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Short-Term Regulation of Crassulacean Acid Metabolism Activity in a Tropical Hemiepiphyte, *Clusia uvitana*

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Diel courses of net CO_2 exchange of leaves were studied in *Clusia uvitana* (Clusiaceae), a tropical Crassulacean acid metabolism (CAM) hemiepiphyte, growing in the crown of a 47-m tall kapok tree on Barro Colorado Island, Panama. Measurements on days without precipitation showed that net uptake of atmospheric CO_2 occurred at night, a feature of CAM, as well as in the early morning and late afternoon. During 36 h of almost continuous rainfall, nocturnal net CO_2 uptake was abolished and the diel pattern of net CO_2 exchange became similar to that of a C_3 plant. Exposing well-watered, potted plants of *Clusia* in the laboratory to temperatures and photosynthetic photon flux densities similar to those during the tropical rainstorm also abolished nocturnal net CO_2 uptake. In contrast, *Kalanchoe pinnata* (Crassulaceae), an obligate CAM plant, still showed net CO_2 dark fixation following the same low-light and moderate-temperature conditions, albeit at decreased rates. During these 12-h photoperiods, titratable acidity in *Clusia* increased slightly above its high level measured at the end of the previous dark period, whereas in *Kalanchoe*, the acid content decreased by about 40%. A survey among outer canopy leaves of *Clusia* on Barro Colorado Island showed that leaves that exhibited little or no nocturnal acidification maintained high levels of H^+ at dawn and dusk. Progressively lower levels of H^+ at dusk were accompanied by progressively higher nocturnal increases in H^+. The data suggest that in *C. uvitana* the rapid switching between CAM- and C_3-type carbon fixation that may occur within 24 h in response to environmental changes is controlled by the acidity status of the leaves in the light. Nocturnal CO_2 fixation is enhanced by conditions that decrease the organic acid content during the light period.

Certain species of plants show an intriguing plasticity in the expression of CAM in response to the environment. The best-studied example is the halophilic therophyte *Mesembryanthemum crystallinum* (Aizoaceae), in which nocturnal net CO_2 uptake and organic acid accumulation, followed by organic acid degradation in the light during the next day, can be experimentally induced by high soil salinity (Winter and von Willert, 1972; Winter, 1985; Bohnert et al., 1988; Schmitt, 1990; Winter and Gademann, 1991). In contrast, photosynthetic characteristics of well-watered *M. crystallinum* in the absence of salt stress may be indistinguishable from those of normal C_3 plants.

In some species of tropical *Clusia*, the proportion of carbon gained in the light and dark is also relatively variable, depending on environmental conditions (Lüttge, 1991). Low water availability, a high leaf-air vapor pressure difference, and reduced ambient CO_2 partial pressures during daytime have been shown to augment CAM activity and to increase rates of dark CO_2 fixation (Schmitt et al., 1988; Winter et al., 1992). Conversely, low light, low temperatures, and high ambient CO_2 partial pressures during daytime negatively affect dark CO_2 fixation and may lead to patterns of diel net CO_2 exchange that approximate those of C_3 plants (Haag-Kerwer et al., 1992; Winter et al., 1992). What is particularly interesting about *Clusia* is that some of these apparent shifts between C_3 and CAM occur very rapidly, i.e. within 24 h. The mechanism underlying these rapid changes, which up to now have not been documented under field conditions, is poorly understood.

Here we report on rapid alterations in CAM activity in a species of *Clusia* under natural tropical conditions on Barro Colorado Island, Panama, and on the analysis of these responses in the laboratory. A possible mechanism for the fast up- and down-regulation of CAM activity in *Clusia* is proposed.

MATERIALS AND METHODS

Field Measurements

Investigations were conducted on Barro Colorado Island (9°10′N, 79°51′W), Republic of Panama. The tropical moist forest on this 15-km^2 biological reserve receives about 2600 mm of rainfall annually with a pronounced dry season from late December to April. Detailed descriptions of vegetation, climate, and ecology were reported by Croat (1978) and Leigh et al. (1982).

