

Induction and reversal of crassulacean acid metabolism in *Calandrinia polyandra*: effects of soil moisture and nutrients

Klaus Winter^{A,C} and Joseph A. M. Holtum^B

^ASmithsonian Tropical Research Institute, PO Box 0843-03092, Balboa, Ancon, Republic of Panama.

^BSchool of Marine and Tropical Biology, James Cook University, Townsville, Qld 4811, Australia.

^CCorresponding author. Email: winterk@si.edu

Abstract. *Calandrinia polyandra* Benth. (Montiaceae), an annual succulent herb endemic to Australia, is an exemplary facultative crassulacean acid metabolism (CAM) plant as demonstrated by continuous whole-plant lifetime CO₂ exchange measurements under controlled conditions in the laboratory. Reduced soil water availability induced a shift from solely daytime CO₂ fixation to dark CO₂ fixation. The shift from C₃ photosynthesis to CAM was reversible either upon rewatering alone, or upon a combination of rewatering and addition of nutrients. These observations highlight the role of edaphic conditions in controlling CAM expression in a plant that has the option of fixing CO₂ either during the day or during the night, providing further evidence that this extreme form of photosynthetic plasticity is primarily controlled by the environment rather than plant ontogeny. The stimulating effect of soil nutrients on CO₂ fixation in the light and its negative effect on dark CO₂ fixation have not been described previously and deserve further attention. In the most widely used CAM model system, the halophytic *Mesembryanthemum crystallinum* L., CAM is typically induced by high salinity, and some metabolic responses may be CAM-unrelated and related to salt stress *per se*. *C. polyandra* could be an excellent complementary system for studying the biochemical and molecular foundations of CAM because drought stress elicits a complete C₃ to CAM transition.

Additional keywords: constitutive CAM, *Parakeelya*, Portulacaceae.

Introduction

Uptake of significant amounts of CO₂ in the dark, the defining characteristic of the water-conserving CAM (crassulacean acid metabolism) pathway of photosynthesis, has been reported in more than 35 families of vascular plants (Kluge and Ting 1978; Smith and Winter 1996; Holtum *et al.* 2007; Nyffeler and Eggli 2010a, 2010b). The expression of CAM is highly variable because CO₂ uptake at night and C₃ photosynthetic uptake of atmospheric CO₂ during the day are not mutually exclusive, and the relative contributions of the two to carbon gain vary with species and, within a species, may respond to environment. In most CAM-equipped plants, following an initial C₃ phase in young tissues (Winter *et al.* 2011), CAM becomes evident as photosynthetic tissues mature. In contrast to this relatively fixed ontogenetically programmed constitutive CAM, some species show CAM only in response to environmental stimuli such as drought stress (facultative CAM), thereby lowering the water expenditure of CO₂ uptake when availability of soil water is low and thus prolonging CO₂ uptake.

Facultative CAM is mainly reported in species from four taxa, the Clusiaceae, the Aizoaceae, the Crassulaceae (*Sedum* spp.) and several lineages previously circumscribed by the Portulacaceae (Smirnoff 1996; Smith and Winter 1996; Herrera 2009; Nyffeler and Eggli 2010a, 2010b). In *Clusia*, facultative CAM is exhibited

by evergreen shrubs and trees, some of which can grow as hemi-epiphytes or epiphytes with reduced contact with soil water (Lüttge 1999, 2007). Although plants of the humid tropics, these perennials experience seasonal drought, during which CAM can become the major pathway of carbon acquisition (Zotz and Winter 1993, 1994; Holtum *et al.* 2004; Winter *et al.* 2005, 2008). In some *Clusia* species, the C₃-to-CAM shift has been shown to be fully reversed following the removal of drought stress (Winter *et al.* 2009).

Within the Aizoaceae and the former Portulacaceae, features of facultative CAM have been reported from both annuals and perennials (Winter and von Willert 1972; Mooney *et al.* 1977; Ting and Hanscom 1977; Martin and Zee 1983; von Willert *et al.* 1992; Veste *et al.* 2001; Guralnick *et al.* 2008). Some of the latter are deciduous. In annuals such as *Mesembryanthemum crystallinum* L. and *Mesembryanthemum nodiflorum* L. (Aizoaceae), a shift from C₃ photosynthesis to CAM accompanies a transition during their lifecycle from an adequate soil water supply when plants germinate and are seedlings, to soil water deficit as plants mature and set seed (Winter and Troughton 1978; Winter *et al.* 1978). Under laboratory conditions, CAM can be readily induced in *M. crystallinum* by high soil salinity and *M. crystallinum* has become an experimental model for studying the biochemistry

of CAM and its molecular components. It has been suggested that this C₃-to-CAM shift represents the acceleration of an ontogenetically pre-programmed process, but there is now strong experimental evidence that the induction and upregulation of the CAM pathway in *M. crystallinum* is primarily under environmental control (Winter and Holtum 2005, 2007), although reversibility of the C₃-to-CAM shift has not been demonstrated for all stages of development. Reversibility has been reported in young leaves of *M. crystallinum* (Winter 1974; Vernon *et al.* 1988) but it is difficult to achieve in older tissues because the leaves are relatively short-lived and reversion may be obscured by tissue senescence. An additional complication of reversibility experiments using a halophyte such as *M. crystallinum* is that the abrupt switch from highly saline to non-saline growth media may osmotically damage roots and further stress the plants.

