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Developmental Control of CAM in *Peperomia scandens*[1]

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ABSTRACT

Experiments were conducted to examine the development of photosynthetic carbon metabolism in *Peperomia scandens*, a tropical epiphyte. Leaves were sampled during a 10-day period when they were between 30 to 165 days old. *P. scandens* exhibits a C_3 to CAM-cycling to CAM shift during maturation with the magnitude of CAM increasing with age. Initially, during both day and night, no significant CO_2 uptake or diurnal acid flux was evident. C_3 gas exchange was detected at 41 days of age with a gradual shift towards CAM gas exchange maximized thereafter. An acidity flux of 130 to 150 microequivalents per gram fresh weight was evident by 41 days. Between 40 and 90 days, the leaves shifted their CO_2 uptake pattern from a daytime to a nighttime peak. After 90 days, the leaves remained in CAM. The $\delta^{13}C$ values became progressively less negative as the leaves matured. In the 30-day-old leaves, the $\delta^{13}C$ value was −21.1% while in the 165-day-old leaves the $\delta^{13}C$ value was −18.3%. The time-dependent shift from C_3 to CAM-cycling to CAM in *P. scandens* does not appear to result from changes in water, light, or temperature regimes since these variables were constant for all leaves sampled.

CAM in succulent plants has been the subject of extensive research during the past 20 years (25). This variation of carbon assimilation is characterized by stomatal opening predominantly during the period from late afternoon to early morning and a diurnal fluctuation of titratable acids, principally malic acid. CO_2 is initially fixed by carboxylation of PEP[2] via PEP carboxylase and is immediately reduced to form malic acid. The malic acid accumulates and is stored overnight in the vacuole until the subsequent light period when it is released to the cytoplasm and decarboxylated. The liberated CO_2 is then assimilated through the C_3 photosynthetic cycle (25).

Two modifications of CAM have been described: CAM-idling and CAM-cycling. The former is characterized by a water stress-induced closure of the stomata which may persist for long periods. There is a constant, albeit reduced, recycling of organic acids through the CAM pathway in both whole plants (16, 19, 23, 29) and in detached stem pieces (5). It is thought that the persistence of a reduced metabolic rate during water-stress maintains the biochemical apparatus until water is no longer a limiting factor for growth (15, 17–19). CAM-idling has been observed in members of the Cactaceae (5, 23), Cucurbitaceae (17, 18), Asclepiadaceae (19), Piperaceae (22, 30), Liliaceae (9), and Aizoaceae (34) but was not detected in various South African CAM plants investigated by von Willert *et al.* (33).

The second modification, CAM-cycling, is typified by predominantly C_3-like gas exchange during the day with a subsequent diurnal cycling of organic acids. CAM-cycling has been observed in members of the Cactaceae (16), Welwitschiaceae (27), Crassulaceae (24), Piperaceae (22, 26), Bromeliaceae (14), and Portulacaceae (13).

Succulents have been observed to shift from a C_3 photosynthetic mode to one of the aforementioned CAM variations. Such shifts are the result of either developmental (34, 36) or environmental (16, 30, 35) changes.

The objective of this study was to investigate the development of CAM in *Peperomia scandens*. *Peperomia scandens* is native to the Caribbean, Mexico, and Central America where it is widespread and exists as an epiphytic or lithophytic vine (32). The genus *Peperomia* exhibits variable photosynthetic gas exchange patterns (22) with *P. scandens* having been shown to be one of the most CAM-like of the peperomias (26). Unpublished observations from our laboratory indicate there are significant differences in gas exchange patterns between the young and old leaves of this plant. Thus we sought to quantitate the CO_2 uptake, malate flux, and carbon isotope composition of the plant during development.

MATERIALS AND METHODS

Plant Material. Specimens of *Peperomia scandens* were propagated from cuttings and grown in a greenhouse in Riverside, CA. PAR did not exceed 135 μmol m^{-2} s^{-1}. The mean RH was 35 to 45% and the mean air temperature was 23 to 29°C. Plants were irrigated frequently using one-quarter strength Hoaglands solution (4) to preclude water and nutrient stress. Experimentation occurred over a 10-d period in August 1985 on leaves initiated between February 1, 1985, and July 16, 1985. Sunrise was at approximately 5 AM and sunset was at approximately 9 PM, thus yielding a 16 h light period. Three leaves, each from separate plants, were sampled in triplicate. Leaf initiation in this study was defined as the point when a leaf was 1 cm long.

