Plant-Water Relations (1) 
Uptake and Transport
Water is a major factor in plant distribution

- Most plants experience occasional water stress
- Losses in growth and yield due to water stress are often cryptic because no unstressed controls exist


Plants have evolved several strategies to manage water

Many bryophytes can tolerate desiccation (i.e., drying to equilibrium with the atmosphere).

Some desert plants evade drought. They survive the dry season as seeds, sprouting and flowering in a brief period of rain.

Most tracheophytes cannot tolerate desiccation – they die.

Some desert plants tolerate dry conditions through adaptations such as deep roots, C₄ photosynthesis, succulence (water storage) and tiny or absent leaves.

Photo credits: Mary Williams; Amrum; Scott Bauer; James Henderson, Golden Delight Honey, Bugwood.org
Outline: Plant - water relations.
Uptake and transport

• Brief history of the study of plant - water relations
• Water movement is governed by physical laws
• Aquaporins: Essential regulators of water movement
• Water moves through the soil-plant-atmosphere continuum (SPAC)
  o Water uptake in roots
  o Water movement through xylem
    o The vulnerable pipeline
  o Water movement within leaves
  o Stomatal control of transpiration
• Hydraulic limits and drought
• Methods
Brief history of the study of plant water relations

1727 Stephen Hales published experiments on the nature of water transport in plants

Do plants pump sap around their bodies?
Does sap circulate?
Is sap pushed up by pressure from the roots?

200 years later, in 1927, the American Society of Plant Biologists established the Stephen Hales Prize for outstanding contributions to plant biology

(1628) William Harvey showed that blood circulates

Malpighi and Grew saw plant conducting tissues (1670 - 1680s)

Marcello Malpighi and Nehemiah Grew both recognized that plant vascular tissues might be conducting tissues, by analogy to similar-appearing structures in animals.
Hales investigated water movement in “Vegetable Staticks” (1727)

Hales measured water uptake by recording the weight of a sealed pot; weight loss was caused by water evaporating from the plant.

Hales showed that transpired liquid is essentially water.

Hales showed that water movement requires leaves exposed to air

"These .. experiments all shew, that .. capillary sap vessels .. have little power to protrude (moisture) farther, without the assistance of the perspiring leaves, which do greatly promote its progress."

The water is not pushed up from the roots, but rather pulled up by transpiration from the leaves

Dixon and Joly: The Cohesion-Tension theory (1890s)

“... the all-sufficient cause of the elevation of the sap (is) ... by exerting a simple tensile stress on the liquid in the conduits.”

Dixon and Joly (1895) suggested that water rising in the plant occurs by an upwards-pulling tension.

Transpiration measured by weight of water removed from tube.

The pressure in the chamber can be elevated to determine tensile strength.

Water uptake and transport are governed by physical laws

The flow of water up a plant, driven by evaporation, can be replicated in a purely physical model.

Like Lego, water is very cohesive, meaning that the molecules stick together and a column of water can be pulled to a great height.

"We may compare this action to the action of a porous vessel drawing up a column of liquid to supply the evaporation loss at its surface"
– Dixon and Joly (1895)

Water has many unusual and important properties

The arrangement of atoms in a water molecule means that charge is distributed asymmetrically; it is polar and it has a high dielectric constant.

Water forms intermolecular hydrogen bonds and so is very cohesive. It sticks together and has unusually high boiling and melting points.

Water’s high dielectric constant means it is a good solvent for polar compounds.

Exobiologists usually consider water a prerequisite for life.

Fick’s law describes the movement of water by diffusion

\[ \frac{dm}{dt} = -D A \frac{dc}{dx} \]

Fick’s Law

\( \frac{dm}{dt} \) = amount of substance moved per unit time

\( D \) = diffusion coefficient (property of substance and matrix)

\( \frac{dc}{dx} \) = gradient of concentration (change in concentration per unit of distance, \( dx \), in a direction perpendicular to the plane \( A \))

\( A \) = area through which flow occurs
Diffusion rate is affected by area, distance, and gradient

\[ \frac{dm}{dt} = - D A \frac{dc}{dx} \]

Diffusion is faster when:
- The concentration gradient \( dc/dx \) is steeper
- The area \( A \) is larger
- The distance \( x \) is smaller
- \( D \) is larger

\( A = \text{area through which flow occurs} \)
Diffusion rate is affected by the properties of material and solvent

\[ \frac{dm}{dt} = -D A \frac{dc}{dx} \]

The diffusion coefficient \( D \) is affected by:
- Molecular shape
- Molecular size
- Molecular charge
- Solvent viscosity
- Solution properties as salt concentration, pH, temperature etc.
Water moves down osmotic gradients

Water moves across a semipermeable membrane into a compartment containing salt.

The water flows inwards until the force of the pressure inside balances the osmotic driving force.

The osmotic potential of pure water is 0. The addition of solutes lowers the water potential. Water moves towards a region with a lower water potential.
Osmotic forces drive the movement of water into and out of cells

Cells have a lower osmotic potential than pure water, (because of the salts and proteins in them), so water moves into them. An animal cell might burst. Salt water has a lower osmotic potential than cells, so water flows outwards. Plant cell walls prevent them from bursting.

Osmotic potential is written as $\Psi_\pi$ and measured in MegaPascals (MPa). For seawater, $\Psi_\pi$ is about -2.5 MPa, and for a typical cell, $\Psi_\pi$ is about -0.8 MPa.
Pressure can be positive or negative

- Pressure washer: 15 MPa
- Household water pressure: 0.3 MPa
- Car tire pressure: 0.25 MPa
- Pressure required to blow up a balloon: 0.01 MPa
- Vacuum cleaner (household): -0.02 MPa
  - Vacuum cleaner (commercial): -0.1 MPa
- Laboratory vacuum: -0.01 MPa
- Inside typical plant cell: 0.5 to 1.5 MPa
- Inside xylem: From +1 MPa to -3 MPa or lower
- Human blood pressure: < 0.02 MPa

* These numbers are relative to atmospheric pressure (0.1 MPa), not absolute.
Turgor pressure supports plant cells and tissues

Young, non-woody tissues are supported by turgor pressure

Water limitation lowers turgor pressure and tissues wilt
The water potential equation incorporates osmotic and pressure potentials

The water potential ($\Psi_w$) is the sum of the pressure potential ($\Psi_p$) and the osmotic potential ($\Psi_\pi$):

$$\Psi_w = \Psi_p + \Psi_\pi$$

Water flows from higher to lower water potential.
Water moves towards lower water potential

Initial conditions:

\[
\begin{align*}
\psi_T &= 0 \\
+ \psi_p &= 0 \\
\psi_w &= 0
\end{align*}
\]

Water moves towards lower water potential

Final conditions:

\[
\begin{align*}
\psi_T &= -2 \\
+ \psi_p &= 0 \\
\psi_w &= -2
\end{align*}
\]

Water is at equilibrium
Water moves by diffusion and bulk flow

\[ \psi_w = -100 \text{ MPa} \]

\[ \psi_w = -0.3 \text{ MPa} \]

1. Water moves from soil to root
2. Water moves through plant
3. Water moves from leaf to air

Diffusion: Random movement of individual molecules (1, 3)

Bulk flow: water in streams, tubes, hoses, water column of xylem (1, 2)
Diffusion rate is affected by the properties of material and solvent.

Cell membranes and walls increase the diffusion coefficient and can drastically lower the rate of diffusion.

Channels between cells called plasmodesmata and water channels called aquaporins can accelerate intercellular diffusion.

A small molecule can diffuse across a 50 μm cell in 0.6 s, but it would take 8 years to diffuse 1 m.
Long-distance water movement occurs by bulk flow

Bulk flow is much faster than diffusion, but requires the input of energy.