In lineages formerly ascribed to the Portulacaceae, facultative aspects of CAM have been described in annuals from *Portulaca* (Koch and Kennedy 1980; Guralnick and Jackson 1993), *Calandrinia* (Winter *et al.* 1981) and *Cistanthe* (most of which were formerly *Calandrinia*; Mooney *et al.* 1974). Within the *Cistanthe*, *Cistanthe maritima* (Nutt.) Carolin ex Hershk. (formerly *Calandrinia maritima*), had $\delta^{13}\text{C}$ values of -24.0 and -22.4‰ towards the end of its lifecycle (Mooney *et al.* 1974). A strong capacity for dark CO₂ fixation measured near the end of the lifecycle suggests that the bulk of CO₂ was fixed via C₃ photosynthesis and that CAM developed late in the lifecycle when the severity of drought increased. Similar C₃-CAM $\delta^{13}\text{C}$ values of -24.0 and -23.2‰ were reported for *Cistanthe ambigua* (S. Wats.) Carolin ex Hershk. (formerly *Calandrinia ambigua*) from Baja California, and *Cistanthe grandiflora* (Lindl.) Carolin ex Hershk. (formerly *Calandrinia grandiflora*) from Chile (Mooney *et al.* 1974; Troughton *et al.* 1974), respectively.

In Australia, *Calandrinia polyandra* Benth. (Bentham 1863; Melville 1959), an annual herb with succulent narrow leaves that grows on sandy or stony nutrient-poor soils, was suspected to exhibit CAM on the basis of leaf $\delta^{13}\text{C}$ values of between -24.7 and -22.2‰ and nocturnal increases in leaf titratable acidity of between 34 and 60 $\mu\text{eq g FW}^{-1}$ (Winter *et al.* 1981). The lifecycle and habit of *C. polyandra* in Western Australia exhibit similarities to *M. crystallinum* in that *C. polyandra* germinates and grows during the cool, wet winter, and flowers and sets seed during the subsequent warmer and drier months (Kapitany 2007, 2010). Young *C. polyandra* plants form a base of leaves from which inflorescences may develop if water supply is of short duration. If sufficient water is available, flowering may be delayed as plants develop decumbent branches that extend radially. Inflorescences subsequently develop along the branches and from the base of the plant. The succulent leaves and branches may persist for weeks after the soil dries out.

Here, we quantify CO₂-exchange throughout the lifecycle of plants, and demonstrate that *C. polyandra* starts as a C₃ plant and that CAM can be induced following drought stress. We exploit certain characteristics of *C. polyandra*, such as the longevity of leaves and the ability to induce substantial activity of CAM by drought stress alone, to explore the capacity to return to C₃ after CAM has been induced. We show for the first time in an annual species that reversibility is possible in young and mature plants,

and highlight the role of nutrient supply in the transition from CAM back to C₃. *C. polyandra* may have potential as a new model system for studying CAM.

Materials and methods

Plant material and net CO₂ exchange

Seeds of *Calandrinia polyandra* Benth. were collected in Western Australia at 27°48'42.29"S, 114°28'26.74"E, a site close to the western edge of the range of *C. polyandra* which extends ~3000 km from the Western Australian coast to the New South Wales–South Australia border, between longitudes 113°E and 141°E, and ~1500 km between latitudes 23°S and 32°S (Australia's Virtual Herbarium 2011). Seeds were germinated in moist Jiffy pellets (Hummert International, Earth City, MO, USA). The seedlings (plus pellets) were transferred to terracotta pots (1.2 L) or plastic pots (3 L) containing potting mix (Cactus, Palm and Citrus Soil, Miracle-Gro Lawn Products, Marysville, OH, USA). Osmocote Plus fertiliser (Scotts-Sierra Horticultural Products, Marysville, OH, USA) was added during fertilisation experiments. Cotyledons attached to the plants were enclosed into a Perspex cuvette (internal dimensions of 30 × 30 × 15 cm) by passing the hypocotyl carrying the two cotyledons through a 1-cm diameter hole in the cuvette base and sealing the hypocotyl–cuvette interface with a non-porous synthetic rubber sealant (Fig. 1; Terostat VII, Henkel-Teroson, Heidelberg, Germany). The roots plus pot remained outside the cuvette.

