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Annual carbon balance and nitrogen-use efficiency in tropical C_3 and CAM epiphytes

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SUMMARY

Over the course of 12 months, diel (24 h) measurements of gas exchange were performed on leaves of three epiphytic species growing in the crown of a kapok tree on Barro Colorado Island, Panama: a drought-deciduous orchid with the C_3 pathway (*Catasetum viridiflavum* Hook.), an evergreen C_3 fern (*Polypodium crassifolium* L.), and an evergreen epiphyte with an intermediate C_3-CAM pathway of photosynthesis (*Clusia uvitana* Pitt.). The gas exchange characteristics of all three species were strongly affected during the four-month dry season. Compared with the rainy season, mean daily carbon gain of *Clusia uvitana* was reduced by almost 40 %, paralleled by a strong decrease in daytime CO_2 uptake and an increase in CO_2 uptake at night. The orchid, growing new leaves in the second half of the dry season, showed markedly decreased stomatal conductances and greatly reduced carbon gain. In the fern, daily carbon balance became negative during the dry season and chronic photoinhibition was indicated by reduced F_V/F_M ratios and a decreased photon-use efficiency of photosynthetic O_2 evolution. Annual carbon gain was similar for the three species (about 1000 g CO_2 m^{-2} yr^{-1}) as was long-term nitrogen-use efficiency (annual carbon gain/mean leaf nitrogen content, about 1·1 g CO_2 mg N^{-1} yr^{-1}). In the C_3-CAM epiphyte, the long-term water use efficiency of net CO_2 uptake was more than twice as high as in the two C_3 epiphytes.

Key words: Crassulacean acid metabolism, carbon balance, epiphytes, nitrogen-use efficiency, tropical rain forest.

INTRODUCTION

Vascular epiphytes are particularly abundant in the wet tropics (Gentry & Dodson, 1987). Water availability is probably the most important environmental factor limiting growth and survival of these plants (Lüttge, 1985, 1989; Benzing, 1990). This is illustrated by the high percentage of epiphytic species which utilize the water-conserving CAM-pathway of photosynthesis, especially those in drier and more exposed habitats (Medina *et al.*, 1977; Griffiths & Smith, 1983; Winter *et al.*, 1983, 1985; Winter, 1985; Griffiths, 1989). Other adaptations like xeromorphic leaves, poikilohydry, deciduousness (Benzing *et al.*, 1982) and general reduction of the shoot (Benzing *et al.*, 1983) permit species with the C_3-photosynthetic pathway to persist in dry, epiphytic sites as well. Comparative field studies on the CO_2 gas exchange and water relations of co-occurring tropical C_3- and CAM-epiphytes have been reported several times (Smith *et al.*, 1985; Griffiths *et al.*, 1986; Goh & Kluge, 1989; Kluge, Avadhani & Goh, 1989), but these previous investigations consisted of measurements taken over only a few days or weeks. The adaptive significance of differences in leaf phenology (evergreen *vs.* deciduous) and photosynthetic pathway (C_3 *vs.* CAM) can only be understood when seasonal and ontogenetic changes in morphological and physiological characteristics are considered. Here, we present the results of a long-term comparative study of the photosynthetic, water and mineral-nutrient relationships of three tropical epiphyte species differing in leaf longevity and mode of carbon assimilation. The objective was to evaluate how phenological and physiological differences would translate into primary productivity and long-term water- and nitrogen-use efficiency at the leaf level.

MATERIALS AND METHODS

Habitat and plant material

The investigations were conducted on Barro Colorado Island (9° 10′ N, 79° 51′ W), Republic of Panama. The forest of this biological reserve is classified as a tropical moist forest (Holdridge *et al.*, 1971). Mean annual rainfall is approximately 2600 mm with a pronounced dry season from late December to late April. Detailed descriptions of vegetation, climate and ecology were reported by Croat (1978) and Leigh, Rand & Windsor (1982). *In situ* field measurements were carried out on three

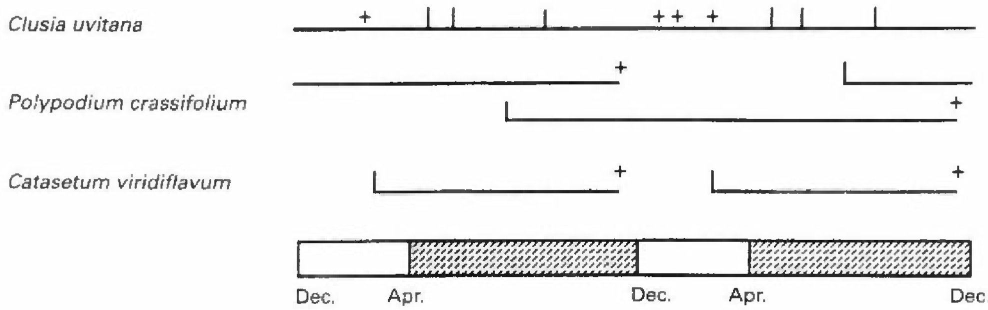

Figure 1. Leaf phenology of three epiphytic species on Barro Colorado Island: vertical lines, production of new leaves; + senescence and abscission; open bars (Dec.–Apr.), dry season; hatched bars (Apr.–Dec.), wet season.

Table 1. *Specific leaf weight (SLW), number of stomata and mineral element content of leaves and fronds, respectively, of three epiphyte species. Values are means* ± SD (*n*). *Samples were taken throughout the year.*

Parameter	*Catasetum viridiflavum*	*Polypodium crassifolium*	*Clusia uvitana*
SLW (g d. wt m^{-2})	41·4 ± 5·2 (5)	113·2 ± 15·1 (7)	173 ± 39·9 (13)
Stomata (mm^{-2})	45 ± 18 (3)	45 ± 12 (3)	134 ± 17 (7)
N (mg m^{-2})	1050 ± 143 (5)	840 ± 270 (7)	1038 ± 174 (13)
P (mg m^{-2})	90 ± 10 (5)	158 ± 108 (7)	132 ± 28 (13)
K (mg m^{-2})	475 ± 118 (5)	3104 ± 354 (7)	3190 ± 879 (13)
S (mg m^{-2})	61 ± 4 (5)	95 ± 24 (7)	214 ± 85 (13)
Mg (mg m^{-2})	66 ± 12 (5)	308 ± 113 (7)	789 ± 235 (13)
Ca (mg m^{-2})	329 ± 181 (5)	808 ± 468 (7)	3944 ± 943 (13)
Na (mg m^{-2})	18 ± 7 (5)	102 ± 55 (7)	169 ± 103 (13)

epiphytes growing about 34 m above-ground in a crotch of an emergent *Ceiba pentandra* (L.) Gaertn., Fruct. & Sem.

