Plant Physiology and Biochemistry

Plant-Water Relation

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Date of Submission: February 28, 2006

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Keywords: Plant Physiology, Plant & Water, Transpiration, Stomata
Introduction

The chapter describes models relating to the question: movement of water and other substances across membranes, throughout the plant, and between the plant and its environment. This question overlaps somewhat with the question of what goes on inside cells, since cellular chemistry influences plant-water relation in interesting ways. Even growth of plants depends on water uptake, and much of plant-water relations depend upon cells interacting with their environment. Many plant functions depend quite directly upon the properties of water and of substances dissolved in the water. Thus, a brief review of water’s properties is required to begin our study on plant-water relation.

1. Physical Properties of Water

More than 70% of the Earth's surface is covered with water. Scientists estimate that the hydrosphere contains about 1.36 billion cubic kilometers of this substance mostly in the form of a liquid that occupies topographic depressions on the Earth. Water is also essential for life and is the major constituent of almost all life forms. Most animals and plants contain more than 60% water by volume. Without water life would probably never have developed on our planet.

Water has a very simple atomic structure. It consists of two hydrogen atoms (H) bonded to one oxygen atom (O). The nature of the atomic structure of water causes its molecules to have unique electrochemical properties. The hydrogen side of the water molecule has a slight positive charge (see Figure 1A). On the other side of the molecule a negative charge exists. This molecular polarity causes water to be a powerful solvent and is responsible for its strong surface tension. Molecules at the surface of liquid water have fewer neighbors and, as a result, have a greater attraction to the few water molecules that are nearby. This enhanced attraction is called surface tension. It makes the surface of the liquid slightly more difficult to break through than the interior. When a small object that would normally sink in water is placed carefully on the surface, it can remain suspended on the surface due to surface tension.

Figure 1 A (© 1999-2005 Michael Pidwirny)
Figure 1B, 1C, 1D (© 1999-2005 Michael Pidwirny)
When water makes a physical phase change its molecules arrange themselves in distinctly different patterns (Figure 1B-1D). The three diagrams above illustrate the distinct patterns of molecular arrangement in water when it changes its physical state from ice to water to gas. When water is frozen its molecules arrange themselves in a particular highly organized rigid geometric pattern that causes the mass of water to expand and to decrease in density. Expansion of water at freezing allows ice to float on top of liquid water. In the liquid phase, water molecules arrange themselves into small groups of joined particles. The fact that these arrangements are small allows liquid water to move and flow. Water in the form of a gas is highly charged with energy. This high-energy state causes the molecules to be always moving reducing the likelihood of bonds between individual molecules from forming.

**Water has several other unique physical properties. These properties are:**

- **Water has a high specific heat.** Specific heat is the amount of energy required to change the temperature of a substance. Because water has a high specific heat, it can absorb large amounts of heat energy before it begins to get hot. It also means that water releases heat energy slowly when situations cause it to cool. Water's high specific heat allows for the moderation of the Earth's climate and helps organisms regulate their body temperature more effectively.

- **Water in a pure state has a neutral pH.** As a result, pure water is neither acidic nor basic. Water changes its pH when substances are dissolved in it. Rain has a naturally acidic pH of about 5.6 because it contains natural derived carbon dioxide and sulfur dioxide.

- **Water conducts heat more easily than any liquid except mercury.** This fact causes large bodies of liquid water like lakes and oceans to have essentially a uniform vertical temperature profile.

- **Water exists as a liquid over an important range of temperature from 0 - 100°C Celsius.** This range allows water to remain as a liquid in most places on the Earth.

- **Liquid water is a universal solvent.** It is able to dissolve a large number of different chemical compounds. This feature also enables water to carry solvent nutrients in runoff, infiltration, groundwater, and living organisms. For example, common table salt, sodium chloride, is an ionic substance that contains alternating sodium and chlorine ions.

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**Figure 1E:** Sodium Chloride contains Na⁺ and Cl⁻ ions (Copyright © 2003 - 2005, Visionlearning, Inc.)

![Sodium Chloride](image)

**Figure 1F:** Table Salt Dissolving in Water (Copyright © 2003 - 2005, Visionlearning, Inc.)

![Table Salt Dissolving in Water](image)
When table salt is added to water, the partial charges on the water molecule are attracted to the Na\(^+\) and Cl\(^-\) ions. The water molecules work their way into the crystal structure and between the individual ions, surrounding them and slowly dissolving the salt. The water molecules will actually line up differently depending on which ions are being pulled into solution. The negative oxygen ends of water molecules will surround the positive sodium ions; the positive hydrogen ends will surround the negative chlorine ions.

In a similar fashion, any substance that carries a net electrical charge, including both ionic compounds and polar covalent molecules (those that have a dipole), can dissolve in water. This idea also explains why some substances do not dissolve in water. Oil, for example, is a non-polar molecule. Because there is no net electrical charge across an oil molecule, it is not attracted to water molecules and, therefore, it does not dissolve in water.

- **Adhesive and Cohesive Forces of Water**: Because of its polar nature, water is attracted to many other substances, that is, it wets them. Molecules of proteins and cell-wall polysaccharides are excellent examples. This attraction between unlike molecules (water and other molecules in this case) is called **adhesion**. In the case of water, it involves hydrogen bonding between the water and the other molecules. The attraction of like molecules for each other (because of hydrogen bonding) is called **cohesion**. Cohesion bestows upon water an unusually high-tensile strength. In a thin, confined column of water such as that in the xylem elements of a stem, this tensile strength can reach high values, allowing water to be pulled to the tops of tall trees.

**Figure 1G**: shows how the water molecules are attracted to each other to create high surface tension. This property can cause water to exist as an extensive thin film over solid surfaces. In the example above, the film is two layers of water molecules thick. (© 1999-2005 Michael Pidwirny)
Figure 1H: The adhesive bonding property of water molecules allows for the formation of water droplets (© 2004 Edward Tsang).

- Water has high surface tension (Figure 1G, 1H). Cohesion between water molecules accounts for surface tension. The molecules at the surface of a liquid are continually being pulled into the liquid by cohesive (hydrogen-bond) forces. The result is that a drop of water acts as if it were covered by a tight elastic skin. It is the surface tension that makes a falling drop spherical. This phenomenon also causes water to stick to the sides of vertical structures despite gravity’s downward pull. The surface tension of water is higher than that of most other liquids. Surface tension often plays a role in the physiology of plants. For example, it allows plants to move water (and dissolve nutrients) from their roots to their leaves. Under normal pressures, the passage of air bubbles through pores and pits in cell walls is prevented because the surface tension of water that surrounds an air bubble is too great to allow deformation of the bubble.

- Water is the only substance on Earth that exists in all three physical states of matter: solid, liquid and gas. Incorporated in the changes of state are massive amounts of heat exchange. This feature plays an important role in the redistribution of heat energy in the Earth's atmosphere. In terms of heat being transferred into the atmosphere, approximately 3/4's of this process is accomplished by the evaporation and condensation of water.

Table 1: Density of water at various temperatures.

<table>
<thead>
<tr>
<th>Temperature (degrees Celsius)</th>
<th>Density (grams per cubic centimeter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (solid)</td>
<td>0.9150</td>
</tr>
<tr>
<td>0 (liquid)</td>
<td>0.9999</td>
</tr>
<tr>
<td>4</td>
<td>1.0000</td>
</tr>
<tr>
<td>20</td>
<td>0.9982</td>
</tr>
<tr>
<td>40</td>
<td>0.9922</td>
</tr>
<tr>
<td>60</td>
<td>0.9832</td>
</tr>
<tr>
<td>80</td>
<td>0.9718</td>
</tr>
<tr>
<td>100 (gas)</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

(© 1999-2005 Michael Pidwirny)
• The freezing of water causes it to expand. When water freezes it expands rapidly adding about 9% by volume. Fresh water has a maximum density at around 4° Celsius (Table 1). Water is the only substance on this planet where the maximum density of its mass does not occur when it becomes solidified.

2. Water and plant cells

2.1. Calculating Capillary Rise

The water properties of cohesion, adhesion and surface tension, give rise to the phenomenon of capillarity, the movement of water for small distances up a capillary tube. The smaller the tube radius, the higher the capillary rises. How far the water will move may be calculated using the following formula:

\[
\text{Capillary rise} = \frac{14.9 \times 10^{-6} \text{ m}^2}{\text{radius}}
\]

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.

Fibrous materials such as cell walls can act like wicks to draw water by capillarity from nearby xylem. This capillary 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 \(10^{-8}\) m, very large physical forces can be generated in the water just below the evaporative surfaces of cell walls.

2.2. Diffusion, Osmosis and Active Transport

Substances move in and out of cells by means of passive or active transport. Examples of passive transport are diffusion and osmosis (the diffusion of water). Important substances like oxygen, glucose, water and mineral salts are needed and must be allowed to enter the cells and metabolic waste products like carbon dioxide and toxins must be allowed to leave the cells. The entry and exit of all substances from the cells is controlled by the cell membrane, which is partially permeable.

2.2.1. Diffusion

Particles in liquids and gases have kinetic energy (energy produces through motion). They move about randomly, at speed, in all directions. In an area of high concentration, some of the particles collide with each other, lose energy and slow down. Others will escape from the concentrated area to places where there are fewer or no particles. Very few particles leave an area of low concentration to go to an area where the concentration is higher.

The result is a concentration gradient, with particles diffusing from an area of high concentration to an area of low concentration. Particles continue to move from high to low concentration for as long as there is a concentration gradient (a difference in concentration between two adjacent areas. Particles will move down the concentration gradient from an area of high concentration to an area of low concentration).
Other example of diffusion across concentration gradients is:

<table>
<thead>
<tr>
<th>Place</th>
<th>Particles move</th>
<th>From</th>
<th>To</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Oxygen</td>
<td>Chloroplast</td>
<td>Air spaces in mesophyll</td>
</tr>
</tbody>
</table>

In the leaf, the air spaces in mesophyll tissues will continue to take in oxygen from the chloroplast, provided that there is more oxygen in the chloroplast than in the air spaces. The oxygen diffuses across the chloroplast walls into the air spaces.

### 2.2.2. Osmosis

Some membranes in plant cells allow certain particles to pass through them and not others. They are partially (or selectively) permeable. Osmosis is simply a special type of diffusion - diffusion of water molecules through a partially permeable membrane.
In the Figure 2.2.2 more water molecules pass from the water into the dilute solution than pass back the other way, because there is a higher concentration of water molecules in the pure water than there is in the solution. This results in a net transfer of molecules down the concentration gradient from the water to the solution. Eventually, the level on the more concentrated side of the membrane will rise, while that on the less concentrated side falls.

When the concentration of water is the same on both sides of the membrane, the movement of water will be the same in both directions. At this point, the net exchange of water is zero and the system is in equilibrium.

Osmosis is vitally important to plants. Plants gain water by osmosis through their roots, and it is osmosis that moves water into plant cells, making them turgid (having turgor; enlarged and swollen with water) or stiff, and thus able to hold the plant upright.

2.2.3. Active transport (Higher Tier)

Active transport is the process by which dissolved molecules (solute) move across a cell membrane from a lower to a higher concentration. In active transport, particles move against the concentration gradient – and, therefore, require an input of energy from the cell.

Sometimes solutes are at a higher concentration inside the cell than outside, but because the organism needs these substances they still need to be absorbed. For this the organism cannot rely on diffusion or osmosis alone. Carrier proteins pick up specific molecules and take them through the cell membrane against the concentration gradient (see Figure 2.2.3).
Examples of active transport

Plants need mineral salts (e.g. nitrates) for making proteins and growth. Nitrates are at a higher concentration inside the root cells than they are when dissolved in the water around the soil particles. If the plant relied on diffusion alone, the vital nitrate salts would drain out of the cells into the soil. So energy is deployed by the cells to actively transport nitrates across the cell membrane into the root cells, against the concentration gradient.
2.2.4. Diffusion, osmosis and active transport compared

<table>
<thead>
<tr>
<th><strong>Diffusion</strong></th>
<th><strong>Osmosis</strong></th>
<th><strong>Active Transport</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Random movement</td>
<td>Random movement of water</td>
<td>Selective movement</td>
</tr>
<tr>
<td>From higher to lower</td>
<td>From higher to lower concentration</td>
<td>From lower to higher</td>
</tr>
<tr>
<td>concentration</td>
<td>Along the concentration gradient</td>
<td>concentration</td>
</tr>
<tr>
<td>Along the concentration gradient</td>
<td></td>
<td>Against the concentration</td>
</tr>
<tr>
<td>No energy needed from</td>
<td>No energy needed from the cell</td>
<td>Energy needed from the</td>
</tr>
<tr>
<td>the cell</td>
<td></td>
<td>cell</td>
</tr>
</tbody>
</table>

2.3. Calculating Half-Times of Diffusion

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

From Fick’s law, one can derive an expression for the time it takes for a substance to diffuse a particular distance. As the substance diffuses away from the starting point, the concentration gradient becomes less steep (Δc decreases) 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 \(t_c = \frac{1}{2}\) is given by the following equation:

\[
t_c = \frac{(\text{distance})^2}{D_s} K
\]

where \(K\) is a constant and \(D_s\) is the diffusion coefficient. The above equation shows that the time required for a substance to diffuse a given distance increases in proportion to the square of that distance. Let us consider two numerical examples. First, how long would it take a small molecule to diffuse across a typical cell? The diffusion coefficient for a small molecule like glucose is about \(10^{-9} \text{ m}^2 \text{ s}^{-1}\), and the cell length may be 50 µm. Thus, for this example:

\[
t_c = \frac{(50 \times 10^{-6} \text{ m})^2}{10^{-9} \text{ m}^2 \text{ s}^{-1}} = 2.5 \text{ s}
\]

