

Carbon isotope ratio and the extent of daily CAM use by Bromeliaceae

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Summary

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- Use of carbon isotope ratio ($\delta^{13}\text{C}$) to resolve photosynthetic pathways (C_3 , C_4 or CAM) has limitations imposed by the use of intermediate photosynthetic modes by certain plant taxa.
- Diel gas-exchange patterns, leaf $\delta^{13}\text{C}$ values and nocturnal tissue acidification were determined for 50 Bromeliaceae.
- $\delta^{13}\text{C}$ values for well watered plants reflected the proportion of daily CO_2 uptake occurring at night. Thirteen per cent of species with $\delta^{13}\text{C}$ values typical of C_3 plants (i.e. from -22.6 to -31.5‰) showed nocturnal acidification and either a small proportion ($< 10\%$) of daily CO_2 uptake occurring nocturnally or internal CO_2 recycling during part of the night. None altered CAM expression in response to short-term drought, but the contribution of CAM to daily carbon gain became proportionally more important as C_3 CO_2 uptake failed.
- Surveys of plant communities using solely the carbon isotope technique underestimate the number of CAM-equipped plants.

Key words: Bromeliad, carbon pathway, crassulacean acid metabolism (CAM), $\delta^{13}\text{C}$, epiphyte, photosynthesis.

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Introduction

Crassulacean acid metabolism (CAM) is a photosynthetic pathway usually associated with succulent plants in arid habitats, characterised by nocturnal CO_2 uptake and storage as organic acids in the vacuole (Winter & Smith, 1996). Organic acids are subsequently decarboxylated in the light, concentrating CO_2 within the leaf and thereby suppressing the oxygenase activity of Rubisco, the enzyme ultimately responsible for CO_2 fixation via the C_3 photosynthetic pathway. Greater water use efficiencies also result from lower evapotranspiration rates at lower night-time temperatures. The extent of CAM expression is highly variable between taxa and is responsive to environmental conditions such as water availability (recent reviews include Cushman, 2001; Cushman & Borland, 2002; Dodd *et al.*, 2002; see also Haslam *et al.*, 2002) and, at least in Bromeliaceae, has allowed radiation into wetter habitats due to an innate adaptability (Pierce *et al.*, 2002).

This variation in CAM expression leads to difficulty in interpreting the carbon isotope composition of plant material

(i.e. the relative abundance of ^{13}C , expressed as $\delta^{13}\text{C}$; Griffiths, 1993), employed as a means of discerning photosynthetic pathways producing plant tissues. This technique is useful for rapid large-scale screening of plant matter, including herbarium material (e.g. Kluge *et al.*, 1991; Hietz *et al.*, 1999). $\delta^{13}\text{C}$ is determined initially by CO_2 diffusion into the leaf, when slight discrimination occurs against the heavy isotope (i.e. against $^{13}\text{CO}_2$ cf. $^{12}\text{CO}_2$), but this is tempered by the much larger discrimination expressed by Rubisco. For CAM plants, the carboxylating enzyme PEPc (phosphoenolpyruvate carboxylase) shows a low discrimination and uses HCO_3^- as substrate (which is enriched in ^{13}C relative to source air), but organic material rarely lies in the theoretical range or that measured instantaneously during photosynthesis (Roberts *et al.*, 1997). More negative $\delta^{13}\text{C}$ values in constitutive CAM plants, similar to those seen for C_4 plants, arise probably because some discrimination is expressed by leakage of CO_2 during decarboxylation. Additionally, the extent of daytime CO_2 uptake directly catalysed by Rubisco can lead to more intermediate $\delta^{13}\text{C}$ values.

Table 1 Life forms or ecophysiological types of Bromeliaceae (Benzing, 2000)

Life form	Characteristics
1	Terrestrial herbs of subfamily Pitcairnioideae (and many Bromelioideae) that use roots to acquire water and nutrients – the leaf hairs being nonabsorbent.
2	Terrestrial Bromelioideae with leaf bases that form a rudimentary watertight 'tank' into which some axillary roots may grow.
3	Terrestrial or epiphytic herbs in subfamily Bromelioideae, the roots of which have reduced importance in water and nutrient acquisition with the leaf bases forming an extensive water-holding tank – predominantly with obligate crassulacean acid metabolism (CAM) and leaf hairs that have some capacity to take up water and nutrients.
4	Tank-forming epiphytes in subfamily Tillandsioideae and some <i>Brocchinia</i> – predominantly C ₃ and with high densities of leaf hairs on the leaf bases that are highly effective at water and nutrient uptake, the roots functioning primarily as holdfasts.
5	Succulent obligate CAM Tillandsioideae that are epiphytic or lithophytic, with leaf hairs taking up water directly over the entire leaf surface (without a tank) and possessing holdfast roots, if any.

Indeed, surveys of plant communities usually reveal a bimodal distribution of $\delta^{13}\text{C}$ values (C₃ plants typically in the range -23 to -36‰ , and C₄ & CAM plants in the range -10 to -18‰ ; Griffiths, 1993), however, some Bromeliaceae exhibit intermediate values around -20‰ suggestive of plants using both C₃ and C₄ carboxylation (Griffiths *et al.*, 1986), as demonstrated by four species of CAM-equipped ferns (Holtum & Winter, 1999). *Werauhia sanguinolenta*, an epiphytic bromeliad with a recorded $\delta^{13}\text{C}$ value of -32.0‰ (Zotz & Ziegler, 1997), exhibits weak CAM evident only as slight nocturnal acidification (Zotz, 1997; Schmidt & Zotz, 2001). In addition to weak CAM in Bromeliaceae, *Guzmania monostachia* exhibits facultative CAM, typically with C₃ gas-exchange (and hence having a $\delta^{13}\text{C}$ value typical of a C₃ plant) but with the ability to express a limited degree of CAM in response to stresses such as drought and high light (Medina *et al.*, 1977; Medina, 1987; Maxwell *et al.*, 1992).