Polypodium crassifolium L. (Polypodiaceae) is probably the most abundant epiphytic fern species on Barro Colorado Island (Croat, 1978). This evergreen C_3 fern is widespread throughout the neotropics in moist forests at 0–1600 m elevation (Lellinger, 1989). Plants flush a new set of fronds in the middle of the rainy season (Fig. 1). These fronds produce sporangia during the following dry period and senesce at the end of the subsequent wet season, thus lasting 14–15 months. *Catasetum viridiflavum* Hook. is a drought-deciduous orchid endemic to Panama (Croat, 1978). Individual plants consist of one pseudobulb carrying leaves and several old leafless bulbs. The leaves show the C_3 photosynthetic pathway (Zimmerman & Ehleringer, 1990). New leaves are produced in the second half of the dry season. Growth occurs until the end of the wet season. Then, leaves are lost and only the pseudobulbs persist during the first part of the dry season (Zimmerman, 1990) (Fig. 1). *Clusia uvitana* Pitt. (Clusiaceae) is a common hemiepiphytic tree species on Barro Colorado Island. Its life cycle includes an initial epiphytic and a subsequent hemiepiphytic phase (roots extending downwards along the trunk of the host tree and into the ground). An epiphyte about 0·6 m tall was studied. *Clusia uvitana* is known to exhibit variable CAM activity in response to environmental conditions (Winter *et al.*, 1992; Zotz & Winter, 1993*a*). New leaves are produced during the wet season, with a peak after the first consecutive rainstorms in April/May (Fig. 1). Leaf longevity may vary from a few months to more than 2 yr.

Gas exchange

Leaf gas exchange was studied from January 1991 to January 1992 with a CO_2/H_2O-Porometer system (CQP 130, Walz, Effeltrich, Germany). The porometer allows the measurement of CO_2 and water vapour exchange, leaf and air temperature, relative air humidity and photosynthetic photon flux density (PPFD). Measuring instruments were kept in two aluminium boxes in the crown of the *Ceiba* tree, no more than 7 m away from the study leaves to minimize the length of the pneumatic system. The gas exchange equipment was used in a continuous open flow mode. The flow rate of air was 300–600 ml min^{-1}. External temperature was automatically tracked inside the leaf cuvette. CO_2 and water vapour exchange were measured with an infrared gas analyzer operating in the differential mode (Binos 100). A CO_2 analyzer in the absolute mode was used to determine ambient CO_2 partial pressure. Zero checks (ambient gas streaming through both the measuring and the reference cells of the differential analyzer) were performed at 1 h (during daytime) and 3–6 h (during nighttime) intervals. The air was passed through a cold trap set at 2 °C before entering the CO_2 analyzers. A second cold trap (KF 18/2,

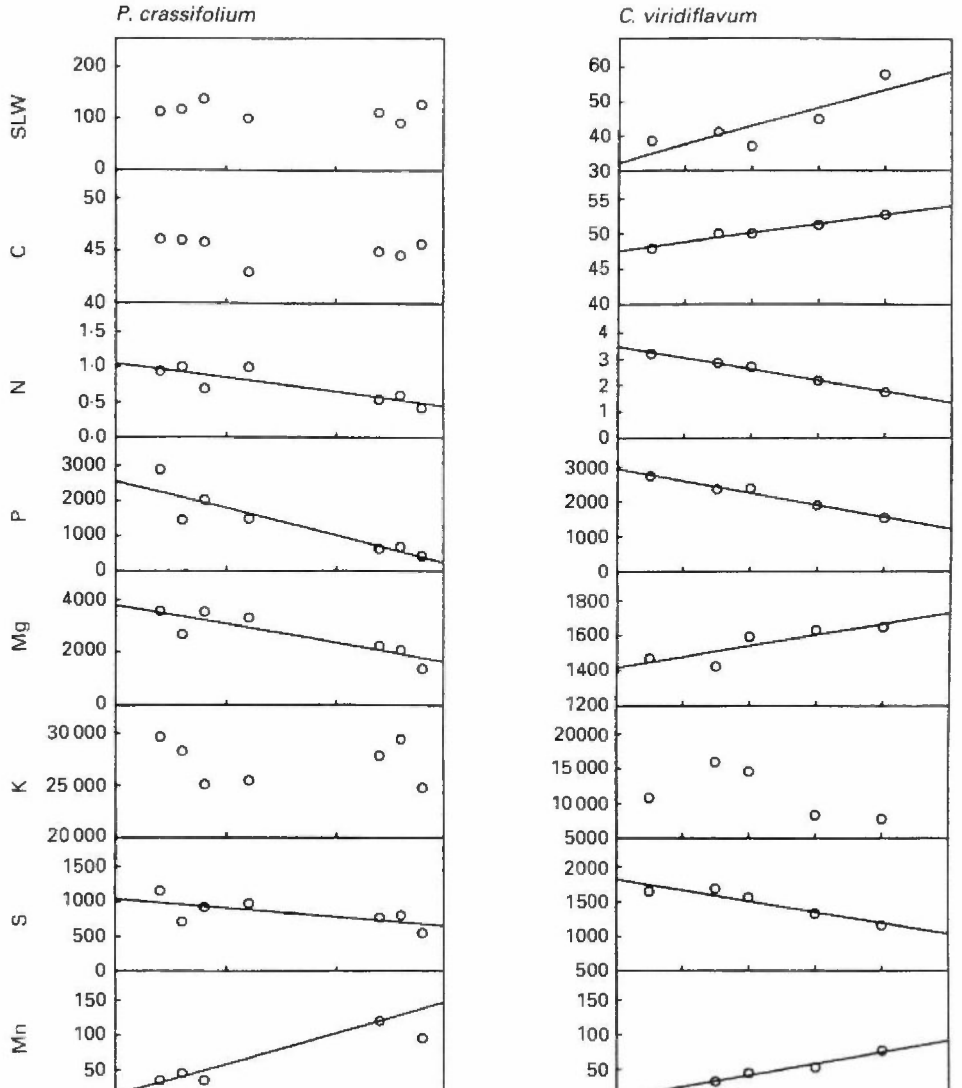

Figure 2. Changes in specific leaf weight (SLW) and mineral element content in leaves of *Catasetum viridiflavum* and fronds of *Polypodium crassifolium*. Data points are means of up to three samples from different specimens growing adjacent to each other in the crown of a *Ceiba pentandra*. Regression lines are given when $r^2 > 0{\cdot}7$ and $P < 0{\cdot}05$. Data are expressed in g d. wt m^{-2} (SLW), % d. wt (C, N) and $\mu g\ g^{-1}$ d. wt (all other elements). Area-based data show similar trends (not shown).

Walz) was used to keep the dew point of the air entering the leaf chamber below ambient to balance transpirational water loss and thus to maintain leaf to air vapour pressure deficits equivalent to ambient conditions. Only when air was saturated with water vapour, e.g. at night or during and after rainstorms, the relative humidity inside the cuvette was decreased below ambient to avoid condensation in the pneumatic system. Leaves were enclosed for 24-h periods from dusk to dusk and full data-sets were registered every 5 min (daytime) and 15 min (night-time), respectively.

Rates of net CO_2 exchange (A), water vapour exchange (E), stomatal conductance to water-vapour

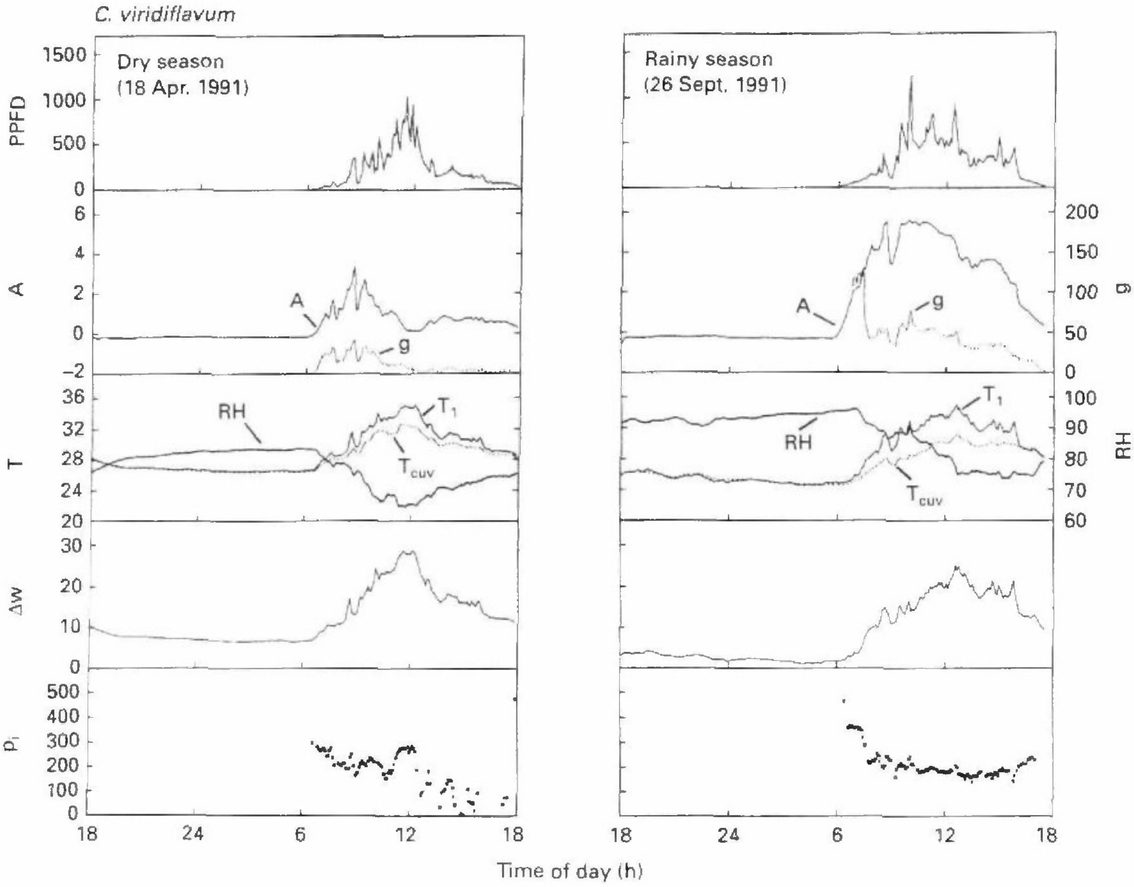

Figure 3. Representative 24-h courses of gas exchange in *Catasetum viridiflavum in situ* during the dry and the wet season. PPFD, photosynthetic photon flux density (μmol m^{-2} s^{-1}); A, rate of net CO_2 assimilation (μmol m^{-2} s^{-1}); g, leaf conductance to water vapour transfer (mmol m^{-2} s^{-1}); T_l, leaf temperature (°C); T_{cuv}, temperature inside leaf-cuvette (= ambient air temperature); RH, relative air humidity (%); Δw, leaf to air vapour pressure difference (mPa Pa^{-1}); p_i, intercellular CO_2 partial pressure (Pa × 10, i.e. μbar).

transfer (g), and intercellular (substomatal) CO_2 partial pressure (p_i), were calculated with a commercial software program (DIAGAS, Walz), which is based on the equations given by Von Caemmerer & Farquhar (1981). Curve integrations (e.g. for determinations of total daytime carbon gain or transpirational water loss) were performed with a digitizing table attached to a microcomputer. Water use efficiency (WUE) refers to the ratio of carbon gained (mmol) *vs.* the amount of water lost (mol) and is based on 24-h measurements.

Photosynthetic O_2 evolution

Ten-cm^2 disks were taken from fronds of *P. crassifolium* and leaves of *C. uvitana* (separate samples from three different plants each) in the early morning after sunny days during the dry season. The disks were immediately placed into the chamber of a Hansatech photosynthesis measuring system (LD-2, Hansatech, Kings Lynn, Norfolk, UK), which was filled with 5% (v/v) CO_2 in normal air. Following illumination at 300 μmol photons m^{-2} s^{-1} for 5–10 min, rates of photosynthetic O_2 evolution were determined for PPFDs between 0 and 300 μmol m^{-2} s^{-1}. The photon-use efficiency of photosynthetic O_2 evolution (Θ), based on absorbed light between 400 and 700 nm, was estimated from the linear portion of the light response curves.

Fluorescence

Leaf disks were taken from plants used in the gas exchange measurements or from comparable plants before dawn following sunny days during the dry and the wet season. Samples were immediately brought to the laboratory and kept in complete darkness for an additional 15 min. Using a PAM modulation fluorometer (Walz) and a flash lamp (Schott, Mainz, Germany), the dark level fluorescence yield (F_0) and the maximum fluorescence yield (F_M), were determined for three to six replicates per species and season. F_V/F_M was used as a measure of the photon-use efficiency of photosystem II electron transport (Kitajima & Butler, 1975), where $F_V = F_M$ minus F_0.

Water relations

Leaf water potential (Ψ) and osmotic pressure (π) of leaf sap were determined psychrometrically using

Table 2. *Light conditions, gas exchange characteristics and water relations of three epiphytes during the dry and wet season. Values are means* ± SD (*n*). A_{24h}, *daily* CO_2 *balance*; R_d, *nocturnal net* CO_2 *loss*; A_{max}, *maximum rate of net* CO_2 *uptake*; g_{max}, *maximum stomatal conductance*; Ψ, *predawn water potential*; π, *predawn osmotic pressure. For the* C_3-*CAM intermediate species* C. uvitana, *the contribution of nocturnal carbon gain to 24 h carbon gain is given as well as the nocturnal increase in titratable acidity* (ΔH^+)

