Journal of Experimental Botany Advance Access published April 24, 2008

Journal of Experimental Botany, Page 1 of 12
doi:10.1093/jxb/ern080

Journal of Experimental Botany
www.jxb.oxfordjournals.org

SPECIAL ISSUE RESEARCH PAPER

On the nature of facultative and constitutive CAM: environmental and developmental control of CAM expression during early growth of *Clusia*, *Kalanchoë*, and *Opuntia*

Klaus Winter[1,*], Milton Garcia[1] and Joseph A. M. Holtum[2]

[1] *Smithsonian Tropical Research Institute, PO Box 0843-03092, Balboa, Ancon, Republic of Panama*

[2] *School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia*

Received 29 January 2008; Revised 21 February 2008; Accepted 26 February 2008

Abstract

The capacity to induce crassulacean acid metabolism developmentally (constitutive CAM) and to up-regulate CAM expression in response to drought stress (facultative CAM) was studied in whole shoots of seven species by measuring net CO_2 gas exchange for up to 120 day–night cycles during early growth. In *Clusia rosea*, CAM was largely induced developmentally. Well-watered seedlings began their life cycle as C_3 plants and developed net dark CO_2 fixation indicative of CAM after the initiation of the fourth leaf pair following the cotyledons. Thereafter, CAM activity increased progressively and drought stress led to only small additional, reversible increases in dark CO_2 fixation. In contrast, CAM expression was overwhelmingly under environmental control in seedlings and mature plants of *Clusia pratensis*. C_3-type CO_2 exchange was maintained under well-watered conditions, but upon drought stress, CO_2 exchange shifted, in a fully reversible manner, to a CAM-type pattern. *Clusia minor* showed CO_2 exchange reponses intermediate to those of *C. rosea* and *C. pratensis*. *Clusia cretosa* operated in the C_3 mode at all times. Notably, reversible stress-induced increases of dark CO_2 fixation were also observed during the developmental progression to pronounced CAM in young *Kalanchoë daigremontiana* and *Kalanchoë pinnata*, two species considered constitutive CAM species. Drought-induced up-regulation of CAM was even detected in young cladodes of a cactus, *Opuntia ficus-indica*, an archetypal constitutive CAM species. Evidently, the defining characteristics of constitutive and facultative CAM are shared, to variable degrees, by all species.

Key words: Carbon dioxide uptake, *Clusia*, constitutive CAM, crassulacean acid metabolism, development, drought stress, environment, facultative CAM, *Kalanchoë*, *Opuntia*.

Introduction

Crassulacean acid metabolism (CAM) is a photosynthetic adaptation to environmental stress that is exhibited in a large number of vascular plants, from at least 343 genera and 34 families, which typically occupy periodically dry habitats in the tropics and subtropics (Smith and Winter, 1996; Holtum and Winter, 1999; Winter and Holtum, 2002; Crayn *et al*., 2004; Silvera *et al*., 2005; Holtum *et al*., 2007). Compared with C_3 and C_4 photosynthesis (Cernusak *et al*., 2007*a*, *b*), CAM photosynthesis significantly reduces the water cost of CO_2 gain by allowing net CO_2 uptake to take place at night when the driving forces for water loss through transpiration are low (Winter *et al*., 2005). The nocturnally fixed carbon is stored as malic acid, which, during the subsequent day, serves as a CO_2 reservoir for C_3 photosynthesis in the light (Winter and Smith, 1996; Holtum *et al*., 2005). The photosynthetic use of CO_2 generated within chloroplast-containing tissues is associated with stomatal closure, minimizing water loss during those parts of the day–night cycle when the driving forces for water loss are strongest.

Much of our early understanding of the metabolic control and functional significance of CAM was derived

* To whom correspondence should be addressed. E-mail: winterk@si.edu

from studies with species, many from the Crassulaceae (e.g. *Kalanchoë daigremontiana*) and Cactaceae (e.g. *Opuntia basilaris* and *Opuntia ficus-indica*), in which CAM is considered to be always expressed in mature photosynthetic tissues (synonyms: constitutive or obligate CAM species; Kluge and Ting, 1978; Osmond, 1978, 2007; Nobel, 1988; Winter and Smith, 1996). A second group of species has subsequently provided insight into how the mechanisms responsible for CAM expression have been fine-tuned by ecological selection to respond to seasonal and shorter term environmental changes. In this latter group, CAM is an option rather than a pre-set mode of carbon assimilation. Over the past three decades, research on these photosynthetically flexible C_3–CAM species (synonyms: C_3/CAM intermediates, inducible CAM species, facultative CAM species) has increased to such an extent that there is now a risk that the ability to choose between the C_3 and CAM option of photosynthetic carbon gain is erroneously perceived as the rule and not as the exception among CAM plants (e.g. Schulze *et al*., 2005).

Well-documented examples of plants in which the expression of CAM is highly flexible include the annual halophytic *Mesembryanthemum crystallinum* (Winter and von Willert, 1972; Winter and Holtum, 2007) and other species in the Aizoaceae (Winter, 1973; Treichel, 1975), species in the Portulacaceae (e.g. *Calandrinia* spp., Mooney *et al*., 1974; Winter *et al*., 1981; other genera, Martin and Zee, 1983; Herrera *et al*., 1991; Guralnick *et al*., 2008) and Crassulaceae (e.g. *Sedum* spp., Kluge, 1977; Borland, 1996; Smirnoff, 1996), and several long-lived woody species of *Clusia* (Borland *et al*., 1992, 1998; Zotz and Winter, 1993, 1994*a*, *b*; Lüttge, 1999, 2006, 2007, 2008; Gehrig *et al*., 2003; Holtum *et al*., 2004).

The common feature of these photosynthetically plastic species is a capacity to induce, or at least to up-regulate, CAM under conditions of stress, particularly drought stress (and/or salinity stress in the case of halophytic species), whereas CO_2 fixation proceeds exclusively or predominantly via the C_3 pathway in the light when ambient conditions are favourable. In contrast, in so-called constitutive CAM species, the CAM cycle is expressed under essentially all conditions that these plants encounter in nature or in the laboratory, i.e. even when environmental conditions are not particularly stressful.

Constitutive CAM species are recognized by the resilience of the CAM cycle to stress rather than by an up-regulation of CAM. For example, following the imposition of drought stress, dark CO_2 fixation in constitutive CAM species usually remains relatively unchanged initially, despite rapid declines of CO_2 fixation in the light (Kluge, 1972; Hanscom and Ting, 1978; Kluge and Ting, 1978; Osmond *et al*., 1979). Although the proportions of light and dark CO_2 uptake change in favour of dark CO_2 fixation under these circumstances, this does not represent a C_3 to CAM shift in the strictest sense, because CAM activity does not increase in absolute terms.

In both constitutive and facultative CAM species, the expression of CAM is most pronounced when photosynthetic tissues are mature. In many constitutive species, a progression from C_3 to CAM occurs as leaves mature (Jones, 1975; Gehrig *et al*., 2005). As a result, young plants, because they have young leaves, tend to function predominantly in the C_3 mode, even if older plants exhibit strong CAM (Avadhani *et al*., 1971). In facultative CAM species, the propensity for the induction, or up-regulation, of CAM also increases as leaves mature, but mature leaves do not necessarily express CAM unless stressed.

The interplay between developmental and environmental factors in controlling the balance between C_3 photosynthetic CO_2 uptake in the light and CAM-type CO_2 uptake in the dark is complex and not well understood. In *M. crystallinum*, the original report of salinity-induced CAM (Winter and von Willert, 1972) was later interpreted as stress-induced acceleration of a genetically controlled developmental programme (Adams *et al*., 1998). It was shown recently that unstressed plants are able to complete their life cycle by operating exclusively in the C_3 mode (Winter and Holtum, 2005, 2007), thus demonstrating that the shift to CAM in *M. crystallinum* is a response to environmental triggers, as was suggested originally. Studies of similar detail are not available for other species.

Using CO_2 exchange data from a body of >1000 complete day–night measurements, here the contributions of development and environment (the latter exemplified by drought stress) to CAM expression in young plants of the genus *Clusia*, the second-most studied C_3–CAM system after *M. crystallinum*, are quantified and the observations are compared with species generally accepted to be constitutively CAM. The *Clusia* species chosen for this study encompass the extraordinary photosynthetic plasticity exhibited by this neotropical group of mostly shrubs and trees. The literature on CAM in *Clusia* ssp. is substantial, and includes a wealth of information on selected spot measurements of daily CO_2 exchange patterns of individual leaves (Lüttge, 2006, 2007). However, in none of these previous studies has the attempt been made to separate carefully, using long-term monitoring of CO_2 exchange, the relative roles of ontogeny and environment in the control of CAM expression at the organismal level.

