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Leaf Water Potentials Measured with a Pressure Chamber

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Summary. Leaf water potentials were estimated from the sum of the balancing pressure measured with a pressure chamber and the osmotic potential of the xylem sap in leafy shoots or leaves. When leaf water potentials in yew, rhododendron, and sunflower were compared with those measured with a thermocouple psychrometer known to indicate accurate values of leaf water potential, determinations were within ± 2 bars of the psychrometer measurements with sunflower and yew. In rhododendron. water potentials measured with the pressure chamber plus xylem sap were 2.5 bars less negative to 4 bars more negative than psychrometer measurements.

The discrepancies in the rhododendron measurements could be attributed, at least in part, to the filling of tissues other than xylem with xylem sap during measurements with the pressure chamber. It was concluded that, although stem characteristics may affect the measurements, pressure chamber determinations were sufficiently close to psychrometer measurements that the pressure chamber may be used for relative measurements of leaf water potentials, especially in sunflower and yew. For accurate determinations of leaf water potential, however, pressure chamber measurements must be calibrated with a thermocouple psychrometer.

A pressure chamber has recently been used to measure what has been termed the sap pressure of plants (9, 10). The method consists of increasing the pressure around a leafy shoot until xylem sap appears at the cut end of the shoot, which extends outside the chamber and is exposed to atmospheric pressure. It has been suggested that the pressure necessary to retain this condition represents negative pressure existing in the intact stem (9, 10), but it is not clear whether the method estimates only hydrostatic pressures or whether other factors are involved (6). Basically, however, the amount of pressure necessary to force water out of the leaf cells into the xylem is a function of the water potential of the leaf cells. Determinations made with the instrument, which have been reported for several species (10), appear qualitatively similar to the water potential expected for leaves of these species. Therefore, experiments were undertaken to determine whether measurements made with the pressure chamber could be used to estimate leaf water potentials.

The thermocouple psychrometer provides a basis for determining the accuracy of the pressure chamber, since an isopiestic modification (4) of psychrometer technique has recently been shown to be an accurate measure of leaf water potentials (2). Comparison of the 2 techniques is complicated, however, by the difference in potential which probably exists between water in the xylem and that in leaf cells as a result of the resistance to water flow between these 2 points in plants in which transpiration is occurring. Thus, measurements obtained with the pressure chamber, which depend on observations of the xylem sap, might not be comparable to those made with a psychrometer, which indicate the water potential of leaves. Unfortunately, no data are available describing the magnitude of the resistance between xylem and leaf. The following comparisons were therefore made with excised shoots in which water loss was zero and the potentials of water in the xylem and leaf were at equilibrium.

Under these conditions, the potential of water in a plant shoot during a measurement with the pressure chamber may be partitioned into the following components:

$$\psi_w = P + \psi_s \qquad (I)$$

where ψ_w is the water potential of the leaf cells, P is the pressure applied by the chamber, and ψ_s represents the effect of solutes in the xylem sap. The terms P and ψ_s represent the total force tending to remove water from the leaf cells and, to the extent that P estimates the hydrostatic or matric forces in the intact xylem, they represent the total force acting on water in the xylem of the intact plant. For convenience, therefore, $P + \psi_s$ will be referred to as xylem ψ_w.

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Although it has been proposed that the effects of solutes are small enough to ignore (9), determinations of leaf water potentials with the pressure chamber must include the osmotic potential of the xylem sap since xylem osmotic potentials may represent 2 or 3 bars in some cases (7, 8).

Materials and Methods

Three species were chosen as examples of a wide range in leaf and stem anatomy: yew (*Taxus cuspidata* Sieb. & Zucc.), rhododendron (*Rhododendron roseum* Rehd.), and sunflower (*Helianthus annuus* L.). Two year old yew and rhododendron were grown in soil in the greenhouse. Sunflower was grown from seed in soil in a constant environment room (temperature 30–31° day, 27–28° night; relative humidity 45–55 %; light 2500 ft-c).

The plant tissue to be sampled was washed and permitted to dry in the air for several hours. Tests with parallel samples indicated that washing had no effect on the potential of the plant tissue. Immediately after sampling and during all subsequent manipulations, the plant tissue was kept in a humid chamber to prevent desiccation.

Pressure Chamber Measurements. A pressure chamber (9, 10) was modified so that nitrogen gas entered the chamber by bubbling through water in the bottom. The moist gas reduced water loss of the plant sample to an undetectable amount during a determination. A baffle prevented the water from splashing on the tissue. The quantity P in equation 1 was estimated with the pressure chamber by applying sufficient pressure to a leafy shoot 10 to 15 cm long (rhododendron and yew) or single leaf (sunflower) to return the meniscus of the xylem sap to the cut surface of the stem or petiole.

Water Potential of the Leaves. After measurements with the pressure chamber, leaves (yew) or interveinal leaf tissues (rhododendron and sunflower) were sampled for the thermocouple psychrometer from the same tissue used for pressure measurements. Leaf water potentials were measured by isopiestic technique (4) and consisted of 2 consecutive determinations, first with water on the thermocouple and then with a sucrose solution on the thermocouple. The potential of the sucrose solution was close to the potential of the leaf tissue. Thermocouple output was plotted as a function of the potential of the water or solution on the thermocouple, and the line was extrapolated to zero output. The potential at zero output was taken as the potential of the leaf tissue. Determinations were corrected for the heat of respiration, which was measured with a dry thermocouple (1).

Values of water potential measured with a thermocouple psychrometer are likely to be in error because water is adsorbed by the chamber walls if the walls are not covered with plant tissue (5). The samples from sunflower and rhododendron were cut so that the wall and bottom of the psychrometer chamber were covered and water adsorption was not a problem. However, the yew leaves did not cover the walls. Preliminary experiments were therefore conducted to reduce the adsorption of water by the walls when leaves of yew were in the psychrometer chamber. The inner surface of the brass psychrometer chamber was made hydrophobic with a coating of vaseline which had been melted and resolidified on the walls and bottom. Under these conditions, the output of the thermocouple with distilled water on the bottom of the chamber and on the thermocouple should have been zero if the adsorption of water vapor on the coated walls was negligible. Figure 1 shows that output was zero after 20 minutes when the walls were coated, but had not reached zero after 2 hours if the walls were uncoated. Evidently wall adsorption was negligible when vaseline covered the walls and all determinations with yew were carried out in chambers coated with this material.

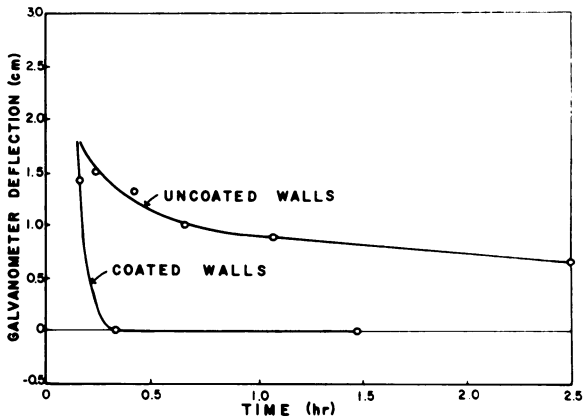

FIG. 1. The effect of a coating of vaseline on the wall of a psychrometer chamber when distilled water covers the bottom of the chamber and the thermocouple. A positive galvanometer deflection indicates that water is evaporating from the thermocouple and is being adsorbed on the chamber walls.

Osmotic Potential of the Xylem Sap. After subsampling for the psychrometer, the shoot or leaf was placed back in the pressure chamber and subjected to a slight overpressure. The first 15 to 30 μl of exudate which appeared had an osmotic potential that was constant to within 0.5 bar in all 3 species. Therefore, the initial 5 μl was collected and placed on a thermocouple for determination of xylem osmotic potential by a micromethod (3).

Results

Figure 2 shows that pressure chamber measurements plus xylem osmotic potentials (xylem ψ_w) were within 2 bars of psychrometer measurements of leaf water potentials (leaf ψ_w) for sunflower

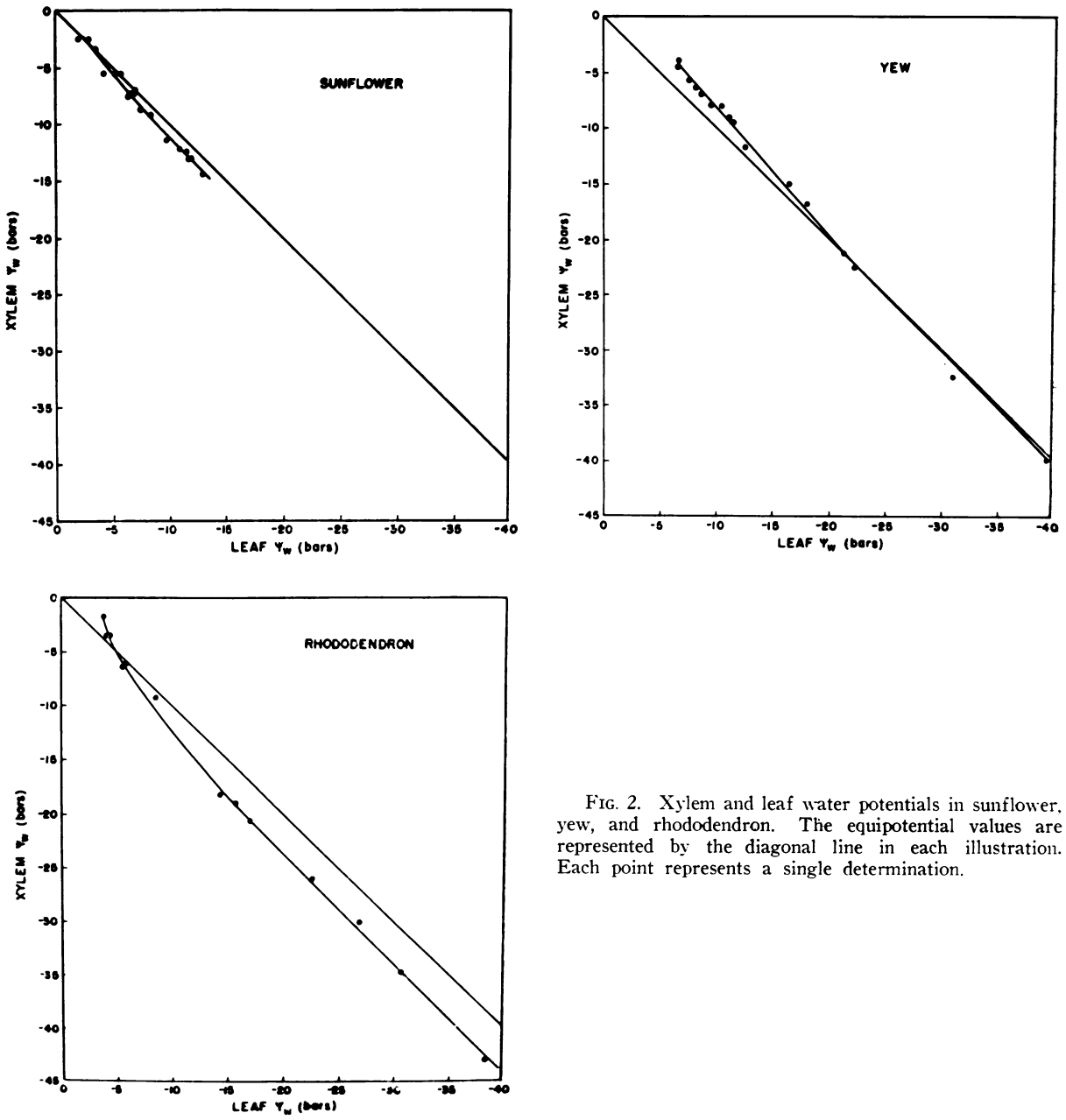

FIG. 2. Xylem and leaf water potentials in sunflower, yew, and rhododendron. The equipotential values are represented by the diagonal line in each illustration. Each point represents a single determination.

and yew. In rhododendron, xylem ψ_w was 2.5 bars less negative to 4 bars more negative than leaf ψ_w. All 3 plant species showed the tendency toward less negative values of xylem ψ_w relative to leaf ψ_w in wet plants and more negative values of xylem ψ_w in dry plants.