	Catasetum viridiflavum		*Polypodium crassifolium*		*Clusia uvitana*	
Parameter	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
PPFD (mol m^{-2} d^{-1})	9·9	16·7 ± 7·4 (7)	12·1 ± 3·3 (6)	16·7 ± 7·4 (11)	12·1 ± 4·8 (12)	14·3 ± 5·8 (21)
A_{24h} (mmol m^{-2} d^{-1})	29·1	108·0 ± 28·0 (7)	35·4 ± 39 (6)	83·1 ± 62·6 (11)	50·2 ± 27·8 (12)	79·9 ± 29·4 (21)
R_d (mmol m^{-2} d^{-1})	7·7	10·3 ± 2·8 (7)	7·1 ± 1·6 (6)	9·5 ± 4·3 (11)	—	—
WUE (mmol CO_2 mol H_2O^{-1})	4·1	4·8 ± 0·9 (7)	5·7 ± 5·8 (6)	4·7 ± 2·5 (11)	13·1 ± 5·3 (12)	9·7 ± 5·6 (21)
A_{max} (μmol m^{-2} s^{-1})	3·1	5·5 ± 0·7 (7)	1·9 ± 1·6 (6)	3·9 ± 2·1 (11)	3·6 ± 1·3 (12)	4·5 ± 1·3 (21)
g_{max} (mmol m^{-2} s^{-1})	34	71 ± 16 (7)	16 ± 6 (6)	49 ± 23 (11)	35 ± 28 (12)	80 ± 63 (21)
Light compensation (μmol m^{-2} s^{-1})[a]	9	13 ± 3 (7)	10 ± 2 (6)	13 ± 4 (11)	—	—
Light saturation (μmol m^{-2} s^{-1})[b]		257 ± 45 (7)	—	236 ± 45 (11)	—	220 ± 33 (12)
Ψ MPa	−0·4	−0·5 ± 0·1 (7)	−1·3 ± 0·6 (7)	−0·8 ± 0·2 (11)	−0·5 ± 0·2 (9)	−0·4 ± 0·1 (14)
π MPa	0·7	0·8 ± 0·1 (7)	1·5 ± 0·6 (7)	1·2 ± 0·2 (11)	0·9 ± 0·1 (9)	0·9 ± 0·2 (14)
Nocturnal carbon gain (% of A_{24h})		—	—	—	49·2 ± 28·5 (12)	17·7 ± 16·4 (21)
ΔH^+ (meq m^{-2})		—	—	—	281 ± 112 (8)	94 ± 93 (11)

[a] The light compensation point was estimated from the early morning data (PPFD 10–100 μmol m^{-2} s^{-1}) of the diel measurements.

[b] Values were obtained from the diel measurements of net CO_2 exchange between 7.30 and 10.00 h during the wet season.

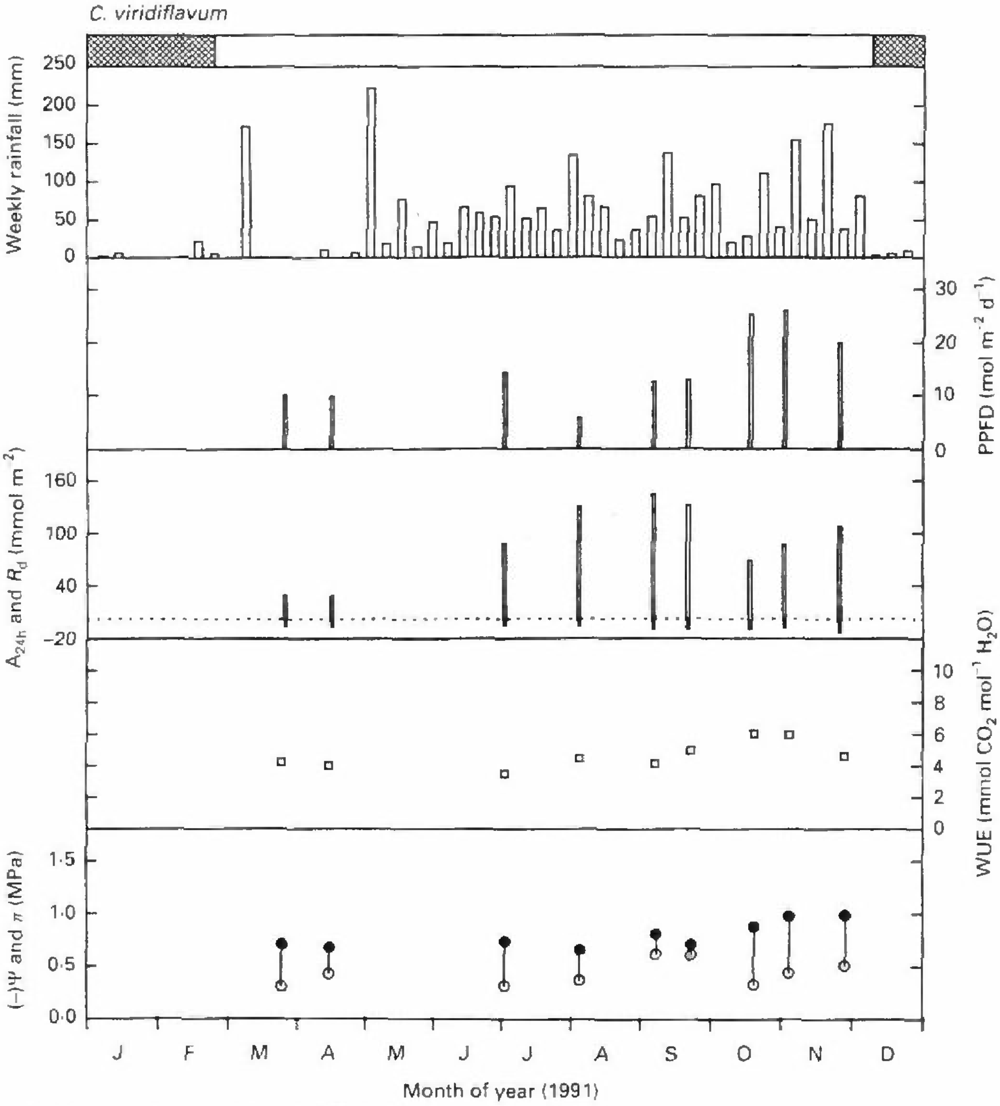

Figure 4. Changes in weekly rainfall, daily integrated PPFD and in gas exchange and water relations parameters in *Catasetum viridiflavum* throughout 1991. A_{24h}, daily (24-h) CO_2 balance (open bars); R_d, nocturnal net CO_2 loss (closed bars). WUE refers to the water use efficiency integrated over 24 h; ○, predawn Ψ; ●, predawn π. The difference between Ψ and π is an estimate of turgor pressure. Data given for Ψ and π are means of 3–5 measurements. The SD ranged from 0·01 to 0·09 MPa. Cross-hatched bars indicate the leafless period.

five sample chambers (C-52, Wescor, Logan, UT, USA) and a Wescor HR-33T dew point microvoltmeter. Samples were collected before dawn from leaves similar in age and size to those used for gas exchange measurements. Following the determination of Ψ, leaf disks were frozen in liquid nitrogen. After thawing and equilibration with room temperature, samples were remeasured for determination of π. Turgor pressure (P) was estimated from the difference between Ψ and π.

Titratable acidity

Determinations of titratable acidity (H^+ content) in *C. uvitana* were made on leaves opposite to the ones used for the gas exchange measurements. Samples were taken with a cork borer from one side of the mid-vein at dusk and from the other side the following dawn. Samples were frozen in liquid nitrogen and then boiled in 60% (v/v) ethanol. Extracts were titrated to pH 7·0 with 20 mM NaOH.

Mineral elements

For determination of specific leaf weight (SLW) and mineral element content, duplicate or triplicate samples were harvested at regular intervals during the study period. Fully developed leaves were considered from plants used in the gas exchange measurements or from similar plants nearby. Samples were dried at 60 °C for 48 h and analyzed at the University of Würzburg using a CHN-O Element Analyzer (Heraeus, Hanau, Germany) and a

ICP Spectrometer JY 70 Plus (ISA, München, Germany).

RESULTS

Seasonal changes in mineral element content

Mineral element contents of the three species (Table 1) were, on average, within the range of values reported previously for other epiphytic species (Benzing & Renfrow, 1974; Benzing, 1990), except for the high levels of N (per unit area) in leaves of *C. viridiflavum* (Fig. 2; see also Zimmerman, 1990). There were age-related changes in several mineral elements in both *C. viridiflavum* and *P. crassifolium* (Fig. 2): N and P decreased with increasing leaf (frond) age, while other elements such as Mn or Na increased. Mg decreased in *P. crassifolium* and slightly increased in *C. viridiflavum*. All these changes were significant ($r^2 > 0{\cdot}70$, $P < 0{\cdot}05$), irrespective of whether data were expressed on an area-basis (Fig. 2) or on a dry-weight basis (not shown). In *C. uvitana*, no clear age-related changes in mineral elements were observed, although very young, 4 to 10-wk-old leaves showed slightly elevated levels of N and P (not shown).

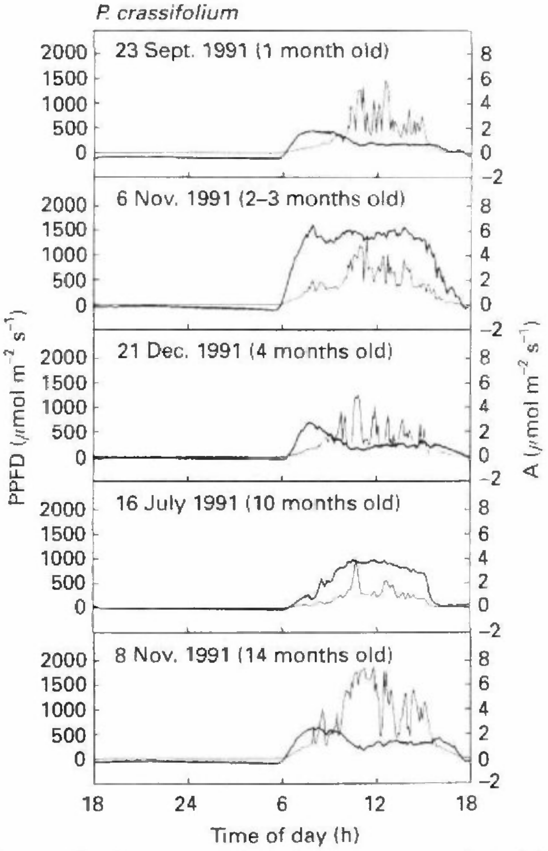

Figure 5. Representative 24-h courses of incident irradiation (PPFD) and net CO_2 exchange (A) *in situ* in fronds of different age of *Polypodium crassifolium* during the wet and the dry season.

Seasonal variation in micro-environmental conditions

Rainfall during the study year (1991) was 2480 mm, which is close to the long-term average for Barro Colorado Island (see Materials and Methods section). Ninety percent of the rainfall occurred in the wet, and 10 % in the dry season. Air temperatures showed little seasonal change. Mean daily maxima and minima were 30–32 °C and 24–26 °C, respectively. The relative humidity of the air was lower in the dry season than in the wet season, occasionally decreasing below 60 % at noon, while maximum values regularly reached 90 %. During the wet season, the air often was close to water saturation, although daily minima sometimes decreased to 70 %. In contrast to the 30 % higher PPFDs reaching the top of the rain-forest canopy during the dry season (Windsor, 1990), the epiphytes received slightly more PPFD per day during the rainy season, owing to the phenology of the host tree *C. pentandra*. The tree flushed new leaves at the beginning of the dry season. During the course of the study, shading of the epiphytes gradually decreased due to wind breakage, herbivory and diseases of *C. pentandra* leaves. In October 1991, the host tree shed its leaves and epiphytes were not shaded for the next 3 months. This phenological pattern of the host tree may be of importance for the growth and survival of these epiphytes: during the potentially most stressful period (second part of the dry season, March–April), the recently fully developed *Ceiba* canopy provides increased protection against high light-irradiance stress, whereas during the potentially most productive part of the year, the rainy season, light limitation of photosynthesis is reduced.

Seasonal changes in photosynthetic and water relationships

Catasetum viridiflavum. In the dry season diurnal courses of net CO_2 exchange and stomatal conductance were characterized by a midmorning maximum, a midday minimum and a minor peak in the afternoon (Fig. 3). In the wet season no midday depression could be observed and the absolute values of A and g were considerably higher (Fig. 3). Rates of net CO_2 uptake reached $6{\cdot}4\ \mu mol\ m^{-2}\ s^{-1}$ (9 August, 1991), about twice the maximum rates of the dry season (Table 2, Fig. 3). Daily carbon gain averaged $108\ mmol\ m^{-2}\ d^{-1}$, almost four times the value of the dry season (Fig. 4, Table 2). Plant water status, however, was similar on all measuring days throughout the year (Fig. 4).

Polypodium crassifolium. In this evergreen fern, 1-month-old fronds (= 1 month after full expansion) and 14-month-old fronds, close to senescence, showed low stomatal conductances and rates of net

