

Cold hardiness and supercooling along an altitudinal gradient in andean giant rosette species

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Summary. Factors affecting supercooling capacity and cold hardiness were investigated in leaves of ten giant rosette species of the genus *Espeletia* (Compositae). These species grow along a 2,800–4,200 m elevation gradient in the Venezuelan Andes. In this high tropical environment, freezing frequently occurs every night, particularly above 3,300 m, but lasts for only a few hours. Supercooling capacity is linearly related to leaf water potential (Ψ_L) in all species; however supercooling is more responsive to Ψ_L changes in *Espeletia* species from high paramos. The rate of change in the species-specific supercooling point and the rate of change of average annual minimum temperature along the elevation and climatic gradient follow the same trend (approximately -0.6 K per 100 m elevation). At a given elevation, the expanded leaves of the different species tend to supercool 8–10 K below minimum air temperatures. Experimentally-induced freezing was accompanied by the formation of intracellular ice and tissue damage. The relative apoplastic water content (RAWC) of the leaves, which may influence the ice nucleation rate or the facility by which ice propagates, was determined by pressure-volume methods. Species from higher sites tend to exhibit lower RAWC (2%–7%) than species from lower sites (7%–36%). A causal relationship between supercooling capacity and RAWC is suggested. In the high tropical Andes, the temperature exotherm plateau of *Espeletia* leaves seems to be sufficiently low to avoid freezing.

Cauliscent giant rosette species of the genus *Espeletia* grow at elevations up to 4,600 m in the high tropical Andes (páramo). This genus, composed mainly of endemic species, has undergone intense radiative speciation (Cuatrecasas 1979). Many species have an erect unbranched stem up to 3 m tall, supporting a single parabolic evergreen rosette of large pubescent leaves. *Espeletia* species of lower elevations tend to be acaulescent, but also have a single terminal rosette. Inside the stem of both caulescent and acaulescent species, a conspicuous central pith provides water to the transpiring leaves, particularly during the early morning hours when soil water is not physiologically available (Goldstein and Meinzer 1983). The stem is insulated from subzero temperatures by a thick layer of marcescent leaves, thus ensuring nightly recharge of the pith reservoir, and preventing frost damage of the pith tissue and formation

of embolisms in the xylem (Smith 1979; Goldstein and Meinzer 1983). The apical meristem is similarly insulated by densely packed layers of expanding leaves that exhibit nyctinastic inward bending at night. This mechanism reduces heat conduction and convection from one leaf to the next (Smith 1974). The temperatures of the tissues inside the stem and the apical bud are therefore partially uncoupled from air temperature variations. Below-freezing temperatures have never been observed in these protected tissues (Smith 1979; Rada 1983; Meinzer and Goldstein 1984). On the other hand mature leaf temperatures frequently drop below 0° C during pre-dawn hours, particularly on clear nights.

Several workers have stressed the peculiarities of tropical high-altitude climates in which diurnal temperature variations by far exceed seasonal ones and freezing temperatures are frequent (Hedberg 1964; Coe 1967; Troll 1968; Beck et al. 1982, 1984). Plants growing in cold tropical environments lacking temperature seasonality are not subjected to the same constraints as those growing in cold temperate climates. For example, giant rosette plants would be expected to exhibit a more or less constant level of physiological activity throughout the year rather than relying on dormancy as a mechanism for withstanding periods of freezing temperatures. The climatic conditions that prevail in the tropical portion of the high Andes, characterized by brief periods of mild nocturnal freezing, make this a likely place where selection for freezing-avoidance mechanisms should be favored (Larcher 1971, 1981). Leaf tissues of *Espeletia* species from high altitudes supercool to relatively low temperatures (Larcher 1975; Rada et al. 1985). If supercooling is selected as the mechanism that allows the avoidance of ice formation in the leaves of high-elevation Andean giant rosette plants, the species-specific supercooling capacity should vary along an altitudinal and climatic gradient. We studied differences in leaf supercooling capacity of ten *Espeletia* species that grow in different narrow ranges of a tropical elevational gradient. We also investigated factors that may partially explain the differences in supercooling capacity and the role these may play in interpreting the altitudinal limits of these species.

