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Negative Pressures Produced in an Artificial Osmotic Cell by Extracellular Freezing[1]

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ABSTRACT

A rigid artificial osmotic cell has been constructed using reverse osmosis membranes that were supported by metal grids from both sides to yield a high elastic modulus of the system. The cell could be subjected to changes of external water potential either by evaporation or by application of hypertonic solutions so that negative internal pressures or tensions (*i.e.* pressures smaller than atmospheric) could be built up. Negative pressures were also obtained by freeze-induced dehydration when the cell was cooled to -1.5°C and ice was formed on the outer surface. Tensions of up to -0.7 megapascals (-7 bars) could be established in the different types of experiments. Smaller tensions could be kept in the cell for several hours. Cavitations caused the pressure to increase instantaneously to values of about -0.1 megapascals (relative to atmospheric pressure) as theoretically expected. Cavitations could be reversed by pressurizing the system. The cell could be cooled to subzero temperatures while the cell solution was under tension. Intracellular freezing could be easily detected from an instantaneous increase in pressure. When the membrane was not supported by a grid from the inside (analogous to the situation in plant cells), no tensions could be built up in the system. The results support the idea of the incidence of negative pressures during freezing, if the wall is sufficiently rigid to prevent cell collapse and if the membrane does not separate from the cell wall.

During freezing, plant cells are dehydrated by the formation of ice in the intercellular spaces causing the protoplasts to shrink and the cell turgor to decrease. Eventually, the cell should plasmolyze or collapse and the cell water freeze as the temperature decreases further. However, in several studies it has been found that the cell sap tends to supercool, preventing cell death by intracellular freezing. By measuring the water potential (Ψ) of frozen tissue and the osmotic pressure of the cell sap (π^i) it has also been found that ($\Psi + \pi^i$) was smaller than zero. Since matric potentials were considered to be rather small, this has been interpreted as evidence for negative pressures in cells of frozen tissue ($P = \Psi + \pi^i$; 1, 6, 9, 17), as has also been proposed during water stress other than freezing in sclerophyllous plants (8, 10, 12, 15). As in the xylem, tensile states of water in cells should be metastable and should lead to cavitation or embolism as soon as seeds for bubble formation are present. Such tensions also require rather rigid cells which are capable of withstanding compressive forces without collapsing. Furthermore, the tensile strength of water may be reduced at low temperature (5), which may also limit the tensions which could develop.

Since negative pressures in plants and the cohesion of water were first postulated by Böhm in 1893 (4), the direct measurement of tensions in plants has been problematical because the introduction of probes would immediately cause cavitations (21). The cell pressure probe in its present state (11, 18, 19) fails to measure tensions except for a range of rather small values. Therefore, an artificial model of a plant cell has been constructed using reverse osmosis membranes (18, 21, 22). The cell consisted of a thin (100–200 μm) layer of an osmotic solution bounded between a pressure transducer (diameter: 4 mm) and the membrane and was, thus, in one dimension similar to the size of plant cells. The system could be used to maintain tensions of a few bars over several hours and also to simulate water and solute relations of plant cells (hydraulic conductivity, solute permeability, reflection coefficients) (21).

As reported in this paper, the osmotic cell was adapted for use at subfreezing temperatures. Freezing dehydration was induced and 'turgor' was measured continuously while tensions were created. The system proved to be an appropriate model for studying freezing responses as long as direct measurements in living plant cells are not possible.

MATERIALS AND METHODS

The Artificial Osmotic Cell

The artificial osmotic cell is shown schematically in Figure 1. It consisted of a Perspex block which was fitted into a metal case. A pressure transducer (XTM 190 from Kulite Semiconductor Products, Leonia, NJ; pressure range: 0–3.5 MPa) was screwed into the perspex block so that a small cavity (0.1–0.2 mm in depth) was formed on the top of the block for the osmotic cell. The surface of the perspex block and of the transducer was covered by a thin layer of silicone rubber which was prepared from liquid material (Xantopren plus, from Bayer, Leverkusen, FRG) prior to filling the cavity with solution. A round slice of a solid film of silicone rubber with an outer diameter of 25 mm and with a hole of 5 mm in diameter in its center was placed on the top of the perspex block. These materials acted as seals when the perspex block was screwed into the metal case. In contrast to the type of osmotic cell used previously (21, 22), in the present version the membrane was open to the outside in order to allow evaporation of water from the surface.

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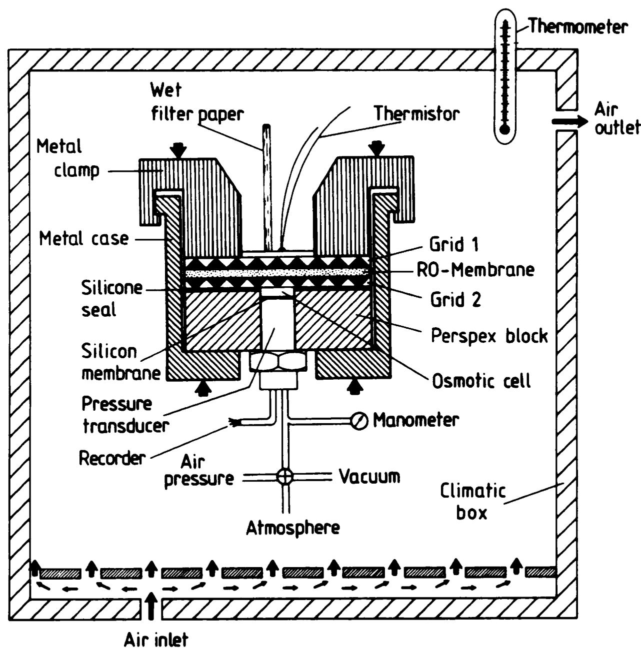

Figure 1. Artificial osmotic cell for creating negative pressures (schematic). The cell was formed from a cavity on the surface of a pressure transducer inserted into a perspex block and was filled with a solution of a nonpermeating solute (360 mOsmol $K_4[Fe(CN)_6]$) and covered by a reverse osmosis membrane sandwiched between two metal grids. The cell was sealed by pressing the grids against the perspex block. The cell was exposed to distilled water to gain a positive pressure. In evaporation experiments, the central part of the upper grid was open to the atmosphere. During osmotic dehydration, a hypertonic solution was applied. During freeze-induced dehydration, a thin layer of ice was formed on a filter disc soaked with water and placed on top of the upper grid to ensure that the membrane was completely covered with water prior to freezing. A wick of wet filter paper was brought into contact with the filter paper disc to trigger extracellular ice formation and to prevent the disc from drying. A thermistor was placed at the upper grid to measure the temperature. The whole equipment was kept in a climatic box.

After filling the cavity with $K_4[Fe(CN)_6]$ solution of 360 mOsmol (measured cryoscopically with an Osmomat 030 from Gonotec, Berlin, FRG), a reverse osmosis membrane (polyamide membrane with a nominal cutoff at 50 D; Reichelt, Heidelberg, FRG) was placed on top of the cavity. To form a rigid cell exhibiting a high coefficient of elasticity, the membrane was supported by metal grids on both sides. The grids had holes with diameters of 0.19 mm (9 holes/mm^2). This allowed creation of both positive and negative pressures in the cell (21, 22). Pressures created in the presence of distilled water or diluted solutions outside the cell were stable for several days, *i.e.* the leak rate of the system was negligible.

The transducer could be used either for measuring positive pressures (concave bending of the membrane) or for measuring negative pressures (convex bending; 21). For the calibration in the range of positive pressures (absolute pressure, $P > 0.1$ MPa = atmospheric pressure), gas pressures were applied to the front plate of the transducer. For the calibration in the range of negative pressures, gas pressures were applied to the opposite side of the membrane *via* a tube at the rear side (Fig. 1). During the experiments, the calibration was checked occasionally by applying pressure pulses of known height to the membrane to prove that the silicon membrane was properly functioning and that no artifacts were measured (*e.g.* by contact of the steel membrane of the transducer with the inner grid; 21).

A thermistor (type K 19, Siemens, FRG; sensitivity: ± 0.1°C) was used to record the temperature at the surface of the cell (Fig. 1). A disc of water-soaked filter paper was placed on the top of the osmotic cell during freezing experiments. To prevent supercooling of the water in the filter paper during freezing, a wick of wet filter paper was in contact with the disc of filter paper. This wick cooled faster than the disc due to a more rapid evaporation so that seeds for the freezing of the water right at the surface of the osmotic cell were created. During freezing experiments, the osmotic cell was placed in a phytobox (PB 500 T.T., BBC YORK, FRG; temperature range: -40 to +10 ± 0.1°C). A temperature of -1.4 ± 0.1°C and a high air flow rate across the chamber were set. Temperature fluctuations of the climatic box were ±0.1°C. The heat capacity of the metal block around the osmotic cell was rather large so that the temperature fluctuations within the block could be controlled within less than ±0.1°C.

Experiments

The osmotic cell could be dehydrated either by evaporation at room temperature, by adding hypertonic solutions at both room temperature and -1.4°C, and by extracellular freezing at -1.4°C. For the evaporation dehydration of the cell, the upper grid was exposed to the air. To dehydrate the cell with hypertonic solution at -1.4°C, methanol was added to the solutions as an antifreeze agent (1210 mOsmol CH_3OH). To simulate the situation in plant cells, where the plasma membrane is not sandwiched between two supporting layers, some experiments were also performed without the inner grid to see whether negative pressures could also be established under these conditions. In addition, we tested whether the negative pressure created by extracellular freezing could have been due to the thermal contraction or expansion of the artificial cell itself (Fig. 2). A negative pressure was set up in the cell, and the system was cooled from room temperature to -0.5°C, while the pressure changes in the cell were recorded in response to the temperature changes. Under these conditions, no extracellular and intracellular freezing took place because of the freezing-point depression of the solutions inside and outside of the cell. It can be seen from Figure 2 that the effects due to thermal expansion or contraction of the system (liquid plus envelope) were small. There was only a small increase in pressure in the early stages of cooling; later on the pressure stayed constant.

RESULTS

Negative Pressure at Room Temperature

Negative gauge pressures (gauge pressure = absolute pressure minus atmospheric pressure) of -0.5 MPa could be established at room temperature (21 ± 2°C) either by evaporation-dehydration or by adding hypertonic solution outside the

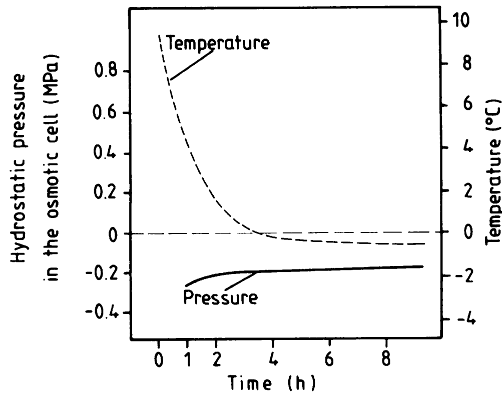

Figure 2. Effects of cooling on the thermal contraction and pressure in the osmotic cell. Pressure changes in the artificial osmotic cell (without freezing) in response to cooling were recorded while the cell was under a tension of -0.3 MPa. Dehydration was induced by a hypertonic solution of K_2HPO_4 outside the cell. The experiment shows that simple effects of thermal contraction or expansion of the system were fairly small. Furthermore, it is demonstrated that tensions could be maintained during cooling to subzero temperatures.

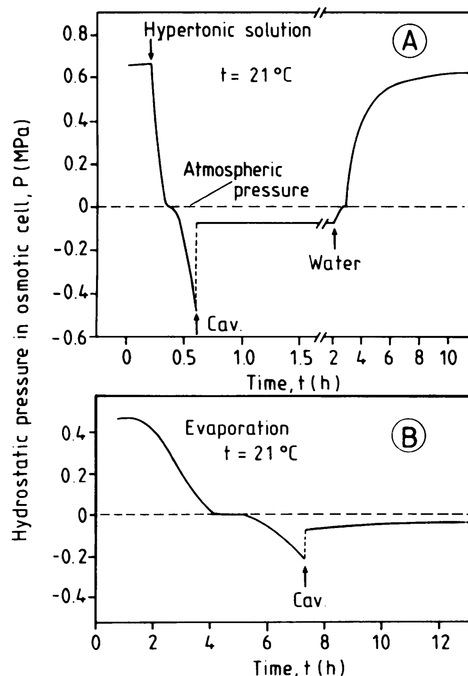

Figure 3. Negative pressures created at room temperature. In (A) tensions were produced by the saturated solution of the nonpermeating solute $K_4[Fe(CN)_6]$ whereas in (B) the negative pressure was caused by evaporation-dehydration (RH about 50%). Cavitations occurred at -0.5 and -0.2 MPa, respectively, and the pressure jumped to values close to -0.1 MPa (*i.e.* close to zero absolute pressure) as is theoretically expected.

membrane of the cell (Fig. 3, A and B). At the beginning of the experiment, the cell had a positive pressure of up to 0.65 MPa. The pressure dropped when a hypertonic solution was added to the top of the upper grid (Fig. 3A) or when evaporation became extensive (Fig. 3B). In both cases, there was a delay in the pressure drop around zero gauge pressure. After the delay, the pressures continued to decrease until cavitation occurred which could be clearly detected by the instantaneous increase in pressure to a value around -0.1 MPa gauge pressure (zero absolute pressure). This is expected because water vapor pressure at 21°C is only 25 hPa (mbar). Tensions of a few bars could be maintained in the cell for several hours (not shown in the figure).

Negative Pressures at Subzero Temperatures

At temperatures below 0°C, negative pressures were created either by applying hypertonic solutions containing an antifreeze agent which was also present in the cell at the same concentration (methanol; Fig. 4A) or by freeze-induced dehydration (Fig. 4B). With a hypertonic solution, the pressure changes in the cell were similar to those obtained at room temperature. When the solution was applied, the pressure dropped and usually showed some delay around atmospheric (zero) pressure. Thereafter, the pressure again decreased until cavitation occurred, which caused the pressure in the cell to increase suddenly. When the hypertonic solution outside was replaced by distilled water and the cell was warmed to room temperature, the cell returned to a positive pressure similar to that at the beginning of the experiment. It should be noted that in the freezing experiments the extent of cooling required to dehydrate the osmotic cell was usually rather small (−1 to −2°C) because the water potential of ice at this temperature is already sufficiently low to cause extensive dehydration.

Presumably, the delay in the pressure drop around zero pressure (Figs. 3 and 4) was caused by elastic properties of the cell, *i.e.* by the switching from slightly concave to convex bending of the grid or, most likely, of the membrane between the grids. These buckling effects could cause considerably large changes in volume and some holdup. In freezing experiments, there was always a delay in the temperature drop around the freezing point due to the formation of ice at the surface which caused a release of the heat of fusion of water (Figs. 4 and 5). However, there was no increase in turgor at the same time which would have occurred if the interior of the cell had become frozen (Fig. 5).

Positive Pressure by Intracellular Freezing

When the cell was cooled rather quickly, *i.e.* to below 0°C within 1 h and further to below -6°C in 2 h, both the extracellular and intracellular water became frozen (Fig. 5). These processes could be seen from a delay in the decline of temperature around 0°C (release of the heat of fusion outside the cell) and from a transient temperature rise at below 0°C (release of the heat of fusion in the cell). Freezing of cell water caused a rapid increase in pressure due to the increase in volume upon formation of ice. Partly, this increase was transient. The reason for the transience is not quite clear, but may have resulted from a bending of the grid at higher pressures, from a rapid exosmotic water flow caused by large pressure, or from a dehydration of the cell as the temperature continued to drop. The processes were reversed upon the addition of warm water when the cell reached the original temperature (Fig. 5).

When the inner grid of the artificial cell was removed, thereby eliminating any resistance to caving in of the mem-

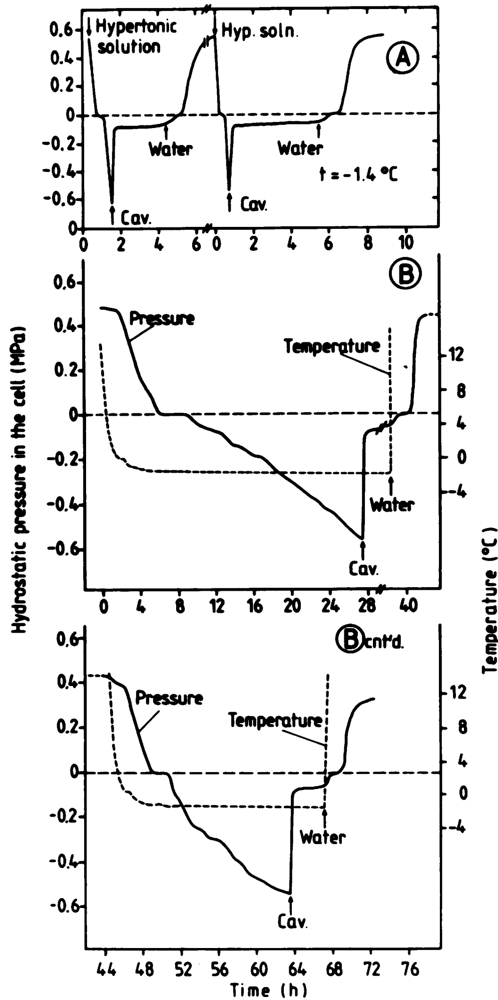

Figure 4. Negative pressure, cavitations, and healing of embolisms at subzero temperatures. A, Negative pressures were created by a hypertonic solution at -1.4°C in the presence of methanol as an antifreeze agent (1210 mOsmol). Cavitation occurred at about -0.6 MPa and the pressure suddenly increased to -0.1 MPa gauge pressure. After cavitation, distilled water was added to the extracellular space, and the cell was warmed to room temperature to restore the positive pressure and to 'heal' the cavitation (embolism) so that the cell could again be subjected to tension. B, Similar results are shown for a freeze-induced dehydration experiment. As in (A), the exposure to positive pressures after a cavitiation reversed embolism so that tensions could be set up again. Note that during the experiments the pressure curves showed a lag at zero gauge pressure (atmospheric pressure).

brane, neither extracellular hypertonic treatment at room temperature (18 ± 1°C; Fig. 6A) nor extracellular freezing (Fig. 6B) resulted in negative pressures. In all experiments, it was found under these conditions that P > -0.015 MPa. This means that in the artificial cell the support of the membrane by the inner grid was essential for building up tensions.

DISCUSSION

Negative pressures of down to -0.7 MPa (-7 bars) could be created by extracellular freezing in an artificial osmotic cell. Artifacts in measuring the tensions could be excluded since

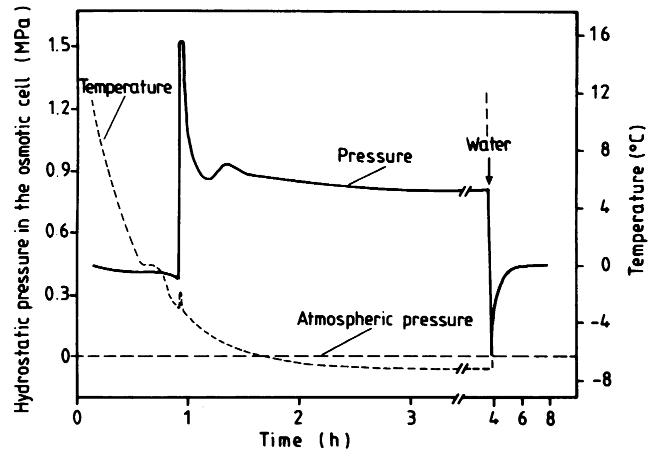

Figure 5. Pressure changes in an artificial cell caused by intracellular freezing. As the cell solution became frozen, the pressure in the cell increased instantaneously because of the larger volume of ice compared to that of water. The subsequent decrease in pressure might have been caused by different processes such as the bending of the grids at higher pressures, an exosmotic water flow due to increased pressure, or the evaporation-dehydration of the cells at lower temperatures. The transients were reversible upon rewarming the cell. In the temperature/time course, there was a retardation at 0°C as ice was formed on the surface followed by a peak caused by the heat of fusion as the cell sap was freezing. Note that in this experiment the cell was frozen to a lower temperature and at a higher cooling rate than in the other experiments.

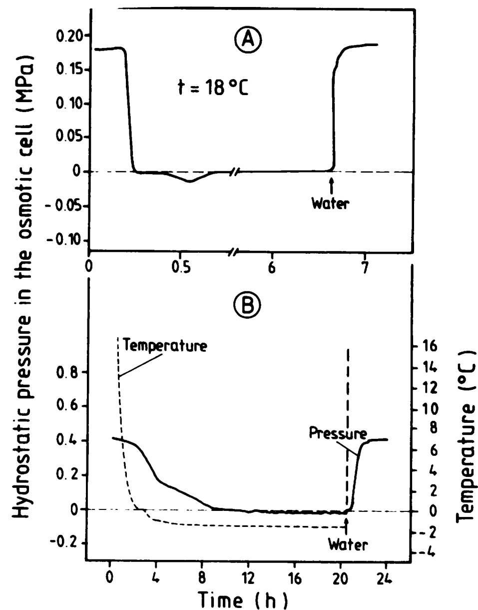

Figure 6. Pressure responses in a cell in which the inner grid (Fig. 1) was omitted. A, A pressure decline was created by an external hypertonic solution at room temperature (t = 18 ± 1°C) and in (B) the cell was dehydrated by extracellular freezing at t = -1.4°C. In both experiments, only very small negative pressures of up to -0.015 MPa (relative to atmospheric pressure) could be obtained. Thus, tensions could only be produced if the membrane was tightly connected with the mechanical support (grid), and the cell could not plasmolyze or collapse.

the freezing of the cell solution as well as cavitations could be followed by direct turgor measurement. In the literature, considerable tensions of up to 300 MPa (3000 bar) have been postulated during freeze-dehydration of plant cells (1, 6, 9, 17). Such tensions would require a considerable rigidity of the cell wall, a lack of separation between cell wall and plasma membrane (16), and a sufficient tensile strength of water at subzero temperatures. Furthermore, seeding by gas bubbles (air, water vapor) would be a problem. At least for the range of low tensions, the results obtained with the artificial system suggest that negative pressures may occur during freezing in cells, although there are differences when comparing the artificial system with a plant cell.

Virtually nothing is known about the rigidity or compressibility of cells in the range of negative turgor pressures which is critical for establishing tensions. Physically, the rigidity of cell walls in the negative range should be different from the elasticity (elastic modulus, ϵ) measured in the positive range, because the rigidity would represent the stiffness of the wall material against bending and cytorrhysis while the elastic modulus is a measure of the elastic stretching of the walls (20). Plant cell walls usually function to withstand extension rather than bending. For the epidermal cells of onion bulbs, Oertli (14) found a very low stiffness and a high tendency to collapse under low tension. However, the same author (15) and others (8, 10, 12) propose a high rigidity of cells of sclerophyllous tissues, a factor believed to be important for the drought resistance of plants.

Another serious problem for the maintenance of negative pressures in plant cells is the fact that plasmolysis should occur as soon as the turgor reaches atmospheric pressure during the evaporation process. In the artificial cell, negative pressures could only be obtained when the membrane was prevented from inward bending. Thus, if the results from plant cells are comparable to those measured with the artificial system, it must be assumed that the plasmalemma is fixed to the cell wall, *e.g.* by the existence of plasmodesmata. However, considering the large tensional forces which develop during freezing-evaporation, this mechanism seems highly unlikely.

From scanning-electron microscopy studies on frost-stressed cereal leaves, Pearce (16) proposed that the plasmalemma would be fixed to the cell wall without chemical bonding thus preventing plasmolysis. When the pores in the cell wall are filled with water, the pore size would reduce the water vapor pressure and prevent a penetration of the wall by gas. Another possibility (also based on capillary forces) would be that in plant tissues the evaporation of water vapor from the wall surfaces causes a tension within the wall pores which is larger than that within the protoplast, thereby maintaining a larger pressure in the protoplast than in the wall pores which would cause the plasmalemma to adhere to the wall. The latter mechanism was proposed by Oertli (15). For the range of positive temperatures, he claimed that in the protoplasts of sclerophyllous tissue this mechanism could create tensions of up to -1.5 MPa. The artificial cell could be used to test this hypothesis, if a material with pores of a diameter similar to those of the intermicrofibrillar spaces (about 10 nm) were used to support the membrane from the outside. These experiments have not yet been performed.

Because of cavitation, the maximum tensions established in the artificial cell were less than those postulated in the literature (see above). Also, the temperatures of the ice formed extracellularly were usually not lower than -1 to -2°C which is equivalent to a water potential of -1.2 to -2.4 MPa driving the dehydration process. Cavitation and gas seeding problems in the artificial system should have been caused mainly by microfissures in the surfaces and/or by the hydrophobic surface of the silicone rubber material used for sealing rather than by the air dissolved in the cell solution (21). In fact, a limiting tension of -0.7 MPa would be equivalent to a size of a spherical air seed of only 0.43 μm in diameter at 0°C. Careful cleaning and boiling of the parts of the equipment, as well as ultrasonic and vacuum treatment, did not substantially reduce the tendency for cavitation. Thus, the removal of seeds remains a serious problem, which must be solved before the technique can be used to create tensions of the order of some MPa, as they occur in the xylem and perhaps also in living cells.

Dehydration of the cell by hypertonic solutions in the range of positive and negative temperatures, as well as during freezing, induced similar responses in the cell. It is remarkable that after cavitation, a gauge pressure of about -0.1 MPa (usually -0.08 MPa) was obtained as theoretically expected, because the water vapor pressure at -1°C would be only some 10^{-4} MPa of absolute pressure. In all cases, cavitations could be healed by pressurizing the cell for some time. During this procedure, gas bubbles (air and/or water vapor) disappeared by dissolving and the cell could be set under tension again. This mechanism may also occur in the xylem during the refilling of cavitated vessels by root pressure as has been already proposed (21).

For the establishment of large negative pressures in frozen tissue (as postulated in the literature), the absolute value of the tensile strength of water is critical. It is usually assumed that the cohesive forces of water are sufficient to sustain large tensions in the xylem and also in plant cells. It has also been assumed that the tensile strength would increase with decreasing temperature (2, 23). However, Briggs (5) found experimentally that the tensile strength had a maximum of about 28 MPa around 10°C and fell dramatically to less than 10% of this value between 5 and 0°C, which may represent another anomaly in the behavior of water in this temperature range. From different theoretical approaches, the absolute value of the tensile strength at normal temperatures has been calculated to range over two orders of magnitude between some 10 and 2000 MPa (2, 7, 13, 23, 25). Experimental values from both artificial and plant systems are substantially lower (1–30 MPa; 3, 5, 24, 26). The discrepancy between experimental and theoretical values of the tensile strength may be explained by the fact that most of the measured values reported for the critical tension are a measure of the 'weakest link' in the system, including interactions between the liquid (water) and the container. They should not necessarily represent the tensile strength of the liquid by itself (25).

This evidence and the temperature dependence of the tensile strength show that the fraction of supercooled water could limit the tensions at temperatures lower than those used in this paper. This would be of interest for plants growing in the

field which are often cooled below 0°C, while the water in the xylem vessels is under tension. To the best of our knowledge, no experimental data for the tensile strength of water at subzero temperatures are available for technical reasons.

As long as direct measurements of negative turgor are not possible *in vivo*, the artificial osmotic cell is useful for simulating the water relations of plant cells, including the conducting elements in the xylem during freezing and other water stresses. Mechanical properties of the cell may be studied at the same time under conditions of both positive and negative pressures. It should also be possible to simulate the properties of the plasmalemma/cell wall complex during evaporation-dehydration. Provided that cavitation can be precluded, data on the temperature dependence of the tensile strength of water may be obtained. These parameters are critically needed to investigate the role of negative turgor pressure and the mechanisms by which supercooled plant cells avoid freezing stress.

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LITERATURE CITED

1. **Anderson JA, Gusta IV, Buchanan DW, Burke MJ** (1983) Freezing of water in *Citrus* leaves. J Am Soc Hortic Sci **108:** 397–400
2. **Benson SW, Gerjouy E** (1949) The tensile strength of liquids. I. Thermodynamic considerations. J Chem Phys **17:** 914–918
3. **Berthelot M** (1850) Sur quelques phenomenes de dilatation forcee des liqiudes. Ann Chim Phys 3e Ser **30:** 232–237
4. **Böhm J** (1893) Capillaritat und Saftsteigen. Ber Deutsch Bot Ges **11:** 203–212
5. **Briggs LJ** (1950) Limiting negative pressure of water. J Appl Phys **21:** 721–722
6. **Burke MF, Rajashekar C, George MF** (1983) Freezing of plant tissues and evidence for large negative pressure potentials (abstract No. 245). Plant Physiol **72:** S-44
7. **Glasstone S** (1966) Textbook of Physical Chemistry. Macmillan, London
8. **Grieve BJ** (1961) Negative turgor pressures in sclerophyll plants. Austr J Sci **23:** 375–377
9. **Hansen J, Beck E** (1988) Evidence for ideal and non-ideal equilibrium freezing of leaf water in frosthardy ivy *(Hedera helix)* and winter barley *(Hordeum vulgare)*. Bot Acta **101:** 76–82
10. **Hellmuth EO** (1969) Ecophysiological studies on plants in arid and semiarid regions in Western Australia. II. Field physiology of *Acacia craspedocarpa* F. Muell. J Ecol **57:** 613–634
11. **Hüsken D, Steudle E, Zimmermann U** (1978) Pressure probe technique for measuring water relations of cells in higher plants. Plant Physiol **61:** 158–163
12. **Kreeb K** (1963) Untersuchungen zum Wasserhaushalt der Pflanzen unter extrem ariden Bedingungen. Planta **59:** 442–458
13. **Nobel PS** (1974) Introduction to biophysical plant physiology. WH Freeman, San Francisco, pp 52–53
14. **Oertli JJ** (1986) The effect of cell size on cell collapse under negative turgor pressure. J Plant Physiol **124:** 365–370
15. **Oertli JJ** (1987) Measurement of the resistance of cell walls to collapse during moisture stress. *In* RJ Hanks, RW Brown, eds, Proceedings of the International Conference on Measurement of Soil and Plant Water Status, Vol 2, Plants. Utah State University, Logan, UT, pp 193–198
16. **Pearce RS** (1988) Extracellular ice and cell shape in frost-stressed cereal leaves: a low-temperature scanning-electron-microscopy study. Planta **175:** 313–324
17. **Rajashekar CB, Burke MJ** (1982) Liquid water during slow freezing based on cell water relations and limited experimental testing. *In* PH Li, A Sakai, eds, Plant Cold Hardiness and Freezing Stress, Vol 2. Academic Press, New York, pp 211–220
18. **Steudle E** (1987) Pressure probe techniques: some recent developments. *In* RJ Hanks, RW Brown, eds, Proceedings of the International Conference on Measurement of Soil and Plant Water Status, Vol 2, Plants. Utah State University, Logan, UT, pp 183–191
19. **Steudle E** (1989) Water flow in plants and its coupling to other processes: an overview. Methods Enzymol **174:** 183–225
20. **Steudle E, Ferrier JM, Dainty J** (1982) Measurements of the volumetric and transverse elastic extensibilities of *Chara corallina* internodes by combining the external force and pressure probe techniques. Can J Bot **60:** 1503–1511
21. **Steudle E, Heydt H** (1988) An artificial osmotic cell: a model system for simulating osmotic processes and for studying phenomena of negative pressure in plants. Plant Cell Environ **11:** 629–637
22. **Steudle E, Stumpf B** (1988) Verfahren und Einrichtung zur Bestimmumg des Gehaltes von in einem Lösungsmittel gelösten Stoffen mittels eines Osmometers. German patent application No P 38 25 208. Date: July 25, 1988
23. **Temperley HNV** (1947) The behaviour of water under hydrostatic tension: III. Proc Phys Soc **59:** 199–208
24. **Temperley HNV, Chambers LLG** (1946) The behaviour of water under hydrostatic tension: I. Proc Phys Soc **58:** 420–436
25. **Trevena DH** (1975) The Liquid State. Wykeham Publications Ltd, London and Winchester
26. **Ursprung A** (1915) Über die Kohäsion des Wassers im Farnannulus. Ber Deutsch Bot Ges **33:** 153–162