DOI: 10.1007/s11099-012-0077-8 PHOTOSYNTHETICA 51 (1): 55-62, 2013

Juvenile tank-bromeliads lacking tanks: do they engage in CAM photosynthesis?

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Abstract

In the epiphytic tillandsioids, *Guzmania monostachia*, *Werauhia sanguinolenta*, and *Guzmania lingulata* (Bromeliaceae), juvenile plants exhibit an atmospheric habit, whereas in adult plants the leaf bases overlap and form water-holding tanks. CO_2 gas-exchange measurements of the whole, intact plants and $\delta^{13}C$ values of mature leaves demonstrated that C_3 photosynthesis was the principal pathway of CO_2 assimilation in juveniles and adults of all three species. Nonetheless, irrespective of plant size, all three species were able to display features of facultative CAM when exposed to drought stress. The capacity for CAM was the greatest in *G. monostachia*, allowing drought-stressed juvenile and adult plants to exhibit net CO_2 uptake at night. CAM expression was markedly lower in *W. sanguinolenta*, and minimal in *G. lingulata*. In both species, low-level CAM merely sufficed to reduce nocturnal respiratory net loss of CO_2. $\delta^{13}C$ values were generally less negative in juveniles than in adult plants, probably indicating increased diffusional limitation of CO_2 uptake in juveniles.

Additional key words: bromeliads; CO_2 exchange; carbon isotope discrimination; crassulacean acid metabolism; drought stress; *Guzmania*; heteroblasty; photosynthesis; *Werauhia*.

Introduction

CAM photosynthesis is a water-conserving mode of CO_2 assimilation present in more than 5% of species of vascular land plants adapted to warm and periodically dry habitats (Winter and Smith 1996, Winter *et al.* 2005, Silvera *et al.* 2005). In CAM, atmospheric CO_2 is taken up at night, at low transpirational water cost, and stored as malic acid within the vacuoles of the chloroplast-containing cells. During the day, malic acid is decarboxylated and the CO_2 thus liberated enters the Calvin cycle *via* Rubisco (Holtum *et al.* 2005, West-Eberhard *et al.* 2011). In the large neotropical family Bromeliaceae, nearly 50% of species exhibit CAM (Crayn *et al.* 2004). In the subgroup Tillandsioideae, the CAM pathway, along with a range of distinct anatomical and morphological xerophytic features, has allowed certain, highly specialized bromeliads to evolve the atmospheric life form and to occupy some of the most extreme arid epiphytic habitats in the tropics and subtropics. In these CAM-performing "air plants" or "atmospherics" (Mez 1904, Tomlinson 1970, Benzing 2000), trichomes on the surfaces of succulent leaves absorb water and nutrients, leaving the roots to function merely as holdfasts (Pierce *et al.* 2001, Givnish *et al.* 2011).

Not all Tillandsioideae are CAM plants. For approximately 70% of them, regular C_3 photosynthesis is the major pathway of CO_2 acquisition (Crayn *et al*. 2004). Of these, many are heteroblastic: as adults, they store water in tanks, structures formed by highly overlapping leaf bases, extending water availability during periods of drought, while as juveniles, they are atmospherics, because tanks are not yet developed. The following questions arise: (*1*) does the CAM pathway contribute to the survival of these species when they are young and more susceptible to drought stress? (*2*) Do these heteroblastic species shift from CAM to C_3 during their development?

Received 1 May 2012, *accepted* 10 October 2012.
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Abbreviations: CAM – crassulacean acid metabolism; DM – dry mass; FM – fresh mass; PFD – photon flux density (400–700 nm); RH – relative humidity; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; STRI – Smithsonian Tropical Research Institute; VPD – leaf-air water vapour pressure difference.
Acknowledgements: J.D.B. was recipient of a short-term fellowship from the Smithsonian Tropical Research Institute and received travel support from the Facultad de Ciencias, Universidad de los Andes. Dayana Agudo performed the $\delta^{13}C$ analyses, Jorge Ceballos helped with microscopy, and Oris Acevedo provided logistical support on Barro Colorado Nature Monument.

In their excellent review on heteroblasty, Zotz *et al.* (2011) do not address these questions. Photosynthetic studies on heteroblastic Tillandsioideae have mainly focused on adult plants (Medina *et al.* 1977, Smith *et al.* 1985, 1986, Griffiths *et al.* 1986, Maxwell *et al.* 1992, Griffiths and Maxwell 1999, Maxwell 2002). Few measurements exist for juvenile plants and they suggest that C_3 photosynthesis is the major photosynthetic pathway (Adams and Martin 1986a, Schmidt and Zotz 2001, Pierce *et al.* 2002, Zotz *et al.* 2004).

