

Foliar resistance to simulated extreme temperature events in contrasting plant functional and chorological types

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Abstract

We analysed leaf resistance of 41 Angiosperms belonging to a wide range of plant functional (PFTs) and chorological types (PCTs) to simulated frost and high-temperature extreme events (EE). Leaf resistance was estimated as percentage of membrane electrolyte leakage under heating and freezing treatments in the lab.

Leaf resistance to heating or freezing was not significantly correlated with the main resource-use characteristics that defined PFTs, such as leaf specific area, toughness, *N* concentration or thickness. Leaf resistance to heating differed among PFTs (graminoids and bromeliads were the more resistant groups), but not among PCTs. In contrast, leaf resistance to freezing significantly differed among PCTs. Along a steep regional climatic gradient, climate variables (annual mean temperature, mean minimum temperature, mean maximum temperature and number of frost-free months) at the locations where the given species were most abundant were also significantly correlated with freezing resistance. Species from colder habitats both at the sub-continental and regional scales showed the highest leaf resistance to freezing. Our work indicates that leaf resistance to climatic EE and resource-use strategy (assessed in previous studies) represent two different, partially decoupled axes of plant specialisation. It also suggests that changes in the frequency of very low temperature events might have regional-scale impacts on vegetation, whereas changes in the frequency of very high temperature events might have more influence at the local scale.

Keywords: comparative ecology, extreme temperature events (EE), % electrolyte leakage (PEL), plant chorological types (PCTs), plant functional types (PFTs)

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Introduction

Climate change projections for the next decades suggest that changes in the average values of climatic variables might be accompanied by changes in the frequency of extreme events (Wigley, 1985; McCarthy *et al.*, 2001). There is already some evidence of a significant lengthening of the frost-free period in most high- and mid-latitude regions during the last decades (Walther *et al.*, 2002). Extreme events, such as unusually severe or untimely frosts, droughts and cold and hot spells, have ecological effects that are disproportionately large in

relation to their duration (Hopkins, 1978; Woodward, 1987). Therefore, they can be important determinants of the nature and rate of vegetation response to climate change (Walker, 1991).

The identification of plant traits that can be associated with species capacity to thrive under different environmental circumstances has always been an important aim of comparative plant ecology (Grime *et al.*, 1988; Keddy, 1992; Westoby, 1998) and has now regained momentum in view of expected environmental changes at the global scale (Chapin *et al.*, 1996; Díaz & Cabido, 1997). There is now an important body of information on individual plant traits that can indicate a species response to resource availability, especially water and nutrients (Chapin *et al.*, 1993; Aerts, 1995; Díaz & Cabido, 1997;

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Grime *et al.*, 1997; Reich *et al.*, 1999; Aerts & Chapin, 2000; Fonseca *et al.*, 2000), and these traits are frequently used in defining plant functional groups. Much less is known about traits related to species resistance to temperature, in particular to extreme temperature events (EE) (Buckland *et al.*, 1997; White *et al.*, 2000, 2001). This represents a major gap in information in order to understand not only present plant distribution, but also to anticipate possible vegetation shifts in the face of global climatic change. This is because species migration in response to a changing climate is supposed to be critically dependent on resistance to EE (Chapin *et al.*, 1996; Woodward & Kelly, 1997), which may not be directly predictable from a species resource-use strategy.

Several authors (Lepš *et al.*, 1982; MacGillivray *et al.*, 1995) have suggested that some functional traits related to life history and resource allocation are of universal value as predictors of resistance of species to extreme climatic events. They argue that traits which promote the tolerance of mineral nutrient stress (long-lived organs, low rates of nutrient turnover) lead to resistance to other forms of damage. Evidence related to these ideas has been obtained mostly from species in communities differing in resource availability but growing under the same climatic conditions (MacGillivray *et al.*, 1995). However, it is uncertain whether these patterns are maintained at a larger spatial scale, e.g. when considering species with different distributions along regional climatic gradients. The few authors who have addressed the issue of responses to climate vs. resource use strategy suggest that these represent two distinct axes of specialisation, which are at least partially decoupled. If these are indeed linked to different traits they should not be easily predictable from each other (Díaz *et al.*, 1999; Grime, 2001).

