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Measurement of Tissue Osmotic Pressure[1]

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ABSTRACT

Osmotic pressure measured by a modified pressure-volume method was compared with that of the mixed sap expressed from frozen and thawed tissue. The error in the latter technique averaged 11 and 16% (too dilute) for greenhouse and field leaves of *Zea mays* L. at all growth stages. These errors were not consistently calculated by a model of simple mixing between the matric and osmotic fractions, both of which decreased with plant age. Some other alternatives to the pressure-volume method are discussed which are based on a more rapid estimate of the zero turgor point.

Osmotic pressure is one of the most basic parameters to be measured in plant water relations. However, there are certain difficulties in the measurement. The Scholander PV[2] technique (5, 6, 8) is theoretically sound, but is so tedious and time-consuming that it is almost impossible to follow rapid changes in osmotic pressure over hours or a day. Techniques based on expressed sap are more rapid but suffer from error due to tissue disruption. This paper reports on the magnitude of that error, and discusses some related alternative methods of estimating component potentials.

There is always some ambiguity when dealing with cell or tissue osmotic pressure. This ambiguity derives from the traditional equation,

$$\psi = P - \pi - \tau, \quad (1)$$

where ψ is total water potential and P, π, and τ are defined as turgor, osmotic, and matric pressures, respectively. The physical meaning of matric pressure in equation 1, other than as a residual, is vague. Moreover, the relative contribution of each component varies spatially in tissue, most importantly between symplast and apoplast. Therefore, equation 1 either must refer to point values of potential, or to volume-weighted averages across cell or tissue. Neither of these is satisfactory, either for measuring potentials or for describing the changes in potential with water volume.

It is more functional to regard the cell water as separate fractions with uniform potentials. The free solution in both cytoplasm and vacuole has uniform osmotic and turgor components. Dilute water in the apoplast can be considered to have a turgor component only. Although this is not true very close to surfaces, where specific components vary, that volume is probably a very small fraction of the apoplast even in cell walls (9). Following its meaning in soils, I will use matric pressure, τ, to denote the normally negative pressure in apoplast water, and distinguish it from the positive turgor pressure, P, in the symplast. The total water volume, V_T, is assumed to be divided between an osmotic volume V_π and a matric volume V_τ, such that the water potential in V_π equals $P - \pi$, and the water potential in V_π equals $P - \pi$, and the water potential in V_τ equals $-\tau$. At equilibrium,

$$\psi_{V_T} = (P - \pi)_{V_\pi} = -\tau_{V_\tau}. \quad (2)$$

It is clear from equation 2 that in order to describe π, and cell turgor pressure by $\psi + \pi$, we need to measure the osmotic pressure of the free solution in cytoplasm and vacuole. It is too difficult in higher plants to extract uncontaminated sap. What has been done instead is either to extract the bulk sap from killed tissue or to force turgor to zero and then measure ψ.

We have argued that the mixed sap expressed from bulk tissue is not what is wanted. Similarly, when pressure is forced to zero by disrupting membranes, the compartmentalization between V_π and V_τ is lost. The osmotic volume may become diluted due to mixing with cell wall water, and water or solutes may bind to new surfaces (3). Techniques which involve sap mixing are nevertheless in wide use. The most common result is apparent dilution, and although the error in π may only be 10 or 20%, this can cause large relative errors in P ($= \psi + \pi$). Tyree (7) has noted that this dilution effect probably explains the frequent reports of negative turgor. Although mixed sap techniques are simple, and potentially rapid, they do not measure the desired variable.

Forcing turgor pressure to zero by removing water is a much sounder procedure which preserves membrane integrity. In the traditional plasmolysis method, water is extracted in graded solutions with a visual determination of the plasmolysis point. That point is fairly qualitative, however, and there can also be problems with solute influx or efflux. The better method is to express water from intact tissue with a pressure bomb, which is the Scholander PV technique (5–8, 11). If the water expressed is almost wholly from the osmotic volume V_π, then zero turgor can be judged as the point at which the tissue begins behaving like a solution only; *i.e.* the remaining volume is inversely proportional to water potential.

