

Freezing tolerance in *Draba chionophila*, a 'miniature' caulescent rosette species

A. Azocar, F. Rada, and G. Goldstein

Departamento de Biología, Facultad de Ciencias, Universidad de los Andes, Merida, Venezuela

Summary. Freezing tolerance as a cold resistance mechanism is described for the first time in a plant growing in the tropical range of the Andean high mountains. *Draba chionophila*, the plant in which freezing tolerance was found, is the vascular plant which reaches the highest altitudes in the Venezuelan Andes (approximately 4700 m). Night cycles of air and leaf temperature were studied in the field to determine the temperature at which leaf freezing began. In the laboratory, thermal analysis and freezing injury determinations were also carried out. From both field and laboratory experiments, it was determined that freezing of the leaf tissue, as well as root and pith tissue, initiated at a temperature of approximately -5.0°C , while freezing injury occurred at approximately -12.0°C for the pith, and below -14.0°C for roots and leaves. This difference in temperature suggests that the plant still survives freezing in the -5.0 to -14.0°C range. Daily cycles of leaf osmotic potential and soluble carbohydrate concentration were also determined in an attempt to explain some of the changes occurring in this species during the nighttime temperature period. A comparison between Andean and African high mountain plants from the point of view of cold resistance mechanisms is made.

Key words: *Draba chionophila* – Freezing tolerance – Cold resistance – High tropical mountains

Tropical high mountain habitats experience very special climatic conditions. Above 3200 m mean daily temperatures are low and subzero air temperatures may occur any night of the year. Under such environmental conditions, plants must not only immediately recover their full photosynthetic capacity after a night frost (Schulze et al. 1985), but also must avoid freezing damage.

The caulescent giant rosette species belonging to the genus *Espeletia* in the Andean Paramos exhibit avoidance mechanisms to resist the low air temperatures (Rada et al. 1985b; Goldstein et al. 1985). Tissues such as the pith and bud are insulated from environmental temperature variations by the marcescent leaves and nocturnal inward bending of fully developed leaves, respectively, which delay cooling and prevent nighttime subzero temperatures (Smith 1974, 1979; Rada 1983). The adult leaves of these plants, on the other hand, are exposed to below freezing temperatures during the night and avoid freezing through their high

supercooling capacity. The leaves of *Polylepis sericea*, a tree species growing in the high Andes, also supercool. In addition, they have a lowered freezing point resulting from the accumulation of soluble carbohydrates in their leaf tissues (Rada et al. 1985a). These results contrast however with those found by Beck et al. (1982) for the giant caulescent rosette species from the African high mountains, which show that freezing of the leaf tissues occurs without any accompanying functional damage.

It is obvious that tropical plants growing in those environments where nighttime freezing temperatures may occur at any time during the year, have been under a strong selective pressure for the evolution of freezing avoidance and/or tolerance mechanisms. The specific adaptive response may depend on the microclimatic temperature regime to which the plants are exposed. In habitats where nighttime temperatures do not drop too far below 0°C and remain there for only short periods of time, the main resistance mechanism should be freezing avoidance (Larcher 1981; Sakai and Larcher 1987). In those tropical habitats where nighttime temperatures drop far below 0°C and stay there for several hours, tolerance should be the selected resistance mechanism (Levitt 1980; Larcher 1981; Rada et al. 1985b; Sakai and Larcher 1987).

In this paper, we document the results of laboratory and field studies of freezing resistance mechanisms in *Draba chionophila* BLAKE, a small rosette species which grows in the Desert Paramo (Monasterio 1980; Pfitsch 1986) being the vascular plant species which reaches the highest altitudes in the Venezuelan Paramos (4700 m) (Vareschi 1970). At this upper limit of vascular plant distribution, the climatic conditions are characterized by temperatures which may reach below -10.0°C any night of the year and are similar to those reported for the giant rosettes in Africa at 4200 m where leaf tissues exhibit freezing tolerance (Beck et al. 1982, 1984; Schulze et al. 1985).

