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The Measurement of the Turgor Pressure and the Water Relations of Plants by the Pressure-bomb Technique

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ABSTRACT

The pressure-bomb technique as developed by Scholander and colleagues is reviewed. A theoretical analysis of the equilibrium water-relations of individual cells of a twig is derived taking due account of the fact that each cell has a unique solute concentration, fluid volume, shape, and unique mechanical constraint by virtue of its cell-wall structure and attachment to nearest neighbours. These equations combine to give a complete description of the whole twig in response to mechanical (air pressure) stress. Our theoretical analysis suggests that the 'pressure–volume curve' can be related quantitatively to meaningful bulk parameters of water relations: viz. the total osmolar content of the symplast N_s, the original volume of the symplast V_0, the volume expressed from the symplast V_e, the gas-pressure of the bomb P, and the volume-averaged turgor pressure (the sum of the products of the relative volume and turgor pressure of each cell). An empirical relation for the volume-averaged turgor pressure of twigs is found which fits all species examined; it also fits the turgor pressure relation for single (*Nitella*) cells.

INTRODUCTION

In an attempt to determine the maximum depression of the water potential (stress) that plants can withstand during transpiration, Dixon (1914) devised an air-pressure chamber. A twig bearing a number of leaves was enclosed in a strong glass cylinder capable of resisting high gas-pressure, and the pressure was raised in Dixon's vessel by means of an air-compression pump, or by directly attaching it to a cylinder containing liquid CO_2. The lower portion of the twig projected through an air-tight seal to the outside where it dipped into a glass vessel containing a weighed quantity of water. Dixon (1914) reasoned that 'for every branch we may expect to find a pressure above which water will be forced back from the leaves into the stem by reasons of the squeezing out of the osmotic cells, and below which water will rise through the conduits to the leaves, on account of the osmotic attraction of the cell-sap'.

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Dixon used his technique to determine the balancing pressure at which water neither entered nor left the twig when it was under transpiration stress and to determine the pressure at which 'flagging' (wilting) commenced. A number of difficulties attended Dixon's experiments, not the least of which was the toxic effect of high CO_2 pressures and two explosions which led Dixon to abandon the technique.

A pressure-bomb was used by Chibnall and Grover (1926) for the limited purpose of sap collection for chemical analysis. In later years Haines (1935) reconstructed much of Dixon's apparatus and again undertook experiments concerning the effect of bomb pressures on the rate of water uptake by a shoot (in a dry pressure-chamber) which took up water through a stem which protruded from the bomb to a water source.

To the best of our knowledge no one else tried to reproduce or improve upon Dixon's experiments until Scholander and colleagues independently devised a similar pressure-bomb. In essence Scholander's pressure-bomb technique involves the measurement of all or part of what is called a 'pressure–volume curve'. A leafy twig is completely enclosed inside a pressure chamber except for the cut end of the stem which protrudes through an air-tight seal into the open air. The nitrogen-pressure of the bomb is increased until fluid flows out of the twig; after a measured quantity has been expressed the pressure is lowered until fluid neither flows in nor out. This pressure is noted and called the balancing pressure, P. The above process is repeated and each time the volume increment and the new balancing pressure is noted. A plot is then constructed of $1/P$ against the total volume expressed, V_e (= the sum of all the volume increments for each P) and this plot is called the 'pressure–volume curve'.

A number of important facts and interpretations have been reported in the literature regarding the pressure-bomb technique (Scholander, Hammel, Hemmingsen, and Bradstreet, 1964; Scholander, Hammel, Bradstreet, and Hemmingsen, 1965; Scholander, Bradstreet, Hammel, and Hemmingsen, 1966; Hammel, 1967, 1968):

(*a*) Sap samples have been expressed from a wide range of species and in all cases the sap has been found fairly dilute even when up to half the water content is expressed. The freezing-point depression of the collected sap is routinely measured and ranges from 0·00 to 0·03 °C on six species of halophytes, 14 species of desert plants, five species of forest plants, and about 10 species of temperate rain-forest plants from Chile. From this observation we conclude that few if any cells are ruptured in the course of the experiments.

(*b*) The 'pressure–volume curve' is at first non-linear for small values of V_e but approaches a linear relation as V_e grows large. See Fig. 1.

(*c*) Since the applied bomb pressure enters into the water-potential balance between the cell-sap (symplasm) and xylem-sap (apoplasm) it is assumed that the initial balancing pressure, P, when no water has been expressed ($V_e = 0$) is equal in magnitude to the water tension present in the apoplast just previous to the time of pressure application.

(*d*) It has been argued that when the linear portion of the 'pressure–volume curve' is reached, the turgor pressure of all (or most) of the twig cells has reached zero

and the balancing pressure, P, is equal to the 'average osmotic pressure' of the cell sap. Indeed, it has been found experimentally that when enough liquid has been expressed to approach the linear part of the curve, the balancing pressure matches closely the osmotic potential of the intracellular sap as determined by the freezing-point depression duly corrected for free space.

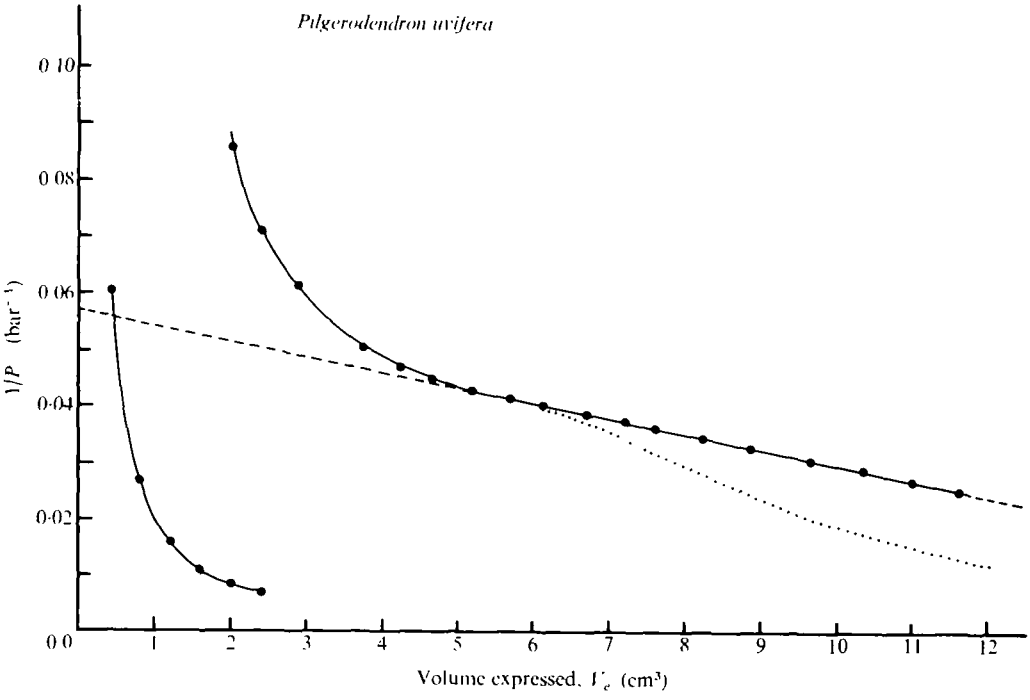

FIG. 1. A typical 'pressure–volume curve' on *Pilgerodendron uvifera* collected from the grand island of Tierra del Fuego on 17 August 1970. The stem stood in water and the leaves were enclosed in a plastic bag for 36 h in order to allow complete rehydration. The dashed line extrapolates to the inverse of the original 'bulk osmotic pressure'; the curve to the lower left is an extension of the 'pressure–volume curve' to the left but the inverse pressures are divided by 10. The dotted line represents what the curve would look like if the stress–strain relation for compression were symmetrical to that for cell expansion.

(*e*) It is argued that the extrapolation of the linear part of the 'pressure–volume curve' to $V_e = 0$ gives the 'initial osmotic pressure' and the extrapolation to $1/P = 0$ gives a measure of the 'intracellular volume' (= symplast volume).

The purpose of this paper is to determine on a more quantitative and theoretical basis what is really meant by the 'initial osmotic pressure', the 'intracellular volume', the 'turgor pressure', and the 'average osmotic pressure' as read from the 'pressure–volume curve'.

THEORY

The purpose of this theoretical section is to determine the quantitative relationship of the 'pressure–volume curve' to certain bulk parameters concerning the water

relations of the twig upon which the determinations are made. In particular our aim is to prove that

$$\frac{1}{P} = \frac{V}{RTN_s - F(V)} = \frac{V_0 - V_e}{RTN_s - F(V)}, \tag{1}$$

where V_0 = the original volume of all the living cells having reasonably pliable walls, V_e = the volume expressed from all the cells, N_s = the total number of osmoles of solute in all the living cells, and $F(V)$ = the volume remaining in all the cells, ($V = V_0 - V_e$) times a turgor pressure function represented solely by a function of V.

Later we will demonstrate by reference to 'pressure–volume curves' for a number of different species that $F(V)/V$ very closely fits the empirical relation

$$\frac{F(V)}{V} = \begin{cases} \epsilon\left(\frac{V - V_p}{V_p}\right)^n, & \text{when } V \geqslant V_p \\ 0, & \text{when } V < V_p \end{cases} \tag{2}$$

where we have defined ϵ as the bulk modulus of elasticity of the twig, n is a coefficient of non-linearity, and V_p is a bulk volume at 'incipient' plasmolysis of the twig.

Let us consider the twig represented diagrammatically in Fig. 2. Several cells are shown clustered around a tracheid; the cells represent all the leaf and stem cells and the tracheid represents all the leaf and stem tracheids. The tracheid of our diagrammatic stem protrudes through an air-tight seal of the pressure-bomb.

The following symbols will be used throughout: P is the air pressure of the pressure chamber; P' is the pressure of the water in the lumen of the conducting element and cell walls (i.e. the tension on the water in the apoplast); and ${}_iC$, ${}_iv$, and ${}_iP_t$ are the osmolar concentration of the solutes, the volume, and the turgor pressure of the ith cell. (All the pressures P, P', and ${}_iP_t$ are measured with respect to standard atmospheric pressure = 1 bar or 0·987 atm.)

We will first show that each cell obeys a relation *of the form of* Eqn. (1). Since Eqn. (1) is an equilibrium relation we must express the water potential of the ith cell and the apoplast in terms of P, P', ${}_iP_t$, ${}_iC$, and ${}_iv$.

(*a*) *The water potential of the* ith *cell*, Ψ_i

The water potential of the ith cell, Ψ_i, is the sum of the water potential of water in the standard state, Ψ^*, the osmotic pressure of the ith cell, $-RT_i C$, and the total pressure on the cell fluid, ${}_iP_{\text{total}}$:

$$\Psi_i = {}_iP_{\text{total}} - RT_i C + \Psi^*. \tag{3}$$

The quantitative dependence on ${}_iP_t$ on ${}_iv$ and the volume at incipient plasmolysis of the ith cell, ${}_iv_p$, is of crucial importance to an understanding of the way in which ${}_iP_t$ and P combine to contribute to ${}_iP_{\text{total}}$ and thus to Ψ_i.

We do know that as ${}_iv$ approaches ${}_iv_p$ then the turgor pressure, ${}_iP_t$, approaches zero. If we could measure this dependence experimentally we could always find an empirical equation of the form

$${}_iP_t = f_i({}_iv) \cong \sum_{j=1}^{n} \epsilon_j \left(\frac{{}_iv - {}_iv_p}{{}_iv_p}\right)^j, \quad {}_iv \geqslant {}_iv_p, \tag{4}$$

where ϵ_j is an empirical constant for each term of the power series. This is a consequence of the mathematical theorem that if a function is continuously differentiable in the interval ${}_iv_0 \geqslant {}_iv \geqslant {}_iv_p$ then it is always possible to approximate the function by a Taylor series expansion. Each cell will have a different set of values for ϵ_j and this will express the fact that each cell will be constrained from completely free expansion by its attachment to neighbouring cells.

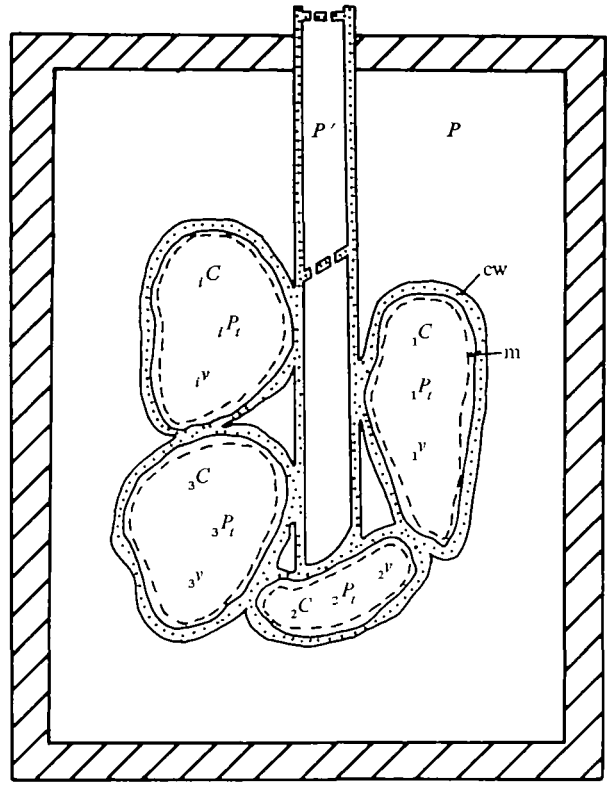

FIG. 2. A diagrammatic representation of a twig in a pressure bomb. Four cells represent the leaf cells and one conducting element represents the xylem. C = the cell osmolar solute concentration; P_t = the cell turgor pressure; v = the cell volume; P = the bomb pressure; P' = the hydrostatic pressure (tension) in the apoplast. Subscripts 1, 2, i, etc., represent individual cells. cw = cell wall. m = semi-permeable membrane.

We can always find a functional relationship for ${}_iP_t$ regardless of how the volume is caused to change, i.e. whether it be by physical compression of the cell or by osmotic stress. The pressure-bomb technique is a means of applying physical compression which permits water to leave the cell via the vascular system, but the pressure-bomb technique could well cause the shape of the cell to change in a way different from that caused by another technique (e.g. the application of osmotic stress as in plasmolysis experiments). If physical stress is applied by a pressure-bomb technique then ${}_iP_t$ is the hydrostatic pressure difference between the cell and the bomb rather than the pressure difference between the cell and the atmosphere

outside the bomb since this pressure difference continuously increases with decreasing ${}_iv$. Clearly the pressure-bomb technique would permit the cell volume to drop below the volume at zero turgor pressure, ${}_iv_p$ (= the volume at incipient plasmolysis). When ${}_iv < {}_iv_p$ we can imagine that the cell wall would take up some of the strain of the applied pressure; therefore the pressure felt in the remaining cell fluid would be less than that applied. We can generalize Eqn. (4) over a wide range of ${}_iv$'s provided we permit ${}_iP_i$ to assume negative values.

We have gone through this long argument to prove that, provided we can in principle write the turgor pressure in the form of ${}_iP_i = f_i({}_iv)$ (Eqn. (4)), then we can express the total pressure as the sum of two terms, P and $f_i({}_iv)$ *which are completely independent of each other.*

Therefore we can write in place of Eqn. (3)

$$\Psi_i = P + f_i({}_iv) - RT_i C + \Psi^* = P + f_i({}_iv) - RT\frac{n_i}{{}_iv} + \Psi^*, \tag{5}$$

where n_i is the total number of osmoles of solute in the ith cell and where $f_i({}_iv)$ assumes positive values for ${}_iv > {}_iv_p$ and negative values when ${}_iv_p > {}_iv$.

(*b*) *The water potential of the apoplast,* Ψ_0

The water potential of water in the apoplast is the sum of the water potential of water in the standard state, Ψ^*, the osmotic pressure of the apoplastic water, $-RTC_0$, and the tension which can be expressed in terms of the radius of curvature of the air-water interface *at the cut end.*

$$\Psi_0 = \Psi^* - \frac{2T}{r} - RTC_0, \tag{6}$$

where r is the radius of curvature of the air-water interface at the cut end of the stem and T is the surface tension of the apoplastic water. The bomb pressure P does not contribute to Ψ_0 because it is balanced by the surface tension effects at the air-water interfaces *inside the bomb.*

At equilibrium the difference in water potentials, $\Delta\Psi = \Psi_i - \Psi_0$ must be zero. Thus from Eqns. (5) and (6)

$$\Delta\Psi = P + f_i({}_iv) - RT({}_iC - C_0) + \frac{2T}{r} = 0.$$

Our criterion for determining the balancing pressure is the pressure, P, at which the cut surface of the stem just beings to wet. At this time the radius of curvature is very large, so $2T/r = 0$ when P is the balancing pressure. Our experience also shows that C_0 is small compared with the expected value of ${}_iC$ so we can reduce the above equation to

$$P + f_i({}_iv) - RT\frac{n_i}{{}_iv} = 0, \tag{7}$$

when P assumes the balancing pressure.

This may be rewritten

$$\frac{1}{P} = \frac{{}_iv}{RTn_i - {}_ivf_i({}_iv)} = \frac{{}_iv_0 - {}_iv_e}{RTn_i - {}_ivf_i({}_iv)}, \tag{8}$$

where ${}_iv_e$ is the volume expressed from the ith cell.

Eqn. (8) is of the form of Eqn. (1). To show that the total population of cells obeys an equation of the form of Eqn. (1) we need only rewrite Eqn. (7),

$$P_i v = RTn_i - {}_i v f_i({}_i v).$$

If the twig has m cells then we must add each equation together for all m cells.

$$\sum_{i=1}^{m} P_i v = \sum_{i=1}^{m} [RTn_i - {}_i v f_i({}_i v)],$$

or since the total symplast volume is

$$V = \sum_{i=1}^{m} {}_i v,$$

and since the total osmoles of symplast solute is

$$N_s = \sum_{i=1}^{m} n_i,$$

we have

$$P = \frac{RTN_s}{V} - \frac{F(V)}{V}, \tag{9}$$

where we have defined $F(V)$ by the parametric equations

$$F = \sum_{i=1}^{m} {}_i v f_i({}_i v) \quad \text{and} \quad V = \sum_{i=1}^{m} {}_i v,$$

solving Eq. (9) for $1/P$ makes it identical with Eq. (1).

It is worth noting that $F(V)/V$ plays a role analogous to $f_i({}_i v)$ in Eqn. (7), and closer inspection demonstrates that $F(V)/V$ is in fact a kind of 'averaged' turgor pressure of all the twig cells; the turgor pressure of each cell, ${}_i P_t = f_i({}_i v)$, is weighted according to its relative volume, ${}_i v/V$. We shall see that this expression for turgor pressure arises quite regularly in water relations and thus is worthy of a name. We shall call it the *Volume-Averaged Turgor pressure* or VAT pressure for short. The VAT pressure should not be confused with an alternative 'averaged' turgor pressure, averaged according to the number of cells regardless of size, $\sum {}_i P_t/m$, which may be termed the *cell-averaged turgor pressure*. (Thermodynamically the VAT pressure is the sounder way of averaging the turgor pressure since V times the VAT pressure is equal to the hydrostatic pressure component of the free energy of the water; this is not generally true for the cell-averaged turgor pressure.)

RESULTS AND DISCUSSION

The meaning and limitation of the theoretical expressions for the 'pressure–volume curve'.

Before proceeding to experimental results it is instructive to point out the consequences of the assumptions and the limitations involved in applying Eqn. (1) to interpret the 'pressure–volume curve' of a real twig.

Firstly, we have tacitly assumed that each cell has an ideal semipermeable barrier surrounding an ideal solute. If we wish to apply Eqn. (1) to a real experiment we must be prepared to allow for the fact that the rational activity coefficient of water, γ_j, will be less than unity and that the membrane will not be completely impermeable to some solutes (i.e. the reflection coefficient, σ_j, is less than one). Thus we must be willing to interpret the bulk osmotic pressure, π_t, as a complex

sum of terms for each solute species

$$\frac{RTN_s}{V} = G(\sigma_j, \gamma_j, N_j, V, \ldots). \tag{10}$$

Secondly, if we apply Eqn. (1) to real results we must recognize the possibility that the volume expressed from the twig under pressure will inevitably include an unknown fraction from the apoplast. This could arise in two ways: (*a*) Some air from the pressure-bomb always escapes through cavities in the wood and from long conducting elements of angiosperms. This would tend to flush out small quantities of water if any were present. This error is, under any circumstances, probably quite small and least in conifers which would not permit flushing of air–water menisci across pit membranes even at high pressures. It can also be argued that vessels accessible to air are probably already embolized and devoid of water. (*b*) Vessels and cell wall spaces could compress under the strain of the bomb pressure and thus yield water. But since conducting elements are capable of withstanding large stresses and since the lumina contain only a small part of the total water content of the twig this error is likewise probably small (Dixon, 1914; Scholander *et al.*, 1964, 1965, 1966).

Finally, we must recognize the limitations in our assumption of thermodynamic equilibrium in a real twig. Each balancing pressure, P, must be a true equilibrium value but, when moving from one equilibrium P_n at volume V_n to another equilibrium P_{n+1} at volume V_{n+1} we must destroy the equilibrium condition by applying an over-pressure, P_0, greater than the previous balancing pressure, P_n. Disequilibrium arises primarily because some cells are more permeable to water than others; the more permeable cells *approach* equilibrium with P_0 more rapidly than the less permeable cells. Consequently when the over-pressure is released the most permeable cells are more dehydrated than the others. The most permeable cells are most responsive to the balancing pressure, so erroneously high values of P_{n+1} will be found until water has exchanged between the cells of greater and lesser water permeability.

We need not be too concerned about the uncertainty of obtaining a true equilibrium value for the balancing pressure because operationally we can follow the approach to equilibrium by taking quasi-balancing pressure readings at regular intervals after releasing the over-pressure until the rate of decline is small. The typical over-pressure is 4 to 8 bar greater than the previous balancing pressure; in most species the rate of decline of the quasi-balancing pressure is small after 45 to 60 min. For *Pilgerodendron uvifera*, for example, we observed a rate of decline of less than 0·2 bar h^{-1} in the time interval of 20 to 40 min after the release of an over-pressure of 4 bars applied for 15 min. Fig. 3 gives the time course for the decline of the quasi-balancing pressure after an unusually large over-pressure (27 bar above the previous balance) had been applied for a period of 13 min to *Pilgerodendron uvifera*.

Cell turgor pressure and its relation to the linear region of the 'pressure–volume curve'

We can now consider the theoretical relation in terms of typical experimental results. Fig. 1 is a 'pressure–volume curve' of a 62-gm *Pilgerodendron uvifera* twig

collected from the grand island of Tierra del Fuego on 16 August 1970; the shape of this curve is typical of all 'pressure–volume curves' although it contains a rather larger number of points. In all cases the relationship between $1/P$ and V_e approaches a straight line of negative slope for sufficiently large values of V_e. Eqn. (1) may be rewritten:

$$\frac{1}{P} = \left(\frac{V_0}{RTN_s - F(V)}\right) - \left(\frac{1}{RTN_s - F(V)}\right)V_e. \qquad (11a)$$

Linearity must mean that for sufficiently large values of V_e ($= V_0 - V$), $F(V)$ must approach a constant (i.e. vary only slightly with respect to the constant RTN_s).

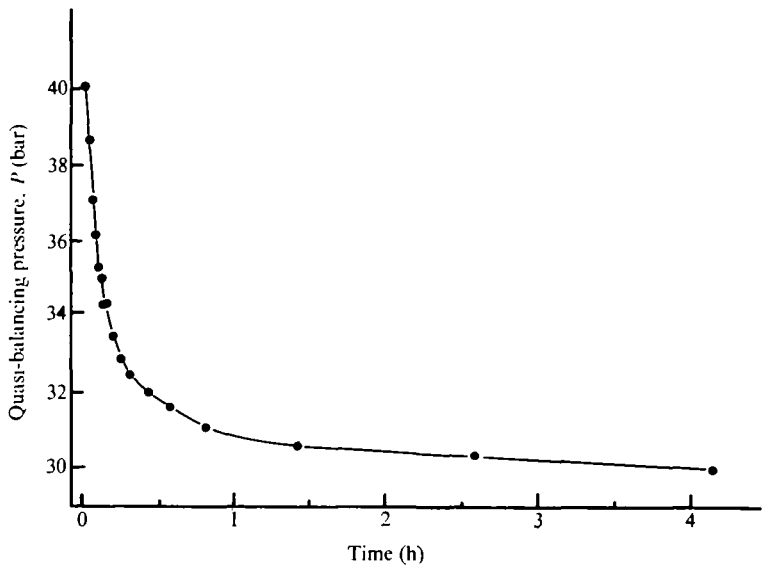

FIG. 3. The time decay of the quasi-balancing pressure in *Pilgerodendron uvifera* after an unusually large over-pressure (27 bar above the previous balance) has been applied for a period of 13 min.

It is important to learn something about the behaviour of $F(V)$ for large values of V_e (i.e. for the smaller values of V); if we can be sure that $F(V)$ is small compared to RTN_s in the linear region of the 'pressure–volume curve' then we can be sure that the intercept of the linear portion extrapolated to $V_e = 0$ is equal to the inverse of what we can call the original 'bulk osmotic pressure'

$$\pi_i^0 = \frac{RTN_s}{V_0}. \qquad (11b)$$

Furthermore, the slope of the linear portion would then approximate to the inverse of RTN_s. Lastly, if $F(V) \ll RTN_s$, every point on the line would give the inverse of the 'bulk osmotic pressure' at each V or volume expressed,

$$\pi_i(V) = \frac{RTN_s}{V} = \frac{RTN_s}{V_0 - V_e}. \qquad (11c)$$

Since $F(V)$ is related to the individual cell turgor pressures we can examine its qualitative behaviour. The turgor pressure of each cell, ${}_iP_i = f_i({}_iv)$, will always

assume positive values above the volume of incipient plasmolysis, ${}_iv_p$, and a value of zero at volume ${}_iv_p$. When ${}_iv$ falls below ${}_iv_p$ under the stress of the pressure bomb, the turgor pressure relation can behave in a number of different ways depending upon the mode of cell wall compression. If the cell wall should gently fold and collapse like the walls of a rubber balloon then the cell walls would take up little of the air-pressure stress and ${}_iP_l$ would only gradually grow negative with decreasing cell volume, ${}_iv$. Alternatively, if the cell wall is rigid (or restrained by neighbouring cells or vessels) then the cell wall will balance part of the air-pressure stress and ${}_iP_l$ would grow negative rapidly with decreasing ${}_iv$. Since each ${}_iP_l$ is a unique function for each cell, the bomb pressure at which ${}_iv_p$ is reached can differ from cell to cell.

$F(V)$ is a linear combination of ${}_iP_l$'s, $F(V) = \sum_{i=1}^{m} {}_iv f_i({}_iv)$; thus, since each $f_i({}_iv)$ eventually reaches zero and either stays nearly zero or grows more negative, $F(V)$ will also reach zero at some bomb pressure and either stay zero or grow more negative. The volume $V = \sum_{i=1}^{m} {}_iv$ at which $F(V)$ reaches zero we can call the volume of bulk incipient plasmolysis, V_p.

From the behaviour of the 'pressure–volume curve' in the linear region we have already concluded that $F(V)$ must reach a constant value. Since $F(V)$ does reach a zero at some $V = V_0 - V_e$ we believe that the constant value reached by $F(V)$ in the linear part of the curve is 'zero' (i.e. some value that is small with respect to RTN_s). Thus $V_p = V_0 - V'_e$ can be read from the 'pressure–volume curve' as being that volume expressed, V'_e, at which the non-linear part just becomes linear.

If we had argued that $F(V)$ reached some negative value comparable with or larger than RTN_s, then we would be faced with the less tenable position that some or all of the cells at first develop increasingly higher negative turgor pressures with decreasing cell volume and that at some volume sufficiently small they compress plastically. It is a more tenable assumption that the cells at first resist compression only slightly at volumes below ${}_iv_p$ and later at very small cell volumes develop resistance to compression; this is in essence what we argue when we assume that $F(V)$ assumes a value near zero. When the volume expressed approaches about two-thirds of the original volume, V_0, we do observe negative deviations from the 'pressure–volume curve' which are consistent with increasingly negative values of $F(V)$ with decreasing V at very small ${}_iv$'s (the dotted line in Fig. 1 shows how much the 'pressure–volume curve' would in theory deviate from linearity below V_p if the stress–strain relation for compression were symmetrical to that for cell expansion above V_p, cf. first section).

Because of the above argument we feel that the linear portion of the 'pressure–volume curve' (and the extrapolation to $V_e = 0$) is approximated by the relation

$$\frac{1}{P} = \frac{V_0}{RTN_s} - \frac{1}{RTN_s} V_e.$$

An empirical expression for the VAT pressure

From Eqn. (11) and our argument concerning the qualitative value of $F(V)$ in the linear region of the 'pressure–volume curve', we conclude that the most reason-

able interpretation of the linear part of the curve and its extrapolation is that it closely approaches the volume dependence of the 'bulk osmotic pressure', π_i (Eqn. (11*c*)).

Combining this interpretation with Eqn. (9) we see that it is possible to 'read' the volume dependence of the VAT pressure for volumes V ($= V_0 - V_e$) above V_p. The relation is

$$\text{VAT Pressure} = \frac{F(V)}{V} = \frac{RTN_s}{V} - P. \tag{12}$$

This expression simply states that at each value of V the volume-averaged turgor pressure is equal to the difference between the 'bulk osmotic pressure' and the balancing pressure; since the balancing pressure is equal to what the apoplastic water tension would be if P were zero, Eqn. (12) is very much like the definition of the turgor pressure of a single cell.

In all those species in which we have examined the volume dependence of the VAT pressure, we have found that the dependence most nearly fits the relation given in Eqn. (2) where the coefficient of non-linearity, n, assumes values in the range of 1·7 to 3·5. The appropriate values of ϵ and n can be obtained by making a log–log plot of the VAT pressure against $(V-V_p)/V_p$. This is done for several species in Figs. 4 and 5. The bulk modulus of elasticity, ϵ, is equal to the VAT pressure extrapolated to $(V-V_p)/V_p = 1{\cdot}0$ and n is given by the slope of the line. These values are recorded in Table 1.

Some uncertainty is involved in determining the values in Figs. 4 and 5. Firstly, we can read P only to within $\pm 0{\cdot}1$ bar. Secondly, the accumulated disequilibrium for some species can be as large as 0·3 or 0·4 bar; this, we think, explains the positive deviation for small relative volumes observed in some cases. Thirdly, a rather large uncertainty is involved in deriving an accurate estimate of V_p; the VAT pressure relation for the 'pressure–volume curve' in Fig. 1 is given in Fig. 4 (squares) yet from Fig. 1 we cannot pinpoint V_p any closer than in the range 15·7 to 16·2 cm^3 (corresponding to $4{\cdot}7 \leqslant V_e \leqslant 5{\cdot}2$ when $V_0 = 20{\cdot}9$). The exact values of ϵ and n and the closeness of the linear fit will depend upon our choice of V_p.

Lastly, we cannot be sure that any one VAT pressure relation will have a general predictive value for the species in question at all seasons. This is because cell walls are both plastic and elastic. During the growing season we might expect that tissues subjected to a prolonged high VAT pressure would experience a greater permanent deformation of the cell wall structure than tissues out of the growing season. This point is borne out by earlier studies in which tissues are subjected to osmotic stress. Frey-Wyssling (1952) has shown that a given tonicity in the bathing medium does not always lead to the same cell volume $_iv$. The cell assumes different volumes in the same solution depending on the osmotic equilibrium previously established and the duration of that equilibrium. Load–extension curves on the primary cell wall (epiderm cells of *Avena* coleoptile) shows that a new load after a release yields quite a different load–extension curve; the cell wall has a higher modulus of elasticity on the second determination than on the first.

Nevertheless, we do not feel that the determination of VAT pressure curves as in Figs. 4 and 5 are entirely fruitless exercises. Out of the growth season, plasticity

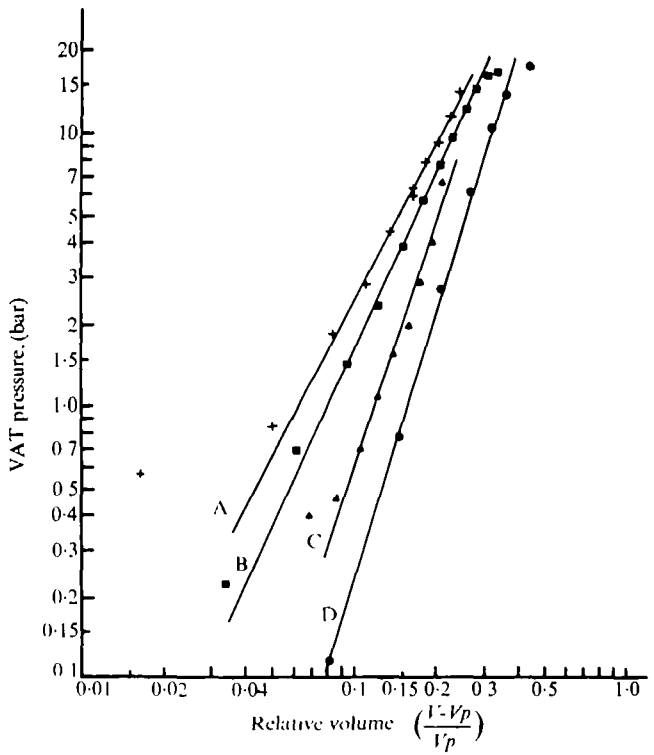

FIG. 4

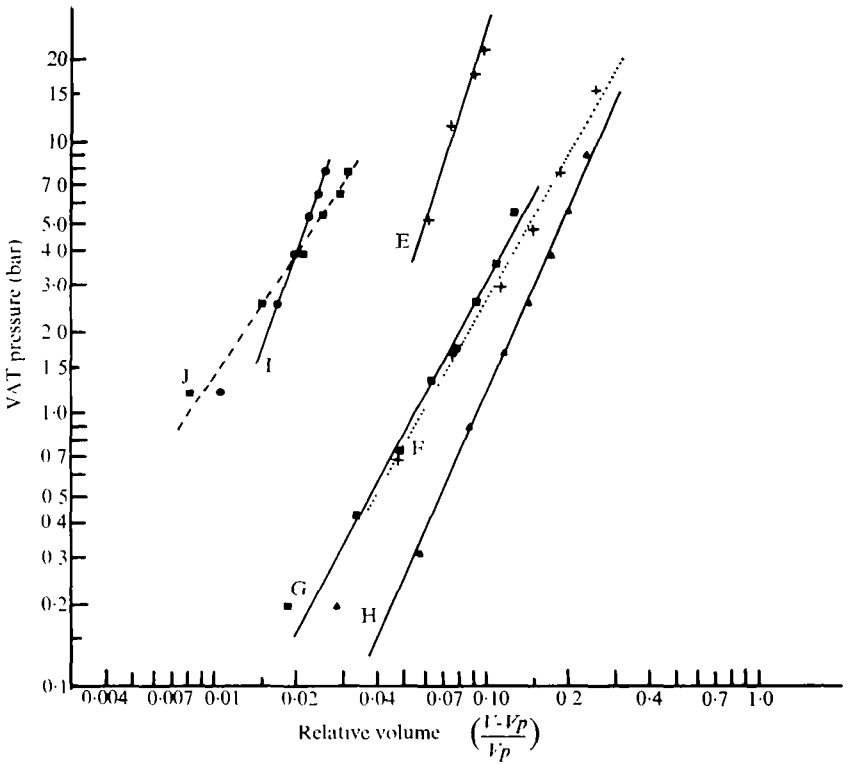

FIG. 5

FIGS. 4 and 5. The dependence of the volume averaged turgor pressure on the relative volume of the symplast. The log-log linearity indicates an empirical dependence given in Eqn. (2). (A) *Pilgerodendron uvifera* twig I, (B) *P. uvifera* twig II from 'pressure–volume curve' in Fig. 1, (C) *Podocarpus nubigenus*, (D) *Nothofagus betuloides*, (E) *Pernettya macronata*, (F) *Weinmannia trichosperma*, (G) Palomar Fir, (H) White spruce, (I) *Nitella translucens* cell II, (J) *N. translucens* cell V.

ought to be at the minimum. Furthermore, by measuring the VAT pressure curves with different equilibration times, at different seasons, or under different conditions, one could obtain some qualitative information on cell wall plasticity.

TABLE 1. *Values of the bulk modulus of elasticity, ϵ, and the coefficient of non-linearity, n, of twigs and cells*

Sample	ϵ (bar)	n
Pilgerodendron uvifera, twig I	$1{\cdot}9\times10^2$	1·9
P. uvifera, twig II	$2{\cdot}8\times10^2$	2·3
Podocarpus nubigenus	$6{\cdot}0\times10^2$	3·0
Nortofagus betuloides	$3{\cdot}7\times10^2$	3·7
Pernettya macronata	$4{\cdot}0\times10^3$	3·2
Weinmannia trichosperma	$1{\cdot}6\times10^2$	1·8
Palomar fir (*Abies concolor*)	$2{\cdot}3\times10^2$	1·9
White spruce (*Picea glauca*)	$1{\cdot}9\times10^2$	2·2
Nitella translucens cell II	$2{\cdot}3\times10^5$	2·8
N. translucens cell V	$1{\cdot}5\times10^3$	1·5

Lastly, it is worth noting that some plasticity has been observed in turgor pressure studies in *Nitella* by Kelly, Kohn, and Dainty (1962–3), but these workers observed that it disappears after exercising the cell wall. So perhaps in twigs subjected to daily water-stress cycles plasticity is at a minimum. Kelly *et al.* (1962–3) plotted the relative cell volume defined as $(V-V_0)/V_0$ (in our notation) versus the tonicity of the bathing medium. They observed a marked non-linearity on these single algal cells. Recalculating some of their data and plotting it as we did the VAT pressure (see Fig. 5) we find that these single cells obey equations of the same form as Eqn. (2). This lends support to our contention that the VAT pressure as defined here is a meaningful quantity.

The original volume of the symplast, V_0

The original volume of the symplast in a twig can be read from the 'pressure–volume curve' either by extrapolating the linear part down to the abscissa or by dividing the ordinate intercept by the slope of the line (cf. Eqn. 11*a*). If V_0 could be measured independently this would constitute a valuable test of the validity of the pressure-bomb theory.

Unfortunately it is rather difficult to estimate V_0 independently without laborious anatomical measurements. The pressure-bomb value of V_0 ranges from 0·65 to 0·90 of the total water content of our twigs (fresh minus dry weight); but we have no reliable measure of the total apoplastic water volume. The pressure-bomb V_0 would not include that volume of water which is bound to polymers. Boyer (1967*b*) estimates the relative cell wall volume (which is probably half water) to be 0·12, 0·14, and 0·28 for sunflower, yew, and rhododendron respectively; so we conclude that our values of V_0 compared to total water content are at least possibly correct. If a discrepancy did exist between V_0 and the true symplast volume it would indicate that either some cell population is excluded from water-potential equilibrium with

the rest of the tissue or that some living semi-permeable cell fraction has very rigid cell walls compared to parenchyma.

Comparison of the pressure-bomb technique with other methods

There is no other technique available which purports to measure the bulk osmotic pressure of the symplast and there is no other technique which gives an estimate of the VAT pressure without concomitant destruction of the cell membranes and without necessitating a correction for free space contamination (e.g. Ehlig, 1962).

There are, however, in common usage a number of techniques which measure the water-potential depression of the apoplastic water (which is in equilibrium with the symplast): viz. the qualitative measure of the water status (Weatherley, 1950) which can be roughly calibrated (Weatherley and Slatyer, 1957); the techniques which involve the steady-state gaseous diffusion from a small quantity of known solution (*a*) from a water drop of known volume (Macklon and Weatherley, 1965), (*b*) from a thermocouple where water is deposited by the Peltier effect (Spanner, 1951), (*c*) from a thermocouple to which known osmotic solutions are added (Richards and Ogata, 1958); by a null technique (isopiestic thermocouple psychrometer) developed by Boyer and Knipling (1965) which avoids complications associated with the leaf resistance to gaseous water diffusion; and by gravimetric techniques involving vapour exchange between leaf samples and salt solutions (Slatyer, 1958). An easy exercise employing the equilibrium relations presented earlier will demonstrate that the psychrometric and gravimetric techniques are intended to measure the apoplastic, Ψ_0, and symplastic, Ψ_i, water potentials given by

$$\Psi_0 = \Psi_i = \frac{RTn_i}{{}_iv_0} - {}_iP_t = \frac{RTN_s}{V_0} - \frac{F(V_0)}{V_0}. \qquad (13)$$

That is to say, these techniques should yield a value identical (in theory) to the initial balancing pressure in the pressure-bomb technique.

There are unfortunately a number of problems associated with reconciling the various experimental determinations in order to test the supposed theoretical identity. Boyer (1967*a*) employed what is perhaps the most accurate psychrometer technique available to compare the leaf water potential, obtained from the sum of the pressure-bomb balancing pressure and the osmotic potential of the xylem sap, with that obtained by the psychrometer. When leaf water potentials in yew, rhododendron, and sunflower were measured by the two techniques, the determinations were within ± 2 bar in sunflower and yew and within $+2{\cdot}5$ to $-4{\cdot}5$ bar in rhododendron. But it is rather difficult to say which of the two values is the more correct. From the time the samples are enclosed in the psychrometer chamber, the recorded values of Ψ_i vary by several bars for the first 6 h until reasonably more stable values are found (Barrs, 1965), but it has never been properly established whether the initial period of change is a true approach to vapour equilibrium or due to real water-potential changes in the leaf in response to cutting. Certainly Slatyer's (1958) gravimetric technique (which does not require vapour-pressure equilibrium for a determination of Ψ_i) indicates a change in Ψ_i of 3 bar for privet (*Ligustrum japonicum*) and 1 bar for tomato (*Lycopersicon esculentum*) between the

4th and 8th h after excision. It is well established that the respiration rate and the slow change to anaerobic conditions found in the psychrometer chamber will effect Ψ_i (Barrs, 1965). Furthermore, it has been observed that minute leaks of air found in psychrometer chambers and slight salt deposits on some leaf surfaces (which act as water-vapour sinks) can produce thermocouple outputs equivalent to a water-potential depression of 4 to 5 bar (Ehlig, 1962). Lastly, in growing tissues a prolonged stress can cause plastic transitions to cell wall structures which in turn could alter Ψ_i values (Frey-Wyssling, 1952). Similar transitions under prolonged stress have been observed in *Nitella* by Probine and Preston (1962).

