

Freezing resistance varies within the growing season and with elevation in high-Andean species of central Chile

Angela Sierra-Almeida^{1,2}, Lohengrin A. Cavieres^{1,2} and León A. Bravo³

¹ECOBIOSIS, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile;

²Instituto de Ecología y Biodiversidad (IEB), Casilla 653, Santiago, Chile; ³Laboratorio de Fisiología Vegetal, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Summary

Author for correspondence:

Angela Sierra-Almeida
Tel: +56 41 2203846
Fax: +56 41 2246005
Email: ansierra@udec.cl

Received: 24 September 2008

Accepted: 6 December 2008

New Phytologist (2009)

doi: 10.1111/j.1469-8137.2008.02756.x

Key words: alpine environments, Andes, drought, freezing resistance, growing season, high-elevation plants.

- Predicted increases in the length of the growing season as a result of climate change may more frequently expose high-elevation plants to severe frosts. Understanding the ability of these species to resist frosts during the growing season is essential for predicting how species may respond to changes in temperature regimes.
- Here, we assessed the freezing resistance of 24 species from the central Chilean Andes by determining their low temperature damage (LT₅₀), ice nucleation temperature (NT), freezing point (FP) and freezing resistance mechanism (i.e. avoidance or tolerance).
- The Andean species were found to resist frosts from −8.2 to −19.5°C during the growing season, and freezing tolerance was the most common resistance mechanism. Freezing resistance (LT₅₀) varied within the growing season, decreasing towards the end of this period in most of the studied species. However, the FP showed the opposite trend. LT₅₀ increased with elevation, whilst FP was lower in plants from lower elevations, especially late in the growing season.
- Andean species have the potential to withstand severe freezing conditions during the growing season, and the aridity of this high-elevation environment seems to play an important role in determining this high freezing resistance.

Introduction

Low temperature is an important determinant of the distribution of plants, and it is the first environmental filter that species have to pass to become high-elevation (Körner, 2003). Thus, plants inhabiting high-elevation environments are adapted to cope with the extreme low temperatures that characterize these habitats (Billings, 1974). Predictable frost events, as they occur during winter, are usually not critical for plant survival as cold hardening and snow cover provide protection against frost damage in most high-elevation species (Körner, 2003). However, when freezing temperatures occur during the growing season, high-elevation plants may lose a substantial fraction of their above-ground tissues or die as a result of frost damage (Körner, 2003; Taschler *et al.*, 2003).

The ability to survive freezing temperatures (i.e. freezing resistance) is closely related to the ambient temperature that plants experience (Beck *et al.*, 2004; Bannister *et al.*, 2005).

This has led some authors to propose that early in the growing season (i.e. after snow melt) high-elevation plants are fully active and the ability to resist freezing temperatures is minimal. Conversely, late in the growing season (i.e. after fruit ripening and/or the seed dispersal phase) plants are hardened to cope with the unfavorable cold season (Sakai & Larcher, 1987; Larcher, 2003). However, freezing temperatures can occur both early and late in the growing season (Greenland & Losleben, 2001), suggesting that, from an evolutionary point of view, the freezing resistance of high-elevation plants should be high both early and late in the growing season.

Climate change is affecting the length of the growing season, and subsequently the survival and reproduction of high-elevation plants (Inouye & McGuire, 1991; Inouye, 2008; Kudernatsch *et al.*, 2008). Some climatic models predict a reduction of snow cover duration and increases in the frequency and intensity of sudden frost events during the growing season (Menzel & Fabian, 1999; Easterling *et al.*,

2000; Cannone *et al.*, 2007), increasing the risk of damage by frost (Neuner *et al.*, 1999). Thus, it seems important to understand the ability of high-elevation plants to withstand freezing conditions and how this changes as the growing season progresses.

Although there have been many studies on freezing resistance in high-elevation plants, most of them were carried out on mesic mountains, where plants do not experience shortages in water availability (Sakai & Ötsuka, 1970; Taschler & Neuner, 2004; Márquez *et al.*, 2007). By contrast, little is known about summer freezing resistance in xeric mountains (Tyurina, 1957 in Sakai & Larcher, 1987) with studies in Mediterranean-type mountains being particularly scarce (Loik & Redar, 2003; Loik *et al.*, 2004). Studies in xeric mountains are particularly important because freezing temperatures and drought share similar physiological responses. For example, plants can counteract cellular dehydration and turgor loss by osmotic adjustment via synthesis of carbohydrates and proline (Blödnér *et al.*, 2005; Nakashima & Yamaguchi-Shimozaki, 2006; Beck *et al.*, 2007). Therefore, the induction of similar physiological responses suggests that the abilities of plants to survive freezing temperatures and drought can be closely connected.

The altitudinal decrease in air temperature may affect the freezing resistance of plants from different elevations (Goldstein *et al.*, 1985; Rada *et al.*, 1987; Halloy & González, 1993; Loik & Redar, 2003). For instance, high-elevation species survive freezing temperatures 4 K lower than low-elevation species in the Northern Chilean Andes (Squeo *et al.*, 1996). Nevertheless, Márquez *et al.* (2007) found that seven out of nine grass species from the Venezuelan Andes did not show higher freezing resistance at higher elevations, suggesting that altitudinal trends in freezing resistance are not universal. Whether freezing resistance is found to vary with elevation can be determined by the time of the year at which altitudinal comparisons are made. For example, Bannister & Fagan (1989) found that the altitudinal difference in freezing resistance for the fern *Blechnum penna-marina* measured in summer was smaller than that measured in winter. This is attributable to an increase in the water deficit in *B. penna-marina* individuals from low elevations, which increases the freezing resistance of the fronds by several degrees (Bannister & Fagan, 1989). Hence, altitudinal trends in the freezing resistance of plants inhabiting xeric mountains may be altered by drought episodes at the end of the growing season, particularly through their effects on plants from low elevations.

