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REVIEW PAPER

Ecophysiology of constitutive and facultative CAM photosynthesis

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Abstract

In plants exhibiting crassulacean acid metabolism (CAM), CAM photosynthesis almost always occurs together with C_3 photosynthesis, and occasionally with C_4 photosynthesis. Depending on species, ontogeny, and environment, CAM input to total carbon gain can vary from values of <1% to 100%. The wide range of CAM phenotypes between and within species is a fascinating example of functional diversity and plasticity, but poses a significant challenge when attempting to define CAM. CO_2 gas exchange experiments designed for this review illustrate key patterns of CAM expression and highlight distinguishing features of constitutive and facultative CAM. Furthermore, they help to address frequently recurring questions on CAM terminology. The functional and evolutionary significance of contrasting CAM phenotypes and of intermediate states between extremes is discussed. Results from a study on nocturnal malate accumulation in 50 species of Aizoaceae exposed to drought and salinity stress suggest that facultative CAM is more widespread amongst vascular plants than previously thought.

Keywords: Acidity, carbon assimilation, evolution, facultative CAM, *Hatiora*, *Kalanchoe*, ontogeny, photosynthesis, photosynthetic intermediate, *Portulaca*.

Introduction

Research on the functional genomics of crassulacean acid metabolism (CAM) plants is rapidly advancing. Elucidating the relationship between genome and phenotype is key to the understanding of CAM evolution and for introducing CAM into C_3 crop plants to enhance their water use efficiency. Concomitant with improving our understanding of the molecular underpinnings of CAM, ecophysiological research is increasing our appreciation for the large phenotypic variation amongst CAM-exhibiting species, in terms of (i) CAM usage for carbon gain relative to C_3 (or C_4); and (ii) how CAM expression is controlled ontogenetically and environmentally. There is hardly a better example of metabolic flexibility amongst vascular plants than facultative CAM (i.e. the reversible induction of CAM in response to drought stress).

This review discusses the current status of research on phenotypic diversity and plasticity of CO_2 assimilation in plants with CAM photosynthesis. The review contrasts the ontogenetic controls of constitutive CAM and the environmental controls of facultative CAM, and features the wide range of CAM expression relative to C_3 or C_4 photosynthesis within and between species. Frequently recurring questions on CAM biology and CAM terminology are addressed, and topics for future ecophysiological CAM research are identified.

Constitutive CAM

In most species with CAM photosynthesis, CAM expression is constitutive (or obligate); that is, the CAM pathway always

manifests itself in mature photosynthetic tissues as part of a developmental routine that typically starts with the C_3 pathway when tissues are young (Box 1). Although generally recognized by CAM researchers and sometimes utilized in comparative physiological and molecular studies (Dever *et al.*, 2015; Hartwell *et al.*, 2016), this ontogenetic C_3–CAM shift has rarely been documented continuously for any species (Winter and Holtum, 2007). Figure 1 shows the ontogenetic progression from C_3 to CAM during the early development of a *Kalanchoe pinnata* leaf. CO_2 exchange measurements started 12 d after leaf emergence, when the area of the leaf under investigation was <5% of the final area. CO_2 fluxes increased with increased leaf size. Up until day 20, the 24 h CO_2 exchange pattern of the leaf was essentially identical to that of a C_3 plant. Nearly constant rates of CO_2 uptake were displayed in the light and were followed predominantly by constant rates of net CO_2 loss in the dark. On day 21, the first signs of CAM appeared: there was a temporary reduction in diurnal CO_2 uptake paralleled by decreased rates of nocturnal CO_2 loss. At the end of day 23, net nocturnal CO_2 fixation was detected for the first time. From that point onward, net dark CO_2 fixation gradually increased while the temporary depression of CO_2 fixation during the first half of the light period gradually became more pronounced. On day 41, nocturnal carbon gain surpassed diurnal carbon gain. On the last day shown in Fig. 1 (day 46), dark CO_2

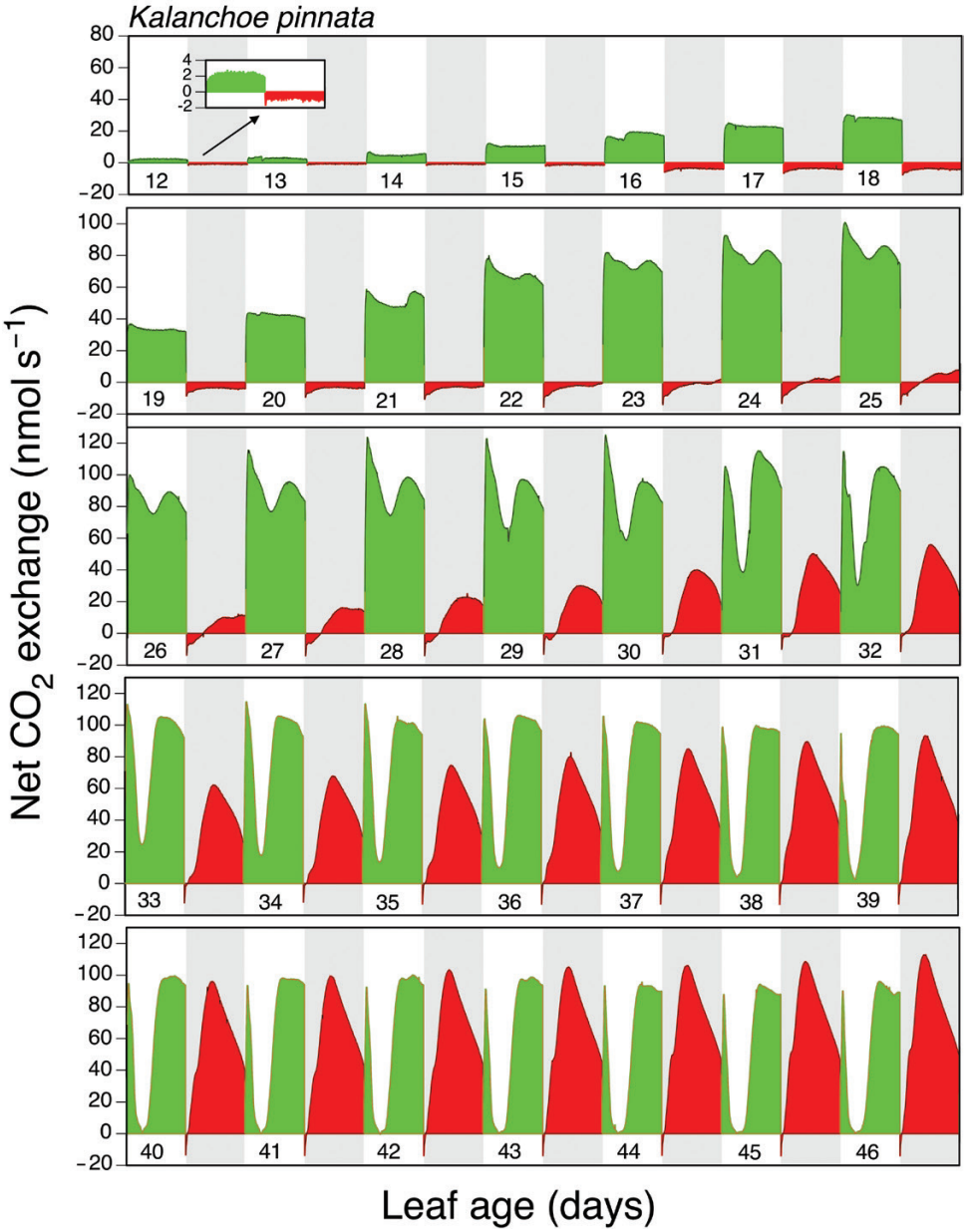

Fig. 1. Net CO_2 exchange during the early development of a *Kalanchoe pinnata* leaf. The youngest leaf attached to a 31 cm tall, well-watered plant was enclosed in a GWK-3M gas exchange cuvette (Walz, Effeltrich, Germany) which was connected to a through-flow gas exchange system. The cuvette was supplied with ambient air. The gas exchange cuvette was placed inside a controlled-environment chamber. Twelve hour light periods (650 µmol photons m^{-2} s^{-1}; 28 °C) alternated with 12 h dark periods (22 °C). Dark periods are indicated by the gray areas. Measurements began 12 d after leaf emergence. Leaf area on day 12 was 9 cm^2, and increased to 190 cm^2 on day 46. Green: CO_2 exchange during light periods. Red: CO_2 exchange during dark periods. Positive values correspond to net CO_2 uptake, and negative values to net CO_2 loss.

Box 1. Key definitions of CAM expression and CAM species terminology

- **Constitutive CAM**

CAM is always expressed in mature photosynthetic tissues as part of a pre-programmed, irreversible ontogenetic process. Environmental conditions influence rates of dark CO_2 fixation, but particular environmental conditions are not necessary to elicit CAM.

- **Obligate CAM**

The terms obligate and constitutive CAM are often used interchangeably. While constitutive CAM refers to the continual operation of CAM in mature tissues, obligate CAM highlights the need for CAM for growth and reproduction (i.e. survival).

- **Facultative CAM**

Facultative CAM is environmentally triggered, optional CAM. CAM is induced or up-regulated in a reversible manner in response to water-deficit stress in plants that, under well-watered conditions, gain carbon exclusively or predominantly via C_3 photosynthesis, or in some instances via C_4 photosynthesis (e.g. *Portulaca*). Even though the CAM phenotype is displayed only under conditions of environmental stress, the ability to do so is hardwired (encoded).

