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CRASSULACEAN ACID METABOLISM IN THE GESNERIACEAE[1]

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ABSTRACT

The occurrence of the Crassulacean acid metabolism (CAM) was studied in four epiphytic species of the Gesneriaceae: two neotropical species, *Codonanthe crassifolia* and *Columnea linearis,* and two paleotropical species, *Aoschynanthus pulcher* and *Saintpaulia ionantha.* Gas exchange parameters, enzymology, and leaf anatomy, including mesophyll succulence and relative percent of the mesophyll volume occupied by airspace, were studied for each species. *Codonanthe crassifolia* was the only species to show nocturnal CO_2 uptake and a diurnal organic acid fluctuation. According to these results, *Codonanthe crassifolia* shows CAM-cycling under well-watered conditions and when subjected to drought, it switches to CAM-idling. Other characteristics, such as leaf anatomy, mesophyll succulence, and PEP carboxylase and NADP malic enzyme activity, indicate attributes of the CAM pathway. All other species tested showed C_3 photosynthesis. The most C_3-like species is *Columnea linearis,* according to the criteria tested in this investigation. The other two species show mesophyll succulence and relative percent of the leaf volume occupied by airspace within the CAM range, but no other characters of the CAM pathway. The leaf structure of certain genera of the Gesneriaceae and of the genus *Peperomia* in the Piperaceae are similar, both having an upper succulent, multiple epidermis, a medium palisade of one or a few cell layers, and a lower, succulent spongy parenchyma not too unlike CAM photosynthetic tissue. We report ecophysiological similarities between these two distantly related families. Thus, the occurrence of CAM-cycling may be more common among epiphytic species than is currently known.

THE CRASSULACEAN ACID METABOLISM (CAM)—pathway of photosynthesis is regarded as an adaptation to arid and semiarid environments (Kluge and Ting, 1978). CAM is characterized by nocturnal CO_2 uptake and malic acid accumulation. Subsequent daytime stomatal closure and decarboxylation of malic acid produces CO_2 as the substrate for photosynthesis, resulting in less water loss under periods of high evaporative demand. The leaves of CAM plants are also characterized by tightly packed chlorenchyma with large vacuoles where the malic acid accumulates (Gibson, 1982). In addition, the mesophyll is generally not differentiated into palisade and spongy parenchyma layers (Kluge and Ting, 1978). These characteristics of CAM are important under water-limiting conditions.

Two new modifications of CAM have recently been reported (Kluge and Ting, 1978). Both are similar to typical CAM metabolism, but show slight modifications in their gas exchange parameters. CAM-cycling is characterized by C_3-type gas exchange accompanied by a diurnal acid fluctuation. Stomatal conductance is high during the day and low at night, but there is still a nocturnal acidification primarily due to recycling of respiratory CO_2. The second modification, called CAM-idling, usually occurs when CAM or CAM-cycling species are water stressed. Exogenous gas exchange is halted due to stomatal closure, but a slight diurnal acid fluctuation is still observed.

Not all CAM species are found in arid environments; in fact, many occur in xeric microenvironments of mesic regions, such as rock outcrops (Martin and Zee, 1983), and others are tropical epiphytes that periodically become drought stressed (Winter et al., 1983; Sinclair, 1984; Ting et al., 1985). The CAM pathway of photosynthesis occurs in tropical epiphytes of the Bromeliaceae (McWilliams, 1970; Medina and Troughton, 1974), Orchidaceae (Knauft and Arditti, 1969; McWilliams, 1970; Neales and Hew, 1975; Sinclair, 1984), Cactaceae (Wiehler, 1982), Rubiaceae (Winter et al., 1983), and the Piperaceae (Sternberg, Deniro and Ting 1984; Sipes and Ting, 1985). CAM is also found in some epiphytic ferns of the Polypodiaceae (Wong and Hew, 1976).

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The largely tropical Gesneriaceae has about 120 genera and 3,000 species, of which 20% are epiphytic in their habitat. A number of the epiphytic gesneriads overlap in range with *Peperomia* spp. of the Piperaceae and, in addition, they both share a similar upper succulent multiple epidermis (Ting et al., 1985). Since it was recently reported that the genus *Peperomia* shows attributes of CAM (Ting et al., 1985), we investigated some epiphytic Gesneriaceae for the occurrence of CAM. In this paper, we report that the Gesneriaceae has epiphytic species showing attributes of CAM. The investigation included a comparison of physiological and anatomical characters of CAM and C_3-related gesneriad species. We wanted to ascertain which anatomical characters might correlate to the CAM pathway. We report here our investigations of the gas exchange parameters, leaf anatomy, and enzymology of four gesneriads: two neotropical and two paleotropical species.

MATERIALS AND METHODS—*Plants*—*Saintpaulia ionantha* (H. Wendl.) plants were a gift from Craig Yoshida of Sunnyside Nursery of Hayward, Calif. *Aeschynanthus pulcher* (G. Don) plants were purchased from Tiegs Nursery of Riverside, California. *Codonanthe crassifolia* (Focke) and *Columnea linearis* (Oerst.) specimens were collected at La Selva, Costa Rica, and propagated in glasshouses at the Univ. of Calif., Riverside. Plants were propagated from cuttings and grown in a glasshouse under natural photoperiods during the course of the year. The glasshouse had a mean annual high temperature of 28 C and a mean low of 18 C. Relative humidity varied between 40 and 60%.

Leaf anatomy—Mature and primordial leaves were collected and fixed in FAA (Johansen, 1940). The tissue was dehydrated in an ethanol series, embedded in glycol methacrylate (Feder and O'Brien, 1968), and sectioned at 4 μm. Sections were stained for 1 min with 0.5% Toluidine Blue O (Feder and O'Brien, 1968). Stereological methods were used to quantify the percentage of mesophyll airspace present in the four species. A plastic overlay with randomly placed points was projected onto a leaf section according to the method described by Parkhurst (1982). Five locations on each leaf section were counted, and a minimum of three separate mature leaves were analyzed for each species. Measurements were taken on entire leaf sections, including both epidermal and mesophyll tissues. In addition, measurements were also taken on the mesophyll tissue alone. Results are expressed as relative percent of the leaf volume occupied by airspace ± SE.

Mesophyll succulence (S_m) was calculated from the ratio of total tissue water to total chlorophyll (Kluge and Ting, 1978) in fresh leaf samples. Tissue water content was determined by drying samples in a microwave oven until dry and subtracting the dry weight from the fresh weight. Total chlorophyll was measured by grinding fresh leaf tissue in 80% acetone and then centrifuging at 1,500 *g*. A 1 ml aliquot was added to 4 ml 80% acetone and then measured spectrophotometrically (Arnon, 1949). S_m was determined for the whole leaf. Results are expressed as g H_2O mg^{-1} Chl ± SE.

Titratable acidity—Three leaves per time point were collected, weighed, and frozen until assayed. Samples were ground in glass distilled water with a tissue grinder and titrated with 0.01 N KOH to a pH 7.0 endpoint. Data are expressed as μeq g^{-1} FW ± SE.

Enzyme assays—Leaf samples were collected in triplicate, weighed, and 1 g FW was ground in a tissue grinder in 10 ml of extraction medium at 4 C, containing 100 mM Hepes-NaOH, 10 mM $MgCl_2$, 10 mM DTT, and 1% PVP 360 adjusted to pH 7.8 for extraction of PEP carboxylase and to pH 7.2 for NADP malic enzyme. The sample was centrifuged at 4,000 rpm at 4 C for 10 min, and a small aliquot was passed through a Sephadex G-25 column, which had been pre-equilibrated with the adjusted Hepes-NaOH buffer. The 4 ml void volume was collected and used as the enzyme preparation.

PEP carboxylase and NADP malic enzyme were both assayed spectrophotometrically by following the oxidation of NADH or the reduction of NADP at 340 nm and 27 C, respectively. The PEP carboxylase assay mixture contained 50 mM Hepes-NaOH (pH 7.8), 10 mM $MgCl_2$, 1 mM $NaHCO_3$, 0.2 mM NADH, 100 μl enzyme preparation, and varying concentrations of PEP. NADP malic enzyme assay mixture contained 50 mM Hepes-NaOH (pH 7.0), 3 mM $MgCl_2$, 0.25 mM NADP, 100 μl enzyme preparation, and varying concentrations of malate.

Gas exchange studies—Gas exchange parameters were measured using a dual isotope porometer (Johnson, Rowlands and Ting, 1979). The porometer passes an airstream of dry $^{14}CO_2$ (330 μl L^{-1} in N_2:O_2 mixture of 80:20) through tritiated water (THO) of known specific radioactivity. Abaxial leaf surfaces of

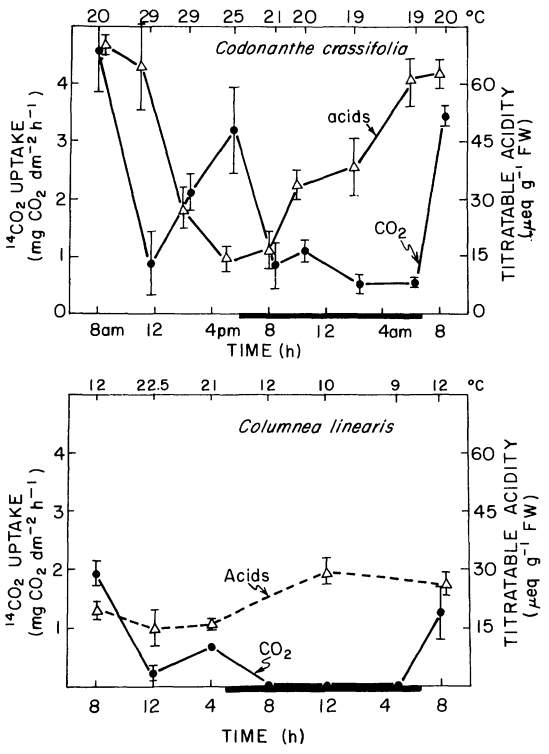

Fig. 1. Diurnal variation of CO_2 uptake (——) and Titratable acidity (– – –). Top: *Codonanthe crassifolia,* Bottom: *Columnea linearis.* Black bar indicates darkness, and each point represents the mean (n = 3) ± SE for Fig. 1–6.

three intact leaves were exposed for 20 s to the radioactive gases. Radioactivity of the samples was determined by liquid scintillation counting. Values for conductances (cm s^{-1}), and CO_2 uptake rates (mg CO_2 $dm^{-2}h^{-1}$) were calculated from THO vapor and $^{14}CO_2$ uptake.

