3 Water and Plant Cells

THIS CHAPTER AND THE FOLLOWING ONE deal with plant water relations. This is an important topic because water plays a crucial role in the life of the plant. For every gram of organic matter made by the plant, approximately 500 g of water is absorbed by the roots, transported through the plant body and lost to the atmosphere. Even slight imbalances in this flow of water can cause water deficits and severe malfunctioning of many cellular processes. Thus, every plant must delicately balance its uptake and loss of water. This balancing is a serious challenge for land plants because their need to draw carbon dioxide from the atmosphere (for photosynthesis) inevitably exposes them to the threat of dehydration by the atmosphere.

Unlike animal cells, plant cells build up a large intracellular pressure, called **turgor pressure**, as a consequence of their normal water balance. Turgor pressure is essential for many physiological processes, including cell enlargement, gas exchange in the leaves, transport in the phloem, and various transport processes in membranes. Turgor pressure also contributes to the rigidity and mechanical stability of nonlignified plant tissues. In this chapter we will consider how water moves into and out of plant cells, emphasizing the physical forces that influence water movement at the cell level. In the next chapter we will examine water transport at the whole-plant level and how land plants cope with the inevitable loss of water to the atmosphere.

But first we will describe the major functions of water in plant life. Water makes up most of the mass of plant cells, as we can readily appreciate if we look at microscopic sections of mature plant cells: Each cell is packed with a large water-filled vacuole. In such cells, the cytoplasm makes up only 5 to 10% of the cell volume; the

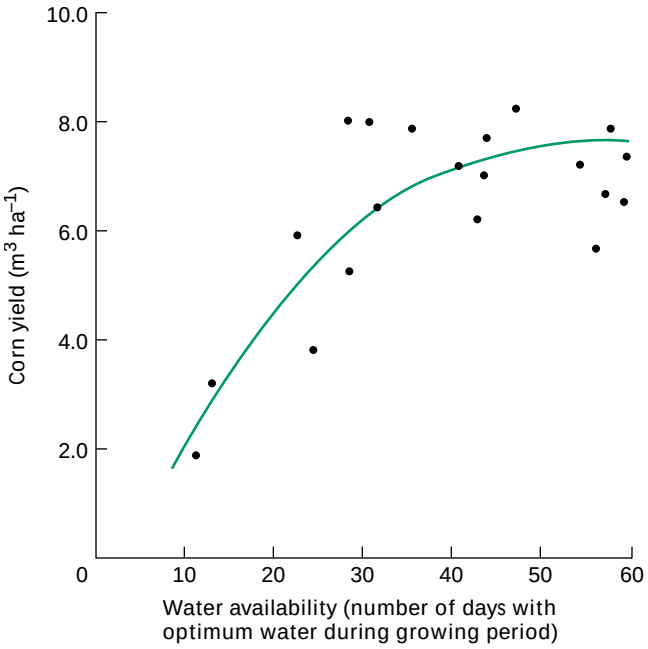


Figure 3.1 Corn yield as a function of water availablity. The data plotted here were gathered at an Iowa farm over a 4-year period. Water availability was assessed as the number of days without water stress during a 9-week growing period. (Data from CAED Report 20.)

remainder is vacuole. Water typically constitutes 80 to 95% of the mass of growing plant tissues. Common vegetables such as carrots and lettuce may contain 85 to 95% water. Wood, which is composed mostly of dead cells, has a lower water content; sapwood, which functions in transport in the xylem, contains 35 to 75% water; and heartwood has a slightly lower water content. Seeds, with a water content of 5 to 15%, are among the driest of plant tissues, yet before germinating they must absorb a considerable amount of water.

Water is the most abundant and arguably the best solvent known. As a solvent, it makes up the medium for the movement of molecules within and between cells and greatly influences the structure of proteins, nucleic acids, polysaccharides, and other cell constituents. Water forms the environment in which most of the biochemical reactions of the cell occur, and it directly participates in many essential chemical reactions, such as those involving hydrolysis and dehydration.

Plants continuously absorb and lose water. On a warm, dry, sunny day a leaf will exchange up to 100% of its water in a single hour. During the plant's lifetime, water equivalent to 100 times the fresh weight of the plant may be lost through the leaf surfaces. Such water loss, called **transpiration**, is an important means of dissipating the heat input from sunlight. Heat dissipates because the water molecules that escape into the atmosphere have higher-than-average energy, which breaks the bonds holding them in the liquid. When these molecules escape, they leave behind a mass of molecules with lower-than-average energy and thus a cooler body of water. For a typical leaf, nearly half of the net heat input from sunlight is dissipated by transpiration. The stream of water taken up by the roots is also an important means of bringing dissolved soil minerals to the root surface for absorption.

Of all the resources that plants need to grow and function, water is the most abundant and at the same time the most limiting for agricultural productivity (Figure 3.1). The fact that water is limiting is the reason for the practice of crop irrigation. Water availability likewise limits the productivity of natural ecosystems (Figure 3.2). Thus an understanding of the uptake and loss of water by plants is very important.

We will begin our study of water by considering how its structure gives rise to some of its unique physical properties. We will then examine the physical basis for water movement, the concept of water potential, and the application of this concept to cell water relations.

The Structure and Properties of Water

Water has special properties that enable it to act as a solvent and to be readily transported through the body of the plant. These properties derive primarily from the polar structure of the water molecule. Here we will examine water's hydrogen bonds and how they contribute to the properties of water that are so necessary for life.

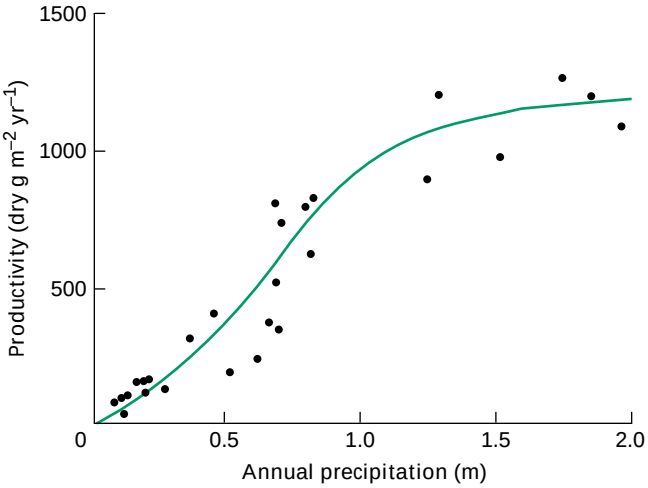


Figure 3.2 Productivity of various ecosystems as a function of annual precipitation. Productivity was estimated as net aboveground accumulation of organic matter through growth and reproduction. (After Whittaker 1970.)

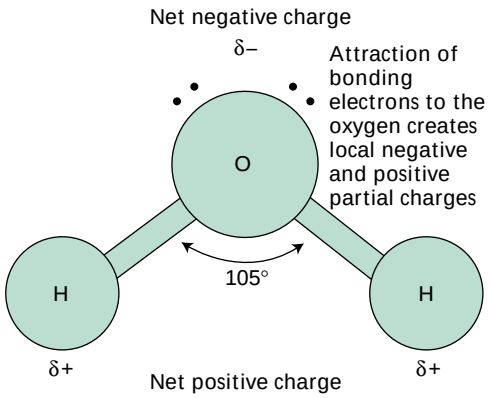


Figure 3.3 Diagram of the water molecule. The two intramolecular hydrogen–oxygen bonds form an angle of 105°. The opposite partial charges (δ– and δ+) on the water molecule result in the formation of intermolecular hydrogen bonds with other water molecules.

The Polarity of Water Molecules Gives Rise to Hydrogen Bonds

The water molecule consists of an oxygen atom covalently bonded to two hydrogen atoms. The two O–H bonds form an angle of 105° (Figure 3.3). Because the oxygen atom is more **electronegative** than hydrogen, it tends to attract the electrons of the covalent bond. This attraction results in a partial negative charge at the oxygen end of the molecule and a partial positive charge at each hydrogen. These partial charges are equal, so the water molecule carries no *net* charge. Nevertheless, this separation of partial charges, together with the shape of the water molecule, makes water a *polar molecule*, and the opposite partial charges between neighboring water molecules tend to attract each other. The weak electrostatic attraction between water molecules, known as a **hydrogen bond**, is responsible for many of the unusual physical properties of water.

Hydrogen bonds can also form between water and other molecules that contain electronegative atoms (O or N). The structure of proteins, polysaccharides, nucleic acids, and other molecules in the cell is strongly influenced by hydrogen bonds. Hydrogen bonding is responsible for the stable base pairing between complementary strands of DNA. In aqueous solutions, hydrogen bonding between water molecules leads to local, ordered clusters of water that, because of the continuous thermal agitation of the water molecules, continually form, break up, and re-form (Figure 3.4).

The Polarity of Water Makes It an Excellent Solvent

The physical properties of water make it uniquely suitable as a medium for life. First, water is an excellent solvent: It dissolves greater amounts of a wider variety of substances than do other related solvents. This versatility as a solvent is due in part to the small size of the water molecule and in part to its polar nature, which makes water a particularly good solvent for ionic substances and for molecules such as sugars and proteins that contain polar —OH or —NH_2 residues.

The water molecules orient themselves around ions and polar solutes in solution and effectively shield their electric charges. This shielding decreases the electrostatic interaction between the charged substances and thereby increases their solubility. Furthermore, the polar ends of water molecules can orient themselves next to charged or partially charged groups in macromolecules, forming **shells of hydration**. Hydrogen bonding between macromolecules and water reduces the interaction between the macromolecules and helps draw them into solution.

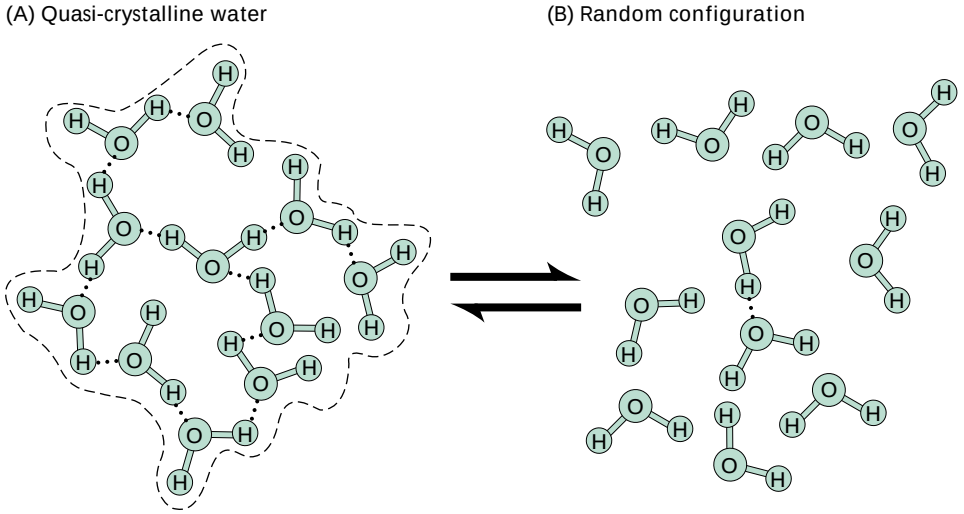


Figure 3.4 Hydrogen bonding between water molecules results in local aggregations of ordered, quasi-crystalline water (A). Because of the continuous thermal agitation of the water molecules, these aggregations are very short-lived; they break up rapidly to form much more random configurations (B).

The Thermal, Cohesive, and Adhesive Properties of Water Result from Hydrogen Bonding

The extensive hydrogen bonding between water molecules results in unusual thermal properties, such as high specific heat and high latent heat of vaporization. **Specific heat** refers to the heat energy required to raise the temperature of a substance by a specific amount. When the temperature of water is raised, the molecules must vibrate faster, so a great deal of energy must be put into the system to break the hydrogen bonds between water molecules. Thus, compared with other liquids, water requires a relatively large energy input to raise its temperature. This large energy input requirement is important for plants because it helps slow potentially harmful temperature fluctuations.

Latent heat of vaporization is the energy needed to separate molecules from the liquid phase and move them into the gas phase at constant temperature—a process that occurs during transpiration. For water at 25°C, the heat of vaporization is 44 kJ mol^{-1}—the highest value known for any liquid. Most of this energy is used to break hydrogen bonds between water molecules. The high latent heat of vaporization of water enables plants to cool themselves by evaporating water from leaf surfaces, which are prone to heat up because of the radiant input from the sun. Transpiration is an important component of temperature regulation in many plants.

Water molecules at an air–water interface are more strongly attracted to neighboring water molecules than to the gas phase on the other side of the surface. As a consequence of this unequal attraction, the air–water interface tends to minimize its surface area. In effect, the water molecules exert a force at the air–water interface. This force not only influences the shape of the surface but also may create a pressure in the rest of the liquid. The condition that exists at the interface is known as **surface tension**. As we will see later, surface tension at the evaporative surfaces of leaves generates the physical forces that pull a stream of water through the plant's vascular system.

In addition, the extensive hydrogen bonding in water gives rise to the property known as **cohesion**, the mutual attraction between molecules. A related property, called **adhesion**, is the attraction of water to a solid phase such as a cell wall or glass surface. Cohesion, adhesion, and surface tension give rise to a phenomenon known as **capillarity**, the movement of water a small distance up a glass capillary tube. The upward movement of the water is due to attraction of water at the periphery to the polar surface of a clean glass tube (adhesion) and to the surface tension of water, which tends to minimize the surface area. Together, adhesion and surface tension exert a tension on the water molecules at and just below the surface, causing them to move up the tube until the force of adhesion is balanced by the weight of the water column. The smaller the tube, the higher the capillary rise, which may be calculated using the following formula:

$$\text{Capillary rise} = \frac{14.9 \times 10^{-6}\,\text{m}^2}{\text{radius}} \qquad (3.1)$$

where both capillary rise and radius are expressed in meters.

For a xylem vessel with 25 μm radius, the capillary rise is about 0.6 m. This distance is much too small to be significant for water transport up tall trees. However, fibrous materials such as cell walls can act like wicks to draw water by capillarity from nearby xylem. This action ensures that cell wall surfaces that are directly exposed to the air, such as those in leaf mesophyll, remain wetted and do not dry out. Because the cell wall capillaries have a tiny radius, about 10 m^{-8}, very large physical forces can be generated in the water just below the evaporative surfaces of cell walls.

Water Has a High Tensile Strength

Cohesion gives water a high **tensile strength**, defined as the ability to resist a pulling force. We do not usually think of liquids as having tensile strength; however, such a property must exist for a water column to be pulled up a capillary tube without breaking.

We can demonstrate the tensile strength of water by placing it in a capped syringe (Figure 3.5). When we *push* on the plunger, the water is compressed and a positive **hydrostatic pressure** builds up. The pressure is measured in units called *pascals* (Pa) or, more conve-

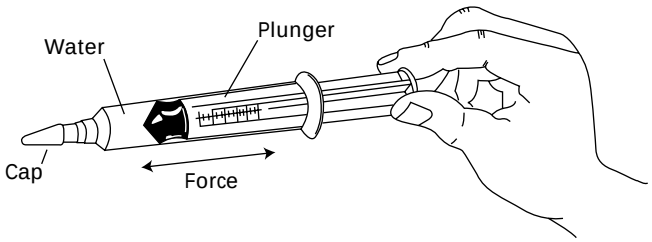


Figure 3.5 A sealed syringe can be used to create positive and negative pressures in a fluid like water. Pushing on the plunger compresses the fluid, and a positive pressure builds up. If a small air bubble is trapped within the syringe, it shrinks as the pressure increases. Pulling on the plunger causes the fluid to develop a tension, or negative pressure. Any air bubbles in the syringe will expand as the pressure is reduced from atmospheric pressure. As a gas bubble in the syringe expands, the pressure never goes below a pure vacuum, because the gas phase can expand indefinitely. If the syringe is filled with a degassed solution and lacks bubbles, pressures below vacuum (i.e., negative pressures) can develop because the hydrogen bonds holding the fluid together can withstand considerable tension before breaking.

TABLE 3.1
Comparison of units of pressure

1 atmosphere = 14.7 pounds per square inch
= 760 mm Hg (at sea level, 45° latitude)
= 1.013 bar
= 0.1013 MPa
= 1.013×10^5 Pa
A car tire is typically inflated to about 0.2 MPa.
The water pressure in home plumbing is typically 0.2–0.3 MPa.
The water pressure under 15 feet (5 m) of water is about 0.05 MPa.

niently, *megapascals* (MPa).* Pressure is equivalent to a force per unit area (1 Pa = 1 N m^{-2}) and to an energy per unit volume (1 Pa = 1 J m^{-3}). Table 3.1 compares pascals with some other units of pressure. If instead of pushing on the plunger we *pull* on it, a tension, or negative hydrostatic pressure, develops in the water to resist the pull. How hard must we pull on the plunger before the water molecules are torn away from each other and the water column breaks? Breaking the water column requires sufficient energy to overcome the forces that attract water molecules to one another.

In the syringe shown in Figure 3.5, small bubbles often interfere with this measurement by expanding as the pressure is reduced. However, careful studies have demonstrated that water in small capillaries can resist tensions more negative than –30 MPa (the negative sign indicates tension, as opposed to compression). This value is only a fraction of the theoretical strength of water computed on the basis of the strength of hydrogen bonds. Nevertheless, it is about 10% of the tensile strength of copper or aluminum wire and is thus quite substantial.

The presence of gas bubbles reduces the tensile strength of a water column. The lowest absolute pressure possible in a gas phase is 0 MPa (pure vacuum) because the intermolecular forces of attraction needed to resist a negative pressure (or tension) do not exist in ideal gases. In contrast, in solids and liquids the intermolecular attractions can resist tensile forces. Therefore, if even a tiny gas bubble forms in a column of water under tension, the gas bubble will expand indefinitely, with the result that the tension in the liquid phase collapses, a phenomenon known as **cavitation**. As we will see in Chapter 4, cavitation can have a devastating effect on water transport through the xylem of trees.

Water Transport Processes

When water moves from the soil through the plant to the atmosphere, it travels through a widely variable medium, and the mechanisms of water transport also vary with the type of medium (cell wall, cytoplasm, membrane, air spaces). We will now consider the two major processes in water transport: molecular diffusion and bulk flow.

Diffusion Is the Movement of Molecules by Random Thermal Agitation

Water molecules in a solution are not static; they are in continuous motion, colliding with one another and exchanging kinetic energy. **Diffusion** is the process by which molecules intermingle as a result of their random thermal agitation. Such agitation gives rise to the random but progressive movement of substances from regions of high free energy to regions of low free energy. As long as other forces are not acting on the molecules, diffusion causes molecules to move from regions of high concentration to regions of low concentration—that is, down a concentration gradient (Figure 3.6). Fick discovered that the rate of diffusional movement is directly proportional to the concentration gradient ($\partial c / \partial x$). In symbols, we write this relation as Fick's first law:

$$J_s = -D_s \frac{\partial c_s}{\partial x} \tag{3.2}$$

The rate of transport, or the **flux density** (J_s), is the amount of substance *s* crossing a unit area per unit time (e.g., J_s may have units of moles per square meter per second [mol m^{-2} s^{-1}]). The **diffusion coefficient** (D_s) is a proportionality constant that measures how easily substance *s* moves through a particular medium. The diffusion coefficient is a characteristic of the substance (larger molecules have smaller diffusion coefficients) and depends on the medium (diffusion in air is much faster than diffusion in a liquid, for example). The concentration gradient ($\partial c_s / \partial x$)is usually approximated as $\Delta c_s / \Delta x$—that is, as the difference in concentration of substance *s* (Δc_s) between two points separated by the distance Δx. The negative sign in the equation indicates that the flux moves down a concentration gradient. Fick's first law says that a substance will diffuse faster when the concentration gradient becomes steeper or when the diffusion coefficient is increased. This equation accounts only for movement in response to a concentration gradient, and not for movement in response to other forces (e.g., pressure, electric fields, and so on).

Diffusion Is Rapid over Short Distances but Extremely Slow over Long Distances

From Fick's law, one can derive (with difficulty) an expression for the time it takes for a substance to diffuse a particular distance. If the initial conditions are such that all the solute molecules are concentrated at the starting position (Figure 3.7A), then the concentration front moves away from the starting position, as shown

* 1 MPa = approximately 9.9 atmospheres.

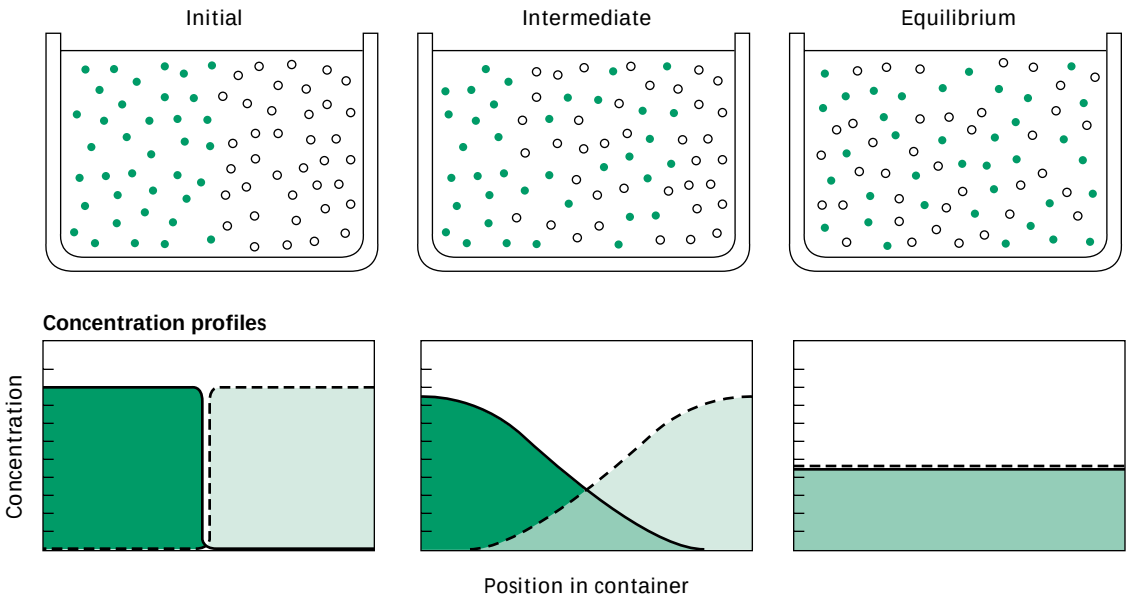


Figure 3.6 Thermal motion of molecules leads to diffusion—the gradual mixing of molecules and eventual dissipation of concentration differences. Initially, two materials containing different molecules are brought into contact. The materials may be gas, liquid, or solid. Diffusion is fastest in gases, intermediate in liquids, and slowest in solids. The initial separation of the molecules is depicted graphically in the upper panels, and the corresponding concentration profiles are shown in the lower panels as a function of position. With time, the mixing and randomization of the molecules diminishes net movement. At equilibrium the two types of molecules are randomly (evenly) distributed.

for a later time point in Figure 3.7B. As the substance diffuses away from the starting point, the concentration gradient becomes less steep, and thus net movement becomes slower. The time it takes for the substance at any given distance from the starting point to reach one-half of the concentration at the starting point ($t_c = 1/2$) is given by the following equation:

$$t_{c=1/2} = \frac{(\text{distance})^2}{D_s} K \qquad (3.3)$$

where K is a constant that depends on the shape of the system (for convenience, we will use a K value of 1 in the following calculations) and D_s is the diffusion coefficient. Equation 3.3 shows that the time required for a substance to diffuse a given distance increases in proportion to the *square* of that distance.