- **CAM cycle**

The core CAM metabolic cycle consists of two phases separated in time. (i) At night: glycolytic breakdown of storage carbohydrate to form the CO_2 acceptor phospho*enol*pyruvate (PEP), nocturnal assimilation of atmospheric CO_2 via cytosolic PEP carboxylase (PEPC), synthesis of malic acid, and vacuolar storage of malic acid. (ii) During the day: release of malic acid into the cytosol, malate decarboxylation, assimilation of liberated CO_2 via Rubisco into the photosynthetic reduction cycle, and gluconeogenic regeneration of storage carbohydrate from the remaining 3-carbon compound (pyruvate or PEP). Note that the four phases of CAM plant gas exchange of Osmond (1978) cover segments of the 24 h cycle that are not necessarily part of the CAM cycle. Late-afternoon phase-4 fixation of atmospheric CO_2 via Rubisco participates in the CAM cycle only insofar as it contributes to carbon reserves available for PEP production.

- **CAM plant**

A species which, throughout its life, gains most of its carbon by dark CO_2 fixation involving CAM. CAM is typically expressed constitutively. $\delta^{13}C$ values are less negative than –20‰. Classic examples are most cacti and agaves.

- **C_3–CAM plant**

A species in which CAM is present but in which C_3 photosynthesis contributes to long-term carbon gain more than CAM does. CAM can be expressed constitutively or facultatively.

- **C_3–CAM intermediate**

Similar meaning to C_3–CAM plant. Sometimes the term is also used for plants with facultative CAM. Given the bimodal distribution of $\delta^{13}C$ values in families with C_3 and CAM species (e.g. Bromeliaceae, Euphorbiaceae) (Winter and Holtum, 2002; Horn *et al.*, 2014; Crayn *et al.*, 2015), C_3–CAM intermediacy with long-term carbon gain derived equally from C_3 photosynthesis and CAM does not seem to be favored ecologically. Possible exceptions from bimodality of $\delta^{13}C$ values are lineages predominantly composed of facultative CAM species which, during their life cycle, can exhibit the full range of isotopic signatures from C_3-type $\delta^{13}C$ values to those reflecting pronounced CAM. The net result is intermediate lifetime means of around –20‰. Lifetime means based on monthly averages for *Mesembryanthemum crystallinum* in Israel and California were –21.1 and –21.6‰, respectively (Winter *et al.*, 1978; Bloom and Troughton, 1979).

- **Strong CAM**

CAM is strongly expressed; that is, the diel CO_2 exchange pattern is characterized by high rates of dark CO_2 fixation in the order of 5 μmol m^{-2} s^{-1}, with much higher values having been reported. Strong CAM is typically associated with constitutive CAM.

- **Full CAM**

The diel CO_2 exchange pattern is characterized entirely by net CO_2 uptake in the dark with essentially no net CO_2 uptake in the light. Rates of dark CO_2 fixation may be high (strong CAM) or low. Plants may exhibit full CAM but not strong CAM in response to drought stress when net CO_2 fixation is restricted to the dark but rates of dark CO_2 fixation are low.

Box 1. Continued

• Weak CAM

CAM is weakly expressed, either facultatively or constitutively, in plants in which C_3 photosynthesis is typically the principal pathway of carbon acquisition. Dark CO_2 fixation contributes less than ~5% to total carbon gain when CAM is expressed constitutively; when expressed facultatively, dark CO_2 fixation is less than ~5% compared with daytime CO_2 fixation in well-watered plants in the C_3 state.

• CAM cycling

An extreme form of weakly expressed CAM in species that exhibit net CO_2 uptake solely in the light. Slightly elevated rates of nocturnal CO_2 fixation result in nocturnal acidification, but nocturnal CO_2 fixation is not sufficient to outweigh nocturnal respiratory CO_2 loss.

• CAM-idling

The term CAM-idling was coined for severely droughted CAM plants that maintain an active CAM cycle by utilizing respiratory CO_2 as a carbon source while gas exchange with the atmosphere has largely ceased. CAM-idling is interpreted as a mechanism that extends survival. The ecological significance of CAM idling is unclear.

fixation contributed 60% to the total daily carbon gain. Nine weeks later, this had increased to 80% of the total daily carbon gain (data not shown), which equates to a further shift from C_3 to CAM. Leaf thickness increased during the course of the experiment, enhancing the storage capacity for nocturnal acid accumulation as leaves matured.

This ontogenetic C_3 to CAM shift is also commonly observed at the organismal level in young plants of constitutive CAM species, in part because young plants carry mostly immature leaves. The shoot of a young plant of *K. pinnata*, propagated from a plantlet that originated from the margins of a mature leaf, exclusively showed C_3-type CO_2 fixation in the light at the onset of measurements (Fig. 2). As the plant grew, CAM-type CO_2 fixation developed (Fig. 2, from day 10 onwards) and eventually exceeded C_3 photosynthetic CO_2 fixation in the light (data not shown). Similar responses have been demonstrated for seedlings of stem-succulent cacti (Winter *et al.*, 2011). The initial C_3 photosynthetic phase may be strongly reduced or absent in young cladodes of platyopuntias that develop on mother cladodes, depending upon the extent to which these young cladodes use carbon supplied from the mother cladode for early growth (Winter and Holtum, 2002; Winter *et al.*, 2008).

In view of the results shown in Figs 1 and 2, one is automatically reminded of Ernst Haeckel's 19th century 'ontogeny recapitulates phylogeny' concept. Although this concept is now considered an oversimplification and is treated by many authors as biological mythology (Gould, 1977), the ontogenetic C_3–CAM shift is nonetheless a fascinating system for modern evolutionary-developmental-biology (evo-devo) research.

Facultative CAM

In contrast to the ontogenetically controlled irreversible constitutive CAM, facultative CAM is environmentally triggered, typically in response to reduced soil water availability caused by drought or high salinity stress. Facultative CAM was discovered by Winter and von Willert (1972), who demonstrated that the halophytic annual *Mesembryanthemum crystallinum* (Aizoaceae) exhibited the day–night CO_2 exchange pattern of a C_3 plant when grown under well-watered, non-saline conditions. In contrast, *M. crystallinum* exhibited the CO_2 exchange pattern of a CAM plant when irrigated with highly saline water containing 400 mM NaCl. The concept of stress-induced facultative CAM was initially met with skepticism as some researchers suggested that the stress treatment merely accelerated a normal irreversible ontogenetic C_3–CAM shift (of the type shown in Figs 1 and 2) (von Willert and Kramer, 1972; Osmond 1978; Adams *et al.*, 1998; Dodd *et al.*, 2002). However, experiments showing that *M. crystallinum* could complete its life cycle and produce seeds by solely operating in the C_3 mode under non-stress conditions disproved the notion that accelerated aging was the principal driver of CAM in *M. crystallinum* (Winter and Holtum, 2007). In order to ensure that CAM is truly facultative in a given species (i.e. optional and environmentally triggered), it has become standard procedure not only to demonstrate the induction or up-regulation of CAM in response to stress, but also to demonstrate the reversion to C_3 upon removal of stress. Such information is now available for a wide range of species (Table 1). Long-lived leaves of *Clusia* ssp. can exhibit multiple C_3–CAM–C_3 swings during repeated wet–dry–wet cycles (K. Winter, unpublished data), emphasizing the crucial role of environmental rather than ontogenetic factors in promoting nocturnal CO_2 fixation in facultative CAM plants.

Figure 3 shows the drought-induced, reversible shift to CAM in a C_4 species, *Portulaca umbraticola*, for which facultative CAM has not been demonstrated previously. CO_2 exchange of an entire shoot (leaves and stems) was continuously monitored for 20 d. As in the experiment shown in Fig. 2, overall CO_2 fluxes increased as the plant inside the gas exchange system increased in size. In the well-watered state, CAM was absent: net CO_2 uptake occurred during the light period only, and during the dark period CO_2 was released at a relatively constant rate. As drought stress developed, CO_2 gain in the light declined over the course of several days, and nocturnal CO_2 exchange gradually changed from net CO_2 loss to net CO_2 gain. Early in the transition to CAM, nocturnal gas exchange passed through a stage typical of CAM

