



Does oxidative stress determine the thermal limits of the regeneration niche of *Vriesea friburgensis* and *Alcantarea imperialis* (Bromeliaceae) seedlings?



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ARTICLE INFO

Keywords:

Thermal limits
Oxidative stress
Nitric oxide
Hydrogen peroxide
Seedling growth
Abiotic stress

ABSTRACT

The predicted environmental changes may be detrimental to initial seedling growth, particularly the expected increase in air temperature. We therefore investigated the thermal limits for growth and development of *Vriesea friburgensis* and *Alcantarea imperialis* seedlings in the context of oxidative stress. The optimal temperatures for the growth of *V. friburgensis* and *A. imperialis* were 25 and 25–30 °C, respectively. Extreme temperatures (15, 30, or 35 °C) induced oxidative stress in both species with significant accumulation of hydrogen peroxide (H₂O₂) and nitric oxide (NO). Under oxidative stress, the amount of chlorophyll decreased in both species, more prominently in *V. friburgensis*, while carotenoid levels dramatically increased in *A. imperialis*. Notably, the activities of superoxide dismutase, catalase (CAT), and ascorbate peroxidase increased in *A. imperialis* at extreme temperatures. Similar results were observed for *V. friburgensis*; however, the activity of CAT remained unaffected regardless of temperature. Seedlings of *A. imperialis* survived at a wider range of temperatures than *V. friburgensis*, which had greater than 40% mortality when growing at 30 °C. Overall, precise control of cellular H₂O₂ and NO levels takes place during the establishment of *A. imperialis* seedlings, allowing the species to cope with relatively high temperatures. The thermal limits of the fundamental niches of the species investigated, determined based on the ability of seedlings to cope with oxidative stress, were distinct from the realized niches of these species. The results suggest that recruitment success is dependent on the ability of seedlings to handle extreme temperature-triggered oxidative stress, which limits the regeneration niche.

1. Introduction

The predicted scenarios based on expected global climatic changes may impose severe limits on the viability of plant populations. Seed germination and post-germination stages are recognized as crucial phases of the plant life cycle, and changes in the environmental conditions to the regeneration niche may severely compromise plant survival (Donohue et al., 2010; Donohue, 2014; Marques et al., 2014; Arana et al., 2015; Fernández-Pascual et al., 2017). Hence, the regeneration niche breadth and the upper and lower thermal limits for germination (Cochrane et al., 2014; Müller et al., 2017; Daibes and Cardoso, 2018; Duarte et al., 2018) and seedling establishment (Klanderud et al., 2017) modulate the initial success of recruitment and thus influence the composition of communities (Walck et al., 2011; Jiménez-Alfaro et al., 2016). Climate change has revealed that functional traits respond to warming along a gradient of local temperatures

spanning the native ranges of species, and that the geographic distributions of species represent the realized, rather than the fundamental, tolerance limits of said species (Drake et al., 2017). The fundamental niche (N_F) of a species comprises the set of environmental conditions that allows it to survive and reproduce not considering its interaction with other living organisms (Soberón and Arroyo-Peña, 2017). However, species can occupy only a portion of the potential area defined by the N_F, which results in a small geographic area known as the realized niche (N_R) (Soberón and Nakamura, 2009).

Most studies on the thermal tolerance of plants focus on seeds, while little is known about seedling performance as a function of temperature variation. Seed germination can be empirically defined as a temperature-dependent process (Porceddu et al., 2013; Batlla and Benech-Arnold, 2015; Arana et al., 2015; Duarte et al., 2018), in which the relationship between temperature and enzyme activity is quite complex (Rasheed et al., 2016). Signaling processes and variation in the activity

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of antioxidant enzymes are biochemical events that may be reliable tools in estimating the thermal limits of the N_F of seedlings. Imbalance in the production of reactive oxygen species (ROS) because of thermal stress (for review see [Szymańska et al., 2017](#)) has been demonstrated to negatively affect cell membrane stability and activity of antioxidant enzymes ([Mittler et al., 2012](#)). Uncontrolled overproduction of ROS (i.e., O_2^- , H_2O_2 , and OH^-) also decreases chlorophyll content and damages the thylakoid membranes, which in turn compromises plant growth ([Nievola et al., 2005](#); [Mollo et al., 2011](#); [Mathur et al., 2014](#)). Notably, plants may benefit from relatively low amounts of ROS because of the ability of ROS to function as signaling molecules ([Mittler et al., 2011](#); [Choudhury et al., 2017](#); [Da Silva et al., 2017](#)). It has been reported that increases in the levels of nitric oxide (NO), a reactive nitrogen species recognized as signaling molecule, boost the antioxidant system and enhance the membrane stability in plant species upon thermal or salt stress ([Fan et al., 2015](#); [Da Silva et al., 2017](#)). The plant antioxidant system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and pigments including carotenoids ([Del Río, 2015](#)).

