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THE GENUS *CLUSIA* AS AN EXAMPLE OF STUDIES ON PLANT RESPONSES TO STRESS IN TROPICAL ENVIRONMENTS

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Resumo:

"O gênero *Clusia* como um exemplo de estudos sobre respostas de plantas ao estresse em ambientes tropicais"

O gênero dióico *Clusia* apresenta uma grande plasticidade de formas de vida e flexibilidade metabólica, ressaltada pela presença de espécies C_3, espécies com metabolismo ácido das crassuláceas (CAM) ou com características intermediárias (C_3-CAM). Em algumas destas, induz-se uma mudança reversível de C_3 para CAM, alterando a intensidade luminosa, o regime hídrico ou temperatura. Em *Clusia* ocorre a acumulação noturna de malato e citrato nos vacúolos. Enquanto a acumulação de malato e sua posterior descarboxilação durante o dia favorece a economia de água e carbono, não está esclarecido qual a importância ecofisiológica das variações diárias nos níveis de citrato. Em plantas de sombra, que não mostraram acumulação noturna de malato, a acumulação de citrato pode resultar em economia de carbono e energia. Em plantas expostas a altas intensidades luminosas, a descarboxilação na fase luminosa de malato e citrato aumenta as concentrações internas de CO_2 e pode evitar a fotoinibição, especialmente para plantas expostas ao déficit hídrico, em que o fechamento estomático reduz o fluxo de CO_2 do ar exterior. Esta flexibilidade metabólica, que permite uma resposta rápida a mudanças ambientais, possivelmente contribuiu para que *Clusia* invadisse uma ampla gama de ambientes tropicais.

Abstract:

The genus *Clusia* is characterized by a great diversity of life forms and the presence of C_3, C_3-CAM and CAM species. Moreover, for some species all these patterns of carbon metabolism can be reversibly induced in a single plant, by changing light levels, temperature or water regime. Another physiological characteristic of this genus is the nocturnal accumulation of both malic and citric acids. While the role of day/night changes in malic acid levels is well established as a mechanism to save water and carbon, the ecophysiological importance of day/night changes in the levels of citric acid are still unclear. It was proposed that for low light plants, which show no day/night changes in malic acid levels, the nocturnal accumulation of citric acid functions as an energy and carbon saving mechanism. In plants exposed to full sunlight, the breakdown of citrate and malate in the presence of light increases the internal CO_2 levels and may prevent photoinhibition especially under drought conditions, when stomatal opening is substantially reduced. This metabolic flexibility allows these plants to rapidly respond to changes in environmental conditions and may have contributed to the successful invasion by *Clusia* of a wide range of tropical habitats.

Introduction

Crassulacean acid metabolism (CAM) plants open their stomata predominantly at night and assimilate CO_2 into malate which is then stored in the cell vacuoles. In the daytime, the stomata close, malate moves back from vacuoles to cytoplasm, is decarboxylated and the CO_2 is refixed through the reductive pentose phosphate cycle. Nighttime CO_2 fixation increases water use efficiency (mmol of CO_2/mol of water loss) because of the reduced evaporative demand of the atmosphere. As a result, CAM is generally considered to be a mechanism to save water and to recycle nighttime respiratory CO_2. It is typically associated with succulent plants in arid and semiarid environments, and epiphytic habitats in tropical regions.

The discovery of CAM in tropical trees of the genus *Clusia* (Hartenburg, 1937; Tinoco Ojanguren & Vásquez-Yánez, 1983) raised some interesting questions about its ecological importance in this genus. *Clusia* has a wide distribution in the tropics, and it can be found in the warm Americas, Madagascar and New Caledonia (Willis, 1973). The ecological amplitude of this dioecious genus composed of about 145 species, is also very large. *Clusia* may occur in sandy coastal plains and coastal rock outcrops, in savannas, in gallery forests, in lowland and montane rainforests and in cloud or fog forests (Lüttge, 1991; Haag-Kerwer & Lüttge, 1993). Several species are also found as epiphytes or growing in the tanks of epiphytic and terrestrial bromeliads (Schimper, 1888; Ball *et al.*, 1991a). Termite mounds in asphalt lakes may also be inhabited (Vareschi, 1980). Seedlings of a given species may germinate both terrestrially and epiphytically. Some species, such as *C. uvitana,* a hemiepiphyte occurring in Central America from Nicaragua to Panama (Croat, 1978), can establish itself in trees up to 40 m above the ground (Zotz *et al.*, 1994). Aerial roots from the plant may take several years to reach the forest floor. In the rooted hemiepiphytic stage it can grow to become a large shrub up to 6 m tall and 1 m diameter (Zotz *et al*, 1994).

