

Freezing avoidance in Andean giant rosette plants

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Received 11 February 1985; accepted for publication 17 April 1985

Abstract. Frost avoidance mechanisms were studied in *Espeletia spicata* and *Espeletia timotensis*, two Andean giant rosette species. The daily courses of soil, air and tissue temperatures were measured at a site at circa 4000 m. Only the leaves were exposed to subzero temperatures; the apical bud and stem pith tissues were insulated by surrounding tissues. The leaf tissues avoided freezing by supercooling rather than by undergoing active osmotic changes. The temperatures at which ice formed in the tissues (the supercooling points) coincided with injury temperatures indicating that *Espeletia* tissue does not tolerate any kind of ice formation. For insulated tissue (apical bud, stem pith, roots) the supercooling point was around -5°C coinciding with the injury temperature. Supercooling points of about -13 to -16°C were observed for leaves. These results contrast with those reported for Afroalpine giant rosettes which tolerate extracellular freezing. The significance of different adaptive responses of giant rosettes to similar cold tropical environments is discussed.

Key-words. *Espeletia spicata*, *Espeletia timotensis*; Compositae; Frailejón; freezing avoidance; supercooling.

Introduction

One of the most striking features of the vegetation of high elevation environments in the tropical Andes is the presence of caulescent giant rosette plants. These plants have an erect unbranched stem up to 3 m tall supporting a single evergreen rosette of large pubescent leaves. This growth form has evolved in several high elevation tropical regions. Several species of the genus *Senecio* and *Lobelia* in the East African mountains and *Espeletia* in the high Andes, for example, have in common a voluminous central pith, marcescent leaves around the stem that insulate internal tissues including the pith reservoir, and a single terminal rosette with large pubescent leaves (Hedberg, 1964; Smith, 1974; Beck *et al.*, 1982; Goldstein & Meinzer, 1983). The independent evolution of these similar structures suggests that they have evolved as an adaptive response to a tropical environment characterized by low mean temperatures and frequent night-time frosts.

High elevation tropical habitats are exposed to very special climatic conditions. Above 4000 m,

mean temperatures are low and subzero temperatures occur very frequently, with practically no seasonality so that protection against freezing injury cannot be based on temperature acclimation, dormancy or leaf fall, characteristic of temperate plants. Beck *et al.* (1982) found that rosette leaves in several African caulescent rosette species were often stiffly frozen after cold nights. The leaf tissue, however, was not injured by freezing. In laboratory studies, Larcher (1975) observed that leaves of *Espeletia semiglobulata*, *Eryngium humboldtii* and *Polylepis sericea* from the Venezuelan páramos have very low supercooling points and are injured upon freezing. This suggests that these species may normally avoid freezing in nature (Larcher, 1981).

This paper describes the mechanisms by which two caulescent giant rosette plants withstand subzero temperatures. Temperature regimes and osmotic characteristics were studied in the field and supercooling capacity and freezing injury were studied in the laboratory.

Materials and methods

The studies were carried out on adult individuals about 1 m tall of *Espeletia spicata* Sch. Bip. and *Espeletia timotensis* Cuatrecasas (Compositae). These species were chosen from different sites in the Piedras Blancas Páramo, Venezuela (ca. $8^{\circ}37' \text{N}$, $70^{\circ}12' \text{W}$) at elevations ranging from 3800 to 4100 m. *E. timotensis* grows on well-drained slopes while *E. spicata* tends to occupy small depressions with fine soil sediments. Both are long-lived caulescent giant rosette plants that reproduce approximately every two years (Estrada, 1984). The mean annual temperature in Piedras Blancas is about 3°C with a difference of only 2.7°C between the mean temperature of the coldest and warmest month. The annual precipitation of 800–900 mm falls mainly between April and December.

Field and laboratory analyses were performed to determine the avoidance mechanisms by which the exposed and insulated tissues of these two species withstand freezing temperatures. We define insulated tissues as those not directly in contact with the air.

Daily cycles of soil, air and plant temperatures

Temperatures were measured with 36-gauge copper-constantan thermocouples which were placed beneath the pubescent layer on the leaves and were

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inserted in the stem and soil inside a syringe with a 10 cm long needle. Complete thermal maps were obtained by placing the thermocouples in the following manner: stem temperatures were measured at two levels (15 cm above ground level and 15 cm below the active rosette growth) in all four cardinal points. Leaf temperatures were measured in young and adult leaves in the same four positions. Apical bud temperature was also measured by inserting a small needle with a thermocouple. Eleven daily cycles were obtained with a data logger or with a microvoltmeter connected to an electronic 0°C reference junction. Figures 1 and 2 represent typical daily cycles for these two species.