Gas Exchange Studies. Gas exchange parameters were measured with a dual-isotope porometer as previously described (6).

Acid Titrations. Leaf samples were collected in triplicate, frozen, and stored on dry ice until assayed. Individual samples were weighed and then ground in glass-distilled water using a coaxial tissue homogenizer with a motor-driven Teflon pestle (Potter-Elvehjem). The resulting homogenate was then titrated to pH 7.0 with 0.01 N KOH.

Isotopic Analysis. Isotopic composition was determined as previously described (26). The precision for the isotope analysis of the whole leaf extract was ±0.2‰ for $\delta^{13}C$ values.

RESULTS

CO_2 uptake patterns of *Peperomia scandens* leaves changed markedly during the course of the study. The leaves exhibited

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[2] Abbreviations: PEP, phosphoenolpyruvate; C_3, Calvin-Benson cycle for CO_2 fixation; FW, fresh weight; RuBP, ribulose bisphosphate; THO, tritiated water.

little CO_2 uptake until 32 d after initiation (Fig. 1A). After 40 d the leaves exhibited a typical C_3 pattern of CO_2 uptake. By 60 d postinitiation, the CO_2 uptake pattern was biphasic with maximum rates at 5 PM and 1 AM (Fig. 2A). By 75 d postinitiation, two broad peaks were evident that were lower in magnitude than observed at 60 d and occurring between 5 to 9 PM and 5 to 9 AM. In older leaves, gas exchange in *P. scandens* assumed a pattern more typical of a CAM plant (Figs. 3A, 4A, and 5A).

The youngest leaves had little or no fluctuation in stomatal conductance over a 24-h period (Fig. 1B). Conductances remained below 0.02 cm s^{-1} for 75 d. After this time, conductances were generally greater than 0.02 cm s^{-1} (Figs. 3B, 4B, and 5B). Conductances did not correlate well with CO_2 uptake until 145 d after initiation (Fig. 5B).

The young leaves (*i.e.* 30–45 d old) of *P. scandens* exhibited no statistically significant diurnal acid fluctuation (Fig. 1C), although the level of tissue acidity remained high. By 45 d postinitiation, acid fluctuation was evident (Fig. 1C). Titratable acid levels reached a minimum at 5 PM and a maximum between 5 AM and 9 AM with a magnitude of 135 to 150 μeq g^{-1} FW (Figs. 2C, 3C, and 4C). This flux was maintained until about 150 d postinitiation after which the leaves began to senesce resulting in a reduced diurnal acid flux (Figs. 5C and 6).

The older leaves of *P. scandens* exhibited less discrimination against ^{13}C than did the younger leaves (Fig. 7). As the leaves matured, $\delta^{13}C$ values became progressively less negative until 130 d postinitiation when $\delta^{13}C$ values stabilized at about −18.1‰.

DISCUSSION

In a previous study, *Peperomia scandens* was reported to be one of the most CAM-like members of the genus analyzed to date (26). The results presented here in more detail confirm the earlier report; however, we now have shown that the magnitude of CAM activity is positively correlated with leaf age.

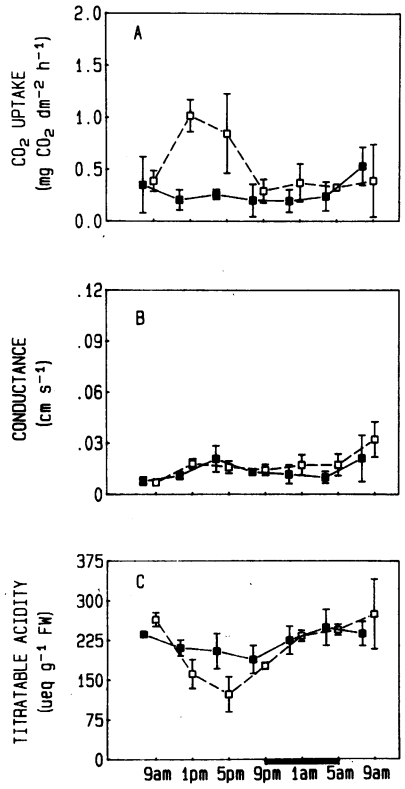

FIG. 1. Diurnal variations in CO_2 uptake (A), conductance (B), and titratable acidity (C) 32 d (■——■) and 41 d (□- - -□) after initiation for leaves of *P. scandens*. Points are means of nine samples ± SE of the mean.

