TEACHING TOOLS IN PLANT BIOLOGY™: LECTURE NOTES

Water Relations 1: Uptake and Transport

This is the first part of two Teaching Tools in Plant Biology to examine plant–water relations. Part 1 examines how plants take up water from their environments and transport it through their bodies. Part 2 looks at plant responses to drought and adaptations to dry environments as well as strategies to produce sufficient food, feed, and fiber within the constraints of water limitation.

INTRODUCTION

Plants, like all living things, are mostly water. Water is the matrix of life, and its availability largely determines the distribution and productivity of plants on earth. To survive in the dry terrestrial environment, plants have evolved two different strategies to manage water uptake and transport. These are best considered as opposite ends of a continuum, where individual plant species may display combinations of both, in varying degrees and at various times.

The first is to manage water relations minimally and to vary cellular water content with environmental water availability. This strategy is called poikilohydry; poikilo means “variable.” (The prefix is also used in poikilothermy, the strategy of varying body temperature with the external environment that is used by reptiles and other “cold-blooded” animals.) Poikilohydrous plants are able to survive periods of extensive desiccation. Most bryophytes (mosses, liverworts, and hornworts) use this strategy, but it is also used by a few vascular plants, including ferns and monocots. The cellular mechanisms of desiccation tolerance are fascinating and covered in detail in Part 2 of this Teaching Tool.

The second strategy is to manage water uptake and transport, in order to maintain relatively constant internal water content. This strategy is called homiohydric; homoio means “same.” Homiohydrous plants, primarily tracheophytes, have evolved complex mechanisms by which to absorb, transport, and retain water; these include roots, xylem, and the water-conducting tracheary elements, and regulated pores (stomata). To understand how water moves in vascular plants, it is important to first understand the properties that make water indispensable for life.

A BRIEF HISTORY OF THE STUDY OF PLANT–WATER RELATIONS

In the 1620s, William Harvey demonstrated that blood in animals is circulated by the action of the pumping heart. In the late 17th century, both Marcello Malpighi and Nehemiah Grew recognized that plant vascular tissues might be conducting tissues, although they assumed that these conduits carried air, so they became known as tracheids or tracheary elements. The most informative early studies of the mechanism of water movement in plants were published in 1727 in the book Vegetable Staticks by the great plant physiologist Stephen Hales. Hales showed that the liquid transpiring from leaves is pure water, and by identifying a way to measure transpiration, he ruled out circulation as a significant form of water movement in plants. He also demonstrated that the upward flow of water in a stem requires evaporation from leaves and that, under most conditions, pressure from the roots is not involved. Eduard Strasburger later demonstrated that living cells are not required for water conduction (although, as we will see later, living cells are nevertheless critical for optimizing and regulating water conduction).

In the 1890s, several scientists, notably Böhm, Dixon, and Joly, converged on the idea that the driving force for the movement of water was the evaporation of water at the leaf surface, transduced through the water column because of water’s chemical cohesion. Dixon and Joly explained, “the all-sufficient cause of the elevation of the sap [is]…by exerting a simple tensile stress on the liquid in the conduits.” This idea, which came to be known as the cohesion–tension theory, elegantly describes our current understanding of the ascent of water. Dixon and Joly showed that the force exerted by the evaporative pull was sufficient to raise water many meters, and later, a similar strategy was used to measure tension in the xylem. By the end of the 19th century, the idea that water moves passively under tension had been proposed and generally accepted, although concerns about this model’s ability to explain the movement of water up very tall trees and vines persisted. Resolving this and other questions required the development of additional methods for measuring xylem tension, and discussions and disagreements about how to interpret these various approaches persist.

WATER UPTAKE AND TRANSPORT ARE GOVERNED BY PHYSICAL LAWS

Water flows into and through plants as a continuous column of water. Ultimately, the energy required to move this column of water comes from sunlight, which drives the evaporation of water at the leaf surface. As water evaporates from the top of the water column, it exerts tension on the column that draws more water in at the bottom of the column. In fact, the movement of water in a plant can be replicated by a physical nonliving object that has an evaporative surface on top of a water-filled tube. The basis for this simple mechanism, which requires no input of energy from the plant, rests on the special properties of water, particularly its cohesiveness.

Special Properties of Water

Water is cohesive, a bit like Lego. Lego bricks attach to each other, so the structures you build with them are durable and stay together; imagine trying to build similarly elaborate structures with
pebbles or playing cards that lack Lego’s cohesive properties. Water molecules stick to other water molecules because each molecule has a partial charge separation, so that the oxygen atom at the center of the molecule has a slight negative charge and flanking hydrogens have a slight positive charge; it is a polar molecule, but with no net charge. Due to the charge separation, water molecules form intermolecular hydrogen bonds that hold them together. The cohesiveness of water means that, compared with other similarly sized molecules, more energy is needed to convert water from a liquid to a gas. Thus, water is a liquid at standard earth temperatures, whereas many other compounds of similar molecular weight are gases. Water’s polar nature also means that it is a very effective solvent for highly polar molecules such as mineral ions as well for somewhat less polar organic peptides and carbohydrates. On earth, if you want a molecule that supports life, there’s no substitute for water.

Factors That Govern the Movement of Water

Diffusion

All matter is in continuous motion. If an element is present in a higher concentration in one region, more molecules move away from that region than toward it. As a consequence, without any input of external energy, gaseous and liquid matter tends to become uniformly distributed; this phenomenon can be described by Fick’s law of diffusion:

\[
\frac{dm}{dx} = -DA \frac{dc}{dx}
\]

Fick’s law states that rate of movement of a substance \((dm/dx)\) is determined by its concentration gradient \((dc/dx)\), the area through which it is diffusing \((A)\), and the properties of the substance and the matrix through which it is diffusing (defined as the diffusion coefficient \(D\)). The negative sign before \(D\) indicates simply that the direction of movement is away from the area of higher concentration.

Osmosis and Osmotic Potential

Osmosis is a special type of diffusion that applies only to water moving across a semipermeable barrier, such as the plasma membrane of a cell. If we imagine a space that contains water with dissolved solutes surrounded by a membrane that is permeable to water but not solutes, then the water inside the membrane will essentially be at a lower concentration than that outside, so water will enter by diffusion. The force that drives water inward is determined by the concentration of solutes. In a constrained volume, the resulting pressure is the osmotic pressure, which is defined as force \((\text{in Newtons})\) per area \((\text{m}^{-2})\); Force per area \((\text{N m}^{-2})\) is also a measure of pressure, for which the standard unit is Pascals \((\text{Pa})\). For use in plant physiology, the megapascal \((\text{MPa}, \text{equivalent to} 10^6 \text{ N m}^{-2})\) is a more useful unit scale. Other units can be used for pressure; 1 MPa is equivalent to 10 bars or 9.87 atmospheres.

Osmotic potential is the inverse of the osmotic pressure and is a property of a solution. The osmotic potential of pure water is defined as 0 MPa, and the addition of any solute lowers the osmotic potential to negative values. The more dissolved solutes, the more negative the osmotic potential and the greater the tendency for water to move into the solution. Thus, one way to move water is to add solutes to the place you want the water to go; the lower osmotic potential will draw the water toward it. A simple way to remember this is through the expression “water follows salt.”

Pressure Potential

Another thermodynamic property of water is its pressure, relative to atmospheric pressure at the earth’s surface. Because it is defined relative to atmospheric pressure, the pressure potential of the water in a container open to the atmosphere is equal to 0 MPa. If that water were put into a pressurized container, its pressure potential would be a positive value, and if it were put into a vacuum, the pressure would be a negative value relative to atmospheric pressure. Water flows from high pressure to low pressure. This can be illustrated by the use of a syringe. When the opening of the syringe is placed into a pool of water and the plunger is pulled up, the pressure inside the barrel is lowered and water is pulled into it. When the plunger is pushed, the pressure inside the barrel is elevated and water is pushed out.

Most plant cells are under a positive pressure, on the order of 0.5 to 1.5 MPa. This is more than the pressure of a car tire (which is \(-0.2\) MPa) and manifold higher than that experienced by animal cells; human arterial blood pressure, for example, is less than 0.02 MPa. This positive pressure, often called turgor pressure, is essential for the support of many tissues. A plant wilts when it cannot absorb enough water to maintain turgor pressure. Positive pressure is also essential for cell growth; for example, the elongation of a root tip into compacted soil is driven by pressure within the cells. Changes in pressure can also affect cell movement, for example, by forcing open, and then releasing, the hinge of a Venus flytrap \((Dionaea muscipula)\) and the motor cells in the leaves of the sensitive plant \((Mimosa pudica)\). By contrast, and as described later, the water inside the conducting tissues of the xylem is normally under a negative pressure (tension).

Water Potential

Water potential provides a convenient quantification of the energy state of water, differences in which can drive water movement, and it is the sum of osmotic, pressure and gravitational potentials. The standard notation to express this is the water potential equation:

\[
\psi_w = \psi_m + \psi_p + \psi_g
\]

where \(\psi_w\) indicates water potential, \(\psi_m\) indicates osmotic potential, \(\psi_p\) indicates pressure potential, and \(\psi_g\) indicates the gravitational potential (\(\psi\) is the Greek letter psi, pronounced “sigh”). The water potential of pure water at standard atmospheric pressure is 0 MPa, because both the osmotic and pressure potentials are zero, by definition. The water potential can be lowered by lowering the osmotic potential (by adding solutes) or the pressure potential (by applying a vacuum or other tensile
stress). The gravitational potential becomes more negative by 0.1 MPa for each 10 m of elevation; this component is often ignored, except in the case of tall trees. The rule governing the movement of water across membranes is that water moves from higher to lower water potential. When no membranes are traversed, for example, in the column of water in the xylem or phloem, water’s movement is driven by the pressure gradient. The movement of water in a transpiring plant involves the continual movement of water from the soil, into the plant tissues, and ultimately out into the atmosphere down a continually decreasing water potential or pressure potential gradient.

Water potential values of plant tissues vary, but as typical examples, the water potential of moist soil is about −0.05 MPa, that of a living cell in the root or leaf tissue is about −0.7 MPa, that of the substomatal cavity is about −7 MPa, and that of the atmospheric air varies with humidity and temperature, from (at 20°C) −15 MPa at 90% relative humidity to −95 MPa at 50% relative humidity and correspondingly lower with drier air.

Water Moves in Plants by Diffusion and by Bulk Flow

The movement of water by osmosis and diffusion occurs at the molecular level and does not require any input of energy. However, the movement of water by diffusion is a relatively slow process. For example, a molecule can diffuse across a 50 μm cell in 0.6 s, but it would take 8 years to diffuse 1 m. Thus, diffusion alone is insufficient to support the needs of the typical plant for water. Instead, vascular plants rely on bulk flow of water. Bulk flow is a much faster form of water movement, but it also requires an energy source to generate the required pressure gradient. Examples of the bulk flow of water are the movement of water in a stream (with gravity providing the energy) or out of a water pipe (with energy provided by a pump) or the movement of blood in the human body (with energy provided by a pump, the heart).

The bulk flow of water through a tube is described by one form of Poiseuille’s law, which states that flow is equal to the hydraulic pressure gradient (ΔP) times r³, the number of tubes (N), and the radius of the tubes to the fourth power (r⁴), divided by 8 times the viscosity of water (η), with units in terms of volume flow per unit of time (e.g., m³ s⁻¹):

\[
\text{Flow} = \frac{\Delta P \pi Nr^4}{8\eta}
\]

An important implication of this equation for plant physiologists derives from the fact that flow is proportional to the fourth power of the tube radius, so a small increase in tube diameter leads to a tremendous increase in flow. We’ll return to this relationship when we look at the movement of water in the conduits of the xylem.

Ohm’s Law Can Model the Flow of Water through a Plant

Another way to think about the flow of water in a plant is through Ohm’s law. Ohm’s law states that current (I) equals voltage (V) divided by resistance (R).

\[
I = \frac{V}{R}
\]

Although this equation describes the flow of electrons (current) through an electrical circuit, it also analogously describes the flow of water. Flow is determined by the difference in water potential between the beginning and end of the path (equivalent to the voltage) divided by the resistance to the flow. An enormous pressure difference can only produce a small flow if the resistance to its flow is very large; think of a giant bucket with a pinprick-sized hole at the bottom. A much larger hole would give less resistance and increase the rate of the flow.

When looking at a complex system such as a plant, it is worth considering how resistance is affected in various segments separately. For example, the resistances of the root system, stem system, and leaf can be measured independently. The resistance of the entire system is the sum of the resistances of each of the segments. Thus, a system in which one component has a very large resistance will tend to be dominated by that resistance. Another way to think about water flow is to consider the conductance of the system; conductance is the reciprocal of resistance (1/R). Although we can measure the conductance of each part of the pathway separately, conductances cannot simply be summed to determine the conductance of the system. Both resistance and conductance are routinely used in plant physiology.

A Note about Nomenclature

The general transport equation, applicable to the transport of water, heat, and electric charge, is that flux = driving force × proportionality constant. Fick’s law, Poiseuille’s law, and Ohm’s law are all variants of the transport equation (see Davies, 1986). Different units can be used for flux (or flow rate) as well as the proportionality constant. One commonly used proportionality constant is the absolute hydraulic conductance (K), which is the ratio of the water flow rate through a plant or plant segment to the pressure driving the flow.

