



Freezing resistance of high-elevation plant species is not related to their height or growth-form in the Central Chilean Andes

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ABSTRACT

The level and mechanism of resistance to damage from freezing temperatures is related to the ambient temperatures experienced by plants. High-elevation habitats exhibit a thermal gradient in the air–soil profile, exposing ground-level species to more rigorous thermal microhabitats than well aboveground species. Such gradients could explain an inverse relationship between freezing temperature damage (LT₅₀) and plant height, such that leaves of smaller plants tolerate freezing conditions, while leaves of taller plants avoid them. However, microclimatic data have not been included in previous studies evaluating those patterns. On the other hand, a proposed trade-off between plant functional traits and freezing resistance suggest that leaves of growth-forms with higher intensity of growth are less freezing resistant than those with lower growth intensity. In this work, we determined whether the ability to resist freezing temperatures is related to plant height or growth-form in 37 high-elevation species from the Central Chilean Andes. We proposed that (1) plants with contrasting heights are exposed to different thermal conditions during freezing events, (2) the level and the mechanism of freezing resistance are inversely related to plant height, and that (3) the level of freezing resistance varied between plants with low- and high-growth intensities. We found that high-Andean species of different heights are exposed to different thermal conditions depending on the distance from the ground. However, neither level nor mechanisms of freezing resistance were related to plant height. Leaves of both short and tall plants showed similar LT₅₀ and their main freezing resistance mechanism was tolerance. Moreover, leaves of growth-forms with high- and low-growth intensity resisted similar freezing temperatures. Our results are discussed in relation to environmental conditions that characterize the Central Chilean Andes.

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1. Introduction

The level and mechanisms by which plants resist freezing temperatures are related to the ambient temperatures that plants experience (Beck et al., 2004; Bannister et al., 2005). Thus, plants can avoid or tolerate extracellular freezing depending on the intensity and duration of the freezing events (Levitt, 1980). Freezing avoidance mechanisms (FA), which prevent the ice formation through freezing point depression or by supercooling, have been found in species inhabiting sites where the intensity of the freezing events is moderate (few degrees below 0 °C) and of short duration. Freezing tolerance mechanisms (FT), defined as the ability of plants to survive the extracellular freezing are frequent in species found in

locations where freezing events are severe (several degrees below 0 °C) and of long duration (Sakai and Larcher, 1987; Larcher, 2003). For example, in tropical and subtropical high-elevation habitats FA is the most common freezing resistance mechanism among species with tall growth-forms (e.g. shrubs of 20–60 cm, giant rosettes >1 m tall; Goldstein et al., 1985; Squeo et al., 1991, 1996), whereas short plants (<20 cm), such as some perennial herbs, rosettes and cushion plants are FT (Azócar et al., 1988; Squeo et al., 1991, 1996). Such trend is based on a thermal gradient in the air–soil profile, where colder air masses are accumulated near soil surfaces during clear sky nights, exposing prostrate species (e.g. rosettes, cushion plants) to more extreme thermal conditions than those with canopies well aboveground (e.g. shrubs) (Larcher, 2003).

Squeo et al. (1991) reported that the freezing temperature damage of Venezuelan Andean species increases (less negative) with the plant height, indicating an inverse relationship between plant height and freezing resistance of Paramo species. Nevertheless, a subsequent study carried out in the Northern Chilean Andes failed to find any relationship between plant height and temperatures inducing freezing damage (Squeo et al., 1996), suggesting that this

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relationship is not universal. Although the plant height-freezing resistance hypothesis is based on the microclimatic differences experienced by plants of different height, none of the studies that tested this hypothesis included microclimatic data to explain the presence or absence of a plant height-freezing resistance relationship.

Plants in high-elevation habitats comprise several growth-forms, such as annuals, perennial rosettes and forbs, and shrubs (Körner, 2003), which differ in their growth intensities. High-growth intensity species are those that produce aboveground organs quickly during snow melts and die before the onset of the unfavorable season (e.g. annuals, bulbous plants and some rosettes). In contrast, plants with low-growth intensity are those that invest resources in aboveground organs (i.e. woody stems, evergreen leaves) that persist throughout the growing season (e.g. shrubs, cushion plants and several perennial herbs). Sakai and Larcher (1987) recognized an inverse relationship between the intensity of growth of a plant species and its freezing resistance during summer. Thus, leaves of high-elevation species with high-growth intensity are expected to be less freezing resistant than leaves of species with low-growth intensity.

Recently, Sierra-Almeida et al. (2009) reported that high-elevation species of Central Chilean Andes are highly resistant to frosts, and that summer drought has considerable effects on their level and mechanisms of freezing resistance. Plant species inhabiting Central Chilean Andes comprise several growth-forms and plant heights, offering an excellent opportunity to test how freezing resistance is related to plant height and growth-form in a xeric mountain system.

The general objective of the present study was to determine whether interspecific differences in the ability of leaves to resist freezing temperatures are related to plant height or growth-form in high-elevation species from the Central Chilean Andes. In particular, using data collected for 37 species, we assessed whether (1) plants with contrasting heights and growth-forms are exposed to different thermal conditions during freezing events, whether (2) the level (i.e. high or low) and the mechanism (i.e. avoidance or tolerance) of freezing resistance are inversely related to plant height, and whether (3) the level of freezing resistance varied between plants with low- and high-growth intensities. We expected that leaves of shorter species were exposed to more prolonged freezing events with lower nadir temperatures than that of taller species. This in turn produces that leaves of shorter species were more freezing resistant than those of taller species, where plants growing closer to the ground were freezing tolerant, whilst well above plants were freezing avoidant. We also expected that leaves of species with high-growth intensity were damaged at higher freezing temperatures than those of species with low-growth intensity.

2. Materials and methods

2.1. Study site and target species

This study was carried out near Farellones, in the Central Chilean Andes, 50 km east of Santiago of Chile. This area is characterized by the dominance of shrubs and the presence of annual species at lower elevations, and cushion plants and perennial herbs dominating at higher elevations (Cavieres et al., 2000). A total of 37 species were collected between 2400 and 2900 m, on a north-facing slope, near La Parva Ski Resort (33°21'S, 70°19'W). Nomenclature of the studied species follows Marticorena and Quezada (1985).

For each species, 20 mature individuals were randomly selected and their plant height (cm) was determined as the distance from the ground level to the uppermost vegetative node. Plant heights ranged from 1 to 60.8 cm (Table 2). The studied species corresponded to six growth-forms: annuals (2), bulbous plants (2),

cushion plants (2), forbs (7), rosettes (12) and shrubs species (12), according to Körner (2003). Classification of species in plants with high- or low-growth intensity was based on phenological behaviours described by Arroyo et al. (1981) and field observations. Sixteen species were considered with high-growth intensity: three forbs, nine rosettes, and all the annual and bulbous plants. These species produce leaves immediately snow melts which die-back before flowering, or leaves die-back after flowering and before fruiting. Twenty one species were included among plants with low-growth intensity: three forbs, four rosettes, and all the cushion plants and shrubs (see Table 2). These species produce new leaves late after snow melts and they maintain aboveground organs throughout growing season (e.g. leaves and woody stems).

2.2. Microclimatic measurements

Thermal conditions of the air–soil profile were characterized by measuring the air temperature at five distances above soil surface: 5, 15, 25, 50, and 75 cm. At each distance, one temperature sensor connected to a mini-logger (HOBO-H8; Onset Corp., Bourne, MA, USA) was fixed to a metallic support, and was partially covered by a plastic roof to protect it from direct exposure to the sun and radiation from the metallic support. Mini-loggers were programmed to record the temperature once every hour for 13 days at the beginning of the growing season, starting in 6 and finishing in 18 November, 2008.

2.3. Plant material collection

Plant material was collected early in the growing season, between 10 and 16 November 2008. For each species, we collected plant samples from different individuals growing in the study area. Plant samples corresponded to small twigs with mature leaves for woody plants (e.g. shrubs, cushion plants), and modules with at least two mature leaves or complete individuals for herbaceous plants (e.g. annuals, forbs and rosettes). Immediately after collection, plant samples were placed into a cooler to avoid changes in tissue water status and transported to a field laboratory for freezing resistance determinations (see below).

