Chapter 2

Pressure Chamber

Pressure chambers are the most widely used field instruments for measuring plant water status. They are portable, allow rapid measurements, and are sturdy. Temperature needs little control and no complex instrumentation is required. The tissue simply is sealed into the top of the chamber in such a way that most is inside and only a small amount extends outside through the top (Fig. 2.1A). The seal gives an airtight barrier between the interior and the atmospheric pressure outside (Fig. 2.1B, C). This allows the tissue to be pressurized inside, forcing water toward the outside. The pressure necessary to hold the water at the outside surface measures the water status of the tissue. The more dehydrated the tissue, the more pressure is required.

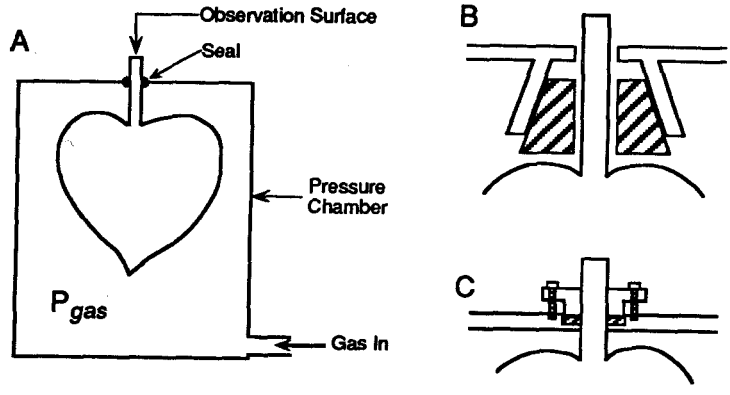

Figure 2.1. Pressure chamber design. A) Basic layout of chamber and tissue during pressurization. B) Enlarged view of a seal that is tightened by gas pressure; the seal around the tissue becomes tighter as pressure drives the rubber stopper (cross-hatched) deeper into the cone below the chamber top. C) Enlarged view of a seal that is tightened manually; tightening the screws forces the rubber seal against the tissue.

The method has been used successfully with leaves, branches, and roots and can provide information about all of the components of the water potential (Scholander *et al.*, 1965; Tyree and Hammel, 1972). Because the chamber is portable, it can be moved to the experimental site, and conditions in the plant can be left undisturbed until the moment of sampling. However, because plants use large pressures to move water, large pressures are needed during measurements. Therefore, pressure chambers must be built strongly and the seal must hold the tissue in place against large forces. Early efforts with pressure chambers were plagued by explosions that prevented the method from being developed (Dixon, 1914). After Scholander and his colleagues built a safer unit (Scholander *et al.*, 1964; 1965), the method became better understood and Boyer (1967a) showed that it could be used to measure the water potential. Commercial versions are now available (Appendix 2.1).

Principles of the Method

The method is based on the concept that the water potential in cells creates a tension (negative pressure) in the cell walls that pulls water toward the cells from the xylem, the root cells, and finally the soil (Fig. 2.2A). Excising a plant part causes the xylem water to pull back into the xylem (Fig. 2.2B). Applying pressure to the tissue raises the cell water potential and forces water out and into the xylem which is open to the atmosphere outside of the chamber. Xylem solution eventually appears at this surface when the applied pressure fully opposes the tension originally in the sample (Fig. 2.2C). Scholander *et al.* (1965) considered the pressure to be a direct measure of the tension in the xylem because of the continuous liquid phase extending into the cell walls.

The liquid moving in the walls and xylem is not pure water. Roots absorb salts from the soil and deliver them to the shoot via the xylem. Together with certain organic constituents traveling in the xylem, the xylem and cell wall solution contain sufficient solute to have osmotic potentials as low as -0.4 MPa (Boyer, 1967a). Boyer (1967a) showed that if solute effects were taken into account, the pressure chamber could be used to measure the tissue water potential. Since then, the pressure chamber has been widely used for measuring tissue water potentials.

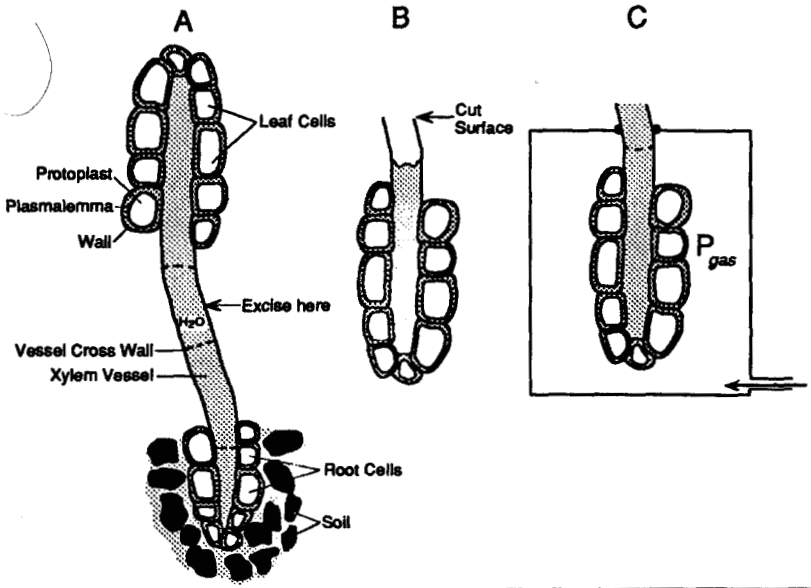

Figure 2.2. Diagram of the water transport system in a plant. A) Liquid continuity occurs between the soil solution and the cells inside the leaf. The cells (protoplasts, open spaces) are water-filled and bounded by a membrane. The cell walls and xylem (apoplast, stippled spaces) are also water-filled. The water in the apoplast is continuous with water in the soil except that in many instances there are waxy substances (Casparian strips, not shown) in the walls of the root cells that force water to flow through the root protoplasts. Leaf protoplasts transmit low water potentials as tensions to the apoplast and the soil. B) Excising a leaf opens the xylem to the atmosphere. The xylem solution retracts to cross walls in the vessels where sufficiently small pores exist to prevent solution from retracting farther. C) Mounting the leaf in a pressure chamber allows pressure to be applied that returns the xylem solution to its position in the intact plant in A. The pressure P_{gas} counteracts the tension (negative pressure) exerted on the xylem/apoplast solution in the intact plant and thus is a measure of the tension.

Pressure Chamber Theory

These concepts can be formalized by considering the components that contribute to the water potential. In cells and tissues, the major ones are solute, pressure, solids (porous solids also termed

matrices), and gravity. The distinction between them sometimes becomes blurred because of the difficulty in categorizing the forces, especially those arising from solutes and solids. In cells, macromolecules can dissolve or sometimes precipitate to form a gel (porous matrix) or form aggregates that may or may not be in solution. Despite this complexity, we will consider aggregates to be in solution unless they precipitate, a practice also followed by J. Willard Gibbs (1931). We will distinguish between pressure generated in pores and pressure applied externally (Gibbs, 1931). Also, as discussed in Chap. 1, we will describe the forces on a unit area basis (pressure) which is proportional to the free energy per mole of molecules (see Chap. 1). Accordingly, the components of the water potential are

$$\Psi_w = \Psi_s + \Psi_p + \Psi_m + \Psi_g, \tag{2.1}$$

where the subscripts s, p, m, and g represent the effects of solute, pressure, matrix, and gravity, respectively, and Ψ_w is the water potential. Each potential refers to the same point in the solution, and each component is additive algebraically according to whether it increases (positive) or decreases (negative) the Ψ_w at that point. The increase or decrease is always relative to pure water at atmospheric pressure, at the same temperature as the solution (see Chap. 1).

The components affect Ψ_w in specific ways. Solute lowers the chemical potential of water by reducing the tendency of water molecules to escape from each other compared to pure water because some of the solution volume is occupied by solute molecules that dilute the water molecules, decreasing the number able to escape. In a similar fashion, porous solids that are wettable occupy volume and cause surface effects that reduce the escaping tendency of the water in the matrix. External pressures applied to the liquid increase the escaping tendency of the water if they are above atmospheric but reduce the escaping tendency if they are below atmospheric. Gravity affects pressures because of the weight of the water, and the escaping tendency of water is increased or decreased depending on whether gravity increases or decreases the local pressure relative to atmospheric pressure. Pressures are high at the bottom of the ocean for this reason but low at the top of a tall tree. Each component decreases Ψ_w (is

negative in Eq. 2.1) except pressure and gravity above the atmospheric level, which increase Ψ_w (are positive in Eq. 2.1).

For most of our purposes, gravitational potentials will be ignored because they become significant only at heights greater than 1 meter in vertical water columns. In these cases, Eq. 2.1 reduces to:

$$\Psi_w = \Psi_s + \Psi_p + \Psi_m. \tag{2.2}$$

Water in plants generally has a negative Ψ_w because Ψ_s and Ψ_m are negative and Ψ_p does not fully compensate for them. Water will move toward more negative Ψ_w or more negative components of Ψ_w, and plants use this principle to extract water from the soil.

We may further conceptualize plant water by recognizing that it is located in two compartments separated by a differentially permeable membrane (Fig. 2.3). The first compartment is the interior of the cells (the protoplasts which collectively are the symplast) and the second is the cell walls and xylem outside of the protoplasts (collectively the apoplast). The membrane separating the compartments is the plasmalemma of each cell, and it allows water to move freely but little solute (i.e., the membrane is differentially permeable and reflects solutes).

Figure 2.4 shows that in the protoplast compartment (Fig. 2.4A), there is a concentrated solution ($\Psi_{s(p)}$) and usually a pressure above atmospheric (the turgor, $\Psi_{p(p)}$) so that the water potential ($\Psi_{w(p)}$) is

$$\Psi_{w(p)} = \Psi_{s(p)} + \Psi_{p(p)}, \tag{2.3}$$

where the subscript *(p)* denotes the protoplast compartment. The matric potential is generally negligible in the protoplast compartment ($\Psi_{m(p)} = 0$; Boyer, 1967b).

In the apoplast compartment (Fig. 2.4B), there is a dilute solution ($\Psi_{s(a)}$) and no turgor. Instead, there are surfaces arising from the porous matrix of the cell walls (Fig. 2.3, inset) and these generate a matric potential $\Psi_{m(a)}$ which is expressed mostly as a tension, i.e., negative pressure when the pores are water-filled. These components are expressed by

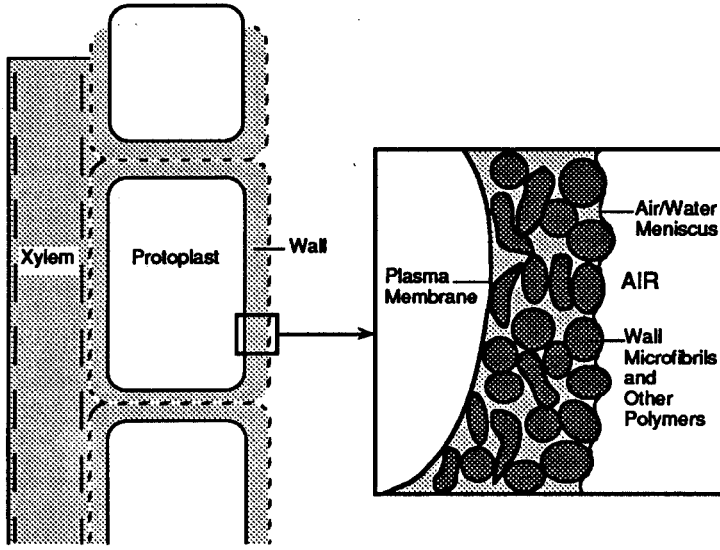

Figure 2.3. Enlarged view of compartmentation in plant tissues. The first compartment is inside the cells (protoplast, open space) and the second is in the cell walls and xylem (apoplast, stippled spaces). The two compartments are separated by the plasmalemma (plasma membrane in magnified inset). The protoplasts are water-filled. The walls contain water in the pores held by the hydrophilic surfaces of the pores and by surface tension at each air/water meniscus. The wall pores are so small that they withstand high tensions without draining.

$$\Psi_{w(a)} = \Psi_{s(a)} + \Psi_{m(a)}, \tag{2.4}$$

where the subscript *(a)* denotes the apoplast compartment.

The water potential in each protoplast is almost always the same as in its own cell wall (Molz and Ferrier, 1982) as shown in Fig. 2.4C:

$$\Psi_{w(a)} = \Psi_{w(p)}. \tag{2.5}$$

Substituting Eq. 2.3 and 2.4 in Eq. 2.5 gives

$$\Psi_{s(a)} + \Psi_{m(a)} = \Psi_{s(p)} + \Psi_{p(p)}, \tag{2.6}$$

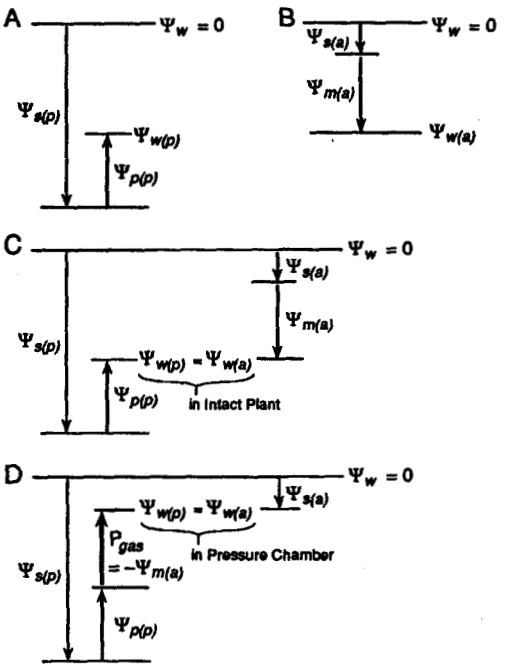

Figure 2.4. Potential diagrams showing water potential and its components inside cells (A) and in walls and xylem (B). The direction of the arrow indicates whether the potential is raised or lowered by each component. The cell interior A is essentially in equilibrium with its wall B so that the diagrams in A and B can be equated as in (C). Pressure (P_{gas}) applied to tissue in the pressure chamber raises the potential of the cell interior as in (D). Water moves out and hydrates the wall, raising its potential. When P_{gas} balances $\Psi_{m(a)}$ as shown in D, liquid appears at the cut surface and does not move at equilibrium.

which shows that the components of the water potential in the protoplasts are different from those in the apoplast but they balance each other locally. Note that the turgor in the cells is positive ($\Psi_{p(p)}$) and the water in the apoplast is under tension ($\Psi_{m(a)}$). This causes a large pressure difference across the plasmalemma. Were it not for the constraining effect of the cell wall, the plasmalemma would burst.

Upon pressurization in a pressure chamber, as in Fig. 2.2C, the water in the cells is uniformly exposed to an external pressure in addition to the turgor (Fig. 2.4D). This raises the cell water potential

above that of the xylem, and water flows into the xylem. By adjusting the pressure, the flow can be stopped when water just fills the xylem. At this balancing pressure P_{gas}, the water has returned to its original position in the intact plant where it forms a stationary flat film without any excess on the cut surface (Fig. 2.2C). This pressure exactly relieves the tension that had been acting on the xylem solution (Fig. 2.4D). The negative of P_{gas} thus measures the tension in the apoplast, i.e., the matric potential of the apoplast:

$$-P_{gas} = \Psi_{m(a)}. \tag{2.7}$$

Substituting Eq. 2.7 in Eq. 2.4, it can be seen that the water potential of the apoplast is the sum of $-P_{gas}$ and the osmotic potential of the apoplast solution $\Psi_{s(a)}$. From Eq. 2.5,

$$\Psi_{s(a)} - P_{gas} = \Psi_{w(a)} = \Psi_{w(p)}, \tag{2.8}$$

and the water potential of the tissue is determined from $\Psi_{s(a)} - P_{gas}$ (Boyer, 1967a). The $\Psi_{s(a)}$ is measured by overpressuring the tissue, collecting a small amount of exudate from the xylem, and determining its osmotic potential in an osmometer (see Chap. 3).

Three basic principles are demonstrated by these relationships. First, the pressure chamber measures the tension in the xylem and cell walls because the applied pressure relieves the tension and the xylem is directly observed. Second, the measurements require an equilibrium between the pressure and the xylem solution (hence the equal signs in Eqs. 2.7 and 2.8). One must make the measurements at equilibrium pressures (no water moves in or out of the tissue) to have a valid measurement. Third, the applied pressure raises the water potential in the tissue. During the measurement, the tissue does not have the same potential as in the intact plant (Fig. 2.4D).

SIGNIFICANCE OF THE THEORY

The ability of water to move depends on its water potential or the components of the water potential, specifically the osmotic potential, pressure, or matric potential. In plants, low potentials are frequent because the shoot tissues become dehydrated on a daily basis. The low potentials create a tension on water in the xylem and, when the pull is

surrounding each cell with uniform pressure (Figs. 2.2 and 2.3). Only the cell to cell contact areas are not in contact with the gas, and liquid can flow from cell to cell through these areas. Thus, the pressure "squeezes" the liquid in the cell toward any region where the cell to cell contact is at a lower potential.

The temperature does not enter any of the above equations because it is uniform in the sample. However, pressure·volume work depends on the Kelvin temperature, and the water potential of the tissue becomes less negative as the temperature decreases within the biological range. You will see this as a slight decrease in the balancing pressure as a leaf sample becomes colder even though there is no change in the water content of the sample. The theoretical basis for the temperature dependence is treated in Kramer and Boyer (1995).

Types of Pressure Chambers

All pressure chambers are constructed similarly except for the seal in the top. Figure 2.1B shows a pressure-activated seal that relies on the slippage of the sealing material, usually rubber, in a cone-shaped cavity built into the underside of the chamber top. As pressure increases inside the chamber, the outward force pushes the sealing material into the cone. The decreasing diameter of the cone tightens the seal around the plant part.

Pressure-driven seals have the advantage that they are quick and automatic. The surface between the seal and the cone needs to be lubricated but otherwise no maintenance is required. The disadvantage is that there is no control over the force applied to the tissue and the seals must be long to align the movement in the cone. The long seal restricts samples to long-stemmed branches and leaves with long petioles. Because the force applied to the tissue can become very large at high pressures, it can damage soft tissues or even interrupt flow. Thus, the design is best for woody stems.

Another type of seal involves a rubber packing gland whose tightness can be adjusted by the operator (Fig. 2.1C). The rubber is enclosed in a well on top of the chamber. A packing plate sits on top of the rubber and can be pushed down. Because the rubber cannot deform outward, it deforms into the center, filling it according to the force on the packing plate. The deformation seals the stem or petiole.

Manually tightened seals have the advantage that a minimum of force is applied to the plant material. The operator listens for escaping gas and tightens just enough to prevent audible leakage. The seal is small so that short-stemmed samples can be used. This type of seal is preferred over the pressure-driven seal because it is less likely to damage the tissue.

Seals can vary in diameter to allow large diameter stems to be used but the stem must be especially secure because pressures exert a force per unit area, and doubling the radius of the sealed tissue increases the force fourfold. In this situation, seals must exert a much larger force on the tissue to hold it in the pressure chamber.

How to Make Measurements

PRELIMINARY CHECKS

The static pressure inside a confining vessel is the same on all the walls, and the pressure gauge may be mounted on the gas feed line rather than on the chamber itself. Before using the instrument for the first time, the gauge may need to be checked for accuracy. Gauges are available at three levels of accuracy: standard, test, and master test. The standard gauge is used on equipment requiring moderately accurate pressure readings. Test gauges are used to calibrate standard gauges and give more reproducible readings. Master test gauges have a calibration traceable to the National Bureau of Standards and are typically used to calibrate test gauges. Of the three, test gauges are preferred for pressure chambers and their accuracy can be assumed. If standard gauges are used, they should be calibrated at least with a test gauge.

Before pressurizing a pressure chamber, test for its ability to withstand high pressures. Measurements with plants usually do not exceed 6 MPa but, whatever the maximum, tests should be at pressures at least twice the maximum. For the test, fill the chamber with water so that there is no air. Seal a metal rod securely in the top in place of the tissue. Pressurize the water and check for leaks. The incompressibility of the water ensures that any failure will not be dangerous.

PROCEDURE

1) After checking that the pressure chamber and seal are in good condition, clean, and dry, check that the incoming gas will enter close to the bottom. Cover the bottom with a layer of water so that the incoming gas passes through the water. Make a baffle to prevent water from splashing onto the tissue (Fig. 2.5). Line the walls with wet filter paper. Connect a cylinder of compressed air to the gas line.

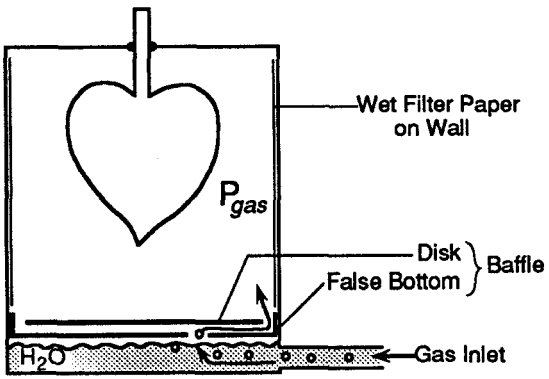

Figure 2.5. Water in the bottom of a pressure chamber reduces overheating of the entering gas and humidifies the air around the leaf. A two-part baffle prevents splashing on the leaf. Normally, the chamber wall is also lined with wet filter paper.

2) Select the sample, avoiding damaged tissue whenever possible. Excise the tissue with a razor blade, insert it swiftly into the seal in the chamber top, and assemble the chamber. The time from excision to sealing the chamber should be no longer than 10 sec to avoid dehydrating the tissue.

If the time is longer than 10 sec, use a humidified glove box to load the sample in the seal and assemble the chamber (see Chap. 3). Work in low light to avoid heating and dehydrating the tissue. Alternately, immediately before excision, enclose the tissue to be pressurized in a flexible plastic bag to retard evaporation (Turner and Long, 1980). Seal the bag enough to inhibit evaporation but allow gas

Figure 2.6. Measuring the xylem tension with a pressure chamber. Note that the observer always stays to the side of the apparatus and wears safety glasses in case tissue is blown out of the chamber.

to escape under pressure, shade the bag, excise the sample, and load into the chamber as above.

3) After the tissue has been placed in the chamber, apply a small amount of pressure and check for leaks. If air is leaking through a manually sealed unit, slowly tighten the seal until audible leakage stops. Raise the pressure slowly and in small steps.

4) Observe the cut surface of the tissue as pressure is being applied. ALWAYS OBSERVE FROM THE SIDE RATHER THAN ABOVE THE

CHAMBER IN CASE THE TISSUE IS BLOWN OUT OF THE SEAL (Fig. 2.6). Do not recut the tissue because the initial excision is the reference position marking the location of water in the xylem in the intact plant. It is to this position that the xylem solution must be returned by the pressure. Increase the pressure until liquid is standing on the cut surface.

5) As pressure increases, it is normal to observe gas bubbles on the surface, but they should be forming slowly enough not to obscure the arrival of the liquid. After liquid appears, reduce the pressure and allow the liquid to be pulled into the tissue until a wet film is all that remains on the cut surface. This is the position of the xylem solution before excising the sample, and it should require a balancing pressure that exactly opposes the tension in the xylem before excision. The meniscus is flat indicating that the water is not constrained by tensions that would otherwise be operating. Adjust the pressure so that the water film remains at the cut surface. For your first measurements, satisfy yourself that the liquid film is maintained for 30 to 60 min without changing the final balancing pressure. During this time, evaporation from the film can be prevented by lining a vial with wet filter paper and inverting it over the cut surface. For routine measurements, it will suffice to observe the meniscus for only 1 or 2 min. The balancing pressure is the negative of the tension in the xylem (Eq. 2.7).

6) After determining the balancing pressure, rinse the surface with water, dry the surface, overpressure, and collect a small (10-20 µl) sample of xylem solution in a microliter syringe for a measurement of the xylem osmotic potential. Measure the osmotic potential with a microliter osmometer or psychrometer (see Chap. 3). If the osmotic potential is sufficiently close to zero, this step can be omitted in subsequent determinations.

7) Release the air in the chamber and remove the tissue. Inspect for damage from pressurizing and sealing.

CALCULATING WATER POTENTIALS FROM PRESSURE CHAMBER DATA

Gauge pressures are converted to megapascals (MPa) or bars according to 1 MPa = 10 bars = 10^6 newtons·m^{-2} = 10^7 dynes·cm^{-2} = 145 lb·in^{-2} = 9.87 atmospheres. The apoplast osmotic potential (negative) is

calculated according to the osmometer instructions (see Chap. 3). The water potential of the tissue is the sum of the apoplast osmotic potential and the negative of the balancing pressure (Eq. 2.8).

Working with Plant Tissue

There are gradients in water potential in plants. As a result, tissue samples are not uniform, and large samples used with pressure chambers may have significantly different water potentials in different parts. The xylem is the source of water for many plant organs and usually is the wetter part of the gradient. After some time elapses in the pressure chamber, the water in the tissue equilibrates and the chamber indicates an average. Usually, the average is approached within 10 min which is the time required for a pressure chamber measurement. However, in some fleshy samples, times can be much longer (hours or days).

Tyree and Hammel (1972) showed that the average is determined by how much water is present in each part of the sample as well as by the potential of the water (also see Fig. 3.19) according to

$$\text{Average } \Psi_w = \frac{\Sigma V^i \cdot \Psi^i_w}{V}, \tag{2.9}$$

where V^i is the water volume in the protoplasm of cell i, Ψ^i_w is the water potential of cell i, and V is the total water volume in the protoplasm of all the cells (the symplasm). The symbol Σ adds the contribution of all the cell V^i and Ψ^i_w in the tissue. Dividing by V gives a volume-weighted average. The volume of water in the apoplast is not considered in this calculation because the xylem and wall matrix are considered to be incompressible (but see section on Changes in Xylem Dimensions).

Equation 2.9 shows that those parts of the tissue containing the largest water volume make the largest contribution to the water potential measured with a pressure chamber. The cells far from the xylem account for a larger volume than the few cells next to the xylem, and the potential of the far cells will dominate the volume average in a pressure chamber. You can observe this by rapidly pressurizing a sample, which often forces a transient show of xylem solution at a low

pressure because of the release of water from the wetter cells immediately around the xylem. However, the solution disappears and the final stable reading is always at a higher pressure reflecting the eventual contribution of the far cells to the volume average. The volume-averaging concept applies to the components of Ψ as well as to any other cell parameters depending on Ψ and measured at the tissue level. With care, the volume average measured with the pressure chamber should be the same as the volume average in the intact plant before sampling.

Other factors can change the Ψ_w, and a particular problem for the pressure chamber is the inherently dehydrating nature of the measurement. Only excised tissue can be used, which eliminates water uptake. The gas entering the pressure chamber is dry and warm as a result of compression, which favors evaporation. Water also evaporates from the cut surface on the outside of the chamber. Steps need to be taken to minimize these problems, specifically by loading the chamber rapidly, humidifying the chamber, and raising the pressure slowly. In addition, you should plan to take the chamber to the plant to be sampled and avoid the temptation to carry the plant or excised sample to the pressure chamber. Not only is loading more rapid, but the plant environment is unchanged and the water potential of the intact system is more readily preserved in the sample.

LEAVES

Most leaves equilibrate rapidly with the applied pressure and are favored material for pressure chamber measurements. Enclose as much of the leaf as possible inside the chamber because pressure chambers measure tensions that extend throughout the sample and pressure must similarly extend over the whole sample insofar as is practical. This ensures that any deformation caused by tensions in the intact plant will be reproduced by the pressure in the chamber.

Leaves having petioles require a round seal but grasses and certain conifer needles require a slit seal. Sampling is similar for both except, for wide grass leaves having a large midrib, the blade is sampled on one side of the midrib. In this case, use a sharp razor blade to cut toward the midrib perpendicularly (Fig. 2.7A). Grip the tissue on the apical side of the cut and tear toward the leaf tip (Fig. 2.7B). This

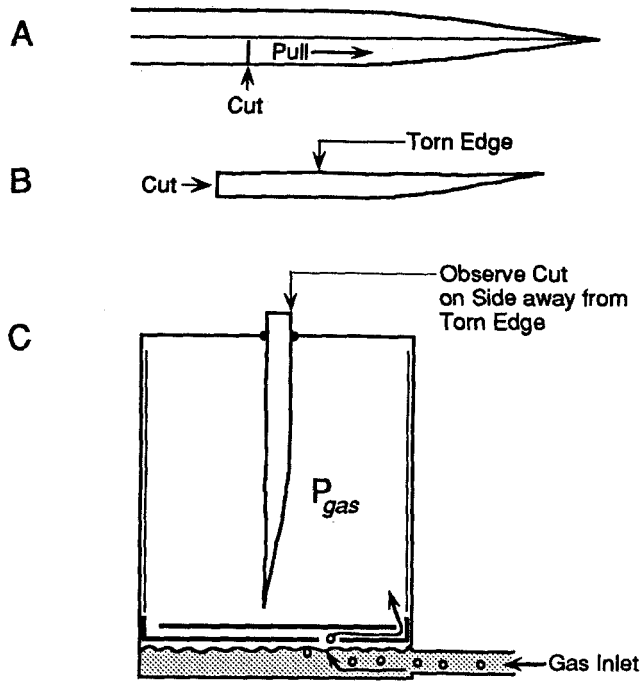

Figure 2.7. Sampling a wide grass leaf. Cut across the leaf almost to the midvein (A), tear the leaf toward the apex from the cut (B), and insert the sample in the pressure chamber (C). Observe the appearance of the xylem solution at the cut surface during pressurization but ignore solution appearing close to the torn edge.

gives a triangular sample with a damaged edge paralleling the midrib. Observe the xylem solution at the cut end but ignore the early appearance in the veins closest to the damaged edge (Fig. 2.7C).

Because leaf tissue is soft and easily crushed, avoid pressure chamber designs with pressure-activated seals where there is no control over how much force the seal applies to the tissue. A slight amount of crushing generally occurs and does not affect the balancing pressure but prolongs the time necessary to make the measurement. If crushing appears severe, test whether the xylem has been affected by leaving the tissue in the seal and excising the edge of the leaf to expose the ends of

the veins. Apply pressure to the leaf in a water-filled pressure chamber. The flow through the tissue in the seal should be much faster than that observed with the intact sample, indicating that the xylem was not constricted by the seal.

Crushing the tissue also can release solution from the crushed cells. This adds liquid to the xylem solution, which appears at the cut surface earlier than if the tissue had not been crushed. Test this effect by loading a leaf into the seal in the usual way, but excise the blade before pressurizing the sample. Pressurize and tighten the seal. Any released liquid will appear on the cut surface and must be from crushing by the seal because there is no other tissue in the chamber. Crushing must be considered whenever the flow of water in or out of the leaf is important especially in pressure activated seals. Sometimes pressure chambers are used to study the rate of water release from leaves (Boyer, 1974; Koide, 1985; Tyree *et al.*, 1981) and care should be taken to avoid pressure activated seals in such studies.

BRANCHES

For woody branches, it may be desirable to strip away the tissues outside of the xylem for a short length so that only woody tissue is inserted into the seal. Regardless of the length of the branch, enclose as much as possible in the pressure chamber.

Branches contain a significant amount of nonleaf tissues such as pith, cortex, cambium, and so on. These often equilibrate slowly with the vascular tissue and pressure readings may be too rapid for complete equilibration. The effect can be demonstrated by measuring the water potential of the intact branch, then removing each leaf, placing Vaseline over the cut surfaces to retard gas entry, and repeating the measurement. If the branch without leaves gives a water potential different from that of the intact branch, equilibration of the entire branch did not occur during the intact measurement. One must then choose whether a "leaf balancing pressure" or "branch balancing pressure" is desired. The leaf balancing pressure is usually achieved in minutes but the whole sample balancing pressure may require hours or days.

ROOTS

Roots are often too fragile to measure individually unless there is extensive secondary thickening. Therefore, one usually uses a whole root system after detaching the shoot and seals the stump of the detopped stem in the chamber. Remove the root medium by placing the root system in a water vapor-saturated glove box and gently allowing the medium to fall away. Assemble the chamber without exposing the roots to dry air. Be sure that the chamber has been prehumidified and note the balancing pressure in the same fashion as with leaves or branches.

ROOTS IN SOILS

It is possible to obtain an average water potential for a root-soil complex by leaving the soil attached to the root system. Pressurize the sample in the same way as with other tissues.

Significant gradients in water potential can be present in the root-soil complex, particularly next to the root surface. The movement of water during pressurization collapses these gradients by forcing water from the bulk soil to the root surface. Thus, pressure readings tend to be weighted toward the potential of the bulk soil. Also, pressurizing wet soils can force water into the intercellular spaces of the root tissues with unpredictable effects (Passioura, 1984). There may be salt gradients next to roots when transpiration is rapid (Kramer and Boyer, 1995) and these can affect the pressure chamber readings.