Measurements of CO_2 and water vapor exchange were performed in situ on fully developed, approximately 4-month-old leaves of a hemiepiphytic tree of *Clusia uvitana* Pittier (Clusiaceae) (=*C. odorata*; Croat, 1978; Hammel, 1986) located in the crown of a 47-m tall kapok tree (*Ceiba pentandra* [L.] Gaertn.). Leaf gas exchange was studied with a CO_2/H_2O porometer (CQP 130; Walz, Effeltrich, Germany) and some additional instrumentation. Leaves were clamped between an aluminum ring and the 16-cm^2 (4.5-cm diameter) opening of a PMK10 gas exchange cuvette (<200-mL volume) (Walz). The leaf itself provided a seal for the cuvette, with the upper leaf surface exposed to ambient conditions, and the lower surface, to which stomates were confined (163 ± 14 mm^{-2}; mean ± SD, n = 8), facing the interior of the gas exchange chamber. The cuvette allowed diffuse light to reach the lower

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leaf surface. All other instruments were kept in two aluminum boxes in the crown of the *Ceiba* tree, no more than 4 m away from the study leaves to minimize the length of the pneumatic system. The gas exchange equipment was used in a continuous open-flow mode. The flow rate of air was 300 to 500 mL min^{-1}. External temperature was automatically tracked inside the leaf cuvette. CO_2 and water vapor exchange were measured with an IRGA operating in the differential mode (Binos 100). A CO_2 analyzer in the absolute mode was used to determine ambient changes in CO_2 partial pressure. Zero checks (ambient gas streaming through both the measuring and reference cells of the differential analyzer) were performed at 1-h (during daytime) and 6-h (during nighttime) intervals. The air was passed through an electronically controlled cold trap set at 2°C before entering the CO_2 analyzers. With a second cold trap (KF18/2; Walz), the dew point of the air entering the leaf chamber was kept below ambient to balance transpirational water loss and to avoid condensation inside the pneumatic system. CO_2 and water vapor exchange were continuously monitored on a dual-channel strip chart recorder. A data logger collected a full data set at 5-min intervals for calculation of gas exchange parameters.

Measurements of leaf water potential and the osmotic pressure of leaf sap were made psychrometrically on leaf discs at 30°C with five thermocouple psychrometers (model C-52) equipped with appropriate electronic circuitry. After leaf water potential was measured, leaf discs were frozen and thawed, which permitted determinations of osmotic pressure. Turgor pressure was estimated from the difference between water potential and osmotic pressure.

Determinations of titratable acidity were made on fully developed 3- to 6-month old, outer canopy leaves in September 1990 and March 1991. Duplicate (3.3 cm^2) samples were taken with a cork borer from one side of the midvein of leaves at dusk and from the other side the following dawn. Samples were frozen in liquid nitrogen and boiled in 60% (v/v) ethanol. Extracts were titrated to pH 7.0 with 20 mM NaOH. A quantum sensor (LI-191SA; Li-Cor, Lincoln, NE) and a LI-1000 data logger were used to measure incident PPFD on the upper leaf surfaces every 5 to 10 min from dawn to dusk, and daily integrals were calculated.

Laboratory Experiments

C. uvitana and *Kalanchoe pinnata* were grown for 12 and 3 months, respectively, in 5-L pots containing Pro-Mix Bx soil (Les Tourbieres Premier LTEE, Quebec, Canada) until plants had reached a height of 30 to 40 cm. Plants were kept outdoors under natural tropical conditions (about 32°C during the day and 25°C at night) on the roof of the Tupper Building (Smithsonian Tropical Research Institute, Panama City). The soil was well watered and flushed with nutrient solution at 2-week intervals. Net CO_2 exchange, transpirational water loss, leaf conductance to water vapor transfer, and intercellular CO_2 partial pressure were determined using an open-flow system that allowed for close control of PPFD, temperature, and CO_2 and water vapor pressures. Recently expanded, attached leaves were enclosed in a GWK-3M Plexiglas chamber (Walz) through which air was passed at a flow rate of 3 L min^{-1}. CO_2 pressures and water vapor pressures of the air entering and leaving the leaf chamber were determined with a Li-Cor 6262 analyzer and a dew point mirror (MTS-MK-1; Walz). For determination of titratable acidity content, duplicate samples were taken at the end of the dark and light periods from leaves enclosed in the GWK-3M chamber.