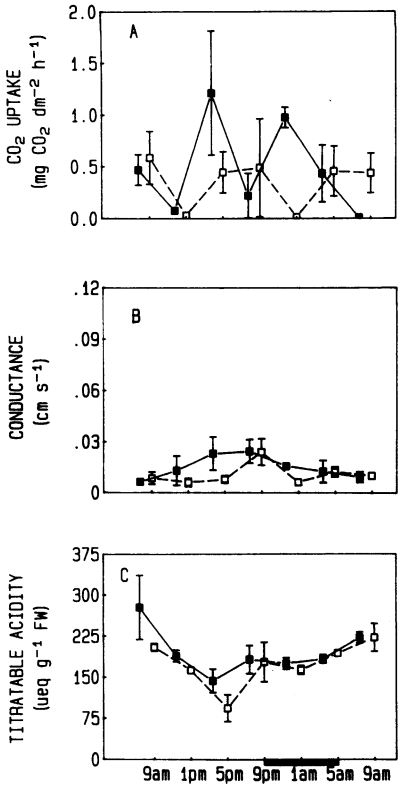

FIG. 2. Diurnal variations in CO_2 uptake (A), conductance (B), and titratable acidity (C) 60 d (■——■) and 75 d (□- - -□) after initiation for leaves of *P. scandens*. Points are means of nine samples ± SE of the mean.

Leaf tissue maturity is a major factor in the induction of CAM in *P. scandens*. This phenomenon of tissue age influencing the mode of carbon fixation has previously been reported in *Cissus quadrangularis* (31), *Mesembryanthemum crystallinum* (34), *Kalanchoe blossfeldiana* and *K. velutina* (1), and *Bryophyllum fedtschenkoi* (7). Shifts from a C_3 to a CAM photosynthetic mode have also been reported to result from changes in environmental conditions such as water status (28, 34), temperature (12), light quality (12), relative humidity (12), and photoperiod (2). Here, the induction of CAM in *P. scandens* occurs under more stable environmental conditions. The constancy of our environmental conditions suggests the development of CAM in *P. scandens* is constitutive. The possibility that environmental signals, in concert with normal developmental signals, effect an accelerated C_3 to CAM shift (as in *Mesembryanthemum* [34]) is certainly plausible in *P. scandens*.

During the maturation of *P. scandens*, the mode of carbon assimilation is quite variable. When quite young, the leaves fix carbon via the C_3 pathway. The high tissue acidity levels present at this time indicate that respiratory CO_2 is being refixed. Thereafter, diurnal acid fluctuation becomes evident before nocturnal CO_2 uptake is observed. Thus, the young leaves of *P. scandens* are performing CAM-cycling prior to the onset of the more typical CAM pattern of a diurnal acid flux coupled with nocturnal CO_2 uptake. The existence of this phenomenon indicates that the enzymatic complement necessary for carbon assimilation through CAM is functional before the stomatal apparatus begins to function at night. The CO_2 source for the CAM-cycling in the young leaves is also presumed to be from respiration. Therefore, these immature leaves possess a mechanism for reducing respiratory CO_2 losses.