Three of the *Clusia* species studied here exhibited different degrees of CAM during their early growth when unstressed, and different degrees of up-regulation of CAM when exposed to drought stress. A major and unexpected observation was that drought-induced up-regulation of CAM was also observed during the developmental progression of CAM in species considered to exhibit CAM constitutively, *Kalanchoë daigremontiana* and *Kalanchoë pinnata*, and in a stem-succulent cactus, *Opuntia ficus-indica*.

These observations provide a new perspective on the concept of constitutive and facultative CAM.

Materials and methods

Plant material

Clusia cretosa Hammel ined. [referred to as *Clusia* sp. A by Gehrig *et al*. (2003) and Holtum *et al*. (2004)], *Clusia minor* L., *Clusia pratensis* Seeman, and *Clusia rosea* Jacq. were grown from seeds collected from plants growing in their natural habitats in Panama, whereas *K. daigremontiana* Hamet. et Perr. and *K. pinnata* (Lam.) Pers. were grown from leaf-borne ramets. For *O. ficus-indica* (L.) Mill., the young cladodes upon which experiments were performed emanated from apical areoles of single cladode mother plants (~5 cm tall, projected areas of 13–16 cm^2).

For laboratory-based experiments, the majority of plants were grown in an 80:20 (v/v) mixture of dark loamy potting soil (Novey Tierra, Albrook Mall, Panama) and Perlite (Good Earth Horticulture, NY, USA) in 2.65 l cylindrical plastic pots. *Clusia rosea* exposed to stress was grown in Schultz Potting Soil Plus (US Home and Gardens, GA, USA). *Opuntia ficus-indica* was grown in Cactus, Palm and Citrus Soil (Miracle-Gro Lawn Products, OH, USA) in either a 0.95 l plastic pot or a 1.7 l terracotta pot. All plants were fertilized with 5 g of Osmocote Plus (Scotts-Sierra Horticultural Products, OH, USA).

In field-based experiments, well-irrigated *C. pratensis* plants in 400 l plastic containers wrapped with reflective insulation were grown outdoors in locally obtained forest top-soil for 2 years at the Smithsonian Tropical Research Institute, Santa Cruz Experimental Research Facility, Gamboa, Republic of Panama (9°07′N, 79°42′W).

Measurements of CO_2 exchange in the laboratory

Intact young shoots or cladodes were sealed inside Plexiglass gas-exchange cuvettes (for *Clusia* and *Kalanchoë* cuvette dimensions were 30 cm×30 cm×15 cm height or 20 cm×20 cm×15 cm height; for *O. ficus-indica* dimensions were 11 cm×11 cm×10 cm height). For shoots of *Clusia* and *Kalanchoë*, transfer into a cuvette of a young shoot involved removal from the soil and threading the roots and lower portion of the stem through a hole in the bottom of the cuvette. The roots were then replaced in the soil and the stem–cuvette interface was sealed with a non-porous synthetic rubber sealant (Terostat VII, Henkel-Teroson, Heidelberg, Germany). For *O. ficus-indica*, the cuvette was sealed around an apical cladode such that ~1.5 cm of the 3.0 cm high cladode was exposed inside the cuvette. The cuvettes were located inside controlled-environment chambers (Environmental Growth Chambers, OH, USA) operating either under 12 h light (28 °C)/12 h dark (22 °C) cycles, or, in the case of *K. daigremontiana*, under 12 h light (23 °C)/12 h dark (17 °C) cycles. Photon flux density (PFD) at the upper outer surface of the cuvettes was 420 μmol m^{-2} s^{-1}. When not stressed, plants were irrigated daily.

Drought treatments were imposed by witholding irrigation and, in a severe water stress treatment of *O. ficus-indica*, removing the plant roots from the soil. The rate at which plants were stressed following the withholding of irrigation was affected by species, plant size, pot size, pot composition, and soil type.

Net CO_2 exchange of the shoots or cladodes in the cuvettes was measured using a LI-6252 CO_2 analyser (Li-Cor, Lincoln, NE, USA) in a flow-through gas-exchange system consisting mainly of Walz components (Walz GmbH, Effeltrich, Germany) (Holtum and Winter, 2003). Ambient air was supplied to the cuvettes at flow rates of between 2.3 l min^{-1} and 4.7 l min^{-1}. The dewpoint of air entering the cuvettes was 15, 18, or 20 °C depending on species and plant size.

Measurements of CO_2 exchange in the field

For monitoring whole-plant gas exchange, a plant was placed inside an aspirated, naturally illuminated chamber constructed of glass panels and an aluminium framework (internal volume: 8.8 m^3). A blower (model 4C054, Grainger Industrial Supply, OH, USA) supplied external air to the chamber at a rate of 10.5 m^3 min^{-1}. Within the chamber, air was circulated by four fans, and a split air-conditioning system (model V1124C2H, Innovair, FL, USA) maintained temperatures at close to ambient.

Whole-plant gas exchange was quantified at 30 min intervals from the rate at which the CO_2 concentration inside the chamber changed when air flow into the chamber was blocked for 5 min, thereby converting the chamber into a closed system. Changes in the CO_2 concentration inside the chamber were measured using a LI-7500 open-path CO_2 analyser (LI-COR). Calculations of net CO_2 exchange were based upon chamber volume that had been corrected for the volumes of the pot, plant, and other pieces of equipment inside the chamber, and the rate at which the CO_2 concentration changed during the period when the chamber was isolated. CO_2 measurements were corrected for changes in temperature and humidity.

Results

Clusia *species*

CO_2 uptake during the light was a major source of carbon in a young *C. rosea* shoot monitored for 73 d under well-watered conditions (Fig. 1). Uptake during the light was

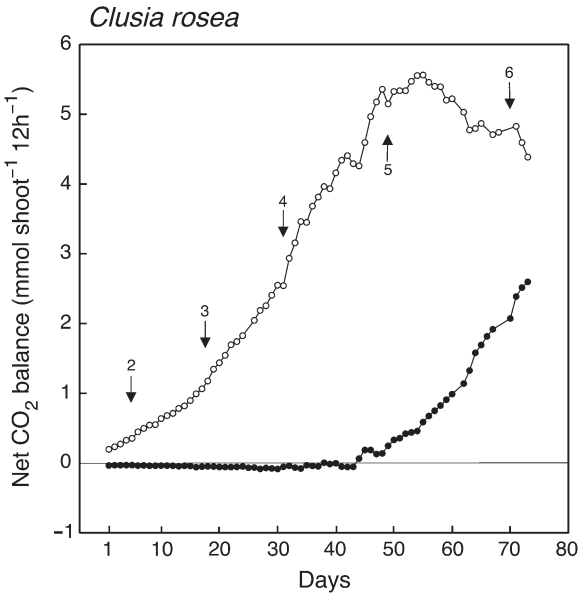

Fig. 1. Net CO_2 balance for a shoot of a well-watered *Clusia rosea* grown for 73 d in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. When sealed in the cuvette, the shoot consisted of a pair of cotyledons and leaf pair 1. Arrows indicate the days upon which numbered leaf pairs were visible (~2 mm). The leaf area of the shoot on the last day of the experiment was 279 cm^2.

the sole contributor to net CO_2 gain until day 37, CO_2 uptake increasing 28-fold between days 1 and 55, and decreasing thereafter by 20%. As the shoot grew, CO_2 uptake in the dark developed. Net CO_2 balance in the dark became positive on day 38, after the initiation of the fourth leaf pair, and progressively increased until day 73.

A second shoot of *C. rosea* exhibited an underlying pattern of CO_2 exchange that was similar to the first, except that the dark CO_2 balance shifted from slightly negative to slightly positive as leaf pair 5 emerged (Fig. 2). Two 13 d drought treatments each resulted in large, reversible decreases of CO_2 uptake in the light. The reductions in CO_2 uptake in the light were accompanied by 1.84-fold and 1.89-fold accelerations, respectively, in the rates of dark CO_2 fixation. Following rewatering after each stress treatment, the daily increment in dark CO_2 uptake returned to the rate observed before the imposition of the stress.

When well watered, net CO_2 uptake by a young shoot of *C. pratensis* was restricted to the light (Fig. 3). Drought treatments of 22 d and 24 d induced pronounced decreases of CO_2 uptake in the light, and shifts from a negative to a positive CO_2 balance at night. The removal of drought stress was rapidly followed by a recovery in CO_2 uptake during the light and the loss of net CO_2 uptake in the dark.

The CO_2 exchange responses to drought of a 2-year-old *C. pratensis* shrub, measured in a naturally illuminated CO_2 exchange chamber under temperature and light conditions close to those in the field (Figs 4, 5), resembled

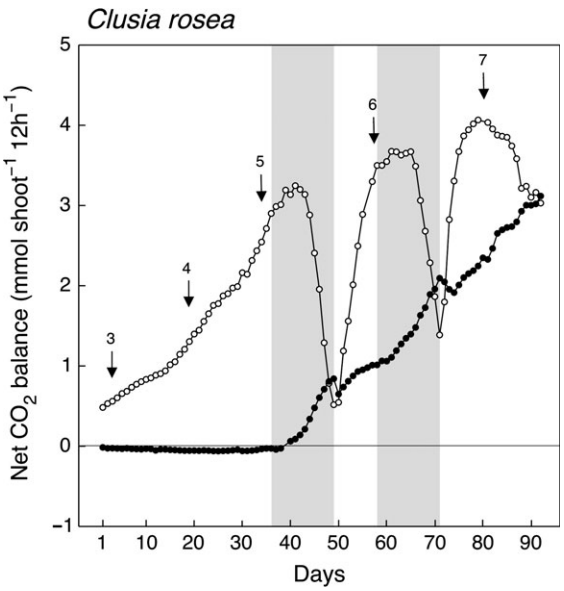

Fig. 2. Net CO_2 balance for a shoot of *Clusia rosea* grown for 92 d in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. The shaded regions represent 13 d periods when irrigation was withheld. Arrows indicate the days upon which numbered leaf pairs were visible (~2 mm). The leaf area of the shoot on the last day of the experiment was 284 cm^2. During the first stress treatment, the decline in CO_2 fixation in the light between days 43 and 48 was accompanied by an increase in nocturnal carbon gain of 0.120 mmol 12 h^{-1} darkness (r^2=0.995), whereas the pre- and post-stress background rate (estimated from days 41–43 and 50–53) was 0.066 mmol 12 h^{-1} darkness (r^2=0.999). During the second stress treatment, the decline in CO_2 fixation in the light between days 65 and 71 was accompanied by an increase in nocturnal CO_2 gain of 0.118 mmol 12 h^{-1} darkness (r^2=0.994), whereas the pre- and post-stress background rate (estimated from days 61–65 and 74–79) was 0.062 mmol 12 h^{-1} darkness (r^2=0.998).

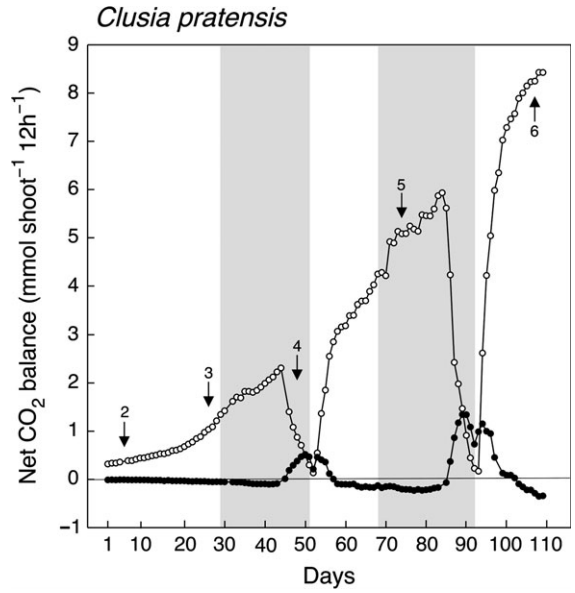

Fig. 3. Net CO_2 balance for a shoot of *Clusia pratensis* grown for 110 d in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. The shaded regions represent periods when irrigation was withheld. Arrows indicate the days upon which numbered leaf pairs were visible (~2 mm). The leaf area of the shoot on the last day of the experiment was 351 cm^2.

Fig. 4. A 2-year-old *Clusia pratensis* in a naturally illuminated whole-plant CO_2 exchange chamber located at the Smithsonian Tropical Research Institute, Santa Cruz Experimental Research Facility, Gamboa, Republic of Panama.

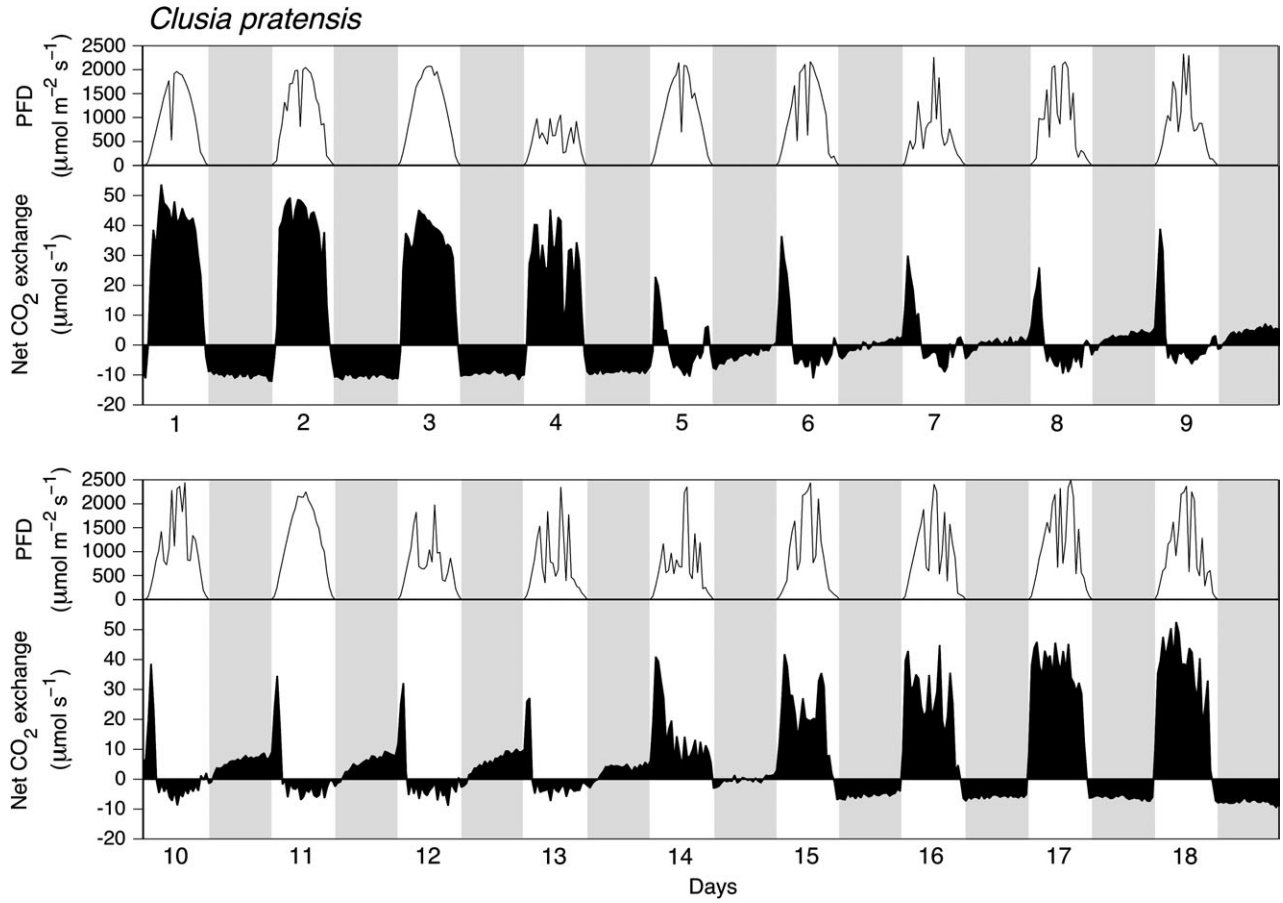

Fig. 5. Photon flux density (PFD) and net CO_2 exchange for a 2-year-old *Clusia pratensis* plant monitored for 18 d in a naturally illuminated chamber (leaf area, 14.3 m^3; dry masses of leaves, stems, and roots were 1.63, 1.83, and 1.84 kg, respectively). PFD was measured outside the chamber. Shading indicates night. Irrigation was withheld between days 2 and 11. The data are for one of four experiments performed.

those observed for the young shoot of *C. pratensis* (Fig. 3). As in the young shoot, the imposition of water stress in the older plant induced changes in CO_2 exchange patterns that were consistent with a C_3 to CAM shift. A rhythm of net CO_2 uptake in the light and net CO_2 loss in the dark was transformed into a rhythm of net CO_2 loss for most parts in the light and net CO_2 uptake during the dark. As water stress unfolded, net CO_2 uptake in the light decreased until it was restricted to a pronounced morning peak and a small afternoon peak between which the CO_2 balance was negative. The accompanying development of CO_2 uptake in the dark was initially observed as a reduction in CO_2 evolution during the night of day 5. Net dark CO_2 fixation was first observed during the following night (day 6) and increased gradually until day 12. Following rewatering (day 12), continual CO_2 uptake during daylight was restored and the net CO_2 balance in the dark became negative again. Because *C. pratensis* produces many stem-borne aerial roots, it was not possible to isolate the pot in order to exclude root and soil respiration from the measurements of net CO_2 exchange. Nevertheless, the inclusion of soil and root respiration in the measurements does not affect the principal observation of a reversible switch from a C_3- to a CAM-type net CO_2 exchange pattern.

