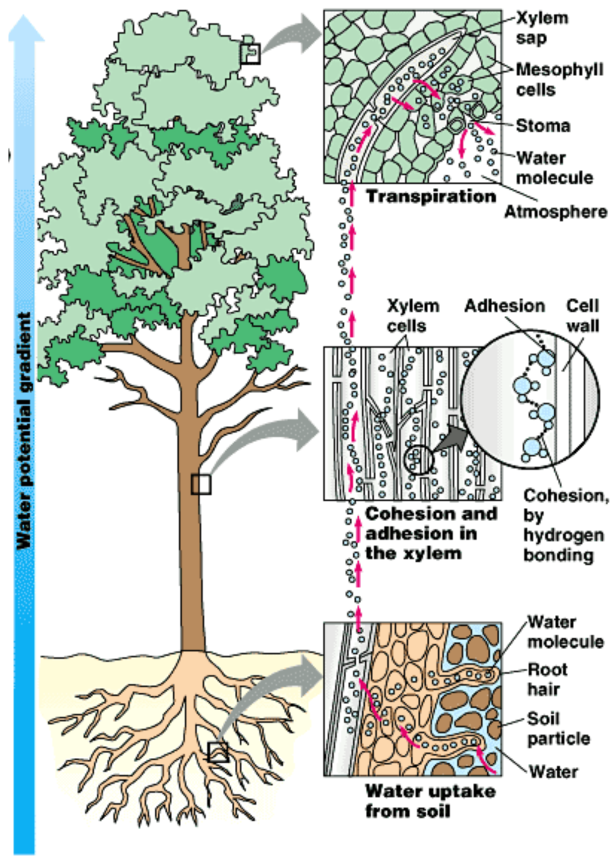
Lab 3: Transpiration

Purpose[1]

The goals of this lab are to (1) observe water movement against gravity from stems to leaves of plants and (2) investigate environmental factors that regulate the rate of transpiration.

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** and **guttation**. If this water is not replaced, the plant will wilt and may die. The transport 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 adhesive and cohesive forces of water molecules.



Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

The overall process: Minerals actively transported into the root accumulate in the xylem, increase solute concentration and decrease 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. The moisture in the air spaces is replaced by water from the adjacent mesophyll cells, lowering their water potential. Water will then move into the mesophyll cells by osmosis from surrounding cells with the 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, (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 **ten-**

[1] Portions of this lab have been adopted from Light and Life, Dr. Bill Eisinger, Santa Clara University.

sion (negative pressure) to form in the xylem, pulling the xylem walls inward. The tension also contributes to the lowering of the 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 the plant. 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 the 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.

Watch the following video clip about transpiration: http://www.youtube.com/watch?v=mc9gUm1mMzc

Part I: Water Transport Through a Carnation or Celery Stalk

As water evaporates from leaves or flower petals, water is pulled up the stem of the plant through the xylem. In this experiment, you will observe the water transport through the xylem of a carnation or stalk of celery.

PROCEDURE:

1. **Observe** the structure of the carnation plant or celery stalk and the color of the carnation leaves or the celery stalk.
2. **Develop a hypothesis** about the movement of food coloring/water from the stem to the flower due to transpiration. Variables you may wish to consider: length of the stem, switching the food coloring, splitting the stem in two different colors. What is the rationale behind your hypothesis?
3. **Design an experiment** to test your hypothesis.
4. Equipment at your disposal: Fresh white carnations or stalks of celery, Scissors, Food coloring (red or blue are best), 250 ml beakers, cups or vases, water bucket to cut stems
5. **Run the experiment and collect data**.
    - **a.** Put one cup of water in a cup or vase and add 6 or more drops of food coloring. The water should turn the color of the food coloring.
    - **b.** Take a white long stemmed carnation (or celery) and diagonally cut the very bottom of the stem under water. This allows fresh plant tissue to transport the dye solution at a relatively fast rate.
    - **c.** Immediately place the carnation into the beaker of colored water and label the beaker with your group name and the date.
    - **d.** Allow 1.5-24 hours for results.
    - **e.** The petals should turn the color of the food dye.
    - **f.** Note how the patterns of dye travel through the flowers. Are the colored areas confined to certain areas of tissue in the flowers?
    - **g.** If time allows: Cut a cross section of the carnation stem and observe the water transport system. What do you notice? Look closely at the arrangement of stained tissues in the stem.
    - **h.** Put a cross section of the stem under a dissection microscope in order to look closer at the xylem system. Recall what you learned about xylem in lab 2.

   i. Describe the results that you observe and briefly record your interpretation and conclusions.

