



How are endemic and widely distributed bromeliads responding to warming temperatures? A case study in a Brazilian hotspot[☆]

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ABSTRACT

The increase in mean global temperature is causing extensive changes in ecosystems. However, little is yet known about the heat tolerance of neotropical plant species. Here, we investigate heat tolerance variation in both restricted and widely distributed bromeliad species co-occurring in *campo rupestre*, a megadiverse ecosystem in central and eastern Brazil. We determined the heat tolerance of the photosynthetic apparatus using chlorophyll fluorescence measurements to test if the endemic species *Vriesea minarum* is more heat sensitive than two widely distributed species, *Vriesea bituminosa* and *Aechmea nudicaulis*. Furthermore, we tested if the distinct photosynthetic metabolisms of the species, sun exposure, and rainfall seasonality of *campo rupestre* influence this outcome. Our results show that, contrary to our expectations, the endemic *campo rupestre* species did not show the greatest heat sensitivity, but did have one of the lowest heat tolerance plasticities. The CAM bromeliad *A. nudicaulis* was more heat tolerant than the other bromeliad species, but both heat tolerance and its plasticity are highly affected by sun exposure and the rainfall seasonality of *campo rupestre*. The low values and plasticity of *V. minarum* thermal tolerance could indicate that the threat of global warming could be greater for this *campo rupestre* endemic species. Our results also indicate that heat tolerance, especially the ability to withstand stressful temperatures for a long time, is an important parameter that differentiates the ecological strategies of these bromeliads species.

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1. Introduction

Temperatures below 10 °C and above 35 °C can cause permanent damages to most plants (Went, 1953; Berry and Bjorkman, 1980). The rise in the mean global temperature observed over the last century (IPCC, 2007) and an increase of ca. 2 °C in the maximum global temperature during the first half of the 21st century (IPCC, 2012) are causing deep alterations to ecosystems, from damages to the chloroplast integrity of plants (Berry and Bjorkman, 1980; Yamane et al., 2000; Hüve et al., 2011; Zhang et al., 2012) to mass extinctions (e.g. Crowley and North, 1987; Malcolm et al., 2006; Carpenter et al., 2008; Weigelt et al., 2016). But prior to going extinct, species may respond to climate change by altering their phenology and other physiological responses (i.e. phenotypic

plasticity); developing new evolutionary strategies; changing their abundance and inter-specific interactions; and shifting their distribution (Holt, 1990; Parmesan, 2006; Blois et al., 2013; IPCC, 2014; Parmesan and Hanley, 2015). Studies have suggested, for instance, that some species have become restricted to refuges (see Rull, 2009; Stewart et al., 2010), shifting their ranges poleward and toward higher elevations due to the warming temperatures since the last glacial maximum (LGM; e.g., Parmesan and Yohe, 2003; Root et al., 2003; Parmesan, 2006; Colwell et al., 2008; Rosenzweig et al., 2008; Chen et al., 2011; Bässler et al., 2013; Pecl et al., 2017). Thus, species currently restricted to interglacial refuges may be more sensitive to warming temperatures than widely distributed species.

Due to its past climatic stability, the *campo rupestre* ecosystem represents an example of interglacial refuge in the Neotropics (Bonatelli et al., 2014; Barbosa et al., 2015). This vegetation complex is endemic to Brazil, and it is usually restricted to altitudes from 900 to over 2,000 m a.s.l., forming a mosaic archipelago-like system on low water-holding rocks of mountaintops surrounded by lowlands (Alves et al., 2014; Silveira et al., 2016). Studies have shown that *campo rupestre* is probably the most ancient open vegetation in eastern South America, comprising a great diversity and rate of endemism (Alves et al., 2014; Silveira et al., 2016). Nevertheless, recent studies indicate that *campo rupestre* vegetation could lose

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from 44% (Bitencourt et al., 2016) to 95% (Fernandes et al., 2012) of its current area and about 25% of its angiosperm species due to climate change (Bitencourt et al., 2016). Such reduction represents the extinction of more than 400 microendemic plant species which are currently restricted to future unsuitable areas (Bitencourt et al., 2016). Such potential scenario is a consequence of the habitat reduction caused by a warming up to 5 °C in temperature and an increase in frequency of extremely dry seasons. The low dispersal ability of most endemic species makes them unable to reach other suitable mountains; and their high specialization level make them more sensitive to climate changes (Christensen et al., 2007; Dawson et al., 2011; Bitencourt et al., 2016). Despite these worrying forecasts, little is known about how these endemic species would respond to overcome the warming temperatures.

Bromeliaceae is one of the most representative plant families in *campo rupestre* and nearly half of the bromeliad species occurring in such environment are endemic (Versieux et al., 2008; Silveira et al., 2016). This family is also one of the best examples of adaptive radiation in the Neotropics (Benzing, 2000; Givnish et al., 2014; Givnish, 2015; Palma-Silva et al., 2016). Crassulacean acid metabolism (CAM), for example, is often noted as a key innovation in the family that allowed the exploitation of arid environments, due to its mechanism that closes the stomata during the daytime and avoids excessive water loss during dry periods (Givnish et al., 2014; Silvestro et al., 2014; Palma-Silva et al., 2016). Global climatic changes may favor species with CAM metabolism, due to their supposed higher thermal tolerance (e.g., Yamada et al., 1996; Weng and Lai, 2005) and the anthropogenic increase of arid areas (Nobel, 1988; Cushman and Borland, 2002; Mercier and Freschi, 2008; Osmond et al., 2008). However, Chaves et al. (2015) showed that the daily organic acid accumulation of CAM plants has a strong negative impact on their thermal tolerance (see also Yamori et al.,

2014a, 2014b). Therefore, it remains unclear how these plants will respond to ongoing climate change.

