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Relationship between Respiration and CAM-Cycling in *Peperomia camptotricha*[1]

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ABSTRACT

Mature leaves of well-watered *Peperomia camptotricha* show Crassulacean acid metabolism (CAM). Young leaves show CAM-cycling in which CO_2 uptake occurs during the day concomitant with a marked diurnal fluctuation of organic acids as in CAM. Evidence is presented suggesting that respiration is the source of CO_2 for nocturnal acid synthesis in leaves exhibiting CAM-cycling. Respiratory quotients for these leaves were consistently much less than unity despite the fact that the leaves metabolize starch. The conservation of CO_2 by refixation into acids at night represents about 17% of the total photosynthetically fixed CO_2 and about 50% of the total respiratory CO_2.

CAM is characterized by gas exchange occurring predominantly at night when the stomata are open (4, 8, 9). CO_2 is fixed via phosphoenolpyruvate carboxylase leading to the formation of oxaloacetate which is reduced to malate by malate dehydrogenase. Malate thus formed accumulates in the vacuole as malic acid where it is stored until the subsequent light period. Then malic acid is decarboxylated and the released CO_2 is refixed via the Calvin-Benson cycle. There are modifications to this basic mode of CAM which occur in response to environmental perturbations and/or as ontogenic differences (4, 12, 13, 19). CAM-idling is one such modification and is characterized by a diminished acid flux with virtually no nocturnal gas exchange with the atmosphere (17, 18). In another variation, CAM-cycling, C_3 gas exchange occurs with stomata open during the day and closed at night, but a significant nocturnal accumulation of organic acids occurs as in CAM (13, 19).

Peperomia camptotricha is an epiphytic plant native to Mexico. The older leaves located at the base of this plant exhibit CAM while the younger apical leaves show CAM-cycling (13, 19). The persistence of CAM-cycling (13, 19) likely results from the nocturnal recycling of endogenous CO_2, and indeed previous studies have assumed refixation of respiratory CO_2 to account for nocturnal acid synthesis when stomata are closed (5–7, 14). The purpose of this study was to estimate the extent to which respiratory CO_2 could be the substrate for nocturnal acid synthesis.

MATERIALS AND METHODS

Plant Material. Plants were propagated from cuttings by rooting in sand. Once the roots were formed, they were transplanted to pots, 15 cm in diameter, containing sandy loam soil and grown in a glasshouse. The average daily maximum temperature in winter was 19°C and the average minimum temperature was 3.5°C. In summer, the average maximum temperature was 34°C, and the average minimum was 14°C. Relative humidity averaged from 52% in the morning to 37% at noon. During the experiments, the following conditions were observed. During midday, PAR reaching the young leaves ranged from 330 to 550 μmol m^{-2} s^{-1}. Day/night relative humidity ranged from 56 to 70%. Mean high temperature was about 28°C and the mean low was about 22°C. Plants were irrigated frequently and fertilized with 0.25 strength Hoagland solution (2).

Gas Exchange Studies. CO_2 assimilation and transpiration parameters were measured using a dual isotope porometer (3). With this instrument, an air stream of $^{14}CO_2$ in an 80:20 mixture of N_2:O_2 and a final concentration of 320 μl L^{-1} CO_2 is passed through a reservoir of tritiated water at 0°C and of known specific activity. After clamping a small chamber onto a leaf, the lower surface was exposed to the gases for 20 s. Leaf samples were taken using a cork borer (8 mm diameter), extracted in 80% methanol, and placed in sunlight to oxidize the Chl. Isotope uptake was determined with a liquid scintillation spectrophotometer equipped with automatic quench correction. Stomatal conductances (cm s^{-1}) and CO_2 uptake rates (mg CO_2 dm^{-2} h^{-1}) were derived from the amount of tritiated water and $^{14}CO_2$ uptake (3).