Materials and methods

This study was carried out on adult individuals of *D. chionophila* (Brassicaceae). Continuous measurements of temperature and osmotic variables of plant tissues were made in the field. Supercooling capacity and freezing injury temperatures were also determined in the laboratory.

Field work was done in the Paramo de Piedras Blancas, Sierra de la Culata (ca. $8^{\circ}56' \text{N}$, $70^{\circ}48' \text{W}$) at an elevation of 4200 m, and in Pico Espejo, Sierra Nevada de Merida (ca. $8^{\circ}35' \text{N}$, $71^{\circ}09' \text{W}$) at 4700 m.

The mean annual temperature in Piedras Blancas is approximately 3°C and the lowest recorded temperature 10 cm above ground was -10°C (Perez 1984) during the dry season. The annual precipitation is 800–900 mm falling mainly between April and November. In Pico Espejo, the mean annual temperature is about -0.3°C, showing very frequent below -5°C temperatures all year round. The annual precipitation of 1137 mm is concentrated between May and December.

D. chionophila is a small, monocarpic rosette with succulent leaves and a mean height of 5 cm (Pfitsch 1986). Its distribution is essentially limited to the highest alpine environments, reaching the highest altitudes (approximately 4700 m) in its range of distribution in the Venezuelan Andes. It grows on very poorly developed and sandy soils, and has a well-developed root system. Individual roots can reach 70 cm long distributed in the first 2 cm of top soil.

Field measurements of air, leaf and soil temperature

Air, leaf and soil temperatures were measured with copper-constantan thermocouples g36 during the night, usually between 18.00 and 7.00 h. Air temperature was measured at 3 cm above the ground and soil temperature at 5 cm below ground. Leaf temperature was measured using 2 to 3 thermocouples placed on different plants in the field. Temperatures were recorded with a continuous chart recorder fitted with an electronic 0°C reference, a 12 V battery and an AC/DC inverter.

Determination of osmotic potential and soluble carbohydrate concentration

During a twenty-four hour period, leaf samples were removed at 2–3 h intervals. Immediately after excision the samples were placed in hermetically sealed test tubes and submerged in liquid nitrogen. These tubes were then placed on ice and transported back to the laboratory. For the osmotic potential determinations, a Wescor HR-33 Dew Point Microvoltmeter with Wescor C-52 chambers were used. Leaf samples were brought to the laboratory and oven-dried at 80°C for 15 minutes and then kept at 40°C for forty-eight hours for the soluble carbohydrate analysis. The Anthrone method described by Allen (1974) was used to determine the soluble carbohydrate concentrations in the leaf samples.

Root and leaf freezing temperature (thermal analysis)

In order to determine the temperature at which leaf and root tissue freezing occurs whole plants were excavated from each of the two field sites, transported to the laboratory and placed in growth chambers with temperature and radiation regimes which simulated field conditions. Leaves and roots were then cut from a plant and immediately enclosed in small, tightly sealed test tubes, thus avoiding changes in tissue water content. Copper-constantan thermocouples were then inserted in the leaf samples and temperature was continuously monitored with a strip chart recorder fitted with an electronic 0°C reference. The tubes were placed in a refrigerated alcohol bath and the temperature was lowered from 10°C to -30°C at a rate of approximately 10°C/h. Since a marked increase in tissue tempera-

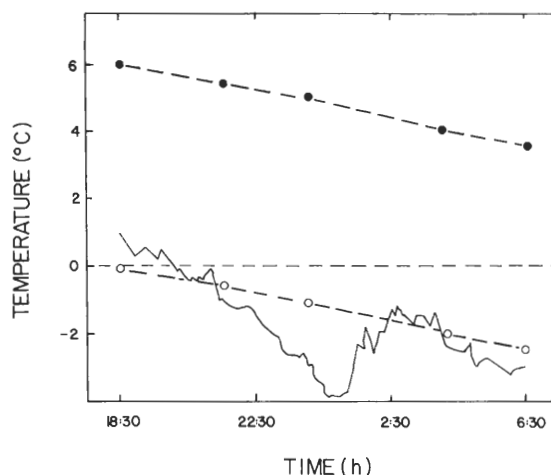


Fig. 1. Night course of leaf (—), air (○) and soil (●) temperatures for *D. chionophila* at Piedras Blancas (4200 m). Air temperature was measured at 5 cm above ground and soil temperature at 5 cm below ground. Leaf temperature was continuously recorded with a strip chart recorder

ture occurs as a result of the exothermic process of ice formation, the freezing temperature of the tissues was readily determined.

Determination of freezing injury

To determine the effects of freezing temperatures on tissues of this species, a refined TTC method (Steponkus and Lanphear 1967) was used. Samples of leaf, root and pith tissue were placed in small test tubes and immediately immersed in a refrigerated alcohol bath. Temperature was lowered from 10°C to -30°C at a rate of approximately 10°C/h. At 5°C intervals, samples were removed and then incubated at 6°C for 8 h. After this incubation period, the TTC solution was added and 15 h later samples were extracted with ethanol and absorbance at 530 nm was measured. Freezing injury was defined as that temperature which caused a 50% reduction in absorbance when compared to the absorbance of a reference sample, in this case the 10°C sample.

Results

Field measurements of air, soil and leaf temperatures

A continuous nighttime temperature cycle for the leaves of *D. chionophila* in the Paramo de Piedras Blancas is shown in Fig. 1. Leaf temperature remained below 0°C, but never dropped below -3.0°C. Leaf temperature remained continuously below air temperature after 21.00 h, except for a brief period between 02.00 and 04.30 h when a slight increase in leaf temperature occurred. This increase was attributed to a brief snowfall which completely covered the ground during this period. The soil temperature at 5 cm below the ground oscillated between 6.6°C and 3.5°C.

Air and leaf temperature for *D. chionophila* in Pico Espejo are shown in Fig. 2. During the night, the air and leaf temperatures fell to much lower values (-9.1°C air temperature and -9.6°C leaf temperature) than those recorded for Piedras Blancas. There was also a sudden temperature rise (exothermic event) at around 23.30 h indicat-

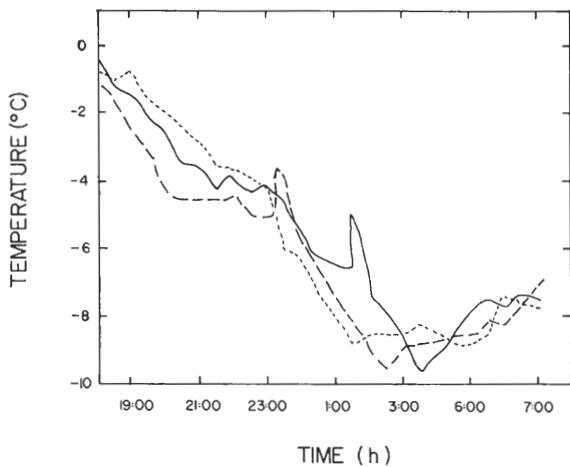


Fig. 2. Night course of leaf and air temperatures for *D. chionophila* at Pico Espejo (4700 m). Air temperature (...) was measured at 5 cm above ground. Two leaves were chosen: (—) and (— · —)

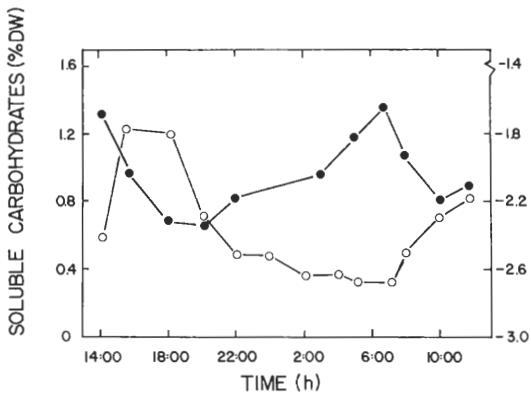


Fig. 3. Daily courses of soluble carbohydrate concentrations (●) and osmotic potential (○) for *D. chionophila*

ing that leaf water froze. Freezing temperature (-5.6°C and -6.5°C) and time of freezing differed in the leaves measured. This is probably due to differences in leaf exposure, maturity or water status. Since these are succulent leaves, in which water storage is probably compartmentalized, we thought that freezing would occur as a series of exothermic events as a consequence of barriers which would stop or delay ice spreading. However, we did not detect more than one exothermic event. Following the release of heat associated with freezing, the leaf tissue temperature began to drop, paralleling the air temperature. This contrasts with results obtained by Krog et al. (1979) for the inflorescence of the Afroalpine *Lobelia telekii*, in which temperature never dropped below 0°C during the night due to a continuous release of heat by freezing within the different inflorescence compartments.

Laboratory experiments and freezing tolerance

In *D. chionophila*, the osmotic potential decreased at night, tending to become more negative as air temperature decreased. On the other hand, the soluble carbohydrate concentration showed an increase during this same period (Fig. 3).

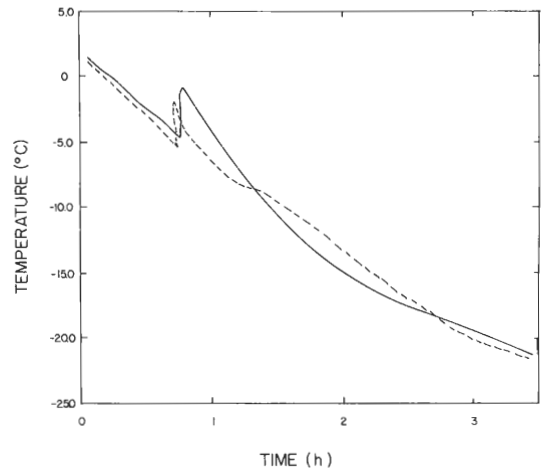


Fig. 4. Temperature changes observed in the laboratory experiments for root (—) and leaf (---) tissues. Exotherms occur at approximately -5.0°C

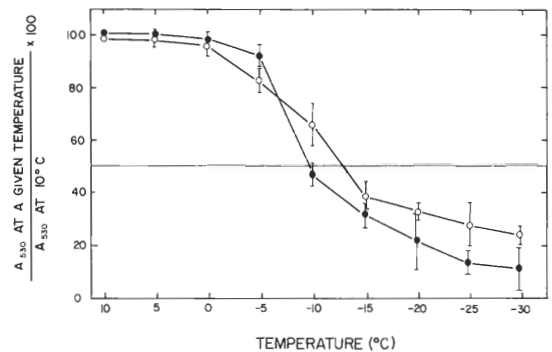


Fig. 5. Percent absorbances at 530 nm (A_{530}) as a function of leaf temperature for *D. chionophila*. Line at 50% indicates injury temperature of leaf (○) and root (●) tissues. Vertical lines indicate one standard error above and below the mean ($n = 5$)

Table 1. Freezing tolerance in different tissues of *D. chionophila*. T_s is supercooling temperature; T_i is injury temperature and T_d is difference between T_s and T_i . Standard errors for T_s and T_i are for $n = 5$

Tissue	T_s ($^{\circ}\text{C}$)	T_i ($^{\circ}\text{C}$)	T_d ($^{\circ}\text{C}$)
Leaves	-5.0 ± 0.3	-14.8 ± 0.7	9.8
Pith	-4.7 ± 0.1	-12.0 ± 0.2	7.3
Roots	-4.0 ± 0.4	-14.0 ± 0.2	10.0

Continuously monitoring of leaf temperatures in the field, revealed that leaf tissues froze between -5.0°C and -6.0°C . In laboratory experiments (Fig. 4), excised leaves also froze at approximately the same temperature range. Freezing injury, on the other hand, occurred at temperatures around -15.0°C for the leaves and at -12.0°C for the roots (Fig. 5). Table 1 shows the values of freezing tolerance for *D. chionophila*. The difference between freezing temperature and the temperature at which injury occurred may indicate that this species is capable of tolerating and recovering from the freezing of water in the intercellular spaces (Larcher 1982).