Medina & Troughton (1974) and Medina *et al.* (1977) predicted that such intermediate modes of CAM could explain the continuous spectrum of $\delta^{13}\text{C}$ values apparent in Bromeliaceae (N.B. Bromeliaceae lack Kranz anatomy typical of C₄ metabolism; Benzing, 2000). The aim of the present study is thus to investigate the proportion of $\delta^{13}\text{C}$ values at the C₃ end of this spectrum representing weak CAM or truly facultative CAM use, rather than entirely C₃ metabolism. This will elucidate the magnitude of uncertainty inherent to $\delta^{13}\text{C}$ surveys. To this end gas-exchange patterns, leaf extract titratable acidity and carbon isotope ratio of 50 taxonomically and ecologically diverse bromeliad species were investigated. Life form categories or ecophysiological types follow Benzing (2000; summarised in Table 1), and nomenclature of Bromeliaceae follows Luther (2000) and subsequent species descriptions of Pierce & Aranda (2000) and Pierce (2001).

Materials and Methods

Plant material and cultivation

Plant material of Panamanian origin (Table 2) was collected from the wild (Pierce & Aranda, 2000; Pierce *et al.*, 2001;

Pierce, 2001), with voucher specimens being held at the University of Panama herbarium (PMA). *Ananas comosus* was grown from meristem culture, with original material provided by the Centre International de Recherche en Agronomie et Développement (Montpellier, France). All other plants were obtained commercially from either Tropiflora (Sarasota, FL, USA) or Bird Rock Tropicals (Carlsbad, CA, USA). All plants were in the adult vegetative phase, with one representative individual of each species investigated. Samples detailed below were taken from a young fully expanded leaf, which in all cases was produced before the beginning of the experiment.

Plants were cultivated in seminatural conditions in an open-sided glasshouse at the main Smithsonian Tropical Research Institute facility in Panama City (*c.* 30 m above sea level), Republic of Panama, for a minimum of 2 months before measurement. Mean daily maximum and minimum temperatures were 32.9 ± 0.3 and $22.2 \pm 0.2^\circ\text{C}$ ($n = 33$), respectively, and mean daily maximum and minimum relative humidity (rh) 98.3 and 52.9%, respectively. Water was supplied via an automatic misting system (Phytotronics Inc., Earth City, MO, USA) and nutrients supplied to terrestrial species as a top-dressing of slow-release fertiliser (Osmocote) or to epiphytes as a 40% Long Ashton nutrient solution (Hewitt, 1966) applied by nebuliser twice weekly.

Gas exchange

For determination of gas-exchange, well-watered plants were placed in a controlled environment cabinet (GEC, Chagrin Falls, OH, USA) with temperatures of 28/22°C (light/dark period), rh of 60/90% (light/dark period), and a photosynthetic photon fluence rate (PPFR) at plant height of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ for a photoperiod of 12 h. Whole plants or mid-leaf portions of young fully expanded leaf blade (depending on plant size and shape) were enclosed in one of the following cuvettes: PLC-3 (ADC Bioscientific, Hoddesdon, Hertfordshire, UK), PMK10 gas-exchange cuvette (Heinz Walz GmbH, Effeltrich, Germany), or one of a range of custom-built whole-plant cuvettes (H. Walz). A

Table 2 Photosynthetic characteristics of fifty well-watered bromeliad species, divided by subfamily

Species	Life form	Leaf blade thickness (mm)	Leaf $\delta^{13}\text{C}$ (‰)	Proportion of CO_2 fixation occurring at night (%)	Titratable acidity (mmol H^+ g^{-1} d. wt _{structural})		Level of significance
					End of light period	End of dark period	
PITCAIRNIOIDEAE							
<i>Brocchinia reducta</i> Baker†	1	0.62 ± 0.03	-22.6	0	0.49 ± 0.04	0.46 ± 0.06	n.s.
<i>Fosterella albicans</i> (Griseb.) L.B. Smith‡	1	0.46 ± 0.03	-28.1	0	0.68 ± 0.04	0.82 ± 0.06	n.s.
<i>Fosterella spectabilis</i> H. Luther†	1	0.49 ± 0.03	-27.1	0	0.68 ± 0.04	0.76 ± 0.04	n.s.
<i>Hechtia guatemalensis</i> Mez‡	1	3.03 ± 0.13	-14.0	66.9	0.58 ± 0.05	1.94 ± 0.20	***
<i>Pitcairnia arcuata</i> (André) André†	1	0.33 ± 0.00	-26.1	0	0.12 ± 0.01	0.12 ± 0.01	n.s.
<i>Pitcairnia atrorubens</i> (Beer) Bakert	1	0.32 ± 0.01	-27.8	0	0.09 ± 0.03	0.14 ± 0.01	n.s.
<i>Pitcairnia integrifolia</i> Ker-Gawler‡	1	0.30 ± 0.01	-27.9	0	0.48 ± 0.16	0.35 ± 0.04	n.s.
<i>Pitcairnia valerii</i> Standley†	1	0.20 ± 0.01	-26.8	0	0.12 ± 0.01	0.12 ± 0.01	n.s.
<i>Pitcairnia xanthocalyx</i> Martius‡	1	0.27 ± 0.01	-27.3	0	0.45 ± 0.03	0.43 ± 0.03	n.s.
<i>Puya laxa</i> L.B. Smith‡	1	3.84 ± 0.16	-15.8	55.7	0.18 ± 0.02	0.65 ± 0.04	***
TILLANDSIOIDEAE							
<i>Catopsis micrantha</i> L.B. Smith†	4	0.23 ± 0.03	-26.2	0	0.12 ± 0.01	0.27 ± 0.08	n.s.
<i>Guzmania circinnata</i> Rauh†	4	0.46 ± 0.03	-27.3	0	0.22 ± 0.03	0.22 ± 0.02	n.s.
<i>Guzmania desautelsii</i> L.B. Smith & R.W. Read†	4	0.48 ± 0.01	-31.5	0	0.09 ± 0.05	0.12 ± 0.04	n.s.
<i>Guzmania glomerata</i> Mez & Wercklé†	4	0.22 ± 0.02	-26.5	0	0.24 ± 0.09	0.47 ± 0.08	n.s.
<i>Guzmania monostachia</i> (L.) Rusby ex Mez var. <i>monostachia</i> †	4	0.32 ± 0.01	-26.7	10.0	0.91 ± 0.06	1.59 ± 0.27	*
<i>Guzmania multiflora</i> (André) André ex Mez§	4	0.29 ± 0.01	-24.0	0	0.68 ± 0.05	0.79 ± 0.09	n.s.
<i>Guzmania musaica</i> (Lind. & André) Mez var. <i>concolor</i> L.B. Smith†	4	0.34 ± 0.02	-28.0	0	0.11 ± 0.01	0.11 ± 0.01	n.s.
<i>Guzmania sanguinea</i> (And.) And. ex Mez var. <i>brevipedicellata</i> Gil.§	4	0.26 ± 0.02	-28.3	0	0.56 ± 0.10	0.82 ± 0.25	n.s.
<i>Tillandsia bulbosa</i> Hook.†	5	–	-13.4	97.2	0.07 ± 0.01	0.80 ± 0.12	**
<i>Tillandsia cretacea</i> L.B. Smith§	4	0.64 ± 0.03	-23.9	0	0.41 ± 0.05	0.90 ± 0.15	**
<i>Tillandsia deppeana</i> Steudel§	4	0.26 ± 0.00	-22.9	0	0.11 ± 0.01	0.12 ± 0.01	n.s.
<i>Tillandsia elongata</i> Kunth var. <i>subimbricata</i> (Baker) L.B. Smith†	4	0.56 ± 0.01	-14.5	100	0.40 ± 0.03	2.34 ± 0.17	***
<i>Tillandsia flexuosa</i> Swartz†	4–5	1.11 ± 0.06	-12.7	96.7	0.13 ± 0.01	1.38 ± 0.26	***
<i>Vriesea barclayana</i> (Baker) L.B. Smith var. <i>barclayana</i> §	4–5	0.87 ± 0.03	-18.1	25.9	0.39 ± 0.03	0.94 ± 0.06	***
<i>Vriesea espinosae</i> (L.B. Smith) Gilmartin§	4–5	1.29 ± 0.03	-18.6	62.6	0.09 ± 0.01	0.19 ± 0.01	***
<i>Vriesea monstrum</i> (Mez) L.B. Smith†	4	0.47 ± 0.06	-25.9	0	0.18 ± 0.05	0.23 ± 0.03	n.s.
<i>Vriesea ospinae</i> H.Luther var. <i>ospinae</i> §	4	0.30 ± 0.01	-28.0	0	0.21 ± 0.02	0.25 ± 0.02	n.s.
<i>Werauhia capitata</i> (Mez & Wercklé) J.R. Grant†	4	0.41 ± 0.01	-27.0	0	0.15 ± 0.04	0.14 ± 0.04	n.s.
<i>Werauhia jenii</i> S. Piercet	4	0.31 ± 0.01	-29.1	0	0.31 ± 0.04	0.37 ± 0.03	n.s.
<i>Werauhia kupperiana</i> (Suess.) J.R. Grant†	4	0.43 ± 0.01	-27.2	0	0.08 ± 0.01	0.08 ± 0.01	n.s.
<i>Werauhia latissima</i> (Mez & Wercklé) J.R. Grant†	4	0.64 ± 0.01	-30.6	0	0.24 ± 0.01	0.17 ± 0.03	n.s.
<i>Werauhia lutheri</i> S. Pierce & J.E. Aranda†	4	0.52 ± 0.03	-28.4	0	0.11 ± 0.04	0.15 ± 0.01	n.s.
<i>Werauhia millennia</i> J.R. Grant†	4	0.43 ± 0.01	-28.1	0	0.12 ± 0.02	0.10 ± 0.01	n.s.
<i>Werauhia paniculata</i> (Mez & Wercklé) J.R. Grant†	4	0.41 ± 0.01	-26.2	0	0.24 ± 0.02	0.24 ± 0.01	n.s.
<i>Werauhia</i> sp. nov.†	4	0.42 ± 0.03	-24.9	0	0.13 ± 0.01	0.12 ± 0.01	n.s.
<i>Werauhia sanguinolenta</i> (Lind. ex Cogn. & March.) J.R. Grant†	4	0.93 ± 0.02	-27.6	0	0.19 ± 0.01	0.24 ± 0.01	*
<i>Werauhia vittata</i> (Mez & Wercklé) J.R. Grant†	4	0.38 ± 0.01	-27.0	0	0.15 ± 0.01	0.14 ± 0.01	n.s.
BROMELIOIDEAE							
<i>Aechmea allenii</i> L.B. Smith†	3	0.54 ± 0.02	-15.6	68.2	0.21 ± 0.01	0.96 ± 0.08	***
<i>Aechmea dactylina</i> Bakert	3	0.76 ± 0.05	-13.6	99.2	0.22 ± 0.01	1.85 ± 0.08	***

Table 2 continued

Species	Life form	Leaf blade thickness (mm)	Leaf $\delta^{13}\text{C}$ (‰)	Proportion of CO_2 fixation occurring at night (%)	Titratable acidity (mmol H^+ g^{-1} d. wt _{structural})		Level of significance
					End of light period	End of dark period	
<i>Aechmea nudicaulis</i> (L.) Griseb. ‡	3	0.88 ± 0.03	-13.1	87.1	0.63 ± 0.05	4.89 ± 1.28	*
<i>Ananas comosus</i> (L.) Merrill cv. Cayenne Lisse'	2	1.40 ± 0.12	-13.9	94.7	0.16 ± 0.02	2.46 ± 0.12	***
<i>Billbergia macrolepis</i> L.B. Smith †	3	0.58 ± 0.06	-14.5	86.5	0.20 ± 0.01	3.62 ± 0.11	***
<i>Cryptanthus bivittatus</i> (Hook.) Regels	1	1.64 ± 0.03	-14.8	63.9	0.26 ± 0.03	0.79 ± 0.16	*
<i>Cryptanthus bromelioides</i> Otto & Dietrichs	1	1.40 ± 0.04	-15.9	89.4	0.18 ± 0.03	0.55 ± 0.03	***
<i>Cryptanthus colnagoi</i> Rauh & Lemeš	1	1.42 ± 0.14	-15.4	75.8	0.24 ± 0.05	0.42 ± 0.03	*
<i>Neoregelia concentrica</i> (Vell.) L.B. Smiths	3	0.75 ± 0.03	-13.6	59.9	0.14 ± 0.02	1.43 ± 0.07	***
<i>Neoregelia cruenta</i> (Graham) L.B. Smith †	3	2.01 ± 0.04	-14.2	96.1	0.28 ± 0.05	1.88 ± 0.09	***
<i>Orthophytum magalhaesii</i> L.B. Smith †	1	2.13 ± 0.20	-16.3	80.5	1.09 ± 0.16	5.79 ± 0.52	***
<i>Portea petropolitana</i> L.B. Smith †	3	0.73 ± 0.04	-12.5	95.5	0.09 ± 0.03	1.74 ± 0.13	***
<i>Ronnbergia explodens</i> L.B. Smith †	1	0.37 ± 0.01	-25.4	8.8	0.13 ± 0.02	0.25 ± 0.02	**