The aim of this work was to investigate differences in leaf resistance to simulated frost and high-temperature events in angiosperm species belonging to a wide spectrum of plant functional (PFTs) and chorological types (PCTs), which grow along a steep climatic gradient in central-western Argentina, and to find out whether resistance to extreme temperatures was associated to, or decoupled from resource-use strategy.

Materials and methods

Selection of species and collection of plant material

Forty-one vascular plant species were selected on the basis of previous studies (Cabido *et al.*, 1993; Díaz *et al.*, 1998; Pérez-Harguindeguy *et al.*, 2000), considering their abundance along a steep climatic gradient, and including as wide a range as possible of plant families and PFTs (Appendix 1). The climatic gradient is located in

central-western Argentina (31°25'–32°S, 64°10'–68°37'W), with a difference in annual precipitation of > 800 mm, a difference in mean annual temperature of > 11 °C, and a difference in altitude of > 1800 m between extreme points. It stretches from the subhumid high plateau of Córdoba Mountains to the western semiarid–arid plains bordered by the Sub-Andean ranges, with rainfall strongly concentrated to the warm season (Díaz *et al.*, 1998). Altitude ranged from 350 to 2155 m, mean annual temperature ranged from 19.6 to 8.08 °C, mean annual precipitation ranged from 84.7 to 911.5 mm yr⁻¹, and the number of frost-free months ranged from 8 to 0 between extreme points.

Plant material was collected between February and March (late summer). Fresh leaves were collected from at least six individuals of each species. Individuals were chosen randomly among healthy looking, sexually mature plants, at those sites along the regional gradient where the abundance (percentage of ground cover; Cabido *et al.*, 1993; Díaz *et al.*, 1998) of the species was highest. Leaves of each individual were chosen randomly among fully expanded, non-senescent sun leaves.

Experimental treatments

We adapted techniques widely applied to crop species and to a lesser extent to wild species (Blum, 1988; Earnshaw *et al.*, 1990; Gurrich, 1998). All leaves were processed on the same day of harvesting in order to minimise natural senescence processes. Five mm-diameter leaf fragments were cut, avoiding the main veins. In some cases, it was impossible to cut 5 mm-diameter fragments, for example in plants with needle-like leaves. In those cases, we cut multiple surfaces of the photosynthetically active tissue until reaching a similar area. The leaf fragments were rinsed for 2 h in deionised water in a shaker and then completely submerged in 1 mL of deionised water in Eppendorf tubes. We applied three treatments to the leaf fragments contained in the tubes, all of them without any acclimation: incubation at 20 °C (control); incubation at 40 °C in an oven, simulating a high-temperature EE; incubation at –8 °C in a freezer, simulating a frost event. All incubations were carried out in complete darkness during 14 h. There were five replicates per treatment per species. After the incubation, we measured the percentage of electrolyte leakage (PEL). PEL, a measure of membrane permeability, is often used as a simple and inexpensive indicator of cell injury (Blum, 1988; Earnshaw *et al.*, 1990). In this paper we used PEL as an inverse indicator of leaf resistance, with high values of PEL indicating low leaf resistance. We measured PEL as follows:

$$\text{PEL (percentage of electrolyte leakage)} = (e_{(s)}/e_{(t)}) * 100$$

where $e_{(s)}$ is the conductivity value of a sample immediately after the treatment, and $e_{(t)}$ is the conductivity value of the same sample after boiling for 15 min. We measured the conductivity of the solutions with a Horiba compact conductivity meter C-172®.

The percentage of electrolyte leakage under the control treatment was expected to be relatively small, and it was considered as an estimator of the damage produced by the manipulations of the samples and the natural membrane permeability of the species, and thus was applied as a 'correction factor' to the results of the treatments (effective PEL in treatment $x = \text{PEL in treatment } x - \text{PEL in control treatment}$). All results in this article refer to effective (= corrected) PEL. Cuticle thickness, leaf shape (i.e. compound vs. entire leaves) did not affect species responses to treatments (Gurvich, 1998).