The gas exchange cuvette was located inside a controlled environment chamber (Environmental Growth Chambers, Chagrin Falls, OH, USA) operating under 12 h light (25°C):12 h dark (17°C) cycles. Photon flux density was 420 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the cuvette. The cuvette was supplied either with ambient air drawn from a 1-m³ container placed on an eight-floor tall building to dampen short-term fluctuations in [CO₂], or with air containing 400 ppm CO₂ generated by a mass-flow controlled CO₂:CO₂-free air mixing unit (GMA-3/10, Walz GmbH, Effeltrich, Germany). The flow



Fig. 1. A flowering *Calandrinia polyandra* growing inside a Perspex CO₂ exchange cuvette in a growth chamber. The roots and pot are outside the cuvette but inside the chamber. Cuvette internal width = 30 cm.

rate of air through the cuvette was between 2.3 and 4.4 L min⁻¹ and the dew point of the air entering the cuvette was 15°C. The dew point of the air leaving the chamber depended upon plant size. Net CO₂ exchange of the developing plants was measured in a flow-through gas exchange system consisting of Walz components (Walz GmbH) and a LI-6252 CO₂ analyzer (Li-Cor, Lincoln, NE, USA) (Holtum and Winter 2003). Drought treatments were imposed by withholding irrigation.

Nomenclatural considerations

Following mounting recognition that the traditional family Portulacaceae (suborder Portulacinae) was an assemblage of different evolutionary lineages (McNeill 1974; Carolin 1987; Nyananyo 1990; Applequist and Wallace 2001), a recent molecular and comparative morphological phylogenetic revision redistributed the taxa previously within the Portulacaceae among eight families: the Anacampserotaceae, Basellaceae, Cactaceae, Didiereaceae, Halophytaceae, Montiaceae, Portulacaceae and Talinaceae (Nyffeler and Eggli 2010a). *Calandrinia*, now placed within the family Montiaceae, had previously been reduced following subdivision into six genera: *Anacampseros*, *Calandrinia*, *Cistanthe*, *Montiopsis*, *Schreiteria* and *Talinum* (Carolin 1987; Hershkovitz 1991, 1993a, 1993b). The remaining *Calandrinia* comprise two separate lineages: a well-defined lineage containing 14 species native to the Americas, and a less well-defined lineage of 35–50 species native to Australia (Hershkovitz 1993a, 1993b; Hershkovitz and Zimmer 1997; Obbens 2006). The New World lineage has retained the name *Calandrinia*, whereas two generic names have been proposed for the Australian species, *Rumicastrum* (Carolin 1987) and *Parakeelya* (Hershkovitz 1998). In the absence of a consensus for either name (Hershkovitz 1998; Obbens 2006), and in the absence of a significant cladistic or genetic study that circumscribes the relatively character-diverse Australian species, here we retain the name *Calandrinia* for the Australian species.

Results

In an experiment that broadly simulated the wet-to-dry transition of its natural environment, *C. polyandra* completed its lifecycle in 4 months (Fig. 2). At first, carbon gain was confined to the light. A following transitional phase of carbon gain in the light and in the dark led to a final extended period dominated by carbon gain in the dark.

Initially, when water supply was adequate and plant size increased rapidly, both CO₂ uptake during the light and CO₂ loss during the dark progressively increased, consistent with the operation of the C₃ pathway. The first obvious response to drought was observed 14 days after watering ceased, when nocturnal CO₂ balance started to become less negative (Day 51). Nocturnal CO₂ balance turned positive following the collapse of carbon gain in the light after Day 55, which coincided with the start of flowering. Dark CO₂ fixation became the predominant mode of CO₂ fixation after Day 60 and remained so. Nocturnal carbon gain maintained a maximum for about 2 weeks and gradually declined thereafter until it approached zero, 80 days after the imposition of drought.

Figure 3 illustrates the contrasting 24 h CO₂ exchange patterns exhibited before and during the stress treatment by the *C. polyandra* portrayed in Fig. 2. The 24 h pattern of CO₂

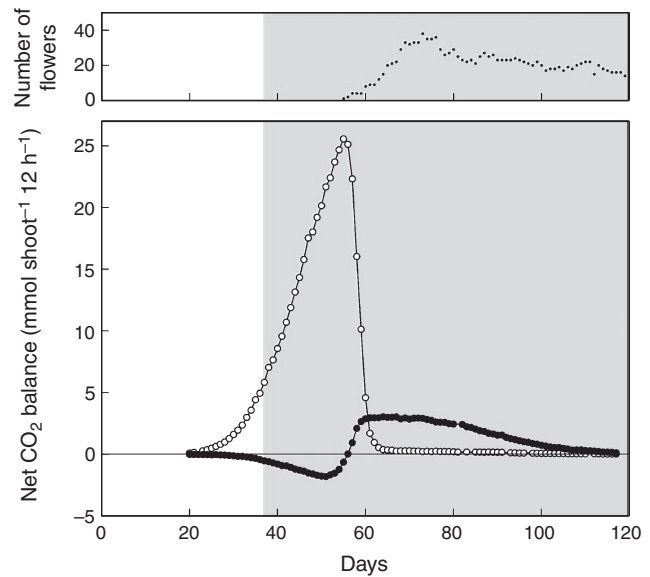


Fig. 2. Net CO₂ balance in the dark (closed symbols) and in the light (open symbols) during the lifecycle of *Calandrinia polyandra* (lower panel). The numbers of open flowers are shown in the upper panel. The shaded area indicates the period without irrigation. The plant was grown in a 3-L plastic pot in fertilised soil.

