

LABORATORY 9. TRANSPIRATION

OVERVIEW

In this laboratory you will apply what you learned about water potential from Laboratory 1 (Diffusion and Osmosis) to the movement of water within the plant. You will measure transpiration under different laboratory conditions and also study the organization of the plant stem and leaf as it relates to these processes by observing sections of tissue.

OBJECTIVES

Before doing this laboratory you should understand:

- how water moves from roots to leaves in terms of the physical/chemical properties of water and the forces provided by differences in water potential;
- the role of transpiration in the transport of water within a plant; and
- the structures used by plants to transport water and regulate water movement.

After doing this laboratory you should be able to:

- test the effects of environmental variables on rates of transpiration using a controlled experiment, and
- make thin sections of stem, identify xylem and phloem cells, and relate the function of these vascular tissues to the structures of their cells.

INTRODUCTION

The amount of water needed daily by plants for the growth and maintenance of tissues is small in comparison to the amount that is lost through the process of **transpiration** (the evaporation of water from the plant surface) and **guttation** (the loss of liquids from the ends of vascular tissues at the margins of leaves). If this water is not replaced, the plant will wilt and may die.

The transport of water up from the roots in the xylem is governed by differences in **water potential** (the potential energy of water molecules). These differences account for water movement from cell to cell and over long distances in the plant. Gravity, pressure, and solute concentration all contribute to water potential, and water always moves from an area of high water potential to an area of low water potential. The movement itself is facilitated by osmosis, root pressure, and adhesion and cohesion of water molecules.

The overall process: Minerals actively transported into the root accumulate in the xylem, increasing solute concentration and decreasing water potential. Water moves in by **osmosis**. As water enters the xylem, it forces fluid up the xylem due to hydrostatic **root pressure**. But this pressure can only move fluid a short distance. The most significant force moving the water and dissolved minerals in the xylem is *upward* pull as a result of **transpiration**, which creates a negative tension. The "pull" on the water from transpiration is increased as a result of cohesion and adhesion of water molecules.

The details: Transpiration begins with evaporation of water through the **stomates** (stomata), small openings in the leaf surface which open into air spaces that surround the mesophyll cells of the leaf. The moist air in these spaces has a higher water potential than the outside air, and water tends to evaporate from the leaf surface (moving from an area of high

water potential to an area of lower water potential). The moisture in the air spaces is replaced by water from the adjacent mesophyll cells, lowering their water potential (since the cytoplasm becomes more concentrated). Water will then move into the mesophyll cells by **osmosis** from surrounding cells with higher water potentials, including the xylem. As each water molecule moves into a mesophyll cell, it exerts a pull on the column of water molecules existing in the xylem all the way from the leaves to the roots. This transpirational pull is caused by (1) the **cohesion** of water molecules to one another due to hydrogen bond formation, and (2) by **adhesion** of water molecules to the walls of the xylem cells which aids in offsetting the downward pull of gravity.

The upward transpirational pull on the fluid in the xylem causes a **tension** (negative pressure) to form in the xylem, pulling the walls of the xylem inward. The tension also contributes to the lowering of water potential in the xylem. This decrease in water potential, transmitted all the way from the leaf to the roots, causes water to move inward from the soil, across the cortex of the root, and into the xylem.

Evaporation through the open stomates is a major route of water loss in plants. However, the stomates must open to allow the entry of CO_2 used in photosynthesis. Therefore, a balance must be maintained between the gain of CO_2 and the loss of water by regulating the opening and closing of stomates on the leaf surface. Many environmental conditions influence the opening and closing of stomates and also affect the rate of transpiration. Temperature, light intensity, air currents, and humidity are some of these factors. Different plants also vary in the rate of transpiration and in the regulation of stomatal opening.

EXERCISE 9A: Transpiration

In this lab, you will measure transpiration under various laboratory conditions using a **potometer**. Four suggested plant species are *Impatiens* (which is a moisture-loving plant), *Oleander* (which is more drought-tolerant), *Zebrina*, and two-week old *Phaseolus vulgaris* (bean seedlings).