Although temperatures applied in the treatments are extreme values that can occur in the field (at least at the coldest and hottest points of the gradient), this experiment was not intended to measure the real values of leaf resistance to EE in the species habitats of origin. It should be interpreted as a comparative method suitable for assessment of large numbers of species under standard conditions (Gurvich, 1998).

Other leaf traits used in this work (specific leaf area, leaf nitrogen concentration, leaf toughness and leaf thickness) were taken from the Ecological Database of Plants from Central Western Argentina (Pérez-Harguindeguy *et al.*, 2000; Vendramini *et al.*, 2000, 2002). See Díaz & Cabido (1997) and Pérez-Harguindeguy *et al.* (2000) for details on measurement of these traits. The PFT classification was taken from Díaz & Cabido (1997). This classification is based on species resource allocation to growth vs. storage/defence, size, specific leaf area and life span, photosynthetic pathway, and shoot phenology. PFTs were graminoids, bromeliads, forbs, woody perennials and woody deciduous. The PCTs classification was taken from Cabido *et al.* (1998). In order to link leaf resistance with species distribution under different climatic conditions, we used a two-scale approach. First, we distinguished different PCTs, which refer to distribution at the sub-continental scale (Cabido *et al.*, 1998). These PCTs were: Andean/Upper montane (distributed at higher elevations in the mountains of north-western Argentina and in Andean habitats of neighbouring countries like Bolivia and Peru), Lower montane (distributed at lower altitude in extra-Andean mountains), Southern-Brazilian (lowlands of central and north-eastern Argentina, and adjacent territories of Paraguay, Brazil and Uruguay), Western Chaquénian (dry Chaco lowlands of central and north-western Argentina) and Cosmopolitan (species with very broad ranges, sometimes introduced).

Second, in order to analyse the relationship between species responses to the treatments and climatic variables (annual mean temperature, mean minimum temperature, mean maximum temperature and number of frost-free months) at a finer scale, we characterised the points along the regional climatic gradient where each species was most abundant (Cabido *et al.*, 1993; Díaz & Cabido, 1997) in terms of climatic variables. The climatic data for all the locations were taken from the official records by the Argentine National Weather Service, Argentine Railways and Palacios & Zamar (1986). Mean values were available from 14 meteorological stations, whereas extreme values were available from five stations. Climatic variables at each plant sampling location were assumed to be those at the closest station.

Data analysis

Since data distribution was not normal, Spearman Rank correlations were employed to test the relationships between percentage of electrolyte leakage, leaf traits, and climatic variables (Hollander & Wolfe, 1972). Kruskal–Wallis tests were used for comparisons among percentage of electrolyte leakage of PFTs and PCTs under different treatments (Hollander & Wolfe, 1972). The multiple comparison method was used for a *posteriori* analysis (Marascuilo & McSweeney, 1977).

Results

In agreement with our expectations, PEL under the control treatment was significantly lower than that under the heating or freezing treatments for most species (Appendix 1). There was no significant correlation between PEL observed in response to freezing and heating ($P > 0.05$).

PEL of different PFTs and PCTs

There were significant differences in PEL among PFTs under the heating treatment (Fig. 1a). Leaves of graminoids were the most resistant to high temperature, followed by those of bromeliads. Leaves of forbs and woody deciduous showed lower resistance, with those of woody perennials being the least resistant. There was no significant difference in leaf resistance under the heating treatment among different PCTs (Fig. 1b).

In contrast, we found no significant difference in PEL under the freezing treatment among different PFTs (Fig. 1c), but observed significant differences among different PCTs (Fig. 1d). Andean species showed the highest resistance, whereas the Western Chaquénian and Cosmopolitan species were the least resistant.

PEL, leaf traits, and species distribution along a regional climatic gradient

Percentage of electrolyte leakage under the heating treatment was not significantly correlated with any climatic variable at the species' preferential site along the regional gradient (Table 1). Species response to freezing treatment, in contrast, was significantly and positively correlated to all the temperature-related variables analysed (Table 1).

Leaf traits (leaf specific area, toughness, *N* concentration and thickness) were not significantly correlated to PEL under either the heating or freezing treatments ($P > 0.05$).