Light and dark CO_2 exchange patterns exhibited during the early development of *C. minor* resembled those in *C. pratensis*, including the switch from a negative to a positive CO_2 balance in the dark following application of drought stress (Fig. 6). However, the CO_2 exchange patterns exhibited by *C. minor* differed from those of *C. pratensis* in two significant respects. Unlike *C. pratensis*, *C. minor* exhibited a slightly positive CO_2 balance during most dark periods even when well watered and, upon rewatering following stress, the dark CO_2 fixation that was induced was not fully reversible. The latter observation is consistent with the onset of developmentally programmed CAM in *C. minor*.

Clusia cretosa exhibited CO_2 exchange patterns typical of a C_3 species (Fig. 7). As in the other *Clusia* spp. studied, drought stress led to a reduction in net CO_2 uptake in the light, but, unlike the other *Clusia* spp., stress was not accompanied by the induction of net CO_2 uptake in the dark.

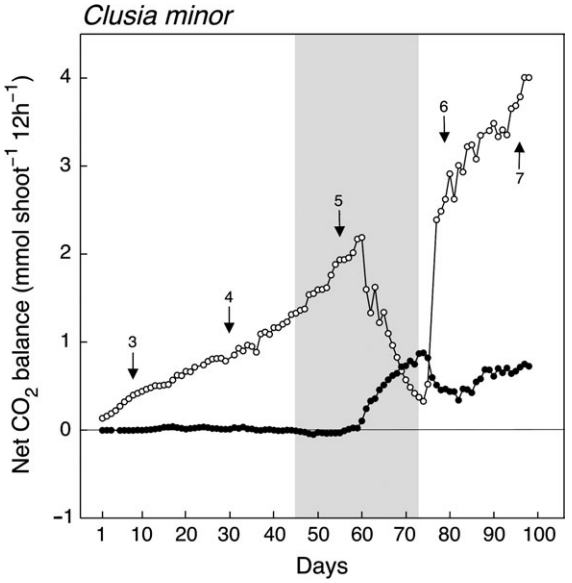

Fig. 6. Net CO_2 balance for a shoot of *Clusia minor* grown for 100 d in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. The shaded region represents the period when irrigation was withheld. Arrows indicate the days upon which numbered leaf pairs were visible (~2 mm). The leaf area of the shoot on the last day of the experiment was 186 cm^2.

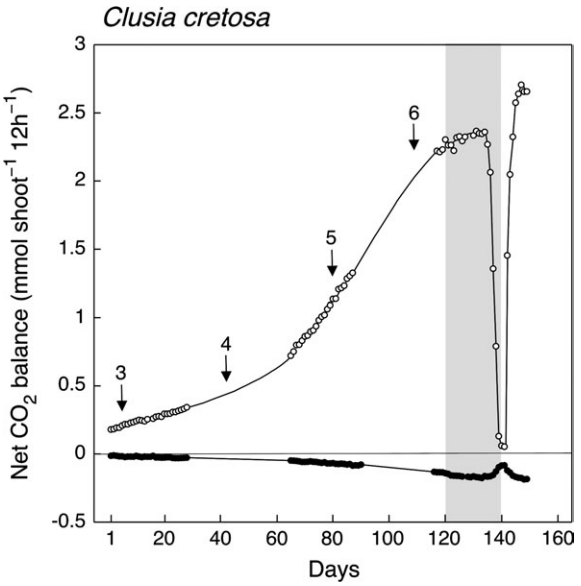

Fig. 7. Net CO_2 balance for a shoot of *Clusia cretosa* grown for 149 d in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. The shaded region represents the period when irrigation was withheld. Arrows indicate the days upon which numbered leaf pairs were visible (~2 mm). The leaf area of the shoot on the last day of the experiment was 99 cm^2.

Kalanchoë *species*

When irrigated, a small *K. daigremontiana* shoot exhibited CO_2 fixation in the light and in the dark (Fig. 8), with light CO_2 uptake contributing the majority

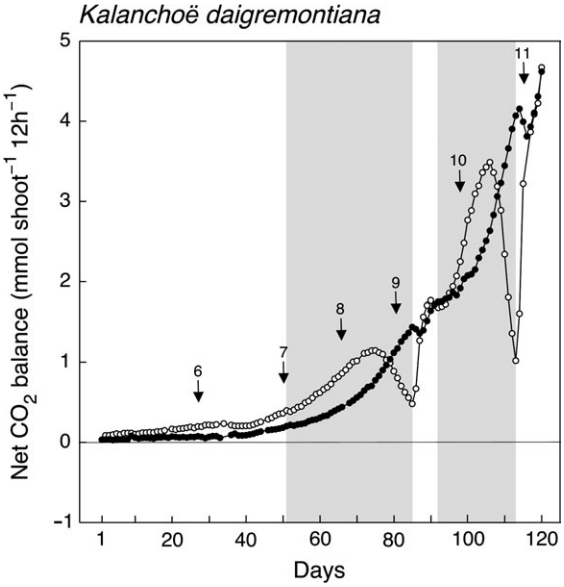

Fig. 8. Net CO_2 balance for a shoot of *Kalanchoë daigremontiana* monitored for 120 d in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. The shaded regions represent the periods when irrigation was withheld. Arrows indicate the days upon which numbered leaf pairs were visible (~2 mm). The leaf area of the shoot on the last day of the experiment was 327 cm^2. During the first stress treatment, the decline in CO_2 fixation in the light between days 74 and 81 was accompanied by an increase in nocturnal carbon gain of 0.067 mmol 12 h^{-1} darkness (r^2=0.999), whereas the pre- and post-stress background rate (estimated from days 72–74 and 87–89) was 0.050 mmol 12 h^{-1} darkness (r^2=0.998). During the second stress treatment, the decline in CO_2 fixation in the light between days 106 and 113 was accompanied by an increase in nocturnal CO_2 gain of 0.202 mmol 12 h^{-1} darkness (r^2=0.997), whereas the pre- and post-stress background rate (estimated from days 103–105 and 116–118) was 0.119 mmol 12 h^{-1} darkness (r^2=0.999).

of the 24 h carbon gain. As the shoot increased in size, the proportion of carbon supplied by CO_2 uptake in the dark increased.

Drought treatments of 34 d and 21 d resulted in substantial reversible decreases of CO_2 uptake in the light. The reductions of CO_2 uptake in the light were accompanied by 1.36-fold and 1.69-fold accelerations, respectively, in the rates of dark CO_2 fixation. Following rewatering after each stress treatment, the daily increment in dark CO_2 uptake returned to the rate observed before the imposition of stress.