Waterfall: Energy provided by gravity

Human circulatory system: Energy provided by mechanical pump (heart)

Water movement up a plant: Solar energy drives evaporation
Bulk flow through a tube is affected by the diameter of the tube

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

Poiseuille’s Law

Where:
- \( \Delta P \) = hydraulic pressure gradient
- \( \eta \) = viscosity of water
- \( l \) = length of capillaries
- \( N \) = number of capillaries
- \( r \) = radius of capillary

Flow increases with the radius to the **fourth** power!
Wider conduits are more conductive

As the vessel radius decreases, the cross-sectional area needed for the same flow increases

- Radius 40 μm
  - 4 x area required
- Radius 20 μm
  - 16 x area required
- Radius 10 μm

Ohm’s law can model water flow, whether diffusion or bulk flow

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

**Ohm’s Law**

Current \((I)\) = voltage \((V)\) / resistance \((R)\)

**Ohm’s law as applied to water flow:**

Water flow = Difference in \(\psi\) / resistance

The water potential difference \((\psi)\) is the difference between water pressure and atmospheric pressure

As the tap opens, resistance decreases and so flow increases

Flow rate is affected by conductance of the root, stem and shoot

This model uses conductance ($K$) which is the inverse of resistance: Higher conductance = higher flow

$$K = \frac{1}{R}$$

It is useful to consider separately how the root, stem and leaves affect conductance.

We can also consider the conductance of individual branches and leaves.

Resistance and conductance are reciprocals & both affect flow

**Example:** Effect of conduit size on resistance

The resistance is higher in the sample with smaller-diameter conduits.

The conductance is higher in the sample with the larger-diameter conduits.

Xylem anatomy affects flow by affecting resistance

Stomatal conductance ($g_s$) is easily measured as the flow of water vapor out of the leaf. When the stomata are open, conductance is higher and resistance is lower.

Stomata affect flow by affecting resistance

Image: Decagon Services
Water flow is governed by the conductance of 3 plant segments

1. **Root radial flow:** From soil to stele and the entry point into the xylem system. Dominated by diffusion and permeability of membranes and cell walls.

2. **Axial flow:** Through the roots and shoots into the leaves via bulk flow through the conducting elements of the xylem.

3. **Leaf radial flow:** From xylem through the mesophyll, conversion to water vapor, and finally release into the atmosphere through regulated stomata.

Summary: Water movement is governed by physical laws

- Water moves by diffusion and bulk flow
- Water moves towards lower water potential ($\psi_w$), or lower pressure potential when no membranes are involved
- Water potential is the sum of pressure and osmotic potentials (and gravitational potential)
  $$\psi_w = \psi_T + \psi_p (+ \psi_g)$$
- Flow is determined by the difference in water potential divided by resistance (Ohm's Law)
  $$I = \frac{V}{R}, \text{ which is analogous to the water potential difference x conductance}$$

Aquaporins are regulated membrane-bound water channels

Expression of an aquaporin in a *Xenopus* oocyte accelerates water uptake

Peter Agre was awarded the Nobel Prize in 2003 for the discovery of aquaporins

Arabidopsis has 35 aquaporin genes in four families

Protoplast swelling assay of cell permeability and aquaporin activity

How fast does the cell swell?

Wild-type cell with many open aquaporins

Loss-of-function of PIP1;2

The rate of volume change after moving into a lower water potential solution indicates membrane water permeability

Loss-of-function of aquaporin PIP1;2 decreases protoplast water permeability ($P_f$), as measured by the change in cell volume


Aquaporin activity is regulated at many different levels, including transcriptional regulation.

Aquaporin activities are also regulated post-transcriptionally.

Aquaporin channels are gated and can be open or closed

Aquaporins are tetramers but each monomer forms a channel

Channel activity is modified by phosphorylation and pH

Aquaporins facilitate rapid plant movements

Cells that need a high rate of water movement such as the motor cells of the sensitive plant can be enriched for aquaporins

Aquaporin expression and activity affect hydraulic conductance

When aquaporin activity is perturbed, conductance and water relations are affected.

Aquaporin genes are expressed throughout the plant are involved in leaf as well as root conductance.

Water moves through Soil – Plant – Atmosphere Continuum (SPAC)

1. Water uptake in roots
2. Transport in the xylem
3. Transport through the leaf and into the air
The driving force for water flow is evaporation powered by solar energy.

The water potential of air is much lower than that of soil, so water is constantly evaporating. The plant harnesses this energy and channels water in, up, and out of it.

\[
\psi_w = -100 \text{ MPa}
\]

\[
\psi_w = -0.3 \text{ MPa}
\]

1. Water moves from soil to root
2. Water moves through plant
3. Water moves from leaf to air
Soil properties affect the movement of water

Soils are made up of particles of various sizes, which affect how water moves and is retained in them.

- **Sand**
- **Silt**
- **Clay**

**Large pore space (sandy soil)**
Gravitational pull dominates

**Small pore space (clayey soil)**
Capillary action contributes to lateral movement

Molecular interactions between water and soil affect water uptake

*Increasing amount of water added to soil*

**Wilting point:** The point at which most plants wilt and will not recover at night

**Field capacity:** The level above which water drains off
The type of soil particle (shape, size) affects soil interactions with water.

Other factors that affect water uptake from soil include organic matter, microbes, and salinity.

Flow = \frac{\Psi_{\text{soil-root interface}} - \Psi_{\text{root xylem}}}{R_{\text{soil-root interface to root xylem}}}

Factors affecting water flow into the root:
- \Psi_w at soil-root interface
- \Psi_w at root xylem
- \( R \) soil-root interface to root xylem

SPAC segment flow equation
Under water deficit, root growth is maintained relative to shoot growth.

As water becomes scarce, the plant’s resources are preferentially allocated to the root.


Water flow into roots is affected by root architecture and conductance.

**Root architecture** (length of roots, branching, angle) affects and is affected by water.

Optimal architecture depends on soil type and water depth, as well as distribution of nutrients and microorganisms.

**Conductance** is affected by root anatomy and morphology.

The distribution of root growth is affected by water availability

Winter wheat grown in regions with different amounts of rainfall

<table>
<thead>
<tr>
<th>Inches</th>
<th>26 – 32 inches</th>
<th>21 – 24 inches</th>
<th>16 – 19 inches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26-32”</td>
<td>21-24”</td>
<td>16-19”</td>
</tr>
</tbody>
</table>

Shallower root systems can be more effective at catching limited rainfall

Non-irrigated soybean plants produce more root mass deeper in the soil

On the other hand, when water is withheld, deeper roots can be more effective at reaching water

Root growth can respond to water gradients (hydrotropism)

Growth of a plant in a uniform water environment is dominated by gravity.

When there is a water potential gradient, the root can exhibit hydrotropic growth – growth towards water.

Growth medium at the bottom of the plate has a very low water potential.

Root conductance is determined by **radial** and **axial** conductance

Radial conductance: From soil to stele

Axial conductance: Through the xylem

Radial conductance is affected by anatomy, morphology, cell wall permeability, activity of water channels, etc.

Axial conductance is affected by the number, diameter and structure of the xylem conduits, as well as formation of embolisms

Water likely moves from soil to stele through multiple paths

- **Apoplastic path (through wall):** No crossing of plasma membrane
- **Symplastic path (through plasmodesmata):** Plasma membrane crossed once
- **Transcellular path:** Plasma membrane crossed many times

Conductance of cell walls and membranes affects water uptake

Key factors that may affect movement through the root cortex:
- The *endodermis*, *exodermis* and water impermeable Casparian strip
- *Aquaporins* (membrane-bound water channels)
- *Plasmodesmata*

The exodermis and endodermis are cell layers with Casparian bands or strips that restrict their conductance of water and solutes.

Plasmodesmata are small plasma membrane–lined cytoplasmic bridges connecting cells.

Aquaporins are regulated water channels in cell membranes.

The Casparian strip forces water to cross a plasma membrane

Water can enter the root through the apoplast (cell wall spaces) until it reaches the Casparian strip, which blocks apoplastic water flow.

Water forced to cross a plasma membrane is effectively filtered.

The root endodermis acts as a filter for incoming water.

The endodermis produces a water-impermeable layer, the Casparian strip, that provides selectivity.

Photo credit: Michael Clayton
Robert Caspary (1865) recognized the significance of this barrier
Summary: Movement of water into and through roots

\[
\text{Flow} = \frac{\Psi_{\text{soil-root interface}} - \Psi_{\text{root xylem}}}{r_{\text{soil-root interface} \rightarrow \text{root xylem}}}
\]

The flow of water into the root is affected by the difference in water potential from the soil-root interface and the root xylem, and the resistance of root tissue to water flow.

Root radial conductance, from soil to stele, is usually lower than axial conductance, and is affected by permeability of membranes and walls, particularly aquaporin activity.
Once the water enters the transpiration stream of the xylem, it moves through the plant by bulk flow, pulled by the tension produced by evaporation at the leaf surface.

