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CAM photosynthesis in desert blooming *Cistanthe* of the Atacama, Chile

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Abstract. When plants of the Atacama desert undergo episodic blooms, among the most prominent are succulent-leaved *Cistanthe* (Montiaceae). We demonstrate that two *Cistanthe* species, the perennial *Cistanthe* sp. aff. *crassifolia* and the annual/biannual *Cistanthe* sp. aff. *longiscapa*, can exhibit net CO_2 uptake and leaf acidification patterns typical of crassulacean acid metabolism (CAM). In *C*. sp. aff. *crassifolia* leaves, CAM expression was facultative. CAM-type nocturnal net CO_2 uptake and acid accumulation occurred in drought-stressed but not in well-watered plants. By contrast, CAM expression in *C*. sp. aff. *longiscapa* was largely constitutive. Nocturnal acid accumulation was present in leaves of well-watered and in droughted plants. Following water-deficit stress, net nocturnal CO_2 uptake was induced and the level of acid accumulated increased. Neither nocturnal CO_2 uptake nor acid accumulation was reduced when the plants were re-watered. $\delta^{13}C$ values of a further nine field-collected *Cistanthe* species are consistent with a contribution of CAM to their carbon pools. In the Portulacinae, a suborder with eight CAM-containing families, *Cistanthe* becomes the sixth genus with CAM within the family Montiaceae, and it is likely that the ancestor of all Portulacineae also possessed CAM photosynthesis. In the stochastic rainfall landscape of the Atacama, carbon uptake in the dark is a water-use efficient mechanism that increases the carbon pool available for seed production or dormancy. The next rain event may be years away.

Keywords: CAM evolution, crassulacean acid metabolism, *Cistanthe*, constitutive CAM, drought, facultative CAM, Montiaceae, $\delta^{13}C$ values, nocturnal, succulent.

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Introduction

When the coastal desert blooms (*desierto florido*) on the Atacama plateau that borders the Pacific coast of northern Chile and southern Peru, as it has 14 times since 1981 (Chávez *et al*. 2019), the combined effects of the increase in vegetation mass and plant cover on evapotranspiration, albedo and heat storage capacity are sufficient to decrease the average diel land surface temperature by 0.29°C ± 0.07°C (He *et al*. 2017). The associated pulse in primary productivity not only affects ecosystem structure and functioning across trophic levels (Holmgren *et al*. 2006) but is also a stimulus for tourism. In 2017, a bloom year, nearly 25% of the 7.6 million tourists to Chile visited the Atacama in northern Chile (World Tourism Organisation 2019). Between them the visitors spent US$4.6 billion.

The bloom-related stimulation of vegetation germination, growth and reproduction that occurs in the hyper-arid, fog and non-fog regions of the Atacama is a response to uncommon, sporadic and often brief rainfall events that tend to be associated with major and minor El Niño episodes (Dillon and Rundel 1989; Rundel *et al*. 1991; Armesto *et al*. 1993). Vidiella *et al*. (1999) suggested that a threshold level of at least 15 mm rainfall is required to trigger a growth pulse, following which the seed banks of annuals, perennials and geophytes are replenished. During the intervals without rainfall, the terrestrial vegetation becomes essentially dormant. Annuals often grow under the protection of nursery shrubs, and leafy perennials lose their leaves, except in those near-shore locations where ocean fog may provide sufficient moisture (Rundel *et al*. 1980, 1991, 1996; Dillon and Rundel 1989; Armesto *et al*. 1993).

The flower colours of the areas in bloom typically change with time as different species flower sequentially (Vidiella *et al*. 1999). In the southern Atacama, flowering shrubs, many with small fleshy leaves, include members of the Aizoaceae, Apocynaceae, Asteraceae Boraginaceae, Frankeniaceae, Malvaceae, Nolanaceae and Verbenaceae; geophytes are represented by Amarylidaceae, Euphorbiaceae, Iridaceae, Liliaceae and Techophilaeceae; and prominent flowering annuals include members of the Asteraceae, Brassicaceae, Chenopodiaceae, Montiaceae, Nolanaceae, Onagraceae and Fabaceae.

Among the most colourful and prominent components of the Atacama blooms are succulent-leaved herbs of the genus *Cistanthe* Spach (Family Montiaceae, Order Caryophyllales) (Fig. 1). With a disjunct North and South American distribution, one species of *Cistanthe* is native to Arizona, California and north-western Mexico, and ~30 species are found in central and northern Chile, north-western and southern Argentina, and Peru (POWO 2020). Of the endemic and native species in Chile, 10 are perennials and 14 annuals (Rodriguez *et al*. 2018).

Long considered a member of the Portulacaceae, *Cistanthe* was transferred to Montiaceae following a molecular- and morphology-based revision of the sub-order Portulacinae (Nyffeler and Eggli 2010*a*, 2010*b*). The genera within the Montiaceae alongside *Cistanthe* are currently *Calandrinia*, *Calyptridium*, *Claytonia*, *Hectorella*, *Lenzia*, *Lewisia*, *Lewisiopsis*, *Lyallia*, *Montia*, *Montiopsis*, *Phemeranthus* and *Schreiteria* (Hernández-Ledesma *et al*. 2015). As a group with not yet fully resolved taxonomy, *Cistanthe* and its fellow Montiaceae remain objects of taxonomic and morphological scrutiny (Hershkovitz 1991*a*, 1991*b*, 1993*a*, 1993*b*, 2018*a*, 2018*b*; Hershkovitz and Zimmer 2000; Stoll *et al*. 2017), with the Australian and New World *Calandrinia* in the process of being split into separate New World (Calandrinia *sensu* stricto) and Australian entities (Carolin 1987; Hershkovitz 1998; Hancock *et al*. 2018; Thiele *et al*. 2018). New species continue to be reported (Obbens 2006, 2011, 2012, 2014, 2018; Ware 2011; Elvebakk *et al*. 2015; Obbens *et al*. 2017; Hershkovitz 2018*a*) and photosynthetic physiology explored (Guralnick *et al*. 2001; Winter and Holtum 2011, 2017; Holtum *et al*. 2016, 2017; Hancock *et al*. 2019).

The carbon-concentrating forms of photosynthesis, crassulacean acid metabolism (CAM) and C_4, have evolved independently several times within the Caryophyllales (Smith and Winter 1996*a*; Edwards and Ogburn 2012; Sage 2016; Edwards 2019; Winter *et al*. 2020*a*). Nine families contain species with CAM and eight families contain C_4 species. The families with CAM include all eight in the reconstructed Portulacinae, including Montiaceae. C_4 photosynthesis is absent from the Montiaceae but is present in the closely related families Portulacaceae and Molluginaceae.