Net CO_2 exchange exhibited by a young well-watered developing shoot of *K. pinnata* was outwardly similar to that expressed by *K. daigremontiana* in that both light and dark CO_2 uptake were present, but the rate of CO_2 uptake in the light greatly exceeded that in the dark (Fig. 9). The rate of dark CO_2 fixation increased markedly when water was withheld and decreased transiently following rewatering. Although the timing of the imposition of water stress evidently coincided with the onset of ontogenetically programmed CAM in the experiment shown in Fig. 9, it was possible to calculate that facultative up-regulation

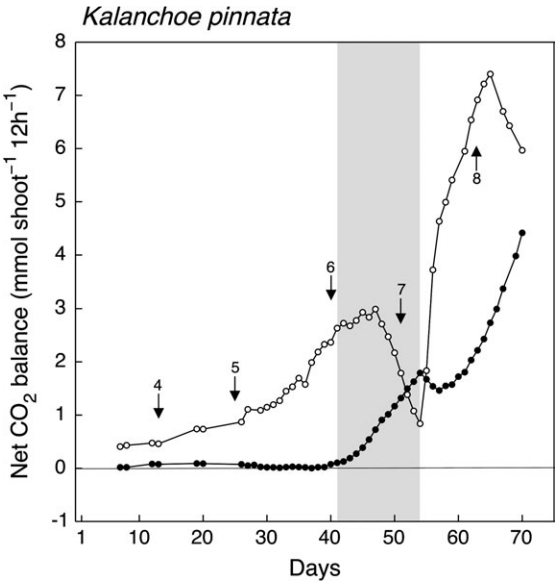

Fig. 9. Net CO_2 balance for a shoot of *Kalanchoë pinnata* grown for 65 d in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. The shaded region represents the period when irrigation was withheld. Arrows indicate the days upon which numbered leaf pairs were visible (~2 mm). The leaf area of the shoot on the last day of the experiment was 536 cm^2. During the stress treatment, the decline in CO_2 fixation in the light between days 47 and 53 was accompanied by an increase in nocturnal carbon gain of 0.151 mmol 12 h^{-1} darkness (r^2=0.998), whereas the pre- and post-stress background rate (estimated from days 45–47 and 57–59) was 0.082 mmol 12 h^{-1} darkness (r^2=0.988).

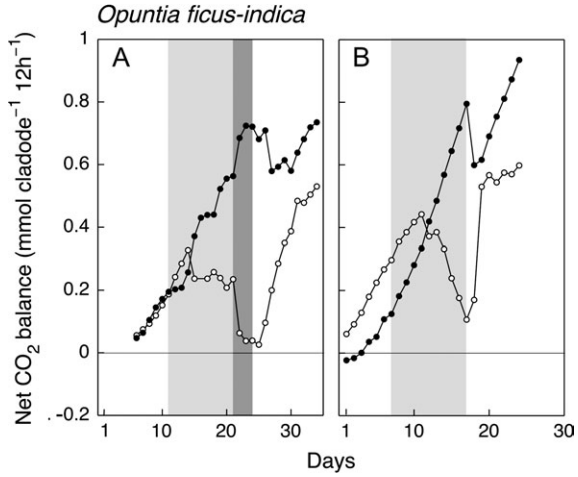

Fig. 10. Net CO_2 balance for cladodes of *Opuntia ficus-indica* grown for 29 d (projected cladode area of 26 cm^2 on the last day) (A) or 24 d (projected cladode area of 26 cm^2 on day 24) (B) in a gas-exchange cuvette under 12 h light (open circles) and 12 h dark (filled circles) cycles. The lighter shaded regions represent periods when irrigation was withheld, whereas the darker shaded region represents a period of intense water stress when a plant was removed *in toto* from the soil. For the shoot shown in (B), the decline in CO_2 fixation in the light during the stress treatment between days 11 and 17 was accompanied by an increase in nocturnal carbon gain of 0.076 mmol 12 h^{-1} darkness (r^2=1.000), whereas the pre- and post-stress background rate (estimated from days 9–11 and 18–20) was 0.040 mmol 12 h^{-1} darkness (r^2=0.995).

resulted in a 1.84-fold acceleration in the rate of CO_2 uptake in the dark.

Opuntia ficus-indica

In a very young cladode of well-watered *O. ficus-indica*, CO_2 balances in the light and in the dark were positive and similar in magnitude until a drought treatment was initiated (Fig. 10A). Droughting involved 10 d without irrigation followed by 3 d of intensive water stress that was imposed by removing the roots from the soil. During the initial part of the water stress treatment, a slight decline in light CO_2 fixation was accompanied by a continuing strong increase in dark CO_2 fixation. The more severe water stress treatment initiated a precipitous decrease in light CO_2 fixation from 0.235 to 0.063 mmol shoot^{-1} 12 h^{-1} and a sharp increase in dark CO_2 uptake. Restoration of the roots to irrigated soil resulted in a substantial increase of CO_2 uptake in the light and a transient 18% reduction in dark CO_2 gain.

In a second experiment, a young cladode of *O. ficus-indica* was grown in a terracotta pot rather than a plastic pot in order to increase the rate of water loss from the substrate during the droughting treatment (Fig. 10B). During the first 12 d in the cuvette, CO_2 uptake in the light markedly exceeded CO_2 uptake in the dark; in fact, during the initial 3 d, carbon balance was positive only in the light. A drought treatment of 11 d led to a substantial decrease of CO_2 uptake in the light and a 1.92-fold acceleration in the rate of dark CO_2 fixation. Following rewatering, the daily increment in dark CO_2 uptake returned to the rate observed before the imposition of the stress.

Discussion

The assessment of developmental and environmental responses of a species ideally requires observation of that species during its entire life cycle. In a previous study, light and dark CO_2 fixation were continuously tracked throughout the life cycle of the annual C_3–CAM species, *M. crystallinum*, demonstrating that the induction of CAM is exclusively under environmental control (Winter and Holtum, 2007). Here, long-term gas exchange measurements are reported of seven perennial species, some of which (*Clusia* spp.) live as trees for decades, rendering life cycle measurements a challenge. Nonetheless, the study of whole shoots during the first few months after germination is meaningful because it provides insights into the relative influences of development and environment on the expression of CAM photosynthesis during a stage critical for establishment and survival.

Reduced water availability was used as a stressor to quantify the degree of environmental control of CAM expression because this is a condition most frequently,

and least ambiguously, linked to changes in the expression of CAM under natural conditions. The responses to drought stress appear to involve regulation at the transcription level, for example, synthesis of phosphoenolpyruvate (PEP) carboxylase and other enzymes of the CAM cycle (Winter *et al*., 1992; Borland *et al*., 1998; Cushman and Bohnert, 1999; Cushman, 2001). The expression of CAM can also be strongly modified in the short term by manipulating temperature, generally by removing day–night temperature fluctuations, or by markedly lowering daytime or increasing night-time temperatures. These changes can lead to rapid shifts from CAM-type to C_3-type 24 h net CO_2 exchange patterns, for example, in *Kalanchoë* spp. (Kluge and Ting, 1978), *Tillandsia* spp. (Kluge *et al*., 1973), and *Clusia* spp. (Haag-Kerwer *et al*., 1992; de Mattos and Lüttge, 2001), but such conditions are only occasionally experienced in the field by land plants (Zotz and Winter, 1993). Moreover, the underlying mechanisms for temperature-induced CAM–C_3 shifts have never been conclusively identified (Brandon, 1967; Kluge and Schomburg, 1996), but certainly include direct effects on membrane fluidity and on catalytic and diffusive processes.

Three major observations derived from this long-term gas exchange study were that (i) C_3 photosynthetic CO_2 uptake in the light was initially the dominant pathway of carbon acquisition in all plants that eventually developed significant CAM activity; (ii) the expression of CAM in all of the species was stimulated by water stress to some degree, including species generally considered to exhibit CAM constitutively; and (iii) in both seedlings and mature plants of one species, *C. pratensis*, the expression of CAM is essentially exclusively under environmental control.

C_3 photosynthesis dominates CO_2 exchange in young plants

In all species, even in those that eventually exhibited CAM after a certain plant age was reached, CO_2 uptake in the light was the predominant mode of carbon acquisition in well-watered young shoots. Net CO_2 uptake in the dark was either absent (*C. rosea* and *C. pratensis*) or very low (both *Kalanchoë* species and *C. minor*). In *O. ficus-indica*, in which the formation of a new cladode originating from a mother cladode was studied, CO_2 uptake in the light was initially equal to or markedly greater than CO_2 uptake in the dark. In a similar study of *O. ficus-indica* by Wang *et al*. (1998), net CO_2 fixation in the light was not observed during the early development of cladodes. Rather, development was characterized by a transition from net CO_2 loss in both the light and the dark to net CO_2 uptake in the dark. Mother cladodes in this previous study were substantially larger, and much of the early growth in the juvenile cladodes was based on carbon transported from the parent tissue. Although 1-d-old cotyledons of cacti can exhibit day–night fluctuations in acidity typical of CAM (Hernández-González and Briones Villareal, 2007), the present study with *O. ficus-indica* suggests that the contribution of CAM to total carbon gain is presumably small at this early stage of development. Consistent with this postulate are observations that in 14 mm seedlings of *Agave deserti* only 27% of carbon was fixed during the dark, a value that increased to 81% in 60 mm seedlings (Nobel, 1988).