Leaf osmotic potential and soluble sugar concentration

Leaf samples were removed from plants in the field at 2–3 h intervals over 24 h cycles. The samples were wrapped in aluminium foil immediately after excision and immersed in liquid nitrogen. They were then placed in hermetically sealed test-tubes and kept in ice during transport back to the laboratory. The thawed samples were placed in a sample chamber (C-52, Wescor, Inc.; Logan, Utah) and after reaching equilibrium (circa 1 h) the tissue osmotic potential was measured with a Dew Point Microvoltmeter (HR33T, Wescor, Inc.; Logan, Utah) operating in the dew point mode. The osmotic potential of at least two samples was measured. If there was a difference between the two values, additional readings were carried out to allow for errors of estimation.

Leaf samples for soluble sugar analysis were removed from the plants at the same time intervals used to determine daily courses of osmotic potential in the field. These were placed in polythene bags completely sealed and immediately placed in ice. In the laboratory, the samples were dried at 40°C during four days. They were then ground and the anthrone method described by Allen (1974) was used to determine the soluble sugar concentrations.

Cold injury in exposed and insulated tissues

Intact plants were excavated with roots and soil, transported to the laboratory and placed in growth chambers with controlled temperature and irradiance simulating field conditions. The refined triphenyl tetrazolium chloride (TTC) method described by Steponkus & Lanphear (1967) was used to determine tissue injury after freezing. Exposed and insulated tissue samples (circa 4 cm long) were cut from these plants and immediately placed in sealed tubes and immersed in an alcohol refrigerated bath (Grant Instruments, Ltd.; Cambridge, England). Temperature was lowered from 10°C to –30°C at a rate of about 10°C h⁻¹. 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 before the final determination. The samples were extracted with ethanol and with the aid of a spectrophotometer the absorbance corresponding to each exposure temperature was recorded. Frost resistance was assumed to correspond to the exposure temperature which resulted in a 50% decrease in absorbance in comparison to the control. This is equivalent to 50% injury in the tissue (Steponkus & Lanphear, 1967).

Thermal analysis (TA)

For thermal analysis, leaf pieces 3 cm long and 1 cm wide were cut and copper-constantan thermocouples (36-gauge) were immediately inserted in the samples. The samples were then tightly sealed in small glass tubes to avoid changes in tissue water content. Prior to immersion in a refrigerated alcohol bath, the tubes were enclosed in an aluminium 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 about 10°C h⁻¹. Changes in temperature were continuously monitored with a strip chart recorder fitted with an electronic 0°C reference. The peaks (exotherms) registered in the chart recorder indicate the moment of freezing as a result of the exothermic heat release during the freezing of water (supercooling point). The warming is temporary, and when the freezing is complete the temperature continues to drop constantly (George *et al.*, 1977).

Results

Daily cycles of plant and air temperature

There were two distinct groups of tissues with respect to the minimum temperatures observed: those which were exposed to subzero temperatures (young and adult leaves), and those which were not (pith, roots and bud). In both daily cycles the air temperature stayed very close to 0°C at night. For *E. spicata* (Fig. 1) temperatures reached 0°C but never dropped below during the night. Leaf temperatures remained below air temperatures throughout the night with the temperature of the young leaves dropping to –1°C (Fig. 1a). The temperature of the insulated apical bud remained more than 2°C above air temperature during much of the night (Fig. 1b). The roots were also well insulated because soil temperatures at 10 cm below ground level never fell below 5°C (Fig. 1b). In the case of *E. timotensis* (Fig. 2a, b) temperature patterns similar to those for *E. spicata* were observed. Young and adult leaf temperature (Fig. 2a) fell below the air temperature at night and, as in Fig. 1, the young leaves reached much lower temperatures (–1.9°C) than the adult ones