Clusia is also one of the most flexible genera we know in terms of environmental responses of gas exchange and metabolism. *Clusia* is characterized by the presence of C_3 species, C_3-CAM intermediates and CAM species. In the laboratory, some species such as *C. minor* are able to switch very rapidly from a C_3-pattern to a CAM-pattern of gas exchange in response to changes in environmental factors such as temperature regime, water and light availability (Franco *et al.*, 1991; Haag-Kerwer *et al.*, 1992). There is also good evidence that several species can show this range in CO_2 uptake patterns in the field (Ting *et al.*, 1987; Ball *et al.*, 1991b; Borland *et al.*, 1992; Zotz & Winter, 1994b). This variability in modes of CO_2 uptake within the same genus has also been observed in other taxa comprising predominantly CAM species, such as bromeliads (Griffiths *et al.*, 1986; Lüttge *et al.*,1986); cacti (Nobel & Hartsock, 1986), and agaves (Nobel & McDaniel, 1988). However, none of these taxa seem to have the flexibility of *C. minor*. In addition, the maximum nighttime assimilation rates measured for this CAM intermediate are much higher than the values measured for other C_3-CAM

intermediates, such as leafy members of the Opuntioideae (Nobel & Hartsock, 1986). In this respect possibly *Clusia* is only matched by another tropical genus, namely *Peperomia* (Sipes & Ting, 1985; Ting *et al.*, 1985, Holthe *et al.*, 1987).

The question of the ecological importance of CAM in *Clusia* is even more critical because of the presence of C_3 and CAM species of *Clusia* within the same habitat (Ting *et al.*, 1987; Franco *et al.*, 1994). As an example, in the *restinga* (sandy coastal plains) of Maricá, Rio de Janeiro, *Clusia criuva* (C_3), *C. lanceolata* (C_3-CAM) and *C. fluminensis* (CAM) are found (Franco *et al.*, 1992; Correia *et al.*, 1993). *Clusia hilariana* (CAM) and *Clusia* aff. *parviflora* (C_3) co-occur in the *restinga* of Carapebús, Rio de Janeiro. The co-occurrence of C_3 and CAM *Clusia* within the same habitat offers an unique opportunity to evaluate the ecological limitations of CAM in tropical environments and the importance of CAM for water use efficiency and carbon recycling in different microenvironments.

Diel patterns of stomatal opening and CO_2-exchange

One of the major characteristics of CAM is nocturnal stomatal opening. Figs. 1 and 2 give a detailed set of measurements of stomatal conductance for a C_3 species, *C.* aff. *parviflora* and a CAM species, *C. hilariana*. Both plants were measured in the restinga of Carapebús, Rio de Janeiro.

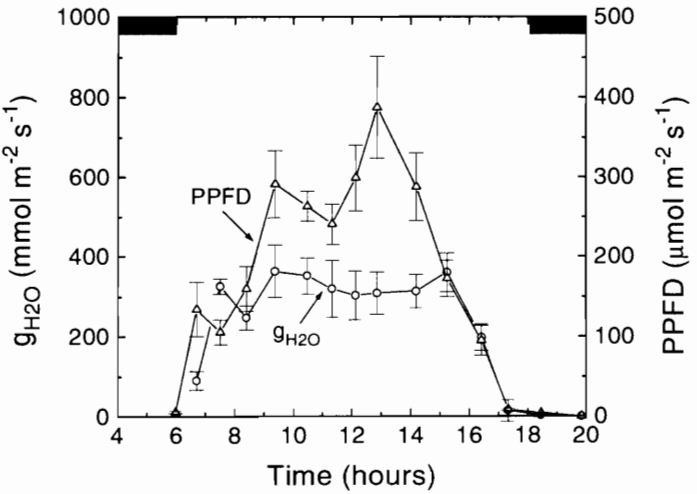

Fig. 1. Diurnal changes in PPFD at the plane of the leaves and leaf conductance to water vapour (g_{H_2O}) for an adult tree of *Clusia* aff. *parviflora* at the restinga of Carapebús, Rio de Janeiro. Data collected on 18 August 1994. Error bars indicate the standard deviation of the means; n = 5 leaves. Dark bars indicate nighttime. A.C. Franco, E.A. Mattos & F.R. Scarano (unpubl. data).

