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Physiological and Isotopic Aspects of Photosynthesis in *Peperomia*[1]

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IRWIN P. TING*, LORETTA BATES, LEONEL O'REILLY STERNBERG, AND MICHAEL J. DENIRO
*Department of Botany and Plant Sciences, University of California, Riverside, California 92521 (*I.P.T., L.B.*); and Department of Earth and Space Sciences (*L.O.S., M.J.D.*) and Archaeology Program (*M.J.D.*), University of California, Los Angeles, California 90024*

ABSTRACT

Physiological and isotopic aspects of several *Peperomia* species were investigated. All but one species had C_3-like stomatal behavior, in that stomata were open during the day and closed during the night. In these species, most atmospheric CO_2 uptake occurred during the day. Concurrent with this stomatal behavior, there were Crassulacean acid metabolism-like acid fluctuations in most species. Carbon and hydrogen isotope ratios of cellulose nitrate from *Peperomia* reflect their physiological behavior. The $\delta^{13}C$ values of cellulose nitrate from *Peperomia* species were similar to values observed in C_3 plants and consistent with the daytime uptake of exogeneous CO_2 via the C_3 photosynthetic pathway. The δD values of cellulose nitrate from *Peperomia* species approach those of Crassulacean acid metabolism plants. These elevated δD values are caused by fractionations occurring during biochemical reactions and not as a consequence of water relations.

The phenomenon of CAM is now fairly well understood (12, 26). In typical CAM, gas exchange occurs predominantly at night when stomata are open. Carbon dioxide is taken up at night by carboxylation of PEP[2], catalyzed by the enzyme PEP carboxylase, to form the first product, oxalacetate. Oxalacetate is rapidly reduced to malic acid, which accumulates in vacuoles. During the subsequent light period, malic acid is decarboxylated, and the CO_2 released into the tissue is assimilated through the C_3 photosynthetic cycle. Because stomata are closed, internal CO_2 concentration may increase to over 1% during the day (4). The ecological interpretation of CAM is that it represents a mechanism by which water is conserved, since most gas exchange takes place at night when evaporative demand is low (26).

A modification which has been known for approximately one decade, termed 'CAM-idling,' occurs when CAM plants are severely water-stressed and stomata close both day and night. In CAM-idling, there is a continual, but low-rate, cycling of organic acids through the CAM pathway (15, 17, 23, 27). Evidently, the low level of metabolism keeps the plant's biochemical activity poised until water is available (13, 15–17). Once water is available, the plants recover, usually within 24 h.

Recently, a modification of CAM, termed CAM-cycling, has been described (27). In CAM-cycling, gas exchange occurs largely during the daylight hours, typical of C_3 plants, yet there is diurnal cycling of organic acids in the manner of CAM. CAM-cycling has been observed in *Pereskia* spp. (14), *Cissus quadrangularis* (28), *Welwitschia* (25), *Talinum* (9), the Bromeliaceae (10), and in some species of the Crassulaceae (24).

It is now well known that carbon isotope ratios ($^{13}C/^{12}C$) can distinguish C_3 plants from C_4 and CAM plants operating in the CAM mode (1). C_3 plants have $^{13}C/^{12}C$ ratios lower than C_4 and CAM plants. It has also been shown that hydrogen isotope ratios (D/H ratios) of cellulose nitrate from CAM plants are much higher than those from C_3 and C_4 plants (19–21). Further, it has been shown that greenhouse-grown CAM-cycling plants have δD values approaching those of CAM plants, although their $\delta^{13}C$ values are typical of C_3 plants (21).

The purpose of this study was to investigate physiological and isotopic aspects of CAM-cycling as they exist in the genus *Peperomia*. There are at least 300 species of *Peperomia* native to tropical and subtropical regions (7). They are found in South America, in the islands of the Caribbean, southeastern United States, and islands of the Pacific including Hawaii. Most species are epiphytic and/or lithophytic. The leaf anatomy of these plants is very distinctive, showing an upper hypodermis (multiple epidermis) that may act as a water storage tissue, a median deep green palisade tissue of perhaps three cell layers, and a lower, lighter green, spongy palisade of many cell layers that has the physical appearance of CAM tissue (7).