RESULTS

From January 1991 to January 1992, 57 complete 24-h cycles of net CO_2 exchange were determined in *C. uvitana*. Diel changes in net CO_2 exchange on March 3 and 4, 1991 (Fig.1), were typical of most days during the dry season. Net dark CO_2 fixation contributed significantly to total 24-h carbon gain. Most CO_2 was taken up during the early morning hours in low light. During the midday period, when leaf temperatures exceeded 35°C, CO_2 was lost to the atmosphere, followed by a short period of net CO_2 uptake before dawn. Beginning in the afternoon of March 4, it rained almost continuously for 36 h (173 mm). Such intense precipitation

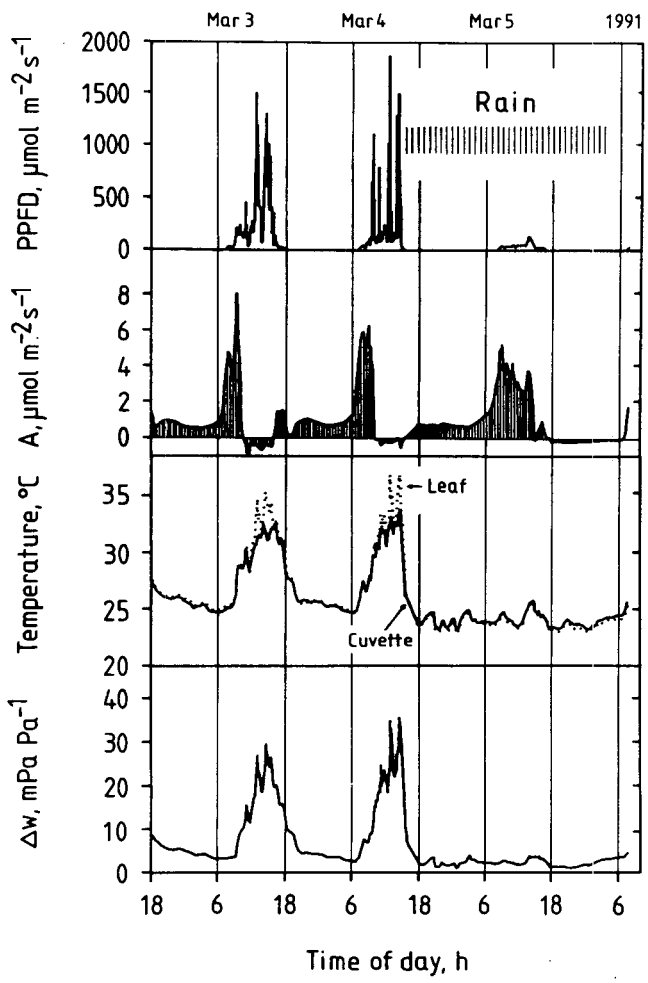

Figure 1. In situ measurements of leaf gas exchange of a hemiepiphytic *C. uvitana* in the crown of a kapok tree on Barro Colorado Island on 3 consecutive days, beginning at 1800 h on March 2, 1991. Shown are diel changes in PPFD, net CO_2 exchange (A), air and leaf temperature, and leaf-air vapor pressure difference (Δw).

is not frequently encountered on Barro Colorado Island during the dry season. Leaf temperatures remained low (25°C) and PPFD barely exceeded 50 μmol m^{-2} s^{-1} between 0600 and 1800 h on March 5. Net CO_2 uptake occurred at relatively high rates throughout the day, whereas nocturnal net CO_2 uptake was abolished, and CO_2 was lost at a low rate to the atmosphere throughout the night. Leaf water potential and turgor pressure did not change during the measuring period from March 3 to March 6. Predawn values ranged between −0.5 and −0.6 MPa (water potential) and between 0.5 and 0.7 MPa (turgor pressure), respectively. By extending its roots downward along the trunk of the host tree and into the ground, *C. uvitana* evidently had ready access to soil water even before onset of the 36-h rainfall.

Under controlled laboratory conditions, nocturnal CO_2 fixation was also negatively affected in well-watered, potted plants of *C. uvitana* exposed to low PPFD and moderate temperatures (Fig. 2), as during the rainstorm (Fig. 1). Following 12-h photoperiods at 320 μmol photons m^{-2} s^{-1} and 30°C (d 1, 3, and 5 in Fig. 2), which was approximately equivalent to the average temperature and light conditions on March 3 and 4 in the field before the rainstorm, rates of nocturnal