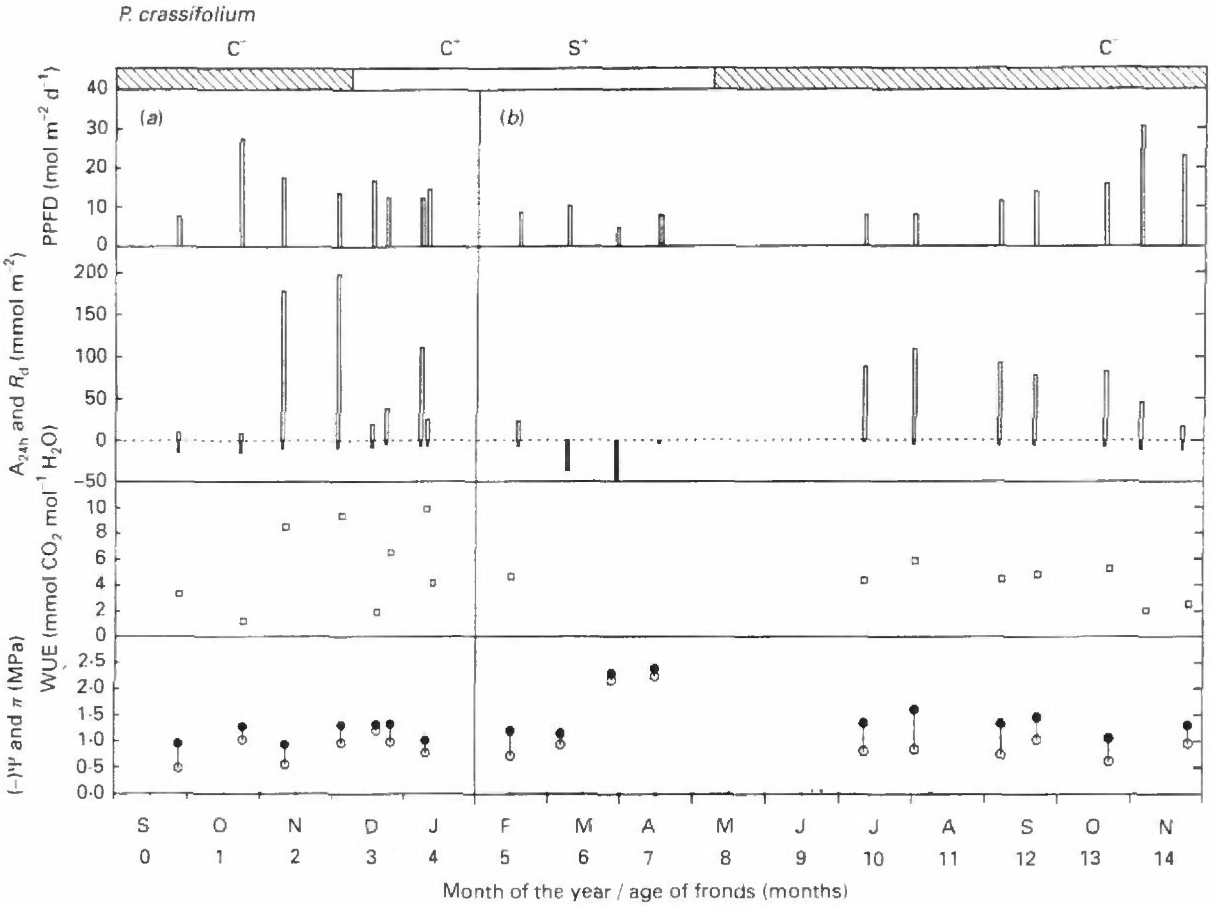

Figure 6. Seasonal changes in gas exchange and water relations parameters in *Polypodium crassifolium*. The left part of the graph (a) depicts fronds formed in 1991, from September 1991 to January 1992. The right part shows, for fronds formed in 1990, data from February to November 1991. This order allows the reconstruction of net carbon gain, water use efficiency and water relations over the whole lifetime of fronds. Negative WUE during the dry season are not shown. Hatched bars indicate the rainy season, open bars the dry season. C^- indicates leaf abscission of the host tree *Ceiba pentandra*, C^+ leaf flush of the host tree; S^+ formation of sporangia. For further explanations see Figure 4.

CO_2 uptake (Fig. 5). The highest rates, up to 7·8 μmol m^{-2} s^{-1}, were measured in 2- to 3-month-old fronds at the end of the wet season (November). Following the onset of the dry season (e.g. 21 December 1991 in Fig. 5), gas exchange was severely reduced. Turgor loss was observed on 3 of 7 measuring days during the dry season (Fig. 6). Towards the end of the dry season, frond water potential decreased by about 1 MPa, stomata remained closed for almost the entire day and 24-h carbon budgets became negative. The extreme negative daily carbon budgets on two measuring days in March 1991 (Fig. 6) were partly due to the presence of non-photosynthetic, respiring sporangia that covered 15–20 % of the lower surface of fronds. During the following rainy season, *A* and *g* increased without reaching the level of 2- to 3-month-old fronds again. Nocturnal carbon loss was highest in recently fully expanded fronds (e.g. 16 mmol CO_2 m^{-2} $night^{-1}$ on 23 September in Fig. 5). Ignoring the measuring days in March, nocturnal carbon loss (R_d) decreased almost linearly with leaf age, independent of season, and was only 3 mmol CO_2 m^{-2} $night^{-1}$ in 10-month-old fronds (Figs 5 and 6). At the end of the fronds life cycle, with 10–14 months, R_d increased again.

Clusia uvitana. In the C_3-CAM species *C. uvitana*, daily carbon gain was less affected by the dry season than in *C. viridiflavum* and *P. crassifolium* (Figs 7 and 8, Table 2). In *C. uvitana*, daily net carbon gain was, on average, 50 mmol m^{-2} d^{-1} during the dry season and 80 mmol m^{-2} s^{-1} during the wet season (Table 2). A_{max} generally occurred during the early morning hours (Fig. 7) and averaged 3·6 μmol m^{-2} s^{-1} in the dry and 4·5 μmol m^{-2} s^{-1} in the wet season (Table 2). During the dry season, leaf gas exchange was almost completely confined to the night and the early morning (Fig. 7) and nocturnal CO_2 fixation accounted for about 50 % of the 24-h carbon gain (Table 2). Overall stomatal conductances were substantially higher during the wet season, exceeding 100 mmol m^{-2} s^{-1} in the early morning several times, but decreased invariably during midday (Fig. 7, 7 August). The highest A_{max} was 6·5 μmol m^{-2} s^{-1} (22 September, 1991). Nocturnal

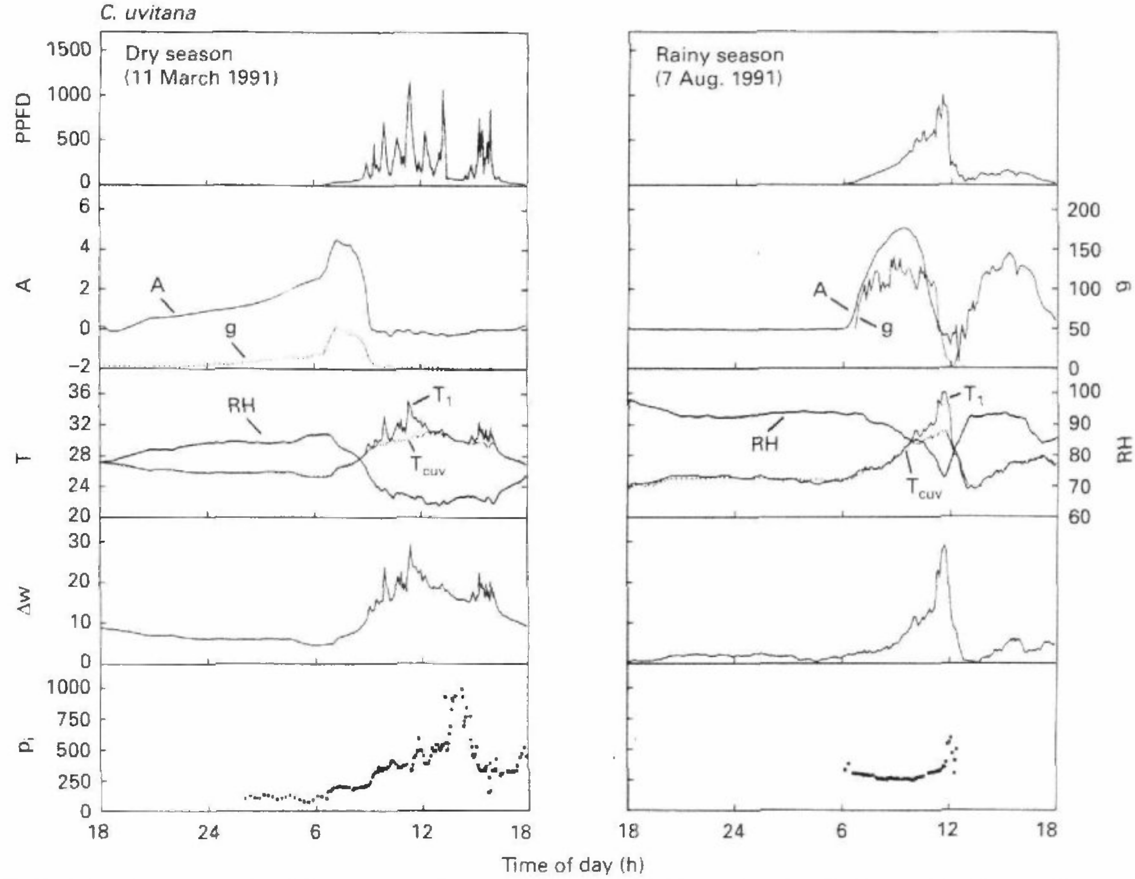

Figure 7. Representative 24-h courses of gas exchange in *Clusia uvitana in situ* during the dry and the wet season. For further explanations see Figure 3.

