

# The role of CAM in high rainfall cloud forests: an *in situ* comparison of photosynthetic pathways in Bromeliaceae

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## ABSTRACT

**Crassulacean acid metabolism (CAM), an advanced photosynthetic pathway conferring water conservation to plants in arid habitats, has enigmatically been reported in some species restricted to extremely wet tropical forests. Of these, epiphytic Bromeliaceae may possess absorbent foliar trichomes that hinder gas-exchange when wetted, imposing further limitations on carbon dioxide (CO<sub>2</sub>) uptake. The hypothesis that the metabolic plasticity inherent to CAM confers an ecological advantage over conventional C<sub>3</sub> plants, when constant rainfall and mist might inhibit gas-exchange was investigated. Gas-exchange, fluorometry and organic acid and mineral nutrient contents were compared for the bromeliads *Aechmea dactylina* (CAM) and *Werauhia capitata* (C<sub>3</sub>) *in situ* at the Cerro Jefe cloud forest, Panama (annual rainfall >4 m). Daily carbon gain and photosynthetic nutrient use efficiencies were consistently higher for *A. dactylina*, due to a greater CO<sub>2</sub> uptake period, recycling of CO<sub>2</sub> from respiration and a dynamic response of CO<sub>2</sub> uptake to wetting of leaf surfaces. During the dry season CAM also had water conserving and photo-protective roles. A paucity of CAM species at Cerro Jefe suggests a recent radiation of this photosynthetic pathway into the wet cloud forest, with CAM extending diversity in form and function for epiphytes.**

*Key-words:* *Aechmea*; *Werauhia*; bromeliad; Crassulacean acid metabolism; epiphyte; montane forest.

## INTRODUCTION

Crassulacean acid metabolism (CAM) is a photosynthetic pathway found in 7% of the angiosperm flora, typically succulent species occupying semi-arid regions; by taking up carbon dioxide gas (CO<sub>2</sub>) at night when evaporation is lowest CAM conserves more water than the common C<sub>3</sub> photosynthetic pathway (Winter & Smith 1996). CAM produces a large proportion of epiphytic biomass in tropical

forests; for example of the epiphytic Bromeliaceae investigated 69% are obligate CAM species (Martin 1994).

Altitudinal transects in tropical latitudes show a greater proportion of CAM plants at lower altitudes, concurrent with lower annual rainfall (Griffiths *et al.* 1986; Kluge *et al.* 1991; Hietz, Wanek & Popp 1999), with only 6% of species in upper montane forests in Mexico possessing obligate CAM (Hietz *et al.* 1999). However, a small number of obligate CAM species are consistently found in high rainfall montane forest habitats in the tropics. Even for plants occupying the potentially drier epiphytic niche, water shortage is unlikely to be severe as these plants store substantial amounts of water (Benzing 2000) and rainfall may be as high as 4–6 m per annum.

Furthermore, the apparent restriction of some obligate CAM species to wet montane sites suggests that certain species may benefit from this seemingly contradictory eco-physiological situation [e.g. the bromeliads *Aechmea aripensis* (N.E. Brown) Pittendrigh, *Aechmea fendleri* André ex Mez, and the wet montane Trinidad endemic *Aechmea downsiana* Pittendrigh; Griffiths *et al.* 1986; as well as *A. lasseri* L.B. Smith in Venezuela]. In the case of Bromeliaceae this is particularly enigmatic as many CAM taxa, including *Aechmea*, possess a layer of water-absorbing leaf hairs (foliar trichomes) that promote the formation of water films over leaf blade surfaces (Pierce *et al.* 2001), potentially hindering gas-exchange in wet weather (Benzing, Seeman & Renfrow 1978; Martin 1994). Pierce *et al.* (2001) determined that C<sub>3</sub> cloud forest dwelling Bromeliaceae possess various morphological mechanisms for shedding excess water (such as repellent trichome or powdery wax layers) that are not shared by sympatric CAM species. However, CAM bromeliads compete effectively and, being at a disadvantage morphologically, a physiological basis of this competitive ability is implied.

CAM confers a number of advantages over C<sub>3</sub> metabolism to bromeliads occupying drier habitats: (1), short-term physiological plasticity via internal recycling of respiratory CO<sub>2</sub> when wetted, followed by a compensatory temporal shift in CO<sub>2</sub> uptake when dry [e.g. *Tillandsia usneoides* (L.) L.; Haslam, Borland & Griffiths 2002]; (2), seasonal plasticity in carbon assimilation and water loss should growing conditions become drier (e.g. *Guzmania monostachia* (L.) Rusby ex Mez; Maxwell *et al.* 1995); and (3) photoprotec-

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tion; on sunny days CAM is thought to provide a steady supply of CO<sub>2</sub> for carboxylation, preventing feedback inhibition of photosystem operation and damage in high light conditions (e.g. *Guzmania monostachia*; Maxwell *et al.* 1995; *Neoregelia cruenta* (R. Graham) L.B. Smith; Fernandes, Chaloub & Reinert 2002).

