Name _____ Class _____ Date_____

Zip Up the Xylem

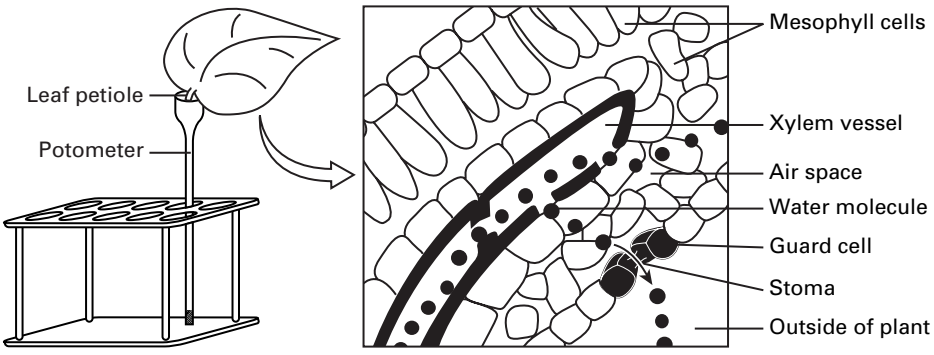
Measuring Transpiration Rates

Question How do plants control the rate at which water is transported through the xylem?

Lab Overview In this investigation you will perform an experiment to measure and compare the transpiration rates of leaves under varying environmental conditions such as intense light, wind, or humidity. You will also make imprints of the bottom surfaces of some of the leaves to observe the guard cells and stomata.

Introduction Stomata (openings in plant leaves) enable carbon dioxide to enter a plant. The openings also allow evaporative cooling, which keeps plant enzymes from breaking down in hot conditions. Environmental conditions influence the number of stomata that are open. For example, low carbon dioxide levels in a leaf cue the guard cells to actively accumulate potassium ions. Due to osmosis, water follows the potassium ions into the guard cells, causing them to swell until gaps (the stomata) open between them. When more water has been lost through transpiration than can be replaced from the soil, the guard cells lose pressure and sag together. The stomata close, preventing more water loss.

Prelab Activity You will make a device called a potometer (puh TAWM ih tur) to measure transpiration rates in the lab. To make it, you will fill four transfer pipettes with water and seal the tips to prevent water loss. Next you will cut off the tops of the bulbs so that a leaf petiole (stalk) can be inserted into each pipette. As water transpires from the stomata, more water will be drawn up through the petioles of the leaves (see diagram below).



You will keep two potometers in "normal" classroom conditions and place two potometers in a different condition such as high humidity, wind, or bright light. You will record how long it takes for water to leave the potometer and calculate the transpiration rate of each sample.

Prelab Questions

1. Explain how the opening and closing of stomata are controlled.

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2. Compared to average environmental conditions, predict how the conditions below would affect a plant's rate of transpiration. Rate each condition using the following scale: 1 = greatly decrease, 2 = slightly decrease, 3 = no effect, 4 = slightly increase, 5 = greatly increase. Explain your predictions on the lines below.

____ High humidity

____ Light wind

____ Heavy wind

____ Moderate light

____ Bright light

_____

_____

Materials

- 2 permanent markers of different colors
- 4 disposable transfer pipettes
- plastic cup of water with food coloring
- safety pin
- petroleum jelly in lip applicator tube
- scissors
- test-tube rack
- large leaves with long petioles or narrow stems
- laboratory balance
- plastic bowl (deep enough to cut the petiole underwater)
- clear, rapid-drying fingernail polish
- clear mailing tape
- microscope slide
- microscope

Procedure

Part A: Making the Potometers

1. With a permanent marker, mark the halfway point between each graduation (marking) on a transfer pipette. For example, between the 0.75 and 1 mL marks, make a mark to represent 0.875 mL. Repeat on the other three transfer pipettes.

