Lab 7: Transpiration

Objectives:
• Examine how environmental factors (light, wind, humidity, temperature, etc.) affect the transpiration rate.
• Learn to identify stomata and determine whether they are opened or closed.
• Practice the scientific method by creating and testing hypotheses, evaluating data, and preparing and presenting your results.

Introduction
Recall from lecture how water is transported from the soil to the roots and from the roots to the rest of the plant. Most of the water taken up by a plant is lost as water vapor through the leaves, a process called transpiration. This water loss occurs through the stomata. Most plants require their stomata to be open during the day in order to obtain carbon dioxide for photosynthesis. Stomata can be opened and closed and help regulate the water balance in a plant. The rate of transpiration is affected by a number of environmental factors as well as leaf morphology. Different environmental factors change the rate of diffusion of water vapor and also affect the opening and closing of stomata.

In this lab you will be investigating the effects of different environmental factors on the rate of transpiration in a plant. You will be using a potometer (a pipette attached to a rubber tube) to measure the rate of water loss in a plant.

General procedure
Work in groups of four. Your objective is to test how different environmental conditions affect the rate of transpiration in plants. Within your group, discuss how different environmental conditions affect the transpiration rate and what triggers stomata to open or close. Determine what one environmental condition your group would like to test. State a hypothesis for the experiment and make a prediction of what you think will happen.

Once you have selected an environmental condition, your instructor will provide you with materials to simulate that environment. You will then follow the methods below to conduct your experiment.

Method
1. Collect branches of the appropriate size to fit the potometer, one branch for each group. Try to select branches with whole, undamaged leaves. (Your instructor may have already done this step).
2. Place branches in the buckets and cut the stems underwater, keeping the cut portion submerged. (Your instructor may have already done this step.)
3. Place the potometer (tubing with pipette attached) under the water and fill it up with water. Try to remove any air bubbles from the tubing. Keeping the potometer and the cut end of the branch submerged, place the end of the branch into the tubing. Be careful not to tear the exterior of the stem. If the stem is too large, recut it underwater and try inserting it into the tubing again.
4. Attach the potometer and branch to the clamp stand as demonstrated by your instructor (see fig. 1). Make sure the tubing is not being constricted by the clamps. If there are leaks where the branch and tubing meet, wrap this area with parafilm.

5. Let the branch **rest for 5 minutes**.

6. Data collection for control treatment:
   a. Record in Table 1 the time and the amount of water in the pipette. This is your start time.
   b. Record the amount of water in the pipette every five minutes for a total of at least 20 min. Record your data in Table 1.

7. To determine whether the stomata were open or closed on your plant at the end of the control treatment, follow the procedure for stomatal impressions below.

8. Change the conditions to your experimental treatment (using the same branch).
   a. If necessary add water to the pipette before beginning.
   b. Allow the branch to **rest for 15 min**.

9. Data collection for experimental treatment:
   a. Record in Table 2 the time and the amount of water in the pipette.
   b. This is your start time. Record the amount of water in the pipette every five minutes for a total of at least 20 min. Record your data in Table 2. If it is necessary to add more water during the experiment, be sure to note how much water was added and account for it in Table 2.

10. To determine whether the stomata were open or closed on your plant at the end of the experiment, follow the procedure for stomatal impressions below.

11. Graph your data (you may begin graphing as soon as you start collecting data).
   a. For the control data plot the total water lost in ml (y axis) over elapsed time (x axis).
   b. Use the slope of a straight, best-fit line through the data points to calculate the rate of water loss (ml/min) (Hint: remember “rise over run”).
   c. Record your data in Table 3.
   d. On the same graph, repeat for the experimental treatment data.

12. Calculate the effect the treatment had on the rate and record it in Table 3.

   Effect of treatment (%) = \frac{\text{experimental rate} - \text{control rate}}{\text{control rate}} \times 100
Making stomatal impression:
1. Apply a very thin layer of clear finger nail polish to a section of the upper epidermis of the leaf.
2. Allow it to dry.
3. Place a piece of clear tape over the finger nail polish and press the tape to the leaf.
4. Remove the tape from the leaf.
5. Place the tape on a slide and examine with a compound microscope. (Hint: for easy removal of tape, fold over a small portion of one end so the tape sticks to itself, providing a “handle” for removal.)
6. You should be able to observe the stomata and whether they are open or closed.
7. Repeat on the lower epidermis of the leaf.
8. When done remove the tape from the slide, clean and dry the slide, and return it to the box.

Results

Table 1: Control data

<table>
<thead>
<tr>
<th>Clock time (min)</th>
<th>Total elapsed time</th>
<th>Pipette reading (ml)</th>
<th>Total water lost (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Were the stomata on your plant open or closed at the end of the control treatment?

Table 2: Experimental data

<table>
<thead>
<tr>
<th>Clock time (min)</th>
<th>Total elapsed time</th>
<th>Pipette reading (ml)</th>
<th>Total water lost (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Were the stomata on your plant open or closed at the end of the experimental treatment?
You may use either just your own data, or include data from the classmates in your paper. **If you are collaborating**, be sure to collect the information for Table 3 **before** you leave lab.

### Table 3: Class results

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Prediction (increase, decrease, no change)</th>
<th>Control rate (ml/min)</th>
<th>Experimental rate (ml/min)</th>
<th>Effect of treatment (%) [\frac{((E.R. - C.R.)}{C.R.}) \times 100]</th>
<th>Stomata open or closed?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each experimental condition above, make sure you are able to explain the reasoning behind each prediction. Make sure you discuss the rate of diffusion of water vapor from the stomata.

Did what was predicted in each experiment occur? Why or why not?

---

**Report**

You lab report for this assignment will consist of a paper. Please see “Paper guidelines for the Transpiration Lab”.

---

*Bio203: Transpiration*