- How does the structure of the xylem cells affect the flow of water?
- Why is the xylem a “vulnerable pipeline”?
Transport in bryophytes – some specialized transport cells

Bryophytes take up water from the air and substrate

Water moves along external surfaces, within cells, between cells, and through specialized waterconducting cells or hydroids (h)

Some bryophytes have food-conducting cells or leptoids (l)

The end walls between food-conducting cells can have enlarged plasmodesmata-

Hydroids (h) in cross section

Hollow conducting tissues allow greater height and CO₂ assimilation

Lycophytes (~ 1200 species)  Ferns (~ 13,000 species)  Gymnosperms (~ 1000 species)  Angiosperms (~ 350,000 species)

Bryophytes  Vascular tissue (> 400 million years ago)  Seeds

Photos by Tom Donald
Fossil record: Xylem conduits became wider and more complex.

There also has been an increase in proportion of lignified, degradation-resistant cell wall material.

The secondary walls are reinforced by lignin, a complex, hydrophobic polymer.

Lignin-strengthened cell walls are one of the major features that lets plants get big.

Lignified xylem provides structural support for vascular plants

The tallest living trees tower over many familiar monuments.

115 m Sequoia sempervirens

St. Paul’s Cathedral 111 m

Statue of Liberty 93 m

Sydney Opera House 65 m

Taj Mahal 65 m
Development and differentiation of tracheary elements

Tracheary elements have been described as “functional corpses”
Most seed plants (and a few others) can produce secondary xylem.

- Lycopods
- Ferns and relatives
- Gymnosperms
  - Angiosperms
  - Monocots

Most cannot produce secondary xylem, but there are exceptions.

The vascular cambium is a lateral meristem that produces new xylem and phloem.

The xylem produced by the vascular cambium allows the plant to grow in the radial dimension and replaces older, less functional xylem.

Secondary thickening produces new xylem and lets plants increase in girth

These trees are *Sequoiadendron giganteum*, giant sequoias
How does xylem structure affect its function?

• Xylem conductance is a function of
  – Distribution of diameters of elements
  – Element length
  – Number of vessels participating
  – Resistance to transfer between elements

Xylem anatomy: Hollow, thickened conducting cells

**Tracheids** (gymnosperm) are narrow, up to 1 cm in length, and perforated by complex pit membranes. 

**Vessels** (angiosperm) are made from **vessel elements**, which are wide, short, perforated by simple pit membranes, and have open or perforated end walls.

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Tracheids and vessels are quite different in size

Poiseuille’s Law: The flow of fluid through a tube scales to the fourth power of the radius of the tube (Flow $\propto r^4$)

Conifer tracheids are significantly shorter and narrower (but with some overlap in the width) than angiosperm vessels.

What other factors are involved in hydraulic conductivity?

Xylem is a “vulnerable pipeline”, prone to embolism

The ability to lift water depends on a continuous column of water.

Air can enter a conduit and expand to form an air-vapor blockage that breaks the water column, a condition known as *embolism*.
Cavitation describes a condition wherein an air bubble moves into a vessel.

Embolisms can spread between vessels

Embolisms (dark; water-vapor filled conduits) in an intact grapevine stem, made visible by high-resolution computed tomography.

Embolisms spread from conduit to conduit; a pathway connecting the embolized vessels is shown in yellow.

Pit membranes permit water flow and limit embolism spread

Some pits are more embolism-resistant than others

Vulnerability curves characterize the sensitivity of a plant to embolism.

Different species and tissues produce different “vulnerability curves”

In most plants the xylem can either have a high hydraulic efficiency or be resistant to embolism formation, but not both, and the functionality of the xylem is correlated with the environment a plant is adapted to.

Many plants experience a seasonal loss and recovery of conductivity

Winter –
Freezing-induced embolisms

Early spring –
Root-pressure refilling

Late spring –
New xylem formation

Freezing-induced embolisms decrease conductivity

Spring activation of cambium increases conductance

Root pressure refills xylem and increases conductance

Vulnerability to embolism is ecologically relevant

A comparison of vulnerability curves for six species from diverse climates:
- Ceanothus megacarpus
- Juniperus virginiana (red cedar)
- Rhizophora mangle (mangrove)
- Acer saccharum (sugar maple)
- Thuja occidentalis (white cedar)
- Populus deltoides (cottonwood)

In general, plants from drier climates tend to have lower $\Psi_{50}$ and be more resistant to cavitation.