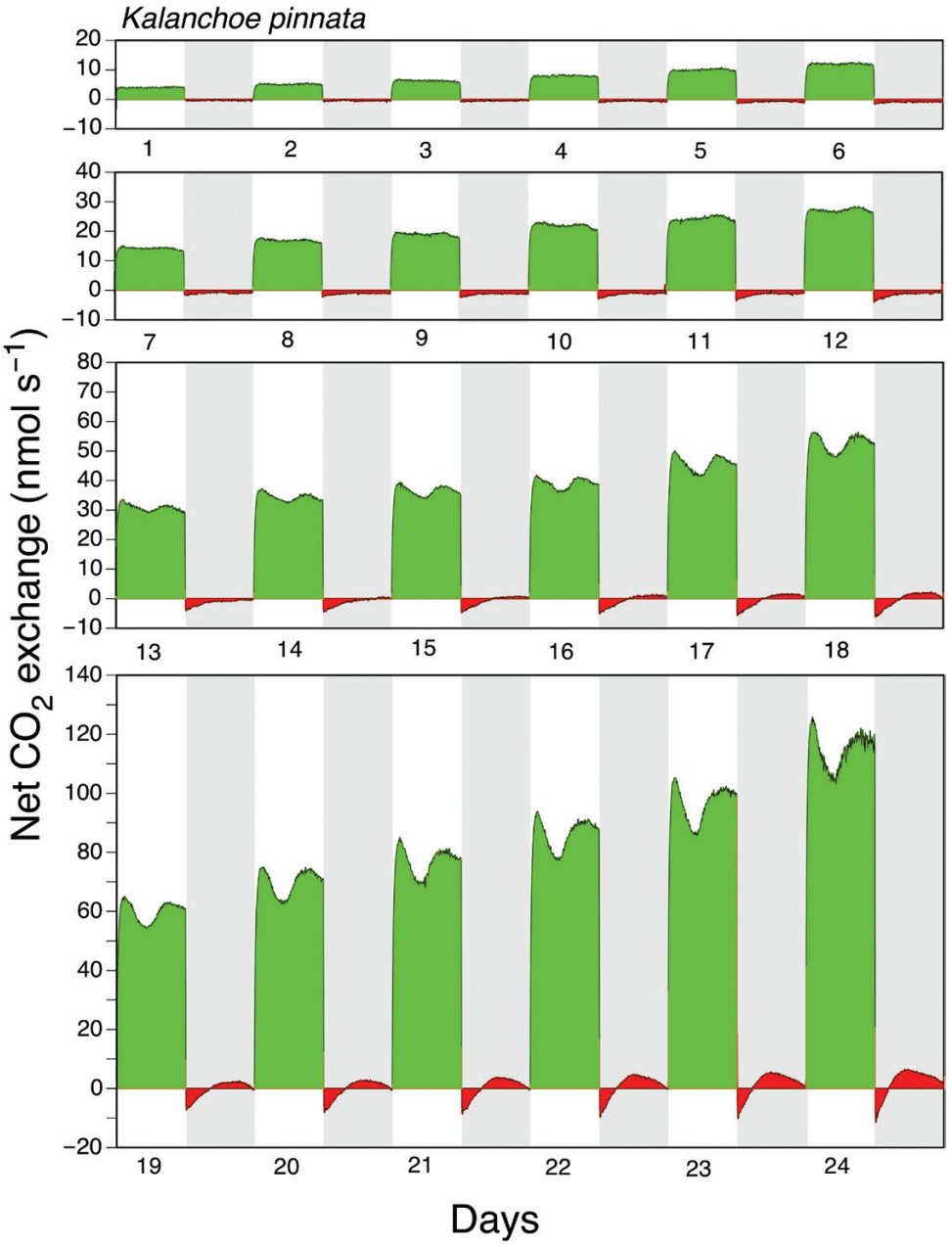

Fig. 2. Net CO_2 exchange during the early development of a well-watered *Kalanchoe pinnata* plant. The shoot of a young plant with recently produced, still immature leaf pairs 2 and 3 was enclosed in a 20×20×15 cm plexiglas cuvette. The cuvette was supplied with ambient air. Roots and the pot were outside the cuvette. Twelve hour light periods (500 µmol photons m^{-2} s^{-1}; 28 °C) alternated with 12 h dark periods (22 °C). Dark periods are indicated by the gray areas. Green: CO_2 exchange during light periods. Red: CO_2 exchange during dark periods. Leaf pairs 4, 5, and 6, emerged on days 5, 12, and 21, respectively.

cycling (Fig. 3, day 12 versus day 11; see also Fig. 1, day 22 versus day 18; Box 1). On days 17 and 18, CO_2 exchange was close to zero for most of the light period, and net dark CO_2 fixation had plateaued. Upon re-watering, the plant rapidly returned to solely net CO_2 fixation in the light. Based on the CO_2 exchange responses in Fig. 3, *P. umbraticula* can be considered a species with facultative, weakly expressed CAM, because nocturnal CO_2 fixation in the stressed state is very small compared with diurnal CO_2 fixation in the non-stressed state (Box 1).

Thus far, facultative CAM has been reported for at least 54 species from 27 genera and 15 families, many in the order Caryophyllales (Table 1). The number of species will increase significantly when previously unpublished results from a CAM survey of 50 species of the large family Aizoaceae (2271 species in total; www.theplantlist.org) are taken into consideration (Table 2). This survey was a direct follow-up to the first demonstration of high-salinity-induced CAM in *M. crystallinum*. Most species in Table 2 belong to the large subfamily Ruschioideae (1939 species in total), with the exception of two species in the subfamily Mesembryanthemoideae [*Mesembryanthemum lanci-folium* and *Phyllobolus prasinus* (formerly *Aridaria prasina*)]. CAM-type nocturnal malate accumulation was observed

Table 1. *Species with facultative CAM*

Family	Species	Reversibility	Reference
Aizoaceae	*Carpobrotus edulis* (L.) N.E.Br.	Yes	Winter (1973); K. Winter (unpublished); Treichel and Bauer (1974)
	Delosperma tradescantioides (P.J.Bergius) L.Bolus	Yes	Herppich *et al.* (1996)
	Disphyma clavellatum (Haw.) Chinnock	ND	Winter *et al.* (1981)
	Mesembryanthemum crystallinum L.	Yes	Winter and von Willert (1972); Winter and Holtum (2014)
	Mesembryanthemum cordifolium L.f.	ND	Treichel (1975)
	Mesembryanthemum nodiflorum L.	ND	Treichel and Bauer (1974)
Anacampserotaceae	*Anacampseros australiana* J.M.Black	Yes	Winter and Holtum (2017)
	Anacampseros coahuilensis (S.Watson) Eggli & Nyffeler	ND	Guralnick *et al.* (2008)
	Grahamia bracteata Gillies ex Hook. & Arn.	ND	Guralnick *et al.* (2008)
Araceae	*Zamioculcas zamiifolia* (Lodd.) Engl.	Yes	Holtum *et al.* (2007)
Basellaceae	*Anredera baselloides* (Kunth) Baill.	Yes	Holtum *et al.* (2018)
	Basella alba L.	Yes	K. Winter *et al.* (unpublished)
Bromeliaceae	*Guzmania monostachia* (L.) Rusby ex Mez	Yes	Medina *et al.* (1977)
	Vrisea sanguinolenta Cogn. & Marchal.	ND	Beltrán *et al.* (2013)
Cactaceae	*Pereskia guamacho* F.A.C.Weber	ND	Diaz and Medina (1984)
Clusiaceae	*Clusia aripoensis* Britton	ND	Borland *et al.* (1998)
	Clusia cylindrica Hammel	Yes	Winter *et al.* (2009)
	Clusia minor L.	Yes	Borland *et al.* (1998); Winter *et al.* (2008)
	Clusia pratensis Seem.	Yes	Winter and Holtum (2014)
	Clusia uvitana Pittier	Yes	Winter *et al.* (1992)
Commelinaceae	*Callisia fragrans* (Lindl.) Woodson	ND	Martin *et al.* (1994)
	Tradescantia brevifolia (Torr.) Rose	ND	Martin *et al.* (1994)
	Tripogandra multiflora (Sw.) Raf.	ND	Martin *et al.* (1994)
Crassulaceae	*Crassula sieberiana* (Schult. & Schult.f.) Druce	Yes	Winter and Holtum (2017)
	Kalanchoe gracilipes (Baker) Baill.	Yes	J. Hartwell *et al.* (unpublished)
	Kalanchoe miniata Hilsenb. & Bojer ex Tul.	Yes	Brulfert *et al.* (1996)
	Kalanchoe porphyrocalyx (Baker) Baill.	Yes	Brulfert *et al.* (1996)
	Sedum acre L.	Yes	Kluge (1977)
	Sedum album L.	Yes	Castillo (1996)
	Sedum pulchellum Michx.	ND	Smith and Eickmeier (1983)
	Sedum sexangulare L.	?	Schuber and Kluge (1981)
	Sedum telephium L.	Yes	Lee and Griffiths (1987)
Didiereaceae	*Ceraria fruticulosa* H.Pearson & Stephens	ND	Veste *et al.* (2001)
	Portulacaria afra Jacq.	Yes	Ting and Hanscom (1977)
Lamiaceae	*Plectranthus amboinicus* (Lour.) Spreng.	Yes	K. Winter (unpublished)
Montiaceae	*Calandrinia creethae* Tratman ex Morrison	Yes	Holtum *et al.* (2017*a*)
	Calandrinia holtumii Obbens & L.P.Hancock	Yes	K. Winter (unpublished)
	Calandrinia pentavalvis Obbens	Yes	Holtum *et al.* (2017*a*)
	Calandrinia polyandra Benth.	Yes	Winter and Holtum (2011)
	Calandrinia quadrivalvis F.Muell.	Yes	Holtum *et al.* (2017*a*)
	Calandrinia reticulata Syeda	Yes	Holtum *et al.* (2017*a*)
	Cistanthe cf. *grandiflora* (Lindl.) Schltdl.	Yes	K. Winter *et al.* (unpublished)
Portulacaceae	*Portulaca australis* Endl.	Yes	Winter and Holtum (2017)
	Portulaca cyclophylla F.Muell.	Yes	Holtum *et al.* (2017*b*)
	Portulaca cryptopetala Speg.	Yes	K. Winter *et al.* (unpublished)
	Portulaca digyna F.Muell.	Yes	Holtum *et al.* (2017*b*)
	Portulaca grandiflora Hook.	ND	Guralnick *et al.* (2002)
	Portulaca molokiniensis Hobdy	Yes	K. Winter *et al.* (unpublished)
	Portulaca oleracea L.	Yes	Koch and Kennedy (1980); Winter and Holtum (2014)
	Portulaca pilosa L.	Yes	Winter and Holtum (2017)
	Portulaca umbraticola Kunth	Yes	This study
Talinaceae	*Talinum fruticosum* (L.) Juss.	Yes	Winter and Holtum (2014)
	Talinum paniculatum (Jacq.) Gaertn.	?	Güerere *et al*. (1996)
Vitaceae	*Cissus trifoliata* (L.) L.	Yes	Olivares *et al.* (1984)

Currently accepted species names are used. Some species names employed in the original publications such as *Aptenia cordifolia* (L.f.) Schwantes, *Werauhia sanguinolenta* (Cogn. & Marchal) J.R.Grant, *Sedum mite* Gilib., and *Talinum triangulare* (Jacq.) Willd. are now considered synonyms of *Mesembryanthemum cordifolium* L.f., *Vriesea sanguinolenta* Cogn. & Marchal., *Sedum sexangulare* L., and *Talinum fruticosum* (L.) Juss., respectively. ND, not determined. ?, not clear whether or not reversibility was examined.