Titratable acidity represents the mean \pm 1 SE of four replicate leaf discs, * denotes significance between means at the end of the light period and the end of the dark period at the $P \leq 0.05$ level, ** denotes $P \leq 0.01$, *** denotes $P \leq 0.001$ as determined by Student's t test. Life forms or ecophysiological types follow the classification of Benzing (2000). † denotes species collected from Panama. ‡ denotes species purchased from Tropiflora (see text), § denotes species purchased from Bird Rock Tropicals, *Ananas comosus* cv. 'Lisse' was obtained from the Centre International de Recherche en Agronomie et Développement (Montpellier, France).

buffer volume of 2 m³ in a shaded situation on the roof of the eight-storey building supplied ambient air. This was scrubbed of CO₂ via a soda-lime column, 350 $\mu\text{mol mol}^{-1}$ CO₂ added via a GMA-4 gas-mixing unit (H. Walz) and then the air dried via a cold trap (H. Walz) and split into reference and sample air streams. After circulation through the cuvette, sample gas was then re-dried. Air was pulled through the system by pumps (H. Walz) at a flow rate of 0.5 l min⁻¹, through a channel switcher (ADC Bioscientific) alternating reference and sample gas streams every two minutes to provide a single input to an LI-6252 CO₂ analyser (Li-Cor Inc., Lincoln, NB, USA). A chart recorder logged differences in CO₂ concentration between reference and sample gas over a 24-h period and net CO₂ exchange (A; units of $\mu\text{mol m}^{-2} \text{s}^{-1}$) was then calculated using the equation of von Caemmerer & Farquhar (1981) for differential systems in which air streams are dried. Where whole plants were used, and the morphology of the plant did not allow accurate determination of leaf area, results are presented on a f. wt basis.

The gas-exchange system described above was also used to investigate the response of species to drought, with the gas-exchange of initially well-watered plants charted over a number of days without water (N.B. water was drained from the 'tanks' or water-storing phytotelmata of epiphytes), followed by re-wetting of the soil and/or leaf blade (depending on life form, i.e. terrestrial or epiphytic).

Leaf tissue titratable acidity and thickness

For determination of titratable acidity, leaf discs were taken from plants in the gas-exchange system. Firstly, at the end of the light period from the area of leaf distal to the cuvette, and then at the end of the dark period from the area inside the cuvette immediately following termination of gas-exchange measurements. These leaf discs were frozen and stored in liquid nitrogen until subsequent hot-water extraction (20 min at 100°C). Extracts were made up to 25 ml with distilled water and titrated against 1 mM NaOH until neutrality as indicated by pH meter. Leaf blade thickness was determined by micrometer for mid-leaf portions of leaf blade (or in the case of the few species such as *Pitcairnia arcuata*, which possess a midrib, immediately adjacent to the midrib in order that typical lamina tissue was investigated), approximately two-thirds along the length of the leaf from the base.

Carbon isotope ratio

Carbon isotope ratios of dried leaf blade samples, relative to Pee Dee belemnite standard, were determined via mass spectrometry as detailed by Crayn *et al.* (2001).

Results

$\delta^{13}\text{C}$ values exhibited a bimodal frequency distribution amongst the bromeliad species investigated (Fig. 1a); species

that showed no nocturnal acidification (28 in total) had $\delta^{13}\text{C}$ values in the range -22.6 to -31.5‰ with a median value of -27.1‰ , and plants that showed nocturnal acidification (22 in total) had values in the range -12.5 to -27.6‰ , with a median value of -14.6‰ . For acidifying species more negative $\delta^{13}\text{C}$ was associated with less nocturnal CO_2 fixation, for example *Vriesea espinosae* ($\delta^{13}\text{C} = -18.6\text{‰}$, proportion of CO_2 uptake occurring at night = 62.6%) and *Tillandsia flexuosa* ($\delta^{13}\text{C} = -12.7\text{‰}$, CO_2 uptake at night = 96.7%).

Non-acidifying species showed diel gas-exchange patterns typical of C_3 plants, with CO_2 uptake restricted to the photoperiod and the extent of respiration constant throughout the dark period (e.g. *Brocchinia reducta*; Fig. 2a). Amongst all subfamilies, acidifying plants exhibited one of four modes of gas-exchange: (a). CO_2 uptake occurring mainly during the photoperiod, but with a lack of net CO_2 losses during at least the early part of the dark period (e.g. *Werauhia sanguinolenta*; Fig. 2b) (b). CO_2 uptake occurring mainly during the photoperiod, but with low and consistent uptake occurring at

night (e.g. *Ronnbergia explodens*; Fig. 2c) (c). CO_2 uptake throughout the day and less so at night (Phase 1), the different phases of CAM apparent but Phase 3 weakly expressed (e.g. *Vriesea barclayana*; Fig. 2) (d). classic 'obligate' CAM, with the majority of CO_2 uptake at night (Phase 1) and three distinct phases apparent by day (Phases 2–4). Species with obligate CAM exhibited differences in the extent and duration of Phase 4 CO_2 uptake during the afternoon; for example *Neoregelia concentrica* (59.9% of daily CO_2 uptake occurring at night) had an extensive Phase 4 (c. $1.5 \mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-1}$ for $9\frac{1}{2}$ h; Fig. 2e), *Aechmea nudicaulis* (87.1% CO_2 uptake at night) less so (Phase 4– $0.4 \mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-1}$ for 6 h; data not shown), and *N. cruenta* (96.1% CO_2 uptake at night) had an almost negligible Phase 4 (c. $0.08 \mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-1}$ for 5 h; Fig. 2f).

