two possible causes for the decrease in the $\delta^{13}C$ value with decreased PFD during growth (Table I). One is that a greater proportion of CO_2 is fixed nocturnally via P-enolpyruvate carboxylase in the higher PFD grown plants, and

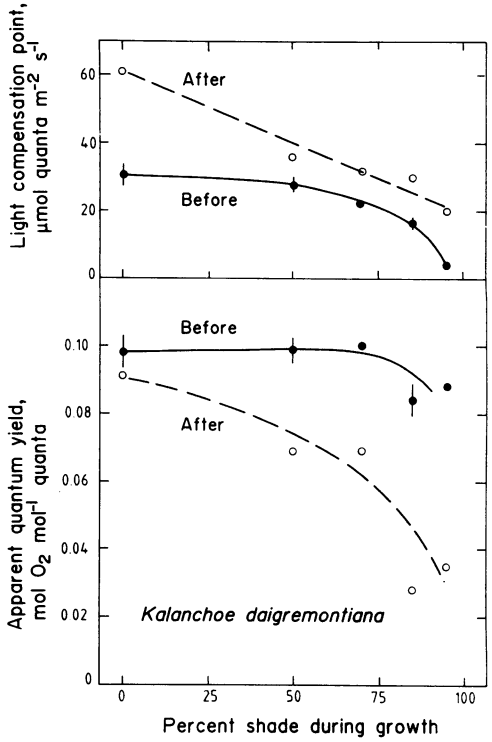

FIG. 5. Changes in the light compensation point and apparent quantum yield of O_2 exchange in *K. daigremontiana* grown at different PFD following a 4 h photoinhibitory treatment. Control values ± SE are taken from Table I.

proportionately more CO_2 is fixed in the light via ribulose-1,5-bisP carboxylase in the shade grown plants (21). The differences in nocturnal acid accumulation (Table I) are indicative of this, but not definitive, as these may simply reflect the differences in available energy. The other possibility is that direct CO_2 fixation by ribulose-1,5-bisP carboxylase in the plants grown at higher PFD occurs at a lower intercellular CO_2 partial pressure than in shade grown plants (9). Analysis of diel gas exchange patterns of CAM plants grown and measured at high and low PFD should help to determine to what extent each may be responsible for the changes in $\delta^{13}C$.

The two leaf succulent CAM plants studied here exhibit many of the same responses to PFD during growth found in other C_3 and C_4 plants. Thus,the respiratory rate and light compensation point were lower in plants grown in deep shade, the rate of light- and CO_2-saturated photosynthesis tended to increase with increased PFD during growth, and quantum yield was relatively unchanged (Tables I and II). An increase in the absolute levels of room temperature fluorescence (P and T; Fig. 1) has also been found in other species grown at low relative to high PFD (12), and in CAM plants under field conditions (24). The increases in the absolute levels of 77K fluorescence (F_o and F_m) observed in *K. daigremontiana* and *H. carnosa* with decreased PFD during growth are likewise similar to those reported in the CAM species *O. basilaris* (2). The constancy of the ratio F_v/F_m at 77K (Tables I and II), despite large differences in F_o and F_m (Fig. 1), is consistent with that observed in a number of other species (2, 5). Chl content on fresh weight or area bases decreased with increased PFD during growth (Tables I and II), as found in other CAM plants (2, 15).

These changes indicate a degree of acclimation in photosynthetic properties to growth PFD, but this seems to be limited. Thus, under the highest PFD, light- and CO_2-saturated photosynthesis is reduced compared with 50% shade, especially in *H.*

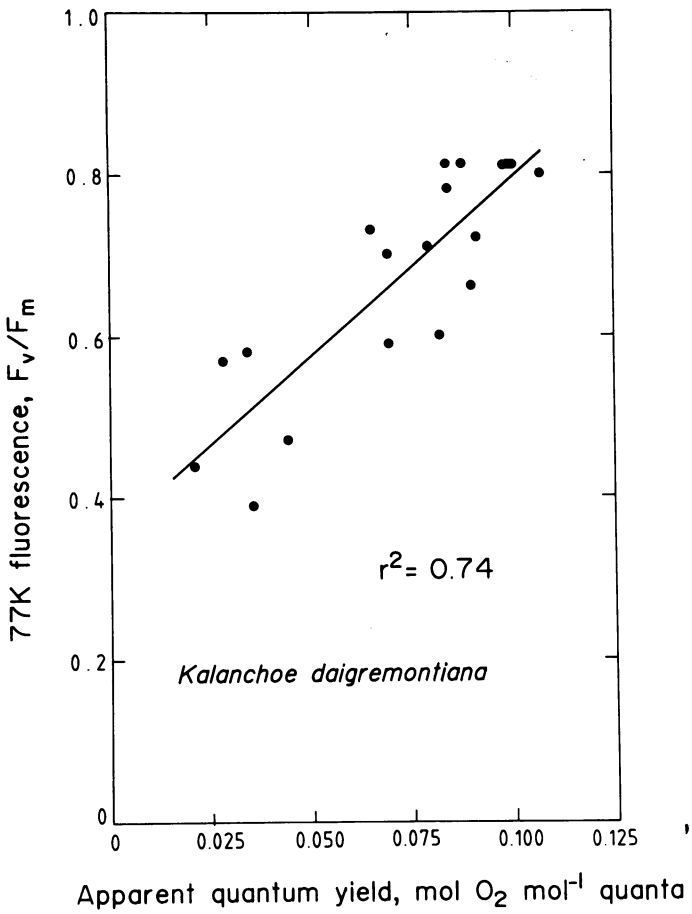

FIG. 6. Relationship between the apparent quantum yield of O_2 exchange and F_v/F_m (77K fluorescence) in *K. daigremontiana* grown at different PFD both before and after a 4 h photoinhibitory treatment.

carnosa which also shows a reduction in quantum yield and 77K fluorescence, as well as a precipitous decrease in Chl (Tables I and II). Similar limited acclimation and low Chl in bright light has been observed in several CAM epiphytes (14, 24), and these responses are confirmed by our preliminary studies of *H. australis* in the field. The failure of *H. carnosa* to acclimate to full sunlight suggests it may suffer chronic photoinhibition.

When shade-grown C_3 plants are transferred to bright light they show light dependent damage to the photosynthetic apparatus which is dependent on time of exposure and PFD (5, 8, 22). Our results with *K. daigremontiana* show that shade-grown CAM plants are no different from C_3 plants in this respect. Decreases in quantum yield, photosynthetic capacity, 77K fluorescence, and room temperature fluorescence were observed after 4 h exposure of shade-grown plants to bright light (Figs. 3–5). Similar responses of quantum yield and room temperature fluorescence were found when CAM plants from deeply shaded natural habitats were exposed to full sunlight (24).

As our measurements of 77K fluorescence were made at 690 nm, they indicate damage to the primary photochemistry of PSII following photoinhibition (22). The correlation between quantum yield and the fluorescence ratio F_v/F_m at 77K (Fig. 6) is similar to those established by Björkman and Demmig (5) and Demmig and Björkman (8). The higher correlations in their experiments may be due to differences in the methods used to establish each correlation. Our quantum yield determinations were preceded by measurements of room temperature fluorescence and a short exposure to high PFD (which might account for the reduced quantum yields in our shade plants), whereas theirs were not. Our quantum yields were also calculated from measurements of net O_2 exchange, while they subtracted the respiratory component of O_2 uptake at each light level. Moreover, their correlations were a function of quantum yields calculated on the basis of absorbed quanta, while ours is on an incident basis.

The relationships between these changes in the photosynthetic apparatus when shade-grown plants are transferred to bright light, and the incomplete acclimation of CAM plants to high PFD remain to be established. It seems likely that rearrangements in the photosynthetic apparatus which accompany acclimation to high PFD make it less susceptible to photoinhibition. However, it is possible that acclimation and repair of photoinhibition may involve conflicting demands on chloroplast function which lead to responses of the sort observed in *H. carnosa*.

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