Although it is not possible to make a firm judgement on whether the psychrometer or pressure-bomb gives the better initial water-potential value ($\Psi_i = \Psi_0$) it is worth noting that only 5 min are required to obtain pressure-bomb readings which are usually stable from the beginning (Boyer, 1967*a*), and therefore the samples are least removed (in time) from their water-potential state *in situ*.

The matric potential, Ψ_m

Boyer (1967*b*) once employed the pressure bomb in order to measure a quantity he called the matric potential of the leaf. He froze branches and leaves of sunflower, yew, and rhododendron to destroy the cell membranes. He then measured the balancing pressures after removing (via an over-pressure) successive volumes of solution; he took these balancing pressures as the measure of the matric potential (see Slatyer, 1967). Rhododendron required a balancing pressure of 4 bar to remove $\frac{3}{10}$ of the fluid whereas yew and sunflower required very much less. Boyer does not clearly define what he means by matric potential, but it is not unlikely that much of it consists of the resistance of the cell wall to mechanical compression. In this case Boyer's matric potential is our negative turgor pressure and is related to the macroscopic mechanical stresses and strains in cell walls as opposed to the microscopic swelling forces associated with the matric potentials in ion-exchange resins (Mears, 1968). If Boyer's matric potential includes terms in addition to negative turgor pressure then we would have to include additional volume-dependent terms ($V\Psi_m(V)$) in the denominator of the right-hand side of Eqn. (1). In view of the clear linearity of the 'pressure–volume curve' these matric potentials would have to be negligible (i.e. $RTN_s \gg V\Psi_m(V)$) for the same reason that $F(V)$ must be negligible.

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LITERATURE CITED

BARRS, H. D., 1965. Comparison of water potentials in leaves as measured by two types of thermocouple psychrometers. *Aust. J. biol. Sci.* **18**, 36–52.

BOYER, J. S., 1967*a*. Leaf water potentials measured with a pressure chamber. *Pl. Physiol., Lancaster*, **42**, 133–7.

—— 1967*b*. Matric potentials of leaves. Ibid. **42**, 213–17.

—— and KNIPLING, E. B., 1965. The isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. *Proc. natl Acad. Sci. U.S.A.* **54**, 1044–51.

CHIBNALL, A. C., and GROVER, C. E., 1926. The extraction of sap from living leaves by means of compressed air. *Ann. Bot.* **40**, 491–6.

DIXON, H. H., 1914. *Transpiration and the ascent of sap in plants*. MacMillan & Co. Ltd., London (see pp. 139 ff.).

EHLIG, C. F., 1962. Measurement of energy status of water in plants with a thermocouple psychrometer. *Pl. Physiol., Lancaster*, **37**, 288–90.

FREY-WYSSLING, A., (Ed.), 1952. *Deformation and flow in biological systems*. North-Holland Publishing Co., Amsterdam.

HAINES, F. M., 1935. Transpiration and pressure deficit. I. Apparatus and preliminary experiments. *Ann. Bot.* **49**, 213–381.

HAMMEL, H. T., 1967. Freezing of xylem sap without cavitation. *Pl. Physiol., Lancaster*, **42**, 55–66.

—— 1968. Measurement of turgor pressure and its gradient in the phloen of oak. Ibid. **43**, 1042–8.

KELLY, R. B., KOHN, P. G., and DAINTY, J. 1962–3. Water relations of *Nitella translucens*. *Trans. bot. Soc. Edinb.* **39**, 373–91.

MACKLON, A. E. S., and WEATHERLEY, P. E., 1965. Vapour-pressure instrument for the measurement of leaf and soil water potential. *J. exp. Bot.* **16**, 261–70.

MEARS, P., 1968. Transport in ion-exchange polymers. In *Diffusion in polymers*. Crank, J., and Park, G. S. (Eds.). Academic Press, London.

PROBINE, M. C., and PRESTON, R. D., 1962. Cell growth and the structure and mechanical properties of the wall in internodal cells of *Nitella opaca*. II. Mechanical properties of the walls. *J. exp. Bot.* **13**, 111–27.

RICHARDS, L. A., and OGATA, G., 1958. Thermocouple for vapor pressure measurement in biological soil systems of high humidity. *Science, N.Y.* **128**, 1089–90.

SCHOLANDER, P. F., BRADSTREET, EDDA D., HAMMEL, H. T., and HEMMINGSEN, E. A., 1966. Sap concentrations in halophytes and some other plants. *Pl. Physiol., Lancaster*, **41**, 529–32.

—— HAMMEL, H. T., HEMMINGSEN, E. A., and BRADSTREET, E. D., 1964. Hydrostatic pressure and osmotic potentials in leaves of mangroves and some other plants. *Proc. natl Acad. Sci. U.S.A.* **51**, 119–25.

—— —— BRADSTREET, EDDA D., and HEMMINGSEN, E. A., 1965. Sap pressure in vascular plants. *Science, N.Y.* **148**, 339–46.

SLATYER, R. O., 1967. *Plant-water relationships*. Academic Press, London.

—— 1958. The measurement of diffusion pressure deficit in plants by a method of vapour equilibrium. *Aust. J. biol. Sci.* **11**, 349–65.

SPANNER, D. C., 1951. The Peltier effect and its use in the measurement of suction pressure. *J. exp. Bot.* **2**, 145–68.

WEATHERLEY, P. E., 1950. Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytol.* **49**, 81–97.

—— and SLATYER, R. O., 1957. Relationship between relative turgidity and diffusion pressure deficit in leaves. *Nature, Lond.* **179**, 1085–6.