Acclimation or stress symptom? An integrated study of intraspecific variation in the clonal plant *Aechmea bromeliifolia*, a widespread CAM tank-bromeliad

F. R. SCARANO*1, FLS, H. M. DUARTE1, G. RÔÇAS1, S. M. B. BARRETO1, E. F. AMADO1, F. REINERT2, T. WENDT2, A. MANTOVANI2, H. R. P. LIMA3 and C. F. BARROS4

1Departamento de Ecologia, Universidade Federal do Rio de Janeiro, CCS, IB, Caixa Postal 68020, cep 21941–970, Rio de Janeiro, RJ, Brazil
2Departamento de Botânica, Universidade Federal do Rio de Janeiro, CCS, IB, cep 21941–590, Rio de Janeiro, RJ, Brazil
3Departamento de Botânica, Universidade Federal Rural do Rio de Janeiro, IB, cep 23851–970, Seropédica, RJ, Brazil
4Jardim Botânico do Rio de Janeiro, Laboratório de Botânica Estrutural, Rua Jardim Botânico 1008, cep 22460–030, Rio de Janeiro, RJ, Brazil

Received July 1999; accepted for publication August 2002

The clonal tank-bromeliad *Aechmea bromeliifolia* (Rudge) Baker was found in four different habitats in a restinga (vegetation mosaic on sandy coastal plains), of south-eastern Brazil. These habitats (swamp forest, dry forest, dry shrubland and herbaceous marsh) lie within a few hundred metres of each other along a gradient extending inland from the coast, and differ markedly in terms of light and flood regime. We compared ramet morphology, leaf anatomy and physiology, and population parameters to examine the amplitude of trait variation of this widespread species in the studied restinga. This integrated approach allowed us to examine which variation conferred acclimation and which was merely a stress symptom. *A. bromeliifolia* showed site-specific differences in abundance, distribution, rosette size and shape, leaf anatomical arrangement and photochemical efficiency (potential quantum yield; $F_v/F_m$) during the day. Most of the variation found seemed to be related to the interaction of light and flooding. The lowest number and size of ramets at the exposed, dry shrubland was matched by a marked leaf photoinhibition, which suggested poor acclimation to local levels of light intensity and limited water supply. In the other habitats, the morphophysiological parameters measured suggested adequate foraging behaviour and site acclimation. © 2002 The Linnean Society of London, Botanical Journal of the Linnean Society, 2002, 140, 391–401.

ADDITIONAL KEYWORDS: clonal growth – foraging behaviour – phenotypic variation – restinga – sandy coastal plains.

INTRODUCTION

Rare and/or endemic species are intrinsically of conservation interest due to the extinction threat to which they are often, but not always, exposed (Mace & Kershaw, 1997). Conversely, widespread, common species are not as easily threatened by extinction. However, populations of the latter can be locally extinguished (McKinney, 1997) and, with those, a wealth of genetic information will no longer be available (Harrison, 1991). Thus, in recent years, several studies have attempted to compare life strategies and ecological functioning of rare vs. common species and of endemic vs. widespread species (for review, see Gaston & Kunin, 1997), both for scientific and for conservation purposes.

Studies comparing ecological features of distinct populations of widespread species of tropical plants under distinct selective pressures are scarce (Araus & Hogan, 1994; Rôças *et al.* 1997; Rôças, Scarano & Barros, 2001), although the tropical region produces many interesting cases of generalist plants (see Scarano *et al.*, 2001; Scarano, 2002). One such case is that of a widespread neotropical bromeliad of the sub-
family Bromelioideae, *Aechmea bromeliifolia* (Rudge) Baker. It occurs as a terrestrial, rupicolous or epiphytic plant, from sea level to 1385 m of altitude, from Central America to Argentina and throughout most Brazilian states (Smith & Downs, 1979), in vegetation as distinct as rain forests, savannas, dunes, marshes and swamps.

The bromeliad family as a whole shows considerable morpho-physiological variation and distribution across a wide range of environmental conditions (Martin, 1994), although at the species level there is a large number of endemisms (Wendt et al., 2001). Thus, the widespread *A. bromeliifolia* provides an interesting case within the family for the study of ecological acclimation to distinct habitats. The marked ecological plasticity of this species, which produces an impounding tank shoot, is seen in a restinga of the state of Rio de Janeiro, south-eastern Brazil. Restinga is the general name collectively given to the varied plant communities covering the sandy coastal plains of Brazil. This ecosystem is a mosaic of habitats which imposes distinct physical constraints such as salinity, flooding, drought or high temperatures on the resident flora (Reinert et al., 1997; Scarano et al., 2001). At least 14 different types of plant community ranging from reptant types to forests occur within the restingsas (Lacerda, Araújo & Maciel, 1993). Different communities frequently lie adjacent to each other. However, very few plants in addition to *A. bromeliifolia* occur in more than one or two of these habitats. In the restinga mentioned above, this species occurs in four different habitats varying in light and flooding regimes, spatially separated by hundreds of metres.

The ecological plasticity of plants such as *A. bromeliifolia* is often linked to high levels of genetic diversity (Benzing, 1983) and/or phenotypic plasticity (Schlichting & Pigliucci, 1998), which confer acclimation to distinct environments, favouring establishment, survival and reproduction. However, acclimation is often difficult to predict based on phenotypic variation alone. In this *in situ* field study, we combined morpho-anatomical, physiological and population parameters in order to assess acclimation of plants of four neighbouring populations of *A. bromeliifolia* under distinct environments. We aimed to assess to what extent variation in traits reflected acclimation by producing changes in foraging behaviour, and to what extent they symptomized stress.

**STUDY AREA**

Surveys were conducted at the State Ecological Reserve of Jacarepiá (22°47′–22°57′S, 42°20′–42°43′W) in the municipality of Saquarema, Rio de Janeiro state, south-eastern Brazil, where some of the most representative vegetation types of the Brazilian restingas occur. Sá (1992) provides a detailed description of this vegetation. Mean annual rainfall is 1000 mm and mean annual temperature ranges from 24 to 26°C.

*Aechmea bromeliifolia* was found in several different types of habitat and, most abundantly, in four types (from inland towards the sea): (i) a swamp forest, in the shaded understory where its rhizomes were flooded; (ii) a dry forest, in the shaded understory growing on sand; (iii) a sparse, open shrubby vegetation, where it was exposed and grew on sand; and (iv) a herbaceous marsh, exposed and flooded.

The swamp forest is a secondary vegetation reduced to a patch of c. 0.5 ha (Rôças et al., 2001). This swamp is c. 200 m distant from the northern limit of the Reserve and c. 1 km in a diagonal line (inland–sea) from the herbaceous marsh described below. It is dominated by *Tabebuia cassinoides* DC (Bignoniaceae). The taller trees reach c. 8 m in height, and the tree species diversity is notably low, as in other swamps in south-eastern Brazil (e.g. Scarano, Rios & Esteves, 1998). The only bromeliad occupying the shaded understory was *A. bromeliifolia*, which densely covered the lowlying areas. Soils remain inundated from 10 to 12 months a year, by standing water 10–30 cm above soil level.

The dry forest is located c. 150 m from the herbaceous marsh, on a straight line parallel to the sea. This forest grows on sand and the population of *A. bromeliifolia* was found at its border. Taller trees reach 10 m in height, and the dominant trees are *Guapira opposita* (Vell.) Reitz (Nyctaginaceae), *Brysonima sericea* DC (Malpighiaceae) and *Tapirira guianensis* Aubl. (Anacardiaceae). *Aechmea bromeliifolia* roots in the shaded understory and also at the ecotone where forest gives way to the dry shrubland described below.

The dry shrubland appears in the transition from the dry forest to the marsh, and remains unflooded throughout the year. *Aechmea bromeliifolia* grows fully exposed, among underdeveloped saplings of trees seeded in from the neighbouring dry forest – *Tapirira guianensis*, *Ocotea* spp. (Lauraceae), *Alchornea triplinervia* (Spreng.) Müll. Arg. (Euphorbiaceae), plus a number of shrubs and herbs common to moist soils in the restingsas. Field sampling for this study was done in 1994, prior to a man-made fire in January 1995, which badly damaged the populations in the dry forest and in the dry shrubland (see Cirne & Scarano, 2001; Scarano et al., 2001).

The herbaceous marsh is permanently flooded at its centre, where it is dominated almost exclusively by *Eleocharis* spp. (Cyperaceae). Near the margin of the marsh, facing the inner beach ridge where flooding is shallow, diversity increases to include a number of graminoids. Isolated saplings of tree species such as
Cecropia spp. (Cecropiaceae), Alchornea triplinervia, and the palm Bactris setosa Mart., dispersed from a neighbouring flood-prone forest are also found. Flooding by still water is continuous.

MATERIAL AND METHODS

POPULATION PARAMETERS AND SHOOT SIZE

150 m² plots were divided into six quadrats of 5 × 5 m at each of the sites. Plot shapes were tailored to maximize the number of ramets (tank shoots) sampled for each bromeliad population. All shoots equal to or taller than 25 cm were counted in each quadrat and shoot height and canopy area were measured. Height was measured as the distance from the soil to the tip of the tallest leaf. Canopy shape was considered to be elliptical, and thus the area occupied by a shoot was estimated by taking two perpendicular measurements of canopy diameter at maximum height. Percent coverage of the species within each plot was calculated based on individual canopy areas.

A fertile individual was collected at each survey site and deposited at the Herbarium of the Rio de Janeiro Botanic Gardens (RB; T. Wendt 272, 273, 274 and 276 & F.R. Scarano).

LEAF ANATOMY

For each population, three pre-adult plants (plants which had not yet flowered) were selected and one fully expanded leaf from each was harvested. Samples of 0.5 cm² were taken from the middle third of each harvested leaf and fixed in FAA (70%), dehydrated in an ethanol series, and infiltrated with metacylate resin (Historesin, Heichert-Jung) following Feder & O’Brien (1968). Sections of 8 µm were stained with astrablue-safranin (4 : 6) in 30% ethanol for 36 h (adapted from Braune, Leman & Taubet, 1979).

Width and number of layers of chlorophyll parenchyma and water parenchyma, and stomata density of the middle third and basal third of the leaf were measured according to Wilkinson (1979) for 39 replicates.

One-way analysis of variance (ANOVA) was used to assess the differences between populations. Two-way ANOVA was used to measure the variation resulting from exposure and soil flooding, and the interaction between these two factors. For multiple comparisons, the Tukey test (Zar, 1996) was used. The software Statistica 4.2 was used to perform the statistical analyses (Statsoft Inc., 1993).

LEAF PHYSIOLOGY

Measurements of variable ($F_d$) to maximal ($F_m$) chlorophyll a fluorescence ($F_d/F_m$), leaf titratable acidity under natural photosynthetic photon flux density (PPFD) and carbon isotope ratios were undertaken in order to assess photosynthetic activity and tolerance to environmental constraints, in the summer of 1994.

Field measurements of $F_d/F_m$ were taken from three plants in each population, three leaves per plant, every 2 h. Prior to measurements, leaves were dark-adapted for 30 min, and then analysed using a Plant Efficiency Analyser (PEA; Hansatech, Norfolk, UK). A $F_d/F_m$ value ≥0.83 indicates total absence of photoinhibition and field readings were compared to this value (Björkman & Demmig, 1987).

The magnitude of crassulacean acid metabolism (CAM), as measured by titratable acidity, was carried out in the leaves of five plants from each population, which were sampled at 2 h intervals on three consecutive days. Leaf discs were immediately placed in ice and subsequently frozen in the laboratory. For titration, leaf discs were boiled for 2 h in 10 mL of distilled water. The aqueous solution was titrated against NaOH with phenolphthalein.

Three composite samples of oven-dried leaves from each population were ground for carbon isotope analysis and combusted in an oxygen bomb. Carbon isotope ratios were determined in a mass spectrometer as described by Osmond et al. (1975), in the Universities of Newcastle (England) and Munich (Germany).

In order to provide an approximation of light regime, PPFD was measured at over 20 points in each habitat, with a LI-COR LI-190SA Quantum Sensor on a clear summer day with no clouds between 11 a.m and 1 p.m.

An attempt was made to obtain gas exchange data for the four populations using an ADC LCA-2 (Cambridge, UK) infrared gas-analyser. However, ambient relative humidity (particularly at the flooded sites) was never lower than 90%, which was beyond the capacity of the equipment to produce reliable readings.