2.4. Thermal analyses

For each species, an expanded mature leaf was removed from each of five plant samples taken in the field, which corresponded to five different individuals. Each leaf was attached to a thermocouple (Gauge 30 copper-constantan thermocouples; Cole Palmer Instruments, Vernon Hills, IL), and immediately enclosed in a small, tightly closed cryotube. The cryotubes were placed in a cryostat (MGW LAUDA RC 20, Lauda-Königshofen, Germany), and the temperature was decreased from 0 to -18°C , at a cooling rate of 2°C h^{-1} . Temperature of individual leaves was monitored every second with a Personal Daq/56 multi-channel thermocouple USB data acquisition module (IOtech, Cleveland, OH). The sudden rise in leaf temperature (exotherm) produced by the heat released during the freezing process was used to determine the Ice Nucleation Temperature (NT).

2.5. Freezing temperature damage (LT_{50})

For each species, five expanded leaves were removed from each of five different plant samples, placed in separate, hermetically sealed plastic bags, and incubated in a previously cooled cryostat. The cryostat was programmed separately at six freezing temperatures: -6 , -9 , -12 , -15 , -18 and -22°C . Samples were kept at each temperature for 2 h to ensure homogeneous cooling. Then, the plastic bags were removed from the cryostat and left at 4°C in the

dark for 24 h. The control treatment consisted of samples placed in plastic bags and directly kept at 4 °C in the dark for 24 h (unfrozen samples). As visual damage was not immediately obvious for all species, leaf damage was assessed after thawing using a chlorophyll fluorimeter (Plant Efficiency Analyzer, Hansatech, Germany) to determine the ratio of variable to maximum fluorescence (F_v/F_m) of dark-adapted photosynthetic organs of each sample (Maxwell and Johnson, 2000). As dead material effectively had a F_v/F_m of zero, damage was calculated as percentage of photoinactivation ($100 \times Phi$), where Phi is the photoinactivation ratio described by Larcher (2000): $Phi = (1 - F_{FT}/F_{max})$, where F_{FT} is the F_v/F_m of the sample exposed to a freezing temperature T and F_{max} is the maximum value of F_v/F_m for all samples of each tested species. The temperature producing 50% damage (LT_{50}) was determined by linear interpolation using the temperature causing the highest Phi of <50% and the temperature causing the lowest Phi of >50% (Bannister et al., 1995, 2005). Although the Phi method focuses on primary reactions in PS II, injuries by cold or frost like destruction of plasma- and tonoplast-membranes can occur earlier and are more responsible for the death of the cell. Hence, plant tissue recovery after freezing would provide more complete view of freezing damage. However, Phi is a good indicator of damage by freezing and it has been used in several studies with a great number of species (e.g. Bodner and Beck, 1987; Neuner and Bannister, 1995; Neuner and Pramsohler, 2006).

2.6. Freezing resistance mechanism

For each species, the freezing resistance mechanism was determined by comparing LT_{50} and NT obtained in the thermal analyses. When LT_{50} occurs at a lower temperature than NT, the plant was classified as freezing tolerant (FT). Conversely, when LT_{50} was not significantly different from NT, the resistance mechanism was classified as freezing avoidance (FA) (Squeo et al., 1991; Bravo et al., 2001).

2.7. Statistical analyses

Thermal differences among distances from the ground surface (i.e. thermal microhabitat) were assessed using Chi-square

Table 1

Climatic conditions for different distances from the ground (i.e. thermal microhabitats) in the Central Chilean Andes. Values correspond to mean \pm 2SE. Different lowercases indicate statistical differences ($P < 0.05$).

	Distance from the ground (cm)		
	<15	15–25	>25
Sampled period (days)	13		
Mean temperature (°C)	12.0 \pm 1.5 ^a	10.5 \pm 1.1 ^a	9.1 \pm 0.8 ^a
Maximum temperature (°C)	30.6 \pm 2.0 ^a	24.1 \pm 1.2 ^b	19.2 \pm 1.3 ^c
Minimum temperature (°C)	-0.9 \pm 1.2 ^a	0.6 \pm 0.8 ^b	1.2 \pm 0.7 ^b
Number of freezing events	9 (69.2%) ^a	3.5 (26.9%) ^b	2.7 (20.5%) ^c
Intensity of freezing events (°C)	-1.7 \pm 1.2 ^a	-2.1 \pm 1.0 ^a	-1.9 \pm 0.7 ^a
Duration of freezing events (h)	4.7 \pm 2.9 ^a	5.6 \pm 2.9 ^a	6.5 \pm 2.5 ^a

tests. Differences in mean, maximum and minimum temperatures among thermal microhabitats were assessed with one-way ANOVA. Post hoc tests were made using Tukey (HSD). Differences in the frequency of freezing events among thermal microhabitats were assessed with Chi-square tests.

