DID YOU KNOW?
Some tracheophytes reproduce with seeds (phanerogams) and some reproduce with spores (cryptogams).

DID YOU KNOW?
Every day, a large tree may lose enough water for you to take eight long showers.

WARD'S

AP Biology Lab 9
Transpiration
Lab Activity
Student Study Guide

BACKGROUND

The plant kingdom can be divided into two major groups: bryophyta and tracheophyta. The major distinction between these two groups is the presence of specialized vascular tissue for the transport of water and food. While bryophytes, such as moss, lack conducting tissues, tracheophytes utilize specialized cells, xylem and phloem, for the transport of water and food. The evolution of vascular tissues and the subsequent ability to transport water and food over greater distances has contributed to the evolution of larger plants with specialized ground tissues and organs: roots for support and uptake of water and minerals, stems for support of leaves and flowers, and leaves for photosynthesis. These adaptations have allowed tracheophytes to become the dominant form of terrestrial plant.

Ground tissues, which comprise the plant body, or cortex, are made up of three types of cells: collenchyma, parenchyma, and sclerenchyma cells. Collenchyma cells are irregularly shaped, with thick cell walls. They are found in a plant stem, where they help support the body of a plant. Parenchyma cells are the photosynthetic cells of a plant, with thin, many-sided walls; they make up the ground tissue of a leaf. Sclerenchyma cells are elongated, with primary and secondary walls, containing lignin, a protein which strengthens the cell. Unlike parenchyma and collenchyma cells, they die at maturity. Sclerenchyma cells are often found near vascular tissue.

According to the generally accepted cohesion–tension theory, water is pulled up to the leaves of a plant by transpirational pull. When stomates are open, water transpires from higher water potential in the mesophyll spaces to lower water potential in the air. Decreasing water potential in the air spaces pulls water from nearby mesophyll cells, which in turn pull water from xylem vessels in nearby veins of the leaf.

Due to the cohesive nature of water molecules, when one water molecule is pulled from the xylem, more and more follow close behind in a chain of water molecules pulled upward from the roots to the leaves. The tension, or negative pressure, caused by the upward pull of the water column is so strong that the diameter of a stem actually decreases when the rate of transpirational pull is very high.

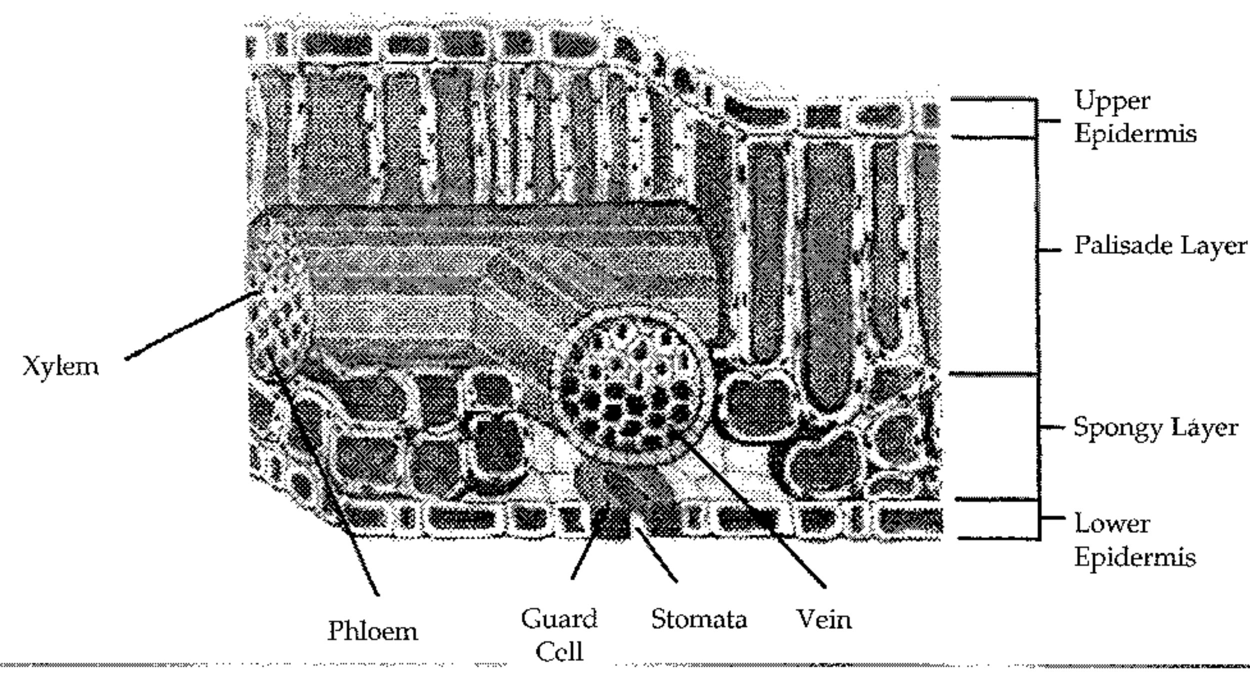
In the root, minerals actively absorbed from the soil increase the solute concentration of cortical cells. This causes water to flow by osmosis from the soil into the root, creating hydrostatic pressure known as root pressure. Water from the cortex of the root continually moves toward the xylem, aided by the push of root pressure, where it is then pulled up by transpiration. Root pressure and transpirational pull together provide more than enough force to offset the pull of gravity and raise water to the top of even the tallest redwood trees.