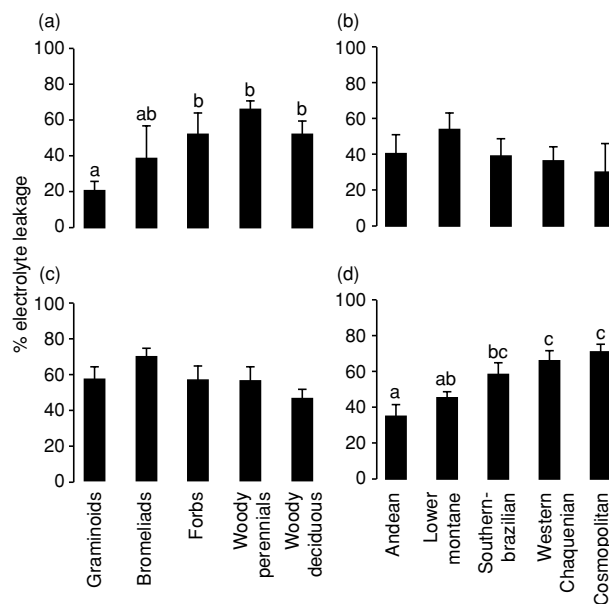


Fig. 1 Percentage of electrolyte leakage (PEL; $x + 1$ SE) of different PFTs under the heating (a) and freezing (c) treatments, and of different PCTs under the heating (b) and freezing (d) treatments. Different letters over bars indicate significant differences among groups ($P \leq 0.05$; Kruskal–Wallis and multiple comparison Test).

Table 1 Spearman Rank correlation coefficient ($N = 41$) among climatic variables and percentage of electrolyte leakage in the freezing and heating treatments. Probabilities are indicated in brackets

	Freezing	Heating
Annual mean temperature ($^{\circ}\text{C}$)	0.54 (< 0.01)	– 0.01 (0.97)
Mean minimum temperature ($^{\circ}\text{C}$)	0.50 (< 0.01)	– 0.04 (0.79)
Mean maximum temperature ($^{\circ}\text{C}$)	0.52 (< 0.01)	– 0.01 (0.96)
No. of frost-free months	0.53 (< 0.01)	– 0.02 (0.90)

Discussion

Limitations of the approach

Our method to assess leaf responses to simulated frost and high-temperature events, although often used in crop plant physiology, can be considered crude. It deals with detached leaf fragments that have lost all connection with the rest of the plant body and it does not allow for plant acclimation, which is a widespread phenomenon in the wild (Levitt, 1980; Larcher, 1995). However, it has proven consistent and robust enough for comparative purposes. It is particularly suitable for large-scale screening of high numbers of plant species, in which speed, ease of procedure and low cost are critical aspects. A more thorough discussion of the advantages and limitations of the approach can be found in Gurvich (1998), but essentially we believe that the method has accurately reflected consistent differences among plants belonging to a wide range of PFTs and PCTs. The method bias may probably lead to conservative rather than over-optimistic predictions of species behaviour in the field, since it is likely to underestimate the resistance of some species to EE (because it does not allow for acclimation), rather than exaggerate it.

Leaf resistance to EE: differences among PFTs and PCTs

Our results showed that leaf resistance to high temperature did not differ between PCTs or between species with different geographical distribution along a regional climatic gradient. In contrast, it differed among PFTs. This was not directly linked to resource-use strategy, however, since there was no significant correlation between leaf resistance to high temperature and the traits considered most important in the definition of PFTs (leaf specific area, toughness, thickness and *N* content; Chapin *et al.*, 1996; Díaz & Cabido, 1997; Grime *et al.*, 1997). We suggest that heat resistance could be related to habit, which influences the microclimatic conditions experienced by a plant, and is not strongly and directly correlated with resource-use traits. The most heat-resistant PFTs were graminoids and bromeliads, whose members are short and stemless. The habit of graminoids, and to a lesser extent bromeliads (i.e. short and compact habit, leaves close to the ground or rocky surfaces, which tend to get very hot or very cold according to air temperature and radiation), may explain their higher heat tolerance as compared to other PFTs, independently of the local macroclimate. This is in accordance with the literature, that suggests that plant resistance to high temperatures is more related to plant habit and microhabitat characteristics than to their geographical distribution (Larcher, 1995; Loehle, 1998;

Körner, 1999). For example, Körner (1999) reported that plants studied *in situ* in very different climatic regions (e.g. the Alps and Mauritania) reach similar leaf temperature and resistance, showing that those plants can cope with heat stress quite independently of the climate where they live. In cold areas, like the Alps, prostrate or cushion-like habit create a warmer, more favourable, microclimate, but can lead to periodic overheating.

