Transpiration LAB

Phaseolus vulgaris L.

(Bean plant)

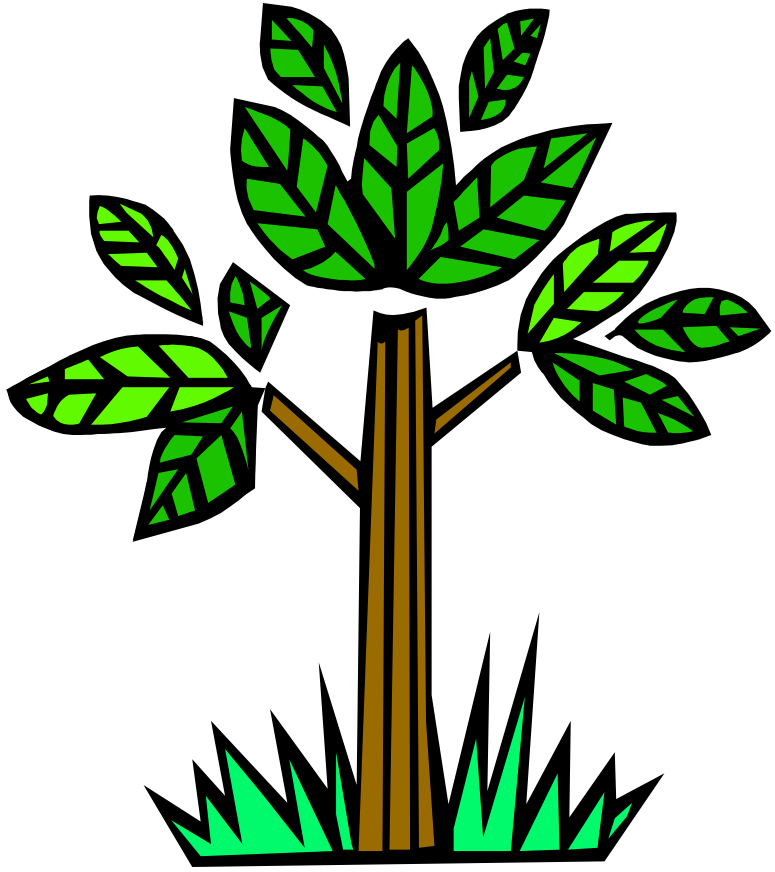
Objectives

- Investigate the effect of low light and high light intensity on the rate of transpiration from leaves.
- Investigate the effect of abscisic acid (ABA) on the rate of transpiration from leaves.
- Make and view slides of plant root, stem, and leaf sections using bright field and fluorescence microscopy.
- Examine epidermal peels under the microscope.

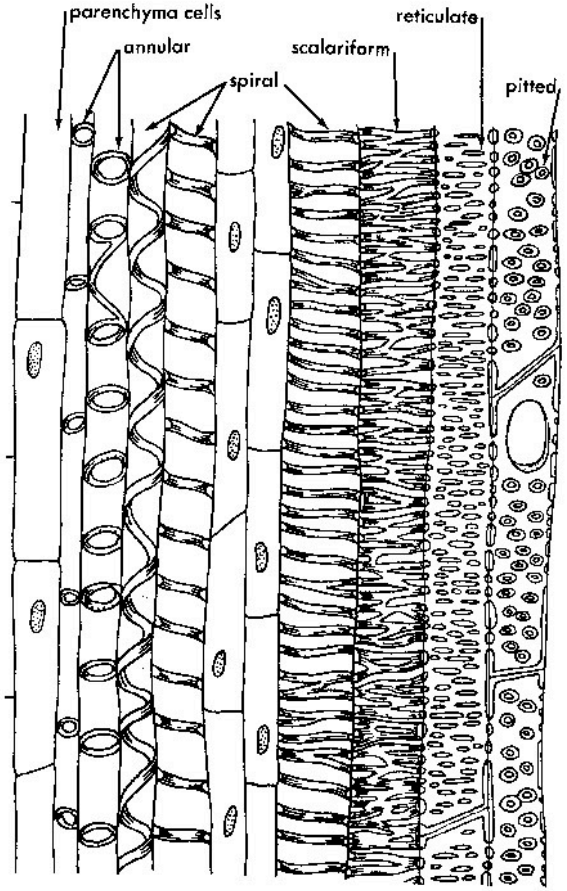
PHOTOSYNTHESIS

- $6CO_2 + 6H_2O + LIGHT \rightarrow C_6H_{12}O_6 + 6O_2$
- CO_2 intake from the air via leaf
- H_2O intake by roots and released at leaf
- LIGHT sunlight on leaf
- O_2 released via leaf

Plant Terms…



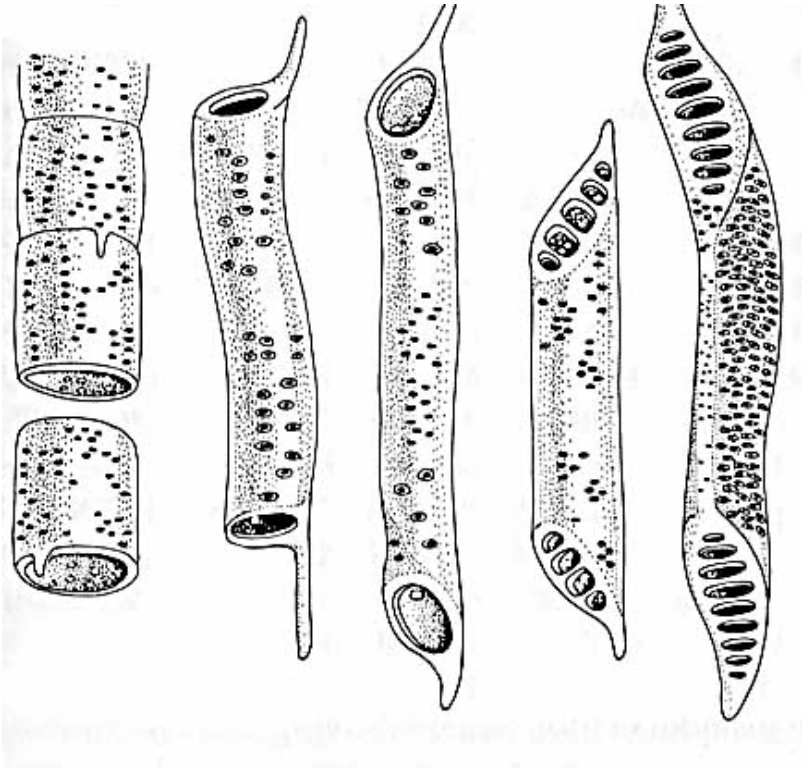
XYLEM



- Complex vascular tissue through which most of the water and minerals are conducted from the roots to other parts of the plant.
- Cells are dead.

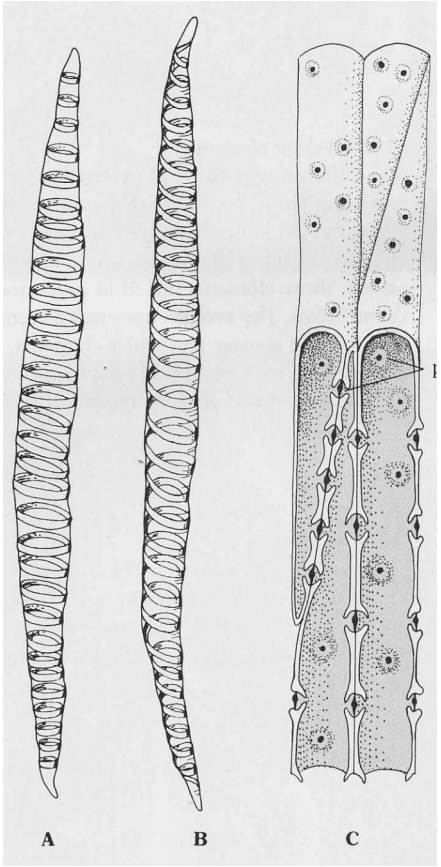
XYLEM ELEMENTS

- ***Vessels**: Elongated, cells placed end to end. Walls at end of vessel members are perforated or are completly missing. Found in most angiosperms.



XYLEM ELEMENTS

- **Tracheid :**
  Elongated, thick-walled conducting cells of xylem, characterized by tapering ends and pitted walls without true perforations.

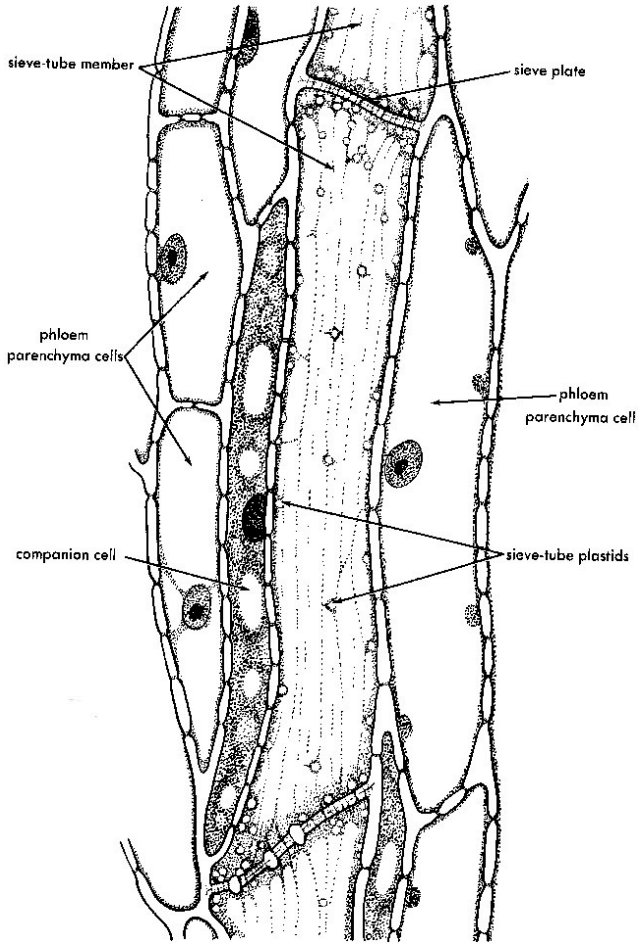


LIGNIN

- Hard supporting material embedded in the cellulose matrix of plant cell walls. Makes up xylem inner layer of cell (secondary) walls
- Second most common plant polymer
- Autofluoresces blue under UV light

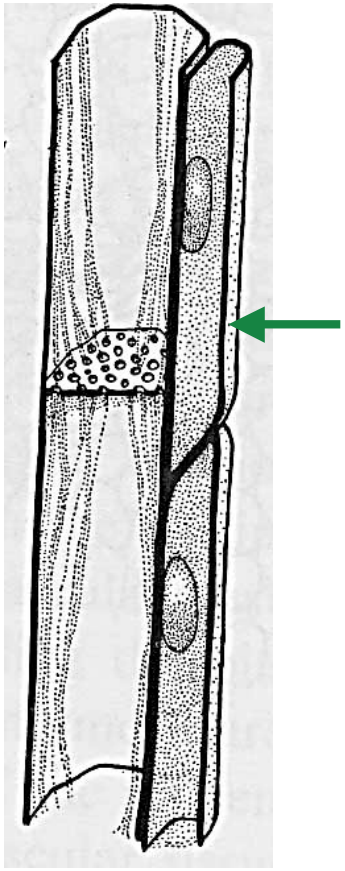
Phloem

- Vascular tissue that conducts sugars and other organic molecules from the leaf to the other parts of the plant.



Sieve tubes

- Cells of phloem that line up connecting leaves, shoots, and roots.
- Cells contain little cytoplasm (which is continuous between cells) and no nucleus.
- Food materials pass from one element to another via sieve plates (perforations).



•Companion cell: a parenchyma* cell that controls metabolic activities of sieve elements.