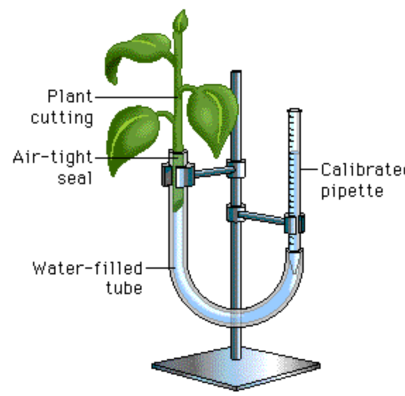
6. **Interpret the results and draw conclusions** about the hypothesis. Was your hypothesis supported by your data? Speculate on why or why not
7. If your hypothesis was not supported, **modify your hypothesis** in view of the experimental results and design more experiments to further test the modified hypothesis.

Part II: Determining the Effects of Environmental Factors on Transpiration Using a Potometer

In this experiment, you will measure transpiration under various laboratory conditions using a **potometer** (from the Greek *poto* to drink). Four suggested plant species are *Coleus, Oleander* **(note: leaves of this plant are poisonous!)**, *Zebrina*, and two week old bean seedlings.

PROCEDURE:

1. **Observe** how a potometer can be used to measure transpiration.
   a. Place the tip of a 1 mL pipette into a 16 -inch piece of clear plastic tubing.
   b. Submerge the tubing and the pipette in a shallow tray of water. Draw water through the tubing until **all** the air bubbles are eliminated.
   c. Carefully cut your plant stem under water. This step is very important, because no air bubbles must be introduced into the xylem.
   d. While your plant and tubing are submerged, insert the freshly cut stem into the open end of the tubing.
   e. Bend the tubing upward into a "U" and use the clamp on a ring stand to hold both the pipette and the tubing.
   f. If necessary use parafilm or petroleum jelly to make an airtight seal surrounding the stem **after** it has been inserted into the tube. **Do not put petroleum jelly on the end of the stem.**
   g. Let the potometer equilibrate for 10 minutes before recording the time zero reading. As water evaporates from the leaves, water will be pulled from the pipette. For example, after 5 minutes the pipette may have lost 0.02 ml of water. We can use this data to calculate the rate of transpiration to be 0.004 ml/min (0.02 ml/5 min).



Potometer

2. **Develop a hypothesis** about variables that may affect the rate of transpiration. Variables you may wish to test: temperature, light intensity, humidity, stem damage, air flow or another variable of your choice. What is the rationale behind your hypothesis?
3. **Design an experiment** to test your hypothesis. Equipment at your disposal: fan, heat lamp, water spray bottle and plastic bags. Test a variable different from the other lab groups. The lab as a whole should consider appropriate controls.
4. **Run the experiment and collect data**.
   a. Read the level of water in the pipette at the beginning of your experiment (time zero) and record your finding.
   b. Continue to record the water level in the pipette every **3** minutes for **30** minutes and record the data.

c. At the end of your experiment, cut the leaves off the plant and mass [weigh] them. Remember to blot off all excess water before massing [weighing]. This will give you an estimate of total leaf surface area; this will be important if you want to compare your results of other students in the class.
d. Share your data with the rest of the class.
e. **Create a graph** of the collective (whole class) data that presents water loss due to transpiration as a function of time. Remember to account for differences in surface area when graphing your data.

5. **Interpret the results and draw conclusions** about your hypothesis. Was your hypothesis supported by your data? Speculate on why or why not.
6. If your hypothesis was not supported, **modify your hypothesis** in view of the experimental results and design more experiments to further test the modified hypothesis.

Lab 3: Transpiration

Name & Date ____

Part I: Water Transport Through a Carnation or Celery Stalk

1. Write your hypothesis and describe your observations for your experiment. Did your observations support your hypothesis? Why or why not?

2. Why is it important to cut the stem or stalk of the plant tissue underwater prior to observing or measuring transpiration?

Part II: Determining the Effects of Environmental Factors on Transpiration

3. Attach a graph of the class data for transpiration as measured using the potometers, which presents water loss due to transpiration as a function of time. Remember to account for differences in surface area when graphing your data. Include an appropriate figure legend.

4. Write your hypothesis and explain whether it was supported by the data collected.

5. For each of the factors tested by the class, explain the biological rationale for the effect on transpiration as indicated by the data.

6. According to your graph, which factor tested in lab had the greatest effect on the rate of transpiration? What evidence did you use to come to this conclusion?