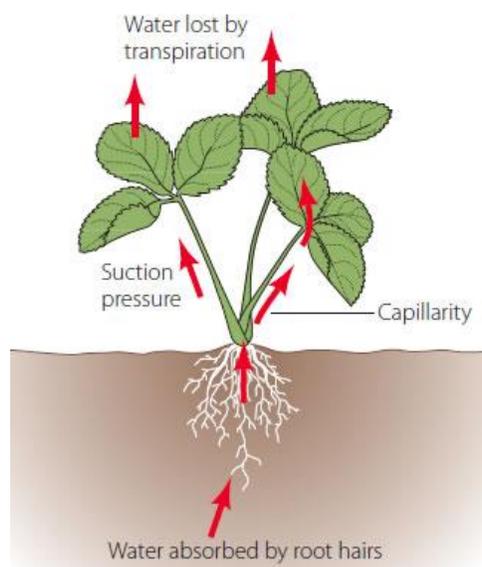# LAB 24 – Transpiration

Objectives:
- To 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.
- To test the effects of environmental variables on rates of transpiration using a controlled experiment.

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 lost 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 (see Lab 4 – Diffusion and Osmosis for a review of Ψ_w). The movement itself is facilitated by osmosis, root pressure, and adhesion and cohesion of water molecules (see Lecture 24 – Regulation and Transport in Plants for a review of water flow through a plant's vascular tissues.

The Overall Process
Minerals actively transported into the root accumulate in the xylem, increasing solute concentration and therefore *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 the 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 tension. The "pull" on the water from transpiration results from the cohesion and adhesion of water molecules (**Figure 1**).


Figure 1: Transpiration model.

The Details

Transpiration begins with the evaporation of water through the **stomata** (stomates), 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 high 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 occurs because of (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.

Plants lose as much as 90% of the water that enters the roots by the process of transpiration. Evaporation through the open stomates is clearly 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 the stomata 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. In this lab, you will measure transpiration under various laboratory conditions using a **potometer**.

Procedure:

1. Obtain 3 pieces of flexible plastic tubing. There should be a 1", 6", and 9" piece.
2. Connect the 6" and 9" pieces 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 that the pipet is firmly inserted. You want to create an airtight system. BE CAREFUL NOT TO SNAP THE GLASS PIPET IN YOUR HANDS!
5. Bend the potometer setup into a J-shape and attach it to a ring stand using two (or more if needed) ring stand clamps. Clamp the potometer in place so that the end of the tubing is about half the height of the graduated pipet (see **Figure 2**).
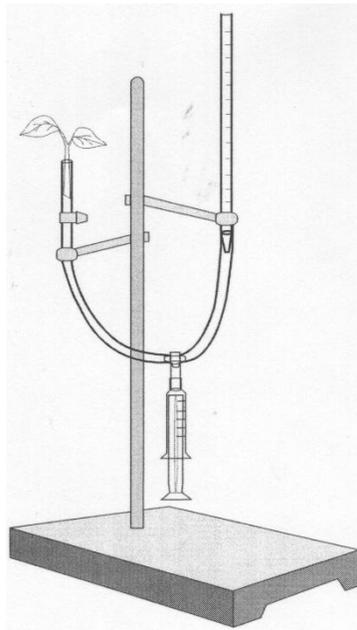
Figure 2: Potometer setup.

6. Fill a 10 mL syringe completely with water. Remove any air bubbles.

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 scalpel, cleanly cut the stem of a bean seedling near the soil. The diameter of the plant stem should fit snugly into the end of the tubing without having to force it in and damaging the tissue. (**It may require several cuts to obtain a suitable piece of plant stem—I recommend first cutting a 'thick' portion, and then trim down to size if necessary**)

10. Insert at least ½" of the stem of the seedling into the flexible tubing. Be sure that 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. "Zero" it by gently depressing the plunger of the syringe until the water level reaches the zero mark in the graduated pipet. (IF THERE IS NOT ENOUGH WATER IN THE SYRINGE TO DO THIS, MAKE SURE YOU RECORD THE STARTING LEVEL OF THE WATER.)

13. Allow the potometer to equalize for approximately 10 minutes.

14. Record your initial reading – time = 0 (which should be near 0.000 mL) for your setup in **Table 1**. Be sure to label the condition that your group was assigned in the data table. Monitor your potometer for 30 minutes. Record data every three minutes.

15. Calculate the amount of water lost from **Table 1** and enter this in **Table 2** for each interval.

16. This procedure will be repeated in three (or more – depending on class size) other environmental conditions. Your group will be assigned one of the following variations:
    a. Setup 1: Study the plant in room conditions.
    b. Setup 2: Place a fan on low setting, approximately three feet from the plant.
    c. Setup 3: Mist the plant leaves with water and place a plastic bag over the plant.
    d. Setup 4: Place the plant in darkness.
    e. Setup 5: Place the plant directly under a fluorescent Gro-Light

17. Once you have completed the experiment, cut the leaves off your seedling and gently blot off any excess moisture. Arranging all the cut-off leaves on the grid (**Figure 3 –** found in the back of the lab), trace the edge pattern directly onto the grid. Count all of the grid boxes that are completely within the tracing and estimate the number of grids that lie partially within the tracing. **Figure 3** has been constructed so that a square of 4 blocks equals 1 cm^2. The total surface area can then be calculated by dividing the total number of blocks covered (totally and estimated sum of all partials) by 4. This will be in cm^2 – you need to convert to m^2 (remember: 1 cm = 0.01 m). Record this value and make sure to indicate this in your results section. You will need this to calculate water loss per m^2.

18. Calculate the water loss per square meter of leaf surface by dividing the water loss at each reading by the leaf surface area you calculated. Record all results in **Table 2**.

19. Finally, record the data from each group in the class in **Table 3**.

Results:

Table 1: Potometer Readings for the Variable: _____

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

Table 2: Individual Water Loss (mL and mL/m^2) for the Variable: _____

Time (min)	0	3	6	9	12	15	18	21	24	27	30
Water Loss (mL)											
Water Loss per m^2											

Table 3: Water Loss in mL/m^2 for each Variable

Time (min)	0	3	6	9	12	15	18	21	24	27	30
Room											
Fan											
Mist											
Darkness											
Gro-Light											
TBD:											

Hints For Your Lab Report:

The <u>Introduction</u> section of your lab should have (but not be limited to) your hypotheses for each of the variables that will be tested. Sequence the hypothesized order of water loss from most to least. A visual – made via an Excel 'drawing' – should be included with your written hypothesis showing the hypothesized respective rates of transpiration.

The <u>Results</u> section of your lab report will include:
- Indicate what total surface area of your group's leaves were. Show all calculations!
- All data tables (properly formatted and labeled).
- Graph of the water loss vs. time of each of the treatments on one graph. You may have to convert data to scientific notation. All numbers should have been recorded to the nearest thousandth. For mL/m^2, go to the nearest hundredth.

Figure 3