Materials 0.1-mL pipette, plant cutting, ring stand, clamps, clear plastic tubing (Tygon® or similar), petroleum jelly, fan, lamp, spray bottle, and plastic bag.

Procedure Each lab group will expose one plant to one treatment.

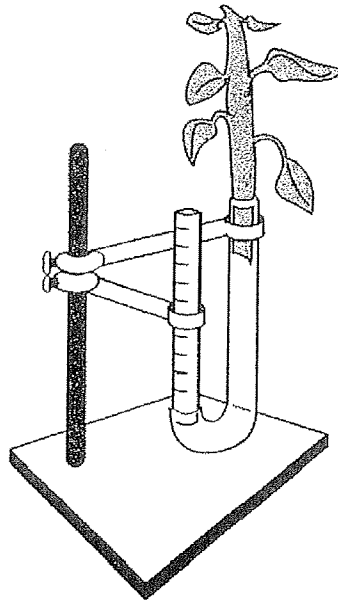
1. Place the *tip* of a 0.1-mL pipette into a 16-inch piece of clear plastic tubing.
2. Submerge the tubing and pipette in a shallow tray of water. Draw water through the tubing until all bubbles are eliminated.
3. Carefully cut your plant stem under water. This step is very important, because no air bubbles must be introduced into the xylem.
4. While your plant and tubing are submerged, insert the freshly cut stem into the open end of the tubing.
5. Bend the tubing upward into a "U" and use the clamp on a ring stand to hold both the pipette and the tubing (Figure 9.1).

6. If necessary use petroleum jelly to make an airtight seal surrounding the stem after it has been inserted into the tube. Make sure that the end of the stem is immersed in the water. **Do not put petroleum jelly on the cut end of the stem.**

Alternative Procedure for Filling Potometer

- (i) Set up the potometer as shown in Figure 9.1.
 - (ii) Use a water bottle or pipette to fill the tubing. Add water until the water comes out of the tube and no bubbles remain.
 - (iii) Quickly cut the plant stem and insert it into the potometer.
7. Let the potometer equilibrate for 10 minutes before recording the time zero reading.

Figure 9.1



8. Expose the plant in the tubing to one of the following treatments (you will be assigned a treatment by your teacher):
 - a. Room conditions.
 - b. Floodlight (place a 100 watt bulb 1 meter from the plant and use a beaker filled with water as a heat sink).
 - c. Fan (place at least 1 meter from the plant, on low speed, creating a gentle breeze).
 - d. Mist (mist the leaves with water and cover with a transparent plastic bag; leave the bottom of the bag open).
9. Read the level of water in the pipette at the beginning of your experiment (time zero) and record your finding in Table 9.1.
10. Continue to record the water level in the pipette every three minutes for 30 minutes and record the data in Table 9.1.

Table 9.1: Potometer Readings

Time (min)	Beginning (0)	3	6	9	12	15	18	21	24	27	30
Reading (ml)											

11. At the end of your experiment, cut all the leaves off the plant and mass them. Remember to blot off all excess water before massing.

Mass of leaves _____ grams.