Here we further investigated the photosynthetic physiology of heteroblastic tillandsioids and presented new data on net CO_2 exchange and other photosynthetic characteristics such as $\delta^{13}C$ value and titratable acidity content in juvenile and adult plants of *G. lingulata*, *G. monostachia*, and *W. sanguinolenta*. By comparing the responses of nonstressed and drought-stressed plants, we explored the possibility of drought-induced up-regulation of CAM (facultative CAM; Winter and Holtum 2007, 2011; Winter *et al.* 2008) in these heteroblastic species, a feature that, amongst Bromeliaceae, has only been demonstrated for adult *G. monostachia* thus far (Medina *et al.* 1977).

Materials and methods

Plant material and collection site: *Guzmania lingulata* (L.) Mez, *Guzmania monostachia* (L.) Rusby ex Mez, and *Werauhia sanguinolenta* (Cogn. & Marchal) J.R. Grant are tank-forming epiphytes in lowland tropical forests of South and Central America. *G. monostachia* and *W. sanguinolenta* grow in exposed habitats, *G. lingulata* grows in shaded habitats (Griffiths and Smith 1983, Griffiths *et al.* 1986, Smith *et al.* 1986, Griffiths and Maxwell 1999). Plants were collected at the Barro Colorado Nature Monument, Panama, in December 2011, at the end of the wet season. *G. monostachia* and *W. sanguinolenta* were taken from sun-exposed *Annona glabra* trees along the shoreline of Gigante peninsula at heights between 3 and 5 m. *G. lingulata* was collected from various tree species at about 3 m height in the subcanopy of Barro Colorado Island. Plants were either immediately dried at 70ºC and analyzed for $^{13}C/^{12}C$ ratio, or transferred to a screen-house at the Tupper Center of the Smithsonian Tropical Research Institute, where they were well watered and maintained at approximately 5% of full sunlight for up to about two weeks prior to experimental treatments.

Anatomy: Hand-made cross-sections of leaves from 27 to 37 plants per species covered the entire ontogenetic spectrum. Sections were taken from the middle of the longest leaf of each plant. The length of that leaf was used as an indicator of plant developmental stage. Sections were photographed under a light microscope, and *ImageJ* software (Abràmoff *et al.* 2004) was used to measure the thicknesses of hydrenchyma and chlorenchyma tissues. The boundary between epidermis and hydrenchyma was not always clearly discernible, and data shown in the Results section for hydrenchyma thickness include the epidermis.

Net CO_2 exchange: For measurements of juveniles, 63 to 98 entire plants were sealed into a translucent *Perspex* gas-exchange cuvette (11 cm × 11 cm × 10 cm). For measurements of adults, one entire plant was sealed into a cuvette (20 cm × 20 cm × 15 cm). Each experiment was repeated once or twice using different plants. Plants were well irrigated before the measurement. The gas-exchange cuvettes were placed inside a controlled-environment chamber (*GC8; EGC*, Chagrin Falls, OH, USA). Plants were exposed to 12 h light (28°C)/12 h dark (22°C) cycles. PFD was 300 μmol m^{-2} s^{-1} (*120 Watt Extreme Flower LED*; *Advanced LED Lights*, Betonville, AR, USA). Net CO_2 exchange was determined using a through-flow system with components of *Walz GmbH* (Effeltrich, Germany) (Holtum and Winter 2003). Air flow through the cuvette was 1.26 dm^3 min^{-1}. The air entering the cuvette had a dew point of 20°C and a CO_2 concentration of 400 ppm (CO_2 mixing system *GMA-3/10; Walz*). Before entering the infrared CO_2 analyzer (*LI-6262, Li-Cor*, Lincoln, NE, USA), the air was dehumidified in a cold-trap at 2°C (*KF-18/2; Walz*).

Titratable acidity: Plants were placed in a *GC15* controlled-environment chamber (*EGC*) and exposed to 12 h light (28°C)/12 h dark (22°C) cycles. PFD was 300 μmol m^{-2} s^{-1} (*300 Watt GrowPanel Pro LED; Sunshine Systems*, Wheeling, IL, USA). RH was 60 and 90% in the light and dark, respectively. For each species, 20 juvenile (5 for each observation) and 3–5 adult plants were used. Whole plants (juveniles) and sections of healthy-looking, mature leaves (adult plants) were harvested at the end of the light period and at the end of the dark period (*1*) following 2 d in the chamber under well-irrigated conditions, and (*2*) after 4 additional days without irrigation. Samples were stored in liquid nitrogen before analyses. Extracts were prepared by boiling samples in 50% ethanol. Extracts were titrated to pH 6.5 with 10 mM KOH using a pH-meter.