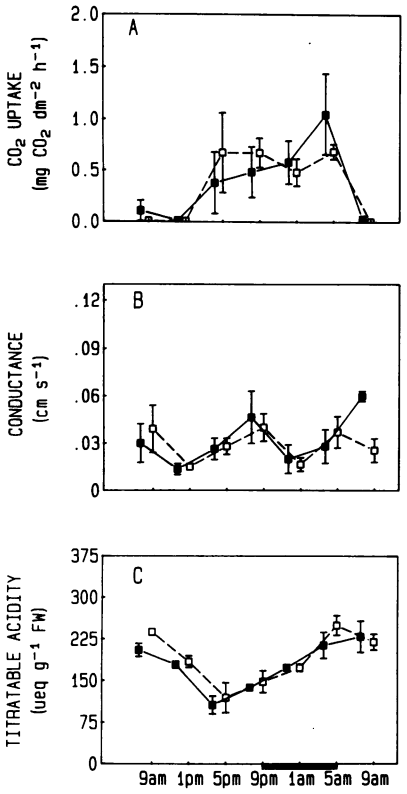

FIG. 3. Diurnal variations in CO_2 uptake (A), conductance (B), and titratable acidity (C) 91 d (■——■) and 131 d (□- - -□) after initiation for leaves of *P. scandens*. Points are means of nine samples ± SE of the mean.

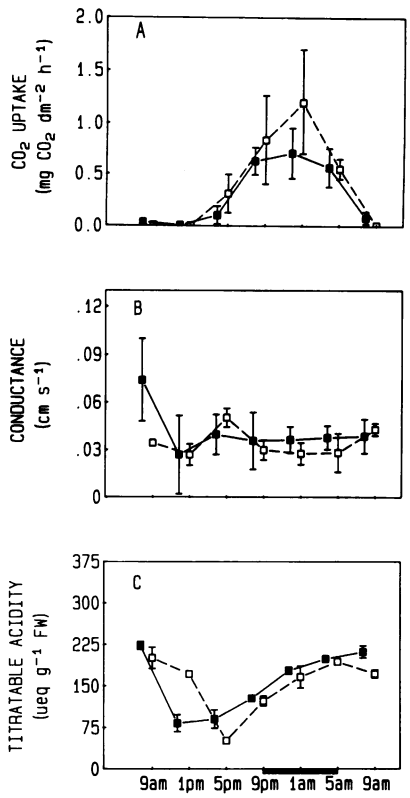

FIG. 4. Diurnal variation in CO_2 uptake (A), conductance (B), and titratable acidity (C) 121 d (■——■) and 131 d (□- - -□) after initiation for leaves of *P. scandens*. Points are means of nine samples ± SE of the mean.

In the genus *Peperomia*, we have measured great variability in stomatal conductance: the highest being in *P. orba* at 0.32 cm s^{-1} and the lowest being *P. scandens* at 0.01 cm s^{-1} (30). In this study we found the young leaves of *P. scandens* had conductances of about 0.02 cm s^{-1} while the old leaves had conductances of 0.04 cm s^{-1}. The low stomatal conductances are probably partially the result of the low irradiances during growth of the experimental plants. Also, conductances of this low magnitude are not uncommon in plants adapted to environments characterized by periodic droughts (8). There was little change in the conductance of *P. scandens* over 24-h periods in this study. The lack of a close correlation between conductance and CO_2 uptake is curious. Sampling on all leaves was conducted in August when the daylength in Riverside is about 16 h. Sipes and Ting (20) have reported that under long days (>14 h), *P. camptotricha* also showed no fluctuation in conductance during the course of a day. It is reasonable, therefore, to assume that the lack of fluctuation in the stomatal conductance of *P. scandens* during this study was a result of daylength. While there was little diurnal fluctuation in conductance, the stomata were nevertheless open and functional (as evidenced by the CO_2 uptake data) during this study.

Young leaves of *P. scandens* exhibit a high level of titratable acids (about 250 μeq g^{-1} FW). Other species of *Peperomia* that have been investigated typically have less than 100 μeq g^{-1} FW of titratable acids (26, 30) although the young leaves of *P. camptotricha* reach a dawn maximum of 160 μeq g^{-1} FW (30). As *P. scandens* matures, the endogenous acid level decreases. This appears to be normal for other CAM plants (7, 30).

The period of peak CO_2 uptake in *P. scandens* differs with the age of the leaf. Young leaves (40–50 d) tend to show a C_3-type daytime gas exchange pattern while mature leaves (>90 d) exhibit a CAM-like nocturnal pattern. Perhaps the most interesting CO_2 uptake patterns were those observed between these two extremes. Between 45 to 90 d, *P. scandens* has a unique CO_2 uptake pattern. There appears to be a progressive shift from daytime to nighttime CO_2 uptake. By 60 d, there are well defined peaks at 5 PM and at 1 AM which are presumably the result of the activity of two different carboxylating enzymes; RuBP-carboxylase and PEP-carboxylase, respectively. By 75 d, the peaks are less well defined, broader, and occur between 5 to 9 PM and 5 to 9 AM. However, the identity of the enzymes responsible for the peaks is unknown.