$\Psi_{50}$ is a measure of the plants’ sensitivity to embolism.

Increasing proportion of cavitated conduits.

Increasing tension (decreasing $\psi_p$).

There is a correlation between habitat and cavitation resistance

Less resistance to cavitation (↑) is correlated with wetter climate (→)

Less resistance to cavitation (→) is correlated with less negative normal xylem pressure experienced (↑). Gymnosperms tend to have larger “hydraulic safety margins”

The environment affects xylem tension

Scholander observed that in spite of their roots being immersed in seawater, the xylem sap of mangroves contains very little salt.

He hypothesized that the xylem pressure must be very low to draw water in against its concentration gradient.

$\psi_{\text{π}} = -2.5 \text{ MPa}$

$\psi_{\text{π}} = -2.5 \text{ MPa}$

$\psi_{\text{π}} = -0.25 \text{ MPa}$

90% of the salt in seawater is filtered out before it reaches the xylem.
Scholander showed that tension in the xylem drives water uptake.

<table>
<thead>
<tr>
<th>Component</th>
<th>Soil Water Potential ($\psi_w$)</th>
<th>Root Pressure Potential ($\psi_p$)</th>
<th>Leaf Turgor Pressure Potential ($\psi_T$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>$-2.5$ MPa</td>
<td>$0$ MPa</td>
<td>$-2.5$ MPa</td>
</tr>
<tr>
<td>Root cortex</td>
<td>$-2.5$ MPa</td>
<td>$0$ MPa</td>
<td>$-0.25$ MPa</td>
</tr>
<tr>
<td>Xylem</td>
<td>$-2.5$ MPa</td>
<td>$-2.3$ MPa</td>
<td>$-2.5$ MPa</td>
</tr>
</tbody>
</table>
Tension in the xylem is balanced by solute accumulation in the leaves.

Water in leaf cells usually has a lower osmotic potential than seawater!

90% of the salt in seawater is filtered out before it reaches the xylem.
Does xylem conductance limit the height of trees?

\[ \Psi_w = \Psi_p + \Psi_{\pi} + \Psi_g \]

Gravitational potential lowers \( \Psi_w \) by -0.01 MPa per m height

Open question:
Does water limitation and its effects on photosynthesis prevent trees from reaching more than ~130 m?
In the winter, the xylem vessels of grapevines are filled with air.

In the summer, transpiration causes tension in the fluid-filled xylem vessels.
Hollow vessels fill with water in the spring due to root pressure

In the spring, before the leaves emerge, the hollow vessels fill with water, driven by pressure generated in the roots.

Pressure is so high, cut vines exude water copiously and are said to "bleed".

In the summer, when the leaves are out and the plant is transpiring, xylem pressure is negative (tension).

Stem pressure and the flow of sap in maple trees (*Acer saccharum*)

In the early spring, when the nights are below freezing and the days are above freezing, in the daytime sweet sap flows from holes drilled into the bark of sugar maples (*Acer saccharum*)

People have been collecting the sugar-rich sap from maple trees in the north east of North America for hundreds of years. *Where does it come from?*

Photo credit: University of Minnesota; T. Davis Sydnor, The Ohio State, Bugwood.org
Clues to the source of maple sap

Maple sap flows in early spring, before leaves emerge, suggesting it is not driven by evaporative transpiration.

When a branch is cut, sap flows from the leafy end, not the root end, suggesting sap flow is not driven by root pressure.
As long as water is provided, sap flow does not require roots or crown

Cut branches set onto dry surfaces, indicating that a source of water is required

Sap flow requires temperatures alternating above and below freezing.

The Milburn & O’Malley model

Cooling: Vapor compresses, water drawn into fibers

Frozen: Ice traps vapor at high pressure

Warming: Thawing ice releases pressurized vapor and forces sap down

In sugar maple the xylem fibers surrounding the vessel elements are usually filled with air.
2008 model: Contribution of sugars in minimally pitted vessels

Pressure from trapped gases aren’t sufficient. Osmotic pressure due to sucrose in the vessel, trapped by minimal pit connections to fibers, can explain the continued flow of sap after thawing.