DID YOU KNOW?
Stomates are sensitive to light quality as well as quantity. They will open in response to red light, but they will open even larger when exposed to the same intensity of blue light.

Although transpiration is the driving force behind water transport, plants have evolved many adaptations to prevent excessive loss of water by transpiration. As expected, the rate of transpiration varies directly with the amount of sunlight, heat, and wind in the environment, and a delicate balance must be maintained between stomates closing to prevent water loss and stomates opening for the exchange of oxygen and carbon dioxide. Structures called guard cells regulate the opening and closing of the stomates. For instance, in desert plants, guard cells often keep the stomates closed during the day when the rate of transpiration is high and open them at night for respiration. In addition, the presence of wax (cutin) on the upper surface of leaves, or small hairs on the lower surface of leaves, prevents the loss of water. The rate of transpiration also varies indirectly with humidity in the environment. Cacti, which live in hot arid environments, have evolved small needle-like leaves to reduce the surface area from which transpiration can occur; whereas tropical plants in humid and shady environments can afford to have very large leaves.

Despite adaptations to limit the loss of water, plants lose as much as 90% of the water that enters their roots by transpiration from stomates. Factors that affect the rate of transpiration in plants and the organization of a typical plant stem as it relates to the transport of water can easily be investigated.

Figure 1
Structure of Leaves



OBJECTIVES

- Explain how the concept of water potential relates to the transport of water from the roots to the stems and to the leaves of a plant
- Define transpiration and relate the process to overall transport of water in plants according to the cohesion-tension theory
- Describe the properties of water as they relate to water transport in plants
- Quantitatively observe the effects of light intensity, wind, and humidity on the rate of transpiration in plants
- Identify and describe the role of vascular and ground tissues in plants

MATERIALS

Part A: Investigating Rates of Transpiration

1 Bean seedling
1 Potometer setup (graduated pipet, T-connector, and tubing)
1 Syringe
Plastic bag
Petroleum jelly
Spray bottle
Ring stand
Ring stand clamps
Floodlight
Electric fan
Balance

Part B: Preparation of Plant Stem Cross-Section

Bean seedling
50% glycerin solution
Toluidine blue
Petri dish
Forceps
Microscope slide
Coverslip
Hand microtome
Razor blade
Paraffin wax
Waterbath
Hotplate
Compound microscope
Thermal protection gloves

DID YOU KNOW?

Ozone, a major constituent of smog, inhibits the ability of guard cells to open the stomates on a plant's leaves and thus negatively impacts the process of photosynthesis by reducing the amount of CO_2 they are able to process and release as O_2.

Potometer:
An apparatus used to measure the rate of uptake of water by a plant and thus, indirectly estimate transpiration.

Porometer:
An instrument used for investigating the opening of stomata by measuring the rate of flow of air (or other gases) through the leaf or the rate of diffusion of water vapor through the leaf.