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:

\[
t_c = \frac{(1 \text{ m})^2}{10^{-9} \text{ m}^2 \text{ s}^{-1}} = 10^9 \text{ s} \approx 32 \text{ years}
\]

a value that exceeds by orders of magnitude the life span of a corn plant, which lives only a few months. This shows that diffusion in solutions can be effective within cellular dimensions but is far too slow for mass transport over long distances. Diffusion is of great importance as a driving force for the water vapor lost from leaves, because the diffusion coefficient for a molecule in air is much greater than in aqueous solutions.
2.4. Alternative Conventions for Components of Water Potential

The components of water potential defined in the text are sometimes given different names and symbols. In particular, the equation

\[ \Psi_w = \Psi_s + \Psi_p \]

is often replaced by the following equivalent equation:

\[ \Psi_w = -\pi + P \]

In this alternative convention, \( P \) is the same as \( \Psi_p \). It is the hydrostatic pressure of the solution, and may be positive, as in turgid cells, or negative, as in xylem water. The symbol \( \pi \) is called osmotic pressure and is the negative of \( \Psi_s \). That is, \( \pi \) has positive values, and \( \Psi_s \) 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 \( \pi \). The negative sign in front of \( \pi \) in the equation above accounts for the reduction in water potential (\( \Psi_w \)) by dissolved solutes. Thus \( \Psi_s = -\pi \).

2.5. The Matric Potential

In discussions of soils, seeds, and cell walls, one often finds reference to yet another component of \( \Psi_w \): the matric potential (\( \Psi_m \)). 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 surface interactions can theoretically be accounted for by an effect on \( \Psi_p \) and \( \Psi_s \) (see Passioura 1980; Nobel 1999). In dry materials, however, this surface interaction effect often cannot easily be separated into \( \Psi_p \) and \( \Psi_s \) components in dry materials, so they are frequently bulked together and designated as the matric potential.

It is generally not valid to add \( \Psi_m \) to independent measurements of \( \Psi_p \) and \( \Psi_s \) to arrive at a total water potential. This is particularly true for water inside hydrated cells and cell walls, where matric effects are either negligible or they are accounted for by a reduction in \( \Psi_p \). For instance, 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 \( \Psi_p \), \( \Psi_s \), and \( \Psi_m \) (Passioura 1980).

2.6. Measuring Water Potential

Plant scientists have 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 \( \Psi_w \), \( \Psi_s \), and \( \Psi_p \) are described here: psychrometer, pressure chamber, cryoscopic osmometer, and pressure probe.

2.6.1. Psychrometer (\( \Psi_w \) 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. 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 2.6.1 A. 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 a standard solution of known solute concentration (known $\Psi_s$ and thus known $\Psi_w$). 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. 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 $\Psi_w$ makes it possible to calculate the water potential of a solution for which the net movement of water between the droplet and the tissue would be zero (Figure 2.6.1 B) signifying that the droplet and the tissue have the same water potential.
Psychrometers can be used to measure the water potentials of both excised and intact plant tissue. Moreover, the method can be used to measure the \( \Psi_t \) of solutions. This can be particularly useful with plant tissues. For example, the \( \Psi_w \) of a tissue is measured with a psychrometer, and then the tissue is crushed and the \( \Psi_s \) 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 \( \Psi_p = \Psi_w - \Psi_s \).

A major difficulty with this approach is the extreme sensitivity of the measurement to temperature fluctuations. For example, a change in temperature of 0.01°C corresponds to a change in water potential of about 0.1 MPa. Thus, psychrometers must be operated under constant temperature conditions. For this reason, the method is used primarily in laboratory settings.

2.6.2. Pressure chamber (\( \Psi_w \) measurement)

A relatively quick method for estimating the water potential of large pieces of tissues, such as leaves and small 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 and coworkers at the Scripps Institution of Oceanography improved the instrument design and showed its practical use (Scholander et al. 1965).
Figure 2.6.2: 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.

In this technique, the organ to be measured is excised from the plant and is partly sealed in a pressure chamber (Figure 2.6.2). Before excision, the water column in the xylem is under tension. When the water column is broken by excision of the organ (i.e., its tension is relieved allowing its $\Psi_p$ to rise to zero), water is pulled rapidly from the xylem into the surrounding living cells by osmosis. The cut surface consequently appears dull and dry. To make a measurement, the investigator pressurizes the chamber with compressed gas until the distribution of water between the living cells and the xylem conduits is returned to its initial, pre-excision, state. This can be detected visually by observing when the water returns to the open ends of the xylem conduits that can be seen in the cut surface. The pressure needed to bring the water back to its initial distribution 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 pressure chamber is often described as a tool to measure the tension in the xylem. However, this is only strictly true for measurements made on a non-transpiring leaf or shoot (for example, one that has been previously enclosed in a plastic bag). When there is no transpiration, the water potential of the leaf cells and the water potential in the xylem will come into equilibrium. The balancing pressure measured on such a non-transpiring shoot is equal in magnitude but opposite in sign to the pressure in the xylem ($\Psi_p$). Because the water potential of our non-transpiring leaf is equal to the water potential of the xylem, one can calculate the water potential of the leaf by adding together $\Psi_p$ and $\Psi_s$ of the xylem, provided one collects a sample of xylem sap for determination of $\Psi_s$. Luckily $\Psi_s$ of the xylem is usually small (>-0.1 MPa) compared to typical midday tensions in the xylem ($\Psi_p$ of -1 to -2 MPa). Thus, correction for the $\Psi_s$ of the xylem sap is frequently omitted.

Balancing pressure measurements of transpiring leaves are more difficult to interpret. The fact that water is flowing from the xylem to the leaf means that differences in water potential must exist. When the transpiring leaf or shoot is cut off, the tension in the xylem is instantly relieved and water is drawn into the leaf cells until the water potentials of the xylem and the leaf cells come into equilibrium. Because the total volume of the leaf cells is
much larger than the volume of sap in the xylem, this equilibrium water potential will be heavily weighted towards that of the leaf. Thus, any measurement of the balancing pressure on such a leaf or shoot will result in a value that is approximately the water potential of the leaf, rather than the tension of the xylem. (To be exact, one would have to add the $\Psi_s$ of the xylem sap to the negative of the balancing pressure to get the leaf water potential.) One can explore the differences between the water potential of the xylem and the water potential of a transpiring leaf by comparing balancing pressures measured on covered (i.e., non-transpiring) versus uncovered (transpiring) leaves.