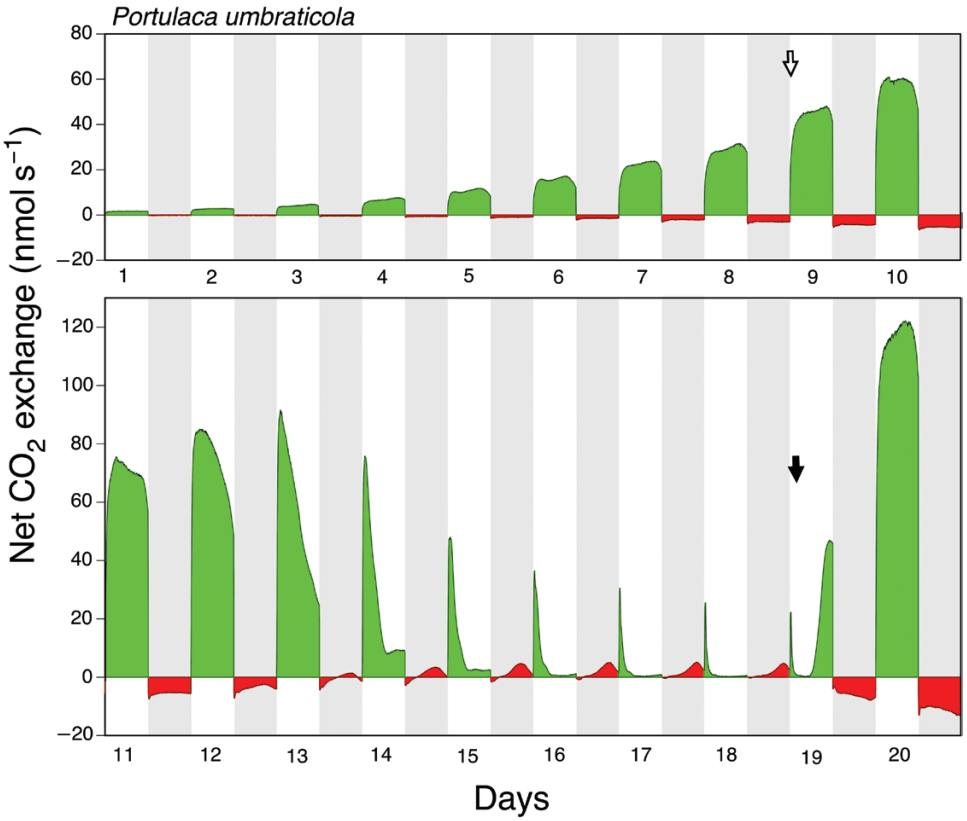

Fig. 3. Net CO_2 exchange of *Portulaca umbraticola* during a wet–dry–wet cycle. Measurements were conducted on a shoot that grew inside a 11×11×10 cm plexiglas cuvette. Roots and the pot were outside the cuvette. [CO_2] of the air entering the cuvette was ~400 µl l^{-1} provided by a Walz CO_2/CO_2-free air mixing system. Twelve hour light periods (600 µmol photons m^{-2} s^{-1}; 28 °C) alternated with 12 h dark periods (22 °C). Green: CO_2 exchange during light periods. Red: CO_2 exchange during dark periods. Irrigation stopped on day 9 (open arrow) and resumed on day 19 (filled arrow). At the onset of the experiment, the plant had cotyledons and the leaf length of the first leaf pair was 7 mm. At the conclusion of the experiment (day 23, not shown), total leaf area was 132 cm^2 and shoot dry mass was 0.602 g.

in 45 of the 50 species studied. Consistent with facultative CAM, 28 species showed nocturnal malate accumulation under conditions of drought and/or drought plus salinity stress, but not when well watered. In the 17 species in which significant nocturnal malate accumulation was already present in well-watered plants, the dawn:dusk malate level ratio (i.e. the fold nocturnal malate increase) was enhanced upon drought and salinity stress, consistent with a facultative CAM component in addition to constitutive CAM. It seems that CAM, especially facultative CAM, is a common feature amongst the Aizoaceae, and that in the Aizoaceae alone, facultative CAM could be present in well over 1000 species.

Do C_3–CAM shifts always reflect facultative CAM?

The answer is no. What distinguishes facultative CAM from other types of C_3–CAM shifts is the fact that upon stress, the magnitude of nocturnal CO_2 uptake increases. Changes in the relative proportions of diurnal to nocturnal carbon gain in favor of nocturnal CO_2 uptake, but without enhanced nocturnal CO_2 assimilation, do not represent facultative CAM *sensu stricto*. Figure 4 depicts the response of a mature leaf of *K. pinnata* to water-deficit stress. The leaf exhibits significant diurnal C_3 photosynthetic CO_2 uptake when well hydrated, in addition to nocturnal CO_2 fixation. Upon the imposition of instantaneous drought stress (petiole cut in the experiment of Fig. 4), night-time CO_2 fixation remains, whereas daytime CO_2 fixation stops almost immediately. In the example of Fig. 4, the shift from a C_3–CAM pattern to an exclusively CAM pattern is driven by a more rapid decline in C_3 photosynthetic CO_2 uptake in the light than in CO_2 uptake in the dark. In this case, the criteria for facultative CAM are not fulfilled.

Sealing entire succulent plants or parts of them into gas exchange cuvettes can be challenging. Therefore, CO_2 measurements are sometimes performed on detached CAM organs, assuming that their gas exchange remains largely unchanged, at least for a day or so upon separation from the mother plant, given the high water-holding capacity of succulent tissues (Boxall *et al.*, 2017). Figure 4 demonstrates that this assumption certainly does not apply to leaves of *K. pinnata*.

During long-term drought stress, net dark CO_2 fixation eventually ceases, but nocturnal acidification may continue at a low magnitude using respiratory CO_2 as substrate for CAM. The recycling of respiratory CO_2 in the absence of gas exchange with the atmosphere has been called CAM-idling and it has been suggested that CAM-idling is an important survival mechanism (Hanscom and Ting, 1978). Supporting evidence for the latter is scarce. Drought experiments were conducted

Table 2. *Leaf malate content end of day (L) and end of night (D) of 50 species of Aizoaceae cultivated under well-watered conditions, or exposed to drought stress, or drought plus salinity stress*

Species	Malate (µmol g^{-1} DM)					
	Control (well-watered)		Drought stress		Drought + salt stress	
	L	D	L	D	L	D
Bergeranthus vespertinus ({Berger) Schwantes	123 ± 37	**280 ± 98**	68 ± 29	**257 ± 43**	36 ± 25	**191 ± 22**
Calamophyllum cylindricum (Haw.) Schwantes	52 ± 21	67 ± 18	11 ± 1	44 ± 12	0 ± 0	15 ± 14
Carpobrotus acinaciformis (L.) L.Bolus	221 ± 67	187 ± 59	46 ± 25	89 ± 25	25 ± 4	**83 ± 8**
Carpobrotus aequilaterus (Haw.) N.E.Br.	183 ± 71	194 ± 62	26 ± 11	**73 ± 7**	ND	ND
Carpobrotus quadrifidus L.Bolus	99 ± 2	104 ± 13	148 ± 42	249 ± 27	21 ± 2	**104 ± 18**
Carruanthus ringens (L.) Boom	205 ± 93	236 ± 87	ND	ND	54 ± 29	**358 ± 27**
Cephalophyllum cupreum L.Bolus	248 ± 167	299 ± 85	106 ± 53	**338 ± 93**	49 ± 14	**217 ± 58**
Cephalophyllum purpureoalbum (Haw.) Schwantes	82 ± 24	73 ± 32	29 ± 6	**52 ± 5**	18 ± 2	**36 ± 10**
Cephalophyllum subulatoides (Haw.) N.E.Br.	92 ± 49	74 ± 24	29 ± 8	**80 ± 21**	13 ± 4	**61 ± 6**
Cephalophyllum tricolorum (Haw.) N.E.Br.	77 ± 18	104 ± 24	25 ± 13	**119 ± 11**	12 ± 1	**67 ± 10**
Chasmatophyllum masculinum (Haw.) Dinter & Schwantes	13 ± 7	**32 ± 9**	7 ± 5	**37 ± 2**	5 ± 3	**23 ± 1**
Conicosia pugioniformis (L.) N.E.Br.	4 ± 1	5 ± 5	ND	ND	ND	ND
Delosperma brunnthaleri (A.Berger) Schwantes ex H.Jacobsen	36 ± 18	51 ± 21	122 ± 15	**205 ± 39**	9 ± 3	35 ± 13
Delosperma hirtum (N.E.Br.) Schwantes	97 ± 3	**219 ± 47**	60 ± 25	**220 ± 22**	51 ± 10	**239 ± 13**
Delosperma luteum L.Bolus	193 ± 59	**325 ± 67**	24 ± 6	**178 ± 26**	25 ± 22	149 ± 80
Delosperma macellum (N.E.Br.) N.E.Br.	120 ± 17	143 ± 39	189 ± 18	**295 ± 19**	61 ± 18	**194 ± 31**
Delosperma mahonii (N.E.Br.) N.E.Br.	114 ± 20	**296 ± 46**	46 ± 16	**184 ± 51**	37 ± 8	**202 ± 19**
Delosperma steytlerae L.Bolus	126 ± 17	196 ± 47	147 ± 45	156 ± 32	36 ±18	60 ± 22
Delosperma wethamae L.Bolus	4 ± 4	3 ± 5	80 ± 30	**152 ± 47**	18 ± 20	**50 ± 6**
Disphyma australe (Sol. ex Aiton) J.M.Black	96 ± 36	93 ± 35	76 ± 24	134 ± 56	102 ± 10	**198 ± 11**
Disphyma crassifolium (L.) L.Bolus	63 ± 18	**100 ± 10**	73 ± 35	177 ± 81	23 ± 9	**59 ± 9**
Erepsia heteropetala (Haw.) Schwantes	48 ± 9	61 ± 10	3 ± 4	**30 ± 4**	ND	ND
Faucaria felina (L.) Schwantes	129 ± 12	**363 ± 74**	91 ± 10	**216 ± 33**	16 ± 14	**84 ± 21**
Faucaria subintegra L.Bolus	337 ± 166	376 ± 153	55 ± 35	95 ± 33	42 ± 16	**92 ± 13**
Faucaria tigrina (Haw.) Schwantes	85 ± 2	**257 ± 2**	112 ± 40	**265 ± 33**	27 ± 9	**151 ± 35**
Glottiphyllum depressum (Haw.) N.E.Br.	186 ± 104	261 ± 95	89 ± 14	**161 ± 23**	36 ± 4	**84 ± 7**
Glottiphyllum difforme (L.) N.E.Br.	228 ± 108	265 ± 65	57 ± 17	**117 ± 17**	45 ± 11	**99 ± 10**
Glottiphyllum longum (Haw.) N.E.Br.	318 ± 134	360 ± 111	156 ± 23	170 ± 81	49 ± 3	**84 ± 3**
Hereroa calycina L. Bolus	105 ± 25	105 ± 9	44 ± 11	**98 ± 13**	9 ± 10	**64 ± 22**
Hereroa gracilis L.Bolus	84 ± 8	**212 ± 36**	40 ± 4	**271 ± 15**	32 ± 8	**198 ± 31**
Hereroa granulata Dinter & Schwantes	130 ± 78	223 ± 82	34 ± 4	**198 ± 18**	0 ± 0	**58 ± 3**
Hereroa stanleyi L. Bolus	63 ± 22	**267 ± 57**	47 ± 21	**183 ± 75**	16 ± 6	**155 ± 29**
Lampranthus curviflorus N.E. Br.	19 ± 6	**35 ± 9**	ND	ND	3 ± 5	**20 ± 8**
Lampranthus deltoides (L.) Glen ex Wijnands	117 ± 68	166 ± 69	26 ± 7	**97 ± 12**	19 ± 7	**62 ± 8**
Lampranthus falciformis (Haw.) N.E.Br.	20 ± 3	**38 ± 2**	6 ± 4	**30 ± 9**	7 ± 3	**31 ± 9**
Lampranthus lunatus N.E. Br.	38 ± 8	63 ± 26	18 ± 6	**71 ± 4**	27 ± 21	62 ± 35
Lampranthus multiseriatus N.E. Br.	14 ± 4	19 ± 2	55 ± 38	38 ± 17	7 ± 7	**34 ± 12**
Lampranthus variabilis N.E. Br.	0 ± 0	12 ± 11	4 ± 3	**32 ± 6**	4 ± 7	**34 ± 16**
Mesembryanthemum lancifolium (L. Bolus) Klak	72 ± 52	**329 ± 111**	145 ± 29	**945 ± 66**	103 ± 19	**848 ± 95**
Nananthus orpenii (N.E.Br.) L.Bolus	196 ± 36	477 ± 103	ND	ND	ND	ND
Phyllobolus prasinus (L.Bolus) Gerbaulet	144 ± 28	**861 ± 83**	98 ± 47	**782 ± 50**	109 ± 6	**862 ± 34**
Pleiospilos compactus Schwantes	898 ± 431	**1430 ±263**	487 ± 71	**1026 ± 37**	227 ± 52	**636 ± 40**
Pleiospilos magnipunctatus Schwantes	646 ± 238	913 ± 312	472 ± 117	785 ± 8	176 ± 0	**439 ± 73**
Rabiea cibdela (N.E.Br.) N.E.Br.	77 ± 23	35 ± 13	ND	ND	ND	ND
Rhinephyllum broomii L. Bolus	17 ± 2	41 ± 33	50 ± 7	**111 ± 18**	ND	ND
Ruschia hexamera L.Bolus	48 ± 36	56 ± 7	28 ± 6	**84 ± 2**	0 ± 0	**53 ± 20**
Ruschia vaginata Schwantes	42 ± 4	**62 ± 6**	15 ± 11	57 ± 1	8 ± 2	**49 ± 0**
Titanopsis calcarea (Marloth) Schwantes	219 ± 82	**405 ± 56**	84 ± 23	106 ± 31	31 ± 5	**77 ± 26**
Trichodiadema barbatum Schwantes	766 ± 78	834 ± 86	151 ± 62	**356 ± 24**	ND	ND
Trichodiadema stelligerum Schwantes	633 ± 131	728 ± 36	116 ± 40	**238 ± 62**	153 ± 14	**302 ± 29**