The present study investigated the above mechanisms *in situ* in a wet cloud forest, comparing sympatric CAM and C<sub>3</sub> bromeliad species. The nomenclature follows Luther & Sieff (1998) and later descriptions of species present at the field site by Grant (2000), (2001), Pierce & Aranda (2000), Pierce (2001), and Pierce & Grant (2002). Classification of life forms or ecophysiological types follows Benzing (2000).

## MATERIALS AND METHODS

### Field site

Investigations were conducted in the cloud forest at the peak of Cerro Jefe (Chief Hill), Chagres National Park, Province of Panama, Republic of Panama (1007 m a.s.l.; 09°13'794" N, 079°22'995" W; Fig. 1A). This region exhibits annual rainfall in excess of 4 m (Carrasquilla 1997). Measurements were taken during the mid-wet season (August 2000), at the end of the wet season (November 2000) and during the short dry season (February 2000). Phorophyte vegetation at Cerro Jefe is stunted by a variety of climatic and edaphic factors (Carrasquilla 1997; R.A. Gottsberger,



**Figure 1.** Cerro Jefe montane cloud forest, Panama province, Republic of Panama. (A) View from the summit looking down hill towards the south-west; (B) *Aechmea dactylina* during the misty wet season (March); and (C), *Werauhia capitata* in the same exposed niche. Photography by Simon Pierce.

pers. comm.), and so access to epiphytes did not require specialist skills or equipment.

### Plant material

*Aechmea dactylina* Baker (Bromeliaceae, Bromelioideae; Fig. 1B) is a Type 3 CAM epiphyte (Pierce *et al.* 2001), with an absorbent trichome layer on both the adaxial and abaxial leaf blade surfaces (Pierce *et al.* 2001). This species has an extravaginal sympodial growth form, with typically one or two daughter shoots produced each growth season. At maturity the young leaves of each shoot do not spread, but remain erect to form a bulbous tube surrounding the scape. Ants of several unidentified species may colonize this domatium, sealing the top of the tube with carton. If the plant is disturbed the ants defend the nest with either painful bites or stings, depending on the ant species (S. Pierce, pers. obs.); an identical myrmecophytism to that recorded for *Aechmea bracteata* (Benzing 2000). *Aechmea dactylina* is pollinated by various hummingbird species (Pierce & Gottsberger 2001a), and although individuals may be found in flower throughout the wet season, the majority of the population appears to flower during August (S. Pierce, pers. obs.) – N.B. individuals used did not flower during the study. *Aechmea dactylina* produces blue succulent berries, probably dispersed in an endozoochorous fashion by birds.

*Werauhia capitata* (Mez & Wercklé) J.R. Grant is a Type 4 member of subfamily Tillandsioideae, also epiphytic in exposed situations at Cerro Jefe. Sympodia arise intravaginally; after flowering a single daughter shoot is initiated close to the crown of the parent shoot. In older plants the consecutive stems in this sympodium may form a woody pseudostem (Fig. 1C), sometimes up to ~50 cm in length (S. Pierce, pers. obs.). The abaxial leaf blade surface possesses a glaucous epicuticular wax powder layer, which possibly aids in self-cleaning, defence or in keeping stomata free of water in wet weather (Pierce *et al.* 2001). *Werauhia capitata* is anemochorous and bat pollinated (Utley 1983), the population flowering at the end of the wet season at Cerro Jefe (Pierce & Gottsberger 2001b). *Werauhia capitata* produces clear mucilage that surrounds all parts of the inflorescence and is thought to protect against herbivory by weevils (Coleoptera, Curculionidae) during reproductive development (Pierce & Gottsberger 2001b).

### Gas-exchange and fluorometry

Gas-exchange measurements were conducted on mid-leaf portions of young fully expanded leaves using an LI-6400 portable infrared gas analysis (IRGA) system (Li-Cor Inc., Lincoln, NE, USA) at intervals of 1.5 h over a diel (24 h) time course. Environmental parameters such as light intensity (photosynthetically active radiation; PAR), temperature and relative humidity were also recorded by the LI-6400. During measurements in November artificial surface water films were induced on target leaves of *A. dactylina* by misting at night with a hand-nebulizer immediately before and after measurements of gas-exchange. This was

observed to completely inundate leaf surfaces. Gas-exchange measurements were carried out without first drying leaves [water mole fractions of reference and sample air were known and used for re-calculation of CO<sub>2</sub> mole fractions of these gas samples; measurements of CO<sub>2</sub> exchange and net photosynthetic rate were then calculated using the equation of von Caemmerer & Farquhar (1981) for differential systems, although parameters such as stomatal conductance ( $g_s$ ) and evapotranspiration (EvT) could not be determined]. Daily net carbon gain was calculated by integrating diel gas-exchange curves (Canvas 3-53 imaging software; Deneba Software, Miami, FL, USA). Fluorometry parameters were measured in concert with gas-exchange using a PAM-2000 portable modulated fluorometer (H. Walz, Effeltrich, Germany) fitted to a PAM adaptor cuvette (Li-Cor Inc.). Absorbance of *A. dactylina* and *W. capitata* leaf blades was previously determined as 0.84 and 0.83, respectively, using a LI-1800 portable spectroradiometer (Li-Cor Inc.), via an 1800–12 s external integrating sphere (Li-Cor Inc.), these values being used by the fluorometer software for calculation of electron transport rate (ETR).

### Biochemical analysis

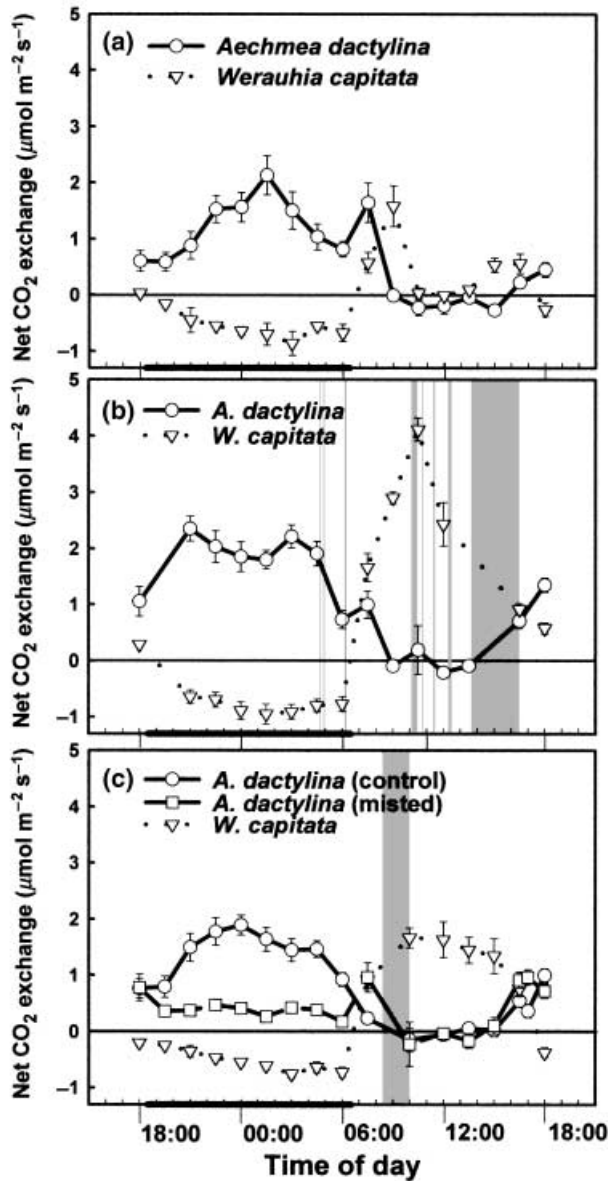
Every 3 h, leaf disc samples were frozen in liquid nitrogen and subsequently assayed enzymatically for malic acid (Gutmann & Wahlefeld 1974) and citric acid (Möllering 1985). Internal CO<sub>2</sub> recycling was then calculated as detailed previously (Griffiths *et al.* 1989). Dried leaf samples were also analysed for nitrogen contents with a CHN element analyser (Heraeus, Hanau, Germany), and analysed for other elements using an ICP JY70 Plus spectroradiometer (ISA, München, Germany). Photosynthetic nitrogen and phosphorus use efficiencies (PNUE and PPUE, respectively) of each species were calculated as a function of leaf blade nitrogen (N) and phosphorus (P) contents and daily carbon gain.

### Carbon isotope ratio

For confirmation of probable CAM or C<sub>3</sub> use, leaf discs were taken from mid-leaf regions of the majority of bromeliad species at Cerro Jefe and analysed for carbon isotope ratio ( $\delta^{13}\text{C}$ ) via mass spectrometry, as described by Crayn, Smith & Winter (2001).

## RESULTS

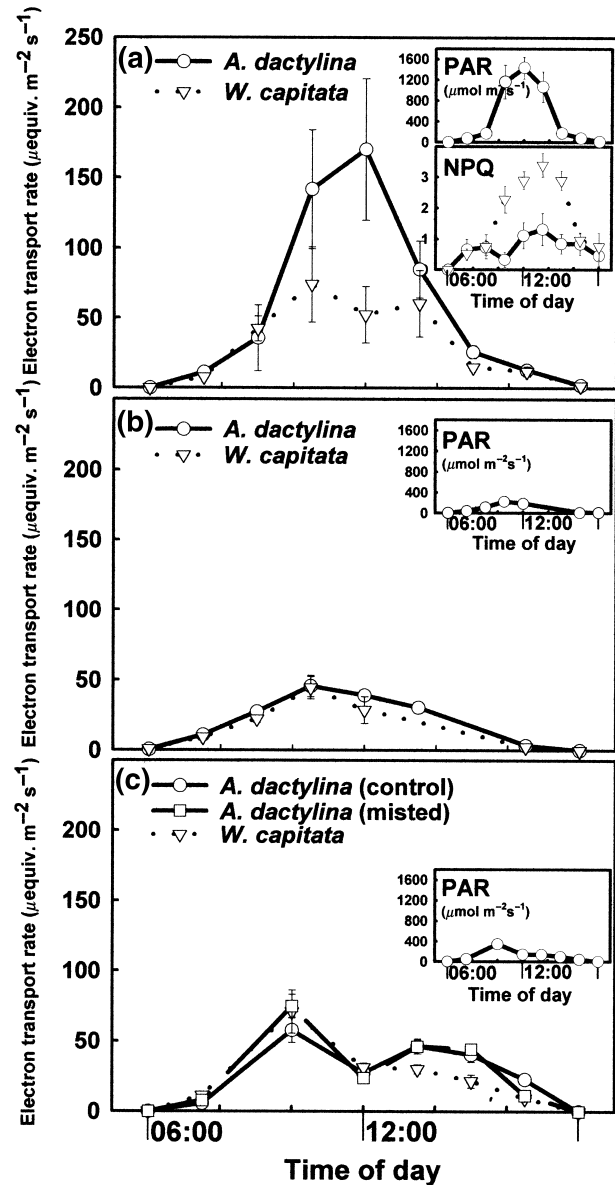
Net photosynthetic rate, photosynthetic electron transport rate and non-photochemical quenching (NPQ) of chlorophyll fluorescence for the two species in both wet and dry seasons are shown in Figs 2 and 3, with diel leaf malic acid contents presented in Fig. 4. For the CAM bromeliad *A. dactylina*, *in situ* measurements showed similar rates of net CO<sub>2</sub> uptake at night (Phase 1) during both seasons (Fig. 2), with additional CO<sub>2</sub> uptake during the early morning and late afternoon (Phase 2 and 4, respectively, of CAM).