Inferences about potential responses of species to climate change from their current distribution can be a good strategy, since some works report a strong relationship between ecological characteristics and niche projections (e.g. Thuiller et al., 2005; Garcia et al., 2014). Here, we aim to test whether an endemic bromeliad species of *campo rupestre* is more sensitive to warming temperatures than species with wider distributions. For such, we measured the fluorescence of photosystem II through two methods: ramping and static temperature assays. We also examine whether morphophysiological traits, such as photosynthetic pathways (i.e., CAM and C₃ photosynthesis), sun exposure, and the marked rainfall seasonality of the *campo rupestre*, can bias the outcome. Finally, we test whether heat tolerance and its plasticity can distinguish the ecological strategies of these bromeliads.

2. Material and methods

2.1. Study site and plant species

This study was carried out in the Piedade Mountains (*Serra da Piedade*), situated within the over-exploited Iron Quadrangle, in the southern-most section of the core area of the *campo rupestre* in the Espinhaço Range, Eastern Brazil (Alves et al., 2014; Silveira et al., 2016). The Piedade Mountains have a maximum altitude of 1,746 m a.s.l., and the highest section presents a typical subtropical high-altitude climate, with a well-defined dry season between April and September, and the frequent occurrence of fog (Brandão and Gavilanes, 1990; Marques and Lemos-Filho, 2008). The surroundings (Belo Horizonte city, 40 km from *Serra da Piedade*) show a well-defined dry season with an average temperature of 19 °C and 6 mm of precipitation in the driest month, and a well-defined

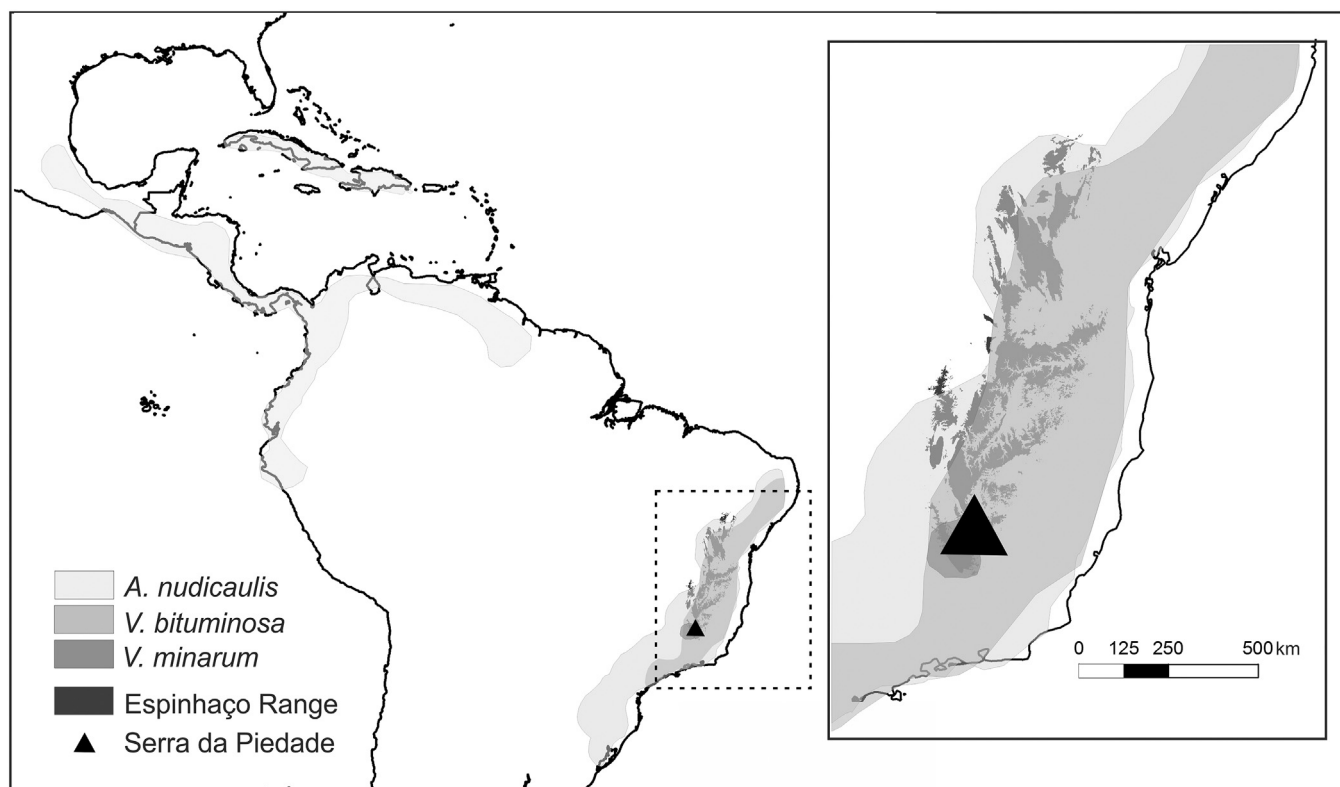


Fig. 1. Schematic representation of the distribution of *Aechema nudicaulis*, *Vriesea bituminosa*, and *Vriesea minarum* according the online database of the Global Biodiversity Information Facility (GBIF; via www.gbif.org).

wet season, with an average temperature of 23 °C and 322 mm of total precipitation in average on the wettest month (historical data from January 1990 to December 2016 achieved in the website www.inmet.gov.br). Frosts and temperatures close to 0 °C are common at the highest altitudes (Brandão and Gavilanes, 1990). Our study area is above 1,400 m.a.s.l. and comprises two microenvironments: a xeric zone, highly exposed to sun and winds, characterized by the occurrence of iron-quartzite rock outcrops and herbaceous-shrubby vegetation (hereafter referred to as sun-exposed environment); and a shaded zone within an altitudinal cloud forest, characterized by the presence of shrubby and arboreal vegetation and high moisture (hereafter, shaded environment).