Conductance can be restricted to a portion of the plant; for example, Ks indicates the conductance of the shoot and Kt the conductance of the leaf. The conductance of the root is sometimes indicated as Kr and also sometimes as Lp. Conductance is also sometimes abbreviated g, which is commonly used when describing the movement of CO₂ and water vapor through the leaf, for example, stomatal conductance (gs), mesophyll cell conductance (gm), and conductance of the intercellular air space (gair). In each case, it can be helpful to remember that conductance is the reciprocal of resistance, so a higher conductance means a greater flow rate per unit of driving force. A related concept is the hydraulic conductivity (e.g., ks [xylem-specific conductivity] or Lp [root hydraulic conductivity]), which is calculated by dividing the flow rate by the pressure gradient over a segment of length or area across which flow is occurring (see Becker et al., 1999).

AQUAPORINS, ESSENTIAL REGULATORS OF WATER MOVEMENT

For many years, the movement of water across cell membranes was hypothesized to involve some sort of water channel, but it
was not until the 1990s that the identity of proteinaceous aquaporin water channels was confirmed, in the laboratory of Peter Agre. For this work, he received the Nobel Prize in Chemistry in 2003. A key experiment that demonstrated the function of a cloned aquaporin involved expressing the protein in Xenopus laevis oocytes. When the aquaporin-expressing cells were transferred into a solution of lower ionic strength, they rapidly took up water and burst within minutes. Shortly after Agre’s studies were completed, plant aquaporins were similarly shown to increase cell permeability to water.

Aquaporins are major intrinsic proteins (and also membrane-intrinsic proteins) that are abundant in many tissues. In plants, there are several families of aquaporins, including the plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPS), that facilitate water movement through the plasma membrane and tonoplast (vacuolar membrane), respectively. Aquaporins are membrane-embedded proteins in which six membrane-spanning domains are arranged to form a gated channel. They are present in membranes as tetramers, but each monomer folds into a separate channel. Water is not the only molecule diffusing through aquaporin channels; several of them represent important membrane-selective pathways for small solutes, such as boron or silicic acid, and CO₂. There are more than 30 genes encoding aquaporins in plants, which fall into four or more families. Some aquaporin gene families are only found in bryophytes, suggesting that they have been selectively lost during tracheophyte evolution.

A functional role for aquaporins in plant–water relations has been demonstrated many times. Aquaporins are especially sensitive to inhibition by mercury, and so treatment of a plant with mercury gives an indication of the flux through aquaporins (although mercury is not totally specific). In various species, mercury reduces root conductance by 30 to 90%. Antisense and mutational approaches are also useful; antisense inhibition of either the PIP1 or PIP2 subclass can reduce root hydraulic conductance by 50%, and loss of function of pip2-2 decreases conductance by 30%. In Arabidopsis thaliana, antisense inhibition plants compensated for this decrease in conductance by increasing their root mass. The cells of plants expressing an antisense construct that interferes with PIP1 expression are less water permeable, and the plants wilt more quickly in response to watering with a high-osmolarity solution. Other studies suggest that the aquaporins have diverse roles in plant development, water flow, and environmental responses in leaves as well as roots.

Aquaporin activity is highly regulated. Many aquaporin genes show differential cell type–specific expression patterns, for example, with preferential expression in the root or the bundle sheath cells surrounding the xylem. Many aquaporin genes are transcriptionally regulated by light, circadian cycles, osmotic stress, or the hormone abscisic acid (ABA). Like some other plasma membrane proteins, including auxin transporter proteins and brassinosteroid hormone receptors, the subcellular localization of aquaporins is regulated. PIPs have been shown to dynamically move from the internal membranes to the plasma membrane and then back to internal membranes in response to osmotic changes. Finally, the water-transporting activity of each channel is gated; that is, the pore can open and close rapidly to control water movements. This occurs through posttranscriptional modifications such as phosphorylation, but there is also sensitivity to pH. Thus, aquaporin activity levels are an important strategy employed by plants to control the movement of water.

WATER MOVES THROUGH THE SOIL–PLANT–ATMOSPHERE CONTINUUM

An average maize (Zea mays) plant transpires 2 to 4 liters of water per day, which means that it lifts that much water up out of the soil, through its body, and out of the pores in its leaves, without any expenditure of its own energy. A tree may lift a few hundred liters of water up to 100 m from the soil in its transpiration stream. These feats are astonishing, and even as late as the end of the 19th century, scientists disputed the veracity of the cohesion–tension model, as it was too difficult to imagine as a viable explanation. The more we learn about water movement in plants, the more it becomes evident that the anatomy and physiology of all stages of the water route have been adapted for efficiency and durability.

The flow rate of water in the plant is determined by the water potential gradient and the conductance of the pathway. Factors that determine the water potential difference include the water content of the soil and the water vapor concentration in the atmosphere, both of which are related to water potential. Other things being equal, plants in arid environments tend to lose water faster because of the very low water potential of the air surrounding them.

Conductance is determined by many different factors. At the end of the pathway, the guard cells of the stomata determine whether or not the plant transpires intensely. When the stomata are fully closed, leaf surface conductance is very low (only a residual conductance exists that is attributable to leakage past the guard cells and through the hydrophobic cuticle), and little water leaves the plant. When the stomata are wide open, transpiration is governed by the vapor pressure gradient between the leaf and the surrounding air. The extent to which the water potential of the leaf is affected by transpiration is determined by the hydraulic conductances of the plant, including the conductance through the root, largely mediated by the activity of aquaporins, the conductance of the xylem conduits, determined by their anatomy and the presence or absence of water vapor obstructions, and the conductance of the living cells of the leaf, as water moves from the xylem to the stomata. All of these are under genetic control and subject to selection, and many are environmentally responsive as well, allowing plants to optimize the rate of transpirational water flow and leaf water potential in many different conditions. To better understand how plants have optimized water uptake and transport, we will discuss separately the contributions of the anatomy and physiology of the roots, xylem conduits, and leaves.

UPTAKE AND TRANSPORT OF WATER BY ROOTS

For the majority of vascular plants, most of the water uptake takes place in roots in contact with soil and is affected by many factors, including the soil properties and soil water content, the architecture of the root system, the abundance of root hairs and mycorrhizal fungi, and the conductance of the root system.
Soil Properties Affect Soil Water Potential and the Transfer of Water into the Root

The ability of soil to retain water and to make it available to roots depends on many factors. One of the most important is the particle size of the soil. Soils are classified into different types, but most soils are very heterogenous mixtures of particles of various sizes. Clays have very fine particles (~0.002 mm) predominating, loam has abundant intermediate-sized particles, and sand has particles as large as 2 mm. Field capacity is a term that defines how much water the soil can hold before additional water runs off. Field capacity is determined by the particle size distribution, with smaller particles being more effective at holding water. Particle size and particle charge also determine how much water is tightly bound to the soil and, therefore, not available to a plant.

The wilting point of a soil can be determined experimentally as the soil water content below which most plants (e.g., wheat or maize) cannot recover from wilting. Besides particle size, the water-holding capacity of a soil is affected by organic matter (e.g., humus) and the presence of mycorrhizal fungi and other biological material.

Root Architecture Affects the Volume and Depth from Which Water Can Be Extracted

Roots have several functions, including water uptake, storage, anchorage, and nutrient uptake. Each of these functions is affected by the distribution and abundance of the surface area of the root. Root architecture, which describes the distribution, length, angle, and number of individual roots and root hairs, is a highly plastic phenotype. Genetically identical plants will produce very different root systems when growing in different environments. In general, when water is limiting to plant growth, shoot growth will be greatly diminished, but root growth, particularly primary root elongation, much less so. However, different kinds of drought can produce different distributions of root growth. When water is withheld, root systems often tend to grow deeper (to reach water deeper in the soil), but when rain is regular and light, the root systems of many plants tend to spread out near the surface, to capture rain before it runs into deeper soil. Roots are able to sense and grow toward water, in a process known as hydrotropism. The mechanisms are still being unraveled, but perception through an unknown sensor seems to take place at the root cap, and the response involves the hormones ABA and cytokinin.

Root Conductance Affects Water Flow

As described earlier, the flow of water is determined in part by the conductance (the inverse of resistance) of the conducting tissue. Conductance into and through the root system consists of two distinct components: radial and axial conductance. Radial conductance affects water movement from the root surface to the transpiration stream in the xylem, and axial conductance affects movement through the xylem along the length of the root. In most conditions, the main limitation to water uptake is due to a limitation in radial rather than axial conductance.

To reach the central vascular cylinder (stele) and the xylem within it, water has to pass through the outer cortical cell layers. Water and its dissolved ions can pass through the porous cell wall matrix (the apoplast) of the root cortical layers by bulk flow. Alternatively, it can pass through a plasma membrane and into the cytoplasm and then move from cell to cell through plasmodesmata; this is known as the symplastic route, because water moves through living cells. Alternatively, the water can take a transcellular route, in which it crosses plasma membranes many times while moving through cell walls and cytoplasm. The plasma membrane is a selective barrier through which water and some small molecules can pass but that acts as a barrier to most larger or charged molecules. Water moves across the plasma membrane by simple and facilitated diffusion, which are slower processes than bulk flow. Thus, the plasma membrane is a source of much of the radial resistance of water entering the root, and the facilitation of water movement by aquaporins is extremely important in the control of water movement into the plant.

Because it has not passed through a permeability barrier, water moving through the apoplastic pathway toward the stele carries in it potentially harmful dissolved ions. The Casparian strip of the endodermis, the cell layer immediately outside of the stele, is a lignified hydrophobic matrix that largely blocks the passage of water through the apoplastic space and forces it to cross the plasma membrane, thus selectively filtering it. It is named after Robert Caspary who recognized its significance and who was a contemporary and correspondent of Charles Darwin. Many older roots produce a similar barrier layer called the exodermis in the root cortex. These barriers are substantial sources of resistance to water movement into the xylem-borne transpiration stream.

FLOW OF WATER THROUGH THE XYLEM

The evolution of tracheary elements, the water-conducting cells of tracheophytes, was one of the most important events in the evolutionary history of plants and our modern biosphere. Flow of water through tracheary elements is much more efficient than through living cells because (1) conduits are formed from dead cells, so there are no plasma membranes to cross, thereby reducing resistance to flow, (2) many tracheary elements tend to have a large diameter, which, as described by Poiseuille’s law, greatly enhances flow, and (3), the thickened secondary walls of tracheary elements can withstand the large tensions associated with the cohesion–tension-based water flow. These efficient conduits can provide much more flow of water to the photosynthetic tissues than if the plant depended on transport through living cells. Under many conditions, the rate of photosynthesis is limited by the uptake of CO₂ into the leaves, and CO₂ uptake is limited by water loss through open stomata. Therefore, a more efficient water transport system means that more CO₂ can be assimilated, potentially leading to a faster rate of growth. A strong selective pressure toward tracheary elements with larger conduits can be seen from the fossil record. The earliest vascular plants, such as Cooksonia, had tracheary element diameters of ~5 μm, whereas some modern vines and lianas have tracheary elements as wide as 500 μm. As taller plants can appropriate a limited light resource, the capacity for long-distance vertical water transport via an efficient xylem has
Development and Differentiation of Tracheary Elements

Tracheary elements are the functional, water-conducting cells of the xylem and are either vessel elements (mostly in angiosperms) or tracheids (mostly in gymnosperms, ferns, and lycophytes). Vessel elements and tracheids differ structurally and functionally. In addition to tracheary elements, xylem tissue can include living cells called xylem parenchyma and xylem ray parenchyma intimately associated with the tracheary elements and, in angiosperms, fibers that are specialized for mechanical tissue support.

The development and organization of primary tissues occur in root and shoot apical meristems, in the distal regions of the plant. Vascular tissue is specified at the meristems and during the development of nascent leaves and lateral roots. The development and differentiation of tracheary elements involves three key stages: patterning, differentiation, and death. Vascular tissue patterning is largely determined by patterns of polar auxin flux, which induces the expression of a transcriptional regulatory cascade leading to cell differentiation. During differentiation, the future tracheary element cells can grow to a very large size; tracheary elements can be 1 cm or more in length and in some plants can reach diameters of 500 μm. Once the cells reach their final size, they lay down elaborately patterned secondary cell walls, which reinforce the primary cell walls. The pattern of secondary wall formation is species-specific and developmentally regulated. Lignin, a polyphenolic polymer, is key to the function of this secondary wall. Unlike the primary wall, which retains malleability and water permeability, the lignified secondary wall is rigid and much less permeable. The final stage of tracheary element differentiation is programmed cell death accompanied by lysis and degradation of the cellular contents, leaving behind a hollow, rigid water-conducting conduit.

Many plants are able to continually produce new vascular tissue, most commonly known as “wood,” in a process called secondary vascular development. The vascular cambium is the meristematic tissue responsible for secondary growth. In seed plants, the vascular cambium is a bifascicular (meaning “two-faced”) pluripotent stem cell population that produces precursors to both xylem and phloem. Most gymnosperms and angiosperms, with the exception of monocots, produce vascular cambia and can produce secondary vascular tissues. Some plants, trees for example, grow extensively in girth during their primary life span. With its massive trunk, the giant sequoia (Sequoiadendron giganteum) is a good example of extensive secondary growth; the General Sherman tree in California is considered the largest living tree by volume and has a trunk diameter of 7.7 m, and the Wawona tunnel tree was famous for having a tunnel cut into it through which cars could drive.

Structure and Function of Tracheary Elements

The structures of tracheary elements are unusual and diverse, and to understand them, we must consider how their structures affect their functions in terms of hydraulic conductance, resistance to blockage, and structural support.