PARENCHYMA

- Plant tissue composed of spongy, living, thin-walled randomly arranged cells with large vacuoles. Usually photosynthetic or storage tissue.
- Most common type of cell in pants

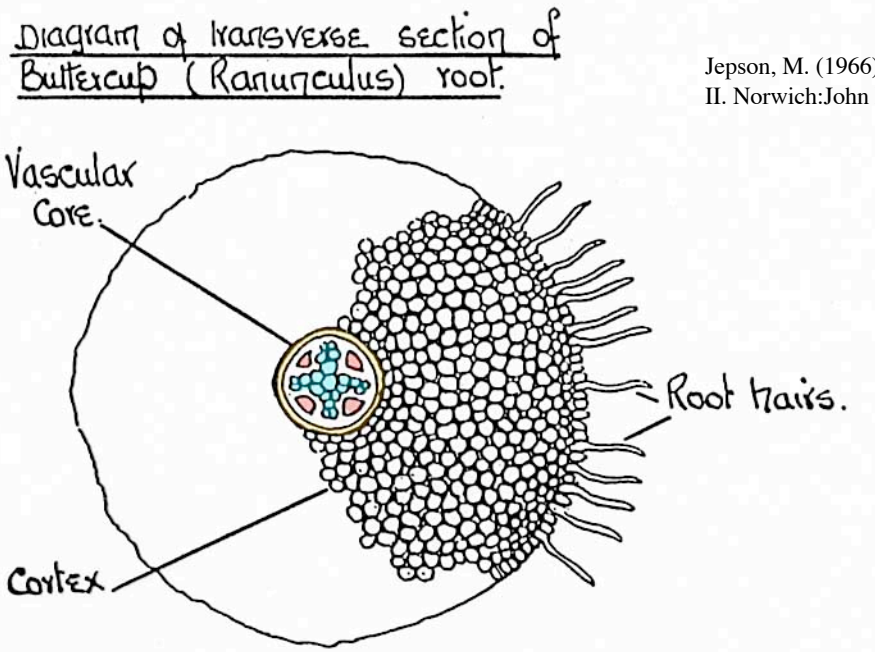
A = Xylem B = Phloem

- Composed of dead cells
- Cytoplasm of one cell joined to adjacent cells
- Carries water
- Carries sugars and organic molecules
- Wall fluoresce blue under UV light

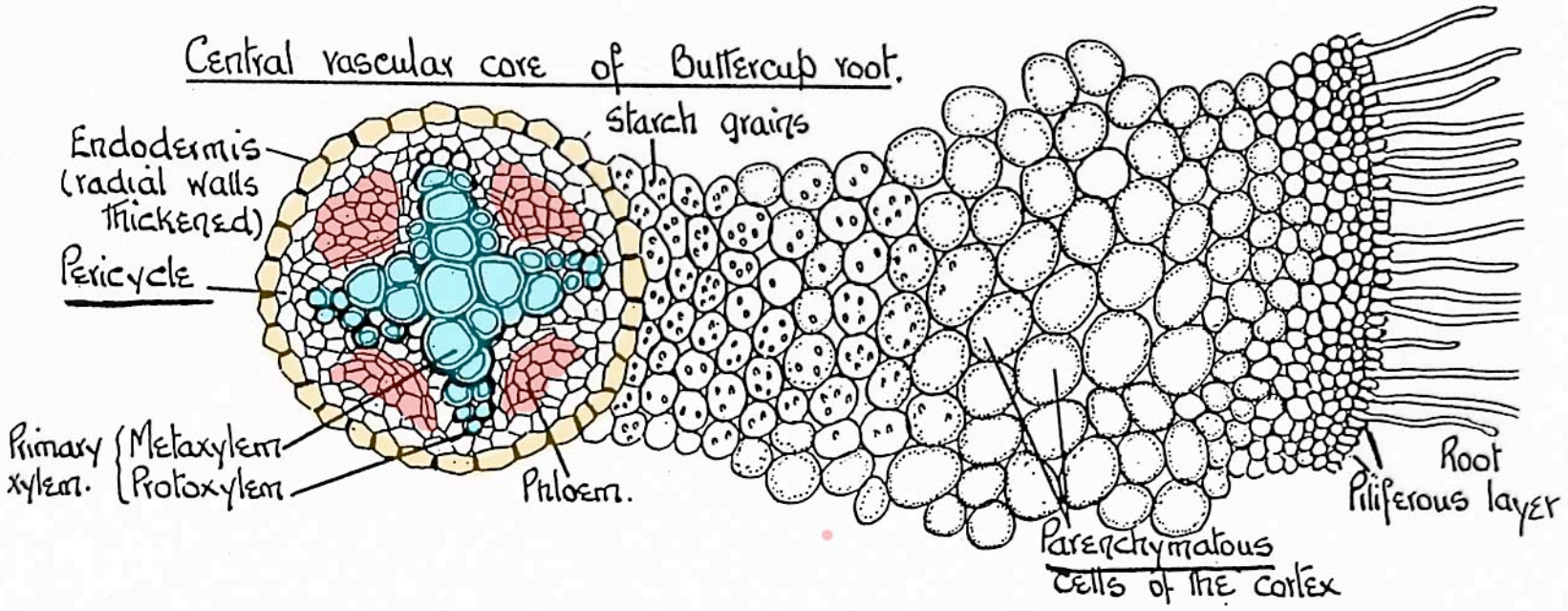
Root Morphology

- **Epidermis** (outer tissue absorbs water and minerals from soil)
- **Cortex** (ground tissue of root, storage parenchyma)
- **Endodermis** (compact cells with no space between them & encircled by continuous band of wax, Casparian strip)
- **Vascular Cylinder** (xylem & phloem)

Casparian strip:
Thickened, waxy strip that extends around and seals the walls of endodermal cells in roots or plants. Restricts diffusion of solutes across the endodermis into vascular tissues of the root.



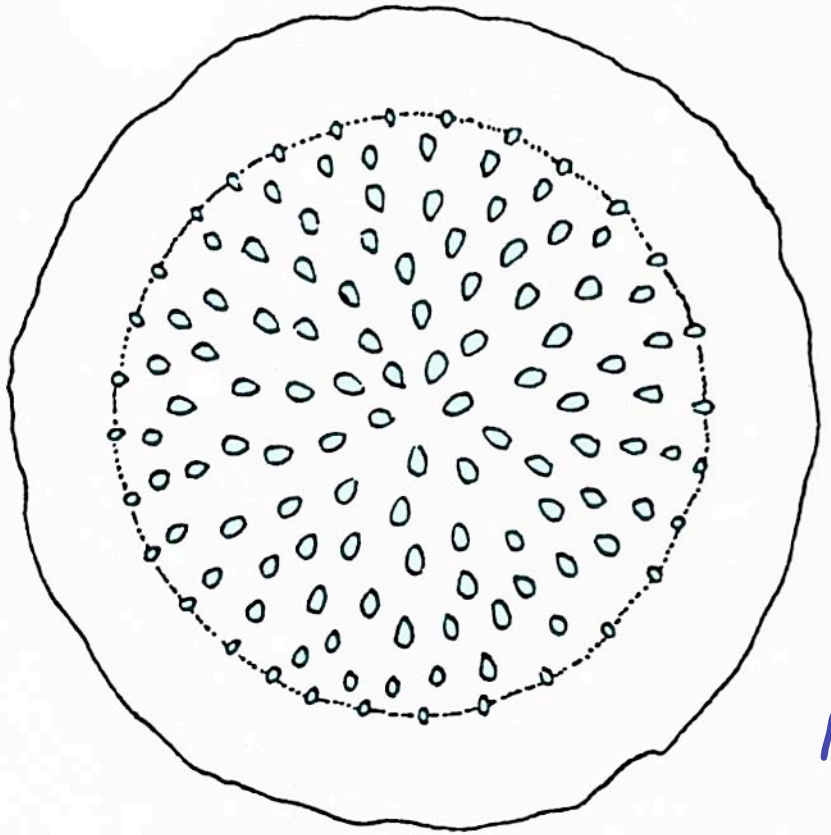
Jepson, M. (1966). *Biological Drawings with Notes*, Parts I & II. Norwich:John Murray (Publishers) Ltd., pp.22-23.



Vascular Bundle

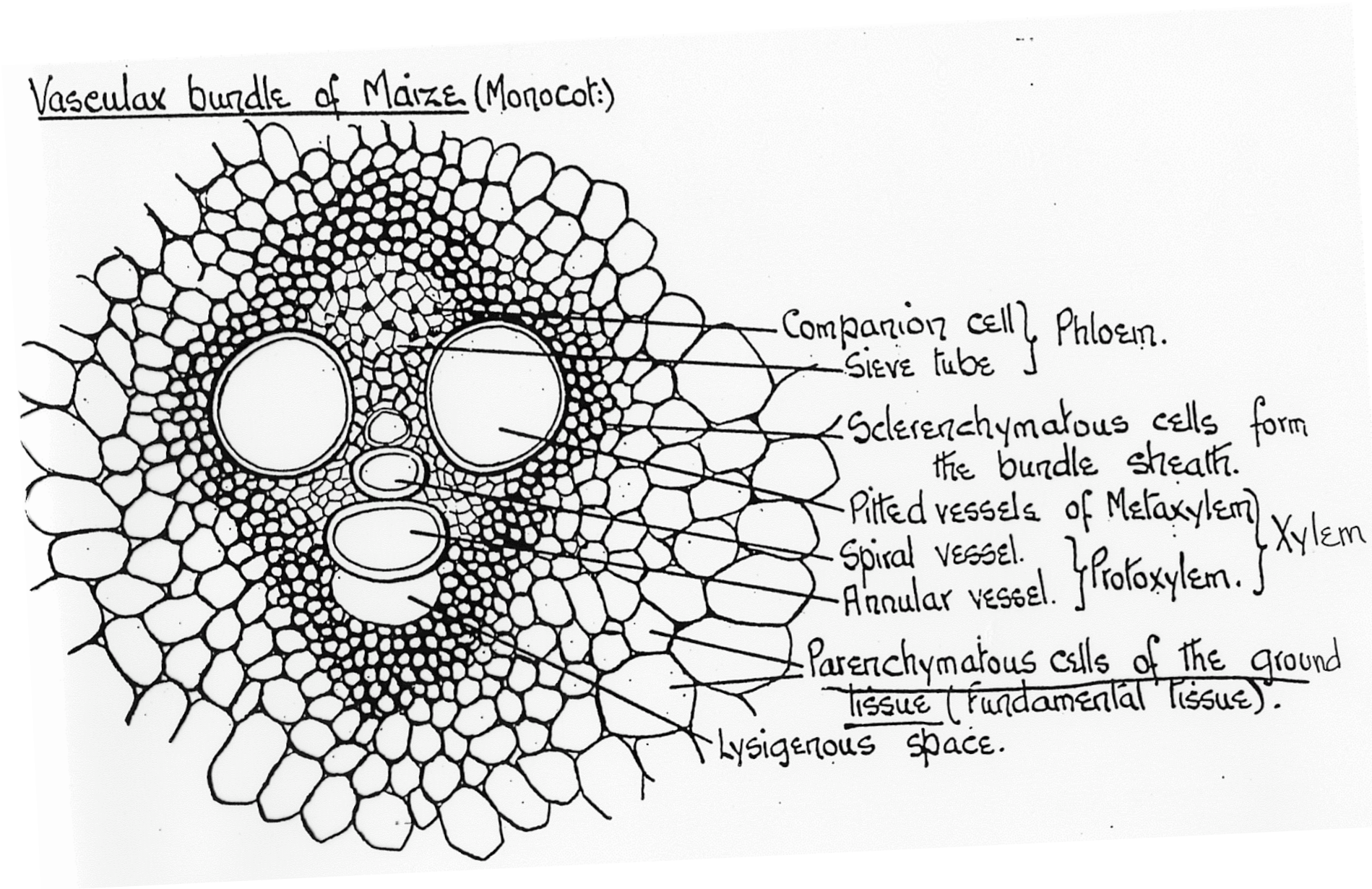
- Long continuous strands of conducting tissue in plants.
- Consist of xylem and phloem tissue close to each other.