A facultative CAM component in 'constitutive' CAM species

After an initial C_3-type phase, plants induce or up-regulate CAM in response to internal or external cues. While internal developmental cues were paramount in the generation of CAM in *C. rosea*, both *Kalanchoë* species, and *O. ficus-indica*, an external cue, in this case water stress, was critical for the induction of CAM in *C. pratensis*. *Clusia minor* exhibited an intermediate behaviour.

A most significant finding is that a facultative component of CAM control was present in all species that were capable of CAM, even in young shoots of the constitutive CAM species *K. daigremontiana* and *K. pinnata*, and in the archetypal platyopuntia, *O. ficus-indica*. The observation of both developmental and environmental effects on the expression of CAM appears to relativize the distinctions between constitutive and facultative CAM plants. Although up-regulation in *Kalanchoë* and *Opuntia* was low in comparison with the background ontogenetically based CAM, the environmental control was generally demonstrable as water stress-stimulated dark CO_2 uptake that could be reversed following rewatering. Comparable responses to drought stress have been seen in fully expanded leaves of *K. daigremontiana*, but reversibility was not documented (Griffiths *et al*., 2002). In the experiment with *K. pinnata*, the stimulation of dark CO_2 fixation was difficult to discern from the developmentally controlled background increase of CAM, but the transient decrease in nocturnal CO_2 uptake upon stress relief is consistent with an up-regulation of CAM by drought stress. Stress-related up-regulation of CAM in *K. pinnata* was confirmed by CO_2 exchange studies with intact attached leaves during their expansion (data not shown).

It has been reported that *A. deserti*, a species generally regarded as a classic constitutive CAM plant, can switch from a strong CAM-type to a C_3-type pattern of CO_2 gas exchange when plants are watered extensively (Hartsock and Nobel, 1976). Although there has never been a follow-up study of this intriguing observation, the data support the notion that strict distinctions between constitutive and facultative CAM are not possible. Increases in dark CO_2 fixation in cacti during the early dry season in the field do not necessarily reflect drought-induced

up-regulation of CAM because night-time temperatures and daily PFDs became more favourable for dark CO_2 fixation (Pimienta-Barrios *et al*., 2000).

C_3 and CAM in Clusia

The four *Clusia* species studied encompass the wide range of photosynthetic options exhibited by this genus, for which there is a considerable, sometimes bewildering, literature on the interactions between CAM and the environment (Lüttge 1999, 2006, 2007, 2008; Dodd *et al*., 2002). Early research on *C. rosea* concluded that 'categorization of this plant as C_3, CAM or an intermediate is impossible' (Schmitt *et al*., 1988), but our long-term gas exchange measurements of shoots, together with other studies of leaf gas exchange, titratable acidity, and carbon isotope ratios, show that the expression of CAM in *C. rosea* is predominantly under developmental control (Ting *et al*., 1985; Ball *et al*., 1991; Lüttge *et al*., 1993; Franco *et al*., 1994; Haag-Kerwer *et al*., 1996; Borland *et al*., 1998; Holtum *et al*., 2004). It is demonstrated for plants with ⩾4 leaf pairs (in addition to the cotyledons) that a facultative component is present but small. We do not know whether younger plants which have not yet started to exhibit the developmental component of CAM can exhibit completely reversible CAM induction when droughted. If so, full facultative CAM control would be present at this early stage of development.

Clusia pratensis represents the most clear-cut example of facultative CAM reported in the genus *Clusia*. Both young shoots and mature plants exhibit drought-induced C_3 to CAM shifts that are totally reversible. The latter observation is unique as it has even yet to be reported for *M. crystallinum*, the most intensely studied C_3–CAM system. Measurements of 2-year-old plants of *C. pratensis*, the first that have integrated whole-plant CO_2 exchange for any *Clusia* species under natural tropical conditions, demonstrate that a developmental component of CAM control may be negligible throughout the life cycle of this species.

In *C. minor*, the CO_2 exchange characteristics and drought stress response were between those of *C. rosea* and *C. pratensis*. The experiment shown in Fig. 6 demonstrates drought-induced up-regulation of CAM activity, but the lack of complete reversibility of CAM is consistent with a developmentally controlled CAM component. In contrast to very young *C. rosea*, a low level of net dark CO_2 uptake was measured in young shoots of *C. minor* during several nights prior to the stress treatment (third and fourth leaf pair following the cotyledons). Other experiments (data not shown) demonstrated the capacity for low-level CAM even in plants with only two leaf pairs and the cotyledons. It is possible that the low level of CAM in such very young *C. minor* is the product of transient transpiration-induced water deficits despite the plant being well watered. We noted that the top pair of leaves, which are initially vertical and connected, wilt transiently as they separate and receive higher levels of radiation. Indeed, low rates of dark CO_2 fixation have previously been reported in 60% expanded young leaves of well-irrigated *C. minor* (Borland *et al*., 1998).

Clusia minor has frequently been used as a model C_3–CAM plant because the expression of CAM appears particularly responsive to environmental change (Borland *et al*., 1994; Lüttge, 2008). In many laboratory experiments, relatively low light intensities, constant day–night temperatures, and high relative humidities have been used to produce an almost exclusively C_3 pattern of day–night net CO_2 exchange (de Mattos and Lüttge, 2001; Grams and Thiel, 2002). Under field conditions, a purely C_3 exchange pattern is less likely and plants would be expected to perform a low background level of CAM under many circumstances, even in the absence of edaphic drought stress. Although field-obtained leaf carbon isotope ratios of between –23‰ and –29‰ are consistent with a predominantly C_3 photosynthetic CO_2 uptake (Ting *et al*., 1987; Borland *et al*., 1992; Franco *et al*., 1994; Holtum *et al*., 2004), such values do not exclude the possibility that up to 30% of plant carbon may be gained by dark CO_2 fixation (Pierce *et al*., 2002; Winter and Holtum, 2002) and, indeed, acid accumulation has been reported for *C. minor* in the field during the wet season (Roberts *et al*., 1998).

Clusia cretosa exhibited C_3-type photosynthesis under both well-watered and stressed conditions. Drought stress, although not accompanied by the induction of CAM as observed in the other *Clusia* species studied, led to a decline in CO_2 fixation in the light and a reduction in nocturnal CO_2 loss. For plants of similar size, dark respiration rates were higher in well-watered *C. cretosa* than in the *Clusia* species capable of performing CAM. This pattern may be species specific, but could also indicate refixation of some respiratory CO_2 by the other *Clusia* species, even when they did not exhibit net CO_2 uptake in the dark.

The absence of net dark CO_2 fixation in *C. cretosa*, even when stressed, further strengthens the postulate that species in the ITS and morphology-based *C. multiflora* clade, in which *C. cretosa* and other higher elevation *Clusia* species have been placed, have little or no capacity for net CO_2 uptake in the dark (Hammel, 1986; Grams *et al*., 1998; Gehrig *et al*., 2003; Holtum *et al*., 2004; Gustafsson *et al*., 2007).

We do not know whether the reversible reduction of nocturnal CO_2 loss in *C. cretosa* which accompanies the strong decrease in diurnal net CO_2 fixation in response to water deficit solely reflects decreased mitochondrial dark respiration of the shoot, or also involves increased nocturnal refixation of respiratory CO_2 via PEP carboxylase. Stress-induced, reversible down-regulation

of mitochondrial respiration, unrelated to the CAM cycle, could contribute to the stress-induced, reversible increase of net dark CO_2 fixation in predominantly constitutive CAM species such as *K. daigremontiana*, *K. pinnata*, and *O. ficus-indica*. Measurements of nocturnal leaf titratable acidity changes, together with expression studies of CAM-associated genes, will thus be crucial to assess the full extent of a facultative CAM component in constitutive CAM species.

Conclusions

Distinguishing between facultative and constitutive CAM is a convenient way to categorize the ability of plants to up-regulate environmentally, in a reversible fashion, the contribution of CAM to total carbon gain and growth. However, this ability is not an all-or-nothing phenomenon. The terms constitutive and facultative CAM are not the manifestations of different CAM pathways, rather they represent extremes on a continuum that ranges from the expression of CAM that is fully controlled by ontogeny to the full control of CAM expression by drought stress. All CAM species, irrespective of whether they are designated constitutive or facultative, are genotypically equipped to perform CAM and, as shown in this study, the proportion of carbon gained in the dark depends on both pre-programmed (i.e. developmental) and environmental forces, albeit to varying degrees. As in most biological systems, few CAM species are expected at the extremes of the scale. *Clusia pratensis* appears close to the environmentally induced, and thus facultative, extreme, but constitutive species that have been studied in detail cannot be placed at the extreme opposite end of the scale because they too exhibit a facultative component.

