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.

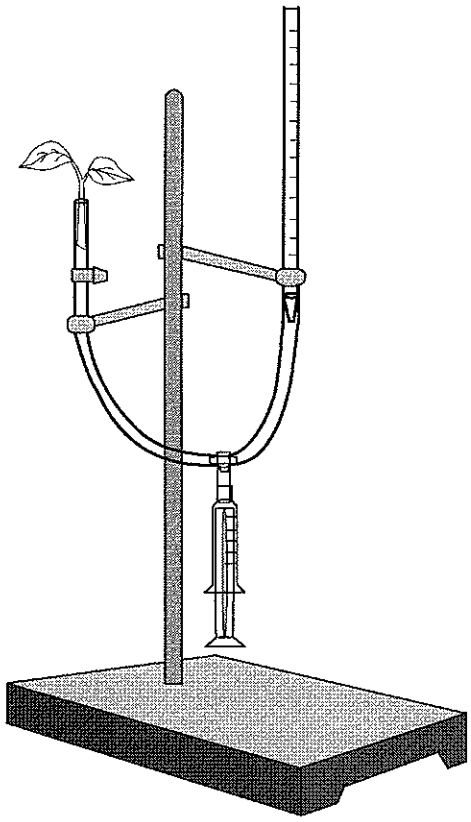
2. Connect a 6" piece of tubing and a 9" piece of tubing to each opposing arm of a T-connector.

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



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.

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.

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.

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.

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

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.

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

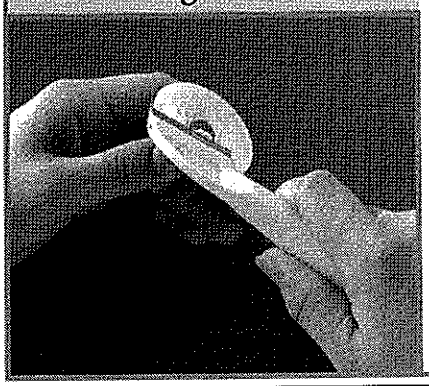
5. Let the wax cool; do not move the specimen while the wax solidifies.

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

Always move the razor blade away from you.

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

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

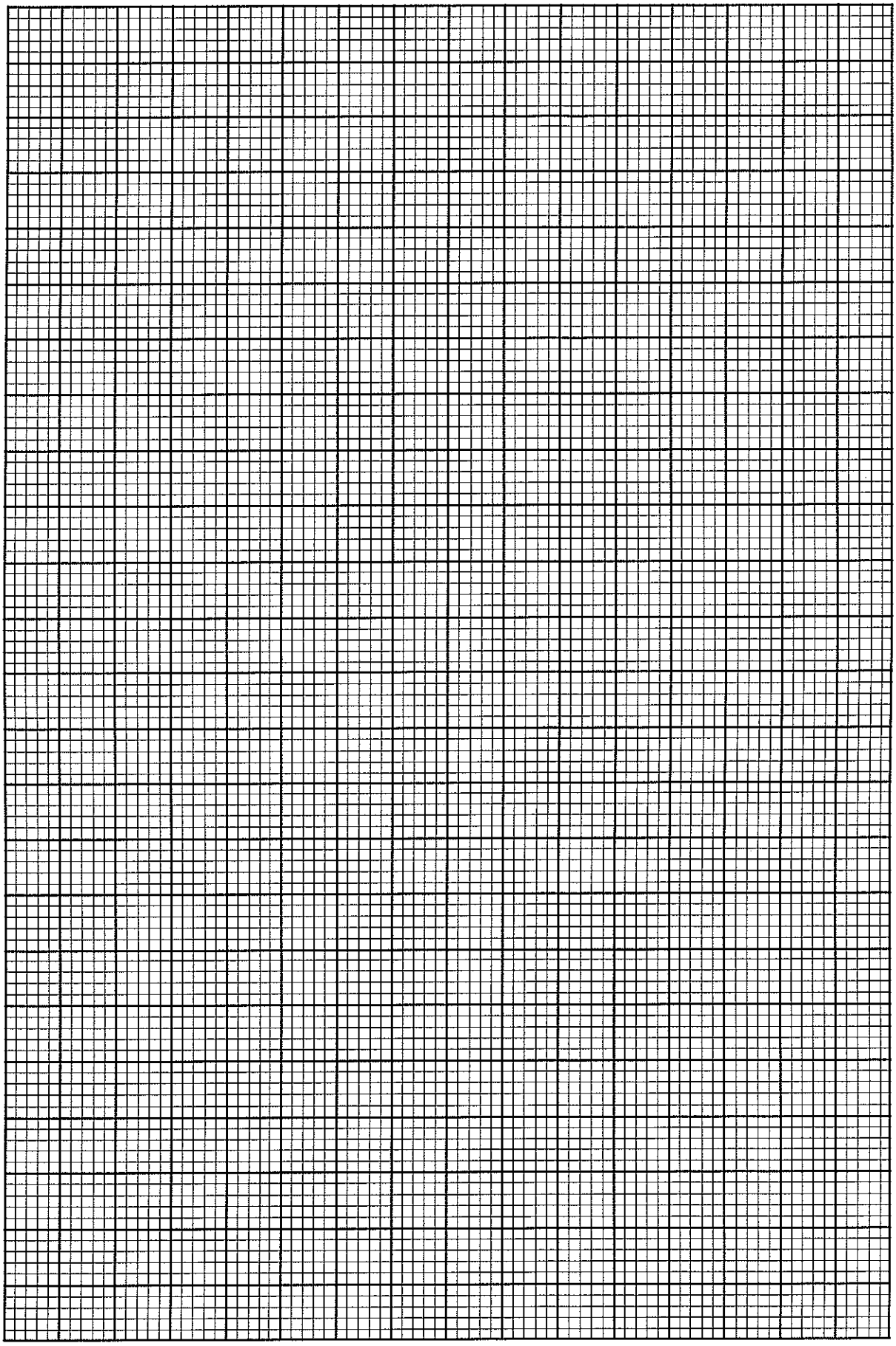
Table 1
Cumulative Water Loss in ml/m²

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

Table 2

Weight of Leaves (g)	
Leaf Surface Area (cm²)	

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: