

1992

Fluorescence quenching in the varied photosynthetic modes of *Portulacaria afra* (L.) Jacq

Lonnie J. Guralnick

Roger Williams University, lguralnick@rwu.edu

Guillermo Goldstein

Robert L. Heath

Irwin P. Ting

Follow this and additional works at: http://docs.rwu.edu/fcas_fp

 Part of the [Biology Commons](#)

Recommended Citation

Guralnick LJ, Guillermo Goldstein, Robert L. Heath, Irwin P. Ting. 1992. "Fluorescence quenching in the varied photosynthetic modes of *Portulacaria afra* (L.) Jacq." *Plant Physiol.* 99 (4):1309-1313.

This Article is brought to you for free and open access by the Feinstein College of Arts and Sciences at DOCS@RWU. It has been accepted for inclusion in Feinstein College of Arts & Sciences Faculty Publications by an authorized administrator of DOCS@RWU. For more information, please contact mwu@rwu.edu.

Fluorescence Quenching in the Varied Photosynthetic Modes of *Portulacaria afra* (L.) Jacq.¹

Lonnie J. Guralnick, Robert L. Heath, Guillermo Goldstein, and Irwin P. Ting*

Division of Natural Sciences and Mathematics, Western Oregon State College, Monmouth, Oregon 97361 (L.J.G.);
Department of Botany and Plant Sciences, University of California, Riverside, California 92521 (R.L.H., I.P.T.);
and Laboratory of Biomedical and Environmental Sciences, University of California,
Los Angeles, California 90024 (G.G.)

ABSTRACT

The kinetics of chlorophyll fluorescence were measured in *Portulacaria afra* (L.) Jacq. when the plants were functioning in either Crassulacean acid metabolism (CAM) or C₃/CAM cycling (called cycling) modes, as determined by fluctuation in titratable acidity and gas exchange properties. Cycling plants showed primarily daytime CO₂ uptake typical of C₃ plants, but with a slight diurnal acid fluctuation, whereas CAM plants showed nocturnal CO₂ uptake, daytime stomatal closure, and a large diurnal acid fluctuation. Results from fluorescence measurements indicated no significant differences in photochemical quenching between cycling and CAM plants; however, sizable differences were detected in nonphotochemical quenching (q_N), with the largest differences being observed during the middle of the day. Cycling plants had lower q_N than CAM plants, indicating altered photosynthetic regulation processes. This q_N difference was believed to be related to reduced internal CO₂ concentration in the CAM plants because of daytime stomatal closure and reduced deacidification rates in the late afternoon when most of the malic acid has been utilized. Experimentally, higher external CO₂ given to plants in the CAM mode resulted in a decline in q_N in comparison to that measured in plants in the cycling mode. No changes were observed in photochemical quenching when CO₂ was added.

Many studies of CAM species have focused on the shift from C₃/CAM cycling (called cycling) to CAM photosynthesis during various stresses (3, 8, 9, 17, 21, 23). These facultative CAM plants change their metabolism from daytime CO₂ uptake with little or no organic acid fluctuations (similar to plants undergoing C₃ photosynthesis) to nocturnal CO₂ uptake with daytime stomatal closure and a larger fluctuation in the organic acid pool, typical of CAM (12, 13, 23).

Variable Chl fluorescence has been used in the past as a noninvasive tool to study the CAM photosynthetic pathway. The amount of variable fluorescence in *Kalanchoë daigremontiana*, *Ananas comosus*, and *Opuntia stricta* changed from morning to evening in concert with the deacidification of the tissue (6, 16). Chl fluorescence with light scattering (a putative measure of the H⁺ fluxes within the chloroplast) has been used to study the day/night cycle of CAM in *K. pinnata* (15).

¹ This research was supported, in part, by National Science Foundation grant DCB-8807860 (to I.P.T.).

The Chl fluorescence transients correlated with increased light scattering. On the other hand, the results of Buschmann and Buchanan-Bollig (4) indicated that Chl fluorescence transients were dependent upon leaf structure, pigment composition, and the organization of the photosystems rather than the CO₂ fixation pathway. The question arises as to how well control of the operation of photosynthesis by internal CO₂ levels (7) can be measured by fluorescence techniques. The levels of CO₂ inside the leaf certainly change between these two photosynthetic modes (5) and so should alter photosynthesis and, concurrently, the fluorescence patterns (18).

A fluorescence technique has been developed that enables the separation of q_P² and q_N quenching of Chl fluorescence (20). This technique uses a weak light beam to measure the fluorescence yield and a high intensity actinic beam to drive photosynthesis. The application of a second light pulse of high intensity is used to overwhelm photochemical quenching, thus permitting direct measurement of q_N. This technique was used here to monitor changes in q_P and q_N in *Portulacaria afra* (L.) Jacq. (a facultative CAM plant) as it shifted from cycling to the CAM mode of photosynthesis under water stress (9, 13) and, in part, to show how internal CO₂ was changing during the metabolic switch. The critical factor in these experiments is that this shift was not accompanied by any changes in pigment composition (11). The effect of external CO₂ levels on these same fluorescence measurements was also examined.

MATERIALS AND METHODS

Plant Material

Portulacaria afra (L.) Jacq. plants were propagated by cuttings from a large shrub growing on the University of California, Riverside, campus. The cuttings were rooted and grown in 2-gallon pots containing loamy sand potting mix. Plants in the cycling mode were watered three times per week and fertilized bimonthly with half-strength Hoagland solution (10). CAM was induced by withholding water during the summer for 2 weeks prior to experimentation. All plants were grown in a glasshouse with a mean high temperature

² Abbreviations: q_P, photochemical quenching; q_N, nonphotochemical quenching; F₀, initial fluorescence (dark adapted); F₀' , initial fluorescence (light adapted); F_m, peak fluorescence (dark adapted); F_m' , peak fluorescence (light adapted).

of 28°C and a mean low of 18°C under natural illumination conditions (maximum photon flux density, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) during the summer months of June through August. All leaves used were mature and fully expanded.

Titrateable Acidity

Twelve to sixteen leaves were collected at various times and frozen (-20°C) until assayed (10). Leaf punches (0.24 cm^2) obtained with a cork borer were ground in glass-distilled water with a tissue grinder. Samples were then titrated with 0.01 N KOH to an endpoint of pH 7.0. Results are expressed as $\mu\text{eq cm}^{-2}$ (\pm SE, $n = 5$).

Gas Exchange

The diurnal course of CO_2 uptake and stomatal conductance was measured with the use of a dual isotope porometer, as previously described (10), with five samples for each time point. These unidirectional conductance measurements were converted to transpiration and photosynthetic rates, also as previously described (10).

Chl Fluorescence

Fluorescence was measured using the Hansatech Modulated Fluorescence System (Decagon, Pullman, WA). This system utilizes a continuous yellow low-intensity beam of 0.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to continuously monitor the fluorescence yield. This light intensity produces no changes in the yield, and so the yield thus induced is taken as F_0 . A second beam of nonmodulated light (white light of high intensity, 320 $\mu\text{mol m}^{-2} \text{s}^{-1}$) required to drive the photochemistry is added to induce an increase in fluorescence yield (F). At 20-s intervals, a third light beam is applied as a pulse (duration of 0.3 s) with saturating intensity (1530 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The application of this pulse allows for the measurement of the flash-saturated fluorescence yield called the peak value of fluorescence (light adapted, F_m') and, under certain conditions, of maximum fluorescence (dark adapted, F_m) (see Fig. 2). Light intensity curves for these plants while in the C_3 mode indicate that CO_2 uptake was saturated between 1000 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The pulse measurements to determine the values of F_m' and F_m were taken at the 10th pulse after which these values were unchanging. This allowed for the determination of q_p and q_N with the use of Equations 1 and 2, taken from Kooten and Snel (14);

$$q_p = [F_m' - F]/[F_m' - F_0'] \quad (1)$$

$$q_N = 1 - [F_m' - F_0']/[F_m - F_0] \quad (2)$$

Leaves of *P. afra* in either the cycling or CAM mode were harvested at 2-h intervals during the day and utilized immediately for the fluorescence measurements (each experimental sequence required about 6–8 min). Detached succulent leaves of *P. afra* have previously been shown to have photosynthetic rates similar to those of attached leaves (10); thus, it was assumed detachment would have a minimal effect on fluorescence. Experimental results supported this assumption.

The standard statistical test of a two-way analysis of variance was applied to timed paired datum points, where applicable, with a level of 5% or less being taken as significant.

CO_2 Supplementation

To elevate the internal CO_2 concentration, some leaves harvested for the fluorescence kinetics were exposed to air containing 1000 ppm CO_2 . The CO_2 supplementation was done in the afternoon during the transition from phase III to phase IV of the CO_2 diurnal uptake pattern (see ref. 16 for a definition of CAM phases). These leaves were placed in a polyethylene chamber with an air/ CO_2 mixture flowing through water-saturated gauze for 5 min before the beginning of the fluorescence measurement. In similar experiments, CO_2 was blown directly onto the leaves placed in the fluorometer during the fluorescence measurements. There were no differences noted in these two types of experiments.

RESULTS

Gas Exchange and Titrateable Acidity

The kinetics of gas exchange for well-watered *P. afra* showed a modified C_3 pattern called CAM cycling or cycling (21), which is marked by predominantly daytime gas exchange with a midday depression of CO_2 uptake (Fig. 1A, + H_2O). Nocturnal CO_2 uptake was absent at the beginning of the night period, but increased slightly during the last 6 h of night. Titrateable acidity showed a small diurnal fluctuation of 17 to 20 $\mu\text{eq cm}^{-2}$, which is typical of *P. afra* (Fig. 1B, + H_2O ; also see ref. 13).

On the other hand, water-stressed *P. afra* plants showed a typical CAM pattern (Fig. 1A, - H_2O) in which CO_2 uptake was highest at sunrise (0600 h) and dropped to near 0 by 0800 h. There was little CO_2 uptake for the remainder of the day. CO_2 uptake became apparent at the beginning of the night period, reaching a maximum by night's end. Titrateable acidity showed a large diurnal fluctuation of 40 $\mu\text{eq cm}^{-2}$ (Fig. 1B). Diurnal acid fluctuations were occasionally smaller, but they were always larger than those of the plants in the cycling mode.

Fluorescence

The Chl fluorescence curves showed two distinct patterns (Fig. 2). The measuring beam elicited a small signal (F_0), which was the same for cycling and for CAM. The light-driven change in fluorescence yield rose to a peak (F_m) in about 1 to 2 s, and then decayed to a relatively low value, slightly above F_0 for both types of plants. The only obvious difference in kinetics between cycling and CAM plants was the appearance of a small, variable peak at about 2 min (called "M" phase in older nomenclature) for plants in the cycling mode. A short light pulse increased the yield to a maximum level (F_m'), which rapidly returned to F after the cessation of the pulse (within 0.5 s).

The kinetics of these pulses enable the calculation of diurnal patterns of q_p and q_N for *P. afra* in each metabolic mode. A stable q_p value was reached at 0800 h, 2 h after sunrise (Fig. 3). The q_p values for the well-watered plants (in the

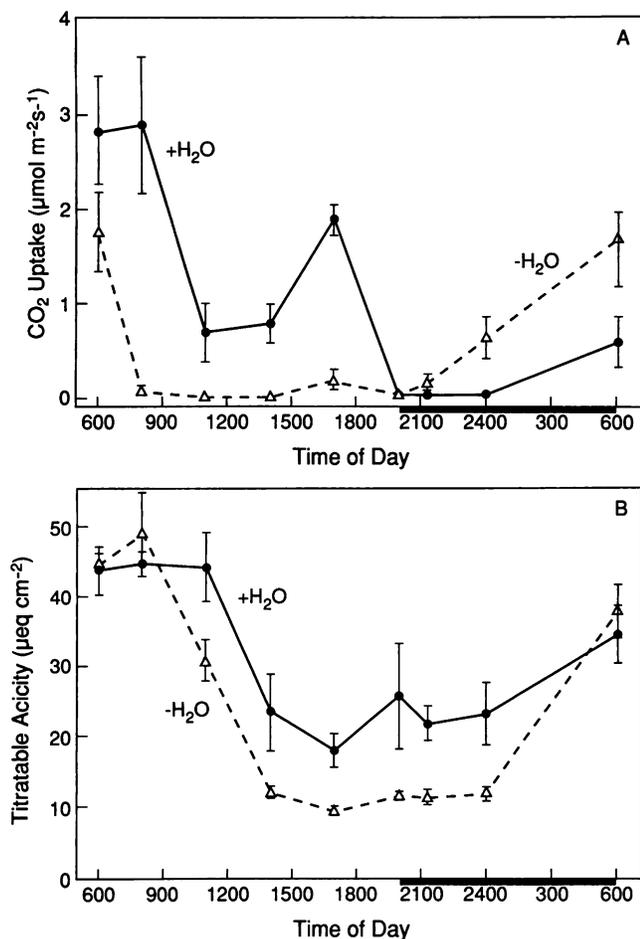


Figure 1. The metabolic parameters characteristic of the cycling and the CAM modes for *P. afra*. The CO₂ uptake rate and leaf acidity were measured and calculated as described in "Materials and Methods." A, CO₂ uptake. A typical diurnal curve of carbon uptake activity. B, Acidity present within the leaf. A typical diurnal curve of titratable acidity. +H₂O, data from well-watered plants showing cycling; -H₂O, data from water-stressed plants showing CAM. Error bars represent 1 SE (n = 6).

cycling mode) were usually slightly lower than those of the plants in the CAM mode; however, the difference between the q_N curves was very small and only slightly above the 5% level statistically.

The largest differences were observed in q_N values. In both cycling and CAM, the q_N values were highest early in the morning, but dropped over the course of the day, rising slightly at sunset, especially for the well-watered plants. Plants in the CAM mode showed q_N values that were typically slightly higher than those of the plants in the cycling mode during the morning hours (0800–1200 h) and significantly higher in the mid-afternoon hours (1600–1800 h).

In the above experiments, leaves were collected in the greenhouse at the indicated time and transported back to the laboratory for the fluorescence measurements (requiring about 5 min); it was thought that dark adaptation of leaves would give a more reproducible signal. Yet, under these circumstances, it seemed that the transportation and dark

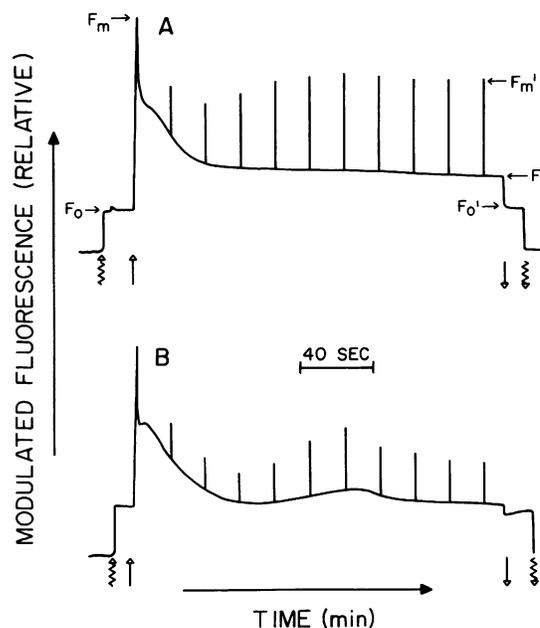


Figure 2. Typical modulated fluorescence signals from *P. afra* leaves in CAM induced by water-stress (A) and cycling (B) modes. Arrows denote onset of modulated light (wavy line), or high intensity actinic light (straight line); upward is light on, downward is light off. The spike of fluorescence yield is caused by a second actinic light given as a pulse (see "Materials and Methods"). Values of F₀, F₀' , F_m, F_m' , and F used in Equations 1 and 2 are indicated on curve A.

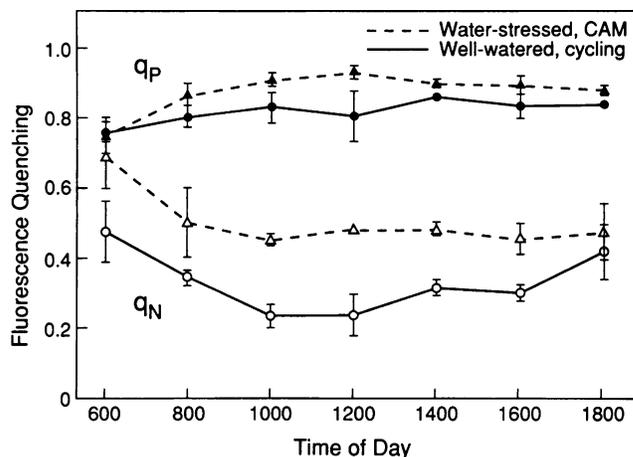


Figure 3. Levels of fluorescence quenching mechanisms in dark-adapted *P. afra*. The plants were shifted to either cycling (well-watered, solid line; ○, ●) or to CAM (drought-stressed, dotted lines; △, ▲). The experiments were conducted in the dark. The levels of q_p (closed symbols) and q_N (open symbols) were measured as described in "Materials and Methods." The data were taken after a dark period of greater than 5 min. Results of four experiments.

adaptation were introducing more variation into the measurements.

Indeed, the q_N difference between CAM and cycling plants noted above became larger with the elimination of a dark adaptation time (Fig. 4). The fluorometer was moved to the greenhouse and the fluorescence from leaves was measured immediately (within 1 min). During midday, the difference observed in q_N between well-watered and water-stressed plants was then accentuated. From 1000 to 1200 h, there was a larger difference in q_N values. Also, the small difference in q_P values apparently reversed; the q_P is lower in CAM plants when measurements were taken on leaves that had not been dark adapted.

CO₂ Supplementation

One source for the difference in q_N values between cycling and CAM plants may be in a difference in internal CO₂ levels in the two modes (5, 18). To test this idea, the CO₂ level of the atmosphere around plants in both modes of metabolism was increased from 330 to 1000 $\mu\text{L L}^{-1}$. This supplementation was carried out in the afternoon, when the plants showed the maximum difference in q_N . As seen in Table I, this addition of CO₂ to plants in the CAM mode significantly lowered the q_N value so that the new level was closer to that of well-watered plants. Thus, CO₂ seems to be an important factor influencing the q_N level, and these data suggested that the CO₂ within these CAM plants was limiting to photosynthesis. In contrast, the higher levels of CO₂ supplied to well-watered plants induced no apparent trend.

DISCUSSION

Gas exchanges observed in these experiments were typical for either the cycling or the CAM modes in *P. afra* (9, 12). The cycling plants showed a pattern of gas exchange termed CAM cycling by Ting (21), with daytime gas exchange and

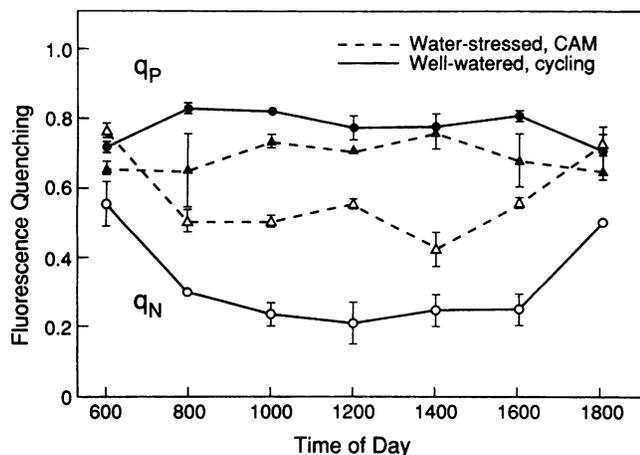


Figure 4. Levels of fluorescence quenching in *P. afra* plant leaves immediately after light exposure. The data were collected and presented as in Figure 3, except that the leaves were held in room light for less than 1 min after removal from daylight. Results of two experiments.

Table I. Change in q_N after CO₂ Supplementation in *P. afra* Undergoing CAM

Treatment	Decline (% Change ^a)
Dark adapted (5 min)	+9.0 (6.0) ^b
No dark adaptation	+19.5 (8.9) ^c

^a Change in q_N is defined as $([q_N \text{ prior} - q_N \text{ after CO}_2 \text{ supplementation}] / q_N \text{ prior})$. Values are means \pm SE. ^b Change in q_N not significantly different from 0 at $P < 0.05$ ($P = .075$, $n = 19$). ^c Change in q_N significantly different from 0 at $P < 0.05$ ($n = 8$).

low diurnal acid fluctuations resulting primarily from the recycling of nocturnal respiratory CO₂. The *P. afra* in the CAM mode showed a much higher daily acid fluctuation, daytime closure and nocturnal opening of stomata, and nocturnal CO₂ uptake typical of CAM plants (13).

The pulsed-light fluorescence technique enables partitioning of fluorescence into q_P and q_N (14, 20). q_P is due to the reduction of Q, the primary electron acceptor of PSII (2, 20). q_N is thought to be due, in part, to a transthylakoid pH gradient (2, 20). High rates of CO₂ fixation, due to a high internal CO₂ level, would result in faster utilization of ATP and NADPH and a decrease in the transthylakoid pH gradient; the combined effect of these processes upon the fluorescence would be a lowering of q_N and, possibly, a slight increase in q_P (19, 22). On the other hand, a lower concentration of internal CO₂ would slow CO₂ fixation and thus give rise to a high level of q_N and, possibly, to a low level of q_P . The exact relations depend upon how close the CO₂ level is to saturating the critical enzymes.

Our results indicated little difference in q_P between cycling and CAM *P. afra*, yet the changes are consistent with the above for plants that have not been dark adapted (Fig. 4). The small differences in q_P cannot be explained by changes in Chl content because Chl remains constant between cycling and CAM when the latter is induced by short-term water deprivation in *P. afra* (11).

The largest fluorescence difference between cycling and CAM was observed in q_N and appears to be related to the CO₂ fixation pathway. CAM plants had a higher q_N throughout the day. The differences in q_N between cycling and CAM plants were smaller in the early morning, possibly reflecting the high deacidification rates in *P. afra* undergoing CAM and, thus, higher levels of internal CO₂ (5). Deacidification rates between 0800 and 1200 h can reach from 8 to 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$, similar to exogenous CO₂ uptake rates in cycling *P. afra* (12). In the early morning and late afternoon, q_N values in both cycling and CAM *P. afra* rose, which indicated that rates of CO₂ fixation were lower due to limitations in light intensity.

The largest difference in q_N between cycling and CAM was observed during the middle of the day. These results correlate with the changes in light scattering found in *K. pinnata*, where the decline in scattering (correlating with higher consumption of ATP) during the middle of the day was smaller than that measured at the beginning or end of the light period (15). However, we believe that this difference in q_N is related to differences in CO₂ fixation and, in the main, to differences

in internal CO₂ level. Cycling plants have two sources of CO₂ during the middle of the day, atmospheric CO₂ and malic acid decarboxylation. A lower q_N in the cycling plants, compared to that of CAM plants, is consistent with the notion that an increased supply of CO₂ within the leaf leads to faster CO₂ fixation. In CAM plants, higher q_N was probably related to stomatal closure, which restricted the supply of exogenous CO₂, coupled with the declining deacidification rate due to a depletion of malic acid within the cell.

CO₂ depletion can close stomata, but there can be more than one signal involved. It is known that ABA from roots (24) can make stomata more sensitive to Ca²⁺, which influences stomatal conductance (1). Thus, the closure of stomata in CAM plants could be due to a combination of signals and not just to CO₂. As the internal CO₂ level drops during the day through depletion of the acidity and its associate CO₂ production, q_N declines. In CAM, neither CO₂ nor q_N declines as low as for cycling plants. There, q_N is very low because internal CO₂ is lower than ambient concentrations during active photosynthesis.

We tested this idea by supplementing CO₂ to CAM leaf tissue in the afternoon, when q_N differences were observed to be the greatest. Higher external CO₂ shifted the value of q_N to a more cycling-like value, *i.e.* lowered it. Yet it was difficult to obtain significant results if a dark adaptation time was allowed; this may reflect either a deactivation of CO₂ fixation or a reversal of photoinhibition. However, data taken without dark adaptation also supported the above concept of limiting internal CO₂ within CAM plants.

Our results may explain differences observed in simple fluorescence kinetics in the cycling mode and for the CAM mode in *Sedum spectabile* (4) and in *K. daigremontiana* (6). Also, the induction kinetics in the CAM plant *Ananas comosus* demonstrating only Q reduction showed no correlation with the CAM pathway (6). Our results, likewise, indicated little or no difference in photochemical quenching, nor in induction kinetics. All of these results indicate that in experiments in which only photochemical quenching is observed, it is found not to correlate well with internal CO₂ levels or with CAM metabolism.

The pulsed fluorescence technique can provide information on the difference in the energy status between cycling and CAM *P. afra* plants that a simple variable fluorescence technique does not readily detect. The pulse fluorescence technique is relatively easy to use and should become a useful research tool with which to study the energetic relationships in photosynthetic carbon metabolism.

LITERATURE CITED

- Atkinson CJ, Mansfield TA, Kean AM, Davies WJ (1989) Control of stomatal aperture by calcium in isolated epidermal tissue and whole leaves of *Commelina communis* L. *New Phytol* 111: 9-17
- Baker NR, Horton P (1987) Chlorophyll fluorescence quenching during photoinhibition. In DJ Kyle, CB Osmond, CJ Arntzen, eds, *Photoinhibition*. Elsevier Science Publishers, New York, pp 145-168
- Bartholomew B (1973) Drought response in the gas exchange of *Dudleya farinosa* (Crassulaceae) grown under natural conditions. *Photosynthetica* 7: 114-120
- Buschmann C, Buchanan-Bollig IC (1983) Changes of the chlorophyll fluorescence induction kinetics of C₃ and CAM plants during day/night cycles. *Photosynth Res* 4: 337-349
- Cockburn W, Ting IP, Sternberg LO (1979) Relationships between stomatal behavior and internal carbon dioxide concentration in Crassulacean acid metabolism plants. *Plant Physiol* 63: 1029-1032
- Everson G, Chen SS, Black CC Jr (1983) Diurnal variations in leaf fluorescence induction kinetics. *Plant Physiol* 72: 455-460
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* 33: 317-345
- Guralnick LJ, Rorabaugh PA, Hanscom Z (1984) Influence of photoperiod and leaf age on Crassulacean acid metabolism in *Portulacaria afra* (L.) Jacq. *Plant Physiol* 75: 454-457
- Guralnick LJ, Rorabaugh PA, Hanscom Z (1984) Seasonal shifts of photosynthesis in *Portulacaria afra* (L.) Jacq. *Plant Physiol* 76: 643-646
- Guralnick LJ, Ting IP (1986) Seasonal response to drought and rewatering in *Portulacaria afra* (L.) Jacq. *Oecologia* 70: 85-91
- Guralnick LJ, Ting IP (1987) Physiological changes in *Portulacaria afra* (L.) Jacq. during a summer drought and rewatering. *Plant Physiol* 70: 85-91
- Guralnick LJ, Ting IP (1988) Seasonal patterns of water relations and enzyme activity of the facultative CAM plant *Portulacaria afra* (L.) Jacq. *Plant Cell Environ* 11: 811-818
- Hanscom Z, Ting IP (1978) Response of succulents to plant water stress. *Plant Physiol* 61: 327-330
- Kooten O, Snell JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth Res* 25: 147-150
- Koster S, Winter K (1985) Light scattering as an indicator of the energy state in leaves of the Crassulacean acid metabolism plant *Kalanchoë pinnata*. *Plant Physiol* 79: 520-524
- Osmond CB (1982) Carbon cycling and stability of the photosynthetic apparatus. In IP Ting, M Gibbs, eds, *Crassulacean Acid Metabolism*. Am Soc Plant Physiol, Rockville, MD, pp 112-127
- Queiroz O (1974) Circadian rhythms and metabolic patterns. *Annu Rev Plant Physiol* 25: 114-134
- Peterson RB, Sivak MN, Walker DA (1988) Relationship between steady-state fluorescence yield and photosynthetic efficiency in spinach leaf tissue. *Plant Physiol* 88: 158-163
- Schreiber U, Bilger W (1987) Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. In JD Tenhunen, FM Catarino, OL Lange, WL Oechel, eds, *Plant Response to Stress*. Springer-Verlag, Berlin, pp 27-53
- Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth Res* 10: 51-62
- Ting IP (1985) Crassulacean acid metabolism. *Annu Rev Plant Physiol* 36: 595-622
- Winter K, Demmig B (1987) Reduction state of Q and non-radiative energy dissipation during photosynthesis in leaves of a Crassulacean acid metabolism plant, *Kalanchoë daigremontiana* Hamet et Perr. *Plant Physiol* 85: 1000-1007
- Winter K, Lüttge U, Winter E, Troughton JH (1978) Seasonal shift from C₃ photosynthesis to Crassulacean acid metabolism in *Mesembryanthemum crystallinum* growing in its natural environment. *Oecologia* 34: 225-237
- Zhang J, Davies WJ (1989) Sequential response of whole plant water regulations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behavior of sunflower plants. *New Phytol* 113: 167-174