Acknowledgements

We thank Jorge Aranda and Aurelio Virgo for technical assistance. This research was funded by the Smithsonian Tropical Research Institute.

References

Adams P, Nelson DE, Yamada S, Chmara W, Jensen RG, Bohnert HJ, Griffiths H. 1998. Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). *New Phytologist* **138,** 171–190.

Avadhani PN, Osmond CB, Tan KK. 1971. Crassulacean acid metabolism and the C_4 pathway of photosynthesis in succulent plants. In: Hatch MD, Osmond CB, Slatyer RO, eds. *Photosynthesis and photorespiration*. New York: Wiley-Interscience, 288–293.

Ball E, Hann J, Kluge M, Lee HSJ, Lüttge U, Orthen B, Popp M, Schmitt A, Ting IP. 1991. Ecophysiological comportment of the tropical CAM-tree *Clusia* in the field. II. Modes of photosynthesis in trees and seedlings. *New Phytologist* **117,** 483–491.

Borland AM. 1996. A model for the partitioning of photosynthetically fixed carbon during the C_3–CAM transition in *Sedum telephium*. *New Phytologist* **134,** 433–444.

Borland AM, Griffiths H, Broadmeadow MSJ, Fordham MC, Maxwell C. 1994. Carbon-isotope composition of biochemical fractions and the regulation of carbon balance in leaves of the C_3–crassulacean acid metabolism intermediate *Clusia minor* L. growing in Trinidad. *Plant Physiology* **106,** 493–501.

Borland AM, Griffiths H, Maxwell C, Broadmeadow MSJ, Griffiths NM, Barnes JD. 1992. On the ecophysiology of the Clusiaceae in Trinidad: expression of CAM in *Clusia minor* L. during the transition from wet to dry season and characterization of three endemic species. *New Phytologist* **122,** 349–357.

Borland AM, Técsi LI, Leegood RC, Walker RP. 1998. Inducibility of crassulacean acid metabolism (CAM) in *Clusia* species; physiological/biochemical characterization and intercellular localization of carboxylation and decarboxylation processes in three species which exhibit different degrees of CAM. *Planta* **205,** 342–351.

Brandon PC. 1967. Temperature features of enzymes affecting crassulacean acid metabolism. *Plant Physiology* **42,** 977–984.

Cernusak LA, Aranda J, Marshall JD, Winter K. 2007*a*. Large variation in whole-plant water-use efficiency among tropical tree species. *New Phytologist* **173,** 294–305.

Cernusak LA, Winter K, Aranda J, Turner BL, Marshall JD. 2007*b*. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *Journal of Experimental Botany* **58,** 3549–3566.

Crayn DM, Winter K, Smith JAC. 2004. Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *Proceedings of the National Academy of Sciences, USA* **101,** 3703–3708.

Cushman JC. 2001. Crassulacean acid metabolism. A plastic photosynthetic adaptation to arid environments. *Plant Physiology* **127,** 1439–1448.

Cushman JC, Bohnert HJ. 1999. Crassulacean acid metabolism: molecular genetics. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 305–332.

de Mattos EA, Lüttge U. 2001. Chlorophyll fluorescence and organic acid oscillations during transition from CAM to C_3-photosynthesis in *Clusia minor* L. (Clusiaceae). *Annals of Botany* **88,** 457–463.

Dodd AN, Borland AM, Haslam RP, Griffiths H, Maxwell K. 2002. Crassulacean acid metabolism: plastic, fantastic. *Journal of Experimental Botany* **53,** 569–580.

Franco AC, Olivares E, Ball E, Lüttge U, Haag-Kerwer A. 1994. *In situ* studies of crassulacean acid metabolism in several sympatric species of tropical trees of the genus *Clusia*. *New Phytologist* **126,** 203–211.

Gehrig HH, Aranda J, Cushman MA, Virgo A, Cushman JC, Hammel BE, Winter K. 2003. Cladogram of Panamanian *Clusia* based on nuclear DNA: implications for the origins of crassulacean acid metabolism. *Plant Biology* **5,** 59–70.

Gehrig HH, Wood JA, Cushman MA, Virgo A, Cushman JC, Winter K. 2005. Large gene family of phospho*enol*pyruvate carboxylase in the crassulacean acid metabolism plant *Kalanchoe pinnata* (Crassulaceae) characterized by partial cDNA sequence analysis. *Functional Plant Biology* **32,** 467–472.

Grams TEE, Herzog B, Lüttge U. 1998. Are there species in the genus *Clusia* with obligate C_3-photosynthesis? *Journal of Plant Physiology* **152,** 1–9.

Grams TEE, Thiel S. 2002. High light-induced switch from C_3-photosynthesis to crassulacean acid metabolism is mediated by UV-A/blue light. *Journal of Experimental Botany* **53,** 1475–1483.

Griffiths H, Helliker B, Roberts A, Haslam RP, Girnus J, Robe WE, Borland AM, Maxwell K. 2002. Regulation of Rubisco activity in crassulacean acid metabolism plants: better late than never. *Functional Plant Biology* **29,** 689–696.

Guralnick LJ, Cline A, Smith M, Sage RF. 2008. Evolutionary physiology: the extent of C_4 and CAM photosynthesis in the genus *Anacampseros* and *Grahamia* of the Portulacaceae. *Journal of Experimental Botany* **59,** (in press)

Gustafsson MHG, Winter K, Bittrich V. 2007. Diversity, phylogeny and classification of *Clusia*. In: Lüttge U, ed. Clusia: *a woody neotropical genus of remarkable plasticity and diversity*. Berlin: Springer, 95–116.

Haag-Kerwer A, Franco AC, Lüttge U. 1992. The effect of temperature and light on gas exchange and acid accumulation in the C_3–CAM plant *Clusia minor* L. *Journal of Experimental Botany* **43,** 345–352.

Haag-Kerwer A, Grams TEE, Olivares E, Ball E, Arndt S, Popp M, Medina E, Lüttge U. 1996. Comparative measurements of gas-exchange, acid accumulation and chlorophyll *a* fluorescence of different species of *Clusia* showing C_3 photosynthesis, or crassulacean acid metabolism, at the same field site in Venezuela. *New Phytologist* **134,** 215–226.

Hammel BE. 1986. New species of Clusiaceae from Central America with notes on *Clusia* and synonymy in the tribe Clusieae. *Selbyana* **9,** 112–120.

Hanscom Z III, Ting IP. 1978. Irrigation magnifies CAM-photosynthesis in *Opuntia basilaris* (Cactaceae). *Oecologia* **33,** 1–15.

Hartsock TL, Nobel PS. 1976. Watering converts a CAM plant to daytime CO_2 uptake. *Nature* **262,** 574–576.

Hernández-Gonzáles O, Briones Villarreal O. 2007. Crassulacean acid metabolism photosynthesis in columnar cactus seedlings during ontogeny: the effect of light on nocturnal acidity accumulation and chlorophyll fluorescence. *American Journal of Botany* **94,** 1344–1351.

Herrera A, Delgado J, Paraguatey I. 1991. Occurrence of inducible crassulacean acid metabolism in leaves of *Talinum triangulare* (Portulacaceae). *Journal of Experimental Botany* **42,** 493–499.

Holtum JAM, Aranda J, Virgo A, Gehrig HH, Winter K. 2004. $\delta^{13}C$ values and crassulacean acid metabolism in *Clusia* species from Panama. *Trees* **18,** 658–668.

Holtum JAM, Smith JAC, Neuhaus HE. 2005. Intracellular transport and pathways of carbon flow in plants with crassulacean acid metabolism. *Functional Plant Biology* **32,** 429–449.

Holtum JAM, Winter K. 1999. Degrees of crassulacean acid metabolism in tropical epiphytic and lithophytic ferns. *Australian Journal of Plant Physiology* **26,** 749–757.

Holtum JAM, Winter K. 2003. Photosynthetic CO_2 uptake in seedlings of two tropical tree species exposed to oscillating elevated concentrations of CO_2. *Planta* **218,** 152–158.