$\delta^{13}C$: Ratios of ^{13}C to ^{12}C were measured at the Stable Isotope Laboratory of the Smithsonian Tropical Research Institute. Leaf dry matter of about 2 mg per sample was combusted in an elemental analyzer (*CE Instruments*, Milan, Italy) and swept by helium carrier gas *via* a constant flow interface into a mass spectrometer (*Delta V; Thermo Fischer Scientific*, Waltham, MA, USA). $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ values, and were calculated relative to the Pee Dee belemnite standard using the

relationship $\delta^{13}C$ (‰) = $[(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1] \times 1{,}000$.

Statistical analyses were performed using the "*R*" statistics package (R Development Core Team 2011). Dry mass data were log-transformed before analysis. Linear regressions were used to analyze relationships among variables. *Student*'s *t*-tests were used to evaluate whether day-night differences in acid content were significant.

Results

The size of the studied plants ranged from 0.3 mg to 3.7 g (*G. monostachia*), from 6 mg to 33 g (*W. sanguinolenta*), and from 0.5 mg to 15 g (*G. lingulata*). Leaf thickness did not change significantly during plant development. The thickness of the longest leaf per plant throughout all developmental stages was 0.44 ± 0.01 mm (mean ± SE) for *W. sanguinolenta*, 0.28 ± 0.01 mm for *G. monostachia*, and 0.21 ± 0.01 mm for *G. lingulata*. In all species the leaf thickness was not significantly correlated with leaf length. The thickness of the upper plus lower epidermis (mean ± SE) was 28 ± 0.3 µm (n = 5) in *W. sanguinolenta*, 27 ± 0.6 µm (n = 5) in *G. monostachia*, and 27 ± 0.3 µm (n = 5) in *G. lingulata*.The ratio of chlorenchyma/ hydrenchyma markedly increased in all three species (Fig. 1). In juveniles, hydrenchyma cells contributed 60 to 80% of leaf volume. In adults the proportion of hydrenchyma cells decreased to about 50%.

$\delta^{13}C$ values of all specimens were more negative than –25‰ (Fig. 2). In *W. sanguinolenta*, $\delta^{13}C$ ranged from –25.2 to –30.8‰, and in *G. monostachia* from –26.1 to –29.6‰. $\delta^{13}C$ values of *G. lingulata* were by 4‰ more negative than those of *W. sanguinolenta* and *G. monostachia*. In all three species, juveniles had less negative $\delta^{13}C$ values than adults. $\delta^{13}C$ values decreased by 2.5‰ during plant development.

Soon after the enclosure of plants into the gas-exchange cuvette, overall net CO_2 fluxes rapidly declined during the course of 4–7 consecutive day/night cycles. In all cases, net CO_2 exchange was initially, when plants were still well hydrated, characterized by relatively high rates of CO_2 uptake in the light and by net CO_2 loss at night, the rate of net CO_2 loss being more or less constant for essentially the entire dark period (*W. sanguinolenta* and *G. lingulata*; Figs. 3, 4), or for at least the second half of the dark period (*G. monostachia*; Fig. 5). As water stress developed, juveniles and adults of *G. monostachia*