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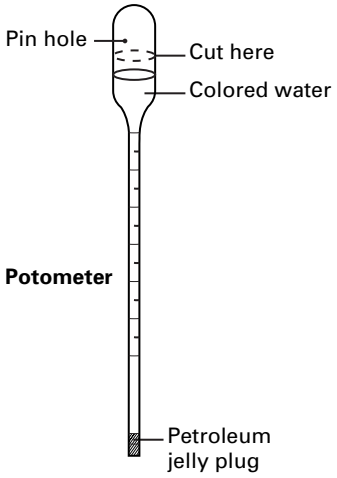
2. Place a transfer pipette in colored water and draw up water past the 1 mL mark. Let go of the bulb before taking the pipette out of the water.
3. Open the petroleum jelly and squeeze the air out of the tip. Place the petroleum jelly tube under the tip of the pipette. Squeeze about 3 mm of petroleum jelly into the pipette to seal it.
4. Without squeezing the pipette bulb, use a safety pin to poke a hole in the bulb. (This keeps pressure from building up in the bulb.) With scissors, cut off the top of the pipette bulb. Repeat steps 1–4 for the other three pipettes. Then place the potometers in a test-tube rack.



5. Find the mass of each leaf using a laboratory balance. Enter the mass of each leaf in Data Table 1 on the next page.
6. Place Leaf 1 in the bowl of water so that its petiole or stem is underwater. Cut off the end of the petiole or stem underwater. Repeat with the other three leaves.
7. Place a leaf petiole or stem in each potometer. Label the leaf samples 1–4 on the remaining portion of the pipette bulb.
8. In Part B you will need to remove the leaves from the potometers to measure how much water has transpired. Because you are starting with only about 1 mL of water in each potometer, the water that sticks to the leaf petioles will affect your measurements. Therefore, you need to know how much water remains in the potometers when the petioles are removed. Remove the leaves and record the initial water levels of each potometer below.

   Leaf 1 potometer initial water level: _____ mL

   Leaf 2 potometer initial water level: _____ mL

   Leaf 3 potometer initial water level: _____ mL

   Leaf 4 potometer initial water level: _____ mL
9. Replace the leaves. Mark the water level on the pipette with a marker of a different color than the one you used before. This mark will help you notice when the water level changes.

Part B: Measuring Transpiration Rates

1. Place Leaf 1 and Leaf 2 in ambient conditions (existing classroom conditions—no special treatment). These two leaves will be your experimental controls. (Testing two leaves in each environment will help verify your results.) Record the time below.

   Start time for Leaf 1 and Leaf 2: _____

2. You will be assigned to test the effect of intense light, wind, or humidity. Record the variable you are testing in Column 1 of Data Table 1. As directed by your teacher, place Leaf 3 and Leaf 4 in your assigned environmental condition. Record the time below.

   Start time for Leaf 3 and Leaf 4: _____

3. To take a measurement on a leaf sample, remove the petiole from the potometer. Read the level of the water and quickly replace the petiole. Subtract the new water level from the initial water level that you recorded in Part A, Step 7. Record the *difference* in Data Table 1. You can take measurements every 10 min or at whatever intervals are appropriate. For instance, if you notice the water level has decreased before 10 min are up, take a measurement and note the time. If nothing has happened in 10 min, take a measurement at 15 min instead. Take measurements for each leaf at three time intervals before calculating the total transpiration rates. To calculate the rate, divide the third water level reading by the mass of the leaf and by the total number of minutes.

Data Table 1

	Mass of Leaf (g)	**Water-Level Difference at ___ min**	**Water-Level Difference at ___ min**	**Water-Level Difference at ___ min**	**Total Transpiration Rate $\left(\frac{\text{mL/g}}{\text{min}}\right)$**
Leaf 1 (control)		____ mL	____ mL	____ mL	
Leaf 2 (control)		____ mL	____ mL	____ mL	
Leaf 3 Variable: _____		____ mL	____ mL	____ mL	
Leaf 4 Variable: _____		____ mL	____ mL	____ mL	

4. Plot 4 line graphs on the same grid on the next page. The *x*-axis should show time (in min) and the *y*-axis should show amount of water transpired (in mL). Be sure to label the different lines and axes and title your graph.