Acid Titrations. Leaf samples were collected and frozen on Dry Ice. They were assayed for total titratable acidity the next day. Tissue samples were weighed and then ground using a coaxial tissue homogenizer with a motor-driven Teflon pestle (Potter-Elvehjem). The extract was titrated to an end point of pH 7.0 using 0.01 N KOH.

O_2 Consumption Measurements. Leaf discs 3.6 mm in diameter were used for the measurement of O_2 uptake. Leaf tissue was placed in a vessel containing 50 mM phosphate buffer (pH 6.8) at 30°C (1). A Clark-type O_2 sensor (Yellow Spring Instruments, OH) was introduced into the vessel and covered with an air-tight lid. The vessel was introduced into a water bath which was maintained at 30°C and covered with three layers of aluminum foil to exclude light allowing measurement of dark respiration. To ensure the penetration of added chemicals, the buffered medium and leaf discs were subjected to vacuum infiltration prior to measurement. The compounds used were malonate, a competitive inhibitor of succinate dehydrogenase; and succinate, a substrate of the tricarboxylic acid cycle. O_2 uptake rates were expressed as μmol O_2 g^{-1} fresh weight min^{-1}. Only one sample per time was used because of equipment limitations; however, each point represents several readings. The experiments were conducted several times and a complete diurnal run was conducted three times, once with malonate as an inhibitor.

Respiratory Quotient Measurements. RQ measurements were performed by the method of Umbreit *et al.* (20) with use of a Gilson Differential Respirometer (General Medical Electronics,

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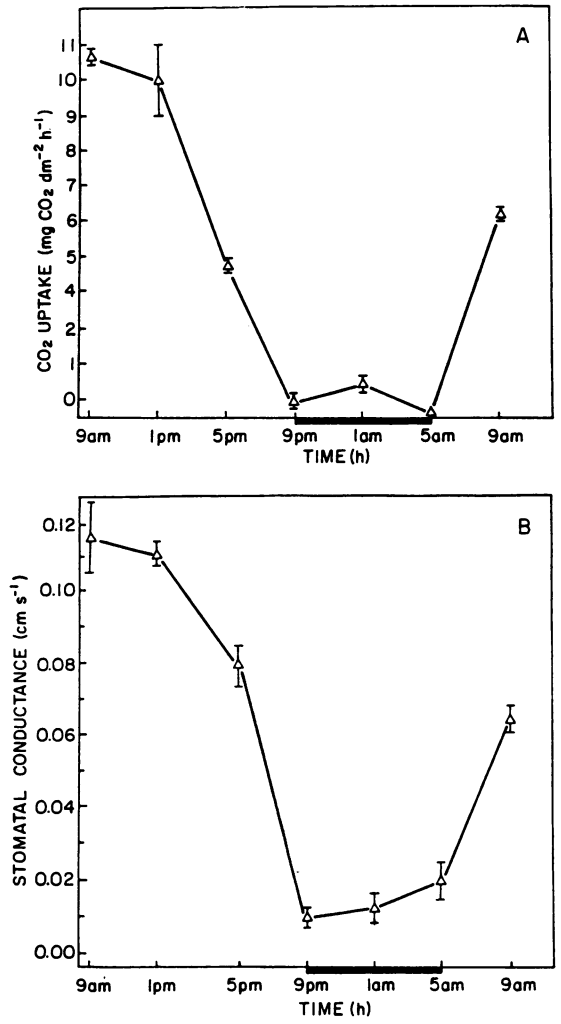

FIG. 1. Diurnal course of CO_2 uptake (A) and stomatal conductance (B) in young leaves of *P. camptotricha*. The dark period is indicated by the black bar on the abscissa. Maximum PAR was 330 to 550 μmol m^{-2} s^{-1}, day/night RH ranged from 56 to 70%, and the temperature from 22 to 28°C. Each datum point represents the mean of three samples ±SE with sampling at 4-h intervals.

