

## SHORT COMMUNICATION

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## Cold resistance mechanisms in high desert Andean plants

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**Abstract** Freezing tolerance and freezing avoidance were studied, during the growing season, in plant species from two different elevations (3200 m and 3700 m) in a desert region of the high Andes (29° 45'S, 69° 59'W) in order to determine whether there was a relationship between plant height and cold resistance mechanisms. Freezing injury and supercooling capacity were determined in plants of different height, from ground-level (<20 cm tall) to tall shrubs (27–90 cm). All ground-level plants showed freezing tolerance as the main mechanism for resistance to freezing temperatures. Tall shrubs avoided freezing temperatures, mainly through supercooling. Supercooling was only present in plants occupying the lower elevation (i.e., 3200 m). Both avoidance and tolerance mechanisms are present in a single genus (i.e., *Adesmia*).

**Key words** Cold resistance mechanisms · Supercooling · Life forms · High desert mountains · Chile

### Introduction

Plant distribution in high mountain habitats is determined, among other factors, by the occurrence of freezing temperatures (Larcher 1973, 1982; Billings 1979). Both freezing tolerance and freezing avoidance have been found in plants inhabiting high mountains (Rada et al. 1985b; Azócar et al. 1988; Körner and Larcher 1988; Scheibe and Beck 1990; Squeo et al. 1991). Some authors (Larcher 1971; Levitt 1980; Sakai and Larcher 1987; Azócar et al. 1988; Squeo et al. 1991) suggest that

freezing tolerance is a better cold resistance mechanism under extreme temperatures than freezing avoidance. In contrast, supercooling and avoidance by insulation could be selected for in regions or microhabitats where less extreme and shorter night-time frost temperatures occur during the period of growth, because these mechanisms can only be effective for a few hours (Goldstein et al. 1985; Rada et al. 1985b).

In the tropical high Andean mountains, supercooling is the most common mechanism of freezing avoidance in arborescent species (Goldstein et al. 1985; Rada et al. 1985a,b, 1987), while ground-level plants show freezing tolerance as the main mechanism of resistance to cold temperatures (Azócar et al. 1988; Squeo et al. 1991). Freezing tolerance has been described as the most important cold resistance mechanism for arborescent plants in high mountain regions of Africa (Beck et al. 1982, 1984; Schulze et al. 1985). In addition, some plants or tissues are insulated from subzero temperatures. For example, some species grow in sites that may be considered thermal refuges (e.g., between rocks, dead fallen plants of other species) (Rada et al. 1985a; Squeo et al. 1991, 1993). At the tissue level, roots may be protected by layers of dead leaves in cushion plant species (Squeo et al. 1991), stems by thick layers of marcescent leaves (Smith 1979; Hedberg and Hedberg 1979; Meinzer and Goldstein 1985; Rada et al. 1985b), and buds by densely packed layers of leaves that exhibit nyctinastic movements at night (Smith 1974; Beck et al. 1982; Rada et al. 1985b; Beck 1994).

In seasonal climates, the winter period is unlikely to be a decisive factor with regard to survival of plant species native to cold regions, since hardening provides sufficient protection (Sakai and Larcher 1987; Körner and Larcher 1988). Since the Chilean high desert Andes, as well as other high mountains in mediterranean climate regions, have low temperatures during the winter season, plants are deciduous and/or are protected by snow cover, and therefore growth is restricted to late spring and summer (Arroyo et al. 1981; Squeo et al. 1993, 1994). Even though during the growing season, diurnal temperatures

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may be favorable for different plant physiological processes, freezing temperatures may occur during any night for a few hours (Ojeda et al. 1990). Therefore, the general objective of this work was to characterize cold resistance mechanisms exhibited by plants growing in the deserts of the high Chilean Andes during their active season.

Similarly to other high mountain regions, these desert regions also exhibit a strong thermal gradient in the air-soil profile. Thus, plants living in this environment would be expected to show a similar relation between plant height and cold resistance mechanisms. If freezing tolerance of the tissues is selected over supercooling ability as the main mechanism to prevent cold damage, we should find freezing tolerance in ground-level species. A similar pattern, freezing tolerance prevailing over freezing avoidance, may be present in plants at higher elevations. To test these two hypotheses, we studied cold resistance mechanisms in the photosynthetic tissues (i.e., leaf or green stem) of several high mountain species with different plant heights in the Cordillera de Doña Ana of north-central Chile.