Pressure chamber measurements provide a quick and accurate way of measuring leaf water potential. Because the pressure chamber method does not require delicate instrumentation or temperature control, it has been used extensively under field conditions (Tyree and Hammel 1972).

### 2.6.3. Cryoscopic osmometer ($\Psi_s$, measurement)

The cryoscopic osmometer measures the osmotic potential of a solution by measuring its freezing point. Solutions have *colligative* properties that collectively depend on the number of dissolved particles and not on the nature of the solute. For example, 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. 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 $–1.86^\circ$C, compared with $0^\circ$C for pure water.

**Figure 2.6.3:** 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.
With a cryoscopic osmometer, solution samples as small as 1 nanoliter ($10^{-9}$ L) are placed in an oil medium located on the temperature-controlled stage of a microscope (Figure 2.6.3). 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 the 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 straightforward to calculate the solute concentration from the freezing-point depression; and from the solute concentration ($c_s$), $\Psi_t$ is calculated as $-RTc_s$. This technique has been used to measure droplets extracted from single cells (Malone and Tomos 1992).

### 2.6.4. Pressure probe ($\Psi_p$ 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. Using a micromanometer, Paul Green at the University of Pennsylvania developed one of the first direct methods 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 2.6.4A). 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 ($\text{pressure} \times \text{volume} = \text{constant}$). This method works only for cells of relatively large volume, such as the giant cell of the filamentous green alga *Nitella*. For smaller cells, the loss of cell sap into the glass tube is sufficient to deflate the cell and this yields artifically low pressures.

**Figure 2.6.4A:** 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.)
For higher plant cells, which are several orders of magnitude smaller 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 2.6.4B). 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.

**Figure 2.6.4B:** Diagram of the simplest pressure probe (not to scale). The primary advantage of this method over the one shown in Web Figure 3.5.E 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.

This method has been used to measure $P_p$ 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 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 $P_p$ in the xylem (Heydt and Steudle 1991).
2.7. Understanding Hydraulic Conductivity

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 ($L_p$) of the membrane.

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

$$\text{Flow rate} = \text{driving force} \times \text{hydraulic conductivity}$$

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. 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:

$$J_v = L_p(\Delta \Psi_w)$$

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$^{-1}$). Please note that 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 $\Delta \Psi_p$ (Nobel 1999).

In our example, $J_v$ has a value of $0.2 \times 10^{-6}$ 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.

The resulting value is the initial rate of water transport. As water is taken up, cell $\Psi_w$ increases and the driving force ($\Delta \Psi_w$) decreases. As a result, water transport slows with time and the rate approaches zero in an exponential manner.

2.8. Wilting and Plasmolysis

Plasmolysis is the separation of plant cell cytoplasm from the cell wall as a result of water loss. It is unlikely to occur in nature, except in severe conditions. Plasmolysis is induced in the laboratory by immersing a plant cell in a strongly saline or sugary solution, so that water is lost by osmosis.

If onion epidermal tissue is immersed in a solution of calcium nitrate, cells rapidly lose water by osmosis and the protoplasm of the cells shrinks (Figure 2.8). This occurs because the calcium and nitrate ions freely permeate the cell wall and encounter the selectively permeable plasma membrane. The large vacuole in the center of the cell originally contains a dilute solution with much lower osmotic pressure than that of the calcium nitrate solution on the other side of the membrane. The vacuole thus loses water and becomes smaller. The space between the cell membrane and the cell wall enlarges and the plasma membrane and the protoplasm within it contract to the center of the cell. Strands of cytoplasm extend to the cell wall because of plasma membrane-cell wall attachment points. Plasmolysed cells die unless they are transferred quickly from the salt or sugar solution to water.
3. Water Balance of Plants

3.1. Irrigation

People have used irrigation to provide water for crops almost as long as they have practiced agriculture. In the harsh, hot deserts of southern Arizona, the prehistoric Hohokam Native American communities dug extensive networks of irrigation canals to divert water from the nearby Salt River to their crops. Thus their society thrived where it would otherwise have perished. Aerial photography reveals the location of these ancient canals, which follow many of the same routes as today's concrete-lined waterways that carry water throughout the area of Phoenix, Arizona.

In the Tigris and Euphrates Valleys of Mesopotamia (modern day Iraq and Syria), which have been called the "cradle of civilization," the irrigation of crops yielded similar results. Civilization may have declined when the irrigated fields were "salted out"—made useless by the continuing addition of salts to the soil from poor-quality water. The ancient Peru also practiced extensive irrigation, including some innovative methods involving raised beds and provision of water from below.

Irrigation as practiced today in world agriculture is governed by the same general principles as in ancient times. Water is expensive and therefore must be used as efficiently as possible. If surface water is used, dams and waterways must be constructed and maintained. If water is pumped from the ground, energy must be expended to raise it to the surface.

Perhaps surprisingly, most of the inefficiency in the use of water is encountered before the water ever reaches the plant in the field. Evaporation from lakes, seepage from canals, transpiration from aquatic weeds that grow in the canals, and uneven application in the fields are just some of the problems faced by irrigation specialists. A system is considered efficient if 50% of the water available is delivered to the root zone of the crop it is supporting.

Various methods have been developed for delivering water to fields. If the land can be graded and sloped appropriately, furrows with a very slight downhill slope can be placed between rows of plants. Water is then applied at the uphill end and allowed to flow through the furrows. Some water is wasted, because the soil at the uphill end becomes saturated before enough has been delivered in the middle of the field. Water may also "puddle" at the downhill end.

Level-basin irrigation is being used increasingly to overcome these problems. Large basins are leveled to within 10 to 20 mm by laser-directed machinery. If the soil is uniformly permeable throughout the basin, this leveling allows water to be applied evenly. If the land cannot be leveled or if the amounts of water applied are small,
sprinklers are often used. However, sprinklers not only require energy to pump and pressurize the water, but they also produce small droplets from which evaporation is excessive. In addition, if the water is of poor quality, sprinkling will deposit salts directly on the leaves, where they can injure the plants. In some cases, sprinkling at night can prevent much of the injury because the salt-laden droplets do not evaporate as fast.

Some soils with very high clay content expand as they are wetted and shrink and crack as they dry. When they are wet they tend to become sealed, and water enters very slowly. For these cracking clay soils, a technique known as surge irrigation has been developed. Water is supplied to the field extremely rapidly so that it can flow down the cracks and enter the root zone before the soil swells and the cracks disappear.

In recent years a technique known as drip irrigation (also called trickle irrigation or microirrigation) has come into use in some areas of the world. Water is pumped directly to the base of a plant by plastic tubing and bled through an emitter at a slow rate that just meets the plant's needs. This approach is very efficient, but it is also very expensive and requires diligent maintenance of the hardware to keep the system working. For instance, the emitters tend to become plugged by both mineral deposits and slime produced by microorganisms. Periodically, the system must be flushed out with acid or with disinfectant. So far, drip irrigation has been used mostly for high-value crops for which quality (and price) depends strongly on a reliable supply of water. Under these conditions the profit from drip irrigation will pay for the extra costs. Fresh fruits such as blueberries and strawberries are irrigated extensively by this method in the United States.