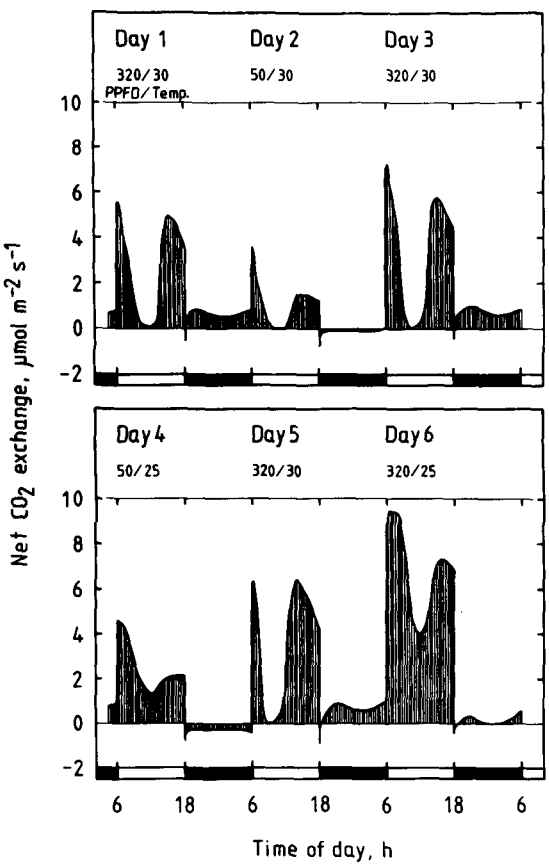

Figure 2. Net CO_2 exchange in *C. uvitana* during 12-h light/12-h dark cycles in the laboratory in response to PPFD (320 or 50 μmol m^{-2} s^{-1}) and temperature (30 or 25°C) during the photoperiods (e.g. 320/30 refers to PPFD/temperature). Temperature during dark periods was always 25°C, and the dew point of the air entering the leaf chamber was 20°C throughout the light/dark cycles.

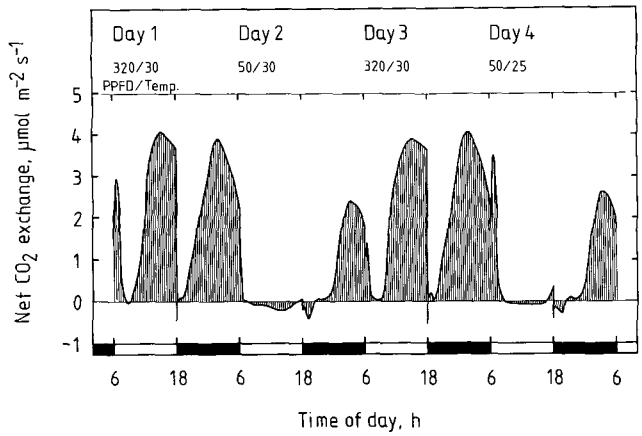

Figure 3. Net CO_2 exchange in *K. pinnata* during 12-h light/12-h dark cycles in the laboratory in response to PPFD and temperature during the photoperiods. Further explanations are given in Figure 2.

CO_2 fixation (measured at 25°C) resembled those under natural conditions. When the temperature was reduced from 30 to 25°C and the PPFD was maintained at 320 μmol m^{-2} s^{-1}, CO_2 uptake in the light markedly increased and rates of net dark CO_2 fixation decreased; yet, the nocturnal carbon balance was still positive (Fig. 2, d 6). Reducing PPFD to 50 μmol m^{-2} s^{-1} while maintaining the temperature at 30°C decreased carbon gain in the light, and the nocturnal carbon balance became negative (Fig. 2, d 2). When temperature and light were reduced together to 50 μmol m^{-2} s^{-1} and 25°C, respectively (i.e. when rainstorm conditions were simulated) (Fig. 2, d 4), nocturnal carbon balance became more negative and CO_2 was lost at an almost constant net rate of 0.4 μmol m^{-2} s^{-1} throughout the dark period. Under these conditions, carbon gain during the 12-h photoperiod was reduced less than if only the light was lowered (and temperature kept at 30°C), and similar to the gas exchange pattern on March 5 during the rainstorm (Fig. 1), the "midday reduction" in net CO_2 uptake was markedly diminished. Thus, the 24-h course of net CO_2 exchange at 50 μmol photons m^{-2} s^{-1} and 25°C (Fig. 2, d 4) closely resembled that of a C_3 plant.

Negative effects of low PPFD and low temperature (e.g. at 15°C day and night) on dark CO_2 fixation were also noticeable in a recent laboratory study on *Clusia minor* (Haag-Kerwer et al., 1992). To evaluate the extent to which these features were unique to C_3-CAM species of *Clusia*, we extended our investigations to *K. pinnata*, an obligate CAM plant with a high capacity for dark CO_2 fixation in fully developed leaves (Fig. 3, d 1 and 3). Decreasing PPFD from 320 to 50 μmol m^{-2} s^{-1} at 30°C abolished net carbon gain in the light and reduced nocturnal carbon gain by 55% (Fig. 3, d 2). When PPFD and temperature were decreased simultaneously to 50 μmol m^{-2} s^{-1} and 25°C, respectively, the effects on carbon gain in the light and dark were similar to those in low light and 30°C, although slightly less pronounced (Fig. 3, d 4).

