Freezing tolerance and avoidance in high tropical Andean plants: Is it equally represented in species with different plant height?

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Summary. Freezing tolerance and avoidance were studied in several different sized species of the tropical high Andes (4200 m) to determine whether there was a relationship between plant height and cold resistance mechanisms. Freezing injury and supercooling capacity were determined in ground level plants (i.e. cushions, small rosettes and a perennial herb), intermediate height plants (shrubs and perennial herbs) and arborescent forms (i.e. giant rosettes and small trees). All ground-level plants showed tolerance as the main mechanism of resistance to cold temperatures. Arborescent forms showed avoidance mechanisms mainly through supercooling, while intermediate plants exhibited both. Insulation mechanisms to avoid low temperatures were present in the two extreme life-forms. We suggest that a combination of freezing tolerance and avoidance by insulation is least expensive and is a more secure mechanism for high tropical mountain plants than supercooling alone.

Key words: Cold resistance mechanisms – Supercooling – Life forms – High tropical mountains

Plant growth and survival in high mountain habitats is determined, among other things, by the occurrence of low temperatures during any time of the year (Larcher 1973, 1981, 1982; Billings 1979; Sarmiento 1986). Freezing tolerance has been described as the most important cold resistance mechanism for plants in African high mountain regions (Beck et al. 1982, 1984; Schulze et al. 1985). Except for the small caulescent rosette *Draba chionophila* (Azocar et al. 1988), the most abundant mechanism reported for the tropical high Andean mountains was supercooling (Goldstein et al. 1985; Rada et al. 1985a, b, 1987). In these last studies, *Polylepis sericea* and several species of *Espeletia,* all with vegetative buds at more than 1 m above ground, showed supercooling as the main mechanism against freezing injury. Despite these differences between African and South American tropical high mountains, some tissues are insulated from

subzero temperatures; for example, stems by thick covered layers of marcescent leaves (Smith 1979; Hedberg and Hedberg 1979; Meinzer and Goldstein 1985; Rada et al. 1985b), and buds protected by densely packed layers of leaves that exhibit nyctinastic movements at night (Smith 1974; Beck et al. 1982; Rada et al. 1985b; Beck 1988).

Some authors (Larcher 1971; Levitt 1980; Sakai and Larcher 1987) suggested that supercooling should be selected as a freezing resistance mechanism in regions where night frosts occur during periods of high metabolic and developmental activity. A similar argument may be used for a particular habitat, but in this case, the specific adaptive response will depend on the microclimatic temperature regime (Azocar et al. 1988).

Several workers have stressed the peculiarities of tropical high altitude climate in which diurnal temperature variation exceeds seasonal variation and freezing temperatures are frequent (Hedberg 1964; Coe 1967; Troll 1968; Beck et al. 1982, 1984). Additionally, a temperature gradient exists along soil and air profiles, and this varies throughout the day (Monasterio 1979; Azocar and Monasterio 1980; Azocar et al. 1988).

If freezing tolerance of the tissues is selected over supercooling ability as the mechanism to prevent cold damage in plants of the coldest near-ground microhabitats in the high elevation Andes, it would not be equally represented in species with different plant height. To test this hypothesis, we studied differences in the cold resistance mechanisms (i.e. freezing and supercooling capacity) in leaf, stem and root tissues of several co-existing high mountain species with different plant heights and belonging to different life-forms, in the Páramo de Piedras Blancas.

Materials and methods

Site characteristics and plant material

Cold hardiness and cold resistance mechanisms in several species were studied with material originating from the Páramo de Piedras Blancas (ca. 8° 37′ N, 70° 12′ W, 4200 m), one of the highest páramos in the Venezuelan Andes, where temperatures may drop below

Table 1. Life-form and altitudinal distribution of species studied in the Venezuelan páramos. All species co-exist in the Páramo de Piedras Blancas, 4200 m (Vareschi 1970)

Code	Species studied	Life-form	Altitudinal distribution (m)
Arj	*Arenaria jahnii* Brake (Caryophyllaceae)	cushion	2400–4350
Azj	*Azorella julianii* Math (Apiaceae)	cushion	3500–4600
Cf	*Castilleja fissifolia* L.F. (Scrophulariaceae)	perennial herb	2000–4300
Dc	*Draba chionophila* Blake (Cruciferae)	small rosette	4300–4600
Em	*Espeletia moritziana* Sch. Bip. (Asteraceae)	giant rosette	3200–4400
Esc	*Espeletia schultzii* Wedd (Asteraceae)	giant rosette	2600–4300
Esp	*Espeletia spicata* Sch. Bip. (Asteraceae)	giant rosette	3800–4300
Et	*Espeletia timotensis* Cuatr. (Asteraceae)	giant rosette	4000–4400
Gm	*Geranium multiceps* Tourcz (Geraniaceae)	perennial herb	3000–4200
Hil	*Hinterhubera lanuginosa* Cuatr et Arist. (Asteraceae)	shrub	3500–4200
Hyl	*Hypericum laricifolium* Juess (Guttiferae)	shrub	2200–4200
Lv	*Lucilia venezualensis* Stmk. (Asteraceae)	cushion	3650–4300
Ps	*Polylepis sericea* Wedd (Rosaceae)	small tree	2400–4600
Sf	*Senecio formosus* H.B.K. (Asteraceae)	perennial herb	2800–4200

zero during any night of the year (Monasterio and Reyes 1980; Goldstein et al. 1985; Azocar et al. 1988). The mean annual temperature in Piedras Blancas is about 3° C with a difference of only 2.7° C between the coldest and warmest month. The lowest recorded temperature 10 cm above ground was −10° C (Perez 1984), and the average minimum annual temperature was −0.02° C (Goldstein et al. 1985). The annual precipitation of 800–900 mm falls mainly between the end of April and early December.

The site has an open vegetation with coexisting giant rosettes (*Espeletia* spp.), shrubs (e.g. *Hypericum, Hinterhubera*), cushions (e.g. *Arenaria, Azorella*), and in protected areas, *Polylepis sericea* with an arborescent growth form (Monasterio 1980; Ricardi et al. 1987). In the present study, we have divided the different life-forms into three groups: (a) plants which grow at ground level (<5 cm) which includes small rosettes, cushion plants and a perennial herb (*Geranium multiceps*); (b) plants which grow at an intermediate level (20–60 cm) which includes perennial herbs and shrubs: and (c) arborescent forms (>100 cm) which include giant rosettes and trees.

The studies were performed with adult individuals of several species with different life-forms (Table 1). The data for *Espeletia* spp., *Polylepis sericea* and *Draba chionophila* have been previously published in Rada et al. (1985a, 1985b, 1987), Goldstein et al. (1985) and Azocar et al. (1988) and are included here for comparison. The data set used in this paper was gathered from the same study site and analyzed with the same methodology (see below).

Several plants ($n \geq 6$) of each species were excavated with roots and surrounding soil from the study site, transported to the laboratory, and placed in a growth chamber.

Determination of cold injury

To determine the effects of subzero temperatures on tissues of these species, a refined triphenyl tetrazolium chloride (TTC) method (Steponkus and Lanphear 1967) was used. Samples of leaf, stem and root tissues were placed in small test tubes and immediately immersed in a refrigerated alcohol bath (Grant Instruments Ltd.). Temperature was lowered from 10° C to −22° 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 (Rada et al. 1987). Four different individuals were used as replicates for each species. At 5 K (or 7 K) intervals, samples were removed, and then incubated at 6° C for 8 h. After this incubation period, the 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 (Spectrophotometer Coleman 55, Perkin-Elmer). Cold injury was defined as the temperature that caused a 50% reduction in absorbance when compared to the absorbance of each reference sample at 10° C (Steponkus and Lanphear 1967; Rada et al. 1985b).

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 with a 16-channel data logger (LIC, Universidad de Los Andes) connected to a microcomputer (Epson HX20). The tubes were placed in a refrigerated alcohol bath and temperature was lowered from 10° C to −30° C at a rate of approximately 10° C/h. A computer program detected any marked increase in tissue temperature due to the exothermic process of ice formation. Therefore, the temperature at which freezing started to occur (appearance of exotherm) in the tissues was readily determined.

Table 2. Relationship between cold injury temperature, appearance of first exotherm (a) and second exotherm (b) for leaf (L), stem (S) and root (R) tissues; cold resistance mechanisms are shown (AI = avoid freezing temperature by insulation, AS = avoid freezing by supercooling, FT = freezing tolerant). Data for $n \geq 6$ individuals, – = data not available, * second exotherm did not appear