Diagram of the transverse section of Butcher's Broom (Ruscus) showing the scattered arrangement of the vascular bundles, which is characteristic of Monocotyledons.



Monocot stem

Jepson, M. (1966). *Biological Drawings with Notes*, Parts I & II. Norwich:John Murray (Publishers) Ltd., pp.22-23.



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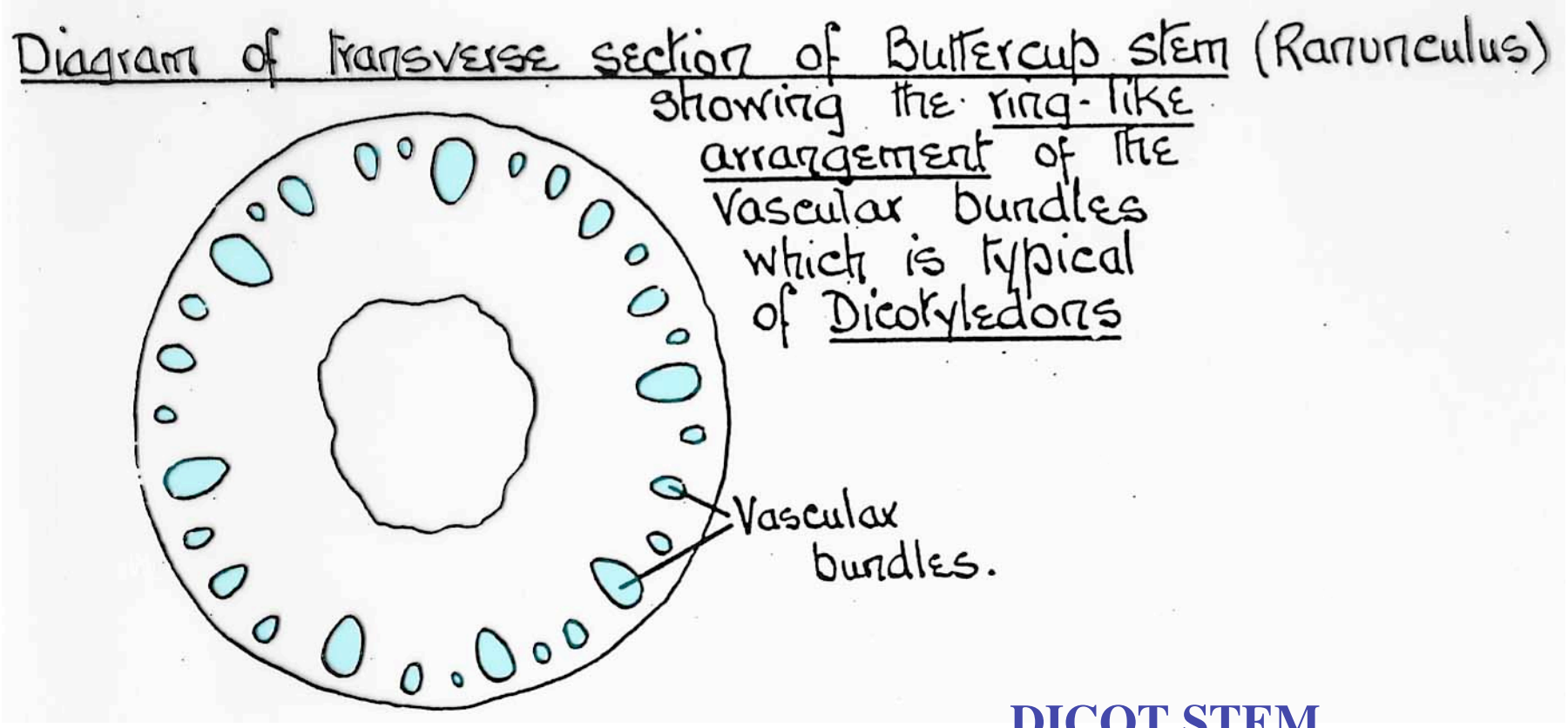
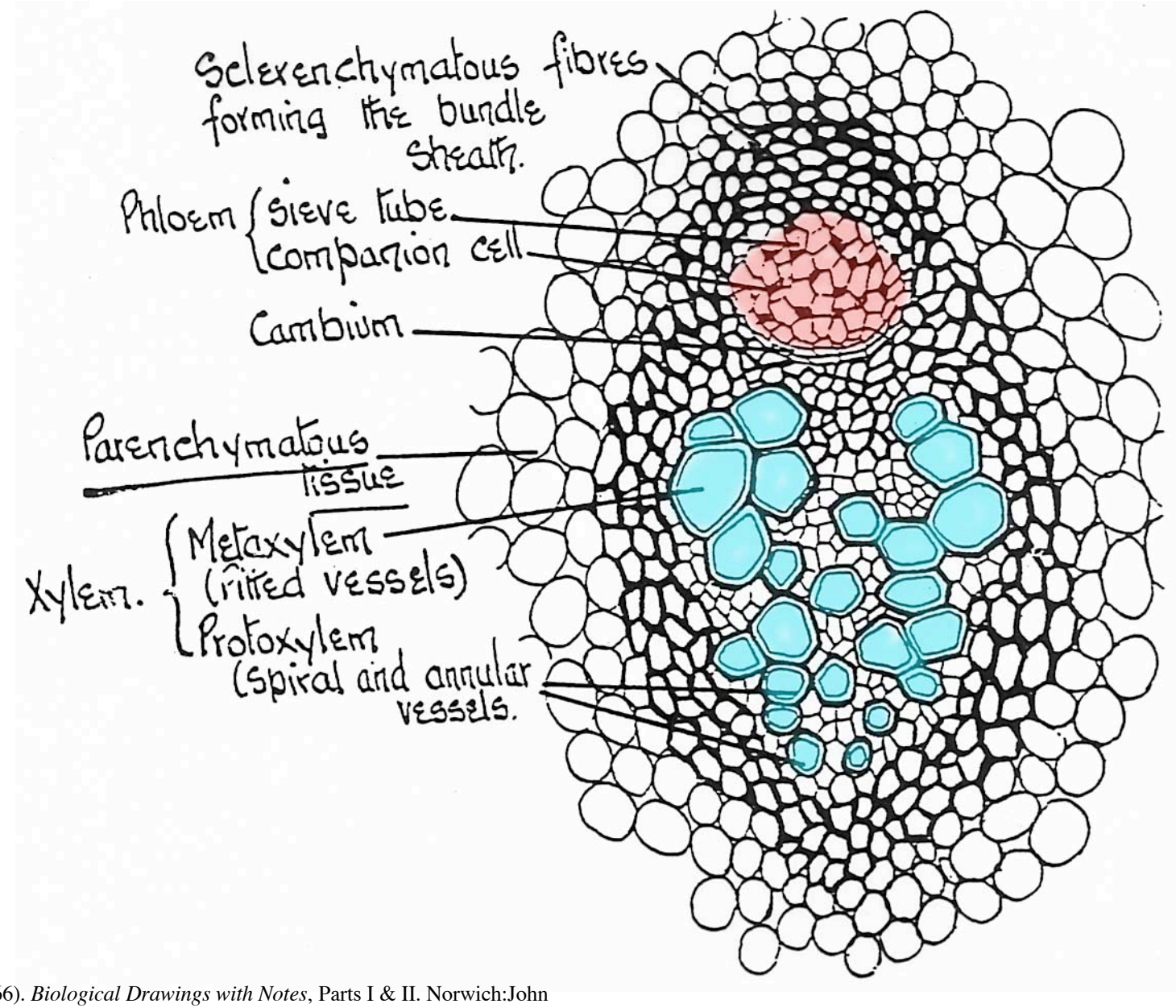


DICOT STEM

Jepson, M. (1966). *Biological Drawings with Notes*, Parts I & II. Norwich:John Murray (Publishers) Ltd., pp.22-23.



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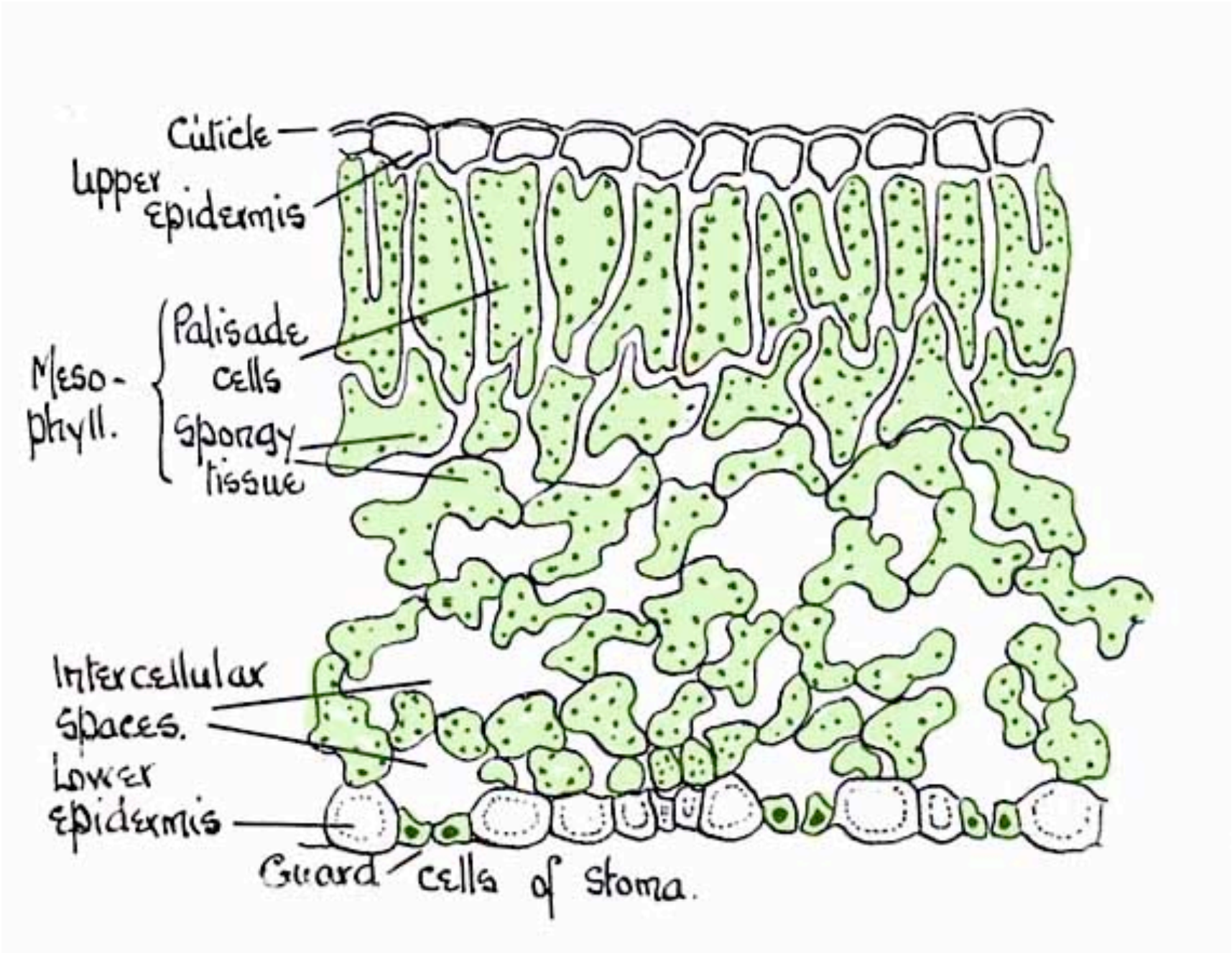
Leaves