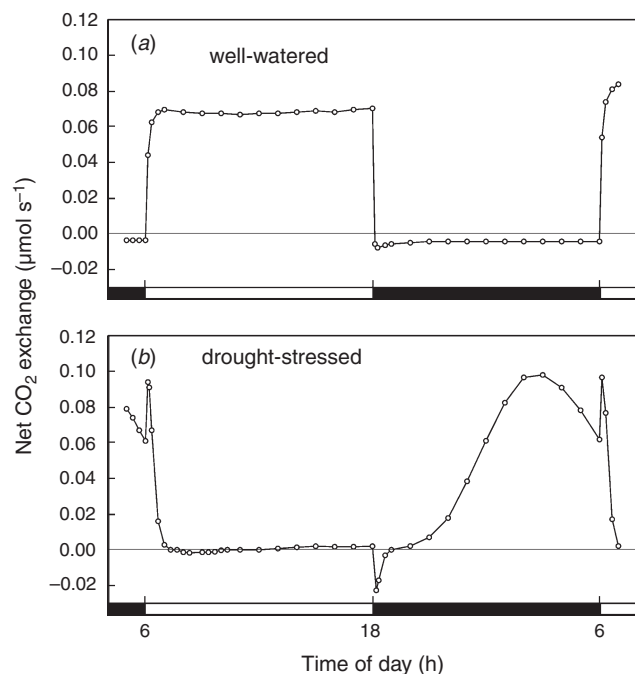


Fig. 3. The diel (24 h) light-dark patterns of net CO₂ exchange of *Calandrinia polyandra* for 2 days of the experiment shown in Fig. 2. (a) The plant after 33 days under well-watered conditions; (b) the 83-day-old plant, from which water had been withheld for 46 days. Open bars, 12 h light periods; closed bars, 12 h dark periods.

exchange on Day 33 was that of a C_3 plant, characterised by a constant rate of CO_2 uptake during the day and a generally constant rate of respiratory CO_2 loss in the dark, reflecting the controlled light and temperature conditions within the gas exchange cuvette. On Day 88, the 24-h pattern of CO_2 exchange was dominated by CO_2 uptake in the dark, characteristic of a plant with pronounced CAM.

Reversibility of the drought-induced C_3 -to-CAM shift was easily demonstrable in relatively young plants (Fig. 4). Following a shift from C_3 to CAM in response to drought, rewatering led to a rapid increase of CO_2 fixation in the light that was accompanied by a change from carbon gain to carbon loss in the dark.

Our attempts to assess the ability of whole shoots of non-stressed *C. polyandra* to operate exclusively as C_3 plants throughout their lifecycles were confounded by the indeterminate extensive growth of well-watered and well fertilised plants that rapidly outgrew the capacity of the gas exchange system to handle the correspondingly large fluxes of CO_2 and water vapour. We tried to overcome this problem by growing well-watered plants in relatively small soil volumes without the addition of extra fertiliser (Fig. 5). Under these conditions (1.2-L terracotta pot), C_3 photosynthetic CO_2 uptake in the light remained the principal pathway of CO_2 fixation. However, despite being well-watered, plants gradually developed low levels of CAM, which either reduced nocturnal carbon losses or led to small positive carbon balances. In the experiment of Fig. 5, when water was withheld from such a mature 120-day-old plant with low-level CAM, CAM activity was again strongly upregulated while light CO_2 fixation collapsed. Again, dark CO_2 uptake eventually diminished as stress intensified. Rewatering of this relatively old plant

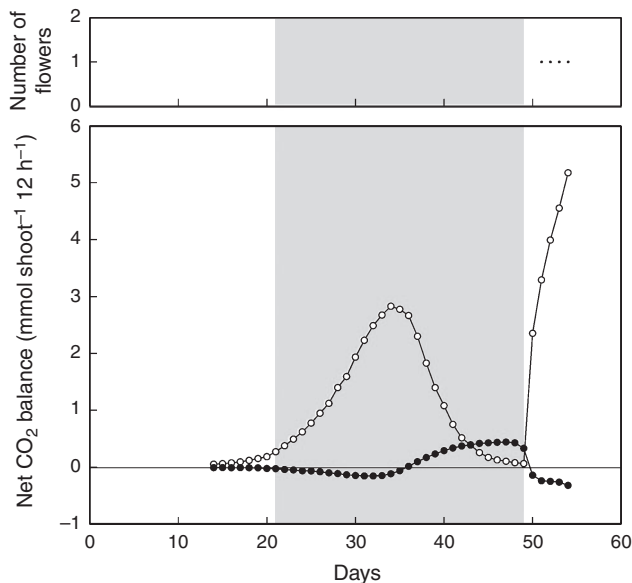


Fig. 4. Effect of drought stress and rewatering on net CO_2 balance in the dark (closed symbols) and in the light (open symbols) in a relatively young *Calandrinia polyandra* (lower panel). The numbers of open flowers are shown in the upper panel. Watering was stopped on Day 21 and resumed on Day 49. The plant was grown in a 1.2-L terracotta pot in soil without additional fertiliser. The shaded area indicates the period without irrigation.

promptly stimulated the C_3 pathway, such that within 4 days, CO_2 uptake in the light had increased to close to pre-stress levels, but there was little change in dark CO_2 fixation. CAM was rapidly lost, however, upon the subsequent application of Osmocote Plus fertiliser (Scotts-Sierra Horticultural Products), a treatment which abolished nocturnal net dark CO_2 fixation and dramatically boosted CO_2 uptake in the light.

