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Measurement of Plant Water Status by the Pressure Chamber Technique

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Summary. The pressure chamber has been widely used in the measurement of total water potential and pressure-volume relations of leaves, twigs and, to a lesser extent, roots. Some of the benefits and precautions in its use in these studies are reviewed and discussed. The pressure chamber has also been used to determine hydraulic resistances of plants, to collect xylem sap, to determine the water potential at various points in the xylem and to establish membrane damage of plants. Developments in this field are reviewed and discussed.

Dixon (1914) was the first to use a pressure chamber to measure the water status of leaves. Perhaps because of the unsafe nature of the glass pressure vessel used, his innovative method for measuring plant water status remained unused for half a century until popularized by Scholander et al. (1964, 1965). In the two decades since Scholander and his colleagues used the pressure chamber to measure the water relations of several trees and shrubs, the technique has been widely adopted as a means of measuring the total water potential and pressure-volume relations of plant tissue. Yet it is more than a decade since Ritchie and Hinckley (1975) published the only comprehensive review of use of the pressure chamber. The purpose of this paper is not to review and integrate all studies using the pressure chamber technique, a formidable task. Rather, the developments in methodology and data interpretation over the past 23 years and the precautions that need to be taken in its use will be discussed. The pressure chamber will be briefly described and then its use in the measurement of total water potential and pressure-volume relations will be covered in detail, before describing its use for other applications.

Pressure Chambers

Pressure chamber design has changed little in two decades (Scholander et al. 1964; Turner et al. 1971). The pressure chamber (Fig. 1) comprises an aluminium, steel or stainless steel pressure vessel (E) that can withstand pressures up to 10 MPa connected to a pressurised supply of inert gas (usually nitrogen) or compressed air (A)

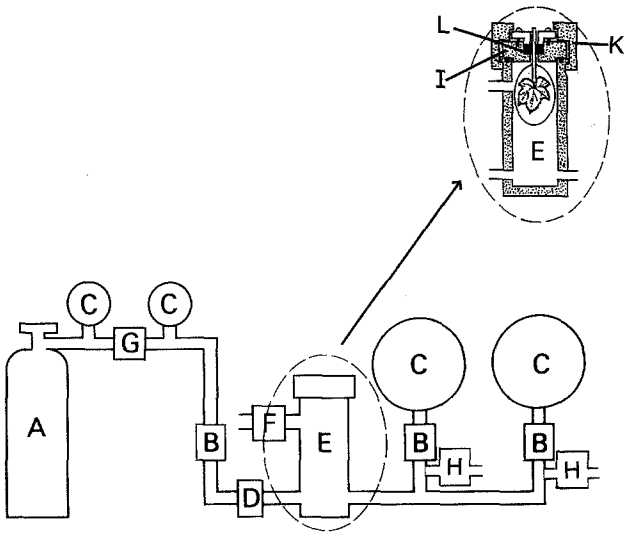

Fig. 1. Schematic diagram of a pressure chamber system: *A*, cylinder of inert gas or compressed air; *B*, shut-off valves; *C*, pressure gauges; *D*, metering valve; *E*, pressure chamber; *F*, exhaust valve; *G*, pressure reduction valve, and *H*, pressure release valves. The insert shows the pressure chamber in greater detail with a leaf inside the chamber enclosed in a polyethylene bag; *I*, chamber top; *K*, cap; and *L*, seal. Modified from Turner (1981 a)

through a pressure-reducing valve (*G*) and metering valve (*D*), and connected to pressure gauges (*C*). In practice I prefer to use two gauges; a more accurate gauge (0.01 MPa) for the more frequent measurements in the range 0 to 2 MPa and a less accurate gauge (0.03 MPa) for the less frequent measurements in the range 2 to 7 MPa. Two improvements that have been made to the original chamber described by Turner et al. (1971) are: *(i)* the addition of a pressure transducer that can provide a digital output of the pressure in the chamber, and *(ii)* the incorporation of pressure-release valves to protect the pressure gauges and to prevent overpressurization of the chamber.

The pressure vessels vary in size and complexity depending on the plant material under study and the desired portability. For measurement of small twigs at remote locations where portability and the use of small gas volumes is essential, a small, light chamber is the most practical. The largest chamber that I have seen in use is one built by Dr. P. Cruiziat at INRA, Clermont-Ferrand, France that can accommodate a whole sunflower plant. For almost all situations a chamber volume of 0.5 l is usually sufficient. To avoid excess use of gas with small leaves, a metal or wooden insert can be placed inside the chamber to reduce its volume.

In the field, speed of measurement is frequently of major importance. A third modification incorporated into most current pressure chambers is the replacement of the screw cap with a cap with a bayonet fitting for quick opening and closing of the chamber. Additionally, the top of the chamber (*I*) can be separated from the cap (*K*) so that chamber tops can be quickly interchanged. Different tops may be necessary for grass leaves, leaves with long petioles, leaves with very short petioles or when

several samples are measured simultaneously. The seal (*L*) itself is important. It should be made of a rubber that is sufficiently elastic to fill in the indentations of irregularly-shaped petioles, but not so soft that it disintegrates under pressure. For very irregular petioles, quick-setting silicon compound can be used, but this slows down the number of leaves that can be measured. With a split rubber stopper as a seal for grass leaves (Turner et al. 1971), it may be necessary to peel back the midrib or take half a leaf excluding the midrib. High-pressure grease on the stopper should be used to prevent or reduce leakage and to prevent leaf damage. Waxed paper placed between the leaf and rubber stopper can also be used to prevent leaf damage. With round petioles a design in which the rubber seal can be tightened to eliminate leaks during pressurization of the chamber has been found to work well in practice. This design also can be used to stop passage of air through the cortex and consequent premature bubbling at the cut surface, a critical feature for the successful use of the pressure chamber with two tropical legumes (McCown and Wall 1979). Milburn (1979) modified the pressure chamber technique in order to enable leaves to be left in the pressure chamber for long periods. He used a clear acrylic chamber filled with paraffin oil and applied hydraulic pressure rather than gas pressure. This modified pressure chamber allowed the leaf to be illuminated during pressurization so that the leaf could be maintained at the carbon dioxide compensation point. The oil-filled chamber also prevented water loss by transpiration to dry air that can occur in the traditional pressure chamber.