The main objectives of this study were to explore the freezing resistance of 24 alpine plant species inhabiting the Mediterranean-type climate of the high Andes of central Chile, and to determine their freezing resistance mechanisms (i.e. avoidance or tolerance). In particular, we assessed whether freezing resistance changes within the growing season, and whether freezing resistance increases with elevation. We hypoth-

esize that freezing resistance of Andean plants does not change within the growing season, but increases with elevation. This information is important for assessing how changes in the length of the growing season and in the frequency and intensity of frost events can affect the survival and distribution of these high-elevation species, and hence for inferring responses in other xeric high-elevation areas.

Materials and Methods

Target species and study site

This study was carried out near the locality of Farellones, in the central Chilean Andes, 50 km east of Santiago. A total of 24 species were selected to include several growth forms, several functional groups and a wide range of families (Supporting Information Table S1). The species studied were collected at between 2500 and 2900 m, on a north-facing slope, located near the La Parva Ski Complex (33°21'S, 70°19'W). This area is characterized by the dominance of shrubs and dwarf shrubs, and the presence of annuals and perennial herbs, and cushion plants such as *Laretia acaulis* (Cav.) Gill et Hook. (Cavieres *et al.*, 2000). Plant samples corresponded to small twigs with mature leaves for shrubs and dwarf shrubs, and modules with at least two mature leaves or complete individuals for rosettes, cushion plants and graminoids. Measurements were conducted at a field laboratory, where plant samples were immediately analyzed after collection in the field. Plant samples were placed into a cooler to prevent changes in tissue water status and then transported to the field laboratory, less than a 10-min drive away. For intraseasonal comparisons, we obtained data for the 24 species sampled early and late in the growing season. The growing season at 2900 m usually starts with the snowmelt in October and finishes in April with the first snowfall. Therefore, the early growing season corresponded to measurements made between 26 October and 4 November 2006. Measurements corresponding to the late growing season were made on 29 March 2007. For altitudinal comparisons, seven of the studied species present at the lower elevation sites were also collected and measured at a second site located at 3600 m elevation, on a northeast-facing slope (33°19'S, 70°15'W). This site is characterized by the cushion plant *Azorella monantha* Clos and the presence of several rosette and graminoid species (Cavieres *et al.*, 2000). The growing season at the high elevation site starts in December and finishes in March. Therefore, measurements of freezing resistance were made on 15 December 2006 and 25 March 2007, corresponding to early and late time-points, respectively. The microclimatic conditions of the low- and high-elevation sites are shown in Table 1. Although some differences in aspect, topography and ground cover exist between the low- and high-elevation sites, the effects of these differences are minor in comparison to the effects of the differences in elevation (Cavieres *et al.*, 2007).

Table 1 Air temperatures and soil water potential ($\Psi\text{H}_2\text{O}$) at 2800 m and 3600 m elevation during the growing season in the Andes of central Chile

	Elevation	
	2800 m	3600 m
Mean minimum temperature ($^{\circ}\text{C}$)	4.4 ± 2.5	0.1 ± 0.6
Mean maximum temperature ($^{\circ}\text{C}$)	20.4 ± 4.0	16.4 ± 2.9
Soil $\Psi\text{H}_2\text{O}$ early in the growing season (MPa)	-1.64 ± 0.09	-0.30 ± 0.19
Soil $\Psi\text{H}_2\text{O}$ late in the growing season (MPa)	-4.51 ± 0.46	-0.25 ± 0.17

Values are means \pm 2 SE. Temperature data were obtained from Cavieres *et al.* (2007).

Thermal analyses

For each species, five expanded mature leaves were removed from the different plant samples taken in the field (each plant sample was taken from different individuals randomly selected), and each leaf was attached to a thermocouple (Gauge 30 copper-constantan thermocouples; Cole Palmer Instruments, Vernon Hills, IL, USA), and immediately enclosed in a small, tightly closed cryotube. The cryotubes were placed in a cryostat (mgw LAUDA RC 20; Königshofen, Germany), and the temperature was decreased from 0 to -18°C at a cooling rate of 2°C h^{-1} . The temperature of individual leaves was monitored every second with a Personal Daq/56 multi-channel thermocouple USB data acquisition module (IOtech, Cleveland, OH, USA). The sudden rise in leaf temperature (exotherm) produced by the heat released during the extracellular freezing process was used to determine two variables: the ice nucleation temperature (NT), which corresponds to the lowest temperature before the exotherm, indicating the onset of ice crystal formation, and the freezing point (FP), the highest point of the exotherm, indicating the freezing of water in the apoplast, including symplastic water driven outwards by the water potential difference caused by the apoplastic ice formation (Larcher, 2003).

Low temperature damage (LT_{50})

For each species, five samples were introduced into separate, hermetically sealed plastic bags, and incubated in a cryostat that had previously been cooled. The cryostat was scheduled 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 kept at 4°C in the dark for 24 h (unfrozen samples). As visual damage was not immediately obvious for all species, damage was assessed after thawing using a chlorophyll fluorimeter (Plant Efficiency Analyzer; Hansatech, King's Lynn, UK) to determine the ratio of variable to maximum

fluorescence (F_v/F_m) of dark-adapted photosynthetic organs of each sample (Maxwell & Johnson, 2000). As dead material effectively had an F_v/F_m of zero, damage was calculated as the percentage of photoinactivation ($100 \times PhI$), where PhI is the photoinactivation ratio described by Larcher (2000):

$$PhI = (1 - F_{FT}/F_{max}) \quad \text{Eqn 1}$$

(F_{FT} , the F_v/F_m of the sample exposed to a freezing temperature T ; F_{max} , 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).

Freezing resistance mechanism

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

Statistical analyses

For each species, differences between NT and LT_{50} to determine freezing resistance mechanisms were assessed using one-tailed t -tests, while seasonal differences in FP and LT_{50} were assessed using paired t -tests. Altitudinal differences among the seven species present at the two elevations were assessed with two-way ANOVA, where elevation and time of measurements (early vs late growing season) were considered as fixed factors. Data were log transformed before statistical analyses when assumptions of normality and homoscedasticity were not met (Dytham, 2003).