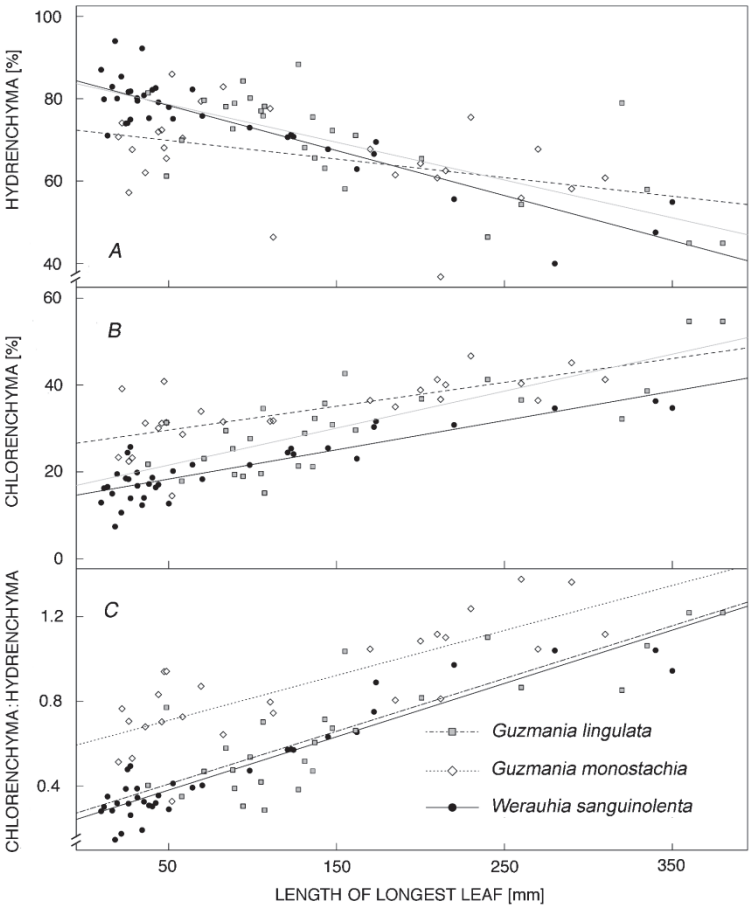

Fig. 1. (*A*) Relationship between length of longest leaf per plant and percentage contribution to cross-sectional area of hydrenchyma (+ epidermis) in *Werauhia sanguinolenta* (r^2 = 0.77, P<0.001, n = 36), *Guzmania monostachia* (r^2 = 0.13, P<0.05, n = 25), and *Guzmania lingulata* (r^2 = 0.45, P<0.001, n = 31). (*B*) Relationship between length of longest leaf per plant and percentage contribution to cross-sectional area of chlorenchyma in *W. sanguinolenta* (r^2 = 0.74, P<0.001, n = 36), *G. monostachia* (r^2 = 0.48, P<0.001, n = 25), and *G. lingulata* (r^2 = 0.54, P<0.001, n = 31). (*C*) Relationship between length of longest leaf per plant and ratio of chlorenchyma to hydrenchyma (+ epidermis) in *W. sanguinolenta* (r^2 = 0.87, P<0.001, n = 36), *G. monostachia* (r^2 = 0.62, P<0.001, n = 25), and *G. lingulata* (r^2 = 0.61, P<0.001, n = 31).

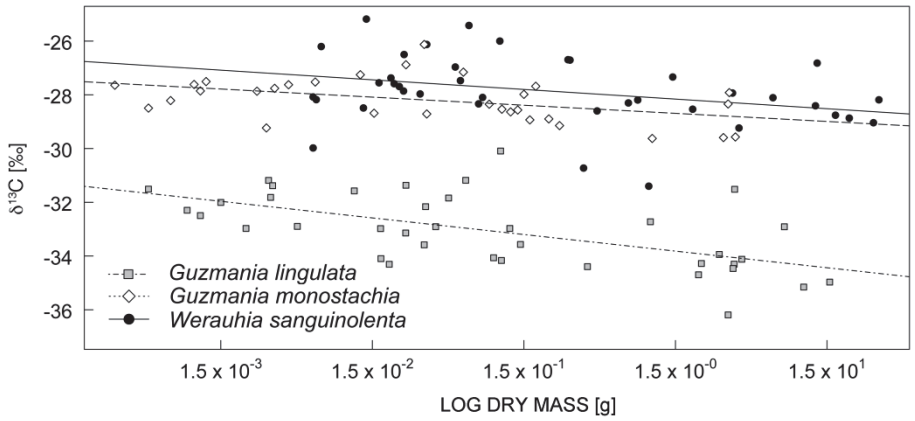

Fig. 2. Relationship between plant size (total dry mass) and $\delta^{13}C$. For stable isotope analysis, dry mass from a representative, mature, healthy leaf of each plant was used. The correlation between log transformed total plant dry mass and $\delta^{13}C$ was significant and negative for *Werauhia sanguinolenta* (r^2 = 0.08, P<0.05, n = 38), *Guzmania monostachia* (r^2 = 0.16, P<0.05, n = 30), and *Guzmania lingulata* (r^2 = 0.33, P<0.001, n = 37).