Pressure chambers are manufactured commercially by PMS Instrument Co. (2750 N.W. Royal Oaks Drive, Corvallis, Oregon 97330, USA), Soil Moisture Equipment Corp. (P.O. Box 30025, Santa Barbara, California 93104, USA), Charles W. Cook and Sons Ltd. (79 Walsall Road, Perry Bar, Birmingham B42 1TT, United Kingdom), Roth Gerätebau (Blumenstraße 5, D-8523 Baiersdorf, West Germany), A.R.I. Kfar Charuv (13 Ben Avigdor Street, Tel-Aviv 67128, Israel) and Daiki-Rikakogyo Co. Ltd. (2-16-2 Machiya, Arakawa-ku, Tokyo 116; available through: Thomas and Co., Ltd., 2-2-4-407 Shibuya, Shibuya-ku, Tokyo 150, Japan). The various manufacturers provide pressure chambers that meet different requirements. For example, PMS Instrument and A.R.I. Kfar Charuv manufacture pressure chambers that are light and very portable, whereas the one manufactured by Soil Moisture Equipment Corporation is less portable, but is available with three different chamber sizes and different tops for use with a wide range of species.

The stored energy of compressed gas is dangerous and care must be taken in the use of the pressure chamber. While pressure release valves that protect the pressure gauges (Fig. 1) also prevent overpressurization of the chamber, care needs to be taken to ensure that the line to the chamber cannot be overpressurized when the input valve (*B*) is closed. Most pressure chambers with a bayonet cap also have a valve that prevents pressurization unless the cap is locked into position. Use of a binocular microscope to observe the cut surface will also protect the operator's eyes if the plant is forced out through the seal or sand grains are blown from the leaf during pressurization. If a microscope is not used, safety glasses should be worn and the cut surface should never be viewed directly from above during pressurization. While there have been no serious accidents from use of modern pressure chambers, it is important that students and support staff are taught adequate safety procedures when first introduced to the technique.

Theory

Scholander et al. (1964, 1965) described the principles of the pressure chamber technique. They showed that due to the evaporation of water from leaf cells by transpiration, coupled with the resistances to flow of water from soil to the leaf, a negative hydrostatic pressure builds up in the xylem. When a transpiring leaf is cut, the xylem sap recedes in the xylem until it becomes restricted by a cross wall (Fig. 2a). Scholander et al. (1964, 1965) suggested that pressurizing the leaf until the water just returns to the cut surface of the xylem tissue gives a measure of the hydrostatic pressure in the xylem (Fig. 2b). Weatherley (1970) and Passioura (1980a) showed that the pressure chamber is analogous to the pressure membrane apparatus used in soil physics, i.e., the pressure chamber measures the matric potential in the apoplast. The matric potential of the apoplast (τ) is similar to the total water potential (ψ) of the adjacent symplast provided that the resistance to flow between the symplast and apoplast is not great and that the osmotic pressure (π_a) of the apoplastic water is near zero, that is:

$$\psi = \tau - \pi_a = P_c - \pi_a , \tag{1}$$

where P_c is the applied pressure in the pressure chamber. In practice, the osmotic pressure of the apoplastic water is usually less than 0.05 MPa (Boyer 1969), so that:

$$\psi \cong \tau \cong P_c . \tag{2}$$

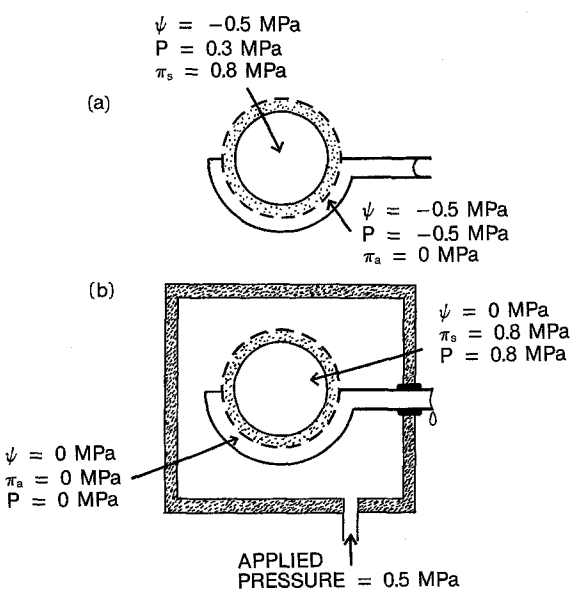

Fig. 2a, b. Schematic concept of the water relations of a cell and adjacent xylem: **a** after severing from a transpiring plant, and **b** in the pressure chamber. ψ is the total water potential, π_a and π_s are apoplastic and symplastic osmotic pressures, respectively, and P is the turgor pressure except that in **b**, P in the symplast (0.8 MPa) is the sum of the turgor pressure (0.3 MPa) and applied pressure (0.5 MPa). Adapted from Scholander et al. (1965)

Scholander et al. (1964, 1965) also showed that once the cells in the tissue reach zero turgor pressure, P_c is related to the cell water:

$$1/P_c = 1/\pi_s = (V_s - V)/RTn, \tag{3}$$

where V_s is the volume of symplastic water in the turgid leaf, π_s is the osmotic pressure of the symplast, V is the volume of water lost from the leaf, R is the universal gas constant, T is the absolute temperature and n is the number of moles of solute in the symplast. This assumes that the cells act as perfect osmometers and that the volume of water in the apoplast does not change during leaf drying or water loss. The theory of the pressure chamber technique was extensively developed by Tyree and Hammel (1972) and elaborated or repeated in subsequent papers (Tyree et al. 1973; Tyree and Dainty 1973; Tyree and Richter 1981; Tyree and Jarvis 1982; Robichaux et al. 1986). Readers are referred to these papers for detailed theory.

Total Water Potential

Comparisons of the pressure chamber against the thermocouple psychrometer generally show good agreement (Ritchie and Hinckley 1975), confirming that in most cases the measured matric potential of the apoplast is closely related to the water potential of the adjacent symplast. Because of this and because of its greater speed and simplicity of operation compared with the thermocouple psychrometer, the pressure chamber is widely used for measuring total water potential.