Relationship between plant height and LT_{50} was assessed using Pearson product-moment correlation. Differences between NT and LT_{50} to determine the freezing resistance mechanism on each species were assessed using t -tests. Difference in LT_{50} between plants with high- and low-growth intensity was assessed using t -test as well. Data were log transformed before statistical analyses when assumptions of normality and homoscedasticity were not met (Dytham, 2003).

3. Results

3.1. Thermal conditions

Our results showed that air temperature varied with the distance from the ground (Fig. 1). Comparisons of distances indicated that leaves of plants <15 cm tall were exposed to different thermal conditions than those growing between 15 and 25 cm from the ground ($\chi^2 = 605.6$, $P < 0.0001$), and that of plants >25 cm tall ($\chi^2 = 736.3$, $P < 0.0001$). Whilst mean air temperature did not differ with the distance from the ground, low and high extreme temperatures were recorded near the ground surface (Table 1). Maximum

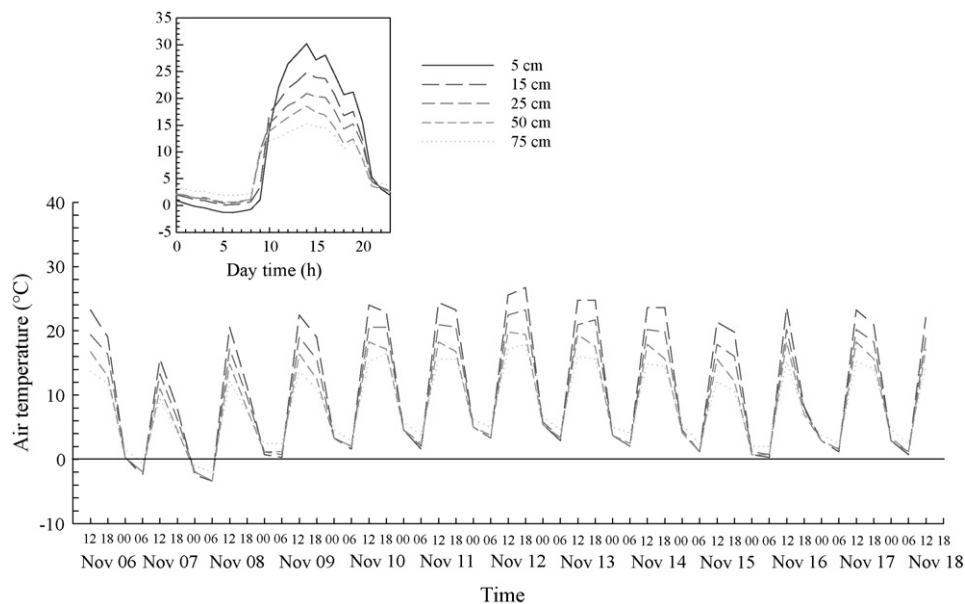


Fig. 1. Air temperature (°C) at five distances from the ground (cm). Small graph shows mean temperature during 24 h. Temperature was measured during 2 weeks early in the growing season, at 2900 m above sea level, in the Central Chilean Andes.

air temperature was 11.4 and 6.5K higher at distances <15 cm than 15–25 cm and >25 cm, respectively ($F_{2,88} = 33.56, P < 0.0001$). Minimum air temperature was 1.8K lower at distances <15 cm than above there ($F_{2,88} = 8.98, P = 0.011$), and the freezing temperatures were more frequent near the ground surface ($\chi^2 = 16.33, P < 0.001$). For example, freezing events occurred in 69.2% of the sampled days at distances <15 cm, with an extreme temperature of -5.3°C . Conversely, freezing events occurred in 26.9% of the days between 15 and 25 cm and only in 20.5% of the days at distances >25 cm, with extreme events of -3.6 and -2.6°C , respectively. Although the magnitude of the extreme freezing events varied among distances from the ground, no differences in their mean intensity and duration were recorded (Table 1; all $P > 0.05$).