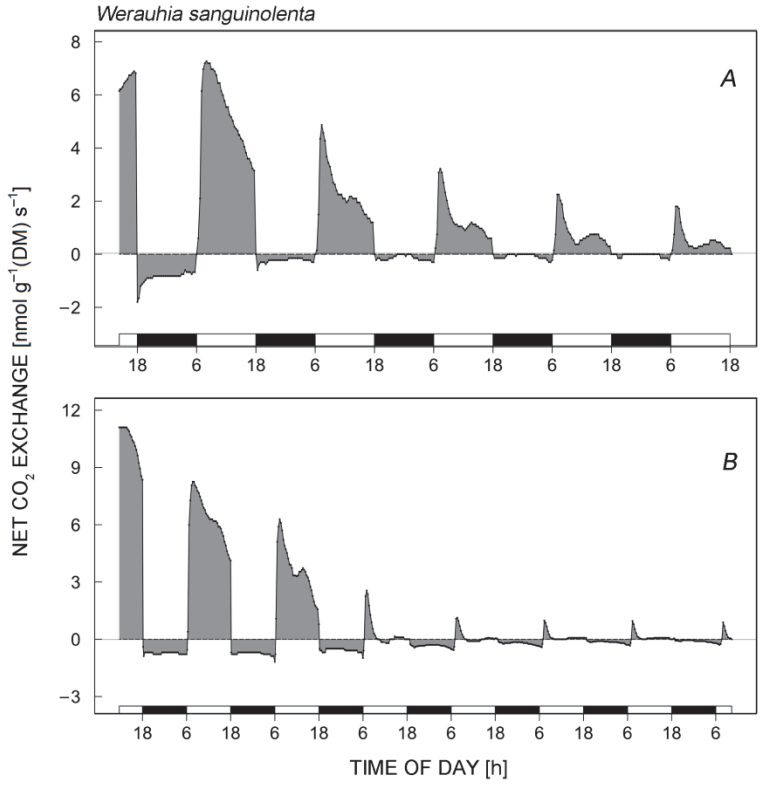

Fig. 3. Effect of drought stress on net CO_2 exchange (*A*) of 61 juvenile plants (total dry mass 624 mg) and (*B*) of one adult plant (dry mass 2.16 g) of *Werauhia sanguinolenta*. Plants were well irrigated when measurements commenced. Plants did not receive additional water during experiments. Open bars represent light periods, closed bars represent dark periods.

exhibited transient midday-reductions in CO_2 uptake during the light periods and net CO_2 uptake at night. Transient midday-reductions of net CO_2 exchange were also observed in stressed juveniles and adults of *W. sanguinolenta* (Fig. 3). In juveniles, this was accompanied by transient reductions in net CO_2 loss during the dark period, whereas in adults net CO_2 loss tended to be lowest during the first part of the dark period, gradually increasing during the second part of the dark period. Patterns of net CO_2 exchange similar to those in *W. sanguinolenta* were observed in juveniles and adults of *G. lingulata* (Fig. 4).

Significant nocturnal increases in a titratable acid content were measured in juvenile and adult plants of *G. monostachia* both at the onset of stress treatments and after 4 d of drought stress (Table 1). Significant nocturnal increases in titratable acidity were also measured in drought-stressed adult plants of *W. sanguinolenta*. Neither nonstressed adult plants nor stressed or nonstressed juvenile plants of *W. sanguinolenta* showed significant day/night changes in titratable acidity. Significant day/night changes in titratable acidity were absent in all plants and treatments of *G. lingulata* (Table 1).

Discussion

Consistent with the classic study on *Tillandsia deppeana* (Adams and Martin 1986a,b), C_3 photosynthesis is the principal pathway of photosynthesis in both tank-possessing adult and in tank-less atmospheric juvenile

plants of the heteroblastic bromeloid species *W. sanguinolenta*, *G. lingulata*, and *G. monostachia*. Because of the rapid development of plant water deficit after the enclosure of plants into the gas-exchange cuvette, it was difficult to capture the initial "steady state" pattern of 24-h net CO_2 exchange in order to fully characterize the CO_2-exchange behaviour of well hydrated plants (Figs. 3–5). Nonetheless, relatively high and more or less constant rates of nocturnal net CO_2 loss in the course of the first night indicated that the CAM cycle did not operate in the well-hydrated juveniles and adults of all species. Increased rates of respiration immediately after onset of the dark period were a temperature effect, because upon the abrupt change of the growth chamber temperature from 28°C to 22°C during the light/dark transition, the temperature inside the gas-exchange cuvette assumed the new lower level with a delay.

