Freezing avoidance in Andean giant rosette plants

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Abstract. Frost avoidance mechanisms were studied in Euphrasia spicata and Euphrasia immutans, 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 sub-zero temperatures; the aerial bud and stem pith tissues were insulated by surrounding tissues. The leaves were protected against 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 Euphrasia tissues do 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.

Keywords: Euphrasia spicata; Euphrasia immutans; Euphorbiaceae; Tropaeolum; 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 caespitose 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 Lactuca in the East African mountains and Euphrasia in the high Andes, for example, have in common a voluminous central pith, mature rosettes leaves around the stem that insulate internal tissues including the pith reservoir, and a single giant rosette with large pubescent leaves (Heilberg, 1964; Smith, 1974; Beck et al., 1982; Godstein & 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,

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mean temperatures are low and subjective temperatures occur very frequently, with practically no reliability to plant response to freezing injury cannot be based on temperature ablation, dormancy or leaf fall, characteristic of temperate plants. Beck et al. (1982) found that rosette leaves in several African caespitose 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 Euphrasia semigladiata, Eryngium lombardii and Polylepis are 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 caespitose giant rosette plants withstand sub-zero 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 Euphrasia spicata Sch. Bip., and Euphrasia immutans Quocholeva (Compositae). These species were chosen from different sites in the Polylepis Blancas Páramo, Venezuela (c. 8°37’ N, 70°12’ W) at elevations ranging from 3000 to 4000 m. E. immutans grows on well-drained slopes while E. spicata tends to occur small depressions with fine soil sediments. Both are long-lived caespitose giant rosette plants that reproduce approximately every two years (Estrella, 1984). The annual mean temperature in Polylepis 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 600-900 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 38-gauge copper-constant thermocouples which were placed beneath the pubescent layer on the leaves and were
insered in the soil and inside a syringe with a 10 cm long needle. Complete thermal maps were obtained by placing the thermocouples at the following locations: stem temperatures were measured at two levels (15 cm above ground level and 10 cm below the active root zone) 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 2:30 h intervals over 24 h cycles. The samples were wrapped in aluminum foil immediately after excision and immersed in liquid nitrogen. They were then placed in thermostatically sealed test-tubes and kept in an oven (39°C) to keep temperature constant during air-drying. The osmotic potential of the aqueous phase was measured with a Dew Point Micrometer (HR33T, Wescor, Inc., Logan, UT) 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 polyethylene bags, completely sealed and immediately placed in ice. In the laboratory, the samples were dried at 40°C during 5-10 min and ground in a clean mortar using 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, transplanted to the laboratory and placed in growth chambers with controlled temperature and illumination simulating field conditions. The refined triphenyl tetrazolium chloride (TTC) method described by Stepenskis & Langhans (1967) was used to determine tissue injury after freezing. Exposed and insulated tissue samples (circumference 4 cm long) were cut from these plants and immediately placed in sealed tubes and immersed in an iced bath (Grant Instruments, Ltd., Cambridge, England). Temperature was lowered from 10°C to 3°C at a rate of about 1°C h⁻¹. These samples 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 95% injury in the tissue (Stepenskis & Langhans, 1967).

Thermal analysis (T4)

For thermal analysis, leaf pieces 3 cm long and 5 cm wide were cut and copper-constantan thermocouples (DG-2000) 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 insertion 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 (Quattum et al., 1972). The temperature of the bath was lowered from 10°C to 3°C at a rate of about 1°C h⁻¹. Changes in temperature were continuously monitored with a strip chart recorder fitted with an electronic B C reference. The peaks (isotherms) registered in the strip chart recorder indicated the moment of freezing as a result of the exotherm prior to the freezing of water (supercooling point). The warming is temporary, and when the freezing is complete the temperature sometimes drops 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 ambient temperatures (young and adult leaves), and those which were hot (stomata, roots and bud). In both daily cycles the air temperature stayed very close to 3°C at night. For L. spinata (Fig. 1a) temperatures reached 1°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 peaks were also well insulated because soil temperatures at 10 cm below ground level were never below 5°C (Fig. 1b). In the case of E. xanthocarpa (Fig. 2a, b) temperature patterns similar to those for L. spinata 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 (1-1.9°C). When the adult ones...
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Figure 1. Daily temperature at (a) young (---), mid adult (- - -), and adult (-----) \textit{E. speciosa} leaves, and (b) mid adult (- - -) and mid adult (-----) leaf and soil temperatures at 15 cm below the surface (- -- -). All temperature readings are relative to air temperatures at 1.5 m above the ground (---) for \textit{E. speciosa} on 13 December 1983.

Figure 2. Daily cycle of leaf osmotic potential (---) and whole-plant sugar concentration (-----) in (a) \textit{E. speciosa} and (b) \textit{E. similis} leaves at 15 cm below the surface (---). Standard errors are shown for $n = 5$.

Figure 3. Daily temperature at (a) young (---), mid adult (- - -), and adult (-----) \textit{E. speciosa} leaves, and (b) mid adult (-----) and leaf and soil temperatures at 15 cm below the surface (- -- -). All temperature readings are relative to air temperatures at 1.5 m above the ground (---) for \textit{E. similis} on 23 April 1984.