Vriesea espinosae had a similar diel gas-exchange pattern to *V. barclayana*, lacking a distinct Phase 3 of CAM (data not shown). Obligatory CAM species with an extensive Phase 4 (in addition to *Neoregelia concentrica*) included *Aechmea allenii*,

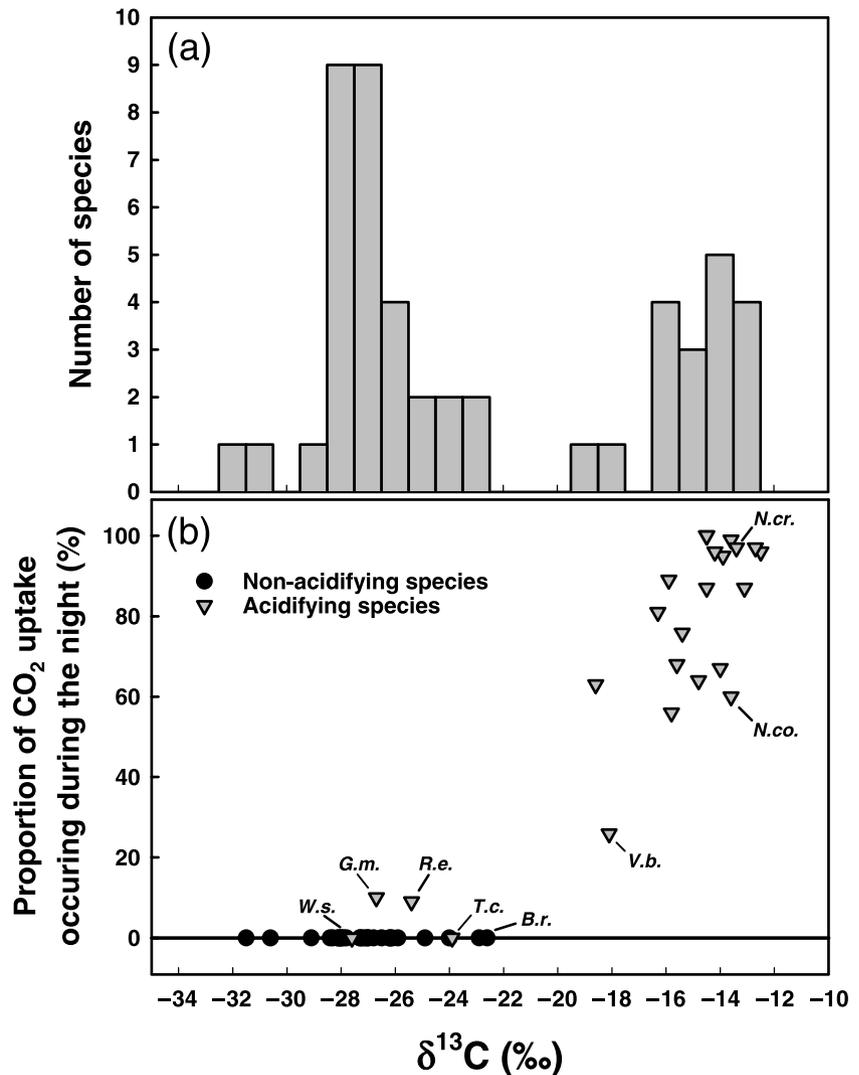


Fig. 1 (a) Frequency of $\delta^{13}\text{C}$ values amongst 50 ecophysiologically diverse bromeliad species (b) the relationship between $\delta^{13}\text{C}$ and proportion of CO_2 uptake occurring at night. B.r. = *Brocchinia reducta*, G.m. = *Guzmania monostachia*, N.co. = *Neoregelia concentrica*, N.cr. = *N. cruenta*, R.e. = *Ronnbergia explodens*, T.c. = *Tillandsia cretacea*, V.b. = *Vriesea barclayana*, W.s. = *Werauhia sanguinolenta*.

