Reprinted from AGRONOMY JOURNAL
Vol. 60, March-April 1968, p. 167-169

Physical State of Water in Plant Xylem Vessels[1]

John W. Cary, Marvin E. Jensen, and Herbert D. Fisher[2]

ABSTRACT

The vapor pressure psychrometer was used as a tool to study the physical state of water in plant xylem vessels. The experimental procedure involved measuring the change in diffusion pressure deficit (DPD) of corn and tomato plants when the stem was cut. When the DPD was greater than 4 bars in tomatoes and 28 bars in corn, the water in xylem vessels no longer appeared to flow in response to hydrostatic pressure gradients. The limiting value of DPD increased as the xylem radius decreased. A mechanism is suggested which describes the physical state and the movement of water through xylem tissue under high DPD. The proposal is based on the pressure difference across a curved air-water interface and on the concept of an electrostatic double layer with its associated osmotic pressure.

Additional index words: **transpiration, DPD, xylem sap.**

WATER in living plants is generally at a lower chemical potential than pure water. This relation (10) may be quantitatively expressed as:

$$\Delta\mu = R\ T\ \ln p/p_o = V\ \Delta P \qquad [1]$$

where $\Delta\mu$ is the difference in chemical potential between pure water and water in the plant; R, the universal gas constant; T, the absolute temperature; p, the equilibrium water vapor pressure associated with the plant; p_o, the saturated vapor pressure of pure water; V, the partial molar volume of water; and ΔP, an effective hydrostatic pressure difference referred to pure water at 1 atmosphere pressure. The chemical potential of water in a plant is normally measured by finding the equilibrium vapor pressure associated with the plant. The result is usually expressed as ΔP in bars of negative pressure, or as DPD, the diffusion pressure deficit.

The large effective negative pressure which can develop in plant cells is known to arise in part from dissolved material; i.e., osmotic pressure. However, since the xylem system consists of very small vessels relatively free from dissolved materials, it has been suggested that the water contained in the vessels may be under a negative hydrostatic pressure (tension) of the same magnitude as the DPD of the cells.

Scholander, et al. (9), conducted extensive studies of the xylem fluid system and concluded that large negative hydrostatic pressures do exist in the vessels. However, their interpretation of experimental results was challenged by Gardner and Rawlins (5).

The question arises: Why doesn't water in the xylem vessels vaporize when the absolute pressure is reduced to the vapor pressure of water? Such vaporization occurs in the laboratory when the water pressure in small tubes is reduced to p_o (i.e., about −1 bar) unless extraordinary precautions are taken to keep the system free from materials which could initiate nucleation of a vapor bubble. Some investigators have suggested that the large negative pressures of water in the xylem are only apparent and are due to physical adsorption to the sides of the vessels. This occurs when a thin water film surrounds finely divided colloids, resulting in a reduced equilibrium vapor pressure. However, if the water is physically adsorbed in the vessels, it is difficult to account for the rapid rate of water transfer up the stem during periods of high transpiration, Zimmerman (12).

An alternate way to study vessel fluid pressure is to determine the water flow in the xylem elements when they are suddenly vented to the atmosphere. Suppose that a plant's water status is in a steady-state condition, such that it may be described by the relation

$$\Delta P = -OP + TP = -DPD \qquad [2]$$

where OP is the osmotic pressure in the cell; TP, the turgor pressure of the cell; and ΔP, the effective pressure of water in the xylem vessels. If the xylem is cut and vented to the atmosphere, ΔP may change according to the air entry value at the cut end. If the DPD were greater than the air entry value of the stems, flow would occur from the xylem vessels into the plant cells. If ΔP is only the "effective" negative pressure and represents primarily the physical adsorption of water to the walls of the xylem vessels, venting the system to the atmosphere will not materially change ΔP and little or no flow will occur. If the flow does occur from the xylem tissue into the plant cells, the osmotic pressure in the cell will decrease and the turgor pressure will rise. The result will be an increase in the equilibrium vapor pressure of the plant. The vapor pressure may be measured with a thermocouple psychrometer as discussed by Rawlins (8).