3.2. Freezing resistance and plant height or growth-form relationships

No linear relationship between plant height and level of freezing resistance (LT_{50}) was found ($r^2 = -0.013, P = 0.51$). Leaves of short as well as tall plant species had similar LT_{50} (Fig. 2 and Table 2). In addition, there was no relationship between plant height and freezing resistance mechanisms of their leaves. While all FA species were <10 cm tall, FT species covered the complete range of plant heights (Fig. 2 and Table 2). Comparisons between growth-forms with high- or low-growth intensity showed that in both groups of plant species leaves resisted similar freezing temperatures ($t = -1.32; P = 0.19$), with a mean LT_{50} ca. -13.4°C (Fig. 3).

Table 2

Freezing resistance in leaves of 37 species from the Andes of Central Chile measured early in the growing season. Parameters: NT, ice nucleation temperature; LT_{50} , temperature producing 50% damage. Values are shown as mean \pm 2SE. Mechanisms: FA, freezing avoidance; FT, freezing tolerance. Plant growth-forms: A, annual; B, bulbous plant; C, cushion plant; F, forb; R, rosette; SHR, shrub; SS, dwarf shrub. Growth intensity: H, high; L, low. Species nomenclature follows Marticorena and Quezada (1985).

Species	Family	Growth-form	Growth intensity	Height (cm)	NT ($^\circ\text{C}$)	LT_{50} ($^\circ\text{C}$)	Mechanism
<i>Acaena pinnatifida</i>	Rosaceae	R	H	8.8 (0.5)	-6.3 (0.5)	-15.2 (0.9)	FT
<i>Acaena splendens</i>	Rosaceae	SS	L	20.5 (0.8)	-3.9 (0.9)	-14.1 (1.0)	FT
<i>Anarthrophyllum cumingii</i>	Fabaceae	SHR	L	11.6 (0.6)	-5.1 (1.7)	-15.8 (0.5)	FT
<i>Astragalus looseri</i>	Fabaceae	L	L	49.4 (2.2)	-6.5 (0.5)	-15.4 (1.1)	FT
<i>Barneoudia major</i>	Ranunculaceae	R	H	5.1 (0.2)	-4.9 (0.6)	-12.7 (0.9)	FT
<i>Barneoudia cf. chilensis</i>	Ranunculaceae	R	H	4.5 (0.3)	-5.1 (1.4)	-11.6 (1.2)	FT
<i>Berberis empetrifolia</i>	Berberidaceae	SHR	L	40.9 (2.0)	-7.1 (1.0)	-16.4 (0.2)	FT
<i>Calandrinia affinis</i>	Portulacaceae	R	H	12.2 (0.9)	-6.3 (0.5)	-14.5 (1.6)	FT
<i>Calceolaria arachnoidea</i>	Scrophulariaceae	F	L	7.5 (0.4)	-5.0 (1.0)	-11.3 (0.4)	FT
<i>Cerastium arvense</i>	Caryophyllaceae	F	H	10 (1.2)	-5.1 (0.7)	-11.3 (0.8)	FT