Plants were grown in terra cotta pots in cold frames at the Botanic Garden of the Technical University of Darmstadt during late spring and summer of 1976. Plants were irrigated daily (well-watered controls), weekly (drought stress), or weekly plus 200 mM NaCl each 4 weeks (drought + salt stress). Values are means ± SD (n=3 samples from different plants, or, in few cases, 2 samples). End-of-night values in bold are significantly higher than end of day values (one-tailed t-test, $P < 0.05$). ND, not determined (K. Winter, previously unpublished results).

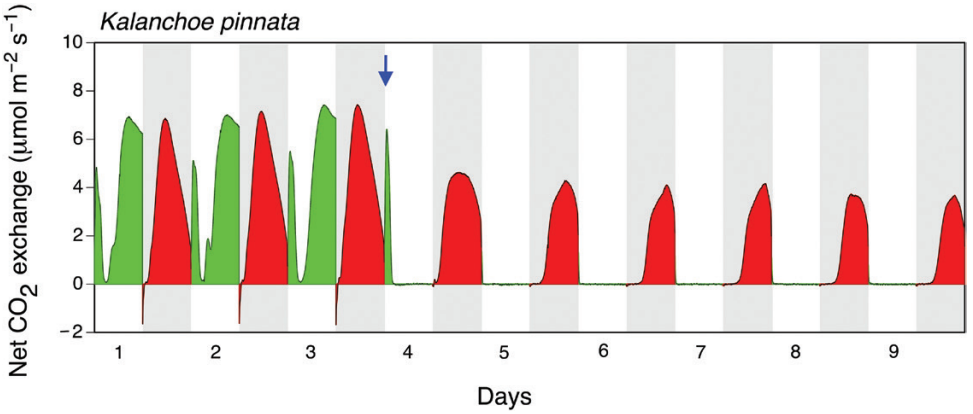

Fig. 4. Effect of abruptly induced water-deficit stress on the net CO_2 exchange of a fully expanded *Kalanchoe pinnata* leaf. The leaf was enclosed in a GWK-3M gas exchange cuvette (Walz, Effeltrich). The cuvette was supplied with ambient air. Twelve hour light periods (650 µmol photons m^{-2} s^{-1}; 28 °C) alternated with 12 h dark periods (22 °C). Dark periods are indicated by the gray areas. Green: CO_2 exchange during light periods. Red: CO_2 exchange during dark periods. From day 1 to day 3, the leaf was attached via its petiole to the plant outside the cuvette. At the beginning of day 4, 15 min into the light period, the petiole was cut (blue arrow). Leaf lamina dry mass at the conclusion of the experiment was 1.53 g and the area was 120 cm^2.

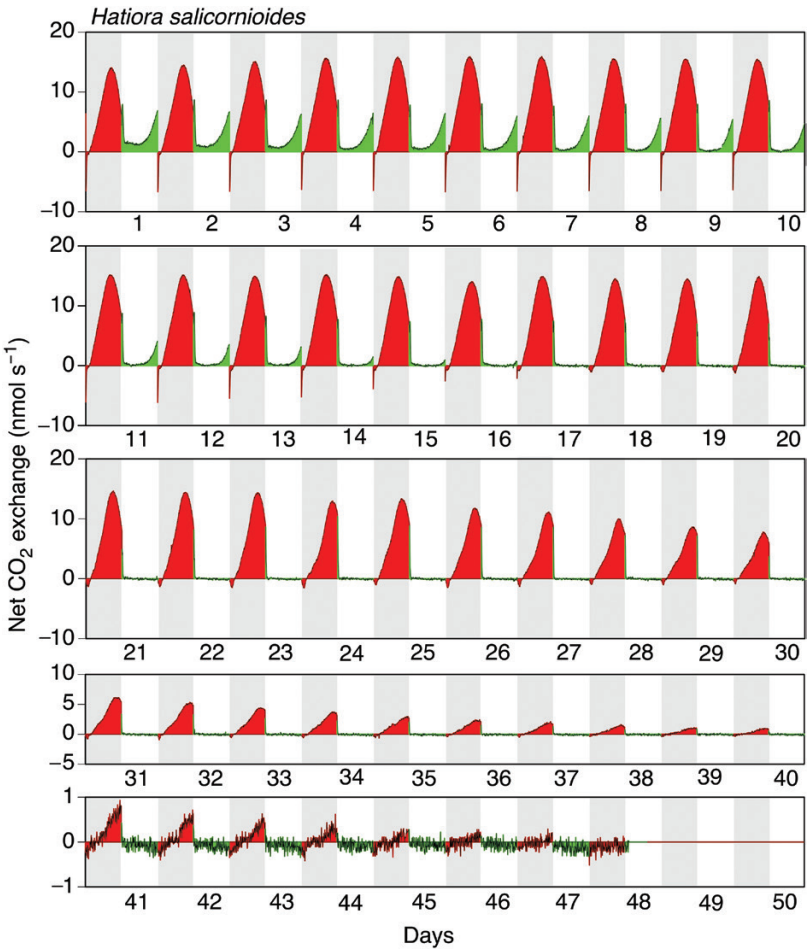

Fig. 5. Effect of prolonged drought stress on the net CO_2 exchange of an intact, attached branch of the epiphytic cactus *Hatiora salicornioides*. The branch was enclosed in a GWK-3M gas exchange cuvette (Walz, Effeltrich). The rest of the plant was outside the cuvette. The cuvette was supplied with ambient air. Twelve hour light periods (650 µmol photons m^{-2} s^{-1}; 28 °C) alternated with 12 h dark periods (22 °C). Dark periods are indicated by the gray areas. Green: CO_2 exchange during light periods. Red: CO_2 exchange during dark periods. Irrigation was stopped at the onset of the experiment and never resumed.

with a range of species where gas exchange was monitored until the exact point in time when net CO_2 uptake in the dark no longer occurred. In the example of Fig. 5, which features an intact attached branch of the epiphytic cactus *Hatiora salicornioides*, nocturnal net CO_2 uptake stopped at exactly 47 d after watering was withheld. Indeed, a small H^+ increase

from 0.4±0.6 μmol g^{-1} to 7.3±1.4 μmol g^{-1} fresh mass was observed during the course of the following night, consistent with CO_2 recycling via CAM. In other species, such as the CAM bromeliad *Tillandsia flexuosa*, a highly drought-resistant epiphytic air plant, nocturnal acid accumulation was no longer detectable once nocturnal net CO_2 uptake had ceased (K. Winter, unpublished data). Notably, dehydrated CAM tissues do not operate as completely closed, airtight systems, and tissues may rapidly shift to continuous diel net CO_2 loss once net CO_2 dark fixation no longer takes place. Furthermore, non-uniform dehydration is a major complication of long-term drought experiments with CAM plants, and partial death of photosynthetic organs occurs in order to assist the remaining tissues in staying active longer. Survival through partial death (Evenari *et al.*, 1971) may well be of greater adaptive significance to drought stress than CAM-idling *per se*.

