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Photosynthesis in epiphytic and rooted *Clusia rosea* Jacq.

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Summary. *Clusia rosea* Jacq. is a hemiepiphyte having Crassulacean Acid Metabolism (CAM). In its natural habitat *Clusia* begins its life cycle as an epiphyte and eventually becomes a rooted tree. These two stages of the life cycle of *Clusia* represent markedly different water regimes. Our CO_2 exchange, stomatal conductance, titratable acidity, and stable carbon isotope ratio measurements indicate that *Clusia* has a flexible photosynthetic mode, where CO_2 is fixed mostly via CAM during its epiphytic stage, when water availability is low, and via both CAM and C_3 during its rooted stage.

Key words: Photosynthesis – Hemiepiphyte – Crassulacean acid metabolism – Carbon isotope ratios.

Crassulacean Acid Metabolism (CAM) is the photosynthetic metabolic pathway of some plants whereby carbon dioxide is fixed into malic acid during the night and released during the day via a decarboxylation reaction (Kluge and Ting 1978; Osmond 1978; Ting 1985). Coupled with this biochemical phenomenon, CAM plants also have the capacity to open their stomata during the night and close them during the day (Kluge and Ting 1978; Osmond 1978). Carbon dioxide uptake in plants having CAM can be divided into the following four phases throughout a 24 h cycle: phase I, CO_2 uptake and fixation into malic acid during the night; phase II, a large increase of CO_2 uptake during the early part of the light period, possibly through the C_3 pathway; phase III, no net CO_2 uptake during the mid-light period; and phase IV, CO_2 fixation presumably some of it through the C_3 pathway at the end of the light period (Osmond 1978). Because of their stomatal behavior, CAM plants transpire mostly during the cooler and more humid part of the 24 h cycle. CAM plants thus transpire less than C_3 plants resulting in a high efficiency in their utilization of water relative to carbon gained and they are frequently found in more xeric environments such as deserts and microenvironments such as those observed where epiphytes are growing (Kluge and Ting 1978). Some CAM plants also have the remarkable property of shifting their photosynthetic mode towards C_3 during humid periods and to CAM during dry periods, typical of facultative CAM plants (Kluge and Ting 1978; Osmond 1978). A recent report indicates that *Clusia rosea*, a hemiepiphyte with a strangling habit, has CAM (Ting et al. 1985). $\delta^{13}C$ values of leaf organic matter from Clusia growing in St. Thomas (Virgin Islands) were in the range of $-17‰$. An obligate CAM plant (*Hylocereus trigonus*) growing in the same area had a $\delta^{13}C$ value of $-13‰$. This suggests that CO_2 in *Clusia rosea* is fixed partially via the C_3 photosynthetic pathway and that *Clusia* may be a facultative CAM plant (Ting et al. 1985). This hypothesis seems reasonable in light of the contrasting water regime *Clusia* individuals experience during their life cycle. At the beginning of their life cycle, when *Clusia* individuals start as epiphytes, their roots do not have access to abundant water. As they become rooted, however, water may become more available. In this study, we examined the possibility that individuals of *Clusia rosea* are facultative CAM. We measured several photosynthetic parameters of epiphytic and rooted plants both during the dry and wet season in southern Florida where *Clusia* is often horticulturally planted as a tree and escapes as an epiphyte.

Materials and methods

One tree and two epiphytes growing at the Gifford Arboretum (University of Miami, Coral Gables, Florida) were chosen for gas exchange measurements. CO_2 exchange and stomatal conductance were measured with a Li-Cor 6000 Photosynthetic System using a one liter chamber. Measurements were taken from two old (six nodes from the stem apex) and two new leaves (one or two nodes from the stem apex) on April 29 through April 30, 1986 (Dry season), and, for a different set of leaves, from July 11 through July 12, 1986 (wet season). The weather in the first set of measurements (dry season) was clear with an average relative humidity of 72% and an average day temperature of 29° C and night temperature of 24° C. The last significant rain had been on April 13, 1986. The weather during the wet season was partly cloudy with an average relative humidity of 75% and an average day temperature of 31° C and night temperature of 27° C. The last rain had been the previous night.

Leaf tissues from several epiphytes and one tree, shortly after gas exchange measurements were collected during the dry season (April 31, 1986, 8:00 PM and May 1, 1986, 7:00 AM) and during the wet season (July 13, 1986, 8:00 PM, and July 14, 1986, 7:00 AM) at the Gifford Arboretum. Leaf disks were cut with a cork borer, weighed, freeze dried, and at a later date malic acid content deter-

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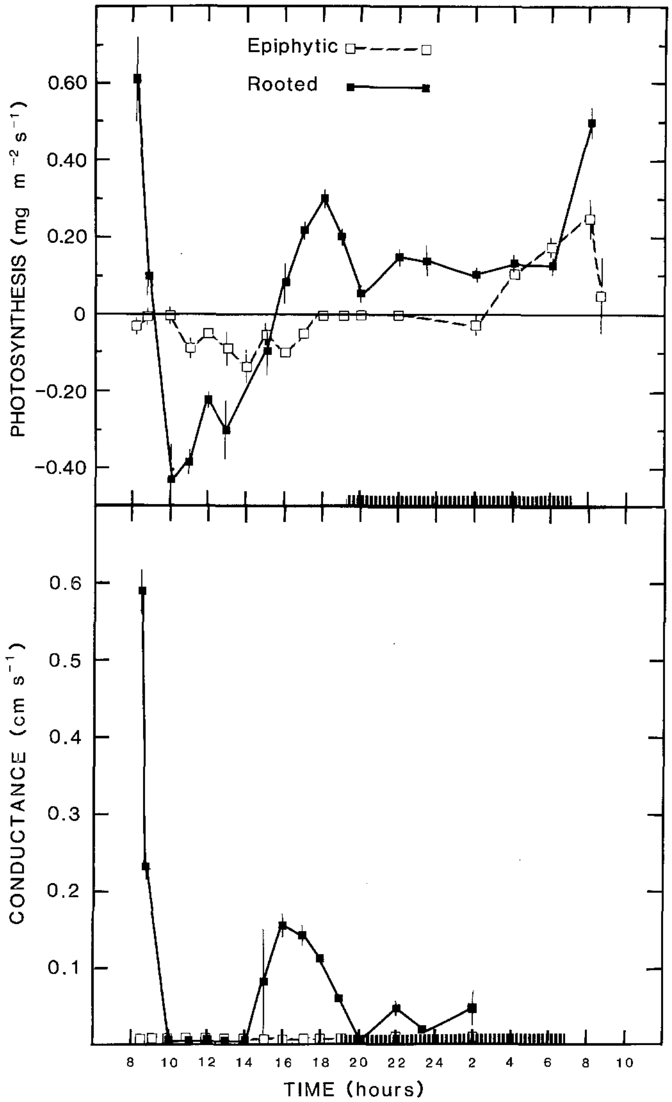

Fig. 1. Carbon dioxide exchange (mg·m^{-2}·s^{-1}) and stomatal conductance (cm·s^{-1}) of leaves from epiphytic (*stipled line*) and rooted (*solid line*) *Clusia rosea* during the dry season in southern Florida. *Squares* represent the means of measurements in two leaves, *bars* represent the range of the measurements. *Striped bar* on the bottom represents the dark period

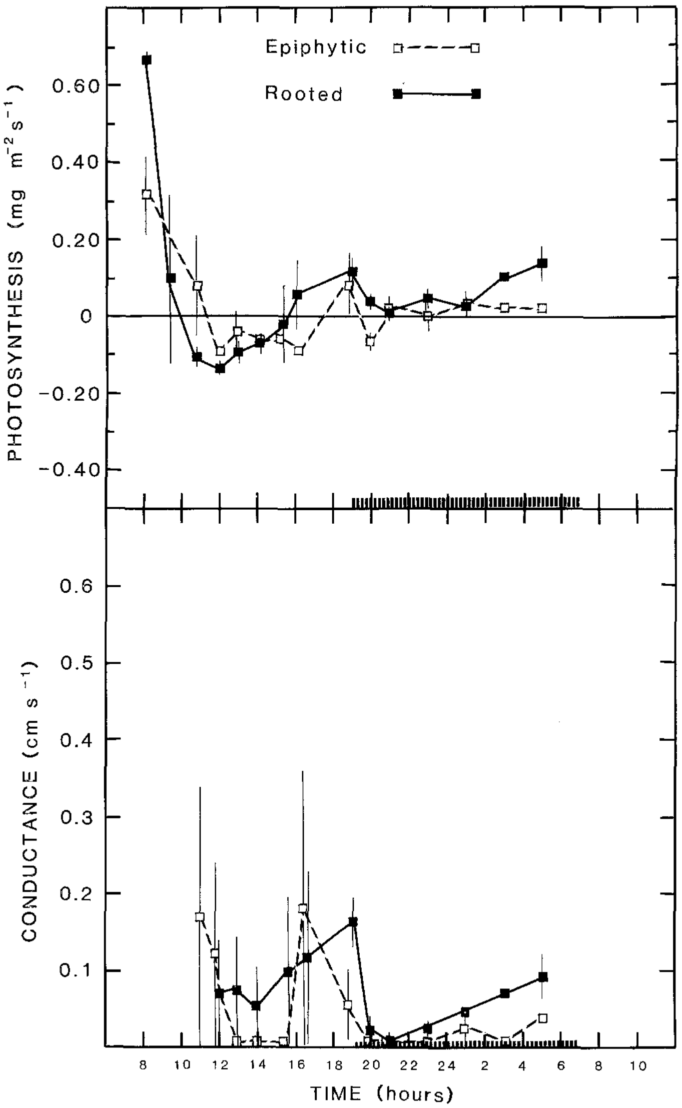

Fig. 2. Carbon dioxide exchange and stomatal conductance of leaves from epiphytic and rooted *Clusia rosea* during the wet season. Symbols are as indicated in Fig. 1

mined enzymatically as previously reported (Williamson and Corkie 1969).

Carbon isotope ratios were measured for two sets of leaf material. The first set was leaf material from leaves used for gas exchange measurement, and the second set, sampled in November, 1986, was leaf material from four trees and six epiphytes growing throughout Miami (Florida). Leaf samples were dried at 50° C, ground to a powder in a Wiley mill and carbon isotope ratios determined as previously reported (Northfelt et al. 1981) and expressed in δ units using PDB carbonate as a standard with a precision of $\pm 0.2‰$.

Results and discussion

Figure 1 shows the diurnal carbon dioxide exchange and stomatal conductance pattern for rooted and epiphytic *Clusia* leaves for the dry season during a 24 hour period. After 2.00 AM stomatal conductance measurements were not reliable due to condensation on the leaf surface and humidity sensor and thus are not reported here. All four phases, or variations thereof, of photosynthesis are represented in measurements of the rooted *Clusia*. Young and old leaves of rooted *Clusia* had a similar pattern throughout a 24 h cycle. Stomatal conductances observed during the day in the rooted *Clusia* comfirm the CO_2 exchange data. Particularly noticeable was the high stomatal conductance during the morning and during the late afternoon period associated with Phase II and IV of carbon dioxide fixation. One unusual characteristic observed in the CO_2 exchange pattern of leaves from the rooted *Clusia* was the large loss of carbon dioxide during the day (in Phase III). This large loss has been previously observed in leaves of *Sempervivum grandifolium* by Andre et al. (1979). With a stomatal conductance in the range of 0.005 cm s^{-1} (when stomata are essentially closed) and the measured CO_2 efflux, one can calculate that

Table 1. Carbon isotope ratios of old and new leaves, photosynthetic phases, and malic acid (μmoles·cm^{-2}) content observed in rooted and epiphytic individuals of *Clusia rosea* during the dry and wet season in southern Florida. Phases II and IV involve CO_2 uptake during the light period and presumably via the C_3 pathway

Growth habit	Season	$\delta^{13}C$ $(^0/_{00})$		Phase				Malic acid		
		Old	New	I	II	III	IV	AM	PM	Δ
Rooted	Dry	−17.0	−17.8	+	+	+	+	13.3±1.9 (10)	3.9±1.6 (8)	9.4
Epiphytic	Dry	−15.4	−14.2	+	−	+	−	12.7±3.8 (7)	1.9±1.1 (8)	10.8
Rooted	Wet	−21.8	−17.7	+	+	+	+	6.1±0.5 (4)	2.2±0.5 (4)	3.9
Epiphytic	Wet	−16.8	−16.6	±	+	+	±	7.1±1.1 (3)	0.7±0.3 (4)	6.4

the intercellular CO_2 concentration was in the range of 5000 ppm, a value similar to those measured for other CAM plants by Cockburn et al. (1982). The loss of carbon dioxide during the day may be typical of epiphytic CAM plants from semitropical and tropical habitats. These plants usually lack the xeromorphic features (such as high cuticular resistance) frequently associated with plants found in temperate deserts (Benzing 1984), thus allowing for the outward diffusion of carbon dioxide. The loss of internal carbon dioxide during phase III may affect the carbon isotope ratios of these plants. $^{12}CO_2$ will preferentially diffuse out of the leaf over $^{13}CO_2$, leaving the internal pool of carbon dioxide enriched in $^{13}CO_2$ (O'Leary 1981). Thus, the overall effect would be to shift the plant carbon isotope ratios towards less negative values.

In contrast to the rooted *Clusia*, epiphytic *Clusia* had only a small amount of carbon dioxide uptake or stomatal conductance throughout the day (Fig. 1). Thus phase II and IV are missing in epiphytic *Clusia rosea* during the dry period. CO_2 uptake in epiphytic *Clusia* starts at about 2:00 AM dropping sharply at sunrise and must be mostly via the CAM pathway.

CO_2 exchange for old and new leaves of rooted *Clusia* during the wet season are similar to that observed during the dry season (Fig. 2). All four photosynthetic phases are represented here. Wet season CO_2 uptake during the late afternoon and night (Phase I and IV), however, was lower than that observed during the dry season. This difference may be due to the inhibitory effects of higher temperatures observed during these measurements (Winter et al. 1986). Much greater variability in stomatal conductance between old and new leaves throughout the day was observed in the wet season measurements. New leaves had an overall higher stomatal conductance than older leaves, and thus a greater amount of carbon dioxide fixation in all CO_2 uptake phases. Leaves of epiphytic *Clusia* in the wet season fixed CO_2 during the early morning hours (phase II), which was not observed during the dry season. In addition, CO_2 was fixed during the late afternoon (at phase IV). As in the rooted individual, much greater variability in stomatal conductance between young and old leaf of epiphytic *Clusia* was also observed during the wet season measurement.

Table 1 shows the results of our malic acid determinations, carbon isotope ratios for leaf samples collected shortly after gas exchange measurements, and summarizes results of the gas exchange measurements. There were clear differences in photosynthesis between epiphytic and rooted *Clusia*, particularly during the dry season. *Clusia rosea* in its rooted form had all four phases typical of CAM during both seasons in southern Florida. This lack of change in photosynthetic behavior between wet and dry season measurements for rooted *Clusia* is expected since the soil will act as a water reservoir and buffer the lower rainfall during the dry season. In contrast, substantial changes occurred between dry and wet season photosynthesis in epiphytic *Clusia*. Most significantly was the presence of phase II and IV during the wet season and the absence of these phases during the dry season. This observation is consistent with previous results from various other plants subjected to drying regimes. Photosynthetic phases IV and II were eliminated in several CAM plants during drought stress (Osmond 1978). Both epiphytic and rooted *Clusia* exhibited an acid flux in the range of 4 to 11 μmole·cm^{-2} which is typical for CAM plants. Early morning malic acid levels and acid flux were greater during the dry season than during the wet season. As previously noticed in the gas exchange measurements, the higher night temperatures during the wet season inhibited CO_2 uptake during the night and consequently the accumulation of malic acid in the leaves. No differences were observed between early morning malic acid content of epiphytic and rooted *Clusia* for each season. Rooted *Clusia*, however, had a higher malic acid content than epiphytic *Clusia* at the end of the day for both seasons. There may be two explanations for this pattern. First, the rooted *Clusia* may not have utilized all of its malic acid during the day, or second, the rooted *Clusia* fixed carbon dioxide during the latter part of the day and may have fixed some via the CAM pathway into malic acid.

It has long been observed that the $\delta^{13}C$ values of total organic matter from CAM plants is an indicator and integrator of the relative proportion of C_3 and CAM operating in the production of plant biomass (Osmond 1978). Plants that are producing biomass solely via the CAM pathway have $\delta^{13}C$ values of about −13‰, those that are operating in the C_3 pathway have $\delta^{13}C$ values close to −27‰, and plants which have both C_3 and CAM have $\delta^{13}C$ values intermediate to those extremes depending on the proportion of C_3 to CAM photosynthesis. Based on our observations of gas exchange summarized in table 1, we would expect that rooted *Clusia* should have $\delta^{13}C$ values approaching those observed for C_3 plants, and epiphytes should have values similar to those observed in obligate CAM plants. Furthermore there should be a shift in biomass $\delta^{13}C$ values towards C_3-like values during the wet season, and to values typical of CAM plants during the dry season. Isotopic measurements of leaf material shortly after gas exchange measurements during both seasons (Table 1) show that indeed epiphytes tend to have $\delta^{13}C$ values closer to those observed for obligate CAM, and that there is a shift towards values typical of C_3 plants during the wet season for both epiphytic and rooted *Clusia*. In addition, $\delta^{13}C$ values of leaf samples from trees collected during November ranged from −20.0‰ to −17.8‰ with an average value of −18.6‰. In contrast, $\delta^{13}C$ values for leaf samples collected from epiphytes at the same time were considerably higher than that of tree samples ranging from −18.0‰ to −14.1‰ with an average value of −16.5‰. These results are consistent with our hypothesis that *Clusia* has a flexible photosynthetic system which produces biomass via CAM when water availability is low, and via CAM and C_3 when water availability is high, and demonstrate the adaptive significance of CAM to the shifting water regime of *Clusia rosea* throughout its life cycle.

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