As with leaves and branches, the pressure chamber measures the tension arising from the matric potential in the apoplast (Eq. 2.4). However, an endodermis separates the stele from the cortex and has Casparian strips that create a hydraulic barrier, and water probably flows mostly through the protoplasm at this barrier. Thus, the apoplast tension measured with the pressure chamber may extend only into the stele, and the cortical apoplast may be under much less tension.

Because soil contains solutes that affect the water potential of the roots, the selectivity of the root system for water is important. Assuming completely selective roots, the water potential of the root is obtained by determining the osmotic potential of the solution in the root xylem and adding the matric potential of the stele of the roots (Eq. 2.4). If the Casparian strips are not completely selective, however, a

correction may need to be applied (see Significance of Reflection Coefficients, Chap. 4).

Roots in hydrated soils often will exude liquid onto the cut surface without any pressure application. This is a normal expression of root pressure, and pressure chamber measurements cannot be made. In dry soil, no exudation takes place and pressure measurements become possible.

Measuring the Components of the Water Potential

With the pressure chamber, the components of the water potential can be measured in the tissues surrounding the xylem provided it is ensured that there is equilibrium between the xylem and the rest of the tissues. The method allows the tissues to remain completely intact in the excised sample, which is an advantage.

OSMOTIC POTENTIAL

When solute is added to water, the free energy of the water decreases because the solute occupies space otherwise occupied by water, diluting the water and decreasing its chemical potential. As discussed in Kramer and Boyer (1995), Ψ_s approximates $-RTC_s = -RTn/V$ for dilute ideal solutions of nondissociating solutes. The C_s is the molar concentration of solute given as n/V (mol·m^{-3} of water), R is the gas constant (m^3·MPa·mol^{-1}·K^{-1}), and T is the temperature (K). This relationship shows that Ψ_s is proportional to the solute concentration, and the pressure chamber can be used to remove water from the cells, leaving the solutes behind and making the cell solution more concentrated. As long as temperature is constant and the number of moles of solute n is a constant inside the cells, $-RTn$ is a constant (k):

$$V \cdot \Psi_s = -RTn = k, \tag{2.10}$$

Rearranging Eq. 2.10 gives the equation of a line with a slope of $(1/k)$:

$$\frac{1}{\Psi_s} = \frac{1}{k} \cdot V. \tag{2.11}$$

The pressure chamber removes water from the cells by overpressuring them and, as the water moves out, the solution in the walls is diluted and its osmotic potential approaches zero. Equation 2.8 becomes $-P_{gas} = \Psi_{w(p)}$ and, with water loss from the cells, turgor becomes zero so that $-P_{gas} = \Psi_{w(p)} = \Psi_{s(p)}$. Replacing Ψ_s in Eq. 2.11 with $-P_{gas}$ gives

$$-\frac{1}{P_{gas}} = \frac{1}{k} \cdot V. \qquad (2.12)$$

Thus, in turgorless tissue, the osmotic potential of the cells is directly measured with the pressure chamber, and a plot of $-1/P_{gas}$ versus V gives a straight line of slope $(1/k)$ because of the concentration dependence of the osmotic potential (Fig. 2.8).

PROCEDURE

1) Apply an overpressure to the tissue, drive out a small volume of water, collect the volume in a syringe, and note the volume.
2) Adjust the pressure to the new balance, and note the pressure. This gives the pressure at the new water volume in the cells after removing the water in 1).
3) Repeat 1) and 2) for about 10 water contents.
4) Determine the final water content of the tissue by releasing the pressure, removing the tissue, excising the veins and any stem, weighing the interveinal tissue, and oven drying the tissue.
5) The difference between the weight of the interveinal tissue before and after oven drying is the volume of water in the tissue at the end of the pressure series (the veins and stem are usually removed because their water content is considered to be relatively constant).
6) Add each removed volume to the volume in the tissue at the end of the pressure series and express each sum as the relative water content at each balancing pressure (Richter, 1978). The relative water content is relative to the maximum water content in fully hydrated tissue expressed as a percentage (see Fig. 2.8). The relative water content is a measure of V in Eq. 2.12.
7) Plot $-1/P_{gas}$ versus the relative water content (Fig. 2.8). The initial part (low pressures) is not straight, but the final part (high pressures)

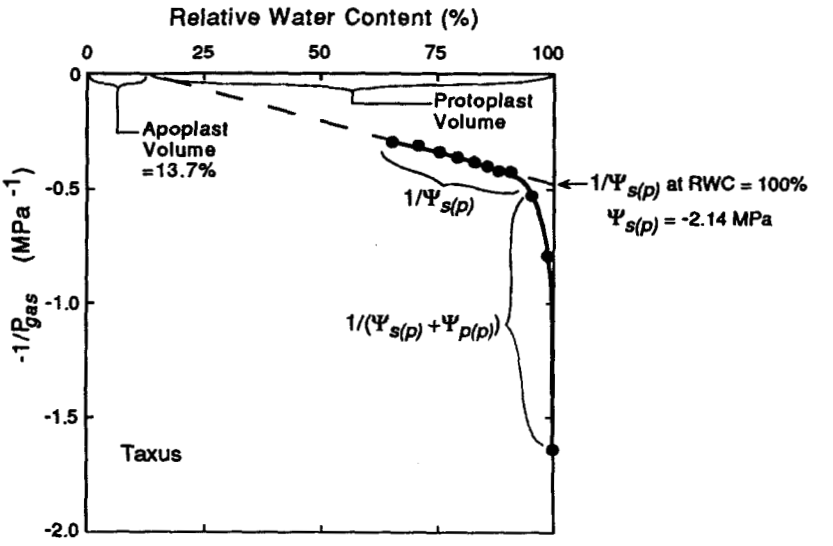

Figure 2.8. Pressure-volume determination for a *Taxus* branch in a pressure chamber. The relative water content is the volume of water in the tissue relative to that in a fully hydrated sample. Overpressures remove water from the leaf in steps, and the balancing pressure and removed volume are noted at each step (individual data points). The relative water content is determined by measuring the volume remaining after all overpressures are finished and adding the volumes that were removed by each overpressure. The dashed line shows the linear relationship governed by the osmotic potential ($\Psi_{s(p)}$, Eqs. 2.11 and 2.12). Extrapolation to the axis on the right gives the osmotic potential at 100% relative water content, and to the axis above gives the apoplast volume. The curvilinear part of the pressure-volume relation (on the right at high relative water contents) shows the effect of the osmotic potential plus the turgor pressure ($\Psi_{s(p)} + \Psi_{p(p)}$) inside the cells. The $\Psi_{s(p)}$ determined from the dashed line is subtracted from the $\Psi_{s(p)} + \Psi_{p(p)}$ to give the turgor (see Fig. 2.9). Data from J. S. Boyer (unpublished).

becomes straight. The straight portion represents the region where Eqs. 2.11 and 2.12 are followed and $-1/P_{gas} = 1/\Psi_{s(p)}$ or, in other words, where $-P_{gas}$ directly measures the osmotic potential of the tissue. Extrapolating the straight portion of the line to other water contents

indicates the osmotic potential at any other water content (Fig. 2.8, dashed line). Extrapolating the straight line to the X axis shows the volume of water remaining after all the protoplast water has been removed (P_{gas} becomes infinite, Fig. 2.8). The remaining water is the volume of the wall and xylem water (apoplast volume), considered to be incompressible.

ASSUMPTIONS

Other ways of analyzing P·V data have been suggested but these are generally less satisfactory than the approach in Eq. 2.12 (Tyree and Richter, 1982). All methods rest on the assumption of a constant solute content in the cells, and Kikuta and Richter (1988) point out that the content may not be constant if solutes are generated by the cells during pressurization. This is not a problem with most tissues but wheat leaves appear capable of enough solute generation to cause an error (Kikuta and Richter, 1988). In general, the cell solutions are assumed to be so dilute that concentrations can be used instead of activities to express solution properties (Tyree and Richter, 1981).

Caution needs to be used in the extrapolation of the straight line. The extrapolations in Fig. 2.8 assume that all the water is released from the cells and none from the walls and xylem. The wall pores and xylem usually are reasonably rigid except in young tissue (Tyree, 1976), but significant water released by these structures can cause extrapolation errors. In particular, the extrapolation to the x axis is long and thus there is a considerable degree of uncertainty (see Changes in Xylem Dimensions).

The method also assumes that the cell walls around the xylem are not rigid and will collapse into the cell compartment without resistance as the tissue dehydrates. In tissues with stiff walls, the collapse may be resisted and $-P_{gas}$ may not equal $\Psi_{s(p)}$, necessitating a correction (see Negative Pressures Inside Protoplasts).