Materials and methods

Site characteristics and plant material

Cold hardiness and factors related to the supercooling capacity of ten *Espeletia* species (Compositae) were studied

Table 1. Physical and climatic characteristics of the sites where *Espeletia* populations were sampled. Species are given with full name and authors according to Aristeguieta (1964). Sources of climatic information: Escuela de Geografía (ULA), Fonaiap (MAC), and Departamento de Hidrología (Ministerio del Ambiente)

Páramo site and elevation (m)	Species studied	Average minimum annual temperature (°C)	Annual precipitation (mm)	Distinct wet-dry season	Number of days with frost
San José (2,850)	<i>Espeletia angustifolia</i> Cuatr. <i>Espeletia lindenii</i> Sch. Bip. ex Wedd.	6.23 ^b	— ^a	no	none
El Batallón (3,100)	<i>Espeletia jahnii</i> Standl. <i>Espeletia marcana</i> Cuatr. <i>Espeletia atropurpurea</i> A.C. Sm.	4.77	1,213	no	very rare
Mucubají (3,560)	<i>Espeletia schultzi</i> Wedd. <i>Espeletia floccosa</i> Standl.	1.66	969	yes	18/month during dry season 3/month during wet season
Piedras Blancas (4,200)	<i>Espeletia lutescens</i> Cuatr. & Aristeg. <i>Espeletia moritziana</i> Sch. Bip ex Wedd. <i>Espeletia spicata</i> Sch. Bip. ex Wedd. <i>Espeletia schultzi</i> Wedd.	−0.02	789	yes	26/month no seasonal variation

^a Information not available

^b Estimated from the regional temperature lapse (−0.58 °C/100 m elevation)

at four sites along an altitudinal gradient (Table 1) in the Venezuelan Andes (approximate coordinates 8°52' N, 70°45' W). The lowest site, at 2,850 m near San José, is a warm and relatively dry páramo, where the caulescent rosettes attain no more than 0.75 m in height and air temperatures stay above freezing. At Piedras Blancas (4,200 m), one of the highest páramos in the Venezuelan Andes, the temperatures can drop below freezing during any night of the year. The vegetation has an open desert-like appearance and the *Espeletia* species can attain 3–4 m in height (Monasterio 1980). The 2,800–4,200 m altitudinal gradient corresponds to a climatic gradient of decreasing temperature with increasing altitude (Fig. 1). The data available indicate that the frequency of night frosts increases sharply with elevation above 3,200–3,300 m (Monasterio and Reyes 1980).

The studies were performed with adult individuals of *Espeletia* that were the dominant rosette species in each páramo (Table 1). Although all the species share a similar growth form, morphological features such as total plant height, pith volume per unit leaf area, and the degree of caulescence change along the altitudinal gradient (Goldstein et al. 1984; Meinzer and Goldstein 1984; Meinzer et al. 1985). Reproductive strategies also differ. Species from higher elevations are polycarpic, reproducing approximately once every 2–3 years (Estrada 1984). Many species from lower páramos, on the other hand, are monocarpic and have relatively shorter life spans (10–30 years).

Frost injury and supercooling

Plants were excavated with intact roots and soil, transported to the laboratory, and kept in growth chambers with temperature and irradiance controls simulating field conditions. Tissue samples from expanded mature leaves were cut, immediately placed in sealed tubes, and immersed in a refrigerated alcohol bath. Temperature was lowered from

10° C to −30° C at a rate of approximately 10° C/h. Three replicas of each sample were taken from the bath at 5 K intervals and incubated at 6° C for 8 h. Following incubation, the refined TTC method described by Steponkus and Lanphear (1967) was used to determine tissue injury.

For quantitative analysis of the freezing process, leaf pieces 3 cm long and 1 cm wide were enclosed in small tightly sealed tubes to avoid changes in tissue water content during the experiment. Copper-constantan thermocouples (36 gauge) were inserted in the leaf sample. Changes in temperature were continuously monitored with a strip chart recorder fitted to an electronic 0° C reference. Prior to immersion in a refrigerated alcohol bath the tubes were enclosed in an aluminum cylinder which acted as a heat sink and provided temperature stabilization during cooling (Quamme et al. 1972). The temperature of the bath was lowered from 10° C to −30° C at a rate of approximately 10° C/h.

Preliminary experiments revealed that it was difficult to establish trends in supercooling capacities unless we were able to use leaf material with a similar water status. However, even small changes in the water status of the leaves of some species produced significant changes in exotherm temperatures. We decided, therefore, to establish the relationship between supercooling capacity and leaf water potential for each species. In order to obtain a wide array of Ψ_L values leaves were removed from the plant and allowed to transpire freely. At set intervals, a leaf was selected, the leaf section to be used in the thermal analysis (TA) experiment excised and prepared as above, and the rest of the leaf immediately placed in a pressure chamber for Ψ_L determination. The small section removed from the leaf did not affect the Ψ_L readings. Measurement of temperature exotherms are complicated not only by their dependence on tissue water status, but because they may also be influenced by the rate of temperature decrease. However, for the ten *Espeletia* species examined in this

study, experimentally varying the cooling rate (within a biologically meaningful range) did not significantly affect the supercooling point.

Components of leaf water potential were estimated in the laboratory by means of the pressure-volume technique (Tyree and Hammel 1972). Curves were analyzed using plots of $1/\Psi_L$ versus relative water content (RWC). A regression of the form $1/\Psi_L = \beta_0 + \beta_1 \times \text{RWC}$ was fitted to the linear portion (Tyree and Richter 1981, 1982).

Results

Figure 1 shows average minimum temperatures for weather stations located within the study area. Seasonal temperature variations are small, usually less than 2.5 K between the coldest and warmest months. The lowest temperatures occur during the short dry season when clear night conditions prevail and ground and plant surfaces cool rapidly due to unimpeded long-wave radiative heat loss. Although lower minimum temperatures prevail during the dry season, the frequency of nights with frost does not fluctuate seasonally at the higher site.

Supercooling appears to be linearly related to Ψ_L (Fig. 2). More negative Ψ_L values tended to lower the temperature of the exothermic event. The linear relationship between Ψ_L and supercooling was significant in all species studied at $p \leq 0.05$. Two other patterns can be established from inspection of Fig. 2: first, at a given Ψ_L , leaves from higher-elevation species supercool more than leaves from lower-elevation species, and second, the slope of the Ψ_L -exotherm temperature relationship tends to increase from lower to higher sites. The latter implies that the species-specific supercooling capacity is more sensitive to leaf water potential changes in *Espeletia* species from high páramos as compared to species of lower elevation.

Although we will discuss the adaptive significance of these patterns in the next section, it is interesting to point out here that *E. moritziana*, which has a smaller supercooling capacity than the other three caulescent giant rosette species found in the Piedras Blancas páramo, only grows on rock outcroppings. There minimum nighttime temperatures are relatively high as compared to the slopes or small depressions where the other three species occur. Similarly, *E. schultzii*, which has the highest supercooling capacity in the Mucubaji páramo, can grow in well drained valley bottoms or near small depressions where nocturnal temperature inversions are frequent. *E. floccosa*, on the other hand, is mostly restricted in Mucubaji to relatively warm moraine slopes.

The rate of change of air temperature with elevation varies from place to place (Schubert and Medina 1982). For example, in northern South America, the altitudinal temperature gradient in continental areas is approximately -0.6 K per 100 m, whereas in the Caribbean islands the gradient is -0.7 K/100 m. These values are -0.3 to -0.4 K/100 m lower than the dry adiabatic lapse rate of approximately -1.0 K/100 m in elevation (Rosenberg 1974). Figure 3 shows the average minimum and absolute minimum temperature gradients in the study area. Nine weather stations with more than 9 years of records were used to obtain an empirical linear relationship between elevation and temperature. The slope was -0.58 K/100 m elevation ($T = 3918.04 + (-171.33 \times E)$, $r = 0.99$; where T is the average

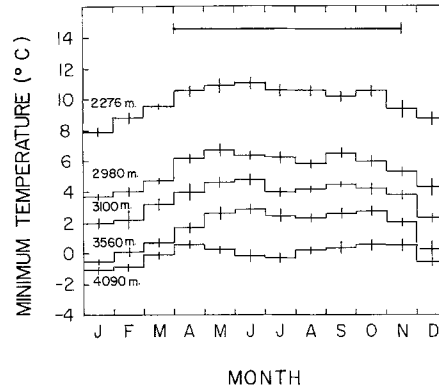


Fig. 1. Seasonal variations in average minimum monthly temperatures for five weather stations located within the study area. Bars represent 1 SE above and below the mean ($5 \leq n \leq 13$). The bar in the upper part of the figure indicates the approximate duration of the wet season

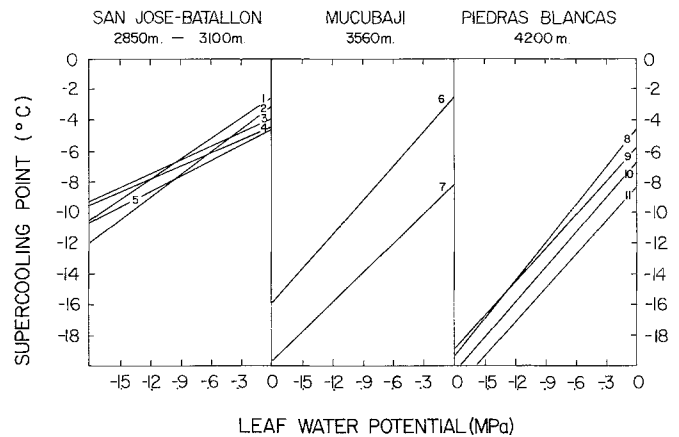


Fig. 2. Relationships obtained by linear regressions between leaf water potential and supercooling for 10 different *Espeletia* species: 1 *E. atropurpurea* ($r = 0.97$, $n = 10$), 2 *E. lindenii* ($r = 0.95$, $n = 11$), 3 *E. jahnii* ($r = 0.84$, $n = 5$), 4 *E. marcana* ($r = 0.99$, $n = 4$), 5 *E. angustifolia* ($r = 0.83$, $n = 6$), 6 *E. floccosa* ($r = 0.98$, $n = 18$), 7 *E. schultzii* ($r = 0.87$, $n = 13$), 8 *E. moritziana* ($r = 0.98$, $n = 7$), 9 *E. spicata* ($r = 0.97$, $n = 7$), 10 *E. lutescens* ($r = 0.91$, $n = 13$), and 11 *E. schultzii* ($r = 0.96$, $n = 8$)

minimum annual air temperature in $^{\circ}\text{C}$ and E is the elevation in m).

The rate of change in the species-specific supercooling temperature with elevation, at a given Ψ_L , follows a similar trend (Fig. 3), but as expected the temperature exotherm was more than 10 K lower than average minimum air temperatures. For every 100 m change in elevation, the supercooling capacity increased by approximately 0.6 K. We calculated this supercooling trend using the regressions between Ψ_L and supercooling capacity illustrated in Fig. 2, and using a Ψ_L of -0.6 MPa, a value frequently observed during early morning hours. Absolute minimum air temperatures were about 2 K lower than average minimum temperatures. Expanded rosette leaves, particularly of small individuals, are expected to experience even colder temperatures (1–2 K below air temperature at night). However, *Espeletia* leaves may supercool to -20.0°C . This temperature exotherm is well below the minimum air temperature at a given elevation making the danger of rapid plasma dehydration or ice formation highly improbable.

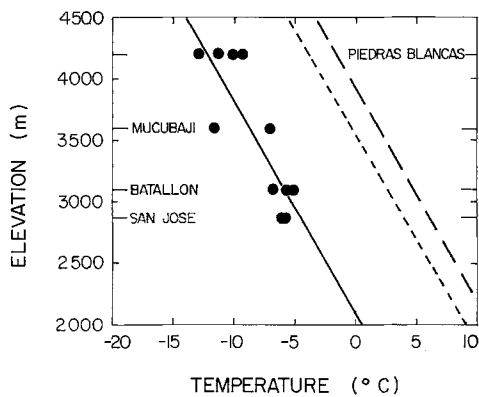


Fig. 3. Average minimum annual temperature lapse (—), absolute minimum annual temperature lapse (---), and supercooling point altitude gradient for *Espeletia* species (— · —) in the central Venezuelan Andes. Data points are species-specific supercooling temperatures at $\Psi_L = -0.6$ MPa

When leaf temperature was experimentally lowered at a constant rate, the point of 50% tissue injury as well as the release of heat of fusion occurred at approximately the same temperature (Table 2). Tissue injury was linearly related to the supercooling point by the equation

$$I = 0.54 + (1.05 \times T) \quad (r = 0.93, \text{ SE of slope} = 0.13)$$

where I is the temperature at 50% tissue injury and T is the last temperature recorded in leaf samples of each species before the release of heat of fusion from freezing water gave a rapid temperature increase. The slope (1.05) does not differ significantly from 1.0, indicating that there is a good agreement between the initiation temperature of the exotherm in each species and the temperature of leaf injury. The type of freezing pattern observed in *Espeletia* leaves suggests that either the freezing process encompasses extra- and intracellular spaces simultaneously, or, if only extracellular ice is formed, the cells do not have the ability to tolerate freeze dehydration. The first alternative seems to be the most likely explanation of freezing injury as only one exotherm was ever observed during thermal analysis in the laboratory. This was true even when leaf temperatures were lowered to -40°C and held there for several hours.