In contrast with the patterns found in the case of resistance to high temperature, and also in accordance with the literature (Sakai & Larcher, 1987; Woodward, 1987; Cabrera, 1996), plant resistance to freezing was related to plant distribution along climatic gradients both at the sub-continental (chorological) and regional scales. It differed among different PCTs and among species with differential distribution along a regional climatic gradient, but it did not differ among PFTs. Our results therefore do not support the conclusions by MacGillivray *et al.* (1995), who found that plants with preferential allocation of resources to storage and defence were more resistant to low temperatures than plants with preferential allocation to growth. This apparent contradiction should be explained by the scale at which these two studies were carried out. MacGillivray *et al.* (1995) studied communities growing under the same climatic conditions, whereas we worked with a much broader spectrum of plants and climatic conditions. This leads to the suspicion that relationships between plant functional traits and freezing resistance may be different at different spatial scales.

Our work also provides empirical support to the idea (Díaz *et al.*, 1999; Grime, 2001) that plant tolerance to different resource-availability situations and leaf resistance to climatic EE represent two different, partially decoupled axes of specialisation, which cannot be easily predicted from each other, and whose assessment requires the measurement of different plant traits.

Implications in the face of climate change

Our results provide the basis for some speculation on the possible responses to vegetation to projected climate change. If along regional climatic gradients leaf resistance to heating can be related to PFTs, but resistance to freezing cannot, then changes in the frequency of EE should have very different consequences, depending on the scale of the analysis. Changes in the frequency of very low temperature events might have regional-scale impacts on vegetation, whereas changes in the frequency of very high temperature events might have more influence at the local scale.

Considering that all the PFTs analysed here are represented along the whole regional climatic gradient, albeit with different relative frequencies (Díaz & Cabido, 1997), and that the PCTs are strongly linked to climatic gradients

(Cabido *et al.*, 1998), changes in the frequency or timing of frost events should be relevant for plant migration across the landscape at the regional scale. In this sense, plant migration into new areas tracking climatic shifts may be limited by species resistance to frost, even if they can tolerate mean temperatures. PFT composition should not be expected to change substantially with changes in the frequency or timing of frost. On the other hand, changes in the frequency of very high temperature events may affect the relative success of plants with different habit and thus indirectly influence the relative abundance of different PFTs, and thus be more relevant to the assembly of communities at the local scale. Plant traits that indicate resource-use strategy are known to be important in the prediction of how natural vegetation might respond to projected changes in climate at the local and regional scale (MacGillivray *et al.*, 1995; Chapin *et al.*, 1996; Díaz & Cabido, 1997). Our work, however, suggests that they may not be sufficient. Other traits, specifically plant habit (linked to microclimatic conditions) and plant traits strongly related to geographical distribution (such as leaf resistance, tested in this article) should also be considered.

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Appendix 1 List of plant species, families, plant functional types (PFTs) and plant chorological types (PCTs), and responses to the freezing and heating treatments, expressed as corrected percentage of electrolyte leakage (PEL). Nomenclature follows Zuloaga *et al.* (1994) and Zuloaga & Morrone (1996a, b). g = graminoids, b = bromeliads, f = forbs, wp = woody perennials and wd = woody deciduous. A = Andean, lm = Lower montane, sb = Southern-Brazilian sb, wch = Western Chaquenan, c = Cosmopolitan

Species	Family	PFTs	PCTs	% electrolyte leakage (PEL)		
				Control 20 °C	Heating 40 °C	Freezing −8 °C
<i>Acalypha communis</i>	Euphorbiaceae	f	sb	16.02	83.46	74.61
<i>Alchemilla pinnata</i>	Rosaceae	f	a	3.09	81.82	27.52
<i>Alternanthera pungens</i>	Amaranthaceae	f	c	4.17	7.33	70.01
<i>Aristida achalensis</i>	Poaceae	g	lm	6.43	30.96	40.39
<i>Aspidosperma quebracho-blanco</i>	Apocynaceae	wp	wch	16.62	68.70	78.09
<i>Bouteloua aristidoides</i>	Poaceae	g	wch	7.80	16.44	88.98
<i>Bromelia urbanianum</i>	Bromeliaceae	b	wch	11.98	9.91	73.80
<i>Buddleja brasiliensis</i>	Buddlejaceae	wd	lm	12.35	60.49	50.60
<i>Capparis atamisquea</i>	Capparaceae	wp	wch	24.13	73.10	44.73
<i>Carex fuscula</i>	Cyperaceae	g	a	5.07	7.92	40.83
<i>Celtis pallida</i>	Celtidaceae	wd	wch	12.16	43.66	53.07
<i>Celtis tala</i>	Celtidaceae	wd	sb	16.59	32.10	36.93
<i>Cortaderia rudiusscula</i>	Poaceae	g	a	13.41	65.48	39.09
<i>Croton sarcopetalus</i>	Euphorbiaceae	wd	sb	16.08	14.80	50.85
<i>Eryngium agavifolium</i>	Apiaceae	f	lm	3.25	15.41	57.00
<i>Eupatorium viscidum</i>	Asteraceae	wd	sb	19.04	74.87	57.54
<i>Festuca tucumanica</i>	Poaceae	g	a	5.86	15.30	21.44
<i>Flourensia campestris</i>	Asteraceae	wd	lm	18.78	79.67	41.97
<i>Gentianella parviflora</i>	Gentianaceae	f	a	19.31	70.54	30.83
<i>Gomphrena pulchella</i>	Amaranthaceae	f	sb	12.25	61.21	66.53
<i>Heterothalamus alienus</i>	Asteraceae	wp	lm	12.52	67.68	54.26
<i>Juncus uruguensis</i>	Juncaceae	g	a	2.03	12.77	17.25
<i>Lithraea molleoides</i>	Anacardiaceae	wp	lm	23.10	53.73	45.86
<i>Maytenus vitis-idea</i>	Celastraceae	wd	wch	53.39	44.47	12.78
<i>Mimozyanthus carinatus</i>	Fabaceae	wd	wch	10.18	60.91	64.41
<i>Muhlenbergia peruviana</i>	Poaceae	g	a	9.58	3.23	65.86
<i>Neobouteloua lophostachya</i>	Poaceae	g	wch	15.07	6.18	64.72
<i>Nicotiana glauca</i>	Solanaceae	wd	c	20.25	74.63	64.30
<i>Nothoscordum gracile</i>	Liliaceae	g	c	6.57	18.51	82.26
<i>Pappophorum caespitosum</i>	Poaceae	g	wch	6.56	31.75	84.88
<i>Paspalum quadrifarium</i>	Poaceae	g	sb	5.17	3.62	24.47
<i>Pithecoctenium cynanchoides</i>	Bignoniaceae	f	sb	12.67	41.08	67.94
<i>Poa stuckertii</i>	Poaceae	g	a	6.65	60.68	66.90
<i>Schinopsis haenkeana</i>	Anacardiaceae	wd	lm	26.35	67.83	32.72
<i>Schizachyrium condensatum</i>	Poaceae	g	sb	6.36	11.81	44.66
<i>Setaria pampeana</i>	Poaceae	g	sb	3.55	30.86	92.31
<i>Sorghum halepense</i>	Poaceae	g	c	7.08	17.36	70.03
<i>Tillandsia duratii</i>	Bromeliaceae	b	wch	15.27	33.85	73.71
<i>Tillandsia capillaris</i>	Bromeliaceae	b	sb	10.24	72.00	59.92
<i>Trichloris crinita</i>	Poaceae	g	sb	10.60	0.00	75.54
<i>Zizyphus mistol</i>	Rhamnaceae	wd	wch	9.99	9.50	40.57