

REVIEW

Crassulacean acid metabolism and fitness under water deficit stress: if not for carbon gain, what is facultative CAM good for?

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- **Background** In obligate Crassulacean acid metabolism (CAM), up to 99 % of CO₂ assimilation occurs during the night, therefore supporting the hypothesis that CAM is adaptive because it allows CO₂ fixation during the part of the day with lower evaporative demand, making life in water-limited environments possible. By comparison, in facultative CAM (inducible CAM, C₃-CAM) and CAM-cycling plants drought-induced dark CO₂ fixation may only be, with few exceptions, a small proportion of C₃ CO₂ assimilation in watered plants and occur during a few days. From the viewpoint of survival the adaptive advantages, i.e. increased fitness, of facultative CAM and CAM-cycling are not obvious. Therefore, it is hypothesized that, if it is to increase fitness, CAM must aid in reproduction.
- **Scope** An examination of published reports of 23 facultative CAM and CAM-cycling species finds that, in 19 species, drought-induced dark CO₂ fixation represents on average 11 % of C₃ CO₂ assimilation of watered plants. Evidence is discussed on the impact of the operation of CAM in facultative and CAM-cycling plants on their survival – carbon balance, water conservation, water absorption, photo-protection of the photosynthetic apparatus – and reproductive effort. It is concluded that in some species, but not all, facultative and cycling CAM contribute, rather than to increase carbon balance, to increase water-use efficiency, water absorption, prevention of photoinhibition and reproductive output.

Key words: Facultative CAM, CAM-cycling, water, crassulacean acid metabolism, deficit.

INTRODUCTION

Crassulacean acid metabolism (CAM), a form of CO₂ assimilation which occurs in approx. 16 000 species of 328 genera in 33 families (Smith and Winter, 1996), has been extensively revised (Osmond, 1978; Ting, 1985; Winter, 1985; Winter and Smith, 1996; Cushman, 2001; among others). Briefly, in CAM plants CO₂ uptake occurs during the night, when stomata open; CO₂ is combined with phosphoenolpyruvate (PEP) through the action of PEP-carboxylase (PEPC) to yield oxaloacetate, which is reduced to malate. Malate is transported passively into the vacuole following the actively transported protons and malic acid accumulates during the night. Nocturnal acid accumulation and nocturnal stomatal aperture are the main diagnostic features of CAM. During the day malate is decarboxylated in the cytoplasm, providing ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) with one of its substrates during C₃ photosynthesis.

The net outcome of the functioning of CAM is that CO₂ is fixed with significant water saving relative to C₃ photosynthesis, since the process occurs at times of lower evaporative demand and a larger air-leaf CO₂ concentration gradient (Winter and Smith, 1996). This means that water-use efficiency (WUE), the ratio assimilation rate : transpiration rate, increases relative to a C₃ plant. In the CAM plant, *Plectranthus marruboides*, WUE increased nearly twice when air water vapour saturation deficit decreased five times, nocturnal CO₂ gain remaining unchanged (Herppich, 1997).

Transpiration ratio (the reciprocal of WUE) has been shown to be 3–10 times higher in CAM than in C₃ plants (Kluge and Ting, 1978).

CAM may operate in different modes: (a) obligate CAM, with high nocturnal acid accumulation (ΔH^+) and CO₂ fixation; (b) facultative or inducible CAM, also known as C₃-CAM, with a C₃ form of CO₂ fixation and nil ΔH^+ in the non-induced state, and small nocturnal CO₂ fixation and ΔH^+ in the induced state; (c) CAM-cycling, with daytime CO₂ fixation and ΔH^+ but no nocturnal stomatal aperture; and (d) idling, with small ΔH^+ and stomatal closure during the entire day and night in severely stressed plants (see Cushman, 2001; Fig. 1). Obligate CAM, in turn, can be differentiated between strong and weak CAM, on the basis of the magnitude of ΔH^+ (for definition and examples, see Cushman and Bohnert, 1999; Winter and Holtum, 2002; Silvera *et al.*, 2005; Holtum *et al.*, 2007).

Facultative CAM and CAM-cycling species typically grow on soil in semi-arid regions, on rocks, on tree branches, and, in general, in habitats where water deficit is frequent. It is widely accepted that CAM is an adaptive mechanism which optimizes water use under conditions of deficient supply; in order to be effective, an adaptation must increase fitness through increased individual survival, increased individual fecundity or both (Futuyma, 1986).