MATERIALS AND METHODS

Plant Material. Isotopic analyses were performed on plants collected at La Selva, Costa Rica. Physiological measurements were conducted on living plants grown in a greenhouse in Riverside, CA. Plants collected at La Selva were propagated from cuttings and grown in the greenhouse. Plants purchased at nurseries or obtained from the University of California Botanic Garden at Berkeley were also grown in the greenhouse. The greenhouse had a mean annual high temperature of 28°C and a mean low of 22°C. Humidity was variable, but in the neighborhood of 40 to 50%. Plants were grown in full glasshouse sun or under shade, depending upon the species. Plants were watered frequently to avoid stress.

Gas Exchange Studies. Gas exchange parameters were determined with a dual-isotope porometer (6) on plants from the greenhouse. The porometer passes an air stream of dry $^{14}CO_2$ (200 μl L^{-1} in N_2:O_2 mixture of 80:20) through THO of known specific radioactivity. Abaxial leaf surfaces were exposed in triplicate to the radioactive gases for 20 s via a small chamber clamped onto the leaves. The resistances to water vapor transfer and CO_2 uptake (cm s^{-1}) were derived from THO vapor uptake and $^{14}CO_2$ uptake data that were determined by liquid scintillation counting. Values for conductances (cm s^{-1}), transpiration

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[2] Abbreviations: PEP, phosphoenolypyruvate; THO, tritiated water; FW, fresh weight.

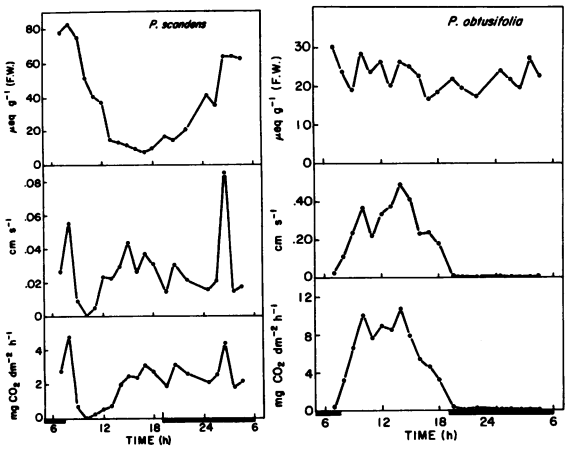

FIG. 1. Gas exchange data for *P. scandens*, a CAM-like species, and for *P. obtusifolia*, a more C_3-like species, both of which were purchased at a Riverside nursery and grown in the greenhouse. CO_2 uptake = mg CO_2 dm^{-2} h^{-1}; titratable acidity = μeq g^{-1} FW; stomatal conductance = cm s^{-1}. Datum points are means of three replicates.

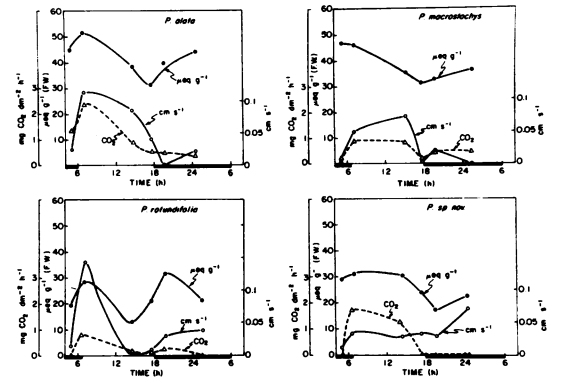

FIG. 2. Gas exchange data for four species of *Peperomia* collected at La Selva and propagated in the greenhouse. See Figure 1 legend for nomenclature and details.

