Influence of Freezing Temperatures on a Cactus, *Coryphantha vivipara*

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**Summary.** *Coryphantha vivipara* (Nutt.) Britton & Rose var. *deserti* (Engelm.) W.T. Marshall (Cactaceae) survived snow and tissue temperatures of −12 °C in southern Nevada. However, the freezing point depression of the cell sap was only about 0.9 °C. When the nocturnal air temperature in the laboratory was reduced from 10 °C to −10 °C for one night, the optimum temperature for CO₂ uptake shifted from 10 °C to 6 °C and uptake was reduced 70%, but full recovery to the original values occurred in 4 days. Nocturnal temperatures of −15 °C killed 2 out of 5 plants and −20 °C killed 5 out of 5, as judged by lack of net CO₂ uptake at night over a 2-month observation period. When the stems were cooled at 2 °C/h, supercooling to about −6 °C occurred followed by an exothermic reaction that presumably represented the freezing of extracellular water. When the subzero temperature was lowered further, no other exothermic reaction was observed and the cells became progressively dehydrated. Freezing-induced tissue death was ascribed to this cellular dehydration, which led to about 94% loss of intracellular water at −15 °C. When the tissue temperature was lowered, the ability of chlorenchyma cells to plasmolyze and to take up a stain decreased, both being nearly 70% inhibited at −15 °C and completely abolished at −20 °C. Some cold-hardening occurred, since lowering the air temperature from 30 °C to −10 °C in 10 °C increments at weekly intervals caused the subzero temperature for 50% inhibition of staining to decrease from −10 °C to −17 °C. Extension of the range of *C. vivipara* to regions with wintertime freezing apparently reflects the tolerance of considerable freeze dehydration by its protoplasts.

**Introduction**

The lowest wintertime temperature can be the most important factor influencing plant distribution (Shreve 1914; Parker 1963). Shreve (1911) stated that “the line which marks the extreme southern limit of frost is the most important climatic boundary in restricting the northward extension of perennial tropical species.” Of the 65 species of tropical and subtropical arborescent cacti in the Sonoran desert, only three (*Carnegiea gigantea, Lemaireocereus thurberi,* and *Lophocereus schottii*) occur further north than the frost line, and frost damage is common on all of them at the northermost part of their ranges in Arizona (Shreve 1911, Uphof 1916; Turnage and Hinckley 1938; Steenbergh and Lowe 1977; Nobel 1980). Moreover, no cacti occur in the colder Mojave or Great Basin Deserts. As is true of cacti in general, *C. gigantea* does not have a very low osmotic potential, values for the stem ranging from −0.4 to −1.0 MPa (Soule and Lowe 1970). Since the freezing point depression for such osmotic potentials is only 0.3 °C to 0.8 °C, freezing would seem inevitable in cold regions. Indeed, succulent plants such as cacti are generally not present in regions with appreciable freezing (Levitt 1980).

For those cacti that can survive freezing temperatures, the actual tolerance varies considerably. Shreve (1911) observed that *C. gigantea* could withstand 19 h of freezing temperature. If no midday thawing occurs, one night’s freezing would persist through the next night, increasing the time frozen from under 24 h to over 36 h. He suggested that *C. gigantea* was restricted to those regions where freezing did not last 24 h, a hypothesis that has received support (Niering et al. 1963). On the other hand, *Echinocereus polyacanthus* and *Opuntia versicolor* can withstand 66 h of continuous freezing and *O. missouriensis* can withstand 24 h to over 36 h. He suggested that *C. gigantea* was restricted to regions where the mean January temperature is above −1 °C (Kinraide 1978). Since the minimum temperatures, especially those during unusually cold periods, would be much lower than −1 °C, *O. imbricata* can withstand considerable freezing. Uphof (1916) noted different freezing sensitivities for a series of opuntias; death occurred at −8 °C for *O. ficus-indica*, −10 °C for *O. fusciculatis*, −17 °C for *O. castilliae*, and −18 °C for *O. ellisihana*. Stems of *C. gigantea* supercool (cool below the equilibrium freezing point) in the range of −3 °C to −12 °C, which has been proposed to protect juveniles from freezing damage on colder slopes (Steenbergh and Lowe 1976). So far very little quantitative information exists relating the low temperature tolerances of cacti to their cellular and physiological properties.

Here the effects of freezing temperature were investigated for a cactus of particularly widespread distribution in North America, *Coryphantha vivipara*. It ranges from northern Mexico (30°N) all the way to Manitoba and Alberta, Canada (50°N) (Earle 1963; Benson 1974) and hence can occur in habitats subjected to considerable freezing. Cellular and physiological properties that permit tolerance of freezing temperatures were studied for this Crassulaceae acid metabolism (CAM) plant. Nocturnal CO₂ uptake was monitored for up to two months after a low temperature treatment to help determine whether a particular subzero temperature was lethal. Evidence was obtained for supercooling of the stem as well as increasing cellular damage as the temperature was progressively lower.

**Materials and Methods**

*Coryphantha vivipara* (Nutt.) Britton & Rose var. *deserti* (Engelm.) W.T. Marshall (nomenclature according to Beatley, 1976) (Cactaceae)
was examined in Mercury Valley at the Nevada Test Site in southern Nevada at 36°40' N, 116°1' W, 1,110 m. The height for 18 field plants measured on March 22, 1979 was 5.3 ± 0.8 (SD) cm and their diameter at midheight was 6.9 ± 1.0 cm. For laboratory studies, plants were transplanted in desert soil and routinely maintained in environmental growth chambers with 12-h days at 10°C and 350 µmol m⁻² s⁻¹ photosynthetically active radiation from 400 to 700 nm (determined with a Lambda Instruments LI-190S quantum sensor). Nighttime air temperature was also 10°C (tissue surface temperatures reached 23°C during the daytime and fell to 10°C at night). Plants were watered weekly with 1/16 Hoagland's solution no. 1 (Hoagland and Arnon 1950) such that the soil water potential near the roots (determined with Wescor PT 51-05 soil thermocouple psychrometers) was generally -0.2 ± 0.1 MPa (1 MPa = 10 bars). The osmotic potential of the tissue was obtained by placing a small amount of macerated tissue in a Wescor C-52 leaf chamber used in conjunction with a Wescor HR-33T microvoltmeter.

Gas exchange was measured as described previously (Nobel and Hartsock 1978). The stem was placed in an assimilation chamber that had 340 µl/l CO₂ and 5 g/m⁵ water vapor in air. The water vapor conductance equaled the net rate of water loss per unit stem surface area divided by the water vapor concentration drop from stem to air. The CO₂ residual conductance equaled the net rate of CO₂ uptake per unit stem surface area divided by the CO₂ concentration in the intercellular air spaces just interior to the stomates (Nobel and Hartsock 1979).

Stems were cooled to subzero temperatures in a Revco ULT-385A Deep Freezer. Temperatures represent the average for three 30-gauge copper-constantan thermocouples (agreement was generally ±0.5°C) placed in the air, within 0.5 mm of the stem surface, or at the center of a stem. To observe cellular properties for a particular subzero treatment, pieces of stem were removed and heated at about 10°C/h (approximately the warming rate observed in the field at sunrise) to 10°C. Plasmolysis and staining were observed for at least 200 chlorenchyma cells on fresh sections 100 µm thick examined at 400 x using a Zeiss phase-contrast research microscope. Plasmolysis was induced by 1 M manitol, and 50 µM neutral red (3-amino-7-dimethyl-amino-2-methylphenazine (HCl)) was used for staining. The fraction of cells that plasmolyzed or took up stain initially increased and approached constancy at about 24 h, and so stem pieces were maintained at 10°C for 24 h (except where indicated) before assaying for plasmolysis or staining ability.

**Results**

Surface temperatures of *C. vivipara* were measured in the field at various times during the winter of 1978/79. On the coldest day examined (February 3, 1979), the minimum air temperature 1 m above the ground was -9.4°C, the minimum tissue surface temperature for two plants 4.4 and 5.1 cm tall under 4 cm of snow was -7.8°C and for two similar-sized plants where the snow had been removed was -11.5°C. All four plants survived this cold spell.

Since the stems could survive subzero temperatures, the effect of such low temperatures on nocturnal CO₂ uptake was examined in the laboratory. A plant was exposed to a night at -10°C air temperature (tissue surface temperatures were within ±0.6°C of air temperature during the second half of the night) and nocturnal CO₂ uptake was then examined on succeeding nights (Fig. 1). On the night after the cold treatment, the optimal temperature for CO₂ uptake had shifted from the usual 10°C down to 6°C and the maximal rate of CO₂ uptake decreased 70%. By the second night the optimum was at about 8°C and the maximum rate had recovered to within 10% of the original value (Fig. 1). On the fourth night after the cold treatment, the plant had essentially returned to the initial CO₂ uptake pattern. Stomatal properties were changed little by the cold treatment, but the CO₂ residual conductance was reduced 75% on the night following the cold treatment and its temperature optimum had also shifted downward (Fig. 1).

To study the low temperature tolerance, plants were exposed to various subzero temperatures for one night and the subsequent nocturnal CO₂ uptake was determined (Fig. 2). Following a cold treatment, gas exchange and tissue condition of the plants were monitored for two months, unless the nocturnal CO₂ uptake became positive sooner. All plants survived -12°C, but two out of five did not survive -15°C (Fig. 2). For the surviving plants, no net CO₂ uptake occurred for 10 nights after the cold treatment (CO₂ exchange between 5°C and 15°C was then always negative). On the 14th night, the CO₂ uptake averaged...