net CO_2 uptake was lower and accounted for only 18% of total daily carbon gain (Table 2). Consistent with higher rates of dark CO_2 fixation during the dry than during the wet season, nocturnal increases in titratable acidity were three times higher during the dry season (Table 2). No clear seasonal changes in leaf water potential, osmotic pressure and turgor pressure were detected (Fig. 8). Most of the time, the water potential was between −0·4 and −0·6 MPa and the turgor pressure between 0·3 and 0·5 MPa. Only occasionally, after several weeks without rain during the dry season, Ψ markedly decreased. For example, on 29 March 1991, Ψ was reduced to −0·95 MPa and turgor pressure was close to zero.

In *C. uvitana*, significant correlations were obtained between integrated PPFD and carbon gain during the early morning hours (phase II of CAM in the terminology of Osmond, 1978; $P < 0{\cdot}05$, $r^2 = 0{\cdot}2$) and during the late afternoon (phase IV; $P < 0{\cdot}05$, $r^2 = 0{\cdot}2$). However, between total daily carbon gain and daily integrated PPFD there was no significant correlation in any of the three epiphytes ($P > 0{\cdot}8$). Water status had a greater influence on gas exchange than light (Fig. 9). In *C. uvitana* and *P. crassifolium*, A_{max} and also daily carbon gain (Zotz & Winter, 1993*b*) were strongly correlated with pre-dawn water potential throughout the year. In these two species, Ψ accounted for approximately 50% of the daily variation in carbon gain. No significant correlation ($P > 0{\cdot}1$) between Ψ and 24-h carbon gain was seen in *C. viridiflavum*. As a consequence of the importance of the water status on the photosynthetic gas exchange there was no correlation between A_{max} or A_{24h} and leaf-N for individual days in *C. uvitana* and *P. crassifolium* ($P > 0{\cdot}6$) or the correlation was significant but negative (*C. viridiflavum*; $r^2 = 0{\cdot}52$, $P < 0{\cdot}02$).

Photosystem II photochemical efficiency (F_V/F_M)

In all species, except for *P. crassifolium* during the dry season, F_V/F_M exceeded 0·8 (Table 3), indicative of a high PSII photochemical efficiency (Björkman & Demmig, 1987). In sun fronds of *P. crassifolium*, F_V/F_M decreased to 0·76 during the dry season suggesting the occurrence of photoinhibition of photosynthesis, whereas fronds growing in shaded parts of the canopy maintained a high F_V/F_M. Sun fronds of *P. crassifolium* also showed a reduced photon-use efficiency of photosynthetic O_2 evolution during the dry season [$0{\cdot}076 \pm 0{\cdot}01$ mol O_2 mol photons^{-1} ($n = 3$)], whilst *C. uvitana* exhibited a high value [$0{\cdot}099 \pm 0{\cdot}012$ mol O_2 mol photons^{-1} ($n = 6$)] typical of non-photoinhibited leaves (Björkman & Demmig, 1987).

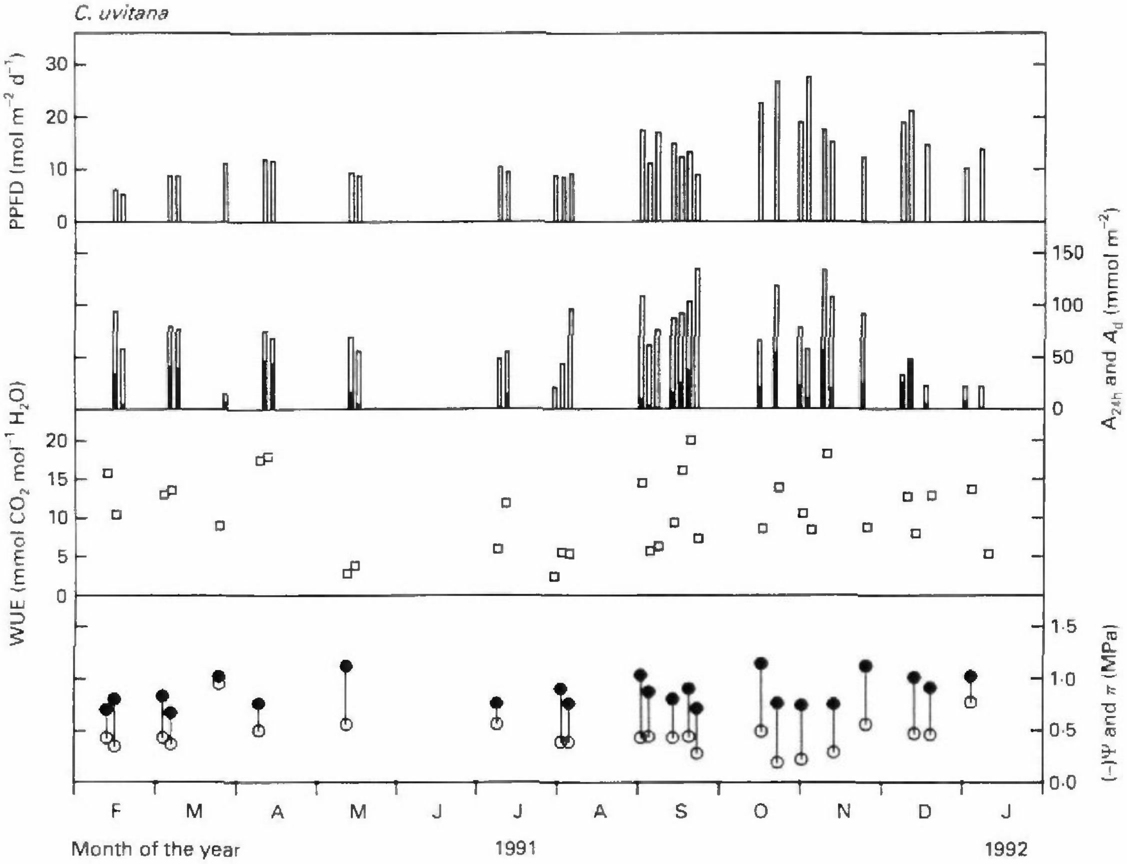

Figure 8. Seasonal changes in gas exchange and water relations parameters in *Clusia uvitana*. A_d, nocturnal carbon gain (closed bars). For further explanations see Figure 4.