RESULTS

POPULATION PARAMETERS AND SHOOT SIZE

Table 1 shows that A. bromeliifolia percent canopy cover (canopy area projection onto the soil) is less related to density than to sun-exposure. A correlation analysis between canopy coverage and plant density showed no significant correlation between these two parameters ($r = 0.59; P > 0.05$). Coverage is markedly reduced for the exposed plants when compared to the shaded ones (Figs 1,2). Indeed, sun-exposed populations are visually more patchy than the shaded ones. This is particularly evident for the exposed/unflooded population, as seen by its frequency of tanks per quadrat as compared to the other populations (Table 1). Despite its higher density, the shaded/unflooded population showed a smaller percent cover
than the flooded one, probably because the latter is less shaded (50–200 μmoles m⁻² s⁻¹) than the former (5–50μmoles m⁻² s⁻¹). For the exposed populations (1500– 2000 μmoles m⁻² s⁻¹), it appears that absence of flooding did not favour establishment and clonal growth of this species, since it showed both the lowest density and percent cover. The absence of a significant correlation between density and percent cover underscores the existence of sun and shade forms in this bromeliad (Figs 1,2).

Table 1. Total number of bromeliad tanks, tank frequency per quadrat (mean ± standard error; N = 6), range of tanks among quadrats (min – max), tank density and canopy coverage area of four populations of Aechmea bromeliifolia (Rudge) Baker in four habitats with different light and inundation regimes. Total area sampled for each habitat: 150 m²

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Total no. of tanks</th>
<th>Frequency per quadrat</th>
<th>Range of tanks among quadrats</th>
<th>Density (N/m²)</th>
<th>Total canopy coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swamp forest (flooded/shaded)</td>
<td>255</td>
<td>41 ± 10</td>
<td>15–81</td>
<td>1.7</td>
<td>127.6</td>
</tr>
<tr>
<td>Dry forest (unflooded/shaded)</td>
<td>460</td>
<td>76 ± 14</td>
<td>37–118</td>
<td>3.1</td>
<td>102.0</td>
</tr>
<tr>
<td>Dry shrubland (unflooded/exposed)</td>
<td>117</td>
<td>20 ± 7</td>
<td>0–39</td>
<td>0.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Herb marsh (flooded/exposed)</td>
<td>291</td>
<td>48 ± 10</td>
<td>21–80</td>
<td>1.9</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Figures 1,2. The morphological differences between shade (Fig. 1) and sun (Fig. 2) rosettes of Aechmea bromeliifolia. The width at the base of the rosette in each case is approximately 10 cm.
INTRASPECIFIC VARIATION IN AECHMEA BROMEIIIFOLIA

Table 2 shows the differences in shoot height and canopy area among the four surveyed populations. Shoot height and canopy area were higher in shadier habitats. Additionally, the foliage of shaded plants was conspicuously darker green compared to the yellowish, more exposed plants.

**LEAF ANATOMY**
Mean values of all anatomical parameters surveyed differed significantly among the populations studied (Table 3). Exposed/flooded plants showed the highest values of chlorophyll parenchyma width (CPW), number of cell layers (CPN) and basal and middle-leaf stomata density (BS and MS). Exposed/unflooded plants showed the lowest values of CPW, CPN, water parenchyma width (WPW) and number of cell layers (WPN). Shaded plants, irrespective of soil water, did not show significant differences for the traits measured, except for MS. Shaded plants also showed lower BS and MS values than the exposed populations. Figures 3–6 show cross sections of the leaves indicating variation in water and chlorophyll parenchyma between sites.

Two-way ANOVA showed that all anatomical traits studied varied significantly in relation to the combination of light-exposure and flooding (Table 3). Flooding alone significantly affected variation of CPW, CPN, WPN and BS, whereas exposure alone affected WPN, WPW, BS and MS.

**LEAF PHYSIOLOGY**
Carbon isotope analysis (Table 4) indicated that plants from all populations sampled are typically CAM (crassulacean acid metabolism). The significantly less negative δ13C values of the dry shrubby population may indicate that these plants have a lower contribution of carbon fixation via the C3 pathway (stomatal reopening during the light period, phase IV of CAM) than, particularly, in the shade populations.

Similarly, variation in leaf titratable acids throughout the day for the four populations under different light and water regimes suggested that CAM is partially inhibited under low light intensity (Fig. 7). The magnitude of CAM as assessed by titratable acidity measurements was considerably higher in sun-exposed (herbaceous marsh and dry shrubland; Fig. 7A,B) than in shaded populations (understorey of swamp forest and of dry forest; Fig. 7C,D). The former showed a difference between dawn and dusk titratable acidity (ΔH+) of c. 120 mmol H⁺ m⁻², whereas the latter showed less ΔH⁺ of c. 40 mmol H⁺ m⁻². Sun-exposed populations showed similar decarboxylating rates throughout the light period.

The photochemical efficiency in the exposed populations of *A. bromeliifolia*, as indicated by $F_v/F_m$ values, was high in the morning (6:00) and evening (16:00) but between these times the efficiency decreased (Fig. 8A,B). In contrast, the photochemical efficiency of *A. bromeliifolia* in the shaded areas was high and constant throughout the day (Fig. 8C,D). The exposed, dry population showed the lowest values (0.7) at dawn (Fig. 8B).

**DISCUSSION**
Acclimation, in the case of *A. bromeliifolia*, was strongly related to foraging tactics as in many other clonal plants (De Kroon & Van Groenendael, 1990). Despite displacements of only a few hundred metres, the surveyed populations of *A. bromeliifolia* differed in terms of spatial array, as well as morpho-anatomical and physiological traits. Such variation has already caused considerable taxonomic controversy. Sá (1992)
Variation in leaf anatomical traits of four populations of *Aechmea bromeliifolia* (Rudge) Baker in four habitats varying in terms of light and inundation regime. Means (± standard error) followed by different letters differ at *P* < 0.05 according to test of multiple comparisons (HSD Tukey test). *F*-values (two-way ANOVA) followed by (*) mean that effect of environmental factor on trait is significant at *P* < 0.05; (**) at *P* < 0.01; (***) at *P* < 0.001. CPW = chlorophyll parenchyma width; CPN = number of cell layers of CP; WPW = number of cell layers of WP; BS = basal stomata density; MS = middle-leaf stomata density.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Trait</th>
<th>Swamp forest</th>
<th>Dry forest</th>
<th>Herb marsh</th>
<th>Dry shrubland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPW (μm)</td>
<td>739.0 ± 32.8</td>
<td>204.0 ± 0.6</td>
<td>579.3 ± 4.4</td>
<td>688.5 ± 9.9</td>
</tr>
<tr>
<td></td>
<td>CPN (μm)</td>
<td>579.3 ± 4.4</td>
<td>204.0 ± 0.6</td>
<td>579.3 ± 4.4</td>
<td>688.5 ± 9.9</td>
</tr>
<tr>
<td></td>
<td>WPN (μm)</td>
<td>153.0 ± 5.7</td>
<td>170.9 ± 5.8</td>
<td>170.9 ± 5.8</td>
<td>170.9 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>WPN (μm)</td>
<td>153.0 ± 5.7</td>
<td>170.9 ± 5.8</td>
<td>170.9 ± 5.8</td>
<td>170.9 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>MS (μm)</td>
<td>1.0 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>MS (μm)</td>
<td>1.0 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Number of</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Number of</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Number of</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Number of</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Number of</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Number of</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
<td>17.0 ± 0.4</td>
<td>22.3 ± 1.1</td>
</tr>
</tbody>
</table>

RESPONSES TO FLOODING

Although many other terrestrial rhizomatous bromeliads (other *Aechmea* and species of *Billbergia*, *Neo-regelia*, *Streptocalyx* and *Vriesea*) inhabit flood-free restinga vegetation at the Jacarepá Reserve (Sá, 1992), *A. bromeliifolia* is the only bromeliad to colonize marshes and swamp forests. Although bromeliads are often associated with drought-tolerance, *Nidularium procerum*, *Nidularium innocentii* and *Quesnelia quesneliana* grow on flooded ground in a swamp forest in northern Rio de Janeiro state (Scarano et al., 1997, 1998, 1999). Rhizome anoxia-tolerance has been reported for a number of temperate plant species (for reviews see Braëndle & Crawford, 1987; Braëndle, 1991).