With the availability of extremely efficient irrigation systems such as drip irrigation, more attention can be shifted to inefficient use of water by the crops themselves and to possible improvements in plant water use efficiency. The physiology of water in plants is an exciting area of research because many problems are waiting to be solved. For example, grain crops are most sensitive to drought when they are flowering, when abortion of the very young embryos can result in a barren plant. The solving of these problems will allow agronomists to make grain yields reliable even without resorting to irrigation.

3.2. Soil Hydraulic Conductivity and Water Potential

Soil hydraulic conductivity is a function of the water potential of the soil. Conductivity measures the ease with which water moves through the soil. As water content (and hence the water potential) decreases, the hydraulic conductivity decreases drastically (Figure 3.2, note the logarithmic scales).
Figure 3.2. Soil hydraulic conductivity as a function of the water potential of the soil. Conductivity measures the ease with which water moves through the soil. The decrease in conductivity as the soil dries is due primarily to the movement of air into the soil to replace the water. As air moves in, the pathways for water flow between soil particles become smaller and more tortuous, and flow becomes more difficult.

The overall shape of this curve is representative of many soils, but the shape for a particular soil may be influenced by the size distribution of its particles and by its organic matter content.

The field capacity (labeled on the curve) is the maximum amount of water the soil is able to retain against gravitational forces. The permanent wilting point (labeled on the curve) is the soil water potential value at which plants cannot regain turgor pressure even at night, in the absence of transpiration.

3.3. Root Hydraulic Conductance

One way to simplify the analysis of the complex process of water movement across the root is to treat the whole multicellular pathway—from the root hairs to the root xylem—as if it were a single membrane with a single hydraulic conductance.

With this simplification one can quantify the root hydraulic conductance \( L_{\text{root}} \) as follows:

\[
L_{\text{root}} = \frac{J_r}{\Delta \Psi_W}
\]

where \( J_r \) is the rate of water flow and \( \Delta \Psi \) is the radial water potential difference across the root.
Researchers have measured the hydraulic conductance of roots under various conditions and found that it is not constant, but varies with flow rate and according to the way in which the radial water potential gradient is established. The explanation for this complex behavior is that roots behave not as simple membranes, but as complex barriers in which pressure differences are more effective as driving forces for water transport than are the differences in osmotic potential (Steudle and Frensch 1996).

3.4. Calculating Velocities of Water Movement in the Xylem and in Living Cells

Let's begin by noting that the velocity with which water travels up the trunk of a tree depends on both the type of tree and the transpirational demand placed on the xylem. For trees with wide vessels (radii of 100 to 200 µm), peak velocities of 16 to 45 m h\(^{-1}\) (4 to 13 mm s\(^{-1}\)) have been measured. Trees with smaller vessels (radii of 25 to 75 µm) have lower peak velocities, from 1 to 6 m h\(^{-1}\) (0.3 to 1.7 mm s\(^{-1}\)). For our calculation we will use a figure of 4 mm s\(^{-1}\) for the xylem transport velocity and 40 µm as the vessel radius. This is a high velocity for such a narrow vessel, so it will tend to exaggerate the pressure gradient required to support water flow in the xylem.

A version of Poiseuille's equation can be used to estimate the pressure gradient (\(\Delta \Psi_p/\Delta x\)) needed to move water at this velocity (4 × 10\(^{-3}\) m s\(^{-1}\)) through a pipe of radius (\(r\)) 40 µm. Dividing the equation by the cross-sectional area (\(\pi r^2\)) of the xylem vessel, we find that the rate of transport (\(J_v\), in m s\(^{-1}\)) is given by the following equation:

\[
J_v = \left(\frac{\text{(radius)}^2}{8 \text{(viscosity)}}\right) \frac{\Delta \Psi_p}{\Delta x}
\]

Taking the viscosity of xylem sap to be that of water (10\(^{-3}\) Pa s), we find that the pressure gradient required is 2 × 10\(^4\) Pa m\(^{-1}\) (or 0.02 MPa m\(^{-1}\)). This is the pressure gradient needed to overcome the viscous drag that arises as water moves through an ideal vessel at a rate of 4 mm s\(^{-1}\). Real vessels have irregular inner wall surfaces and constrictions, such as perforation plates, at the points where vessel elements meet. Tracheids, with their smaller diameters and pitted walls, offer even greater resistance to water flow. Such deviations from an ideal pipe will increase the frictional drag above that calculated from Poiseuille's equation, but since we selected a low value for vessel radius, our estimate of 0.02 MPa m\(^{-1}\) should be in the correct range for pressure gradients found in real trees.

Let's now compare this value (0.02 MPa m\(^{-1}\)) with the driving force that would be necessary to move water at the same velocity through a layer of living cells. We will ignore water movement in the apoplasm pathway in this example and focus on water moving from cell to cell, crossing the plasma membrane each time. The velocity (\(J_v\)) of water flow across a membrane depends on the membrane hydraulic conductivity (\(L_p\)) and on the difference in water potential (\(\Delta \Psi_w\)) across the membrane:

\[
J_v = L_p(\Delta \Psi_w)
\]

A high value for the \(L_p\) of higher plant cells is about 4 × 10\(^{-7}\) m s\(^{-1}\) MPa\(^{-1}\). Thus, to move water across a membrane at 4 × 10\(^{-3}\) m s\(^{-1}\) would require a driving force (\(\Delta \Psi_w\)) of 10\(^4\) MPa (4 × 10\(^{-3}\) m s\(^{-1}\) divided by 4 × 10\(^{-7}\) m s\(^{-1}\) MPa\(^{-1}\)). This is the driving force needed to move the water across a single membrane. To move through a cell, water must cross at least two membranes, so the total driving force across one cell would be 2 × 10\(^4\) MPa. If we estimate the cell length as 100 µm (10\(^{-4}\) m, a generous estimate), then the water potential gradient needed for water to move at a velocity of 4 mm s\(^{-1}\) through a layer of cells would be 2 × 10\(^7\) MPa divided by 10\(^{-4}\) m, or 2 × 10\(^{10}\) MPa m\(^{-1}\). This is an enormous driving force, and it illustrates that water flow through the xylem is exceedingly more efficient than water flow across the membranes of cells. Comparing the two driving forces (for open vessel and cell transport), we see that the two pathways show a difference of a factor of 10\(^{10}\). This is a huge difference indeed.
3.5. Leaf Transpiration and Water Vapor Gradients

Transpiration from the leaf depends on two major factors: the difference in water vapor concentration between the leaf air spaces and the external air and, the diffusion resistance ($r$) of this pathway. This concept of transpiration is analogous to the flow of electrons in an electric circuit. Indeed, an electrical analog is commonly used as a model for water vapor loss from the leaf.