- Usually the site of photosynthesis and transpiration.
- Epidermal cells
- Mesophyll (middle leaf)
  - **Palisade parenchyma**
  - **Spongy parenchyma**
- Stomata (composed of guard cells)



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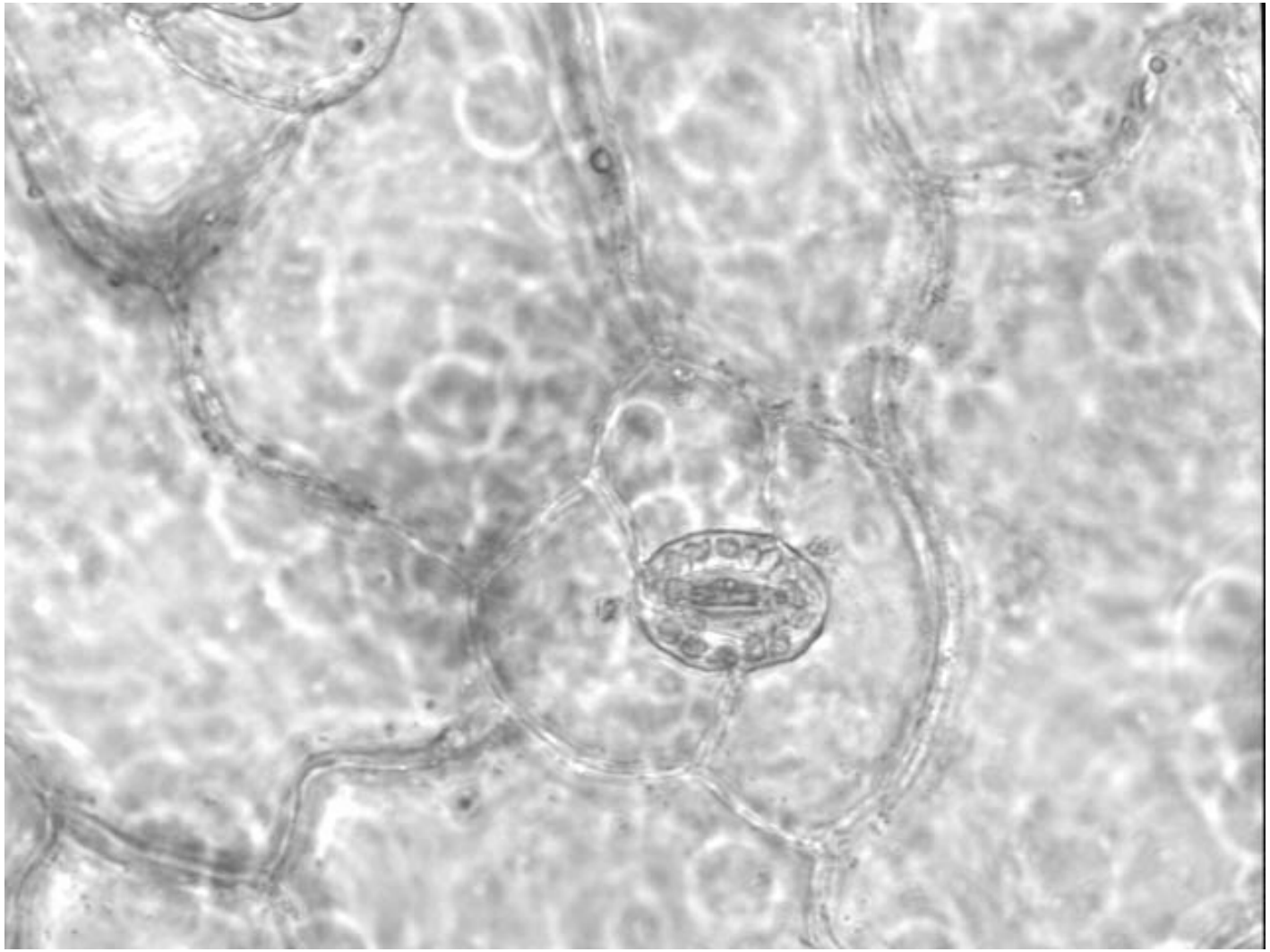
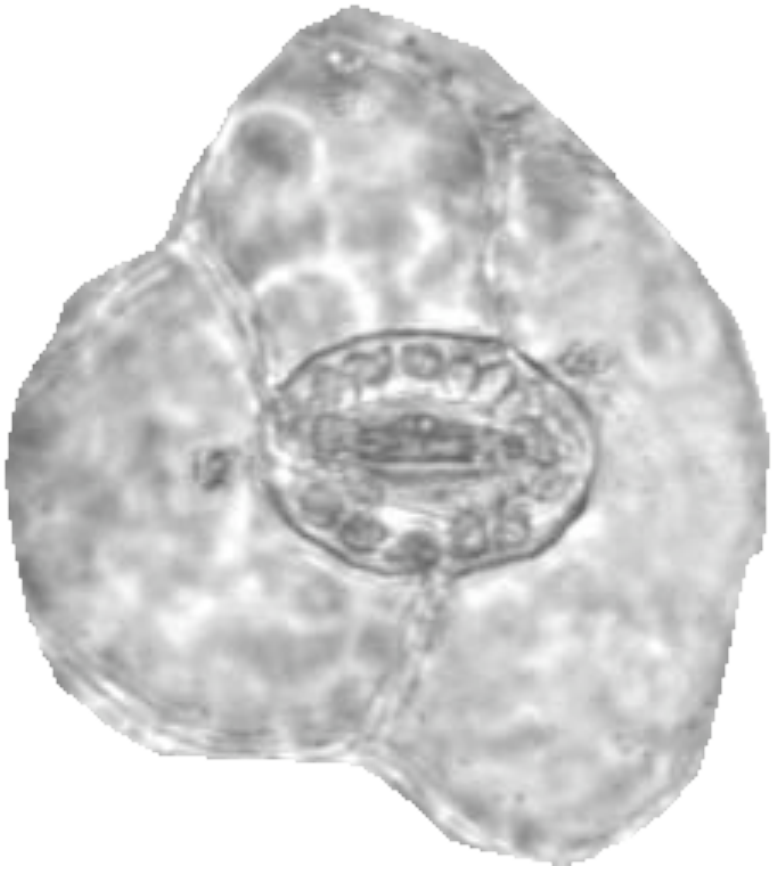
Stomata



Guard Cells

- Guard cells (special type of epidermal cell)
- Paired cells attached to each other at ends
- Stoma/stomata (opening)
- Stoma/stomata (paired guard cells plus pore)
- Guard cells contain chloroplasts



Guard Cells

Changes within guard cells control stoma opening.

- **FACTORS that cause changes**
  - **Light (blue)**
  - **Temperature**
  - **Relative humidity**
  - **Abscisic acid**
  - **Carbon dioxide**

Factors causing stomata to open

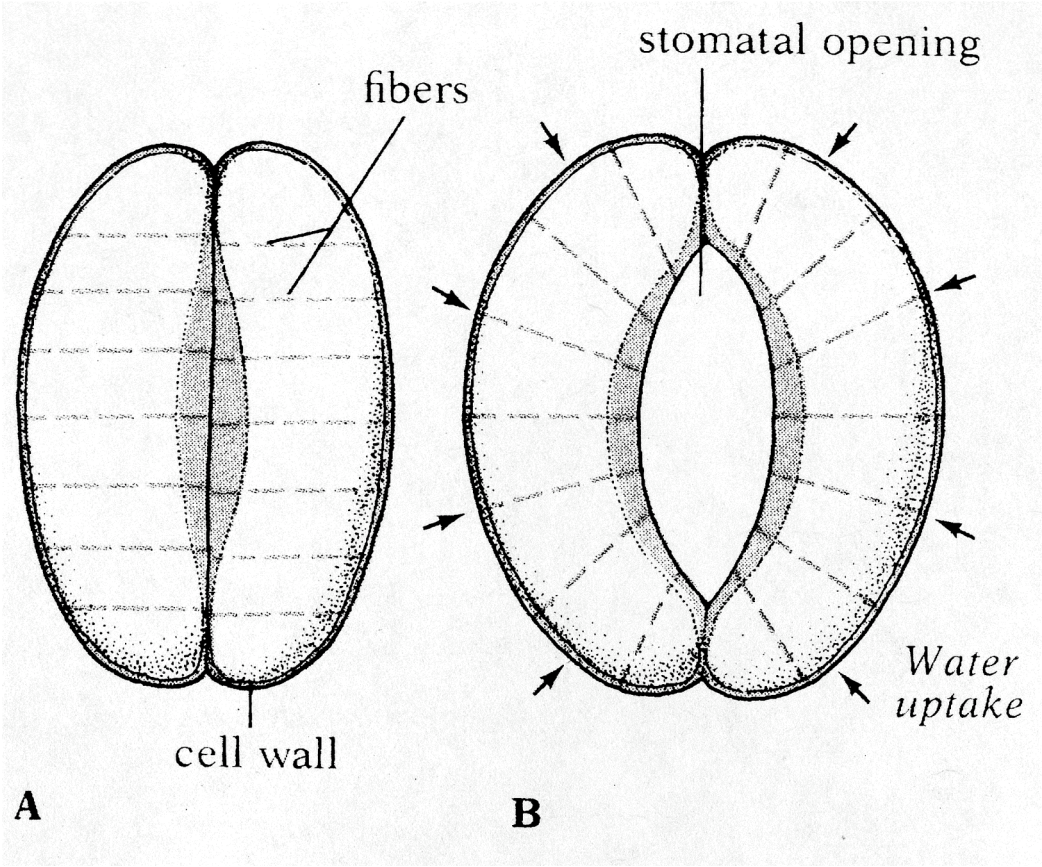
- General: Open in day and close at night
- Cues to open at dawn
  - Blue-light receptors in guard cell
    - Stimulate proton pumps promoting uptake of K^+
    - Drive photosynthesis and lower [CO_2]
  - Low [CO_2]
  - Circadian rhythms (internal clock)

Factors causing stomata to close

- Environmental stress (excessive transpiration)
  - Dehydration
  - ABA
- High [CO_2]
- Temperature (works both ways)
  - Increase rate of photosynthesis = ↓ [CO_2]
  - Increase rate of respiration = ↑ [CO_2]

Cellulose microfibrils arranged in loops around guard cells and uneven thickness of walls prevent radial expansion but allows lengthwise expansion.

flaccid



turgid

Keeton, WT & Gould, JL (1986). Biological Science, NY: WW Norton, p. 276.

*Where are the Stoma?

	Ave. # Stomata/cm^2	Ave. # Stomata/cm^2
Leaf type	Upper side	Lower side
Bean	4000	28,100
Apple	0	29,400
Tomato	1,200	13,000

*Robbins, Weier & Stocking (1966). *Botany: An introduction to plant science*, NY: John Wiley & Sons, p. 145.

Hydropassive Closure

- Low humidity air dehydrates guard cells.
- Guard cells lose tugor and close.

Hydroactive Closure

- Guard cells have CO_2 sensors
- Leaf needs CO_2 for photosynthesis.
- When CO_2 concentration drops, guard cells gain tugor pressure and stomata open.
- When CO_2 concentration increases, guard cell lose tugor pressure and stomata close.

Hydroactive Closure

- Guard cells have ABA sensors.
- ABA accumulates in chloroplasts of mesophyll cells.
- Mesophyll becomes mildly dehydrated:

  a. Stored ABA released outside of cell so it can stream to guard cells.

  b. Rate of ABA synthesis increases

Abscisic Acid (ABA)

- Originally thought to cause abscission of fruits.
- ABA can be transported in xylem and phloem and thus move up and down the stem.
- ABA production is initiated by stresses such as water loss or freezing temperatures.