## Materials and methods

### Site characteristics and plant material

Cold resistance mechanisms were studied in several species from the Cordillera de Doña Ana (c. 29° 45' S, 69° 59' W), a mountain system of the Cordillera de Los Andes. These high elevation mountains show an arid mediterranean climate with cold, wet winters and dry, warm summers. At 3750 m elevation, the mean annual temperature is 4.3 °C. July is the coldest month (-1.8 °C) while January and February are the warmest months (9.9 °C) (Squeo et al. 1994). The annual precipitation of 242 mm falls mainly (97%) from May to October, mostly as snow.

Three vegetation belts are present in the Cordillera Doña Ana (Squeo et al. 1993, 1994): subalpine (2700–3500 m) with shrubs 0.5–1.5 m tall; low alpine (3500–4250 m) with sub-shrubs and cushion plants; and high alpine (4250 m to vegetation limit at 4450 m) with small rosettes. We selected two stations located in the subalpine belt (c. 3200 m) and low alpine belt (c. 3700 m). Vegetation cover at the subalpine station (SA) was 40%, where shrubs (e.g., *Adesmia hystrix*, *Ephedra breana*), sub-shrubs (e.g., *Adesmia aegiceras*, *Viviania marifolia*), perennial herbs (e.g., *Astragalus cruckshanksii*, *Phacelia cumingii*) and annuals (e.g., *Viola chrysantha*) coexist. Vegetation cover at the low alpine station (LA) was 27%, with sub-shrubs (e.g., *Adesmia aegiceras*, *A. echinus*), cushion plants (e.g., *Calceolaria pinnifolia*, *Adesmia subterranea*) and perennial herbs (e.g., *Viola* spp., *Chaetanthera* spp.) present.

The studies were performed during January and February 1991, middle of the growing season which extends from the end of November to early April (Arroyo et al. 1981; Squeo et al. 1994). Samples of adult individuals of several species with different life-forms growing in the field were collected in the field and transported to a nearby laboratory located at 3200 m.

### Determination of cold injury

To determine the effects of subzero temperatures on tissues, a refined triphenyl tetrazolium chloride (TTC) method was used (Steponkus and Lanphear 1967). Samples of fully expanded leaves (or stems in *Ephedra breana*) were placed in small test tubes and

immediately immersed into a refrigerated alcohol bath. The temperature was lowered from 10 °C to -20 °C at a rate of 10 °C/h. This cooling rate was similar to the maximum temperature change at the end of the day in the field. Five to eight different individuals were used as replicates for each species. At 10°, 3°, -2°, -7°, 12°, -16°, 20 °C, samples were removed, and then incubated at 6 °C for 8 h. After this incubation period, TTC solution was added, infiltrated under vacuum for 30 min and left at 6 °C for 15 h. Afterwards, samples were extracted with ethanol and absorbance at 530 nm was measured. Cold injury was defined as the temperature that caused a 50% reduction in absorbance compared to the absorbance of each reference sample at 10 °C (Steponkus and Lanphear 1967; Rada et al. 1985b; Squeo et al. 1991).

### Thermal analysis

To determine the temperature at which tissue freezing occurred, small pieces of each tissue were cut and immediately enclosed in small, tightly sealed test tubes, thus avoiding changes in tissue water content. Copper-constantan thermocouples were inserted in the tissue samples and temperature was continuously monitored. The tubes were placed in a refrigerated alcohol bath and temperature was lowered from 10 °C to 20 °C at a rate of approximately 10 °C/h. Therefore, the temperature at which freezing started to occur (increase in tissue temperature due to the exothermic process of ice formation) in the tissues was readily determined.