Table I. *Carbon and Hydrogen Isotopic Compositions from Selected Peperomia spp.*

Species	Locality[a]	$\delta^{13}C$	δD
		‰	
P. alata (form *b*)[b]	CR	−29.4	+31
P. serpens	CR	−33.8	+13
P. panamensis	CR	−31.6	+18
P. macrostachys	CR	−28.5	−5
P. rotundifolia (form *a*)[b]	CR	−27.6	−9
P. sp. nov.[c]	CR	−30.5	+5
P. alata (form *a*)[b]	CR	−24.7	+9
P. rotundifolia (form *b*)[b]	CR	−29.2	−0
P. globella	CR	−31.0	−2
P. oerstedii	CR	−28.4	−19
P. orba	UCR	−29.5[d]	−1[d]
P. peltifolia	UCR	−25.5[d]	−12[d]
P. obtusifolia	UCR	−24.9[d]	−14[d]
P. camptotricha	UCR	−27.7[d]	+23[d]
P. scandens	UCR	−22.3[d]	+14[d]

[a] CR, Costa Rica; UCR, University of California at Riverside. [b] There are two different morphological forms of *P. alata* and *P. rotundifolia*; form *a* for both species is the form used in physiological experiments reported here. [c] This species of *Peperomia* could not be identified. [d] Previously reported (21).

rates (g water loss dm^{-2} h^{-1}), and CO_2 uptake rates (mg CO_2 dm^{-2} h^{-1}) were also calculated from the gas exchange data.

Acid Titrations. Leaf samples from greenhouse plants were collected, frozen with dry ice, and stored in a freezer until assayed. Individual samples were weighed, ground in glass-distilled H_2O with a motorized Teflon tissue homogenizer, and titrated to a pH 7.0 endpoint with 0.01 N KOH. Data are expressed as μeq acid g^{-1} FW or as μeq acid cm^{-2}.

Isotope Analyses. Samples of plant material for isotopic analyses were dried in an oven at 50°C, further desiccated in a freeze dryer, and ground to a fine powder in a Wiley mill. cellulose was extracted as previously described (11). Cellulose oxygen isotope ratios were determined by the method of Rittenberg and Ponticorvo (18) as modified by Burk (3). Carbon and hydrogen isotope ratios of cellulose nitrate prepared from cellulose (11) were determined by a modified version of the Stump and Frazer method (11, 22). Isotope ratios are expressed as δ values, where

$$\delta = \left(\frac{R \text{ sample}}{R \text{ standard}} - 1\right) \times 1000‰$$

and R represents $^{18}O/^{16}O$ for $\delta^{18}O$ values,D/H for δD values, and $^{13}C/^{12}C$ for $\delta^{13}C$ values. The standards are standard mean ocean water for $\delta^{18}O$ and δD values and the belemnite from the Peedee formation of South Carolina carbonate for $\delta^{13}C$ values. The precision of the isotope analyses of cellulose and cellulose nitrate were ±2°/oo for δD values, ±0.05°/oo for $\delta^{18}O$ values, and ± 0.2°/oo for $\delta^{13}C$ values.

RESULTS AND DISCUSSION

Peperomia scandens is one of the most CAM-like *Peperomia* species that we have tested (Fig. 1). There is a marked diurnal fluctuation of organic acids identical to that of CAM plants. CO_2 fixation shows a definite early morning burst comparable to CAM plants (12), a depression during the day, followed by an increase toward the end of the light period. There is substantial CO_2 fixation at night in comparison to the light period. Also shown in Figure 1 are data for *Peperomia obtusifolia*, one of the least CAM-like of the *Peperomia* species that we have studied. For this species, there is little evidence of dark CO_2 fixation or dark stomatal conductance, even though organic acids tended to be high. We have, however, observed diurnal acid fluctuation for this species previously (6).