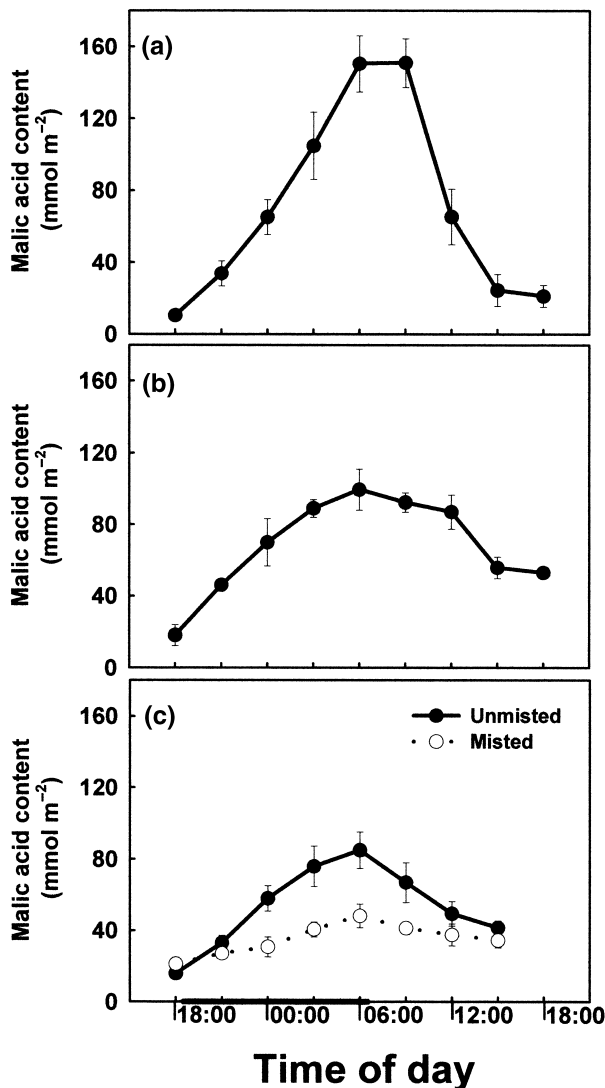
**Figure 2.** Diel gas-exchange of the epiphytic bromeliads *Aechmea dactylina* (CAM photosynthetic pathway) and *Werauhia capitata* ( $C_3$  pathway) *in situ* in a Panamanian cloud forest during (a), dry season (February); (b), mid wet season (August); and (c), late wet season (November). During the late wet season measurements further leaves of *Aechmea* were also artificially misted during the night. Values represent the mean  $\pm 1$  SE. of six replicates. Pale grey vertical bars represent the timing and duration of rain. Horizontal black bar represents night-time.

Although the overall integrated nocturnal  $CO_2$  uptake was slightly lower in the dry season (Table 1), malic acid accumulation was higher (Fig. 4), with carbon recycled from respiration accounting for a little over half the carbon fixed at night (Table 1). Although  $C_3$  *W. capitata* showed consistent net  $CO_2$  uptake in the wet season (Fig. 2b & c), relatively high rates of respiration were apparent at night, and a pronounced mid-day depression of  $CO_2$  uptake evident in the

dry season (Fig. 2a). During both seasons daily carbon gain via the CAM pathway was consistently higher than that via the  $C_3$  pathway and, most notably, was approximately twice as effective as  $C_3$  assimilation during wet season measurements (Table 1). Wetted CAM leaves were the equal of unwetted  $C_3$  leaves in terms of daily carbon gain (Table 1).



**Figure 3.** Diurnal photosynthetic electron transport rate of the epiphytic bromeliads *Aechmea dactylina* (CAM photosynthetic pathway) and *Werauhia capitata* ( $C_3$  pathway) *in situ* in a Panamanian cloud forest during (a), dry season (February); (b), mid wet season (August); and (c), late wet season (November). During late wet season measurements further leaves of *Aechmea* were also artificially misted during the night. Values represent the mean  $\pm 1$  SE. of six replicates. Insets show respective light intensities (photosynthetically active radiation; PAR), and for the dry season, non-photochemical quenching (NPQ) of chlorophyll fluorescence.



**Figure 4.** Malic acid content of *Aechmea dactylina* leaf blades throughout a diel time-course during (a), dry season (February); (b), mid wet season (August); and (c), late wet season (November). During late wet season measurements further leaves of *Aechmea* were also artificially misted during the night. Values represent the mean  $\pm$  1 SE. of six replicates. Horizontal black bar represents night-time.

Thus, CAM allowed CO<sub>2</sub> uptake for a greater proportion of each day and prevented respiratory losses of CO<sub>2</sub> (Fig. 2; Table 1).

Artificial wetting of leaves at night did not completely inhibit gas-exchange (Fig. 2c), resulting in nocturnal CO<sub>2</sub> uptake of  $16.6 \pm 1.3$  mmol C m<sup>-2</sup> (cf.  $60.6 \pm 6.3$  mmol C m<sup>-2</sup> in unmisted controls; Table 1). Internal recycling of CO<sub>2</sub> was higher in misted leaves (36.3 cf. 8.1% in unmisted controls; Table 1), with malic acid accumulating steadily throughout the night (Fig. 4c). The wetted leaves subsequently showed markedly higher morning (CAM Phase 2) and afternoon (Phase 4) net CO<sub>2</sub> uptake in comparison with unmisted control leaves on the same plants (Fig. 2c).

During the wet season net photosynthetic rate of both species was light limited to the same extent (Fig. 2b & c), with low electron transport rates tracking low light intensities (Fig. 3b & c) and no NPQ apparent. Low light intensities resulted mainly from cloud cover and, until about 1100 h each day, wind-borne mist. Light intensities (PAR) at midday were  $273.3 \pm 78.3$  and  $141.8 \pm 13.1$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  during mid- and late-wet season measurements, respectively (Fig. 3b & c), with air temperatures reaching midday maxima of 27.2 and 29.7 °C, respectively. Despite this potential limitation to the CAM cycle, carbon uptake at night matched malic acid accumulation (Table 1), with malic acid accumulating at a constant rate throughout the night (Fig. 4b & c). Citric acid was not accumulated in either season by *A. dactylina*, and leaves of *W. capitata* did not acidify over night (data not shown).

During the dry season, electron transport rates were much higher for *A. dactylina*, with NPQ gradually increasing during the light period (Fig. 3a). In contrast, the midday depression of gas-exchange in *W. capitata* was associated with lower ETR in concert with a high NPQ, that was typical of high light stress (Fig. 3a). Indeed, mean PAR at midday was  $1398.0 \pm 220.1$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 3a) and the maximum air temperature recorded was 35.1 °C. The carbon gain of the C<sub>3</sub> species balanced, but did not exceed respiratory losses of carbon (Table 1).

Leaf blades of *A. dactylina* had significantly higher absolute contents of the mineral nutrients Ca, Mg, Mn and P than *W. capitata* (Table 2). The different mineral composition was associated with greater photosynthetic nitrogen and phosphorus use efficiencies for the CAM species (Table 1), representing yet another advantage for the CAM pathway, in addition to carbon balance and light utilization (Fig. 2; Table 1).

Carbon isotope ratios greater (more positive) than -19‰ were interpreted as denoting CAM as the principal photosynthetic pathway (S. Pierce, unpubl. results), and were found in only three of 39 species at Cerro Jefe (8% of the bromeliad flora); *A. allenii*, *A. dactylina* and *Tillandsia bulbosa* (Table 3). All members of the genus *Werauhia*, including *W. capitata*, had carbon isotope ratios more negative than -19‰. The  $\delta^{13}\text{C}$  values indicated C<sub>3</sub> photosynthesis in *Tillandsia anceps* and *Tillandsia monadelphica*, and all members of the genera *Catopsis*, *Guzmania*, *Pitcairnia*, *Ronnbergia* and *Vriesea* found at Cerro Jefe (Table 3).

## DISCUSSION

Crassulacean acid metabolism provides leaf blades of *A. dactylina* with greater daily carbon gains than those of *W. capitata* via a number of mechanisms not shared by the C<sub>3</sub> pathway. The CAM pathway endowed a CO<sub>2</sub> uptake period longer than the photoperiod, compensated for wetting of the leaf by taking up more CO<sub>2</sub> when subsequently dry, recycled CO<sub>2</sub> from respiration, and acted as a photo-protective and water-conserving mechanism during drier

**Table 1.** Carbon assimilation characteristics of the epiphytic bromeliads *Aechmea dactylina* (CAM photosynthetic pathway) and *Werauhia capitata* (C<sub>3</sub> pathway) *in situ* in a Panamanian cloud forest during representative days in the dry season (February), mid wet season (August) and late wet season (November). During late wet season measurements further leaves of *Aechmea* were also artificially misted during the night

Species	Daily carbon gain (mmol C m <sup>-2</sup> d <sup>-1</sup> )	Nocturnal carbon gain (mmol C m <sup>-2</sup> )	ΔMalic acid (mmol m <sup>-2</sup> )	Internal CO <sub>2</sub> recycling as percentage of carbon fixed	PNUE (mol C mol N m <sup>-2</sup> d <sup>-1</sup> )	PPUE (mol C mol P m <sup>-2</sup> d <sup>-1</sup> )
Dry season						
<i>A. dactylina</i>	63.1 ± 8.6 <sup>b</sup>	53.5 ± 7.4	139.9 ± 16.7	59.6	–	–
<i>W. capitata</i>	–7.1 ± 6.9 <sup>a</sup>	–	–	–	–	–
Mid wet season						
<i>A. dactylina</i>	97.4 ± 8.3 <sup>b</sup>	79.6 ± 9.4	81.2 ± 8.7	0.2	–	–
<i>W. capitata</i>	55.6 ± 6.6 <sup>a</sup>	–	–	–	–	–
Late wet season						
<i>A. dactylina</i> (control)	69.8 ± 6.3 <sup>b</sup>	60.6 ± 6.3	69.0 ± 9.3	8.1	17.7 ± 3.4	39.1 ± 7.5
<i>A. dactylina</i> (misted)	29.7 ± 3.2 <sup>a</sup>	16.6 ± 1.3	26.7 ± 2.9	36.3	7.5 ± 1.4	16.6 ± 3.2
<i>W. capitata</i>	26.6 ± 5.5 <sup>a</sup>	–	–	–	7.9 ± 1.6	17.4 ± 3.5

Values represent the mean ± 1 SE of six replicates. ΔMalic acid represents nocturnal accumulation of malic acid. Percentage recycling denotes the proportion of malic acid stored during the night derived from respiratory CO<sub>2</sub> (cf. exogenous CO<sub>2</sub> uptake). PNUE, photosynthetic nitrogen use efficiency; PPUE, photosynthetic phosphorus use efficiency.

<sup>a, b</sup> Indicate significant differences between means of daily carbon gain within each season at the  $P \leq 0.01$  level, as determined by Tukey's multiple comparison procedure (ANOVA).

days. These mechanisms are all exhibited by CAM bromeliads in drier habitats (Maxwell *et al.* 1995; Fernandes *et al.* 2002; Haslam *et al.* 2002) in which xeric life forms in the family originally evolved (Medina 1974). Greater carbon gains via these mechanisms also allow more efficient use of mineral nutrients comprising photosynthetic machinery. For plants with nitrogen and phosphorus contents an order of magnitude less than those typical of deficient angiosperm leaves (Marschner 1999), photosynthetic nutrient use efficiencies are undoubtedly of critical importance in such a nutrient-poor niche. The greater absolute contents of Ca, Mg, Mn and P in *A. dactylina* leaves may also give this species an advantage over *W. capitata*; possibly myrmecophytism may explain these higher nutrient contents, as demonstrated by the epiphytic myrmecophyte *Dischidia major* Merril (Asclepiadaceae; Treseder, Davidson & Ehleringer 1995).

Gas exchange was not completely inhibited for wetted

leaves of *A. dactylina*, possibly as absorbent trichomes are less densely distributed over leaf surfaces of this species compared to lowland *Tillandsia* species (Pierce *et al.* 2001) and presumably some stomata remain unblocked even when wetted trichomes hold water on the leaf surface. Although this may be true of the two *Aechmea* species at Cerro Jefe, the third obligate CAM species present, *T. bulbosa*, is unusual for the atmospheric life form (Type 5) in lacking a layer of absorbent trichomes on exposed leaf blade surfaces. Benzing (1980) suggests that this limits reflectance of light by the leaf, thereby allowing the plant to capture more light and inhabit darker cloud forest habitats. This could alternatively represent an adaptation of the atmospheric life form to wet conditions that would otherwise starve the leaf of CO<sub>2</sub>. However, the data do indicate that during the wet season light-dependent electron transport, and not CO<sub>2</sub> availability, is the more fundamental limitation to carbon assimilation in cloud forest-dwelling

**Table 2.** Mineral nutrient contents of *Aechmea dactylina* and *Werauhia capitata* (Bromeliaceae) from Cerro Jefe, central Panama

Species	Mineral nutrient content (mg g <sup>-1</sup> DWT)											
	N	P	K	Al	B	Ca	Fe	Mg	Mn	Na	S	Zn
<i>Aechmea dactylina</i>	8.6 ± 0.7	0.4 ± 0.03	15.7 ± 4.2	0.4 ± 0.27	0.003 ± 0.0009	5.7 ± 0.9	0.5 ± 0.1	2.7 ± 0.3	1.1 ± 0.23	3.6 ± 0.5	0.8 ± 0.1	0.03 ± 0.004
<i>Werauhia capitata</i>	7.8 ± 0.8	0.2 ± 0.01	7.8 ± 1.2	0.2 ± 0.03	0.005 ± 0.0006	1.6 ± 0.3	0.5 ± 0.1	1.8 ± 0.2	0.1 ± 0.02	4.9 ± 0.5	1.5 ± 0.3	0.03 ± 0.008
Significance	NS	***	NS	NS	NS	**	NS	*	**	NS	NS	NS

Data represent the mean ± 1 SE of five replicates. Significant differences in each nutrient content between species were determined by Student's *t*-test: \* $P = 0.05$ , \*\* $P = 0.01$ , \*\*\* $P = 0.001$ ; NS, not significant.