Malic acid assay—Organic acids were extracted from leaf tissue (0.5–1.0 g FW) by grinding in 2 ml of glass distilled water. The sample was then transferred to a centrifuge tube containing 100 mg cation exchange resin (Bio-Rad AG 50x-X8) and mixed. After centrifugation at 1,500 *g* for 10 min, the supernatant fluid was filtered through a 2 μm pore filter. The filtrate was used for malic acid analysis on a Beckman model 330 HPLC system. The column was a Bio-Rad HPX-874 organic acid column with an ultraviolet detector at 214 nm. The solvent was 5% acetonitrile in 0.01 N H_2SO_4.

RESULTS—*Gas exchange parameters and titratable acidity* —The diurnal pattern of gas exchange in all but one species (*Codonanthe crassifolia*) was typical for C_3 photosynthesis indicated by only daytime CO_2 uptake (Fig. 1, 3). Daytime stomatal conductance on the order 0.05 to 0.09 cm s^{-1} was observed for *A. pulcher, S. ionantha,* and *Columnea linearis,* while nighttime stomatal conductance varied from 0.002 to 0.04 cm s^{-1}. Little or no organic acid fluctuation was measured. In *Codonanthe crassifolia,* in addition to daytime CO_2 uptake, low levels of nocturnal CO_2 uptake (gross) on the order of 0.5 to 1.1 mg CO_2 $dm^{-2}h^{-1}$ was observed (Fig. 1). Stomatal conductance in *Codonanthe crassifolia* was higher in the daytime than during the night, except for a midday depression at noon (Fig. 2). This was accompanied by nighttime acidification and a 60 μeq g^{-1} FW fluctuation of titratable organic acids. *A. pulcher,* sampled under similar environmental conditions, exhibited low organic acid levels and no nocturnal CO_2 uptake (Fig. 3). *Columnea linearis* (Fig. 1) showed similar gas exchange parameters and acid levels to those of *A. pulcher,* though there was a slight acid fluctuation. *S. ionantha* (Fig. 3) showed typical C_3 gas exchange parameters, but twice the organic acid levels noted in a *A. pulcher.*

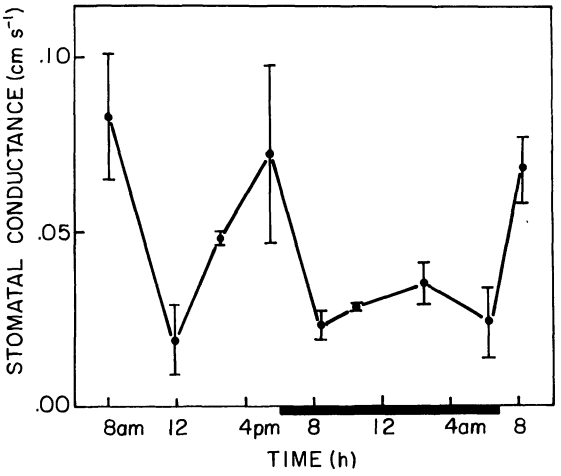

Fig. 2. Diurnal variation of stomatal conductance in *Codonanthe crassifolia.*

Codonanthe crassifolia was compared to *S. ionantha* under water-stressed conditions, since leaves of *S. ionantha* had high organic acid levels, and we suspected that it could shift to CAM. By withholding water for 21 days, exogenous CO_2 uptake in both *S. ionantha* (Fig. 5) and *C. crassifolia* (Fig. 4) was completely stopped. However, *C. crassifolia* still exhibited a 40 μeq g^{-1} FW diurnal fluctuation of organic acids. Control plants of both species maintained C_3 photosynthesis, but *C. crassifolia* did

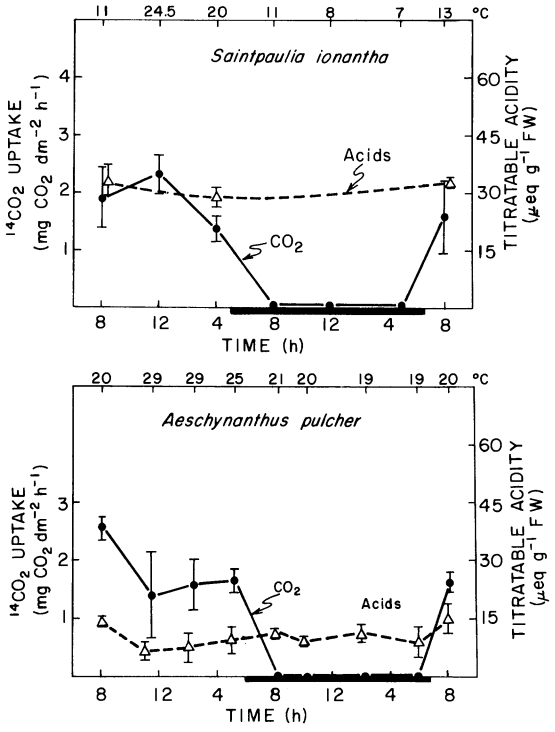

Fig. 3. Diurnal variation of CO_2 uptake (——) and titratable acidity. Top: *Saintpaulia ionantha*, Bottom: *Aeschynanthus pulcher*.

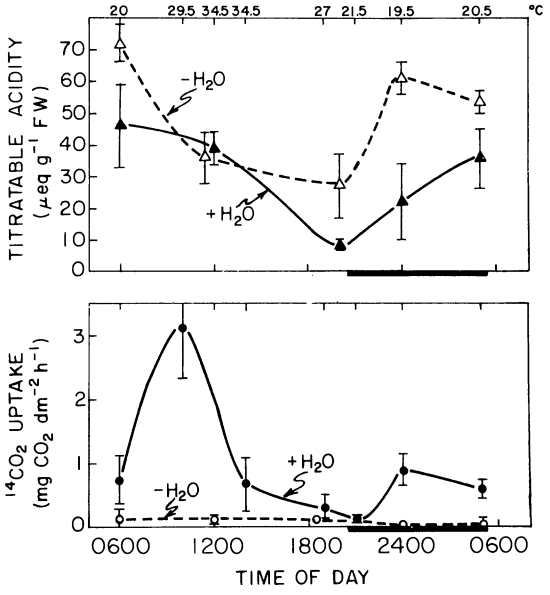

Fig. 4. Diurnal variation of titratable acidity (top) and CO_2 uptake (bottom) in *Codonanthe crassifolia*. Plants were irrigated every third day (——) or water was withheld for 21 days (– – –).

exhibit nocturnal CO_2 uptake between 0.5 to 1.0 mg CO_2 $dm^{-2}h^{-1}$ and an organic acid fluctuation of 35 μeq g^{-1} FW.

Organic acid analysis indicated a fluctuation of malic acid in *Codonanthe crassifolia* from 9.2 mg g^{-1} (68.7 μmol g^{-1} FW) in the morning to 7.2 mg g^{-1} (53.7 μmol g^{-1} FW) in the afternoon (Fig. 6). Though there was no significant difference between morning and evening malic acid levels, the results indicate that the total titratable acid fluctuation was attributable to malic acid. There was a 22.0 μeq g^{-1} FW fluctuation which is not quite large enough to account for the 15.0 μmol malate g^{-1} FW fluctuation if one assumes a $2H^+$:1 malate stoichiometry. The levels of malic acid in the other species were significantly lower than that observed in *C. crassifolia* and either no or slight diurnal fluctuations of malic acid were observed.

Enzyme analysis—The enzymes of the CAM pathway, PEP carboxylase and NADP malic enzyme, were analyzed for activity in all four species. PEP carboxylase activity was highest in *Codonanthe crassifolia* and lowest in *Columnea linearis* (Table 1). NADP malic enzyme activity was highest in *Codonanthe crassifolia* and lower in both *S. ionantha* and *Columnea linearis*. Further enzymatic studies of *Codonanthe crassifolia* showed that the K_m (PEP) for PEP carboxylase and K_m (malate) for NADP malic enzyme were 0.150 mM and 0.810 mM, respectively.

Comparative leaf anatomy—With the exception of *S. ionantha*, all species have a similar leaf anatomy with four tissue layers present–an upper succulent multiple epidermis, a middle palisade parenchyma, a lower succulent spongy parenchyma not unlike CAM tissue, and a lower uniseriate epidermis (Fig. 7, 11, 12, 15). *S. ionantha* (Fig. 11) lacks the upper multiple epidermis. The succulent tissue on the adaxial side of the leaf in the other three species was established as being derived from periclinal divisions in the protoderm layer and, thus, is an example of a true multiple epidermis and not a hypodermis (Fig. 9, 10, 13, 14, 16, 17). The epidermis varied from three to four cells thick in *Columnea linearis* (Fig. 7), and to six cells thick in *Codonanthe crassifolia* (Fig. 15). The internal epidermal cells are enlarged and highly vacuolated, as are the cells of the single adaxial epidermal layer in *S. ionantha* (Fig. 10). The single, lower epidermal layer is similar anatomically to the external epidermal layer of the multiple epidermis in all species (Fig. 7,

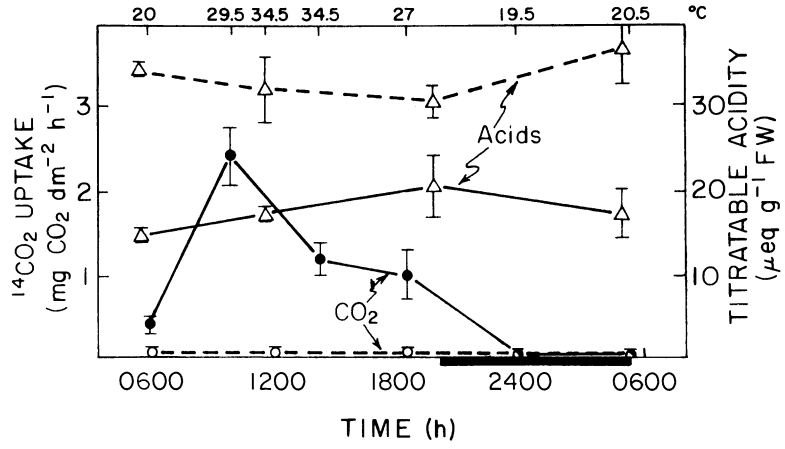

Fig. 5. Diurnal variation of CO_2 uptake and titratable acidity in *Saintpaulia ionantha.* Plants were irrigated every third day (——) or water was withheld for 21 days (– – –).

11, 12, 15). Stomata were found only on the abaxial surface in all species. The palisade parenchyma layer was uniformly one cell thick, except in *A. pulcher,* where the palisade layer graded into the spongy parenchyma (Fig. 12).

The relative percent of leaf volume occupied by airspace in the four species was not significantly different (Table 2). In the mesophyll tissue alone, however, there were differences between the four species in cell shape and amount of intercellular space. *Columnea linearis* contained the largest intercellular airspaces of the spongy parenchyma (Fig. 7, 8), where most cells had the lobed appearance typical of the spongy mesophyll of a mesophytic leaf. The other species were significantly lower in spongy mesophyll airspace with cells more isodiametric in shape than *C. linearis* (Table 2). There were no intercellular spaces in the multiple epidermis.

There was a significant difference in mesophyll succulence (S_m) in whole leaf tissue (Table 3) among the 4 species. *Columnea linearis* had a significantly lower value of S_m, less than 1.0 g H_2O/mg Chl, than all other species. *Codonanthe crassifolia* was the most succulent of all species with a S_m of 3.37 g H_2O/mg Chl.

Discussion—Of the species studied, *Saintpaulia ionantha* and *Aeschynanthus pulcher* are from the Old World. *Saintpaulia ionantha* occurs naturally on shaded limestone cliffs on the coastal plain of Tanzania, Africa, where the climate is characterized by rainfall all year, high humidity, and annual temperatures ranging between 20 and 30 C (Johansson, 1978). *Aeschynanthus pulcher* is an epiphytic subshrub endemic to India, Malaysia, Sri Lanka, and southern China (Moore, 1957). We have no climatological data for *A. pulcher.* The neotropical species, *Codonanthe crassifolia,* is a small epiphytic vine occurring from southern Mexico through Central America, and south to Brazil (Kleinfeldt, 1978). The other neotropical species, *Columnea linearis,* is an epiphytic shrub occurring mostly in Costa Rica and Panama (Skog, 1978). At La Selva, Costa Rica, where both species were collected, the average rainfall is 4,000 mm yr^{-1} with a maximum in July and minimum in February and September. The mean daily temperature is 24 C.

There are no previous reports of the occurrence of CAM in the Gesneriaceae (Szarek and Ting, 1977; Szarek, 1979). We report here that *Codonanthe crassifolia* exhibits many attributes of CAM photosynthesis. Under well-watered conditions, *C. crassifolia* showed a low level of nocturnal CO_2 uptake (gross), diurnal fluctuation of organic acids attributable to malic acid, and daytime CO_2 uptake. This pattern of gas exchange is typical of CAM-cycling species (Sipes and Ting, 1985). CAM-cycling of *C. crassifolia* is further supported by the carbon and deuterium isotope composition values of −24.6‰ and +12.0‰, respectively (as reported by Ting et al., 1985), and these isotope composition values are comparable to other CAM-cycling species (Sternberg et al., 1984).

Codonanthe crassifolia responded to water stress by switching from CAM-cycling to CAM-idling, as noted by an absence of exogenous CO_2 uptake, but a continued diurnal fluctuation of organic acids. Thus, *C. crassifolia* exhibits C_3 photosynthesis, CAM-cycling, and CAM-idling.

In addition to gas exchange parameters, *Co-*

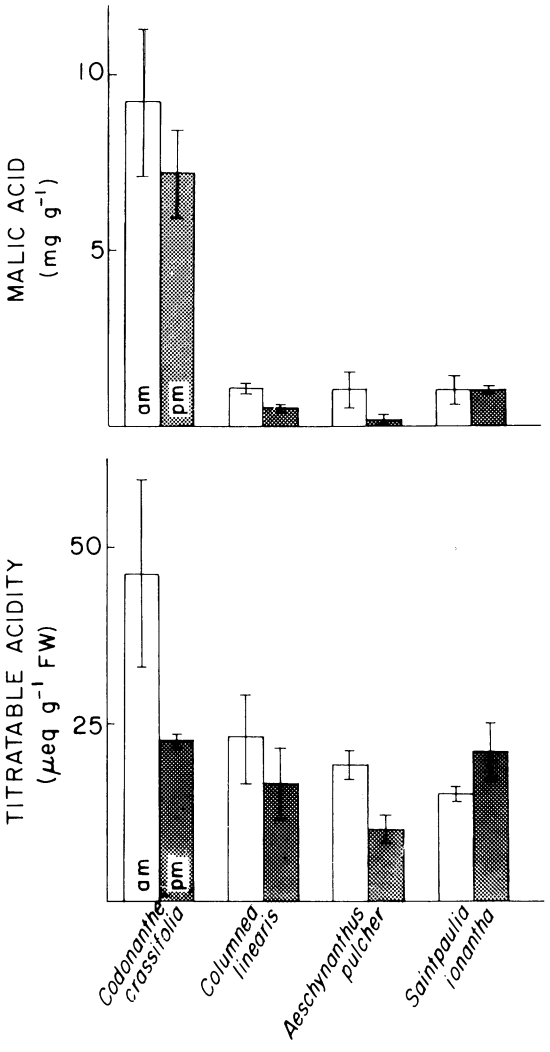

Fig. 6. Diurnal fluctuations of malic acid (top) and total titratable acids (bottom). Samples were taken at 8:00 a.m. (white areas) and at 5:00 p.m. (shaded areas).

donanthe crassifolia has other characteristics of the CAM pathway, including high PEP carboxylase and NADP malic enzyme activities with substrate K_m's typical for CAM species (Kluge and Ting, 1978). The leaf anatomy of this species is CAM-like in its succulence (Gibson, 1982) with a relatively low-level of internal airspace (Warmbrodt, 1984). In addition, the S_m values are within the range reported for CAM species (Kluge and Ting, 1978).

The other species tested all exhibited C_3 photosynthesis. The most C_3-like species is *Columnea linearis.* Although most CAM criteria tested here were negative for *C. linearis,* possibly under conditions not tested, this species may yet exhibit CAM. *Aeschynanthus pulcher* and *S. ionantha* had S_m values in the CAM range (Kluge and Ting, 1978), although they did not show any other physiological CAM characteristics. Enzymic characteristics of PEP carboxylase and NADP-malic enzyme of *A. pulcher* were similar to *Codonanthe crassifolia,* but maximum enzymic velocities were lower. In addition, malic acid levels in both *S. ionantha* and *A. pulcher* were much lower than observed for *C. crassifolia,* and little diurnal fluctuation was observed. .

TABLE 1. *V_{max} of PEP carboxylase and NADP malic enzyme for four species of the Gesneriaceae*

	PEP carboxylase (μmol min⁻¹ mg⁻¹ Chl)	NADP malic enzyme (μmol min⁻¹ mg⁻¹ Chl)
Codonanthe crassifolia	7.46	7.04
Aeschynanthus pulcher	3.39	5.55
Saintpaulia ionantha	2.13	0.90
Columnea linearis	0.66	1.38

The leaf anatomical differences appear related to the carbon metabolism in *Codonanthe crassifolia,* which is CAM-like and *Columnea linearis,* which is C_3. The shape of the cells in *Columnea linearis* results in the spongy mesophyll having a large percent volume of airspace (32%), which is in the range of other C_3 species (20–50%; Byott, 1976). Since the mesophyll tissue represents such a small percent of the total leaf volume (37.2 ± 6.6%), due to the large multiple epidermis the total leaf airspace percentage (9.7 ± 3.2%) is deceptively low. This leaf anatomy of *Columnea linearis* has been noted previously in other *Columnea* spp. by Wiehler (1982). In contrast, the isodiametrically shaped mesophyll cells of *Codonanthe crassifolia* result in close packing and little intercellular space and, thus, the total leaf airspace for *Codonanthe crassifolia* is the lowest of the four species. However, the values for all four species are in the range of typical CAM plants (range 2–11%) (Smith and Heuer, 1981). *A. pulcher* was similar to *Codonanthe crassifolia* in having a closely packed spongy parenchyma, but exhibited a slightly higher percent airspace volume. In earlier work by Herat and Theobold (1979), transections of leaves of other *Aeschynanthus* spp. indicate that they may have a higher airspace volume than has been observed in this study of *A. pulcher.* It is interesting to note that *A. pulcher* has a spongy parenchyma layer anatomically similar to that of *Codonanthe crassifolia,* but a lower mesophyll chlorophyll content.

Although *C. crassifolia, S. ionantha,* and *A. pulcher* are similar anatomically in having a multiple epidermis and closely packed mesophyll tissue, biochemical and physiological dif-

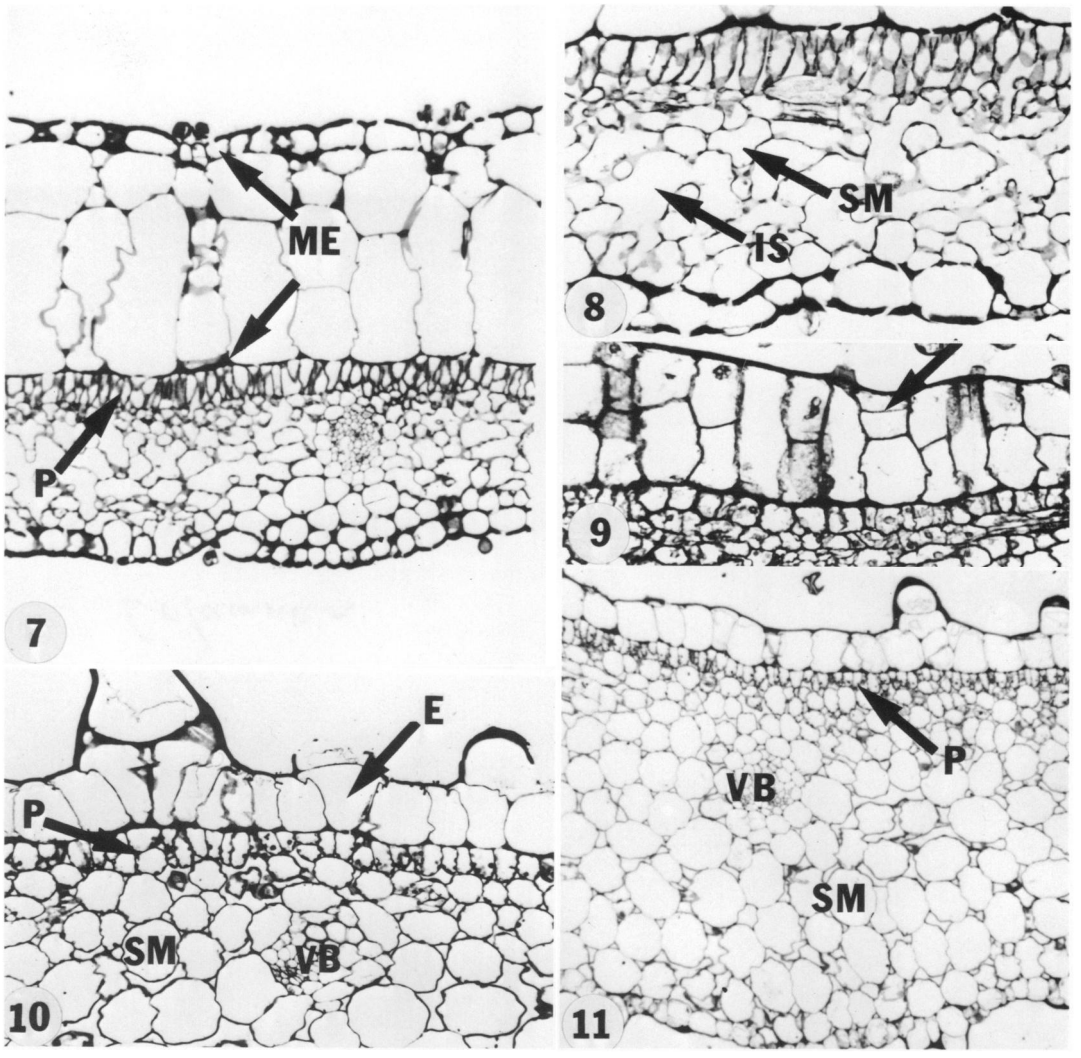

Fig. 7–9. Transections of *Columnea linearis* leaves. **7.** Mature leaf cross section. The arrows delimit the multiple epidermis (ME) above the single chlorophyllous palisade mesophyll layer (P). ×95. **8.** Higher magnification of spongy mesophyll (SM); arrow on lobed cell; note extent of intercellular airspace (IS). ×130. **9.** Transection of leaf primordium showing initiation of multiple epidermis by periclinal divisions in the protoderm (arrow). ×250.

Fig. 10, 11. Sections through mature leaves of *Saintpaulia ionantha.* **10.** Note uniseriate epidermis (E) above single palisade mesophyll layer (P). ×160. **11.** Spongy mesophyll (SM) with vascular bundles (VB). ×100.

ferences attributable to the CAM pathway separate *Codonanthe crassifolia* from *S. ionantha* and *A. pulcher.* Thus, leaf anatomical features such as succulence and relative airspace volume are, in themselves, not sufficient to indicate CAM metabolism. However, the significance of the low intercellular airspaces observed in CAM plants may aid in trapping CO_2 that is decarboxylated from malic acid. During decarboxylation, internal CO_2 levels can exceed 2% (Cockburn, Ting and Sternberg, 1979). In CAM-cycling species where stomata are open during the day, the potential loss of CO_2 may be lowered by a reduction in the amount of intercellular airspace in the mesophyll.

The multiple epidermis in some of the Gesneriaceae described here is also present in *Peperomia* spp. (Piperaceae) (Pfitzer, 1872; Haberlandt, 1914). The term multiple epidermis connotes periclinal divisions in the protoderm, giving rise to a true multiseriate epidermis. The term hypodermis, often used to describe the distinct adaxial layer in *Peperomia,* implies

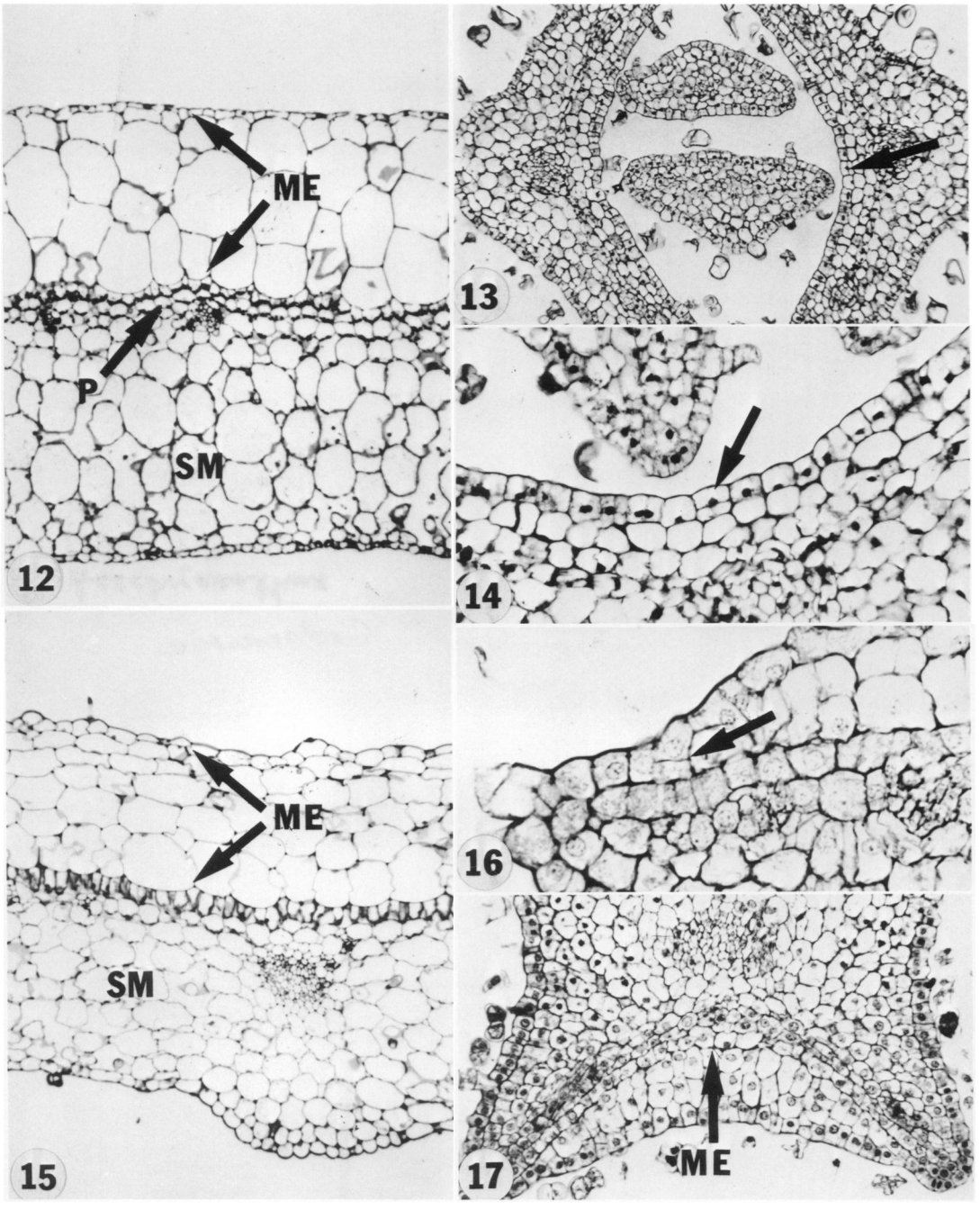

Fig. 12–14. Sections through leaves of *Aeschynanthus pulcher*. **12.** Mature leaf cross section. The arrows delimit the multiple epidermis (ME). SM, spongy mesophyll. ×92. **13.** Cross section of shoot apex showing leaf primordia. Small arrow on youngest leaf showing an intact protoderm with no periclinal divisions observed; larger arrow on site of initiation of multiple epidermis. ×120. **14.** Higher magnification of Fig. 13 showing periclinal divisions in protoderm (arrow). ×200.

Fig. 15–17. Sections through leaves of *Codonanthe crassifolia*. **15.** Mature leaf transection. The arrows delimit the multiple epidermis (ME). SM, spongy mesophyll. ×90. **16.** Cross section of developing leaf blade. Periclinal division (arrow) indicates initiation of multiple epidermis. ×500. **17.** Leaf primordium cross section. Arrow on innermost layer of multiple epidermis (ME). Periclinal divisions are restricted mainly to leaf margins. ×140.

TABLE 2. *Relative percent of leaf volume occupied by airspace in four species of the Gesneriaceae*

	Total leaf airspace[a]	Mesophyll airspace[b]	Area of mesophyll/ total leaf area
Codonanthe crassifolia	8.34 ± 2.7	15.7 ± 3.1	56.4 ± 6.4
Aeschynanthus pulcher	10.0 ± 3.3	17.1 ± 1.2	60.2 ± 9.5
Saintpaulia ionantha	9.1 ± 3.1	11.9 ± 2.3	88.4 ± 3.9
Columnea linearis	9.7 ± 3.2	32.2 ± 2.4	60.2 ± 9.5

[a] Total leaf airspace is the amount of airspace in the multiple epidermis, mesophyll, and lower epidermis.
[b] Mesophyll airspace is the amount of airspace in the mesophyll and the lower epidermis.

origin independent from the protoderm (Linsbauer, 1930) and is inappropriate for either members of the Piperaceae or Gesneriaceae. In addition to the similar leaf anatomy, *Codonanthe crassifolia* exhibits ecophysiological properties similar to those of *Peperomia camptotricha* (Sipes and Ting, 1985) and other *Peperomia* spp. (Ting et al., 1985). These families are distantly related, the Piperaceae being more primitive and the Gesneriaceae relatively advanced (Cronquist, 1981), but among the dicotyledonous plants, these two families have the highest number of epiphytic species (Wiehler, 1982). It is also interesting that both *Codonanthe crassifolia* and some *Peperomia* spp. overlap both in range and epiphytic habit. Thus, *Codonanthe crassifolia* and the *Peperomia* spp. present a unique case of convergent evolution in leaf structure and physiology among tropical epiphytes. The *Peperomia* spp. that have been tested show a range of gas exchange characteristics from C_3 photosynthesis to CAM-cycling to full CAM metabolism (Ting et al., 1985). A more intensive physiological survey is needed to establish the occurrence of CAM among the Gesneriaceae.

A number of criteria have been used in this report to establish whether CAM photosynthesis is present among the gesneriads. In addition to gas exchange parameters, organic acid fluctuations, and carbon isotope composition values, enzyme activity, malic acid flucutations, deuterium isotope composition values, and leaf anatomy are useful as CAM criteria. Further comparative structural studies on CAM species and their C_3 relatives are needed to better establish whether the anatomical features discussed here do indeed relate to photosynthetic metabolism. CAM-cycling is probably common in other tropical epiphytes. Using additional criteria, the species *Guzmania monostachia* (Bromeliaceae), which showed nocturnal CO_2 uptake, but a $-23.7‰$ carbon isotope composition, may be considered a CAM-cycling species (Medina and Troughton, 1974). In addition, CAM-cycling may occur in epiphytic ferns which show slight organic acid fluctuations with daytime stomatal opening (Sinclair, 1984). CAM-cycling may be more prevalent than is presently known at this time. Further research is needed to better understand the phenomenon of CAM-cycling and its relation to the evolution of CAM in tropical epiphytes.

TABLE 3. *Mesophyll succulence of whole leaves and the mesophyll for four species of the Gesneriaceae*

	Whole leaf ($g\ H_2O\ mg^{-1}$ Chl)
Codonanthe crassifolia	3.37 ± 0.69
Aeschynanthus pulcher	2.84 ± 0.33
Saintpaulia ionantha	2.25 ± 0.18
Columnea linearis	0.76 ± 0.04

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