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Matric Potentials of Leaves

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Summary. A pressure chamber was used to measure matric potentials of frozen and thawed leaves. Significant matric potentials were demonstrated in sunflower (*Helianthus annuus* L.), yew (*Taxus cuspidata* Sieb. and Zucc.), and rhododendron (*Rhododendron roseum* Rehd.). Matric potentials were particularly negative in rhododendron and were correlated with the amount of cell wall present and with the volume of water outside the leaf protoplasts at comparable matric potentials. It was concluded that matric forces in leaves are associated mainly with cell walls, at least within the physiological range of water contents. Calculations indicated that the water potential of the solution in the cell wall could be estimated for living tissue from the sum of matric and osmotic potentials acting on water outside the protoplasts.

The availability of water to soil-grown plants is determined in largest part by the interaction of water with the surfaces of soil particles and by the effects of soil solutes. It has been convenient to group the surface forces, usually adsorptive and capillary forces, in a single term, matric potential (2, 9, 15, 16). Plants, like soil, have large areas of surface which may interact with water, e.g., cell walls and particles or organelles in the protoplasm. Wiebe (17) has shown that matric forces exist in fleshy stems (asparagus) and storage organs (potatoes and mangels) but he concludes that they are small in these organs over the physiological range of water contents. Matric potentials have been postulated for plant leaves (6, 16, 17) but they have not been measured. In this report, I show that plant leaves have significant matric potentials and present evidence that the matric potentials which are observed are associated mainly with the cell walls.

A pressure chamber (12, 13) has been used to estimate leaf water potentials (4) and, in certain instances, the hydrostatic or adsorptive forces affecting water in the xylem of the intact plant (4, 7, 12, 13). Basically, measurements are made by applying pressure to a leafy shoot until sap appears at the cut end of the shoot, which extends outside the chamber and is exposed to atmospheric pressure. The pressure necessary for the appearance of sap represents the amount by which the water potential of the leaf cells must be raised to equal the potential of the xylem sap at atmospheric pressure. This paper reports matric potentials measured by a similar technique but using frozen and thawed plant tissues rather than live material.

Measurements of matric potential with a pressure chamber were based on the equation describing the state of water in plant tissue in terms of the component potentials affecting the total water potential, ψ_w, of the system at equilibrium and constant temperature:

$$\psi_w = \psi_p + \psi_g + \psi_s + \psi_m \qquad (I)$$

where the subscripts p, g, s, and m signify the effects of pressure, gravity, solutes, and matrix, respectively. It is known that plant cells, when frozen and thawed, are disrupted (11) with resultant loss of turgor (5). Water in the system is then affected only by matric and osmotic potentials (gravitational effects are negligible in the excised shoot). If pressure is applied to frozen and thawed tissue in the pressure chamber, the potential of the released cell sap rises until it equals the osmotic potential of the same solution at atmospheric pressure, at which point the sap appears at the cut end of the shoot. The resultant pressure is a function of matric forces alone; the solute concentration of the bulk solution derived from the cells does not affect the measurement, and the pressure and gravitational components of equation I are essentially zero.

Although the distinction between matric and other types of forces is arbitrary, matric potential has been defined as that arising from forces exerted by adsorbed water, adsorbed solutes, and surface tension (2). However, it is difficult to measure matric potential as a function of these parameters. Since they vary as a function of water content which is easily determined, matric

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potential has usually been studied in systems with varying water contents (10, 16) and this is the approach adopted here.

Materials and Methods

Three species having leaves and stems of widely different anatomy were chosen for study: sunflower (*Helianthus annuus* L.), yew (*Taxus cuspidata* Sieb. and Zucc.), and rhododendron (*Rhododendron roseum* Rehd.). Two year old yew and rhododendron were grown in soil in the greenhouse. Sunflower was grown from seed in soil in a controlled environment room (temp, 30–31° day and 27–28° night; relative humidity, 45–55 %; light, 2500 ft-c).

Matric potentials were measured with a pressure chamber (12, 13) in leafy shoots 20 to 30 cm long (rhododendron and yew) and leaves (sunflower) that had been frozen at −20° and slowly thawed. The chamber was slightly modified by bubbling the incoming nitrogen gas through water in the bottom of the chamber to prevent drying of the tissue. A baffle prevented the water from splashing on the tissue.