In this analog, resistances are associated with each part of the pathway; the major ones are the resistance at the stomatal pore ($r_s$) and the resistance due to the layer of unstirred air at the surface of the leaf ($r_b$) (the so-called boundary layer). Transpiration rate ($E$, in mol m$^{-2}$ s$^{-1}$) may be related to diffusional resistances ($r$, in s m$^{-1}$) by the following equation (Equation 3.5.1):

$$E = \frac{c_{wv(leaf)} - c_{wv(air)}}{r_s + r_b}$$

Let's examine the factors in Equation 3.5.1 in greater detail. The difference in water vapor concentration is expressed as $c_{wv(leaf)} - c_{wv(air)}$. Sometimes vapor pressures are used instead of concentrations, and the difference is called the water vapor pressure deficit.

Water vapor pressure ($\rho_{wv}$) is measured in kilopascals (kPa) and is proportional to water vapor concentration (see Table 3.5.1) where $c_{wv(leaf)}$ is the water vapor concentration inside the leaf and $c_{wv(air)}$ is the water vapor concentration of the air outside the leaf, both expressed in moles per cubic meter (mol m$^{-3}$). Resistance ($r$) is the inverse of conductance; that is, resistance = 1/conductance. Thus, a high resistance is the same as a low conductance. Expression of this value in terms of resistances is preferred over expression in terms of conductances in some instances because resistances in series may be summed to calculate a total resistance, as in the above equation, whereas a similar calculation of conductance in series is more complicated. In the leaf, the total resistance is due mostly to the diffusion limitation imposed by the stomatal pore, but other parts of the pathway for water vapor loss, such as the boundary layer, may contribute significantly to $r$.

Vapor pressures and concentrations are equivalent; in our analysis we will use the latter. The water vapor concentration of bulk air ($c_{wv(air)}$) can be readily measured, but that of the leaf ($c_{wv(leaf)}$) is more difficult to assess. We can estimate it by assuming that the air space in the leaf is close to water potential equilibrium with the cell wall surfaces. This approximation is not strictly true, because water is diffusing away from these surfaces. However, it introduces little error because the major resistance to vapor loss is at the stomatal pore. Moreover, the volume of air space inside the leaf is small, whereas the wet surface from which water evaporates is comparatively large. Air space volume is about 5% of the total leaf volume for pine needles, 10% for corn leaves, 30% for barley, and 40% for tobacco leaves. The internal surface area from which water evaporates may be from 7 to 30 times the external leaf area. This high ratio of surface area to volume makes for rapid vapor equilibration inside the leaf.

Making the assumption of equilibrium, we can calculate the water vapor concentration in the leaf air spaces if we know (1) the water potential of the leaf (which is the same as the water potential of the wall surfaces from which water is evaporating) and (2) the leaf temperature. Let's take as an example a leaf with a water potential of $-1.0$ MPa. To reach vapor equilibrium, water evaporates from the cell wall surfaces until the water potential of the air inside the leaf equals the water potential of the leaf. The water potential of the air is given by the following equation (Equation 3.5.2):

$$\psi_w = \frac{RT}{V_w} \ln (RH)$$

where $R$ is the gas constant, $T$ is temperature (in degrees Kelvin), $V_w$ is the partial molar volume of liquid water, and $RH$ is the relative humidity of the air.
Relative humidity is the water vapor concentration expressed as a fraction of the saturation water vapor concentration, $c_{wv}$(sat.) (Equation 3.5.3):

$$RH = \frac{c_{wv}}{c_{wv}(sat.)}$$

$RH$ varies between 0 and 1; $RH$ multiplied by 100 is the percentage relative humidity.

**Table 3.5.1**: Relationship among water vapor concentration ($C_{wv}$), water vapor pressure ($P_{wv}$), relative humidity ($RH$), and water potential ($\Psi_w$).

<table>
<thead>
<tr>
<th>$C_{wv}$ (mol m$^{-3}$)</th>
<th>$P_{wv}$ (kPa)</th>
<th>RH</th>
<th>$\Psi_w$ (Mpa)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.961</td>
<td>2.34</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>0.957</td>
<td>2.33</td>
<td>0.996</td>
<td>-0.54</td>
</tr>
<tr>
<td>0.951</td>
<td>2.32</td>
<td>0.990</td>
<td>-1.36</td>
</tr>
<tr>
<td>0.923</td>
<td>2.25</td>
<td>0.960</td>
<td>-5.51</td>
</tr>
<tr>
<td>0.865</td>
<td>2.11</td>
<td>0.900</td>
<td>-14.2</td>
</tr>
<tr>
<td>0.480</td>
<td>1.17</td>
<td>0.500</td>
<td>-93.6</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-infinity</td>
</tr>
</tbody>
</table>

Note: These data are for 20°C

$^a$Calculated using equation 3.5.2, with a value of 135 Mpa for RT/$\Psi_w$.

**Table 3.5.1** shows $RH$ values as a function of water potential, calculated from Equation 3.5.2. This table shows that the air spaces of living leaves must have a high $RH$, a value of nearly 1 (100%), when water potentials are in the physiological range. Moreover, outside air, with $RH$ of, for example, 0.5 (50%), has a remarkably low water potential.

To convert from $RH$ to $c_{wv}$, we need to know $c_{wv}$(sat). The saturation water vapor concentration is strongly dependent on temperature. As the air temperature rises, the water-holding capacity of air increases sharply. In the range of 10 to 35°C, an increase in air temperature of 12°C doubles the water vapor concentration of saturated air. This is an important observation. If our leaf with a water potential of –1.0 MPa warms up abruptly from 20°C to 32°C, the relative humidity in the leaf air space drops abruptly from 99.3 to almost 50%. This drop in $RH$ results because the water-holding capacity of the air, $c_{wv}$(sat), doubles. As a result of the drop in $RH$, water will evaporate in the air space until $RH$ returns to a value of 99.3% and the air is again in water potential equilibrium with the leaf. As a consequence of this change in temperature, $c_{wv}$(leaf) (the water vapor concentration of the leaf air space) increases from 0.95 to 1.87 mol m$^{-3}$, which makes for a steeper concentration difference driving the diffusional loss of water from the leaf. For this reason, leaf temperature is an important determinant of the transpiration rate.

4. Transport of water and Transpiration

During the summer of 1974, John Hanks, a soil scientist at Utah State University, kept careful track of the amount of water required to grow a crop of maize (corn). To mature the crop, water equivalent to 600mm (23.6 in) of rain was added to the field. A fourth of this evaporated from the soil, but most of the remaining 450 mm passed through the plants into the atmosphere. This evaporation of water from plants is called transpiration. In plants, it refers to internal water loss through stomata, cuticle, or lenticels. Hanks showed that 600 kg of water were transpired by the maize plants for each 1 kg of dry maize (grain) produced. In fact, 225 kg of water were transpired to produce 1 kg of dried plant material, including leaves, stems, roots, cobs and seeds. This large amount of transpired water is typical, although there are substantial differences among species.