The effects of flooding on population structure and individual phenotype were apparent from tank density, height and site percent coverage data. The lowest values for all these parameters were found at the unflooded, exposed site. Flooding did not seem to be deleterious to this species, in fact much to the contrary. Freitas, Scarano & Wendt (1998) proposed that the facultative epiphytes *Nidularium procerum* and *N. innocentii* (also tank-bromeliads of the Bromeliaceae subfamily) behave as epiphytes on a ‘muddy phorophyte’, referring to the swampy, muddy soil upon which they grew. These bromeliads had shoot autonomy for feeding and the rhizome acted mostly as an anchoring organ, as it would do on a tree trunk for instance. Such seems to be the case of *A. bromeliifolia*: flood-tolerant rhizomes are anchoring structures, while the leaves are the feeding sites. Flooding is likely to ameliorate local air humidity, which could account for longer-term retention of rain water in the leaf-tanks. Although local air humidity and tank water content were not measured, these seem to be obvious consequences to expect.

RESPONSES TO LIGHT

Although shoots were always aggregated, light exposure influenced distributions. Populations subjected to direct sunlight (marsh and transition forest-marsh)
formed scattered colonies of various sizes whereas those in shade did not. Shaded populations showed slightly different patterns of spatial distribution. In the swamp forest (5–50 μmoles m⁻² s⁻¹ irradiance reaching the understorey), the rosettes formed confluent covers, fully covering the ground floor of this forest, giving the visual impression of being one large group of rosettes in contrast to the fragmented groups of the populations growing in full sunlight. The contact between groups diminished in the dry forest where irradiance was higher (50–200 μmoles m⁻² s⁻¹). Hutchings & Slade (1988) claim that alterations in light quality may change the foraging behaviour of clonal herbs. In *Glechoma hederacea*, they found that

Figures 3–6. Cross-sections of leaves of *Aechmea bromeliifolia* collected in situ at distinct soil water/light regimes. Fig. 3. Flooded/exposed; Fig. 4. Unflooded/exposed; Fig. 5. Unflooded/shaded; Fig. 6. Flooded/shaded. ✽ = water parenchyma; ★ = chlorophyll parenchyma. Scale bars in all figures = 50 μm.

Table 4. Carbon isotope discrimination of leaves of four populations of *Aechmea bromeliifolia* (Rudge) Baker in four habitats varying in terms of light and inundation regime. Means ± standard deviation (N = 3) followed by different letters differ at P < 0.05 according to test of multiple comparisons (HSD Tukey test). F (one-way ANOVA) was significant at P < 0.05

<table>
<thead>
<tr>
<th></th>
<th>Swamp forest (flooded/shaded)</th>
<th>Dry forest (unflooded/shaded)</th>
<th>Dry shrubland (unflooded/exposed)</th>
<th>Herb marsh (flooded/exposed)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ¹³C</td>
<td>−13.37 ± 0.40 a</td>
<td>−13.38 ± 0.07 a</td>
<td>−12.55 ± 0.09 b</td>
<td>−13.06 ± 0.03 ab</td>
<td>4.29</td>
</tr>
</tbody>
</table>
when light quality was spectrally biased by an overhanging canopy of leaves, the clones tended to expand linearly and forage extensively. Under full light, branching and foraging increased. This could explain the distribution gradient of *Aechmea bromeliifolia* from exposed scattered colonies to shaded more confluent colonies.

Furthermore, light intensity often affects plant form and structure (Björkman, 1981; Chazdon & Kaufmann, 1993; Strauss-Debenedetti & Berlyn, 1994). The rosettes of *A. bromeliifolia* varied from wide open tanks with a large canopy area in the shade, to almost totally closed tanks when exposed. This variation often comes as a result of different leaf size, width and angle in response to light regime, which has been extensively reported for bromeliads (Lee *et al*., 1989; Martin, 1994). The consequence of this acclimation process is that sun plants are capable of avoiding direct light incidence upon most of the leaf surface, whilst simultaneously storing and keeping larger amounts of rain water in its central tank. Conversely, shade plants reach out for sunflecks by resorting to a more open tank formation and reduced leaf overlapping. Indeed, in these populations carbon fixation probably occurs mostly during sunflecks: photosynthesis is limited by low light, as nocturnal acid accumulation is restricted by the previous day’s integrated PPFD (see Winter, 1985; Chazdon & Pearcy, 1986; Griffiths, 1988).