Fig. 1. The blooming of the Atacama Desert in October, 2017, on an alluvial plain between Vallenar and Copiacó, Chile, at latitude 27.521583°S, longitude –70.432833°W. Plants with pink flowers are *Cistanthe* sp.

Within the Montiaceae as it is currently circumscribed, CAM-defining CO_2 exchange and/or nocturnal acidification patterns have been demonstrated in *Calandrinia sensu* stricto, the Australian *Calandrinia* clade, *Claytonia*, *Lewisia* and *Phemeranthus* (Winter *et al*. 1981; Harris and Martin 1991*a*, 1991*b*; Guralnick and Jackson 2001; Guralnick *et al*. 2001; Winter and Holtum 2011, 2017; Holtum *et al*. 2017; Hancock *et al*. 2019). Note that the *Talinum* species studied by Harris and Martin (1991*a*, 1991*b*) are now considered species of *Phemeranthus* (POWO 2020). Many of the Australian *Calandrinia* exhibit CAM facultatively, in that CAM is absent from well-watered plants but is present in plants subjected to water-deficit stress (Hancock *et al*. 2019; Winter 2019).

CAM has not been measured experimentally in *Cistanthe*, but some carbon isotope ratios of tissues collected from plants growing in their natural environments are intermediate between values expected for full-CAM and C_3 plants and have been tentatively interpreted as 'CAM-type' (Mooney *et al*. 1974; Troughton *et al*. 1974; Arroyo *et al*. 1990; Ehleringer *et al*. 1998). The interpretations are tentative because plants fully operating in the C_3 mode under conditions of particularly constrained stomatal diffusion, as might be predicted for plants operating in low-rainfall and low humidity environments or at high elevations, may exhibit such intermediate isotopic ratios in the absence of CAM (Farquhar *et al*. 1982; Cernusak *et al*. 2013).

In order to test whether the carbon isotope ratios reported for *Cistanthe* growing in their native desert environments truly reflect a contribution of CAM photosynthesis to plant carbon gain we measured CO_2 exchange patterns during the day and night in *Cistanthe* growing under well-watered and drought-stressed conditions. We also quantified the capacity of leaves to accumulate titratable acidity at night, a CAM-defining attribute.

We report here the presence of CAM in two species of *Cistanthe*, the perennial *Cistanthe* sp. aff. *crassifolia* (Phil.) Peralta & D.I.Ford, and the annual/biannual *Cistanthe* sp. aff. *longiscapa* (Phil.) Peralta & D.I. Ford (Fig. 2). In *C*. sp. aff. *crassifolia* the expression of CAM, as determined by measurements of CO_2 exchange and nocturnal acidification, was overwhelmingly facultative. Nocturnal acidification and net CO_2 uptake was induced following water-deficit stress and essentially disappeared when plants were rewatered. In *C*. sp. aff. *longiscapa*, low-level CAM present in well-watered plants was upregulated following water-deficit stress. Following rewatering, the rates of CO_2 uptake in the light increased but nocturnal net CO_2 uptake and acidification remained elevated.

Materials and methods

Plant material

During the Atacama desert bloom of 2017, seed of *Cistanthe* sp. aff. *crassifolia* (Phil.) Peralta & D.I.Ford (Fig. 2) was

Fig. 2. An 11-week-old perennial *Cistanthe* sp. aff. *crassifolia* (top panel), an annual *Cistanthe* sp. aff. *longiscapa* with flowering stem (bottom left panel) and a flower of *Cistanthe* sp. aff. *longiscapa* (bottom right panel). Pots are 14 cm (top panel) and 10 cm in diameter, respectively, and the flower is 2.5 cm in diameter.

collected from a coastal desert plain Llanos de Challe, Atacama Region, Chile (–28.175892°S, –71.158455°W, 31 m above sea level), and seed of *Cistanthe* sp. aff. *longiscapa* (Phil.) Peralta & D.I. Ford (Fig. 2) was collected from an alluvial plain ~20 km south of Copiapó, Atacama region, Chile (–27.521583°S, –70.432833°W, 730 m above sea level).

Plants germinated under 50% sunlight were transferred, when ~3 cm tall, to 0.4 or 1 L pots (one plant per pot; terracotta for the experiments using *C*. sp. aff. *crassifolia* or plastic for the experiments using *C*. sp. aff. *longiscapa*) containing a commercial potting mix (Miracle Gro) and were grown-on under 12 h light/12 h dark photoperiods in externally ventilated controlled-climate cabinets (models GC8-T or GC15, EGC, USA; model FH-1200, Hi-Point). Unless specified otherwise, plants were watered daily to field capacity.

Gas-exchange measurements of shoots and leaves

Gas exchange by shoots of whole plants with 2–8 fully-expanded leaves was measured using custom-built gas-exchange systems incorporating LI-COR, Qubit and Walz elements. For measuring CO_2 exchange an entire shoot of a plant was sealed in a 12 cm × 12 cm × 12 cm (ID) polished Perspex chamber (Walz) such that the roots and pot were outside the chamber but within the growth cabinet. The entry point of the shoot into the chamber was sealed with Terostat VII (Henkel AG). Air entering the chamber was sourced from either a 2 or a 3 m^3 container situated above the roof of the laboratory building. $[CO_2]$ and $[H_2O]$ of the incoming and outgoing air were measured by infrared gas analysis (model LI-7000 or LI-6262, LI-COR Biosciences). Air dewpoint was controlled using a cool-trap (model KF-18/2B, Walz). Flow rates, day/night temperatures and light intensities for each experiment are listed in the legends of Figures 4–8.

Plants were initially watered daily to field capacity with rainwater and CO_2 exchange was monitored until the daily exchange pattern was either constant or increased as the plant grew. Watering was then withheld until net CO_2 exchange during the light was reduced to close to zero. The period without watering depended upon the size of the plant and the pot. The plant was then rewatered daily until CO_2 exchange patterns were close to those at the beginning of the experiment, were similar from day-to-day, or increased as the plant grew. Gas-exchange of the watering-stress response for each species was repeated between 3–5 times.