In order to investigate factors that may explain species-specific differences in supercooling capacity the relative volume of apoplastic water in leaves was determined by means of pressure-volume (P-V) methods. The P-V approach was preferred because it not only gives a quantitative value of intercellular water content, but also averages apoplastic volumes from different leaf tissues. We are assuming here that solute concentration of the very dilute intercellular water is similar among different species and, thus, that the volume of apoplastic water should significantly influence the ice nucleation rate or the facility by which ice propagates throughout the cells. Figure 4 shows P-V curves from a high-elevation and a low-elevation *Espeletia* species. Extrapolating the linear portion of the curve to the abscissa results in an estimate of the relative apoplastic water content (Tyree and Richter 1982). The slope of the line was steeper for the low-elevation species and therefore intercepted the abscissa before the other species, indicating a larger relative volume of apoplastic water. Considering

Table 2. Summary of the temperature at which 50% injury was observed, supercooling points (obtained during injury assessment experiments), and the relative apoplastic water content of the leaves for 10 *Espeletia* species occurring along an altitudinal gradient. Leaf water potentials for the supercooling and injury determinations were within the -0.4 to -0.9 MPa range. The x -intercepts of 3–5 pressure-volume curves were averaged to provide the apoplastic water content value

Species	Elevation (m)	Supercooling point ($^\circ\text{C}$)	50% injury ($^\circ\text{C}$)	Rel. apoplastic water cont (%)
<i>E. lindenii</i>	2,850	-7.5	-6.5	7.0
<i>E. angustifolia</i>	2,850	-6.6	-6.1	35.8
<i>E. atropurpurea</i>	2,850	-6.4	-5.9	26.2
<i>E. marcana</i>	3,100	-9.1	-8.0	20.5
<i>E. atropurpurea</i>	3,100	-7.3	-8.1	19.9
<i>E. jahnii</i>	3,100	-5.7	-5.6	25.1
<i>E. schultzii</i>	3,560	-10.8	-10.0	16.0
<i>E. floccosa</i>	3,560	-8.5	-9.3	7.3
<i>E. schultzii</i>	4,200	-10.0	-11.2	3.9
<i>E. moritziana</i>	4,200	-10.6	-11.3	4.0
<i>E. spicata</i>	4,200	-10.0	-9.5	7.4
<i>E. lutescens</i>	4,200	-10.5	-10.2	2.2

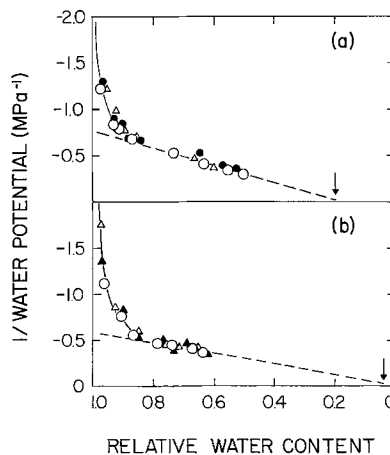


Fig. 4. Pressure-volume curves for expanded leaves of **a** *E. marcana* and **b** *E. moritziana*, a low- and a high-elevation species respectively. The symbols on each curve are from three different samples. Arrows indicate the relative apoplastic water content

the whole set of species under study, Table 2 shows that species from higher páramos tend to exhibit lower relative apoplastic water contents than species from lower páramos.

Discussion

One of the most notable features of high tropical mountain environments is the high degree of seasonal temperature constancy combined with low prevailing temperatures. This poses a series of constraints for plant survival and growth, not only because temperatures may be suboptimal for processes such as water uptake, translocation of assimilates, and growth, but also because subzero temperatures may cause injury in exposed tissue and even plant death.

The results of this study show that, at a given elevation, *Espeletia* species supercool several degrees below minimum

air temperature. The supercooled state in plants, however, is usually very labile in tissues that exhibit transient supercooling (Larcher 1982) and, therefore, can seldom be maintained under field conditions for more than a few hours. Furthermore, it has been proposed (Levitt 1980) that because freezing after marked supercooling is far more likely to be fatal than the gradual freezing that occurs when there is no marked supercooling, it is not likely to be selected as a survival factor.