Discussion

Due to their small size, *D. chionophila* rosettes grow in much more extreme microclimates than do the larger rosette species. The daily temperature within 5 cm of the ground, the zone where these plants are found, varies from approximately 20° C to below -10° C (Azocar and Monasterio 1980; Perez 1984). The long periods during the night with subzero temperatures accompanied by frost (Perez 1984) also makes this environment more extreme than that found for giant rosette plants such as adult *Espeletias*. Hedberg (1964) has suggested that the leaf tissues of these small rosette plants should be able to delay cooling and maintain higher than ambient nighttime temperatures since they are very dense rosettes and therefore radiation emission at night is reduced. Our results show that leaf temperature in *D. chionophila* reaches lower than ambient values, demonstrating that these rosettes are not insulated from low nighttime air temperatures. These plants also show little supercooling capacity compared to the giant caulescent rosette plants. *Espeletia* species have exposed leaves that exhibit supercooling temperatures between -13° C and -16° C. These species, however, have a lower distributional limit than *D. chionophila*. The tissues of the leaves, pith and roots freeze at around -5.0° C for *D. chionophila*. Ice formation occurs about 10° C before freezing injury is observed, which indicates that *D. chionophila* tolerates freezing without damage for the range of temperatures to which this species is usually exposed to in the field.

The increased concentration of soluble carbohydrates and the concomitant decrease in osmotic potential during periods of low nighttime temperature (Fig. 3) may have a clear adaptive advantage. Soluble carbohydrates and in particular sucrose, are known to be membrane cryoprotectants (Steponkus et al. 1977). On the other hand, this increase in carbohydrate concentration, and therefore a decrease in the osmotic potential could enhance an even greater supercooling capacity of the leaf tissues (Zachariassen and Husby 1982). If this actually happens it would contradict the freezing resistance syndrome observed in *D. chionophila*, where avoidance of supercooling is a prerequisite to prevent the formation of intracellular ice upon freezing (Beck et al. 1982). These two hypotheses on the significance of the observed carbohydrate concentration increase at low temperatures have to be experimentally tested. Similar patterns of carbohydrate concentration changes were observed in *Polylepis sericea* (Rada et al. 1985a) but in this case they were due to active osmotic temperature dependent changes. Measurements of water potential components and soluble carbohydrates in *P. sericea* revealed an osmotic adjustment of up to 1.0 MPa during the predawn hours. A linear relationship was also found between minimum osmotic potential and leaf temperature for various cycles indicating that temperature may significantly affect the magnitude of the predawn osmotic potential drop (Rada et al. 1985a).

Troll (1966) has noted the great similarity in climatic conditions and plant life forms of tropical high mountains around the world. A classic example is the giant caulescent rosette plants of the African and Andean mountains, where convergency in morphology has occurred due to similar climatic regimes (Coe 1967). Nevertheless, the results of our work, together with those of others (Beck et al. 1982, 1984; Schulze et al. 1984; Rada et al. 1985b; Goldstein

et al. 1985), show that at the physiological level, plants may respond in different ways to below freezing temperatures. The response seems to depend partially on the specific nature of the microclimate in which they grow. In tropical high mountains, freezing tolerance to survive freezing may be selected for in areas where temperatures drop far below 0° C and remain there for most of the night. This has been shown to occur in some of the giant rosette species of the African high mountains and we are reporting it for the first time for an Andean species which grows at the highest altitudes of any plant occurring in these mountains. Supercooling, in these habitats, may be of great risk to the plant because temperatures are low and last for a relatively long nighttime period. Freezing is associated, in the tissues of giant rosette species that supercool, with rapid dehydration of the cells and even the formation of intracellular ice. Both are lethal for these plants. It is possible that the altitudinal distribution of the genus *Espeletia* may be partially attributed to their inability to maintain supercooling in their tissues when exposed to the same extreme temperature conditions near ground level where *D. chionophila* is found.

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