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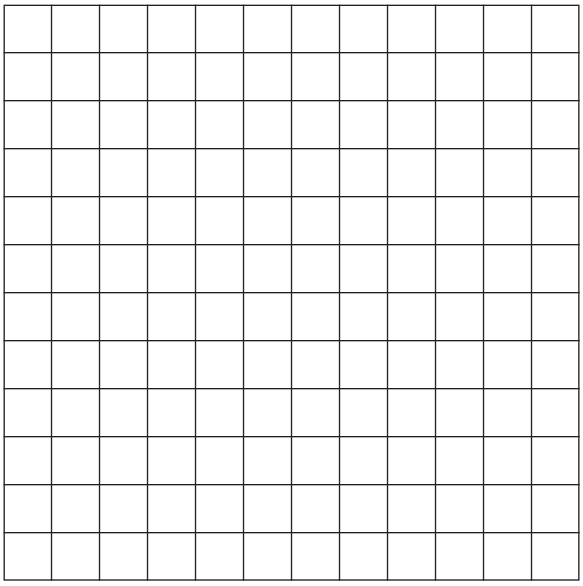
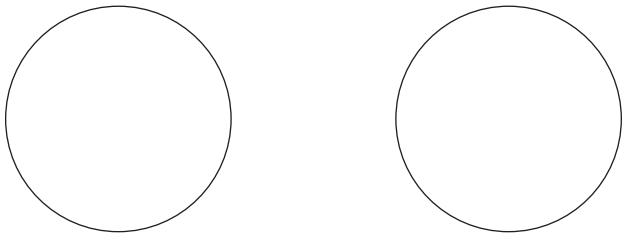


Part C: Observing Stomata and Guard Cells

1. Apply clear fingernail polish to a 1-cm^2 section of the underside of Leaf 2 to make an impression of the stomata and guard cells. Repeat with Leaf 4.
2. Follow your teacher's instructions to allow time for the fingernail polish to dry. When it is dry, cut out two 2-cm^2 pieces of clear mailing tape. Place the sticky side of the tape over the dry polish on each leaf and then gently pull it off. With a permanent marker, label one corner of the tape with the corresponding number of each leaf.
3. Place both pieces of tape sticky side down on a microscope slide.
4. Focus first on low power, then switch to medium power to observe the impression of the leaves' epidermal cells and guard cells. Draw and label sketches of the impressions of both leaves below.



5. Count the numbers of open and closed stomata in Leaf 2 that you can see in a field of view at 100× power (medium power on most microscopes). Record the numbers of open and closed stomata and the total number of stomata in Data Table 2 on the next page. Repeat with Leaf 4. Calculate the percentage of open stomata by dividing the number of open stomata by the number of total stomata. Then multiply by 100%.

Data Table 2

	Open Stomata	Closed Stomata	Total Stomata	% Open Stomata	Transpiration Rate (mL/g/min) (from Part B)
Leaf 2 (control)					
Leaf 4 (wind)					
Leaf 4 (humidity)					
Leaf 4 (bright light)					

6. To fill in the rest of the table, gather data from your classmates about leaves in the conditions that you did not test.

Analysis and Conclusion

1. Compare the predictions you made in the Prelab Activity to the data. Do the data support your prediction? Explain.

_____

_____

2. What can you conclude about the response of plants to wind and humidity?

_____

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_____

3. A gardener noticed that her plants were more wilted on sunny days even when it wasn't very hot. Explain why this might have occurred, based on the results of your experiment.

_____

_____

Extension

With permission from your teacher, repeat the procedure in Part C to make impressions of leaves from plants adapted to specific conditions, such as drought-tolerant plants that are adapted to very dry conditions (smooth-leaved species such as aloe, cape honeysuckle, and Indian hawthorne). First predict how you think the results will compare to what you observed in this lab. Then count the numbers of open and closed stomata in one field of view under 100× power, and compare the number with your previous observations.