Single leaf gas exchange of the third emerged leaf of *C*. sp. aff. *longiscapa* was performed using a portable photosynthesis analyser (model LI-6400, LI-COR Biosciences). Light intensity and temperature within the measuring chamber tracked the conditions in the controlled environment chamber within which the experiment was performed (model FH-1200, Hipoint): 12 h light (220 µmol m^{-2} s^{-1}) at 28°C/12 h dark at 20°C. Air, sourced from the 3 m^3 buffer mentioned above, was passed through a 3 × 2 cm chamber at 300 µmol s^{-1}. Throughout the experiment, CO_2 and H_2O-exchange were determined at 1 s intervals and 10 min averages were logged.

Measurements of titratable acidity

For *C*. sp. aff. *crassifolia*, leaf samples from 12 plants harvested at dawn and dusk were weighed, leaf area determined (model LI- 3100-C leaf area meter, LI-COR Biosciences) and frozen in liquid nitrogen. Samples were transferred from liquid nitrogen to a freeze-drier (Freezone 4.5, Labconco). After 72 h, samples were reweighed for dry mass determination.

For *C*. sp. aff. *longiscapa*, leaf samples harvested at dawn and dusk were weighed, photographed against a scale, and frozen in liquid nitrogen. Dry mass was estimated by sampling opposite leaf pairs similarly harvested at dawn and dusk, and by weighing the tissue before and after drying in an oven for 7 days at 70°C. Leaf areas were quantified by image-analysis of the photographs (ImageJ).

Titratable acidity was quantified by extracting tissues sequentially in boiling 50% ethanol and in water and titrating the extracts with 5 mM KOH to pH 6.5 (Winter and Holtum 2017).

Statistical analyses of the *C*. sp. aff. *longiscapa* acidity data utilised the R package (R Core Team 2020). The influence of experimental treatments on fresh mass and area were compared using a one-way anova and Tukey's post-hoc tests as variances between treatment levels were homogeneous (Levene's test, $P > 0.05$). The influence of experimental treatments on dry mass were compared using a Welch one-way ANOVA and Games-Howell post-hoc tests as variances between treatment levels were heterogenous (Levene's test, $P < 0.05$).

Estimation of soil water content

Volumetric water content of the soil in gas-exchange experiments with *C.* sp. aff. *longiscapa* shoots was measured using a soil capacitance sensor (Teros-12, Meter Group) permanently inserted through the side of the pot parallel to its base, approximately two-thirds of the distance from the bottom to the top of the pot. The probe was within the rooting zone of the plant. One hundred percent soil water content (field-water capacity) was defined as the average volumetric water content of the soil 1 h after watering on 5 consecutive days.

Results

Carbon isotope values

The published δ^{13}C values for nine species of *Cistanthe*, growing in their natural environments between sea-level and 4000 m above sea level, range between –18.0 and –24.1 ‰ (Fig. 3; Table 1). The mean δ^{13}C values of perennial *Cistanthe* (–21.9 ± 1.4 ‰, $n = 5$) do not differ from the annual species (–22.6 ± 1.8 ‰, $n = 3$; $P = 0.48$) and no altitudinal correlation with δ^{13}C values was uncovered. The average isotopic value of –21.6 ± 1.9 ‰ for all of the *Cistanthe* spp. is 3.6 ‰ more less negative than the –25.2 ± 1.0 ‰ mean for four species of *Montiopsis*, a sister clade to *Cistanthe*. The *Montiopsis* samples were collected from natural populations in Chile that grew

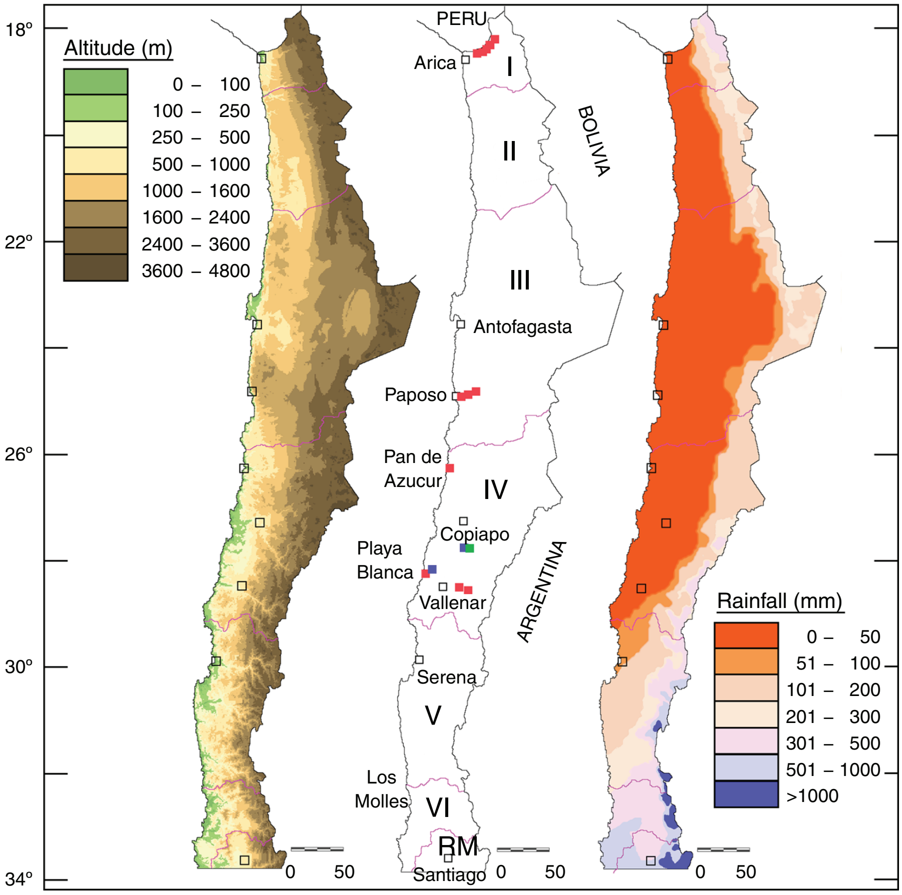

Fig. 3. Northern Chile: maps show topography (left panel), sites near which the Chile-collected *Cistanthe* shown in Table 1 were collected (middle panel, red, blue and green squares), and mean annual rainfall (right panel). The maps show the location of towns mentioned in the text and Table 1 (open squares), the borders of administrative regions of Chile (pink lines), and latitude south of the Equator. The Latin numerals refer to Chilean administrative regions: I = Arica and Parinacota, II = Tarapicá, III = Antofagasta, IV = Atacama, V = Coquimbo, VI = Valparaíso, RM = Metropolitano de Santiago. In the middle panel, the green square indicates where the photo in Fig. 1 was taken, the red squares are sites near which the Chile-collected *Cistanthe* for which isotope values are shown in Table 1 were collected, and blue squares are the sites where seeds of the two species used for gas exchange were collected.