PROCEDURE

Part A: Investigating Rates of Transpiration

1. Obtain a piece of flexible plastic tubing. Cut a 1″ piece and a 6″ piece from one end of the tubing. You should now have three pieces (1″, 6″, and 9″) of tubing.

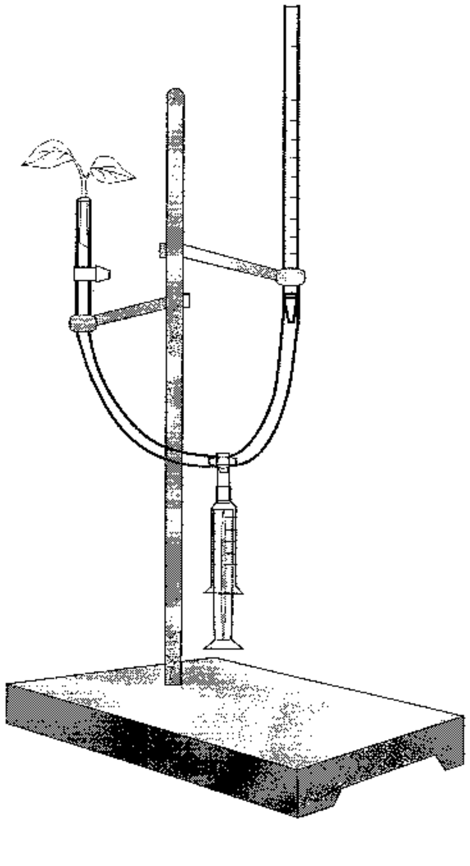
2. Connect a 6″ piece of tubing and a 9″ piece of tubing to each opposing arm of a **T**-connector.

3. Attach the remaining 1″ piece of tubing to the bottom of the T-connector.

4. Insert the tapered end of a graduated pipet into the 6″ piece of tubing. Ensure the pipet is firmly inserted. You want to create an airtight system.

5. Bend the potometer setup into a J-shape and attach to a ring stand using two ring stand clamps. Clamp the potometer in place so that the end of the tubing is about half the height of the graduated pipet (Figure 2).

Figure 2



6. Remove the tip from a 10 ml syringe and fill with water.

7. Attach the tip of the syringe to the end of the 1″ piece of tubing on the T-connector.

8. Charge the potometer by adding water with the syringe until the level of water forms a bead on top of the 9″ piece of tubing.

9. Using a razor blade or scalpel, cleanly cut the stem of a bean seedling near the soil.

NOTE *Make sure that your plant stem fits snugly into the end of the tubing without having to force it in and damaging tissue. It may require several cuts to obtain a suitable piece of plant material.*

10. Insert at least 1/2″ of the stem of the seedling into the flexible tubing. Be sure there are no air bubbles in the potometer at the base of the seedling. Remember, you want an airtight system. If air bubbles are present, remove the seedling, cut a couple of millimeters off the end, and reinsert it into the tubing.

11. Dry the area at the base of the stem near the tubing and seal it with petroleum jelly. Your completed potometer should resemble Figure 2.

12. Allow the potometer to equilibrate for approximately 10 minutes.

13. Once the potometer has equilibrated, zero it by gently depressing the plunger of the syringe until the water level reaches the zero mark in the graduated pipet.

14. Record your initial reading (0 ml) for Setup 1 in Table 1 in the Analysis section of the lab. Monitor your potometer for 30 minutes. Take readings at 10, 20, and 30 minutes and record them in Table 1.

15. Repeat the procedure in three different environments. Be sure to re-zero the potometer before beginning each experiment. Take readings at 0, 10, 20, and 30 minutes and record each reading in Table 1.

    - Setup 2: Place the plant under a 100 W floodlight.
    - Setup 3: Place a fan on low setting, approximately three feet from the plant.
    - Setup 4: Mist the plant leaves with water and place a plastic bag over the plant. Do not seal the bag.

DID YOU KNOW?

Individual plants of the same species, or even parts of the same plant, can adapt to the chronic light conditions peculiar to a location. For example, the leaves of a mature cherry bark red oak that grows in the shade appear dramatically different than the leaves exposed to full sun at the top of the tree.

16. Once you have completed the experiments, cut the leaves off your seedling and blot off any excess moisture. Weigh the leaves and record the weight in Table 2.