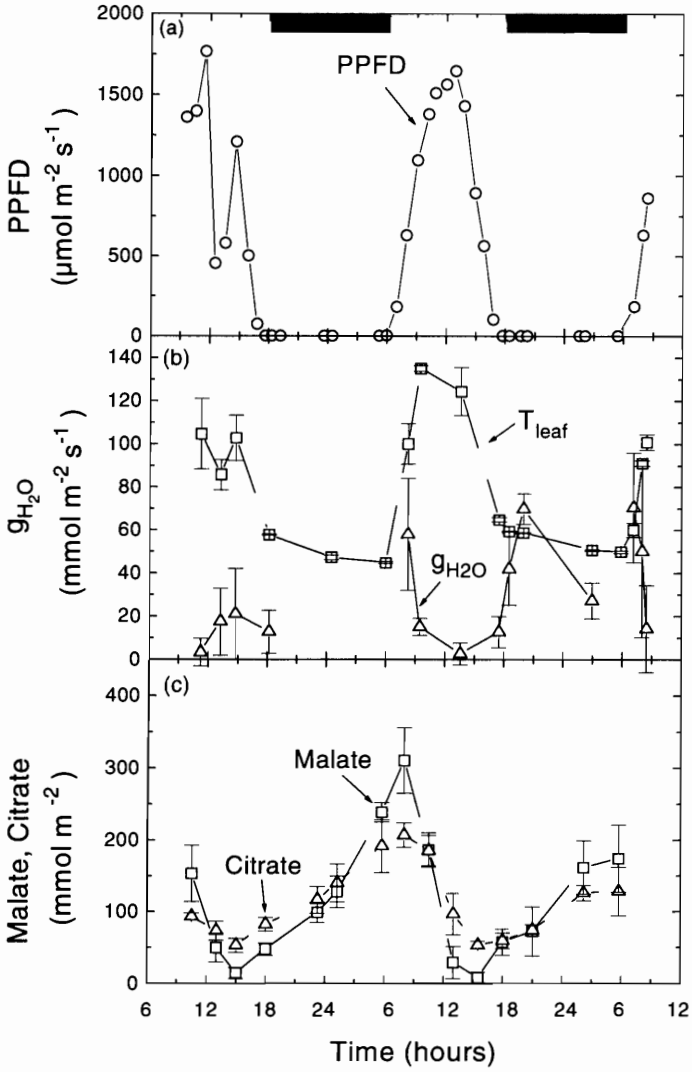

Fig. 2. Daily course of photosynthetic photon flux density (PPFD), leaf conductance, leaf temperature and organic acid levels for an adult tree of *Clusia hilariana* during a 45-hour period (15 August to 17 August 1995). (a) PPFD on a horizontal plane. (b) Leaf temperature (T_{leaf}) and leaf conductance to water vapour (g_{H_2O}). (c) Citrate and malate levels. Error bars indicate the standard deviation of the means; n=4 for gas exchange measurements and n=5 for organic acid determinations. Dark bars indicate the nighttime. Adapted from Franco *et al.* (1996).

As expected, the stomata of *C.* aff. *parviflora* opened only during daylight hours (Fig. 1). The stomata of *C. hilariana* showed a typical CAM pattern , with an early morning peak, midday stomatal closure, and a later afternoon recovery (Fig.2b). Although the stomata of *C. hilariana* opened during nighttime, maximum nighttime leaf conductances were considerably lower than the maximum values reached during the morning peak. *C.* aff. *parviflora* showed no nocturnal accumulation of malate or citrate (data not shown), whereas *C. hilariana* showed considerable nighttime accumulation of both organic acids (Fig. 2c). Under certain environmental conditions some C_3-CAM species such as *C. minor* are capable of maintaining substantial stomatal opening and positive net CO_2 uptake throughout a complete day-night cycle (Franco *et al.*, 1990).

Maximum nighttime conductance (g_{wv}) of the studied species was generally between 10 to 80 mmol m^{-2} s^{-1} (Franco *et al.*, 1990, 1994; Ball *et al.*, 1991b, Zotz & Winter, 1994b). However, Ting *et al.*, (1987) recorded maximum g_{wv} of about 120 mmol m^{-2} s^{-1} for *C. minor* and about 160 mmol m^{-2} s^{-1} for *C. rosea* in the field. Nighttime g_{wv} of 25 to 100 mmol m^{-2} s^{-1} was measured for CAM plants such as agaves and cacti under optimal conditions (Nobel, 1988). Maximum nighttime CO_2 uptake rates of *Clusia* spp. were lower (1-5 µmol m^{-2} s^{-1}; Ting *et al.*, 1987, Franco *et al.*, 1990, 1994, Ball *et al.*, 1991b, Zotz & Winter, 1994b) than the maximum rates measured for cacti and agaves (3-11 µmol m^{-2} s^{-1}; Nobel, 1988) but comparable to rates found in bromeliads (Griffiths, 1988; Borland & Griffiths, 1989; Fetene *et al.*, 1990) or *Kalanchoe* spp. (Medina, 1982; Lüttge, 1987; Ritz & Kluge, 1987).

Nighttime respiration rates (as O_2 evolution) in *Clusia* were high, when compared to rates for C_3 plants (Table 1; for respiration rates in C_3 plants, see Larcher, 1980; Sims & Pearcy, 1989) and most CAM plants (Lüttge & Ball, 1987; Griffiths *et al.*, 1989), but similar to rates measured for the bromeliads *Aechmea nudicaulis* and *A. fendleri* (Griffiths, 1988). Recycling of respiratory CO_2 (nighttime malate accumulation - nighttime CO_2 uptake from the atmosphere) corresponded to 65% to 98% of the nighttime O_2 consumption (Franco *et al.*, 1990). This high degree of respiratory CO_2 recycling suggested that CAM in *Clusia* is an important mechanism to recover the CO_2 released by respiration (Franco *et al.*, 1990).

Table 1. Daytime and nighttime dark respiration rates (µmol m^{-2} s^{-1}) for *Clusia* species. Data expressed as mean ± SD, n=3 leaves. Respiration rates measured at 30°C for daytime rates and 20°C for nighttime rates. Adapted from Franco *et al.* (1990). C_3 means that the plants showed only daytime stomatal opening and there was no nighttime accumulation of malate. C_3-CAM refers to plants that maintained the stomata opened during daytime and nighttime. CAM means that the plants showed nighttime stomatal opening and a morning peak in CO_2-uptake that was followed by stomatal closure.