Nevertheless, in all three species, CAM was not entirely absent. Facultative CAM has been demonstrated in adult *G. monostachia* (Medina *et al.* 1977). However, in the only previous study that compared CO_2 exchange of young (size not specified) and mature plants of *G. monostachia* (Pierce *et al.* 2002), evidence for facultative CAM was not conclusive. Studies on *G. monostachia in situ* indicate that daily PFD may be at least as important as plant water status in determining CAM expression (Smith *et al.* 1985), and positive nocturnal CO_2 balances have not yet been documented for plants in the field (Lüttge *et al*. 1986a, Maxwell *et al*. 1994). Here, we clearly demonstrated the drought-stress-induced nocturnal net CO_2 uptake not only in adult, but also in juvenile *G. monostachia* (Fig. 5). In juveniles the shift from nocturnal CO_2 loss to nocturnal CO_2 gain was rapid and occurred within 24 h. In adult plants, whose tanks were filled with water prior to the stress treatment, the change was gradual, involving intermediate CAM expression stages in which CAM-typical midday depressions of CO_2 uptake in the light (Phase 3: Osmond 1978) were accompanied by midnight reductions in respiratory CO_2 loss owing to transiently increased rates of dark CO_2 fixation.

Indications of weakly expressed facultative CAM were also observed in juvenile and adult plants of *W. sanguinolenta* (Fig. 3). Even in *G. lingulata* (Fig. 4), a species previously considered strictly C_3 (Smith *et al.* 1985, Maxwell 2002), both juvenile and adult plants showed features of very weak CAM when drought-stressed (*i.e.* temporary reductions in daytime CO_2 fixation; transient reductions in net CO_2 loss at night and/or markedly reduced rates of net CO_2 uptake during the first part of the night; Holtum and Winter 1999). PFD and temperature were held constant during 12-h light periods. It is therefore safe to assume that midday-depressions in CO_2 uptake reflected leaf internal processes related to Phase 3 of CAM, and were not related to stomatal closure caused by external cues. Midday-depressions of CO_2-uptake, unrelated to CAM, can occur in C_3 performing plants on sunny days in the field, when stomatal

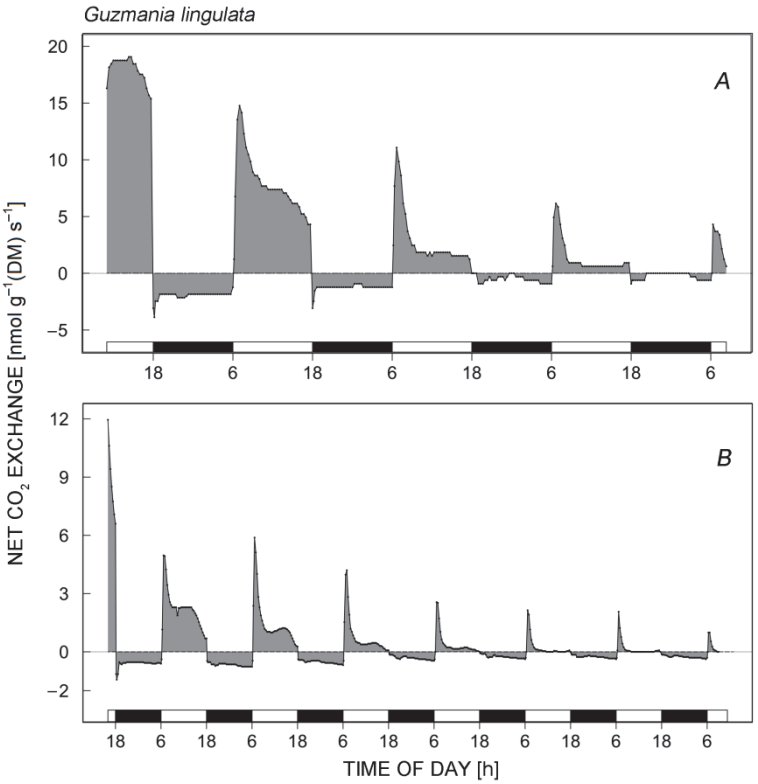

Fig. 4. Effect of drought stress on net CO_2 exchange (*A*) of 80 juvenile plants (total dry mass 1625 mg) and (*B*) of one adult plant (dry mass 2.22 g) of *Guzmania lingulata*. Plants were well irrigated when measurements commenced. Plants did not receive additional water during experiments. Open bars represent light periods, closed bars represent dark periods.