## Results

### Cold injury

Photosynthetic tissues in most of the species showed cold injury below -11 °C (Table 1). The exception to this pattern were *Cristaria andicola* (-4.7 °C), *Adesmia hystrix* (-7.8 °C) and *Viola montagnei* (-9.0 °C). Since leaves of *C. andicola* showed a marginal supercooling capacity [freezing temperatures down to -5 °C are common in freezing-sensitive species due to solutes inside cells (Levitt 1980; Sakai and Larcher 1987)] which may be associated with other strategies, we have excluded this species from the following analysis. Independent of plant height, low alpine (LA) plant species showed lower injury temperatures compared to subalpine (SA) plant species (mean  $\pm$  SD = 16.3 $\pm$ 3.5 °C,  $n$  = 7 for LA; -12.3 $\pm$ 2.9 °C,  $n$  = 8 for SA;  $P$  < 0.05).

However, in the subalpine station, injury temperature in short plants (i.e., below 20 cm in height) was 2.5 K lower than in tall plants (i.e., higher than 20 cm) (-13.2 and -10.7 °C, respectively). In the case of short plants, injury temperature in species from the subalpine station (-13.2 °C) was 3.1 K higher than in species from low alpine station located 500 m higher (-16.3 °C). This temperature decrease with altitude was very close to environmental temperature decreases in the northern Chilean Andes (i.e., 6.5 K/1000 m) (Squeo et al. 1994). These results suggest that there is a relation between plant height and injury temperature, and that cold temperature resistance is greater in short plants and in plants growing in higher elevations.

**Table 1** Cold injury temperature and cold resistance mechanisms for photosynthetic tissues of species from Subalpine station (SA, 3200m) and Low alpine station (LA, 3700 m). Cold resistance mechanisms, either by supercooling (AS, avoiding freezing) or freezing tolerance (FT), were determined by the difference be-

tween injury temperature and the first exotherm (a). A second exotherm (b) appears in some cases. Mean  $\pm$  SD,  $n \geq 6$  individuals, \* = second exotherm not found (life-form: S shrub, SS sub-shrub, C cushion, PH perennial herb, A annual)

Altitudinal level	Species studied	Life-form	Plant height (cm)	Injury temp. (°C)	Freezing temp. (°C)		Cold resistance mechanism
					a	b	
SA	<i>Cristaria andicola</i>	PH	10.6	-4.7 $\pm$ 0.9	-5.5 $\pm$ 0.6	*	-
SA	<i>Adesmia hystrix</i>	S	70.7	-7.8 $\pm$ 0.5	-7.5 $\pm$ 0.1	*	AS
SA	<i>Ephedra breana</i>	S	89.3	-13.3 $\pm$ 0.5	-11.5 $\pm$ 1.0	*	AS
SA	<i>Tetraglochin alatum</i>	S	27.0	-11.1 $\pm$ 0.6	-12.0 $\pm$ 1.9	*	AS
SA	<i>Adesmia aegiceras</i>	SS	11.5	-15.3 $\pm$ 0.7	-5.8 $\pm$ 0.5	-18.1 $\pm$ 0.7	FT
SA	<i>Astragalus cruckshanksii</i>	PH	15.2	-16.0 $\pm$ 0.6	-3.1 $\pm$ 0.1	*	FT
SA	<i>Phacelia cumingii</i>	PH	18.1	-13.9 $\pm$ 0.3	-3.7 $\pm$ 0.5	-19.0 $\pm$ 0.4	FT
SA	<i>Viola montagnei</i>	A	3.5	-9.0 $\pm$ 1.2	-3.8 $\pm$ 0.8	*	FT
SA	<i>Viviania marifolia</i>	SS	15.3	-12.0 $\pm$ 0.7	-3.5 $\pm$ 0.1	-15.2 $\pm$ 1.4	FT
LA	<i>Adesmia echinus</i>	SS	10.0	-14.2 $\pm$ 1.2	-4.1 $\pm$ 0.4	-17.6 $\pm$ 0.4	FT
LA	<i>Adesmia subterranea</i>	C	1.2	-12.3 $\pm$ 0.5	-5.1 $\pm$ 0.8	-18.1 $\pm$ 0.3	FT
LA	<i>Calceolaria pinifolia</i>	C	6.4	-20.0 $\pm$ 0.7	-4.1 $\pm$ 0.6	*	FT
LA	<i>Chaetanthera acerosa</i>	PH	1.2	-19.0 $\pm$ 0.6	-5.3 $\pm$ 0.6	-15.2 $\pm$ 0.7	FT
LA	<i>Gymnophyton spinosissimum</i>	C	1.0	-12.0 $\pm$ 0.6	-2.7 $\pm$ 0.3	*	FT
LA	<i>Menonvillea cuneata</i>	PH	14.0	-16.3 $\pm$ 0.7	-2.7 $\pm$ 0.8	*	FT
LA	<i>Viola chrysantha</i>	A	0.5	-20.0 $\pm$ 0.7	-2.4 $\pm$ 0.2	-17.0 $\pm$ 0.6	FT