We noted a distinct difference between *C. uvitana* and *K. pinnata* in the manner titratable acid content changed in response to the low-light/moderate-temperature treatment.

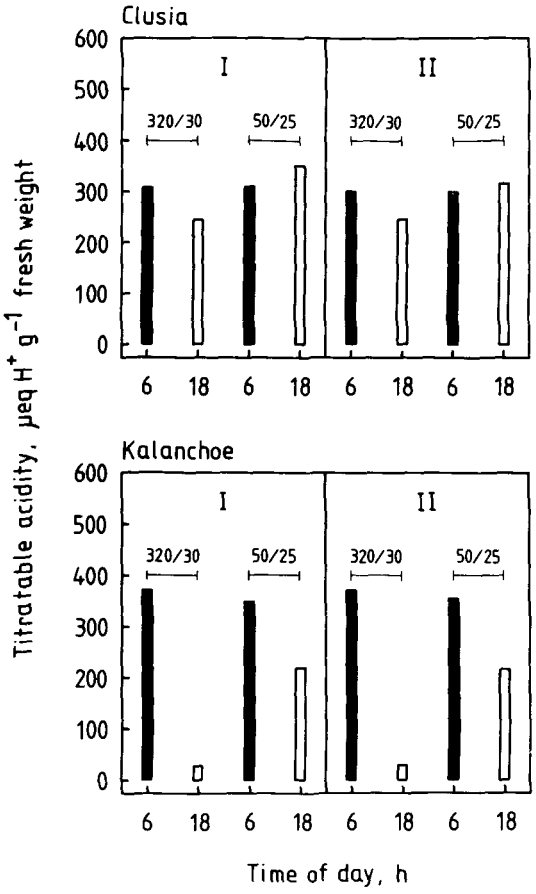

Figure 4. Titratable acidity in leaves of *C. uvitana* and *K. pinnata* at the end of a 12-h dark period (0600 h, filled bars) and at the end of a 12-h light period (1800 h, open bars). PPFD and temperature were 320 μmol m^{-2} s^{-1} and 30°C, respectively, during the first photoperiod and 50 μmol m^{-2} s^{-1} and 25°C during the second photoperiod. Data are shown for two independent experiments (I and II). Experimental leaves were enclosed in a gas exchange chamber, and acidity determinations were with discs taken from the leaves with a cork borer. The leaf punching had no effect on the general gas exchange pattern shown in Figures 2 and 3.

Under standard conditions (12 h light: 320 μmol photons m^{-2} s^{-1} and 30°C/12 h dark: 25°C), *K. pinnata* showed marked acid fluctuations during light/dark cycles, and H^+ content decreased from about 370 to 30 μeq g^{-1} fresh weight in the course of the light period (Fig. 4). Consistent with lower rates of dark CO_2 fixation, fluctuations in acid content were smaller in *C. uvitana;* yet overall H^+ levels remained high at the end of the light period. In *K. pinnata,* the loss of titratable acidity was slowed during 12 h at 50 μmol m^{-2} s^{-1} and 25°C. In *C. uvitana,* net organic acid consumption did not occur under these low-light, moderate-temperature conditions; H^+ content even slightly increased, on average by 18% (=55 ± 47 μeq g^{-1} fresh weight; mean increase ± SD [n = 6]; paired t test, P = 0.035).

The importance of acid levels in the light for the regulation of CAM activity was also suggested in a field survey on Barro Colorado Island, in which day/night changes in H^+ content were studied in sun and shade leaves of *C. uvitana* in response to natural daily light. Figure 5 depicts total acid content in 41 sun leaves at dusk (A) and dawn (B). Some leaves had high H^+ levels throughout day and night (approximately 500 μeq g^{-1} fresh weight) and showed little or no nocturnal acidification. In leaves in which nocturnal acidification was well pronounced, H^+ levels were reduced at dusk, in the most extreme case to 100 μeq g^{-1} fresh weight. The proportion of sun leaves showing marked diel acid fluctuations increased during the dry season (Fig. 5). Overnight acid accumulation was 238 ± 88 μeq H^+ g^{-1} fresh weight (mean ± SD, n = 16) during the dry season (March 1991) and 136 ± 89 (n = 25) μeq H^+ g^{-1} fresh weight during the wet season (September 1990) (t test, $P < 0.01$). Shade leaves were studied during the dry season only and had lower overall acid values than did sun leaves (Fig. 5, C and D). Nocturnal acidification ranged from 0 to 100 μeq g^{-1} fresh weight.

In shade leaves, nocturnal acid accumulation was linearly correlated with daily incident PPFD (Fig. 6A). In their natural

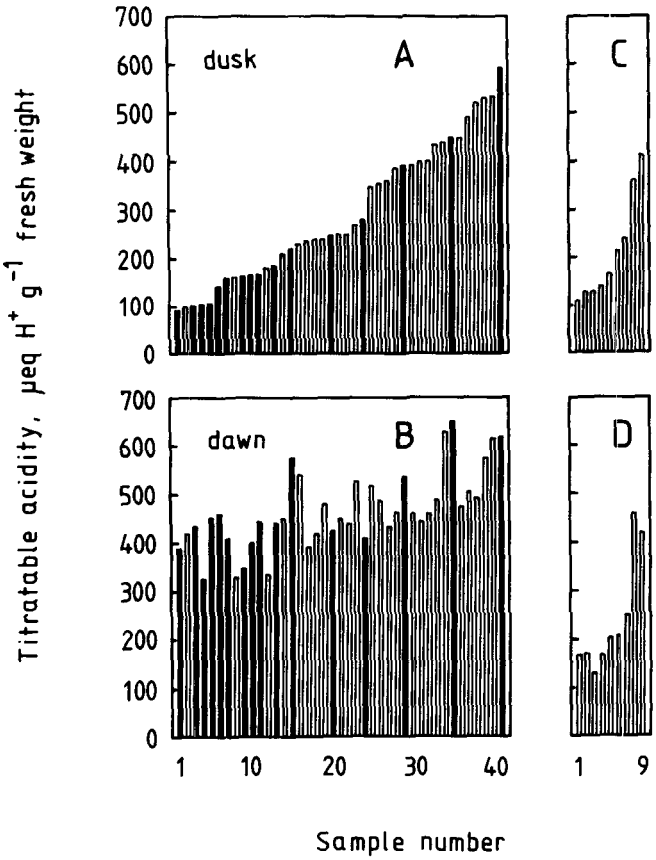

Figure 5. Titratable acidity levels in 41 sun leaves (A and B) and nine shade leaves (C and D) of *C. uvitana* on Barro Colorado Island at dusk (A and C) and corresponding levels at dawn (B and D). Measurements at dusk (A and C) were ranked from left to right according to increasing H^+ content. Open bars, Wet season (September 1990); closed bars, dry season (March 1991). Leaf weight of sun leaves: 791 ± 78 g fresh weight m^{-2}, 177 ± 14 g dry weight m^{-2}; leaf weight of shade leaves: 670 ± 57 g fresh weight m^{-2}, 121 ± 11 g dry weight m^{-2}.

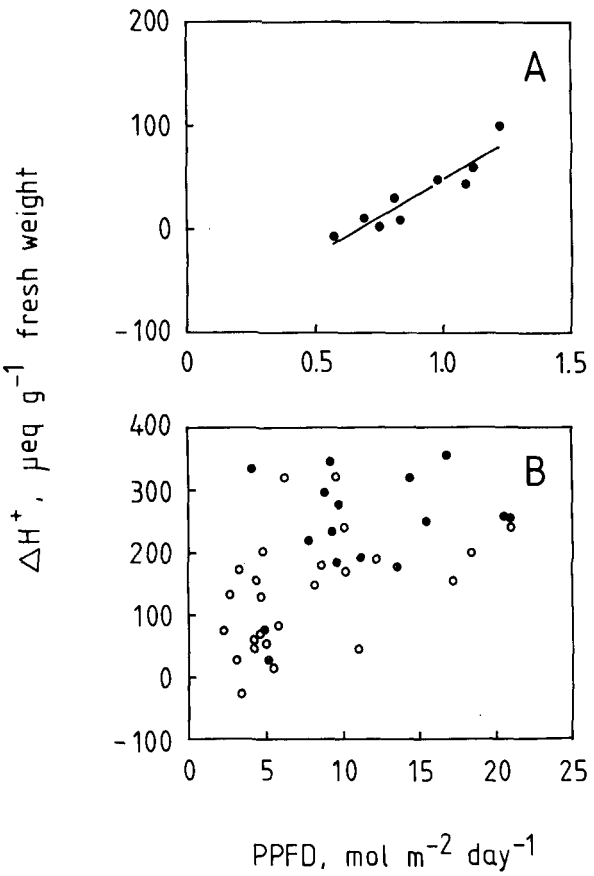

Figure 6. Nocturnal increase in titratable acidity ($\Delta H^+ = H^+$ at dawn minus H^+ at dusk) in response to daily PPFD in shade (A) and sun (B) leaves of *C. uvitana* on Barro Colorado Island. O, Wet season; ●, dry season. A, $R^2 = 0.87$, $P < 0.001$.

