Sap Concentrations in Halophytes and Some Other Plants

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Summary. Freezing point depression in xylem sap of mangroves was found to range from 0.05 to 0.50°. In desert plants from 0.01 to 0.16°. In crush juices from leaves of Batis and Salicornia, 90% or more of the freezing point depression was made up of sodium and chlorine ions in mangroves they constituted 50 to 70%, the rest probably being organic solutes. Plants growing in seawater have -39 to -60 atmospheres pressure in the xylem sap. As shown earlier, at zero turgor pressure the intracellular freezing point of the parenchyma cells matches closely the negative pressure in the xylem sap. This agrees with the present data, that the fluid which exudes from the xylem by applying gas pressure on the leaves is practically pure water; freezing point is rarely above 0.01 to 0.02°. To perform this ultrafiltration, the plasma membrane is subjected to a hydrostatic pressure gradient which in some cases may exceed 100 atmospheres.

Methods and Materials

(A) Samples of xylem sap were obtained by a field version of the method of Bennett, Anderssen and Milad(2). The end of a piece of stem is stripped of bark and fitted airtight into a suction cup which connects to an automobile tire pump with reversed piston valve (fig 1). When the handle is pulled out and fixed by a clamp, suction is produced, and short pieces are cut off from the stem, allowing the sap to descend stepwise into the cup. After repeated evacuations and cuttings the sample is removed with a syringe, and cleared with Millipore filtration if necessary. Sap obtained in this way is slightly contaminated by severed cells, for bark, phloem and undoubtedly living cells in the xylem have high salinity, like leaves.

In most species the sap was colorless and the chloride could be titrated on the spot(5). However, in Laguncularia, Rhizophora and some others, the sap usually turned brown and necessitated acid digestion and Volhard titration(4). Sodium, potassium and magnesium were determined by flame photometry. Freezing points were determined by modification of the micro method by Pounder and Masson(9). Provided the 10 to 20 mm³ sample in the freezing tube is stirred and both incipient melting and incipient freezing are observed, the determinations are true to within 0.01 to 0.02°, when concentration is low.

(B) Crush juices from leaves were obtained by removing the rachis and stuffing pieces into a Tygon tubing which was closed at both ends and frozen on carbon dioxide ice. When thawed, the tubing was
put into the jaws of a powerful vise and crushed while the sap was removed with a syringe and cleared through a Millipore filter. Strips of bark and phloem were similarly treated.

(C) Xylem sap extruded from the leaves was obtained by means of the pressure bomb (fig 2). The twig was placed in the bomb with the cut stem protruding. Nitrogen pressure was applied, and the clear fluid was collected in a small vial (6). Repeated flushings rapidly made the bomb completely anaerobic.

**Fig. 2.** Pressure bomb for determination of the negative pressure of xylem sap. Lid, screw cap and chamber are made of aluminum alloy. Nitrogen pressure is supplied by a high pressure reduction valve provided with a tap for release of pressure. A light blow on a screw head releases the cap if sticking. Pressure seal of the xylem stem is maintained by an adjustable rubber compression gland. Useful dimension of chamber (ID) has been 4.5 × 15 cm.

**Xylem Sap in Mangroves and Other Plants.** The relation between sodium chloride and freezing points in stem sap from various halophytes, obtained by the vacuum technique, is given in figure 3. The sap concentration in Avicennia is much higher than in any of the other species, ranging from 4 to 8 mg per ml. Rhizophora, Laguncularia, Conocarpus and several common beach plants hold only 1.2 to 1.5 mg NaCl per ml. Salicornia and Batis have so far never yielded sap by vacuum cutting, but the closely related Alnus cordifolia yielded samples with only 0.08 to 1.0 mg...
per ml. This plant has been deemed the most extreme halophyte of the desert (3). The osmotic concentration of sap from a series of plants from various habitats will be seen in table I. The freezing point depression is mostly below 0.1 to 0.2°.

Table I. Freezing Point in Xylem Sap

<table>
<thead>
<tr>
<th>Species</th>
<th>Stem cutting</th>
<th>Leaf exudate</th>
<th>Extrusion pressure atm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halophytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Avicennia nitida</em></td>
<td>0.3–0.5</td>
<td>0.03–0.04</td>
<td>75–86</td>
</tr>
<tr>
<td><em>Rhizophora Mangle</em></td>
<td>0.05–0.12</td>
<td>0.01–0.04</td>
<td>54–67</td>
</tr>
<tr>
<td><em>Laguncularia racemosa</em></td>
<td>0.12–0.15</td>
<td>0.00–0.01</td>
<td>54–75</td>
</tr>
<tr>
<td><em>Salicornia pacifica</em></td>
<td>0.01–0.03</td>
<td>58–105</td>
<td></td>
</tr>
<tr>
<td><em>Allenrolfea occidentalis</em></td>
<td>0.06–0.10*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Batis maritima</em></td>
<td>0.00–0.05</td>
<td>61–102</td>
<td></td>
</tr>
</tbody>
</table>

Desert Plants

*Juniperus californica* | 0.01–0.03 | 68–107 |
*Juniperus miltioides*  | 0.02      | 82     |
*Laurea divaricata*     | 0.05      | 61–85  |
*Tamarix aphylla*        | 0.15–0.16 | 34–48  |
*Atriplex polycarpa*     | 0.12      | 68–82  |
*Dalca spinosa*          | 0.01      | 27     |
*Prosopis juliflora*     | 0.15      | 48     |
*Prosopis miltioides*    | 0.01–0.02 | 48–68  |
*Chilopsis linearis*     | 0.01      | 34     |
*Acacia Greggii*         | 0.12      | 44     |
*Fouquieria splendens*   | 0.02      | 20–48  |
*Idria columnaris*       | 0.01–0.03 | 8–34   |
*Bursera Hindsiana*      | 0.01      | 30     |
*Bursera miltioides*     | 0.01–0.02 | 24     |

Forest

*Sequoia sempervirens*    | 0.00–0.03 | 18–39   |
*Pseudotsuga Menziesii*  | 0.00–0.03 | 22–50   |
*Tsuga canadensis*       | 0.00–0.02 | 17–72   |
*Vaccinium atrocinun*    | 0.01–0.02 | 20–65   |

* Estimated from chloride determinations.

Unpublished data from our Australian material (8) did not reveal any diurnal variation in the salt concentration of the xylem sap (fig 4), and we were likewise unable to detect any gradient from base to top of the plants.

*Osmotic Concentration in Leaf Cells.* In figure 5 is given the total osmotic concentration derived from freezing point depressions, in relation to the sum of ionic components, found in the juices of frozen and crushed leaves. In the 3 mangroves, only about 50 to 70% of the freezing point depression is produced

![Figure 3](https://academic.oup.com/plphys/article-abstract/41/3/529/6090745/6090745-fig3)

**Fig. 3.** Sodium chloride (based on Cl) and freezing point in samples of xylem sap from various halophytes (*Avicennia nitida, Rhizophora Mangle, Laguncularia racemosa, Conocarpus erecta, Suriarnia maritima, Canavalia lineata, Scaevola Plummeri, and Ipomea pes-caprae*).

![Figure 4](https://academic.oup.com/plphys/article-abstract/41/3/529/6090745/6090745-fig4)

**Fig. 4.** Sodium chloride concentration (based on Cl) in xylem sap from Australian mangroves at various times of day: *Avicennia marina, Aegiceras corniculatum, Rhizophora mucronata, Sonneratia alba.*

![Figure 5](https://academic.oup.com/plphys/article-abstract/41/3/529/6090745/6090745-fig5)

**Fig. 5.** Solute concentration of crush juices from leaf parenchyma cells. In the diagram the ion columns are stacked one on top of the other to give the total molar concentration. The same quantity determined by the freezing point depression is given as a broken line. In *Salicornia* and *Rhizophora* it was necessary to pool samples from several plants.
by sodium and chloride ions; most of the remaining solutes presumably are organic. However, in *Batis* sodium chloride alone made up to 90% or more of the total osmotic concentration. This was confirmed by dry weight determinations which again left very little, if any, room for other constituents.

Composition of the Sap Expressed from the Leaves by External Gas Pressure. When a twig is put into the bomb and nitrogen is admitted beyond the balancing pressure, the cells yield fluid by osmosis. With few exceptions, this sap is almost pure water with a freezing point depression of only 1 or 2 hundredths of 1 degree. It is remarkable that the plasma membrane in some species remains functional while being subjected to a hydrostatic gradient of 100 atmospheres or more (table 1). When enough liquid has been yielded to drop the turgor pressure to zero, the balancing pressure of the xylem sap matches closely the osmotic potential of the intracellular sap, such as determined by the freezing point depression duly corrected for free space (6). This simple osmotic relation holds true for all ordinary plants tested, as well as for extreme halophytes and desert plants.

Discussion

It was shown in an earlier paper (6) that there is always enough tension in the xylem sap of mangroves to effect a separation of water from the seawater by simple osmosis. This negative sap pressure is balanced by a similar or even greater reduction in water potential of the parenchyma cells due to solutes. The sodium chloride concentration in the leaf cells may thus be 100-fold or more greater in the cells than in the xylem sap. We are back to the classical question: What maintains such concentration gradients? One possibility is active transport, presumably by a sodium pump.

Another possibility would be if the ions, while free to enter the cells by simple diffusion, were somehow prevented from getting out, for instance, by becoming enlarged by hydration or other means. We know from the close match in *Batis* and *Avicennia* between freezing point depression and NaCl concentration that each ion must be separate, and also that there is not enough organic material present to combine separately with each of them. Comparing drops of filtered cell sap from *Batis* and *Avicennia* with controls of equal strength of NaCl, it was found that both had the same electrical conductivity and that corresponding ions moved at identical rates when tested by chromatography as well as by electrophoresis. Cellulose acetate desalination membranes partially permeable to NaCl did not significantly differentiate between the solutions. It seemed, therefore, that the mobility of the ions in vitro was the same. Although suggestive, this does not necessarily preclude that conditions in the living cell could be different. Whatever is the concentrating mechanism, the holding mechanism is certainly operative in an all-nitrogen atmosphere, and in the case of *Avicennia* it was not altered after the leaves had been immersed in a saturated solution of dinitrophenol for 30 minutes, although in this case we do not know the penetration.

No clear diurnal variation was found in the concentration of xylem sap in mangroves (fig 4), but the rate of salt secretion by the leaves of *Avicennia* varied 10-fold or more, and even greater variations were observed in *Aegialitis* (7). It would seem, therefore, that the desalination process in the root system produces a sap of rather constant concentration which is largely independent of the transpiration rate, and that it is up to the salt glands to eliminate the considerable quantities of salts left behind by the transpiration.

Acknowledgments

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Literature Cited