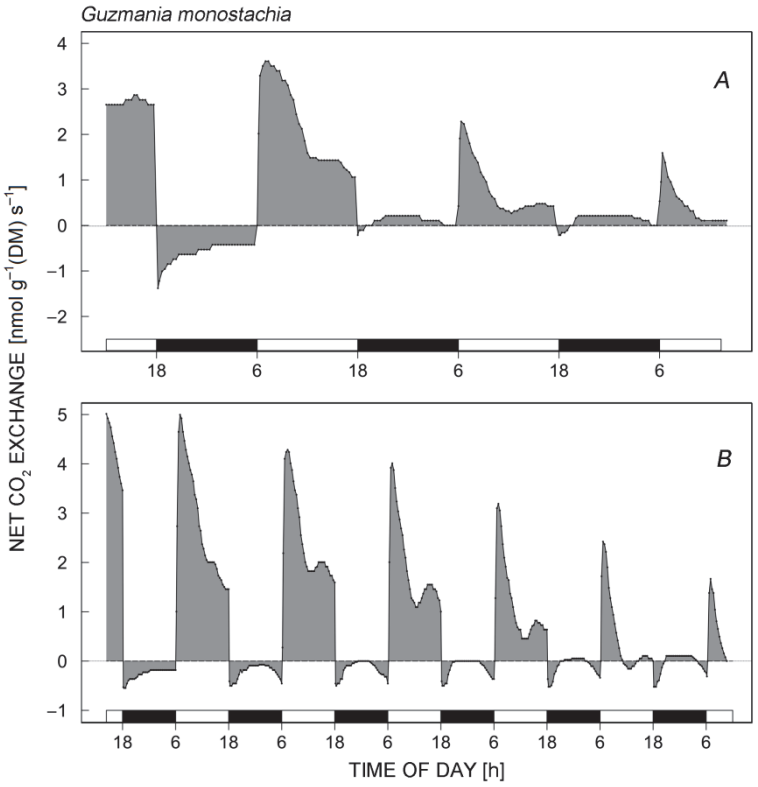

Fig. 5. Effect of drought stress on net CO_2 exchange (*A*) of 98 juvenile plants (total dry mass 686 mg) and (*B*) of one adult plant (dry mass 3.26 g) of *Guzmania monostachia*. Plants were well irrigated when measurements commenced. Plants did not receive additional water during experiments. Open bars represent light periods, closed bars represent dark periods.

Table 1. Titratable acidity in juvenile and adult plants of *Guzmania lingulata*, *Guzmania monostachia*, and *Werauhia sanguinolenta*, before, and 4 d after irrigation was withheld. Values are means ± SE (n = 5). L – end of light period; D – end of dark period.

Species	Stage	Drought stress	Titratable acidity [μmol(H^+) g^{-1}(FM)] L	D	D-L	*P* value
Guzmania lingulata	Juvenile	Before	2.3 ± 1.0	2.8 ± 1.2	0.5	0.7581
		After	0 ± 0	1.9 ± 1.9	1.9	0.3739
	Adult	Before	2.0 ± 0.3	2.9 ± 0.4	0.9	0.0931
		After	2.9 ± 0.2	2.6 ± 0.2	–0.3	0.1943
Guzmania monostachia	Juvenile	Before	5.0 ± 1.3	10.7 ± 1.0	5.7	0.0236
		After	4.2 ± 1.5	15.8 ± 1.6	11.6	0.0183
	Adult	Before	12.9 ± 1.0	27.1 ± 1.4	14.2	0.0009
		After	12.6 ± 2.2	39.0 ± 1.6	26.4	0.0007
Werauhia sanguinolenta	Juvenile	Before	1.2 ± 0.8	3.4 ± 0.5	2.2	0.1382
		After	0.5 ± 0.5	3.3 ± 1.1	2.8	0.1304
	Adult	Before	5.6 ± 1.4	9.3 ± 0.8	3.7	0.1512
		After	5.3 ± 0.6	9.8 ± 0.9	4.5	0.0127

conductance decreases in a response to high temperatures and high VPDs (Zotz *et al.* 1995, Lüttge *et al*. 1986b, Schmidt and Zotz 2001).