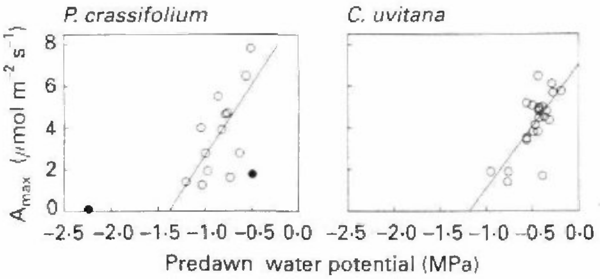

Figure 9. Relationship between maximum rate of net CO$_2$ uptake during diel measurements and predawn water potential in leaves of *Clusia uvitana* and fronds of *Polypodium crassifolium*. In *P. crassifolium*, the two data points with closed symbols (very young developing frond, day with almost complete stomatal closure) were not used for the calculation of the regression line. *C. uvitana*: $A_{max} = 5{\cdot}95\ \Psi + 7{\cdot}04$, $r^2 = 0{\cdot}52$, $P < 0{\cdot}05$; *P. crassifolium*: $A_{max} = 6{\cdot}78\ \Psi + 9{\cdot}41$, $r^2 = 0{\cdot}46$, $P < 0{\cdot}05$.

Primary productivity, water-use efficiency and nitrogen-use efficiency

Annual leaf carbon budgets were estimated by integration of the daily carbon balances shown in Figures 4, 6 and 8. On a leaf area basis, the carbon gain was similar for all three species and ranged from 840 to 1090 g CO$_2$ m^{-2} yr^{-1} (Table 4). On a dry weight basis, the relatively thin-leaved *C. viridiflavum* fixed about 3 to 4 times more CO$_2$ than *C. uvitana* and *P. crassifolium*, respectively. The long-term water-use efficiency (WUE) of net CO$_2$ uptake in the C$_3$-CAM intermediate *C. uvitana* was 11·0 mmol CO$_2$ mol H$_2$O^{-1} and thus more than twice as high than in the two C$_3$ epiphytes (Table 4). The long-term nitrogen-use efficiency (NUE), estimated as annual CO$_2$ gain per mean leaf-nitrogen (Table 1) was essentially identical for all three epiphytes (Table 4).

DISCUSSION

The study presented here shows that different adaptations to environmental stress associated with a tropical epiphytic habitat can be similarly beneficial in terms of annual leaf carbon gain: (i) the option to perform CAM and to survive the dry season by aid of dark CO$_2$ fixation (*C. uvitana*), (ii) the option of stress tolerance including loss of turgor and partial photoinhibition during the dry season (*P. crassifolium*), and (iii) the option of stress avoidance characterized by the loss of leaves during parts of the dry season (*C. viridiflavum*).

The estimates of the annual leaf carbon gain of these epiphytes (Table 4) equal or even exceed those of many deciduous trees and shrubs from temperate zones like Central Europe (Schulze, 1970; Küppers, 1984). This difference may be related to the longer growing season in the tropics. No estimates of long-

Table 3. *Ratio of variable to maximum fluorescence yield in dark-adapted leaf and frond samples, respectively, of three epiphytes during the dry and wet season. Values are means* ± SD (*n*)

Species	Season (exposure)	F_V/F_M
Catasetum viridiflavum	Wet season (sun)	0·804 ± 0·016 (3)
	Dry season (sun)	—
Polypodium crassifolium	Wet season (sun)	0·819 ± 0·016 (5)
	Dry season (sun)	0·760 ± 0·015 (6)
	Dry season (shade)	0·850 ± 0·003 (3)
Clusia uvitana	Wet season (sun)	0·818 ± 0·008 (3)
	Dry season (sun)	0·801 ± 0·006 (4)

Table 4. *Annual carbon balance, water-use efficiency (mean* ± SD [*n*] *of 24-h measurements throughout the study year) and annual nitrogen-use efficiency of leaves and fronds, respectively, of three epiphyte species. Estimates for carbon balances were derived from Figures 4, 6 and 8 by linear interpolation and planimetry*

	Annual CO_2 balance			
Species	(g CO_2 g^{-1} d. wt yr^{-1})	(g CO_2 m^{-2} yr^{-1})	Water-use efficiency (mmol CO_2 mol H_2O^{-1})	Nitrogen-use efficiency (g CO_2 mg^{-1} N yr^{-1})
Catasetum viridiflavum	26·3	1090	4·8 ± 0·9 (9)	1·04
Polypodium crassifolium	7·4[a]	840[a]	5·0 ± 3·8 (17)[a]	1·13
Clusia uvitana	6·1	1060	11·0 ± 5·6 (33)	1·11

[a] Estimated without the measuring days in March 1991, when sporangia were present.

term carbon gain are available for other epiphytes. Therefore we can only use the results of short-term measurements of A_{max} and A_{24h} with other species for a preliminary comparison. Overall net rates of CO_2 uptake and daily carbon gains were slightly higher in *C. uvitana* than in epiphytic plants of congeneric *Clusia rosea* in Florida (Sternberg *et al.*, 1987) or *Clusia minor* in Venezuela (Ting *et al.*, 1987). A_{max} of *P. crassifolium* markedly exceeded values reported previously for other epiphytic ferns (Hew & Wong, 1974; Nobel, Calkin & Gibson, 1984; Lüttge *et al.*, 1986; Kluge *et al.*, 1989), while the maximum net rates of CO_2 uptake in *C. viridiflavum* were almost identical with the A_{max} reported in a greenhouse study with *Catasetum integerrimum* (Benzing *et al.*, 1982).

Consistent with the well established water-conserving nature of CAM (Kluge & Ting, 1978), the long-term water-use efficiency (WUE) of photosynthesis was more than twice as high in *C. uvitana* than in the two C_3 epiphytes. In the C_3 species the 24-h carbon gains were much stronger reduced during the dry season than in *C. uvitana* (Table 2). This difference might be a consequence of the better water economy of the CAM plant. Even so, WUEs in *C. viridiflavum*, *P. crassifolium* and in previously studied tropical C_3 epiphytes on Trinidad (Griffiths *et al.*, 1986) are higher than WUEs from many non-tropical terrestrial C_3 plants (Nobel, 1991), probably because the latter generally experience greater leaf–air vapour pressure differences and thus higher water losses during periods of net CO_2 uptake.

Another major finding of this study is the very similar long-term nitrogen-use efficiency of all three species (Table 4). This suggests that it is possible to estimate annual leaf carbon gain from leaf N content using the same conversion factor for all species. It is possible that this relationship will be applicable for other species as well. This conclusion is supported by several observations: (a) The photosynthetic capacity A_{max} and leaf-nitrogen correlate closely in a large number of field-grown plants (Field & Mooney, 1986; Reich *et al.*, 1991), and (b) it is possible to extrapolate from measurements of A_{max} to 24-h carbon gain (Zotz & Winter, 1993*b*). Hence, a relationship between leaf-nitrogen and annual carbon gain could be expected. In the particular case of the epiphytes in this study, drought stress imposed strong limitations on photosynthesis (Fig. 9) and there was no positive correlation of A_{max} and leaf N on a day-to-day basis. Nevertheless, the annual nitrogen-use efficiencies were almost identical in all three epiphyte species. This shows that leaf nitrogen has to be treated as a long-term investment that can only be fully understood in the context of the entire growing season or the entire leaf life span. Given the close correlation of A_{max} and A_{24h} (Zotz & Winter, 1993*b*) it now seems possible to demonstrate a relationship between time-integrated photosynthesis and leaf nitrogen by screening a large number of species in their natural environment.

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