Can facultative and constitutive CAM co-occur?

The answer is yes. Under well-watered conditions prior to CAM induction, not all species with facultative CAM exhibit pure C_3 physiology with zero nocturnal acidification. Even if they do, full reversibility to C_3 does not always occur. In many species, facultative CAM is observed as stress-induced up-regulation of CAM, superimposed on a pre-existing background of weakly expressed constitutive CAM. Upon the removal of stress, plants either fully or partly return to the pre-stress CAM level. Even plants with pronounced constitutive CAM have been shown to exhibit small transient increases in nocturnal CO_2 uptake in response to water-deficit stress (Winter *et al.*, 2008, 2014). In all these cases, the facultative component of CAM refers to the stress-induced reversible increase of nocturnal CO_2 uptake and nocturnal acidification, irrespective of the magnitude of a possible constitutive CAM background.

Is facultative CAM a transitional state between C_3 and full CAM?

The adaptive significance of facultative CAM in annuals such as *M. crystallinum* or *Calandrinia* ssp. appears obvious (Winter *et al.*, 1978; Winter and Ziegler, 1992; Herrera, 2009; Winter and Holtum, 2014). C_3 photosynthesis promotes initial rapid vegetative growth when it rains, while water-use-efficient CAM prolongs the life cycle and aids reproduction during the subsequent drought. This is photosynthetic plasticity par excellence and seems like a perfect strategy for these annuals in their particular habitats. It is hard to imagine that these plants are on their way to becoming perennials with full CAM. Nonetheless, such reasoning does not exclude the possibility that extant species showing pronounced constitutive CAM derived from ancestors with facultative CAM. Recent research demonstrated facultative CAM in leaves and stems of the annual C_3–C_4 intermediate *Portulaca cryptopetala* (K. Winter *et al.*, unpublished data). Similarly, combinations of weakly expressed facultative and constitutive CAM were detected in leaves and stems of the tropical vine *Basella alba* (K. Winter *et al.*, unpublished data) and the pan-tropical coastal *Sesuvium portulacastrum* (Winter *et al.*, 2019). Life forms with these attributes could give rise to long-lived stem succulents with full CAM through the thickening of stems and their green cortex, accompanied by the shift of stem photosynthesis from facultative to strong constitutive CAM, in addition to the permanent loss of leaves. In the Cactaceae, leafy forms with C_3 photosynthesis or facultative CAM are ancestral to non-leafy stem succulents with full CAM (Diaz and Medina, 1984; Edwards and Diaz, 2006). In the genus *Euphorbia* (Euphorbiaceae) containing leafy and cactiform growth forms, CAM has evolved multiple times in a complex phylogenetic pattern (Horn *et al.*, 2012, 2014). Weakly expressed CAM occurs in *Euphorbia milii* (Herrera, 2013), but facultative CAM has not yet been demonstrated conclusively in this species-rich genus (Mies *et al.*, 1996; Hastilestari *et al.*, 2013). Within the large genus *Kalanchoe* (Crassulaceae), species in the basal Kitchingia group are capable of facultative CAM, whereas the most derived taxa exhibit strong constitutive CAM (Hartwell *et al.*, 2016).

Should all plants with CAM be considered CAM plants?

The answer to this recurring question is no. The CAM cycle is believed to be present in well over 5% of vascular plant species (Winter and Smith, 1996). As noted above, in most cases CAM co-occurs with C_3 photosynthesis. Depending on the species, developmental stage, and environmental conditions, the contribution of CAM to daily carbon gain may range from <1% to 100%. The term CAM plant should be reserved for species which, throughout their lives, obtain the majority of their carbon through the CAM pathway (Box 1; Winter *et al.*, 2015). Many cacti and agaves seem to operate at or close to the full-CAM end of the phenotypic C_3–CAM spectrum (Nobel, 1988) and are traditionally and rightly considered CAM plants. *Kalanchoe pinnata* would also qualify for the CAM plant category: despite significant C_3-type CO_2 exchange during early development, CAM eventually becomes the major contributor to leaf life cycle carbon gain (Fig. 1). On the other hand, the term CAM plant would be inappropriate for species with weakly expressed CAM in which C_3 rather than CAM is the principal mode of carbon assimilation. This is particularly true for species such as *Welwitschia mirabilis* with very minor roles of CAM relative to C_3. In such circumstances, the term C_3–CAM plant is an option (Winter *et al.*, 2015).

In general, it is much easier to define distinct functional properties (e.g. facultative CAM) of a plant than to categorize organisms on the basis of a specific trait (e.g. facultative CAM plant), because this trait may co-occur in multiple combinations with other traits during different phases of the life cycle. Therefore, when characterizing a species in the context of CAM, detailed case-by-case descriptions of CAM expression relative to C_3 (or C_4) may be more informative than trying to tally a species to one of a multitude of categories created to accommodate all possible C_3/C_4–CAM trait combinations. Even categories that researchers have agreed upon such as constitutive (or obligate) CAM versus facultative CAM are

still compromises. For example, besides indicating that a process is always active, the term constitutive also has the connotation of something that is hardwired. Thus, strictly speaking, the ability to engage in facultative CAM is also constitutive. Furthermore, the term obligate, which has the meaning of being biologically essential for survival, also applies to facultative CAM annuals, where the dry season switch to CAM aids reproduction.

All these terminological complications can be largely disregarded in phylogenetic studies on CAM evolution, where the presence of CAM (irrespective of the degree of CAM usage relative to C_3 usage) and the absence of CAM are the key binary traits of the most basic analysis.

How to identify weakly expressed CAM

In recent years, there has been growing awareness of the significant number of species in which the CAM cycle (although present) contributes little to overall CO_2 assimilation when compared with C_3 or C_4 photosynthesis. Carbon isotopic signatures ($\delta^{13}C$ values) of dried plant material are not suitable indicators of weakly expressed CAM because the amount of CAM-derived carbon is usually too small to be detected against the large background of C_3 carbon. Similarly, isotopic measurements do not reveal weakly expressed CAM in C_4 plants such as *Portulaca* spp., since the C_4 and CAM isotopic signals are largely identical.

Weakly expressed CAM can only be diagnosed in living material. Highly replicated measurements of titratable acidity of samples from mature tissues, when repeatedly collected at dusk and dawn over the course of several days, can conclusively demonstrate low levels of nocturnal acidification. Acid titrations are highly sensitive and can resolve day–night differences of as low as 1–2 μmol H^+ g^{-1} fresh mass. It is advisable to demonstrate nocturnal acidification not only on a fresh mass, but also on a dry mass and area basis. In some species with highly elastic fleshy leaves, leaf fresh mass decreases in the course of the day even in the absence of soil-water deficit stress; this leads to an overestimation of acidity levels at dusk and thus an underestimation of nocturnal acidification. In extreme cases, decreases in fresh mass during the day can completely mask nocturnal increases in H^+.

Measurements of malate levels at dawn and dusk (e.g. enzymatically or through HPLC) can also be used to detect low-level CAM. However, malate assays do not distinguish between malic acid involved in CAM and malate anions that are electrochemically balanced by cations such as K^+ and that do not participate in CAM. It is noteworthy that the leaves of some non-CAM species in the Brassicaceae accumulate substantial amounts of malate in the course of the day (i.e. not at night!) (Winter *et al.*, 1976, 1982), when NO_3^- taken up as KNO_3 is metabolized in the light and malate^{2-} is synthesized to balance the positive charges of the remaining K^+. Such diurnal increases in malate are completely unrelated to CAM. Potassium malate, unlike malic acid, is not detectable by titration. Suggestions that nocturnal net acid accumulation is a feature of C_3 species (fig. 2A of Bräutigam *et al.*, 2017) are unfounded.

In order to confirm the presence of weakly expressed CAM in a species, acidity measurements should be accompanied by continuous CO_2 gas exchange measurements during 12 h light/12 h dark cycles. In the past, such measurements generally required elaborate, laboratory-installed custom-built gas exchange systems. Nowadays, miniaturized modern portable photosynthesis systems, when programmed for automatic long-term CO_2 logging, are suitable tools for CAM studies as well. Weakly expressed CAM may or may not be associated with net CO_2 dark fixation, and in many cases the nocturnal carbon balance remains negative. If nocturnal net CO_2 fixation does occur, it is often restricted to a brief phase of the dark period. In the case when dark CO_2 fixation capacity—although elevated when compared with regular C_3 plants—is not sufficient to support net CO_2 fixation, weak CAM nonetheless results in characteristic, curved CO_2 exchange patterns of net CO_2 loss during the course of the night, with lowest rates of net CO_2 loss typically in the middle of the night. Weak, presumably constitutive, CAM has been reported for a large number of species in the Orchidaceae (Silvera *et al.*, 2005). Furthermore, in many species with facultative CAM, the degree of drought-induced CAM is very low compared with C_3 photosynthetic CO_2 uptake in the light in unstressed plants (Box 1).