Since frozen leaves or freezing injury have never been observed in *Espeletia* species in the wild, the above statement does not apply to high Andean tropical environments. Rada et al. (1985) working with *E. spicata* and *E. timotensis* at approximately 4,200 m found that supercooling is the principal mechanism for leaf survival and that, typically for markedly supercooled organs, they are unable to tolerate ice formation. The leaves of giant caulescent rosette plants from Equatorial Africa, on the contrary, are often stiffly frozen by morning (Beck et al. 1982). These authors found that these plants can tolerate extracellular ice in the bulk of the rosette leaves and that the exotherm temperatures of expanded leaves are closer to 0° C than exotherms of high-elevation *Espeletia* plants. There exist, however, important differences in the temperature regimes between high elevation equatorial African and South American regions that may help explain the differences in cold resistance mechanisms. Minimum nighttime temperatures at approximately 4,200 m often drop to -10° C in the East African mountains, while in the American páramos nighttime temperatures stay very close to 0° C at the same elevation.

Under the conditions of temperature constancy and moderate freezes of short duration (usually no more than 3–4 hours) that prevail in the Venezuelan páramos, the supercooling capacity by the different *Espeletia* species could be considered a significant survival factor. Neither extracellular nor intracellular freezing are likely to occur under such conditions. Several characteristics of *Espeletia* leaves may further help to stabilize the supercooling state for a few hours: sessile leaves and nyctinastic inward bending of the rosette leaves during night hours may reduce leaf movement and buffer the rate of temperature change, pubescent hydrophobic layers on both leaf sides may create a barrier against external ice nucleators, and little intercellular space (see below) would impede or delay ice crystal formation.

The water status of a tissue or plant organ is known to affect its supercooling capacity (Larcher 1973; Timmis and Worrall 1975; Burke et al. 1976; Levitt 1980). Salt and Kaku (1967) for example have shown that the number and magnitude of the exothermic events during freezing depends on the water content of *Picea pungens* needles. Supercooling in *Espeletia* leaves is also responsive to changes in the water status of leaf tissues. Supercooling points in general tend to decrease linearly with decreasing leaf water potentials, but higher elevation species, with small relative apoplastic water content, are particularly sensitive to changes in Ψ_L (Fig. 2). High-elevation species exhibit a very strong change in supercooling points with relatively moderate changes in water potential. This pattern undoubtedly has an adaptive value. Minimum air and leaf temperatures in high páramos occur during the dry season when leaf water potentials tend to be more negative, especially in young *Espeletia* individuals which are less buffered from daily and seasonal fluctua-

tions in soil water availability (Goldstein et al. 1984). The nature of the mechanisms coupling supercooling points with leaf water status is not known, however, calculating the decrease in the osmotic potential brought about by changes in leaf water potential does not fully explain the observed supercooling capacity increase.

According to Levitt (1980), the ability of a plant tissue to exist in a supercooled state depends on several physical factors, such as small cell size and little intercellular space available for ice nucleation. The leaf apoplastic water content (an indirect measure of intercellular spaces) in the ten *Espeletia* species considered here vary along an altitudinal and climatic gradient. Species from higher elevations tend to have a smaller apoplastic water content (Table 2). A strong relationship was found between relative apoplastic water content and the amount of intercellular spaces observed in leaf sections of *E. schultzei* individuals growing at different altitudes (Rada, Goldstein and Azocar, unpublished information). Tyree and Richter (1981, 1982), in a discussion of the merits of different transformations of water potential isotherms, conclude that plotting $1/\Psi_L$ versus relative water content (used in this work) is less prone to bias estimates of the relative amount of apoplastic water in leaf tissues. They expect, however, that even using this transformation the estimation of the apoplastic water content cannot be made very accurately. For this reason the effect of the apoplastic volume on leaf supercooling capacities in *Espeletia* species should be considered with caution, and although not conclusive in themselves, these results may lead to future research in the field of causal factors of supercooling in plant tissues.

In a few species from temperate regions low temperature exotherms (deep supercooling) of parenchymatous xylem rays were observed to be strongly correlated with the minimum temperatures of the altitudinal or latitudinal distribution limit (George et al. 1974; Kaku and Iwaya 1978), indicating that supercooling may indeed play an important role not only in the cold resistance mechanisms of these species, but also in explaining, at least partially, their ecological and geographical patterns of distribution. In this work we document a species-specific trend in supercooling capacity of leaf organs along an altitudinal gradient. Given that the supercooling points of *Espeletia* species at each elevation are several degrees lower than minimum air temperatures and that the mild freezes last only a few hours, supercooling in giant caulescent rosette plants seems likely to be an adaptive mechanism to avoid the effects of freezing in cold tropical Andes ecosystems.

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