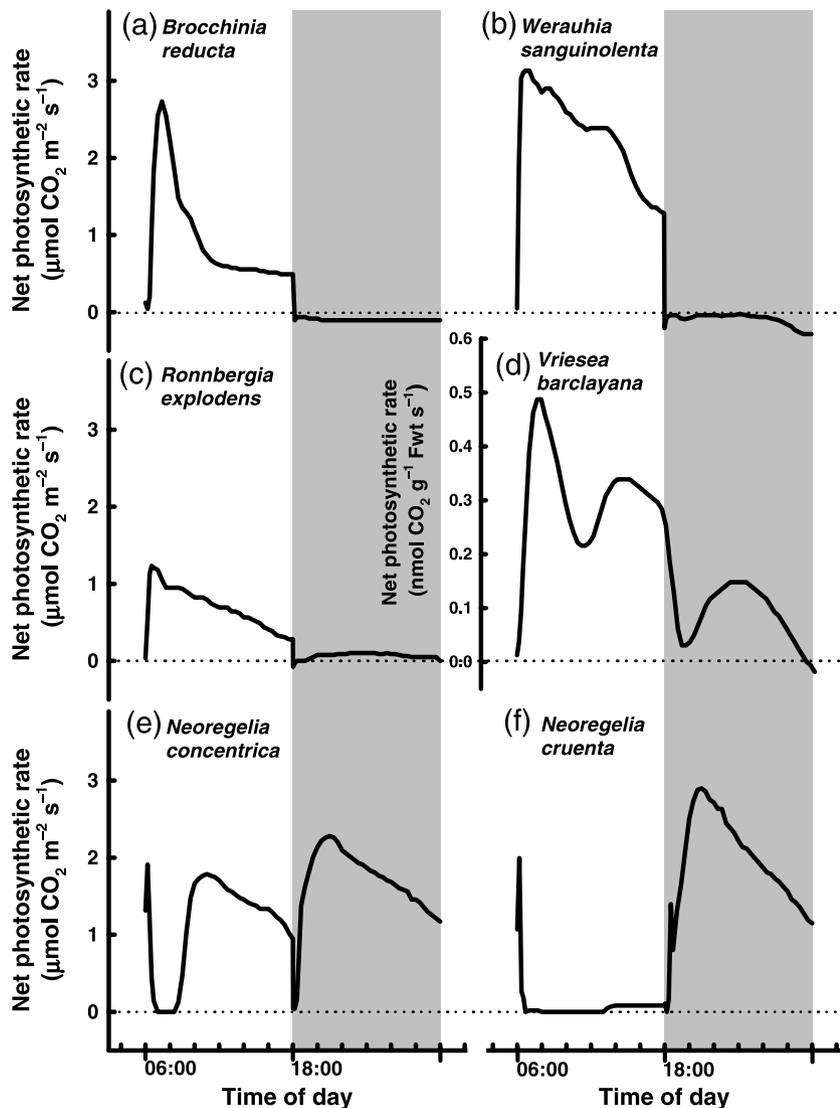


Fig. 2 Representative diel patterns of gas-exchange in well-watered Bromeliaceae: (a) *Brocchinia reducta* (typical C_3 photosynthesis; no nocturnal acidification of leaf tissues) (b) *Werauhia sanguinolenta* (C_3 -like gas-exchange, but with nocturnal recycling of respiratory CO_2 associated with acidification of leaf tissues) (c) *Ronnbergia explodens* (net CO_2 uptake mainly during the photoperiod, but with limited uptake throughout the night associated with acidification) (d) *Vriesea barclayana* (CAM with extensive Phases 2 & 4, indistinct Phase 3, and a relatively small Phase 1) (e) *Neoregelia concentrica* (CAM with well defined Phase 3 and extensive Phase 4) (f) *Neoregelia cruenta* (obligate CAM with limited Phase 4).

Cryptanthus brivittata, *C. colnagoi*, *Hechtia guatemalensis* and *Puya laxa* (data not shown). *Ananas comosus* and *Billbergia macrolepis* had moderate Phase 4 CO_2 uptake, similar to *Aechmea nudicaulis*. *Aechmea dactylina*, *Cryptanthus bromelioides*, *Orthophytum magalhaesii*, *Portea petropolitana*, *Tillandsia bulbosa*, *T. elongata* and *T. flexuosa* had similar diel patterns of gas-exchange to *Neoregelia cruenta*, with almost no Phase 4 CO_2 uptake (data not shown) and the majority of uptake occurring during the night (Table 2).

Two members of subfamily Pitcairnioideae with succulent leaves (*Hechtia guatemalensis*, leaf blade thickness = 3.0 ± 0.1 (SE) mm; *Puya laxa*, 3.8 ± 0.1 mm; $n = 4$) showed night-time CO_2 uptake, a nocturnal increase in leaf tissue acidity, and carbon isotope ratios of -14.0 and -15.8 ‰, respectively (Table 2). Remaining Pitcairnioideae had C_3 photosynthetic characteristics (*Brocchinia reducta*, *Fosterella* spp. and *Pitcairnia* spp.; Table 2; Fig. 2a), thinner leaves, ranging between 0.2

and 0.6 mm in thickness, and carbon isotope ratios more negative than -22.6 ‰ (Table 2).

Within subfamily Tillandsioideae a similar situation was apparent; plants with thinner leaves (0.2–0.6 mm) generally lacking nocturnal CO_2 uptake, having $\delta^{13}C$ values more negative than -22.9 ‰, and with no changes in titratable acidity apparent. *Tillandsia bulbosa*, *T. elongata*, *T. flexuosa*, *Vriesea barclayana* and *V. espinosae* had all the indicators of CAM including more succulent leaves (0.6–1.3 mm thick; Table 2), and all possess water-absorbing trichomes over leaf blade surfaces. *Tillandsia deppeana* is a 'soft leaved' (0.3 mm thick) member of the genus and has photosynthetic characteristics typical of C_3 plants and the Type 4 life form. However, three tillandsioid species showed carbon isotope ratios typical of C_3 plants, but accumulated acid overnight and showed gas-exchange characteristics atypical of C_3 metabolism. Well-watered *Guzmania monostachia* performed 10% of

daily carbon uptake at night (Table 2) using thin leaves (0.3 ± 0.01 mm; $n = 4$), whereas *Tillandsia cretacea* and *Werauhia sanguinolenta* had thicker leaves (0.6 ± 0.05 and 0.9 ± 0.01 mm, respectively) and showed a relief of respiratory losses of carbon during the early hours of the night (*T. cretacea*) or throughout the majority of the night (*W. sanguinolenta*; Fig. 2b). Two further individuals of *Werauhia sanguinolenta* also showed day/night changes in titratable acidity and variable respiratory CO_2 loss throughout the night (data not shown; N.B. non-acid accumulating *Werauhia* spp. had leaf blade thicknesses in the range 0.3–0.6 mm and are highland species).

Within subfamily Bromelioideae all species accumulated acid during the night, and all except *Ronnbergia explodens* had $\delta^{13}\text{C}$ values less negative than -16.3‰ as a result of CO_2 fixation mainly during the night. *Ronnbergia explodens*, despite a $\delta^{13}\text{C}$ value of -25.4‰ , showed almost constant CO_2 uptake throughout the diel period, although during the night this was only *c.* $0.1 \mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-1}$ and accounted for just

8.8% of daily carbon gain (Table 2; Fig. 2c). When *Ronnbergia explodens* was droughted for 10 d the extent of nocturnal CO_2 uptake did not differ over this period (denoted by both gas-exchange and titratable acidity; Fig. 3a,b), although as the absolute amount of daytime uptake decreased nocturnal uptake provided proportionally more carbon each day (Fig. 3c). On re-wetting, daytime CO_2 uptake recovered quickly (Fig. 3a). Young fully expanded leaves of *Guzmania monostachia* showed a response to drought identical to that of *Ronnbergia explodens*, with consistent nocturnal CO_2 uptake, whether well watered or droughted (Fig. 4a). When whole plants were investigated a different response was observed, with respiration apparent in well-watered tissues but with gas-exchange shifting to a pattern of nocturnal CO_2 uptake as drought progressed, and returning to net CO_2 loss at night when watering resumed (Fig. 4b,c). Of the mature CAM-equipped species with C_3 -like carbon isotope ratios (*Guzmania monostachia*, *Tillandsia cretacea*, *Ronnbergia explodens*, *Werauhia sanguinolenta*), none showed variation in the