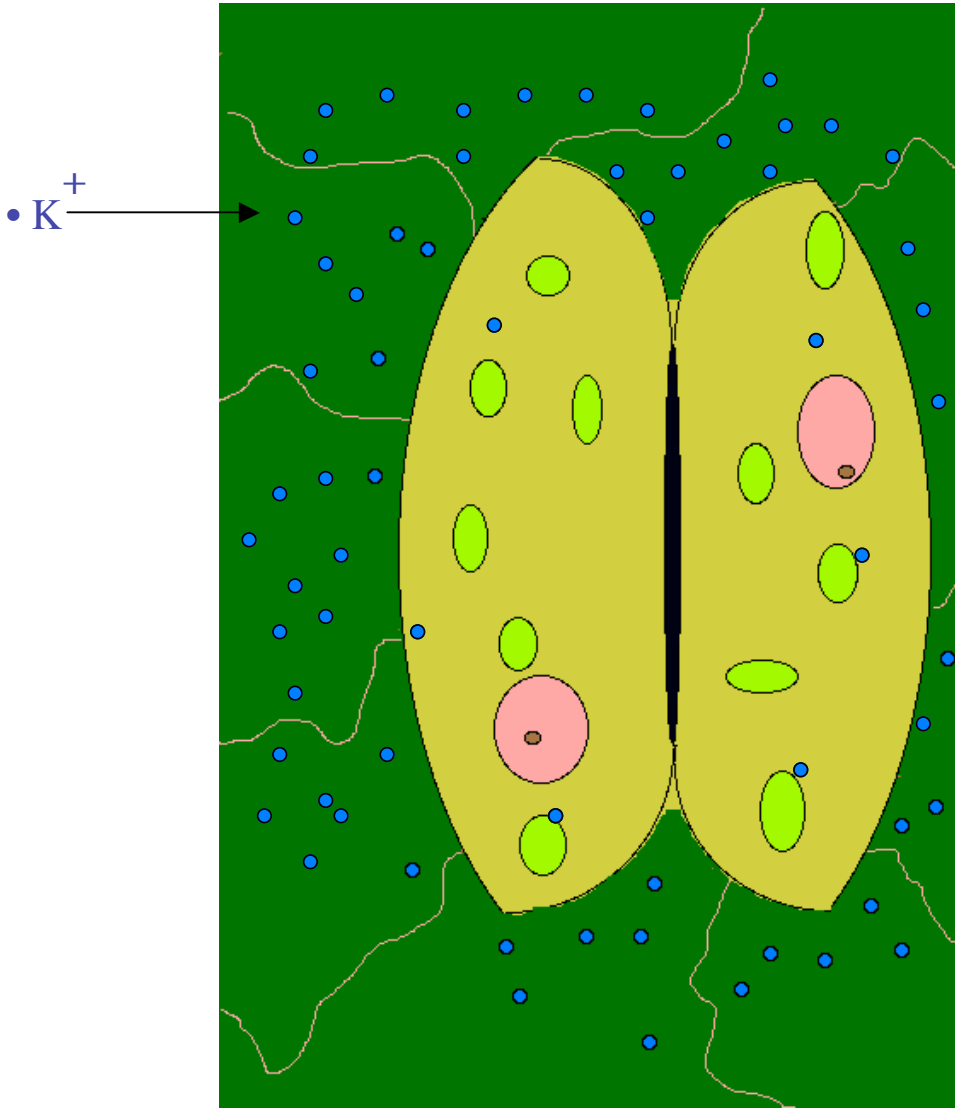
Some Functions of Abscisic Acid

- 1. Stimulates the closure of stomata.
- 2. Inhibits shoot growth (not root).
- 3. Induces seed to synthesize storage proteins.
- 4. Inhibits the affect of gibberellins.
- 5. Initiates and maintains dormancy.
- 6. Induces gene transcription for proteinase inhibitors in response injury.

What actually causes water to flow in and out of cells?

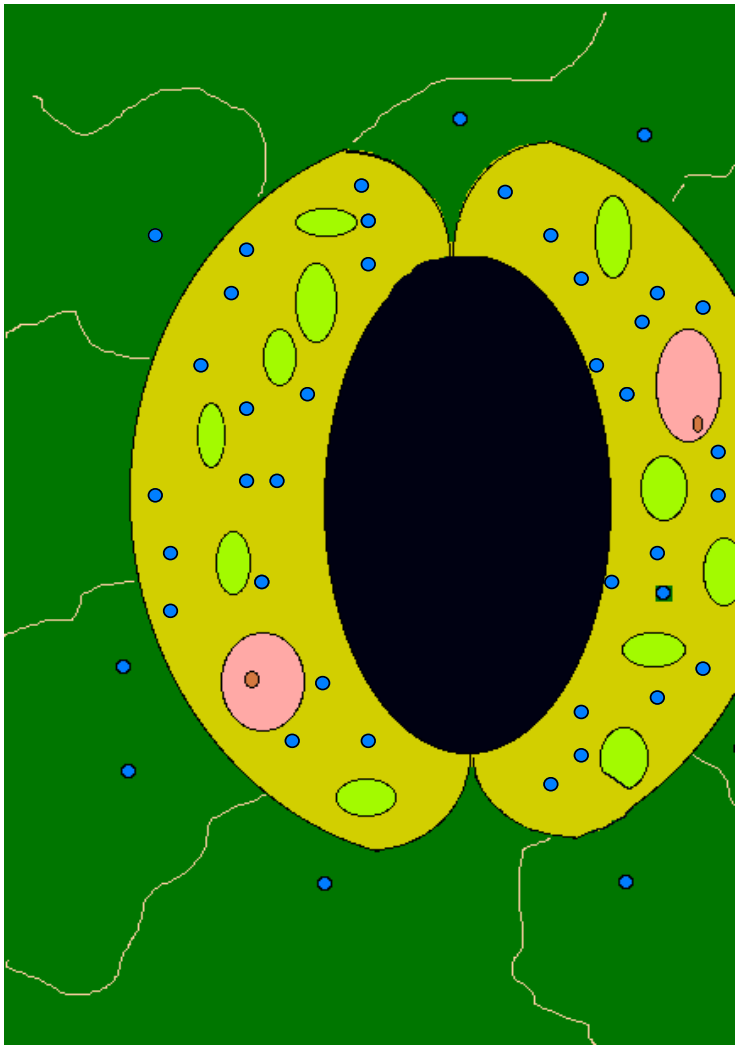
- Active transport moves K^+ ions in or out of guard cell.
- Water flows from hypotonic solutions to hypertonic solutions.

POTASSIUM ION (K^+) CONCENTRATED OUTSIDE OF GUARD CELLS.
WATER LEAVES GUARD CELLS. CELLS BECOME FLACCID.



Stoma closed

INFLUX OF POTASSIUM IONS INTO CELL. WATER FOLLOWS. Guard cells become turgid.

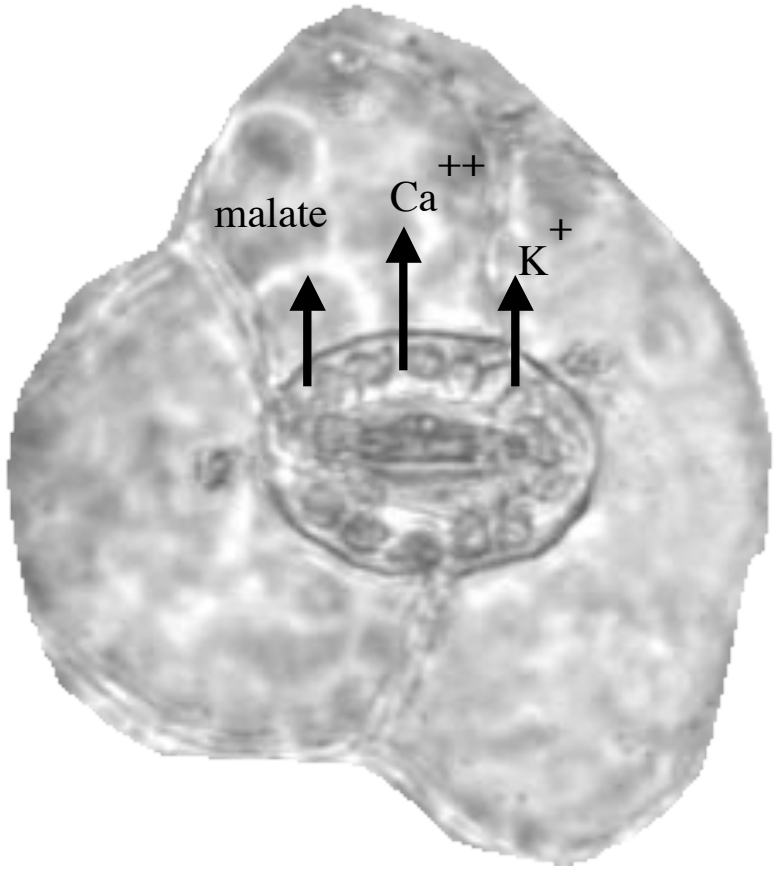


Stoma open

http://www.tvdsb.on.ca/westmin/science/sbioac/plants/stoma.htm

Guard Cells & ABA

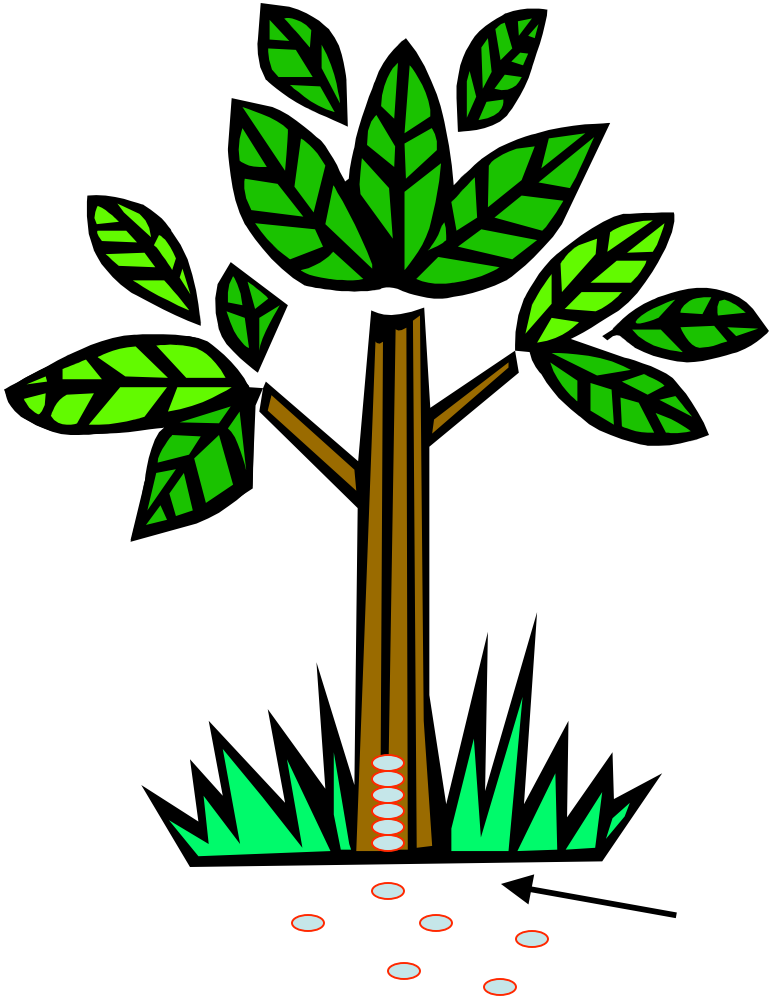
- Plant water stressed
- ABA released by plant cells
- ABA binds to guard cell receptors
- Activates signal transduction pathway
- Lowers solute concentration in guard cells
- Lowers cell tugor
- Stoma close