The limits of tolerance are species-specific ([Davis et al., 2005](#)) and vary according to the plant's capacity to metabolically adjust to temperatures outside of the optimal range. Relatively little is known about the potential effects of rising temperatures on bromeliads ([Chaves et al., 2015, 2018](#); [Duarte et al., 2018](#); [Müller et al., 2017](#)), as only three reports have so far focused on plant cultivation under low temperatures ([Pedroso et al., 2010](#); [Mollo et al., 2011](#); [Carvalho et al., 2013, 2014](#)). Thus, the lack of knowledge on the effect of temperature on bromeliad seedling growth makes the prediction of recruitment success of this family following climate change difficult. Recent studies of thermal tolerance have suggested that the capacity for evolutionary adaptation is greater for minimum rather than maximum thermal tolerances, with maximum temperature tolerance remaining relatively unchanged ([Araújo et al., 2013](#)). In addition, species with narrow N_R are restricted to a narrow N_F , whereas N_R can either be broad or narrow when the N_F is broad ([Bush et al., 2017](#)). In this context, we evaluated the performance of *Vriesea friburgensis* (a widely distributed species) and *Alcantarea imperialis* (a species with a narrow geographic range) based on the activation of the antioxidant system in seedlings to determine the thermal niche breadths of both bromeliads. The seedlings' growth and survival were investigated at optimal and extreme temperatures to correlate their performance with metabolic changes associated with oxidative stress.

2. Material and methods

2.1. Selected plant species

Vriesea friburgensis Mez var. *friburgensis* is a medium size bromeliad, with terrestrial and epiphytic ([Marques et al., 2012](#)) and saxicolous habitats ([Versieux and Wendt, 2006](#)). It is mainly distributed in the Brazilian Atlantic Forest ([Versieux and Wendt, 2006](#); [Martinelli et al., 2008](#)) from extremely southern regions (Rio Grande do Sul) to the northeast region (Pernambuco) ([Fig. 1](#)) at altitudes above 900 m asl. The average minimum air temperature registered during the dry season by the nearest weather station to the place of plant occurrence was slightly below 15 °C, whereas the average maximum temperature during the wet season was above 30 °C ([Fig. 1](#)). Its occurrence in the Cerrado, Campos Rupestres, and Caatinga is incipient except in their borders with the Atlantic Forest ([Lima and Soares-Silva, 2016](#)). In the Campos Rupestres of the Espinhaço Mountain Range (EMR), *V. friburgensis* is found in mesic habitats at altitudes between 1400 and 1700 m asl. ([Marques et al., 2012](#)). *Alcantarea imperialis* (Carriere) Harms is a large bromeliad that grow up to 3 m tall ([Negrelle et al., 2012](#)). The species occurs on rocky outcrops ('inselbergs') at altitudes above 800 m asl in the Atlantic Forest of Southeast Brazil ([Safford and Martinelli, 2000](#)) ([Fig. 1](#)), and it is categorized as an endangered species

([Paula et al., 2016](#)). The species also occurs in Campos Rupestres throughout the EMR ([Versieux and Wanderley, 2007](#)). Within the geographic limits of its occurrence, the average minimum temperature registered during the dry season is slightly above 15 °C, while the average maximum temperature during the wet season is below 30 °C ([Fig. 1](#)).

Seeds of at least 10 *V. friburgensis* plants (~2500 seeds) grown in Campo Rupestre of Serra da Piedade (Minas Gerais State) and 10 *A. imperialis* plants (~2500 seeds) grown in a garden in São Paulo (São Paulo State) were collected in June (the dry season) and October (onset of the wet season), respectively, during their dispersal periods. Freshly harvested seeds from mature fruits of each species were mixed to avoid maternal effects and were set to germinate in 9 mm Petri dishes containing a sufficient amount of water for imbibition. Afterwards, plates were maintained in a germination chamber (FANEN, model 347 CDG, São Paulo, Brazil) at 25 °C, with a photosynthetic photon flux density (PPFD) of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a 12 h photoperiod. The temperature of 25 °C was chosen because both species exhibited the highest germination percentage and rate at this temperature. Five samples, each composed of 100 seeds, were dried out at 60 °C for 72 h in order to determine seed biomass.

2.2. Seedling growth and quantification of photosynthetic pigments

Three hundred seeds with protruding roots were selected and maintained on moisturized paper inside Plexiglass boxes (15 per box) at a PPFD of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (12 h photoperiod) and different air temperatures (15, 25, 30, or 35 °C) with five replicates per treatment. Seedling mortality and number of leaves were determined for all individuals on the last day of experimentation (day 30). The effect of temperature on biomass accumulation in seedlings of *Vriesea friburgensis* (n = 12 per replicate) and *Alcantarea imperialis* (n = 12 per replicate) was determined as later described in [Section 2.1](#). Photosynthetic pigments were extracted from three seedlings from each replicate using dimethyl sulfoxide and quantified as described by [Wellburn \(1994\)](#). The results were expressed as μg of pigment per g of fresh weight (FW).

2.3. Quantification of endogenous H_2O_2 , NO and activity of antioxidant enzymes

Five samples of both plant species, each constituting 20 fresh seedlings, were grown at 15, 25, 30, or 35 °C and harvested 30 days after root protrusion to quantify NO and H_2O_2 . Plant homogenates were obtained by grinding tissues (20–120 mg) in liquid nitrogen following the addition of 50 mM phosphate buffer (pH 6.8) (400–1500 μL). The amounts of H_2O_2 and NO were assessed using a TBR4100 Free Radical Analyzer (WPI, USA) equipped with the amperometric sensors ISO-NOP and ISO-HPO-2, which allowed the quantification of NO and H_2O_2 at detection limits lower than 1 nM and 100 nM, respectively. The H_2O_2 and NO levels were determined based on standard curves of H_2O_2 (Sigma) and S-nitroso-N-acetylpenicillamine (Sigma), respectively.