Matric potentials were determined as a function of the water content of the plant tissue by placing a frozen and thawed plant sample in the pressure chamber with the cut stem protruding through the top of the chamber. Several aliquots of cell sap were expressed by raising the pressure around the plant sample. After each aliquot was removed, the balancing pressure was determined and represented the matric potential at that water content. Following the measurement of matric potentials, the total water in the sample was determined by drying at 100° and adding the water loss during drying to the volume of cell sap in aliquots collected while the sample had been subjected to pressure.

The average cell wall volume of the leaf mesophyll cells was determined after staining fresh leaf cross sections with Schiff reagent by the PAS method (8). The average volume of the protoplast and protoplast plus cell wall was computed from measurements taken from photomicrographs. The volume occupied by the cell wall was the difference between the 2 values.

Pressure applied to living plant material is related to the volume of water in the cells at constant temperature (1) by

$$P\ V = n\ k \qquad \text{(II)}$$

where n is the number of moles of solute within the cells and k is a constant (liter bars mole^{-1}). Scholander, et al. (12, 13) showed that, in the pressure chamber, living shoots approximate this relationship and demonstrated that the data from leafy shoots having zero turgor fitted a linear form of equation (II):

$$\frac{1}{P} = \frac{k}{n}\ (v^\circ - v) \qquad \text{(III)}$$

where v° is the initial volume of water in the cells and v is the volume of water removed from the shoots at successively higher pressures. A plot of $1/P$ versus v, when extrapolated to $1/P$ equal to zero, indicates the total volume of liquid that can be expressed by pressure. It has been suggested (12, 13) that this volume represents the volume of water within the leaf protoplasts.

The following procedure was used to determine the volume of water which occurred outside the leaf protoplasts in living shoots: Measurements of water volume within the protoplasts were made according to equation III by expressing sap from living shoots at successively higher pressures. After an aliquot of sap was removed, the corresponding balancing pressure was determined when the shoot had come to equilibrium (4) at the new pressure. The volume of water occurring outside the protoplasts was then calculated from the difference between the volume removable by pressure (equation III) and the total water volume in the sample (obtained by drying the leaf tissue at 100° without the main veins and adding to the weight loss the volume of sap which had been removed by exposing the shoot to high pressures).

Results and Discussion

Figure 1 shows the matric potentials of sunflower, yew, and rhododendron at different water contents. Before the samples were frozen for determination of matric potential, the cut ends of the stems (rhododendron and yew) or petioles (sunflower) had been placed in water until the water potential of the tissue reached −1 to −3 bars (4). The data indicate that rhododendron had a measurable matric potential, −0.8 bar, even though no sap had been removed from the sample and the water potential of the sample when living was −1 to −3 bars. Matric potentials as low as

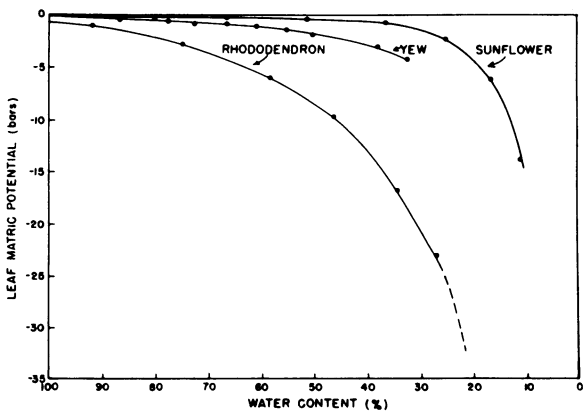

FIG. 1. Matric potentials of frozen and thawed leaves from sunflower, yew, and rhododendron measured at various water contents. The water potentials of the samples were —1 to —3 bars before freezing.

−23 bars were recorded for rhododendron after 73 % of the water in the sample had been removed. Both yew and sunflower had less negative matric potentials than rhododendron. Matric potential in these 2 species could not be detected with the pressure chamber when the frozen samples had a water content corresponding to −1 to −3 bars in the living tissue but matric potential became more negative after a small amount of water had been expressed from the tissue (in a separate experiment, yew and sunflower having water potentials of −10 and −7 bars had −0.6 and −0.3 bar matric potentials, respectively). These data indicate that all 3 plant species have significant matric potentials when the water potential of the living tissue is within the range normally met in nature and that matric forces in rhododendron are significant even in plants that are well watered.

The exudate that was obtained from the once frozen samples in the above experiment was clear but colored light to dark brown. Apparently, soluble compounds were expressed in the sap but cell organelles and denatured proteins were retained in the leaf matrix. Thus, the pressure chamber measured matric potentials arising both from cell walls and from any solid material derived from the protoplast.

Matric potentials are affected by the configuration of the matrix as well as the moisture content of the system. Deformation of plant tissues is known to occur in the pressure chamber (4). The extent to which differences in configuration may have affected measurements of matric potentials is not known. However, determinations of water potentials (4) and osmotic potentials (Boyer, unpublished data) with the pressure chamber at high pressures agree fairly closely with measurements made with a thermocouple psychrometer, where deformation does not occur.

At any one water content (table I), a matric potential series could be written for the 3 species: |rhododendron| > > |yew| > |sunflower|. The low negative values of matric potential in rhododendron imply that, per unit water, the leaves of this species have considerably greater surface area for matric effects than either yew or sunflower. The relationship of surface to matric potential may be indicated by a differential form of equation I:

$$dG = VdP + Mgdh + d\omega_o + d\omega_a + d\alpha + \sigma dO \qquad \text{(IV)}$$

where G is the partial molal Gibbs free energy of water in the system, V is the partial molal volume of water, P is the local pressure (excluding pressures due to surface tension), $Mgdh$ denotes the effects of gravity, ω_o is the osmotic effect of solutes in the bulk solution, ω_a is the effect of adsorbed solutes, α is the energy of water adsorption by the solid phase, σ is the surface tension of water, and O is the area of the air-water interface per mole of water. The pressure, gravitational, and osmotic terms in equation IV are analogous to the same terms in equation I. The last 3 terms in equation IV show the matric effects of the surfaces associated with any 3 phase system, solid-liquid-air, in which water is considered only in the liquid phase. An increase in surface area per unit of water generally will affect all 3 surface terms, cause a decrease in the free energy of water in the system, and result in a lower matric potential. Thus, measurements of surface area in rhododendron, yew, and sunflower should provide information about the differences in matric potential shown in figure 1.

It is difficult to measure the surface area of solid protoplasmic constituents, but the micellar nature of cell walls makes it possible to estimate the relative area of cell wall surfaces from measurements of the quantity of cell wall present, i. e., the volume of cell wall per total water volume for each cell. Correspondence between matric potentials and the volume of cell wall would implicate cell walls as the major source of matric forces. On the other hand, if matric forces arise mainly in the protoplast, there should be little relationship between the volume of cell wall and the matric potential of the tissue.

These 2 alternatives were tested by computing the cell wall volume of the photosynthetic tissues of the 3 species from photomicrographs. Rhododendron had twice the cell wall volume of sunflower and yew when expressed as the percent of cell wall volume relative to the water volume of the protoplast plus cell wall (table I). A cell wall series for the 3 species would be the same as that

Table I. *Matric Potential at 50 % Water Content, Average Cell Wall Volume, and Average Volume of Water Outside Protoplasts in Leaves of Sunflower, Yew, and Rhododendron*

Cell wall volumes were obtained from photomicrographs of leaf photosynthetic tissues.

	Matric potential at 50 % water content	Avg cell wall volume/total water volume in cell	Avg water volume outside protoplasts/total water volume in leaf
Sunflower	—0.6 bar	12 %	9 %
Yew	—2.0	14	11
Rhododendron	—10	28	26

written for matric potential and provides evidence that matric potential arises mainly in the cell wall.

The association of matric potential with cell wall surfaces was tested further by measuring the amount of water outside the leaf protoplasts in the 3 species. Thus, at any given matric potential, more water should be present outside the protoplasts in species having a greater area of cell wall than in those having less cell wall providing matric forces arise largely in the wall.

The method of measuring the volume of water outside the protoplasts in living tissue requires measurements over a range of pressures (equation III) and, in order to base the data on the same potentials for the 3 species, a similar range of pressures was used for all measurements (15–35 bars). The data (table I) indicate that the volume of water outside the protoplasts, when expressed on the basis of the total water in the leaf, is correlated with the quantity of cell wall present. Thus, the same series may be written for the volume of water outside the protoplasts as for the cell wall volume and matric potential for the 3 species.

The data collected according to equation III assume that all the water expressed by the pressure chamber arises from leaf protoplasts. However, additional water is undoubtedly expressed from cell walls when pressures are applied to the tissue, especially at low pressures. Filter paper has been shown to lose water most rapidly at matric potentials above −4 bars (17). Below that potential, water loss was negligible. If the water retentivity curve for cell walls is similar to that for filter paper, water loss from the cell walls would have negligible effect on water volumes measured with the pressure chamber since they were carried out at potentials well below −4 bars.