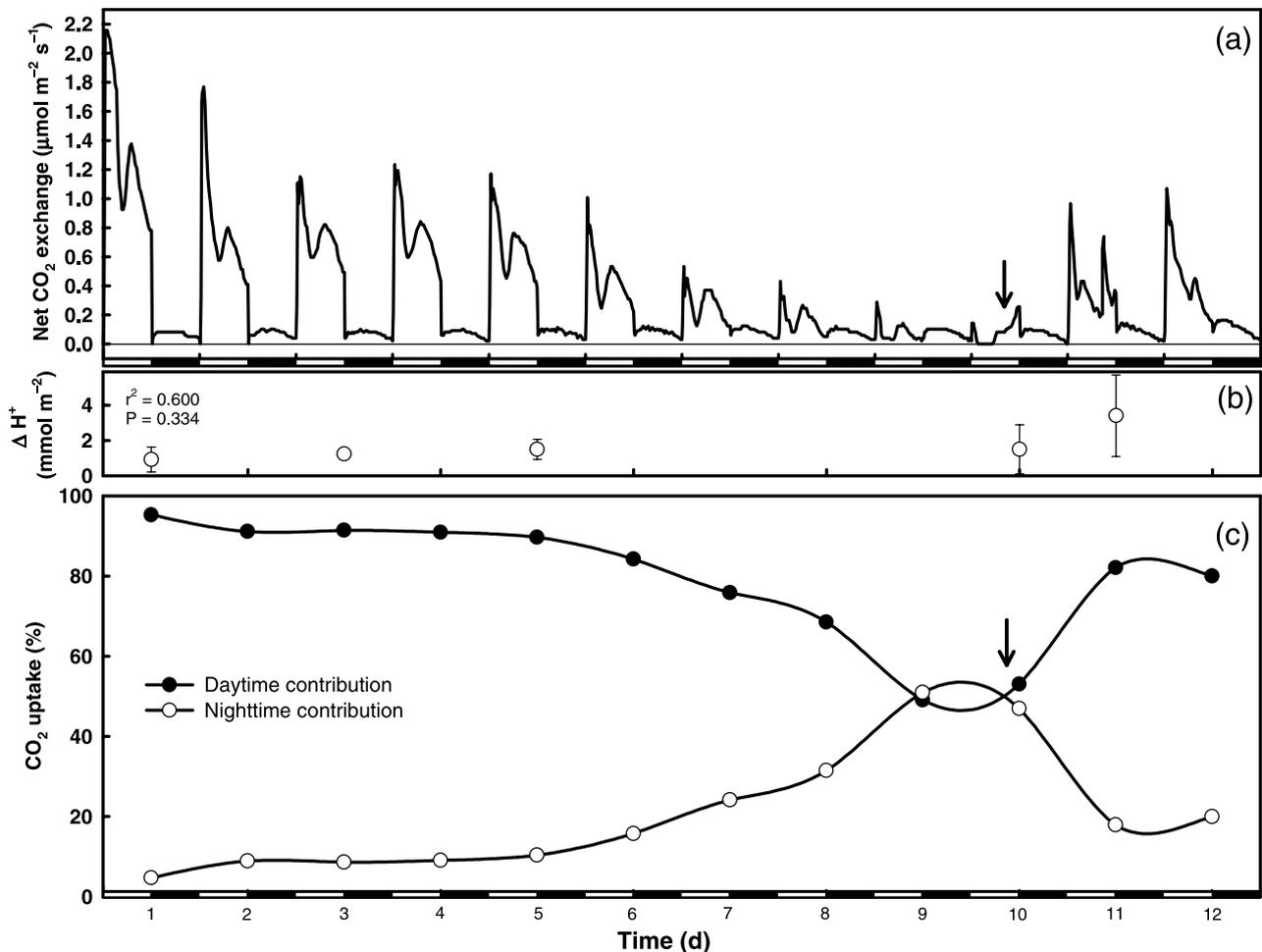


Fig. 3 Diel CO_2 exchange characteristics of the terrestrial bromeliad *Ronnbergia explodens* during 10 days of drought: (a) net CO_2 exchange (b) extent of nocturnal acidification (ΔH^+) and (c) the relative contribution of daytime and nighttime CO_2 uptake to daily carbon gain. Arrow indicates time at which watering was resumed. Dark bars indicate dark period. P and r^2 values in (b) refer to linear regression fitted to acidification data.