Calculation of Leaf Surface Area

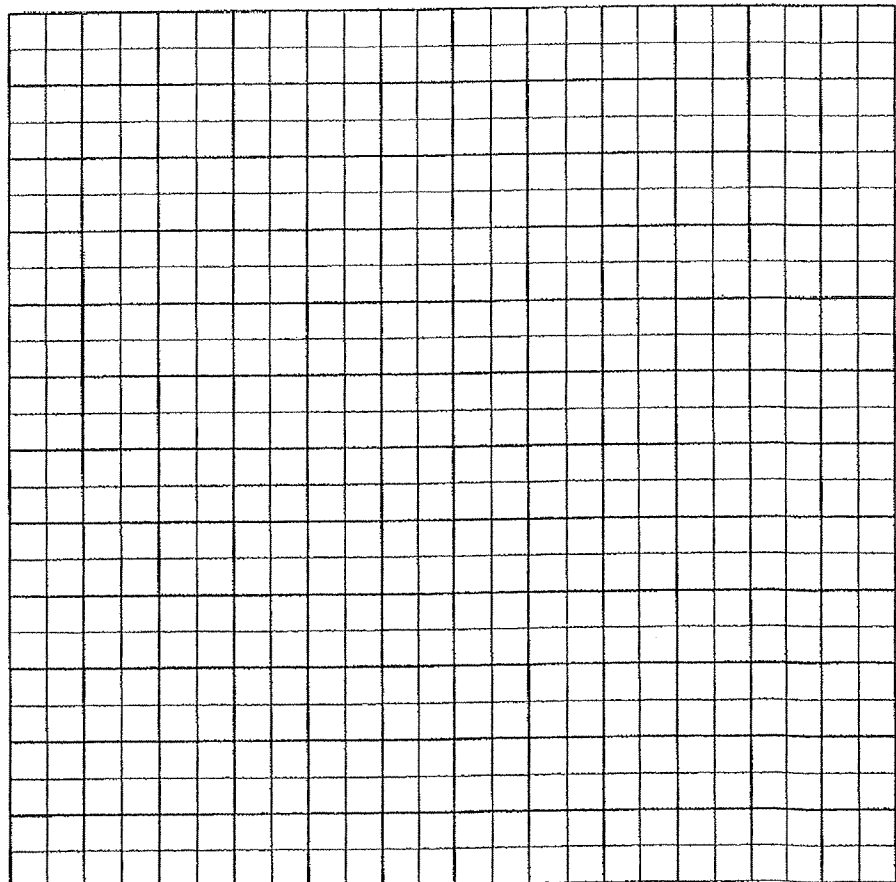
The total surface area of all the leaves can be calculated by using *one* of the following procedures.

_____ = Leaf Surface Area (m²)

Leaf Trace Method:

After arranging all the cut-off leaves on the grid below, trace the edge pattern directly on to the grid. Count all of the grids that are completely within the tracing and estimate the number of grids that lie partially within the tracing. The grid has been constructed so that a square of four blocks equals 1 cm². The total surface area can then be calculated by dividing the total number of blocks covered by 4. Record this value above.

Grid 9.1



14. For each treatment, graph the average of the class data for each time interval. You may need to convert data to scientific notation. All numbers must be reported to the same power of ten for graphing purposes.

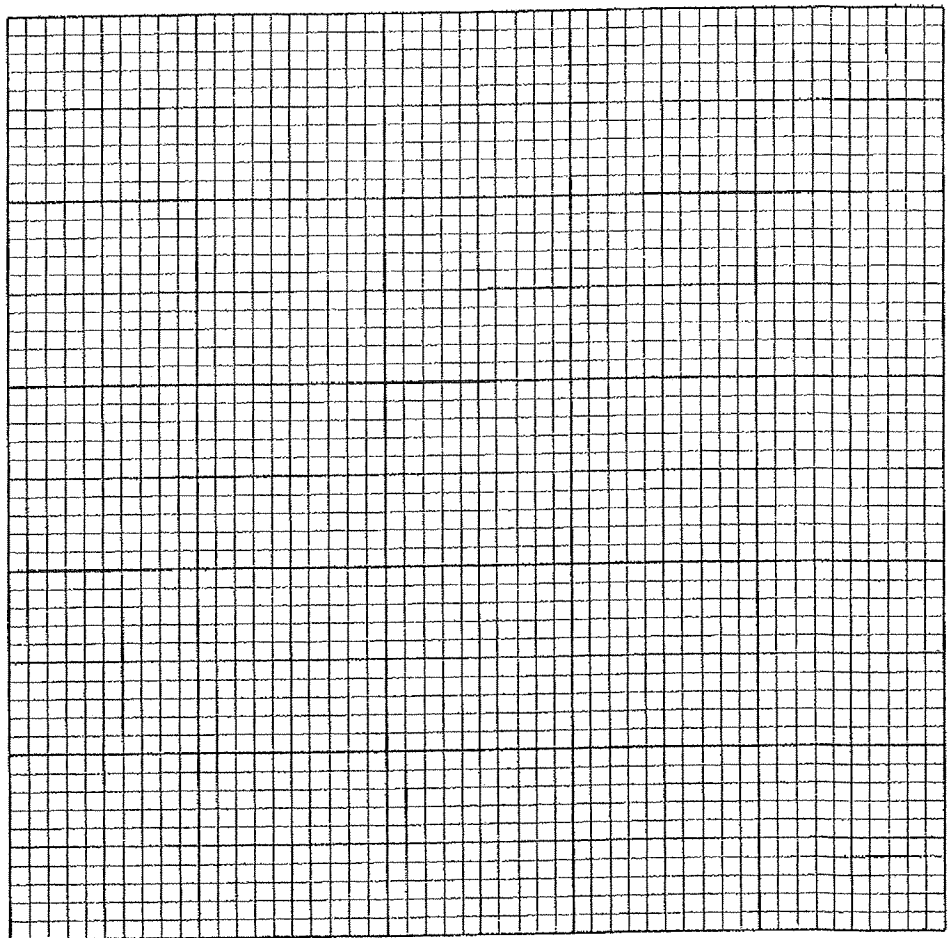
For this graph you will need to determine the following:

a. the *independent* variable _____
Use this to label the horizontal (X) axis.

b. the *dependent* variable _____
Use this to label the vertical (Y) axis.

Graph Title: _____

Graph 9.1



Analysis of Results

1. Calculate the average **rate** of water loss per minute for each of the treatments:

Room: _____

Fan: _____

Light: _____

Mist: _____

2. Explain why each of the conditions causes an increase or decrease in transpiration compared with the control.

CONDITION	EFFECT	REASONS
Room		
Fan		
Light		
Mist		

3. How did each condition affect the gradient of water potential from stem to leaf in the experimental plant?

4. What is the advantage to a plant of closed stomata when water is in short supply? What are the disadvantages?

5. Describe several adaptations that enable plants to reduce water loss from their leaves. Include both structural and physiological adaptations.

6. Why did you need to calculate leaf surface area in tabulating your results?

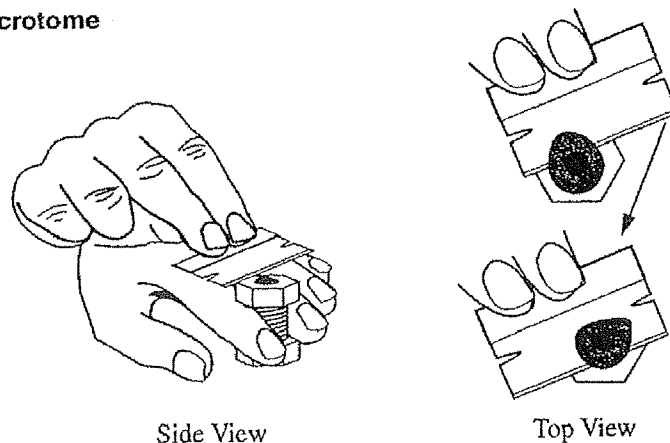
EXERCISE 9B: Structure of the Stem

The movement of fluids and nutrients throughout the plant occurs in the vascular tissue: the xylem and phloem of the roots, stems, and leaves. In this exercise you will study the structure of the plant stem by preparing sections of the stem from the plant that you used in Exercise 9A. If your teacher provides you with prepared slides, proceed to Step 15.

Procedures

1. Obtain a nut-and-bolt microtome from your teacher.
2. Turn the nut until it is almost at the end of the bolt, forming a small "cup."
3. Using a new, single-edge razor blade, cut a short piece of plant stem (approximately 5mm—slightly longer than the depth of the "cup" in the nut) from the base of your plant. Make two cuts so that both ends are freshly cut. Make sure that this portion of the stem is free of petroleum jelly if you are using the same plant that you used for Exercise 9A.
4. Stand the stem up on its end in the opening of the nut and carefully pour melted paraffin into the nut until it fills the opening, completely covering the stem. Your teacher will direct you in safely melting and pouring the paraffin. (Be careful that the paraffin is not too hot when you pour it or you will cook your stem!) This assembly will allow you to hold your stem upright and cut thin slices.
5. Hold the head of the bolt horizontal on the table with one hand. Holding the razor blade in your other hand, remove the excess wax on top by slicing down to the nut. This technique keeps your fingers out of the way of the razor blade (see Figure 9.2).

Figure 9.2: Using the Nut-and-Bolt Microtome



6. Twist the bolt just a little, so a thin core of paraffin and stem sticks up above the surface of the nut.
7. Use a slicing motion to cut this section down to the nut. Use as much of the edge of the razor blade as possible by starting on one end and sliding down to the other with each slice.
8. Put the slice in a dish containing 50% ethanol.
9. Twist the bolt a bit more to get another slice. Remember: you are trying to get the thinnest possible slice. It is better to get part of a thin slice than a thick slice that is entirely round. As you cut each slice, put it in the dish of 50% ethanol. Obtain 8 -10 thin sections.
10. Leave the sections in 50% ethanol for five minutes. Free the plant tissue from paraffin, if necessary.
11. Using forceps, move the sections to a dish of toluidine blue O stain and leave them there for between one and two minutes.
12. Rinse the sections in a dish of distilled water.
13. Mount the sections in a drop of 50% glycerin on a microscope slide.
14. Add a coverslip and observe the sections using a compound microscope.
15. Make a drawing of your sections in the space provided in Figure 9.3. Identify and label the cell and tissue types described below.

Cell Types

Parenchyma. The most abundant cell type is parenchyma. Parenchyma cells are relatively unspecialized and retain their protoplasts throughout their existence. They have primary cell walls. They make up the mesophyll of leaves (where most of the photosynthetic activity takes place), the flesh of fruits, the pith of stems, and the root and stem cortex. Many parenchyma cells are used for food storage (mainly starch). Starch, you will recall, is a polymer of glucose. Starch forms grains within the parenchyma cells. These grains can be seen inside the cells. Re-examine your section and label the parenchyma cells in your drawing.

Sclerenchyma. Elongated sclerenchyma cells make up fibers and have thick secondary cell walls. They are often lignified, and the protoplasts die at maturity. Fibers may be found in leaves, stems, and fruits. Usually fibers are in bundles, serving a support function, and often are associated with vascular tissue. Check your stem cross section for fibers. They will be found just outside the vascular bundles, their thick walls stained bright blue.

Collenchyma. Many young stems and leaves contain collenchyma cells for support. These cells are living at maturity and characteristically have primary cell walls that are thickened at the corners. Locate collenchyma cells in your cross section.

Tissue Types

Xylem. Xylem is a tissue composed of several different cell types. It is the water-conducting tissue that conveys water and minerals from the soil through the plant. The earliest xylem cells to evolve were fiberlike with thick lignified secondary walls arranged with overlapping ends, with a series of membrane-covered "pits" for passing water from one cell to the next. These are the tracheids. The cells that actually carry the water were misnamed "tracheary elements" in the seventeenth century — "trachea" means air duct — and the name was never corrected. Vessel elements evolved later, first appearing in flowering plants, and are larger in diameter, have holes rather than pits, and offer less resistance to water flow than tracheids. Both vessel elements and tracheids are dead at maturity. Xylem tissue may also contain parenchyma cells and fibers. Look at your cross sections and label the xylem in your drawing.

Phloem. Phloem is a tissue that distributes the carbohydrate products of photosynthesis throughout the plant. This is achieved in flowering plants by the sieve tube members, which have primary cell walls and living protoplasts at maturity but lack nuclei. Companion cells are associated with sieve tube members. These companion cells have nuclei and play an important role in the transfer of substances from cell to cell. Phloem may also contain parenchyma cells and fibers. Look at your cross section. The phloem is located outside the xylem. This aggregation of xylem and phloem is called the vascular bundle. Monocots and dicots have different arrangements of the xylem and phloem tissues, but the cell and tissue types involved are the same.

Epidermis. The epidermis is the outermost layer of cells that serves as a covering for the above-ground plant parts. Some epidermal tissues are covered with a layer of cutin which prevents water loss. The specialized guard cells of the epidermis open and close the stomates. Locate the epidermis on your stem section, then locate guard cells on the leaf section.

Figure 9.3: Stem Cross Section