Table 1. Collated δ^{13}C values (‰), habit and site information of species within the Montiaceae in desert ecosystems of the southern USA, north-west Mexico and in South and Central America
Current names (POWO 2020) and the names under which the data was originally published are shown

Current name	Name as published	Perrenial (P) or annual (A)	Site	Altitude (m above sea level)	δ^{13}C (‰)
Calandrinia acaulis Kunth	*Calandrinia acaulis*	P	East of Arica, 18°S	3700	–25.5[A]
		P	Chile East of Arica, 18°S, Chile	4400	–26.4[A]
Calandrinia compacta Barnéoud	*Calandrinia occulta*	P	East of Vallenar, 18°S, Chile	3500	–24.3[A]
Calandrinia spicata Phil.	*Calandrinia spicata*	A	East of Vallenar, 18°S, Chile	1800	–16.2[A]
Cistanthe aff. *longiscapa* (Barnéoud) Carolin ex Hershk.	*Calandrinia aff. longiscapa*	A	East of Arica, 18°S, Chile	3600	–24.1[A]
Cistanthe aff. *thyrsoidea* (Reiche) Peralta & D.I.Ford	*Calandrinia aff. thyrsoidea*	A	East of Arica, 18°S, Chile	1950	–21.9[A]
Cistanthe amarantoides (Phil.) Carolin ex Hershk.	*Cistanthe amaranthoides*	P	Paposo, 25°S, Chile	920–1010	–19.7[B]
	Philippiamra amaranthoides	P	East of Arica, 18°S, Chile	3000	–21.6[A]
Cistanthe ambigua (S.Watson) Carolin ex Hershk.	*Calandrinia ambigua*	A	n/a	n/a	–24.0[C]
Cistanthe celosioides (Phil.) Carolin ex Hershk.	*Philippiamra celosioides*	A	East of Arica, 18°S, Chile	2700	–22.4[A]
		A	East of Arica, 18°S, Chile	3100	–19.1[A]
Cistanthe grandiflora (Lindl.) Schltdl.	*Calandrinia grandiflora*	P	Los Molles, 32°S, Chile	Coastal	–23.2[D]
		P	Paposo, 25°S, Chile	490–650	–22.1[B]
		P	Pan de Azucar, 26°S, Chile	Coastal	–21.4[B]
		P	East of Vallenar, 28°S, Chile	2600	–20.6[A]
Cistanthe maritima (Nutt.) Carolin ex Hershk.	*Calandrinia maritima*	A	San Quintin, 30°N, Mexico	Coastal	–24.0[D]
		A	El Rosario, 30°N, Mexico	Coastal	–22.4[D]
Cistanthe picta (Gillies ex Arn.) Carolin ex Hershk.	*Calandrinia picta*	P	East of Vallenar, 18°S, Chile	4000	–23.9[A]
Cistanthe sp.	*Cistanthe* sp.	n/a	Paposo, 25°S, Chile	770–840	–18.0[B]
Lenzia chamaepitys Phil.	*Lenzia chamaepitys*	P	East of Vallenar, 18°S, Chile	3900	–25.9[A]
Montiopsis capitata (Hook. & Arn.) D.I.Ford	*Calandrina capitata*	A	East of Arica, 18°S, Chile	3300	–25.3[A]
Montiopsis copiapina (Phil.) D.I.Ford	*Calandrinia copiapina*	P	East of Vallenar, 18°S, Chile	3500	–26.5[A]
Montiopsis glomerata (Phil.) D.I.Ford	*Calandrinia leucocephala*	P	East of Vallenar, 18°S, Chile	4000	–24.5[A]
Montiopsis parviflora (Phil.) D.I.Ford	*Calandrinia bandurriae*	A	East of Vallenar, 18°S, Chile	3400	–24.3[A]

[A] Arroyo *et al*. (1990).
[B] Ehleringer *et al*. (1998).
[C] Troughton *et al*. (1974).
[D] Mooney *et al*. (1974).

between 3300 and 4000 m above sea level, often in the vicinity of *Cistanthe* spp. (Ehleringer *et al*. 1998).

Cistanthe *sp. aff.* crassifolia*: CO_2 exchange and titratable acidity*

The diel gas-exchange of an intact shoot of well-watered *C*. sp. aff. *crassifolia* exhibited a C_3-like pattern (Fig. 4). Net CO_2 uptake was restricted to the light and net CO_2 loss was observed in the dark. The kinetics of CO_2-exchange in the dark involved an initial increase in CO_2 efflux, a phenomenon often observed when plants and their enclosing gas-exchange cuvette undergo temperature equilibration following an abrupt change to a lower night-time set temperature (see Winter and Holtum 2014; for gas-exchange traces of C_3, C_4 and CAM plants), followed by a prolonged period during which the temperature was constant and the rate of CO_2 loss remained essentially constant. The 24 h carbon gain increased daily as the plant grew and the biomass of tissues in the cuvette increased.

Following the termination of watering on day 2, the plant continued to grow until, by day 9, the amount of water in the pot became limiting (Fig. 4). Subsequently, as water continued to be removed from the soil by the plant and by evaporation through the porous terracotta walls of the pot, daytime carbon gain was reduced progressively, and a pronounced mid-day depression in CO_2 exchange appeared on day 11. CO_2 uptake in the afternoon decreased more rapidly than CO_2 uptake in the morning. Concomitant with the overall daily reduction in CO_2 gain was a change in shape of the night-time CO_2 efflux trace. A small curvature in the efflux pattern, detectable during the night of day 9, became more pronounced such that from the night of day 10 until the night of day 17, net CO_2 uptake was present at night. On day 16, CO_2 uptake at night exceeded CO_2 uptake during the light which had been reduced to a small spike at the onset of the light period and negligible afternoon exchange.