For the antioxidant enzyme assays, frozen seedlings were ground into powder with liquid nitrogen and proteins were extracted using 50 mM phosphate buffer (pH 6.8) containing 100 μM EDTA, 10% (w/w) polyvinylpyrrolidone, and a protease inhibitor cocktail (Sigma). Seedling extracts were centrifuged at 10,000 $\times g$ for 15 min at 4 °C and the supernatant was collected for further analysis of SOD, CAT, and APX activities. SOD activity was measured at 575 nm, and 1 unit of activity was defined as the amount of enzyme required to inhibit the reduction of nitro blue tetrazolium by 50% ([Giannopolitis and Ries, 1977](#)). CAT activity was determined by incubating plant samples with 50 mM phosphate buffer (pH 6.8) and 125 μM H_2O_2 . The enzyme activity was estimated using the equation $\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ from measurements of H_2O_2 degradation at 240 nm ([Cakmak and Horst, 1991](#)). The medium used to measure APX activity was comprised of

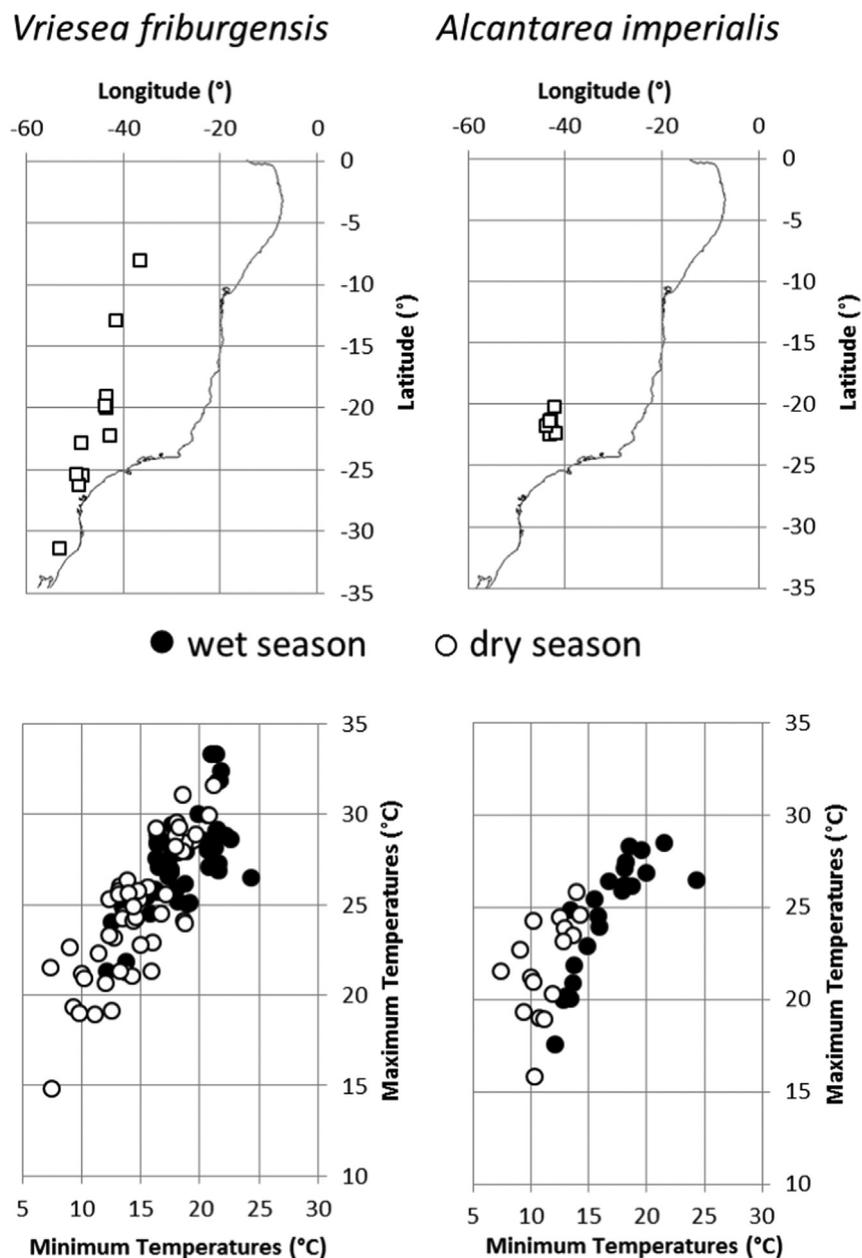


Fig. 1. Geographical distribution of *Vriesia friburgensis* and *Alcantarea imperialis* and the average temperatures registered by the nearest weather station during the wet (Oct–Mar) and dry season (Apr–Sep). Coordinates were obtained from www.splink.cria.org.br and the average temperatures from INMET (www.inmet.gov.br).