Bordered pits $P_{[b]}$ and half-bordered pits $P_{[hb]}$ could allow sugar (orange arrows) to move into but not out of the vessel elements, and water (blue) to pass freely.

Net influx of water generates pressure.
Summary: Water flow through xylem, a low-resistance pathway

- Xylem evolution reflects trade-offs between safety and efficiency
- Angiosperms sometimes have larger conduits
- Gymnosperms can move water from conduit-to-conduit more efficiently
- Preventing embolisms is critical to plant success
Soil – Plant – Atmosphere Continuum 3: From leaf to air

Three elements to water flow in leaves:
1. Through veins
2. From vein to stomata
3. Out through stomata

Resistance diagram for water flow in a leaf. From the end of the vein to the stomatal pore, water can move through an apoplastic or symplastic pathway.

Leaf conductance ($K_{leaf}$): Xylem and out-of-xylem conductance

Leaf conductance ($K_{leaf}$) is affected by xylem conductance ($K_x$) and out-of-xylem conductance ($K_{ox}$).

$K_x$ is determined by xylem conduit diameter, abundance, anatomy, etc.

$K_{ox}$ is determined by the conductance of the non-vascular cells, the path length from vein to stomata, and stomatal density and conductance.

Leaf xylem anatomy affects leaf hydraulic properties

Dicot leaves with reticulate vein patterns, showing increasing vein densities

Monocot leaves with parallel veins at two different densities

Dichotomously branching veins of ferns

The distance from the end of the conducting cells to the air matters

\[ K_{leaf} \] of angiosperms is correlated with a short distance from the end of the vein xylem to the gas-exchange epidermis \((D_m)\)

Water conducting sclereids shorten $D_m$ and increase $K_{leaf}$

Some gymnosperms (circled) produce lignified transfusion tissue (a.k.a. water-conducting sclereids) that serve as hydraulic shortcuts.

The water-conducting sclereids (stained blue) provide a hydraulic shortcut from the vein (V) to the stoma (*) in this *Podocarpus dispermis*.

Leaf and small branch xylem can act as “disposable fuses”

Increased vulnerability to embolism in small (< 6 mm diameter) vs large (> 6 mm) branches in Acer saccharum

Increased vulnerability to embolism in leaves (●) vs stems (○) in two tree species

In conifer tracheids, xylem collapse in the needles protects stem xylem

$K_{ox}$: Water leaving the xylem passes through bundle sheath cells

Water leaving the xylem moves into bundle sheath cells, and from there into the symplast or other cells. Bundle sheath cells may contribute to controlling water and solute flow into the leaf tissues.

Aquaporins contribute to radial conductance in the leaf

Aquaporin activity regulates resistance of the outside-of-xylem pathway

The flow of water from bundle sheath cells to the stomata is affected by aquaporin activities

Guard cells are the gatekeepers of transpiration

Water flow requires tension produced by water exiting the leaf, and water can only* exit the leaf when the guard cells are open (*with an effective cuticle very little water is lost via non-stomatal transpiration)

As an analogy, it doesn’t matter how quickly you can jump out of your airplane seat, until the door opens, you’re not going anywhere

Closed stomata – no transpiration, no flow

Open stomata – flow regulated by other factors
Guard cells are the portals through which CO$_2$ enters and H$_2$O exits.
Guard cells movements are controlled by ion currents

When signalled to close, ion channels in the vacuolar and plasma membranes open, releasing ions from the cell.
Exiting ions draw water out of the cell

Water follows by osmosis
The guard cells lose turgor pressure and relax, closing the pore

The cell volume shrinks and the cell shape changes, closing the stomatal pore
What regulates stomatal aperture?

Guard cells respond to the hormone abscisic acid (ABA), light, and [CO₂]

Guard cells also respond to hydraulic signals and a change in ψ_w

Stomatal phylogeny, anatomy and morphology affect function

Stomatal crypts increase humidity outside stomata, reducing flow when the stomata are open.

In some grasses, subsidiary cells participate in guard cell movement and make the pores more efficient.