When a plant is transpiring, the pressure chamber does NOT measure the potential of the xylem at the point of severance. This can be demonstrated by data from De Roo (1969) who severed tobacco (*Nicotiana tabacum* L.) plants near the soil surface and measured the water potential of the shoot and root. The root had a 0.6 to 0.8 MPa higher water potential than the shoot when well watered. While this discrepancy between the water potential of the root and shoot may have arisen partly from damage to the roots (Gee et al. 1974), in a transpiring plant there is a gradient in water potential from root to shoot and this is reflected in the data of De Roo (1969). Similarly, Janes and Gee (1973) observed that the water potential of a leaf was about −0.3 MPa when the water potential of the xylem in the petiole was positive due to pressurisation of the roots (Gee et al. 1973). On severing the leaf, water exuded from the petiole. However, in a non-transpiring plant the water potential throughout the plant will be in equilibrium and the water potential at the point of severing should closely approximate the potential of the leaves and roots (De Roo 1969). Consequently, in transpiring plants it is inappropriate to refer to the potential measured by the pressure chamber as the xylem pressure potential (Ritchie and Hinckley 1975). Not only does the pressure chamber measure the matric potential of the apoplast, but xylem pressure potential is a mixture of both mechanical and thermodynamic terminology and is thermodynamically incorrect (Passioura 1982). Because of the close similarity between the matric potential of the apoplast and the total water potential of the symplast, the measured value obtained from the pressure chamber will be referred to as total water potential.

Acceptance of the conclusion that the pressure chamber measures the matric potential of the apoplast does not overcome a problem in defining the site of measurement in a complex organ such as a leaf, twig or root. From studying the kinetics of

water movement, Tyree and Dainty (1973) concluded that there were three distinct cell types in hemlock (*Tsuga canadiensis* L. Carr.) that differed in their cell hydraulic conductivities. Further studies by Tyree et al. (1975) and Turner et al. (1984) suggested that the resistance to water flow in the xylem can be significant and can lead to water being extracted from cells close to the cut surface before it is extracted from cells further away from the cut surface. Thus, in sunflower (*Helianthus annuus* L.) the leaf water potential measured by the pressure chamber was much more closely correlated with the water potential measured by in situ psychrometry near the midrib than that at the leaf periphery (Turner et al. 1984).

Passioura (1982) suggested that the water potential measured by the pressure chamber is a capacitance-weighted average of the variation in total water potential throughout the whole leaf. The resistance to flow within the xylem of the leaf and petiole may result in slow attainment of equilibrium of water potential throughout the entire leaf (as opposed to a rapid equilibration between symplast and apoplast) leading to the pressure chamber tending to measure the water potential of the wettest cells. This may explain why in sunflower and sorghum leaves severed from their water supply, the leaf water potential did not change after an initial decrease (Turner et al. 1978; Turner and Singh 1984; Turner 1986). While some uncertainty still exists concerning the site of measurement in a leaf, there is greater uncertainty about the location of measurement in the root because of the isolation of the stele by the endodermis. Gee et al. (1973) described a pressure chamber designed to measure root water potentials, but no comparative measurements of root water potential by pressure chamber and psychrometric techniques have been reported.

For the measurement of leaf water potential, a leaf or twig is detached from the shoot and placed in the pressure chamber with the cut end protruding from the chamber and exposed to atmospheric pressure (Fig. 1). The xylem sap that receded away from the cut surface into the cells on severing, returns to the cut surface when the applied pressure equals the tension in the leaf cells at severing (Fig. 2). This balancing pressure is a measure of the leaf water potential. With roots, either plants have to be grown in sufficiently small pots so that the whole root system can be inserted in the pressure chamber (De Roo 1969; Gee et al. 1973), or a portion of the root is exposed, severed from the plant and placed in the pressure chamber (De Roo 1969; Hellkvist et al. 1974).

A number of precautions are necessary if reliable results are to be obtained from the pressure chamber (Turner 1981 a):

(i) In transpiring plants, water loss between sampling and measurement must be prevented, particularly in plants with a high bulk modulus of elasticity (Turner and Long 1980). This may be especially important in roots. When the bulk modulus of elasticity is high, a small loss of water will lead to a large decrease in water potential. Turner and Long (1980) showed that water loss in the first 20 s after severing can lead to a lowering of the water potential by 0.7 MPa in rapidly transpiring leaves. Enclosing the leaf in a plastic sheath or film immediately prior to severing will eliminate the error (Wenkert et al. 1978; Turner and Long 1980; O'Toole and Moya 1981; Leach et al. 1982; Kobata and Takami 1984).

(ii) Condensation of water on the sample before measurement should be prevented as droplets of free water on the leaf can increase the water potential by 0.1 to 0.2 MPa. While bubbling the incoming dry gas through water (Boyer 1969) or lining

the chamber with damp filter paper or towelling (Turner et al. 1971) have been recommended to prevent evaporative water loss, the latter can be avoided by keeping the sample enclosed in plastic while in the pressure chamber (Turner and Long 1980). This avoids having to moisten the air in the chamber. The plastic should only be loosely sealed with a paper or spring clip so that the pressure applied is effective on all cells.

(iii) Recutting of the petiole or leaf must be avoided as this can lead to error (Scholander et al. 1965). The reason why recutting leads to errors is not known.

(iv) The portion of the leaf, root or petiole external to the seal must be minimised to reduce exclusion errors (Millar and Hansen 1975). Errors arise in measurement when water expressed from the cells inside the chamber fills up the free-space outside the chamber. It is particularly important to reduce the tissue external to the chamber in small samples such as leaflets or conifer needles. Some commercial pressure chambers, particularly ones with a bayonet top and internal lugs, have the disadvantage of needing long petioles external to the chamber to clearly see the cut surface.

(v) Pressurization of the chamber should be slow (0.003 to 0.005 MPa s^{-1}) for accurate measurements of the capacitance-weighted average water potential and to prevent large temperature changes in the chamber. Waring and Cleary (1967) showed that rapid pressurization of Douglas fir (*Pseudotsuga menziesii* L.) twigs led to water potential values that were lower than those at slow rates of pressurization. They recommended use of fast initial rates of pressurization, but suggested that the rate be slowed down to 0.07 MPa s^{-1} within 0.7 MPa of the the endpoint as a compromise between the need for slow pressurization and the need for many measurements in a short time. M. M. Jones and N. C. Turner (unpublished) showed that this procedure still resulted in lower water potentials than if slow rates of pressurization were used throughout. Although Blum et al. (1973) used rates only half of those suggested by Waring and Cleary (1967), they showed that fast pressurization rates (0.04 MPa s^{-1}) led to higher water potentials than slow rates of pressurization (0.03 MPa s^{-1}). Turner (1986) suggested that fast rates of pressurization can lead to underestimates or overestimates of water potential depending on the gradients of water potential in the leaf. With long equilibration times required for certain cell types (Tyree and Dainty 1973), it is impractical to wait for equilibration across a leaf or through a complex tissue and some error may have to be accepted in determining the water potential of such tissues. Turner (1981 a) suggested a pressurization rate of 0.025 MPa s^{-1} was acceptable in most situations.

(vi) Identification of the endpoint is critical to accurate estimation of the water potential. The correct endpoint is when the xylem sap just returns to the cut surface of the xylem. In some species the severed ends of the xylem vessels darken just prior to the endpoint, but use of a binocular microscope is required to detect this. If there is any uncertainty about the endpoint, the gauge should be read and then the pressure increased by 0.1 MPa. If the correct endpoint has been reached, this overpressurization will result in xylem sap gushing from the cut surface. Reducing the pressure after overshooting an endpoint, followed by re-pressurization, can only be used as a guide to the value of the initial endpoint. Once tissue has been pressurized beyond the balancing pressure, the measured endpoint will almost always give a lower water potential, presumably because some of the xylem sap evaporated or infiltrated into non-xylem tissue during overpressurization.

(vii) Gas leaks from the chamber through the plant tissue must be minimised. In some species, especially cotton (*Gossypium hirsutum* L.), oleander (*Nerium oleander* L.) and some legumes, gas from the chamber passes through the intercellular spaces of the leaf and/or petiole and escapes from the cut surface giving a false endpoint. Drying the cut surface with filter paper or lintless tissue, particularly at high water potentials, and/or constriction of the stem (McGown and Wall 1979) will aid in detection of the correct endpoint.

Generally values of water potential obtained by thermocouple psychrometry are used as a standard in evaluating values of water potential obtained with the pressure-chamber (Boyer 1967a; Ritchie and Hinckley 1975; Blum et al. 1973; Millar 1982). However, errors can arise with both methods (Kikuta et al. 1985) and it is not clear that one technique is more reliable as a standard than the other. For example, Klepper and Barrs (1968) showed that considerable discrepancy existed between the two techniques in cotton, but this was due to secretion of salt on the leaf during equilibration in the thermocouple psychrometer and not from any lack of reliability of the pressure chamber technique. Likewise, Turner et al. (1984) observed a wide discrepancy between the leaf water potential measured by in situ psychrometry and that measured by the pressure chamber in hazel (*Corylus avellana* L.), oleander (*N. oleander*) and pistachio (*Pistacea vera* L.). This poor correlation between the psychrometer and pressure chamber arose from the high resistance to water flux between the psychrometer chamber and leaf interior and hence the inability of the leaf and chamber to equilibrate quickly enough for reliable readings to be obtained with the psychrometer (Turner et al. 1984). On the other hand, loss of water after excision in the pressure chamber technique (Turner and Long 1980) may account for some of the discrepancies in the early comparisons between the two methods.

Pressure-Volume Relations

Another advantage of the pressure chamber technique is that it can also be used to measure the pressure-volume relations of plant tissue. Scholander et al. (1964, 1965) showed that if additional pressure (P_c) is applied to a leaf or twig beyond the initial balancing pressure, xylem sap is expressed; this sap can be collected and its volume determined. By doing this in a step-wise manner, a pressure-volume curve can be established (Fig. 3). Once the turgor pressure reaches zero, the plot of $1/P_c$ against V becomes linear as predicted by Eq. (3). Establishing the pressure-volume curves of species has become popular because a wide range of tissue water parameters can be derived including:

(i) Total Water Content. Assuming the density of water is unity, and provided that the leaf or twig is fully rehydrated before pressurization and its initial mass (M_t) is measured and a final oven-dry mass (M_d) is obtained, the total water content of the leaf (V_t) is given by:

$$V_t = M_t - M_d . \tag{4}$$

(ii) Turgid Mass/Dry Mass Ratio. The turgid mass/dry mass ratio (M_t/M_d) or water content on a dry mass basis (V_t/M_d) may give some indication of solute accumu-

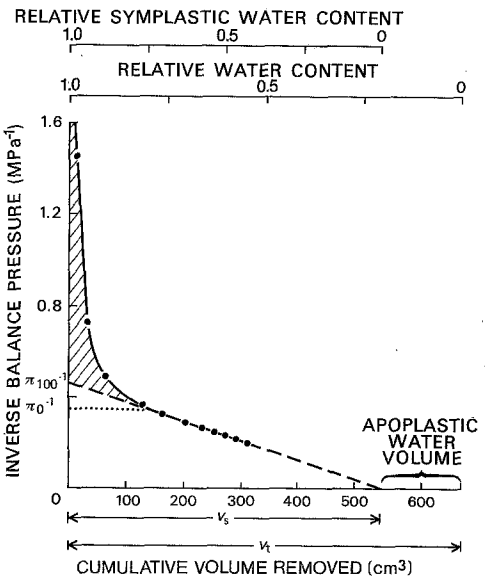

Fig. 3. A pressure-volume curve that depicts the relationship between the inverse of the balance pressure and the cumulative volume of cell sap removed from wheat leaves. V_s is the volume of symplastic water and V_t is the total volume of water in the leaf. π_{100}^{-1} and π_0^{-1} are the inverses of the osmotic pressures at full and zero turgor, respectively. The hatching indicates the inverse of turgor pressure. Modified from Turner (1981 a)

lation. Changes in M_t/M_d have recently been shown to correlate well with changes in osmotic adjustment in some species (Sobrado and Turner 1983 b; Turner et al. 1987).

(iii) Relative Water Content. The relative water content (R_t) for any part of a pressure-volume curve is given by:

$$R_t = (V_t - V)/V_t. \tag{5}$$

(iv) Apoplastic and Symplastic Water Contents. Extrapolation of the straight line relating $1/P_c$ against V to where $1/P_c = 0$, that is to infinite pressure, gives the relative volumes of water in the symplastic (V_s) and apoplastic ($V_t - V_s$) fractions (Fig. 3). An assumption of the pressure-volume method is that the absolute volume of apoplastic water does not change during pressurization. While Tyree (1976) argues that this is a realistic assumption over the pressure range employed in pressure-volume studies, Acock (1975) has questioned the validity of this assumption. If Tyree's assumption is correct, the volume of water left when $1/P_c$ is extrapolated to zero is the apoplastic water. Actual pressurization to infinite pressure should remove the apoplastic water leaving only 'bound' water. Consequently, apoplastic water, rather than bound water, is the more scientifically correct term for the residual water fraction estimated by extrapolation.

(v) Relative Symplastic Water Content. The relative symplastic water content (R_s) at any part of a pressure-volume curve is given by:

$$R_s = (V_s - V)/V_s. \tag{6}$$

(vi) Osmotic Pressure at Full Turgor. Extrapolation of the straight section of the line relating $1/P_c$ against V to where $V = 0$ (or to where $R_t = 1.0$) gives the balance pressure equivalent to the osmotic pressure at full turgor (π_{100}^{-1} in Fig. 3). Comparison of π_{100} in adequately watered plants and those subjected to a water deficit is one method of estimating osmotic adjustment (e.g. Jones and Turner 1980).

(vii) Osmotic Pressure at Zero Turgor. By determining the point at which the pressure-volume curve becomes linear, the osmotic pressure at zero turgor can be determined (π_0^{-1} in Fig. 3). The degree of osmotic adjustment at zero turgor can be determined by comparing π_0 in an adequately watered plant with that in a plant subjected to a water deficit.

(viii) Relative Water Content at Zero Turgor. R_t or R_s at zero turgor can also be determined once the point of zero turgor has been established.

(ix) Bulk Modulus of Elasticity. The change in turgor pressure (ΔP) with change in volume (ΔV) is dependent on the elasticity of the cell walls (ε):

$$\varepsilon = (\Delta P/\Delta V)\, V. \tag{7}$$

In the region of positive turgor (Fig. 3), the pressure chamber technique gives a mass-averaged bulk modulus of elasticity, $\bar{\varepsilon}$ (Tyree and Jarvis 1982):

$$\bar{\varepsilon} = (\Delta \bar{P}/\Delta M)\, M_s \tag{8}$$

where $\Delta \bar{P}$ is the change in bulk tissue turgor pressure, ΔM is the change in mass and M_s is the mass of symplastic water. As the density of symplastic water is assumed to be unity, the bulk modulus of elasticity is usually calculated as:

$$\bar{\varepsilon} = (\Delta \bar{P}/\Delta V)\, V_s, \tag{9}$$

or approximated by:

$$\bar{\varepsilon} = (\Delta \bar{P}/\Delta R_t)\, R_t, \tag{10}$$

where ΔR_t is the change in relative water content. Quantitatively ε and $\bar{\varepsilon}$ are usually similar, especially at high turgor pressures, but they can be different at low turgor when some cells have reached zero turgor and no longer contribute to $\Delta \bar{P}$, but continue to lose water and contribute to ΔR_t.

(x) Water potential Isotherms. The relationship between water potential and relative water content is termed the water potential isotherm or moisture release curve for a particular tissue. These relationships have been used to determine the drought resistance characteristics of species (Jones et al. 1981).

(xi) Höfler Diagram. A pressure-volume curve provides all the parameters to plot a Höfler (1920) diagram (Fig. 4).

Overpressurization studies showed that it may take up to 2 h for all the cells in a leaf to come to equilibrium after a step increase in pressure (Tyree and Dainty 1973). This makes the determination of pressure-volume curves very slow. Hellkvist et al. (1974) suggested a fixed collection time at a particular pressure to speed up collection. However, Tyree et al. (1978) showed that this technique overestimated the osmotic pressure by 0.2 MPa to 0.8 MPa in several species; the magnitude of the error depended on the internal resistance to flow of the leaves and shoots. Moreover, collection of all the expressed sap proved difficult due to water loss both from the leaf inside the chamber (particularly if a gas leak occurred at the seal) and from the collection vessel. To overcome this problem, Wilson et al. (1979) measured the loss of mass of the leaf during overpressurization by weighing the leaf before and after pressurization and relating the volume of water expressed to the balance pressure after weighing and not to the holding pressure during overpressurization.

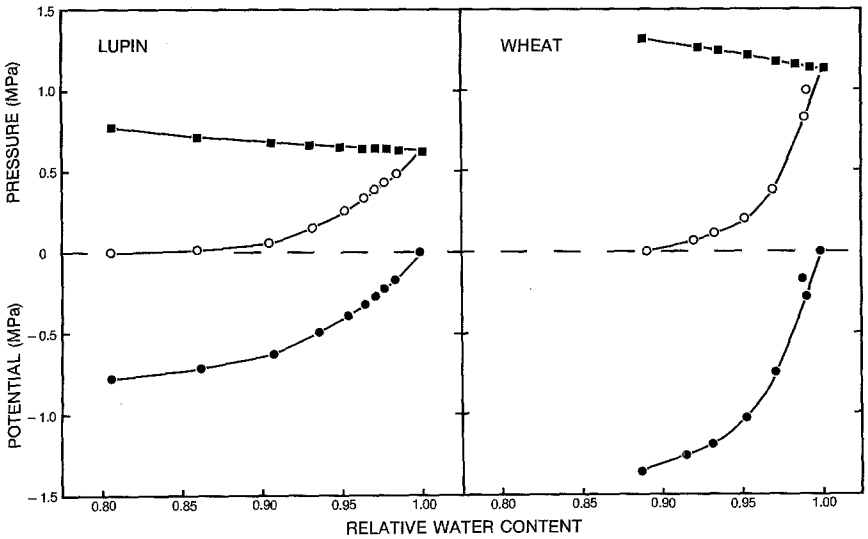

Fig. 4. A contemporary Höfler diagram for lupin and wheat leaves from irrigated field plants: ●, leaf water potential; ■, osmotic pressure; and ○, turgor pressure

Currently, a method described by Hinckley et al. (1980) and Henson (1982) is being widely used. This method involves leaving the leaf or twig to dry on a bench between readings. After an initial balance pressure is obtained, the leaf is quickly weighed and then left to dry until a new balance point and mass are established. The pressure chamber is not monopolized by one leaf during overpressurization and allows the measurement of several leaves at the same time. This method not only avoids the problem of overpressurization forcing dehydration of complex tissues at different rates depending on their internal resistance to flow, but also allows measurement of 6–8 samples per day per pressure chamber instead of 1–2 samples per day.

In practice, a leaf or twig or root is cut under water and allowed to rehydrate to near to full turgor. A leaf or branch from the sample is then covered by a plastic sheath or film of known mass and the balance pressure is measured. The leaf is then immediately weighed and left on the bench to dry. At intervals during the drying, the leaf is quickly weighed and then placed in the pressure chamber, and a new balancing pressure is established. To speed the rate of drying, the plastic sheath or film can be removed when the leaf is on the bench (Henson 1982), but it must be replaced before weighing and measurement in the pressure chamber. Finally after sufficient data points have been obtained, the leaf or branch is placed in an oven at 80 °C and dried to constant mass.

For reliable results the following precautions need to be taken:

(i) The tissues must be nearly fully rehydrated before the pressure-volume relationship is established. This is usually achieved by placing the leaf or twig in a dark, humid chamber for several hours. Alternatively, the leaves can be quickly rehydrated by expressing the air from the xylem by slight pressurization and then placing a supply of water over the cut surface and quickly releasing the pressure (Cheung et al. 1975).

Rapid rehydration can also be achieved by dipping the cut leaf surface into water in the pressure chamber and applying pressure until the leaf guttates (Campbell et al. 1979). The rehydration time must not be too long, especially in plants adapted to water deficits, because the plants may lose or gain solutes during rehydration (Jones and Turner 1980; Takami et al. 1981; Brown and Tanner 1983; Thomas 1986). Studies with most crop species suggest that rehydration for 3 to 4 h is sufficient to regain full turgor without any marked loss of solutes. After rehydration, if the leaf is not at full turgor, and this is likely, the fresh mass at zero water potential can be determined by linear extrapolation from the first three or four readings (Ladiges 1975). This requires that the water potential be between 0.0 and −0.2 MPa and that first readings be close together to enable linear extrapolation.

(ii) Crushing of the stem must be minimised, otherwise the relationship between $1/P_c$ against V deviates from linearity at high pressures or low water potentials (Wilson et al. 1979). Wenkert et al. (1978) suggested that fast-setting silicone compound in a stepped and tapered hole reduces the lateral forces extended on the petiole. Turner (1981 a) suggested the use of fast-setting silicone compound that was strengthened and given greater adherence to the petiole by wrapping the petiole with cotton thread.

(iii) Extended exposure to nitrogen gas or compressed air must be avoided to prevent death of the tissue or a change in the permeability of membranes to water that leads to little change in water content with considerable change in applied pressure (Tyree et al. 1973). This was a problem, when long overpressurization times were utilised, but should not be a problem with current methods (Hinckley et al. 1980; Henson 1982; Sobrado and Turner 1983 a, b; Robichaux 1984).

Several methods of data presentation have been suggested. Scholander et al. (1964, 1965) plotted $1/P_c$ against the percentage of intracellular water removed, similar to Figure 3. Richter (1978) suggested that plotting applied pressure (P_c) against the reciprocal of the relative water content ($1/R_t$) was an alternative method that allowed better depiction of data in the positive turgor range. The two forms of presentation are presented in Figure 5 (a and b) for the same data set. Estimates of the osmotic pressures at full turgor by the two methods, however, can give different results (Richter et al. 1980). Analyses showed that the difference in osmotic pressure at full turgor between the two methods of linearization increased with the fraction of apoplastic to symplastic water (Tyree and Richter 1982). The amount of apoplastic water does not influence the plots of $1/P_c$ against R_t provided that the apoplastic water content does not change during pressurization. However, the greater the apoplastic water fraction, the lower the estimated osmotic pressure at full turgor when P_c is plotted against $1/R_t$ (Tyree and Richter 1981). Consequently, for reliable estimates of the osmotic pressure at full turgor plotting of $1/P_c$ against R_t is preferable.

Another concern in analyzing the results is in the determination of the point of zero turgor. Including data above zero turgor when fitting a linear regression of $1/P_c$ against R_t can influence the estimates of osmotic pressure at full turgor and apoplastic water content. While the correlation coefficient for the regression may improve, the osmotic pressure at full turgor will decrease and the apoplastic water content increase with the inclusion of additional data near zero turgor.

Because the extrapolation to full turgor is small relative to the extrapolation to $1/P_c = 0$, estimates of the apoplastic water fraction are much less reliable than estimates of the osmotic pressure at full turgor (Wilson et al. 1979; Wenkert 1980; Tyree and

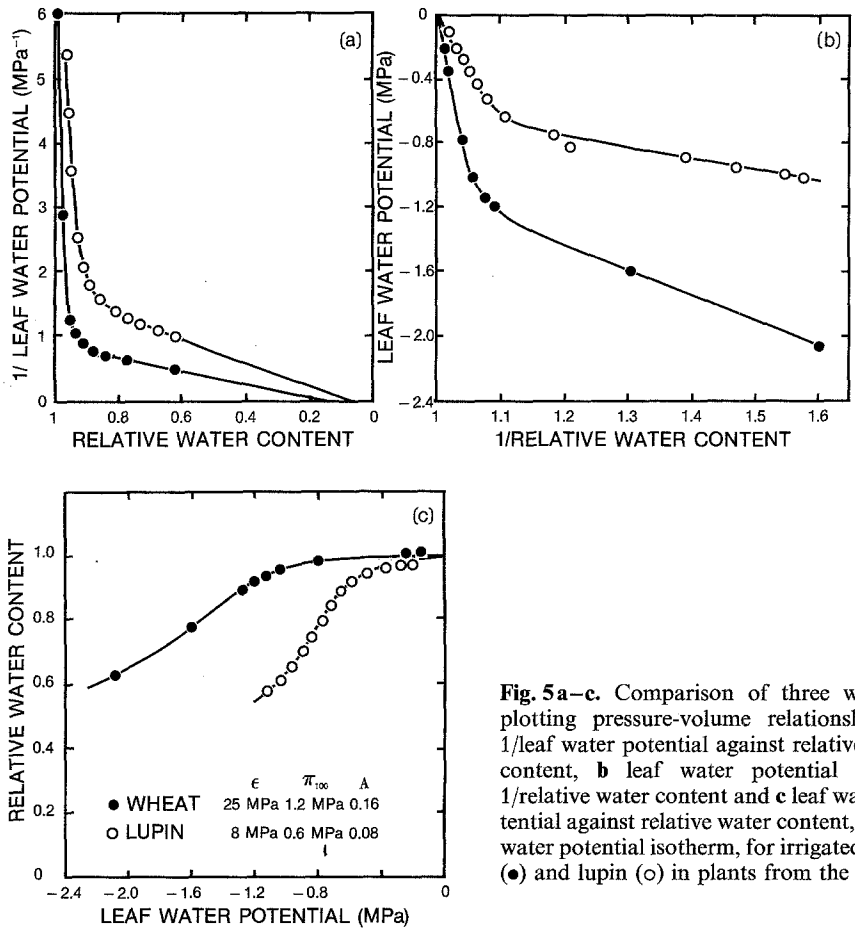

Fig. 5a–c. Comparison of three ways of plotting pressure-volume relationships: **a** 1/leaf water potential against relative water content, **b** leaf water potential against 1/relative water content and **c** leaf water potential against relative water content, i.e. the water potential isotherm, for irrigated wheat (●) and lupin (○) in plants from the field

Richter 1981, 1982). Plotting the water potential isotherm as suggested by Melkonian et al. (1982) often provides a clearer estimate of the point of zero turgor (Fig. 5c). This method has been used as a standard analytical procedure for pressure-volume relations by Turner et al. (1986, 1987) and is preferred to the method of Bahari et al. (1985).

Because the osmotic pressure estimated from pressure-volume relations is not subject to dilution of symplastic solution with dilute apoplastic water, as is the case with measurements of osmotic pressure by psychrometry (Tyree 1976; Wenkert 1980), it has been heralded as a preferred method (Tyree and Jarvis 1982). While this may be true in most situations, the several hours required to establish pressure-volume curves may preclude their use. The apoplastic water content and its change with dehydration prior to measurement can be estimated from pressure-volume relations and successfully used to correct for the effects of dilution (Turner et al. 1986). Sobrado and Turner (1983a) and Turner et al. (1987) compared the osmotic pressure at full turgor obtained by pressure-volume relations with that calculated from values of osmotic pressure measured by psychrometry and measured values of relative water

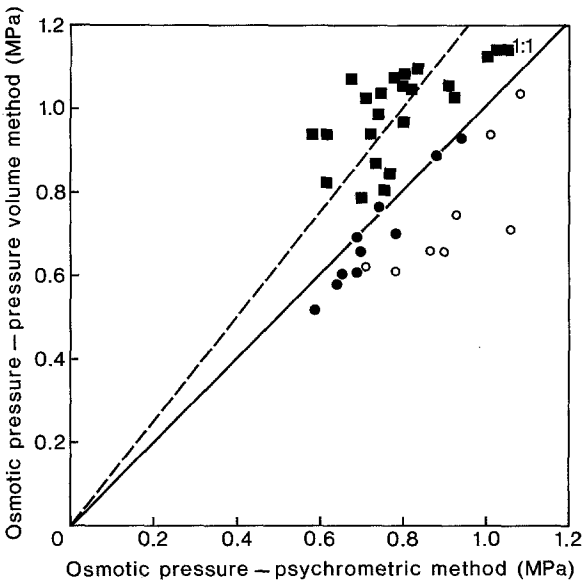

Fig. 6. Relationship between the osmotic pressure at full turgor determined by the pressure-volume method and that determined by psychrometry and relative water content for leaves of sunflower (■) and lupin (●, ○) plants that had either been adequately watered (●) or had been slowly dried to a predawn leaf water potential of −1.6 ±0.1 MPa (○) before rehydration. The 1:1 line (solid) and line assuming 20% dilution of symplastic solution by apoplastic water (dashed) are also shown. Adapted from Sobrado and Turner (1983 a) and Turner et al. (1987)

content on the same tissue. Figure 6 shows that while the comparisons were reasonable for sunflower, assuming a 20% dilution of symplastic solution with apoplastic water, the comparison was poor for lupins (*Lupinus angustifolius* L.) especially those that had undergone a soil drying cycle before rehydration. Turner et al. (1987) concluded that for lupins crushing of the petiole and cellular damage made the use of pressure-volume curves unreliable for estimating the osmotic potential at full turgor. This was particularly the case for *L pilosus* where it was impossible to obtain reliable values of the balance pressure once the leaves reached zero turgor.

Davis and Mooney (1986) have pointed out a problem with the pressure-volume technique in heterogeneous tissue. To obtain sufficient material of the xeromorphic chaparral shrub *Adenostoma fasciculatum* for use in the pressure chamber, the terminal portion of a shoot was required. This terminal shoot had leaves with a range of ages and comprised 65% stem tissue and 35% leaf tissue on a dry weight basis. Because the development of leaves occurred over the season, changes in osmotic pressure due to water deficits could not be distinguished from those due to ontogenetic development. Irrigation did not overcome this problem because the ratio of young to old leaves varied from irrigated to unirrigated plants. The authors had to use psychrometry on individual leaves to discriminate between osmotic changes induced by water deficits and those induced by age.

Finally, rehydration of tissue to full turgor can become a problem with the pressure-volume technique. Apart from the loss of osmotica during rehydration mentioned previously, rehydration may cause cellular damage. In wheat in which the water potential had fallen to −3.5 MPa and in which the degree of osmotic adjustment at full turgor was 1.0 MPa, water-soaking of the interveinal areas was observed after 3 h rehydration (N. C. Turner, unpublished). When the pressure-volume curve was established, the loss of a considerable volume of water for little change in water potential, similar to partial membrane damage (Turner 1976), was initially detected.

The high turgor pressures in the rehydrated cells may have caused them to burst. Similar results may occur if leaves or twigs overimbibe during rehydration. This not only affects the measured values of bulk modulus of elasticity, but also the total water content of the tissue.

Recently the pressure chamber technique has been used to establish the pressure-volume relations of roots (Turner et al. 1987). Although the root represents a heterogenous range of cells in which the cortical apoplast is isolated from the stele by the endodermis with its Casparian strip, osmotic pressures at full turgor obtained from pressure-volume relations compared favourably with those obtained by psychrometry (Turner et al. 1987). Nevertheless, the bulk modulus of elasticity of the root cells could not be measured because initially considerable change in water content took place with little change in water potential, presumably as a result of root pressure.

Other Uses of The Pressure Chamber

Resistances to Flow in Plants

The pressure chamber has been used to apply pressure to a leaf, root or stem segment in order to measure the flow rate of liquid water for a given pressure drop between inside and outside the chamber. For example, Tyree et al. (1973, 1975) and Tyree and Cheung (1977) used the pressure-volume technique to estimate the relative magnitudes of the membrane and extracellular resistances of hemlock (*T. canadiensis*) and beech (*Fagus grandifolia*).

Van Alfen and Turner (1975a, b) used a segment of stem dipping into water within the pressure chamber to estimate the resistances to flow in healthy stems of elm (*Ulmus americana* L.) and lucerne (*Medicago sativa* L.) and in stems after exposure to toxins produced by the disease agents *Ceratocystis ulmi* (Buisman) C. Moreau and *Corynebacterium insidiosum* (McCull.) Jensen, respectively. The toxins were shown to quickly block the xylem vessels and increase the hydraulic resistance of the stem.

Passioura (1980b), Passioura and Munns (1984) and Passioura and Tanner (1985) have pressurized the roots of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), lupin (*L. albus* L.) and cotton (*G. hirsutum* L.) grown in special pots that fit inside the pressure chamber to study the hydraulic resistances of *transpiring* plants. Passioura (1980b) found no major resistance to water flow at the root: soil interface as the soil dried, but Passioura (1980b) and Passioura and Munns (1984) observed a diurnal change in the relationship between applied pressure and rate of transpiration in wheat, barley and lupin similar to that found in sunflower by other methods (Turner 1981b). They showed that the system could not be used with soils near saturation or with solution culture because the long periods of pressurization filled the air spaces in the roots with water. Since long exposure to nitrogen gas kills the tissue (Tyree et al. 1973), roots should be pressurized with a mixture of compressed air and nitrogen maintained at a partial pressure of oxygen of 21 kPa (Passioura and Munns 1984).

Measurement of the Xylem Water Potential

Two methods of measuring the xylem water potential by the pressure chamber technique have been proposed. Begg and Turner (1970) covered leaves with aluminium foil

over a plastic bag to measure the water potential of the xylem at the point of attachment of the leaf to the stem. The covered leaf, when equilibrated, acts like a tensiometer plugged into the stem (Passioura 1982). The technique had been used in several species (Begg and Turner 1970; Hellkvist et al. 1974; Meyer and Ritchie 1980; Turner 1981 b) to calculate the gradient of water potential along the pathway of water flow and the hydraulic resistances to flow in the pathway.

The root pressurization technique can also be used to determine the water potential of the xylem at different points along the pathway. For example, if the xylem at the tip of a leaf is exposed and the pressure on the roots is slowly increased until the water in the xylem just returns to the cut surface, adjustment of the pressure so that the drop of moisture at the cut surface neither grows nor shrinks gives the water potential of the xylem at that point. By cutting the leaf or penetrating the xylem with a needle at various points, the water potential of the xylem at those points can be measured. Thereby, the gradient in water potential along the xylem can be determined.

Passioura and Tanner (1985) described a system for controlling the pressure in the chamber to maintain the xylem water potential at zero. By using this technique, Gollan et al. (1986) were able to follow the influence of soil drying on stomatal conductance while the leaf water potential was maintained near zero.

Collection of Sap

The pressure chamber provides a simple method of collecting xylem sap from the plant. Decapitation of a plant with its roots inside the chamber following by overpressurization allows collection of the sap flowing from the roots. Similarly by tapping into the xylem at different points along the pathways, sap flowing in a naturally transpiring plant can be collected by slight (100 kPa) overpressurization once a balancing pressure has been determined (Munns and Termaat 1986) or by reducing the transpiration of the leaves while maintaining the balancing pressure constant. The latter avoids dilution of xylem sap by the increased flow resulting from overpressurization. Slight overpressurization has been used to determine the concentration of ions in the xylem sap at various points along the stem and leaf in lupin (*L. albus*) and barley (*H. vulgare*) plants exposed to saline root media (Munns 1985).

By gradual overpressurization of a leaf in the pressure chamber, the apoplastic water in the major vessels of a leaf can be expressed and its constituents determined. This technique has been used to determine the osmotic pressure of the xylem sap showing that it is usually less than 0.05 MPa (Boyer 1969), and the nutrient relations of the xylem sap, thereby providing information on the nutrient supply to leaves and twigs (Schulze et al. 1984; Stark et al. 1985). However as overpressurization increases, the apoplastic water is displaced with membrane-filtered symplastic water. Recent measurements of the osmotic pressures of sap expressed from a sunflower (*H. annuus*) leaf showed that initially the osmotic pressure was 0.05 MPa and then decreased to a value of 0.02 MPa before decreasing to zero (Jachetta et al. 1986). Jachetta et al. (1986) suggested that the initial fraction came from the petiole and midrib, and the second fraction came from the minor veins and cell walls. However, without much greater knowledge of the pore diameters in the various portions of the leaf apoplast

and the hydraulic resistance to flow within the different cells of the leaf, this interpretation is difficult to substantiate.

Membrane Damage of Plants

Turner (1976) showed that the pressure-volume technique could be used to demonstrate damage to cell membranes. When membranes were damaged, the relationship between $1/P_c$ and R_t was not linear. The technique was used to show that membrane damage was induced by *Helminthosporium victoriae* Mechan and Murphy toxin in a susceptible, but not in a resistant oat (*Avena sativa* L.) cultivar. The technique, however, proved useful only when the majority of cells in a leaf were damaged. No difference in the pressure-volume relations was detected when ozone damage to maize (*Zea mays* L.) leaves was sufficient to cause visible water-soaked areas (Turner 1976). However, Kyriakopoulos and Richter (1981) showed that use of P_c against $1/R_t$ was a more useful way of demonstrating partial membrane damage arising from drought injury.

Matric Potential of Leaves

Boyer (1967b) suggested that the pressure chamber could be used to measure the matric potential of leaves if the leaves were frozen and thawed to break the cell membranes prior to insertion in the pressure chamber. The validity of this technique was questioned by Weatherley (1970) who argued that the purported differences in matric potential may simply reflect differences in the compressibility of the dead tissue. Subsequently, Passioura (1980a) questioned the meaning of matric potential as applied to whole tissues. Further, Campbell et al. (1979) and Campbell (1985) have indicated that the relative water content of a frozen leaf at the osmotic pressure of the leaf gives the apoplastic water content. Values of apoplastic water content obtained by this method appear rather high compared with values obtained by other methods. These criticisms of matric potentials measured by the pressure chamber do not invalidate the use of the pressure chamber to detect membrane damage.

Conclusion

The pressure chamber is widely used in studies of plant water relations because of its relative ease of operation and versatility. It will continue to be used, particularly in field studies, for the measurement of total water potential. Additionally, the use of the pressure chamber in documenting the pressure-volume relationships will continue to provide a wide range of water relations parameters.

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