CHAPTER

17

Stem Anatomy and Pressure–Volume Curves

In this chapter we consider pressure–volume curves, which are used to determine osmotic potential and turgor potential. They are constructed from data obtained from the volume of sap exuded from the cut end of a stem protruding from a pressure chamber. Therefore, we need to understand stem anatomy before we turn to pressure–volume curves.

17.1 STEM ANATOMY

17.1.1 General Structure

The close association of the stem with the leaves makes the aerial part of the plant axis structurally more complex than the root (Esau, 1977, p. 257). The term *shoot*, which refers to the stem and leaves as one system, serves to express this association. The stem, like the root, consists of three tissue systems: the dermal (epidermis), the fundamental or ground (pith and cortex), and the fascicular or vascular. The variations in the primary structure in stems of different species are based chiefly on differences in the relative distribution of the fundamental and vascular tissues (Esau, 1977, p. 257).

17.1.2 Dicotyledonous Stem

In dicotyledons, the vascular system of the internode commonly appears as a hollow cylinder delimiting an outer and an inner region of ground tissue, the cortex and the pith, respectively. Figure 17.1 shows bird's foot trefoil, which has a typical dicotyledonous stem. The subdivisions of the vascular system, the vascular bundles, are separated from each other by more or less wide panels of ground parenchyma—the interfascicular parenchyma—that interconnects the pith and the cortex.

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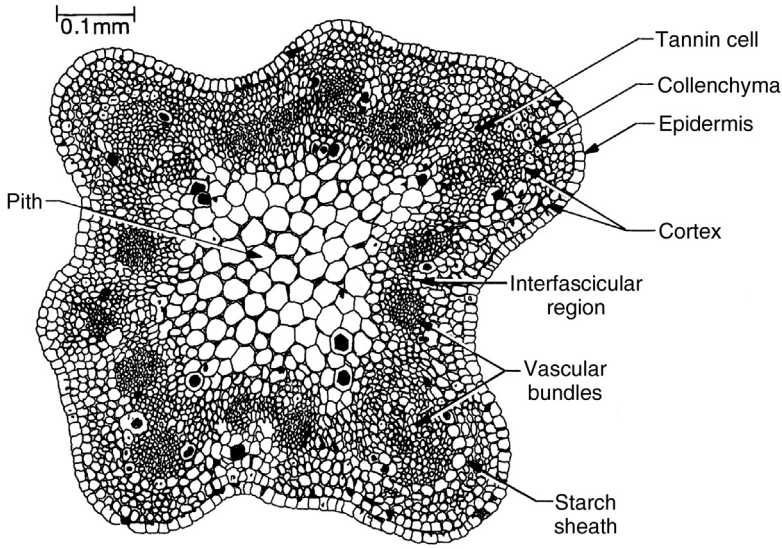

FIGURE 17.1 Cross section of an herbaceous dicotyledon stem, *Lotus corniculatus* or bird's foot trefoil (Leguminosae family or legume family), in primary state of growth. The phloem is on the outside of each vascular bundle, and the xylem is on the inside. *From Esau (1977). This material is used by permission of John Wiley & Sons, Inc.*

This tissue is called interfascicular because it occurs between the bundles or fascicles (Esau, 1977, pp. 257–258).

17.1.3 Monocotyledonous Stem

Stems of most monocotyledons have a complex arrangement of vascular tissues. The bundles may occur in more than one ring or may appear scattered throughout the cross section. The stems (culms) of the Poaceae (grass family), seen in cross section, have widely spaced vascular bundles not restricted to one circle (Esau, 1977, p. 313). The bundles are either in two circles (*Avena*, oat; *Hordeum*, barley; *Secale*, rye; *Triticum*, wheat; *Oryza*, rice) or scattered throughout the section (*Bambusa*, bamboo; *Saccharum*, sugarcane; *Sorghum*, sorghum; *Zea*, corn). Figure 17.2 shows a cross section of a corn stem. The delimitation of the ground tissue into cortex and pith is less precise or does not exist when the vascular bundles do not form a ring in cross sections of internodes.

Monocotyledons other than Poaceae also have vascular bundles scattered or in rings near the periphery, as seen in stem transections. In *Tradescantia* (spiderwort) (Commelinaceae; spiderwort family), the central cylinder has scattered bundles. Compaction of vascular tissue characteristic of hydrophytes is common in aquatic monocotyledons. In *Potamogeton* (pondweed) (Potamogetonaceae; pondweed family), for example, a wide

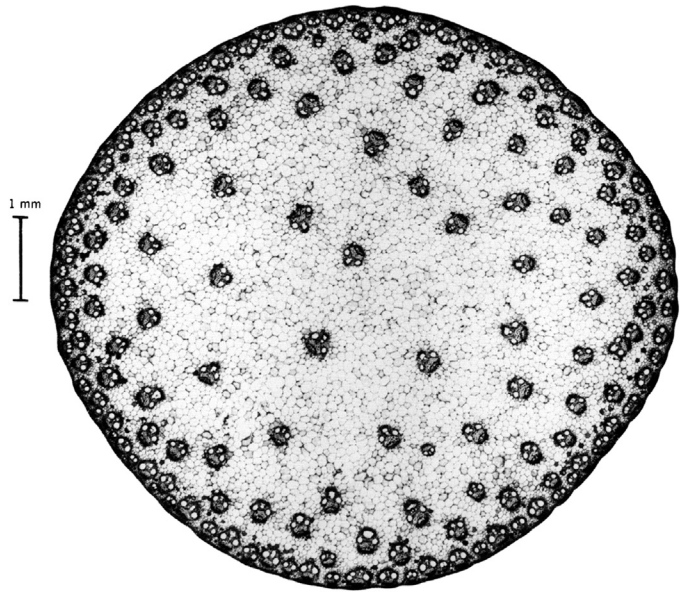

FIGURE 17.2 Cross section of a monocotyledonous stem, *Zea mays* or corn (Poaceae family or grass family). Note vascular bundles are distributed throughout the section, but are more numerous near the periphery. In each vascular bundle, the xylem is oriented toward the center of the stem and three large vessels ("monkey faces"), part of the metaxylem, are visible in each bundle. The phloem in each vascular bundle is oriented toward the outside of the stem. *From Esau (1977). This material is used by permission of John Wiley & Sons, Inc.*

cortex consisting of aerenchyma encloses a compact vascular cylinder delimited by a small-celled endodermis. Variable amounts of pith tissue occur in different species of the genus (Esau, 1977, p. 314).

In general, we can say that dicotyledonous stems usually have a pith at the center of the stem surrounded by vascular bundles. Monocotyledonous stems often have scattered vascular bundles in a ground tissue, and no pith and cortex are delineated.

17.1.4 Stomata, Cortex, Pith, and Vascular Bundles in Primary Xylem

Stomata can be present on stems, but constitute a less prominent epidermal component in the stem than in the leaf (Esau, 1977, p. 259). The stem epidermis commonly consists of one layer of cells and has a cuticle and cutinized walls. It is a living tissue capable of mitotic activity, an important characteristic in view of the stresses to which the tissue is subjected during the primary and secondary increase in thickness of the stem. The epidermal cells respond to these stresses by enlargement and divisions (Esau, 1977, p. 259).

The cortex of stems contains parenchyma, usually with chloroplasts. Intercellular spaces are prominent, but sometimes are largely restricted to the median part of the cortex. In many aquatic angiosperms, the cortex develops as an aerenchyma with a system of large intercellular spaces (Esau, 1977, p. 259). The peripheral part of the cortex frequently contains collenchyma (Figure 17.1). *Collenchyma* is a supporting tissue composed of more or less elongated living cells with unevenly thickened, nonlignified primary walls. It is in regions of primary growth in stems and leaves. In some plants, notably grasses, sclerenchyma rather than collenchyma develops as the primary supporting tissue in the outer region of the stem. *Sclerenchyma* is a tissue composed of sclerenchyma cells. A sclerenchyma cell is a cell variable in form and size and having more or less thick, often lignified, secondary walls. It is a supporting cell and may or may not be devoid of a protoplast at maturity.

As noted when we studied root anatomy (Chapter 15), the innermost layer of the cortex (endodermis) of roots of vascular plants has the casparian strip. Stems commonly lack a morphologically differentiated endodermis. In young stems, the innermost layer or layers may contain abundant starch and thus be recognized as a starch sheath (Figure 17.1). Some dicotyledons, however, do develop casparian strips in the innermost cortical layer of the stem, and many lower vascular plants have a clearly differentiated stem endodermis (Esau, 1977, p. 259).

The pith of stems is commonly composed of parenchyma, which may contain chloroplasts. In many stems, the central part of the pith is destroyed during growth. Frequently, this destruction occurs only in the internodes, whereas the nodes retain their pith. The pith has prominent intercellular spaces, at least in the central part. The peripheral part may be distinct from the inner part in having compactly arranged small cells and greater longevity (Esau, 1977, p. 261).