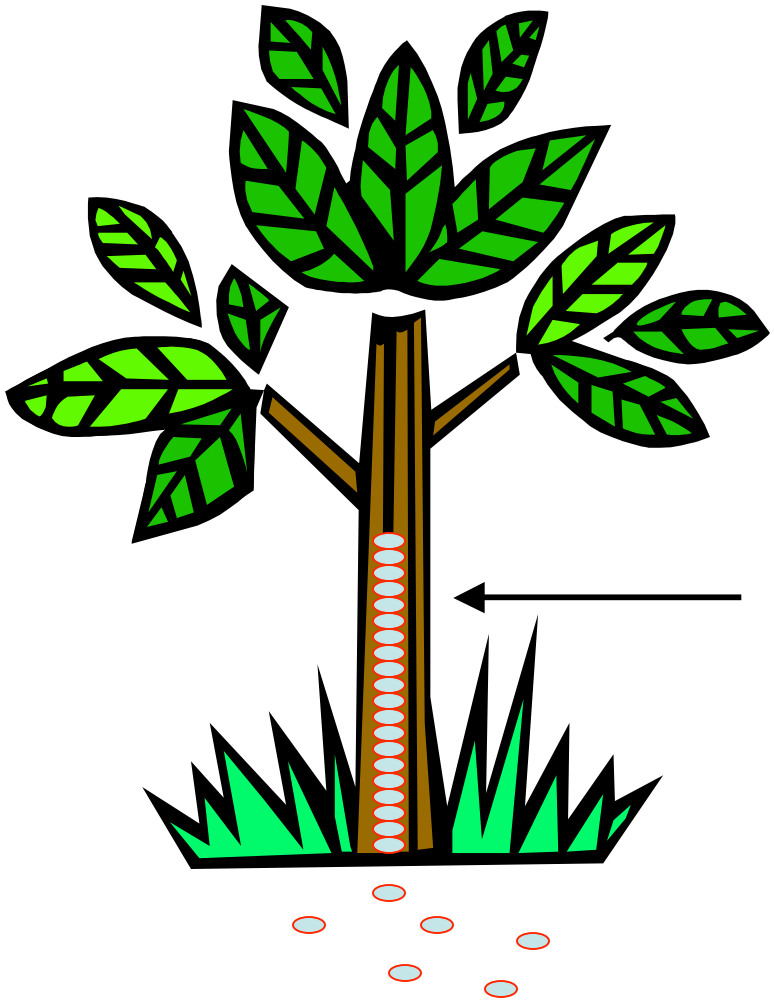
Transpiration

- Loss of water from plant by evaporation.
- Water loss mainly from leaves.
- Energy for process from sun

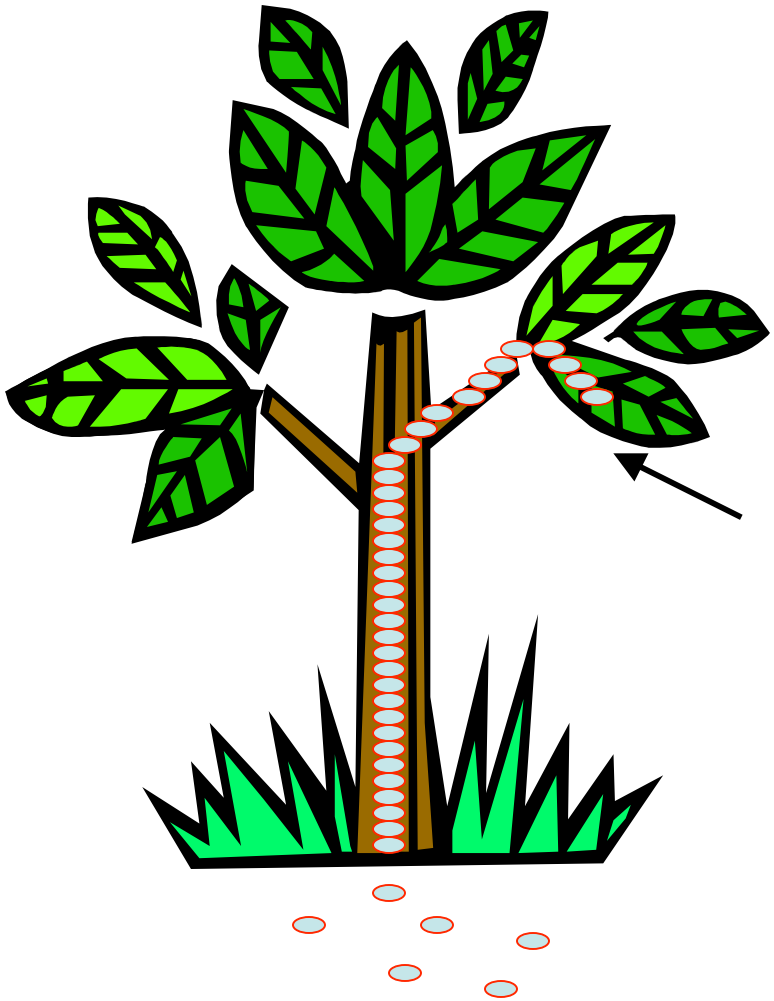
Water moves from soil into the root.



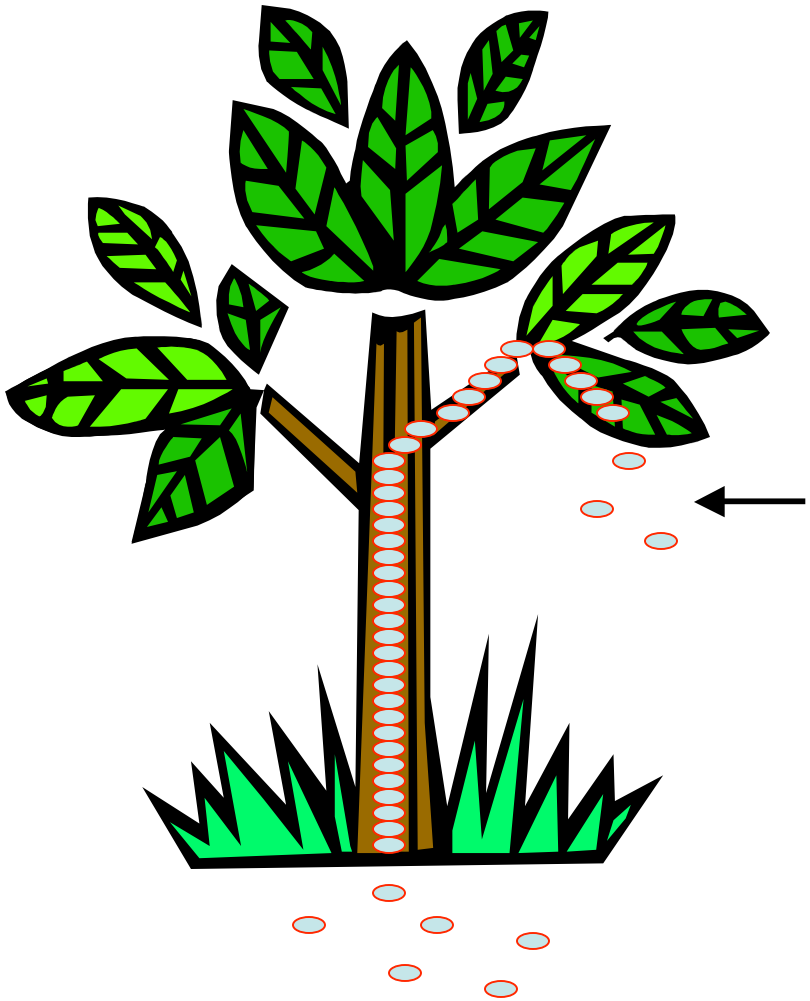
- Water moves from soil into the root.



- Water moves from root xylem into the stem xylem.



- Water moves from leaf xylem into mesophyll cells



- Water moves from leaf xylem into mesophyll cells.
- Water vapor inside leaf is lost via diffusion through stomata.

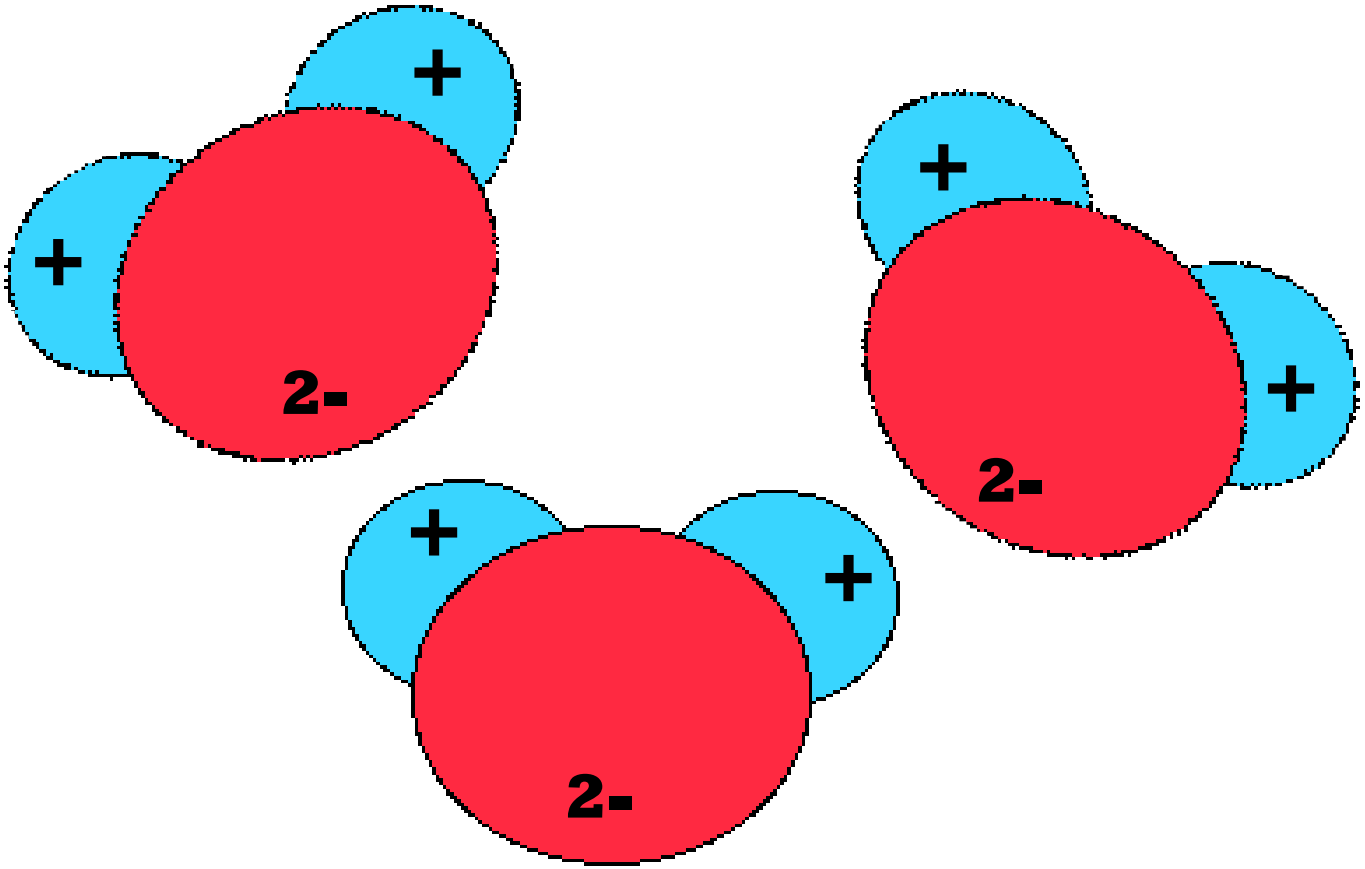
How is water transported up xylem?