**Table 3.** Life forms, photosynthetic pathways and pollination syndromes of 39 species comprising the known bromeliad flora of Cerro Jefe, central Panama

Species	Life form	$\delta^{13}\text{C}$ value (‰)	Photosynthetic pathway	Pollination syndrome	Level of endemism
<i>Aechmea allenii</i> L.B. Smith	III	-15.6	CAM	Bird	Panama
<i>Aechmea dactylina</i> Baker	III	-13.6	CAM	Bird <sup>a</sup>	
<i>Catopsis micrantha</i> L.B. Smith	IV	-26.2	C <sub>3</sub>	Insect	Panama
<i>Guzmania armeniaca</i> H.E. Luther	IV	-	C <sub>3</sub> (?)	Bird	Cerro Jefe
<i>Guzmania calamifolia</i> André ex Mez var. <i>calamifolia</i>	I	-23.7	C <sub>3</sub>	Bird <sup>a</sup>	
<i>Guzmania circinnata</i> Rauh	IV	-27.3	C <sub>3</sub>	Bird	Panama
<i>Guzmania coriostachya</i> (Grisebach) Mez	IV	-29.2	C <sub>3</sub>	Bird	
<i>Guzmania desautelsii</i> L.B. Smith & R.W. Read	IV	-31.5	C <sub>3</sub>	Bird	
<i>Guzmania filiorum</i> L.B. Smith	IV	-31.3	C <sub>3</sub>	Bird	Panama
<i>Guzmania flagellata</i> S. Pierce & J.R. Grant	IV	-	C <sub>3</sub> (?)	Bird	Cerro Jefe
<i>Guzmania glomerata</i> Mez & Wercklé	IV	-26.5	C <sub>3</sub>	Bird	
<i>Guzmania lingulata</i> (Linnaeus) Mez var. <i>minor</i> (Mez) L.B. Smith & Pittendrigh	IV	-27.0	C <sub>3</sub>	Bird	
<i>Guzmania loraxiana</i> J.R. Grant	IV	-	C <sub>3</sub> (?)	Insect	Panama
<i>Guzmania macropoda</i> L.B. Smith	IV	-27.5	C <sub>3</sub>	Bird	Cerro Jefe
<i>Guzmania musaica</i> (Linden & André) Mez var. <i>concolor</i> L.B. Smith	IV	-28.0	C <sub>3</sub>	Bird <sup>a</sup>	
<i>Guzmania musaica</i> (Linden & André) Mez var. <i>discolor</i> H.E. Luther	IV	-	C <sub>3</sub> (?)	Bird <sup>b</sup>	
<i>Guzmania musaica</i> (Linden & André) Mez var. <i>musaica</i>	IV	-27.1	C <sub>3</sub>	Bird	
<i>Guzmania scherzeriana</i> Mez	IV	-	C <sub>3</sub> (?)	Bird	
<i>Guzmania sprucei</i> (André) L.B. Smith	IV	-29.1	C <sub>3</sub>	Bird	
<i>Guzmania subcorymbosa</i> L.B. Smith	IV	-27.4	C <sub>3</sub>	Insect <sup>b</sup>	
<i>Pitcairnia arcuata</i> (André) André	I	-26.1	C <sub>3</sub>	Bird	
<i>Pitcairnia valerii</i> Standley	I	-26.8	C <sub>3</sub>	Bird	
<i>Racinaea spiculosa</i> (Grisebach) M.A. Spencer & L.B. Smith var. <i>spiculosa</i>	IV	-28.5	C <sub>3</sub>	Insect	
<i>Ronnbergia explodens</i> L.B. Smith	I	-25.4	C <sub>3</sub>	Bird	
<i>Tillandsia anceps</i> Loddiges	IV	-	C <sub>3</sub> *	Insect	
<i>Tillandsia bulbosa</i> Hooker	V	-13.4	CAM	Bird	
<i>Tillandsia monadelpha</i> (E. Morren) Baker	IV	-25.2	C <sub>3</sub>	Insect	
<i>Vriesea monstrum</i> (Mez) L.B. Smith	IV	-25.9	C <sub>3</sub>	Bird	
<i>Werauhia</i> cf. <i>apiculata</i> (L.B. Smith) J.R. Grant	IV	-	C <sub>3</sub> (?)	Bat	
<i>Werauhia capitata</i> (Mez & Wercklé) J.R. Grant	IV	-27.0	C <sub>3</sub>	Bat	
<i>Werauhia greenbergii</i> (Utley) J.R. Grant	IV	-	C <sub>3</sub> *	Bat	
<i>Werauhia hygrometrica</i> (André) J.R. Grant	IV	-28.2	C <sub>3</sub>	Bat	
<i>Werauhia jeni</i> S. Pierce	IV	-29.1	C <sub>3</sub>	Bat	Panama
<i>Werauhia kupperiana</i> (Suessenguth) J.R. Grant	IV	-27.2	C <sub>3</sub>	Bat	
<i>Werauhia lutheri</i> S. Pierce & J.E. Aranda	IV	-27.2	C <sub>3</sub>	Bat	Panama
<i>Werauhia millennia</i> J.R. Grant	IV	-28.1	C <sub>3</sub>	Bat	Panama
<i>Werauhia panamaensis</i> (E. Gross & Rauh) J.R. Grant	IV	-27.6	C <sub>3</sub>	Bat	Panama
<i>Werauhia</i> sp. nov. 1	IV	-24.9	C <sub>3</sub>	Bat (?)	Cerro Jefe
<i>Werauhia</i> sp. nov. 2	IV	-27.5	C <sub>3</sub>	Bat	Cerro Jefe
<i>Werauhia</i> aff. <i>viridiflora</i> (Regel) J.R. Grant	IV	-	C <sub>3</sub> (?)	Bat	
<i>Werauhia vittata</i> (Mez & Wercklé) J.R. Grant	IV	-27.0	C <sub>3</sub>	Bat	