The discrete individual strands of the primary vascular system of seed plants are commonly referred to as vascular bundles. The phloem and xylem show variations in their relative position in vascular bundles. The prevalent arrangement is *collateral*, in which the phloem occurs on one side (*abaxial*, or directed away from the axis) of the xylem (Figures 17.1 and 17.2). That is, the phloem is closest to the outside of the stem, even in monocots with scattered vascular bundles (Figure 17.2). The xylem in the corn plant shown in Figure 17.2 makes "monkey faces" (two eyes and one large mouth) and is directed toward the center of the stem (away from the epidermis). In some dicotyledons (e.g., Cucurbitaceae, the squash family, and Solanaceae, the nightshade family, which includes potato), one part of the phloem occurs on the outer side and another on the inner side of the xylem. This arrangement is called *bicollateral*, and the two parts of the phloem are referred to as the *external* (abaxial) and the *internal* (adaxial) phloem (Esau, 1977, p. 261). *Adaxial* means directed toward the axis.

17.1.5 Structure of Secondary Xylem

In Chapter 15, Section 15.4, we considered secondary xylem when making calculations of Poiseuille law flow through wood. Here we look at the structure of secondary xylem. A study of a block of wood reveals the presence of two distinct systems of cells (Figure 17.3) (Esau, 1977, p. 101): the *axial* (longitudinal or vertical) and the *radial* (transverse or horizontal) or *ray* system. The axial system contains files of cells with their long axes oriented vertically in the stem or the root, that is, parallel to the main, or longitudinal, axis of these organs. The radial system is composed of files of cells oriented horizontally with regard to the axis of the stem or root.

Each of the two systems has its characteristic appearance in the three kinds of sections employed in the study of wood (Esau, 1977, p. 102). In the *transverse* section, that is, the section cut at right angles to the main axis of stem or root, the cells of the axial system are cut transversely and reveal their smallest dimensions. The rays, in contrast, are exposed in their longitudinal extent in a cross section. When stems or roots are cut lengthwise, two kinds of longitudinal sections are obtained: the *radial* (parallel to a radius) and the *tangential* (perpendicular to a radius) (Figure 17.3).

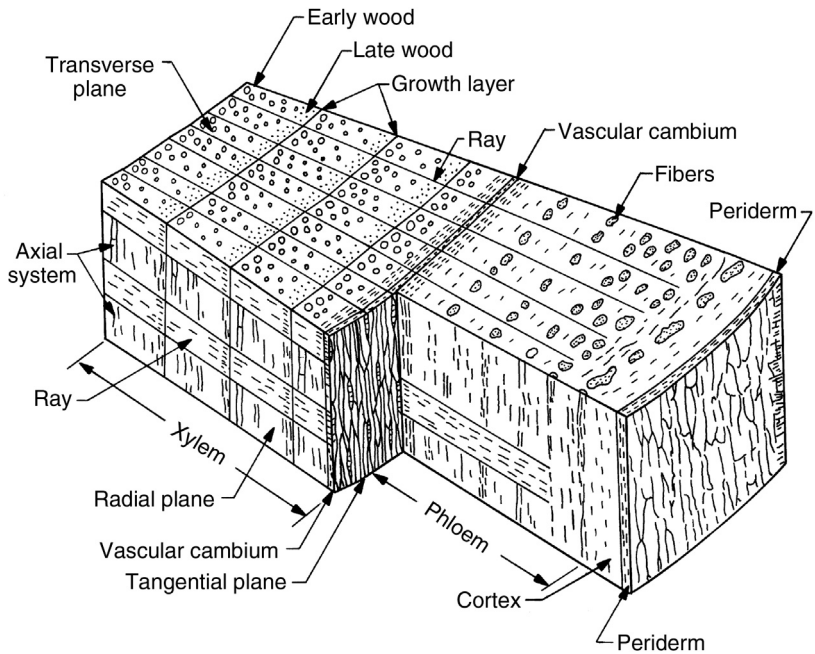

FIGURE 17.3 Block diagram illustrating the basic features of secondary vascular tissue. *From Esau (1977). This material is used by permission of John Wiley & Sons, Inc.*

With little or no magnification, the wood shows the layering resulting from the presence of more or less sharp boundaries between successive growth layers (Figure 17.3). Each growth layer may be a product of one season's growth, but various environmental conditions may induce the formation of more than one growth layer in one season (Esau, 1977, p. 103). When conspicuous layering is present, each growth layer is divisible into early and late wood. The early wood is less dense than the late wood, because wider cells with thinner walls predominate in the early wood and narrower cells with thicker walls occur in the late wood.

Although woody stems are usually not used to make pressure–volume curves, we are interested in the structure of wood when we consider the rise of sap in plants. Its structure is a key part of the cohesion theory (see Chapter 20), which explains how water can ascend to the top of tall trees.

17.2 MEASUREMENT OF THE COMPONENTS OF THE WATER POTENTIAL

Differences in total water potential, osmotic potential, pressure potential, matric potential, and gravitational potential can develop in the water of part of a plant, for example, a leaf. (We say "potential," when we recognize that we mean "potential energy". "Potential" is shorter than "potential energy" and saves spaces in printing.) As we saw in Chapter 4 (Eqn 4.3), when we were focusing on soil water, these five potentials for water at a particular point in a plant or soil are related by the equation:

$$\Psi = \Psi_s + \Psi_p + \Psi_m + \Psi_g \tag{17.1}$$

in which the total water potential Ψ for a particular unit mass of water (say a milligram) at a particular point is composed of four components, that is the potentials due to solutes, Ψ_s, pressure, Ψ_p, matrix, Ψ_m, and gravity, Ψ_g. The term Ψ_m is associated with capillary or adsorption forces, which in a plant are forces such as those at the cell walls. Equation (17.1) can be compared, term by term, with the classical equation of plant physiology (Meyer et al., 1960, p. 56):

$$\mathrm{DPD} = \mathrm{OP} - \mathrm{TP}, \tag{17.2}$$

where

DPD = diffusion pressure deficit
OP = osmotic pressure
TP = turgor pressure

and terms corresponding to Ψ_m, the matric potential, and Ψ_g, the gravitational potential, are neglected. If Ψ_m and Ψ_g are ignored, the

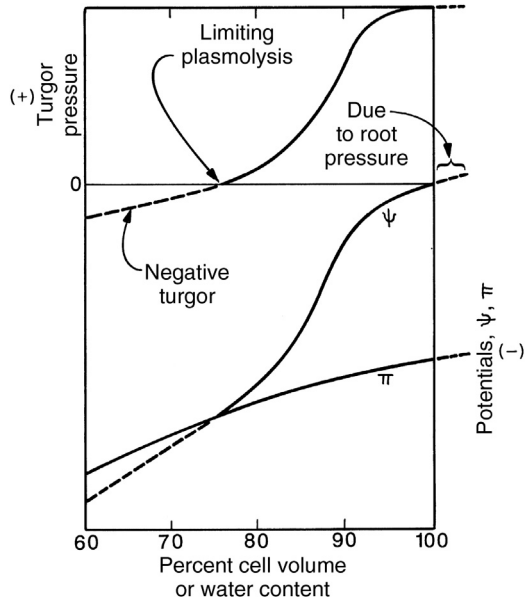

FIGURE 17.4 A type of Höfler diagram; relationship between cell volume or water content and total water potential or its components for a single cell. *From Barrs (1968). Reprinted by permission of Academic Press.*

relation of Ψ, Ψ_s, and Ψ_p to water content or cell volume may be described by means of a Höfler-type diagram (Höfler, 1920). In a Höfler-type diagram, the three potentials—water potential, osmotic potential, and turgor potential—are shown together. Figures 17.4 and 17.5 show two types of Höfler diagrams. A quantitative estimate of Ψ is possible if the sum of Ψ_s and Ψ_p is known (Barrs, 1968, p. 236). In this chapter, we wish to discuss methods to measure Ψ_s and Ψ_p.