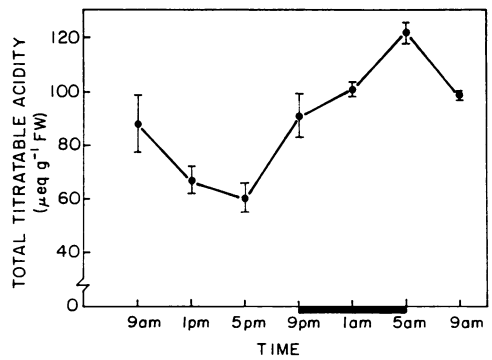

FIG. 2. Diurnal course of organic acid accumulation. The dark period is indicated by the black bar on the abscissa. Each datum point represents a mean of three samples ±SD.

WI). Manometeric flasks used for the measurement of O_2 uptake contained 0.2 ml of 10% (w/v) KOH and a small piece of filter paper folded in pleats and placed in the center well. Flasks used for the measurement of net O_2 and CO_2 exchange did not contain KOH in the center well (15, 16). Experimental points were replicated three times.

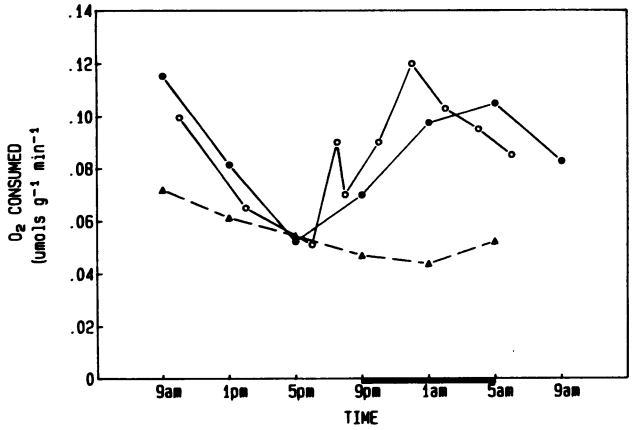

FIG. 3. Diurnal course of the rate of respiration in *P. camptotricha* leaf discs. (●——●), Control 1; (○——○), control 2; (▲- - -▲), 20 mM malonate-treated samples. The dark period for control 1 and malonate-treated samples was from 9:00 PM to 5:00 AM. For control 2, the dark period was from 6:00 PM to 6:00 AM. The mean nocturnal respiration rate reported in text was estimated as the average of the rates shown in control 1 and control 2.

Table I. *Respiratory O_2 Exchange by Leaf Discs of P. camptotricha in the Presence of Malonate and Succinate*

Measurements were made between 10:00 AM to 1:00 PM.

Treatment	O_2 Consumed
	μmol g^{-1} fresh wt min^{-1}
Control	0.046 ± 0.0053 (n = 3)
Succinate	0.062 ± 0.018
Malonate	0.013 ± 0.0062
Succinate + malonate	0.015 ± 0.0047

Table II. *Estimated Day and Night CO_2 Exchange Taken from Figures 1A, 2, and 3 by Integrating Appropriate Areas Under the Curves*

	By CAM[a]	By Respiration[b]	By Exogenous CO_2 Fixation
		μmol CO_2 g^{-1} fresh wt^{-1}	
Day[c]	−26	−64.8	182.9
Night	31	−60	3.9

[a] Estimated from rate of organic acid synthesis (night) or degradation (light). [b] Estimated from O_2 consumption measurements assuming that RQ = 1. [c] Negative values represent CO_2 liberated.

Table III. *Respiratory Quotient (CO_2 produced/O_2 consumed) Calculated from Four Experiments*

Measurements were made between 1:30 AM and 3:00 AM.