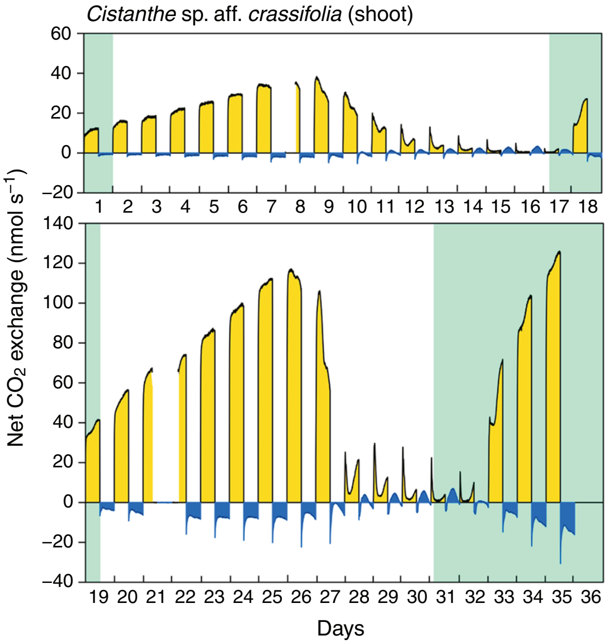

Fig. 4. Thirty-five days of net CO_2 exchange by a shoot of a potted *Cistanthe* aff. *crassifolia* growing in a 1 L pot under 12 h light (25°C, 700 µmol m^{-2} s^{-1})/ 12 h dark (17°C) periods. Flow rate was 1.26 L min^{-1}. During the experiment, the plant was subjected to two drying/re-watering cycles. Watering was withheld on days 2 and 21 and recommenced on days 17 and 31. Green background indicates days upon which the plant was watered to field capacity and the white background indicates days when no water was supplied to the plant. Net CO_2 exchange during the light is shown by yellow shading whereas net CO_2 exchange during the dark is shown by blue shading.

After rewatering on day 17, a recovery of CO_2 uptake in the light to pre-droughting rates was observed within 3 days. Associated with the recovery in rates of daytime CO_2 uptake, nocturnal respiratory CO_2 loss increased to rates greater than observed before the water-deficit treatment. A similar pattern of water-deficit-dependent nocturnal CO_2 uptake was observed during a second watering-droughting-watering cycle and during watering-droughting-watering treatments of other plants (cf. Supplementary material Fig. S1).

For *C*. sp. aff. *crassifolia* that had been well watered, no significant differences were observed between the titratable acidity of leaf samples harvested at the end of the day and at the end of the night (Fig. 5). In contrast, after water had been withheld for 13 days, titratable acidity at dawn was significantly greater than at dusk, irrespective of whether the ΔH^+ is expressed on a fresh mass, dry mass or area basis. The difference between dawn and dusk values is ~8- and 17-fold when expressed on fresh mass and dry mass bases respectively. By 7 days after rewatering, the dawn acid levels had decreased markedly but had not yet fully returned to the original well-watered levels.

Cistanthe *sp. aff.* longiscapa*: CO_2 exchange and titratable acidity*

CO_2-exchange by shoots of well-atered *C*. sp. aff. *longiscapa* was characterised by net CO_2 uptake during the light and CO_2 loss in the dark (Fig. 6). The rate of nocturnal CO_2 loss was not constant throughout the night, it either gradually decreased as the night progressed or exhibited a small decrease during the middle of the night. As water-deficit stress was imposed, CO_2 uptake in the light was reduced and a mid-day depression of CO_2 assimilation appeared and became more pronounced. Nocturnal CO_2 loss was reduced overall and the middle of the night reduction in CO_2 loss became more pronounced until, beginning at day 13, net nocturnal CO_2 uptake occurred. Following rewatering on day 29, the soil water content rapidly returned to close to field-capacity (Fig. 6, upper panels), daytime CO_2 uptake increased 6-fold (Fig. 6, lower panels), but net nocturnal CO_2 uptake was maintained at ~95% of the water-stressed level.

The CO_2-exchange responses of a single leaf of *C*. sp. aff. *longiscapa* exposed to a well-watered/water-deficit/well-watered treatment (Fig. 7) were similar to the shoot responses (Fig. 6) except that recovery of the daytime CO_2 uptake rates of the leaf following re-watering was more rapid and recovery more extensive. CO_2 uptake during the light increased 216-fold following rewatering whereas CO_2 uptake at night was 94% of the water-deficit level.

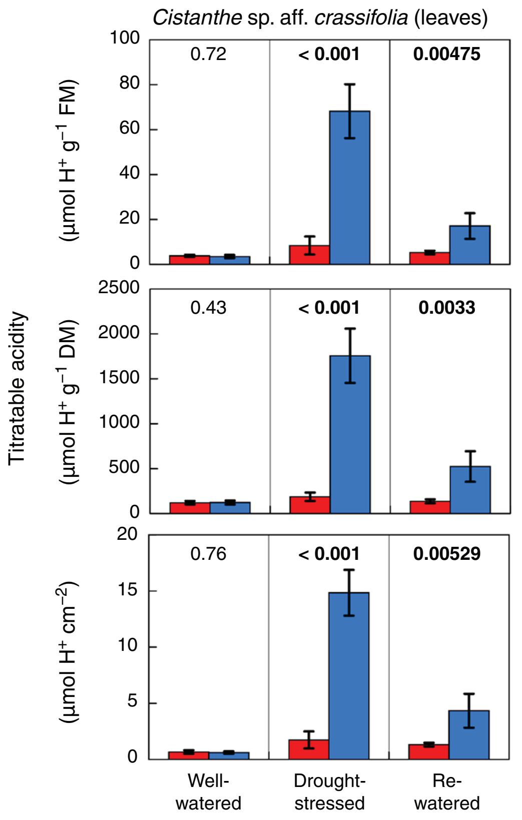

Fig. 5. Titratable acidity at dusk (red columns) and dawn (blue columns) in leaves of *Cistanthe* sp. aff. *crassifolia* cultivated in 1 L pots (one plant per pot) under 12 h light (25°C, 450 µmol m^{-2} s^{-1})/12 h dark (17°C) periods. Well-watered and re-watered = daily watering. Data are shown for well-watered plants (left-hand columns), droughted plants (middle columns; 13 days without irrigation) and for plants that had been droughted and re-watered (right-hand columns; 7 days with irrigation). The values are expressed on a fresh mass basis (upper panels), dry mass basis (middle panels), and leaf area basis (lower panels). Bars are s.d. ($n = 5$ leaves from different plants) and numbers are P-values (one-tailed t-test, bold letters indicate that the values at dawn were significantly greater than those at dusk at $P \leq 0.05$).