You can test whether any of these problems affect your data by calculating $\Psi_{s(p)} \cdot V$ at various V, that is, $\Psi_{s(p)}$ multiplied by the relative water content at any relative water content. This is a measure of the solute content of the tissue (Eq. 2.10) and establishes whether the solute content has remained constant over a wide range of water contents, as required by the theory. It also shows whether the xylem and cells

follow solution behavior. The $\Psi_{s(p)} \cdot V$ should not vary along the straight line portion of Fig. 2.8. It is wise to make this test on all measurements of Ψ_s.

Another feature of the method requiring caution is the considerable time needed for making a series of overpressures. It is important to use compressed air so that oxygen is available to the tissue during the measurements and to minimize evaporation by humidifying the chamber, collecting the removed liquid quickly, and determining each new balancing pressure quickly (but be sure to wait long enough for a true balance). Avoid pressures above 3 to 4 MPa if possible because cell membranes can be disrupted and release cell solutes to the vascular system, which will cause the plot to deviate from linearity. If you have difficulty achieving linearity, measure the osmotic potential of the solution exuding from the cut surface. An osmotic potential significantly below zero at high pressures means that cell membranes have broken and cell solutes are being released to the apoplast. In this situation, the measurement must be abandoned.

TURGOR

Turgor results in pressure on the cell solution, and the balancing pressures in a pressure chamber are less than in comparable turgorless tissue. When turgor is present, the balancing pressures do not follow Eqs. 2.11 and 2.12, and a plot of Eq. 2.12 curves downward (on the right close to the Y axis, Fig. 2.8). By comparing the downwardly deviating line (which describes $-1/P_{gas} = 1/(\Psi_{s(p)} + \Psi_{p(p)})$ with the linear extrapolation (which describes $-1/P_{gas} = 1/\Psi_{s(p)}$), the turgor can be determined by difference (Eq. 2.3), and the turgor can be found at any tissue water content (Fig. 2.9).

MATRIC POTENTIAL

Matric potentials occur because the surface of a liquid has properties that differ from those in the interior. In any porous medium wettable by water, solids extend the surface so that a larger share of the molecules have surface properties. The wettability results mostly from hydrogen bonding between water and OH groups on the surfaces and from surface charges that attract the water dipole. The surface charges also attract ions in the water. The total effect is to constrain water and

solute next to the surfaces. The wettability attracts water from the air and any liquid water and, because water forms strong bonds with other water molecules, the pores in the matrix tend to fill. Electrically constrained ions next to the pore surfaces also move water into the matrix with osmotic-like force. As a consequence, the water content of the matrix can become very large. Pressures are generated next to the surfaces and the whole matrix can swell.

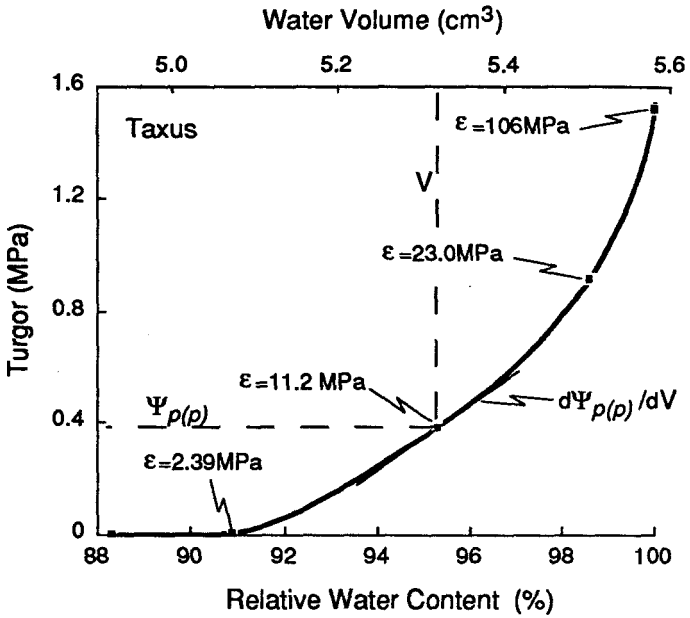

Figure 2.9. The turgor ($\Psi_{p(p)}$) and volume (V) of water in the cells of the *Taxus* branch shown in Fig. 2.8. The slope $d\Psi_{p(p)}/dV$ was measured at each $\Psi_{p(p)}$ and V, and the elastic modulus (ε) also was calculated. In the example shown, the slope is drawn through a point and ε = (0.380 MPa/0.180 cm^3)·5.32 cm^3 = 11.2 MPa. Data from J. S. Boyer (unpublished).

For most plant cells, the walls are the major site of the matric potential (Boyer, 1967b). The surfaces are highly wettable, and water fills the pores. Because of the small pore diameter, tensions (negative

pressures) to about -58 MPa can be present without draining the water. Of course the tension on pore water varies between zero and -58 MPa, and the various pressures measured with a pressure chamber demonstrate this principle. Accordingly, pressure chambers give a direct measure of the matric potential in the walls of the living tissue ($\Psi_{m(a)}$ in Eqs. 2.4 and 2.7).

The pressure chamber also can measure the matric potential in leaves killed by freezing and thawing (Boyer, 1967b), which is sometimes useful (see Figs. 2.10 and 2.11). In this situation, there is no turgor and the osmotic potential is virtually without effect because there are no membranes. The only force holding water in the tissue is the matric potential resulting from surface interactions. As pressure is applied to the system, water and solute move out of the cells and exude from the cut end of the petiole. The walls tend to collapse into the cell compartment and resist collapse according to the strength of the wall, but the measured matric potential is accurate regardless of how much the walls collapse.

The forces holding water in the dead matrix are of the same physical nature as those holding water in the apoplast of living tissue. However, freezing and thawing flood the walls with protoplast solution and change the matric potential of the apoplast to a higher value (less negative) than in the living tissue (Boyer, 1967b). Only when the water content in the frozen/thawed tissue is the same as in the walls of living tissue do the matric potentials approach those in the living tissue, as pointed out by Boyer (1967b) and Passioura (1980).

With frozen/thawed tissue, it is usually desirable to keep the petiole alive so that it retains its usual strength in the seal in the top of the chamber. Wrap the petiole in wet cotton while the leaf blade is being frozen and, with care, a freezing time can be found that allows the blade to be frozen but not the petiole. Mount the petiole in the seal of the pressure chamber in the usual way and pressurize the tissue slightly. Increasing pressures will cause the cell solution to exude onto the cut surface and decreasing pressures will cause the exudate to move back into the tissue. Because the matrix is flooded with cell solution released by freeze/thawing, the pressures are small and rates of exudation are slow.

ELASTIC MODULUS OF PLANT TISSUE

As turgor is generated by the walls pressing on the cell contents, the walls are under strain much as the cover of a ball comes under strain at high internal pressures. The strain is elastic and reversible. The elasticity of the strained cell wall can be measured at various water contents using a pressure chamber (Fig. 2.9). Because pressure applies a force in three dimensions, the elasticity is determined as the bulk modulus of elasticity (ε, MPa) defined by

$$\varepsilon = \frac{d\Psi_{p(p)}}{dV} \cdot V \tag{2.13}$$

or

$$d\Psi_{p(p)} = \varepsilon \cdot \frac{dV}{V} \tag{2.14}$$

The bulk modulus is a proportionality constant indicating how much change occurs in the relative cell volume dV/V when the pressure inside the cell changes by an amount $d\Psi_{p(p)}$. The more elastic the cell wall, the smaller is the value of ε. The ε is maximum when turgor is at its maximum but becomes zero when the turgor is zero for a cell having thin elastic walls. Figure 2.9 shows this effect using data from Fig. 2.8. Because the pressure chamber measures volume-averaged tissue $\Psi_{p(p)}$, the ε is a volume-averaged parameter.

Precautions

SAFETY

Pressure chambers can be dangerous because of the large gas volumes involved. There can be explosive release of the tissue from the seal or failure of a chamber component. Always work at the side of the chamber and never view the cut surface from overhead (Fig. 2.6). To avoid the failure of a chamber component, pressure test the sealed and water-filled chamber before the first use as described earlier. All components should withstand pressures at least double the maximum expected to be used.

DEHYDRATION DURING LOADING

Excised tissue continually dehydrates. In normal air, its potential decreases significantly in a few seconds. It is generally impossible to prevent this completely but placing the tissue in a saturated atmosphere and keeping it as isothermal as possible minimizes the problem (plant tissue produces small amounts of heat metabolically and thus loses water slowly even in saturated air).

With these principles in mind, one must work swiftly and place the pressure chamber next to the plant to minimize the time between excision and chamber loading. Because the chamber contains saturated air, loading reduces the rate of evaporation. If loading times are longer than 10 sec, quickly place the tissue into a glove box with a saturated atmosphere so that loading can be extended without dehydrating the tissue. A convenient glove box can be constructed from a Styrofoam box with a Plexiglas sheet on top (see Chap. 3). An alternative is to quickly place a thin polyethylene bag around the sample immediately prior to excision (Turner and Long, 1980). Seal the bag loosely, shade, excise the tissue, and insert the sample into the seal of the chamber top. Apply pressure to the tissue in the bag.

DEHYDRATION DURING PRESSURIZATION

After sealing in the chamber, the tissue is exposed to dehydration from the dry gas used to pressurize the sample. Pressure chambers should be humidified by bubbling the incoming gas through water in the bottom and past walls lined with wet filter paper. The water in the bottom not only humidifies but also cools the incoming gas.

A baffle can be inserted in the pressure chamber just above the liquid to prevent water droplets from splashing onto the tissue (Fig. 2.5). A simple baffle can be made from the bottom of a suitably sized polyethylene bottle which is forced into the pressure chamber and held by the walls just above the surface of the water. Place one or two small holes in this false bottom to allow compressed air to move into the main body of the chamber. From another plastic bottle, cut a flat disc that is slightly smaller than the inside diameter of the chamber. Place this disc on top of the false bottom. The incoming air will bubble through the water, pass through the small holes in the false bottom, and go around the disc to enter the main portion of the chamber. The disc

breaks up any water droplets that are blown through the holes. If your pressure chamber is not designed for gas to enter the bottom, insert a tube inside the chamber to lead the gas from the inlet to the bottom.

MEASURING AT EQUILIBRIUM

Pressure chambers generally use large tissue samples. Because there are water potential gradients in plants and soils (see Boyer *et al.*, 1980, for an example), it is important to make measurements slowly enough to allow the gradients in the sample to equilibrate and form a volume-averaged potential. For the most part, gradients in leaf samples will equilibrate adequately in 10 min. In larger samples (e.g., branches), the time is longer.

Pressure chambers are useful for making water potential measurements rapidly, and it is tempting not to wait for a true balancing pressure. Indeed, some pressure chambers are designed to allow air to enter at a steady rate and be shut off at the first sign of liquid on the cut surface. This method is not recommended because it does not allow balancing pressures to be achieved. Moreover, the tissue can be heated by rapid air entry into the chamber (Puritch and Turner, 1973). To ensure that equilibrium occurs, the pressure should be adjusted to give a stable, flat water film at the cut xylem surface. The film should not grow or shrink, indicating that water is neither exiting nor entering the tissue.

OVERHEATING

When pressure chambers are used continuously at high ambient temperatures, spurious readings can result. The problem is most often encountered at temperatures above 30°C in the field when large amounts of heat are produced by frequent compression of the incoming gas (Puritch and Turner, 1973). The errors in the readings are caused initially by excessive dehydration of the tissue and ultimately by breakdown of the membranes because of the heat. The breakdown causes readings to be too low. The effect can be minimized by wrapping a wet paper towel around the outside of the chamber to allow evaporative cooling of the chamber walls. Also, pressurizing slowly and bubbling the gas through water on the chamber bottom will help keep the chamber cool.

AVOIDING TISSUE HYDRATION BY SURFACE WATER

Plant tissue sometimes may be coated with dew or have droplets of water on the surface. When pressurized, the liquid water is forced into the tissue and raises its water potential. If water is present on the tissue to be pressurized, blot the tissue dry. If possible, allow some time for the tissue to dry completely before sampling. Avoid tissue contact with wet filter paper on the walls by folding the leaf loosely and holding the folds in place with a rubber band or tape. Water splashing from the bottom of the chamber can be avoided by constructing a baffle in the bottom (Fig. 2.5).

ANATOMICAL ERROR

Pressure chambers use the cut surface of the sample as a reference position for the measurement. Therefore, never recut the tissue after removing the sample from the plant. Tissues comprising the cut surface can have hollows or pith that allow the xylem solution to spill over and be trapped after it first appears on the cut surface (Boyer, 1967a). More pressure is necessary to maintain the liquid at the surface in this situation and the reading will be spuriously high. In this case, pressure readings are considered to be relative rather than absolute measurements (Boyer, 1967a).

BUBBLING IN XYLEM SOLUTION

In a typical sample, pressurization does not cause much movement of gas through the tissue. Of that appearing at the cut surface, most travels through the intercellular spaces. A small amount moves through the xylem probably because gas has been forced into solution at high pressure and bubbles out of solution as atmospheric pressure is encountered.

Wounding of pressurized samples can allow gas to enter the vascular tissue more rapidly. Liquid in the xylem appears prematurely at the cut surface and bubbling can be so severe that the water films are difficult to observe or are dissipated as a fine spray. Wherever possible, avoid using wounded tissue for measurements. If wounds cannot be avoided, it is sometimes possible to coat the wounded area with petrolatum (Vaseline) and decrease the rate of gas entry.

Xylem solutions sometimes contain compounds that have surfactant properties, and the solution foams on the cut surface. If

foaming is excessive, the measurement must be abandoned. If it is moderate, increase the pressure until liquid accumulates underneath the foam on the cut surface. Then withdraw the liquid into the tissue and determine the balance pressure. If the foam fails to break up during this procedure, touch it with your finger.

CHANGES IN XYLEM DIMENSIONS

Mature plant xylem in secondary tissues withstands large pressures. Immature xylem, protoxylem, and metaxylem do not nor do tissues around the xylem, and their volume decreases as water flows out of the cells. The xylem is often under large tensions, and when the tissue is excised and pressurized, some of the xylem can change dimensions or can exchange water with the surrounding tissues, particularly if the sample contains growing tissues. To avoid error caused by these effects, the xylem should be pressurized along as much of its length as possible to force the surrounding tissue to the same water content during the measurement as it had when the xylem was under tension in the plant. You can test whether these effects are a problem by changing the position of the sample in the seal. Use alternating measurements with most of the xylem pressurized (most of stem inside of chamber) or the least amount of xylem pressurized (most of stem outside). If balancing pressures are less when most of the xylem is pressurized, stem tissues are sensitive to pressure and need to be considered.

POTENTIAL GRADIENTS

In addition to the need to let potential gradients equilibrate in the sample, there are long-distance gradients that need to be considered (e.g., Boyer *et al.*, 1980). The two largest contributors to gradients are the distance of the sample from the water supply and the degree of illumination of the sample. In most cases, the variability between samples can be markedly reduced by knowing where gradients exist and by sampling in the same part of the gradient. Comparisons of potentials between plants under these conditions require samples from the same part of the canopy, similarly illuminated, and at a similar stage of development.

Depending on the research question, one should sample at an appropriate position in the plant gradient. For example, to measure the

water potential of leaves that are doing most of the photosynthetic work for the plant, sample at the top of the canopy using leaves that are recently fully expanded and oriented perpendicular to the incoming light.

Enclosing leaves in gas exchange cuvettes poses special problems because the enclosure generally changes the leaf water potential. Always measure the water potential of the leaf in the cuvette if you wish to relate leaf performance in the cuvette to the water status of the leaf. Avoid sampling leaves outside the cuvette and assuming that the water potential is the same as inside the cuvette. If you cannot sacrifice the leaf in the cuvette for the pressure chamber measurement, consider using a thermocouple psychrometer on a small leaf sample (see Chap. 3).

NEGATIVE PRESSURES INSIDE PROTOPLASTS

Pressures rise and fall in cells according to changes in cell water contents. Turgor is high in the protoplasts when water contents are high, and tensions are small in the apoplast. As water contents decrease, turgor diminishes and tensions become greater. A question then arises: if water contents continue to decrease, does turgor disappear and tension begin to extend into the protoplasts and, if so, does this affect pressure chamber measurements? The answer appears to be yes for both questions under particular conditions.

The effect depends on how much the cell walls resist collapse under tension. In most tissues, the cell walls are thin and follow the shrinkage of the cell solution without significant resistance as water contents decrease. In sunflower, for example, the walls occupy only 9-12% of the total cell volume (Boyer, 1967b), and they tend to fold and follow the shrinking protoplasts as the cells dehydrate, which is clearly visible under the electron microscope (Fellows and Boyer, 1978). This suggests that they do not exert a significant counterforce to the shrinkage, and Fig. 2.10 shows that the volume changes readily in frozen/thawed tissue, and the matric potential ($\Psi_{m(t)}$) is small when compared to that in the living tissue ($\Psi_{m(a)}$). The frozen/thawed tissue has no turgor and the small $\Psi_{m(t)}$ indicates that the walls do not resist shrinkage as water is lost from the matrix. This lack of much counterforce continues until relative water contents fall below 10-20%. Accordingly, at a higher water content of 60%, the free energy diagram

on the right of Fig. 2.10 shows that $\Psi_{m(t)}$ is a very small component compared to P_{gas} in the living tissue. Therefore, in living sunflower, at these higher water contents, tensions in protoplasts can be neglected for pressure chamber measurements.

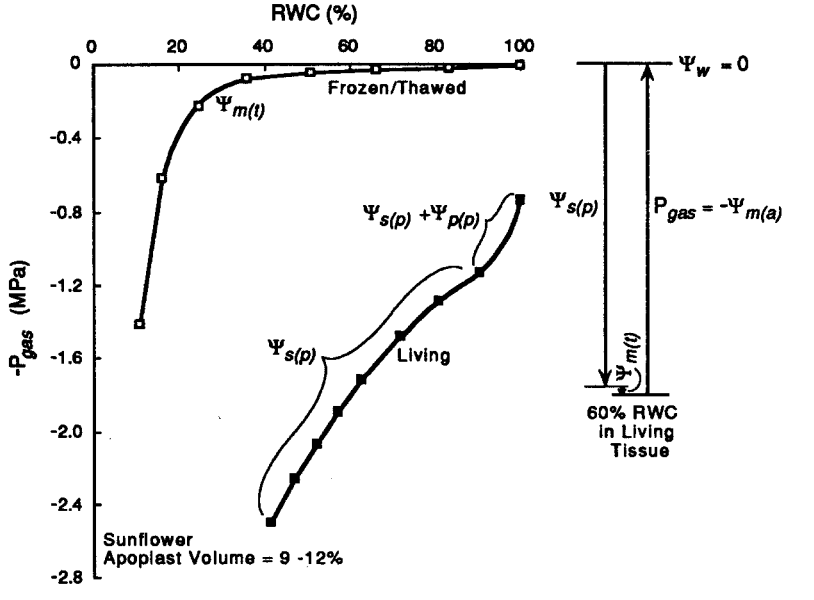

Figure 2.10. Comparison between pressure-volume data in living and frozen/thawed leaves of sunflower. In living tissue, the pressure chamber ($-P_{gas}$) measures the matric potential $\Psi_{m(a)}$ of the apoplast (Eq. 2.7). In frozen/thawed tissue, the pressure chamber measures the matric potential $\Psi_{m(t)}$ of the entire nonliving tissue. The $\Psi_{m(t)}$ is mostly caused by the cell walls. Note that between 40 and 100% RWC, $\Psi_{m(t)}$ changes little and large amounts of solution can be removed indicating that the walls collapse into the shrinking cell compartments. The cell walls occupy 9-12% of the total cell volume and thus have little resistance to the shrinkage. The diagram on the right gives the components measured by the pressure chamber in living tissue at 60% RWC taken from the graph on the left. In living tissue, a 60% water content is unable to generate turgor, and the $-P_{gas} = \Psi_{m(a)} = \Psi_{s(p)}$. The $\Psi_{m(t)}$ is so small that it can be neglected. Data from J. S. Boyer (unpublished).

However, tissues having thick cell walls behave differently. Figure 2.11 shows that in rhododendron, the apoplast volume was 26 to 28% of the total water volume in the tissue (Boyer, 1967b). The walls were relatively rigid and resisted collapse and in consequence the frozen/thawed tissue showed a substantial $\Psi_{m(t)}$ at all water contents. Therefore, when turgor disappeared in living leaves, the tension in the xylem extended into the surrounding protoplasts.

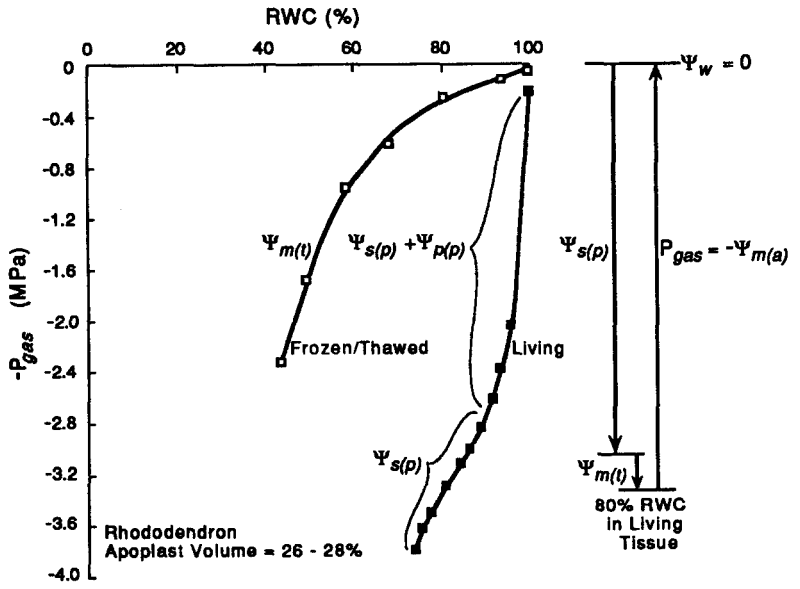

Figure 2.11. Same comparison as in Fig. 2.10 but for rhododendron instead of sunflower. Rhododendron leaves have stiffer cell walls (26-28% of cell volume) than sunflower (9-12% of cell volume), and $\Psi_{m(t)}$ is a significant component of $-P_{gas}$ at most water contents (e.g., diagram on right for 80% RWC). As shown on the right, $\Psi_{m(t)}$ contributes so much to the measurement of $-P_{gas}$ that $\Psi_{m(t)}$ must be subtracted from $-P_{gas}$ to obtain a valid measure of $\Psi_{s(p)}$ in living tissue. This becomes important for measurements of $\Psi_{s(p)}$ in any tissue having rigid cell walls. For a test to determine whether the subtraction is necessary, see the text. Data from J. S. Boyer (unpublished).

A simple test for this effect is to determine whether $\Psi_{s(p)} \cdot V$ is constant over the linear range of volumes in Eq. 2.12 (see Measuring the Components of the Water Potential, Osmotic Potential). If it is not, a possible cause is a tension in the protoplast compartment caused by the resistance of cell walls to collapse during dehydration of the tissue. The tension can be subtracted from the P_{gas} to give a more accurate osmotic potential (Fig.2.11). Freeze/thaw a comparable sample and, from measurements of $\Psi_{m(t)}$ at various relative water contents as shown in Fig. 2.11, subtract the pressure used to measure $\Psi_{m(t)}$ from P_{gas} in the living tissue (Fig. 2.11 on the right). This subtraction should give a corrected $\Psi_{s(p)} \cdot V$ that is constant and thus provide a true $\Psi_{s(p)}$ at each water content.

It should be recognized that in the living tissue, the tension begins to extend into the protoplasts only after turgor decreases to zero. Tyree (1976) recognized that this tension could be present but considered it to be a "negative turgor" (an unfortunate misnomer) that was negligible in most cases. His test was based on the straightness of the slope of the line generated by the data, as in Fig. 2.8. He recognized that a better test would have been $\Psi_{s(p)} \cdot V = k$ to indicate that the solution in the cells behaved ideally. However, the test could not be made because the required data were not available. For the measurements shown in Figure 2.8, however, the test could be made and $\Psi_{s(p)} \cdot V = k$ at all relative water contents showing that negative pressures were not a factor.

Appendix 2.1-Pressure Chamber Manufacturers

Skye Instruments, Inc.
P.O. Box 278
Perkasie, PA 18944
USA
Telephone: (215) 453-9484

PMS Instrument Company
2750 NW Royal Oaks Drive
Corvallis, OR 97330
USA
Telephone: (503) 752-7926

Pacific Agricultural Services, Inc.
4325 West Avenue
Fresno, CA 93722
USA
Telephone: (209) 275-0775

Soilmoisture Equipment Corporation
P.O. Box 30025
Santa Barbara, CA 93105
USA
Telephone: (805) 964-3525