Here, we selected three bromeliad species: two C₃ species, *Vriesea minarum* L.B. Sm and *Vriesea bituminosa* Wawra, from the Tillandsioideae subfamily, and a CAM species, *Aechmea nudicaulis* (L.) Griseb., from the Bromelioideae subfamily. *Vriesea minarum* is a rupicolous species endemic to the *campo rupestre* (Fig. 1; Forzza et al., 2012), occurring in sun-exposed environments of the Piedade Mountains. *Vriesea bituminosa* occurs as a rupicolous or epiphytic bromeliad in sections of forests associated with outcrops in eastern Brazil and in Venezuela (Fig. 1; Coser, 2008; Forzza et al., 2012). At the study site, this species is often found in shaded areas, where we collected all samples. The CAM species, *A. nudicaulis*, is widely distributed throughout the Neotropics, from Mexico to Peru, the Caribbean, eastern Brazil, and Guyana (Fig. 1; Smith and Downs, 1979). In Brazil, the species occurs in sandy coastal zones, moist forests, rock outcrops in the Atlantic Forest, and *campos rupestres* (Bert and Luther, 2005). In our study site, *A. nudicaulis* were sampled in both sun-exposed and shaded environments, designated as “sun-exposed *A. nudicaulis*” and “shaded *A. nudicaulis*”, respectively.

2.2. Microclimatic information

To measure the seasonal climate differences of the study site, we recorded photosynthetic photon flux density (PPFD) and air temperature data from sun-exposed and shaded environments on all sampling days during the dry and rainy seasons (Fig. 2). We also measured relative humidity in the shaded environment and the dry-season surface temperature of a sun-exposed rocky outcrop. All measurements were made using sensors coupled with data loggers (LI-1400, LI-COR Inc. and TD-880, ICEL).

2.3. Sampling

We employed two different methods to determine photosynthetic thermal tolerance of the studied species: a ramping assay (under increasing temperatures) and a static assay (under constant high temperature; see following sections). For the ramping assay, we collected ~1.5 cm² leaf samples from the top (upper third) and basal (bottom third) sections of one young and totally expanded leaf from five individuals of each studied group (sun-exposed *A. nudicaulis*, shaded *A. nudicaulis*, *V. minarum*, and *V. bituminosa*). We sampled these individuals during the dry and rainy seasons, at 08:00 and 15:00, when organic acids reach their maximum and minimum accumulations in *A. nudicaulis* (due to CAM photosynthesis; data not shown). We also sampled the same individuals to determine morphophysiological traits (see section 2.6). For the static assay, we collected ~1.5 cm² samples from the top and basal leaf regions of five individuals from each studied group during the rainy season, at 15:00; see follow sections).

2.4. Heat tolerance of photosystem II (PSII): ramping assay

To determine the thermal tolerance of photosystem II (P_{siII}) under increasing temperatures (ramping assay), we placed each of

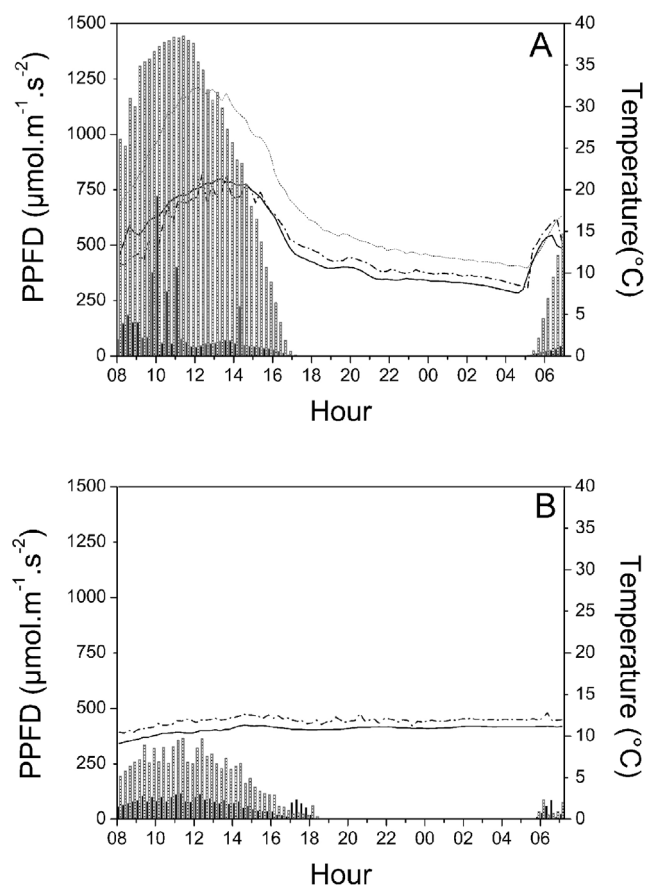


Fig. 2. Photosynthetic photon flux density (PPFD) and average temperature of a day in the dry (A) and rainy seasons (B). Full and dashed bars represent the PPFD of shaded and exposed environments, respectively. Solid and dashed lines represent, respectively, the temperature of shaded and exposed environments. The dotted line, in A, represents the temperature of rock outcrop.

the collected samples in a hermetically sealed plastic bag and kept it under natural light, partially shaded, for 24 h. We proceeded with a thermal tolerance test in the laboratory, using an *ex vivo* procedure. Previous tests of this procedure have shown that it does not significantly influence the measured potential quantum yield (variable fluorescence/maximum fluorescence; F_v/F_m) of the leaves (data not shown). Subsequently, we removed the samples from the plastic bags and kept them in the dark and at room temperature (~25 °C) for 15 min; we then measured the initial F_v/F_m using a modulate fluorometer (MINI-PAM, Walz). The thermal tolerance test was conducted as described by Godoy et al. (2011), but using a thermostatic water bath as a heater (214D2, QUIMIS). We sealed the aluminum plate containing the samples with a plastic bag and used a thermocouple linked to a digital thermometer (TD-880, ICEL) to check the temperature of samples. Leaf samples were subjected to increasing temperatures, from 35 °C—the upper limit of the optimal temperature range for most plants (Went, 1953; Berry and Bjorkman, 1980)—to 65 °C, with an average increase of 1 °C every 3 min. We measured F_v/F_m once after every increase of 2 °C. The data were used to determine the critical temperatures that promote a 15% (T_{15}) and 50% (T_{50}) reduction of the initial F_v/F_m , using a sigmoidal equation (Fig. 3A) as described by Knight and Ackerly (2003). This analysis was performed using a purpose-built function in R statistical software version 3.0.1 (see Supplementary Material; R Core Team, 2014).

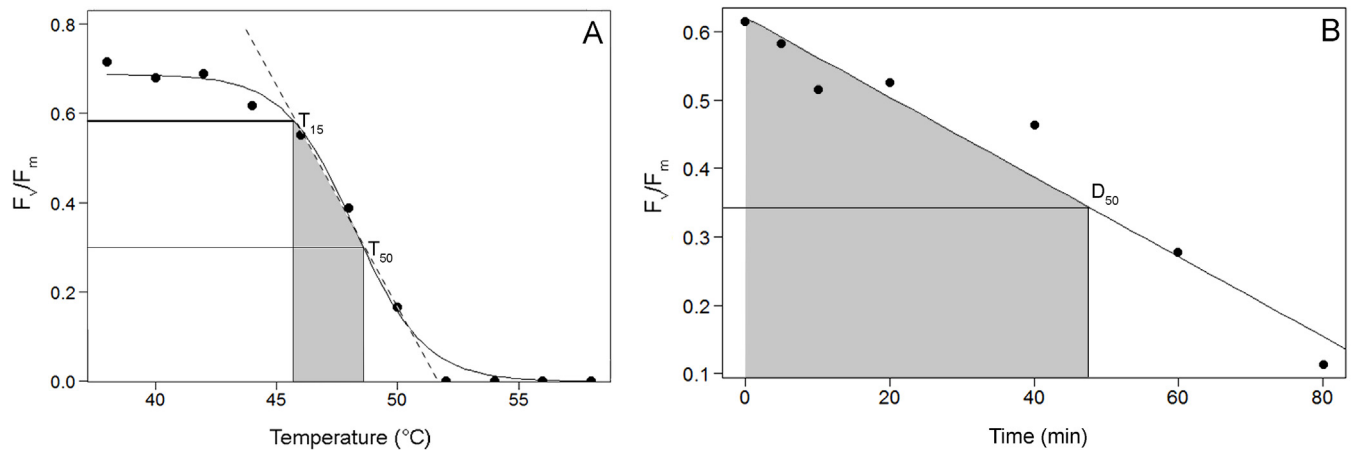


Fig. 3. Schematic representation showing the F_v/F_m decrease (filled lines) under increasing temperature (A) and under time of exposure to a constant high temperature (43 °C; B), highlighting the critical parameters (T_{15} , T_{50} , and D_{50}). The dashed line in A represents the linear function of F_v/F_m decrease between T_{15} and T_{50} . Shaded areas in A and B represent, respectively, the temperature differences and the time elapsed between the decay of 15% and 50% on initial F_v/F_m .

2.5. Heat tolerance of PSII: static assay

To test if the species most tolerant to increasing temperatures is also the most tolerant to constant stressful temperatures, we followed an experimental procedure similar to the one described above, except that the F_v/F_m measures were made before immersing the samples in a water bath and again after 1, 5, 10, 20, 40, 60, and 80 min, at a constant temperature of 43 °C (which represents the average of T_{15} values obtained in the ramping assay for all the samples of *Vriesea* species, the less heat tolerant groups; see Table S1). We estimated the critical time, in minutes, that would promote a 50% reduction of the initial F_v/F_m (D_{50}), using a linear function (Fig. 3B). This analysis was also performed using a purpose-built function in R statistical software version 3.0.1 (see Supplementary Material; R Core Team, 2014).

2.6. Morphophysiological traits and heat tolerance

To verify the relationships between heat tolerance and other morpho-physiological traits, and to distinguish the ecological strategies of each experimental group (sun-exposed *A. nudicaulis*, shaded *A. nudicaulis*, *V. minarum*, and *V. bituminosa*), we determined the daily variation of titratable acidity (as per Hartsock and Nobel, 1976), succulence index (SI; as per Ogburn and Edwards, 2010, 2012), and relative water content (RWC) of all samples. Moreover, we estimated the stomata and scale densities of the top and basal sections of the leaves from each experimental group. To do this, leaf samples were fixed in FAA50 (Johansen, 1940) and stored in ethanol 70% for subsequent analysis. The epidermises were excised by immersion in 66% commercial sodium hypochlorite for three days. We then stained the epidermises with safranin-astra blue (Bukatsch, 1972) and mounted them in Kaiser gelatin (Kraus and Arduin, 1997). The adaxial and abaxial stomata and scales within six 0.06-mm² fields on each sample were counted using a microscope with a camera lucida (Stemi, 2000, Zeiss). We considered the scale density as the sum of both faces of each sample.

To overcome the variability of each physiological trait, we estimated the overall phenotypic plasticity based on the relative distance phenotypic index (RDPI), as described by Valladares et al. (2006). For each experimental group (sun-exposed *A. nudicaulis*, shaded *A. nudicaulis*, *V. minarum*, and *V. bituminosa*), we calculated a combination of the relative distances between the values for these traits for all collection times of day and seasons, generating only one distance value.

Table 1

Means and standard errors of projected D_{50} values of all experimental groups at 15:00 in the rainy season. Same letters represent values statistically equal among all groups.