We further corroborated the nutrient effect on CAM reversibility using a 77-day-old well-watered plant that had developed low-level CAM while growing in a small volume of soil without the addition of extra fertiliser (Fig. 6). Flushing of the soil with 20 mM KNO_3 resulted in the loss of net dark CO_2 fixation within 48 h (Fig. 6, Day 80). After 1 week, the daily CO_2 exchange pattern was characterised by the C_3 photosynthetic pattern of constant rates of CO_2 uptake in the light and relatively constant rates of respiratory CO_2 loss in the dark.

Discussion

These data highlight the predominant role of soil conditions in the control of the expression of photosynthetic pathway in a plant that has the option of fixing CO_2 either during the day or during the night, and strengthen the argument that facultative CAM is principally under environmental governance.

Our continuous long-term measurements provide a detailed record of the response kinetics of daytime and night-time fixation in *C. polyandra* to changes in the environment. When *C. polyandra* switches from C_3 photosynthesis to CAM, it

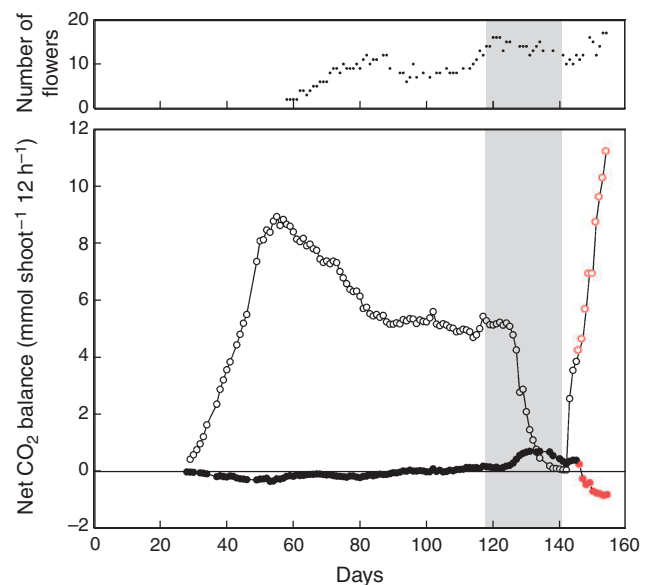


Fig. 5. Net CO_2 balance in the dark (closed symbols) and in the light (open symbols) during 154 days of the lifecycle of a *Calandrinia polyandra* (lower panel). The plant was grown in a 1.2-L terracotta pot without additional fertiliser and kept well watered until day 117. The plant was not watered between Day 118 and Day 141. The plant was rewatered on Day 142; on Day 146, 5 g Osmocote Plus fertiliser were added (red symbols). The shaded area indicates the period without irrigation. Due to technical problems with the controlled environment chamber, between Days 34 and 47 night-time temperatures were between 20 and 22°C instead of 17°C.