Species	Day	Night
C. venosa (C_3)	1.0 ± 0.1	0.5 ± 0.1
C. minor (C_3-CAM)	2.4 ± 0.6	0.8 ± 0.1
C. rosea (CAM)	2.2 ± 0.3	1.0 ± 0.2
C. alata (CAM)	4.3 ± 0.6	3.6 ± 0.5

Day/night changes in organic acids, titratable acidity and osmolality

Under controlled conditions several species showed considerable change in the levels of titratable acidity, malate, citrate and osmolality, when exposed to moderate light levels (Table 2). Day/night changes reached values of over 1000 mol m^{-3} for *C. rosea* and *C. minor* (Borland *et al.*, 1992; Franco *et al.*, 1992), the highest values measured for any CAM plant, such as bromeliads (Smith *et al.*, 1986; Griffiths, 1988; Borland & Griffiths 1989), cacti (Nobel, 1988) or agaves (Nobel & McDaniel, 1988; Nobel, 1988). For most CAM plants nocturnal oscillations in titratable acidity are the result of changes in malate levels (Lüttge *et al.*, 1982). However, in *Clusia*, nocturnal variations in titratable acidity are related to variations in the levels of both malate and citrate. Indeed dawn/dusk changes in malate + citrate levels closely matched the changes in titratable acidity, considering that there were two titrated protons per molecule of malic acid and three titrated protons for citric acid (Table 2). Similar results were reported for field conditions (Franco *et al.*, 1994, 1996; see however Ball *et al.*, 1991b).

Table 2. Dawn and dusk levels of titratable protons, malate, citrate and osmolality for the leaf sap of five species of *Clusia*. Plants were well-watered, and PPFD levels ranged from 220 to 300 μmol m^{-2} s^{-1} during a 12-h period. All data (mol m^{-3}) are the mean of three leaves. 2Mal+3Citr is the calculated nighttime accumulation of protons considering that there were two titrated protons per molecule of malic acid and three titrated protons for citric acid (see Lüttge, 1988). Adapted from Franco (1993).

	Malate	Citrate	Titratable protons	2Mal+3Citr	Osmolality
C. venosa					
Dusk	17	63	334	223	457
Dawn	71	82	474	388	419
Dawn-Dusk	54	19	140	165	-38
C. lanceolata					
Dusk	10	212	604	656	510
Dawn	133	294	968	1148	607
Dawn-Dusk	123	82	364	492	97
C. alata					
Dusk	8	67	147	217	337
Dawn	135	135	527	675	431
Dawn-Dusk	127	68	380	458	94
C. minor					
Dusk	22	63	167	233	337
Dawn	174	128	687	735	475
Dawn-Dusk	152	66	520	502	138
C. rosea					
Dusk	22	49	82	191	594
Dawn	336	175	1080	1197	772
Dawn-Dusk	314	126	998	1006	178

These nocturnal variations in the levels of organic acids may affect the osmotic pressure of the vacuolar sap (Table 2). However the magnitude of the influence depends on the precursors for the synthesis of these organic acids. In the case of species such as *K. daigremontiana*, where the synthesis of malate is the result of the breakdown of starch molecules, the relationship between the variations in the levels of osmolality and changes in the levels of malate is approximately 1:1 (Smith & Lüttge 1985). However in *Clusia* the carbon skeletons for the synthesis of both malic and citric acids are derived mostly from the degradation of free sugars (Popp *et al.*, 1987; Ball *et al.*, 1991b; Franco *et al.*, 1994). In this case each hexose can originate two molecules of malate or one molecule of citrate (Lüttge, 1988). There is also some evidence that small day-night oscillations in starch levels occur in several species (Popp *et al.*, 1987; Ball *et al.*, 1991b; Franco *et al.*, 1994). As a result, the nocturnal variations of osmotic pressure in *Clusia* depend not only on variations in the levels of both organic acids but also on the variations of levels of free sugars and starch. Because of nighttime stomatal opening, transpirational water loss may also affect tissue and cell water relations during nighttime, depending on the evaporative demand of the atmosphere and soil water availability.

Organic acids: Synthesis, breakdown and energetics

The transport of organic acids into the vacuoles requires energy supplied by respiratory ATP (Lüttge, 1987; Lütge & Ball, 1987). In *Clusia*, the precursors involved in nocturnal malate and citrate synthesis are mostly derived from the consumption of free sugars. This results in a lower ATP production per unit of respiratory O_2, compared to CAM species where the synthesis of malate is the result of breakdown of starch molecules (Lüttge, 1987; Lüttge & Ball, 1987). The analysis of metabolic pathways and transport energy requirements indicated that the nocturnal accumulation of 1 mol of citric acid (starting with glucose-P as precursor) leads to a net gain of 8 mol ATP (Lüttge 1988). The nighttime synthesis and transport into the vacuole of 1 mol of malate leads to a net loss of 0.5 mol ATP when the synthesis of malate via PEP carboxylase (PEPc) is the result of dark fixation of atmospheric CO_2. Much work has been presented showing that substantial recycling of respiratory CO_2 occurs via malate synthesis in *Clusia* (Franco *et al.*, 1990; 1991; 1992; 1994) as well as in other CAM plants (Griffiths, 1988, 1989). In this case the nocturnal energy balance of carbon recycling via both acids in fact would be identical when the nocturnal synthesis of malate is mainly the result of re-fixation of respiratory CO_2 (see Franco *et al.*, 1992 for an analysis of metabolic pathways of malate and citrate).