<i>Chaetanthera euphrasioides</i>	Asteraceae	A	H	1.8 (0.1)	-8.9 (0.3)	-8.3 (0.3)	FA
<i>Chuiriraga oppositifolia</i>	Asteraceae	SHR	L	21.1 (1.0)	-5.7 (1.6)	-13.6 (1.1)	FT
<i>Colobanthus quitensis</i>	Caryophyllaceae	R	L	1 (0.2)	-7.8 (0.9)	-16.2 (0.4)	FT
<i>Discaria nana</i>	Rhamnaceae	C	L	23 (2.5)	-5.9 (0.3)	-13.9 (0.5)	FT
<i>Erigeron andicola</i>	Asteraceae	R	L	4 (0.2)	-5.6 (0.7)	-12.4 (2.6)	FT
<i>Euphorbia collina</i>	Euphorbiaceae	F	H	10.2 (0.4)	-5.5 (0.7)	-12.3 (0.8)	FT
<i>Haplopappus anthyllioides</i>	Asteraceae	SS	L	5.4 (0.2)	-7.2 (2.3)	-12.1 (1.3)	FT
<i>Haplopappus schumannii</i>	Asteraceae	SS	L	28 (1.0)	-7.5 (1.5)	-10.3 (0.4)	FT
<i>Hordeum comosum</i>	Poaceae	F	L	10 (1.2)	-11.0 (1.0)	-11.5 (0.4)	FA
<i>Hypochaeris clarionoides</i>	Asteraceae	R	H	3.3 (0.8)	-8.7 (1.4)	-8.2 (0.3)	FA
<i>Laretia acaulis</i>	Apiaceae	C	L	4.5 (0.6)	-7.0 (1.5)	-10.3 (0.9)	FT
<i>Microsteris gracilis</i>	Polemoniaceae	A	H	6 (0.3)	-7.6 (0.9)	-11.6 (1.6)	FT
<i>Nassauvia looseri</i>	Asteraceae	SS	L	9.6 (0.1)	-8.6 (1.2)	-12.4 (0.9)	FT
<i>Nastanthus spathulatus</i>	Calyceraceae	R	L	4.2 (0.5)	-5.4 (1.4)	-18.0 (0.0)	FT
<i>Noccaea magellanica</i>	Brassicaceae	R	H	4 (0.5)	-4.1 (0.6)	-12.9 (1.9)	FT
<i>Perezia carthamoides</i>	Asteraceae	R	H	3.8 (0.3)	-3.3 (0.3)	-14.7 (0.7)	FT
<i>Phacelia secunda</i>	Hydrophyllaceae	F	L	9.1 (0.8)	-3.1 (1.0)	-16.8 (0.7)	FT
<i>Pozoa coriacea</i>	Apiaceae	R	L	4.3 (0.1)	-8.2 (0.3)	-17.3 (0.9)	FT
<i>Quinchamalium chilense</i>	Santalaceae	F	H	1.9 (0.2)	-8.4 (1.5)	-10.3 (1.5)	FA
<i>Rhodophiala rhodolirion</i>	Amaryllidaceae	B	H	8.7 (0.8)	-5.5 (1.0)	-22.0 (0.1)	FT
<i>Sanicula graveolens</i>	Apiaceae	R	H	15 (1.4)	-6.8 (0.7)	-13.2 (0.8)	FT
<i>Senecio bustillosianus</i>	Asteraceae	SHR	L	14.6 (1.0)	-6.8 (0.3)	-11.9 (0.6)	FT
<i>Senecio erucaeformis</i>	Asteraceae	SHR	L	26.1 (1.0)	-5.6 (0.4)	-13.4 (1.0)	FT
<i>Senecio cf. polygaloides</i>	Asteraceae	SS	L	8.3 (1.2)	-5.3 (1.3)	-13.0 (2.0)	FT
<i>Taraxacum officinale</i>	Asteraceae	R	H	3.9 (0.2)	-5.7 (1.6)	-16.6 (1.2)	FT
<i>Tetraglochin alatum</i>	Rosaceae	SHR	L	60.8 (2.3)	-1.6 (0.3)	-11.7 (0.7)	FT
<i>Tristagma bivalve</i>	Alliaceae	B	H	8.5 (1.1)	-7.1 (1.2)	-17.4 (0.8)	FT