The low values of tank density, height and site percent coverage in the exposed, unflooded site, when compared to the other three sites, can be better under-

**Figure 7.** 24-h course of titratable acidity (H⁺ mmol m⁻²) in leaves of four populations of the CAM bromeliad *Aechmea bromeliifolia* (*N* = 5). Bars represent standard errors. (A) Herbaceous marsh (flooded/exposed). (B) Dry shrubland (unflooded/exposed). (C) Swamp forest (flooded/shaded). (D) Dry forest (unflooded/shaded). Numbers inside the graphs represent ΔH⁺ values (mmol m⁻²).
stood by examining the leaf anatomy and physiological data.

RESPONSE TO THE LIGHT/FLOODING INTERACTION
The exposed, unflooded population had the lowest values for number of cell layers and width for chlorophyll and water parenchyma, suggesting stress and malfunctioning under these conditions (see Tood, Richardson & Sengupa, 1974). It has often been reported that, as light intensity increases, the thickness of mesophyll tissue (chlorophyll + water parenchyma) increases, conferring a higher degree of acclimation to high light (e.g. Chazdon & Kaufmann, 1993). This pattern of development was matched by the rosettes studied, except for those established in the exposed, unflooded site. Considering that the leaves of the exposed, flooded rosettes conformed to the above pattern, the lower development of mesophyll tissues in the leaves of the exposed, dry rosettes is probably a symptom of stress rather than of acclimation to high light levels. The stress is probably due to the interaction between high light and absence of flooding, rather than to high light alone, since the exposed, flooded population showed the largest mesophyll development. The similarity of the small values for chlorophyllous tissue parameters observed between exposed, unflooded plants and the shaded ones suggests that this response, typically a plastic acclimation response to

Figure 8. Field measurements from dawn to dusk of variable to maximal fluorescence ($F_v/F_m$) in dark-adapted leaves of four populations of the CAM bromeliad Aechmea bromeliifolia ($N = 5$). Bars represent standard errors. (A) Herbaceous marsh (flooded/exposed). (B) Dry shrubland (unflooded/exposed). (C) Swamp forest (flooded/shaded). (D) Dry forest (unflooded/shaded).
low light (Björkman, 1981), may be symptomizing stress in the former case and acclimation in the latter.

The stress imposed on *A. bromeliifolia* by the exposed, unflooded conditions was further confirmed by the lowest photochemical efficiency of this population. Throughout the whole day, $F_v/F_m$ were always considerably below optimum (0.83). However, after a marked depression at midday, early morning values were recovered later in the afternoon ($F_v/F_m = 0.7$ in both cases) showing that photoinhibition in this population may have been a dynamic rather than a chronic event (Osmond, 1994). In the exposed, flooded population photochemical efficiency was also below optimum during the whole day; however, it was higher in the early morning and late afternoon (0.75) than its unflooded counterpart. Although the presence of CAM is often associated with extreme conditions such as water limitation (Lüttge, 1997), and is known to alleviate photoinhibition under light or water stress (Griffiths et al., 1989), this CAM plant suffers photoinhibition for most of the day in exposed, unflooded conditions in the restiga. The other physiological traits investigated further confirm this pattern: the exposed, flooded population was less photoinhibited, had a higher $\Delta F^+$, as well as a more negative $\delta^{13}$C than its unflooded counterpart.

**CONCLUSION**

In conclusion, the phenotypic variation found among the four populations indicates the high plasticity of *A. bromeliifolia*, even within a very narrow geographical range. However, the joint analysis of population patterns, and morphological, anatomical and physiological characteristic of the individuals showed that while three populations are acclimated to distinct combinations of light and flooding regime, one, the exposed, unflooded population, was clearly under stress. This integrated approach was essential to establish which variation conferred acclimation and which was a mere stress symptom. Future transplant experiments and studies on genetic diversity will be necessary to differentiate phenotypic and genotypic variation.

**ACKNOWLEDGEMENTS**

We thank D. Benzing for valuable suggestions on an earlier version of the manuscript; D.S.D. Araujo for comments and linguistic advice; U. Lüttge, H. Ziegler and H. Griffiths for the carbon isotope analyses; H. Griffiths for lending us the PEA and the infrared gas analyser; R.C. Vieira for laboratory facilities; R.C. Reis and C.F.C. de Sá for accommodation and logistic support during our field excursions; Fundação O Botânico and Pronex-Finep for funding; CNPq and The Margaret Mee Amazon Trust for research grants. FRS thanks the hospitality of all colleagues in Prof. Lütte’s working group at Darmstadt University of Technology, Germany, where this final version was produced.

**REFERENCES**


