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Determination of the Volumetric Modulus of Elasticity of Wheat Leaves by Pressure-Volume Relations and the Effect of Drought Conditioning[1]

Jeffrey J. Melkonian, Joe Wolfe, and Peter L. Steponkus[2]

ABSTRACT

A pressure-volume (P-V) method was modified to facilitate the rapid determination of more points in the positive turgor region of P-V dehydration curves of vernalized winter wheat (*Triticum aestivum* L. cv. Yorkstar) leaves. This allowed for a more accurate estimate of the volumetric modulus of elasticity (ϵ) and its dependence on turgor. P-V relations during dehydration in a pressure bomb were characterized for leaves from either well-watered plants (control, solute potential, $\psi_s = -1.0$ MPa) or plants previously subjected to two stress cycles (conditioned, $\psi_s = -1.4$ MPa). Prior to P-V determinations, the plants were brought to maximum hydration by thorough watering and maintenance in the dark for 12 hours (water potential $\Psi = -0.02$ MPa and -0.07 MPa for control and conditioned leaves, respectively). Additionally, P-V relations of leaves from conditioned plants subjected to a third stress ($\psi_s = -1.9$ MPa) were determined. In this case, leaves were rapidly rehydrated in the pressure bomb prior to the P-V measurements. For leaves of both control and conditioned plants, ϵ was constant and non-hysteretic over a range of applied pressure of 0.0 to 0.8 MPa, i.e., at high values of turgor. The turgor dependence of ϵ at higher applied pressures (i.e., lower turgor pressures) was not considered because of the unresolvable influence of variations in ψ_s of individual cells in the leaf. Leaves of conditioned plants had a significantly higher ϵ (28 MPa) than leaves of control plants (22 MPa). The value of ϵ of leaves of plants subjected to three stress cycles and rapidly rehydrated in the pressure bomb before determination of the P-V relations increased to 40 MPa—significantly different from plants subjected to two stress cycles and slow rehydration.

Additional index words: *Triticum aestivum* L., Pressure bomb, Turgor potential, Solute potential, Capillary system.

THE pressure-volume (P-V) technique, originally introduced by Scholander et al. (1964), has been frequently used to measure water potential (Ψ) and it components, solute potential (ψ_s) and turgor potential (ψ_p) of a wide variety of tissues (Tyree and Hammel, 1972); Goode and Higgs, 1973; Hellkvist et al., 1974; Richter, 1978; Wenkert et al., 1978). The technique has also been used to estimate the volumetric modulus of elasticity (ϵ) (Tyree and Hammel, 1972; Cutler et al., 1979). Theoretical aspects of the technique have been detailed by Tyree and Hammel (1972) with potential sources of error, especially in the determination of ϵ, discussed by Cheung et al. (1976).

Typically, P-V relations are plotted as the inverse of the applied pressure (1/P) vs. the cumulative volume or weight of the expressed sap or residual weight of the tissue. Such plots exhibit a rapid curvilinear decline at low applied pressures, a region where turgor is maintained, with a slow linear decline at higher applied pressures, when turgor has been reduced to zero. This behavior is interpreted as the osmometric behavior of the protoplasts (Tyree and Hammel, 1972) assuming that under compression the cell walls have negligible mechanical strength (i.e., assuming substantial negative values of ψ_p are not possible). Estimates of ψ_s are made from the linear region which is described by the Boyle-van't Hoff relation, i.e., volume expressed varies linearly with 1/P. Estimates of ϵ are made from the curvilinear region where positive turgor is maintained. Two major difficulties arise, however, in such determinations of ϵ: a limited number of observations at low applied pressures and difficulty in estimating the point of zero turgor (Cheung et al., 1976). Frequently it is assumed that the region where positive turgor is maintained extends to the point where the relationship is described by the Boyle-van't Hoff law, i.e., the linear region of a plot of 1/P vs. V. This point is, however, an extreme value (Cheung et al., 1976) and at lower applied pressures varying proportions of the cells will be at zero turgor (Virgin, 1955) and estimates of ϵ will be confounded.

Generally, a dependence of ϵ on ψ_p is inferred from such P-V relations (Hellkvist et al., 1974; Cutler et al., 1979; Jones and Turner, 1980). This is consistent with results obtained by direct measurements of individual cells with pressure probes (Zimmerman and Steudle, 1975; Steudle et al., 1977; Hüsken et al., 1978). While Cheung et al. (1976) also reported that values of ϵ obtained from P-V relations of tissues exhibited a strong dependence on the volume averaged turgor (VAT) pressure, and in several instances varied roughly linearly; at higher values of VAT pressure, ϵ was nearly constant. Similarly, although direct measurements of individual cells with pressure probes show that ϵ increases linearly

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[2] Graduate research assistant, research associate, and professor, respectively, Dep. of Agronomy, Cornell Univ., Ithaca, N.Y. 14853.

with increases in turgor, this is usually restricted to the lower values of turgor and ϵ appears constant at higher values of turgor in both giant algae cells (Zimmerman and Steudle, 1975) and *Capsicum annuum* cells (Hüsken et al., 1978). Only in leaf bladder cells of a halophyte, *Mesembryantheum crystallinum,* did the pressure and volume dependence of ϵ extend up to the highest turgor pressures. Since models of the P-V relations of hypothetical shoots composed of cells with varied values of ψ_s but similar and constant values of ϵ closely approximate typical P-V curves (Cheung et al., 1976), a differential loss of turgor in the population of cells contributes to the curvilinear relation—and is especially pronounced at lower turgor pressures, i.e., higher applied pressures. Thus, there is the dilemma that while direct measurements of individual cells suggest a pressure dependency of ϵ at low turgor pressures, this dependency is difficult to resolve from P-V relations of tissues. Hence estimates of ϵ which encompass all of the data points in that region of a 1/P vs. V plot which is not linear are not appropriate and the conclusions regarding the dependence of ϵ on ψ_p not necessarily valid. Recently, Campbell et al. (1979) assumed a differential loss of turgor and determined that ϵ was constant, i.e., independent of changes in turgor, at higher turgors in wheat. In other previous reports, P-V relations for leaves have lacked sufficient detail to resolve the issue due to the difficulty of collecting a large number of accurate data in a relatively short time over which evapotranspiration may be safely neglected. We have modified the pressure-volume technique reported by Cutler et al. (1979) to obtain a large number of points in the positive turgor range while minimizing the time required for the measurements.

Accurate estimates of ϵ are important for the elaboration of plant water relations under drought stress since cell wall elasticity influences the relation between water content and water potential (Dainty, 1972; Cheung et al., 1975, 1976; Zimmerman, 1978). Genotypic differences and the influence of drought conditioning on ϵ are of special concern. Estimates of ϵ for various species vary widely (Dainty, 1976), and there are conflicting reports of the change, if any, in ϵ as a result of drought conditioning. For example, although Sanchez-Diaz and Kramer (1971) inferred that ϵ is higher for sorghum (*Sorghum bicolor* L.) than for maize (*Zea mays* L.) similar values of ϵ were inferred for two varieties of sorghum which reputedly differed in drought resistance (Jones and Turner, 1978). While water stress decreased ϵ in *Vicia faba* (Kassam and Elston, 1974; Elston et al., 1976), a doubling of ϵ in drought-conditioned sorghum was reported (Jones and Turner, 1978). In contrast, no significant change in ϵ as a result of drought conditioning was observed in either cotton *(Gossypium hirsutum)* (Hsiao et al., 1978), rice (*Oryza sativa* L.) (Cutler et al., 1979), or sunflower (*Helianthus annuus* L.) (Jones and Turner, 1980). Campbell et al. (1979) reported no significant difference in ϵ of winter wheat (*Triticum aestivum* L.) grown under relatively dry vs. relatively wet field conditions. In this paper, we report a modified P-V method which facilitates the rapid determination of more points in the positive turgor region and the use of this method to determine: 1) the P-V relations for wheat leaves, 2) whether ϵ varies with turgor pressure, and 3) whether drought conditioning and subsequent water stress alters ϵ.

MATERIALS AND METHODS

Seeds of a winter wheat (cv. Yorkstar) were soaked for 24 hours in aerated tap water, sown in vermiculite, and placed in a greenhouse (24 C day/ 16 C night). One week after planting, the seedlings were vernalized at 2 C for 12 weeks (10-hour daylength). After vernalization, seedlings were transplanted to 12-inch clay pots containing a silt loam, four plants/pot. These were placed in a greenhouse (24 C day/16 C night) receiving supplemental light from metal halide lamps on a 12-hour light/12-hour dark schedule. After 10 days, the plants were thinned to two plants/pot. The pots were thoroughly watered twice daily and fertilized weekly with soluble fertilizer. Plants were drought conditioned at the five to six leaf stage (three to five tillers/plant). Water was withheld until predawn leaf water potentials ($\Psi_{predawn}$) were approximately -1.0 MPa. The plants were then rewatered for 2 days and the treatment repeated. During these stress treatments, control plants were well-watered. After completion of the second stress treatment, the stress plants were rewatered for 2 days. At that time, both control and conditioned plants were subjected to a severe drought stress. Water was withheld until $\Psi_{predawn}$ ranged from -1.5 MPa to -2.0 MPa (approximately 10 days after initiation of the stress). During this 10 day stress, leaf water potentials (Ψ) were measured with a pressure bomb on four conditioned plants and two control plants daily.

Leaf osmotic potentials (ψ_s) were determined by the P-V method described by Cutler et al. (1979), and turgor potentials (ψ_p) calculated by the difference between Ψ and ψ_s. The (apparent) bulk modulus of elasticity (ϵ) was determined from P-V relations obtained by the modification presented in the Results section. All leaves used in this experiment were the most recently mature leaves on main tillers. Leaves were excised at predawn, immediately wrapped in aluminum foil, encased (3 to 4 cm of the cut end exposed) in two plastic bags to which a small amount of water had been added and then placed in the pressure bomb.

RESULTS AND DISCUSSION

Procedure for Determining P-V Relations

Typically, when used to estimate Ψ and its components, ψ_s and ψ_p, P-V methodology requires only a good estimate of the linear portion of a 1/P vs. V plot that represents Boyle-van't Hoff behavior. Usually only a few points in the initial region, and not more than 15 or 20 points over the entire range, are recorded. Generally, an equilibrium balance pressure is obtained by waiting for an appropriate period of time that is unique for different tissues and must be empirically determined. Such an approach limits the number of observations that may be obtained and increases the possibility of desiccation; and there is often considerable spread in the data about the Boyle-van't Hoff line, suggesting experimental inaccuracies.

Alternatively, if the time course of sap efflux is known, balance points can be predicted and then quickly measured by applying an excessive pressure and reducing it to the predicted value. A capillary system (Fig. 1) was used to measure the time constants of sap efflux and influx during rehydration. The cut end of the leaf protruded from the pressure bomb into a water-filled antechamber which was secured to the top of bomb and connected to a water-filled capillary. Seals between the pressure bomb, the antechamber, and capillary were air tight. The level of water in the capillary was determined with a linear scale attached to the capillary. Following stepwise increments in pressure, the rise of the water column in the capillary was measured with time until an equilibrium was established, i.e., the meniscus stopped moving. (This procedure is only possible in healthy leaves. When pressures are applied to insect-scarred or broken leaves, gas bubbles are pumped through the xylem and make this measurement difficult. For such leaves, however, the time course of sap influx may be assessed after a step decrement to zero in the applied pressure). The response of the sap exudation to a step change in pressure was well fitted by an exponential approach to its new equilibrium value (Fig. 2a). Plots of the log of disequilibrium displacement against time are approximated by straight lines, the slope of which defines τ, the time constant of the response to pressure changes (Fig. 2b). Note, however, that the fit is pragmatic or descriptive, rather than the manifestation of a physical law. We observe that if the hydrodynamic resistance from each cell to the cut end of the leaf was the same, τ would be the ratio of this resistance to the volumetric modulus per unit volume. This resistance, however, is likely to be different for cells in different parts of the leaf. Thus the quality of the exponential fit is fortuitous, but is of practical use in the obtaining of P-V plots and also yields qualitative information about the resistance to flow in the leaf. (A detailed analysis of efflux curves requires many parameters,

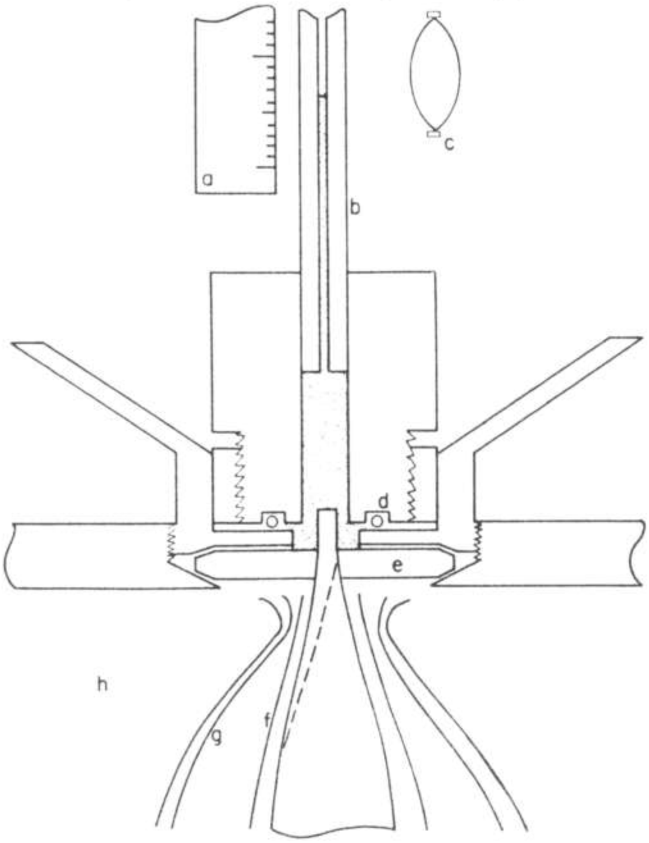

Fig. 1. The pressure-bomb capillary apparatus used to observe sap efflux from leaves under applied pressure: a) scale, b) glass capillary, c) lens, d) "O" ring seal, e) pressure bomb collar seal, f) aluminum foil, g) two plastic bags, h) pressurized N_2 atmosphere.

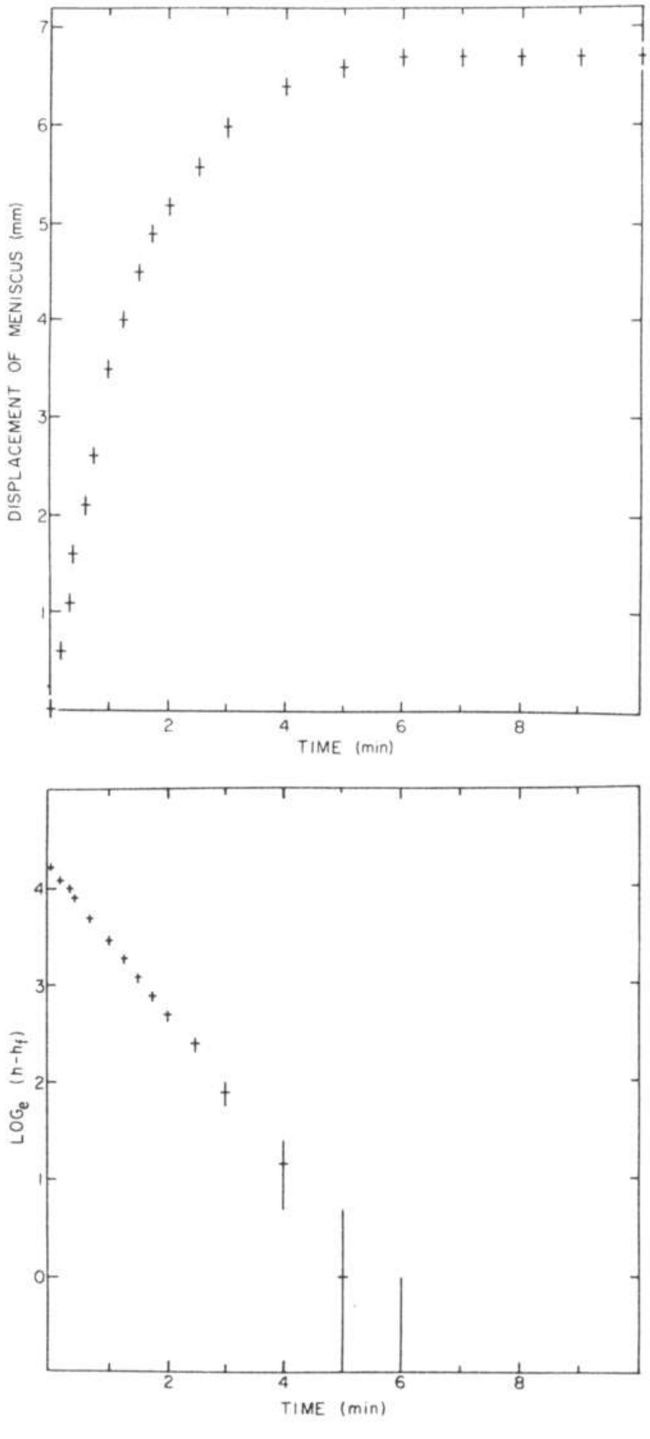

Fig. 2. A. The efflux of sap with time from a leaf to which is applied a step change in pressure of 0.5 MPa. Bars represent estimated errors, as described in Results and Discussion.

B. The same data plotted logarithmically to demonstrate the suitability of the descriptive equation.

$$h = h_f - (h_i - h_f)\, e^{-t/\tau}$$

where h_i and h_f are the initial and final values of the meniscus displacement h and τ the time constant thus defined.

some of which are impossible to measure (Stroshine et al., 1979.)). The average time constant of sap efflux (τ) for leaves of control plants at maximum hydration ($\Psi = -0.02$ MPa) was 20 ± 5 sec ($n = 11$). For leaves of conditioned plants, $\tau = 27 \pm 5$ sec ($n = 8$) for plants at maximum hydration ($\Psi = -0.07$ MPa) and 41 ± 4 sec ($n = 6$) for plants in a stressed state ($\Psi = -1.6$ MPa). It should be noted that the application of this technique for determining P-V relations in other species will be determined by the time constants for efflux and whether efflux follows a simple exponential.

Following the determination of time constants of sap efflux, P-V relations were determined as follows:

1) An initial balance point was found at which an applied pressure produced no movement of the sap. 2) Pressure, ΔP, was rapidly increased from this balance pressure, P, and maintained for a period T. 3) Pressure was then lowered to $P + \Delta P(1 - e^{-T/\tau})$. If ΔP was small, the new pressure was very close to a new balance pressure and fine adjustments were made to stop the flow. 4) The exuded sap was either collected in a small (2 dram) vial containing dry cotton and weighed or the volume of exuded sap was determined by using the capillary system. This procedure was repeated several times in the positive turgor range. Measurement errors in P and V were estimated by repeated measurements of the pressure needed to produce a balance point with a given exudation of sap and by repeated weighing of vials or measurements of a volume displacement in the capillary tube. A representative plot of the P-V relations so obtained is shown in Fig. 3. Using this approach it was routinely possible to obtain approx. 15 data points in the non-linear region. With control leaves this required ~5 min.

Calculation of ϵ

Tyree and Hammel (1972) define a parameter which they call the "bulk modulus of elasticity, ϵ" with the equations

$$(RTN_s/V) - P = F(V)/V = \epsilon[V - V_p)/V_p]^n, V \geq V_p$$

where R is the gas constant, T the absolute temperature, N_s the total number of osmoles of solute in all the living cells, P is the applied pressure, V is "the original volume of all the living cells having reasonably pliable walls (less) the volume of water expressed from all the cells", V_p is "a bulk volume at 'incipient' plasmolysis", n is a coefficient of non-linearity and where the equation on the left defines F(V), which they prove to be solely a function of volume, and the equation on the right is an empirical relation in which ϵ and n are chosen to fit the data. This unusual definition of bulk modulus of elasticity may be rationalized as follows: if $n = 1$, and if the apoplastic water volume does not change when pressue is applied, and if F(V)/V represents the volume averaged turgor, and if V_p is a well defined parameter of the tissue, then ϵ is minus one times the bulk modulus of the symplastic volume when the tissue is "at incipient plasmolysis", and where "volume averaged turgor" replaces "pressure" in the usual definition of bulk modulus (e.g., Weast, 1972).

Cheung et al. (1976) define a bulk modulus ϵ by $\epsilon = dP_{VAT}/dF$ where P_{VAT} is the volume averaged turgor pressure and $F = (V_o - V_p - V_e)/V_p$ where V_e is the volume of water expressed and V_o is the tissue symplast volume at $V_e = 0$. Thus

$$\epsilon_{CHEUNG} = -V_p(dP_{VAT}/dV_e)$$

Both ϵ_{TYREE} and ϵ_{CHEUNG} are defined in terms of derived quantities, and in particular depend on the rather uncertain estimation of V_p. We have therefore chosen to define the tissue-water bulk modulus, defined by

$$\epsilon = V_w(\Delta P/\Delta V)$$

where V_w is the volume of water in the leaf at full hydration (calculated from the difference between the dry weight and the fresh weight of the leaf after the experiment plus the weight of water expressed since full hydration) and ΔV is the volume expressed between balance pressures differing by ΔP. This is simply the bulk modulus of the volume of water in the tissue, calculated at full hydration, and where "applied pressure" replaces "pressure" in the usual definition of bulk modulus (Weast, 1972). It is an unambiguously determined parameter of the leaf.

Because Tyree and Hammel choose $n \neq 1$, ϵ_{TYREE} cannot be easily compared with ϵ_{CHEUNG} or ϵ as defined above. The latter pair, however, may be easily compared:

$$\epsilon/\epsilon_{CHEUNG} = (V_w/V_p)(\Delta P/-\Delta P_{VAT})$$

The first factor (the ratio of water volume at full hydration to water volume at "incipient plasmolysis" less apoplastic volume) may be estimated with only that accuracy with which V_p may be estimated, and is constant for any leaf. For cereal leaves it would be about 1.12 to 1.25. (In our study it was very nearly constant for all leaves, since those leaves with the highest ϵ had the highest $|\psi_s|$, and conversely: i.e., leaves generally lost turgor at around the same fractional volume, independent of $|\psi_s|$, though this is difficult to quantify because of the difficulty in estimating V_p.)

The second factor is greater than unity because of the volume dependence of the osmotic pressure. In the Appendix we show that this factor is nearly constant and ≈ 1.06. Thus, in order to compare with ϵ_{CHEUNG}, ϵ ought to be reduced in all cases by about 18%.

When plotted in the traditional manner of 1/P vs. V (Fig. 3), the P-V relations exhibit the typical curvilinear behavior with a steep non-linear decline at low applied pressures and a gradual linear decline at higher applied pressures. Because V varies linearly with 1/P in the zero turgor region, ψ_s can be readily inferred from such a plot. If ϵ, defined as V(dP/dV), is of interest, however, a plot of P vs. V is more useful.

Figure 4 illustrates the same data as Fig. 3, but plotted in the form of P vs. V. In this and all other P-V plots we have constructed, the region between 0.0 and 0.8 MPa appears linear, i.e., ϵ is independent of turgor in this range. To quantify this observation we have also fitted this region with curves of the form $\Delta P = \epsilon_o(V/V)^{1+n}$ where n

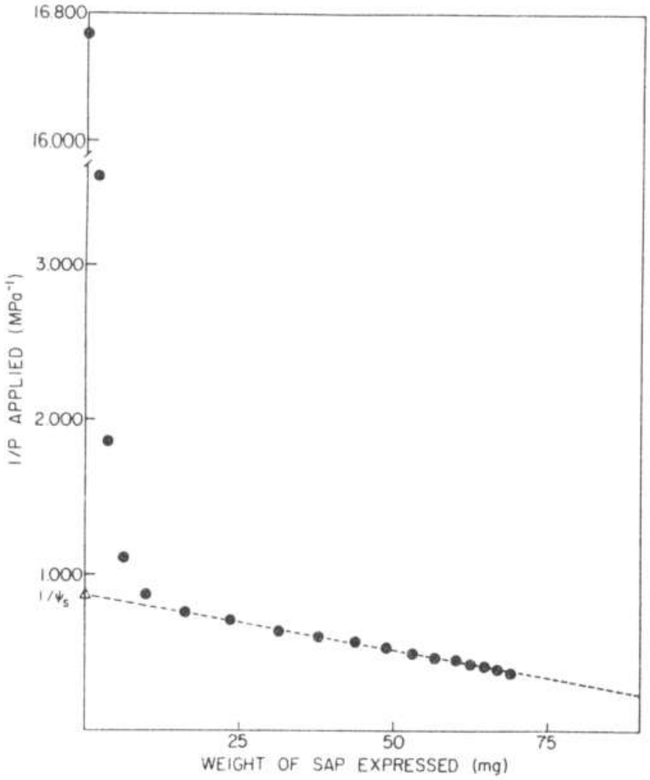

Fig. 3. P-V relation of a single leaf plotted as 1/P applied vs. weight of sap expressed. γ-intercept of the dashed line is the inverse of ψ_s at full hydration.

is a coefficient of non-linearity (after Tyree and Hammel, 1972). Pooling data from 20 experiments, we have applied the residue test of Wolfe and Bagnall (1979) and conclude that the hypothesis that $n \geq 0.1$ may be rejected at the 99% confidence level.

We note in the Appendix that P_{VAT} is very nearly proportional to P, and thus the linear dependence of V on P at high turgor implies a linear dependence of V on P_{VAT}. (Explicitly, linear regressions of P_{VAT} vs. V_e over the range $P = 0.0$ to 0.8 MPa gave r^2 values greater than 0.99). Thus we conclude that the modulus of elasticity, whether defined as V(dP/dV) or ϵ_{CHEUNG}, is independent of turgor over the range of applied pressure 0.0 to 0.8 MPa. In routine measurements of ϵ, however, we did not continue past this range, and thus did not compute ψ_s, V_p, P_{VAT} for all specimens.

The Boyle-van't Hoff relation is a rectangular hyperbola on a P-V plot and at high applied pressures such a curve is an excellent fit to the data over a wide range. In the P-V plot (Fig. 4) there is a curvilinear region between the linear elastic region and the Boyle-van't Hoff region. This curvilinear region may be attributable to differences in ψ_s among the individual cells in the leaf and/or a turgor dependence of ϵ. Since it is reasonable to expect a variation in ψ_s among individual cells, we concur with Virgin (1955) and Campbell et al. (1979) that the curvilinear region could result from a varying proportion of the cells reaching zero turgor at higher water potentials than others. That is, in this region some cells have zero turgor and exhibit characteristic Boyle-van't Hoff behavior whereas other cells are turgid and their volume is limited by the

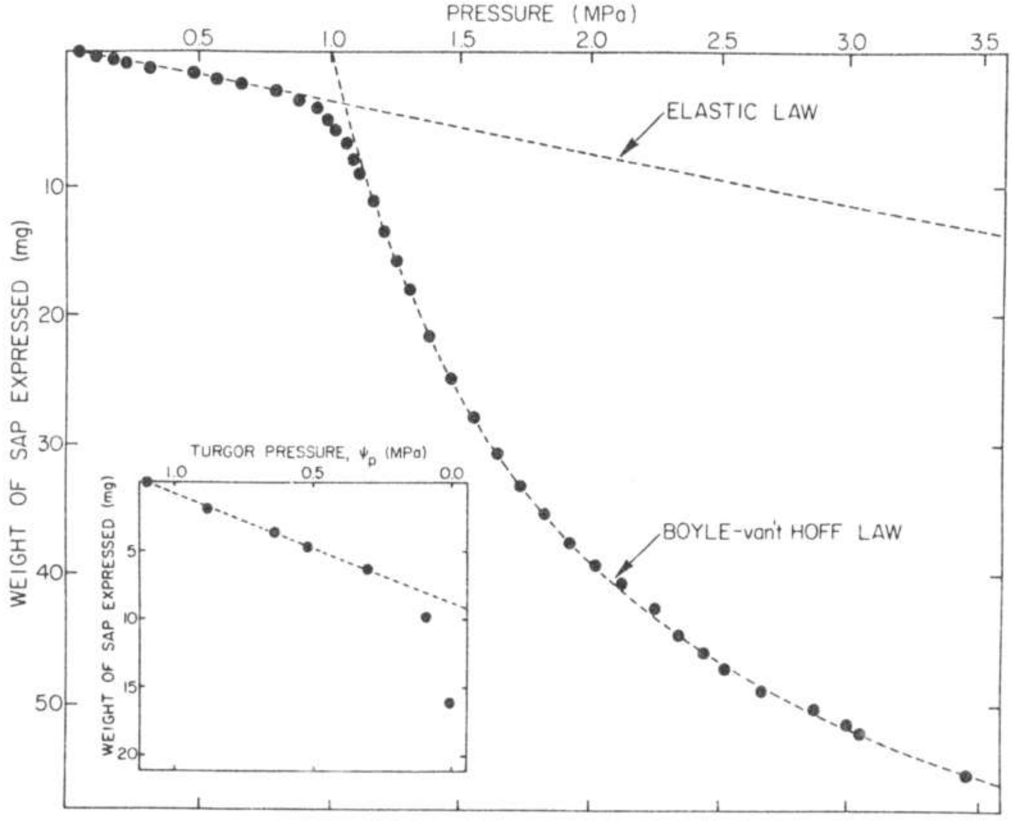

Fig. 4. Detailed pressure-volume relation for a leaf from control plants. The vertical axis effectively shows the volume of the leaf inferred from the weight of sap expressed. Inset: ψ_p vs. weight of sap expressed for the same leaf.

properties of the cell wall. This portion would therefore reflect variation in solute potentials among different cells in the leaf, although a treatment of this distribution would require a more detailed experimental measurement of this region of the P-V relation. Although it is within this region that a turgor dependency of ϵ is inferred from direct measurements of single cells with a pressure probe (Zimmerman and Steudle, 1975; Hüsken et al., 1978), the extent to which the curvilinear region of P-V relations of tissues is a function of variations in ψ_s in the population of cells vs. a turgor dependence of ϵ cannot, at this time, be resolved. [Note added in proof—Tyree (1981) calculates bulk tissue moduli for several model systems in which solute potentials are varied between cells.] Hence, it would seem appropriate, for the present, not to include data points from this region in the estimation of ϵ. The value of ϵ inferred solely from the linear region of P-V plots corresponds to the ϵ_{max} of Cheung et al. (1976). As they have suggested that the point of zero turgor inferred from 1/P vs. V plots (i.e., the point at which the relationship becomes linear) is an extreme value (when the last cell(s) lose turgor), the point of departure from the linear relationship in a P vs. V plot may represent the other extreme—when the first cells lose turgor. Hence, a range over which a varied proportion of cells are at zero turgor may be defined.

To determine whether ϵ was a well defined parameter for a particular leaf, we investigated its short-term time dependence; specifically, whether it depended on the rate of deformation (i.e., exhibited viscoelasticity) or whether it varied in successive determinations (i.e., exhibited hysteresis). Successive dehydrations of the same leaf were conducted between rehydrations. Modification of the pressure bomb with the capillary system (Fig. 1) allowed

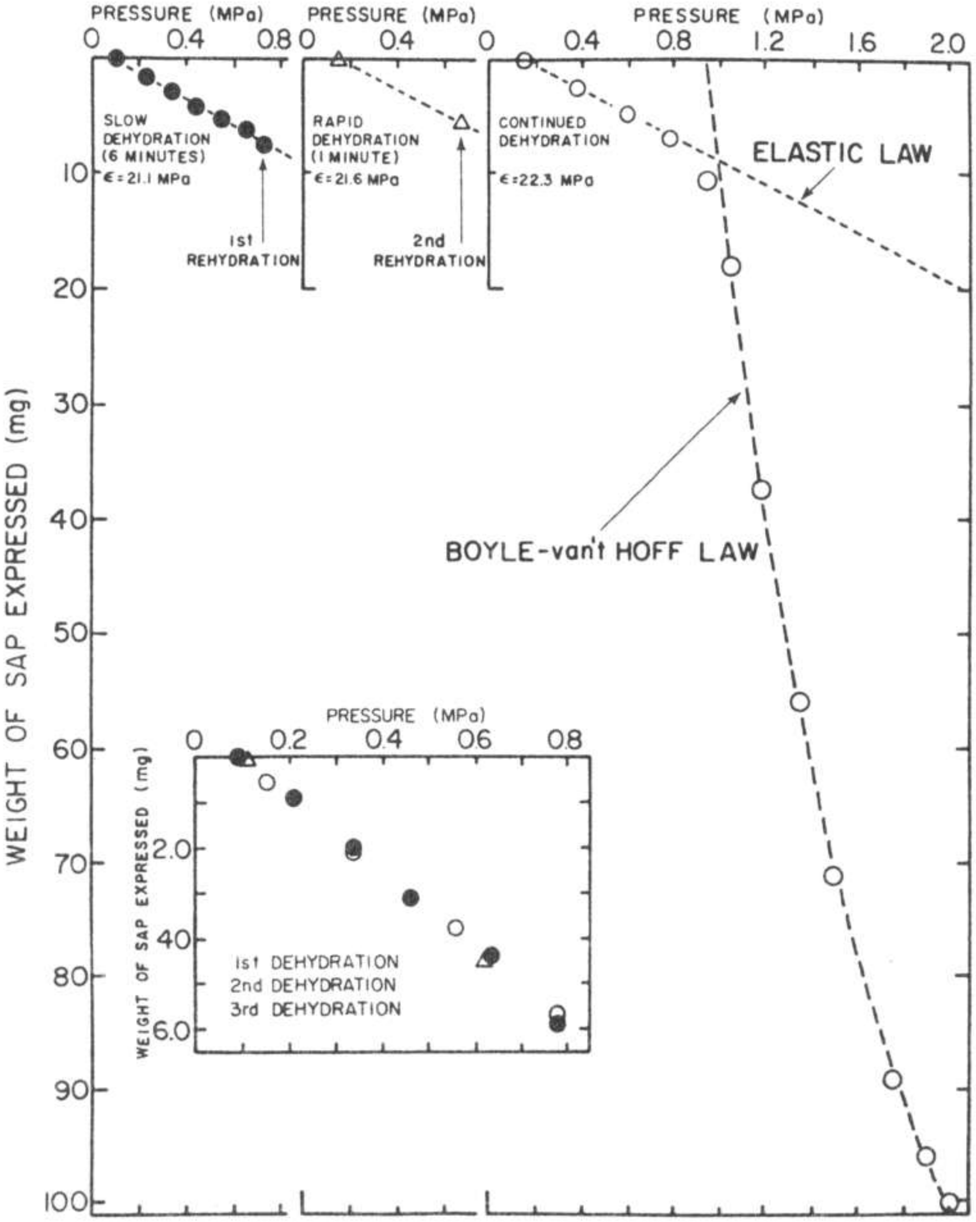

Fig. 5. Successive dehydrations of a leaf from a control plant at different dehydration rates.

for rapid rehydration of the leaf while it remained in the pressure chamber. By decreasing the applied pressure, water in the capillary and antechamber was reabsorbed by the leaf. A period of 10 min was adequate if the leaf was rehydrated from 1.5 MPa of applied pressure. In each of the three hydrations similar values of ϵ were inferred, independent of the period of the measurement (Fig. 5). Furthermore, while the pressure increments, and therefore the elapsed time, were varied during successive dehydrations, the values of ϵ were constant to the accuracy of the experiment. Thus, over the pressures and rather brief times imposed, the P-V relations did not reflect either hysteresis or viscoelasticity in spite of repeated dehydration or rapid rehydration of the leaf while it remained in the pressure bomb.

We have thus demonstrated that a value of ϵ (ϵ_{max} of Cheung et al., 1976) can be determined with confidence in the region of high turgor by the method described. In contrast, measurement of ϵ from the slope between points in the positive turgor and a point inferred to be zero turgor (start of the linear Boyle-van't Hoff region in a 1/P vs. V plot; cf., Wenkert et al., 1978; Cutler et al., 1979; Jones and Turner, 1980) would give a very different value of ϵ, and a value which decreased at low turgors.

Effect of Drought Conditioning on ϵ

For plants maintained under well watered conditions (ψ_s = −1.0MPa), a value of ϵ of 22.3 ± 4.7 MPa (n = 11) was determined for the most recently matured leaf. For plants subjected to two conditioning stress periods and subsequently watered for 2 days (ψ_s = −1.4 MPa), a value of ϵ of 27.9 ± 8.2 MPa (n = 10) was determined. The difference was significant at the 90% level. When plants were subjected to a third stress period and rehydrated in the pressure bomb using the capillary attachment (ψ_s = −1.9 MPa), a value of ϵ of 39.5 ± 12.0 MPa (n = 13) was determined. The difference between this value and that for plants exposed to two stress cycles was significant at the 98% level. We conclude that, in wheat leaves, exposure to drought stress decreases ψ_s and increases ϵ. These changes would collectively reduce the volume of water removed from a conditioned leaf at any given water potential in a turgid state.

It must be emphasized that the apparent modulus measured by the P-V technique is a tissue parameter. It is unkown whether the changes in it are a result of water stress induced changes in the mechanical properties of the cell wall per se, changes in cell size, or a combination of factors.

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NOTE ADDED IN PROOF

Tomos et al. (Plant Physiol. 68:1135-1143, 1981) recently reported in studies using the pressure probe that "within the limits of accuracy is not influenced by cell turgor and cell volume." This further diminishes the assumption that there is a dependence of on (see p. 121).

APPENDIX

A general equation describing applied pressure (P) in terms of its osmotic and turgor components is given by Cheung et al. (1976):

$$P = [\pi_o V_o/(V_o - V_e)] - (\epsilon/V_p)\,(V_o - V_e - V_p)$$

Where

π_o = tissue osmotic potential at $V_e = 0$,
V_o = tissue symplast volume at $V_e = 0$,
V_e = volume expressed,
V_p = tissue symplast volume at incipient plasmolysis,
ϵ = bulk elastic modulus of the symplast volume at incipient plasmolysis, where minus one times the volume averaged turgor pressure replaces pressure in the usual definition of bulk modulus and where the osmotically inactive volume of the symplast is assumed to be negligible.

Differentiating,

$$dP/dV_e = [\pi_o V_o/(V_o - V_e)^2] + \epsilon/V_p$$

hence

$$dP/dV_e\big|_{V_{e=0}} = \pi_o/V_o + \epsilon/V_p$$

Using data representative of a control leaf, (π_o = −1.14 MPa, V_o = 0.1245g, V_p = 0.1153g) we calculated ϵ = 17.4 MPa from the slope of the turgor pressure vs. $V_{expressed}$ plot for this leaf (Fig. 4). From the above equation, the osmotic potential contributed 5.8% to the slope of the P vs. $V_{expressed}$ plot of the leaf at $V_e = 0$ (P = 0.0 MPa). At the largest V_e used in calculating ϵ (P = 0.91 MPa), the osmotic potential contributed 6.5%. Alternatively, when the slope of a P vs. V_e plot for this leaf was used to calculate ϵ, the value of ϵ was 18.7 MPa. Again, the volume dependence of the osmotic pressure contributed 7% to ϵ.

Thus our values of ϵ should be reduced by about 18% for comparison with the ϵ of Cheung et al. They cannot easily be compared with the ϵ of Tyree and Hammel, except in the case where n = 1.

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