environment, these leaves are evidently operating in the light-limited portion of the light-response curve. In sun leaves (Fig. 6B), H^+ accumulation was light saturated at approximately 10 mol photons m^{-2} d^{-1}; yet the relationship between daily PPFD and nocturnal acidification was less consistent than in shade leaves. The light-response curve, in contrast to reports for several species of *Agave* and cacti (Nobel, 1988), was only vaguely hyperbolic, suggesting an additional controlling factor in addition to light. Nocturnal acidification in sun leaves was well described by the H^+ level present at the end of the day (Fig. 7B), i.e. there was a good correlation between the degree of deacidification and the nocturnal accumulation of H^+. No such correlation was observed in shade leaves (Fig. 7A).

DISCUSSION

Similar to other species of *Clusia* (Ball et al., 1991; Franco et al., 1991) and to some species in the genera *Portulacaria, Peperomia,* and *Pereskia* (Ting and Rayder, 1982), leaves of *C. uvitana* that exhibit little CAM activity also contain high organic acid levels both day and night. This is different from the inducible CAM plant *M. crystallinum*. When operating in the C_3 mode, *M. crystallinum* has a low content of organic acids, a feature generally observed in C_3 species. In *C. uvitana*, acidity levels during daytime decrease as CAM develops and nocturnal carbon gain increases (Fig. 5A). The linear relationship between H^+ at dusk and ΔH^+ overnight points to the mechanism underlying the high short-term flexibility in diurnal versus nocturnal CO_2 fixation. The shift from a CAM-type to a C_3-type pattern of diel net CO_2 exchange in response to low PPFDs and moderate temperatures, observed both in the field and in the laboratory, was accompanied by an increase in tissue acidity during daytime. Evidently, decarboxylation of organic acids was inhibited at 50 μmol photons m^{-2} s^{-1} and 25°C, and uptake of atmospheric CO_2 in the light was favored by the reduced leaf-air vapor pressure difference (resulting from the temperature decline from 30 to 25°C). We propose that part of the CO_2 fixation in the light occurred via phospho*enol*pyruvate carboxylase, allowing acid synthesis to continue for significant parts of the photoperiod. This could reduce the capacity to further add organic acids to the high pool of H^+ that already existed at the onset of night, inhibiting dark CO_2 fixation.

Conversely, dark CO_2 fixation in *C. uvitana* is stimulated by water stress, high leaf-air vapor pressure differences, and

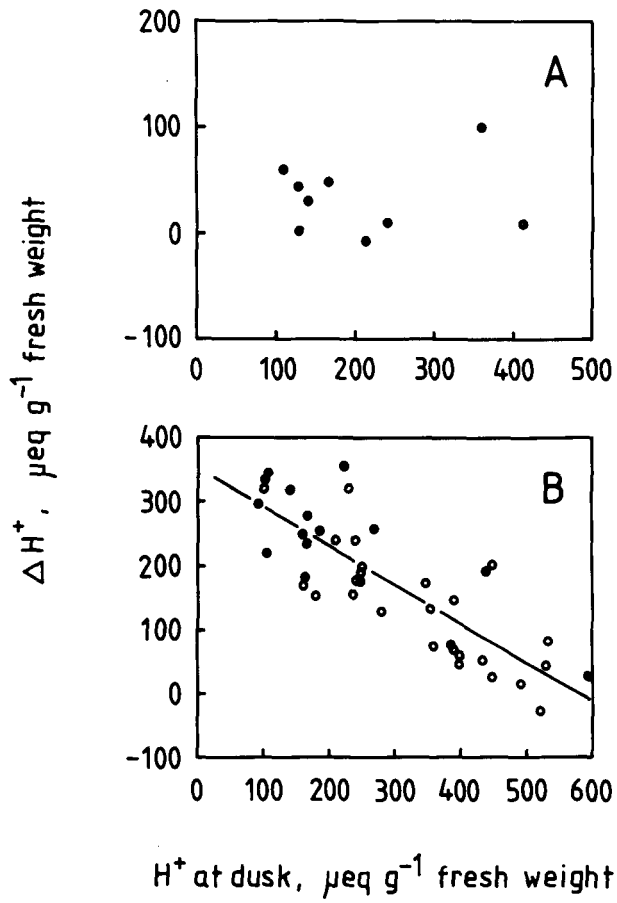

Figure 7. Nocturnal increase in titratable acidity (ΔH^+) as a function of the acid content at dusk in shade (A) and sun (B) leaves of *C. uvitana* on Barro Colorado Island. O, Wet season; ●, dry season. B, $R^2 = 0.68$, $P < 0.001$.