17. Determine the surface area of the leaves using the sheet of graph paper in the Analysis section of the lab. To estimate the area of the leaves: Trace the leaves on the 1 cm x 1 cm graph paper and count the number of square centimeters. Record the total area of the leaves in Table 2.

Part B: Preparation of a Plant Stem Cross-Section

Wear protective gloves of heavyweight cotton Kelnit, safety goggles, and aprons or smocks when handling melted wax.

DID YOU KNOW?
Paraffin wax is obtained as a residue from the distillation of petroleum.

1. Place the paraffin granules into a 100 ml beaker and place in a boiling waterbath until completely melted.

NOTE

The paraffin should be melted but not boiling. Overheated wax will damage the stem tissue.

2. Cut a piece of bean stem approximately 1″ long with the included razor blade.

3. Unscrew the threaded bolt of the hand microtome so that you create a well approximately 1″ deep. Place the stem section upright in the microtome so that a small portion protrudes from the top.

4. Carefully pour the melted wax into the well, surrounding the specimen with melted wax.

CAUTION

Avoid contact with skin; melted wax can cause second- and third-degree burns.

5. Let the wax cool; do not move the specimen while the wax solidifies.

6. Using the razor, shave the protruding section until it is flush with the top of the microtome (Figure 3).

CAUTION

Always move the razor blade away from you.

Figure 3

DID YOU KNOW?
Toluidine blue is classified as a basic metachromatic stain (i.e., the stained material will take on a color that is different from that of the dye employed). For example, nucleic acids may stain blue while sulfated polysaccharides may stain purple.

7. Twist the threaded bolt slightly so the section rises to just above the top of the microtome.

8. Shave as thin a specimen as possible from the section.

9. Place the cross-section in a Petri dish of distilled water.

10. Repeat this procedure until you have obtained eight to ten cross-sections.

11. Using forceps, remove the stem cross-sections from the distilled water. Carefully remove the paraffin from the cross-section.

12. Place a section on a microscope slide. Using a pipet, add one or two drops of toluidine blue stain.

13. Wait 30 seconds, then remove the excess stain with a paper towel, taking care not to damage the cross-section.

14. Using a pipet, cover the cross-section with two or three drops of 50% glycerin and add a coverslip.

15. Observe the slide under a microscope, first at 100X and then at 400X. Draw what you see in the Analysis section of the lab, and identify the different types of cells in your cross section.

Name: ______________________
Group: ______________________
Date: ______________________

ANALYSIS

Table 1
Cumulative Water Loss in ml/m²

	Reading			
Setup	**Initial**	**10 min.**	**20 min.**	**30 min.**
1				
2				
3				
4				

Table 2

Weight of Leaves (g)	
Leaf Surface Area (cm^2)	

Cross-Section of Stem

Name: ____________________
Group: ____________________
Date: ____________________

ASSESSMENT

1. Determine the plant's rate of transpiration per minute for each of the enviromental conditions.

2. Name three functions of transpiration.

3. How is the molecular structure of water significant to the transport of water in plants?

4. Explain the purpose of each of the following with regard to transpiration:

   Cuticle –

   Guard cells –

   Leaf hairs –

5. Place the following terms in the proper column:

   found in stems
   thin-walled, many-sided cells
   dead at maturity
   contain lignin
   thick-walled, irregularly shaped
   photosynthetic cells
   leaf ground tissue
   found near vascular bundles

Collenchyma	Parenchyma	Sclerenchyma

6. You came in to class one morning and one of the classroom plants was looking wilted and unhealthy. You watered the plant and a couple of hours later, the plants appeared rigid and healthy. What happened after you watered it?

7. Create a Venn diagram comparing bryophytes and tracheophytes.

8. Do you think the number of stomata on the underside of a leaf is more, less, or the same as on the upper surface of a leaf? Design an experiment to determine the answer.

9. One of the following statements is not true. Decide which one is false and explain your answer.

'The guard cells of non-desert plants often close during the daytime and open at night to conserve water'

'The guard cells of desert plants often close during the daytime and open at night to conserve water'

10. When you receive a bouquet of flowers, it is recommended that you cut a couple of inches off of the stems before placing them in a vase. Why do you think this is?

11. Create a concept map about transpiration: