Crassulacean Acid Metabolism in the Strangler *Clusia rosea* Jacq.

Abstract. *Observations of malic acid fluctuation, leaf anatomy, and stable carbon isotopic composition showed that the epiphytic strangler* Clusia rosea, *growing on Saint John, U.S. Virgin Islands, has crassulacean acid metabolism. This hemiepiphyte may be the only woody dicotyledonous tree species among the many thousands of flowering species in the 30 or more plant families that shows this type of metabolism. The finding has implications with respect to water balance during the process whereby* Clusia rosea *establishes itself as a tree, since crassulacean acid metabolism is a photosynthetic adaptation to water-stressed environments.*

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Crassulacean acid metabolism (CAM) is an adaptation of certain plants, such as cacti, to arid and water-stressed environments. CAM is characterized by nocturnal uptake of CO_2 through open stomata, carboxylation of phosphoenolpyruvate to form oxalacetate, and reduction of the latter to malate. The malate is stored at night in large central vacuoles as malic acid. During the next day, the stomata are closed and malate is decarboxylated to produce CO_2 and a three-carbon fragment. This CO_2 is trapped in the tissues and then fixed photosynthetically through the Calvin cycle, which is used by all plants (*1*, *2*). The consequence of this modification of photosynthesis is that the stomata are open when the evaporative demand is low, and hence plants showing CAM lose less water during CO_2 uptake than do other plants (*1*). The criteria used to determine whether or not a plant has CAM include the presence of photosynthetic tissue with large vacuolated cells that have chloroplasts, diurnal fluctuation of acidity attributable largely to malic acid, stomatal opening and gas exchange at night, and a characteristic stable carbon isotopic composition (*1*, *3*).

Many, or perhaps most, tropical holoepiphytes (*4*), including epiphytic orchids, bromeliads, and cacti, show CAM or a modification thereof (*5*, *6*). These holoepiphytes, which are abundant in the tropical flora, reach host plants by wind or animal transport of seeds. After germination, the growing plant receives all its water and inorganic nutrition from rain runoff from the host plant, not from the soil (*7*). The occurrence of CAM in tropical holoepiphytes that experience frequent and periodic drought is thus not unexpected.

The photosynthetic mode used by another group of tropical epiphytes, the hemiepiphytes (*4*), has not been studied to our knowledge. These plants are epiphytic in their seedling stages, but as adults are rooted in the forest floor. The stranglers, such as species of *Ficus* (Moraceae) and *Clusia rosea* (Clusiaceae) (Fig. 1), are examples of hemiepiphytes. These plants produce two types of roots, one that serves to anchor them to a host tree by encircling its trunk and another that grows down to the forest floor and penetrates the soil. Shading by the hemiepiphyte and restriction of the host's secondary growth in girth by the hemiepiphyte's root system eventually kills the host, after which these woody, aerial roots serve as a "trunk" for the strangler. In this manner, *Clusia* replaces its host in the forest (*7*). Here we describe our study of *C. rosea*, which grows in the moist forests on Saint John, U.S. Virgin Islands, as well as in other tropical localities. Our evidence indicates that *C. rosea* shows CAM metabolism. This observation is important because of its implications for the unique life cycle of *Clusia* and because it may be the first observation of CAM in a woody dicotyledonous tree.

Leaf samples were collected morning and evening for studies of diurnal fluctuations in acid levels. Organic acid levels were determined by grinding fresh leaves in water with a hand-held glass homogenizer and titrating to *p*H 7.0 with 0.01*N* KOH. Duplicate leaf samples were placed in ethanol and returned to the laboratory, where they were analyzed