Cohesion-Tension Theory

- "…Transpiration of a water molecule results in a negative (below 1 atmosphere) pressure in the leaf cells, inducing the entrance from the vascular tissue of another water molecule, which, (because of the cohesive property of water), pulls with it a chain of water molecules extending up from the cells of the root tip."

- Curtis & Barnes (1989). *Biology, G-5.*

WATER IS A POLAR MOLECULE!



Properties of Water (H_2O)

- Water is a polar molecule.
- Polarity aids water movement in plant:
  - **Cohesive strength**
    - (hydrogen bonding, molecules stick to each other)
  - **Adhesive strength**
    - (water sticks to other things)
  - **Tensile strength**
    - (pulls chain of water molecules sticking to each other)

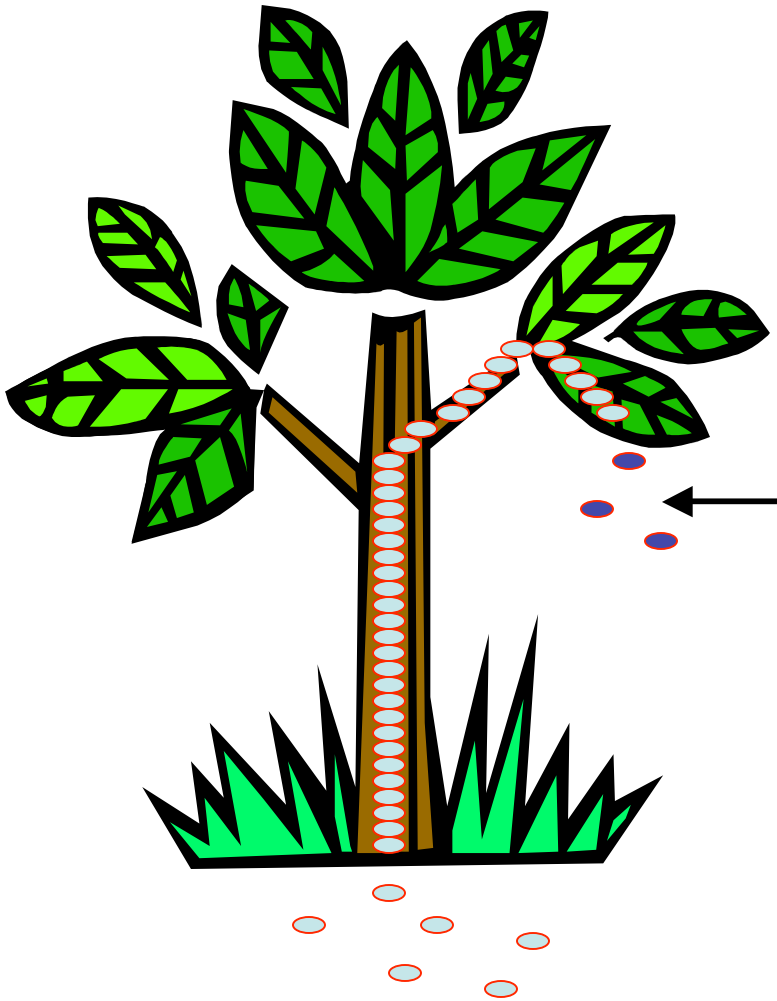
TACT

{Cohesion-tension theory}

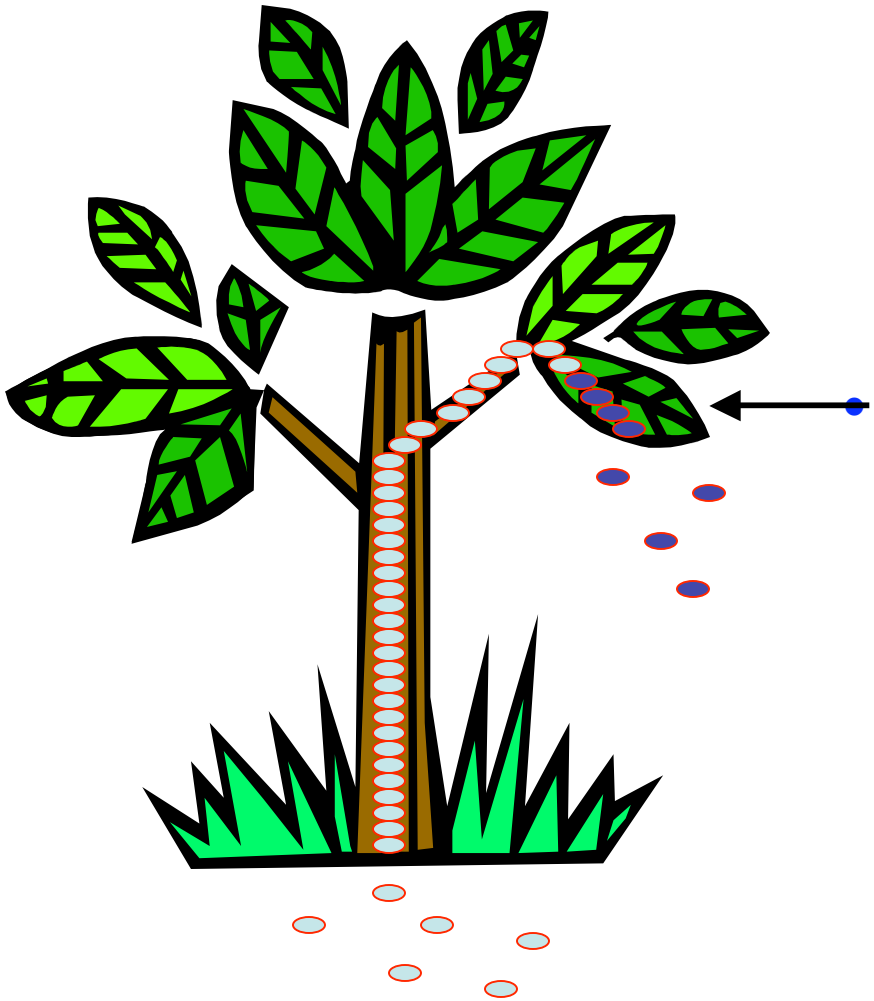
- **T= Transpiration** (loss of water from plant)
- **A= Adhesion** (hydrophilic attraction to vessel walls)
- **C= Cohesion** (hydrogen bonding twixt H_2O molecules)
- **T= Tensile** (upward pull creates negative pressure)

Pressure vs. Tension

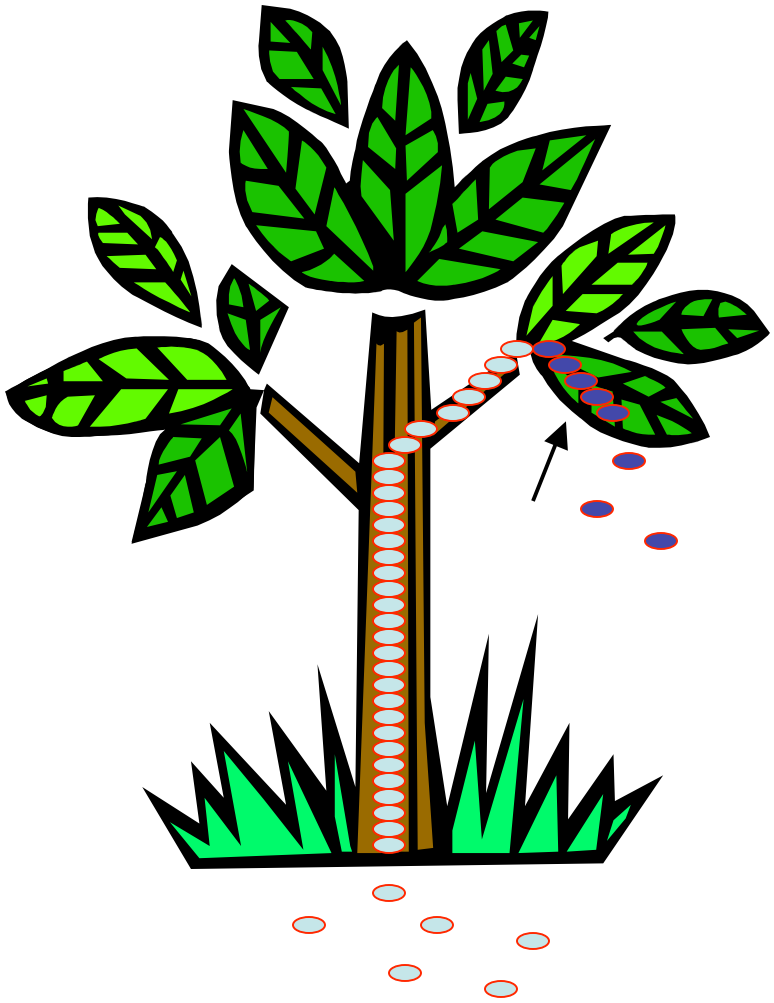
- Tension opposite of pressure.
- **Pressure** is exerted in every direction. Causes a cell wall to swell.
- **Tension** is a negative pressure. Tends to pull in walls of a cell.



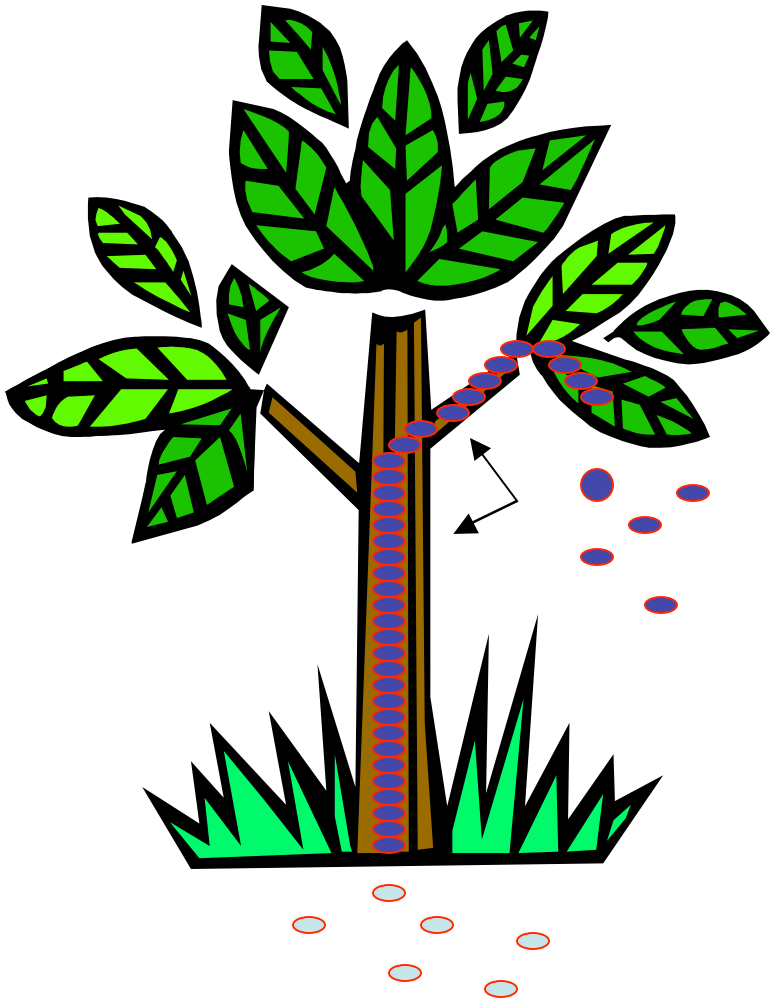
- Water vapor inside leaf is lost, molecule by molecule. via diffusion through stomata.



Water evaporates, molecule by molecule, from surface of mesophyll cells into the intercellular air spaces.