Malic and citric acid transport into the vacuoles of CAM cells essentially depends on the proton-eletrochemical-gradient at the tonoplast. The free energy available from ATP hydrolysis in the cytoplasm was estimated to be about -58kJ mol^{-1}, which would allow the transport of $2H^+$/ATP for *Kalanchoë* species (Smith *et al.*, 1982). This corresponded to a vacuolar pH at the end of the dark period of about 3.3 for *K. tubiflora* and 3.5 for *K. daigremontiana* (Lüttge *et al.*, 1981). The curve of malate + citrate levels

for *C. minor* approached a pH of 3.0 and a pH of 2.6 for *C. rosea*. For a nighttime temperature of 20°C the transport of $2H^+$/ATP with 1 $malate^{2-}$ following electrophoretically would still be feasible if we assume a transmembrane electro-potential gradient at the tonoplast of less than + 15 mV and a cytoplasmatic pH of 7.5 as reasonable values (Rona *et al.*, 1980). Thus, it is possible that it is the divalent form of citrate that is being transported. At a cytoplasmatic pH of 7.5, about 10% of the citric acid would still be dissociated to a divalent anion (Lüttge, 1988). Since citrate is a well-known buffer, and *Clusia* spp. show the largest day-night changes in organic acids levels measured in any CAM plant, it is possible that citrate increases the buffer capacity of the vacuoles (Franco *et al.*, 1992). Indeed malate and titratable acidity levels are positively related to citrate levels (Franco *et al.*, 1992). Moreover, *Clusia* species that show the highest nocturnal accumulation of organic acids are also the ones that show the greatest changes in citric acid levels (Table 2; Franco *et al.*, 1992).

It is well-established that the nocturnal formation of malate in CAM occurs as a result of carboxylation of phosphoenolpyruvate (PEP) in the cytoplasm, a reaction catalyzed by the enzyme PEPc. On the other hand, the metabolic pathways involved in the nocturnal synthesis of citrate in *Clusia* are not well-established. In $^{14}CO_2$-pulse/chase experiments with *C. minor*, $^{14}CO_2$-pulse during the last part of the light period and early part of the night period resulted in incorporation of $^{14}CO_2$ almost exclusively into malate (Olivares *et al.*, 1993). During a chase with normal air, the label was subsequently transferred from malate into citrate (Olivares *et al.*, 1993). This suggests that during accumulation of citric acid in the dark period of CAM in *C. minor*, citrate is synthetized in the mitochondria from malate and oxaloacetate after formation of malate in the cytosol and its intermediate storage in the vacuole. Analysis of overnight enrichment in ^{13}C of organic acids (90% of which was citrate) in leaves of *C. minor* during the wet season in Trinidad further supported this route of synthesis of citrate (Borland *et al.*, 1994). The acetyl-CoA required for citrate synthetase could be derived from pyruvate, although it cannot be excluded that it comes from fatty-acid breakdown. These observations also imply that there is considerable export of citrate from the mitochondria during CAM in *C. minor* after uptake of pyruvate, malate and/or oxaloacetate. Studies with isolated spinach leaf mitochondria have shown that export of citrate does occur. In C_3-plants *in vivo* it may mainly serve the formation of 2-oxaloglutarate via cytosolic aconitase and NADP-isocitrate dehydrogenase as required for N-assimilation in the glutamine/glutamate cycle (Hanning & Heldt, 1993).

Most of the degradation of both malic and citric acid occurs during the period of midday stomatal closure characteristic of phase III of CAM (Osmond, 1978). However, Franco *et al.* (1996) reported that in *C. hilariana*, the accumulation of both acids started in phase IV of CAM, before sunset, and it was persistent in the early morning hours (phase II of CAM) suggesting a limited contribution of PEPc activity in the light (Kluge, 1979). On-line carbon isotope discrimination measurements have also indicated CO_2 fixation via PEPc during phases II and IV of CAM for *C. minor* in Trinidad (Borland *et*

al., 1993). Although malate and citrate levels in leaves of *C. rosea* still increased in phase II, there was no detectable accumulation of these organic acids in phase IV of CAM (Ball *et al.*, 1991b; Franco *et al.*, 1994).

The effects of light, temperature and water availability on CO_2-exchange in *Clusia*

Nighttime CO_2 fixation increases water use efficiency (mmol of CO_2/mol of water loss) because of the reduced evaporative demand of the atmosphere. Indeed, water use efficiency of predominatly CAM species is much higher, when compared to the more C_3-like species, both under controlled conditions (Franco *et al.*, 1990; 1992) and in the field (Franco *et al.*, 1994).

In *Clusia*, as in other CAM plants, water shortage resulted in a significant decrease in stomatal opening during the daytime, and thus a reduction in the CO_2-exchange during this period (see for instance Franco *et al.*, 1993). Water shortage induced a CAM pattern of gas exchange in C_3-CAM intermediates and a significant increase in the absorption of atmospheric CO_2 during the night for CAM species (Table 3). As expected, the predominance of nocturnal CO_2 uptake, when water is limiting, resulted in an increase in water use efficiency (Table 3). Controlled water shortage experiments with the C_3-CAM *C. uvitana* have shown that this stimulation of dark CO_2 fixation involved the synthesis of PEP-carboxylase protein (Winter *et al.*, 1992).