In facultative species, CAM may be induced by factors such as drought (Borland and Griffiths, 1990; Herrera *et al.*, 1991; Olivares *et al.*, 1984), salinity (Winter and von Willert, 1972), photoperiod (Brulfert *et al.*, 1988), high photosynthetic

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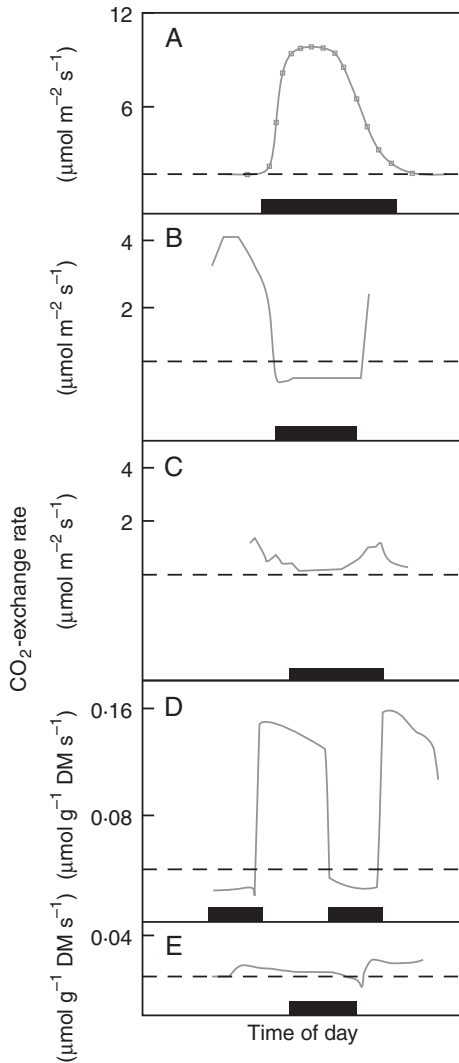


FIG. 1. Daily gas-exchange of plants with different modes of CAM: (A) Obligate CAM in watered plants of *Opuntia ficus-indica* (Nobel and Hartscock, 1983); (B) C_3 CO_2 -uptake in well-watered plants of the inducible-CAM species, *Talinum triangulare* (Herrera *et al.*, 1991); (C) small dark CO_2 fixation in plants of *T. triangulare* drought-stressed for 10 d (Herrera *et al.*, 1991); (D) CAM-cycling in watered plants of *Talinum calycinum* (Martin *et al.*, 1988); (E) CAM-idling in plants of *Talinum calycinum* drought-stressed for 3 d (Martin *et al.*, 1988). The broken line indicates no net CO_2 -exchange; the dark bar on the abscissa indicates the duration of the dark period. The corresponding values of nocturnal acid accumulation were: (A) $85 \mu\text{mol H}^+ \text{cm}^{-2}$ (droughted plants had a value of $16 \mu\text{mol H}^+ \text{cm}^{-2}$); (B) $8 \mu\text{mol H}^+ \text{g}^{-1} \text{FM}$ ($0.2 \mu\text{mol H}^+ \text{cm}^{-2}$); (C) $100 \mu\text{mol H}^+ \text{g}^{-1} \text{FM}$ ($2.0 \mu\text{mol H}^+ \text{cm}^{-2}$); (D, E) $56 \mu\text{mol H}^+ \text{g}^{-1} \text{FM}$. Redrawn with permission from the authors.

photon flux (PPF) (Maxwell, 2002), nitrogen deficiency (Ota, 1988) and phosphorus deficiency (Paul and Cockburn, 1990), among others. Induction by external factors is generally rapid and reversible; in a classic example of plasticity, opposite leaves of *Clusia minor* may or may not perform CAM depending on the relative humidity of the air surrounding them (Schmitt *et al.*, 1988); also, the CO_2 exchange pattern of droughted plants of *Talinum triangulare* 24 h after re-watering reverted from idling to purely daytime assimilation (Herrera *et al.*, 1991).

In perennial obligate CAM plants, such as cacti, up to 99 % of CO_2 assimilation occurs during the night (Nobel, 1988), thus supporting the hypothesis that CAM is adaptive because it allows CO_2 fixation during the part of the day with lower evaporative demand. Additionally, in many species of obligate CAM plants, watering enhances nocturnal CO_2 fixation, as in the case of *Opuntia basilaris* (Hanscom and Ting, 1978). By comparison, the adaptive advantages, i.e. increased fitness, of facultative CAM and CAM-cycling are not obvious, since, even though it may be the sole means of CO_2 acquisition in stressed plant, drought-induced dark CO_2 fixation is either a small proportion of C_3 CO_2 assimilation in watered plants, as in *Delosperma tradescantoides* (Herppich *et al.*, 1996) or a significant proportion but occurs only during a relatively short period (days), as in *C. minor* (Franco *et al.*, 1990).

This review discusses examples of facultative CAM and CAM-cycling under water deficit and the implications of the functioning of these modes, distinguishing between traits associated mainly with survival or reproduction. Included will be some cases of CAM-cycling, since the relationship between increased WUE and fitness of individuals of species with this mode is, in my opinion (see also Martin, 1996), not fully understood. Also, in some reports authors seem to have had difficulty in distinguishing between facultative CAM and CAM-cycling [e.g. Martin *et al.* (1990, in reference to *T. paniculatum* as opposed to Güerere *et al.* (1996)], given the occurrence of ΔH^+ together with very low nocturnal CO_2 uptake under water deficit. Some of these cases may also be encompassed in the category of weak CAM. Obligate weak CAM is not touched because this mode may operate in watered plants and this review is centred on the question of CAM induction.

One of the criteria for assessing the operation of facultative CAM is the $\delta^{13}C$ value of dry mass of photosynthesizing organs. The $\delta^{13}C$ alone does not distinguish between field-grown C_3 plants and plants that acquire up to one-third of their carbon through nocturnal CO_2 fixation, as is the case for weak-CAM and facultative CAM plants (Winter and Holtum, 2002). Since the low dark CO_2 fixation rates measured in the field may be at the end of the resolution of currently used portable infrared gas analysers, the limit between facultative CAM, CAM-cycling and weak CAM plants become blurry and modes are arguable.

In the rest of this review, the relationship between drought-induced CAM and carbon balance, reduction of respiratory CO_2 loss, succulence, water saving, water absorption, photo-protection and reproduction will be examined, as well as the impact of the duration of induced CAM. Many authors have interpreted facultative CAM and CAM-cycling as mechanisms for avoiding respiratory CO_2 losses, enabling water saving and extending the life cycle, hence favouring reproduction under stressful conditions. Evidence in support of these hypotheses is discussed below.

Aspects related to CAM induction by factors other than water deficit, such as photoperiod, mineral nutrition and submergence – important as they are – will not be covered here since they do not always imply stress. The emphasis will be on terrestrial plants exposed to environmental conditions that propitiate plant water deficit, with its consequences on carbon acquisition, water status and metabolism.

TABLE 1. Maximum proportion of C balance contributed by nocturnal CO₂ fixation relative to daytime carbon balance of watered plants and the relative carbon isotope composition (where available) of several facultative CAM species measured under controlled (C) and field (F) conditions

Species*	C balance (%) [†]	Condition [‡]	δ ¹³ C (‰) [‡]	Reference [§]
<i>Delosperma tradescantioides</i>	2	C	na	Herppich <i>et al.</i> (1996)
<i>Sedum album</i>	2	C	-24	Castillo (1996), Earnshaw <i>et al.</i> (1985)
<i>Carpobrotus edulis</i>	3	C	-25	Winter (1973), Earnshaw <i>et al.</i> (1987)
<i>Talinum paniculatum</i>	8	C	-27	Güerere <i>et al.</i> (1996), Martin <i>et al.</i> (1990)
<i>Clusia aripoensis</i>	8	F	-25	Borland <i>et al.</i> (1998), Lüttge (2007)
<i>Mesembryanthemum crystallinum</i>	10	F	-14	Bloom and Troughton (1979)
	6	C	-16	Winter and Gademann (1991)
<i>Sedum pulchellum</i>	10	F	-30	Smith and Eickmeier (1983)
<i>Sedum mitte</i>	12	F	-26	Schuber and Kluge (1981)
	5	C		Schuber and Kluge (1981)
<i>Callisia fragans</i>	12	C	-23	Martin <i>et al.</i> (1994), Martin <i>et al.</i> (1990)
<i>Talinum triangulare</i>	12	C	-23	Herrera <i>et al.</i> (1991)
<i>Sedum nuttallianum</i>	12	C	-30	Martin and Jackson (1986)
<i>Grahamia coahuilensis</i>	13	C	-24	Guralnick <i>et al.</i> (2008)
<i>Grahamia bracteata</i>	14	C	-24	Guralnick <i>et al.</i> (2008)
<i>Talinum calycinum</i>	14	C	-28	Martin and Zee (1983)
<i>Peperomia campotricha</i>	15	C	-28	Helliker and Martin (1997), Ting <i>et al.</i> (1985)
<i>Cissus trifoliata</i>	23	C	na	Olivares <i>et al.</i> (1984)
<i>Portulacaria afra</i>	23	F	-17	Guralnick <i>et al.</i> (1984a), Guralnick <i>et al.</i> (1984b)
	29	C		Guralnick <i>et al.</i> (1984b)
<i>Sedum acre</i>	26	F	-27	Schuber and Kluge (1981)
<i>Ceraria fruticulosa</i>	39	C	-21	Veste <i>et al.</i> (2001), Rundel <i>et al.</i> (1999)
<i>Clusia uvitana</i>	47	F	-23	Zotz and Winter (1993), Winter <i>et al.</i> (2005)
	21	C		Winter <i>et al.</i> (1992)
<i>Sedum telephium</i>	57	C	-30 to -25	Lee and Griffiths (1987), Borland (1996)
	7	C		Borland (1996)
<i>Sedum wrightii</i>	66	C	-23 to -14	Gravatt and Martin (1992), Kalisz and Teeri (1986)
<i>Clusia minor</i>	76	F	-27	Franco <i>et al.</i> (1990), Lüttge (2007)
	59	F		Borland <i>et al.</i> (1998)

* Species are listed in order of increasing proportion of nocturnal carbon balance.

[†] Values of relative carbon balance are either reported in or calculated from publications.

[‡] Values or δ¹³C are rounded-up averages of values reported under field (F) or controlled (C) conditions. na, Not available.

[§] Where two references are given the first is for C balance, the second for δ¹³C.

CARBON BALANCE

Eighty-three per cent of 23 facultative CAM and CAM-cycling species examined fix during the night in the field or in experiments with controlled water deficit only up to one-quarter, with a mean of 11 %, of the daily CO₂ balance of watered plants (Table 1). The adaptive value of such a relatively small contribution to carbon budget has been questioned by Zotz (2002), who also proposed that a continuum between C₃ and CAM exists, and considered that terminology may obscure our understanding of CAM. This is further supported by the observation that plants such as *Sedum telephium* (see Table 1) show either a very small or a very large proportion of the daily carbon balance fixed during the night, denoting extreme variability in CAM performance.