Species	Leaf			Stem			Root			Cold resistance mechanism		
	Injury temp. (°C)	Freezing temp. (°C)		Injury temp. (°C)	Freezing temp. (°C)		Injury temp. (°C)	Freezing temp. (°C)		L	S	R
		a	b		a	b		a	b			
Espeletia moritziana[a]	−11.3	−10.6	*	−			−	−	−	AS	AI?	?
E. schultzii[a,c]	−12.0	−11.6	*	−	−	−		−	−	AS	AI?	?
E. spicata[a,b]	−11.3	−12.8	*	−5.0	−4.6	*	−5.0	−4.1	*	AS	AI	AI
E. timotensis[a,b]	−11.9	−11.7	*	−6.5	−5.5	*	−4.0	−5.4	*	AS	AI	AI
Polylepis sericea[d]	−8.0	−7.5	*	−9.4	7.6	*	−	−	−	AS	AS	?
Hinterhubera lanuginosa	−12.3	−12.7	*	−13.8	−12.8	*	−	−4.4	*	AS	AS	AI?
Hypericum laricifolium	−10.9	−9.4	*	−11.2	−8.4	*	−	−4.1	*	AS	AS	AI?
Senecio formosus	−9.3	−3.5	−9.9	−7.9	−3.0	−11.2	−3.7	−3.4	*	FT	FT	AI
Castilleja fissifolia	−14.8	−4.1	−9.3	−11.7	−4.2	*	−	−4.5	*	FT	FT	AI?
Arenaria jahnii	−18.8	−3.2	−7.3	−19.1	−3.4	−8.6		−3.3	−8.1	FT	FT	FT?
Azorella julianii	−10.6	−3.7	−8.5	−9.2	−4.2	*	−4.0	−3.3	*	FT	FT	AI
Draba chionophila[e]	−14.8	−5.0	*	−12.0	−4.7	*	−14.0	−4.0	*	FT	FT	FT
Geranium multiceps	−	−3.5	−6.1	−	−3.6	−7.8	−	−3.8	*	FT?	FT?	?
Lucilia venezualensis	−14.3	−4.4	−11.4	−11.7	−3.5	*	−9.8	−3.5	−7.5	FT	FT	FT

[a] Goldstein et al. 1985 [b] Rada et al. 1985b [c] Rada et al. 1987 [d] Rada et al. 1985 [e] Azócar et al. 1988
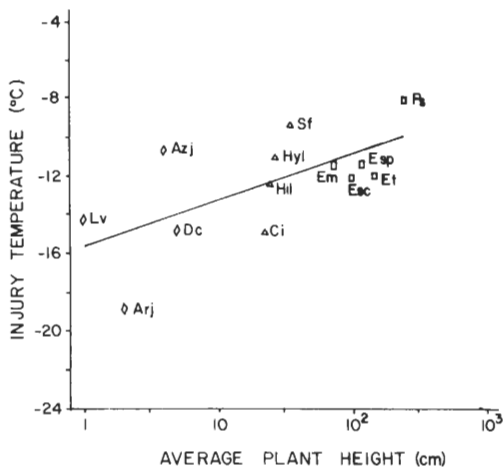
Fig. 1. Relationship between average plant height and injury temperature. Regression function is Injury temperature (°C) = −15.659 + 1.052 Ln (Avg plant height); n = 13 spp, r = 0.66 ($P < 0.05$). (◊) cushions and small rosettes; (△) shrubs and perennial herbs; (□) giant rosettes and small trees. See species codes in Table 1

Results

Cold injury

Leaves of all species analyzed in this work showed cold injury below −10° C, with the exception of *Polylepis sericea* and *Senecio formosus* (−8.0° C and −9.3° C, respectively) (Table 2). *Arenaria jahnii* showed the lowest injury temperature (−18.8° C). Injury temperature in ground level plants was approximately 3 K lower than for the other plant heights. These results show a relation between plant height and injury temperature, and that cold temperature resistance is greater in life-forms closer to the ground (Fig. 1). This may be explained by the lowest nighttime temperatures which occur at ground level in this area (Monasterio 1979). The broad deviation from the regression line for *Arenaria jahnii*, in contrast to the other species, could be correlated with the greater availability of different microhabitats for the ground level species in these ecosystems. Sakai and Larcher (1987) suggest that potential frost resistance in temperate mountain prostrate species is connected with the characteristic microsite preference of the species.

For stems, in general, the same trend was observed for all species (Table 2). Root data are not sufficient to show a trend, although the lowest injury temperatures do occur in *Draba chionophila* and *Lucilla venezuelensis*, both plants with a shallow root system and growing closer to the ground where lowest nighttime soil temperatures are found.

Cold resistance mechanisms: tolerance or avoidance?

To determine cold resistance mechanisms for each species and, consequently, each life-form, we conducted thermal analysis. The difference between freezing temperature and the temperature at which injury occurs may indicate the capacity to tolerate or avoid freezing of water in the intercellular spaces (Larcher 1982).

Previously studied life-forms with plant heights greater than 1 m (Goldstein et al. 1985; Rada et al. 1985a, 1985b, 1987) showed very small differences be-

tween injury and freezing temperature. These authors conclude that these species avoid freezing and are not able to resist ice formation in any of their tissues (Table 2). *Espeletia* spp. present two different avoidance mechanisms: supercooling of leaves and low temperature avoidance of stem and root tissues; while *Polylepis sericea* shows supercooling of all its tissues.