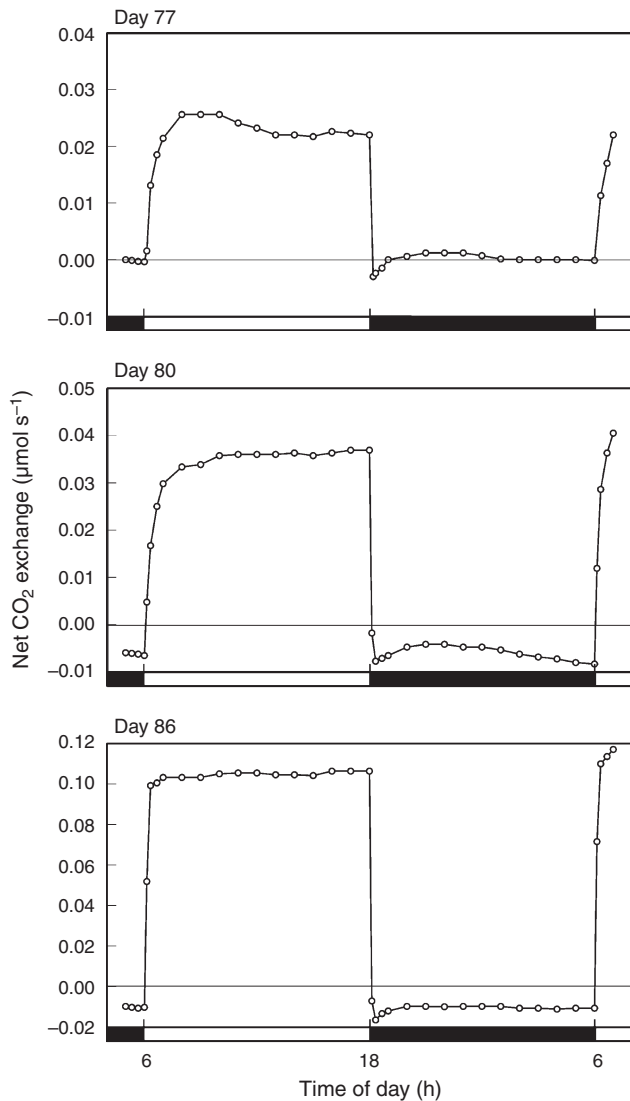


Fig. 6. The effect of KNO₃ fertilisation on diel (24 h) light–dark patterns of net CO₂ exchange of a well-watered *Calandrinia polyandra*. The upper panel depicts CO₂ exchange on Day 77 after germination, before the KNO₃ treatment. The soil was flushed with 20 mM KNO₃ on Days 78 and 79. The middle and lower panels show day–night CO₂ exchange 2 days and 8 days after initiation of the KNO₃ treatment respectively. The plant was grown in a 1.2-L terracotta pot without additional fertiliser until the KNO₃ treatment. Open bars, 12 h light periods; closed bars, 12 h dark periods.

does so quite rapidly and, if conditions are favourable, the switch back can also be rapid. Once in the CAM mode, plants can have a positive carbon balance for weeks without any supply of water.

The gradual development of low-level CAM observed in well-watered plants in some experiments appears, at first sight, to support the idea that development of CAM in *C. polyandra* has an ontogenetic age-related component that is independent of environmental influences. However, in these experiments, plants were grown in small 1.2-L pots that were flushed with 0.6 L H₂O every two days, which raises the possibility that the slow appearance of low-level CAM is the response to nutrient leaching. Indeed – and this is the most remarkable result of this

study – addition of nutrients either in the form of Osmocote Plus (Scotts-Sierra Horticultural Products) or in the form of KNO₃ eliminated this ‘age-related’ CAM and also enabled the reversibility of CAM in old, previously drought-stressed plants when rewatering alone was insufficient. Furthermore, plants that were well fertilised remained in the C₃ mode for as long as we were able to monitor their CO₂ exchange, i.e. for up to about 3 months, when flowering had commenced (data not shown). Although generally considered an annual plant, when well watered and well fertilised, vegetative growth in *C. polyandra* can markedly exceed 1 year, making it difficult to demonstrate that plants remain in the C₃ mode throughout their lifecycle, which is the ultimate proof for showing that CAM induction in *C. polyandra* is solely under environmental control.

This study is the first to show that soil nutrient supply affects the balance between CAM and C₃ photosynthesis. Previous studies with constitutive CAM plants have shown that nocturnal acidification increases in nutrient-rich soil (Winter 1985; Nobel 1988), but this is the first demonstration of an effect on the balance between light and dark CO₂ fixation in a facultative species. Future research needs to demonstrate whether the N that is added leads to a preferential increase in Rubisco protein as compared with phosphoenolpyruvate carboxylase, and/or whether the nutrient effect is based upon an increased supply of K⁺ that is required for osmotic adjustment and turgor maintenance.

Calandrinia may be of use as a CAM model system in biochemical and ecological contexts. Hitherto, salinity-induced CAM in the halophytic aroid *M. crystallinum* has been widely used as a study system to assist the identification of the biochemical and molecular requirements for CAM. However, it has not always been clear whether some of the changes occurring during the C₃-to-CAM shift are CAM-unrelated salinity effects *per se*. Using *C. polyandra* as a complementary study system, in which CAM can be readily induced by drought stress, will reduce such ambiguities.

In an ecological context, Australian *Calandrinia* species have been collected across the continent from the east coast, through the arid inlands to the west coast, and between latitudes ranging from temperate Tasmania to the monsoon-dominated tropics. A detailed survey of these species, which include annual, perennial and tuberous forms (Pate and Dixon 1981; Obbens 2006; Kapitany 2007), correlating life-form, seasonal activity, carbon isotopic signal and other photosynthetic features could add to our understanding of the functional significance of CAM. It is noteworthy that a *C. polyandra* collected by an English privateer, William Dampier, during his second visit to ‘New Holland’ in 1699 (Dampier 1703) is one of the oldest Australian plants in a herbarium (OXF; Fielding-Druce Herbarium, Oxford) and is the first CAM plant collected by a European in Australia.

Acknowledgements

This research was supported by funds from the Smithsonian Tropical Research Institute. JAMH was supported by the JCU Special Studies Program. We acknowledge the informed advice about *Calandrinia polyandra* and its habitats provided by Kingsley Dixon (Director, Science, Kings Park and Botanic Garden, Perth, WA) and Frank Obbens (Western Australian Herbarium, Perth, WA).