In four of the species examined, dark CO₂ fixation under water deficit represents from 39 % to 76 % of the carbon balance of watered plants (Table 1) but this process goes on for only a matter of days (Brulfert *et al.*, 1988; Franco *et al.*, 1990; Zotz and Winter, 1993). Therefore, the impact of CAM on growth of facultative plants is, in comparison with obligate CAM plants, only marginal. Nevertheless, a low dark CO₂ fixation and/or recycling of respiratory CO₂ may help maintain a positive carbon balance (or at least no C losses) under stress. Exceptions are the facultative species,

Mesembryanthemum crystallinum (Bloom and Troughton, 1979) and *M. nodiflorum* (Winter and Troughton, 1978), in which, once induced, CAM is the sole CO₂ fixation pathway and operates for a considerable length of time (months).

The great majority of species examined (Table 1) show low δ¹³C values that indicate little contribution of CAM to biomass production, but there are exceptions. In *M. crystallinum* dark CO₂ fixation rate in the field satisfactorily accounted for relative growth rate and was higher than the photosynthetic rate of sympatric C₃ species (Bloom and Troughton, 1979). Values of δ¹³C increased during the life cycle of *M. crystallinum* and *M. nodiflorum* from around -26 ‰ to -14 ‰ (Winter and Troughton, 1978; Bloom and Troughton, 1979), highlighting the importance of nocturnal CO₂ fixation for biomass production in these species.

REDUCTION OF RESPIRATORY CO₂ LOSS

Recycling of respired CO₂ during the night seems to be a universal role of CAM. Even though no nocturnal CO₂ uptake may be observed, in facultative and cycling CAM species the imposition of water deficit results in maintenance or increases in ΔH⁺, indicating that an internal source of CO₂ is being used for the carboxylation of PEP. A small respiration

rate (<14 % of maximum daytime assimilation rate) or no nocturnal CO₂ loss is found in facultative and CAM-cycling species, such as *Sedum acre* (Schuber and Kluge, 1981), *Sedum pulchellum* (Smith and Eickmeier, 1983; Martin *et al.*, 1988), *Cissus trifoliata* (Olivares *et al.*, 1984), *T. triangulare* (Herrera *et al.*, 1991), *Delosperma tradescantioides* (Herppich *et al.*, 1996) and *Grahamia bracteata* (Guralnick *et al.*, 2008). Particularly under stressful conditions, recycling has been attributed the important role of reducing CO₂ loss due to nocturnal respiration, which partially ameliorates damage to plant metabolism. Calculations made with published data on these species indicate that nocturnal CO₂ loss of watered plants amounts to 6–20 % of diurnal balance. Since recycling of respiratory CO₂ in droughted plants may be as high as 100 % (e.g. in *T. triangulare*; Herrera *et al.*, 1991), this process is potentially saving on average 10 % CO₂ that would otherwise be lost. In contrast, the value of recycling in watered CAM-cycling plants has been challenged by Martin (1996) on the grounds of the parity of energetic costs of daytime CO₂ fixation versus recycling.

Internal recycling of respiratory CO₂ may also contribute to growth. In plants of *S. telephium* subjected to water deficit under high or low PPF, an enhancement of CAM-cycling at high PPF not only helped water conservation but also allowed continued export of carbon to sinks and possibly continued growth (Borland, 1996). Under severe water deficit and low PPF which may limit photosynthesis, CAM served mainly as a maintenance mechanism providing a positive carbon balance through recycling of carbon skeletons at the expense of growth.

SUCCULENCE AND WATER SAVING

Survival may be increased in CAM plants thanks to increased WUE and succulence, which may delay dehydration. When four populations of the inducible species, *Sedum wrightii*, were compared, there was a correlation between leaf thickness and $\delta^{13}\text{C}$, the population with the thicker leaves showing the highest values of $\delta^{13}\text{C}$, and the population with the thinner leaves the lowest (Kalisz and Teeri, 1986). In contrast, in a survey of 214 orchid species, many of which showed obligate weak CAM (plants were watered daily), it was found that thick-leaved species had high $\delta^{13}\text{C}$ and ΔH^+ but there were exceptions in which thin leaves had high ΔH^+ and the species with the thickest leaves, medium ΔH^+ (Silvera *et al.*, 2005). Re-examination of data provided by these authors shows that $\delta^{13}\text{C}$ values grouped relative to a leaf thickness range of 0.2–4.0 mm in two clusters, one in the range of –18.1 to –11.8 ‰ and the other cluster in the range of –32.3 to –18.9 ‰, indicating that leaf thickness had apparently little to do with the degree of CAM. The authors acknowledge that anatomical studies are necessary to rule out that succulence is due to the presence of non-photosynthetic water-accumulating tissues.