Table 2 Freezing resistance in leaves of 24 species from the Andes of central Chile measured early in the growing season

Species	Growth form	NT	FP	LT ₅₀	Thermal difference	Mechanism
La Parva (low elevation)						
<i>Acaena pinnatifida</i>	PH	-6.3 (0.5)	-3.9 (0.5)	-15.2 (0.9)	8.9**	FT
<i>Acaena splendens</i>	SS	-3.9 (0.9)	-2.1 (0.5)	-14.1 (1)	10.2**	FT
<i>Anarthrophyllum cumingii</i>	SHR	-5.1 (1.7)	-2.6 (1.9)	-15.8 (0.5)	10.7**	FT
<i>Astragalus looserii</i>	SHR	-6.5 (0.5)	-1.8 (0.7)	-15.4 (1.1)	8.9**	FT
<i>Berberis empetrifolia</i>	SHR	-7.1 (1)	-3.7 (0.8)	-16.4 (0.2)	9.3*	FT
<i>Calceolaria arachnoidea</i>	PH	-5.0 (1)	-1.3 (0.5)	-11.3 (0.4)	6.3**	FT
<i>Cerastium arvense</i>	PH	-5.1 (0.7)	-1.4 (0.5)	-11.3 (0.8)	6.2***	FT
<i>Chuquiraga oppositifolia</i>	SHR	-5.7 (1.6)	-4.4 (1.3)	-13.6 (1.1)	7.9**	FT
<i>Colobanthus quitensis</i>	PH	-7.8 (0.9)	-5.4 (1.2)	-16.2 (0.4)	8.4*	FT
<i>Erigeron andicola</i>	PH	-5.6 (0.7)	-1 (1)	-12.4 (2.6)	6.8**	FT
<i>Euphorbia collina</i>	PH	-5.5 (0.7)	-1.4 (0.3)	-12.3 (0.8)	6.8*	FT
<i>Haplopappus anthylloides</i>	SS	-7.2 (2.3)	-4.04 (1.3)	-12.1 (1.3)	4.9*	FT
<i>Haplopappus schumannii</i>	SS	-7.5 (1.5)	-4.9 (1.3)	-10.3 (0.4)	2.8**	FT
<i>Hordeum comosum</i>	PH	-11 (1)	-9.2 (1.1)	-11.5 (0.4)	0.5	FA
<i>Nassauvia looserii</i>	PH	-8.6 (1.2)	-6.5 (0.7)	-12.4 (0.9)	3.8**	FT
<i>Nastanthus spathulatus</i>	PH	-5.4 (1.4)	-0.1 (0.3)	-18.0 (0)	12.6**	FT
<i>Perezia carthamoides</i>	PH	-3.3 (0.3)	-1.0 (0.3)	-14.7 (0.7)	11.4**	FT
<i>Phacelia secunda</i>	PH	-3.1 (1.0)	-0.5 (0.3)	-16.8 (0.7)	13.7**	FT
<i>Pozoa coriacea</i>	PH	-8.2 (0.3)	-3.5 (0.8)	-17.3 (0.9)	9.1**	FT
<i>Quinchamalium chilense</i>	PH	-8.4 (1.5)	-3.9 (1.9)	-10.3 (1.5)	1.9	FA
<i>Senecio bustillosianus</i>	SHR	-6.8 (0.3)	-2.0 (0.8)	-11.9 (0.6)	5.1*	FT
<i>Senecio erucaeformis</i>	SHR	-5.6 (0.4)	-1.8 (0.5)	-13.4 (1.0)	7.8**	FT
<i>Senecio pachyphyllos</i>	SS	-5.3 (1.3)	-1.9 (0.8)	-13.0 (2.0)	7.7**	FT
<i>Taraxacum officinale</i>	PH	-5.7 (1.6)	-2.5 (0.7)	-16.6 (1.2)	10.9*	FT
Cerro Franciscano (high elevation)						
<i>Colobanthus quitensis</i>	PH	-7.4 (1)	-3.6 (0.7)	-14.4 (1)	7**	FT
<i>Erigeron andicola</i>	PH	-5 (1)	-2.1 (0.8)	-11.6 (1.4)	6.6***	FT
<i>Hordeum comosum</i>	PH	-8.1 (1.7)	-3.9 (1.2)	-17 (1.1)	8.9***	FT
<i>Perezia carthamoides</i>	PH	-5.5 (0.8)	-1.3 (0.6)	-17 (0.7)	11.5*	FT
<i>Phacelia secunda</i>	PH	-8.3 (1.5)	-1.9 (1.5)	-16.5 (0.7)	-8.2**	FT
<i>Pozoa coriacea</i>	PH	-9.3 (0.6)	-1.2 (0.5)	-16.1 (0.6)	6.8**	FT
<i>Taraxacum officinale</i>	PH	-8.4 (0.8)	-1.2 (0.9)	-17.8 (0.2)	9.4**	FT

Samples were collected at two elevations: La Parva (2500–2900 m) and Cerro Franciscano (3600 m). We measured the ice nucleation temperature (NT), the freezing point (FP), and the temperature producing 50% damage (LT₅₀), where values are mean ± 2 SE. Thermal difference corresponds to the difference between NT and LT₅₀. When NT and LT₅₀ were similar, the mechanism of freezing resistance was freezing avoidance (FA), but when NT and LT₅₀ were different plants were classified as freezing tolerant (FT). Level of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Plant species were categorized according to growth form: SHR, shrub; SS, dwarf shrub; PH, perennial herb. Species nomenclature follows Marticorena & Quezada (1985).