Osmotic pressure can be defined at constant temperature by

$$\pi = \frac{nk}{V_\pi} = \frac{nk}{v_\pi V_T} \quad (3)$$

where n is the number of moles of osmotically active solute, k is a constant, and v_π is the osmotic fraction of the total water volume. If all water loss occurs from this volume, and all the remaining water is associated with a matric fraction v_τ ($\equiv V_\tau/V_T$), such that $v_\tau + v_\pi = 1$, then the osmotic fraction at any water content is given by

$$v_\pi = 1 - \frac{v_\tau^o}{(V_T/V_T^o)} \quad (4)$$

where the o superscript denotes the original values. Substituting equation 4 into equation 3 gives

$$\frac{1}{\pi} = \frac{(V_T/V_T^o) - v_\tau^o}{nk'} \quad (5)$$

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[2] Abbreviation: PV: pressure-volume.

where k′ is another constant (= k/V_T°). This demonstrates that relative water volume is linear with $1/\pi$ (with slope $d(V_T/V_T^\circ)/d(1/\pi) = nk'$ and intercept v_τ° at $1/\pi = 0$), as long as the matric fraction and the number of solute molecules is constant. This has seemed to hold in the standard PV measurement. Nonideality of the solution does not upset the linearity, but will cause an increase in the observed v_τ°. For sucrose solutions of −1.0 and −2.0 mPa, there is an apparent v_τ° of about 3 and 7%, respectively. This concentration effect will be ignored here, but it would have to be considered for more accurate estimates of v_τ° at low π.

Since the PV technique is theoretically sound, it may be used to assess the error in more rapid techniques which involve tissue disruption. Scholander *et al.* (6) compared the PV measurement of π with the osmotic pressure of the mixed sap expressed from frozen and thawed tissue. The two agreed fairly well for most species, if what we will call the "mixed" osmotic pressure, π_m, was corrected by assuming full mixing and dilution with nonosmotic water:

$$\pi = \pi_m/v_\pi \quad (6)$$

Similarly, Boyer and Potter (2) found an error of 11% in π for intact frozen and thawed sunflower leaf tissue, and this was very close to the matric fraction determined by an earlier study (1) with this species. The matric fractions given in Boyer (1) have since been used by a few others to correct π_m with the assumption that equation 6 applied. However, there is no reason to suppose *a priori* that the osmotic fraction is constant, that dilution is complete, that the mixed fraction remains constant with water content and other factors, or that no other changes in free solute levels occur with mixing.

Using leaves of corn (*Zea mays* L.), I first determined the osmotic pressure error associated with mixed sap at all water contents. This was done simply by measuring π_m at each step in a modified PV technique. This was determined for both field and greenhouse plants at all ages. I then examined the adequacy of the mixing model described by equation 6. Finally, I suggest some alternative techniques for measuring π, which are related to the PV principle.

MATERIALS AND METHODS

The PV relation has always been based on the expressed sap from single samples of tissue. This has the advantage of allowing very precise measurements of the small changes in water volume associated with changes in turgor. It has disadvantages, however, in not representing a large number of samples, and in requiring somewhat specialized equipment and techniques for measuring the expressed sap. Also, some species apparently form air emboli sms or other blockages to axial flow out of the leaf, leading to very long equilibration times (10). I used a modified technique employing a number of matched leaves—one for each PV point—drying in air on the laboratory table outside of the bomb. I was not interested in changes in turgor, and the large changes in leaf water content with osmotic pressure could be easily measured by weighing the leaves on a balance. Water loss should be relatively uniform over the surface of the drying leaves, and this probably avoids large gradients of water potential within the tissue. After water potential measurement, the same tissue was available for a matching measurement of π_m.

Between 20 and 30 leaf strips, each about 30 cm long, were cut from the distal ends of leaves with similar irradiance and orientation. The youngest mature leaves (with ligule) were routinely chosen. When dew was on the leaf it was blotted dry immediately before cutting. The cut strips were rapidly rolled, wrapped tightly in plastic, and stored in the dark until measurement 30–60 min later. All leaf strips were initially weighed, then unrolled and left on the bench to dry. Water potential initially changed very rapidly while turgor was present. Turgor was generally zero within a few minutes, after which drying rates ranged from 0.004 to 0.025 mPa min^{-1}. Every 5–10 min 2 strips were chosen and reweighed, wrapped in a damp cloth to prevent further desiccation, and their water potential measured in the pressure bomb. The initial balance pressure was repeatable, indicating approximate equilibrium within the tissue. The balance pressure was equated with leaf water potential with the assumption that xylem osmotic potential was negligible. After removal from the bomb, the leaves were folded tightly, placed in vials made of tygon tubing, and frozen on dry ice. The whole procedure generally took 2–3 h. At any time later these samples were thawed on the bench until reaching room temperature, placed in a vise, and the expressed sap measured in a vapor pressure osmometer.

The maize variety used was a short season hybrid (Schlessman H99–H95, A632). Samples were taken at all plant stages, both in greenhouse and field. The field plants were from four different planting dates, ranging from May 26 to August 2, and therefore represented a range of growth conditions. All results are from irrigated plots which never exhibited midday stomatal closure. Greenhouse plants were grown in 15- to 20-cm diameter pots with a 1:1 mixture of sand and peat and watered with half-strength Meyer solution (4). Natural light was supplemented with high pressure sodium lamps to achieve midday irradiances on upper leaves of about 600–1,000 $\mu E\ m^{-2}s^{-1}$. Night temperatures were 18–20 C. Midday temperatures ranged from 23 to 38 C and RH from 30 to 60%.

Samples were generally taken early in the morning, with dew still on those taken from the field. Although the original water content was not far from full turgidity, the relative water volume (V_T/V_T°) described here is not necessarily equivalent to the traditional relative water content. The former is calculated as (fresh weight − dry weight)/(original fresh weight − dry weight). Relative water content, however, is (fresh weight − dry weight)/(fully hydrated fresh weight − dry weight), with full hydration achieved artificially by floating on water. This technique can only assume that the solute level and associated water volume, after hydration, is the same as that in the natural fully hydrated state.

RESULTS

The success of the PV technique requires that there are no changes in solute level during drying. This appeared to be the case with the modification used here, since $1/\psi$ was linear with relative water volume, excluding the range where turgor was present. In fact, there was no significant osmotic adjustment anytime after the leaf strips were removed. Leaf strips wrapped in plastic and stored in the dark had no change in osmotic potential over 3 h. In another experiment, in which the folded tissue in tygon vials was placed in the dark but not on ice, π decreased only 0.04 mPa or 3% over 2 h, and 0.08 mPa or 7% overnight.

A good least-squares fit of $1/\psi$ on (V_T/V_T°) was found for 15 trials, with a minimum r^2 of 0.91. In five other trials, the pressure bomb readings in the dry range either became scattered or approximately constant despite water loss. There apparently was some tissue damage here although it was not correlated with either growth environment or plant stage.

The osmotic pressure of the expressed mixed sap from the PV leaves was analyzed in the same way as the pressure bomb readings. A typical trial is shown in Figure 1. In each of 20 such trials, $1/\pi_m$ was linear with water content. However, the fitted regression lines usually were not parallel with the 15 linear $1/\pi$ relationships. In five trials the lines were parallel, although in ten trials the slope nk′ of $1/\pi_m$ was greater than $1/\pi$ (as in Fig. 1). The average difference in slope for all trials was 8% with a SD of 14%.

The intercepts from all linear regressions are shown as v_π° (= 1 − v_τ°) and $v_{\pi_{-m}}$ (= 1 − $v_{\tau_{-m}}$) (Fig. 2). All values were plotted *versus* days from emergence, with the results for the slower growing

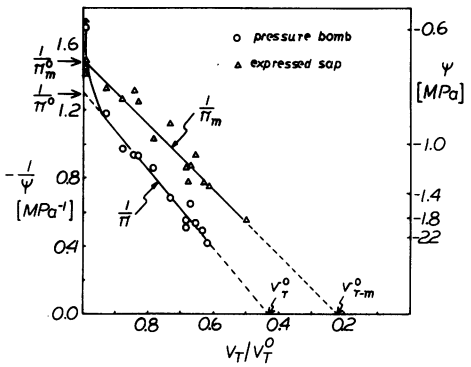

FIG. 1. A typical pressure volume measurement (○) using the mean of two leaf strips for each point. These same leaves were then sampled for π_m (△).

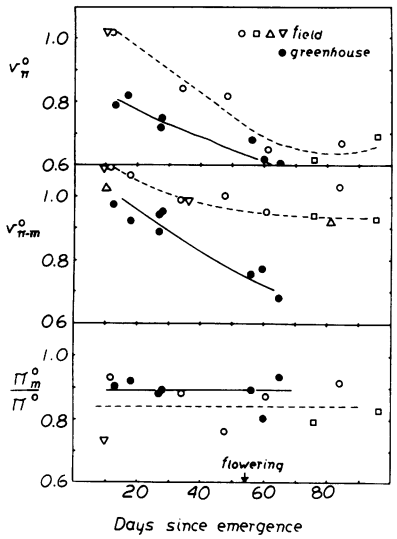

FIG. 2. A summary of season's results showing the original osmotic fraction (v_π^o, upper block), mixed osmotic fraction ($v_{\pi-m}^o$, middle), and ratio of the original-mixed and original actual osmotic potentials (π_m^o/π^o, lower). Different open symbols denote different planting dates in field.

plants—the late plantings and greenhouse pots—normalized so that pollination began at 54 days. The pressure bomb results (upper block) demonstrate that the osmotic fraction decreased greatly with plant age and that greenhouse plants always had lower v_π^o. Surprisingly, the mixed osmotic fraction (middle block, Fig. 2) also showed a decrease over the season, especially for greenhouse leaves. A $v_{\pi-m}^o$ close to one might be interpreted as evidence of complete mixing between matric and osmotic fractions. This was approximately the case for field plants, except early in the season when $v_{\pi-m}^o$ was greater than 1.0.

The ratio π_m^o/π^o for all trials is shown in the lower block of Figure 2. If the regressions of $1/\pi$ and $1/\pi_m$ had all been parallel, then π_m could have been corrected to π at any water content by $\pi_m/\pi = [(V_T/V_T^o) - v_\tau^o]/[(V_T/V_T^o) - v_{\tau-m}^o]$. At full hydration this is simply $v_\tau^o/v_{\tau-m}^o$. Since the lines often were not parallel, we report only the observed corrections at full hydration π_m^o/π^o. These are relatively constant over the season, although the field values are fairly variable. The means and SD were 0.84 ± 0.07 and 0.89 ± 0.04 for field and greenhouse leaves, respectively, indicating an apparent dilution error of 16 and 11% in the expressed sap osmotic potential.

DISCUSSION

Correction for Mixed Sap Techniques. It is evident that a correction is required when osmotic pressure is measured using mixed expressed sap. However, the correction based on the model of complete mixing between matric and osmotic fractions (equation 6) can evidently lead to larger error than if none at all were used. This is due to the fact that the mixed osmotic fraction $v_{\pi-m}^o$ was not 1.0, and in fact roughly paralleled the changes in v_π^o over the season (Fig. 2).

The trials for which $v_{\pi-m}^o$ was less than 1.0, primarily later in the season, are presumably due to incomplete mixing between the matric and osmotic volumes. This is probably the most important factor contradicting equation 6. The few field results early in the season, for which $v_{\pi-m}^o$ was greater than 1.0, are more difficult. These could be explained only by changes in solute level, or the mixed fraction, which parallel the changes in water content and hence maintain the observed linearity.

There were also large unexplained differences between plants grown in bulk soil or pots. Both the mixed and unmixed osmotic fractions were consistently lower in greenhouse leaves, and this was not related to obvious differences in succulence. Both leaf types showed large decreases in fresh weight/dry weight over the season (Fig. 3). This paralleled the trends of v_π^o and $v_{\pi-m}^o$, but the difference between field and greenhouse plants was always small.

Another problem, although of lesser importance, was the variability in the degree of parallelism between $1/\pi$ and $1/\pi_m$. This was responsible for the variability seen in π_m^o/π^o for the early season field results, as both v_π^o and $v_{\pi-m}^o$ were fairly uniform (Fig. 2). A larger slope nk′ was generally found for the expressed sap implying a possible increase in solute levels following mixing. Why this occurred in some instances and not in others is not clear; the lack of parallelism was not associated with plant age, the initial values of π, v_π, or $v_{\pi-m}$, or with the rate of drying on the bench. The measurement techniques themselves were fairly systematic. Tyree and Karamanos (9) have suggested that incomplete cell disruption may be a major source of variability in freeze and thaw techniques, but the results reviewed by Brown (3) suggest that disruption is nearly complete. Also, this would not affect π_m if the relative volume of intact cells was paralleled by an intact and unmixed volume of matric water.

We can conclude that an empirical correction should be applied when mixed sap is used to measure osmotic pressure. Even if the relative uncertainty in this correction is large, it should much improve the estimate of turgor from $\psi + \pi$, and may therefore allow a rapid and sufficiently accurate sampling of P. Improving the generality of the correction will require more information about what the different water fractions really represent, and how they interact upon mixing.

When osmotic potential is determined by freezing and thawing whole tissue discs in a psychrometer, the errors involved are likely to be similar to those discussed here, but they are not necessarily the same. Membranes are disrupted in both instances, but mixing may be more complete when sap is expressed, and cell surfaces are no longer present in this case.

Alternative Techniques. The PV measurement requires a num-

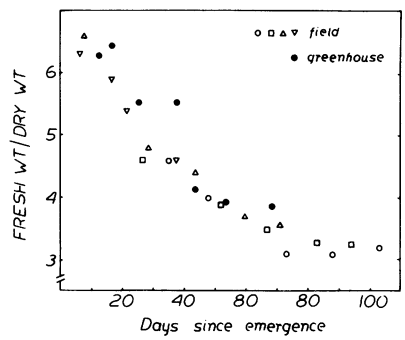

FIG. 3. Decrease in succulence with plant age. Different open symbols denote different planting dates in field.

ber of points, extended to very low water contents, in order to determine accurately the original matric fraction v_τ°. An accurate measure of the original osmotic pressure does not require points at low water content since it involves a very short extrapolation from the wettest P = O point (see, for example, Fig. 1). The osmotic pressure at any relative water volume (V_T/V_T°) is related to the original osmotic pressure by

$$\frac{\pi}{\pi^\circ} = \frac{1 - v_\tau^\circ}{(V_T/V_T^\circ) - v_\tau^\circ} \quad (7)$$

This demonstrates the insensitivity of π/π° to v_τ° as long as (V_T/V_T°) is relatively high. If turgor falls to zero at a high water content, then π° may be adequately estimated with a much abbreviated PV curve.

In fact, if a single water content at which turgor is certainly zero can be determined, π at that point can be measured in the pressure bomb and π° calculated from equation (7) with only an estimate for v_τ°. Almost all the values of v_τ° found here, and those measured and reviewed in the literature by Wenkert *et al.* (11), are encompassed by $v_\tau^\circ = 0.25 \pm 0.15$. Zero turgor always occurred at between 90 and 94% relative water volume for corn leaves in this study which were initially fully hydrated. If π could be accurately measured at $V_T/V_T^\circ = 0.90$, then the maximum error in π° from assuming that $v_\tau^\circ = 0.25$ would only be about 4%. This procedure might require multiple measurements at the single zero turgor point, due to normal variability, but it would still allow a much more rapid measure of π° than the traditional PV method.

The abbreviated technique described would be even more rapid if the point of zero turgor, and the (V_T/V_T°) at which this occurred, could simply be estimated. This could be done fairly readily with the corn leaves studied, since the upper surface became dull and pale near zero turgor. This happened long before any leaf-rolling and was a well defined point in the drying process when viewed under natural light. The loss of sheen begins first in the interveinal areas, particularly near the margin of the leaf. There is often some mottling at this point, but it progresses rapidly to a uniform dull and pale surface. Turgor at this point was measured for field leaves, both drying naturally and as excised strips. Water potential measured with the pressure bomb was compared with the corrected osmotic pressure from the expressed mixed sap. For 5 days spread over the season, and 21 measurements, the mean $\psi + \pi$ (apparent turgor) was 0.03 ± 0.09 mPa.

This type of procedure might be considered whenever there is a good relationship between zero turgor and some easily measured surface or mechanical change. Osmotic and turgor pressures could be estimated rapidly by successive measurement of ψ in the pressure bomb before and after the required amount of drying. This has the advantage, like the PV technique, of measuring both ψ and π, and therefore turgor, with the same instrument. Although judgement of the zero turgor point may be partly subjective, and there is some uncertainty in the estimate of V_T/V_T° at that point, the error in the estimate of original turgor pressure may still be smaller than in any of the mixed sap techniques.

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LITERATURE CITED

1. Boyer JS 1967 Matric potential of leaves. Plant Physiol 42: 213–217
2. Boyer JS, JR Potter 1973 Chloroplast response to low leaf water potentials. I. Role of turgor. Plant Physiol 51: 989–992
3. Brown RW 1972 Determination of leaf osmotic potential using thermocouple psychrometers. *In* RW Brown, BP VanHaveren, eds, Psychrometry in Water Relations Research, Utah State University, pp 198–209
4. Meyer BS, DB Anderson, CA Swanson 1955 Laboratory Plant Physiology. D. Van Nostrand, Princeton, pp 120–122
5. Scholander PF, HT Hammel, ED Bradstreet, EA Hemmingsen 1965 Sap pressure in vascular plants. Sci 148: 339–346
6. Scholander PF, HT Hammel, EA Hemmingsen, ED Bradstreet 1964 Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc Nat Acad Sci USA 52: 119–124
7. Tyree MT 1976 Negative turgor pressure in plant cells: fact or fallacy? Can J Bot 54: 2738–2746
8. Tyree MT, HT Hammel 1972 The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J Exp Bot 23: 267–282
9. Tyree MT, AJ Karamanos 1979 Water stress as an ecological factor. *In* Plants and Their Atmospheric Environment. Harvest House, Reading, England
10. Tyree MT, ME MacGregor, A Petrov, MI Upenieks 1978 A comparison of systematic errors between the Richards and Hammel methods of measuring tissue-water relations parameters. Can J Bot 56: 2153–2161
11. Wenkert W, ER Lemon, TR Sinclair 1978 Water content-potential relationship in soya bean: changes in component potentials for mature and immature leaves under field conditions. Ann Bot 42: 295–307