As expected, measurements of the titratable acidity demonstrated CAM in mature drought-stressed *G. monostachia* and *W. sanguinolenta*. However, not all H^+ data were fully consistent with CO_2 exchange. For example, in the irrigated adult *G. monostachia*, nocturnal acidification occurred despite the lack of gas-exchange-based evidence of CAM, and in the drought-stressed juveniles of *W. sanguinolenta*, nocturnal acidification was not observed although CO_2 gas-exchange clearly suggested the presence of weak CAM. There may be two possible causes for these apparent discrepancies. (*1*) Samples for H^+ determination were not taken from the same plants monitored in the gas-exchange system, and while CO_2 gas-exchange was recorded continuously over the course of several days, ΔH^+ refers to two time points only, *i.e*. before and 4 d after irrigation was withheld. (*2*) RH was relatively low (about 60%) during the light period inside the controlled-environment chamber to which plants had been transferred to be sampled for H^+ determination. Because of this and owing to the high air movement within the chamber, some stress may have developed, despite irrigation, during the first 2 d prior to the actual drought treatment.

$\delta^{13}C$ values for all species collected *in situ* were in the range typical of C_3 plants (Winter and Holtum 2002) (Fig. 2). $\delta^{13}C$ values of *G. lingulata* were more negative than those of *G. monostachia* and *W. sanguniolenta*. This is in accordance with the shaded, less stressful habitat occupied by *G. lingulata* (Smith *et al.* 1986), promoting the photosynthetic usage of atmospheric CO_2 with a somewhat more negative isotopic signature than in the exposed habitats of *G. monostachia* and *W. sanguinolenta* (Medina and Minchin 1980, Holtum and Winter 2005). In all species, $\delta^{13}C$ values became more negative as plants matured (Fig. 2; *see* also Zotz *et al*. 2004 for *W. sanguinolenta*). Less negative $\delta^{13}C$ values in the juvenile than in adult plants could, in principal, be caused (*1*) by increased contribution of the CAM cycle to overall carbon gain in juveniles and/or (*2*) by increased diffusional (stomatal or mesophyll) limitation of CO_2 uptake (Farquhar *et al.* 1989; Cernusak *et al.* 2007, 2008) in juveniles. The observation that size-related changes in $\delta^{13}C$ value were of similar magnitude in all three species despite significant differences in the capacity of CAM expression (*G. monostachia* > *W. sanguinolenta* > *G. lingulata*) suggests that the differences in $\delta^{13}C$ between juveniles and adults were primarily caused by changes in the diffusional component of isotope fractionation. The least negative $\delta^{13}C$ values (close to –25‰) were noted in *W. sangiunolenta* and not in *G. monostachia*, despite the greater demonstrated ability of *G. monostachia* to employ CAM. For nonstressed plants, a $\delta^{13}C$ of –25‰ could indicate that as much as 15% of total carbon gain is derived *via* nocturnal CO_2 fixation (Winter and Holtum 2002). Either field-grown *W. sanguinolenta* engaged in more, or *G. monostachia* engaged in less CAM than previously thought, or plants of both two species experienced different degrees of stress resulting in differences in the proportional stomatal limitation of CO_2 uptake; and/or both two species showed intrinsic differences in leaf mesophyll conductance.

In all three species, photosynthetically active chlorenchyma cells were embedded into an upper and lower hydrenchyma, which decreased in size relative to the chlorenchyma as plants matured (Fig. 1). The greater presence of hydrenchyma in juveniles was consistent with their atmospheric life form and was expected to slow dehydration. Water flow from hydrenchyma to chlorenchyma cells under conditions of drought stress has been reported for *Peperomia magnoliaefolia* (Schmidt and Kaiser 1987) and *Opuntia ficus-indica* (Goldstein *et al.* 1991). Juveniles of *G. monostachia* had a higher proportion of chlorenchyma tissue than juveniles of *G. lingulata* and *W. sanguinolenta* (Fig. 1). This may partly explain the greater capacity for CAM in *G. monostachia* as the operation of the CAM cycle is restricted to chloroplast-containing cells.

It is important to note that even the smallest juveniles used in the present study were not strictly young in the sense of being characterized by immature tissues. We did not know the exact age of these juveniles, but we estimated that they might be 6–12 months old. Immature developing leaf tissue would have largely precluded the operation of the CAM cycle (Winter *et al.* 2011).

Conclusion: C_3 photosynthesis is the principal pathway of CO_2 uptake in well hydrated atmospheric juveniles and tank-forming adults of the tillandsioid bromeliads *G. monostachia*, *W. sanguniolenta*, and *G. lingulata*. CO_2 gas-exchange measurements indicated that under conditions of drought stress, juvenile and adult plants of all three species could induce low-level CAM. The ability to do so was the greatest in *G. monostachia* and smallest in *G. lingulata*. Further research is needed to validate the presence of low-level facultative CAM in *G. lingulata*.

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