Results

Early in the growing season, the majority of the studied species could resist temperatures as low as -11°C (LT₅₀). Exceptions were *Quinchamalium chilense* Mol. and *Haplopappus schumannii* (Kuntze) G.K. et W.D. (both with LT₅₀ = -10.3°C). In this period, *Nastanthus spathulatus* (Phil.) was the most freezing-resistant species (LT₅₀ = -18°C) (Table 2). Twenty-two out of the 24 investigated species were classified as FT, while only two species (*Hordeum comosum* (J. Presl.) Löve and *Q. chilense*) were classified as freezing avoidant (FA) (Table 2). Late in the growing season, most of the studied species resisted temperatures below -10°C (LT₅₀), except for *Astragalus looserii* I.M. Johnst. (LT₅₀ = -8.2°C) and

Calceolaria arachnoidea Graham (LT₅₀ = -8.6°C). *Erigeron andicola* D.C. was the most freezing-resistant species (LT₅₀ = -19.5°C). In this period, 16 species were classified as FT and eight species as FA (Table 3). Thus, the ability to resist freezing temperatures changed during the growing season, and several patterns emerged depending on the variable analyzed (Fig. 1). Contrary to expectations, no intraseasonal changes in LT₅₀ were detected in only seven out of the 24 studied species (29.2%). Twenty-five per cent of species (six species) were more freezing resistant (more negative LT₅₀) late than early in the growing season, while 45.8% of species (11 species) showed the opposite trend (Fig. 1a). In this last case, the average of freezing resistance early in the growing season was significantly higher than that late in the growing season

Table 3 Freezing resistance in leaves of 24 species from the Andes of central Chile measured late in the growing season

Species	Growth form	NT	FP	LT ₅₀	Thermal difference	Mechanism
La Parva (low elevation)						
<i>Acaena pinnatifida</i>	PH	-9.4 (1.5)	-7.2 (1.0)	-14.6 (0.8)	5.2*	FT
<i>Acaena splendens</i>	SS	-8.2 (1.6)	-5.9 (1.3)	-10 (0.7)	1.8	FA
<i>Anarthrophyllum cumingii</i>	SHR	-10.6 (2.4)	-8.9 (2.9)	-10.6 (0.5)	0.0	FA
<i>Astragalus looserii</i>	SHR	-9.4 (0.5)	-3.0 (0.4)	-8.2 (0.6)	1.2	FA
<i>Berberis empetrifolia</i>	SHR	-9.1 (1.5)	-7.6 (1.6)	-11.5 (0.2)	2.4	FA
<i>Calceolaria arachnoidea</i>	PH	-5.4 (0.8)	-3.0 (1.3)	-8.6 (0.2)	3.2**	FT
<i>Cerastium arvense</i>	PH	-4.5 (1)	-2.8 (0.7)	-9.7 (1.1)	5.2***	FT
<i>Chuquiraga oppositifolia</i>	SHR	-11.5 (1.5)	-8.4 (1.0)	-15.4 (0.8)	3.9**	FT
<i>Colobanthus quitensis</i>	PH	-8.4 (1.5)	-2.1 (1.5)	-10.6 (1.4)	2.2	FA
<i>Erigeron andicola</i>	PH	-11.6 (1.3)	-6.5 (1.1)	-19.5 (0.4)	7.9**	FT
<i>Euphorbia collina</i>	PH	-5.0 (0.9)	-2.7 (1.2)	-17.2 (0.9)	12.2**	FT
<i>Haplopappus anthylloides</i>	SS	-8.1 (1)	-5.1 (0.5)	-15.4 (1.2)	7.3**	FT
<i>Haplopappus schumannii</i>	SS	-10.5 (1.4)	-7.9 (0.5)	-13.3 (0.3)	2.8*	FT
<i>Hordeum comosum</i>	PH	-11.6 (2.2)	-10 (2.5)	-12 (1)	0.4	FA
<i>Nassauvia looserii</i>	SS	-6.9 (0.9)	-5.2 (0.5)	-13.9 (0.6)	7*	FT
<i>Nastanthus spathulatus</i>	PH	-6.7 (1)	-3.4 (0.5)	-13.2 (0.6)	6.5**	FT
<i>Perezia carthamoides</i>	PH	-9.7 (0.6)	-6.0 (0.6)	-13 (0.9)	3.3*	FT
<i>Phacelia secunda</i>	PH	-5.8 (0.7)	-2.4 (0.5)	-12.8 (1.1)	7**	FT
<i>Pozoa coriacea</i>	PH	-6.3 (0.5)	-2.4 (0.5)	-14.1 (0.3)	7.8**	FT
<i>Quinchamalium chilense</i>	PH	-10.2 (0.7)	-5.8 (1)	-9.2 (1)	1	FA
<i>Senecio bustillosianus</i>	SHR	-7.9 (1.3)	-4.1 (1.8)	-12.6 (1.4)	4.7*	FT
<i>Senecio erucaeiformis</i>	SHR	-3.8 (0.3)	-0.6 (0.2)	-8.4 (0.2)	4.6*	FT
<i>Senecio pachyphyllos</i>	SS	-7.9 (1.3)	-4.6 (1.2)	-12.1 (1.2)	4.2*	FT
<i>Taraxacum officinale</i>	PH	-10.6 (1.5)	-6.6 (2.2)	-12.3 (0.7)	1.7	FA
Cerro Franciscano (high elevation)						
<i>Colobanthus quitensis</i>	PH	-8.5 (1.9)	-1.8 (2.4)	-13.4 (0.9)	4.9*	FT
<i>Erigeron andicola</i>	PH	-3.7 (0.4)	-1.4 (0.4)	-18.1 (1)	14.4*	FT
<i>Hordeum comosum</i>	PH	-7.8 (0.3)	-6.6 (0.3)	-20.4 (1)	12.6*	FT
<i>Perezia carthamoides</i>	PH	-6.4 (0.6)	-3.3 (0.6)	-13.3 (0.7)	6.9**	FT
<i>Phacelia secunda</i>	PH	-3.3 (0.7)	-1.7 (0.8)	-14.7 (1)	11.4**	FT
<i>Pozoa coriacea</i>	PH	-6.1 (0.5)	-2.3 (0.5)	-13.3 (0.8)	7.2**	FT
<i>Taraxacum officinale</i>	PH	-5.9 (0.6)	-3.1 (1.1)	-14.1 (0.7)	8.2*	FT

Samples were collected at two elevations: La Parva (2500–2900 m) and Cerro Franciscano (3600 m). We measured the ice nucleation temperature (NT), the freezing point (FP), and the temperature producing 50% damage (LT₅₀), where values are mean ± 2 SE. Thermal difference corresponds to the difference between NT and LT₅₀. When NT and LT₅₀ were similar, the mechanism of freezing resistance was freezing avoidance (FA), but when NT and LT₅₀ were different plants were classified as freezing tolerant (FT). Level of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Plant species were categorized according to growth form: SHR, shrub; SS, dwarf shrub; PH, perennial herb. Species nomenclature follows Marticorena & Quezada (1985).

(LT₅₀ = $-15.5 \pm 0.6^\circ\text{C}$ vs $-10.9 \pm 0.6^\circ\text{C}$; $t = 6.05$ (paired t -test), $P < 0.0001$).

Regarding FP, 8.3% of the species showed apoplastic freezing at lower temperatures early than late in the growing season. Conversely, in 62.5% of the species apoplastic freezing occurred at lower temperatures late than early in the growing season (Fig. 1b). Mean FP significantly decreased from $-2.1 \pm 0.4^\circ\text{C}$ early to $-5.6 \pm 0.6^\circ\text{C}$ late in the growing season ($t = 6.91$ (paired t -test), $P < 0.0001$). Twenty-nine per cent of the species did not change FP within the growing season.

Altitudinal differences in LT₅₀ were found in four out of seven studied species (57.1%) (Table 4). Mean freezing resistance was $c.$ 2 K higher (lower LT₅₀) at high than at low elevations. Three species (*Colobanthus quitensis* (Kunth) Bartl.,

E. andicola, and *Phacelia secunda* J.F. Gmel) did not change their LT₅₀ with elevation.

In six studied species (85.7%) apoplastic freezing (FP) occurred at lower temperatures in plants from low than from high elevations (Table 5). Only *C. quitensis* did not change its FP with elevation. However, a significant interaction between elevation and time of measurements within the growing season was detected in some species, both for LT₅₀ and for FP (Tables 4, 5), suggesting that altitudinal differences in freezing resistance changed through the growing season.

Discussion

Although our results show a wide range of summer freezing resistance in high-elevation species from the Mediterranean-

Table 4 Results of two-way ANOVA performed to assess the effect of elevation (low and high) and time of measurements within the growing season (early and late) on freezing resistance (the temperature producing 50% damage (LT_{50})) in leaves of Andean species from central Chile

Species	$F_{\text{elevation}}$	F_{time}	$F_{\text{elevation} \times \text{time}}$
<i>Colobanthus quitensis</i>	0.962 ns	38.17****	18.95***
<i>Erigeron andicola</i>	1.27 ns	262.84****	13.65**
<i>Hordeum comosum</i>	205.49****	16.24***	8.97**
<i>Perezia carthamoides</i>	6.39*	47.59****	3.49 ns
<i>Phacelia secunda</i>	2.91 ns	36.42****	5.28*
<i>Pozoa coriacea</i>	8.1*	73.03****	0.49 ns
<i>Taraxacum officinale</i>	6.56**	114.19****	0.66 ns

Significance levels: ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

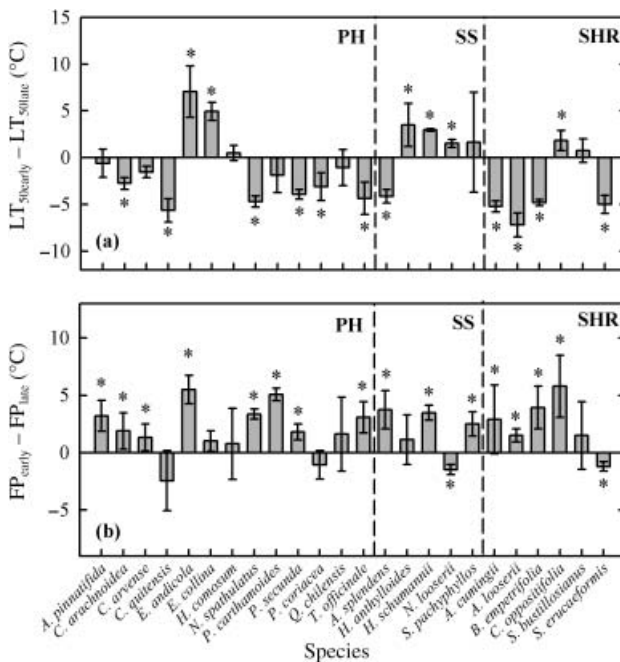


Fig. 1 Intraseasonal changes in freezing resistance and apoplastic ice formation: (a) the temperature (°C) producing 50% damage (LT_{50}); (b) the freezing point (FP; °C). Data are shown for each variable as the difference between measurements made early and late in the growing season in 24 species (mean \pm 2 SE). *, $P < 0.05$. Positive values indicate a lower temperature late than early in the growing season, while negative values indicate a higher temperature late than early in the growing season. Species were grouped by growth form: PH, perennial herbs; SS, dwarf shrubs; SHR, shrubs.

type zone of central Chile (from -8.2 to -19.5°C) (Tables 2, 3), most of the species were found to be able to resist temperatures below -10°C . Thus, our results indicate that the 24 species studied here are among the high-elevation species with the highest summer freezing resistance reported to date. High-elevation species from Mt Kurodake (Japan) and Mt Patscherkofel (Austria), mountains located in mesic areas, resist freezing temperatures from -3 to -7°C and from -4.5 to -14.6°C , respectively (Sakai & Ötsuka, 1970; Taschler & Neuner, 2004). In mountains located in xeric areas, it has

been found that high-elevation species resist temperatures from -7.5 to -16°C in East Pamir (Tyurina, 1957 in Sakai & Larcher, 1987) and from -4.7 to -20°C in the Doña Ana Mountains, northern Chile (Squeo *et al.*, 1996). While the mean summer freezing resistance for lowland species in the Northern Hemisphere is *c.* 4 K greater than that for species in the Southern Hemisphere, high-elevation plants from the Southern Hemisphere are more freezing resistant than high-elevation plants from the Northern Hemisphere (Bannister, 2007), and our results support this trend. Some authors have suggested that freezing resistance assessed in attached and detached leaves varies depending on the techniques employed (see Bannister, 2007 for a complete review). In particular, Taschler & Neuner (2004) found that the freezing resistance of Austrian Alp species measured on detached leaves was underestimated with respect to attached leaves. Our estimations were based on detached plant material, suggesting that the actual freezing resistance of the high-elevation species of the central Chilean Andes may be even greater than the high values found here.