In a study undertaken in the succulent Karoo – the very dry desert in South Africa and Namibia where most plants are succulent – the assumption that obligate CAM species have more succulent leaves than facultative CAM plants did not prove true; of three species studied, the least flexible one in the

expression of CAM had the least succulent leaves (Veste *et al.*, 2001). Examining the relationship between leaf anatomical traits and the operation of CAM, Nelson *et al.* (2005) found that leaves were thicker and with larger cells in CAM species than in C₃ or C₄ species, but in five out of 17 CAM species this variable did not differentiate them from C₃ or C₄ species; these CAM species with intermediate thickness were possibly C₃-CAM. Facultative CAM is present mostly, with the possible exception of the arboreal genera *Clusia*, in which many species have a leaf hydrenchyma (Lüttge, 2007) and *Pereskia* (Edwards and Díaz, 2006), in ephemeral or deciduous perennial herbs and shrubs in which leaves tend to be less fleshy than in obligate CAM plants such as many Crassulaceae. The conclusion is that small succulence is not a characteristic feature of facultative CAM.

One variable that has proved useful in assessing the operation of CAM is the mesophyll succulence index, S_m , i.e. the ratio g leaf water : mg leaf chlorophyll (Kluge and Ting, 1978); a plant is considered CAM when S_m is higher than unit. In the inducible species *C. trifoliata* (Olivares *et al.*, 1984), *T. triangulare* (Herrera *et al.*, 1991) and *T. paniculatum* (Güerere *et al.*, 1996), S_m was always higher than 1. The determination of S_m in the photosynthetic tissues of facultative CAM species should help establish the relationship between succulence and facultative CAM.

The operation of CAM is commonly induced by a few days of water deficit and CO₂ fixation rapidly reverts to C₃ upon watering (Herrera *et al.*, 1991; Zotz and Winter, 1993). Under extended drought, the idling mode sets in. Thus, in the case of short duration of the CAM phase, the period during which a water-saving mechanism of carbon acquisition operates may be too short to contribute significantly to survival or leaf maintenance.

Potential water saving through the operation of CAM was only 5 % in plants of *T. calycinum* (Martin *et al.*, 1988), whereas in *S. telephium* it ranged from 4 % to 77 % of daytime transpiration (Borland, 1996); in *T. paniculatum* water saved through recycling was between 5 and 12 times the amount lost by transpiration (Güerere *et al.*, 1996). In contrast, Gravatt and Martin (1992) found no evidence of water conservation associated with CAM-cycling in several species of *Sedum*; similarly, Helliker and Martin (1997) found no significant differences in integrated WUE between a C₃ and a C₃-CAM species of *Peperomia* with similar life forms.

Morphological adaptations in CAM plants that contribute to increase WUE may be common to CAM and non-CAM species, thus obscuring the interpretation of the impact of CAM on WUE (Helliker and Martin, 1997). CAM induction ran parallel to increased leaf rolling in both *T. triangulare* (Herrera *et al.*, 1991) and *T. paniculatum* (Güerere *et al.*, 1996) and both mechanisms may have aided in the increased WUE. Unless manipulations of morphological characteristics, or comparison with congeners lacking them, are carried out, distinguishing between their effect and those of CAM on plant performance (leaf duration, drought tolerance, among others) may lead to erroneous conclusions.

The increase in WUE due to the operation of CAM may range from nothing to 5-fold and a clear correlation between the magnitude of CAM induction and the resulting daily WUE has not proved universal in facultative species

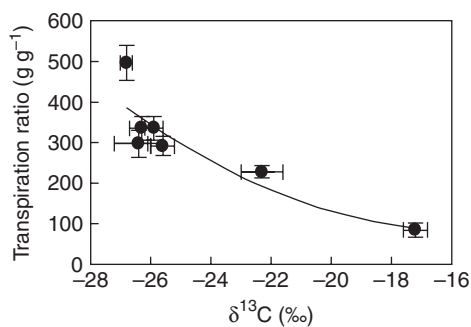


FIG. 2. Relationship between transpiration ratio (mass H₂O transpired : biomass) and carbon isotopic composition in several species of *Clusia*. Values are mean \pm 1 s.e. The determination coefficient is $r^2 = 0.79$. Data in Winter *et al.* (2005).

(Cushman and Borland, 2002). WUE was 6 (watered plants) and 12 (droughted plants) times higher during the night than the day in the obligate CAM species, *Cissus quadrangularis* (Virzo de Santo and Bartoli, 1996); in contrast, no difference in WUE was found between droughted plants of *Cotyledon orbiculata* (obligate CAM) and *Othonna opina* (C₃), two succulents with similar growth form (Eller and Ferrari, 1997). These observations draw attention to the risks of over-generalizations. It is noteworthy, though, that in seven facultative species of *Clusia*, there was a high ($r^2 = 0.79$) curvilinear negative regression between transpiration ratio (transpired water to biomass) and $\delta^{13}\text{C}$ (Fig. 2), supporting the hypothesis that CAM increases WUE in facultative CAM species (Winter *et al.*, 2005).