	R CO_2	R O_2	R CO_2/R O_2
	μmol g fresh wt^{-1} min^{-1}		*ratio*
1.	0.016	0.046	0.3478
2.	0.048	0.094	0.5106
3.	0.055	0.12	0.4583
4.	0.103	0.18	0.5722
			$\bar{X}$ = 0.47 ± 0.0951

RESULTS AND DISCUSSION

The young leaves of *Peperomia camptotricha* (*i.e.* the leaves closest to the apex up to plastochron 6) show C_3 gas exchange

(Fig. 1A) and a marked diurnal fluctuation of titratable tissue acidity (Fig. 2). During the light period, stomata were open and CO_2 was taken up whereas in the dark period there was little or no CO_2 uptake. Stomatal conductances paralleled the CO_2 uptake patterns in these leaves (Fig. 1B). Young leaves exhibited a diurnal fluctuation of the rate of respiration (Fig. 3). This increase in the rate of respiration at night may be due to an increase in nocturnal activity of certain respiratory enzymes (10). To confirm that the changes in O_2 consumption were the result of mitochondrial respiration, we examined O_2 exchange in the presence of malonate and succinate. As expected in the presence of malonate, a decrease in O_2 consumption was observed whereas succinate increased the rate of O_2 uptake (Table I; Fig. 3). These findings indicate that the observed O_2 uptake was the result of mitochondrial respiration.

The mean nocturnal respiration rate was 0.091 μmol O_2 consumed g^{-1} fresh weight min^{-1} estimated from the average of the rates shown in control 1 and control 2 of Figure 3. The rate of nocturnal acidification was 0.04 μmol acid synthesized g^{-1} fresh weight min^{-1} estimated from the slope of the line in Figure 2 from 5:00 PM to 5:00 AM. This acidification rate was calculated assuming that the titrations required 2 μeq of KOH per μmol of malic acid. Acid fluctuations in this species have previously been shown to be due to malic acid (19). We calculated from the data of Table II that the amount of CO_2 taken up nocturnally was 17% of the CO_2 fixed photosynthetically. Our data also indicate that approximately 50% of the CO_2 released from respiration was refixed into acid (Table II).

The nocturnal CO_2 was estimated as follows: the RQ was assumed to be 1, since these plants primarily store and metabolize carbohydrates (4, 11). The difference between the mean measured RQ of 0.47 ± 0.095 (Table III) and unity should represent the CO_2 fixed into acid. If the ratio $CO_2/O_2 = 0.47$ then $R\ O_2 \times (0.47) = R\ CO_2$ (rate of CO_2 evolution). $R\ CO_2$ calculated in this manner was subtracted from $R\ O_2$ (rate of O_2 consumption) to estimate a rate of 0.048 μmol g^{-1} min^{-1} CO_2 fixed into acid. This estimate of the CO_2 fixation rate is approximately equal to the measured rate of acid synthesis (0.04 μmol g^{-1} min^{-1}).

Previous studies have indicated the occurrence of carbon recycling in CAM plants (5–7). In droughted plants of *Opuntia basilaris* Engelm. and Bigelov., stomata remained continuously closed (14). It was hypothesized that recycling of endogenous CO_2 prevented photooxidation of photosystems in high light (17). Martin and Zee (6) also proposed that *Talinum calycinum* Englem., a C_3 plant with CAM characteristics, has the ability to refix respiratory CO_2 at night. Their studies imply that the acid flux under nighttime stomatal closure results from refixation of respiratory CO_2. They estimate that without this recycling of CO_2, the nocturnal respiratory losses would be double those observed.

Winter *et al.* (21) examined the temperature effects on nocturnal carbon gain and nocturnal acid accumulation in three species of plants showing CAM. Their studies imply that conservation of carbon by recycling respiratory CO_2 is temperature dependent.

Our data indicate that organic acid synthesis at night when stomata are closed in CAM-cycling plants is the result of refixation of respiratory CO_2. This refixation represents about 50% of the total nocturnal respiratory CO_2 and amounts to about 17% of the total photosynthetically fixed CO_2. Thus there is a substantial conservation of CO_2 in this CAM-cycling species.

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