In addition to water availability, light and temperature regime also affected the expression of CAM in *C. minor* (Franco *et al.*, 1991, Haag-Kerwer *et al.*, 1992). For plants exposed to low light levels, large differences in day/night-temperatures were necessary to induce a CAM-pattern of gas exchange and nighttime malate accumulation (Table 4). On the other hand, plants exposed to moderate light levels and a constant day/night temperature regime, showed substantial nighttime accumulation of malate, without showing nighttime uptake of atmospheric CO_2 (Haag-Kerwer *et al.*, 1992). In some temperature regimes, plants exposed to low light levels showed considerable increase in titratable protons, due to an increase in the levels of citric acid only (Haag-Kerwer *et al.*, 1992). This was the first time that nocturnal variations in the levels of titratable protons in CAM plants were not due to nighttime accumulation of malic acid. In this case the CO_2 for the formation of malate was probably derived form CO_2 released in nighttime respiration. This induction of C_3 to a CAM pattern of gas-exchange in response to changes in light levels or temperature regime in *C. minor* is reversible (Franco *et al.*, 1991, Haag-Kerwer *et al.*, 1992).

Similar light effects on patterns of CO_2-exchange were reported for *C. rosea* (Franco *et al.*, 1992). In *C. uvitana*, moderate light levels induced a CAM-pattern of gas exchange, at a constant temperature regime of 25°C or 30°C (Zotz & Winter, 1993). Mature leaves of this species in the field performed a CAM-pattern of gas exchange only during the dry season, when light levels were also higher, whereas during the rainy

season low light levels due to clouds and well-watered conditions resulted in a C_3-pattern of gas exchange (Zotz & Winter 1994b). This shift to a C_3-pattern of gas exchange during the wet season was also reported for *C. minor*, growing in the savannas adjacent to Barinas, Venezuela (Ting *et al.*, 1987). Exposed and shaded plants of *C. minor* showed only C_3-activity during the wet season in a seasonal forest on the island of Trinidad, whereas both leaf types showed considerable CAM activity during the dry season (Borland *et al.*, 1993).

Table 3. Influence of water stress on integrated net CO_2 uptake (CO_2, mmol m^{-2}), water vapour loss (H_2O, mol m^{-2}), and water use efficiency (WUE) in five *Clusia* species under simulated field conditions in the laboratory. "Dry" is a period of 6 to 10 days without watering. Day/night temperatures averaged 26/20°C or 25/19°C (*C. lanceolata*). Photosynthetic photon flux density (PPFD) ranged from 250 to 350 µmol m^{-2} s^{-1} during a 12-h period. Adapted from Franco *et al.* (1990; 1992) and Franco (1993).

		Wet			Dry	
Species	CO_2	H_2O	WUE	CO_2	H_2O	WUE
C. venosa						
Day	190.8	27.0	7.1	8.1	0.1	62.3
Night	2.2	0.8	2.8	16.8	0.3	54.2
Total	192.9	27.8	6.9	24.9	0.4	56.6
C. lanceolata						
Day	18.2	4.3	4.2	4.2	1.0	4.3
Night	32.3	2.3	14.1	44.1	2.0	22.2
Total	50.5	6.6	7.6	48.3	3.0	16.3
C. minor						
Day	103.8	16.2	6.4	8.0	0.6	14.4
Night	54.1	1.6	33.8	66.2	1.1	57.7
Total	157.8	17.9	8.8	74.2	1.7	43.6
C. alata						
Day	27.6	2.4	11.5	8.9	1.6	5.6
Night	91.2	3.1	29.4	86.9	2.9	30.0
Total	118.8	5.5	21.6	95.8	4.5	21.1
C. rosea						
Day	42.1	3.1	13.6	-0.5	0.21	-2.4
Night	109.4	4.4	24.9	14.3	0.02	715.0
Total	151.6	7.5	20.2	13.8	0.23	60.0

Table 4. The influence of temperature and light levels on the induction of CAM in *C. minor*. Photosynthetic photon flux density (PPFD) at the plane of the leaves was 30 to 50 μmol m^{-2} s^{-1} or 260 to 300 μmol m^{-2} s^{-1} during a 12-h period. After a new leaf pair developed (after at least four weeks) two plants of each light regime were acclimated for at least one week to a particular temperature regime before gas exchange measurements and samples for organic acid analyses were taken. C_3 means that the plants showed a C_3-pattern of gas exchange and there was no nighttime accumulation of malate. C_3-CAM refers to plants that maintained relatively constant daylight photosynthetic rates and showed nighttime malate accumulation. CAM means that the plants showed nighttime CO_2-uptake from the atmosphere and a morning peak in CO_2-uptake that was followed by stomatal closure. In all cases the daylight temperature was 30°C and leaf-to-air vapour pressure deficit (VPD) was 10 mbar bar^{-1}. Adapted from Haag-Kerwer *et al.* (1992).