Holtum JAM, Winter K, Weeks MA, Sexton TR. 2007. Crassulacean acid metabolism in the ZZ plant, *Zamioculcas zamiifolia* (Araceae). *American Journal of Botany* **94,** 1670–1676.

Jones MB. 1975. Effect of leaf age on leaf resistance and CO_2 exchange of the CAM plant *Bryophyllum fedtschenkoi*. *Planta* **123,** 91–96.

Kluge M. 1972. Die Sukkulenten: Spezialisten im CO_2-Gaswechsel. *Biologie in unserer Zeit* **4,** 120–129.

Kluge M. 1977. Is *Sedum acre* a CAM plant? *Oecologia* **29,** 77–83.

Kluge M, Lange OL, von Eichenauer M, Schmid R. 1973. Diurnaler Säurerhythmus bei *Tillandsia usneoides*: Untersuchungen über den Weg des Kohlenstoffs sowie die Abhängigkeit des CO_2-Gaswechsels von Lichtintensität, Temperatur and Wassergehalt der Pflanze. *Planta* **112,** 357–372.

Kluge M, Schomburg M. 1996. The tonoplast as a target of temperature effects in crassulacean acid metabolism. In: Winter K, Smith JAC, eds. *Crassulacean acid metabolism*. Berlin: Springer, 72–77.

Kluge M, Ting IP. 1978. *Crassulacean acid metabolism*. Berlin, Heidelberg: Springer.

Lüttge U. 1999. One morphotype, three physiotypes: sympatric species of *Clusia* with obligate C_3 photosynthesis, obligate CAM and C_3-CAM intermediate behaviour. *Plant Biology* **1,** 138–148.

Lüttge U. 2006. Photosynthetic flexibility and ecophysiological plasticity: questions and lessons from *Clusia*, the only CAM tree, in the neotropics. *New Phytologist* **171,** 7–25.

Lüttge U. 2007. Clusia*: a woody neotropical genus of remarkable plasticity and diversity*. Ecological Studies 194. Berlin: Springer.

Lüttge U. 2008. *Clusia*: Holy Grail and enigma. *Journal of Experimental Botany* **59,** (in press)

Lüttge U, Ziegler H, Ting IP. 1993. Ecophysiological comportment of the tropical C_3/CAM-intermediate tree *Clusia rosea* in the field as assessed by analyses of stable carbon- and hydrogen-isotope ratios. *Journal of Plant Physiology* **142,** 497–501.

Martin CE, Zee AK. 1983. C_3 photosynthesis and crassulacean acid metabolism in a Kansas rock outcrop succulent, *Talinum calycinum* Engelm. (Portulacaceae). *Plant Physiology* **73,** 718–723.

Mooney HA, Troughton JH, Berry JA. 1974. Arid climates and photosynthetic systems. *Carnegie Institute Year Book* **73,** 793–805.

Nobel PS. 1988. *Environmental biology of agaves and cacti*. Cambridge: Cambridge University Press.

Osmond CB. 1978. Crassulacean acid metabolism: a curiosity in context. *Annual Review of Plant Physiology* **29,** 379–414.

Osmond CB. 2007. Crassulacean acid metabolism: now and then. *Progress in Botany* **69,** 3–32.

Osmond CB, Nott DL, Firth PM. 1979. Carbon assimilation patterns and growth of the introduced CAM plant *Opuntia inermis* in eastern Australia. *Oecologia* **40,** 331–350.

Pierce S, Winter K, Griffiths H. 2002. Carbon isotope ratio and the extent of daily CAM use by Bromeliaceae. *New Phytologist* **156,** 75–84.

Pimienta-Barrios E, Zañudo J, Yepez E, Pimienta-Barrios E, Nobel PS. 2000. Seasonal variation of net CO_2 uptake for cactus pear (*Opuntia ficus-indica*) and pitayo (*Stenocereus queretaroensis*) in a semi-arid environment. *Journal of Arid Environments* **44,** 73–83.

Roberts A, Borland AM, Maxwell K, Griffiths H. 1998. Ecophysiology of the C_3–CAM intermediate *Clusia minor* L. in Trinidad: seasonal and short-term photosynthetic characteristics of sun and shade leaves. *Journal of Experimental Botany* **49,** 1563–1573.

Schmitt AK, Lee HSJ, Lüttge U. 1988. The response of the C_3-CAM tree, *Clusia rosea*, to light and water stress. I. Gas exchange characteristics. *Journal of Experimental Botany* **39,** 1581–1590.

Schulze ED, Beck E, Müller-Hohenstein K. 2005. *Plant ecology*. Berlin: Springer.

Silvera K, Santiago LS, Winter K. 2005. Distribution of crassulacean acid metabolism in orchids of Panama: evidence of selection for weak and strong modes. *Functional Plant Biology* **32,** 397–407.

Smirnoff N. 1996. Regulation of crassulacean acid metabolism by water status in the C_3/CAM intermediate *Sedum telephium*. In: Winter K, Smith JAC, eds. *Crassulacean acid metabolism*. Berlin: Springer, 176–191.

Smith JAC, Winter K. 1996. Taxonomic distribution of crassulacean acid metabolism. In: Winter K, Smith JAC, eds. *Crassulacean acid metabolism*. Berlin: Springer, 427–436.

Ting IP, Hann J, Holbrook NM, Putz FE, Sternberg LSL, Price D, Goldstein G. 1987. Photosynthesis in epiphytic species of *Clusia* and *Ficus*. *Oecologia* **74,** 339–346.

Ting IP, Lord EM, Sternberg LSL, DeNiro MJ. 1985. Crassulacean acid metabolism in the strangler *Clusia rosea* Jacq. *Science* **229,** 969–971.

Treichel S. 1975. Crassulaceensäurestoffwechsel bei einem salztoleranten Vertreter der Aizoaceae: *Aptenia cordifolia*. *Plant Science Letters* **4,** 141–144.

Wang N, Zhang H, Nobel PS. 1998. Carbon flow and carbohydrate metabolism during sink-to-source transition for developing cladodes of *Opuntia ficus-indica*. *Journal of Experimental Botany* **49,** 1835–1843.

Winter K. 1973. NaCl-induzierter Crassulaceensäurestoffwechsel bei einer weiteren Aizoaceae: *Carpobrotus edulis*. *Planta* **115,** 187–188.

Winter K, Aranda J, Holtum JAM. 2005. Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. *Functional Plant Biology* **32,** 381–388.

Winter K, Holtum JAM. 2002. How closely do the $\delta^{13}C$ values of crassulacean acid metabolism plants reflect the proportion of CO_2 fixed during day and night? *Plant Physiology* **129,** 1843–1851.

Winter K, Holtum JAM. 2005. The effects of salinity, crassulacean acid metabolism and plant age on the carbon isotope composition of *Mesembryanthemum crystallinum* L., a halophytic C_3–CAM species. *Planta* **222,** 201–209.

Winter K, Holtum JAM. 2007. Environment or development? Lifetime net CO_2 exchange and control of the expression of crassulacean acid metabolism in *Mesembryanthemum crystallinum*. *Plant Physiology* **143,** 98–107.

Winter K, Osmond CB, Pate JS. 1981. Coping with salinity. In: Pate JS, McComb AJ, eds. *The biology of Australian plants*. Nedlands: University of Western Australia Press, 88–113.

Winter K, Smith JAC. 1996. An introduction to crassulacean acid metabolism. Biochemical principles and ecological diversity. In: Winter K, Smith JAC, eds. *Crassulacean acid metabolism*. Berlin: Springer, 1–13.

Winter K, von Willert DJ. 1972. NaCl-induzierter Crassulaceensäurestoffwechsel bei *Mesembryanthemum crystallinum*. *Zeitschrift für Pflanzenphysiologie* **67,** 166–170.

Winter K, Zotz G, Baur B, Dietz KJ. 1992. Light and dark CO_2 fixation in *Clusia uvitana* as affected by plant water status and CO_2 availability. *Oecologia* **91,** 47–51.

Zotz G, Winter K. 1993. Short-term regulation of crassulacean acid metabolism activity in a tropical hemiepiphyte, *Clusia uvitana*. *Plant Physiology* **102,** 835–841.

Zotz G, Winter K. 1994*a*. Annual carbon balance and nitrogen-use efficiency in tropical C_3 and CAM epiphytes. *New Phytologist* **126,** 481–492.

Zotz G, Winter K. 1994*b*. A one-year study on carbon, water and nutrient relationships in a tropical C_3-CAM hemi-epiphyte, *Clusia uvitana* Pittier. *New Phytologist* **127,** 45–60.