We can see what this means by considering two numerical examples. First, how long it would take a small molecule to diffuse across a typical cell? The diffusion coefficient for a small molecule like glucose is about 10^{-9} m^2 s^{-1}, and the cell size may be 50 µm. Thus, for this example:

$$t_{c=1/2} = \frac{(50 \times 10^{-6}\,\text{m})^2}{10^{-9}\,\text{m}^2\text{s}^{-1}} = 2.5\ \text{s}$$

This calculation shows that small molecules diffuse over cellular dimensions rapidly. What about diffusion over longer distances? Calculating the time needed for the same substance to move a distance of 1 m (e.g., the length of a corn leaf), we find:

$$t_{c\,=\,1/2} = \frac{(1\ \text{m})^2}{10^{-9}\,\text{m}^2\ \text{s}^{-1}} = 10^9\ \text{s} \approx 32\ \text{years}$$

a value that exceeds by orders of magnitude the life span of a corn plant, which lives only a few months.

From these numerical examples we see that diffusion in solutions can be effective within cellular dimensions but is far too slow for mass transport over long distances. As we'll see in Chapter 4, diffusion is of great importance during loss of water vapor from leaves because the diffusion coefficient in air is much greater than in aqueous solutions.

Pressure-Driven Bulk Flow Drives Long-Distance Water Transport

A second process by which water moves is known as **bulk flow** or **mass flow**. Bulk flow is the concerted movement of groups of molecules en masse, most often in response to a pressure gradient. Among many common examples of bulk flow are convection currents, water moving through a garden hose, a river flowing, and rain falling.

If we consider bulk flow through a pipe, the rate of volume flow depends on the radius (r) of the pipe, the

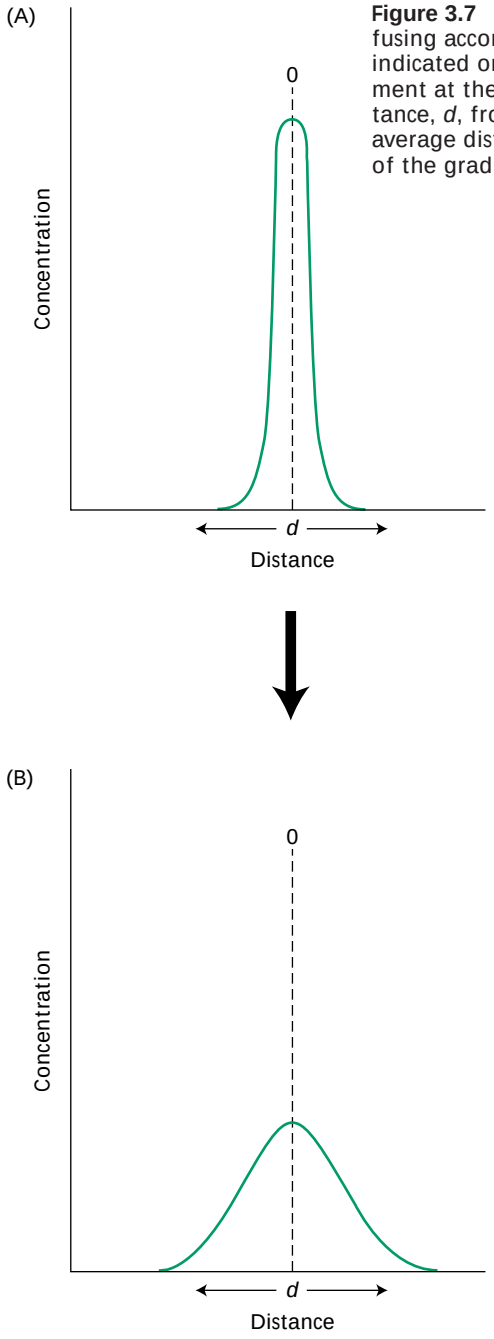


Figure 3.7 Graphical representation of the concentration gradient of a solute that is diffusing according to Fick's law. The solute molecules were initially located in the plane indicated on the x-axis (0). (A) The distribution of solute molecules shortly after placement at the plane of origin. Note how sharply the concentration drops off as the distance, *d*, from the origin increases. (B) The solute distribution at a later time point. The average distance of the diffusing molecules from the origin has increased, and the slope of the gradient has flattened out. (After Nobel 1991.)

viscosity (η) of the liquid, and the pressure gradient ($\partial\Psi_z / \partial x$) that drives the flow. This relation is given by one form of **Poiseuille's equation**:*

$$\text{Volume flow rate} = \left(\frac{\pi r^4}{8\eta}\right)\left(\frac{\partial \Psi_p}{\partial x}\right) \qquad (3.4)$$

expressed in cubic meters per second ($m^3\ s^{-1}$). This equation demonstrates that bulk flow is very sensitive to the radius of the pipe. If the radius is doubled, the volume flow rate increases by a factor of 2^4 (that is, 16).

Pressure-driven bulk flow of water is the predominant mechanism responsible for long-distance transport of water in the plant via the xylem. It may also account for much of the water flow through the soil and through the cell walls of plant tissues. In contrast to diffusion, pressure-driven bulk flow is independent of solute concentration gradients, as long as viscosity changes are negligible.

Osmosis Is Driven by a Water Potential Gradient

Movement of a solvent such as water through a membrane is called **osmosis**. Although water can be taken up and lost by plant cells relatively quickly, uptake and loss are significantly limited by the plasma membrane, which acts as a barrier to the movement of most substances. Membranes of plant cells are **selectively permeable**; that is, they allow the movement of water and other small uncharged substances across them more readily than the movement of larger solutes and charged substances. To facilitate the transport of inorganic ions, sugars, amino acids, and other metabolites across the various cell membranes, special transport proteins are required. This aspect of membrane transport will be discussed in Chapter 6. Here we restrict our discussion to the movement of water across membranes.

Like molecular diffusion and pressure-driven bulk flow, osmosis occurs spontaneously in response to a driving force. In simple diffusion, substances move down a concentration gradient; in pressure-driven bulk flow, substances move down a pressure gradient; in osmosis, both types of gradients influence transport. The direction and rate of water flow across a membrane are determined not solely by the concentration gradient of water or by the pressure gradient, but by the sum of these two driving forces. This observation leads to the concept of a composite or total driving force, representing the free-energy gradient of water. In practice, this driving force is expressed as a **chemical-potential gradient** or, more commonly by plant physiologists, as a **water potential gradient**.

In the case of water movement into plant cells, the mechanism of osmosis involves a combination of (1) diffusion of single water molecules across the membrane bilayer and (2) bulk flow through tiny water-filled pores of molecular dimensions (Figure 3.8). Whether water

* Jean-Léonard-Marie Poiseuille (1797–1869) was a French physician and physiologist.

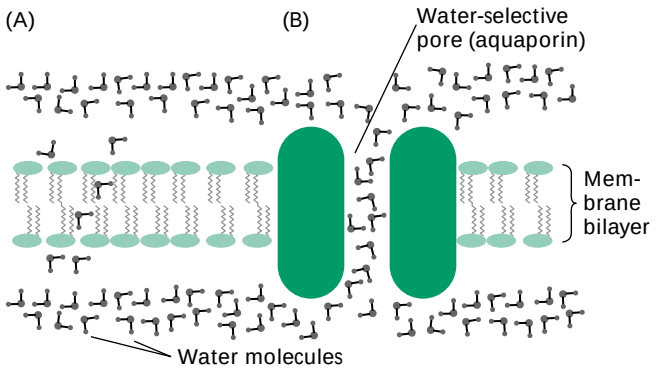


Figure 3.8 Water can cross plant membranes (A) by diffusion of individual water molecules through the membrane bilayer and (B) by bulk flow of files of water molecules through a water-selective pore formed by integral membrane proteins such as aquaporins.

moves by diffusion or by microscopic bulk flow, the relevant driving force is the water potential gradient, for reasons that were worked out in a theoretical analysis of osmosis by Peter Ray (1960). According to Ray's analysis, the exclusion of solutes from the water-filled pore in the membrane creates a pressure gradient within the pore. Thus, gradients in concentration and pressure give rise to equivalent transport across the membrane. This equivalence of pressure and concentration is strictly true only for ideal membranes—that is, membranes with much greater permeability for water than for dissolved solutes. For nonideal membranes, more advanced analyses are needed (Stein 1986; Finkelstein 1987).

For many years there was much uncertainty about whether water actually moved through microscopic pores within plant membranes. Some studies indicated that simple diffusion was not sufficient to account for osmosis, but the evidence in support of microscopic pores was not compelling. This uncertainty was put to rest with the recent discovery of **aquaporins** (see Figure 3.8B). These are integral membrane proteins that form a water-selective channels across the membrane (see also Chapter 6). Such channels facilitate water movement across the membrane (Weig et al. 1997; Schäffner 1998). The ability of aquaporins to transport water may be regulated by their phosphorylation state (Maurel et al. 1995). In other words, the cell can regulate its membrane permeability to water by adding or removing phosphate groups to specific amino acid residues on the aquaporin protein. Such a modulation of aquaporin activity may alter the *rate* of water movement across the membrane; however, it does not change the direction of transport or the driving force for water movement.

To begin our detailed discussion of cell water relations, consider the following "thought experiment": Place a flaccid cell—that is, one with negligible turgor pressure—in water. The cell takes up water, initially quickly and then more slowly, and eventually reaches an equilibrium where net water uptake ceases. At this point, the free energy of water is the same inside and outside the cell. If we measured cell volume and turgor pressure, we would find that they increase and then stabilize with a similar time course. Now we may ask the following questions: What is the nature of this equilibrium and how is it defined quantitatively? What are the values for the changes in cell volume and cell turgor? What is the time course for reaching equilibrium and on what does it depend? These issues are examined in the sections that follow.

The Chemical Potential of Water Represents the Free-Energy Status of Water

The **chemical potential** of water (or *water potential*) is a quantitative expression of the free energy associated with water. In thermodynamics, free energy represents a potential for performing work. All living things, including plants, require a continuous input of free energy in order to maintain and repair their structures and their organized states, as well as to grow and reproduce. Processes such as biochemical reactions, solute accumulation, and long-distance transport are all driven by an input of free energy into the plant.

The concepts of free energy and chemical potential derive from thermodynamics, the study of the transformations of energy. As we discussed in Chapter 2, thermodynamics provides a useful framework for studying the energetics of many processes essential to life. Here we will restrict ourselves to a discussion of the thermodynamic basis for transport of water in plants.

Osmosis is an energetically spontaneous process. That is, water moves down a chemical-potential gradient, from a region of high chemical potential to a region of low chemical potential. In the thought experiment described in the previous section, this means that water moves from outside to inside the cell. No net work is involved in osmosis; rather, free energy is released. To understand water movement by osmosis, we need to examine more closely what influences the chemical potential of water.