EXPERIMENTAL PROCEDURE

Tomato seedlings were grown in a nutrient solution and corn seedlings in distilled water. When the plants reached a height of 6 to 15 cm, they were gently folded and placed into a psychrometer cup 4 cm in diameter and 5 cm deep. In cases where a high DPD was desired, the corn roots were presoaked in aerated 0.4 to 0.8 molar KCl solutions for 3 hours prior to placing them in the psychrometer cup.[3] The psychrometer was

[1] Contribution from the Northwest Branch, Soil and Water Conservation Research Division, Agricultural Research Service, USDA; Idaho Agr. Exp. Sta. cooperating. Received May 27, 1967.

[2] Research Soil Scientist, Research Investigations Leader, and Mathematician, respectively, Snake River Conservation Research Center, Kimberly, Idaho 83341.

[3] Drying the seedlings for a few minutes in air to create the desired stress was satisfactory for the tomatoes, but failed to affect the corn until the root system became severely dessicated, thus the KCl solution was used instead.

placed in the constant temperature bath and time for reaching equilibrium was allowed. This required 3 or 4 hours for the tomatoes, and 12 to 16 hours for the corn. After reaching steady state, the DPD was measured, and a piece of razor blade was inserted through a port in the cup to sever the stem. Within 30 minutes the psychrometer system recovered from the perturbation caused by the insertion of the blade. The change in DPD caused by severing the stem was then measured over a 2-hour period.

RESULTS AND DISCUSSION

Results typical of the 143 seedlings tested are shown in Table 1. Since each sample had a slightly different geometry and since this geometry could not be exactly copied with the calibrating solution on filter paper strips, an uncertainty of $\pm 1/2$ bar exists in the absolute value of these measurements. Uncertainties associated with the change in DPD of each single sample were much less because the geometry was not disturbed by the cutting operation. Measurements of DPD were made on each seedling for several hours prior to cutting. From these data the standard error of the mean was found to be 0.04 bar or less for all observations reported in Table 1. Consequently, all differences between measurements of DPD before and after cutting are real. Of all the 143 seedlings used, none decreased after cutting by more than their standard error so long as the initial DPD was less than 0.8 bar or greater than 4 bars in the case of tomatoes, or when the DPD was less than 18 bars or greater than 28 bars in the case of corn.

Both the corn and tomato plants showed a definite region of DPD values in which a decrease in DPD followed severing of the stem. The lower limit of this range of DPD values could have been due to biological plugging of the vessels by some substance such as callose (3), but more likely it was a result of the air entry value of the xylem vessels combined with osmotic effects.

The relation between the air entry pressure, P_c, and the vessel's radius, r, is given by the capillary equation when $r_1 = r_2$, and the contact angle is 0

$$P_c = -\sigma \left(\frac{1}{r_1} + \frac{1}{r_2} \right) \qquad [3]$$

where σ is the surface tension of water, and r_1 and r_2 represent the radius of the curvature. In the case of the tomato seedlings, the lower limit of pressure response was 0.9 bar, which gives a vessel radius of about 1.7 microns. This is an order of magnitude less than values reported by Dimond (4). The apparent discrepancy may be attributed to the osmotic pressure of the xylem fluid which develops in part from electrostatic double-layer phenomena. Assuming that the xylem vessel's wall has a charge density of 1 electron per $50A^2$, the concentration of protons and cations associated with these charges will be in the neighborhood of $1/2$ milliequivalent per liter of vessel volume. These cations and protons will be dissolved in the xylem fluid. Such a system could easily create several tenths of a bar of osmotic pressure within 100 A or so of the cell wall (1, 7). In addition, the water in the xylem vessels has a small amount of dissolved solutes which creates an additional osmotic pressure, although this would generally be less than 1 bar. The solutes in the xylem fluid are displaced toward the center of the pore by the cations which dissociate from the wall, thus increasing the concentration per unit volume and consequently the osmotic pressure of the fluid in the pore.

Examination of the tomato vessels with a calibrated microscope indicated their radius to be about 10 to 20 microns, which gives an air entry value of about 0.1 bar. Since the observed air entry was 0.9 bar, the effective osmotic pressure in the xylem vessel would have been about 0.8 bar.

The failure of the DPD to respond to cutting at stresses above 4 bars may be due to the effects of the vessel's radius and the solution's osmotic pressure on the stability of a two-phase liquid-vapor system. For example, when the tomato seedling was under a zero moisture stress (DPD = 0), the osmotic pressure of the solution in the xylem was 0.8 bar. This osmotic pressure must be balanced by a real positive hydrostatic turgor pressure contained by the walls of the vessel (6), similar to that which occurs in a turgid plant cell. With an increase in DPD due to increased evaporation, higher soil moisture stress, or both, the positive hydrostatic pressure exerted by the walls of the xylem vessels would decrease and eventually go to zero as the DPD reached the osmotic pressure of the fluid in the xylem. With a greater increase in DPD, the water in the xylem would undergo increasing negative hydrostatic pressure until the absolute pressure reached the vapor pressure of water, i.e., approximately —1 bar. At this point one might expect nucleation to occur and a water vapor phase to develop in the center of the vessel. However, when the gas phase does appear, the smallest pressure difference across the interface, as determined by equation [3], which could exist in the tomato xylem would be about 0.05 bar if r_1 were to be a maximum of 15 microns and if constraints were such that $r_2 >> r_1$. It is likely r_2 would be similar to r_1 and the lower limit for a stable two-phase system would require a pressure drop of about 0.1 bar. Since the pressure in vapor phase is fixed at a value near that of the vapor pressure of water, it follows that pressures in the liquid phase must approach a value of P_c.

In order for the vapor phase to develop, liquid must be displaced from the vessels. This most likely occurs by water flow across semipermeable membranes into surrounding cells, resulting in an increased concentration of solutes in the xylem and a compression of the electrostatic double layer. The result should be a significant rise in the osmotic pressure of the fluid and a bubble radius less than the radius of the vessel.

Table 1. Typical observations of the change in DPD caused by severing the stems of small corn and tomato plants. Standard errors are 0.04 bar or less and discussed in detail in the text.

DPD before cutting		DPD after cutting	DPD before cutting		DPD after cutting
Bars	Tomato	Bars	Bars	Corn	Bars
0.3		0.3	0.2		0.2
0.8		0.8	4.0		4.0
1.0		0.5	18.0		18.0
1.2		0.5	22.0		19.5
1.5		1.0	22.5		22.5
2.4		2.0	25.3		23.0
3.0		2.0	26.5		24.0
4.0		4.0	28.0		28.0
5.5		5.5*	33.5		33.5
			39.5		40.0†

* Tomato seedlings stressed above 5 or 7 bars of DPD did not come to equilibrium in the psychrometer, evidently because of restricted water transfer between the leaves and roots. † Of the many seedlings tested, several showed a small rise in DPD upon cutting. This did not seem to be confined to any particular range of DPD values.

The data in Table 1 would indicate that the vapor phase did not develop until the DPD reached about 4 bars in the tomato seedlings. This suggests that until this point was reached the total stress in excess of 0.8 bar was dependent on negative hydrostatic pressure in the xylem fluid and at these negative pressures the system was stable because of the interrelation between the osmotic pressure of the liquid and the hydrostatic pressure drop across a curved air-water interface.

The apparent air entry value for the corn seedling stem was about 20 bars, indicating a vessel diameter of approximately 0.15 micron. Microscopic examination of the xylem elements indicated that they were only about ¼ the size of the tomato vessels with diameters of 5 to 10 microns. The air entry value would be about 0.2 bar. One possible source of this difference is the pretreatment of corn seedlings with KCl solutions to achieve stresses above 12 bars. If the solution "leaked" into the xylem elements, the higher DPD measurements for corn in Table 1 could have been shifted upward due to higher than normal osmotic pressure in the vessel fluid. Three other factors which should be considered are: (a) the surface tension of the xylem fluid was taken as that of pure water, (b) the contact angle was taken as zero, and (c) the radius of the corn xylem vessels under negative pressure stress may have been significantly less than the radius observed in the dissected tissue under the microscope. In any case, the same general events described for the tomato seedlings likely occur in corn seedlings. However, because of the possibility of a higher concentration of xylem solutes coupled with smaller conducting vessels, and consequently a greater charge per unit volume, a stable liquid vapor system was not reached until a DPD of about 28 bars, which halted the "rapid" viscous flow of water through the stem.

If this theory describes the state of water in the xylem, then other implications are involved. For example, when the water vapor phase comes into existence, the flow of water through the vessel may decrease sharply because of the increased viscosity (7) and reduced cross-sectional area of the liquid phase. This event may be followed by wilting. If desiccation continues, the moisture stress could eventually reach the air entry value of the vessel walls, allowing a flow of atmospheric gas into the vapor phase contained in the vessel (see the discussion by Teoh et al. (11). At this point, the gas phase may not readily disappear upon relaxation of the environmental moisture stress. However, as DPD in the plant proceeds from high stress values toward zero, the gas pressure in bubbles in the xylem will approach P_e. The increasing gas pressure accelerates the solution of gas into the xylem fluid. This is a key factor in the reestablishment of fluid flow in woody plants after thawing, a phenomenon recently reported on by Hammel (6).

CONCLUSIONS

If the preceding explanation is correct, even in part, it implies that the transpiration stream from the roots to the leaves could be maintained under increasingly high stress conditions by reducing the radius and increasing the number of xylem vessels, by increasing the charge density in the xylem vessels, and by introducing monovalent cations into the xylem to develop maximum diffuse double-layer effects. Consequently, xylem elements may deserve much more than the usual passing acknowledgment in studies of plant-water relations. While the data presented here are by no means complete proof of the proposed system, and while the calculations are of an "order of magnitude" nature due to simplifying assumptions, the mechanism does present a qualitative explanation of many plant-water relations and is based on known physical phenomena.

In any event, the data do suggest that plants have an upper stress limit above which water will not flow freely through the xylem vessels under a hydrostatic pressure gradient. It also appears that the "effective" radius of the xylem vessels may vary widely between plant species. Furthermore, it may be noted that sampling plants may cause a significant decrease in DPD of the sample. This should be considered when interpreting data such as that recently reported by Barrs (2).

ACKNOWLEDGMENTS

The authors would like to acknowledge the help of Richard Allen in assembling and calibrating the psychrometer used in this work. We are also indebted to Dr. J. H. Smith for help in setting up the solution cultures used in growing the tomatoes.

LITERATURE CITED

1. Anonymous. 1954. Agriculture Handbook No. 60. Diagnosis and improvement of saline and alkali soils. USDA, U. S. Gov. Print. Off.
2. Barrs, H. D. 1966. Root pressure and leaf water potential. Science 152:1266-1268.
3. Currier, H. B., and S. Strugger. 1956. Aniline blue and fluorescence microscopy of callose in bulb scales of *Allum cepa* L. Protoplasma 45:552-559.
4. Dimond, A. E. 1966. Pressure and flow relations in vascular bundles of the tomato plant. Plan Physiol. 41(1):119-131.
5. Gardner, W. R. and S. L. Rawlins. 1965. Sap pressure in plants. Science 149:920.
6. Hammel, H. T. 1967. Freezing of xylem sap without cavitation. Plant Physiol. 42(1):55-66.
7. Kemper, W. D. 1960. Water and ion movement in thin films as influenced by the electrostatic charge and diffuse layer of cations associated with clay mineral surfaces. Soil Sci. Soc. Amer. Proc. 24:10-16.
8. Rawlins, S. L. 1966. Theory for thermocouple psychrometers used to measure water potential in soil and plant samples. Agr. Meteorol. 3:293-310.
9. Scholander, P. F., H. T. Hammel, E. D. Brabstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339-346.
10. Slatyer, R. O. and S. A. Taylor. 1960. Terminology in plant- and soil-water relations. Nature 187(4741):922-924.
11. Teoh, T. S., L. A. G. Aylmore, and J. P. Quirk. 1967. Retention of water by plant cell walls and implications for drought resistance. Australian J. Biol. Sci. 20:41-50.
12. Zimmermann, M. H. 1965. Water movement in stems of tall plants. *In:* The state and movement of water in living organisms. Academic Press, Inc., New York, pp. 151-155.