WATER ABSORPTION

CAM may not only serve for water saving but also for water collection. In droughted plants of the obligate CAM species *Senecio medley-woodii*, an increase in morning osmotic pressure and malate content correlated significantly with water uptake (Ruess *et al.*, 1988). Also, accumulation of malate in the vacuole of the obligate CAM species *Kalanchoë daigremontiana* caused a reduction in leaf sap osmotic potential, which may favour water absorption (Smith and Lüttge, 1985). A nocturnal decrease of 2.2 MPa in leaf sap osmotic potential calculated from data of ΔH^+ and sugar contents in *C. minor* (Lüttge, 2007) suggests that night-time water absorption is favoured by CAM.

Although examples of facultative CAM species similar to the findings in *S. medley-woodii* are not abundant in the literature, it is possible that the operation of CAM in facultative and CAM-cycling plants serves the same end. In *C. minor*, the proportion of daily sap flow occurring at night was strongly and positively correlated with the concentration of acid in sap taken from leaves at dawn, suggesting that the induction of CAM aided in water absorption through a decrease in leaf sap osmotic potential (Herrera *et al.*, 2008).

PHOTOPROTECTION

Ever since Osmond (1982) suggested that internal recycling of respiratory CO₂ might contribute to the protection of the

photosynthetic machinery against high irradiance with closed stomata, an appreciable amount of evidence has been gathered, some of it in facultative CAM plants, in favour of this hypothesis.

Plants of *C. minor* grown under controlled conditions and subjected to water deficit for 16 d showed a significant, albeit small, decrease in maximum quantum yield of photosystem two (PSII; $F_v/F_m = 0.80$ down to 0.75), which was interpreted as a sign of chronic photoinhibition; this was reverted after 2 d of re-watering, highlighting the potential of CAM for photoprotection and recovery in this species (Mattos *et al.*, 1999). Decreases in relative quantum yield of PSII (Φ_{PSII}) and increases in the Stern–Volmer coefficient of non-photochemical quenching (NPQ) were pronounced in phases II and IV of CAM but did not occur on phase III, when decarboxylation of organic acids probably sustained a high intercellular CO₂ concentration (C_i). The reduction in F_v/F_m was observed at a stage during the drought cycle when no CO₂ fixation took place on phase IV and quenching by slow-relaxing components (q_s) was highest.

When plants of the epiphytic bromeliads *Guzmania lingulata* (C₃) and *G. monostachia* (CAM) without or with CAM induced by high PPF were compared, electron transport rate at midday suffered a decrease of 60 % (*G. lingulata*) and 30 % (*G. monostachia* in the C₃ mode) relative to early morning and late afternoon values but remained nearly stable in *G. monostachia* in the CAM mode (Maxwell, 2002). These results lend support to the photoprotective role of internal recycling of CO₂ produced by decarboxylation of acids when stomata are closed.

In *T. triangulare*, the operation of CAM appeared to contribute to the maintenance of photosynthetic capacity, since chlorophyll content remained unchanged under drought and photosynthetic rate was, a few hours after re-watering, even higher than at the beginning of drought (Herrera *et al.*, 1991). Similarly, in plants of *T. paniculatum* droughted for 20 d, light-saturated photosynthetic rate and apparent quantum yield of PSII recovered to the values of watered plants after 24-h re-watering (Güerere *et al.*, 1996). A high and positive correlation was found in droughted plants of *T. triangulare* between de-epoxidation state of xanthophylls, NPQ and ΔH^+ (Pieters *et al.*, 2003), supporting the hypothesis of the existence of a daytime sink for the products of photochemical activity when no external CO₂ is fixed.

Although the possible relationship between zeaxanthin synthesis and ΔH^+ is not clear from these results, it is tempting to speculate that it may be at the heart of a mechanistic explanation for the observed changes in these variables with drought. The operation of CAM in *M. crystallinum* inhibits the accumulation of zeaxanthin in guard cells, thus preventing stomatal opening in response to blue light (Tallman *et al.*, 1997). The fact that CAM species close their stomata during the day may be the cause of increasing C_i and abscisic acid (ABA) (Tallman, 2004). Daytime decarboxylation combined with high rate of respiration due to high temperature would increase C_i and favour Calvin cycle activity in guard cell chloroplasts and consumption of NADPH, and prevent destruction of endogenous guard cell ABA, thus ensuring stomatal closure during the day (Tallman, 2004). This, together with the fact that zeaxanthin is a precursor of ABA

(Tallman, 2004), could help link the observed lack of zeaxanthin synthesis in the guard cell chloroplast with the decrease in whole-leaf zeaxanthin in plants of *T. triangulare* in the idling mode.

Photoprotection through the xanthophyll cycle may not necessarily be more effective in CAM than in C_3 species, as shown in a comparison of *Clusia multiflora* (obligate C_3) and *C. minor* (Lüttge, 2007). Both species have the same life form and similar large, leathery leaves. Zeaxanthin content and NPQ were equally large in both species subjected to high PPF, precluding a specific role of CAM in energy dissipation. Nevertheless, leaves of *C. multiflora* became necrotic and died when plants were maintained for a long period under high PPF, whereas leaves of *C. minor*, by switching to CAM, did not.