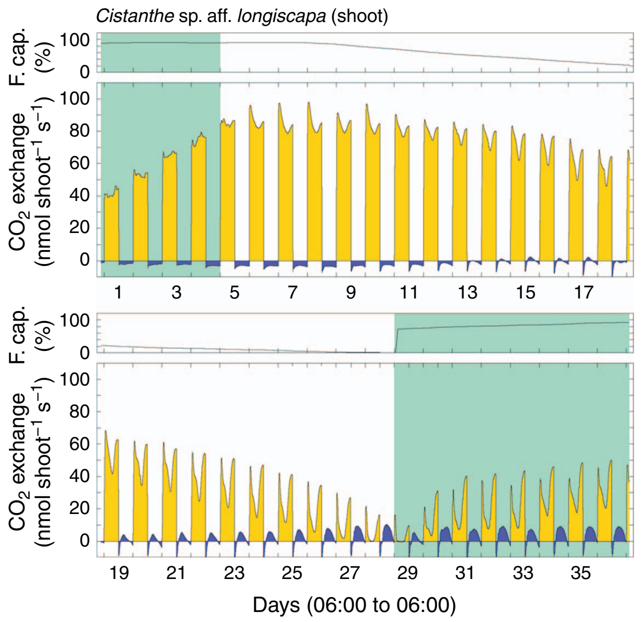

Fig. 6. Thirty-six days of net CO_2 exchange by an attached shoot of *Cistanthe* aff. *longiscapa* growing in a 0.4 L pot under 12 h light (26°C, 300 µmol m^{-2} s^{-1})/ 12 h dark (20°C) periods. During this experiment, the plant was subjected to a single watering/drying/re-watering cycle. Soil water content, expressed as the proportion of water-holding capacity of the growth medium, is shown in the upper panels. Green background indicates days upon which the plant was watered to field capacity and the white background indicates days when no water was supplied to the plant. Net CO_2 exchange during the light is shown by yellow shading whereas net CO_2 exchange during the dark is shown by blue shading. On the last day of the experiment: leaf and stem fresh masses were 18.506 and 2.022 g, respectively, and leaf and stem dry masses were 1.143 and 0.331 g, respectively.

Nocturnal acidification was detected in leaves of *C.* sp. aff. *longiscapa* under all treatments, well-watered, water-deficit and rewatered, irrespective of whether the data was expressed on a fresh mass, dry mass or area basis (Fig. 8). Acidification increased following water-deficit stress (fresh mass: one-way

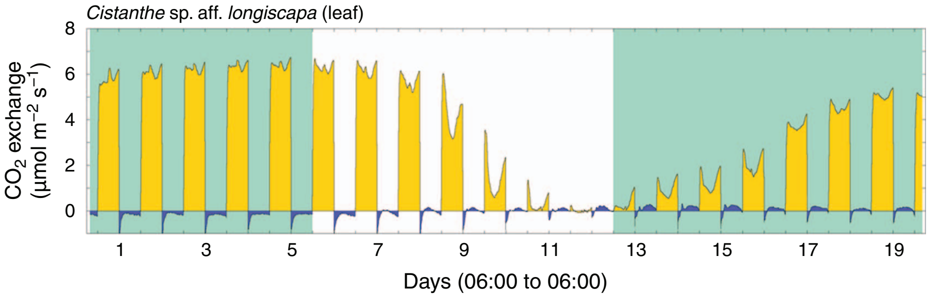

Fig. 7. Nineteen days of net CO_2 exchange by an attached fully expanded leaf (3rd from bottom of the rosette) of a *Cistanthe* aff. *longiscapa* growing in a 0.4 L pot under 12 h light (26°C, 220 µmol m^{-2} s^{-1})/12 h dark (20°C) periods. During this period, the plant was subjected to a single watering/drying/re-watering cycle. Green background indicates days upon which the plant was watered to field capacity and the white background indicates days when no water was supplied to the plant (7 days). Net CO_2 exchange during the light is shown by yellow shading whereas net CO_2 exchange during the dark is shown by blue shading. On the last day of the experiment, total leaf area was 11.52 cm^2 and fresh and dry masses were 1.680 and 0.092 g, respectively.

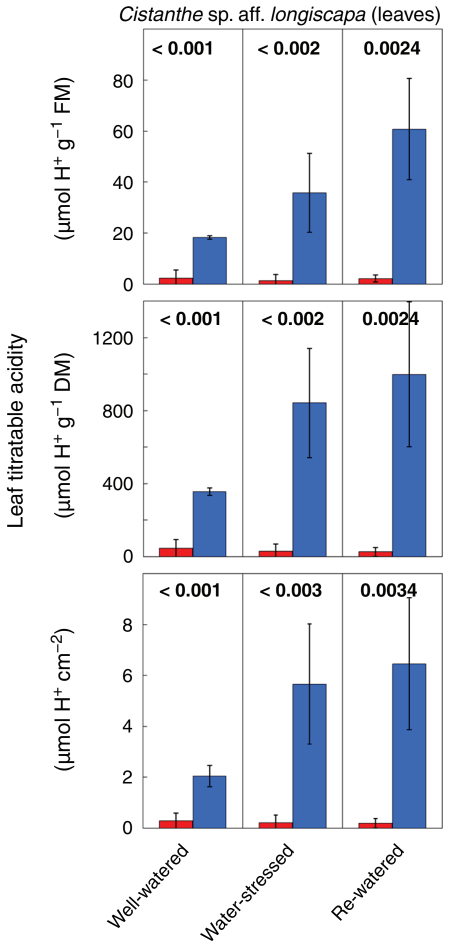

Fig. 8. Titratable acidity at dusk (red columns) and dawn (blue columns) in leaves of *Cistanthe* sp. aff. *longiscapa* growing in 0.4 L pots under 12 h light (26°C, 300 µmol m^{-2} s^{-1})/12 h dark (20°C) periods. The plants were subjected to a watering/drying/re-watering cycle (well-watered and re-watered = daily watering.) Data are shown for well-watered plants (left-hand columns), droughted plants (middle columns; 18 days without added water) and for plants that had been watered, droughted and re-watered (right-hand columns; 16 days with irrigation). Well-watered and re-watered = daily watering to field capacity. The values are expressed on a fresh mass basis (upper panel), dry mass basis (middle panel), and leaf area basis (lower panel). Bars are standard deviations (n = 5 plants) and numbers are P values (one-tailed, paired t-test that tested whether values at dawn were greater than those at dusk; bold letters indicate that the values at dawn were significantly greater than those at dusk at $P \leq 0.05$).