17.3 OSMOTIC POTENTIAL (Ψ_S)

Two methods are commonly used to determine osmotic potential of plant leaves. In the first method, leaves are frozen, which breaks cell membranes and releases the solutes in the cell. Alternatively, the tissue can be crushed instead of frozen to break the cell membranes, but one has to devise a crushing device. With freezing, one is assured of breaking all the membranes after a tissue has been in a freezer overnight or in dry ice for a few minutes. Some people freeze the tissue and then squeeze out sap from the frozen tissue. With this method it is certain that the sap contains the solute component of the total water potential. The concentration of the

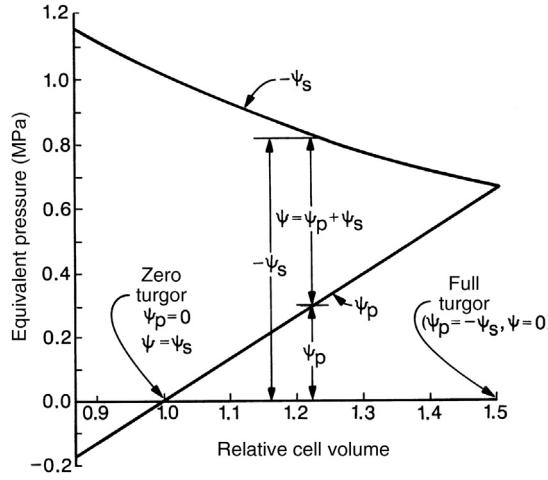

FIGURE 17.5 A type of Höfler diagram; relationship between pressure potential (Ψ_p), solute potential (Ψ_s), and the resultant water potential (Ψ) in an idealized (elastic) plant cell. *From Baker (1984). Reprinted by permission of Pearson Education Limited, Essex, United Kingdom, and Dennis A. Baker.*

solutes in the sap from crushed tissue or from the frozen and crushed tissue then can be measured with a thermocouple psychrometer or an osmometer (see this chapter, Section 17.8, for a discussion of the osmometer).

Tissue can be handled in two ways in the frozen tissue method. First, a piece of tissue can be put into a thermocouple psychrometer. Water potential, Ψ, is determined. The chamber with the tissue is removed from the thermocouple, corked, and frozen. After freezing of the tissue, the chamber with the tissue is reattached to the thermocouple, and osmotic potential of the same tissue for which water potential was determined (same geometry, same cells), is measured. Second, water potential can be analyzed on a sample of a plant by using one instrument (e.g., a pressure chamber). The osmotic potential then is determined on another sample of the plant by freezing the sample, exuding sap from it, putting the sap on a piece of filter paper, and measuring its osmotic potential with a thermocouple psychrometer or an osmometer (Clarke and Simpson, 1978). This second way has the disadvantage that, because water potential and osmotic potential are not determined on the same piece of tissue, osmotic potential readings can be higher than water potential readings (Singh et al., 1983). This is not possible and is due to experimental error or ignoring potentials other than the solute potential and turgor potential that contribute to the total water potential. Osmotic potential is lower or equal to the water potential, unless matric or gravitational potentials are significant.

17.4 THEORY OF SCHOLANDER PRESSURE–VOLUME CURVES

The second method for determination of osmotic potential employs a pressure chamber to create a pressure–volume curve. We need to know the background of pressure–volume curves to understand them. The method now used is based on concepts developed by Scholander and colleagues (Scholander et al., 1964, 1965). We first need to define the term *hydrostatic pressure*, which Scholander et al. (1964, 1965) use. It is generally accepted that water is under tension (negative pressure) in the vessels in the xylem tissue. Scholander et al. (1964, 1965) refer to this pressure as "hydrostatic pressure". The *Handbook of Chemistry and Physics* (Weast, 1964, p. F-45) defines hydrostatic pressure as follows:

Hydrostatic pressure at a distance h from the surface of a liquid of density d, is

$$P = hdg. \tag{17.3}$$

The total force on an area A due to hydrostatic pressure is

$$F = PA = Ahdg, \tag{17.4}$$

where

F = force (dynes)
P = hydrostatic pressure (dynes/cm^2)
h = distance (cm)
d = density (g/cm^3)
g = acceleration due to gravity (cm/s^2).

We sometimes denote d by the Greek letter rho, ρ. Nobel (1974, p. 38; 1983, p. 41) and Barrs (1968, p. 336) equate the hydrostatic pressure with turgor pressure. There is, however, no turgor pressure in mature xylem vessels, because cell membranes have disintegrated.

Scholander et al. (1964) show a leaf cell with vessel, as it exists outside and inside a pressure chamber, in a fresh state (Figure 17.6) and in a wilted state (Figure 17.7). They assume that negative hydrostatic pressure exists in a vessel connected to a living cell (Figures 17.6 and 17.7(A)). They further assume that ambient air cannot enter the system because of surface tension. Water is extruded from the cut end of the stem when pressure is applied by using the pressure chamber (Figures 17.6 and 17.7(B)). They assume that the membrane surrounding the cell (Figures 17.6 and 17.7) is semipermeable and that no solutes come out of the cell when pressure is applied. Therefore, they assume that the extruded liquid is plain water and that the rise in intracellular solute concentration (which occurs as water is pushed out of the cell) is proportional to the rise in the equilibrium pressure.

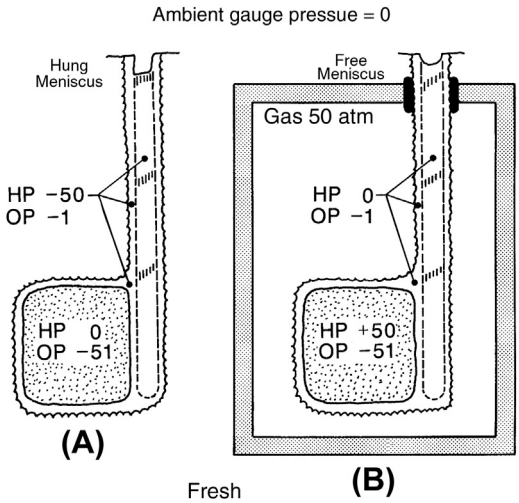

FIGURE 17.6 (A): Leaf cell with vessel. Air cannot enter the system because of surface tension (indicated as concave menisci). HP = hydrostatic pressure, OP = osmotic potential. (B): Balancing pressure on the same system, produced by compressed nitrogen. Notice the free meniscus at the cut end of the vessel. *From Scholander et al. (1964). Reprinted by permission of Harold T. Hammel.*

We now need to define *equilibrium pressure.* When the vessels (capillary tubes) are cut in a stem to put a leafy shoot in a pressure chamber, the water recedes from the cut (Figures 17.6 and 17.7(A)). The reason for the recession is because atmospheric pressure is higher on the outside of the cut end than on the inside. If the same difference in pressure were reestablished, the meniscus would move back exactly to the cut. The cut shoot, therefore, is placed in a pressure chamber, leaving the vessels (capillaries) protruding. Gas pressure (usually nitrogen gas) is applied,

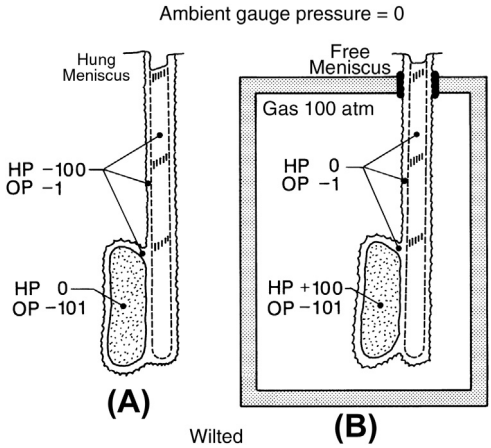

FIGURE 17.7 Same system as in Figure 17.6, but wilted, with about half of the intracellular water extruded. *From Scholander et al. (1964). Reprinted by permission of Harold T. Hammel.*

and when the meniscus is back at the cut, we have the equilibrium pressure (Scholander et al., 1965, p. 340).

Using these assumptions, Scholander et al. (1965) provide the following analysis: If external gas pressure is applied in excess of the balancing pressure, pure water runs out, and at zero turgor the molal concentration in the cell should, therefore, be proportional to the sap pressure, according to the following equation:

$$S/(I - V) = KP \tag{17.5}$$

or

$$I - V = K_1 P^{-1} \tag{17.6}$$

where S stands for the intracellular solutes, I the cell volume, V the water that has run out, and P the equilibrium pressure. They do not define K or K_1. But they must be constants, and K_1 must equal S/K. If the inverse of pressure ($1/P$) is plotted against liquid removed (V), Scholander and colleagues say that a straight line results whenever the concentration is proportional to the pressure, and the intercept on the abscissa gives the volume of water that is being concentrated—that is, the intracellular water (I). The studies by Scholander et al. (1964, 1965) have been cited many times, as has a subsequent paper by Tyree and Hammel (1972).

We can refer to physical chemistry textbooks for the foundation of pressure–volume curves (see, for example, Moore, 1962, p. 135; Daniels and Alberty, 1966, p. 170). Let us follow the analysis of Daniels and Alberty (1966, pp. 170–172). When a solution is separated from the solvent by a semipermeable membrane, which is permeable by solvent but not by solute, the solvent flows through the membrane into the solution, where the chemical potential of the solvent is lower. This process is known as osmosis. This flow of solvent through the membrane can be prevented by applying a sufficiently high pressure to the solution. The osmotic pressure π is the pressure difference across the membrane required to prevent spontaneous flow in either direction across the membrane. Figure 4.1 shows a diagram of an osmometer, which can be used to measure osmotic pressure.

The phenomenon of osmotic pressure was described by Abbé Nollet in 1748. Pfeffer, a botanist, made the first direct measurements of it in 1877. (For a biography of Pfeffer, see the Appendix, Section 17.9.) Van't Hoff analyzed Pfeffer's data on the osmotic pressure of sugar solutions and found empirically that an equation analogous to the ideal gas law gave approximately the behavior of a dilute solution, namely (Daniels and Alberty, 1966, p. 170)

$$\pi \overline{V} = RT \tag{17.7}$$

where $\overline{V}$ is the volume of solution containing a mole of solute and R is the ideal gas constant and T is the absolute temperature. The origin of the pressure is different from that for a gas, however, and the equation of the form of the ideal gas equation is applicable only in the limit of low concentrations. See Figure 17.8 for the ideal gas law.

Daniels and Alberty (1966) then proceed to derive the van't Hoff law using calculus, which we shall not do. (For a biography of van't Hoff, see the Appendix, Section 17.10.) The van't Hoff law, which J.H. van't Hoff developed in 1885 (Moore, 1962, p. 135; see Hammel and Scholander, 1976; for references by van't Hoff) and which applies only to dilute solutions, is as follows (Daniels and Alberty, 1966, p. 171):

$$\pi = (cRT)/M \tag{17.8}$$

where c is the concentration of solute in grams per unit volume and M is the molecular weight of the solute. This is the approximate equation that van't Hoff found empirically. Moore (1962, p. 135) writes the van't Hoff law as follows:

$$\pi = cRT \tag{17.9}$$

where $c = n/V$ and $n =$ number of moles (g/M) and V is volume. We can write Eqn (17.9) as follows:

$$\pi = (nRT)/V. \tag{17.10}$$

Equation (17.10) is similar in form to Eqn (17.6), developed by Scholander et al. (1965). That is, we have pressure inversely related to

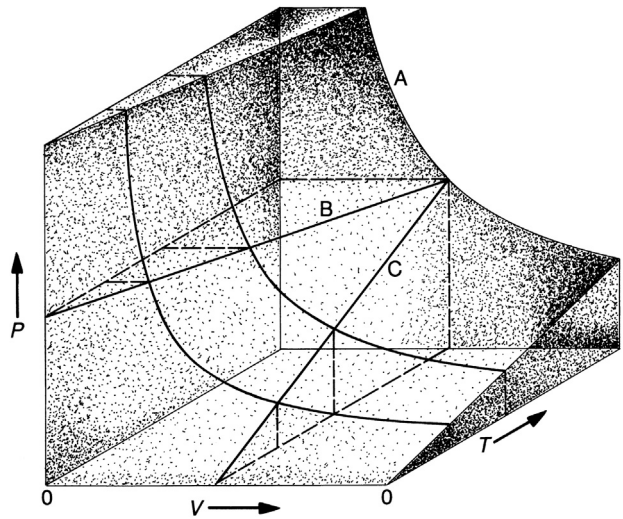

FIGURE 17.8 Pressure–volume–absolute temperature relation for an ideal gas. *From Daniels and Alberty (1966, p. 8). This material is used by permission of John Wiley & Sons, Inc.*

volume, if we are considering a dilute solution. However, we must note that Scholander et al. (1964, 1965) plot $1/P$ versus volume exuded (not volume left in the plant), and $1/P$ in their curves is inversely related to V. Note in Eqn (17.10) and in Figure 17.8 that P (or π), not $1/P$, is inversely related to V. Also, at constant temperature, P versus V is a rectangular hyperbola (Daniels and Alberty, 1966, p. 9) and the curve never touches the y or x axis, as it does in the curves developed by Scholander and colleagues (e.g., see Figure 17.9 from Hammel and Scholander (1976); note the right-hand side ordinate is $1/P$).

Gardner and Rawlins (1965), who discussed the paper by Scholander et al. (1965), said that their procedure measured the difference in free energy per unit volume between water in the plant and the same water outside of the plant. The pressure chamber operates on the same principle as the pressure-membrane apparatus used to measure the potential energy of water in soils (see Chapter 5, Figure 5.5). Gardner and Rawlins said:

> When air pressure is applied to the sample chamber, the free energy of the water is raised. If this pressure increase is carried out isothermally, the free energy of the water would be raised by approximately $V\Delta P$, where V is the volume of water in the sample and ΔP is the pressure increase necessary to establish equilibrium between water in the system and that outside. It is common practice to express this energy difference in terms of energy per unit volume (the water potential), which, of course, is dimensionally the same as pressure. In the experiment of Scholander et al., the plant itself provides the membrane which is permeable to water but not to air.

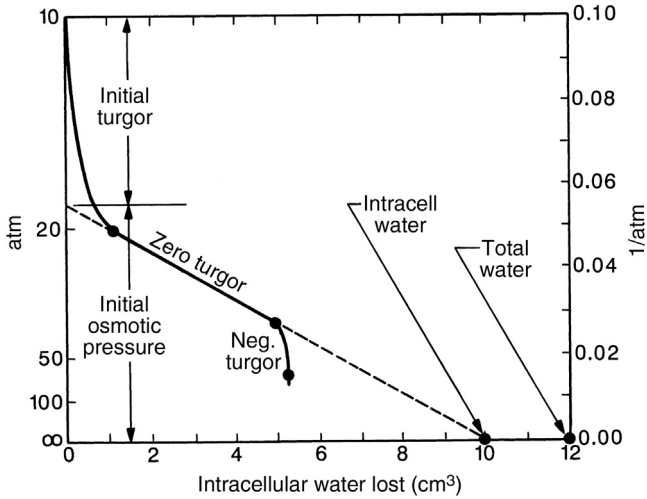

FIGURE 17.9 Schematic presentation of a pressure–volume curve. *From Hammel and Scholander (1976, Figure 24, p. 36). Reprinted by permission of Springer-Verlag and Harold T. Hammel.*

17.5 HOW TO ANALYZE A PRESSURE–VOLUME CURVE

Now let us return to the actual measurement of osmotic potential using a pressure–volume curve. Pressure is applied incrementally to a plant sample. After each increase in pressure, the volume of exudate from the cut end of the plant (e.g., stem, petiole) is collected and measured, and a curve of the reciprocal of pressure versus cumulative volume exuded is plotted (Figures 17.10 and 17.11). From this pressure–volume curve, the osmotic potential at full turgor and the osmotic potential at zero turgor are indirectly determined by reading values on the ordinate (Figure 17.10). Sometimes, instead of plotting pressure versus volume exuded, pressure versus water content (or relative water content) of the plant is plotted. In this case, the plot is called a water-release curve instead of a pressure–volume curve, and is similar to water–release curves developed for soils, in which pressure

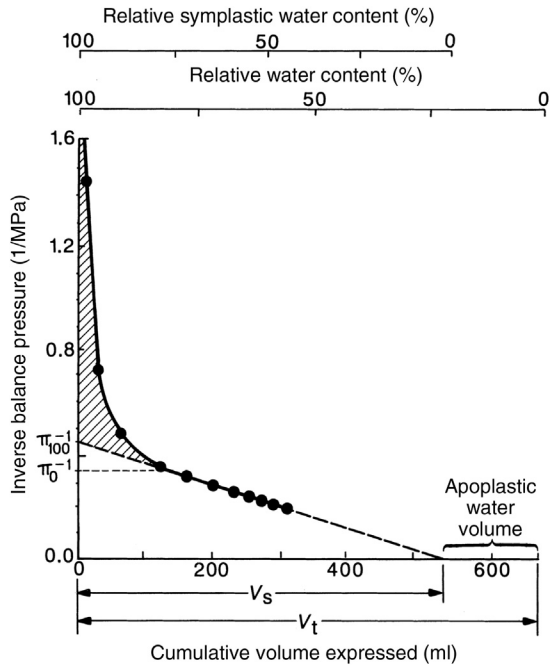

FIGURE 17.10 A pressure–volume curve, i.e., the relationship between the inverse of the balance pressure and the cumulative volume of cell sap expressed, for wheat leaves. V_s is the volume of symplast water and V_t is the total volume of water in the leaf. π_{100}^{-1} and π_0^{-1} are the inverse of the osmotic potentials at full and zero turgor, respectively. The hatching indicates the inverse of the turgor pressure. *From Turner (1981, Figure 7, p. 355). With kind permission of Kluwer Academic Publishers and Neil C. Turner.*

(or potential) is plotted versus soil water content. Turner (1981) (Figure 17.10) illustrates how to determine osmotic potential from either a pressure–volume curve or a water–release curve.