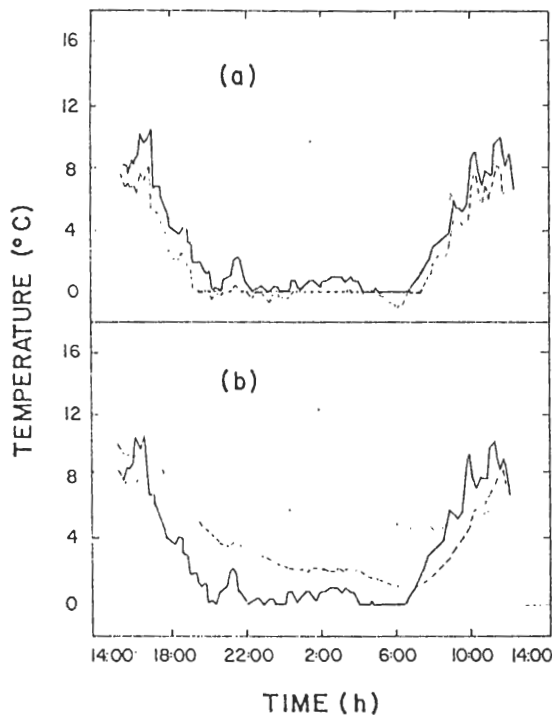


Figure 1. Daily temperature of (a) young (---) and adult (· · ·) leaves, and (b) bud (—) and soil temperature at 15 cm below the surface (---), in relation to air temperature at 1.5 m (---) for *Espeletia spicata* on 15 December 1983.

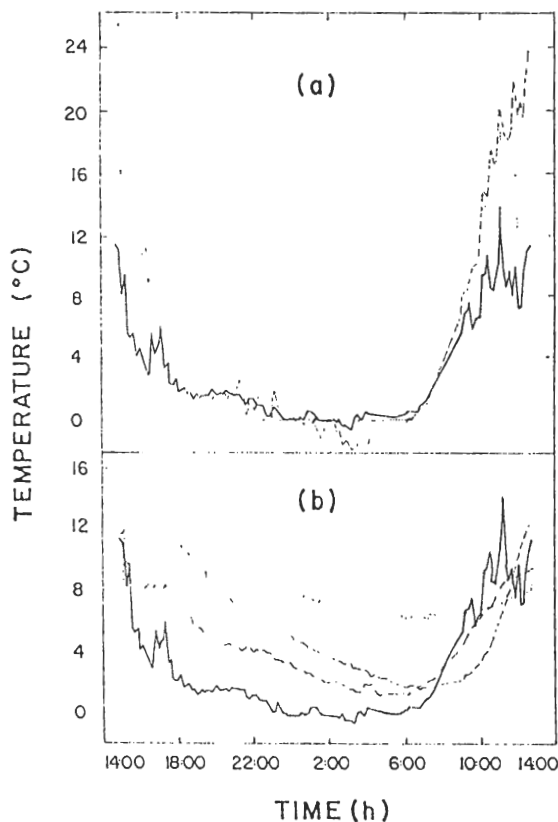


Figure 2. Daily temperatures of (a) young (---) and adult (· · ·) leaves, and (b) bud (—), pith (---) and soil temperature at 15 cm below the surface (---), in relation to air temperature at 1.5 m (---) for *Espeletia timotensis* on 23 April 1984.

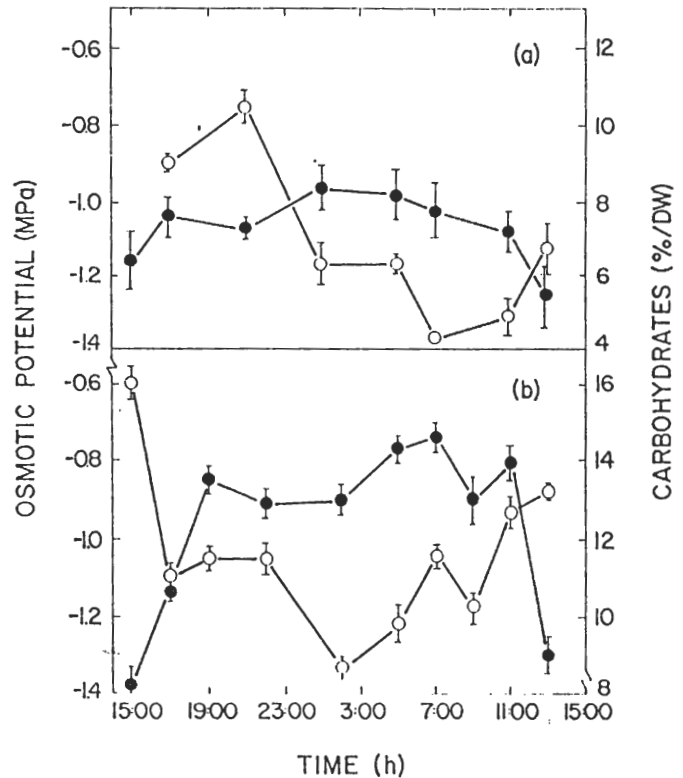


Figure 3. Daily cycles of leaf osmotic potential (●) and soluble sugar concentration (○) in (a) *Espeletia spicata* and (b) *Espeletia timotensis*. Standard errors are shown for $n = 3-5$.

(-0.4°C). Apical bud and stem temperatures remained well above that of the air throughout the night (Fig. 2b).

Osmotic potential and sugar concentration

For *E. spicata*, the osmotic potential decreased during the day and increased at night (Fig. 3a). The sugar concentration reached its maximum in the afternoon hours and began to drop at night. A similar situation was observed for *E. timotensis* (Fig. 3b). The osmotic potential increased during the afternoon and was more or less constant through the night until the next day when it dropped again.

These results suggest that the osmotic potential changes were passive and not active, and were due to the greater water stress during the day and a much lesser stress at night when the water demand was much lower. Because osmotic potentials were highest and sugar concentrations lowest during the night we can exclude lowering of the freezing point by accumulation of osmotically active solutes as a freezing avoidance mechanism in *E. spicata* and *E. timotensis*. This behaviour contrasts with that of another Venezuelan páramo species, *Polylepis sericea*, which shows large, active night-time increases in osmotic and sugar concentrations (Rada *et al.*, 1985).

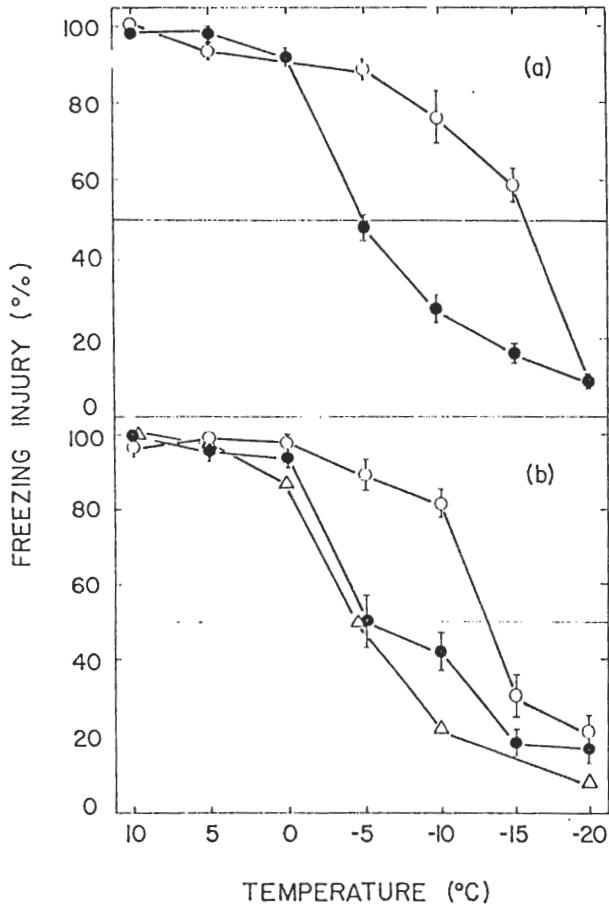


Figure 4. Freezing injury expressed as a percentage of living tissue at different temperatures for *Espeletia spicata*. (a) Young leaves (O) and pith (●). (b) Adult leaves (O), roots (Δ) and bud (●). Bars are standard errors for n = 3.

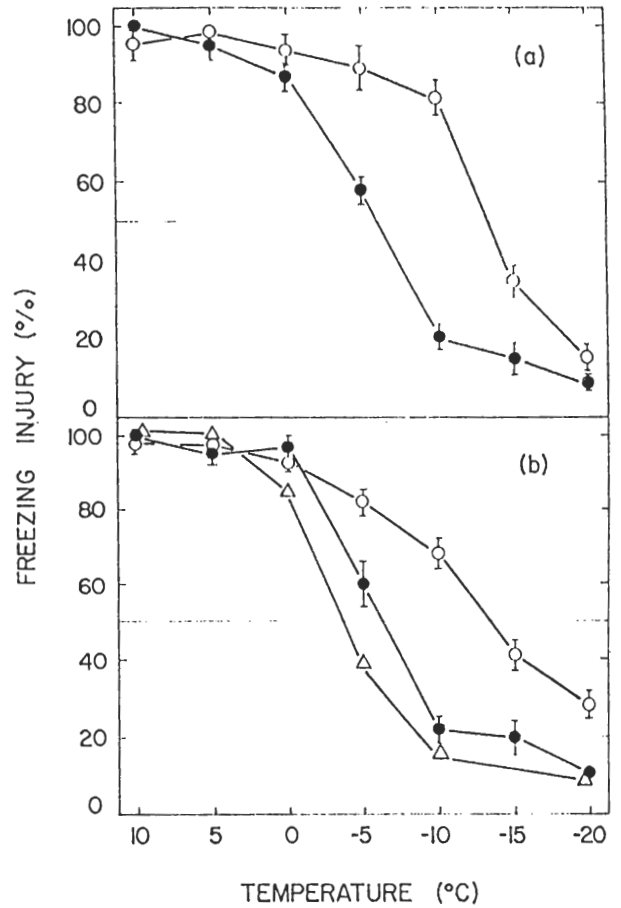


Figure 5. Freezing injury expressed as a percentage of living tissue at different temperatures for *Espeletia timotensis*. (a) Young leaves (O) and pith (●). (b) Adult leaves (O), roots (Δ) and bud (●). Bars are standard errors for n = 3.

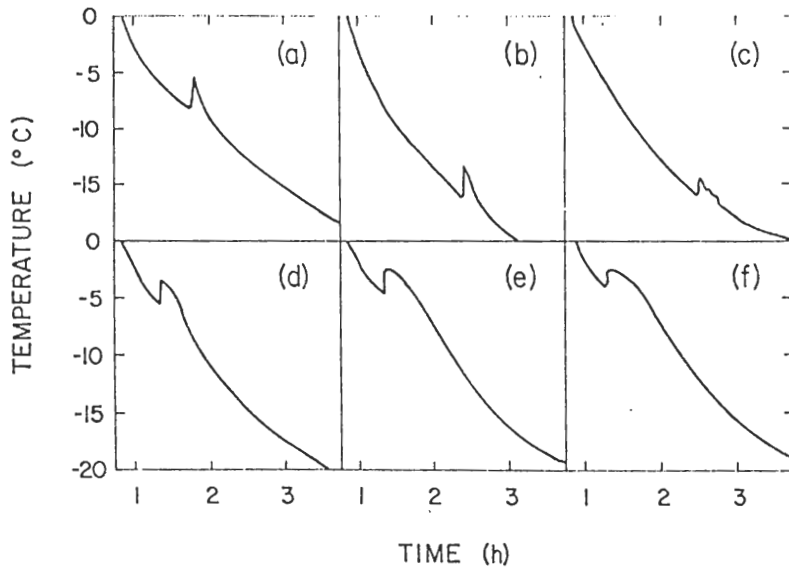


Figure 6. Exotherm appearance in six different tissues of *Espeletia spicata* (a) Unexpanded leaf, (b) young leaf, (c) adult leaf, (d) bud, (e) pith and (f) roots.

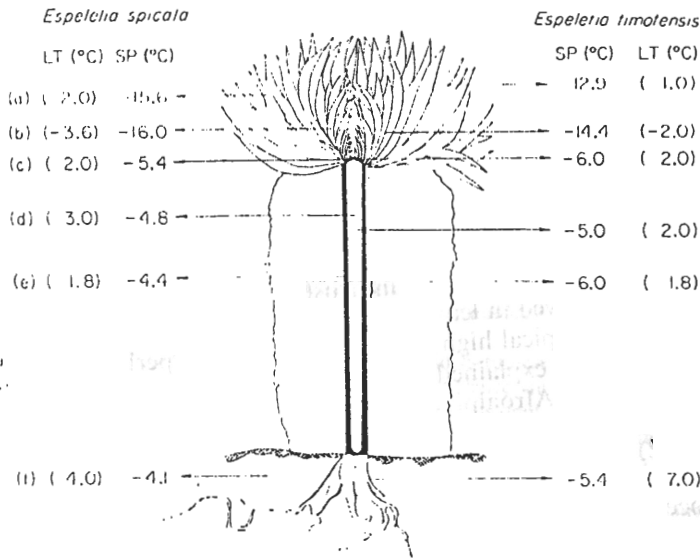


Figure 7. Schematic representation of a caulescent giant rosette plant showing lowest temperatures (LT) recorded in the field during the measurement days, and supercooling points (SP) for different organs and tissues. (a) Adult leaves, (b) young leaves, (c) apical bud, (d) stem pith, (e) phloem and periderm tissue, and (f) roots at approximately 10 cm in depth.

Freezing injury and supercooling

In exposed young leaves of *E. spicata* 50% injury occurred at approximately -16°C while in adult leaves the 50% injury temperature was -13°C (Fig. 4). Under natural conditions the young leaves reached lower temperatures than older leaves at night (Figs 1 & 2). The normally insulated bud, pith and root tissues experienced 50% freezing injury at a significantly higher temperature of about -5°C (Fig. 4b). Similar patterns of freezing injury versus temperature were observed for *E. timotensis* (Fig. 5) with the exception that both young and adult leaves yielded similar 50% injury temperatures of -13.5°C.

Figure 6 shows typical TA profiles for different *E. spicata* tissues. The peaks shown in each of the panels of the figure represent the appearance of the only exotherm observed down to -30°C. The exotherm is displaced downward in exposed tissues as compared to insulated ones. Figure 7 summarizes the exotherm temperatures for the tissues studied in the two species, and demonstrates that the tissues insulated from freezing temperatures have supercooling points closer to zero than those tissues frequently exposed to freezing temperatures. In addition, a very high correlation was found between supercooling and 50% tissue injury temperatures. The regression line (supercooling point = 0.084 + (1.0104 × Freezing injury)) has a slope that does not differ significantly from one, indicating that the temperature at which 50% injury was observed corresponded to the exotherm appearance. Therefore none of these tissues seem to exhibit the ability to tolerate ice formation.

Discussion

High-elevation tropical giant rosette plants occur in habitats where the temperatures are constantly low throughout the year and there is a high probability of frost every night. These conditions have favoured the development of certain resistance mechanisms that include both avoidance and tolerance of freezing. The development of morphological features together with physiological adaptations that permit these plants to resist the freezing stress has been suggested by previous authors (Hedberg, 1964; Larcher, 1975; Smith, 1974; Beck *et al.*, 1982; Goldstein & Meinzer, 1983). Differentiation of frost resistance mechanisms is best seen comparing what we have called exposed and insulated tissues in both Andean and African giant rosette plants.

Insulated tissues

The roots of *E. spicata* and *E. timotensis* are well protected against freezing under the ground because soil temperatures at rooting depth remain above 0°C throughout the daily cycle. The pith is also very well insulated by the marcescent leaves surrounding the stem. Stem temperatures of *E. spicata* and *E. timotensis* never dropped below 0°C. This behaviour has been mentioned for species of *Lobelia* and *Senecio* in the Afroalpine vegetation (Hedberg, 1964; Beck *et al.*, 1982); and *E. schultzei* in the Andean páramos (Smith, 1979). This protection of the stem by the marcescent leaves is a very important freezing avoidance mechanism because the pith acts as a water reservoir for the plant (Goldstein *et al.*, 1985), permitting these species to have a constant water supply even when the soil is cold and the air temperature is below zero.

The bud in *E. spicata* and *E. timotensis* also shows a novel frost avoidance mechanism which can also be observed in plants of the same life-form in the Afroalpine vegetation (Hedberg, 1964; Beck *et al.*, 1982). The bud is well protected by the very young developing leaves forming a many layered cone-shaped structure. Together with this cone-shaped bud, the outer surrounding leaves show nyctinastic bending inward during the night period resulting in an additional protection (Smith, 1974; Rada, 1983). The higher heat capacity of this massive structure and the additional layers of insulation seem to be sufficient to delay cooling during the short low temperature period.

In another study, Krog *et al.* (1979) found that the inflorescence of a *Lobelia* species has a fluid filled center which acts as a thermal buffer by liberating heat of fusion when ice begins to form. This avoidance mechanism was not found in *Espeletia* species.

Exposed tissues

Our results suggest that plants from Andean tropical high mountains and those from the Afroalpine

regions utilize the same mechanisms to protect bud, pith and root tissue from frost damage. With respect to frost resistance of exposed leaf tissue, however, the results of the present study indicate that there is a difference with regard to the mechanisms used by the same life-form of the Andean and African high mountains. The pubescence, as already shown by previous authors (Beck *et al.*, 1982; Meinzer & Goldstein, 1985) has no role in keeping the leaves warmer as they reach much lower temperatures than the air during the night due to boundary layer effects on convective heat transfer. Our results show that the leaves of Andean giant rosette plants avoid freezing by means of supercooling and that these species do not tolerate any kind of ice formation within their tissues. We believe that in *Espeletia* leaves when freezing is induced in the laboratory, ice spreads very rapidly throughout the tissue. The appearance of only one exotherm down to temperatures below -30°C suggests that extra and intracellular ice formation occur simultaneously. The close agreement between appearance of exotherms and induction of 50% injury supports this idea. This contrasts with the results obtained by Beck *et al.* (1982, 1984) in which extracellular ice formation occurred readily in leaves of Afroalpine giant rosettes and resulted in no apparent damage. These authors have further suggested that avoidance of supercooling in Afroalpine plants is a prerequisite for their survival. Beck *et al.* (1984) have found that when freezing occurs, leaf water potential reaches very negative values (-5.0 MPa). On the other hand, Goldstein *et al.* (1985) have shown that irreversible damage in *E. timotensis* leaves begins to occur at a relatively high relative water content and very close to the turgor loss point (-1.5 MPa) therefore tolerating a lesser degree of dehydration compared to the Afroalpine plants.

Persistent supercooling (Larcher, 1982) is now known to be an effective avoidance mechanism in xylem tissues, seeds, flower buds and leaves of some woody plants (Weiser, 1970; Quamme *et al.*, 1972; George & Burke, 1977; Larcher, 1982). In our study, the expanded leaves of both species supercool down to -13 to -16°C . This supercooling temperature is rather low for leaf tissue. Moderate to extreme supercooling temperatures observed in nature fall in the -10 to -43°C range (Levitt, 1980). We have not studied the mechanisms that allowed these leaf tissues to be relatively strong supercoolers; however, several characteristics that were observed in *Espeletia* leaves may be responsible for these supercooling temperatures: small cell size (particularly the small size of chlorenchyma cells), small intercellular spaces for ice nucleation and the presence of a dense pubescence layer (2–3 mm). Dense leaf trichomes may help to prevent dew water or rainfall from wetting the surface. Supercooling of *Eucalyptus urnigera* was possible down to -10°C if the leaf

surface was dry, but only to -2 to -4°C when wet (Thomas & Barber, 1974).

We did not observe in Andean giant rosette plants the secretion of large volumes of viscous mucilage from the leaves as did Beck *et al.* (1982) in *Lobelia* and *Senecio* species. These authors suggested that this substance may fulfil a protective role in the cells once extracellular ice formation occurs.

The clear differences in frost resistance mechanisms observed in leaves of the same life-form in two different tropical high mountain regions can perhaps be partially explained by the different temperature regimes. In Afroalpine regions, temperatures below -10°C are fairly common (Coe, 1967; Beck *et al.*, 1982); while in the tropical high Andes, the temperatures stay very close to 0°C and only on rare occasions they drop below -5°C .

Freezing avoidance would seem to give an adaptive advantage to the *Espeletia* species with regard to biomass production. A plant which is subjected to ice formation in its leaves any night of the year should be less productive than plants which never freeze. Beck *et al.* (1980) have found annual leaf production values around 166 g m^{-2} of leaf area for *Senecio* plants in the 0.6–1.3 m range, compared to 671 g m^{-2} of leaf area for *E. spicata* and 370 g m^{-2} of leaf area for *E. timotensis* in the same height range (Estrada, 1984). These results show that leaf production is doubled in *E. timotensis* and in the case of *E. spicata* four times greater than the values for *Senecio* in Africa, probably because this last species reaches considerable photosynthetic capacity up to two hours after thawing of the leaves (Beck *et al.*, 1982).

Overall, the results of this work show that *Espeletia* species rely exclusively on avoidance mechanisms to withstand frequent subzero temperatures: (1) pith, roots and bud are partially insulated from air temperature variations and therefore exhibit low temperature avoidance and (2) the supercooling temperatures of the leaves are low enough to avoid freezing without the dangers of a rapid plasma dehydration and intracellular ice formation.

Acknowledgments

The work was supported by the Universidad de Los Andes (CDCH) Grant No. C-179-81 and by a CONICIT Grant to the Postgrado de Ecología Tropical.

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