ANOVA, $F_{2,12} = 7.69$, $P < 0.01$, Levene's test $P < 0.05$; dry mass: Welch one-way ANOVA, $F_{2,5.59} = 7.99$, $P < 0.05$, Games-Howell test $P < 0.05$; area: one-way ANOVA, $F_{2,12} = 4.98$, $P < 0.05$, Levene's test $P < 0.05$) but did not significantly decrease following rewatering (fresh mass: Levene's test $P = 0.64$; dry mass: Games-Howell test $P = 0.99$; area: Levene's test $P = 0.67$).

Discussion

CO_2 uptake at night and nocturnal acidification patterns typical of CAM were detected in *C.* sp. aff. *crassifolia* and in *C.* sp. aff. *longiscapa* but the manifestation of CAM differed between the species. In *C.* sp. aff. *crassifolia* the expression of CAM was overwhelmingly facultative in that no CAM-type CO_2 exchange or acidity signals were detected in well-watered plants, but both CAM CO_2 exchange and nocturnal acidification of leaves were present in plants subjected to water-deficit stress. Rewatering of stressed plants was accompanied by loss of most of the CAM signal i.e. the expression of CAM was coupled to environmental water-deficit.

In *C.* aff. *longiscapa*, the traces of whole shoot and single leaf photosynthesis in well-watered plants shown in Figs 6 and 7 are C_3-like but two CAM-associated features are consistently present. During the daytime, CO_2 uptake dips following a decrease in stomatal conductance during the middle of the day. In plants undergoing CAM, the daytime stomatal closure has been linked to the increase in the internal CO_2 concentration produced by the decarboxylation of malate (Phase III in the terminology of Osmond (1978). In plants undergoing stress-induced C_3-to-CAM transitions, such as *Mesembryanthemum crystallinum* and *Clusia minor*, the depression is overwhelmingly a response to changes in leaf water status (Winter and Gademann 1991; Borland and Griffiths 1996). Presumably, when CAM and stress are present both contribute to the response.

At night, a slight reduction of CO_2 loss, indicated by curved rather than flat-line kinetics, occurs following the initial CO_2-loss overshoot that is associated with temperature equilibration within the gas-exchange chamber and the outside cabinet. The two CAM-type gas-exchange signals were substantiated by measurements of CAM-type nocturnal acidification of leaves (Fig. 8). The nocturnal CO_2 uptake and acid accumulation increased significantly following the imposition of water-deficit stress. Rewatering was associated with an increase in CO_2 uptake during the light but not with substantial changes to nocturnal CO_2 exchange and acidification. The presence of low-level CAM in tissues of the well-watered plants depicted in Figs 6 and 7 and the irreversible upregulation of CAM following stress are both indicators of CAM expression that is largely constitutive, i.e. the environment may affect rates of dark CO_2 fixation but particular environmental conditions are not necessary to elicit CAM (Winter 2019).

The demonstration of CAM in the two taxa of *Cistanthe* measured here, coupled with CAM-type $\delta^{13}C$ values for nine species of *Cistanthe* growing in their natural environments in Chile, south-west USA and north-west Mexico (Table 1), indicates that CAM may be widespread in *Cistanthe*. CAM has now been documented within all of the clades in Montiaceae, but not within all genera. It is present in *Phemeranthus* (Harris and Martin 1991*a*; Guralnick and Jackson 2001), the Australian *Calandrinia* (Winter *et al.* 1981; Winter and Holtum 2011; Holtum *et al.* 2017; Hancock *et al.* 2019) and *Calandrinia s.s.* in the New World (Guralnick and Jackson 2001). Within the Montiae clade, Guralnick and Jackson (2001) demonstrated CAM in one species of *Lewisia* (~15 spp. known) and one species of *Claytonia* (32 spp. known) but not in *Montia* (20 spp. known; their *Montia perfoliata* = *Claytonia perfoliata*) nor in *Lewisiopsis* (1 sp.). With the description here of CAM in *Cistanthe*, CAM is now documented in two of the three genera of the *Calyptridium*, *Cistanthe* and *Montiopsis* clade of the Montiaceae. CAM is documented here in two species of *Cistanthe*. CAM has been reported in *Calyptridium* (*C. monandrum*, Guralnick and Jackson 2001) but has yet to be reported in any of the 18 species ascribed to *Montiopsis*.

It can be estimated, using the relationship generated by Winter and Holtum (2002), that up to ~30% of the carbon in *Cistanthe* growing in the field may have been obtained via nocturnal CO_2 uptake. It is noted that the relationship can overestimate the contribution of dark CO_2 uptake in field-grown plants because it was derived from non-stressed plants. If increased diffusional limitation of CO_2 uptake occurs (resulting in low c_i/c_a ratios during photosynthesis), as might be expected in environments characterised by high leaf-to-air vapour pressure difference and low soil moisture, the $\delta^{13}C$ values of plant tissue will be less negative than predicted. Nevertheless, the model is useful in providing an estimate of the uppermost likely contribution of CAM to biomass. Ehleringer *et al*. (1998) noted that at Pan de Azucar, a hyper-arid area ~250 km north of the sites from which seeds of the *Cistanthe* species examined here were collected, annuals, short-lived herbaceous species and longer-lived perennials exhibit reasonably similar carbon isotope ratios consistent with low operational leaf intercellular CO_2 concentrations. Presumably the values, less negative than might be expected for a C_3 plant growing under well-watered conditions, reflect the low moisture content of the soil and indicate that few opportunities exist for plants to operate at higher intercellular CO_2 concentrations. The observation that the mean $\delta^{13}C$ value for four species of *Montiopsis*, at –25.2 ± 1.0 ‰, is 3.6 ‰ more negative than the average for the eight or nine presumably CAM *Cistanthe* species suggests at face value that, if the isotopic values listed in Table 1 are representative of all species of *Montiopsis*, the expression of CAM in *Montiopsis*, if present, will be low. An extreme example of an arid-environment associated shift towards less-negative $\delta^{13}C$ values independent of CAM is seen in *Nolana moltis* (Nolanaceae) growing at Pan de Azucar and elsewhere in northern Chile. It exhibits CAM-like $\delta^{13}C$ values of –19.8 to –23.1 ‰ (Mooney *et al*. 1974, 1980; Ehleringer *et al*. 1998), yet the species is considered C_3 (PW Rundel and B Palma, unpubl. data cited in Ehleringer *et al*. 1998).