Nighttime Temperature (°C)	30 to 50 μmol m^{-2} s^{-1}	260 to 300 μmol m^{-2} s^{-1}
15	CAM	CAM
20	C_3	CAM
25	C_3	CAM
30	C_3	C_3-CAM

For C_3-CAM intermediate species, short term switches between the two modes of photosynthesis are primarily a mechanism to save water and for recycling of respiratory CO_2. Most of the carbon gain occurs as a result of the C_3-pathway (Borland *et al.*, 1992; 1993; Franco *et al.*, 1992; Zotz & Winter, 1994b). Indeed $^{14}CO_2$ pulse-chase experiments with *C. minor* have shown that no labelled compounds are exported from leaves in the CAM-mode during the dark period, while the roots of *C. minor* in the C_3-mode were strong sinks (Olivares *et al.*, 1993). Carbon isotope composition of structural material of *C. minor* leaves did not change significantly between the wet and dry seasons, indicating that most of the carbon used in growth was derived from C_3 carboxylation (Borland *et al.*, 1994). This is clearly not the case for *Clusia* species that are predominantly CAM in the field in which nocturnal CO_2 uptake accounts for 50% to 75% of total CO_2-uptake (Ball *et al.*, 1991b; Franco *et al.*, 1994). However, a comparison of the water use efficiency of *C. uvitana* growing as an epiphyte with two other C_3 epiphytes (*Polypodium crassifolium* and *Catasetum viridiflavum*) has shown that the C_3-CAM intermediate *C. uvitana* has a much higher water use efficiency, although annual carbon gain was similar among these three species (Zotz & Winter, 1994a).

Organic Acids: Photochemical efficiency, potential quantum yields and photoinhibition

Nocturnal fixation of CO_2 in the form of malate can apparently protect photosynthetic tissues of CAM plants adequately from photoinhibition during daylight deacidification when stomata are closed (Adams & Osmond, 1988). The importance of decarboxylation of malate in CAM plants to alleviate the danger of photoinhibition

under high light exposure has been shown for several species of cacti and in the epiphytic bromeliad *Guzmania monostachia* in the field (Adams *et al.*, 1989; Maxwell *et al.*, 1992; Winter & Lesch, 1992). This is particularly expressed in species of *Clusia*, that show large day/night changes in both malic and citric acid levels (Popp *et al.*, 1987; Franco *et al.*, 1990; 1992; Borland *et al.*, 1992), that are unsurpassed by any other CAM plant. It has been suggested that citric acid decarboxylation may be an important mechanism to alleviate photoinhibition (Franco *et al.*, 1992; Haag-Kerwer *et al.*, 1992).

The breakdown of citric acid to pyruvate in the light period releases three molecules of CO_2, while the breakdown of malic acid releases only one CO_2 per pyruvate formed. Thus citric acid should be more effective than malic acid as a mechanism to increase CO_2 concentration and may help prevent photoinhibition. In fact, as the drought developed in laboratory conditions and the stomata remained closed during most of the day, citric acid accumulation remained the same or increased, while malate accumulation substantially decreased for *C. lanceolata*, *C. minor* and *C. rosea* in the laboratory. Malate/citrate ratios also decreased for *C. alata* and *C. rosea* during the dry season (Popp *et al.*, 1987).

Well-watered plants of *C. rosea* and *C. uvitana* under constant moderate light levels (respectively 200 and 450 μmol m^{-2} s^{-1}, 12-h photoperiod) showed the highest values of effective quantum yield of photosystem II ($\Delta F/F'_m$) during phase III, when malic and citric acid decarboxylation probably provide saturating levels of intercellular CO_2 for photosynthesis (Winter *et al.*, 1990; 1992). The increase in $\Delta F/F'_m$ during phase III for plants under constant moderate light levels gradually disappeared for *C. minor* leaves exposed to increasingly higher PPFD (Haag-Kerwer, 1994). In the three species mentioned above, $\Delta F/F'_m$ declined during phase IV of CAM. The magnitude of the decline during phase IV was accentuated at higher light levels and in water-stressed plants (Winter *et al.*, 1992, Haag-Kerwer, 1994).

Field measurements of fluorescence parameters and organic acid levels in *C. hilariana* suggested that high levels of CO_2 fixation via organic acid decarboxylation and of radiationless energy dissipation resulted in only a moderate decrease in photochemical efficiency at the high irradiance levels that are generally observed around midday in tropical sandy coastal plains (Franco *et al.*, 1996). In *C. rosea*, increased rates of radiationless energy dissipation were at least in part, the result of changes in the relative proportion of the components of the xantophyll cycle (Winter *et al.*, 1990). However, the depletion of the organic acid pool in the afternoon exposed *C. hilariana* leaves to the danger of photoinhibition. Values of potential quantum yields (F_v/F_m) of dark adapted leaves declined significantly during phase IV of CAM in partially shaded and exposed *C. hilariana*, when lower internal CO_2 levels probably prevailed (Franco *et al.*, 1996). Therefore, phase IV of CAM is the most prone to photoinhibition, as reported for cacti in northern Venezuela (Adams *et al.*, 1989). For several species of *Clusia* exposed to water stress, the stomata remained closed in the late afternoon so that phase IV CO_2-

uptake was not expressed (Franco *et al.*, 1992), which might enhance the potential for photoinhibition especially in periods of water stress accompanied by high irradiance levels. Diurnal light responses of photosynthetic O_2-exchange for leaves of *C. minor* in Trinidad have indicated a marked reduction in photochemical efficiency during a drought period (Borland *et al.*, 1992).

