Relationship between Respiration and CAM-Cycling in *Peperomia camptotricha*

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**ABSTRACT**

Mature leaves of well-watered *Peperomia camptotricha* show Crassulacean acid metabolism (CAM). Young leaves show CAM-cycling in which CO₂ 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 CO₂ 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 CO₂ by refixation into acids at night represents about 17% of the total photosynthetically fixed CO₂ and about 50% of the total respiratory CO₂.

CAM is characterized by gas exchange occurring predominantly at night when the stomata are open (4, 8, 9). CO₂ 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 CO₂ 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, C₃ 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 CO₂, and indeed previous studies have assumed refixation of respiratory CO₂ 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 CO₂ 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.** CO₂ assimilation and transpiration parameters were measured using a dual isotope porometer (3). With this instrument, an air stream of ¹⁴CO₂ in an 80:20 mixture of N₂:O₂ and a final concentration of 320 μL L⁻¹ CO₂ 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 CO₂ uptake rates (mg CO₂ dm⁻² h⁻¹) were derived from the amount of tritiated water and ¹⁴CO₂ 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 coxial 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.

**O₂ Consumption Measurements.** Leaf discs 3.6 mm in diameter were used for the measurement of O₂ uptake. Leaf tissue was placed in a vessel containing 50 mL phosphate buffer (pH 6.8) at 3°C (1). A Clark-type O₂ 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. O₂ uptake rates were expressed as μmol O₂ 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 Umbrecht et al. (20) with use of a Gilson Differential Respirometer (General Medical Electronics, 1987 American Society of Plant Biologists. All rights reserved.)
for the paper folded contained 0.2 a mean is indicated with sampling to the black (B) in s-', day/night of 2.

FIG. 1. Diurnal course of CO2 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.

W1). Manometric flasks used for the measurement of O2 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 O2 and CO2 exchange did not contain KOH in the center well (15, 16). Experimental points were replicated three times.

RESULTS AND DISCUSSION

The young leaves of *Peperomia camptotricha* (i.e. the leaves closest to the apex up to plastochron 6) show C3 gas exchange...
(Fig. 1A) and a marked diurnal fluctuation of titratable tissue acidity (Fig. 2). During the light period, stomata were open and CO\textsubscript{2} was taken up whereas in the dark period there was little or no CO\textsubscript{2} uptake. Stomatal conductances paralleled the CO\textsubscript{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\textsubscript{2} consumption were the result of mitochondrial respiration, we examined O\textsubscript{2} exchange in the presence of malonate and succinate. As expected in the presence of malonate, a decrease in O\textsubscript{2} consumption was observed whereas succinate increased the rate of O\textsubscript{2} uptake (Table I; Fig. 3). These findings indicate that the observed O\textsubscript{2} uptake was the result of mitochondrial respiration.

The mean nocturnal respiration rate was 0.091 \textmu mol O\textsubscript{2} consumed g\textsuperscript{-1} fresh weight min\textsuperscript{-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 \textmu mol acid synthesized g\textsuperscript{-1} fresh weight min\textsuperscript{-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 \textmu eq of K\textsubscript{OH} per \textmu mol 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\textsubscript{2} taken up nocturnally was 17% of the CO\textsubscript{2} fixed photosynthetically. Our data also indicate that approximately 50% of the CO\textsubscript{2} released from respiration was refixed into acid (Table II).

The nocturnal CO\textsubscript{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 \pm 0.095 (Table III) and unity should represent the CO\textsubscript{2} fixed into acid. If the ratio CO\textsubscript{2}/O\textsubscript{2} = 0.47 then R O\textsubscript{2} \times (0.47) = R CO\textsubscript{2} (rate of CO\textsubscript{2} evolution). R CO\textsubscript{2} calculated in this manner was subtracted from R O\textsubscript{2} (rate of O\textsubscript{2} consumption) to estimate a rate of 0.048 \textmu mol g\textsuperscript{-1} min\textsuperscript{-1} CO\textsubscript{2} fixed into acid. This estimate of the CO\textsubscript{2} fixation rate is approximately equal to the measured rate of acid synthesis (0.04 \textmu mol g\textsuperscript{-1} min\textsuperscript{-1}).

Previous studies have indicated the occurrence of carbon recycling in CAM plants (5–7). In droughted plants of *Opuntia basilaris* Engelm. and Bigelow., stomata remained continuously closed (14). It was hypothesized that recycling of endogenous CO\textsubscript{2} prevented photooxidation of photosystems in high light (17). Martin and Zee (6) also proposed that *Talinum calycinum* Engelm., a C\textsubscript{3} plant with CAM characteristics, has the ability to refix respiratory CO\textsubscript{2} at night. Their studies imply that the acid flux under nighttime stomatal closure results from refixation of respiratory CO\textsubscript{2}. They estimate that without this recycling of CO\textsubscript{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\textsubscript{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\textsubscript{2}. This refixation represents about 50% of the total nocturnal respiratory CO\textsubscript{2} and amounts to about 17% of the total photosynthetically fixed CO\textsubscript{2}. Thus there is a substantial conservation of CO\textsubscript{2} in this CAM-cycling species.

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