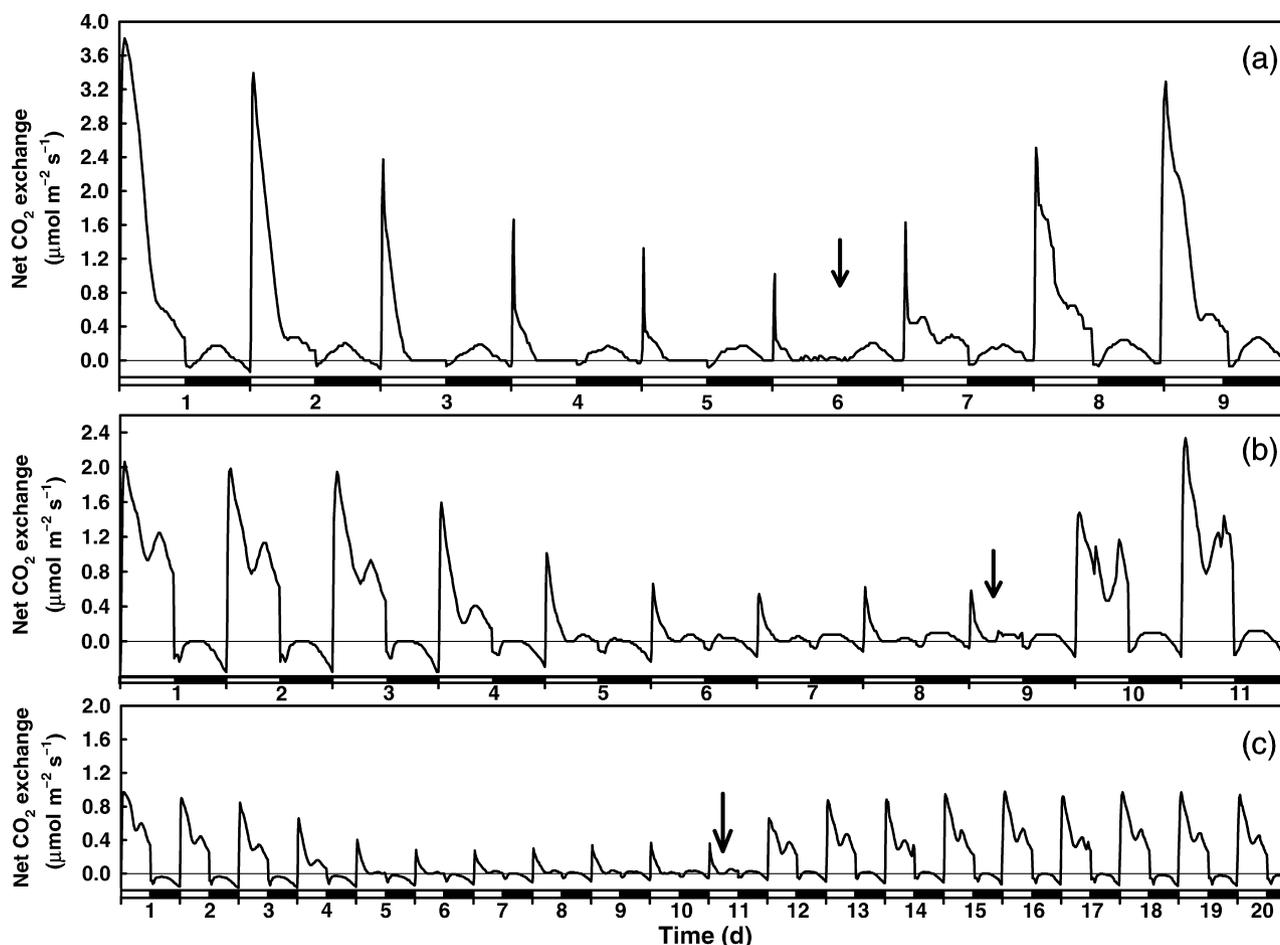


Fig. 4 Diel CO_2 exchange characteristics of the epiphytic Type 4 bromeliad *Guzmania monostachia* during drought: (a) young fully expanded leaf of mature plant (b) whole mature plant and (c) whole seedling. Arrow indicates time at which watering was resumed. Dark bars indicate dark period.

magnitude of CAM in single leaf blades in response to at least three days drought (data not shown).

Discussion

As predicted by Medina *et al.* (1977) carbon isotope ratio reflects the degree of C_4 carboxylation in acid-accumulating bromeliad species. Indeed, 13% of species with $\delta^{13}\text{C}$ values typical of C_3 plants were capable of limited nocturnal CO_2 uptake or CAM cycling (i.e. *Guzmania monostachia*, *Ronbergia explodens*, *Tillandsia cretacea* and *Werauhia sanguinolenta*). Despite a lack of plasticity in CAM expression for these species, Phase 1 CO_2 uptake contributed proportionally more to daily carbon gain as drought progressed; a characteristic of undoubted adaptive significance for plants in arid habitats. In light of these weak CAM species, surveys employing carbon isotope ratios to investigate the frequency of CAM-equipped plants within populations should be regarded as conservative estimates.

Indeed, Crayn *et al.* (2001) investigated herbarium material of Rapateaceae (close relatives of Bromeliaceae) and concluded

that a small number of species, having $\delta^{13}\text{C}$ values in the order of -20‰ , could be CAM-equipped (Rapateaceae are so rare in cultivation that confirmation from living material was not possible). The present study supports this view. Also, a forthcoming survey of bromeliad herbarium material (D. M. Crayn, A. J. C. Smith & K. Winter, unpublished data) will probably include some C_3 -like $\delta^{13}\text{C}$ values masking weak CAM; particularly in subfamily Bromelioideae and some soft-leaved *Tillandsia* and *Vriesea* species (Tillandsioideae).

Guzmania monostachia appears to be exceptional as a soft-leaved species with the ability to up-regulate CAM seasonally (Maxwell *et al.*, 1992; Maxwell *et al.*, 1995), with induction occurring within days of drought/high-light stress (K. Maxwell, unpublished data). However, despite exhibiting weak CAM in the present study (also unusual for the genus), facultative CAM was not unequivocally demonstrated for *G. monostachia* in response to short-term drought, only exhibiting an apparent response of night-time CO_2 uptake when whole plants were measured (the technique employed by Medina & Troughton, 1974) but with individual leaves unresponsive. These patterns of whole-plant gas-exchange could result from

changes in relative rates of respiration amongst leaves. The above workers have investigated *G. monostachia* only from Trinidad and neighbouring Venezuela, populations far removed from the Panamanian ecotype used in the present study. The present data thus indicate that true facultative CAM is rare in this family and modes of CAM use could vary even between different populations of the same species.

Some tillandsioids, such as *Vriesea barclayana* (epiphytic in lowland dry scrub forest in Ecuador; Smith & Downs, 1977), are ecophysiologicaly intermediate between Type Four and Five life forms in terms of both photosynthetic pathway (fixing a small proportion of CO₂ at night) and morphology (possessing substantial phytotelmata but also with absorbent trichome-covered and succulent leaf blades). Thus a gradient in life form is evident, with the extent of CAM expression correlating with the degree of adaptation to xeric niches.

In conclusion, carbon isotope ratio of leaf material in acid-accumulating Bromeliaceae reflects the proportion of daily carbon gain fixed by C₃ or C₄ carboxylation. A small number of bromeliad species with C₃-like carbon isotope ratios are capable of weak CAM use, either taking up a small amount of CO₂ at night, or with the ability to recycle CO₂ from respiration. δ¹³C surveys of plant populations in the tropics will underestimate the number of CAM-equipped species.

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