All ground level plants studied showed differences of 7–15 K between injury and the appearance of the first exotherm, indicating that they can resist ice formation in their tissues and therefore are tolerant of freezing (Table 2). For the first time in tropical mountains, a second exotherm was recorded in many cases. This exotherm was slightly higher than the injury temperature, suggesting a relationship between them. The only exception to this pattern in ground level plants was the roots of *Azorella julianii*, which had similar injury and freezing temperatures close to 0° C (ca. −4.0° C) suggesting some kind of low temperature avoidance mechanism through dead leaves which remain under the active leaf canopy playing a protecting role, similar to that reported for *Espeletia* stems (Rada et al. 1985b).

Intermediate level plants do not have a unique behavior showing both avoidance and tolerance patterns. Two of four species studied, *Hypericum laricifolium* and *Hinterhubera lanuginosa* (both shrubs), presented similar injury and freezing leaf temperatures (ca. −10° C and −13° C, respectively) suggesting that they avoid freezing through supercooling. The other two species, *Castilleja fissifolia* and *Senecio formosus* (perennial herbs), showed two exotherms, the first close to −4° C and the second similar to injury temperature (ca. −9.5° C). The results suggest that these last species can cope with extracellular ice formation and therefore are freezing tolerant. Although stem and root data were not available for all species, a trend similar to that described for leaves was observed, except for *Senecio formosus* roots which have high injury and freezing temperatures (ca. −3.5° C). This suggests a low-temperature resistance mechanism which may be explained by the presence of this species in protected microsites (i.e. between rocks, dead fallen *Espeletia* individuals) and not by an internal protecting mechanism.

Discussion

Our results show that different resistance mechanisms against freezing injury are found in different plant strata. Arborescent forms (*Espeletia* spp. and *Polylepis sericea*) show avoidance mechanisms only. The genus *Espeletia* shows supercooling of leaves and low temperature avoidance in stems by marcescent leaves around it, and protection of the apical bud by nyctinastic movements of the rosette leaves (Smith 1979; Rada et al. 1985b). *Polylepis sericea* shows supercooling of all its tissues (Rada et al. 1985a). If temperature conditions were more extreme or lasted for longer in the microhabitats where species with freezing avoidance mechanisms are found, this strategy would be very risky, because super-

cooling and/or insulation of tissues can only be endured for a few hours (Goldstein et al. 1985; Rada et al. 1985b). Following this reasoning, seedlings and juveniles of avoidant species would be strongly limited during this stage of their life-cycles. This has been shown for *Espeletia timotensis* (Guariguata and Azocar 1988) and *E. spicata* (Estrada and Monasterio 1988), both giant rosettes of Venezuelan paramos. Nevertheless, young *Espeletia* individuals avoid ice formation by lowering leaf water potentials (i.e. lower supercooling point) (Goldstein et al. 1984) permitting them to reach adult stages in some cases. A complementary avoidance mechanism for tall-growing species is found in *Polylepis*. These species grow in sites that may be considered thermal refuges, and therefore freezing temperatures are less frequent than in the surrounding paramo (Simpson 1979; Azocar and Monasterio 1980; Rada et al. 1985a). The combination of this and supercooling (Rada et al. 1985a) may explain the wider altitudinal distribution of *Polylepis* in the Andes.

In the case of plants growing closer to the ground, where a more rigorous thermal microhabitat exists, not only are temperatures lower than at greater heights above the ground, but also they remain below zero longer (Azocar and Monasterio 1980). Under these conditions, ground level plants (small rosettes, cushion plants and *Geranium multiceps*, a perennial herb) show freezing tolerance as the main mechanism against injury. Additionally, insulation mechanisms to avoid very low temperatures may be present in some tolerant species. For example, *Azorella julianii* avoids low temperatures in root tissues, which may be explained by an inversion of the thermal gradient in the soil (i.e. less rigorous microhabitat) and an additional protection by layer of dead leaves and canopy.

As stated in our initial hypothesis, ground level plants showing cold tolerance are different from arborescent forms exibiting cold avoidance. Plants of intermediate height show both avoidance and tolerance mechanisms. Since at this level microclimatic conditions are also intermediate between the other two levels, life-form seems to be more important than plant-height. In other words, plant structure seems to be the determinant, according to our results: *Hypericum laricifolium* and *Hinterhubera lanuginosa*, both shrubs, show avoidance mechanisms, while *Senecio formosus* and *Castilleja fissifolia*, both perennial herbs, show tolerance. Finally, from these results we suggest that for colder environments and in cost/benefit terms, a combination of freezing tolerance and avoidance through protective structures is a less expensive and more secure mechanism than supercooling alone.

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