Most of the species studied here tolerated some extracellular ice formation in their leaves, as indicated by the significant difference between NT and LT_{50} (Tables 2,3). Therefore, freezing tolerance is the main freezing resistance mechanism in the species studied. Given that freezing avoidance mechanisms can only be effective for a few hours (Goldstein *et al.*, 1985; Rada *et al.*, 1987), these types of mechanism may be less effective than freezing tolerance mechanisms in coping with the extremely low and long lasting freezing temperatures that occur during the growing season in the central Chilean Andes, and therefore, FA species were poorly represented in the species studied. This result is consistent with those of Sakai & Larcher (1987), who suggested that, in areas with severe frosts during the growing season, plants must be capable of tolerating extracellular ice formation even when they are metabolically active.

Given that freezing temperatures can occur early and late in the growing season (Greenland & Losleben, 2001), we expected that the freezing resistance of Andean plants would be similar at the two extremes of the growing season. However, our results indicated that only 29.2% of the studied

Table 5 Results of two-way ANOVA performed to assess the effect of elevation (low and high) and time of measurements within the growing season (early and late) on the freezing point (FP) in leaves of Andean species from the Andes of central Chile

Species	$F_{\text{elevation}}$	F_{time}	$F_{\text{elevation} \times \text{time}}$
<i>Colobanthus quitensis</i>	2.99 ns	19.09**	1.51 ns
<i>Erigeron andicola</i>	79.34****	7.33*	21.35***
<i>Hordeum comosum</i>	37.79****	6.16*	1.91 ns
<i>Perezia carthamoides</i>	42.77****	95.48****	6.71*
<i>Phacelia secunda</i>	0.63*	4.3 ns	5.45*
<i>Pozoa coriacea</i>	18.51***	0.42 ns	15.48**
<i>Taraxacum officinale</i>	12.71**	20.18***	2.56 ns

Significance levels: ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

species resist similar freezing temperatures early and late in the growing season. By contrast, while 25% of the studied species were more freezing resistant late than early in the growing season, c. 45% of the studied species showed higher freezing resistance early than late in the growing season. Data from a climate station located at 2650 m above sea level showed that the mean minimum air temperature increased from $3.6 \pm 0.5^\circ\text{C}$ in November 2004 to $7.9 \pm 0.4^\circ\text{C}$ in March 2005 (early and late growing seasons, respectively). Similar variation of the mean minimum air temperature was reported by Casanova-Katny *et al.* (2006), suggesting that the decrease in freezing resistance towards the end of the growing season in the majority of studied species was related to the higher temperature conditions that plants experienced during this period. In addition, a high proportion of the species in our study site lose part or all of their above-ground tissues before the onset of the unfavorable season (e.g. *Perezia carthamoides*, *Quinchamalium chilense* and *Taraxacum officinale*), suggesting that they invest resources to protect the regenerating tissues located below-ground and/or to accumulate reserves in below-ground tissues instead of cold hardening the above-ground organs.

In contrast to freezing resistance trends (LT_{50}), an FP depression at the end of the growing season was observed in 62.5% of the studied species, suggesting that plants are also responding to environmental factors other than temperature. In the central Chilean Andes, soil moisture decreases during the growing season (Table 1), exposing plants to prolonged drought episodes at the end of this period (Cavieres *et al.*, 2006, 2007). FP and NT depend on specific properties of the plant tissues and may vary according to the cell sap concentration and/or the accumulation of water-binding substances inside the cell (Sakai & Larcher, 1987). Several studies have reported that water-soluble carbohydrates depress FP, and their accumulation is positively related to drought survival (Streeter *et al.*, 2001; Merchant *et al.*, 2006; Monson *et al.*, 2006). Moreover, other studies reported that the lower FP of water-stressed plants result from a higher of solute concentration in their tissues (Chen *et al.*, 1977; Goldstein *et al.*, 1985; Anisko & Lindstrom, 1996). Hence, decreases in the FP at the end of the growing season are likely to be related to plant responses

to drought rather than to ambient temperatures alone. Although further studies are needed, it seems that the loss of above-ground tissues shown by many plant species in this high-elevation zone is in response to drought occurring at the end of the growing season. Accelerated senescence and leaf abscission are associated with drought in perennial plants, as a strategy that contributes to the survival of the plant and the completion of the plant life cycle under drought stress (Munné-Bosch & Alegre, 2004; Rivero *et al.*, 2007).

As expected, most of the plants from higher elevations resisted 2 K more than plants from lower elevations, which is consistent with results reported in previous studies (Goldstein *et al.*, 1985; Squeo *et al.*, 1996; Taschler & Neuner, 2004). However, our altitudinal difference in LT_{50} is lower than the altitudinal difference in mean minimum and maximum temperatures reported by Cavieres *et al.* (2007) (see also Table 1). Our altitudinal difference in freezing resistance is also narrower than the 5.6 K expected from the altitudinal lapse rate, which is c. 7°C km^{-1} in the summer in the central Chilean Andes (Cavieres & Arroyo, 1999). In the Venezuelan Andes, with an altitudinal lapse rate of $5.8^\circ\text{C km}^{-1}$, *Espeletia schultzei* plants resist temperatures 5.9 K lower at 4200 m than at 2600 m (Rada *et al.*, 1987), indicating that the altitudinal difference in freezing resistance in this species is narrower than the 9.3 K expected. Loik & Redar (2003) found that freezing resistance of *Artemisia tridentata* seedlings differed c. 0.9 K between low and high sites, in Bishop Creek, California where the altitudinal lapse rate is $2.6^\circ\text{C km}^{-1}$. They attributed the narrow altitudinal difference to microclimatic effects, whereby plants at higher elevations avoid low temperatures beneath the snow pack, while plants at lower elevations are directly exposed to frost events occurring through the growing season. In our case, plants from lower elevations are exposed to drought, which reinforces plant responses to frost events, mitigating the expected altitudinal differences in freezing resistance during the summer. For instance, in the majority of the studied species (85.7%), low-elevation plants had a lower FP compared with those from the higher elevation, with the magnitude of this altitudinal difference increasing at the end of the growing season (Table 5). Hence, the lower FP