Another aspect in which the operation of CAM can confer photoprotection is the processing of reactive oxygen species that appear when PSII is over-energized during phase III behind closed stomata with high PPF, low C_i , low NADP content and high intercellular O_2 concentration (Niewiadomska and Borland, 2008). Plants of *M. crystallinum* with CAM induced by salinity and exposed to high ozone concentration in the air – in order to cause oxidative stress – showed no signs of oxidative damage, in contrast to plants operating in the C_3 mode, which showed severe necrosis and a 31 % reduction in F_v/F_m ; photoprotection in CAM plants was attributed to the up-regulation of CuZn-superoxide dismutase, one of the enzymes of the antioxidative response system (Hurst *et al.*, 2004). When plants of a CAM-less mutant of *M. crystallinum* and the wild type were subjected to salinity, the activities of several isoforms of superoxide dismutase used as markers for the production of reactive oxygen species increased in both genotypes, but this increase was greatest in the mutant, indicating potentially smaller oxidative load in the wild type (Borland *et al.*, 2006). However, a causal relationship between increased activity of enzymes of the antioxidative response system, the xanthophyll cycle and the operation of CAM awaits disclosure.

REPRODUCTION

The operation of CAM may be associated with an increase in fitness by increasing reproduction under drought. Exposing plants of *M. crystallinum*, with CAM induced, to CO_2 -free air during the night caused a reduction in both ΔH^+ and reproductive output, suggesting a direct relationship between the operation of CAM and fecundity (Winter and Ziegler, 1992). Moreover, CAM-less mutants of this species had lower fecundity than the wild type (Cushman *et al.*, 2008). In plants of *M. nodiflorum* (Sayed and Hegazy, 1994) both ΔH^+ and reproductive output increased during summer, and CAM induction was interpreted as a strategy to extend resource allocation to reproductive biomass and maximize reproductive yield before the end of the life-span.

Similarly, in five CAM-cycling species of *Talinum*, the ratio droughted : watered plants for reproductive, but not for vegetative, biomass was correlated with ΔH^+ (Harris and Martin, 1991). A high positive correlation was found in *T. triangulare* between ΔH^+ and reproductive effort (reproductive mass : leaf mass) under both laboratory (Taisma and

Herrera, 1998) and field conditions (Taisma and Herrera, 2003). Also, the fecundity of plants of *T. triangulare*, in which induction of CAM by drought was prevented by manipulation of the photoperiod, was drastically reduced relative to plants with CAM induced by drought under normal photoperiod (Herrera, 1999).

Another role of CAM induction by drought may be to provide an important source of energy for reproduction. Fruits of *M. crystallinum* exhibited higher $\delta^{13}C$ values than leaves (-11% vs. -13%), indicating that carbon used for inflorescence development was primarily fixed through CAM late in the growing season (Winter, 1985). In contrast, no difference was found in $\delta^{13}C$ of fruits and leaves of plants of *T. triangulare* under natural drought, suggesting that the source of carbon for fruit set was not different from that for leaf production (Taisma and Herrera, 2003) and that the contribution of dark CO_2 uptake to reproduction was as small as it was to carbon balance of leaves.

An increase in WUE of plants of *T. triangulare* subjected to natural water supply in the field, possibly due to the joint effect of the operation of CAM, leaf rolling and increased reflectance and angle, may help explain the observed increase in reproductive effort (Taisma and Herrera, 2003) and so far its is not possible to pinpoint any of the variables studied as causing increased reproduction.

DURATION OF INDUCED CAM DURING THE LIFE CYCLE

The period during which drought-induced nocturnal CO_2 fixation occurs may be long (months), as in *M. crystallinum* (Bloom and Troughton, 1979), or short (hours to a few days), as in *Clusia uvitana* (Zotz and Winter, 1993), *T. triangulare* (Herrera *et al.*, 1991), *C. minor* (Lee and Griffiths, 1987), *S. nuttallianum* (Martin and Jackson, 1986), *S. acre* and *S. mitte* (Schuber and Kluge, 1981). Inducible CAM as well as CAM-cycling may help extend the time the plant is physiologically active during prolonged drought (Martin and Jackson, 1986; Sayed and Hegazy, 1994). A frequently cited example of long-term functioning of induced CAM is that of *M. crystallinum*, in which plants begin exhibiting CAM as the dry summer season approaches and remain in this mode until the end of the life cycle. Evidence in favour of the adaptive character of the operation of CAM in plants of *M. crystallinum* and *M. nodiflorum* under dry and saline conditions is very convincing and the induction and operation of CAM by drought has been closely followed in the field (Winter and Troughton, 1978; Bloom and Troughton, 1979).

Nevertheless, many facultative CAM species, such as *C. trifoliata*, *T. triangulare* and *T. paniculatum*, are deciduous perennials which lose their leaves under extreme drought, yet show CAM after as little as 1 d under drought, and resort to idling after 10–20 d of drought. What is real life like for these species? For how long under natural conditions may induced CAM play its role in the prevention of the impairment of, or help optimize physiological performance under drought?