Figure 17.11 can be used to learn how one gets Ψ, Ψ_s, and Ψ_p from the $1/P$ values obtained with the pressure chamber. The top part of Figure 17.11 has the data and the bottom part has the converted data. There are 11 data points that line up in the top and bottom part of the figure. The first 6 data points, starting in the upper left in the top part of the figure, are in the region of turgor potential. This is the region of turgor, because the relationship between $1/P$ and volume of sap exuded is curvilinear. (As we shall calculate, Point 6 is right at the break between the region of turgor and no turgor.) Points 7, 8, 9, 10, and 11 are in the region of zero turgor potential where a straight line relation exists between $1/P$ and volume of sap expressed (compare the straight line in Figure 17.11, top, with the straight line for zero turgor in Figure 17.9). First we determine P (from $1/P$) and get the curve for Ψ_w (this is the total water potential or Ψ in Eqn (17.1)). The six values (in the region of turgor) are about −0.71, −1.11, −1.43, −1.82, −2.00,

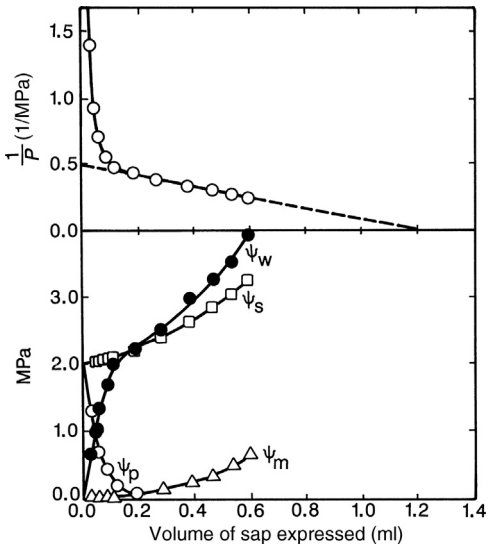

FIGURE 17.11 The upper graph is a pressure–volume curve for *Ilex opaca* (American holly). The ordinate is the reciprocal of the pressure, and the abscissa is the volume of water expressed, in milliliters. The dashed line is the calculated extension of the linear part of the curve. It intersects the ordinate at 0.51, equal to an osmotic potential of −1.96 MPa, and the abscissa at 1.23 ml. The data in the lower graph were obtained by analysis of the pressure–volume curve. Curves are shown for turgor (Ψ_p), osmotic (Ψ_s), and matric (Ψ_m) potentials, and total water potential (Ψ_w). The turgor potential is positive, and all other potentials are negative. The abscissa of the lower graph represents water volume in milliliters, and the ordinate is given in megaPascals. *From Kramer (1983, p. 53). Reprinted by permission of Academic Press.*

and −2.13 MPa. The water potential, Ψ_w, is negative, so we need to add negative signs in front of these values. Then we determine $1/\Psi_s$ by measuring down from the data point in the top part of the figure to the dashed line and then reading the value on the ordinate. We then take the reciprocal of $1/\Psi_s$ to get Ψ_s. The six values for Ψ_s in the region of turgor in Figure 17.11 are about −1.98, −2.00, −2.02, −2.04, −2.08, and −2.13 MPa. We add negative signs because they are the solute potential. Subtracting Ψ_s from Ψ_w, we get the six values for turgor potential, Ψ_p, which are 1.27, 0.89, 0.59, 0.22, 0.08, and 0.00 MPa (positive values). The last value is zero, showing that Point 6 is in the region of zero turgor.

In addition to water potential, osmotic potential, and turgor potential, the pressure–volume curves can be used to find the modulus of elasticity (Melkonian et al., 1982; Sinclair and Venables, 1983). (In Chapter 23 we will determine modulus of elasticity of leaves, but not using pressure–volume curves.) Pressure–volume or water-release curves also can be obtained with dew point hygrometers instead of pressure chambers (Richter, 1978; Wilson et al., 1979).

The osmotic potential of the sap exuded during a pressure-chamber measurement can be determined by placing the sap on filter paper and measuring its osmotic potential with a thermocouple psychrometer or an osmometer (Meyer and Ritchie, 1980). The sap in the dead cells that conduct water (vessel members or tracheids) has a much higher osmotic potential (less negative, i.e., it is very dilute) than that in the sap of living cells (Scholander et al., 1964; Ike et al., 1978).

There is disagreement as to which method (frozen tissue; or pressure–volume curve, or water-release curve) provides the more reliable results. Brown and Tanner (1983) used alfalfa (*Medicago sativa* L.) to compare the two methods for determining osmotic potential. Osmotic potential of sap expressed from thawed tissue was 0.21–0.89 MPa lower than the osmotic potential obtained from water-release curves. They felt that the difference was due primarily to the production of solutes in thawed tissue by enzymatic hydrolysis and suggested that the water-release curve method was a better way to measure osmotic potential than the frozen tissue method. In contrast, Rakhi et al. (1978) said that, because the pressure chamber dehydrated the tissues that they studied (*Carex physodes* M. Bieb. (sedge) and *Populus tremula* L. (European aspen)), the values for osmotic potential determined by freezing the tissue were more reliable than the ones derived from water-release curves. Walker et al. (1983) investigated wheat (*Triticum aestivum* L.) and found that the osmotic potential, as measured by the pressure–volume method, compared favorably with the osmotic potential, as measured on frozen tissue with psychrometers. Experiments with other plants are needed to find out if these two methods for determining osmotic potential give similar results.

17.6 TURGOR POTENTIAL (Ψ_P)

Turgor potential normally is determined in one of two ways. First, turgor potential can be calculated, if the water potential and osmotic potential are measured with thermocouple psychrometers. The turgor potential is the difference between osmotic potential and water potential, assuming that matric potential (Barrs, 1968, p. 337) and gravitational potential are negligible. (Kirkham (1983) discusses situations in which gravity can be important.) Second, turgor potential can be estimated by using pressure–volume curves (Turner, 1981, Figure 17.10; Melkonian et al., 1982; Sinclair and Venables, 1983). Both of these methods require excised samples.

For many years, people have tried to measure turgor potential (pressure) directly by using probes (Barrs, 1968, p. 336). The practical difficulties, however, have been enormous, because of the small size of most plant cells. Most of the work, until recent years, was done with large-celled algae (e.g., *Nitella*, *Chara*). Now a pressure probe has been developed to measure turgor potential of higher plants directly. According to Wei et al. (2001), the contemporary cell pressure probe was designed in Germany by Steudle and Zimmermann (1971). In the United States, Nonami and Boyer (1984) were probably the first to publish on the pressure probe, and they reported the turgor potential of a higher plant, soybean (*Glycine max* L. Merr.). Boyer (1995) has described in detail the pressure probe. The equipment is expensive, complex, requires extensive training to learn how to use it, and takes precise control. The smallest vibration (such as an air movement or vibration of the floor) can cause pressure probe measurements to fail. A vibration damping table is advised.

Tomos and Leigh (1999) review how the pressure probe has evolved from an instrument for measuring cell turgor into a device for sampling the contents of individual higher plant cells in situ in the living plant. In addition, the probe is being used to measure root pressure, xylem tension (negative pressure), hydraulic conductivity, the reflection coefficient of solutes, elasticity, solute concentrations, and enzyme activities at the resolution of single cells. Tomos and Leigh (1999) also review the controversy surrounding the interpretation of measurements of xylem tension obtained with the pressure probe. It is critical to know what these tensions (negative pressures) are, if we are to confirm or refute the cohesion theory for the rise of sap in plants (see Chapter 20). Tensions in the xylem measured with the pressure probe under transpiring conditions in both small plants and tall trees have been less negative than those obtained with the pressure chamber, and in many cases are positive, which would refute the cohesion theory, because it assumes negative pressures (tensions) in the xylem. Wei et al. (2001) state that

controversy over the cohesion theory resulting from measurements made with the pressure probe is due to improper probing technique. They point out that handling and methodology of the probe are critical to its success.

Despite the questions surrounding the pressure probe, its cost, and the difficulty in learning how to use it, the instrument gives us a basic understanding about water and solute relations at the cellular level. Using the probe, Thomas et al. (2006) measured cell turgor in grape (*Vitis vinifera* L.) before and after veraison. Veraison is a term used in viticulture. It comes from the French word *véraison* and refers to fruits, especially grapes, when they begin to change color at maturity. They found that cell turgor in berries at predawn was 0.02 MPa preveraison and was reduced by an order of magnitude to 0.02 MPa postveraison. The fact that a measurable cell turgor of about 0.02 MPa was exhibited in postveraison berries indicated that cell membranes remained intact after veraison, contrary to current hypotheses that veraison is associated with a loss of membrane function and cellular compartmentation in the grape berry. Bouchabké et al. (2006) used the pressure probe to measure cell turgor in the elongating zone of maize (*Zea mays* L.) leaves and showed that leaf elongation rate was positively correlated with turgor.

Calbo et al. (2010) described a portable instrument, called a wiltmeter, to estimate turgor pressure by measuring the flatness of a leaf. They compared their wiltmeter measurements with those made with a pressure probe. The wiltmeter produced leaf pressure estimates that were close to the leaf cell turgor pressure as measured by the pressure probe. They suggested that the wiltmeter would be a practical field substitute for the pressure probe and could be used to determine leaf freshness before harvest or to schedule irrigations.

17.7 MEASUREMENT OF PLANT WATER CONTENT AND RELATIVE WATER CONTENT

Höfler-type curves plot plant potentials versus water content (Figure 17.4) and pressure–volume curves plot the inverse balance pressure versus relative water content (Figure 17.10). Therefore, we need to know how to measure both plant water content and relative water content.

Plant water status is usually described by two basic parameters: the content of water in the plant or the energy status of the water in the plant, expressed as the (total) water potential, Ψ (Barrs, 1968, p. 236). We have studied the water potential and its components in this chapter, in

Sections 17.2 through 17.6. Here we determine how to measure plant water content and relative water content.

Old techniques to measure plant water content expressed the water content on the following bases: dry weight, fresh weight, or leaf area. These are not acceptable because they are not stable. They change diurnally and seasonally, and the leaf area is reduced with drought.

Soil water content is expressed on a dry weight basis as:

$$\text{soil water content (\%)} = [(\text{wet weight} - \text{dry weight})/\text{dry weight}] \times 100. \tag{17.11}$$

The soil water content is almost always less than 100% when expressed on a dry weight basis. Exceptions are highly organic soils, such as those that occur on forest floors. For example, the 0–5, 5–10, and 10–15 cm layers of forest soil from the Craigieburn Range in the South Island of New Zealand had water contents of 382.3%, 134.4%, and 89.1%, respectively (Kirkham and Clothier, 2000).

If we do express plant water content on a weight basis, then we choose the fresh weight basis:

$$\text{plant water content (\%)} = [(\text{fresh weight} - \text{dry weight})/\text{fresh weight}] \times 100. \tag{17.12}$$

If we put the water content on a dry weight basis, the water content always would be greater than 100%, because of the high water content of plants.

Plant water content changes with age and condition of the plant. Therefore, some standard must be used in determining water content. The water content at full turgor has been used as a standard since the work of Stocker (1928, 1929) in Germany. He determined plant water content using the following equation:

$$\begin{aligned}\text{WD (\%)} &= \text{WSD (\%)} \\ &= [(\text{turgid weight} - \text{fresh weight})/(\text{turgid weight} - \text{dry weight})] \times 100\end{aligned} \tag{17.13}$$

where WD is the water deficit and WSD is the water saturation deficit; the two terms are equivalent. He determined the turgid weight by cutting off a whole leaf, or, when working with conifers, a small branch, and standing it in a little water in a closed container for 48 h (Barrs, 1968, p. 243). The fresh weight is the weight at time of sampling. The dry weight is the weight after oven drying.

The method that Stocker developed was a reliable one to determine plant water content. However, some scientists ignored its importance and used the following equation (Barrs, 1968, p. 243):

$$\text{“WD”} = [(\text{turgid weight} - \text{fresh weight})/\text{dry weight}] \times 100. \quad (17.14)$$

This equation neglects the importance of using the fully turgid weight as the basis for water content. Note that turgid weight does not appear in the denominator of Eqn (17.14).

The following equation was used to avoid getting dry weight:

$$\text{RSD} = \text{DSH} = [(\text{turgid weight} - \text{fresh weight})/\text{turgid weight}] \times 100, \quad (17.15)$$

where RSD = relative saturation deficit and DSH is the *déficit de saturation hydrique* (the French term for water saturation deficit). The DSH was confused in English with the WSD. However, the RSD or DSH method is not reliable. Dry weight needs to be determined. These many methods (WD, “WD”, WSD, RSD, DSH) resulted in chaos in the literature and no standard technique existed.

In 1950, Weatherley (1950) standardized the technique. He used punched disks instead of standing whole leaves in water. He floated the disks for 24 h (sometimes 48 h) in closed Petri dishes that were exposed to diffuse daylight and a constant temperature. He then calculated relative turgidity, RT, as follows:

$$\text{RT} = [(\text{fresh weight} - \text{dry weight})/(\text{turgid weight} - \text{dry weight})] \times 100. \quad (17.16)$$

The relative turgidity is related to the water saturation deficit (or water deficit), as follows:

$$100 - \text{RT} = \text{WSD}. \quad (17.17)$$

Later Barrs and Weatherley (1962) revised Weatherley’s (1950) method. They noted that there are two phases for water uptake: Phase I, which is in response to the initial water deficit and during which rapid uptake of water occurs, and Phase II in which a continued slow uptake of water continues due to growth of the tissue, even though it is excised (Figure 17.12). The aim of the Barrs and Weatherley (1962) method to determine relative turgidity was to measure only Phase I. One needs to determine for each species when rapid uptake of water ceases (end of Phase I) and then float disks for this length of time. If an initial experiment to determine this time is not done, leaves can be floated for 3–6 h, which is the normal time for Phase I for most plants. Four hours is the time most often chosen.

The Barrs and Weatherley (1962) technique is the standard method to measure relative turgidity, which is now called relative water content

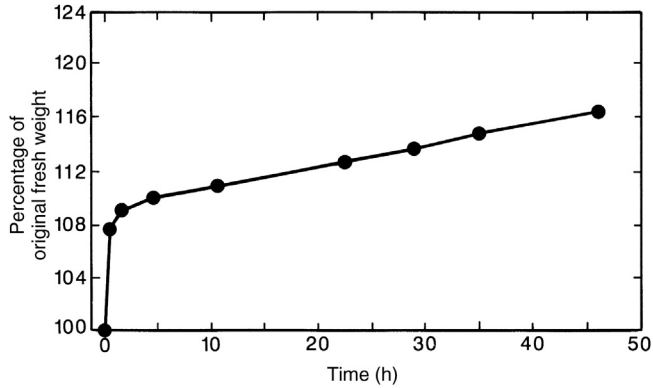

FIGURE 17.12 Change over time in fresh weight of floating leaf disks. The leaves are from the castor-oil plant (*Ricinus communis* L.). Note the two phases in water uptake: a first phase in which water is taken up rapidly (0–5 h) and a second phase in which water is taken up slowly (5–46 h). *From Barrs and Weatherley (1962). Permission to reprint granted by CSIRO Publishing, Collingwood, Victoria, Australia, the original publisher of the information.*

(RWC). The old term *relative turgidity* has been abandoned because early workers confused relative turgidity with turgor pressure (turgor potential) (Barrs, 1968, pp. 244–245). For example, Box and Lemon (1958) referred to "turgor pressure" instead of "relative turgidity" in discussing Weatherley's (1950) results with cotton. The turgor potential is an energy-based measurement. Relative turgidity (or relative water content) is a measure of plant water content.

The Barrs and Weatherley (1962) RWC technique consists of doing the following:

1. A punch is used to punch disks out of a leaf. The punch must be sharp to minimize cut-edge effects. When plant tissue is cut, cells are damaged, causing infiltration of water. This creates excessive uptake of water and gives spuriously low RWCs. These errors are hard to quantify and appear not always to be present (Barrs, 1968, p. 247). A sharp punch, along with a good-sized leaf disk, avoids the cut-edge effect. Small disks are more prone to higher RWCs than are larger disks, and disks should not be smaller than 8 mm in diameter. A Number 8 cork borer, which has a diameter of 14 mm, is a good size to use.
2. The same diameter disks must be used in an experiment; 12 disks are recommended (Barrs, 1968, p. 251).
3. Mature or nearly mature leaves should be used rather than rapidly growing or senescent leaves. If mature leaves are used (e.g., third, fourth, or fifth leaf from the top of a plant), a time of 3–4 h for

floating should be sufficient and will coincide with Phase I (Barrs, 1968, p. 246).

4. Leaf disks must be placed in a closed Petri dish to maintain a constant humidity.
5. A constant temperature must be used. A thermometer should be placed on the laboratory bench to record the temperature. Results will differ depending on the temperature used (Figure 17.13). Barrs and Weatherley (1962, p. 415) used 20 °C in a constant temperature room.
6. The leaf disks must be exposed to diffuse light, such as that on a laboratory bench out of direct sunlight, to minimize growth and heating due to the sun.
7. After floating, the leaf disks are removed using tweezers from the Petri plate, blotted dry on a paper towel, and then put in a drying oven at 85 °C (Barrs, 1968, p. 239).
8. All weights should be weighed on an analytical balance to the fourth decimal point and then rounded off to the third decimal point.
9. For conifer needles and grass leaves, which are not wide enough for punched disks, razors mounted on a block are used to cut constant-length segments. A length of 15–25 mm is normally used (Barrs, 1968, p. 250). Grass leaves can be floated, but conifer leaves should be placed upright in a beaker of water, because needles can become waterlogged and sink.