*Why is so much water lost by transpiration to mature a crop?* Because an essential part of those dry corn seeds and all other plant parts are the carbon atoms that form the skeletons of the organic molecules of which those plant parts consist, and virtually all of this carbon must come from the atmosphere.
It enters the plant as carbon dioxide (CO$_2$) through the stomatal pores, mostly on leaf surfaces, and water exits by diffusion through these same pores as along as they are open. This is the dilemma faced by the plant: How to get as much CO$_2$ as possible from the atmosphere in which it is extremely dilute (about 0.03 % by volume), and at the same time retain as much water as possible, water that must fill and keep turgid all the cells and provide the medium in which the CO$_2$ can be photosynthetically combined with water to form the molecules of life.

Environmental factors influence not only the physical processes of evaporation and diffusion but also the stomata on the leaf’s surfaces and their apertures, through which over 90 percent of the transpired water and the CO$_2$ pass. Increased leaf temperature promotes evaporation considerably and diffusion slightly but may cause the stomata to close, or to open wider, depending on species. At dawn, stomata open in response to the increasing light, and the light increases the temperature of the leaf. The increasing temperature allows the air to hold more moisture, so evaporation is promoted, and perhaps stomatal aperture is affected. Wind brings more CO$_2$ and blows away the water vapor, causing an increase in evaporation and CO$_2$ uptake. But if the leaf is warmed above air temperature by sunlight, wind will lower its temperature, causing a decrease in transpiration. When soil moisture becomes limiting, transpiration and CO$_2$ uptake are inhibited because stomata close.

### 4.1. Measurement of Transpiration

CO$_2$ concentration in the atmosphere is fairly constant, while transpiration studies must consider evaporation as well as diffusion. Understanding transpiration provides the foundation for understanding CO$_2$ uptake. The water molecules diffuse 1.56 times as fast as CO$_2$ molecules (because of their lower molecular weight) and that the atmosphere normally contains much more water vapor than CO$_2$ (at 35°C, H$_2$O = 0.3 to 1.4 mol m$^{-3}$; CO$_2$ = 0.0156 mol m$^{-3}$).

*How could we measure transpiration?* A much simpler approach is to weigh at intervals on a sensitive balance a potted plant with its soil sealed against water loss. This is called Lysimeter method. Since the amount of water used in plant growth is less than 1 percent of the final dry weight of the plant, virtually all the change in weight can be ascribed to transpiration.

Lysimeter is placed on a large plastic bag buried beneath it and filled with fluid (water and antifreeze) that extends into a standpipe above the surface. The level of liquid in the pipe is a measure of the weight of the lysimeter, so it changes with the water content of the soil in the lysimeter and the growing plants, but their weight is small compared to the soil. Water in the soil depends only upon evaporation from the soil surface and transpiration from plants. Evaporation from soil and transpiration combined are called evapotranspiration. Lysimeters provide the most reliable field method for studying evapotranspiration.

A more convenient method is to remove a leaf from a plant in the field and put it immediately on a sensitive balance. The loss of weight during the first minute or two has often been used as an indication of transpiration. Since the very act of detaching the leaf from the plant often changes the rate of transpiration, this method has only limited usefulness in comparative studies.

Another laboratory approach is to detach a plant and insert the cut stem into a device that allows easy measurement of the water absorbed by the plant. Sometimes, the stem is attached to a burette, or water absorption might be measured as an air bubble moves through a capillary tube connected to the closed water reservoir from which the plant is absorbing water (*a potometer*). This can be useful when relative transpiration rates must be studied for brief time intervals.

### 4.2. Stomatal Mechanics

The waxy cuticle on leaf surfaces restricts diffusion so that most water vapor and other gases must pass through the openings between the guard cells (*Figure 4.2A*). Technically, these openings are called stoma (*pl. stomata*), but often the term is applied to the entire stomatal apparatus, including the guard cells. Adjacent to each guard cell are usually one or two other modified epidermal cells called accessory or subsidiary cells (*Figure 4.2 B,C*). Water evaporates inside the leaf from the palisade parenchyma and spongy parenchyma cell walls into the intercellular spaces (*Figure 4.2D*), which are continuous with the outside air when the stomata are open. Carbon dioxide follows the reverse diffusional path into the leaf.
Figure 4.2 C

Figure 4.2, D:
Stomata sometimes occur only on the lower surface of leaves but often are found on both upper and lower surfaces (with perhaps more on the lower surface). Waterlily pads have stomata only on upper surface, and submerged plants have none at all. Grasses usually have about equal numbers on both sides. Sometimes, stomata occur in a substomatal crypt such as in the oleander or pine. Such sunken stomata are apparently an adaptation to reduce transpiration.

Typical stomata of dicots consist of two kidney-shaped guard cells; grass guard cells tend to be more elongate (dumbbell shaped). Guard cells contain a few chloroplasts, whereas their neighboring epidermal cells seldom do.

Figure 4.2 E: Schematic drawing of the two stomata, showing radial micellation. Left, a dicot leaf stomata (dashed line show guard cells after loss of water, hence closing); right, a monocot (grass) stomata. (©Plant Physiology, Ist Ed., F.B. Salisbury, C.W. Ross, Wadsworth Pub. Belmont, CA, 1986)

Figure 4.2 F: The stomatal mechanism: The radial orientation of microfibrils and the strategic fusion of facing inner walls in the paired guard cells translate increasing turgor into cell bending, which enlarges the stoma. (© Biology: The Science of Life; R.A. Wallace, G.P Sanders, and R.J. Ferl, Harper-Collins, New York, 1991)

Stomata open because the guard cells take up water and swell. Swelling guard cells would force the inside walls of the stomata together. Stomata function the way they do because of special features in the submicroscopic anatomy of their cell walls. The cellulose microfibrils, or micelles, that make up the plant cell walls are arranged around the circumference of the elongated guard cells as though they were radiated from a region at the center of the stomata (Figure 4.2 E, F). The result of this arrangement of microfibrils, called radial micellation, is that when a guard cell expands by taking up water, it cannot increase much in diameter, because the microfibrils do not stretch much along their length. But the guard cell can increase in length; therefore, because two guard cells are attached to each other at both ends, they bend outward when they swell, which opens the stomata.
The guard cells of some species were slightly more thickened along the concave wall adjacent to the stomatal openings. It is suggested that the thickening was responsible for the opening when the guard cell takes up water.