Nutrient effects

Information on nutrient effects in *Clusia* is limited. Nutrient levels may be particularly limiting for *Clusia* spp. growing epiphytically. Indeed, leaf nitrogen concentrations of *C. rosea* epiphytic seedlings growing in small humus pockets were significantly less (ca. 1.3%) than leaf N concentrations of terrestrial seedlings (ca. 1.7%; Ball *et al.*, 1991a). Terrestrial seedlings also had higher leaf N levels than adult trees or CAM bromeliads, but they were lower than the levels encountered in C_3-Araceae at the same sites (Ball *et al.*, 1991a). Zotz & Winter (1994a) compared gas exchange characteristics and nutrient levels of *C. uvitana* and two other epiphytic species (a C_3 orchid *Catasetum viridiflavum* and the C_3 fern *Polypodium crassifolium*) growing in the crown of a kapok tree on Barro Colorado Island, Panama. Their results showed that annual nitrogen use efficiencies were almost identical in all three epiphytic species (ca. 1.1 g CO_2 mg $N^{-1}yr^{-1}$). However *C. uvitana* had much higher leaf S, Mg, Ca and Na concentrations compared to the two other species. A considerable proportion (20% to 60%) of N, P, K and Mg were recycled in both sun and shade laves, while the content of S did not change and Ca even increased by about 20% in recently shed leaves (Zotz & Winter, 1994b). Relatively high Ca concentrations were also measured in leaves of *C. rosea* collected at several sites on St John Island, Lesser Antilles (Ball *et al.*, 1991a). In two field sites in Northern Venezuela the C_3 *C. multiflora* had the lowest N and P levels, when compared to four other *Clusia* species that showed CAM activity (Franco *et al.*, 1994).

In a laboratory study, Franco *et al.* (1991) were able to show that light levels and N nutrition interacted in their effect on leaf structure of *C. minor* leaves. In N deficient conditions, total daily net CO_2 uptake and total leaf area were slightly less for high-light-grown plants. However, N deficient plants exposed to high light levels had smaller leaves with a very high specific leaf dry weight. In contrast, high-light-grown plants supplied with N showed about a 4-fold higher total daily CO_2 uptake and about twice the total leaf area of low-light-grown plants. N deficiency did not stimulate CAM activity. More detailed studies are necessary to determine the effects of nutrients on net CO_2 uptake and productivity of *Clusia* species in natural conditions.

Conclusions

Although the ecophysiological significance of CAM in *Clusia* is quite clear (Table 5), we still need to better evaluate the ecological limitations of CAM in tropical environments. The following two examples illustrate this point.

Table 5. The possible role of malic and citric acid accumulation during the CAM cycle in *Clusia*. Modified from Franco *et al.* (1992).

	Δmal	Δcitr
Precursor	PEP + CO_2	Malate + Acetyl-CoA?
CO_2 acquisition	yes	no
Increase in water use efficiency	yes	probably not
Recycling (daytime)	CO_2	CO_2
Recycling (nighttime)	CO_2	C-skeleton
Prevention of photoinhibition	yes	yes (more effectively)
Generation of reducing power	not always (depends on CO_2 source)	yes
Osmotic changes	yes	limited
Buffer capacity	low	high

Sun and shade plants of *C. hilariana* in the restinga of Carapebús, Rio de Janeiro, showed a typical diel CAM-pattern of stomatal opening and of changes in organic acid levels. Values of carbon isotope ratio for both exposed and shaded plants were also typical of obligatory CAM plants (Franco *et al.*, 1996). The high levels of organic acid accumulation even in the shade and the capacity to tolerate high light levels suggest that plants of *C. hilariana* are capable of growing successfully under a fairly large range of irradiance levels in the field. This is particularly important for the young seedlings which already showed a distinct capacity for nocturnal acid accumulation and where recovery from irradiance elicited reduction of photosynthetic efficiency, *i.e.* from reduced F_v/F_m indicating photoinhibition, was even more rapid than in the adult plants. This enables seedlings to become established under variable light conditions and it may explain the dominance of *C. hilariana* in the sandy coastal areas of Northern Rio de Janeiro state. However in wetter areas, this species co-occurs with the C_3 *C.* aff. *parviflora*, which is also commonly found in coastal rock outcrops of Rio de Janeiro state (Mattos *et al.*, 1997).

In contrast, both *C. multiflora* and *C. minor* are common in the first stages of forest succession on mountain slopes of Venezuela (E. Medina, pers. comm.). Although their altitudinal range overlaps somewhat, C_3 *C. multiflora* is found in cloud forests and subparamo altitudes of up to 2200 m, whereas C_3-CAM *C. minor* is found at lower altitudes (Steyermark & Huber, 1978). Indeed CAM species of *Clusia* were not reported above 1500 m in Venezuela (Diaz *et al.*, 1996). In a site where both species were found, *C. multiflora* apparently performed well in relatively exposed sites, while *C. minor* seemed to perform better in partially shaded habitats (Franco *et al.*, 1994), even though it apparently colonizes relatively dry sites successfully (Steyermark & Huber, 1978). Maximal net CO_2 uptake rates of *C. multiflora* were similar to values reported for the tropical C_3 pioneer trees cited in the review of Medina (1986). Water use efficiency was definitely higher for *C. minor*, but *C. multiflora* had higher daily CO_2 gain and lower leaf P and N levels.

In conclusion, the study of water and carbon economy coupled with biochemical analyses certainly provided extremely important insights in the physiological adaptations of *Clusia* to the environment (Table 5). It is also quite clear that the plasticity in their physiological responses to the environment has contributed to the successful invasion of *Clusia* in most neotropical environments. However, they are not the sole mechanisms or adaptations that may allow this genus to successfully grow in tropical environments.

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