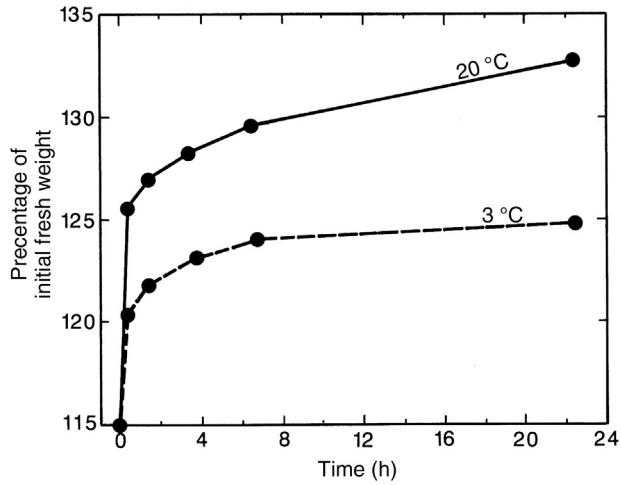

FIGURE 17.13 Effects of floating on water at 20 and 3 °C on changes of fresh weight of leaf disks from the castor-oil plant (*Ricinus communis* L.). *From Barrs and Weatherley (1962). Permission to reprint granted by CSIRO Publishing, Collingwood, Victoria, Australia, the original publisher of the information.*

The advantages of the Barrs and Weatherley method (1962) are:

1. It standardizes sampling.
2. It is sparing of tissue.
3. It allows some leaves to be sampled more than once.
4. It can be done in a shorter time (3–6 h) than previous methods (24–48 h).
5. Even though the measurements are tedious, the relative water content method is simple and requires only an analytical balance, Petri dishes, paper towels, a thermometer, tweezers, and a drying oven. No special skills other than carefulness and patience are needed to take the measurements.

Relative water content measurements are important for several reasons. First, they are used to construct pressure–volume curves (Figure 17.10). Second, relative water content can be used as a guide to irrigation. Ehrler and Nakayama (1984) in Arizona found that relative water content measurements were a good guide to schedule irrigations of guayule (a small shrub of northern Mexico and the southwestern part of the United States cultivated for the rubber obtained from its sap). Third, the measurements show variation of water status in different portions of a leaf, if the leaf is large enough for such sampling. Slavík (1963) measured water saturation deficit (see Eqn (17.17)) across a tobacco leaf and found that the WSD was least at the base of the leaf and most at the center edge and tip of the leaf. The high WSD at the tip of the leaf was associated with a low transpiration rate. The transpiration rate was highest at the base of the leaf.

Diaz-Perez et al. (1995) state that the relative water content method is a good measure of water status and is easier to measure than water potential. The value of relative water content measurements versus water-potential measurements has been debated (Kramer, 1988; Passioura, 1988; Schulze et al., 1988). Each has its place in measurement of plant water status. However, only by measuring plant water potential can we determine the direction of movement of water in the plant. Water moves according to a potential-energy gradient (from high to low potential energy). The same holds true for water in the soil. It is important to know soil water content, but only measurements of soil water potential (or the total head) tell us the direction of movement of water (e.g., see Figure 4.3).

In sum, by using the Barrs and Weatherley (1962) technique, the problems associated with measurements of plant water content (i.e., dry weight increases with time, continued increase in water content after attainment of full turgidity, and injection of water into the intercellular spaces at the cut edge) can be minimized. The method provides a standard technique that can be replicated by workers at any location. When it is used, the paper by Barrs and Weatherley (1962) always should be cited.

17.8 OSMOMETER

We have mentioned that osmotic potential can be determined with an osmometer. The osmometer measures osmolality, not osmotic pressure, so we must learn how we can relate osmolality to osmotic pressure (or its negative value, osmotic potential).

Osmolality expresses the total concentration of dissolved particles in a solution without regard for the particle size, density, configuration, or electrical charge (Wescor, 1989). All these items listed are *particle characters*. A *colligative property* depends on the number of solute and solvent particles present in a solution, not their character. Osmotic pressure is a colligative property, not a cardinal property, which we shall now discuss.

Consider a solvent and a solute (a solution). There are three cardinal properties of a solvent (e.g., water): freezing point, which is lowered by solutes; boiling point, which is raised by solutes; and vapor pressure, which is lowered by solutes. Measurement of solution concentration or osmolality can be made indirectly by comparing a colligative property of the solution (solute + solvent) with a corresponding cardinal property of the pure solvent (e.g., water).

The first instruments to measure osmolality were based on freezing point depression. The Wescor osmometer (5500 Series; Logan, Utah; Figure 17.14) measures osmolality through measurement of vapor pressure by using thermocouple hygrometery (same principle that we shall see for measuring water potential with thermocouple psychrometers in Chapter 18). The dew point depression is determined using the Peltier effect. The relationship between vapor pressure depression and dew point temperature depression is given by (Wescor, 1989) as:

$$\Delta T = \Delta e / S, \tag{17.18}$$

where ΔT is the dew point temperature depression in °C, Δe is the difference between saturated and chamber vapor pressure, and S is the slope

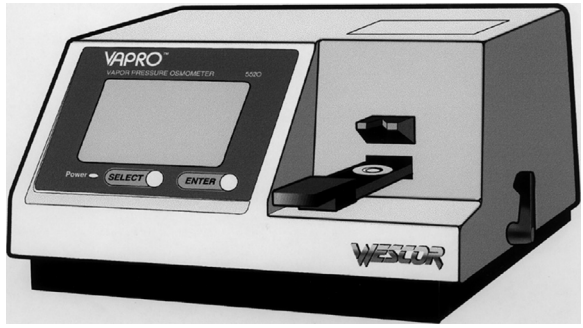

FIGURE 17.14 The vapor pressure osmometer made by Wescor, Inc., Logan, Utah. *From a Wescor, Inc., Logan, Utah, brochure. Reprinted by permission of Wescor, Inc., Logan, Utah.*

of the vapor pressure–temperature function at ambient temperature (37 °C in the osmometer). (The osmometer probably operates at 37 °C, because it is used in medical clinics and the human body is normally at this temperature.) The Clausius–Clapeyron equation gives S as a function of temperature (T in °K), saturation vapor pressure (e_0), and the latent heat of vaporization (λ), as follows (Wescor, 1989)

$$S = (e_0\lambda)/(RT^2), \tag{17.19}$$

where R is the universal gas constant. (For a biography of Clausius, see the Appendix, Section 17.11.)

Figure 17.15 shows the relation between vapor pressure and $1/T$ from a physical chemistry textbook (Daniels and Alberty, 1966, p. 127). Note the slopes for the different compounds are essentially linear.

The vapor pressure depression is a linear function of osmolality. A calibration line is obtained with two solutions that come with the equipment: 290 and 1000 mmol. The salt in the calibrating solutions is not given because it does not matter, but the calibrating solutions are probably NaCl solutions.

The Wescor instrument gives us osmolality, but we would like to relate these values to osmotic pressure. Let us determine a relationship between osmolality and osmotic pressure. We know the van't Hoff law from Eqn (17.8), $\pi = (cRT)/M$, or from Eqn (17.10), $\pi = (nRT)/V$.

Example: What is the osmotic pressure, π, for 2 mol of NaCl ($58.5 \times 2 = 117.0$ g)?

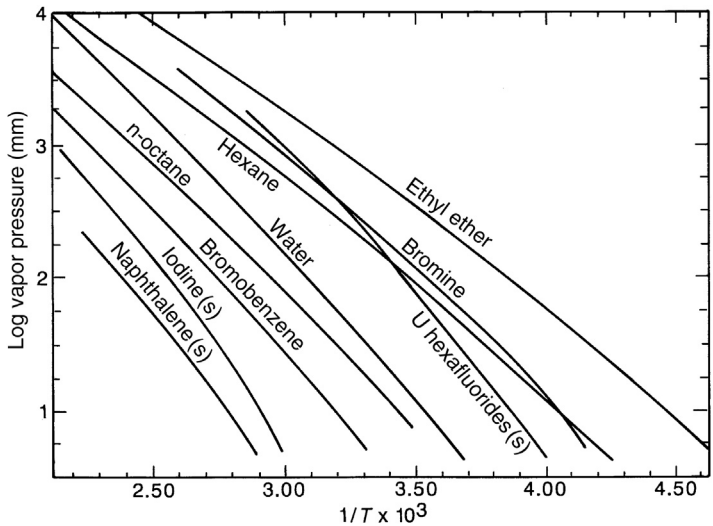

FIGURE 17.15 Log of vapor pressure versus $1/T$ for various vaporization processes. T = temperature in degrees Kelvin. *From Daniels and Alberty (1966, p. 127). This material is used by permission of John Wiley & Sons, Inc.*

If we have NaCl, we have 2 ions from one molecule of salt.

$$\pi = (nRT)/V = (\text{grams/volume})\ RT/M$$

$$\pi = (117.0/1\ \text{l})(0.0821\ \text{l} - \text{atm/mol/deg})(310\ \text{K})/58.5 = 50.84\ \text{atm}.$$

$$50.84\ \text{atm} \times 2\ \text{ions} = 101.68\ \text{atm} = 103.02\ \text{bars}.$$

The table published by Lang (1967) and reproduced by Barrs (1968, p. 288) gives 101.60 bars at 35 °C. The value of 103.02 bars we calculated is close to the value given by Lang (1967).