The complete equation describing the water potential of the cell wall and protoplast in equilibrium with the surroundings may be written:

$$\psi_w^{cell} = \psi_p^{wall} + \psi_g^{wall} + \psi_s^{wall} + \psi_m^{wall} = \psi_p^{proto} + \psi_g^{proto} + \psi_s^{proto} + \psi_m^{proto} \quad (V)$$

where the superscript, proto, refers to the protoplasm. The correspondence in relative terms between cell wall volume, water outside the protoplasts, and matric potential is evidence that matric potential originates largely within the walls of leaf cells rather than the protoplast. Therefore, ψ_m^{proto} probably may be ignored, at least in the physiological range of water contents for highly vacuolated leaf cells. The gravitational terms in wall and protoplast are equal and therefore cancel. Since the pressures in the wall include only those other than pressures caused by surface tension (equation IV), ψ_p^{wall} is zero and equation V reduces to:

$$\psi_w^{cell} = \psi_s^{wall} + \psi_m^{wall} = \psi_p^{proto} + \psi_s^{proto} \quad (VI)$$

Thus, calculations of turgor which have ignored ψ_m^{proto} are probably correct (3, 6, 14). However, those studies which measured leaf osmotic potentials with psychrometers by freezing and thawing the tissue (3, 6) probably measured a combination of the osmotic potential of the protoplast contents and the matric potential of the cell walls. In sunflower and yew, the matric potentials are fairly high but in rhododendron, matric potential would be an important component of the osmotic potential indicated by a psychrometer.

It is possible to test equation VI from the data in this study. Since the water volume outside leaf protoplasts is measured at a range of pressures and the plant material comes to equilibrium at each pressure, the data may be converted to a range of approximate water potentials for the tissue (4). Thus, simultaneous estimates may be obtained for the water potentials of the shoots and the water volume outside the protoplasts. Matric potentials for the cell wall can then be estimated from figure 1 at these water volumes. Since the osmotic component of the solution outside the cells is known for the 3 species (4), the water potential of the solution in the wall may be estimated from equation VI and compared with the water potential of the tissue as a whole in the pressure chamber.

In rhododendron, for example, pressures equivalent to water potentials of −17 to −31 bars gave estimates of the water volume outside the protoplasts that ranged from 25 to 32 % in different samples of tissue. The data in figure 1 show that the matric potential of samples at 25 to 32 % water content would have ranged from −20 to −28 bars. When combined with an osmotic component of −1 to −2.5 bars in the solution outside the cell (4), equation VI indicates a range of water potentials in the wall of −21 to −30 bars. Although each estimate of wall water potentials is for a different shoot which was exposed to a range of pressures, the wall potentials for all of them fall within the range of water potentials for the tissue in the pressure chamber.

The same results were found for sunflower, which had a water volume outside the protoplast ranging from 5 to 14 % in different samples. This represented a range of osmotic plus matric potentials in the walls of approximately −13 to perhaps −25 to −35 bars, which is fairly close to the range of water potentials (−15 to −30 bars) of the tissue. A similar argument was not possible for yew because matric potentials could not be measured at high pressures due to a rapid escape of gas through the tissue.

If appreciable matric potentials arise from water in the protoplasm, it should not be possible to predict the water potential of cell walls by estimating matric potentials solely as a function of water volume outside the protoplasts. Although estimates of water potentials and the water volume outside the protoplasts are only approximate, it would appear from the data that the water potential of the solution in cell walls may be accounted for by 2 components in living tissue, matric potential and

osmotic potential. The sum of the 2 potentials is similar to the water potential of the tissue when it is in equilibrium with its surroundings. The test of equation VI therefore supports the idea that matric forces arise primarily in the walls of leaf cells and that those arising in the protoplasm may be neglected at the higher water contents.

In addition to the limitations already discussed, the test of equation VI makes 2 other assumptions. First, freezing and thawing had a negligible effect on the matric potentials of the cell walls. Thus, the matric component of the water potentials present in living tissue during estimates of water volume were assumed to be comparable to the matric potentials measured with frozen and thawed tissue. Wiebe (17) has shown that freezing and thawing had no appreciable effect on matric potentials of agar below −4 bars and thus it appears that this assumption is justified. A second assumption is also made that the release of soluble protoplasmic contents to the cell walls after freezing and thawing did not affect the matric forces which act there (mainly through changes in the ω_a term of equation IV). However, the effect of adsorbed solutes on matric potentials was not tested.

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