Life forms or ecophysiological types follow Benzing (2000).  $\delta^{13}\text{C}$  values less negative than -19‰ denote CAM (\*determination of photosynthetic pathway based on unpublished  $\delta^{13}\text{C}$  data of Crayn, Winter & Smith).

Pollination syndromes: bird = brightly coloured (usually red) bracts, odourless flowers with white or yellow corolla; bat = large hooded pale crepuscular flowers with musky/fishy scent; insect = large numbers of small, pale flowers and bracts.

<sup>a</sup>Visits by potential pollinators observed by Pierce & Gottsberger (2001a).

<sup>b</sup>Visits observed by main author. ? denotes pathways/syndromes typical of the life form and genus.

bromeliads. Similarly, the suggested exploitation of elevated atmospheric CO<sub>2</sub> concentrations at night by CAM in wet montane habitats (Carter & Martin 1994) is unlikely to be of principal importance, with carbon dioxide concentrations only 6–20  $\mu\text{mol mol}^{-1}$  higher at night in the present study (data not shown).

A long-term comparative study of leaf life-span carbon gain would allow more precise quantification of the relative performance of CAM and C<sub>3</sub> photosynthetic pathways over greater time scales. However, in the present study, CAM was consistently more effective for carbon acquisition under a range of environmental conditions. Despite this, C<sub>3</sub>

bromeliads dominate the flora at Cerro Jefe, with only 8% of species using obligate CAM (Table 3). Eleven described  $C_3$  species at Cerro Jefe are endemic, most of these belonging to the two largest genera present, *Guzmania* (15 spp.) and *Werauhia* (13 spp.); at least three of which have only ever been found on this single hill top (*Guzmania armeniacana*, *Guzmania flagellata*, and *Guzmania macropoda*, and possibly also *Werauhia jenii*; Luther 1971, 1993; Pierce 2001; Pierce & Grant 2002; Table 3). Remaining  $C_3$  taxa include ecophysiologicaly more primitive life forms with broad ranges (e.g. Type 1 *Pitcairnia arcuata*; Costa Rica-Peru). This diversity and endemism suggests that the  $C_3$  flora has been present for sufficient time for extensive speciation to occur (partly in response to competition for pollinators, as evidenced by the range of pollination syndromes present; Table 3). Epiphytic *Aechmea* species at Cerro Jefe appear to be more recent arrivals; molecular phylogenetics indicates that *Aechmea* species are extant in relation to sub-family Tillandsioideae (Terry, Brown & Olmstead 1997; Horres, Kahl & Weising 2000; Crayn, Winter & Smith, unpubl. data), which dominates the bromeliad flora at Cerro Jefe (Table 3). *Aechmea allenii* is endemic and the geographic range of *A. dactylina* also centres on Panama (Smith & Downs 1979). Both possess advanced life forms (Benzing 2000) including the most advanced breeding system in the family (Smith & Downs 1974) and a 'more recently acquired' CAM physiology typical of younger taxa developing in arid habitats (Medina 1974). Thus, the scarcity of CAM species in the wet cloud forest appears to be the result of a relatively recent movement of CAM taxa from drier lowland forests into a habitat that is already dominated by  $C_3$  life forms, with the plasticity inherent to CAM pre-adapting xeromorphic species to wetter habitats. This may have profound implications for the future composition and diversity of montane tropical forests, in which species with highly flexible crassulacean acid metabolism may be favoured.

To conclude – CAM is a relatively efficient mode of growth underpinning the competitive abilities of *Aechmea* species in an extremely wet cloud forest in Panama. CAM species possess a number of ecophysiological advantages over  $C_3$  counterparts in wet cloud forest habitats, and a scarcity of CAM taxa suggests a recent radiation of this photosynthetic pathway into cloud forests.

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