Species	Environment	Leaf region	D_{50} (min.)
<i>A. nudicaulis</i>	Sun-exposed	Top	80.86 ± 11.8 ^b
		Basal	103.17 ± 5.3 ^b
	Shaded	Top	157.87 ± 17.4 ^a
		Basal	163.66 ± 28.1 ^a
<i>V. minarum</i>	Sun-exposed	Top	73.19 ± 4.8 ^b
		Basal	81.56 ± 2.3 ^b
<i>V. bituminosa</i>	Shaded	Top	70.59 ± 1.8 ^b
		Basal	73.02 ± 0.9 ^b

2.7. Statistical analysis

We performed linear fixed-effect models (LMEs) to test whether the variation in thermal tolerance (T_{50} , D_{50} , and $RDPI_{T50}$ values) is associated with the measured morphophysiological traits, and whether thermal tolerance and the morphophysiological traits are related to each other, according Fig. 4A and 4B, respectively. We used the explanatory variables as fixed effects, and individual labels and their leaf regions as random variables. We tested the significance of the models through an analysis of variance (ANOVA), sequentially removing all non-significant variables ($p > 0.05$) with the greatest p -values. To test if the plants with the highest thermal tolerance to increasing temperature are also the most thermal tolerant to static stressful temperatures, and to test whether both assays are related, we performed a simple regression between T_{50} and D_{50} values of all studied groups.

Finally, we performed a canonical discriminant analysis (CDA) followed by general linear models (GLM; see Fig. S1) to evaluate whether thermal tolerance, together with the measured morphophysiological variables, could discriminate the ecological strategies of each species. All statistical analyses were performed with the R packages “nlme”, “candisc”, and “glmulti” (Wagenmakers and Farrell, 2004; Friendly, 2007; Kenny and Hoyt, 2009).

3. Results

3.1. Heat tolerance of PSII

Generally, shaded *Aechmea nudicaulis* and *Vriesea bituminosa* individuals showed the highest and the lowest heat tolerances, respectively, under both ramping and static thermal tolerance assays (Table 1, Fig. 4, and Tables S1 and S2). Considering the mean

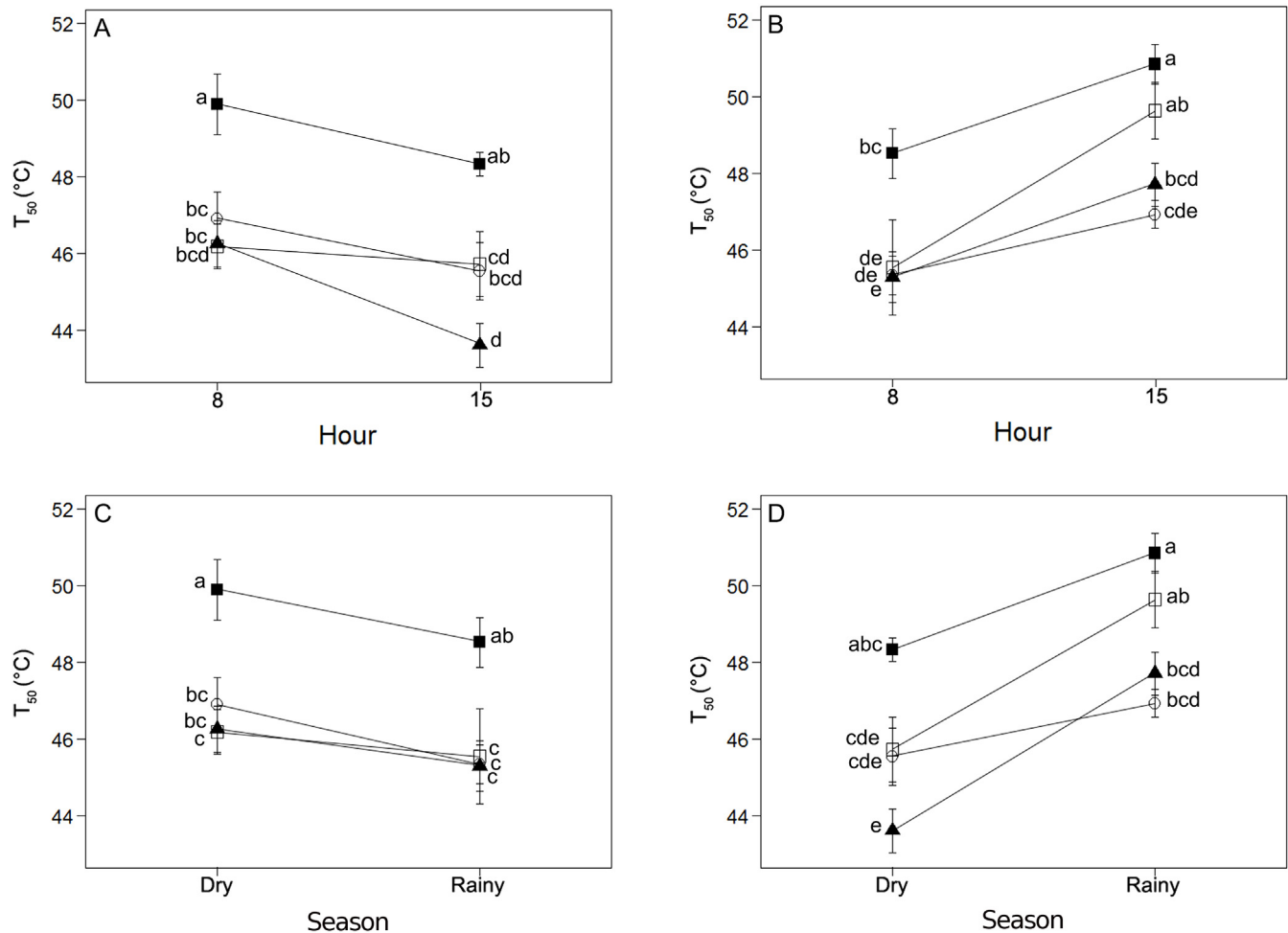


Fig. 4. Norms of reaction of T_{50} variations of sun-exposed (open squares) and shaded (closed squares) *A. nudicaulis*, *V. minarum* (open circles) and *V. bituminosa* (closed triangles), between times of day in the dry (A) and rainy (B) seasons, and between seasons, at 08:00 (C) and 15:00 (D). Same letters represent no statistical differences ($p > 0.05$). Each average showed also group the values of top and basal leaf sections of each individual.

of the T_{50} values of all studied groups, we found an interaction among day times and seasons, with greater values at 15:00 in the rainy season and lower values at 15:00 in the dry season (Fig. 4 and Table S1). In the ramping assay performed in the morning, as well as in the static assay, we detected a significantly higher thermal tolerance in shaded *A. nudicaulis* than in sun-exposed plants, which had a thermal tolerance similar to that of *Vriesea* species (Tables 1, and S1). No significant differences were detected between leaf regions ($p > 0.05$; Table 1). We observed directly proportional relationships between T_{50} and D_{50} (Fig. 5).