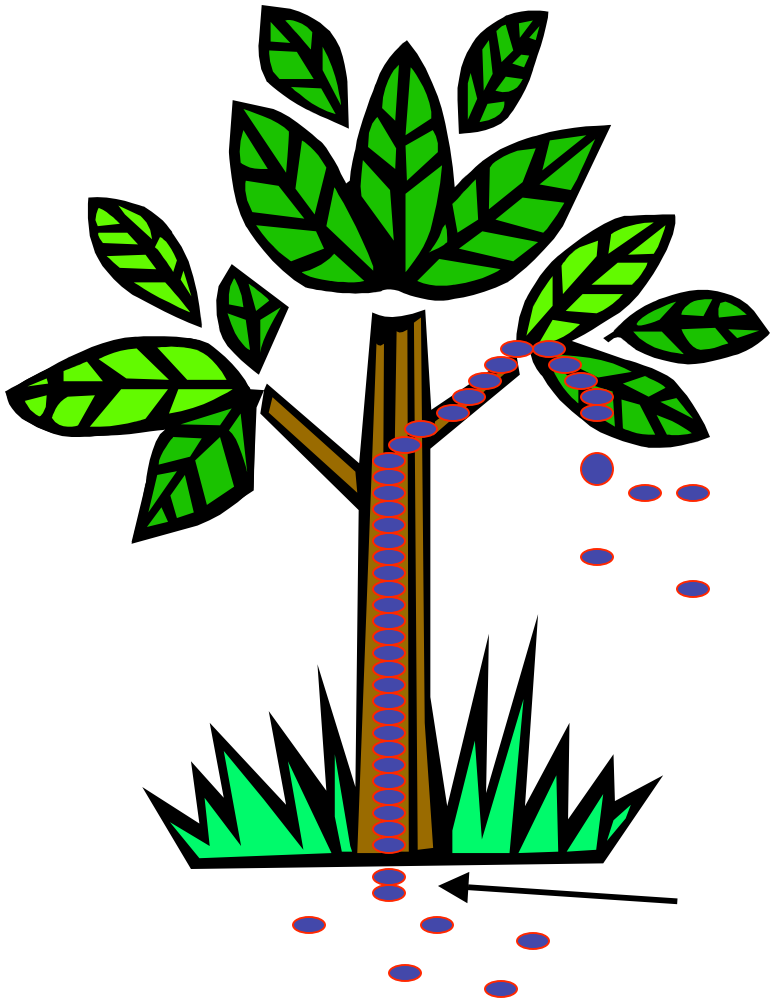
- As water potential of leaf cell decreases, water moves, molecule by molecule, from leaf xylem into mesophyll cells.



Water moves from stem xylem into leaf xylem.

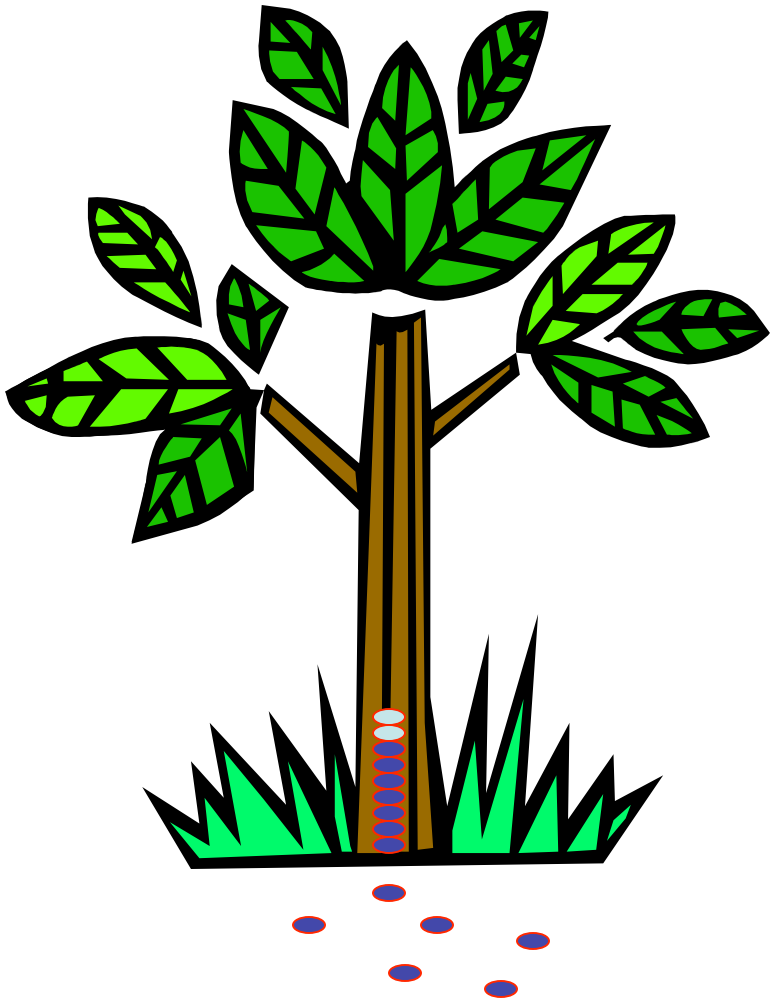
Cohesion between water molecules creates large tensile strength (140 kg/cm) on thin continuous column of water.

Water, molecule by molecule, adhere to inner surface of xylem vessels.



Water moves , molecule by molecule, from soil into the root.

A break in the continuous column of water stops the water from rising.



State of Stoma
A = Opens B = Closes

- Bright Light?
- Low level of CO_2 in leaf?
- Water stress?
- High ABA?
- High Temperature?
- K^+ high in guard cells?

STATE OF STOMATA

STOMA OPEN	STOMA CLOSED
Guard cells turgid	Guard cell flaccid
Bright light	Darkness
CO_2 Low	CO_2 High
[ABA] low	[ABA] high
K^+ High in guard cells	K^+ Low in guard cells
Temperature High	Temperature Low
Photosynthesis High	Photosynthesis Low
Water Plentiful	Water Stressed

Measuring Transpiration Rate

- Water moves in response to a driving force:
  - Concentration gradient (diffusion)
  - Water potential gradient (osmosis)
  - Pressure gradient (bulk flow)
- Flow rate determined by several factors:
  - Driving force/resistance
  - Driving force x conductance

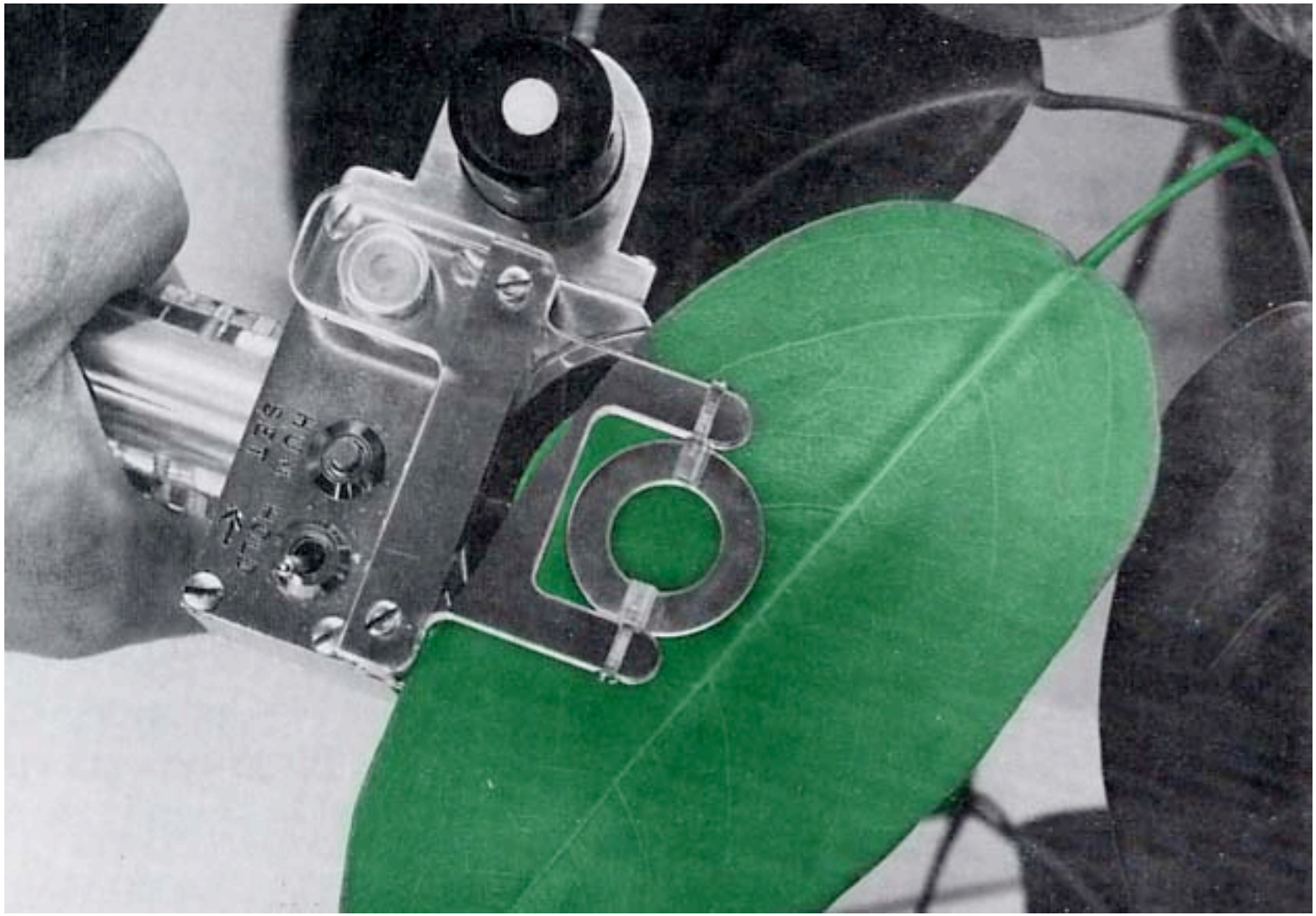
Stomatal Conductance

- As measure of how freely water vapor can pass from inside a leaf to the outside.
- Conductance in pipes depends upon the resistance (length of pipe, its radius and viscosity of the water).
- Conductance in leaves depends upon the size of the stomata (open/closed) and the density of stomata on the leaves.

Measuring Transpiration Rate

- Porometer measures the stomatal resistance of plant leaves (C=1/R)
  - Measures transpiration rate by measuring the resistance to the loss of water vapor through the stomata.
  - Instruments measures the time it takes for a leaf to release sufficient water vapor to change the relative humidity in a small chamber by a fixed amount.
  - Using unifoliate leaves

Using Porometer



Stomatal Conductance Measurements

- Conductance = **m s^{-1}** or **cm s^{-1}**
  - (in velocity units)

- Conductance = **mmol H_2O m^{-2} s^{-1}**
  - (as mole units)

fini

Movement of water up plant

- Water moves from soil into the root.
- Water moves from root xylem into the stem xylem.
- Water moves from stem xylem into leaf xylem.
- Water moves from leaf xylem into mesophyll cells.
- Water evaporates from surface of mesophyll cells into the intercellular air spaces.
- Water vapor inside leaf is lost via diffusion through stomata.

Transpiration

1. Water vapor inside leaf is lost, molecule by molecule. via diffusion through stomata.

2. Water evaporates, molecule by molecule, from surface of mesophyll cells into the intercellular air spaces.

3. As water potential of leaf cell decreases, water moves, molecule by molecule, from leaf xylem into mesophyll cells.

4. Water moves from stem xylem into leaf xylem. Cohesion between water molecules creates large tensile strength (140 kg/cm) on thin continuous column of water. Water, molecule by molecule, adhere to inner surface of xylem vessels.

5. Water moves, molecule by molecule, from root xylem into the stem xylem.

6. Water moves , molecule by molecule, from soil into the root.