Demonstrating the absence of the CAM cycle in a given species, especially when the species under investigation belongs to a lineage that contains confirmed CAM species, can be as challenging as demonstrating the presence of weakly expressed CAM in a species. Excluding CAM is of major concern in phylogenetic studies on CAM evolution that may require 100% pure C_3 ancestral material for comparative purposes. It may also be useful to keep in mind the dictum that 'absence of evidence is not evidence of absence' when exploring CAM to C_3 reversals in lineages.

Is there a C_3–CAM continuum?

The answer is: phenotypically yes, genotypically no. The entire range of diel CO_2 exchange patterns is possible from 0% CAM (i.e. 100% C_3 or C_4) to 100% CAM, as demonstrated by comparative gas exchange studies of C_3-, C_4-, and CAM-exhibiting species, particularly of CAM species transitioning from C_3 to CAM ontogenetically or facultatively (Figs 1–5). Hence, phenotypically, there is undoubtedly a C_3–CAM continuum. Genotypically, this is not the case as the evolutionary transitioning to CAM is based on discrete changes in the genetic makeup of species resulting in changes in protein sequence and/or gene expression (Yang *et al.*, 2016, 2017). Enhanced succulence in some lineages may have a potentiating effect, increasing the likelihood for CAM to evolve (Heyduk *et al.*, 2016). Even if the development of CAM were to be entirely based on the up-regulation of pre-existing C_3 genes in some species, the ability to do so is a heritable trait that would be encoded in the genome. Unraveling the molecular basis of CAM is one of the most active areas of current CAM research (e.g. Goolsby *et al.*, 2018; Heyduk *et al.*, 2018), although emphasis is largely on iconic CAM species with pronounced CAM such as *Agave* ssp., *Opuntia* ssp., and *Ananas comosus* (Ming *et al.*, 2015; Abraham

et al., 2016). Genome and transcriptome studies of plants with weakly expressed constitutive and facultative CAM are currently less in vogue (Brilhaus *et al.*, 2016), but hold particular promise for capturing the early steps of CAM evolution.

Underscoring the distinctness of CAM is the fact that CAM (including weakly expressed CAM) is currently noted in 35 families of angiosperms, plus five families of lycopods, ferns, and gymnosperms (Smith and Winter, 1996; J.A.C. Smith *et al.*, unpublished data), whereas CAM is not known to occur in over 370 angiosperm families. Unquestionably, CAM will be discovered in additional families in the future, but this is unlikely to alter the predominance of non-CAM taxa. One possible candidate for a 'new' CAM (or C_4) family is the Capparaceae. For one of its members, *Cadaba aphylla*, a leafless, succulent-branched shrub from tropical Africa to South Africa and Namibia, Schulze *et al.* (1976) reported a $\delta^{13}C$ value of –16.9‰. Further studies into the photosynthetic pathway operating in this species would be rewarding.

Bioengineering CAM into C_3

Introducing CAM into C_3 crops may make them more resilient to hotter and drier conditions in the face of concurrent man-made climate change (Yang *et al.*, 2015). However, even if CAM-into-C_3 engineering would become technically feasible, there is more to the CAM 'syndrome' than merely its CAM cycle biochemistry. Full, strongly expressed CAM is typically associated with highly succulent leaves or photosynthetic stems, and hence relatively low surface to dry mass ratios of individual photosynthetic organs. These morphological features tend to lower the ratio of total photosynthetic surface area to total dry mass of plants, thereby lowering rates of growth, even though CAM plants are known for maximizing available surface areas for CO_2 uptake. Considerations of this kind are useful when planning to bioengineer CAM into C_3 plants, especially C_3 trees (Borland *et al.*, 2015), which in terms of potential growth rates are already disadvantaged due to substantial dry mass allocation to non-photosynthetic stem tissue. Leaves with facultative CAM are likely to have lower construction costs in comparison with leaves with full, strongly expressed CAM. Thus, facultative CAM may be the preferred form of CAM to be introduced into C_3 trees, after having evaluated the benefits of improved water use efficiency versus potential constraints on rates of biomass accumulation.

Topics for future ecophysiological CAM research

A range of topics and questions appear to be of particular interest for future ecophysiological CAM research. These include (i) whole-plant *in situ* CO_2 gas exchange measurements of archetypal constitutive CAM species, such as columnar cacti and platyopuntias, combined with measurements of plant productivity. Does CO_2 fixation during the early morning contribute to growth? Is there substantial net CO_2 loss during hot daytime hours? How 'airtight' are these plants under severe drought stress? (ii) How do the net assimilation rate and leaf or stem area ratio determine relative growth rates of leaf and stem succulent CAM plants? Thus far, CAM-exhibiting species have been largely omitted from global analyses of plant functional traits that describe the performance of species along the fast–slow lifestyle continuum (Osnas *et al.*, 2013; Huber *et al.*, 2018; Males and Griffiths, 2018). (iii) How does eddy-flux-based CO_2 and water vapor exchange of CAM-dominated vegetation and of cultivated CAM crops respond to seasonal change (see, for example, Owen *et al.*, 2016)? (iv) How do daily patterns of C_3 and CAM photosynthesis vary during the life cycle of annual and perennial facultative CAM species *in situ*? This requires the monitoring of C_3 and CAM activities at close (e.g. weekly) intervals, combined with measurements of plant phenology, microclimate, and edaphic conditions. (v) What is the adaptive significance of weakly expressed CAM? (vi) How abundant is CAM in orchids—one of the two largest families of vascular plants—especially in the relatively understudied Asian species? (vi) How many species of the large family Aizoaceae engage in facultative or constitutive CAM? CAM may have been a key innovation that facilitated the rapid, recent diversification of this clade (see, for example, Klak *et al.*, 2004). (vii) How do CAM species respond to atmospheric and climate change? Is CAM down-regulated in favor of C_3 photosynthesis as atmospheric CO_2 concentrations rise? (viii) Eco-transcriptomics: how does the expression of CAM genes and CAM-related genes change during natural day–night cycles with varying diurnal photon flux density (PFD), especially in genes postulated to be under circadian control?

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References

Abraham PE, Yin H, Borland AM, *et al.* 2016. Transcript, protein and metabolite temporal dynamics in the CAM plant *Agave*. Nature Plants **2,** 16178.

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.

Beltrán JD, Lasso E, Madriñán S, Virgo A, Winter K. 2013. Juvenile tank-bromeliads lacking tanks: do they engage in CAM photosynthesis? Photosynthetica **51,** 55–62.

Bloom AJ, Troughton JH. 1979. High productivity and photosynthetic flexibility in a CAM plant. Oecologia **38,** 35–43.

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.

Borland AM, Wullschleger SD, Weston DJ, Hartwell J, Tuskan GA, Yang X, Cushman JC. 2015. Climate-resilient agroforestry: physiological responses to climate change and engineering of crassulacean acid metabolism (CAM) as a mitigation strategy. Plant, Cell & Environment **38,** 1833–1849.

Boxall SF, Dever LV, Kneřová J, Gould PD, Hartwell J. 2017. Phosphorylation of phosphoenolpyruvate carboxylase is essential for maximal and sustained dark CO_2 fixation and core circadian clock operation in the obligate crassulacean acid metabolism species *Kalanchoë fedtschenkoi*. The Plant Cell **29,** 2519–2536.

Bräutigam A, Schlüter U, Eisenhut M, Gowik U. 2017. On the evolutionary origin of CAM photosynthesis. Plant Physiology **174,** 473–477.

Brilhaus D, Bräutigam A, Mettler-Altmann T, Winter K, Weber AP. 2016. Reversible burst of transcriptional changes during induction of crassulacean acid metabolism in *Talinum triangulare*. Plant Physiology **170,** 102–122.

Brulfert J, Ravelomanana D, Güçlü S, Kluge M. 1996. Ecophysiological studies in *Kalanchoë porphyrocalyx* (Baker) and *K. miniata* (Hils et Bojer), two species performing highly flexible CAM. Photosynthesis Research **49,** 29–36.

Castillo FT. 1996. Antioxidative protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. Oecologia **107,** 469–477.

Crayn DM, Winter K, Schulte K, Smith JAC. 2015. Photosynthetic pathways in Bromeliaceae: phylogenetic and ecological significance of CAM and C_3 based on carbon isotope ratios for 1893 species. Botanical Journal of the Linnean Society **178,** 169–221.

Dever LV, Boxall SF, Kneřová J, Hartwell J. 2015. Transgenic perturbation of the decarboxylation phase of crassulacean acid metabolism alters physiology and metabolism but has only a small effect on growth. Plant Physiology **167,** 44–59.

Diaz M, Medina E. 1984. Actividad CAM de cactaceas en condiciones naturales. In: Medina E, ed. Eco-fisiologia de plantas CAM. Caracas: CIET (IVIC-UNESCO), 98–113.

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

Edwards EJ, Diaz M. 2006. Ecological physiology of *Pereskia guamacho*, a cactus with leaves. Plant, Cell & Environment **29,** 247–256.

Evenari M, Shanan L, Tadmor N. 1971. The Negev. The challenge of a desert. Cambridge, MA: Harvard University Press.

Goolsby EW, Moore AJ, Hancock LP, De Vos JM, Edwards EJ. 2018. Molecular evolution of key metabolic genes during transitions to C4 and CAM photosynthesis. American Journal of Botany **105,** 602–613.

Gould SJ. 1977. Ontogeny and phylogeny. Cambridge, MA: The Belknap Press of Harvard University Press.

Güerere I, Tezara W, Herrera C, Fernández MD, Herrera A. 1996. Recycling of CO_2 during induction of CAM by drought in *Talinum paniculatum* (Portulacaceae). Physiologia Plantarum **98,** 471–476.

Guralnick LJ, Cline A, Smith M, Sage RF. 2008. Evolutionary physiology: the extent of C_4 and CAM photosynthesis in the genera *Anacampseros* and *Grahamia* of the Portulacaceae. Journal of Experimental Botany **59,** 1735–1742.

Guralnick LJ, Edwards G, Ku MS, Hockema B, Franceschi VR. 2002. Photosynthetic and anatomical characteristics in the C_4–crassulacean acid metabolism-cycling plant, *Portulaca grandiflora*. Functional Plant Biology **29,** 763–773.

Hanscom Z, Ting IP. 1978. Responses of succulents to plant water stress. Plant Physiology **61,** 327–330.

Hastilestari BR, Mudersbach M, Tomala F, Vogt H, Biskupek-Korell B, Van Damme P, Guretzki S, Papenbrock J. 2013. *Euphorbia tirucalli*—comprehensive characterization of a drought tolerant plant with a potential as biofuel source. PLoS One **8,** e63501.

Hartwell J, Dever LV, Boxall SF. 2016. Emerging model systems for functional genomics analysis of crassulacean acid metabolism. Current Opinion in Plant Biology **31,** 100–108.

Herppich WB, Midgley G, von Willert DJ, Veste M. 1996. CAM variations in the leaf-succulent *Delosperma tradescantioides* (Mesembryanthemaceae), native to southern Africa. Physiologia Plantarum **98,** 485–492.

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.

Herrera A. 2013. Crassulacean acid metabolism-cycling in *Euphorbia milii*. AoB Plants **5,** plt014.

Heyduk K, McKain MR, Lalani F, Leebens-Mack J. 2016. Evolution of a CAM anatomy predates the origins of crassulacean acid metabolism in the Agavoideae (Asparagaceae). Molecular Phylogenetics and Evolution **105,** 102–113.

Heyduk K, Ray JN, Ayyampalayam S, Leebens-Mack J. 2018. Shifts in gene expression profiles are associated with weak and strong crassulacean acid metabolism. American Journal of Botany **105,** 587–601.

Holtum JAM, Hancock LP, Edwards EJ, Winter K. 2017*a*. Facultative CAM photosynthesis (crassulacean acid metabolism) in four species of *Calandrinia*, ephemeral succulents of arid Australia. Photosynthesis Research **134,** 17–25.

Holtum JAM, Hancock LP, Edwards EJ, Winter K. 2017*b*. Optional use of CAM photosynthesis in two C_4 species, *Portulaca cyclophylla* and *Portulaca digyna*. Journal of Plant Physiology **214,** 91–96.

Holtum JAM, Hancock LP, Edwards EJ, Winter K. 2018. Crassulacean acid metabolism (CAM) in the Basellaceae (Caryophyllales). Plant Biology **20,** 409–414.

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

Horn JW, van Ee BW, Morawetz JJ, Riina R, Steinmann VW, Berry PE, Wurdack KJ. 2012. Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). Molecular Phylogenetics and Evolution **63,** 305–326.

Horn JW, Xi Z, Riina R, Peirson JA, Yang Y, Dorsey BL, Berry PE, Davis CC, Wurdack KJ. 2014. Evolutionary bursts in *Euphorbia* (Euphorbiaceae) are linked with photosynthetic pathway. Evolution **68,** 3485–3594.

Huber J, Dettman DL, Williams DG, Hultine KR. 2018. Gas exchange characteristics of giant cacti species varying in stem morphology and life history strategy. American Journal of Botany **105,** 1688–1702.

Klak C, Reeves G, Hedderson T. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. Nature **427,** 63–65.

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

Koch K, Kennedy RA. 1980. Characteristics of crassulacean acid metabolism in the succulent C_4 dicot, *Portulaca oleracea* L. Plant Physiology **65,** 193–197.

Lee HSJ, Griffiths H. 1987. Induction and repression of CAM in *Sedum telephium* L. in response to photoperiod and water stress. Journal of Experimental Botany **38,** 834–841.

Males J, Griffiths H. 2018. Economic and hydraulic divergences underpin ecological differentiation in the Bromeliaceae. Plant, Cell & Environment **41,** 64–78.

Martin CE, Gravatt DA, Loeschen VS. 1994. Crassulacean acid metabolism in three species of Commelinaceae. Annals of Botany **74,** 457–463.

Medina E, Delgado M, Troughton JH, Medina JD. 1977. Physiological ecology of CO_2 fixation in Bromeliaceae. Flora **166,** 137–152.

Mies B, Jiménez MS, Morales D. 1996. Ecophysiology and distribution of the endemic leafless spurge *Euphorbia aphylla* and the introduced *E. tirucalli* (*Euphorbiaceae*, *Euphorbia* sect. *Tirucalli*) in the Canary Islands. Plant Systematics and Evolution **202,** 27–36.

Ming R, VanBuren R, Wai CM, *et al*. 2015. The pineapple genome and the evolution of CAM photosynthesis. Nature Genetics **47,** 1435–1442.

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

Olivares E, Urich R, Montes G, Coronel I, Herrera A. 1984. Occurrence of crassulacean acid metabolism in *Cissus trifoliata* L. (Vitaceae). Oecologia **61,** 358–362.

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

Osnas JLD, Lichstein JW, Reich PB, Pacala SW. 2013. Global leaf trait relationships: mass, area and the leaf economics spectrum. Science **340,** 741–744.

Owen NA, Choncubhair ÓN, Males J, Del Real Laborde JI, Rubio-Cortés R, Griffiths H, Lanigan G. 2016. Eddy covariance captures four-phase crassulacean acid metabolism (CAM) gas exchange signature in *Agave*. Plant, Cell & Environment **39,** 295–309.

Schuber M, Kluge M. 1981. *In situ* studies of crassulacean acid metabolism in *Sedum acre* L. and *Sedum mite* Gil. Oecologia **50,** 82–87.

Schulze ED, Ziegler H, Stichler W. 1976. Environmental control of crassulacean acid metabolism in *Welwitschia mirabilis* Hook. Fil. in its range of natural distribution in the Namib desert. Oecologia **24,** 323–334.

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.

Smith JAC, Winter K. 1996. Taxonomic distribution of crassulacean acid metabolism. In: Winter K, Smith JAC, eds. Crassulacean acid metabolism. Biochemistry, ecophysiology and evolution. Berlin Heidelberg: Springer, 427–436.

Smith TL, Eickmeier WG. 1983. Limited photosynthetic plasticity in *Sedum pulchellum* Michx. Oecologia **56,** 374–380.

Ting IP, Hanscom Z. 1977. Induction of acid metabolism in *Portulacaria afra*. Plant Physiology **59,** 511–514.

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

Treichel S, Bauer P. 1974. Unterschiedliche NaCl-Abhängigkeit des tagesperiodischen CO_2-Gaswechsels bei einigen halisch wachsenden Küstenpflanzen. Oecologia **17,** 87–95.

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.

Von Willert DJ, Kramer D. 1972. Feinstruktur und Crassulaceen-Säurestoffwechsel in Blättern von *Mesembryanthemum crystallinum* während natürlicher und NaCl-induzierter Alterung. Planta **107,** 227–237.

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

Winter K, Garcia M, Holtum JA. 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.

Winter K, Garcia M, Holtum JAM. 2009. Canopy CO_2 exchange of two neotropical tree species exhibiting constitutive and facultative CAM photosynthesis, *Clusia rosea* and *Clusia cylindrica*. Journal of Experimental Botany **60,** 3167–3177.

Winter K, Garcia M, Holtum JA. 2011. Drought-stress-induced up-regulation of CAM in seedlings of a tropical cactus, *Opuntia elatior*, operating predominantly in the C_3 mode. Journal of Experimental Botany **62,** 4037–4042.

Winter K, Garcia M, Holtum JA. 2014. Nocturnal versus diurnal CO_2 uptake: how flexible is *Agave angustifolia*? Journal of Experimental Botany **65,** 3695–3703.

Winter K, Garcia M, Virgo A, Holtum JAM. 2019. Operating at the very low end of the crassulacean-acid-metabolism (CAM) spectrum: *Sesuvium portulacastrum* (Aizoaceae). Journal of Experimental Botany **70,** 6561–6570.

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

Winter K, Holtum JA. 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, Holtum JAM. 2011. Induction and reversal of crassulacean acid metabolism in *Calandrinia polyandra*: effects of soil moisture and nutrients. Functional Plant Biology **38,** 576–582.

Winter K, Holtum JA. 2014. Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. Journal of Experimental Botany **65,** 3425–3441.

Winter K, Holtum JAM. 2017. Facultative crassulacean acid metabolism (CAM) in four small C_3 and C_4 leaf-succulents. Australian Journal of Botany **65,** 103–108.

Winter K, Holtum JA, Smith JA. 2015. Crassulacean acid metabolism: a continuous or discrete trait? New Phytologist **208,** 73–78.

Winter K, Lüttge U, Winter E, Troughton JH. 1978. Seasonal shift from C_3 photosynthesis to crassulacean acid metabolism in *Mesembryanthemum crystallinum* growing in its natural environment. Oecologia **34,** 225–237.

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. Biochemistry, ecophysiology and evolution. Berlin Heidelberg: Springer, 1–13.

Winter K, Troughton JH, Evenari M, Läuchli A, Lüttge U. 1976. Mineral ion composition and occurrence of CAM-like diurnal malate fluctuations in plants of coastal and desert habitats of Israel and the Sinai. Oecologia **25,** 125–143.

Winter K, Usuda H, Tsuzuki M, Schmitt M, Edwards GE. 1982. Influence of nitrate and ammonia on photosynthetic characteristics and leaf anatomy of *Moricandia arvensis*. Plant Physiology **70,** 616–625.

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

Winter K, Ziegler H. 1992. Induction of crassulacean acid metabolism in *Mesembryanthemum crystallinum* increases reproductive success under conditions of drought and salinity stress. Oecologia **92,** 475–479.

Yang X, Cushman JC, Borland AM, *et al*. 2015. A roadmap for research on crassulacean acid metabolism (CAM) to enhance sustainable food and bioenergy production in a hotter, drier world. New Phytologist **207,** 491–504.

Yang X, Hu R, Yin H, *et al*. 2017. The *Kalanchoë* genome provides insights into convergent evolution and building blocks of crassulacean acid metabolism. Nature Communications **8,** 1899.