References

- Applequist WL, Wallace RS (2001) Phylogeny of the portulacaceous cohort based on *ndhF* sequence data. *Systematic Botany* **26**, 406–419.
- Australia's Virtual Herbarium (2011) Council of Heads of Australasian Herbaria Inc.: Canberra. Available at <http://avh.rb.g.vic.gov.au/avh/> [Verified 22 January 2011].
- Bentham G (1863) 'Flora Australiensis: a description of the plants of the Australian territory. Volume 1: Ranunculaceae to Anacardiaceae.' (Lovell Reeve and Co: London)
- Carolin RC (1987) A review of the family Portulacaceae. *Australian Journal of Botany* **35**, 383–412. doi:10.1071/BT9870383
- Dampier WA (1703) 'A voyage to New Holland etc in the year 1699.' (James Knapton: London)
- Guralnick LJ, Jackson MD (1993) Crassulacean acid metabolism activity in the family Portulacaceae. *Plant Physiology* **102**, 139.
- Guralnick LJ, Cline A, Smith M, Sage RF (2008) Evolutionary physiology: the extent of C₄ and CAM photosynthesis in the genera *Anacampseros* and *Grahamia* of the Portulacaceae. *Journal of Experimental Botany* **59**, 1735–1742. doi:10.1093/jxb/ern081
- Herrera A (2009) Crassulacean acid metabolism and fitness under water deficit stress: if not for carbon gain, what is facultative CAM good for? *Annals of Botany* **103**, 645–653. doi:10.1093/aob/mcn145
- Hershkovitz MA (1991) Phylogenetic assessment and revised circumscription of *Cistanthe* Spach (Portulacaceae). *Annals of the Missouri Botanical Garden* **78**, 1009–1021. doi:10.2307/2399741
- Hershkovitz MA (1993a) Revised circumscriptions and subgeneric taxonomies of *Calandrinia* and *Montiopsis* (Portulacaceae) with notes on phylogeny of the portulacaceous alliance. *Annals of the Missouri Botanical Garden* **80**, 333–365. doi:10.2307/2399789
- Hershkovitz MA (1993b) Leaf morphology of *Calandrinia* Kunth and *Montiopsis* Kuntze (Portulacaceae). *Annals of the Missouri Botanical Garden* **80**, 366–396. doi:10.2307/2399790
- Hershkovitz MA (1998) *Parakeelya*: a new genus segregated from *Calandrinia* (Portulacaceae). *Phytologia* **84**, 98–106.
- Hershkovitz MA, Zimmer EA (1997) On the evolutionary origins of the cacti. *Taxon* **46**, 217–242. doi:10.2307/1224092
- Holtum JAM, Winter K (2003) Photosynthetic CO₂ uptake in seedlings of two tropical tree species exposed to oscillating elevated concentrations of CO₂. *Planta* **218**, 152–158. doi:10.1007/s00425-003-1089-1
- Holtum JAM, Aranda J, Virgo A, Gehrig HH, Winter K (2004) δ¹³C values and crassulacean acid metabolism in *Clusia* species from Panama. *Trees – Structure and Function* **18**, 658–668.
- 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. doi:10.3732/ajb.94.10.1670
- Kapitany A (2007) 'Australian succulent plants.' (Kapitany Concepts: Boronia, Australia)
- Kapitany A (2010) The Australian *Calandrinia*. Spinette July 2010. (The Cactus and Succulent Society of Australia Inc.: Braeside)
- Kluge M, Ting IP (1978) 'Crassulacean acid metabolism.' (Springer-Verlag: Berlin)
- Koch K, Kennedy RA (1980) Characteristics of crassulacean acid metabolism in the succulent C₄ dicot, *Portulaca oleracea* L. *Plant Physiology* **65**, 193–197. doi:10.1104/pp.65.2.193
- Lüttge U (1999) One morphotype, three physiotypes: sympatric species of *Clusia* with obligate C₃ photosynthesis, obligate CAM and C₃-CAM intermediate behaviour. *Plant Biology* **1**, 138–148. doi:10.1111/j.1438-8677.1999.tb00237.x
- Lüttge U (2007) 'Clusia: a woody neotropical genus of remarkable plasticity and diversity.' (Springer: Berlin)
- Martin CE, Zee AK (1983) C₃ photosynthesis and crassulacean acid metabolism in a Kansas rock outcrop succulent, *Talinum calycinum* Engelm (Portulacaceae). *Plant Physiology* **73**, 718–723. doi:10.1104/pp.73.3.718
- McNeill J (1974) Synopsis of a revised classification of the Portulacaceae. *Taxon* **23**, 725–728. doi:10.2307/1218433
- Melville R (1959) The identity of *Calandrinia polyandra* Bentham. *Kew Bulletin* **13**, 400–401. doi:10.2307/4118106
- Mooney HA, Troughton JH, Berry JA (1974) Arid climates and photosynthetic systems. *Carnegie Institution Yearbook* **73**, 793–805.
- Mooney HA, Troughton JH, Berry JA (1977) Carbon isotope ratio measurements of succulent plants in southern Africa. *Oecologia* **30**, 295–305. doi:10.1007/BF00399762
- Nobel PS (1988) 'Environmental biology of agaves and cacti.' (Cambridge University Press: Cambridge, UK)
- Nyananyo BL (1990) Tribal and generic relationship in the Portulacaceae (Centrospermae). *Feddes Repertorium* **101**, 237–241. doi:10.1002/fedr.19901010504
- Nyffeler R, Eggli U (2010a) Disintegrating Portulacaceae: a new familial classification of the suborder Portulacineae (Caryophyllales) based on molecular and morphological data. *Taxon* **59**, 227–240.
- Nyffeler R, Eggli U (2010b) An up-to-date familial and suprafamilial classification of succulent plants. *Bradleya* **28**, 125–144.
- Obbens FJ (2006) A review of the tuberous *Calandrinia* species (section Tuberosae), including three new species for Western Australia. *Nuytsia* **16**, 95–115.
- Pate JS, Dixon K (1981) Plants with fleshy underground storage organs. In 'The biology of Australian plants'. (Eds JS Pate, AJ McComb) pp. 181–215. (University of Western Australia Press: Perth)
- Smirnoff N (1996) Regulation of crassulacean acid metabolism by water status in the C₃/CAM intermediate *Sedum telephium*. In 'Crassulacean acid metabolism'. (Eds K Winter, JAC Smith) pp. 176–191. (Springer-Verlag: Berlin)
- Smith JAC, Winter K (1996). Taxonomic distribution of crassulacean acid metabolism. In 'Crassulacean acid metabolism.' (Eds K Winter, JAC Smith) pp. 427–436. (Springer-Verlag: Berlin)
- Ting IP, Hanscom Z III (1977) Induction of acid metabolism in *Portulacaria afra*. *Plant Physiology* **59**, 511–514. doi:10.1104/pp.59.3.511
- Troughton JH, Card KA, Hendy CH (1974) Photosynthetic pathways and carbon isotope discrimination by plants. *Carnegie Institution Yearbook* **73**, 768–780.
- Vernon DM, Ostrem JA, Schmitt JM, Bohnert HJ (1988) PEPCase transcript levels in *Mesembryanthemum crystallinum* decline rapidly upon relief from salt stress. *Plant Physiology* **86**, 1002–1004. doi:10.1104/pp.86.4.1002
- Veste M, Herppich WB, von Willert DJ (2001) Variability of CAM in leaf-deciduous succulents from the Succulent Karoo (South Africa). *Basic and Applied Ecology* **2**, 283–288. doi:10.1078/1439-1791-00056
- von Willert DJ, Eller BM, Werger MJA, Brinckmann E, Ihlenfeldt HD (1992) 'Life strategies of succulent plants in deserts, with special reference to the Namib desert.' (University of Cambridge Press: Cambridge, UK)
- Winter K (1974) NaCl-induzierter Crassulaceen-Säurestoffwechsel bei der Salzpflanze *Mesembryanthemum crystallinum*. *Oecologia* **15**, 383–392. doi:10.1007/BF00345435
- Winter K (1985) Crassulacean acid metabolism. In 'Photosynthetic mechanisms and the environment'. (Eds J Barber, NR Baker) pp. 329–387. (Elsevier: Amsterdam)
- 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 C₃-CAM species. *Planta* **222**, 201–209. doi:10.1007/s00425-005-1516-6
- Winter K, Holtum JAM (2007) Environment or development? Lifetime net CO₂ exchange and control of the expression of crassulacean acid metabolism in *Mesembryanthemum crystallinum*. *Plant Physiology* **143**, 98–107. doi:10.1104/pp.106.088922
- Winter K, Troughton JH (1978) Carbon assimilation pathways in *Mesembryanthemum nodiflorum* L. under natural conditions. *Zeitschrift für Pflanzenphysiologie* **88**, 153–162.