### Cold resistance mechanisms

To determine if the cold resistance mechanism for each species was tolerance or avoidance, we conducted thermal analysis experiments. The difference between freezing temperature and the temperature at which injury occurred may indicate the capacity to tolerate or avoid freezing of water in the intercellular spaces (Larcher 1982; Sakai and Larcher 1987).

Of the 16 species 12 showed differences of 5–18 K between injury temperature and the appearance of the first exotherm, indicating that they can resist ice formation in their photosynthetic tissues and therefore are tolerant to freezing (Table 1). All of these species were below 20 cm in height, including life-forms from annual to sub-shrubs, and they grew at both elevations (i.e., 3200 and 3700 m). A second exotherm, in average 12.9 $\pm$ 1.9 K lower than the first exotherm, was recorded in 7 of these 12 species. The difference between injury temperature and second exotherm for these species was, on average, as close as 3.9 $\pm$ 1.1 K.

For four species, injury temperature and the first exotherm were separated by only 0.3–1.8 K. These results suggest that supercooling is the main avoidance mechanism against freezing injury for these species, with the exception of the leaves of *Cristaria andicola* which did not resist freezing temperatures below -5 °C. These species are shrubs taller than 20 cm and their distributional limit is lower than 3500 m.

### Discussion

Our results show that different mechanisms to protect against freezing injury are found in different vegetation

strata and altitudinal levels in the desert region of the high Andes. Tall shrubs show avoidance mechanisms and species below 20 cm show tolerance mechanisms. A similar relationship between plant height and cold resistance mechanisms has been described for high tropical Andean plants in Venezuela (Squeo et al. 1991). In the Venezuelan Andes, plants with arborescent forms showed supercooling capacity in their leaves while all ground-level plants showed tolerance as the main resistance mechanism to cold temperatures.

In addition to the supercooling capacity shown in this work, tall shrubs grow exclusively on equator-facing slopes on rocky sites of the subalpine belt that may be considered as thermal refuges (Squeo et al. 1993). Avoidance mechanisms by growing in protected microsites has been described for species with supercooling capacity such as *Polylepis sericea* (Rada et al. 1985a) and in tolerant species such as *Senecio formosus* (Squeo et al. 1991).

An additional strategy could be used by *Cristaria andicola*, a perennial herb that represents 13% of the total plant cover in the subalpine belt (Squeo et al. 1993). Marginal leaf supercooling capacity and high injury temperature could be associated with high photosynthetic rates and fast growth during the warmest summer months (unpublished data). In *C. andicola*, all the above-ground biomass dies each year, thus avoiding persistent and more extreme freezing temperatures which start in early fall.

In the desert region of the high Andes, both avoidance and tolerance mechanisms are present in a single genus. In *Adesmia* spp., a tall shrub species (i.e., *A. hystrix*) shows freezing avoidance while sub-shrubs (i.e., *A. aegiceras* and *A. echinus*) and cushion (i.e., *A. subterranea*) species are freezing-tolerant. This result suggests that

phylogenetic constraint on change between supercooling and freezing tolerance mechanisms is low. In the Venezuelan Andes, the twelve dominant species of *Espeletia* show only avoidance mechanisms (Smith 1979; Goldstein et al. 1985; Rada et al. 1985b, 1987; Squeo et al. 1991). All of the *Espeletia* species studied were caulescent giant rosette species and many had an erect unbranched stem up to 3 m tall.

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