Superooling along an Altitudinal Gradient in *Espeletia schultzi*, a Caulescent Giant Rosette Species

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ABSTRACT

Tropical high Andes plants may be exposed to sub-zero temperatures any night of the year. These plants have to rely on mechanisms which protect them from these environmental conditions but at the same time allow their growth and development. Superooling has been found to be the principal avoidance mechanism in leaves of the caulescent giant rosette genus *Espeletia* in the Andes. We report here the differences in supercooing capacity and cold injury in several *Espeletia schultzi* populations growing along an altitudinal gradient. The relationships between supercooling, water potential and leaf anatomy were also investigated. The supercooling capacity increased and injury temperature decreased from lower to higher elevation populations. These changes may be explained in terms of physiological, morphological and anatomical characteristics of the leaves.

Key words: *Espeletia schultzi*, supercooling, freezing avoidance, mechanisms.

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INTRODUCTION
Superooling is known to be an effective mechanism against freezing injury in only a limited number of plant species (Larcher, 1982; Georges, Burke, Pellet, and Johnson, 1974; Kaku and Iwats, 1982; Larcher, 1977) and Levitt (1980) suggested that supercooling should be selected as a freezing resistance mechanism in regions where light frost occurs during periods of high metabolic and developmental activity. In some high tropical environments where these characteristics are present due to the possibility of below zero temperatures during any night of the year, however, frost tolerance as opposed to its avoidance has been observed. This is the case of the giant caulescent rosette species of tropical high mountains in Africa, where freezing of the leaves occurs without damage (Beck, Senser, Scheibe, Steiger, and Pontgranz, 1982; Beck, Schlote, Senser, and Scheibe, 1984). In the high Andes, leaf tissues of *Espeletia* species supercool relatively low temperatures, avoiding extracellular freezing of leaf tissues (Rada, Goldstein, Azocar, and Meiner, 1985; Goldstein, Rada, and Azocar, 1985). The species-specific supercooling points coincide with the temperature at which significant tissue damage was observed, indicating that these species are not capable of tolerating extracellular ice formation.

*Espeletia schultzi* Weid (Compositae) is a giant caulescent rosette species occurring along an elevational gradient from 2600 m to 4200 m in the Venezuelan Andes. These plants © Oxford University Press 1987
consist of a stem covered by marcescent leaves which maintain the stem temperature above 0 °C, and an apical rosette of densely pubescent leaves. The apical bud is insulated by layers of expanding and mature leaves, which at night bend inward by means of nystanic movements (Smith, 1974). Temperatures below 0 °C were never observed in these protected stems, pith and bud tissues (Smith, 1974; 1979; Goldstein and Meister, 1983; Rada, 1983). A series of morphological characteristics of *Espeletia schultzi* change along the altitudinal gradient: total plant height as elevation increases, pith volume per unit leaf area increases with elevation resulting in a larger water storage capacity at higher elevations (Goldstein, Meinerz, and Monastario, 1984; Meinerz and Goldstein, 1984), smaller and more erect leaves with a thicker pubescent layer occur as elevation increases (Meinerz, Goldstein, and Rundel, 1985).

The purpose of the present study was to determine the supercooling capacity and freezing injury in *Espeletia schultzi* populations growing under different temperature regimes. The relationships between supercooling, leaf water potential components and leaf anatomy are also discussed.

**MATERIALS AND METHODS**

Five *Espeletia schultzi* Wedd populations were studied along an altitudinal gradient (Table 1) in the Venezuelan Andes (approximate coordinates lat. 8° 52' N, long. 70° 45' W). The lowest population, Santo Domingo, is found at an elevation of 2600 m where the plants occur in abandoned fields. The remaining four populations exist as components of the natural paramo (tropical alpine) vegetation: Los Platanos (3 100 m), Mucasbub (3 550 m), Trasandina (3 800 m) and Piedra Blancas (4 200 m). This 2600-4200 m altitudinal gradient corresponds to a climatic gradient of decreasing temperature with 3 200 m marking a rapid increase in the frequency of night frosts (Monastario and Reyes, 1980). Plants were excavated from each site with roots and soil intact, transported to the laboratory and placed in a growth chamber with controlled temperature and irradiance simulating field conditions.

**Cold injury**

The refined TTC method described by Steponkus and Lamphere (1967) was used to determine tissue injury. Tissue samples from mature leaves were cut and immediately placed in sealed tubes and immersed in an alcohol refrigerated bath (Grant Instruments Ltd.). Temperature was lowered from 10 °C to -30 °C at a rate of 10 °C h^-1_. This cooling rate was selected because it was similar to the maximum temperature change at the end of the daytime period observed in the field. Three replicates of each sample were taken from the bath at 5 °C intervals and incubated at 6 °C for 8 h. After this incubation period, the TTC solution was applied and left for 15 h. An ethanol extract was then made and the absorbance at 530 nm measured (Spectronic 20, Bausch and Lomb). Freezing injury was defined as the amount of TTC reduced by the samples which resulted in 50%, absorbance of the amount of TTC reduced by the unfrozen reference sample at 10 °C. This temperature was selected as 100%, absorbance because it was the temperature at which the plants were maintained under control conditions before measurements were made. Steponkus and Lamphere (1967) have used 5 °C as 100%, absorbance because they have used tissue samples from plants that were acclimated at this temperature. The slight decrease in absorbance between 10 °C and 0 °C may have been caused by sampling errors although this decrease was very small compared to the one observed below 0 °C.

**Thermal analysis**

In order to determine the temperature at which leaf tissue freezing occurred, pieces 30 cm long and 10 cm wide were cut and immediately enclosed in small tightly sealed tubes to avoid changes in tissue water content. Copper-constantan thermocouples (36 gauge) were inserted in the leaf sample and temperature changes were continuously monitored with a strip chart recorder fitted with 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 the cooling process (Quaasne, Stainshoff and Weiss, 1972). The temperature of the bath was lowered from 10 °C to -30 °C at a rate of 10 °C h^-1_. The temperature at which freezing of the leaf tissue occurred was determined by the marked increase in leaf temperature due to the exothermic process of ice formation.
Leaf water potential ($\Psi_w$) and supercooling capacity

The effect of $\Psi_w$ on supercooling was investigated by determining the supercooling capacity of leaves of different $\Psi_w$. Leaves were cut from the rosette and allowed to transpire freely for different lengths of time in order to obtain a wide range of $\Psi_w$ values. At each time interval a small section of the leaf was placed in the bath tubes and the thermal analysis procedure carried out. The rest of the leaf was immediately put in the pressure chamber for $\Psi_w$ determination.

Relative apoplastic water content

Relative apoplastic water content was obtained in a manner similar to the one suggested by Richter, Duhme, Glazert, Hinckley, and Karlic (1980). Rehydrated leaves were submerged in liquid nitrogen and then slowly dehydrated while $\Psi_w$ was measured at regular intervals. From the relative water loss needed to make $\Psi_w = \Psi_{w0}$ we calculated the relative apoplastic water content.

RESULTS

Figure 1 presents the relationship between supercooling and $\Psi_w$ for the five *Espeletia schultzi* populations. Supercooling capacity increased linearly with decreasing leaf water potentials, with plants from higher elevations being more sensitive to changes in $\Psi_w$. At a given leaf water potential the supercooling capacity increased from the lowest elevation (Santo Domingo, 2600 m) to the highest one (Piedras Blancas, 4200 m). The slopes ($b$) of the relationship for the two lower elevations, Santo Domingo ($b = 0.40$) and Los Planitos ($b = 0.54$), were lower relative to the other three higher elevation sites: Mucubají ($b = 0.82$), Trasandina ($b = 0.77$) and Piedras Blancas ($b = 0.85$) ($P < 0.05$, Q(4 82) = 7.75, Sokal and Rohlf, 1981).
A strong linear relationship between elevation and supercooling capacity (Fig. 2) was also obtained for the five populations. For every 100 m change in elevation, the supercooling capacity increased approximately 0.5°C. The supercooling points in this figure were selected from Fig. 1 using a leaf water potential of −0.06 MPa, a value which generally occurs during the early morning hours when the lowest air temperatures are observed. This rate of change in supercooling with elevation is comparable to the rate of change of minimum air temperature with elevation. Such rates vary from place to place in tropical areas (Schüchter and Medina, 1982) with the rate for the Venezuelan Andes being −0.58°C/100 m (obtained from nine weather stations with at least nine years of continuous records). The rate of change in the supercooling capacity observed in *E. schultii* as well as the correlation coefficient of the regression (r = 0.98) can be even further improved if the Santo Domingo site is removed from the regression analysis. This population is found more than 500 m below the limit of night frost (3200 m) and therefore, supercooling may not be as important as a resistance mechanism against freezing. Omitting this population, the rate of change of −0.5°C/100 m in the supercooling capacity is obtained, which corresponds closely to the −0.58°C/100 m value for minimum air temperature.

The temperature at which freezing injury occurred also showed a trend along the altitudinal gradient (Fig. 3). 50% tissue injury occurred at −7.4°C for Santo Domingo and at −13.3°C for Piedras Blancas, with temperatures within this range for the other populations.

The ability of a plant tissue to exist in a supercooled state depends on physical factors such as cell size and the intercellular spaces available for ice nucleation (Levitt, 1980). Epidermal and mesophyll cell size decreased with increasing altitude, as did the relative apoplastic water content, which is an index of intercellular volume (Table 1). Plants from the Santo Domingo site (2600 m) had a relative apoplastic water content of more than 30%, while plants from Piedras Blancas (4200 m) had values less than 4%. Relative apoplastic water content values were comparable to those obtained with the pressure-volume method described by Tyree and Hammel (1972). With respect to cell size, both epidermal and mesophyll cells are much larger in the Santo Domingo plants, approximately 50 μm and 30 μm, respectively.
Fig. 3. Percent absorbance at 330 nm (A330) as a function of leaf temperature for five *Espeletia schultzi* populations: Santo Domingo, 2,400 m; Los Planzons, 3,000 m; Munchij, 3,550 m; Trasandina, 3,800 m; and Piedra Blancas, 4,200 m. Dashed line indicates S0.1 injury.

Compared to Piedra Blancas individuals where the epidermal cells are approximately 30 μm and the mesophyll cells are close to 20 μm, there is a clear correlation between epidermal cell size and elevation (r = 0.92) and for mesophyll cell size and elevation (r = 0.94).

**Discussion**

Supercooling appears to play an important role in the cold resistance mechanisms of Andean giant rosette plants. These results for *Espeletia schultzi* support those obtained for other *Espeletia* species where a very close relationship was found between freezing injury and supercooling capacity (Rada et al., 1985; Rada et al., 1985; Goldstein et al., 1985). Freezing after a marked supercooling, however, is far more likely to be fatal than the gradual freezing that occurs when there is no marked supercooling leading Levitt (1980) to consider supercooling an unlikely survival strategy. The relatively constant temperatures and moderate freezes of short duration which characterize high Andean environments, make it possible for *Espeletia schultzi* to utilize supercooling as an important resistance mechanism for the protection of the leaves from freezing.

The Santo Domingo and Los Planzons populations exhibit a relatively low supercooling capacity and higher freezing injury temperatures as compared to the other three populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Elevation (m)</th>
<th>Minimum average air temp (°C)</th>
<th>Supercooling (°C)</th>
<th>Injury temp (°C)</th>
<th>RWC (%)</th>
<th>Epidermal cell size (μm)</th>
<th>Mesophyll cell size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santo Domingo</td>
<td>2400</td>
<td>-10</td>
<td>-5.3</td>
<td>-7.4</td>
<td>3.1</td>
<td>20.8 × 159</td>
<td>30.9 × 238</td>
</tr>
<tr>
<td>Los Planzons</td>
<td>2150</td>
<td>-14</td>
<td>-9.7</td>
<td>-10.6</td>
<td>3.0</td>
<td>15.1 × 169</td>
<td>30.9 × 238</td>
</tr>
<tr>
<td>Munchij</td>
<td>2750</td>
<td>-7</td>
<td>-9.2</td>
<td>-12.6</td>
<td>3.1</td>
<td>31.4 × 139</td>
<td>28.9 × 231</td>
</tr>
<tr>
<td>Trasandina</td>
<td>3000</td>
<td>-2</td>
<td>-10.3</td>
<td>-15.8</td>
<td>7.7</td>
<td>26.8 × 75</td>
<td>28.7 × 235</td>
</tr>
<tr>
<td>Piedra Blancas</td>
<td>4300</td>
<td>-9.2</td>
<td>-15.2</td>
<td>-15.9</td>
<td>9.3</td>
<td>26.8 × 169</td>
<td>28.7 × 235</td>
</tr>
</tbody>
</table>

**Table 1.** Relationship between supercooling temperature, freezing injury temperature and anatomical characteristics for five *Espeletia schultzi* populations.

For epidermal and mesophyll cell size the mean ± standard error for n > 30 cells/leaf obtained from two leaves of three different individuals are shown. RWC = Relative apoplastic water content.
found above the limit of night frosts (Figs 1, 3). Based on these differences in freezing resistance capacity the populations may be grouped into two types: Santo Domingo (2600 m) and Los Plantios (3100 m) as type 1; and Musubaji (3550 m), Trasandina (3800 m) and Piedras Blancas (4200 m) as type 2. It is likely that there has been a stronger selection for differences in supercooling ability in type 2 populations which are frequently subjected to night-time freezing.

Tissue water status is known to affect supercooling capacity (Burke, Gusta, Quamme, Weiser, and Li, 1974; Timmis and Worrell, 1977). Furthermore, the number and magnitude of the exothermic events during freezing depend on the water content of the leaves (Salt and Kaku, 1967). In the genus Espeletia, Goldstein et al. (1985) have shown that water content influences the supercooling capacity of the leaves. The results of this study for a single species, Espeletia schultzi, support these previous findings. Supercooling capacity increases linearly with decreasing leaf water potentials, with plants from higher elevations being more sensitive to changes in °F, than lower ones (Fig. 1). These patterns have an adaptive value since minimum air and plant temperatures °F during the dry season when leaf water potentials are lowest (Goldstein et al., 1984).

The necessary qualifications for supercooling are not fully understood but they include: the absence of internal nucleators, barriers against external nucleators, the presence of anti-nucleators (substances which oppose the formation of nucleators), a relatively low moisture content, small cell size and little or no intercellular space for nucleation (Shearman, Olijen, Marchetti, and Everson, 1973). We have observed that the water content and the leaf water potential influence the supercooling capacity of the leaves of Espeletia schultzi. We have also shown that cell size and both epidermis and mesophyll, decrease as elevation increases. And finally the probability of ice formation is reduced when the intercellular spaces are reduced since sites for ice nucleation and spreading will be reduced (Levitt, 1980). This is another characteristic which changes along the gradient and together with the other qualifications suggest that anatomical differences may account for changes in the supercooling capacity of the leaves and show that selective pressures in Espeletia schultzi act towards a more efficient avoidance mechanism through supercooling as the plants reach higher altitudes.

George et al. (1974) have shown that in some temperate region species the appearance of the exotherm was strongly correlated with the minimum temperatures of their altitudinal and latitudinal distribution limits. This suggests the importance of supercooling as a cold resistance mechanism in the geographical and ecological distribution of these species. The supercooling characteristics observed in Espeletia schultzi show a clear example of its importance in the distribution of this species along the altitudinal gradient in the Andean Paramos.

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LITERATURE CITED