In general, when present, the expression of CAM in members of the Montiaceae is at a low level, with no species known as yet that fixes carbon predominantly at night when well-watered. In most species of *Calandrinia* studied so far, water-deficit induced upregulation of CAM expression was noted (Winter and Holtum 2011, 2017; Holtum *et al*. 2017; Hancock *et al*. 2019), either facultatively in form of a relatively clean, reversible shift from C_3 to CAM, or as facultative CAM on top of a low-level constitutive CAM (see also Winter *et al*. 2020*b*). In some cases, the drought-stimulated expression of CAM was not or only partially reversible upon rewatering. In five species of *Phemeranthus* (previously described as *Talinum*) low-level constitutive CAM was present in well-watered plants (Harris and Martin 1991*a*, 1991*b*). Nocturnal acidification slightly increased under water-deficit conditions, but reversibility was not tested. As for *Calandrinia*, and probably in *Phemeranthus*, the evidence provided here for *Cistanthe* is that two manifestations of CAM are present in the genus: low level facultative CAM that is reversible as demonstrated for *C*. sp. aff. *crassifolia*, and low-level constitutive CAM that is upregulated following stress in a non-reversibel manner as demonstrated for *C*. sp. aff. *longiscapa*. In both species, when CAM is operating net CO_2 uptake at night was detected. A more complete screening for photosynthetic pathway of species within the Montiaceae, across moisture as well as altitudinal gradients, would provide an understanding of the patterns and limits of CAM expression across the family. A detailed study of life-time CO_2 exchange of *C*. sp. aff. *longiscapa* at both the leaf- and shoot-levels would reveal the extent to which the irreversible upregulation of CAM by water-deficit stress can be interpreted as an accelerated ontogenetic process. It is worth noting that late in the life cycle of the facultative annual CAM species *Calandrinia polyandra*, following a period of CAM gas-exchange under water-deficit conditions, rewatering alone stimulated C_3 photosynthetic CO_2 fixation in the light but did not alter CO_2 dark fixation. It was only upon additional application of soil nutrients that CAM was abolished (Winter and Holtum 2011).

Within *Portullugo*, a clade that includes the sub-order Portulacineae plus Molluginaceae (Edwards and Ogburn 2012), CAM is known in every family except the Molluginaceae and the monotypic family, Halophytaceae, although in the latter, *Halophytum ameghinoi* has a CAM-type $\delta^{13}C$ value of –18.6 ‰ (EJ Edwards, unpubl. data in Holtum *et al*. 2018). The demonstration of facultative CAM in multiple genera within the Montiaceae, and in the other families of the Portulacinae, Anacampserotaceae, Basellaceae, Cactaceae, Didieraceae, Portulacaceae and Talinaceae (see Winter 2019; and references therein) highlights growing evidence for the widespread capacity for facultative CAM across the Portulacinae. Outside the Portullugo, but within the Caryophyllales, induction/upregulation of CAM by drought-stress have been demonstrated only in the Aizoaceae (Winter 2019), whereas more extensively across the Angiosperm phylogenetic tree facultative CAM species are present in the Alismatales (Araceae), Commelinales (Commelinaceae), Lamiales (Lamiaceae), Malphigales (Clusiaceae), Poales (Bromeliaceae) and Vitales (Vitaceae) (Winter 2019). It is notable that in the Asparagales, the order with the most known CAM species, and which includes the CAM-species rich Orchidaceae, facultative CAM has yet to be reported (K Silvera and K Winter, unpubl. data).

In our experience, despite several higher-level taxonomic treatments (Hershkovitz 1991*a*, 1991*b*, 2018*a*, 2018*b*), identification of *Cistanthe* in the field is difficult, particularly of the larger fleshy-leaved species. In the field, characters such as plant size, degree of succulence, leaf shape and colour, and flower colour can vary over short distances and respond markedly to the nature of rainfall which is episodic, patchy and variable in intensity. In addition, morphological characteristics were often not preserved well in the herbarium specimens to which we referred. Presumably, over multi-year time-scales, stochastic rains can create spatially discrete plant communities that are isolated during the intervening dry periods (Dillon and Rundel 1989). Should subsequent selection secure differences between populations, the isolation provided by the surrounding desert might effectively maintain these differences. The systematic and taxonomic understanding of *Cistanthe* would benefit greatly from an integrated molecular and morphological assessment of plants that had been well sampled across their ranges.

Vegetation such as *Cistanthe*, which germinates, or emerges from dormancy in the case of perennials and geophytes, to bloom following episodic rain in the Atacama desert has a limited period within which to grow and reproduce. For example, following a 15 mm rain event at Carrizal Bajo in September 1989, plant cover increased to a maximum of ~23% after 9 weeks but had retreated to a baseline 6% by 19 weeks (Vidiella *et al*. 1999). The capacity of the upper soil layers to retain plant available water is generally poor as they tend to be sandy with low organic carbon contents, and soil moisture is rapidly lost or complexes with salts (McKay *et al*. 2003). Species with the C_4 pathway of photosynthesis, which are typically well represented in other arid and semiarid environments in the New World, are not well represented in the Atacama desert flora (Rundel *et al*. 1996), possibly because temperatures are low after rain events which tend to occur in winter. Most species, even perennial shrubs, appear to be C_3 with succulent or fleshy leaves that, based on carbon isotope ratios, probably operate photosynthetically under low intercellular CO_2 concentrations (Ehleringer *et al*. 1998).

The perennial vegetation of the Atacama contains several prominent CAM species, such as the stem-succulent *Copiapoa* and *Eulychnia* cacti, but smaller herbaceous CAM species are conspicuous following rain (Arroyo *et al*. 1990). When water is only abundant for a short period, and nights are cool, there is presumably an ecological advantage in operating initially as a C_3 plant and then switching to a more water-use efficient dark CO_2 uptake of CAM. It is probably of significance that the form of CAM present in the *Cistanthe* species studied is not just carbon-conserving CAM-cycling or CAM-idling but involves net carbon gain during the dark. When water is limiting, the plants are not marking time until the next rain event, they are actively increasing the plant carbon pool required for seed production or energy storage. The next rain event may be years away.

Conflicts of interest

The authors declare no conflicts of interest.

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