For determinations with the pressure chamber, all plant samples were exposed to balancing pressures for 5 minutes in order to detect whether equilibration of water potentials in xylem and leaf cells had occurred. In every case, there was negligible change in the balancing pressures, which remained constant in tests which extended to half an hour in some cases. Apparently, equilibration of the potential of water in leaf cells and xylem occurred rapidly.

The pressures necessary for measurement compressed the vascular tissue of sunflower and rhododendron. The stem that protruded outside the chamber necessitated additional pressures equivalent to 0.2 to 0.3 bar cm^{-1} of protruding stem in order to obtain balancing pressures. The effect was not present in yew but, for uniformity, samples of each of the 3 species were mounted in the chamber so

Table I. *Comparison of Xylem and Leaf Water Potentials in Long and Short Stemmed Rhododendron Samples at Various Leaf Water Potentials*

Each pair of long and short stemmed samples was taken from the same plant.

Long stems			Short stems		
Xylem ψ_w	Leaf ψ_w	Difference	Xylem ψ_w	Leaf ψ_w	Difference
—15.0 bars	—13.0 bars	2.6 bars	—14.0 bars	—15.6 bars	—1.6 bars
—21.5	—19.0	2.5	—21.7	—20.4	1.3
—22.1	—16.6	5.5	—20.5	—16.5	4.0
—28.4	—23.6	4.8	—27.2	—23.3	3.9
—32.1	—28.6	3.2	—32.1	—29.6	2.5
—32.6	—29.5	3.1	—32.3	—29.5	2.8
	Average	3.6		Average	2.2

that a maximum amount of the stem or petiole was exposed to pressures inside the chamber.

While using the pressure chamber with rhododendron, which showed the greatest discrepancy between xylem and leaf ψ_w, it was observed that the pith filled with sap during a determination. The free liquid surface of the xylem was continuous with that of the pith at balancing pressure. In such a situation, greater pressure would be required to fill both xylem and intercellular spaces in the pith, resulting in erroneously low values of xylem ψ_w.

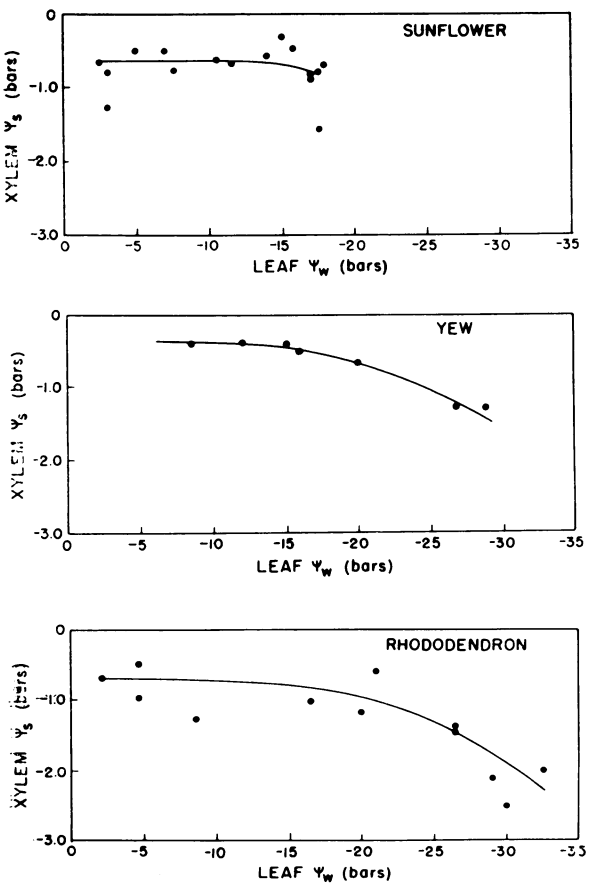

FIG. 3. Xylem osmotic potentials (xylem ψ_s) measured at various leaf water potentials in sunflower, yew, and rhododendron. Each point represents a single determination.

To test this hypothesis, comparisons of xylem and leaf ψ_w were made with short stemmed (3–5 cm) and long stemmed (10–12 cm) shoots of dry rhododendron taken from the same plant. The branches had approximately the same number of leaves, while the amount of pith tissue in the sample varied with the different stem lengths. The results, table I, indicate that in every case xylem ψ_w was nearer leaf ψ_w when short stemmed rather than long stemmed samples were observed. The average difference between xylem and leaf ψ_w for the short stemmed samples was 2.2 bars; for long stemmed, 3.6 bars. This difference was highly significant.

Sap concentrations at various leaf water potentials remained essentially constant (fig 3) at -0.4 to -1.0 bar in all 3 species when leaf water potentials were -15 bars or less negative. Below that potential, however, the sap became more concentrated in rhododendron and yew and had osmotic potentials as low as -2.5 bars.

Discussion

The pressure chamber does not measure xylem potentials directly. The pressure applied to the leafy shoot raises the potential of water in the leaf cells of the shoot but the xylem sap is exposed only to atmospheric pressure. The method therefore measures the pressure necessary to raise the potential of water in the leaf cells to the point at which it equals the potential of the xylem sap at atmospheric pressure. To extend this measurement to xylem potentials occurring in the intact plant, 2 assumptions must be made. First, the water potentials of the xylem sap and leaf cells must be in equilibrium during the time of measurement. Equilibration apparently occurred in the experiments reported here, so rapidly in fact that a significant change in potential could not be detected after the initial balancing pressures were

applied to the tissue and for intervals as long as a half hour.

Secondly, the assumption must be made that water is arranged spatially in the same manner in the shoot under pressure as it is in the shoot while intact on the plant. This assumption implies that the dimensions of the conducting system during measurement represent those in the intact plant and that the stem tissues are filled with water in the same manner in the 2 situations.

Both of the latter restrictions pose problems in certain plants. In rhododendron, for example, deviations between xylem and leaf water potentials were the largest of the 3 species tested. Both stem deformation and the filling of stem tissues other than xylem (probably pith tissues) occurred in this species. On the other hand, there was relatively good agreement between xylem and leaf water potentials in yew, which was not subject to stem deformation and had only a small amount of pith. The large amount of ground parenchyma in sunflower also may have affected the values obtained with this species, but the effect of ground parenchyma was not tested.

Regardless of the errors involved, the agreement between leaf and xylem ψ_w provides evidence that the pressure chamber estimates the nonosmotic component of xylem ψ_w, whether it arises from matric or hydrostatic forces (6). Agreement is close enough so that pressure chamber measurements plus xylem osmotic potentials may be used to predict relative values of leaf ψ_w, particularly in sunflower and yew. Fairly accurate estimates of leaf ψ_w in the absolute sense are possible in all 3 species if the pressure chamber method is first calibrated with a thermocouple psychrometer (2), although there is more variability in the data obtained with the pressure chamber [in sunflower, a range of ± 0.1 bar may be obtained with the psychrometer (2) whereas pressure chamber measurements had a range of ± 0.3 bar in this species].

Determinations made with the pressure chamber are rapid and simple. For certain studies, the rapidity of determinations might justify its use in estimating relative values of leaf ψ_w without accounting for xylem osmotic potentials. Field measurements, for example, often contain sources of variability which are larger than the discrepancies between leaf ψ_w and relative measurements made with the pressure chamber. The pressure chamber would find wide use in such studies.

Although the comparisons were made with shoots or leaves which were not losing water, the rapidity with which equilibrium occurred between xylem and leaf cells in the pressure chamber may indicate that resistance to water flow between xylem and leaf cells is low. If such is the case, the results presented here could be extended to shoots in which transpiration is occurring.

The osmotic potentials of the xylem sap of the 3 plants are similar to those reported for other plants (7,8). Although xylem osmotic potential is often small relative to pressure chamber measurements, it constitutes as much as half of xylem ψ_w when plants are well watered. The increase in concentration which occurred in rhododendron and yew as these plants dried has not been reported before. Scholander, et al. (8) tested for diurnal changes in xylem osmotic potentials but found that sap concentrations remained about the same throughout the day, although diurnal changes in leaf ψ_w probably occurred.

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