in plants from lower elevations at the end of the growing season suggests that FP is reflecting plant responses to the water shortage conditions, which are greater at lower elevations (Cavieres *et al.*, 2006). This is reinforced by the statistical interaction between elevation and time of measurement within growing season for FP (Table 5). For example, *E. andicola* from the low elevation had an FP 1.6 K lower than plants from the high elevation, early in the growing season. However, this altitudinal difference increased to 5 K for plants measured late in the growing season. Additionally, *C. quitensis*, the only species that did not show altitudinal changes in FP, is a perennial herb inhabiting bogs that provide water availability throughout the entire growing season.

The change in the freezing resistance mechanism from FT to FA observed both in intraseasonal and in altitudinal comparisons was mainly related to a decrease in NT, indicating that tissues have less water to freeze, reducing the probability of ice nucleation (Pearce, 2001). As far as we know, changes in the freezing resistance mechanism within the same species have not been previously reported, suggesting that future studies should consider the time during the growing season at which freezing resistance measurements are conducted before drawing final conclusions. As discussed above, changes from FT to FA are more closely related to plant water status than to low temperatures *per se*, suggesting that water shortage conditions are altering patterns of freezing resistance in several ways in plants inhabiting xeric mountains.

In conclusion, our results demonstrate that Andean plants have a high ability to resist freezing temperatures during the growing season, and that the main freezing resistance mechanism is freezing tolerance. Freezing resistance of the Andean species varies within the growing season and with elevation, and both intraseasonal and altitudinal variations seem to be strongly affected by water availability. Further studies are needed to separate drought from thermal effects on summer freezing resistance in high-elevation plants from the xeric Andes. Climate models predict an increment of frost events and severe droughts for Mediterranean-type mountains, and thus a better knowledge of the mechanisms underlying freezing and drought resistance will improve any prediction of plant responses to global warming in this and other xeric high-elevation ecosystems.

Acknowledgements

We thank the staff of La Parva and Valle Nevado Ski Resorts for their help with access to our study sites, and also Mauricio Castro and Valeria Neira for their technical assistance in the field and with lab work. We thank C. Lortie and Jürgen Hacker for providing useful comments which improved the manuscript. We also thank Victor and Angélica Rojas from Valparaiso Lodge, our second home. This study was supported by FONDECYT 1060710, 1060910 and P05-002 ICM

(Center for Advanced Studies in Ecology and Research on Biodiversity). AS-A is supported by a CONICYT Doctoral Scholarship.

References

- Anisko T, Lindstrom OM. 1996. Cold hardiness and water relations parameters in *Rhododendron* cv Catawbiense Boursault subjected to drought episodes. *Physiologia Plantarum* 98: 147–155.
- Bannister P. 2007. A touch of frost? Cold hardiness of plants in the Southern Hemisphere. *New Zealand Journal of Botany* 45: 1–33.
- Bannister P, Colhoun CM, Jameson PE. 1995. The winter hardening and foliar frost resistance of some New Zealand species of *Pittosporum*. *New Zealand Journal of Botany* 33: 409–414.
- Bannister P, Fagan B. 1989. Frost resistance of fronds of *Blechnum penna-marina* in relation to season, altitude, and short-term hardening and dehardening. *New Zealand Journal of Botany* 27: 471–476.
- Bannister P, Maegli T, Dickinson K, Halloy S, Knight A, Lord J, Mark A, Spencer K. 2005. Will loss of snow cover during climatic warming expose New Zealand alpine plants to increased frost damage? *Oecologia* 144: 245–256.
- Beck EH, Fettig S, Knake C, Hartig K, Bhattarai T. 2007. Specific and unspecific responses of plants to cold and drought stress. *Journal of Biosciences* 32: 501–510.
- Beck EH, Heim R, Hansen J. 2004. Plant resistance to cold stress: mechanisms and environmental signals triggering frost hardening and dehardening. *Journal of Biosciences* 29: 449–459.
- Billings WD. 1974. Adaptations and origins of alpine plants. *Arctic Antarctic and Alpine Research* 6: 129–142.
- Blödner C, Skroppa T, Johnsen O, Polle A. 2005. Freezing tolerance in two Norway spruce (*Picea abies* [L.] Karst.) progenies is physiologically correlated with drought tolerance. *Journal of Plant Physiology* 162: 549–558.
- Bravo LA, Ulloa N, Zúñiga GE, Casanova A, Corcuera LJ, Alberdi M. 2001. Cold resistance in Antarctic angiosperms. *Physiologia Plantarum* 111: 55–65.
- Cannone N, Sgorbati S, Guglielmin M. (2007) Unexpected impacts of climate change on alpine vegetation. *Frontiers in Ecology and the Environment* 5: 360–364.
- Casanova-Katny MA, Bravo LA, Molina-Montenegro MA, Corchera LJ, Cavieres LA. 2006. Photosynthetic performance of *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) in a high-elevation site of the Andes of central Chile. *Revista Chilena de Historia Natural* 79: 49–53.
- Cavieres LA, Arroyo MTK. 1999. Tasa de enfriamiento adiabático del aire en el Valle del Río Molina, Provincia de Santiago, Chile central (33S). *Revista Geográfica de Chile Terra Australis* 44: 79–86.
- Cavieres LA, Badano EI, Sierra-Almeida A, Gómez-González S, Molina-Montenegro MA. 2006. Positive interactions between alpine plants species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of central Chile. *New Phytologist* 169: 59–69.
- Cavieres LA, Badano EI, Sierra-Almeida A, Molina-Montenegro MA. 2007. Microclimatic modifications of cushion plants and their consequences for seedling survival of native and nonnative herbaceous species in the High Andes of central Chile. *Arctic Antarctic and Alpine Research* 39: 229–236.
- Cavieres LA, Peñaloza A, Arroyo MTK. 2000. Altitudinal vegetation belts in the high-Andes of central Chile (33S). *Revista Chilena de Historia Natural* 73: 331–344.
- Chen PM, Li PH, Burke MJ. 1977. Induction of frost hardiness in stem cortical tissues of *Cornus stolonifera* Michx. by water stress. *Plant Physiology* 59: 236–239.
- Dytham C. 2003. *Choosing and using statistics: a biologist's guide*. Oxford, UK: Blackwell Publishing Company.

- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR. 2000. Climate extremes: observations, modeling, and impacts. *Science* 289: 2068.
- Goldstein G, Rada F, Azócar A. 1985. Cold hardiness and supercooling along an altitudinal gradient in Andean giant rosette species. *Oecologia* 68: 147–152.
- Greenland D, Losleben M. 2001. Climate. In: Bowman WD, Seastedt TR, eds. *Structure and function of an alpine ecosystem, Niwot Ridge, Colorado*. Oxford, UK: Oxford University Press, 18–19.
- Halloy S, González JA. 1993. An inverse relation between frost survival and atmospheric pressure. *Arctic and Alpine Research* 25: 117–123.
- Inouye DW. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89: 353–362.
- Inouye DW, McGuire AD. 1991. Effects of snowpack on timing and abundance of flowering in *Delphinium nelsonii* (Ranunculaceae): implications for climate change. *American Journal of Botany* 78: 997–1001.
- Körner C. 2003. *Alpine plant life*. Berlin, Germany: Springer.
- Kudernatsch T, Fischer A, Bernhardt-Römermann M, Abs C. 2008. Short-term effects of temperature enhancement on growth and reproduction of alpine grassland species. *Basic and Applied Ecology* 9: 263–274.
- Larcher W. 2000. Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosystems* 134: 279–295.
- Larcher W. 2003. *Physiological plant ecology: ecophysiology and stress physiology of functional group*. Berlin, Germany: Springer-Verlag.
- Loik ME, Redar SP. 2003. Microclimate, freezing tolerance, and cold acclimation along an elevation gradient for seedlings of the Great Basin Desert shrub *Artemisia tridentata*. *Journal of Arid Environment* 54: 769–782.
- Loik ME, Still J, Huxman TE, Harte J. 2004. *In situ* photosynthetic freezing tolerance for plants exposed to a global warming manipulation on the Rocky Mountains, Colorado, USA. *New Phytologist* 162: 331–341.
- Márquez EJ, Rada F, Fariñas MR. 2007. Freezing tolerance in grasses along an altitudinal gradient in the Venezuelan Andes. *Oecologia* 150: 393–397.
- Marticorena C, Quezada M. 1985. Catálogo de la flora vascular de Chile. *Gayana Botanica* 42: 1–151.
- Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* 51: 659–668.
- Menzel A, Fabian P. 1999. Growing season extended in Europe. *Nature* 397: 659.
- Merchant A, Tausz M, Arndt SK, Adams MA. 2006. Cyclitols and carbohydrates in leaves and roots of 13 *Eucalyptus* species suggest contrasting physiological responses to water deficit. *Plant, Cell & Environment* 29: 2017–2029.
- Monson RK, Rosentel TN, Forbis TA, Lipson DA, Jaeger CH. 2006. Nitrogen and carbon storage in alpine plants. *Integrative and Comparative Biology* 46: 35–48.
- Munné-Bosch S, Alegre L. 2004. Die and let life: leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology* 31: 203–216.
- Nakashima K, Yamaguchi-Shimozaki K. 2006. Regulons involved in osmotic stress responses and cold stress-responsive gene expression in plants. *Physiologia Plantarum* 126: 62–71.
- Neuner G, Ambach D, Aichner K. 1999. Impact of snow cover on photoinhibition and winter desiccation in evergreen *Rhododendron ferrugineum* leaves during subalpine winter. *Tree Physiology* 19: 725–732.
- Pearce RS. 2001. Plant Freezing and damage. *Annals of Botany* 87: 417–424.
- Rada F, Goldstein G, Azócar A, Torres F. 1987. Supercooling along altitudinal gradient in *Espeletia schultzii*, a caulescent giant rosette species. *Journal of Experimental Botany* 38: 491–497.
- Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E. 2007. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of the National Academy of Sciences, USA* 104: 19631–19636.
- Sakai A, Larcher W. 1987. *Frost survival of plants: responses and adaptation to freezing stress*. Berlin, Germany: Springer-Verlag.
- Sakai A, Ötsuka K. 1970. Freezing resistance of alpine plants. *Ecology* 54: 665–671.
- Squeo FA, Rada F, Azócar A, Goldstein G. 1991. Freezing tolerance and avoidance in high tropical Andean plants: is it equally represented in species with different plant height? *Oecologia* 86: 378–382.
- Squeo FA, Rada F, García C, Ponce M, Rojas A, Azócar A. 1996. Cold resistance mechanisms in high desert Andean plants. *Oecologia* 105: 552–555.
- Streeter JG, Lohnes DG, Fioritto RJ. 2001. Patterns of pinitol accumulation in soybean plants and relationships to drought tolerance. *Plant, Cell & Environment* 24: 429–438.
- Taschler D, Beikircher B, Neuner G. 2003. Frost resistance and ice nucleation in leaves of five woody timberline species measured *in situ* during shoot expansion. *Tree Physiology* 24: 331–337.
- Taschler D, Neuner G. 2004. Summer frost resistance and freezing patterns measured *in situ* in leaves of major alpine plant growth forms in relation to their upper distribution boundary. *Plant, Cell & Environment* 27: 737–746.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Plant species included in the study of summer freezing resistance in the Andes of central Chile

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than about missing material) should be directed to the *New Phytologist* Central Office.