Tracheids: Tracheary Elements of Gymnosperms, Ferns, and Lycophytes

The water-conducting cells of most gymnosperms, ferns, and lycophytes are tracheids: hollow conduits largely reinforced by lignified secondary walls. Tracheids are best characterized from conifers, so we will restrict our discussion to these. Tracheids are long cells with tapered ends. They range from less than 0.1 cm to more than 0.5 cm long and have diameters from 5 to 80 μm. As water flows from tracheid to tracheid, it moves through partially digested regions of the cell walls, known as bordered pits. The pit membrane is within the bordered pit and separates adjacent cells. Pit membranes are not lipid bilayers or plasma membranes but instead are thin, water-permeable regions composed of cellulose, which is derived from the primary walls of adjacent cells and the middle lamella between them. In conifers, the pit membrane has a center torus that is thick and water impermeable, surrounded with a margo that is highly permeable. As compared with the pit membrane of angiosperms, the conifer pit membrane has a relatively low resistance to water flow.

Vessels, Vessel Elements, and Fibers: Xylem of Angiosperms

In the xylem of most angiosperms, the functions of support and water transport are distributed to two cell types, the fibers and vessel elements, respectively. Fibers are hollow, lignified cells that are not normally water-conducting cells and will not be discussed here further. The conduit of water transport is the vessel, which is composed of several vessel elements stacked one upon the other. Vessel elements tend to be roughly cylindrical and less tapered than tracheids. End walls are either entirely open (with no primary or secondary walls) or partially enclosed by secondary walls. Thus, there is little resistance to water flow from vessel element to vessel element, so the functional unit is the multicellular vessel. Vessels can be very long, from 1 cm to more than 1000 cm, with diameters from 15 to 500 μm. The greater length of open, unimpeded vessels as compared with tracheids, as well as their larger diameter, should mean that angiosperm tracheary elements are less resistant to flow than those in conifers. However, the greater conductance of the conifer pits (as well as the greater total area often occupied by tracheids in conifers) compensates somewhat for the morphological differences, which leads to the question, “How does xylem structure affect its function?”

Xylem Conductance

As described by Ohm’s law, the flow of water through the xylem is the product of its driving force (the difference in pressure potential between the two ends) and the xylem conductance. As described by Poiseuille’s law, flow scales with the radius of individual conduits to the fourth power, so the maximal xylem conductance
generally is higher in species with larger diameter conduits. Conductance can be quantified in living tissues in several ways. Conductance can be measured in excised stem segments by measuring the amount of water that moves through it (by weighing the efflux over a period of time) for a given pressure gradient or it can be measured in vivo by applying a heat pulse to a branch and determining the time it takes to reach another point downstream to determine the flow rate, which, when divided by the pressure gradient, provides the conductance.

Xylem conductance is affected by the anatomy of the tracheary elements, but nevertheless, it can vary with conditions. Recently, xylem conductance has been shown to be sensitive to the ionic composition of the xylem sap; specifically, the presence of cations in the xylem sap increases pit membrane conductance relative to pure water. Imaging of the pit membranes with atomic force microscopy revealed that their thickness decreases when cations are present, which increases their conductance. Other studies suggest that this ionic effect is adaptive and functionally relevant, although to what extent it is under the control of the plant is not certain.

“The Vulnerable Pipeline”: Cavitation and Embolism Formation

The cohesion–tension mechanism of water transport requires little or no internal energetic expenditure. There is an associated cost, however, which is that the system is vulnerable to potentially catastrophic embolism formation. This occurs when air enters through the pit membranes from an adjoining air-filled conduit, a phenomenon referred to as cavitation. Because of the tension in the surrounding sap, the air expands to form an air-vapor blockage that breaks the water column, a condition known as embolism. A fully gas-filled conduit is no longer able to contribute to water flow. Under some conditions, including drought and freezing temperatures, water flow can be extensively impeded by embolized xylem, even to the extent of the death of the plant.

The anatomy of the pit is important in resistance to drought-induced cavitation and in the resistance to embolism spread. Drought causes increased tension in the xylem conduits, because water is held at greater tension in the soil and transpirational demand will increase this tension as water flows across much steeper water potential gradients than would develop in a well-watered plant. Increased tension most likely induces cavitation to occur by air seeding from an adjacent gas-filled tracheary element or the atmosphere at the pits between conduits. Pits that resist air seeding can reduce drought-induced losses of hydraulic conductance. Some xerophytes produce vested pits, which have an elaboration of the secondary wall filling the pit cavity and which are thought to aid in resisting cavitation. The thickness and size of the pit membrane, as well as its interactions with adjacent secondary cell walls, also can affect resistance to embolism spread. The torus-margo arrangement of bordered pits found in conifers can be particularly efficient at resisting the spread of embolisms between cells. Anatomies that resist embolism formation or spreading are found in plants that regularly experience drought or freezing stress.

The diameter of the conduits also contributes to resistance or sensitivity to embolism. For example, species with an average conduit diameter of greater than 30 μm are particularly susceptible to cavitation induced by freezing and thawing. When water in the xylem freezes, dissolved air becomes less soluble and comes out of solution as gas. Upon thawing and the reestablishment of tension in the xylem sap, the gas bubbles can either dissolve again or expand and form an embolism. Smaller bubbles usually dissolve, larger ones usually embolize. Narrow conduits prevent large bubbles from forming, thus conferring resistance to freeze–thaw-induced embolism. Consequently, large conduits are rare in high-latitude or high-elevation species, in spite of the greater conductivity they provide. In contrast, coniferous species, with their small-volume tracheids, are more resistant to permanent embolism and thus well adapted to cold regions. However, due to the important roles of pit membranes in resisting cavitation and embolism, the relation between conduit size and cavitation resistance is not simple.

Many methods have been developed with which to measure embolism formation. These include the quantification of acoustic emissions (sounds) that occur with cavitation and imaging methods involving scanning electron microscopy or x-ray-based computer-assisted tomography (these are described in more detail under Methods Used in Plant–Water Relations Research). One of the more widely used methods is to determine the conductivity of a tissue at several different water potentials and from these data construct a vulnerability curve or a percentage loss of conductivity curve. Percentage loss of conductivity curves provide useful information about the sensitivity of the plant to environmentally induced embolisms, which generally correlate with where the plant can survive. For example, plants endemic to humid areas lose their hydraulic conductivity at higher (less negative) water potentials than those from dry regions.

The conductance of a plant can decrease over a growing season as more and more xylem vessels are blocked by embolism. In some species, root pressure can refill the embolized vessels (as described further below). Gymnosperms and angiosperms are able to replace nonfunctional xylem by the action of the vascular cambium, which produces new xylem. Most ferns and lycophytes are not able to generate secondary xylem. Interestingly, studies have demonstrated that ferns have a very conservative strategy to prevent embolization of their vascular tissues. Relative to the maximum xylem tension that each can endure, fern stomata close in response to less xylem tension than angiosperm stomata; they stop transpiration, and therefore photosynthesis, before there is a great risk of xylem failure due to embolism formation. In other words, if you can’t replace it, you’d better take care of it!

Thus, in xylem construction, there has to be a balance between hydraulic efficiency and safety from embolism. These safety–efficiency tradeoffs are found at the level of the pit, the conduit, and the whole plant. Essentially, plants cannot do everything well. Hydraulically efficient species such as water birches are susceptible to embolism, while drought-resistant species such as Ceanothus shrubs have a very low hydraulic efficiency but high cavitation resistance.