3.2. Morphophysiological traits and heat tolerance

The relationships between heat tolerance and morphophysiological variables were significant only for titratable acidity of the basal leaf regions of *A. nudicaulis* sampled in the rainy season. Titratable acidity was inversely proportional to the T_{50} values (Fig. 6). Regarding the plasticity of morphophysiological traits, sun-exposed *A. nudicaulis* showed some of the greatest significant values for most of them, except for Titratable acidity and SI in the top and basal leaf regions, respectively (Table 2). In these cases, shaded *A. nudicaulis* was the most plastic. For most of the other cases, shaded *A. nudicaulis* was, together with *V. minarum*, the less plastic group (Table 2).

The top of sun-exposed *A. nudicaulis* leaves had the greatest daily variation of titratable acidity (ΔH^+ ; Table S2). Their SI val-

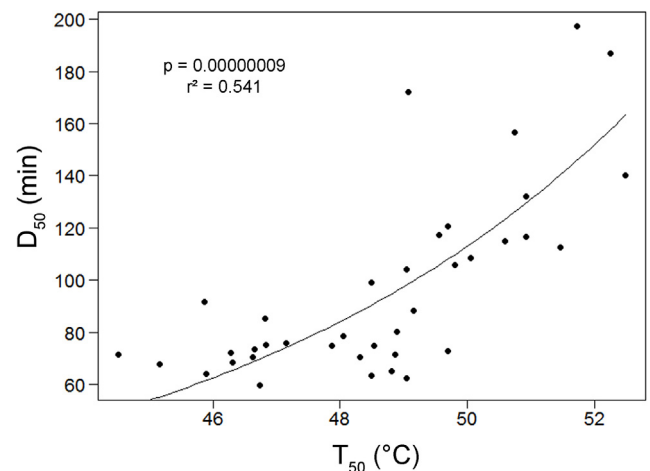


Fig. 5. Exponential relationship between T_{50} (ramping assay) and D_{50} (static assay) values of all studied groups obtained during the rainy season at 15:00.

ues, however, were not statistically distinct from the other studied groups (Table S2). For stomata density, *V. minarum* and *V. bituminosa* had, respectively, the lowest and the highest absolute values among the top regions of the leaves (Table S3). For scale density, *V. minarum* and *V. bituminosa* had, respectively, the lowest and the

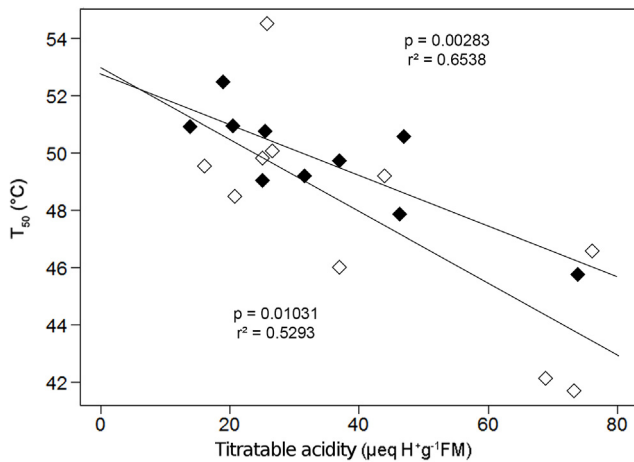


Fig. 6. Negative relationships between T_{50} and titratable acidity in basal region of sun-exposed (open diamond) and shaded (closed diamond) *A. nudicaulis*.

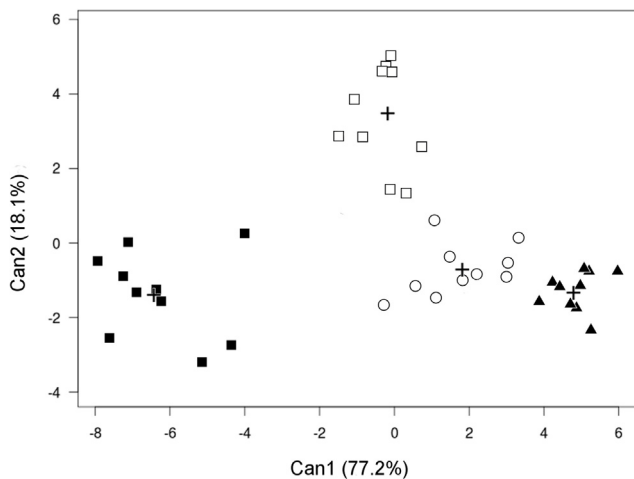


Fig. 7. Biplot representation of the scores on the first two axes of the canonical discriminant analysis for 13 morphophysiological variables of sun-exposed (open squares) and shaded (closed squares) *A. nudicaulis*, *V. minarum* (open circles), and *V. bituminosa* (closed triangles). The centroids of each group are represented by a cross.

highest absolute values in the basal regions of the leaves (Table S3). However, stomata and scale density of C_3 species were not statistically distinct from those of *A. nudicaulis* (Table S3).