low ambient CO_2 partial pressures during daytime (Winter et al., 1992), but previously, the mechanism was not clear. All these treatments reduce daytime carbon gain by decreasing the availability of atmospheric CO_2 for photosynthesis. Increased use of stored organic acids for photosynthetic CO_2 reduction in the light seems likely, providing more favorable conditions for nocturnal acid accumulation by dark CO_2 fixation. This form of short-term control of CAM activity is based on the concept of a limited vacuolar storage capacity for H^+ and may not require increases in the amount of phospho*enol*pyruvate carboxylase for stimulation of dark CO_2 fixation, but this has not yet been studied. Under prolonged drought, however, increased net synthesis of phospho*enol*pyruvate carboxylase does occur (Winter et al., 1992). In that case (long-term regulation), the molecular processes that lead to the stimulation of CAM in *C. uvitana* may be similar to those during the induction of CAM in *M. crystallinum* in response to high soil salinity (Bohnert et al., 1988; Winter et al., 1992).

Dark CO_2 fixation was also adversely affected in the obligate CAM plant *K. pinnata* by the combined decrease in PPFD and temperature (Fig. 3). In *Kalanchoe daigremontiana* and *Kalanchoe tubiflora*, organic acid degradation is reduced at low PPFD and low temperature, delaying or preventing the onset of phase IV of CAM (Kluge, 1968, 1969, 1971; Osmond, 1978). These earlier experiments have focused almost exclusively on acid and carbohydrate metabolism in the light. Later, quantitative analyses by Nobel (1988) on species of *Agave* and *Opuntia* showed that nocturnal acidification increased in a hyperbolic fashion with increases in integrated PPFD of the previous day. Our results with *K. pinnata* are consistent with these findings. Unlike *K. pinnata*, however, in which CO_2 fixation in the light was almost completely abolished at 50 μmol photons m^{-2} s^{-1} and 25°C, carbon gain in the light was hardly affected in *Clusia*. Furthermore, acid levels did not decrease in *C. uvitana* during the photoperiod at low light/reduced temperature, whereas they did in *K. pinnata*. Factors other than the H^+ status of the leaves in the light such as reduced availability of carbohydrates for nocturnal synthesis of phospho*enol*pyruvate seem more important in explaining the reduction of dark CO_2 fixation in *K. pinnata*.

It has been suggested that stomatal closure during daytime, caused by water stress or ABA treatment, enhances dark CO_2 fixation via decarboxylation of organic acids in the light for *Portulacaria afra* (Ting, 1981; Ting and Rayder, 1982). Experiments in which *P. afra* was kept at different CO_2 partial pressures during daytime (Huerta and Ting, 1988), however, failed to support the idea that the water stress-induced CAM stimulation is related to diurnal reduction in stomatal conductance. By contrast, manipulation of the ambient CO_2 partial pressure strongly influenced nocturnal CO_2 uptake in *C. uvitana* (Winter et al., 1992). A major difference between *C. uvitana* and *P. afra* is that original acid levels are much higher in *C. uvitana*. This may allow for a larger flexibility in *C. uvitana* than in *P. afra* in controlling dark CO_2 fixation by organic acid decarboxylation in the light.

We propose that the rapid changes in the phenotypic expression of CAM in response to short-term changes in the environment, as reported here and elsewhere for members of *Clusia*, are related to the effects of these environmental changes on the H^+ content of the leaves during the light period. Whenever organic acids are maintained at a high level during day and night, C_3 photosynthetic CO_2 uptake in the light is favored. Conditions that lead to increased usage of organic acids for photosynthesis during the day (and which are usually accompanied by reduced uptake of atmospheric CO_2 in the light) will enhance dark CO_2 fixation and will result in a pattern of diel net CO_2 exchange that is increasingly CAM-like. The extent to which this possible mechanism applies to species from other genera, such as *P. afra*, which also maintain high organic acid levels in their leaves when operating in the C_3 mode, warrants renewed consideration. Experiments are underway to study the molecular aspects of the short- and long-term regulation of CAM activity in *C. uvitana*.

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