Species of *Sedum* and *Talinum* inhabit rock outcrops, and semi-arid as well as seasonally dry environments, and may experience short periods of drought followed by rains in an

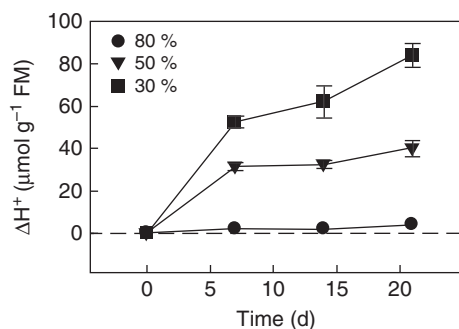


FIG. 3. Time-course of changes in nocturnal acid accumulation on a fresh mass basis in plants of the facultative CAM plant, *Talinum triangulare*, watered to 80 % (circles), 50 % (triangles) and 30 % (squares) field capacity. Values are mean \pm 1 s.e. Modified from Irazábal (2005).

unpredictable manner. The possibility exists that they spend a significant length of time in the CAM mode, given an adequately low water soil content. Watering plants of *T. triangulare* to 80 % field capacity prevented the induction of CAM, whereas 50 % and 30 % field capacity increasingly promoted nocturnal acid accumulation; ΔH^+ in the 50 % treatment tended to a plateau, whereas at 30 % it tended to increase with the duration of the dry period (Irazábal, 2005; Fig. 3). Similarly, low rates of nocturnal CO_2 uptake were observed for 80 d in plants of *S. pulchellum* maintained at a soil water potential of -5.0 to -8.5 MPa but not at -1.5 to -4.0 MPa, when either no net loss or gain or respiration were observed during the night (Smith and Eickmeier, 1983).

The above-described experiments may reflect the actual situation in the field: C_3 photosynthesis under abundant rainfall, induced CAM at a moderate level in partly wet soil for a considerable length of time, and CAM idling in a dry soil until leaf shedding occurs. As in the case of *C. minor*, in which it is so plastic, CAM may in rooted individuals make a major contribution to biomass by operating for a longer period than short-term water-deficit experiments (Franco *et al.*, 1990) or measurements on hemi-epiphytic individuals (Zotz and Winter, 1993) suggest.

Many of the data reported in the references in Table 1 were gathered in experiments done in pots to which watering was withheld and pots let to dry out. Clearly, operation of CAM in the field, with a very large rooting volume (equivalent to a very large 'pot' volume) and slow drying, continues for much longer than in pot experiments. This brings us to the issue of low $\delta^{13}\text{C}$ values in facultative CAM plants.

With the possible exception of *M. crystallinum*, facultative CAM plants tend to show $\delta^{13}\text{C}$ values which are more C_3 -than CAM-like, the extreme being found in one experiment on *S. nuttallianum* under controlled conditions, in which $\delta^{13}\text{C}$ was -30‰ [but see comment by Martin and Jackson (1986) on the carbon isotope composition of the air in this experiment] and in the study of orchids in Panama, in which a $\delta^{13}\text{C}$ value as low as -30.0‰ was associated with a significant ΔH^+ (Silvera *et al.*, 2005). Even in facultative species of *Clusia*, which may easily and reversibly shift from C_3 to CAM in a matter of days throughout the seasons, values of $\delta^{13}\text{C}$ much higher than -21‰ have not been found (Lüttge, 2007). Such low values indicate that carbon is mostly acquired

through C_3 photosynthesis and dark CO_2 fixation makes only a small contribution to $\delta^{13}\text{C}$.

Values of $\delta^{13}\text{C}$ as high as -14‰ , typical of a full CAM plant, were found in low-altitude populations of *S. wrightii*, whereas those populations at higher altitudes tended towards -23‰ , a more C_3 -like value (Kalisz and Teeri, 1986). This points to the possibility that the lowland populations spent more of their life cycle in the CAM mode than the highland ones, although the effect of altitude on air $\delta^{13}\text{C}$ and C_i should be evaluated.

CONCLUSIONS AND FUTURE RESEARCH

In the great majority of facultative CAM and CAM-cycling species examined, dark CO_2 fixation represented $<30\%$ of the daily carbon balance of watered plants. Examination, by no means exhaustive, of evidence suggests that these modes of carbon acquisition have an important role in water balance, photo-protection and reproduction. In order to aid in our understanding of these complex forms of carbon acquisition and elucidate the potential of facultative CAM for biomass (vegetative as well as reproductive) production, the following are needed.

- (1) Experiments under controlled conditions. Subjecting to water deficit CAM-deficient mutants, such as those obtained for *M. crystallinum* (Cushman *et al.*, 2008) or wild-type plants under environmental manipulations that prevent CAM induction, such as *T. triangulare* under interrupted dark periods (Herrera, 1999), and determining the impact of the lack of CAM operation on biomass maintenance/growth, photoprotection and reproduction.
- (2) Field studies. More long-term field studies are necessary on the performance of facultative, especially drought-deciduous, CAM plants when they remain for long periods under the induced state; determination of 24-h carbon balance, WUE, productivity, reproductive effort and survival in static cohorts of natural populations subjected to abiotic and biotic factors (Taisma and Herrera, 1998, 2003) could help clarify the role of induced CAM on fitness.

Among matters pending is whether facultative CAM and CAM-cycling merely represent an intermediate step between C_3 and obligate CAM possessing neutral characters that neither promote nor hinder adaptation; this issue could be settled by extended research into the evolution of CAM.

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