- Winter K, von Willert DJ (1972) NaCl-induzierter Crassulaceensäurestoffwechsel bei *Mesembryanthemum crystallinum*. *Zeitschrift für Pflanzenphysiologie* **67**, 166–170.
- Winter K, Lüttge U, Winter E, Troughton JH (1978) Seasonal shift from C₃ photosynthesis to crassulacean acid metabolism in *Mesembryanthemum crystallinum* growing in its natural environment. *Oecologia* **34**, 225–237. doi:10.1007/BF00345168
- Winter K, Osmond CB, Pate JS (1981) Coping with salinity. In 'The biology of Australian plants'. (Eds JS Pate, AJ McComb) pp. 88–113. (University of Western Australia Press: Perth)
- 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. doi:10.1071/FP04123
- Winter K, Garcia M, Holtum JAM (2008) On the nature of facultative and constitutive CAM: environmental and developmental control of CAM expression during early growth of *Clusia*, *Kalanchoë*, and *Opuntia*. *Journal of Experimental Botany* **59**, 1829–1840. doi:10.1093/jxb/ern080
- Winter K, Garcia M, Holtum JAM (2009) Canopy CO₂ exchange of two neotropical tree species exhibiting constitutive and facultative CAM photosynthesis, *Clusia rosea* and *Clusia cylindrica*. *Journal of Experimental Botany* **60**, 3167–3177. doi:10.1093/jxb/erp149
- Winter K, Garcia M, Holtum JAM (2011) Drought-stress induced up-regulation of CAM in seedlings of a tropical cactus, *Opuntia elatior*, operating predominantly in the C₃ mode. *Journal of Experimental Botany* doi:10.1093/jxb/err106
- 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 one-year study on carbon, water and nutrient relationships in a tropical C₃-CAM hemi-epiphyte, *Clusia uvitana* Pittier. *New Phytologist* **127**, 45–60. doi:10.1111/j.1469-8137.1994.tb04258.x

Manuscript received 25 January 2011, accepted 28 April 2011