In general, the chemical potential of water may be influenced by three major factors: *concentration*, *pressure*, and *gravity*. Note that chemical potential is a relative quantity: It is usually expressed as the difference between the potential of a substance in a given state and the potential of the same substance in a standard state. The unit of chemical potential is energy per mole of substance ($J\ mol^{-1}$).

For historical reasons, plant physiologists have most often used a related quantity called **water potential**, defined as the chemical potential of water divided by the partial molal volume of water (the volume of 1 mol of water): $18 \times 10^{-6}\ m^3\ mol^{-1}$. Water potential is a mea-

TABLE 3.2
Values of *RT* and osmotic potential of solutions at various temperatures

Temperature (°C)	RT[a] (L MPa mol^{-1})	Osmotic potential (MPa) of solution with solute concentration in mol L^{-1} water			Osmotic potential of seawater (MPa)
		0.01	0.10	1.00	
0	2.271	–0.0227	–0.227	–2.27	–2.6
20	2.436	–0.0244	–0.244	–2.44	–2.8
25	2.478	–0.0248	–0.248	–2.48	–2.8
30	2.519	–0.0252	–0.252	–2.52	–2.9

[a] R = 0.0083143 L MPa mol^{-1} K^{-1}.

sure of the free energy of water per unit volume (J m^{-3}). These units are equivalent to pressure units such as pascals, which is the common measurement unit for water potential. We will now consider more fully the important concept of water potential.

Three Major Factors Contribute to Cell Water Potential

Water potential is symbolized by Ψ_w (Greek letter psi), and the water potential of solutions may be dissected into individual components, usually written as the following sum:

$$\Psi_w = \Psi_s + \Psi_p + \Psi_g \qquad (3.5)$$

The terms Ψ_s, Ψ_p, and Ψ_g denote the effects of solutes, pressure, and gravity, respectively, on the free energy of water. The reference state used to define water potential is liquid water at ambient pressure and temperature. This means that Ψ_w is proportional to the work required to move 1 mol of pure water at ambient pressure and temperature to another state at the same temperature. In most cases Ψ_w inside plant cells is negative, because pure water has a higher potential than the water inside the cell. Let's consider each of the terms on the right side of Equation 3.5.

Solutes. The term Ψ_s, called the **solute potential** or the **osmotic potential**, represents the effect of dissolved solutes on water potential. Solutes reduce the free energy of water by diluting the water. This effect is primarily an entropy effect; that is, the mixing of solutes and water increases the disorder of the system and thereby lowers free energy. The entropy effect of dissolved solutes is revealed in other physical effects known as the **colligative properties** (called "colligative" because they all occur together, or collectively; colligative properties depend on the number of dissolved particles and not on the nature of the solute) of solutions: Solutes reduce the vapor pressure of a solution, raise its boiling point, and lower its freezing point. The specific nature of the solute does not matter. For dilute solutions of nondissociating substances, the osmotic potential may be estimated by the **van't Hoff equation**:

$$\Psi_s = -RTc_s \qquad (3.6)$$

where R is the gas constant (8.32 J mol^{-1} K^{-1}), T is the absolute temperature (in degrees Kelvin, or K), and c_s is the solute concentration of the solution, expressed as **osmolality** (moles of total dissolved solutes per liter of water [mol L^{-1}]). The minus sign indicates that dissolved solutes reduce the water potential of a solution. Table 3.2 shows the values of RT at various temperatures and the Ψ_s values of solutions of different solute concentrations.*

For ionic solutes that dissociate into two or more particles, c_s must be multiplied by the number of dissociated particles to account for the increased number of dissolved particles. Thus, if we dissolve 0.1 mol of sucrose in 1 L of water, we obtain a solution with an osmolality of 0.1 mol L^{-1}. If instead we dissolve 0.1 mol of salt (NaCl) in 1 L of water, we obtain an osmolality of 0.2 mol L^{-1} because salt dissociates into two particles. At 20°C (293 K), these values translate into the following osmotic potential (Ψ_s) values: –0.244 MPa for the sucrose solution and –0.488 MPa for the salt solution.

Equation 3.6 is valid for "ideal" solutions at dilute concentration. Real solutions frequently deviate from the ideal, especially at high concentrations—for example, greater than 0.1 mol L^{-1}. In our treatment of water

* The great German plant physiologist Wilhelm Pfeffer (1845–1920) made the fundamental discoveries about solutes, osmotic potentials, and the need for a membrane for osmosis to work. Pfeffer's results led to the Dutch chemist Jacobus Hendricus van't Hoff's (1852–1911) theory of solutions, for which van't Hoff received a Nobel prize in 1901. See Bünning 1989 for a fascinating biography of Pfeffer.

BOX 3.1

Alternative Conventions for Components of Water Potential

STUDENTS PLANNING FURTHER STUDY of plant water relations should note that the components of water potential defined in the text are sometimes given different names and symbols. In particular, the equation

$$\Psi_w = \Psi_s + \Psi_p$$

(Equation 3.7 in the text) is often replaced by the following equivalent equation:

$$\Psi_w = -\pi + P$$

In this alternative convention, P is the same as Ψ_p. It is the hydrostatic pressure of the solution, and may be positive, as in turgid cells, or negative, as in xylem water. The symbol π is called **osmotic pressure** and is the negative of Ψ_s. That is, π has positive values, and Ψ_s has negative values. "Osmotic pressure" is the term that physical chemists, zoologists, and many others use to denote the effect of dissolved solutes on the free energy of water. Most handbooks of physics and chemistry use the term "osmotic pressure" and the symbol π. The negative sign in front of π in the equation above accounts for the reduction in water potential (Ψ_w) by dissolved solutes. Thus $\Psi_s = -\pi$. A very interesting, if somewhat unconventional, account of the history and physical meaning of osmotic pressure is given by Hammel and Scholander (1976).

Unfortunately, some authors have mixed the conventions for Ψ_s and π, leading to unnecessary confusion about what is meant by the symbol π. Thus, π is sometimes incorrectly called osmotic potential instead of osmotic pressure, and it may be used either as a positive quantity or as a negative quantity. Let the reader beware!

potential, we will assume that we are dealing with ideal solutions.* Readers are referred to more advanced treatments for further discussion of nonideal behavior (see Friedman 1986; Nobel 1991).

Pressure. The term Ψ_p is the **hydrostatic pressure** of the solution. Positive pressures raise the water potential; negative pressures reduce it. Sometimes Ψ_p is called *pressure potential*. When referring to the positive hydrostatic pressure within cells, Ψ_p is usually called **turgor pressure**. The value of Ψ_p may be negative—for example, in the xylem and in the walls between cells, where a *tension*, or a *negative hydrostatic pressure*, can develop. As we will see, negative pressures outside cells are very important in moving water long distances through the plant.

Hydrostatic pressure is measured as the deviation from ambient pressure (Box 3.2). Remember that water in the reference state is at ambient pressure, so by this definition $\Psi_p = 0$ MPa for water in the standard state. Thus, the value of Ψ_p for pure water in an open beaker is 0 MPa, even though its absolute pressure is approximately 0.1 MPa (1 atmosphere). Water under a perfect vacuum has a Ψ_p value of –0.1 MPa; its absolute pressure is 0 MPa. It is important to keep in mind the distinction between absolute pressure and Ψ_p, which will be relevant to later discussions.

Gravity. Gravity causes water to move downward, unless the force of gravity is opposed by an equal and opposite force. The potential for water movement thus depends on height. The effect of gravity on water potential, Ψ_g, depends on the height (h) of the water above the reference-state water, the density of water (ρ_w), and the acceleration due to gravity (g). In symbols, we write these relationships as follows: $\Psi_g = \rho_w g h$, where $\rho_w g$ has a value of 0.01 MPa m^{-1}. Thus, a vertical distance of 10 m translates into a 0.1 MPa change in water potential.

Matric potential. In discussions of dry soils, seeds, and cell walls, one often finds reference to yet another component of Ψ_w: the **matric potential** (Ψ_m).† The matric potential is used to account for the reduction in free energy of water when it exists as a thin surface layer, one or two molecules thick, adsorbed onto the surface of relatively dry soil particles, cell walls, and other materials. The matric potential does not represent a new force acting on water, because the effect of the surface interaction can theoretically be accounted for by its effects on Ψ_s and Ψ_p (see Passioura 1980; Nobel 1991). As a practical matter, however, this surface interaction effect often cannot be easily be separated into Ψ_p and Ψ_s components in dry materials, so frequently they are bulked together and designated as the matric potential.

It is generally not valid to add Ψ_m to independent measurements of Ψ_s and Ψ_p to arrive at a total water potential. This is particularly true for water inside

* Reference books of chemistry and physics often give tables listing exact osmotic potentials of actual solutions. These are usually given as osmotic pressures (usually the symbol π). Osmotic pressure and osmotic potential differ only in sign: $\Psi_s = -\pi$. In other words, osmotic potentials are negative, and osmotic pressures are positive (see Box 3.1).

† In the alternative naming system discussed in Box 3.1, matric potential is usually designated by the symbol t.

hydrated cells and cell walls, where matric effects are either negligible or they are accounted for by a reduction in Ψ_p. For instance, what we describe in this chapter as the negative pressure in water held by cell wall microcapillaries at the evaporative surfaces of leaves is sometimes described as a wall matric potential. Care is needed to avoid inconsistencies when accounting for this physical effect in definitions of Ψ_p, Ψ_s, and Ψ_m (Passioura 1980).

Simplifications for cell water relations. When dealing with water transport at the cell level, Equation 3.5 is usually simplified as follows:

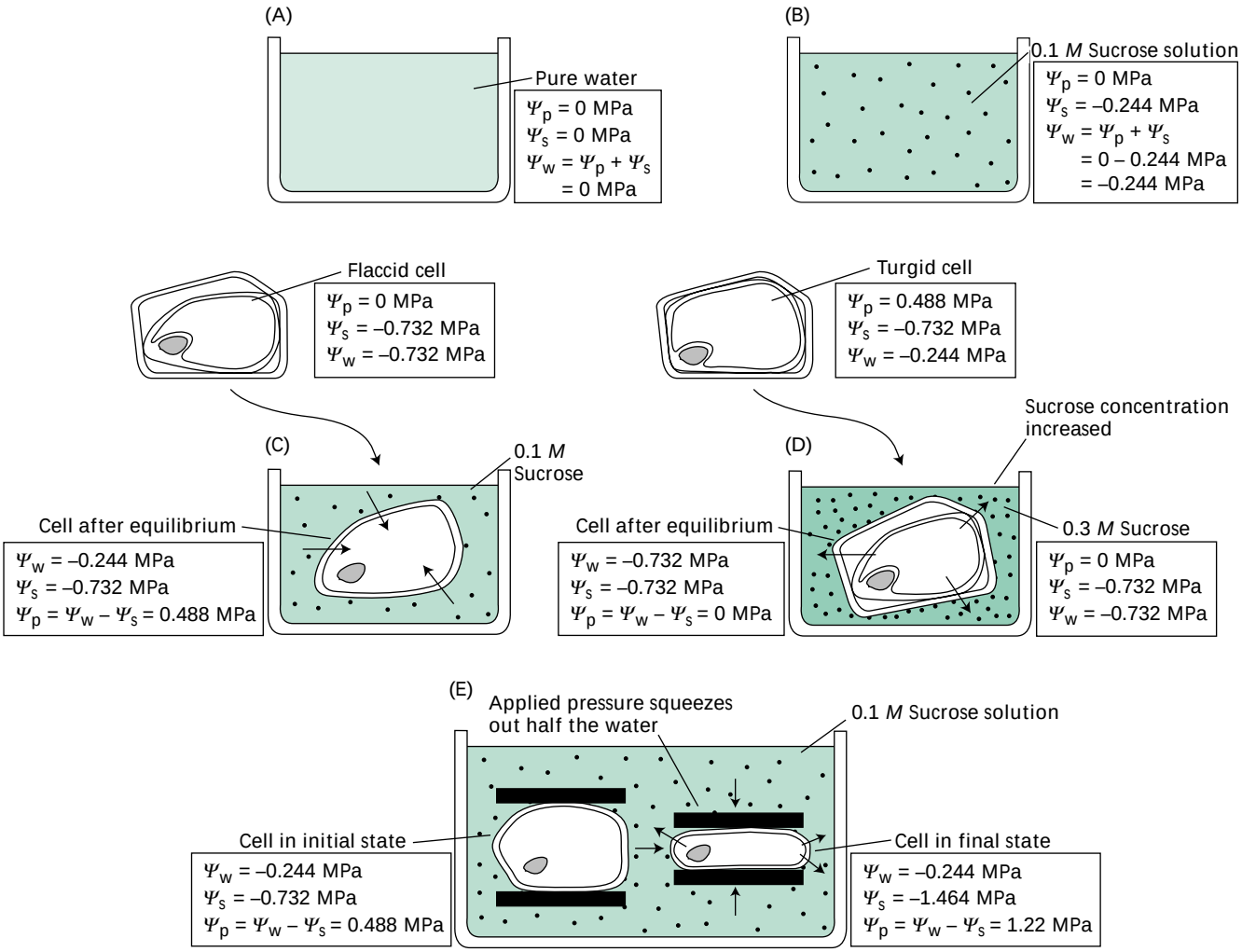
$$\Psi_w = \Psi_s + \Psi_p \tag{3.7}$$

or sometimes $\Psi = \Psi_s + \Psi_p$. The gravitational component (Ψ_g) is ignored because it is negligible when vertical distances are small (say, less than 5 m). Thus the only significant components of cell Ψ_w are due to dissolved solutes and hydrostatic pressure.

Water Enters the Cell along a Water Potential Gradient

Now let's extend our thought experiment by illustrating the osmotic behavior of plant cells with some numerical examples. First, imagine an open beaker full of pure water at 20°C (Figure 3.9A). Since the water is open to

Figure 3.9 Five examples illustrating the concept of water potential and its components. (A) Pure water. (B) A solution containing 0.1 *M* sucrose. (C) After a flaccid cell is dropped in the 0.1 *M* sucrose solution, because the starting water potential of the cell is less than the water potential of the solution, the cell takes up water. After equilibration, the water potential of the cell rises to equal the water potential of the solution, and the result is a cell with a positive turgor pressure. (D) Increasing the concentration of sucrose in the solution makes the cell lose water. The increased sucrose concentration lowers the solution water potential, draws water out from the cell, and thereby reduces the cell's turgor pressure. (E) Another way to make the cell lose water is by slowly pressing it between two plates. In this case, half of the cell water is removed, so cell osmotic potential increases and turgor pressure increases correspondingly.



BOX 3.2

Measuring Water Potential

CELL GROWTH, photosynthesis, and crop productivity are all strongly influenced by water potential and its components. Like the body temperature of humans, water potential is a good overall indicator of plant health. Plant scientists have thus expended considerable effort in devising accurate and reliable methods for evaluating the water status of a plant. Four instruments that have been used extensively to measure Ψ_w, Ψ_s, and Ψ_p are described here: psychrometer, pressure chamber, cryoscopic osmometer, and pressure probe.

Psychrometer (Ψ_w measurement)

Psychrometry (the prefix "psychro-" comes from the Greek word *psychein*, "to cool") is based on the fact that the vapor pressure of water is lowered as its water potential is reduced. This is one of the *colligative properties* of solutions. Psychrometers measure the water vapor pressure of a solution or plant sample, on the basis of the principle that evaporation of water from a surface cools the surface.

One psychrometric technique, known as *isopiestic psychrometry*, has been used extensively by John Boyer and coworkers (Boyer and Knipling 1965) and is illustrated in Figure 1. Investigators make a measurement by placing a piece of tissue sealed inside a small chamber that contains a temperature sensor (in this case, a thermocouple) in contact with a small droplet of water. Initially, water evaporates from both the tissue and the water droplet, raising the humidity of the air inside the sealed chamber.

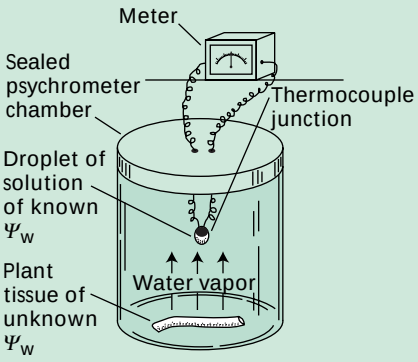


Figure 1 Diagram illustrating the use of isopiestic psychrometry to measure the water potential of a plant tissue.

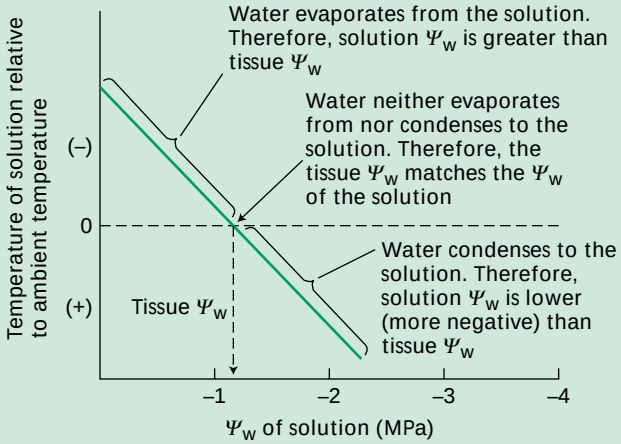


Figure 2 The temperature of the sensor in the psychrometer shown in Figure 1 depends on the water potential of the solution relative to the water potential of the tissue sample.

Evaporation continues until the air becomes saturated or nearly saturated with water vapor. At this point, if the plant tissue and the water droplet have the same water potential, the net movement of water from the droplet stops, and the temperature of the droplet, measured with the temperature sensor, is the same as the ambient temperature. But if the tissue has a lower water potential than that of the droplet, water evaporates from the droplet, diffuses through the air, and is absorbed by the tissue. This slight evaporation of water cools the drop. The larger the difference in water potential between the tissue and the droplet, the higher the rate of water transfer and hence the cooler the droplet.

Rather than placing pure water on the temperature sensor, one may place a standard solution of known solute concentration (known Ψ_s and thus known Ψ_w). If the standard solution has a lower water potential than that of the sample to be measured, water will diffuse from the tissue to the droplet, causing warming of the droplet. Measuring the change in temperature of the droplet for several solutions of known Ψ_w makes it possible to match exactly the water potential of the solution with that of the sample (Figure 2). When the match is perfect, the change in temperature of the droplet is zero.

Psychrometers have been used to measure the water potentials of excised and intact plant tissue. Moreover, the method is applicable to solutions in which Ψ_w equals Ψ_s. Thus psychrometry can measure both the water potential of living tissue and the osmotic potential of a solution. Frequently, the Ψ_w of a tissue is measured with a psychrometer, and then the tissue is crushed and the Ψ_s value of the expressed cell sap is measured with the same instrument. By combining the two measurements, researchers can estimate the turgor pressure that existed in the cells before the tissue was crushed ($\Psi_p = \Psi_w - \Psi_s$).

This method is very useful, but it is very sensitive to temperature fluctuations. For example, a change in temperature of 0.01°C may correspond to a change in water potential of about 0.1 MPa (the value varies with the type of temperature sensor). Since it is often desirable to have a resolution of 0.01 MPa, the instrument must be kept under stringent conditions of constant temperature. For this reason, the method is used primarily in laboratory settings and has found only limited use for fieldwork, where temperature is not easily controlled. There are many variations in psychrometric technique; interested readers should consult Brown and Van Haveren 1972 and Slavik 1974.

Pressure chamber (Ψ_w measurement)

A relatively quick method for estimating the water potential of large pieces of tissues, such as whole leaves and shoots, is by use of the **pressure chamber**. This method was pioneered by Henry Dixon at Trinity College, Dublin, at the beginning of the twentieth century, but it did not come into widespread use until P. Scholander

BOX 3.2 (*continued*)

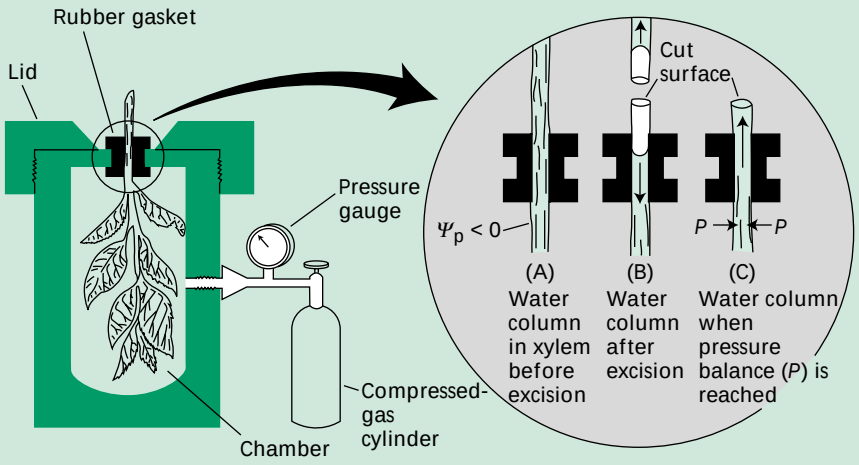


Figure 3 The pressure chamber method for measuring plant water potential. The diagram at left shows a shoot sealed into a chamber, which may be pressurized with compressed gas. The diagrams at right show the state of the water columns within the xylem at three points in time: (A) The xylem is uncut and under a negative pressure, or tension. (B) The shoot is cut, causing the water to pull back into the tissue, away from the cut surface, in response to the tension in the xylem. (C) The chamber is pressurized, bringing the xylem sap back to the cut surface.

and coworkers at the Scripps Institution of Oceanography improved the instrument design and showed its practical use (Scholander et al. 1965).

The pressure chamber measures the negative hydrostatic pressure (tension) that exists in the xylem of most plants. The water potential of the xylem is assumed to be fairly close to the average water potential of the whole organ—an assumption that is probably valid, because (1) in many cases the osmotic potential of the xylem solution is negligible, so the major component of the water potential in the xylem is the (negative) hydrostatic pressure in the xylem column, and (2) the xylem is in intimate contact with most cells in the plant.

In this technique, the organ to be measured is excised from the plant and is partly sealed in a pressure chamber (Figure 3). Before excision, the water column in the xylem is under some tension. When the water column is broken by excision of the organ, the water is pulled into the xylem capillary by the now unopposed tension. The cut surface consequently appears dull and dry. To make a measurement, the investigator pressurizes the chamber with compressed gas until the water in the xylem is brought back to the cut surface. The pressure needed to bring the water back to the surface is called the *balance pressure* and is readily detected by the change in the appearance of the cut surface, which becomes wet and shiny when this pressure is attained.

The balance pressure is equal in magnitude (but opposite in sign) to the negative pressure that existed in the xylem column before the plant material was excised. For example, if a balance pressure of 0.5 MPa is found, then Ψ_p in the xylem before excision was –0.5 MPa. If we know Ψ_s for the xylem sap from other measurements, we may calculate the water potential of the xylem, which, as already stated, is assumed to be close to the water potential of the whole organ.

In many outdoor plants, Ψ_p in the xylem may be –1 to –2 MPa, whereas Ψ_s may be only –0.05 to –0.2 MPa. Therefore, in many situations pressure chamber measurements by themselves provide an adequate estimate of the water potential of the plant. Because the pressure chamber method is rapid and does not require delicate instrumentation or elaborate temperature control, it has been used extensively under field conditions to estimate water potential (Tyree and Hammel 1972).

Cryoscopic osmometer (Ψ_s measurement)

The **cryoscopic osmometer** measures the osmotic potential of a solution by measuring its freezing point. One of the *colligative properties* of solutions is the decrease in the freezing point as the solute concentration increases. For example, a solution containing 1 mol of solutes per kilogram of water has a freezing point of –1.86°C, compared with 0°C for pure water.

Various instruments can be used to measure the freezing-point depression of solutions (for two examples, see Prager and Bowman 1963 and Bearce and Kohl 1970). With a cryoscopic osmometer, solution samples as small as 1 nanoliter (10^{-9} L) are placed in an oil medium located on a temperature-controlled stage (Figure 4). The very small sample size allows sap from single cells to be measured and permits rapid thermal equilibration with the stage. To prevent evaporation, the investigator suspends the samples in oil-filled wells in a silver plate (silver has high thermal conductivity). The temperature of the stage is rapidly decreased to about –30°C, which causes the sample to freeze. The temperature is then raised very slowly, and the melting process in the sample is observed through a microscope. When the last ice crystal in the sample melts, the temperature of the stage is recorded (note that the melting and freezing points are the same). It is a straightforward job to calculate the solute concentration from the freezing-point depression; and from the solute concentration (c_s), Ψ_s is calculated as $-RTc_s$.This technique has been used to measure droplets extracted from single cells (Malone and Tomos 1992).

Pressure probe (Ψ_p measurement)

If a cell were as large as a watermelon or even a grape, measuring its hydrostatic pressure would be a relatively easy task. Because of the small size of plant cells, however, the development of methods for direct measurement of turgor pressure has been slow. Paul Green at the University of Pennsylvania developed one of the first direct methods, using a micromanometer, for measuring turgor pressure in plant cells (Green and Stanton 1967). In this technique, an air-filled glass tube sealed at one end is inserted into a cell (Figure 5). The high pressure in the cell compresses the trapped gas, and from the change in volume one can readily calculate the pressure of the cell from the ideal gas law (pressure × volume = constant). This method works only for cells of relatively large volume, such as the giant cell of the filamentous

BOX 3.2 (*continued*)

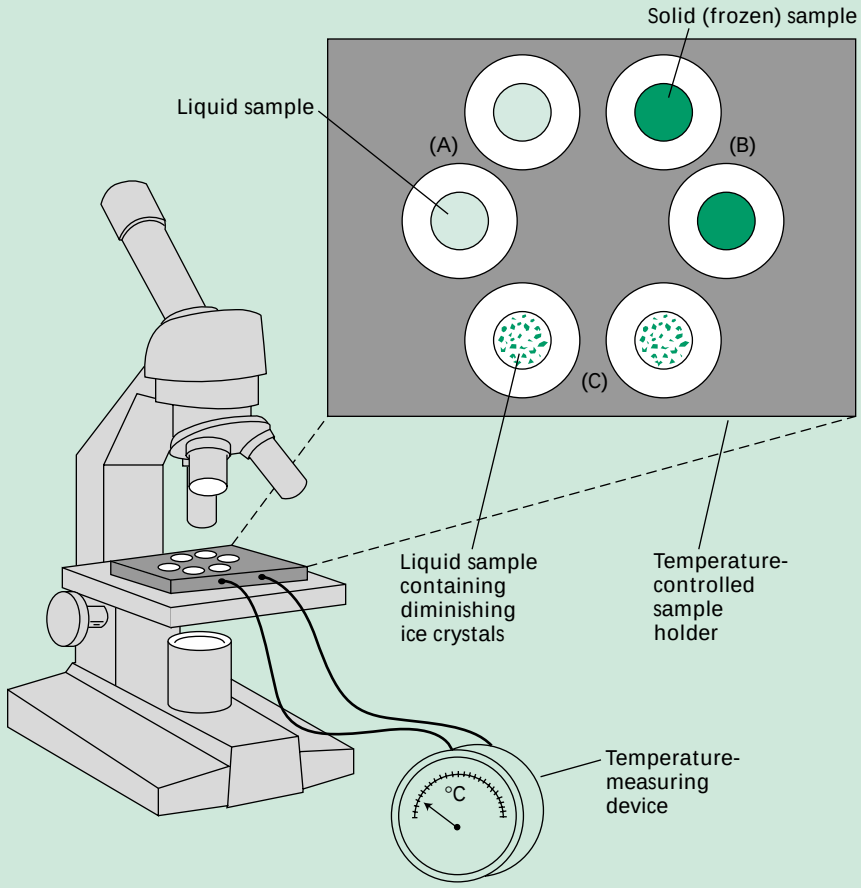
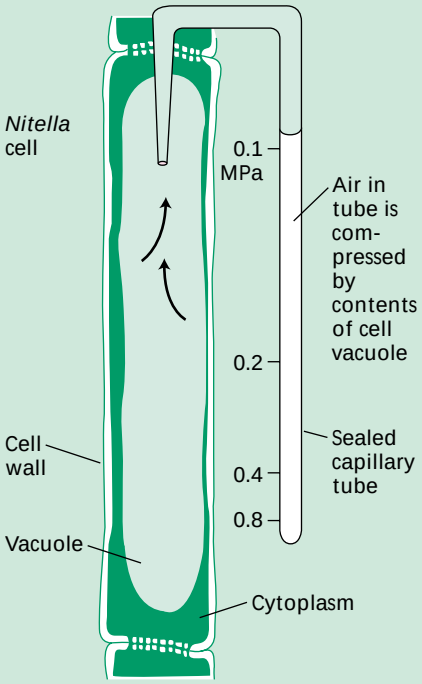


Figure 4 A cryoscopic osmometer measures the concentration of total dissolved solutes by measuring the freezing-point depression of a solution. (A) Very small liquid samples are loaded onto the temperature-controlled stage of a microscope. (B) When the temperature is quickly reduced, the samples supercool and freeze. (C) Slowly warming the stage causes the samples to thaw. The temperature at which the last ice crystal melts provides a measure of the melting point of the sample.

Figure 5 Use of the micromanometer, a pressure probe, to measure cell turgor pressure. *Nitella* cells (which are particularly large—about 100 mm in diameter and many centimeters long) were used for these measurements. (After Green 1968.)



green alga *Nitella*. For smaller cells, the loss of cell sap into the glass tube is sufficient to deflate the cell and result in artifactually low pressures.

For higher plant cells, which are several orders of magnitude smaller

the atmosphere, the hydrostatic pressure of the water is the same as atmospheric pressure (Ψ_p = 0 MPa). There are no solutes in the water, so Ψ_s = 0 MPa; therefore the water potential is 0 MPa ($\Psi_w = \Psi_s + \Psi_p$). Now imagine dissolving sucrose in the water to a concentration of 0.1 *M* (Figure 3.9B). This addition lowers the osmotic potential (Ψ_s) to –0.244 MPa (see Table 3.2) and decreases the water potential (Ψ_w) to –0.244 MPa.

Next, consider a flaccid plant cell (i.e., a cell with no turgor pressure) that has a total internal solute concentration of 0.3 *M* (Figure 3.9C). This solute concentration gives an osmotic potential (Ψ_s) of –0.732 MPa. Because the cell is flaccid, the internal pressure is the same as ambient pressure, so the hydrostatic pressure (Ψ_p) is 0 MPa and the water potential of the cell is –0.732 MPa. What happens if this cell is placed in the beaker containing 0.1 *M* sucrose (see Figure 3.9C)? Because the water potential of the sucrose solution (Ψ_w = –0.244 MPa; see Figure 3.9B) is greater than the water potential of the cell (Ψ_w = –0.732 MPa), water will move from the sucrose solution to the cell (from high to low water potential).

Even a slight increase in cell volume causes a large increase in the hydrostatic pressure within the cell, because plant cells are surrounded by relatively rigid cell walls. As water enters the cell, the cell wall is stretched by the contents of the enlarging protoplast. The wall resists such stretching by pushing back on the cell. This phenomenon is analogous to inflating a basketball with air, except that air is compressible, whereas water is nearly incompressible. Thus, as water moves into the cell, the hydrostatic pressure or turgor pressure (Ψ_p) of the cell increases. Consequently, the cell water potential (Ψ_w) increases and the difference between inside and outside water potentials ($\Delta\Psi_w$) is reduced.

BOX 3.2 (*continued*)

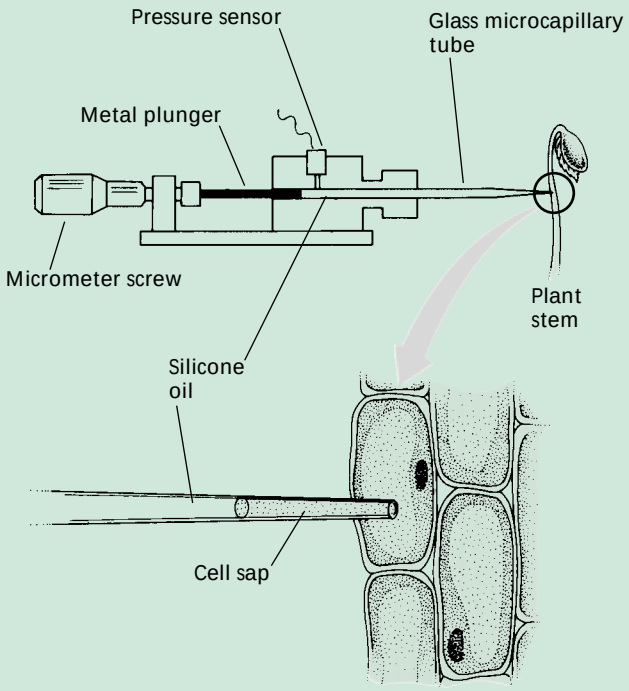


Figure 6 Diagram of the simplest pressure probe (not to scale). The primary advantage of this method over the one shown in Figure 5 is that cell volume is minimally disturbed. Minimal disturbance is of great importance for the tiny cells that are typical of higher plants, in which loss of even a few picoliters (10^{-12} L) of fluid can substantially reduce turgor pressure.

in volume than *Nitella*, a more sophisticated device, the **pressure probe**, was developed by Ernest Steudle, Ulrich Zimmermann, and their colleagues in Germany (Husken et al. 1978). This instrument is similar to a miniature syringe (Figure 6). A glass microcapillary tube is pulled to a fine point and is inserted into a cell. The microcapillary is filled with silicone oil, a relatively incompressible fluid that can be readily distinguished from cell sap under a microscope. When the tip of the microcapillary is first inserted into the cell, cell sap begins to flow into the capillary because of the initial low pressure of that region. Investigators can observe such movement of sap under the microscope and counteract it by pushing on the plunger of the device, thus building up a pressure. In such fashion the boundary between the oil and the cell sap can be pushed back to the tip of the microcapillary. When the boundary is returned to the tip and is held in a constant position, the initial volume of the cell is restored and the pressure inside the cell is exactly balanced by the pressure in the capillary. This pressure is measured by a pressure sensor in the device. Thus the hydrostatic pressure of individual cells may be measured directly.

This method has been used to measure Ψ_p and other parameters of water relations in cells of both excised and intact tissues of a variety of plant species (Steudle 1993). The primary limitation of this method is that some cells are too small for current instruments to measure. Furthermore, some cells tend to leak after being stabbed with the capillary, and others plug up the tip of the capillary, thereby preventing valid measurements. The pressure probe has also been adapted to measure positive and negative values of Ψ_p in the xylem (Heydt and Steudle 1991). However, technical problems with cavitation (see Chapter 4) limit the measurement of negative Ψ_p by this technique.

Eventually, cell Ψ_p increases enough to raise the cell Ψ_w to the same value as the Ψ_w of the sucrose solution. At this point, equilibrium is reached ($\Delta\Psi_w = 0$ MPa), and net water transport ceases. The tiny amount of water taken up by the cell does not significantly affect the solute concentration of the sucrose solution, the volume of which is very much larger than that of the cell. Hence, Ψ_s, Ψ_p, and Ψ_w of the sucrose solution are not altered. Therefore, at equilibrium $\Psi_{w(cell)} = \Psi_{w(solution)} = -0.244$ Mpa.

The calculation of cell Ψ_p and Ψ_s is straightforward. If we assume that the cell has a very rigid cell wall, then very little water will enter. Thus we can assume to a first approximation that $\Psi_{s(cell)}$ is unchanged during the equilibration process and that $\Psi_{s(solution)}$ remains at –0.732 MPa. We can obtain cell hydrostatic pressure by rearranging Equation 3.7: $\Psi_p = \Psi_w - \Psi_s = (-0.244) - (-0.732) = 0.488$ MPa.

Water Can Also Leave the Cell in Response to a Water Potential Gradient

Water can also leave the cell by osmosis. If, in the previous example, we remove our plant cell from the 0.1 *M* sucrose solution and place it in a 0.3 *M* sucrose solution (Figure 3.9D), $\Psi_{w(solution)}$ (–0.732 MPa) is more negative than $\Psi_{w(cell)}$ (–0.244 MPa), and water will move from the cell to the solution. As water leaves the cell, the cell volume decreases. As the cell volume decreases, cell Ψ_p and Ψ_w decrease also until $\Psi_{w(cell)} = \Psi_{w(solution)} = -0.732$ MPa. From the water potential equation (Equation 3.7) we can calculate that at equilibrium $\Psi_p = 0$ MPa. As before, we assumed that the change in cell volume is small, so we can ignore the change in Ψ_s.

If we slowly squeeze the cell by pressing it between two plates (Figure 3.9E), we effectively raise the cell Ψ_p, consequently raising the cell Ψ_w and creating a $\Delta\Psi_w$ such

that water now flows *out* of the cell. If we continue squeezing until half the cell water is removed and then hold the cell in this condition, the cell will reach a new equilibrium. As in the previous example, at equilibrium $\Delta\Psi_w = 0$ MPa, and the amount of water added to the external solution is so small that it can be ignored. The cell will thus return to the Ψ_w value that it had before the squeezing procedure. However, the components of the cell Ψ_w will be quite different.

Because half of the water was squeezed out of the cell while the solutes remained inside the cell (the membrane is selectively permeable), the cell solution is concentrated twofold, and thus Ψ_s is lower ($-0.732 \times 2 = -1.464$ MPa). Knowing the final values for Ψ_w and Ψ_s, we can calculate the turgor pressure from Equation 3.7 as $\Psi_p = \Psi_w - \Psi_s = (-0.244) - (-1.464) = 1.22$ MPa. This final example is unusual in that we used an external force to change cell volume without a change in water potential. In most cases, the water potential of the cell's environment changes and the cell gains or loses water until its Ψ_w matches that of its environment.

One point common to all these examples deserves emphasis: *Water flow is a passive process. That is, water moves in response to physical forces, toward regions of low water potential or free energy*. There are no metabolic "pumps" (reactions driven by ATP hydrolysis) that push water from one place to another. This rule is valid as long as water is the only substance being transported. When solutes are transported, however, as occurs for short distances across membranes (see Chapter 6) and for long distances in the phloem (see Chapter 7), then water transport may be coupled to solute transport and this coupling may move water against a water potential gradient. For example, when phloem transports phloem sap from the leaves to the roots, it moves water against a water potential gradient (see Nobel 1991: 516–520).

On another scale, the movement of sugars, amino acids, or other small molecules by various membrane transport proteins was found to be coupled directly to the movement of up to 260 water molecules across the membrane per molecule of solute (Loo et al. 1996). Such exceptions defy no laws of thermodynamics, because the loss of free energy by the solute more than compensates for the gain of free energy by the water. Thus, the net change in free energy remains negative. *These exceptions notwithstanding, the vast majority of water transport in plants is energetically downhill, toward lower water potential.*

Small Changes in Plant Cell Volume Cause Large Changes in Turgor Pressure

Because plants cells have rigid walls, a change in cell (protoplast) volume and Ψ_w is usually accomplished by a change in Ψ_p, with little change in cell Ψ_s. This phenomenon is illustrated in greater detail in the **Höfler diagram** of Figure 3.10, which plots Ψ_w, Ψ_p, and Ψ_s as a function of relative cell volume for a hypothetical cell. In this example, as the cell volume is reduced by 5%, Ψ_w decreases from 0 to about –1.8 MPa. Most of this decrease is due to a reduction in Ψ_p (by about 1.5 MPa); Ψ_s decreases by about 0.3 MPa as a result of water loss by the cell and consequent increased concentration of cell solutes.

The exact shape of the curves in Figure 3.10 depends on the rigidity of the cell wall. If the wall is very rigid, the pressure–volume curve is very steep and a small change in volume gives a large change in cell turgor pressure. The wall rigidity is usually measured as **volumetric elastic modulus**, symbolized by ε (Greek epsilon). This wall property is given by the change in pressure ($\Delta\Psi_p$) divided by the relative change in volume ($\Delta V / V$); that is,

$$\varepsilon = \frac{\Delta\Psi_p}{\Delta V / V} \tag{3.8}$$

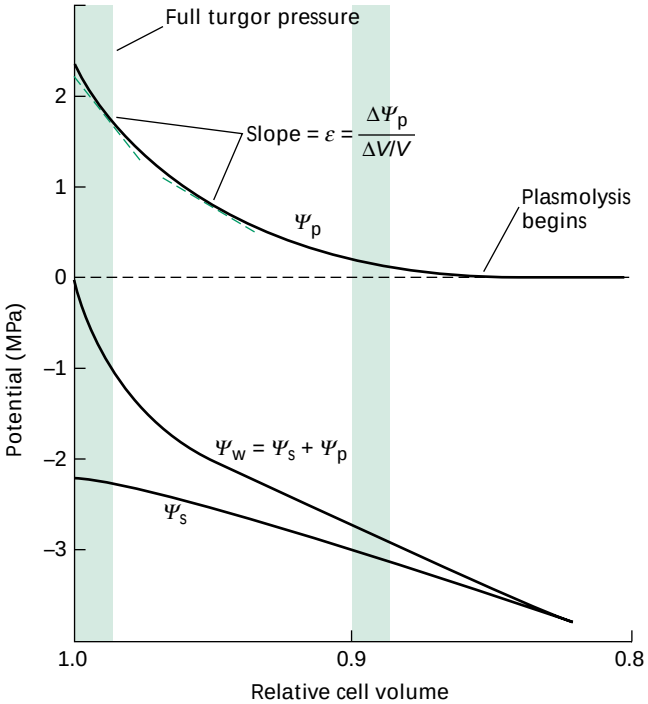


Figure 3.10 Modified Höfler diagram, showing that turgor pressure (Ψ_p) decreases steeply with the initial decrease in cell volume. In comparison, osmotic potential (Ψ_s) changes very little (see screened area at left). As cell volume decreases below 0.9 in this example, the situation reverses: Most of the change in water potential is due to a drop in cell Ψ_s accompanied by relatively little change in turgor pressure (see screened area at right). The slope of the curve that illustrates Ψ_p versus volume relationship is a measure of the cell's volumetric elastic modulus (ε). Note that ε is not constant but decreases as the cell loses turgor. (After Tyree and Jarvis 1982, based on a shoot of Sitka spruce.)

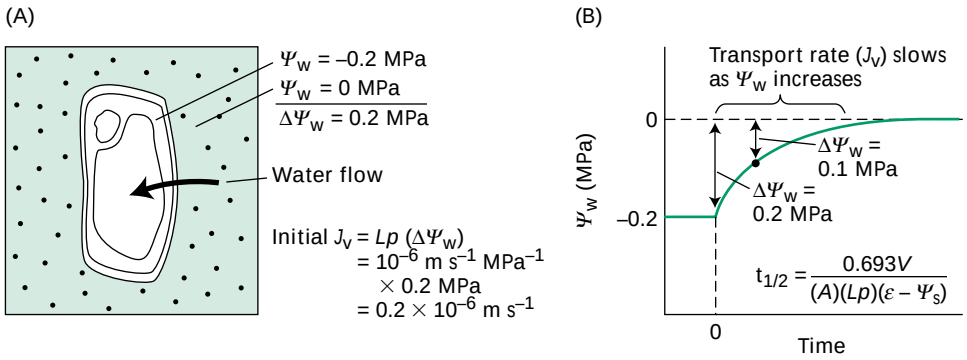


Figure 3.11 The rate of water transport into a cell depends on the water potential difference ($\Delta\Psi_w$) and the hydraulic conductivity of the cell membranes (Lp). In this example, (A) the initial water potential difference is 0.2 MPa and Lp is 10^{-6} m s^{-1} MPa^{-1}. These values give an initial transport rate (J_v) of 0.2×10^{-6} m s^{-1}. (B) As water is taken up by the cell, the water potential difference decreases with time, leading to a slowing in the rate of water uptake. This effect follows an exponentially decaying time course with a half-time ($t_{1/2}$) that depends on the following cell parameters: volume (V), surface area (A), Lp, volumetric elastic modulus (ε), and cell osmotic potential (Ψ_s).

The volumetric elastic modulus, ε, is the slope of the Ψ_p curve in Figure 3.10 and has units of pressure, with typical values on the order of 10 MPa.

Equation 3.8 shows that when ε = 10 MPa, a 1% increase in volume increases turgor pressure (Ψ_p) by 0.1 MPa. This increase is equivalent to a 10% increase in turgor pressure for a typical cell (in which Ψ_p = 1.0 MPa). In contrast, a 1% increase in volume would result in only a 1% increase in cell Ψ_s. Thus, when the water potential of turgid cells increases as a result of water uptake, most of this change is effected through an increase in Ψ_p rather than an increase in Ψ_s.

Figure 3.10 illustrates additional aspects that are characteristic of plant cell wall relations. First, turgor pressure (Ψ_p) approaches zero as cells lose a mere 10 to 15% of their cell volume. (For cells with very stretchy walls, such as guard cells, this volume fraction may be substantially larger.) Second, note that ε is not constant but decreases as turgor pressure is lowered. This effect is most evident in the flatness of the Ψ_p curve in the region where cell volume is 85 to 90% (see Figure 3.10). Third, when ε and Ψ_p are low, changes in water potential are due mostly to changes in Ψ_s (look at the Ψ_p and Ψ_s curves where volume = 85%). In fact, this relationship is characteristic of animal cells that lack cell walls and occurs because nonlignified plant cell walls usually are rigid only when turgor pressure puts them in tension. Such cells act like a basketball: The wall is stiff (has high ε) when the ball is inflated, but becomes soft and collapsible (ε = 0) when the ball loses pressure.