The ecological strategies of each experimental group (sun-exposed *A. nudicaulis*, shaded *A. nudicaulis*, *V. minarum*, and *V. bituminosa*) were clearly discriminated by CDA analysis (Fig. 7). The first canonical axis accounted for 77.2% of trait variation and discriminated almost all studied groups (Fig. 7). This axis grouped the variation of mainly SI, D_{50} , and the RDPI of titratable acidity (Table

Table 3

Results of AIC_i (Akaike criterion for model i), $\Delta_i(AIC)$ ($AIC_i - \min AIC$), and $w_i(AIC)$ (rounded Akaike weights) for all competing models. Bold rows indicate the best fit models, based on $\Delta_i(AIC) < 2.0$.

Model	AIC_i	$\Delta_i(AIC)$	$w_i(AIC)$
D50 + RDPI.acidity + acidity + RDPI.SI	18.280	0.000	5.26E-001
SI + D50 + RDPI.acidity + acidity + RDPI.SI	19.387	1.108	3.02E-001
D50 + RDPI.acidity + acidity	21.997	3.718	8.20E-002
SI + D50 + RDPI.acidity + acidity	23.303	5.024	4.27E-002
SI + D50 + acidity + RDPI.SI	24.089	5.809	2.88E-002
RDPI.acidity + acidity + RDPI.SI	28.013	9.734	4.05E-003
D50 + acidity + RDPI.SI	28.551	10.272	3.09E-003
SI + D50 + acidity	28.907	10.627	2.59E-003
RDPI.acidity + acidity	29.038	10.759	2.43E-003
SI + RDPI.acidity + acidity + RDPI.SI	30.006	11.726	1.50E-003
acidity + RDPI.SI	30.797	12.518	1.01E-003
D50 + acidity	30.927	12.647	9.43E-004
SI + RDPI.acidity + acidity	31.025	12.745	8.98E-004
SI + acidity + RDPI.SI	31.161	12.881	8.39E-004
acidity	32.549	14.269	4.19E-004
SI + acidity	33.985	15.705	2.05E-004
SI + D50 + RDPI.acidity + RDPI.SI	39.168	20.888	1.53E-005
SI + D50 + RDPI.SI	39.755	21.476	1.14E-005
SI + D50 + RDPI.acidity	39.772	21.492	1.13E-005
SI + RDPI.SI	41.875	23.596	3.96E-006
SI + D50	43.112	24.833	2.13E-006
SI + RDPI.acidity + RDPI.SI	43.451	25.172	1.80E-006
D50 + RDPI.acidity	43.477	25.198	1.78E-006
D50 + RDPI.acidity + RDPI.SI	44.236	25.956	1.22E-006
RDPI.acidity	44.839	26.559	8.99E-007
RDPI.acidity + RDPI.SI	45.042	26.763	8.12E-007
SI	45.116	26.836	7.83E-007
SI + RDPI.acidity	45.275	26.995	7.23E-007
RDPI.SI	46.300	28.020	4.33E-007
NULL	46.987	28.707	3.07E-007
D50 + RDPI.SI	48.089	29.810	1.77E-007
D50	48.695	30.415	1.31E-007

S4). The second canonical axis — most associated with variation of titratable acidity, D_{50} and the RDPI of SI — accounted for 18.1% of the trait variation (Table S4) and differentiated sun-exposed *A. nudicaulis* from the other groups (Fig. 7). The best model to describe the variances of discriminated functional groups (based on $\Delta AIC < 2.0$) included the average of D_{50} , the mean and RDPI of titratable acidity, and the RDPI of SI (Table 3).

4. Discussion

Our results did not corroborate the hypothesis that the bromeliad species restricted to the *campo rupestre*, *Vriesea minarum*, is more heat sensitive than the other studied species. Instead, we demonstrated that its heat tolerance was similar to the individuals of the most widely distributed species, *Aechmea nudicaulis*, growing under the same conditions of *V. minarum*. Despite this result, *V. minarum* showed a low thermal tolerance plasticity that was statistically equal to those of shaded individuals of *A. nudicaulis*. On the other hand, the low thermal tolerance and the plasticity of *V. minarum* indicate a great level of specializa-

Table 2

Mean of total plasticity index (RDPI) of T_{50} , titratable acidity, SI and RWC of each experimental group. Same superscript letters represent values statistically equal between all experimental groups, for each leaf region. Asterisks represent differences statistically significant ($p < 0.05$) between leaf regions.

Parameter measured	Leaf region	Sun-exposed <i>A. nudicaulis</i>	Shaded <i>A. nudicaulis</i>	<i>V. minarum</i>	<i>V. bituminosa</i>
T_{50}	Top	0.031 ^a	0.020 ^c	0.022 ^{bc}	0.026 ^b
	Basal	0.038 ^a	0.024 ^c	0.021 ^c	0.031 ^b
Titratable acidity	Top	0.219 ^b *	0.350 ^a	0.190 ^b	0.182 ^b
	Basal	0.410 ^a *	0.310 ^b	0.208 ^c	0.219 ^c
SI	Top	0.119 ^a *	0.116 ^a *	0.101 ^a	0.119 ^a
	Basal	0.074 ^c *	0.162 ^a *	0.093 ^c	0.119 ^b
RWC	Top	0.125 ^a	0.058 ^b *	0.111 ^a *	0.137 ^a
	Basal	0.138 ^a	0.102 ^{bc} *	0.077 ^c *	0.125 ^{ab}

tion of this species to the current conditions of *campo rupestre*, and the extra susceptibility of this species to climate change (see Vié et al., 2009). Extrapolating these results and considering the high endemism rate and specialization of *campo rupestre* species (see Silveira et al., 2016), our results may presage a worrying scenario for this vegetation profile under global warming.