A lively debate is taking place right now about the extent to which embolisms can be refilled when the xylem is under...
tension. We refer the reader to the articles cited in Recommended Reading for an elaboration of the arguments for and against this model.

A FEW EXAMPLES TO ILLUSTRATE WATER MOVEMENT IN XYLEM

Mangroves and Ultrafiltration of Seawater

Like Stephen Hales, Per Scholander studied the physiology of both plants and animals, and much of his work was on the diving physiology of marine mammals. We remember him for his studies of xylem tension, using the Scholander pressure bomb method, in plants from several habitats, including mangroves. Mangroves are diverse plants from many different families that share the feature of growing with their roots in brackish water or seawater. Mangroves must obtain water from a salty substrate with a low water (osmotic) potential. This is analogous to growing in a perpetually very dry soil. Scholander reasoned that the highly negative osmotic potential of seawater would pose a significant challenge to the ability of these plants to take in water. In 1962, he asked, “What balance, if any, exists between osmotic potential of seawater, roots, and leaves on one hand and hydrostatic pressure and osmotic potential of the xylem sap on the other?” Mangroves have adapted to their environment through the development of very low osmotic potentials in living cells (by the accumulation of compatible solutes such as proline) that allows them to develop low leaf and root water potentials sufficient to pull water into the roots. They also must possess sufficiently strong resistance to xylem tension. In 1964, Scholander measured xylem tension in mangroves at about −3 to −5 MPa, much lower than that of a “typical” plant. As Scholander observed, mangroves face an exaggerated version of the old problem of how sap rises in tall trees.” In other words, both mangroves and tall trees require very low water potentials in the xylem to bring water to their leaves.

Xylem under Pressure: The Roles of Root and Stem Pressure on Water Movement

Early plant physiologists wondered if the flow of water in the xylem was a result of pressure in the roots forcing water upward. Although Hales and others demonstrated that this is not the case most of the time, there are cases in which root or stem pressure is associated with water flow.

Refilling of Grapevines by Root Pressure

Grapevines (Vitis vinifera) have long been used as a model system for the study of plant–water relations. Not only are they economically important, but they also have some of the largest diameter vessels of any plants. Large vessels mean that water flows efficiently, but they also mean an increased vulnerability to freezing-induced cavitation. Because of this, grapevine vessels are empty (air filled) in the winter. Prior to leaf emergence, the vine vessels fill with water, which is accompanied by a high positive xylem pressure. The pressure is such that a cut vine exudes copious amounts of water and is said to “bleed.” Pressurized vessel filling is a short-lived effect, and after the buds open and leaves form, transpiration begins and the vessels show a negative pressure. The springtime positive pressure originates from osmotic pressure in the root xylem, produced by the active transport of solutes. Note that guttation, or the forcible extrusion of water from the hydathodes at the edges of leaves, is also a product of root pressure, which can occur when plants are not transpiring and the water potential gradient nevertheless draws water into the roots from the soil.

A Sweet Enigma: Stem Pressure and Flow of Maple Sap

The flow of maple sap is an interesting example of an unusual form of sap movement, driven by stem pressure. Sugar maples (Acer saccharum) are common in the northeast part of North America and have been used for centuries as a source of sugar and syrup. During early spring, sweet sap flows from a spike, called a spile, driven into the trunk of a tree. The mechanism of sap flow has long puzzled plant physiologists and relies in large part on the unusual anatomy of the trees. In sugar maple wood (and in many other trees), the structural fibers that are interspersed with the vessels are air filled. Sap flows only under very specific conditions, which gives clues to its mechanism. Sap flows from barren, leafless trees, indicating that it is not a transpiration-driven process. In contrast with grapevines, if a maple branch is cut off, the sap flows out of the distal “leaf” end rather than the proximal “root” end of the cut; this suggests that it is not root pressure that drives sap flow. In fact, an isolated segment of branch without any roots can release sap. Also unlike grapevines, maple sap flow is temperature dependent and occurs when below-freezing nights are followed by above-freezing days. An isolated branch takes up water into its cut end when the temperature is below freezing and exudes it when the temperature is above freezing. This cycle argues against sap flow being driven by the change in volume of freezing water, because water expands when frozen. Instead, the current model suggests that the freezing temperatures draw water into the air-filled cavity of the fibers, which then freezes, compressing and pressurizing the air. When the ice
thaws, the pressurized air forces the water back into the vessels
and down the tree trunk. The continuing sap flow probably
requires additional osmotic pressure in the vessels. Again, the anatomy
of the maple supports this possibility, because the vessels are unusually
void of pits connecting them to the fibers, which raises the
possibility that sucrose can move into the vessels but not out of
them, leading to an increased pressure driven by the influx of water.
Unfortunately, sap flow’s dependence on freezing temperatures
means that climate change is expected to restrict sap production in
traditional sugar maple-growing regions.

**MOVEMENT OF WATER FROM XYLEM TO LEAF CELLS TO THE STOMATAL PORE**

The movement of water within the leaf takes place as liquid
water moving through the xylem and the living leaf cells and as
water vapor within the intercellular spaces of the leaf. The rate of
flow is determined in part by the conductance of each of these
segments of the pathway: within the vascular tissues, through
the nonvascular tissues (via apoplastic, symplastic, and trans-
cellular pathways), and stomatal conductance.

**Leaf Vein Anatomy and Conductance**

Within the leaf, the xylem provides an efficient and low-resistance
pathway for water movement. In most leaves, the vein pattern is
such that the photosynthetic cells are all located very close to a
vein, ensuring them both a steady supply of water and a relatively
short pathway to return photosynthate to the vascular tissues. Vein density is notably greater in angiosperms than in non-
angiosperms. The fossil record shows that increased vein density
appeared 100 million years ago, and this higher density may be
essential to support the high rates of transpiration and photosyn-
thesis of which angiosperms are capable. Veins in dicots tend to
form branching reticulated networks, whereas in monocots, there
is a tendency for parallel veins or parallel secondary veins branching
off a single midvein; these redundancies ensure that there are
alternative pathways for water to flow if one gets damaged or
embolized.

Because of the relatively lower conductance of mesophyll cells
as compared with xylem, conductance is negatively correlated with
distance from vein to evaporating surface, so the greater vein
density of angiosperms means shorter vein-to-stomata distances
and is correlated with higher maximum photosynthetic rates. Some
gymnosperms have additional non-xylem-conducting tissues called
water-conducting sclereids that facilitate water movement through
the mesophyll. Aquaporin activity in the leaf mesophyll can also
increase the conductance of the pathway and has been shown to be
regulated by many environmental parameters, including light,
temperature, and drought stress.