The Rate of Water Transport Depends on Driving Force and Hydraulic Conductivity

So far, we have seen that water moves across a membrane in response to a water potential gradient. The direction of flow is determined by the direction of the Ψ_w gradient, which is the **driving force** for transport. But what determines the *rate* at which the water moves?

Consider a cell with an initial water potential of –0.2 MPa, submerged in pure water. From this information we know that water will flow into the cell and that the driving force is $\Delta\Psi_w$ = 0.2 MPa, but what is the initial rate of movement? The rate depends on permeability of the membrane to water, a property usually called the **hydraulic conductivity** (Lp) of the membrane (Figure 3.11).

Driving force, membrane permeability, and flow rate are related by the following equation:

$$\text{Flow rate} = \text{driving force} \times \text{hydraulic conductivity} \qquad (3.9)$$

Hydraulic conductivity expresses how readily water can move across a membrane and has units of volume of water per unit area of membrane per unit time per unit driving force (for instance, m^3 m^{-2} s^{-1} MPa^{-1} or m s^{-1} MPa^{-1}). The larger the hydraulic conductivity, the larger the flow rate. In our example in Figure 3.11, the hydraulic conductivity of the membrane is 10^{-6} m s^{-1} MPa^{-1}. The transport (flow) rate (J_v) can then be calculated from the following equation:*

$$J_v = Lp(\Delta\Psi_w) \qquad (3.10)$$

where J_v is the volume of water crossing the membrane per unit area of membrane and per unit time (m^3 m^{-2} s^{-1} or, equivalently, m s^{-1}). In this example, J_v has a value of 0.2×10^{-6} m s^{-1}. Note that J_v has the physical meaning of a velocity. We can calculate the flow rate in volumetric terms (m^3 s^{-1}) by multiplying J_v by the surface area of the cell.

* This equation assumes that the membrane is ideal—that is, that solute transport is negligible and water transport is equally sensitive to $\Delta\Psi_s$ and $\Delta\Psi_p$ across the membrane. Nonideal membranes require a more complicated equation that separately accounts for water flow induced by $\Delta\Psi_s$ and by $\Delta\Psi_p$ (see Nobel 1991).

The resulting value is the *initial* rate of water transport. As water is taken up, cell Ψ_w increases and the driving force ($\Delta\Psi_w$) decreases. As a result, water transport slows with time. The rate approaches zero in an exponential manner (see Dainty 1976), with a half-time given by:

$$t_{1/2} = \left(\frac{0.693}{(A)(Lp)}\right)\left(\frac{V}{\varepsilon - \Psi_s}\right) \qquad (3.11)$$

where V and A are the volume and surface area of the cell, respectively. A small value for t means fast equilibration. This equation shows that the rate of water potential equilibration is affected by cell geometry (V and A) and by hydraulic conductivity (Lp) of the membrane. The quantity $1/(A)(Lp)$ is sometimes called the cell **hydraulic resistance** and $V/(\varepsilon - \Psi_s)$ is its **hydraulic capacitance**. Hydraulic capacitance determines the total volume of water that must move for any given change in water potential. For instance, if the cell walls are very rigid (meaning ε is large), very little water transport is required to effect a change in water potential, and thus the cell half-time is small. Cell half-times typically range from 1 to 10 s, although some are much shorter (Steudle 1989). These low half-time values mean that single cells come to water potential equilibrium with their surroundings in less than 1 minute. For multicellular tissues, the half-time values may be much larger.

The Water Potential Concept Helps Us Evaluate the Water Status of a Plant

The concept of water potential has two principal uses. First, water potential is the quantity that governs transport across cell membranes, as we have described. Second, water potential is often used as a measure of the *water status* of a plant. Because of transpirational water loss to the atmosphere, plants are seldom fully hydrated. They suffer from water deficits that lead to inhibition of plant growth and photosynthesis, as well as to other detrimental effects. Figure 3.12 lists some of the physiological changes that plants experience as they become dry. The process that is most affected by water deficit is cell growth. More severe water stress leads to inhibition of cell division, inhibition of wall and protein synthesis, accumulation of solutes, closing of stomata, and inhibition of photosynthesis. Water potential is one measure of how hydrated a plant is and thus provides a relative index of the *water stress* the plant is experiencing (see Chapter 25).

Figure 3.12 also shows representative values for Ψ_w at various stages of water stress. In leaves of well-watered plants, Ψ_w ranges from –0.2 to about –0.6 MPa, but the leaves of plants in arid climates can have much lower values, perhaps –2 to –5 MPa under extreme conditions. Because water transport is a passive process, plants can take up water only when the plant Ψ_w is less than the soil Ψ_w. As the soil becomes drier, the plant similarly becomes less hydrated (attains a lower Ψ_w). If this were not the case, the soil would begin to extract water from the plant.

The Components of Water Potential Vary with Growth Conditions and Location within the Plant

Just as Ψ_w values depend on the growing conditions and the type of plant, so too the values of Ψ_s can vary considerably. Within cells of well-watered garden plants (examples include lettuce, cucumber seedlings, and bean leaves), Ψ_s may be as high as –0.5 MPa, although values of –0.8 to –1.2 MPa are more typical. The upper limit for cell Ψ_s is set probably by the minimum concentration of dissolved ions, metabolites, and proteins in the cytoplasm of living cells. At the other extreme, plants sometimes attain a much lower Ψ_s. For instance, water stress typically leads to an accumulation of solutes in the cytoplasm and vacuole, thus maintaining turgor pressure despite low water potentials.

Plant tissues that store high concentrations of sucrose or other sugars, such as sugar beet roots, sugarcane stems, or grape berries, also attain low values of Ψ_s. Val-

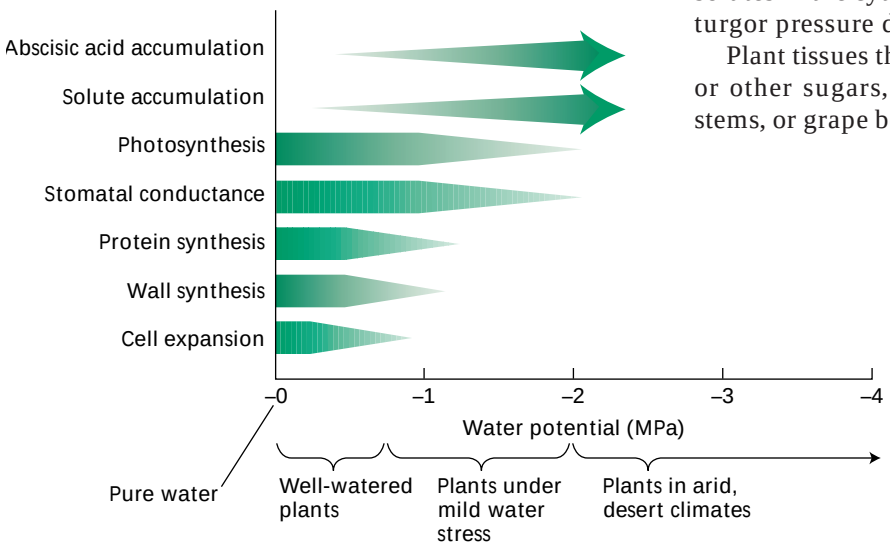


Figure 3.12 Water potential of plants under various growing conditions, and sensitivity of various physiological processes to water potential. The intensity of the bar color corresponds to the magnitude of the process. For example, cell expansion decreases as water potential falls (becomes more negative). Abscisic acid is a hormone that induces stomatal closure during water stress (see Chapter 23). (After Hsiao 1979.)

ues as low as –2.5 MPa are not unusual. **Halophytes**, plants that grow in saline environments, typically have very low values of Ψ_s; by this means they reduce cell Ψ_w enough to extract water from salt water. Most crop plants cannot survive in seawater which, because of the dissolved salts, has a lower water potential than the tissues of the plant can attain while maintaining their functional competence. Although Ψ_s *within* cells may be quite negative, the apoplastic solution surrounding the cells—that is, in the cell walls and in the xylem—may contain only low concentrations of solutes. Thus Ψ_s of this phase of the plant is typically much higher—for example, –0.1 to 0 MPa. Negative water potentials in the xylem and cell walls are usually due to negative Ψ_p (in the wall, this is sometimes attributed to a low matric potential, Ψ_m).

Values for Ψ_p within cells of well-watered garden plants may range from 0.1 to perhaps 1 MPa, depending on the value of Ψ_s inside the cell. A positive turgor pressure (Ψ_p) is important for two principal reasons. First, growth of plant cells requires turgor pressure to stretch the cell walls. The loss of Ψ_p under water deficits can explain in part why cell growth is so sensitive to water stress (see Chapter 25). Second, turgor pressure increases the mechanical rigidity of cells and tissues. This function of cell turgor pressure is particularly important for young nonlignified tissues, which cannot support themselves mechanically without a high internal pressure. A plant **wilts** when the turgor pressure inside the cells of such tissues falls toward zero. In cells submerged in a solution, **plasmolysis** may result when additional water is removed. Plasmolysis is the condition in which the membrane pulls away from the wall.

Whereas the solution inside cells may have a positive and large Ψ_p, the water outside the cell may have negative values for Ψ_p. In the xylem of rapidly transpiring plants, Ψ_p is negative and may attain values of –1 MPa or lower. The magnitude of Ψ_p in the cell walls and xylem varies considerably, depending on the rate of transpiration and the height of the plant. During the middle of the day, when transpiration is maximal, xylem Ψ_p reaches its lowest, most negative values, whereas it tends to increase at night, when transpiration is low, and the plant rehydrates.

Summary

Water is important in the life of plants because it makes up the matrix and medium in which most biochemical processes essential for life take place. The structure and properties of water strongly influence the structure and properties of proteins, membranes, nucleic acids, and other cell constituents.

In most land plants, water is continually lost to the atmosphere and taken up from the soil. The movement of water is driven by a reduction in free energy, and water may move by diffusion, by bulk flow, or by a combination of these fundamental transport mechanisms. Water diffuses because molecules are in constant thermal agitation, which tends to even out concentration differences. Water moves by bulk flow in response to a pressure difference, whenever there is a suitable pathway for bulk movement of water. Osmosis, the movement of water across membranes, depends on a gradient in free energy of water across the membrane—a gradient commonly measured as a difference in water potential.

Solute concentration and hydrostatic pressure are the two major factors that affect water potential, although gravity is also important when large vertical distances are involved. These components of the water potential may be summed: $\Psi_w = \Psi_s + \Psi_p + \Psi_g$. In soils, seeds, and dry cell walls, the reduction in water potential is usually attributed to a matric potential (Ψ_m). Plant cells quickly come into water potential equilibrium with their local environment by absorbing or losing water. Usually this change in cell volume results in a change in cell Ψ_p, accompanied by minor changes in cell Ψ_s. The rate of water transport across a membrane depends on the water potential difference across the membrane and the hydraulic conductivity of the membrane.

In addition to its importance in transport, water potential is a useful measure of the water status of plants. As we will see in Chapter 4, diffusion, bulk flow, and osmosis all help move water from the soil through the plant to the atmosphere.

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* Indicates a reference that is general reading in the field and is also cited in this chapter.

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