From leaf to air: Summary

Water movement in leaves occurs through three distinct segments:

- Movement through the vascular tissues
- Movement through the non-vascular tissues
- Movement through the stomata
Drought, hydraulic failure and what it all means

Plants are well adapted for their own environment, but may not tolerate a drier one

As an example, two species of Quercus (oak) showing different sensitivities to water deficit. Quercus robur is drought sensitive and in Europe shows a more northern distribution.

A disturbing and widespread increase in tree mortality is occurring

This trend is taking place world-wide

Dots show locations of documented mortality that have been associated with drought since 1970

Examples shown in Korea, China and Turkey

Drought has pleiotropic effects and increases vulnerability to pests

The anticipated combination of drier and hotter climate will be particularly detrimental to plants

Water Uptake and Transport: Summary

From **dynamic root growth patterns**, to **regulated aquaporin channels**, with an amazing **integration of xylem structure and function** and **sophisticated leaf anatomy**, the mechanisms by which plants take up and transport water are **spectacular**.

Transpiration rate can be measured with a porometer, which measures water vapor release from the leaf.

Transpiration rate can be determined by water loss from a sealed pot (by weighing), or measuring water removed from a source using a potometer ("drink" meter).

Pressure to balance xylem tension can be measured in pressure bomb

The Scholander “pressure bomb”

The total water potential of leaf cells is transduced into tension at the cut surface

A single xylem conduit showing water pulling away from the cut surface

Using the pressure bomb, the internal tension of the xylem can be measured by the balancing pressure required to extrude water from the cut surface

A pressure probe can also measure xylem tension, but it is difficult.

The probe records tension when the xylem is impaled.

Flow rate can be measured by the rate at which heat moves.

Heat-based methods can determine the rate of xylem flow. The rate of heat movement from a source is correlated with flow rate.

Granier Sap Flow System (PS-TDP8, PlantSensors)
Conductance can be measured as flow through a segment

Conductance = Flow / Δ Pressure
For a given pressure, more flow (more water passed through the segment) indicates more conductance

Flow calculated by volume of water moving through the segment

The height of the water source can be raised or lowered to change the pressure.

Balance to weigh water

Stem segment

Protoplast-swelling assay measures cell membrane water conductance

How fast does the cell swell?

Wild-type cell with many open aquaporins

Loss-of-function of PIP1;2

The rate of volume change in response to a change in water potential indicates membrane water permeability.

Loss-of-function of aquaporin PIP1;2 decreases protoplast water permeability ($P_f$), as measured by the change in cell volume.


Percentage loss of conductivity can be measured by lowering $\psi_w$

A comparison of vulnerability curves for six species from diverse climates:
- *Ceanothus megacarpus*
- *Juniperus virginiana* (red cedar)
- *Rhizophora mangle* (mangrove)
- *Acer saccharum* (sugar maple)
- *Thuja occidentalis* (white cedar)
- *Populus deltoides* (cottonwood)

Plants from drier climates tend to lose conductivity at lower $\psi_w$, indicating less vulnerability to embolism formation

Xylem $\psi_w$ can be lowered by drying or centrifugation

The spinning method allows measurements to be carried out more quickly. A stem segment is rotated at various speeds, exerting tension on the xylem sap.

The original method involved letting the segment slowly dehydrate. Flow was measured repeatedly as the stem dried and xylem water pressure decreased.

% Loss xylem conductance

Increasing proportion of cavitated conduits

Xylem water pressure (MPa)

Stem segments in centrifuge rotor

Maximal conductance can be measured after refilling embolisms

Step 1. Measure flow in partially embolized segment

Step 2. Push water through segment at high pressure to clear embolisms

Step 3. Measure flow in de-embolized segments

Embolism visualization by Cryo-SEM and dye uptake studies

Cryo-SEM involves freezing tissue and then slicing it to see if air or ice is in the conduits.

- **Ice** (indicates embolism)
- **Air**

Ultrasonic acoustic emissions – sound produced when embolisms form

- Dye is drawn into functioning conduits
- Dye does not enter embolized conduits

3D and 4D images of vascular tissues can be obtained using X-rays.

2D scans are reconstructed into 3D forms using a computer.