**Figure 4.2 G:** Both ends of balloon glued, inflated 50%. (©Plant Physiology, 4th Ed., F.B. Salisbury, C.W. Ross, Wadsworth Pub. Belmont, CA, 1992)

**Figure 4.2 H:** Further inflated, showing stomatal pore. (©Plant Physiology, 4th Ed., F.B. Salisbury, C.W. Ross, Wadsworth Pub. Belmont, CA, 1992)

The balloon models (**Figure 4.2 G, H**) show that the radial micellation is much more important in stomatal opening than the thickening of the inside walls and that radial micellation is as important in grass as in dicot guard cells.

**4.3. Stomatal Mechanisms**

*What is it that causes guard cells to take up water so that the stomata open?*

**Environmental Effects on Stomata:** Many changes in environmental factors influence stomatal apertures. Stomata of most plants open at sunrise and close in darkness, allowing entry of the CO₂ needed for photosynthesis during the daytime. Opening generally requires about an hour, and closing is gradual throughout the afternoon. Stomata close faster if plants are suddenly placed in darkness. Certain succulents that are native to hot, dry conditions (e.g., Cacti, Bryophyllum) act in an opposite manner: they open their stomata at night, fix CO₂ into organic acids in the dark, and close their stomata during the day. This is an appropriate way to absorb CO₂ through open stomata at night, when transpiration stress is low, and conserve water during the heat of the day. The
minimum light level for the opening of stomata in other plants is about 1/1000 to 1/30 of full sunlight, just enough to cause some net photosynthesis, which reduces the CO₂ concentration in the leaf. Light level influences not only the rate of opening but also the final aperture size, bright light causing a wider aperture.

Low concentrations of CO₂ in the leaves cause stomata to open, and removal of CO₂ by mesophyll cells during photosynthesis is the main reason for stomata of most species to open in light. Succulents fix CO₂ into organic acids at night, diminishing CO₂ and causing stomatal opening. If CO₂-free air is blown across leaves even in darkness, then their slightly open stomata open wider. Conversely, high CO₂ concentration in the leaves causes the stomata to close partially, and this occurs in the light as well as in the dark. When the stomata are completely closed, which is unusual, external CO₂-free air has no effect. The environmental factors that influence photosynthesis and respiration probably affect stomatal opening and closing by acting indirectly on the internal CO₂ concentration. Such coupling of stomatal action to photosynthesis has obvious survival rule.

The water potential within a leaf also has a powerful effect on stomatal opening and closing. As water potential decreases (water stress increases), the stomata close. This effect can override low CO₂ levels and bright light. Its protective value during drought is obvious.

High temperatures (30°C to 35°C) usually cause stomatal closing. This might be an indirect response to water stress, or else a rise in respiration rate might cause an increase in CO₂ within the leaf. High CO₂ concentration in the leaf is probably the correct explanation for high-temperature stomatal closing in some species, because it can be prevented by flushing the leaf continuously with CO₂-free air. In some plants, however, high temperatures cause stomatal opening instead of closing. As a result, the increased transpiration removes heat from the leaf.

Sometimes stomata partially close when the leaf is exposed to gentle breezes, probably because more CO₂ is brought close to the stomata, increasing its diffusion into the leaf. Wind can also increase transpiration, leading to water stress and stomatal closing.

Guard-Cell Uptake of Potassium Ions: Since stomata open because the guard cells take up water, and water uptake is caused by more solute and hence a more negative osmotic potential. What is the solute and where does it come from? Accumulated experimental evidence has made it clear that potassium ions (K⁺) move from the surrounding cells into the guard cells when stomata open.

The quantities of K⁺ that build up in the vacuoles of guard cells during stomatal opening are sufficient to account for the opening. Increases of up to 0.5 M in K⁺ concentration are observed, enough to decrease the osmotic potential by about 2.0 MPa. Stomatal opening and K⁺ movement into the guard cells are closely correlated. Light causes a buildup of K⁺ in guard cells, as does CO₂-free air. When leaves are transferred to the dark, K⁺ moves out of guard cells into the surrounding cells, and stomata close.

When strips of epidermal tissue are removed from the leaves of broadbean (Vicia faba), most epidermal cells are broken, but the guard cells remain intact. When these strips are floated on solutions, stomata will not open, unless the solutions contain K⁺. So guard cells must normally obtain K⁺ ions from adjacent epidermal cells. Stomata also close in response to the application of abscisic acid, a plant growth regulator, which causes loss of K⁺ from the guard cells. K⁺ transport from accessory cells to guard cells is the cause of the more negative osmotic potentials and hence stomatal opening, and that reverse transport is the cause of closing.

The Abscisic Acid (ABA) Effect on Stomata: Another observation of the early 1970s was almost as revolutionary as that of K⁺ uptake. When the growth regulator abscisic acid, is applied at extremely low concentrations (e.g. 10⁻⁶ M), it causes stomata to close. Furthermore, when leaves are subjected to water stress, the ABA in their tissues builds up. When leaves dry at normally slow rates, ABA builds up before stomata close, suggesting that stomatal closure in response to leaf water stress is mediated by ABA.

Light quality and Stomatal Response: Since stomata open in the dark in response to CO₂-free air, low oxygen levels, and other factors, light is not directly essential for their opening. When light is effective, it is partially because photosynthesis in the leaf mesophyll cells lowers the CO₂ in the intercellular spaces, and the guard cells respond by taking up more K⁺ ions.

4.4. Significance of Transpiration

Transpiration is important for plants because it directly influences the absorption of water from the soil. The evaporation of water during transpiration contributes to the cooling of leaves (and also the surrounding air) and protects leaves from heat, injury, particularly under conditions of high temperature and intense sunlight.
Transpiration is also important because it causes the movement of water and minerals absorbed by the roots to the other parts of the plant. Leaves are ideally suited to capture sun’s energy and absorb CO2 through the stomata. These features tend to increase transpiration rates.

**Study Questions**

1. What is the importance of the water potential concept in plant physiology? What are the components of water potential?
2. List three unique properties of water that make it such a good medium for cellular functioning, and explain how each property is useful to the plant.
3. What is turgor pressure? Can plant cells have negative turgor pressure values? Can you calculate the turgor pressure of a cell from water potential and osmotic potential values?
4. How does water move across a largely hydrophobic plasma membrane?
5. Define and give an example of osmosis in a plant cell. What is the driving force for osmosis?
6. Describe the path followed by water from the soil, through the plant and into the atmosphere. Where in the path are the important resistances to water movement?
7. What is the driving force for the movement of water from the soil to the top of a tree and into the atmosphere?
8. Describe root pressure and indicate when it might occur.
9. How does air relative humidity affect the transpiration rate of a leaf? If the air surrounding a transpiring leaf gets warmer, how will such a change affect the transpiration rate of the leaf?
10. What are guard cells? Explain their role in regulating transpiration.

**Suggested Readings**

**Text Book:**


**References:**