50 mM phosphate buffer (pH 6.0), 1 mM ascorbic acid, and 2 mM H_2O_2 (Nakano and Asada, 1981). The rate of ascorbic acid oxidation was monitored at 290 nm and the quantification was performed using the equation $\varepsilon = 2.8 \text{ mM cm}^{-1}$ (Nakano and Asada, 1981).

2.4. Experimental design and statistical analysis

Treatments (five replicates) were arranged with a completely randomized design. Data were submitted to normality and homoscedastic tests prior to ANOVA analysis. Means were compared using the Tukey test ($P < 0.05$) and all data were analyzed using the Sisvar software (Sisvar 5.3 Build 77). Data were analyzed using SigmaPlot 11.0 and curves were plotted using nonlinear regression.

3. Results

3.1. Seedling growth and photosynthetic pigments

The number of leaves and seedling biomass for both species was significantly ($P < 0.001$) higher at 25 °C, henceforth referred to as the control air temperature (Figs. 2 and 3). Additionally, *A. imperialis* exhibited more growth at 30 °C (Fig. 3), and no seedlings of this species died even at extreme temperatures (15 and 35 °C; Table 1). Along with the significantly lower number of leaves at 15 (3.8-fold) and 30 °C (2.7-fold) compared to the control air temperature (Fig. 3), *V. friburgensis* seedlings were highly sensitive to heat as attested by a mortality of over 40% at 30 °C and no seedling survival at 35 °C (Table 1). Although the number of leaves for *A. imperialis* individuals grown at 15 (2-fold) and 35 °C (2.3-fold) was lower compared to seedlings grown at 25 °C (Fig. 3), no dead plants had been registered at the end of the experiment. Both species had a significant increase in biomass ($P < 0.001$),

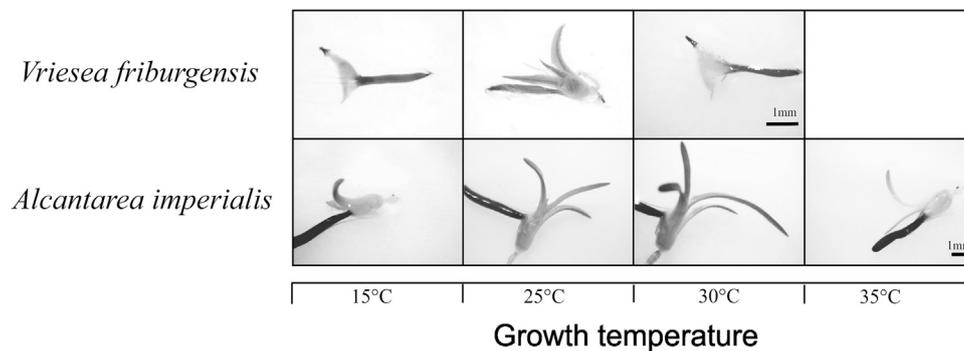


Fig. 2. Seedlings of *Vriesea friburgensis* and *Alcantarea imperialis* 30 days after growing at various air temperatures at $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically photon flux density and 12 h photoperiod. Seedlings of *V. friburgensis* failed to cope with 35 °C.

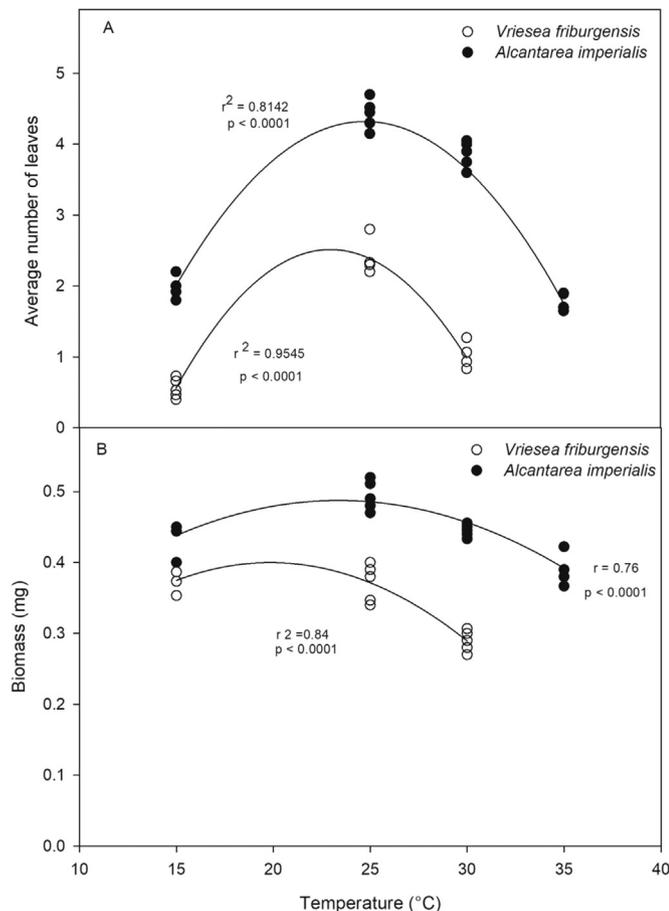


Fig. 3. Relationships between temperature and number of leaves per seedlings (A) and seedling biomass accumulation (B). Seedlings were subjected to temperatures in the range from 15 to 35 °C. Samples were harvested 30 days after the beginning of experiments for the analyses of the number of leaves and dry mass. Each point represents the mean values for 15 seedlings (for the number of leaves) or 12 seedlings (for biomass accumulation).

reaching higher masses than the average dry seed biomass regardless of temperature ($0.13 \pm 0.003 \text{ mg/V. friburgensis}$ seed and $0.33 \pm 0.001 \text{ mg/A. imperialis}$ seed).

Higher levels of total chlorophyll ($\text{Chl}_{\text{total}}$) were detected in both species grown at 25 °C, and *A. imperialis* also presented high $\text{Chl}_{\text{total}}$ content at 30 °C (Table 1). The carotenoid (Ck) content in leaves of *V. friburgensis* grown at 30 °C was 2-fold lower than of seedlings at the control air temperature. However, an increase of at least 50% was noted for *A. imperialis* leaves under similar experimental conditions. A significant difference in the proportion of pigments in the antenna

complex was recorded in both species; *V. friburgensis* showed about twice as much $\text{Chl } a/b$ and $\text{Ck}/\text{Chl}_{\text{total}}$ ratios compared to seedlings grown at the control air temperature, while a 9.7% decrease (on average) in the ratio of $\text{Chl } a/b$ and a 1.5- to 2.5-fold increase in $\text{Ck}/\text{Chl}_{\text{total}}$ levels were observed for *A. imperialis* at 15 and 35 °C (Table 1).

3.2. Effect of temperature on NO and H_2O_2 levels and enzymatic antioxidant system

The levels of H_2O_2 were 1.5- and 2.0-fold higher in *V. friburgensis* seedlings at 15 and 30 °C, respectively, compared to seedlings grown at the control air temperature (Fig. 3; $P < 0.001$). A negative correlation between H_2O_2 level and the average number of leaves was observed, regardless of plant species (Fig. 4). The amount of NO in seedlings growing at 15 °C was ca. 10-fold higher than in seedlings grown at the control air temperature or at 30 °C (Fig. 5). Seedlings of *A. imperialis* showed significantly lower levels of both H_2O_2 and NO at 30 °C and at the control air temperature.

Different patterns of activities of the antioxidant enzymes SOD, CAT, and APX were observed for seedlings as a function of temperature (Fig. 6). Interestingly, in relation to the control, *A. imperialis* presented the highest enzyme activity at 15 °C and the lowest activity at 30 °C. The SOD activity was also higher in seedlings at 35 °C than at the control air temperature, although this value was 5-fold lower than that observed in seedlings growing at 15 °C. No differences were observed in the activity of CAT in seedlings of *V. friburgensis* growing at different temperatures, while APX and SOD presented higher activities at 15 and 30 °C.

4. Discussion

Extreme temperatures limit the growth of *V. friburgensis* and *A. imperialis* seedlings to different extents. Exposure to low or high temperatures increased oxidative stress due to the accumulation of H_2O_2 (in the order of $\mu\text{mol g}^{-1} \text{ DW}$), which in turn led to a decrease in the number of leaves and biomass. Seedlings of *A. imperialis* were more vigorous and showed a wider optimum temperature range for growth than *V. friburgensis*, as the latter did not survive at 35 °C. The geographically widespread *V. friburgensis* exhibits higher thermal amplitude for the realized N_R compared to the thermal limits of the N_F determined by seedling growth. In contrast, *A. imperialis*, which has a narrower geographical distance between populations than the N_F , presents narrow thermal limits of the N_R . These results suggest that, along the gradient of the geographical distribution, distinct populations of *V. friburgensis* can present genetic differences that determine local adaptation, which results in higher thermal amplitude of the N_R . The N_R may also have been overestimated if species were sheltered from broader temperature extremes by landscape refuges, i.e., microclimates (De Frenne and Rodríguez-Sánchez, 2013). The narrow N_R compared to the N_F for *A. imperialis* seedlings may also be the result of differences

Table 1

Effect of growth temperature on total chlorophyll (Chl_{total}) and carotenoids (Ck) levels and mortality (%) in seedlings of *Vriesea friburgensis* and *Alcantarea imperialis*.

Temperature (°C)	Chl _{total} (µg g ⁻¹)	Chl a/b	Ck (µg g ⁻¹)	(Ck)/Chl _{total}	Mortality (%)
<i>V. friburgensis</i>					
15	126.0 ± 18.1 b	2.1 ± 0.2 b	36.4 ± 4.2 b	0.29 ± 0.0 b	0.0
25	204.3 ± 15.7 c	1.1 ± 0.1 a	38.1 ± 3.2 b	0.18 ± 0.0 a	9.3 ± 3.6
30	55.7 ± 12.4 a	2.4 ± 0.7 b	18.7 ± 3.4 a	0.34 ± 0.0 c	44.0 ± 3.6
35	–	–	–	–	100.0
<i>A. imperialis</i>					
15	90.9 ± 14.7 a	2.7 ± 0.4 b	45.5 ± 3.8 c	0.50 ± 0.1 c	0
25	180.4 ± 18.4 b	3.1 ± 0.1 c	30.2 ± 0.8 a	0.20 ± 0.1 a	0
30	158.6 ± 12.7 b	2.9 ± 0.1 b	37.4 ± 2.3 b	0.30 ± 0.1 b	0
35	96.1 ± 5.0 a	1.1 ± 0.2 a	52.4 ± 2.7 d	0.60 ± 0.1 c	0

Values indicate the mean ± standard deviation of independent experiments carried out with five replicates. Distinct letters indicate significant differences between treatments (Tukey; *P* < 0.05).

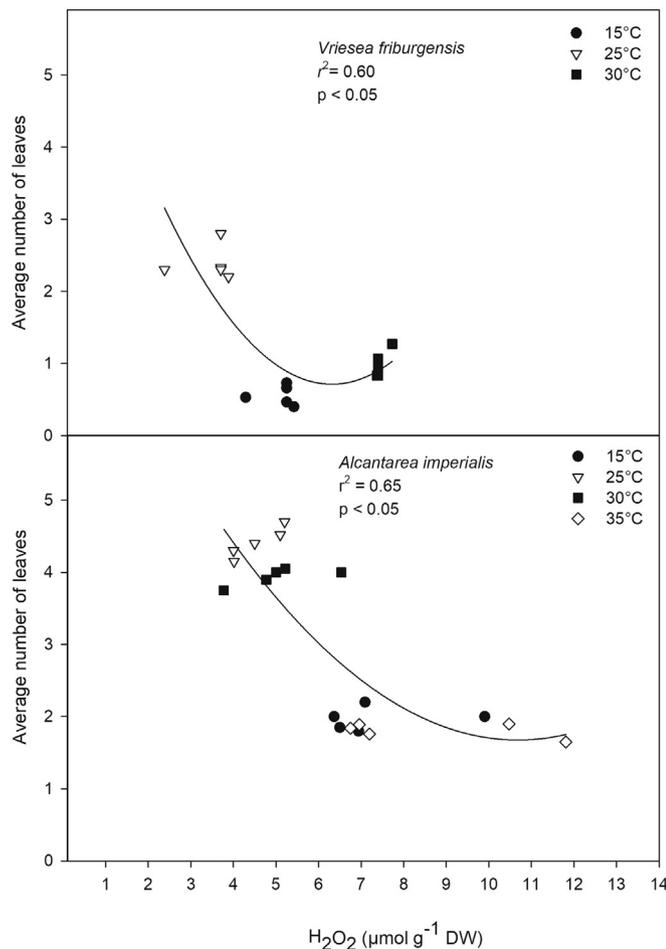


Fig. 4. Relationship between H₂O₂ and number of leaves in seedlings of *Alcantarea imperialis* and *Vriesea friburgensis* growing at different temperatures. Seedlings were subjected to temperatures in the range from 15 to 35 °C. Samples were harvested 30 days after the beginning of experiments for the analyses of H₂O₂ and number of leaves. Data are significantly different from each other (Tukey test; *P* < 0.05).

between the micro-climate experienced by plants and the temperature data from weather stations used to establish the thermal limits for the natural distribution of the species. *A. imperialis* is a rupicolous species and thus can be exposed to air temperatures higher than those recorded by meteorological stations. In a study comparing micro-habitats of bromeliads, Chaves et al. (2018) demonstrated that the temperature of rocks was up to 10 °C higher than the air temperature. Due to the habitus and habitat, *A. imperialis* can experience a wide temperature range

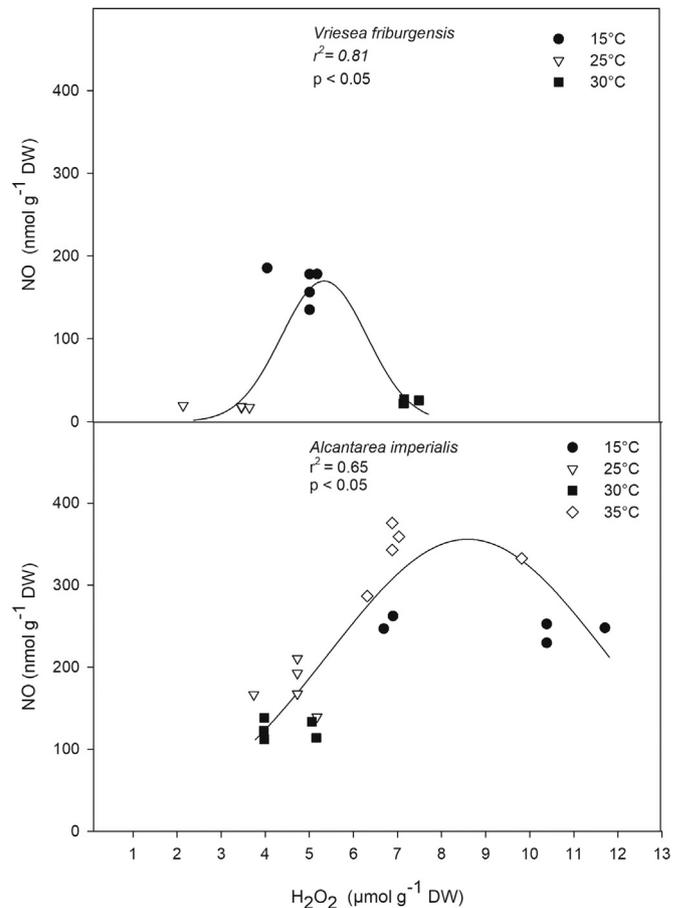


Fig. 5. Relationships between NO and H₂O₂ in *Alcantarea imperialis* and *Vriesea friburgensis* seedlings as a function of temperature. Seedlings were grown at temperatures ranging from 15 to 35 °C for 30 days and afterwards analyzed for nitric oxide (NO) and hydrogen peroxide (H₂O₂) accumulation.

from 5 to 40 °C in a single day on rocky outcrops (Mollo et al., 2011). Species that already experience higher maximum temperatures are more likely to be close to their thermal tolerance limit (Bush et al., 2017). Thus, *A. imperialis* seedlings are probably experiencing their upper thermal limit already at the start of the wet season, at which time the highest air temperatures are recorded.

The high susceptibility of *V. friburgensis* seedlings to heat (100% mortality at 35 °C), contrasts findings for other monocotyledonous species such as wheat, rice, and maize, which have lethal temperatures above 42 °C (Sánchez et al., 2014). Although no seedling mortality was observed for either species at 15 °C, seedling growth decreased significantly compared to those seedlings grown at the control air

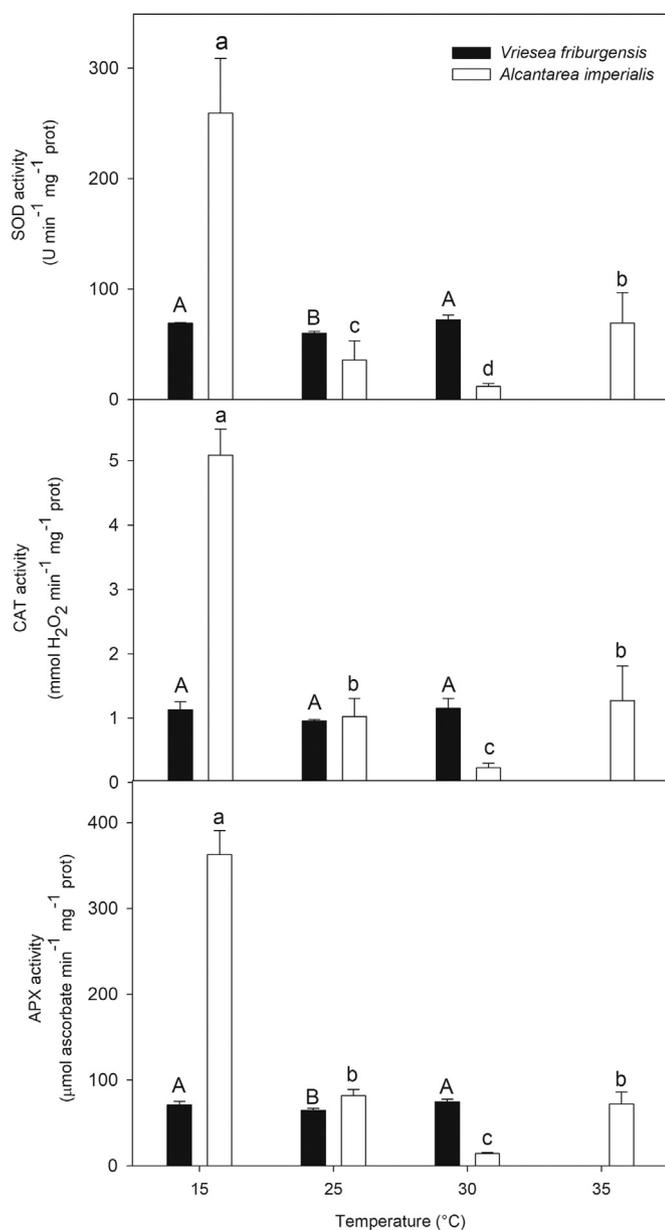


Fig. 6. Effect of temperature on the activity of antioxidant enzymes in *Alcantarea imperialis* and *Vriesea friburgensis* seedlings. Seedlings were grown at temperatures ranging from 15 to 35 °C for 30 days and afterwards analyzed for the activities of superoxide dismutase (SOD; A), catalase (CAT; B) and ascorbate peroxidase (APX; C). Distinct uppercase letters indicate significant difference between temperatures within *A. imperialis* while distinct lowercase letters indicate significant difference within *V. friburgensis* (Tukey test; $P < 0.05$). Data are the means \pm standard deviation of independent experiments done in quintuplicate.

temperature. These results support those reported by Mollo et al. (2011), in which *in vitro* cultivation of *A. imperialis* at low temperatures resulted in lower number of leaves and less root growth. A decrease in leaf number and biomass accumulation was also reported for *Acanthostachys strobilacea* grown at low temperatures *in vitro* (Carvalho et al., 2014).

The oxidative stress caused by extreme temperature in seedlings of both bromeliads studied was accompanied by an increase in the amount of NO. Seedlings of *A. imperialis* produced leaves even at 15 °C and accumulated higher amounts of NO than plants grown at 25 or 30 °C. The signaling role of NO during plant response to abiotic stress is well known (Choudhury et al., 2017; Da Silva et al., 2017). For instance, the

amount of NO and H₂O₂ in tobacco plants under salt stress was in the same range as in *A. imperialis* seedlings reported in this study, and was determined to be important for tobacco tolerance to salt stress (Da Silva et al., 2017). The increase in NO was also reported in the woody species *Swietenia macrophylla* (mahogany) under drought conditions during seedling establishment (Horta et al., 2014). There is strong evidence that NO may fulfill an important role during CAM-signaling in bromeliads under water deficit (Freschi et al., 2010). Additionally, NO has been implicated in the activation of antioxidant enzymes that mitigate oxidative damage triggered by abiotic stress (Da Silva et al., 2017). The increase in NO in *A. imperialis* was accompanied by increases in the activity of some antioxidant enzymes (SOD, CAT, APX) at all temperatures, except at 30 °C. This indicates that at the optimum temperature for seedling establishment, *A. imperialis* produces lower amounts of ROS (e.g., low H₂O₂ levels) without great activation of the antioxidant enzyme system. The lowest temperature tested (15 °C) exacerbated the activity of SOD, CAT, and APX in *A. imperialis* only, a phenomenon not observed to a similar extent in seedlings at the highest temperature tested (35 °C). Similarly, *Cucumis sativus* presented higher APX and SOD activity under chilling stress (Lee and Lee, 2000). A significant increase in the activity of antioxidant enzymes was reported in the bromeliads *Tillandsia brachycaulos* and *Bromelia karatas* during the dry season, a consequence of water deficit and higher light conditions (González-Salvatierra et al., 2010).

The lack of mortality of *A. imperialis* seedlings at extreme temperatures (15 or 35 °C) suggests that the antioxidant system was able to keep ROS at non-lethal levels, a characteristic that was not observed in *V. friburgensis*. The degradation of ROS by APX and CAT in *V. friburgensis* at 30 °C was not enough to control the oxidative stress caused by H₂O₂ accumulation, as attested by the high seedling mortality at this temperature. Low efficiency of the antioxidant enzyme system has been reported in plant species sensitive to heat (Almeselmani et al., 2009). Indeed, it has been shown that mahogany seedlings under increasing water deficit are able to cope with such abiotic stress during the first 45 days of plant development by increasing NO production (Horta et al., 2014). Likewise, the temporal and spatial regulation of ROS (such as H₂O₂) and reactive nitrogen species (such as NO) production were later shown to be critical for the success of seedling development under stress-free conditions (Airaki et al., 2015). Equally important is the contribution of non-enzymatic antioxidant molecules in the mitigation of oxidative stress. Oxidation of carotenoids, such as β -carotene, leads to the production of a volatile substance that functions as a signaling molecule in plant defense against singlet oxygen (Ramel et al., 2012). The carotenoid content in the most sensitive bromeliad species investigated in this study (*V. friburgensis*) dropped upon temperature stress, while the levels of these antioxidant pigments increased in the more tolerant species (*A. imperialis*), regardless of the temperature tested. Similar contrasting results were reported for *Brassica oleracea* ‘Winterbor’ (kale) and *Spinacia oleracea* ‘Melody’ (spinach) at air temperatures ranging from 10 to 30 °C (Lefsrud et al., 2005). The total chlorophyll levels were also reported to decrease in two bromeliad species grown under relatively low temperatures (Carvalho et al., 2013, 2014). High temperatures rapidly decreased photosystem II activity, which affected Chl-binding proteins (LHCII and type II-LHCI) to different extents (Xu et al., 1995). Oxidative stress dramatically reduced the total chlorophyll content in the most sensitive bromeliad species, which resulted in an increased Chl a/b ratio. A decrease in LHCII of PSII was observed in plants upon increased Chl a/b ratio (Anderson et al., 1988).

Climate change projections for the upcoming decades estimate an increase in the temperature and in the number of drought episodes (IPCC, 2014), conditions that will determine the success of recruitment (Jiménez-Alfaro et al., 2016). Here, it has been demonstrated that a fine control of H₂O₂ and NO levels seems to be required for seedling establishment in species with the ability to cope with temperature stress. The seeds of both bromeliad species studied are dispersed during the

dry season, while seed germination and seedling initial growth takes place in the rainy season. Increases in water availability and air temperature occur during the latter period (also known as spring equinox), a condition that favors the establishment of seedlings of both bromeliad species.

The lower thermal limits of the N_F , defined by the capacity of seedlings to deal with the oxidative stress caused by extreme temperatures, could indicate that seedlings of the epiphytic *V. friburgensis* have a higher risk of mortality in the face of heat waves compared to *A. imperialis* seedlings. *A. imperialis* occurs in inselbergs where seedlings probably experience high temperatures close to those of the limits of their N_F and may also be at risk if they cannot cope with the oxidative stress imposed by temperatures higher than the current temperature.

Acknowledgements

This study was financed in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - (CAPES) - Finance Code 001. LVM and JPLF are supported by research fellowships from CNPq and AAD is a recipient of a graduate scholarship from CAPES and FAPEMIG.

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