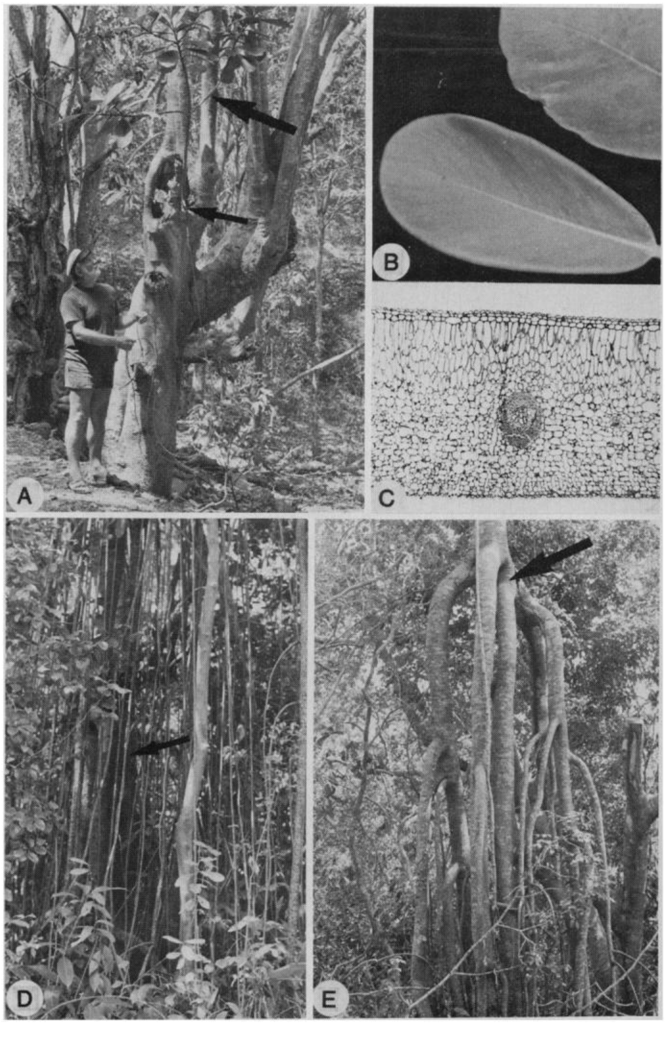

Fig. 1. Epiphytic *C. rosea*. (A) Young plant established on host tree (arrows point to epiphyte shoot), aerial roots not yet established in soil. (B) Mature leaf (×0.3). (C) Leaf cross section (×33). (D) Extensive aerial root system of established epiphyte. (E) Aerial root system of epiphyte after much secondary growth; host plant has died and is no longer evident. Arrow points to transition between root and shoot system.

for malic and citric acids with a Beckman model 330 high-pressure liquid chromatograph and an Ultrasphere octadecylsilane C_{18} column. For studies of photosynthetic structure, a third sample of leaves was fixed in formalin and acetic acid, dehydrated in 2,2-dimethoxypropane, embedded in glycol methacrylate, and sectioned at 4 μm. Sections were stained with toluidine blue and stereological methods were used on a sample of leaf sections (n = 20 from three different leaves) to determine the relative volume of air space in the leaf (*8*). Finally, the carbon isotopic composition of the total organic matter from *Clusia* leaves and from the leaves of other species growing nearby was determined (*9, 10*).

The leaves of *Clusia* are tough and not apparently succulent (Fig. 1B) until their internal structure is revealed (Fig. 1C). The cells are closely packed, with little differentiation into spongy and palisade parenchyma, and have enlarged vacuoles. The intercellular air spaces are restricted to the lower half of the leaf and represent only 9.5 ± 1.9 percent (mean ± standard deviation; n = 20 leaves) of the total leaf volume, a low value compared to that for C_3-type leaves (range, 20 to 50 percent) (*11*), but in the range for other CAM species (2 to 11 percent) (*12, 13*).

Figure 2A shows the diurnal fluctuation in total titratable acidity of *Clusia* seedlings growing on a rock (lithophyte) and on the host tree, *Melicocca bijugata* (Genip), as a true hemiepiphyte. Consistent with most studies of CAM (*1*), older leaves were more CAM-like than younger leaves. Figure 2B shows that malic acid tracks the fluctuation in titratable acidity. As in other CAM plants (*1*), there is a smaller fluctuation in citric acid.

The $\delta^{13}C$ value (*10*) for *Clusia* leaves is dissimilar to those of nearby terrestrial and epiphyte species that utilize the C_3 pathway (Table 1), and in the range observed for CAM plants (*3*). The difference in $\delta^{13}C$ values between *Clusia* and another CAM plant (*Hylocereus trigonus*) that grew near it suggests that *Clusia* is assimilating most, but not all, of its CO_2 through the CAM pathway. It remains to be determined whether *Clusia* is a facultative CAM plant (assimilating CO_2 through the C_3 pathway or CAM pathway depending on environmental conditions) or whether *Clusia* assimilates some of its CO_2 through the CAM pathway and the rest through the C_3 pathway, regardless of environmental conditions.

We were unable to measure gas exchange in *C. rosea* because of a lack of equipment on Saint John, but nocturnal stomatal opening, along with diurnal fluctuation in titratable acidity, have been demonstrated in a related species, *Clusia lundelli*, by Tinoco and Vazquez-Yanes in Veracruz, Mexico (*14*). Although gas exchange measurements would be useful, such studies would not be inconsistent with the measurements reported here demonstrating CAM in *C. rosea*. There are no known examples of plants that show leaf succulence, have diurnal titratable acidity and malic acid fluctuations, and have a carbon isotopic composition such as observed here that are not CAM.

Table 1. Carbon isotopic composition of *Clusia rosea* and of known C_3 and CAM plants growing nearby.

Species	Habit	$\delta^{13}C$ (per mil)
Clusia rosea	Epiphytic	−17.9
*Anthurium cordatum**	Terrestrial	−29.7
*Anthurium selloum**	Terrestrial	−26.6
*Anthurium crenatum**	Terrestrial	−27.7
*Anthurium crenatum**	Epiphytic	−28.9
Hylocereus trigonus†	Epiphytic	−13.3

*C_3. †CAM.

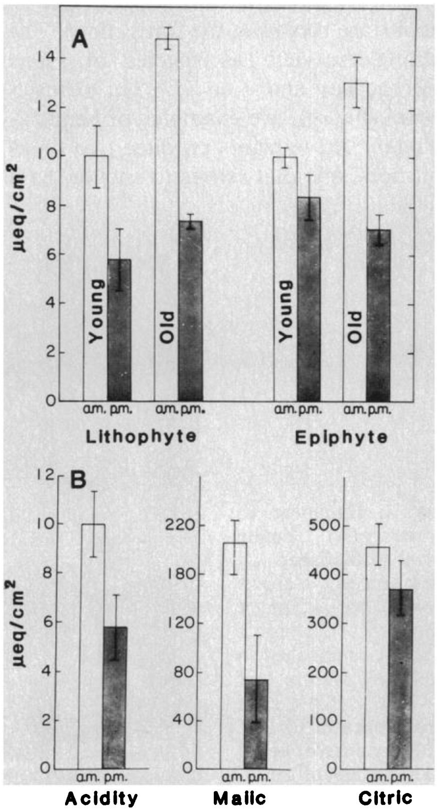

Fig. 2. (A) Early morning and late afternoon total titratable acidity from young and old leaves of *C. rosea* collected from plants growing epiphytically and lithophytically. Samples are averages of three determinations; the error lines represent standard errors. (B) Total titratable acidity as determined in (A), and determination of malic and citric acids from samples collected in the field and stored in ethanol before analysis by high-performance liquid chromatography. Malic and citric acids are expressed as micrograms rather than microequivalents because of uncertainties about the amount of malate present and the number of titratable groups on malic acid in the tissue. Young leaves were sampled from plants growing on a rock wall. Data represent determinations from three samples; the error lines represent standard errors.

Thus, all the data support the conclusion that the stranglers in the genus *Clusia* have CAM. This epiphyte has the same photosynthetic adaptation as many other tropical epiphytes that are subject to the drought and water-stress problems peculiar to their habitat. It is an intriguing possibility that *Clusia* shows CAM only when it is growing epiphytically and is subject to water stress. Finally, we note that *Clusia* may be the only dicot tree among the thousands of flowering plant species spread over 30 or more families that shows CAM.

Note added in proof. In June 1985, a second field session sponsored by the University of California Research Expedition Program was conducted on Saint John with the purpose of studying further aspects of photosynthesis in *C. rosea*. The diurnal patterns of gas exchange in a free-standing tree, a lithophyte, and an epiphyte were measured with a LICOR-6000 Portable Photosynthesis System. The results indicate typical CAM-type gas exchange with nocturnal stomatal conductance and little or no CO_2 uptake during the day for all three forms of *C. rosea*. The gas exchange data indicate that *Clusia* can continue to function as a CAM plant after it becomes a free-standing tree. (This observation may have been influenced by the drought conditions that prevailed when the measurements were made.) The 1985 expedition was led by I. P. T. Hann and J. Hann, with L. Blose, R. E. Bonning, J. C. Broyles, D. W. Stewart, and J. A. Zabilski as participants.

References and Notes

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4. An epiphyte is a plant that starts its life cycle growing nonparasitically on another plant. Epiphytes do not have root systems that extend to the ground. Holoepiphytes are epiphytic during their entire life cycle, while hemiepiphytes are epiphytic for only the first part of their life cycle, being rooted in the soil for the rest.
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10. The carbon isotope ratios are expressed in the δ notation, where

$$\delta^{13}C = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 100 \text{ per mil}$$

where R represents the $^{13}C/^{12}C$ ratio and the standard is the Pee Dee belemnite carbonate. The precision of the determinations of $\delta^{13}C$ values, based on replicate analyses of individual samples, was ±0.2 per mil.
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15. The field research was conducted during a University of California Research Expeditions Program to the U.S. Virgin Islands in July 1984. Participants were M. Becker, J. Brandenberger, L. Johnson, L. Labos-Simpao, S. McMahon, L. Poli, and J. Simmons. We thank the National Park Service, Virgin Islands National Park, Saint Thomas, for allowing us to work in the national park on Saint John. We also thank J. Hann for assistance in the laboratory at Riverside and H. Ajie and D. Winter for assistance in determining the $\delta^{13}C$ values. Supported by NSF grants PCM82-00366 (I.P.T.), DBM84-05003 (M.J.D.), and PCM 8303-619 (E.M.L.).

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A 1500-Year Record of Tropical Precipitation in Ice Cores from the Quelccaya Ice Cap, Peru

Abstract. *Two ice cores, covering 1500 years of climatic information, from the summit (5670 meters) of the tropical Quelccaya ice cap, in the Andes of southern Peru, provide information on general environmental conditions including droughts, volcanic activity, moisture sources, temperature, and glacier net balance. The net balance record reconstructed from these cores reflects major precipitation trends for the southern Andes of Peru. These records indicate extended dry periods between 1720 and 1860, 1250 and 1310, and 570 and 610; wet conditions prevailed between 1500 and 1720. Establishing a tropical precipitation record may help explain climatic fluctuations since the tropical evaporation-precipitation cycle is a principal mechanism driving the atmospheric circulation.*

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Since 1976 a glaciological research program (*1*) has been conducted each summer on the tropical Quelccaya ice cap in the Andes of southern Peru. In 1983 two cores were recovered, one 154.8 m long with 1350 years of climatic information and the other 163.6 m long with 1500 years. Visibly distinct annual dust layers (Fig. 1) made it possible to date these cores accurately. Because the site is too remote and too high for the use of a conventional drill system, a newly designed portable light-weight, solar-powered drill was used (*2*).

A mono-pulse radar unit (*3, 4*), was used to determine ice thickness (Fig. 2). The 163.6-m core (core 1) was drilled to bedrock and the 154.8-m core (summit core) was drilled to some undetermined distance above the bedrock after penetration of an unconformity at 153.7 m (Fig. 2). This unconformity consisted of dust layers inclined 18° from the horizontal and contained elongated air bubbles just above two distinct bubble-free ice layers, one 13 cm thick and the other 8 cm thick. Below the bubble-free ice, the distinct annual dust layers were horizontal with round air bubbles. The unconformity may represent a shear zone between stagnant ice in the depression (Fig. 2) just east of the summit and the upper active ice. Therefore, core 1 is considered the better record before A.D. 1200 because it is continuous from the surface to bedrock. The bedrock is relatively flat at the core 1 site and all the visible dust layers were horizontal from top to bottom.

The temperature profile for the Quelccaya ice cap is unusual. Previous temperature measurements to a depth of 37.5 m suggested that Quelccaya is temperate–that is, the ice is at the pressure melting point (0.0°C). However, from 42.5 to 160 m in the core, between 0.5 and 0.8 cm of new bubble-free ice would freeze at the bottom of the borehole overnight. Both boreholes contained meltwater derived in part from the temperate upper layer of the ice cap and in part from the use of a thermal drill. Thermodynamic calculations indicate that ice temperature between 42.5 and 160 m must be 1° to 3°C below the pressure melting point.

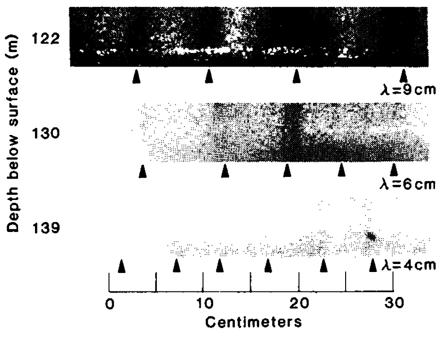

Fig. 1. Three representative core sections show the distinct annual dry season dust layers (triangles) used to date the cores. The average thickness (λ) of the annual layers is shown; annual layer thinning with depth is evident (see Fig. 4).

Three representative core sections (Fig. 1) show the distinct annual dust layers used to date these cores and the thinning of annual layers occurring with depth. Annual-layer thickness (ice equivalent) ranges from 1.2 m at the surface to 0.01 m at the base. The time scale, based solely on visible dust layers, is subject to errors in the upper firn sections, where the core is not transparent and dust layers are more difficult to see. Additionally, annual layers may be missed when the visible dust band is thin and occurs where the core is broken into sections during drilling. Individual core sections were 2 m in length and 8 cm in diameter on average. Selected core sections were analyzed in the field for both liquid and solid conductivity. Six thousand samples were collected for microparticle and oxygen-isotope analyses, and 1500 samples for total beta radioactivity and chemical measurements.

Little is known about the relation between tropical glaciers and long-term variations in tropical climate. Quelccaya (latitude 13°56′S, longitude 70°50′W), in the outer tropics on the western margin of the Amazon Basin, is located in a climatically sensitive region (*5*). The annual mean temperature at this high ice cap (elevation 5670 m) is −3°C, precluding significant melting and percolation. The 55-km^2 ice cap sits on an extensive and relatively flat ignimbrite plateau, with simple radial flow causing little distortion of the ice layers (*1, 6*).

Snow pits and shallow ice cores have been sampled each year since 1976 to determine seasonal variations in microparticles, oxygen isotopes, and beta radioactivity (*1, 7*). Automatic weather stations (RIMCO Mark III) have provided records of temperature, wind speed and direction, and sunshine duration for periods extending from 3 to 8 months each year since 1976. These data, although incomplete, provide a link between the meteorological conditions on Quelccaya