(-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 \textit{E. speciosa}, 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 \textit{E. similis} (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 \textit{E. speciosa} and \textit{E. similis}. This behaviour contrasts with that of another \textit{E. speciosa} pithramo species, \textit{Polybiopsis sericea}, which shows large, active night-time increases in osmotic and sugar concentrations (Ruda et al., 1985).
Figure 4: Freezing injury expressed as a percentage of living tissue at different temperatures for Euphorbia spinosa. (a) Young leaves, (b) Adult leaves, (c) roots, and bud.

Figure 5: Freezing injury expressed as a percentage of living tissue at different temperatures for Euphorbia spinosa. (a) Young leaves, (b) Adult leaves, (c) roots, and bud.

Figure 6: Ectotherm appearance in six different times of Euphorbia spinosa. (a) Unexposed leaf, (b) young leaf, (c) adult leaf, (d) bud, (e) pistil, and (f) roots.
Freezing injury and supercooling

In exposed young leaves of *E. spinosa* 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. tinctorius* (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. spinosa* 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 tissue as compared to insulated ones. Figure 7 summarizes the exothermic temperatures for the tissues studied in the same species, and demonstrates that the insulated tissues (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 = 0.0164 + 0.0104 × Freezing injury) has a slope that does not differ significantly from 0, indicating that the temperature at which 50% injury was observed corresponded to the exothermic appearance. Therefore none of these tissues seem to exhibit the ability to tolerate ice formation.

Discussion

High-elevation tropical giant rosette plants occur in areas where the frost-free period 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 these mechanisms is the area of current research. In the future, it is expected that freezing tolerance will be better understood and that more effective methods of frost protection will be developed. The development of new and improved frost-resistant cultivars is likely to be a key area of future research.

Insulated tissues

The roots of *E. spinosa* and *E. tinctorius* have developed to avoid freezing. In the future, it is expected that freezing tolerance will be better understood and that more effective methods of frost protection will be developed. The development of new and improved frost-resistant cultivars is likely to be a key area of future research.

Exposed tissues

Our results suggest that plants from Andean tropical high mountains and those from the Afrotropical
regions unlike the same mechanisms to protect bud, path 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 Afromontane high mountains. The phytocenosis, as already shown by previous authors (Buck et al., 1986; Mottier & Goldstein, 1985) has no role in keeping the leaves warm 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 these tissues. We believe that in Erythrina leaves when freezing is induced in the laboratory, ice spreads very rapidly throughout the tissue. The appearance of only one exothermic down to temperatures below -30°C suggests that exists intracellular ice formation occur simultaneously. The close agreement between appearance of exotherm and induction of 90% injury supports this idea. This contrasts with the results obtained by Buck et al. (1982, 1984) in which extracellular ice formation occurred readily in leaves of Afromontane giant rosettes and resulted in no apparent damage. These authors have further suggested that avoidance of supercooling in Afromontane plants is a prerequisite for frost survival. Buck et al. (1986) have shown that when freezing occurs, leaf water potential reaches very negative values (-5.0 MPa). On the other hand, we have shown that the irreversible damage in E. tinctoria leaves begins to occur at a relatively high relative water content and very close to the tissue loss point (-1.5 MPa) therefore tolerating a lesser degree of dehydration compared to the Afromontane plants.

Perissidi supercooling (Larchet, 1982) is now known to be an effective avoidance mechanism in xylem tissues, seeds, flower buds and leaves of some woody plants (Winter, 1970; Quantrou et al., 1972; George & Burke, 1978; Larchet, 1982). In our study, the expanded leaves of both species supercool down to -13 to -10°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 (Levis, 1980). We have observed that these species to be relatively strong supercooling however, several characteristics that were observed in Erythrina leaves may be responsible for these supercooling temperatures: small cell size (particularly the small size of chloroplasts 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 Erastyrus tinctorius was possible down to -10°C if the leaf surface was dry, but only to -2 to -4°C when wet.

We did not observe in Andean giant rosette plants the variation in large volumes of viscous mistletoe from the leaves as did Buck et al. (1982) in Erythrina and Soherul publica species. These authors suggested that this substance may facilitate 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 Afromontane regions, temperatures below -10°C are rather common (Col, 1987; Buck et al., 1984); while in the tropical high Andes, the temperatures stay very close to 0°C and only on rare occasions they drop below -5°C. Frost avoidance would seem to give an adaptive advantage to the Erythrina 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. Buck et al. (1986) have found annual leaf production values around 106 g m-2 of leaf area for Senecio plants in the 8-1.3 m range, compared to 171 g m-2 of leaf area for E. tinctoria and 371 g m-3 l of leaf area for E. tinctoria in the same height range (Mattia, 1986). These results show that leaf production is doubled in E. tinctoria and in the case of E. tinctoria is almost five times greater the values for Senecio in Africa, probably because this last species resides considerable photosynthetic capacity up to 2 hours after thawing of the leaves (Buck et al., 1982).

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

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References