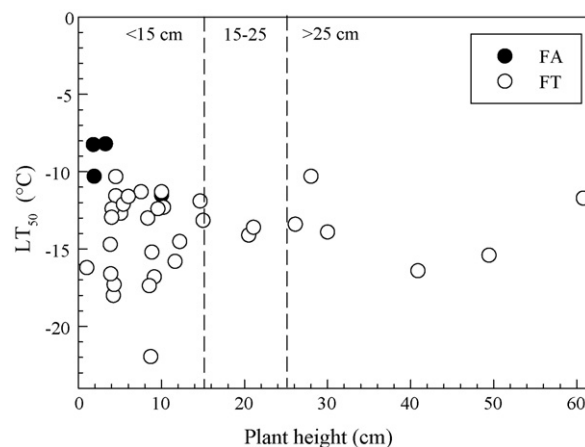


Fig. 2. Relationship between plant height (cm) and leaf low temperature damage, LT_{50} ($^\circ\text{C}$) of 37 high-Andean plants species from Central Chile. White circles are freezing tolerant species (FT), black circles are freezing avoidant species (FA). Dashed lines indicate the distances from the ground where different thermal conditions were significant ($P < 0.05$).

4. Discussion

Microclimatic measurements showed that high-Andean species of different height are exposed to different thermal conditions depending on the distance from the ground (Fig. 1). Our results are in concordance with previous studies reporting that lower freezing temperatures are more frequent near the ground (Leuning and

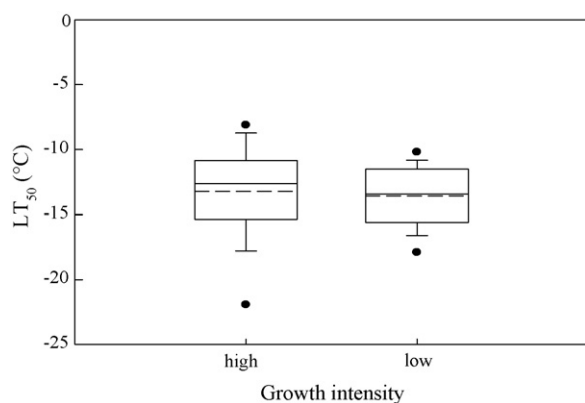


Fig. 3. Low temperature damage, LT_{50} ($^{\circ}C$) measured in leaves of species with low- ($n = 21$) and with high-growth intensity ($n = 16$). For each functional group, box plots median (solid line), mean (dashed line), 25th and 75th percentiles and SE. Points indicate 5th and 95th percentiles. High-Andean plants species that were included in each functional group are detailed in Section 2.

Cremer, 1988; Jordan and Smith, 1995). However, the level of freezing resistance in high-Andean species was not related to the plant height, indicating that leaves of short as well as of tall plants species are damaged to similar freezing temperatures (Fig. 2). For example, the 40.9 cm tall shrub *Berberis empetrifolia* Lam. and the 9.1 cm tall forb *Phacelia secunda* J.F. Gmel. were both damaged at ca. $-16^{\circ}C$ (Table 2). Leaf temperatures during frosts are determined by a combination of environmental (i.e. surrounding surface, soil moisture) and plant morphological factors (i.e. plant height, leaf size and orientation) that affect the net energy balance between a plant and its environment (Schulze et al., 2005; Lambers et al., 2008), and consequently its ability to resist freezing temperatures. For example, plants surrounded by grass or litter layers are exposed to cooler temperatures than those surrounded by bare ground (Leuning and Cremer, 1988; Ball et al., 2002). In our study area, dead plant material drawn by wind accumulates under *B. empetrifolia*, like under other tall shrub species (e.g. *Anarthrophyllum cumingii* (Hook. & Arn.) Phil., *Chuquiraga oppositifolia* D. Don, *Tetraglochin alatum* (Gill. ex H. et A.) Kuntze). Consequently, the leaves of these tall plant species are exposed to lower freezing temperatures than expected by the distance aboveground, increasing their freezing resistance, and reducing the difference in LT_{50} among leaves of tall and of small plants species.

Similar LT_{50} between species with contrasting plant heights have been previously reported. For instance, the 89.3 cm tall shrub *Ephedra breana* and the 18.1 cm tall forb *Phacelia cumingii* were both damaged at ca. $-13^{\circ}C$, in the Desert Chilean Andes (Squeo et al., 1996). Lack of relationship between plant height and LT_{50} in xeric mountain systems suggests that environmental factors other than air temperature may also be affecting the freezing resistance of high-Andean species. In contrast to the Venezuelan tropical Andes, where Squeo et al. (1991) reported a linear relationship between plant height and LT_{50} , Central Chilean Andes are characterized by severe drought episodes during summer (Cavieres et al., 2006). Such drought episodes can alter patterns of freezing resistance of high-Andean species in several ways. For instance, Sierra-Almeida et al. (2009) reported that the freezing point (i.e. freezing of water in the apoplast) decreased when drought increased, with that variation strongly affecting the ability of several high-Andean species to resist frosts. Plant responses to freezing temperatures and drought share similar physiological pathways (e.g. osmotic adjustment via synthesis of carbohydrates and proline; but see Beck et al., 2007), producing a strong relationship between plant water status and the ability to resist freezing temperatures (Goldstein et al., 1985; Rada et al., 1987). In our study, several tall plant species have mor-

phological features related to drought resistance (e.g. small and sclerophyllous leaves, thorns), suggesting that those drought adaptations in tall plants may increase the freezing resistance of their leaves to similar levels of short plant species.