The shift in $\delta^{13}C$ values from −21.1% towards a more CAM-like value of −18.1% is consistent with the observed shift in the CO_2 uptake pattern from C_3 to CAM-cycling to CAM. Other workers have reported changes in the $\delta^{13}C$ value with increasing age in CAM plants (3, 10). A $\delta^{13}C$ value of −21.1% indicates that a portion of *P. scandens*' carbon fixation is through the CAM pathway. Although the leaves appear to be in the C_3 mode, there is a small amount of nocturnal CO_2 uptake (Fig. 1A). This nocturnal carbon gain along with translocation from more mature leaves could account for the −21.1% value. The observed steady decrease in ^{13}C discrimination with leaf maturity is indicative of a greater reliance on PEP-carboxylase for carbon fixation.

In *Kalanchoe*, environmental parameters can influence carbon isotope values. Increased diurnal temperature fluctuations, longer photoperiods, water stress, and salt stress induce a shift to a more CAM-like $\delta^{13}C$ value (21). Water and salt stress were not factors during this study. The leaves assayed were initiated between February and July such that the older leaves may have been influenced by the greater daily temperature fluctuations and longer photoperiods associated with the onset of summer. However, one would expect the young leaves to also exhibit a $\delta^{13}C$ value similar to the older leaves since both experienced the same environmental conditions. Therefore, we believe that the

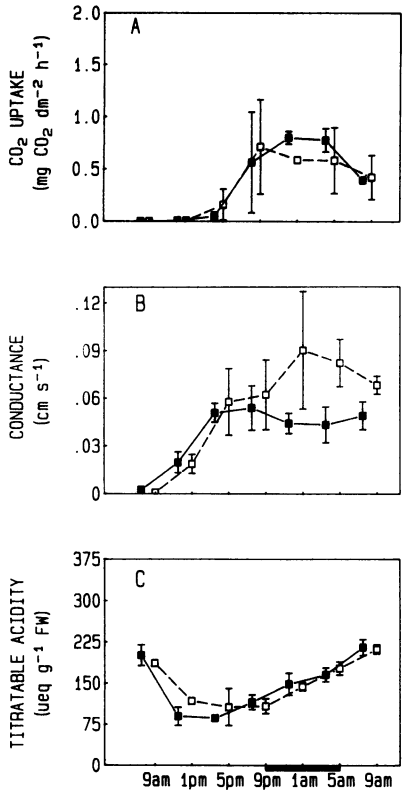

FIG. 5. Diurnal variation of CO_2uptake (A), conductance (B), and titratable acidity (C) 145 d (■——■) and 158 d (□- - -□) after initiation for leaves of *P. scandens*. Points are means of nine samples ± SE of the mean.

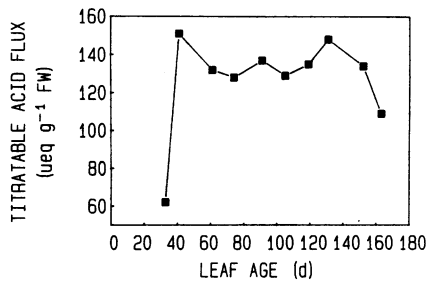

FIG. 6. Variation in diurnal acid flux as a function of leaf age for leaves of *P. scandens*. Points are means of nine samples.

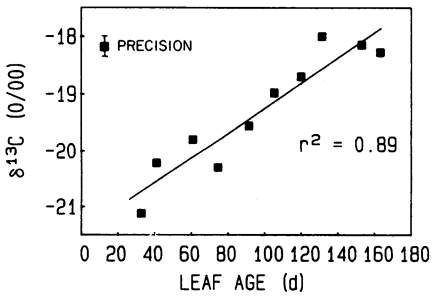

FIG. 7. Variation of ^{13}C composition of leaves from *P. scandens* as a function of leaf age. The precision for the isotope analysis was ±0.2%.

shift in $\delta^{13}C$ value is largely ontogenetic in origin but may be environmentally enhanced.

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