Figure 2 shows gas exchange properties for four species of *Peperomia* collected at La Selva and grown in the greenhouse at Riverside. For the most part, CO_2 uptake is typical of C_3 plants, occurring largely during the day. However, in all cases, there appears to be some exogenous CO_2 fixation at night, although rates are rather low. Observations of stomatal conductance expressed in cm s^{-1} are consistent with CO_2 uptake patterns. Care must be taken in interpretation of the CO_2 fixation data presented here because they were obtained with $^{14}CO_2$ and, thus, net CO_2 fixation was not measured. It is highly likely and indeed probable that the small amount of CO_2 fixation at night, sometimes reaching about 1 mg dm^{-2} h^{-1}, is less than the amount of CO_2 lost through respiration. Nevertheless, there appears to be substantial dark CO_2 fixation, since there is a definite, but small, diurnal fluctuation of organic acids similar to that observed in CAM plants, with levels of organic acids reaching values as high as 50 μeq g^{-1} FW.

We do not have sufficient data to calculate the contribution to acid synthesis by dark CO_2 fixation. However, for *Peperomia* sp. nov., there was no apparent CO_2 uptake at night, yet an organic acid fluctuation of about 15 μeq g^{-1} FW was measured. Thus, in this species, dark CO_2 fixation seems insufficient to acocunt for acid synthesis.

In summary, the gas exchange and acid flux data (Figs. 1 and

Table II. *Carbon and Hydrogen Isotopic Compositions of C_3, C_4, CAM, and Peperomia spp.*

Plant Type	Greenhouse[a]		La Selva, Costa Rica	
	$\delta^{13}C$	δD	$\delta^{13}C$	δD
	‰		‰	
C_3	−28.2 ± 1.3[b] (10)[c]	−80 ± 35	−28.3 ± 1.7 (7)	−45 ± 11
C_4	−12.6 (2)	−25	−11.3 (2)	−41
CAM	−15.4 ± 3.2 (21)	+30 ± 19	−16.2 ± 2.3 (6)	+27 ± 13
Peperomia	−26.0 ± 2.7 (5)	+2 ± 16	−29.5 ± 2.5 (10)	+4 ± 14

[a] Previously reported (21). [b] Data are given as means ± 1 SD. [c] Numbers in parentheses indicate number of species analyzed.

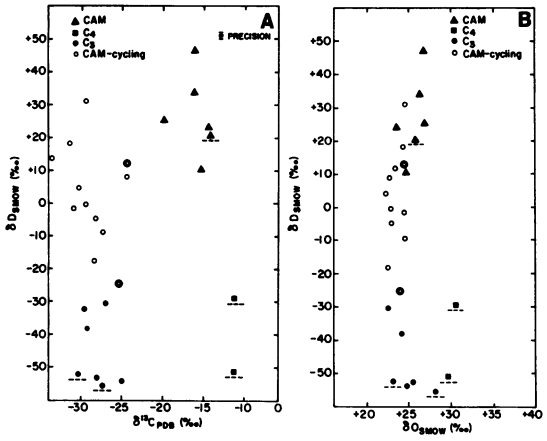

FIG. 3. a, δD values *versus* $\delta^{13}C$ values of cellulose nitrate from plants collected at La Selva, Costa Rica. The symbols indicate C_3 plants (●), C_4 plants (■), CAM plants (Δ), *Peperomia* species (○),and species from the family Gesneriaceae (⊙). Datum points which are underlined with a dashed line are values measured from nonepiphytic terrestrial plants. All other plants sampled were epiphytes. b, δD values of cellulose nitrate *versus* $\delta^{18}O$ values of cellulose for the same plants shown in Figure 3a. SMOW, standard mean ocean water; PDB, belemnite from the Peedee formation of South Carolina.

2) indicate that *Peperomia* spp. may have CAM (*P. scandens*) or CAM-cycling (all species in Fig. 2), but that some species may show little evidence of these photosynthetic modes (*P. obtusifolia*).

Carbon and hydrogen isotope ratios for cellulose nitrate from five species of *Peperomia* bought at a local nursery and maintained in the greenhouse and nine species of *Peperomia* collected at La Selva, Costa Rica, are given in Table I. Inspection of the data shown in Table II indicates that $\delta^{13}C$ values of *Peperomia* are comparable to those observed for C_3 plants and dissimilar to values of C_4 and CAM plants. Cellulose nitrate from *Peperomia* species is enriched in deuterium relative to that in C_3 and C_4 plants for both greenhouse and Costa Rican samples, approaching values seen in CAM plants in both cases (Table II).

As shown in Figure 3, a plot of δD values *versus* $\delta^{13}C$ values of samples collected in La Selva allows separation of the *Peperomia* showing CAM-cycling from C_3, C_4, and CAM plants. Thus, our previous observations that plants showing CAM-cycling can be identified based on their hydrogen and carbon isotopic compositions are confirmed (21). In addition to *Peperomia*, Figure 3 displays data for two epiphytes in the Gesneriaceae family (*Codananthe crassifolia* and *Columnea linearis*), which were also collected at La Selva, Costa Rica. These two plants have the same leaf morphology as *Peperomia* species. Indeed *Codananthe*, with the more positive δD value of the two, shows the same carbon metabolism properties as the *Peperomia* species showing CAM-cycling, whereas *Columnea*, with the more negative δD value, has carbon metabolism properties more similar to a typical C_3 plant (unpublished observations). Thus, δD values of cellulose nitrate from these two plants reflect the two different types of photosynthetic metabolisms.

The similarity of the $\delta^{13}C$ values of the *Peperomia* species and the C_3 plants, sampled either in the greenhouse or at La Selva (Table II), indicates that exogenous carbon fixation in *Peperomia* is through the C_3 pathway. In those *Peperomia* species showing CAM-cycling, we assume that all CO_2 fixed by the CAM pathway at night (when stomata are closed) is the result of refixation of respiratory CO_2. This respiratory CO_2, coming from carbohydrates synthesized by C_3 photosynthesis, would have $\delta^{13}C$ values which reflect C_3 photosynthesis. Thus, these plants have $\delta^{13}C$ values similar to those of C_3 plants, and not of CAM plants.

The deuterium enrichment we observed in CAM-cycling *Peperomia* spp. could be caused by isotopic fractionations occurring during H_2O metabolism. Briefly, the enrichment would occur if the leaf water in these plants becomes enriched in deuterium during evapotranspiration and if this H_2O then labels the organically bound hydrogen. If this hypothesis is true, cellulose from CAM-cycling plants should also be enriched in ^{18}O relative to C_3 and C_4 plants, since evapotranspiration enriches leaf water in both D and ^{18}O (2, 5). Measurement of oxygen isotope ratios for the field-grown Costa Rican sample set shows no such oxygen enrichment in CAM-cycling plants (Fig. 3b). In fact, in plants with the various photosynthetic modes, all have similar oxygen isotope ratios. Thus, we conclude that elevated δD values of cellulose nitrate from CAM-cycling plants, like those in CAM plants (19, 20), are due to fractionations occurring during biochemical reactions, perhaps those associated with acid fluctuation.

The contribution of the CAM-cycling phenomenon to the physiology and/or ecology of a plant is not apparent at this time. *Peperomia* species in their natural environment, being mostly epiphytic, are subjected to frequent periods of drought. Root systems are underdeveloped and thus the plants tend to be hydrated only during and immediately after precipitation. Plants, particularly those species that grow in partial or full sun, become water stressed soon after a precipitation event. We suspect that the CAM-cycling phenomenon is similar to the CAM-idling phenomenon, in which there is organic acid recycling during severe drought when stomata are closed day and night (15, 16). We suggest that drought occurring between precipitation episodes is accompanied by stomatal closure in CAM-cycling plants. If organic acid cyclic continues, the plants would be in the CAM-idling phase and, thus, metabolically poised to resume full photosynthetic activity when water is available. Further investigations are being conducted to ascertain these aspects of CAM-cycling.

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