The shaded individuals of *A. nudicaulis* showed the highest heat tolerance among the studied groups. In fact, we found a wide variation in heat tolerance between sun-exposed and shaded individuals of this species (more than 3 °C). Overall, the sun-exposed individuals of *A. nudicaulis* showed similar values to *Vriesea* species. Regarding these differences in *A. nudicaulis* responses, the more constant conditions along days and years of shade environments seems to increase and stabilize (i.e. reduce the plasticity) the thermal tolerance of this species. These results are consistent with the findings that light conditions interact strongly with heat stress and can, at high intensity, damage the photosynthetic apparatus (Valladares and Pearcy, 1997; but see Krause et al., 2016). On the other hand, since *A. nudicaulis* is from Bromelioideae and *V. minarum* and *V. bituminosa* are from Tillandsioideae subfamily, the similar thermal tolerance observed among the last two species may be explained not only by the influence of their photosynthetic pathway (i.e. C₃ photosynthesis), but also by a phylogenetic signal, as shown by Marques et al. (2012) and Müller et al. (2016) for seed germination of bromeliads.

The low plasticity of heat tolerance found for all studied groups in this study (RDPI < 0.04) corroborated the findings of Araújo et al. (2013), which show that plants often exhibit low variability in heat tolerance. The authors suggest that this is a consequence of the limited variation in the ability of organisms to avoid the destabilizing effects of elevated temperatures on membranes and proteins, since the changes in lipid composition of membranes and the increased production of heat shock proteins are normally not enough to enable them to deal with temperatures above 45 °C. Thus, despite being small, the observed differences in T₅₀ plasticity among tested groups are ecologically relevant for *campo rupestre* bromeliads.

Among the few studies providing estimates of photosynthetic heat tolerance in CAM plants, Weng and Lai (2005) — who assessed the temperature at which the minimal fluorescence (F₀) starts to increase sharply (T_c) — found that the CAM bromeliad *Ananas comosus* showed greater heat tolerance than some C₃ and C₄ species. In another study comparing the same species to other C₃ species, *A. comosus* was again the most heat tolerant (Yamada et al., 1996). Transforming the T_c values obtained by Weng and Lai (2005) into T₅₀, using the equations provided by Knight and Ackerly (2003), we obtained a T₅₀ close to 49.5 °C for *A. comosus*. This value is quite similar to the greatest T₅₀ values observed for *A. nudicaulis* in the current study (T₅₀ = 50.8 °C), and to previously published values of C₃ species from a hot Californian desert in USA (Knight and Ackerly, 2002). In another study, based on a T₅₀-like index for electrolyte leakage, Didden-Zopf and Nobel (1982) show that the CAM cactus *Opuntia bigelovii*, from the same Californian desert, had a T₅₀-like index similar to the greatest values recorded by Knight and Ackerly (2002) (closed to 60 °C). In summary, although *A. comosus* and *A. nudicaulis* did not usually experience such high temperatures, they showed a heat tolerance similar to that of most C₃ plants native to this desert. Therefore, it is likely that CAM plants are essentially more capable of tolerating increasing temperatures, but their heat tolerance can be diminished by other environmental stresses such as sun exposure. Furthermore, the assumed greater heat tolerance of CAM plants, together with their water conservative mechanism, agree with previous assumptions that global increase in average temperature and arid areas may favor these plants (e.g. Graham and Nobel, 1996; Reyes-García and Andrade, 2009).

The negative relationship between heat tolerance and titratable acidity of shaded and sun-exposed *A. nudicaulis* was reflected in the

daily variations observed for both physiological parameters during the rainy season. This relationship was reported for other CAM plants (Larcher, 1980; Lösch and Kappen, 1983; Kappen and Lösch, 1984; Krause et al., 2016) as well as for another CAM bromeliad, for which Chaves et al. (2015) showed that, under controlled conditions, leaf acidity decreases and thermal tolerance increases as temperature increases. This mechanism may have evolved due to the increase in tonoplast permeability and acid remobilization from vacuoles in response to higher temperatures (Friemert et al., 1988; Behzadipour et al., 1998; Savchenko et al., 2002; Lin et al., 2006), avoiding acid leakage during a warming event. On the other hand, in agreement with our observations for *V. bituminosa*, other studies have also reported daily variations of thermal tolerance in C₃ plants (e.g., Braun et al., 2002; Froux et al., 2004; Campos, 2011). This variation may be related to changes in sun-exposure or leaf temperature throughout the day (Braun et al., 2002; Froux et al., 2004), or may even be due to sensitivity to the water stress experienced at the hottest time of day during the dry season (see Goltsev et al., 2012; Rollins et al., 2013; Zivcak et al., 2013; Zivcak et al., 2014).

Heat tolerance was a very important variable for distinguishing the ecological strategies of the studied groups. Thus, this parameter could be used as a trait to differentiate the functional roles of plants. Furthermore, the strong correlation between both assays of heat tolerance demonstrated that the studied bromeliads do not have the thermal trade-off described for animals by Rezende et al. (2014). That is, the most tolerant bromeliad species to increasing temperature was also the one able to withstand stressful temperatures for longer periods. This lack of thermal trade-off may be common in plants, since their physiological strategies are distinct from those of animals; or it may be a consequence of the pronounced daily and annual temperature variation in *campo rupestre* formations (see Alves et al., 2014; Silveira et al., 2016).

Our results showed that bromeliads' ability to overcome hot temperatures, together with its plasticity, may be relevant for explaining their current distribution. In this case, the species currently restricted to the inter-glacial refuge (i.e. *campo rupestre*), may be more threatened by climate changes due to their lower heat tolerance and plasticity. Our study also indicated that CAM bromeliads may potentially be more heat tolerant than C₃ plants, but their heat tolerance and its plasticity are highly affected by sun exposure conditions and climate seasonality. Furthermore, our results also indicated that heat tolerance, especially the ability to withstand stressful temperatures for a long time, is an important parameter that differentiates the ecological strategies of these bromeliads species. This is the first case study evaluating the ability of *campo rupestre* species to overcome warming temperatures. Further investigation on this topic will certainly improve our understanding about the effects of climate change on this megadiverse ecosystem.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.flora.2017.05.003>.

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