It is known that the freezing resistance of high-elevation plants with green overwintering leaves varies intra-seasonally in the Central Chilean Andes (Sierra-Almeida et al., 2009). However, both short and tall plants varied in a similar way their LT_{50} throughout the growing season. For example, while *B. empetrifolia* and *P. secunda* had LT_{50} ca. $-16^{\circ}C$ early in the growing season, their LT_{50} increased to ca. $-12^{\circ}C$ late in the growing season (Sierra-Almeida et al., 2009), suggesting that the lack of relationship between plant height and LT_{50} is a general trend among the high-Andean plants examined.

Mechanisms of freezing resistance were not related to plant height (Fig. 2). Most of the studies species were FT and covered the entire range of plant heights, while only four species were FA and all were ≤ 10 cm tall (Table 2). These results contrasted with those reported by Squeo et al. (1991, 1996), where a plant height threshold at 50 and 20 cm separated FT from FA species in Venezuelan and Northern Chilean Andes, respectively. FT is a common freezing resistance mechanism in areas where freezing temperatures are several degrees below $0^{\circ}C$ and last for a long time (Sakai and Larcher, 1987; Larcher, 2003). Dominance of FT among studied species suggests that, although leaves of short and tall plants are exposed to different thermal conditions, freezing events are severe at all distances. For example, freezing events are less frequent at distances >25 cm from the ground, but their duration was in average 6.5 h, exposing even tall plants to prolonged episodes of freezing temperatures. Thus, although tall plants experience a lower frequency of freezing events than short plants, such freezing events are severe due to their long duration, and therefore, their leaves need to have an effective mechanism to protect them from frost damage. Finally, only four species were classified as FA species (Table 2). Leaves of these species showed the lowest NT among studied species, which were at least 3 K lower than $-5.3^{\circ}C$, the most extreme freezing event recorded in the study area. In terms of intensity of freezing events, FA species could effectively protect their leaves from frost damage through this mechanism, because ice crystals are formed in their tissues at lower temperatures than recorded during freezing events in the field. However, a higher frequency of freezing events near the ground, and their long duration in general suggest that FA mechanisms may be a risky strategy for these species, when FA protection is of limited duration (i.e. few hours), time after which freezing take place abruptly, producing dehydration of plant tissues (Sakai and Larcher, 1987). Thus, freezing survival of these species during the growing season could improve if they combine physiological FA mechanisms (e.g. supercooling) with other strategies, such as to grow in sites considered thermal refuges (e.g. between rocks) and/or by a high capacity to regenerate and replace damaged tissues (Körner, 2003).

Our results do not support Sakai and Larcher (1987), who proposed an inverse relationship between growth intensity and freezing resistance. In Central Chilean Andes, leaves of high-elevation plants with high- and low-growth intensities have similar LT_{50} (Fig. 3). For example, the annual herb *Microsteris gracilis* (Douglas ex Hook.) Greene and the shrub *Senecio bustillosianus* Remy were both damaged at ca. $-11^{\circ}C$ (Table 2). Several authors have proposed 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 (Lepš et al., 1982; MacGillivray et al., 1995). However, Gurvich et al. (2002) found that plant traits and resistance to different environmental stresses represent two distinct axes of specialization, which are at least partially decoupled. Thus, growth intensity is not a useful trait to predict freezing resistance of high-elevation species, when leaves

of species with high- and low-growth intensity are damaged to similar freezing temperatures during summer. Freezing resistance of high elevation species seems to be more related to plant distribution along climatic gradients than to growth-forms (Gurvich et al., 2002; Taschler and Neuner, 2004). In this sense, changes in the frequency and severity of extreme freezing events should be relevant for plant migration into new areas within climatic gradients (Grabherr et al., 1994; Guisan and Theurillat, 2000; Cannone et al., 2007), but should not be expected to substantially affect functional group composition of plant communities in high-elevation habitats, at least in terms of leaf freezing resistant. Further studies are needed to evaluate the effects of freezing temperatures on other plant tissues, like flowers and belowground organs for predicting how high-elevation plants may respond to changes in the freezing temperature regimes.

In conclusion, we found that high-Andean species with contrasting plant heights were exposed to different thermal conditions during freezing events. Nevertheless, the level and mechanisms of freezing resistance were not related to plant height. Apparently, environmental factors other than air temperature affecting the ability of leaves of tall plants to resist frosts to similar levels of short plant species. Dominance of FT species seemed to be related to the long duration of freezing events at all distances from the ground. Finally, growth intensity was not related to freezing resistance of high-Andean species, suggesting that climatic conditions are better predictors of freezing resistance than plant functional traits.

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