Several studies have suggested that one function of the xylem
within leaves may be to protect the xylem in the stem. For
example, in some deciduous plants, the xylem of the leaves is less
resistant to cavitation than that of stems or branches, suggesting
that the conduits in these leaves may act as disposable “fuses”
that help protect tissues in branches that the plant has invested in
more heavily. In some nondeciduous conifer leaves (which are not
as short-lived as deciduous leaves), high xylem tension can cause
the leaf tracheids to collapse reversibly, which by sealing off the
stem xylem system from the transpirational pull may protect it
from embolism.

**Outside-of-Xylem Conductance**

In angiosperms, the leaf vasculature is surrounded by specialized
mesophyll cells called bundle sheath cells. In C₄ photosynthetic
plants such as maize, these cells have been thoroughly studied for
their contribution to CO₂ fixation, but they are found in C₃ plants
as well. Water leaving the xylem passes through these cells,
which are thought to vary in conductance with environmental
conditions. Furthermore, there is some evidence suggesting that
the bundle sheath cells may have a similar role to that of the
endodermis in controlling the flow of solutes into the leaf tissues.
The hydraulic conductance of the radial pathway from vein to
stomatal pore may be at least partially regulated by ABA and by
drought signals, which are likely to be mediated at least partially
through aquaporin activities.

The flow of water from the xylem to the mesophyll requires that
the water potential of the mesophyll cells is lower than that of the
xylem. Xylem water potential is primarily determined by tension,
whereas mesophyll cells have a positive turgor pressure. If the
mesophyll cells had the same osmotic potential as the sap in the
xylem, water would flow backward into the xylem. The fact that it
does not is due to the lower osmotic potential of the mesophyll
cells. For a well-watered plant, the osmotic potential of mesophyll
cells can be on the order of −0.5 to −1.5 MPa. However, for
plants adapted to low-water environments or plants under drought
stress, the water potential of the xylem can be considerably lower,
−3 MPa or lower, so the mesophyll cells must compensate by
accumulating additional solutes, such as ions or organic solutes.
Thus, the leaf adjusts its osmotic milieu to ensure that the water
potential of the xylem sap is not lower than that of the living leaf cells.

**Stomatal Conductance**

Stomata are the tiny openings in the leaf epidermis and the adjacent
pairs of guard cells that act as gatekeepers of transpiration/gas
exchange. (In some species, the stomatal complex includes
additional neighboring cells that support the activities of the
guard cells.) When conditions are right for the guard cells to open,
ions are pumped into the cytoplasm, which leads to the
movement of water into the cell by osmosis. Because the guard
cells have a rigid inner wall and radial bands of cellulose, as they
increase in volume their outer radial wall expands, which leads
to the pair of cells moving away from each other, opening the pore
that they cover. When the stomatal pore is open, CO₂ enters and
water vapor is released by diffusion. When water needs to be
conserved, plasma membrane-localized ion channels open,
ions and water flow out, and the guard cells lose turgor and
relax together, covering the pore and blocking transpiration.

Most guard cells are extremely sensitive to the hormone ABA,
which promotes stomatal closure and inhibits stomatal opening.
Downstream of ABA are a family of receptor proteins that operate
as dimers and, when bound to ABA, regulate both transcription
factor and ion channel activities. The immediate effect of ABA on guard cells is to open potassium and anion channels, releasing ions from the cytoplasm, followed by water, and pore closure. Decreases in stomatal aperture and transpiration can be recorded within minutes of ABA application to a leaf or epidermal strip.

ABA can be synthesized in guard cells and their neighbors in response to decreasing relative humidity. ABA can also be carried through the transpiration stream from drying roots to guard cells. Furthermore, ABA can affect transpiration by altering the conductance of nonstomatatal cells of the leaf and root. Other studies have shown that a hydraulic signal can contribute to guard cell responses directly. The relative importance of hydraulic signals and ABA is still being debated; some studies have suggested that guard cells of earlier emerging plants such as ferns and lycophytes are insensitive or less sensitive to ABA than are those of angiosperms. Interestingly, the genomes of these earlier emerging plants reveal that genes required for ABA responses are present. How and if hydraulic signals integrate with ABA signals in the regulation of water transport continue to be fascinating questions. Stomata also respond in an adaptive fashion to other environmental signals. For example, guard cells respond to light (opening) and dark (closing) and low humidity (closing) stimuli, all of which promote a greater ratio of CO₂ molecule capture in photosynthesis per molecule of water loss.

**DROUGHT, HYDRAULIC FAILURE, AND WHAT IT ALL MEANS**

Plants tend to have a system for water uptake and transport that is well suited for the niche they occupy and for competing with species occupying similar niches. Fast-growing species in moist environments may have large conduits and numerous stomata to support a high rate of transpiration and carbon uptake but that makes them vulnerable to drought stress. Arid-climate species may have a more conservative approach to transpiration and gas exchange that limits their competitiveness in moist environments but allows them to tolerate arid ones. Root and shoot size and architecture can also be optimized to support the balanced needs for water uptake and photosynthesis in a given niche. Most species have some range of tolerances to account for seasonal and annual variations in environmental conditions. However, prolonged conditions of reduced water availability (drought) are often lethal. In some cases, particularly managed and agricultural systems, drought can be ameliorated by irrigation. In others, particularly forest and natural systems, a lack of rainfall can push a plant beyond its tolerances. Drought lowers the water potential of the soil, leading to lower water potentials in the xylem. Beyond a threshold level of hydraulic tolerance, depending on the interplay between xylem anatomy, stomatal responsiveness, and root uptake efficiency, a droughted plant can experience hydraulic failure, which may result in death. In recent years, there have been documented increases in drought-induced vegetation mortality associated with climate change. This condition is often exacerbated by the increased vulnerability of drought-stressed trees to further damage by pests such as bark beetle and opportunistic pathogens. Because forests store vast amounts of carbon, widespread mortalities of forest trees could further exacerbate the rate of carbon emission and climate destabilization, potentially leading to a positive feedback cycle that we must avoid.

**SUMMARY AND ONGOING RESEARCH**

The cohesion–tension model states that water moves through plants without any mechanical pumping required, and this can be demonstrated by a simple clay pot on a tube. When you consider how much energy would have to be extended to move water up a 100-m tree if pumping were involved, this feat seems remarkable, and acceptance of the cohesion–tension model was slow. Nevertheless, it is now the widely accepted explanation for water uptake and transport in plants. Plants are much more complicated than clay pots on sticks, and the results of millions of years of evolutionary selection underpin every step of the water-flow process. From the far ends of the roots to the highly sensitive guard cells, living plant cells sense and respond to water, allowing them to maximize CO₂ uptake while minimizing dehydration injury and damage to their vulnerable conducting tissues. Millennia of evolution have produced highly efficient tracheary elements, with anatomical features that can be genetically and perhaps physiologically fine-tuned to provide greater hydraulic conductance or embolism resistance. The living tissues of the shoot and root are more resistant to water movement than the hollow conduits of the xylem but also provide more opportunities for regulatory controls.

There is still much that we do not understand yet about water uptake and transport, and the field is as rich with conflicting models and hypotheses as it was when the cohesion–tension model was put forth more than 100 years ago. Exciting new methods and technologies such as high-resolution computed technology can test established models in new ways and pave the way for new models. The genetic resources of Arabidopsis as well as other model species, and the power of transcriptomics, proteomics, and metabolomics methods, are being applied toward these questions. Our forests may be the most important beneficiaries of these studies, as long-lived, tall trees are particularly vulnerable to the kinds of dramatic changes in rainfall patterns and water availability that are being postulated in coming years. As the authors of an influential paper (Choat et al., 2012) point out, “If the tight link between embolism resistance and water availability is the product of natural selection over many generations and adaptation is limited by a long generation cycle of perennial plants, then the rapid pace of climate change may outstrip the capacity of populations to adapt.”

**METHODS USED IN PLANT–WATER RELATIONS RESEARCH**

Some of the liveliest discussions that take place among plant scientists are those about the relative merits of various methods for measuring a plant’s hydraulic properties. Simply put, there is no perfect method, as these properties are extremely challenging to measure. Without getting too deeply into the controversies, we will introduce a few of the methods commonly used and refer the reader to Recommended Reading for more thorough discussions.
Transpiration, Water Uptake and Release, and Water Potential

A simple method to measure the movement of water at the whole-plant level was developed by Stephen Hales in the 1700s. This involves weighing a plant in a sealed pot at various times, with the loss of mass being attributed to the movement of water from the pot to the atmosphere through the plant. This method is still widely used today, particularly for small, pot-grown plants like Arabidopsis and rice (Oryza sativa), but it has several limitations in addition to the need for the plant to be grown in a pot. In particular, the method measures whole-plant flow but does not differentiate which segments of the plant are responsible for any variation in flow rate.

Alternatively, a porometer can be used to measure transpiration or stomatal conductance in real time. A porometer has a chamber that can be clamped onto a leaf, and the amount of water vapor that moves from the leaf into the device is measured by a change in chamber humidity. Porometers are portable and widely used in the field. A similar principle is used in thermocouple psychrometers, which determine the water potential of a sample placed into a chamber. Psychrometers can be used in determining the water potential of plant or soil samples.

Transpiration can also be indirectly measured by a change in leaf temperature, as the water evaporation withdraws heat energy from the leaf. Temperature changes can be measured through infrared imaging of single plants or leaves or of entire fields or ecosystems using satellites.

A potometer can give an indirect measurement of transpiration rate by recording the amount of water drawn into a cut branch that is immersed in water; the name means “drink meter,” and it records how much the plant “drinks.” The simplicity of this method makes it a popular one for school science laboratories.

Pressure and Tension

Dixon and Joly (1895) and later Scholander (1960s) pioneered the use of “pressure bombs” to measure xylem tension. Cut branches or leaves are placed into sealed chambers so that only the cut surface protrudes. Because the xylem system is under tension, water retreats from the cut surface into the severed branch or leaf. The balancing pressure applied to the chambered tissues that is sufficient to force the xylem stream back to the level of the cut surface can be interpreted as the negative of the xylem tension if the leaf was not transpiring. Another method to estimate tension is to measure the diameter of the stem, with more tension leading to a narrower stem. More recently, a pressure probe method has been developed that measures pressure or tension within a tissue by impaling individual cells with a fluid-filled needle and measuring the force exerted on the needle.

Flow

The flow rate of sap within a plant can be measured by heat-flow measurements. Heat is introduced into a stem or branch, and the amount of time required for the heat pulse to be detected at a sensor farther along the transpiration stream is measured. The longer the time the pulse takes to reach the sensor, the slower the rate of flow in the xylem. A more sensitive variation of this technique involves adjusting the rate with which heat has to be introduced to the stem in order for a constant temperature to be maintained. As the flow rate increases, the rate of heat addition has to be increased as well. Dyes or tracers can be used instead of heat.

Conductance

Hydraulic conductance can be measured by measuring the flow through a branch, branch or root segment, or root system, driven by various pressure gradients. Tyree and colleagues developed a high-pressure flow meter method for measuring root conductance in situ, in which a root or root system is excised from the shoot (but left in the soil) and a pressurized water source is attached to the cut end. The flow rate at which water enters the root system can be plotted against the pressure applied to determine conductance. In Tyree’s method, the water was forced from the root base (near the shoot) to the distal end (i.e., rootward), the opposite direction to the normal water flow. Root conductance also can be measured by placing a root system into a pressure chamber and measuring water flow from the cut surface (i.e., shootward flow).

Hydraulic conductance or conductivity across a cell membrane can be measured by stripping off the cell wall, immersing the protoplast in a solution of defined osmotic potential, and measuring the change in cell volume. Because water movement across a membrane occurs largely through aquaporins, this method is useful in determining aquaporin activity.

Vulnerability curves can indicate how well a plant tissue resists cavitation at various xylem tensions. Vulnerability curves are determined by measuring the flow through an excised stem segment at progressively lower xylem tensions. As the tension increases, flow will progressively decrease due to embolism blockages. To determine the maximal, 100% flow, the embolized vessels can be forcefully refilled with high-pressure water. Various methods can be used to lower the xylem water potential, including progressively drying the segment to lower the xylem water potential or spinning the segment in a centrifuge, and conductivity is measured at several water potential values. These “percentage loss of conductivity” curves are widely used to compare the hydraulic properties of various plants and plant parts. The water pressure at which a segment embolizes can also be measured by plotting the number of cavitations (monitored by sound) occurring at various water potentials.

Imaging methods have been developed that reveal the presence of embolisms in living tissues. A relatively simple approach is to cut a segment and put the cut end into a dye, which labels the actively transpiring conduits. Another method, cryoscanning electron microscopy, involves freezing stem segments and slicing them while frozen. Air-filled (embolized) conduits are hollow in the resulting sections, whereas water-filled ones remain filled with ice, although there is some concern about the extent to which sample processing introduces artifacts.

More recently, medical imaging techniques have been adapted for use in plants. Magnetic resonance imaging can distinguish
water from air, but the resolution of this method is insufficient to resolve single conduits. High-resolution computed tomography is a noninvasive imaging method that can resolve fine structures and has been used in the past few years to image embolism spread and repair in grapevines. See Cochard et al. (2013) for a discussion of the reliability of these different methods.

Understanding plant responses to water limitation is complex, and uptake and transport is just part of the picture. In Part 2 of this article, we examine how water limitation affects photosynthesis and growth and how plants respond to the stress of water deficit.

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RECOMMENDED READING
(This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included.)

Books

History


Water Movement Is Governed by Physical Laws

Aquaporins
Xylem Structure and Function and Resistance to Embolism


Examples: Mangroves, Tall Trees, Grapevines and Maple Syrup


Drought, Hydraulic Failures, and What It All Means


Methods Used in Plant–Water Relations Research