If we want to convert osmolality to osmotic potential, and we do not know the number of ions (as we do with an NaCl or KCl solution), we use the following equation (Taiz and Zieger, 2002, p. 40):

$$\Psi_s = -C_s RT, \tag{17.20}$$

where

C_s = osmolality (in mol/kg or mol/l).

So if we measure with the osmometer 2 mmol/1 kg = 0.002 molal

$$\Psi_s = -0.002\ RT =$$

$$(-\ 0.002\ \text{mol/l})$$

$$(0.0821\ \text{l atm/mol/deg})(310\ \text{K}) = -0.05\ \text{atm}.$$

Note that (0.0821 l-atm/mol/deg) (310 K) = a constant = 25.45 or (0.0832 l-bar/mol/deg) (310 K) = a constant = 25.8.

To summarize the method to use the osmometer:

1. With the osmometer, ΔT is measured to get Δe, and we know

$$\Delta T = \Delta e/S,$$

where we get S from the Clausius–Clapeyron equation.

2. Then Δe is related to osmolality (linear function). Vapor pressure depression is a linear function of osmolality.
3. We make a calibration line with two solutions. Wescor provides 290 and 1000 mmol/kg for the calibrating solutions.
4. We report the reading as osmolality (mmol/kg) or as osmotic pressure using

$$\Psi_s = -C_s RT.$$

17.9 APPENDIX: BIOGRAPHY OF WILHELM PFEFFER

Wilhelm Friedrich Philipp Pfeffer (1845–1920) was a German physiological botanist. He was born in Grebenstein, where his father owned a

chemist's shop (pharmacy) (Frommhold, 1996), on March 9, 1845 (McIlrath, 1971). There he learned fundamental knowledge and manual skills for his later profession. After earning a degree in botany and chemistry from Göttingen University in 1865, he spent the next several years studying botany and pharmacy at Marburg University. He continued his botanical studies at Berlin (1869–1870) and Würtzburg University (1870–1871). In 1871 he returned to lecture at Marburg, and in 1873 he was appointed lecturer at Bonn University. In 1877 he became a professor at Basel University and later at the universities of Tübingen (1878) and Leipzig (1887). In Leipzig, Pfeffer began his scientific research which lasted more than three decades (Frommhold, 1996). There he was director of the Botanical Institute and the Botanical Gardens. In 1884, Pfeffer married Henrika Volk (Frommhold, 1996), and in May of 1885 his son Otto was born.

His laboratories in the Botanical Institute in Leipzig were modern for the time. He had microscopes and a room with a constant temperature (the precursor of the growth chamber). He positioned measuring instruments so they were free from vibration. He installed a dark room and made devices by which alteration of light and dark conditions could be done automatically. He improved equipment such as the clinostat and the auxanometer. Pfeffer's institute attracted students and visiting scientists from around the world. One of his famous students was Carl Correns, who studied mutants in the Botanical Gardens. Pfeffer knew Wilhelm Ostwald, who also went to Leipzig in 1887.

Pfeffer made significant contributions in the following areas of plant physiology: respiration, photosynthesis, protein metabolism, and tropic and nastic movements. In 1881 he published the first part of his *Handbuch der Pflanzenphysiologie* (English translation by A.J. Ewart, *Physiology of Plants*, three volumes, 1906), which was an important text for many years.

During his life Pfeffer received numerous awards. His seventieth birthday (March 9, 1915) and his golden doctor's jubilee (February 10, 1915) were both celebrated on the same day in the first year of the First World War (1914–1918). The hardest blow in Pfeffer's life hit shortly before the end of the war. A few weeks before the armistice, he was informed that his son Otto was missing, and in the middle of 1919 he learned that Otto had been killed in France. He also suffered physically, because he, like many other Germans at the end of the war, did not have enough to eat. He destroyed all his scientific manuscripts because he felt that he could not complete his work. His research publications ended in 1916. However, he kept teaching, and in 1919–1920, his lectures often had to be offered twice, as the rooms were overcrowded with returned soldiers (Frommhold, 1996).

His last year of life was difficult. In addition to the loss of his son, he also faced the loss of his official residence upon enforced retirement in

1920 (Frommhold, 1996). On the day of his last physiology lecture on January 31, 1920, he died without having been seriously ill.

17.10 APPENDIX: BIOGRAPHY OF JACOBUS VAN'T HOFF

Jacobus Hendricus van't Hoff (1852–1911) was a Dutch physical chemist, who received the first Nobel Prize in chemistry (1901) for his work on chemical dynamics and osmotic pressure in solutions (Preece, 1971a). He was born in Rotterdam on August 30, 1852, and studied at the Polytechnic at Delft and at the University of Leiden. He then studied under Friedrich A. Kekulé von Stradonitz (1829–1896; German chemist) at Bonn, Charles A. Wurtz at Paris in the École de Médecine, and G.J. Mulder at Utrecht, where he obtained his doctorate in 1874. He was a lecturer in physics at the veterinary school in Utrecht (1876); professor of chemistry, mineralogy, and geology in Amsterdam University (1878); and professor at the Prussian Academy of Sciences in Berlin (1896), accepting an honorary professorship in the university so that he might lecture if he wished. He was elected a foreign member of the Royal Society in 1897 and awarded its Davy medal in 1893. He died in Berlin on March 1, 1911.

van't Hoff's earliest important contribution was made in 1874. Starting with the results of the work of Johannes Wiclicenus (1835–1902; German chemist, who studied isomers), he showed that the four valencies of the carbon atom were probably directed in space toward the four corners of a regular tetrahedron. In this way optical activity, shown to be always associated with an asymmetric carbon atom, could be explained. An identical idea was put forward two months later (November 1874), independently, by Joseph Achille Le Bel (1847−1930; French chemist). van't Hoff and le Bel had been fellow students under Wurtz but had never exchanged a word about the carbon tetrahedron. The concept was attacked by Hermann Kolbe (1818–1884; German chemist), but its value was soon universally realized, and it laid the foundation stone of the science of stereochemistry (Preece, 1971a).

In 1877 van't Hoff published *Ansichten über die organischen Chemie*, which contains the beginnings of his studies in chemical thermodynamics. In *Études de dynamique chimique* (1884) he developed the principles of chemical kinetics, described a new method of determining the order of a reaction, and applied thermodynamics to chemical equilibriums. In 1886 he published the results of his study of dilute solutions and showed the analogy existing between them and gases, because they both obey equations of the type $pv = RT$. During the next nine years he developed this work in connection with the theory of electrolytic dissociation enunciated by Svante August Arrhenius (1859–1927; Swedish chemist).

With Wilhelm Ostwald (1853–1932; German chemist who won the 1909 Nobel Prize in chemistry), he started the important *Zeitshrift für physikalische Chemie* in 1887, the first volume of which contained the famous paper by Arrhenius on electrolytic dissociation, along with the fundamental paper by van't Hoff (Preece, 1971a).

17.11 APPENDIX: BIOGRAPHY OF RUDOLF CLAUSIUS

Rudolf Julius Emanuel Clausius (1822–1888) was a German physicist who made important contributions to molecular physics (Preece, 1971b). He was born in Köslin in Pomerania. In 1848 he got his degree at Halle, and in 1850 he was appointed professor of physics in the royal artillery and engineering school at Berlin and *Privatdocent* in the university. In 1855 he became an ordinary professor at the Zürich Polytechnic and professor at the University of Zürich. Clausius moved to Würzburg in Germany in 1867 as professor of physics, and two years later he was appointed to the same chair at Bonn, a position that he held until his death.

The work of Clausius, who was a mathematical rather than an experimental physicist, was concerned with many of the most abstruse problems of molecular physics. He made thermodynamics a science; he enunciated the second law, in a paper contributed to the Berlin Academy in 1850, in the well-known form, "Heat cannot of itself pass from a colder to a hotter body". He applied his results to an exhaustive development of the theory of the steam engine.

The kinetic theory of gases owes much to his researches. He raised it to the level of a theory, and he carried out many numerical determinations in connection with it, such as determining the mean free path of a molecule. Clausius also made an important advance in the theory of electrolysis, suggesting that molecules in electrolytes are continually interchanging atoms. This view found little favor until 1887, when it was taken up by S.A. Arrhenius, who made it the basis of the theory of electrolytic dissociation.

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