INVESTIGATION 11

TRANSPIRATION*

What factors, including environmental variables, affect the rate of transpiration in plants?

BACKGROUND

Cells and organisms must exchange matter with the environment to grow, reproduce, and maintain organization, and the availability of resources influences responses and activities. For example, water and macronutrients are used to synthesize new molecules, and, in plants, water is essential for photosynthesis. Organisms have evolved various mechanisms for accumulating sufficient quantities of water, ions, and other nutrients and for keeping them properly balanced to maintain homeostasis.

Plants absorb and transport water, nutrients, and ions from the surrounding soil via osmosis, diffusion, and active transport. Once water and dissolved nutrients have entered the root xylem, they are transported upward to the stems and leaves as part of the process of transpiration, with a subsequent loss of water due to evaporation from the leaf surface. Too much water loss can be detrimental to plants; they can wilt and die.

The transport of water upward from roots to shoots in the xylem is governed by differences in water (or osmotic) potential, with water molecules moving from an area of high water potential (higher free energy, more water) to an area of low water potential (lower free energy, less water). (You may have studied the concept of water potential in more detail when exploring the processes of osmosis and diffusion in Investigation 4 in this manual.) The movement of water through a plant is facilitated by osmosis, root pressure, and the physical and chemical properties of water. Transpiration creates a lower osmotic potential in the leaf, and the TACT (transpiration, adhesion, cohesion, and tension) mechanism describes the forces that move water and dissolved nutrients up the xylem.

* Transitioned from the *AP Biology Lab Manual* (2001)

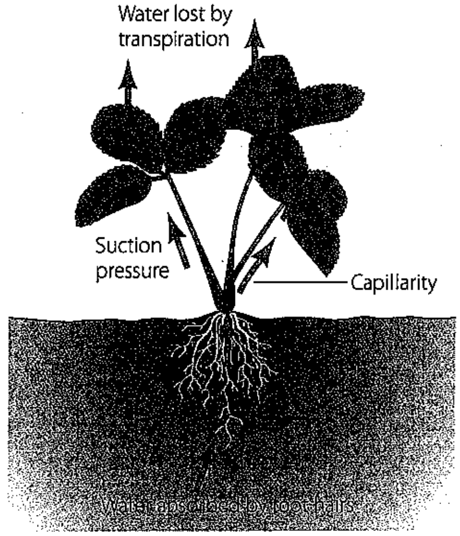

Figure 1. Transpiration Model

During transpiration, water evaporating from the spaces within leaves escapes through small pores called stomata. Although evaporation of water through open stomata is a major route of water loss in plants, the stomata must open to allow for the entry of CO_2 used in photosynthesis. In addition, O_2 produced in photosynthesis exits through open stomata. Consequently, a balance must be maintained between the transport of CO_2 and O_2 and the loss of water. Specialized cells called guard cells help regulate the opening and closing of stomata.

In this laboratory investigation, you will begin by calculating leaf surface area and then determine the average number of stomata per square millimeter. From your data, several questions emerge about the process of transpiration in plants, including the following:

- Do all plants have stomata? Is there any relationship between the number of stomata and the environment in which the plant species evolved?
- Are leaf surface area and the number of stomata related to the rate of transpiration? What might happen to the rate of transpiration if the number of leaves or the size of leaves is reduced?
- Do all parts of a plant transpire?
- Do all plants transpire at the same rate? Is there a relationship between the habitat in which plants evolved and their rate of transpiration?
- What other factors, including environmental variables, might contribute to the rate of transpiration?
- What structural features and/or physiological processes help plants regulate the amount of water lost through transpiration? How do plants maintain the balance between the transport of CO_2 and O_2 and the amount of water lost through transpiration?

You will then design an experiment to investigate one of these questions or a question of your own. As a supplemental activity, you can examine microscopically thin sections of stems, identify xylem and phloem cells, and relate the function of these vascular tissues to observations made about the structure of these cells.

The investigation also provides an opportunity for you to apply and review concepts you have studied previously, including the relationship between cell structure and function; osmosis, diffusion, and active transport; the movement of molecules and ions across cell membranes; the physical and chemical properties of water; photosynthesis; and the exchange of matter between biological systems and the environment.

Learning Objectives

- To investigate the relationship among leaf surface area, number of stomata, and the rate of transpiration
- To design and conduct an experiment to explore other factors, including different environmental variables, on the rate of transpiration
- To investigate the relationship between the structure of vascular tissues (xylem and phloem) and their functions in transporting water and nutrients in plants

General Safety Precautions

If you investigate transpiration rates using a potometer, you should be careful when assembling your equipment and when using a razor blade or scalpel to cut the stem of a plant, cutting to a 45° angle.

When appropriate, you should wear goggles for conducting investigations. Nail polish used in the investigation is toxic by ingestion and inhalation, and you should avoid eye contact. All materials should be disposed of properly as per your teacher's instructions.

THE INVESTIGATIONS

Getting Started

These questions are designed to help you understand concepts related to transpiration in plants before you design and conduct your investigation(s).

1. If a plant cell has a lower water potential than its surrounding environment, make a prediction about the movement of water across the cell membrane. In other words, will the cell gain water or lose water? Explain your answer in the form of a diagram with annotations.

2. In the winter, salt is sometimes spread over icy roads. In the spring, after the ice has melted, grass often dies near these roads. What causes this to happen? Explain your answer in the form of a diagram with annotations.

3. Prepare a thin section of stem from your plant and examine it under the microscope to identify the vascular tissues (xylem and phloem) and the structural differences in their cells. Describe how the observed differences in cellular structure reflect differences in function of the two types of vascular tissue.

4. If you wanted to transplant a tree, would you choose to move the tree in the winter, when it doesn't possess any leaves but it's cold outside, or during the summer, when the tree has leaves and it's warm and sunny? Explain your answer.

Procedure

Materials

- Living representative plant species available in your region/season, such as *Impatiens* (a moisture-loving plant), *Coleus*, oleander (more drought tolerant), *Phaseolus vulgaris* (bean seedlings), pea plants, varieties of *Lycopersicon* (tomato), peppers, and ferns
- Calculator, microscope, microscope slides, clear cellophane tape, clear nail polish, and scissors
- Additional supplies that you might need after you choose a method to determine leaf surface area (Step 1 below). Ask your teacher for advice.

Record data and any answers to questions in your lab notebooks, as instructed by your teacher.

Step 1 Form teams of two or three and investigate methods of calculating leaf surface area. (You will need to calculate leaf surface area when you conduct your experiments.) Think about and formulate answers to the following questions as you work through this activity:

a. How can you calculate the total leaf surface area expressed in cm^2? In mm^2?

b. How can you estimate the leaf surface area of the entire plant without measuring every leaf?

c. What predictions and/or hypotheses can you make about the number of stomata per mm^2 and the rate of transpiration?

d. Is the leaf surface area directly related to the rate of transpiration?

e. What predictions can you make about the rate of transpiration in plants with smaller or fewer leaves?

f. Because most leaves have two sides, do you think you have to double your calculation to obtain the surface area of one leaf? Why or why not?

g. Water is transpired through stomata, but carbon dioxide also must pass through stomata into a leaf for photosynthesis to occur. There is evidence that the level of carbon dioxide in the atmosphere has not always been the same over the history of life on Earth. Explain how the presence of a higher or lower concentration of atmospheric carbon dioxide would impact the evolution of stomata density in plants.

h. Based on the data in the following table, is there a relationship between the habitat (in terms of moisture) to which the plants are adapted and the density of stomata in their leaves? What evidence from the data supports your answer?

Table 1. Average Number of Stomata per Square Millimeter (mm^2) of Leaf Surface Area

PLANT	IN UPPER EPIDERMIS	IN LOWER EPIDERMIS
Anacharis	0	0
Coleus	0	141
Black Walnut	0	160
Kidney Bean	40	176
Nasturtium	0	130
Sunflower	85	156
Oats	25	23
Corn	70	88
Tomato	12	130
Water Lily	460	0

Step 2 Make a wet mount of a nail polish stomatal peel to view leaf epidermis using the following technique:

a. Obtain a leaf. (The leaf may remain on the plant or be removed.)

b. Paint a solid patch of clear nail polish on the leaf surface being studied. Make a patch of at least one square centimeter.

c. Allow the nail polish to dry completely.

d. Press a piece of clean, clear cellophane tape to the dried nail polish patch. Using clear (not opaque) tape is essential here. You might also try pulling the peel away from the leaf without using any tape and then preparing a wet mount of the peel with a drop of water and a cover slip.

e. Gently peel the nail polish patch from the leaf by pulling a corner of the tape and peeling the nail polish off the leaf. This is the leaf impression that you will examine. (Make only one leaf impression on each side of the leaf, especially if the leaf is going to be left on a live plant.)

f. Tape the peeled impression to a clean microscope slide. Use scissors to trim away any excess tape. Label the slide as appropriate for the specimen being examined and label the side of leaf from which the peel was taken.

g. Examine the leaf impression under a light microscope to at least 400X (or highest magnification). Draw and label what you observe. Can you observe any stomata? Search for areas where there are numerous stomata.

h. Count all the stomata in one microscopic field. Record the number.

i. Repeat counts for at least three other distinct microscopic fields and record the number of stomata.

j. Determine an average number of stomata per microscopic field.

k. From the average number per microscopic field, calculate the number of stomata per 1 mm^2. You can estimate the area of the field of view by placing a transparent plastic ruler along its diameter, measuring the field's diameter, and then calculating area by using πr^2. (Most low-power fields have a diameter between 1.5–2.0 mm.)

l. Trade slides with two other lab teams so you examine three different slides under the microscope using the same procedure described above.

Designing and Conducting Your Investigation

The procedure should have raised several questions about factors that relate to the rate of transpiration in plants. Some possible questions are listed below, but you may have others.

- What environmental variables might affect the rate of transpiration?
- Do all parts of a plant transpire?
- Do all plants transpire at the same rate?
- Is there a relationship between the habitat in which plants evolved to their rate of transpiration?

Rate of transpiration can be measured by a variety of methods, including the use of a potometer with or without a gas pressure sensor and computer interface or the use of the whole plant method. These methods are detailed in this investigation, but your teacher may help you substitute another procedure.

If using a gas pressure sensor and computer interface to measure transpiration rate, your teacher likely will provide instructions. If you are unfamiliar with the use of probes with computer interface, it is suggested that you spend about 30 minutes learning how to collect data using the equipment.

Step 1 Design an experiment to investigate one of the aforementioned questions or one of your own questions to determine the effect of an environmental variable(s) on the rate of transpiration in plants. When identifying your design, be sure to address the following questions:

- What is the essential question being addressed?
- What assumptions are made about the questions being addressed?
- Can those assumptions be easily verified?
- Will the measurement(s) provide the necessary data to answer the question under study?
- Did you include a control in your experiment?

Step 2 Make a hypothesis/prediction about which environmental factors will have the greatest effect on transpiration rates. Be sure to explain your hypothesis.

Step 3 Conduct your experiment(s) and record data and any answers to your questions in your lab notebooks or as instructed by your teacher. Write down any additional questions that arose during this study that might lead to *other* investigations that you can conduct.

Option 1: Potometer with or Without Gas Pressure Sensor

Materials

- Representative plant species available in your region/season, such as *Impatiens* (a moisture-loving plant), *Coleus*, oleander (more drought tolerant), *Phaseolus vulgaris* (bean seedlings), pea plants, varieties of *Lycopersicon* (tomato), peppers, and ferns
- Potometer, which you assemble from clear plastic tubing, a ring stand with clamp, and a 0.1-mL or 1.0-mL pipette, depending on the diameter of the stem of the plant you choose. Your teacher will have several different sizes of plastic tubing available. (The tubing can be filled using a water bottle or plastic syringe *without a needle.*) If using a syringe, attach it to the end of the pipette and pull water into the potometer. (Why should the tubing be free of air bubbles? Why must the stem be completely immersed in the water?) If using a gas pressure sensor, the tubing is inserted directly into the device, with no pipettes required. (The potometer assembly is illustrated in Figure 2.)
- Fan, heat lamp, water, small plastic bag, spray bottle with water, salt, and other materials provided by your teacher to simulate an environmental variable
- Petroleum jelly to make an airtight seal between the cut end of stem and tubing filled with water (You can also use small clamps to seal without the "goop.")

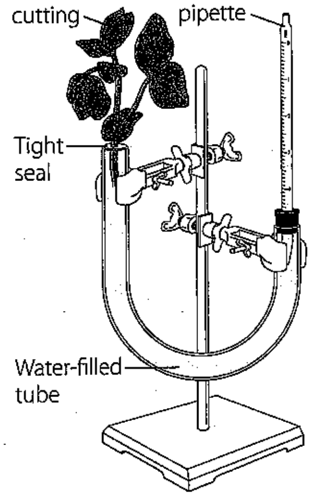

Figure 2. Potometer Assembly

Using a razor blade, carefully cut the plant stem so that its diameter will fit into the piece of plastic tubing in the potometer assembly. Note that it is often helpful to cut the stem while it is submerged under water to prevent air bubbles from being introduced into the xylem. Your teacher will provide additional instructions, if necessary. Please be careful when using the razor blade!

If using the gas pressure sensor to collect data, connect the gas pressure sensor to the computer interface. Prepare the computer for data collection by opening the file "10 Transpiration" from the *Biology with Vernier* folder of Logger*Pro*. If using a pipette to measure water loss, you will have to determine your method of data collection.

Option 2: Whole Plant Method

Materials

- Small potted plant (*Impatiens*, tomato seedling, bean seedling, pea plant, etc.) with many leaves and few flowers
- One-gallon size plastic food storage bag without zipper
- String

Step 1 Saturate the plant with water the day/night before beginning your investigation.

Step 2 Carefully remove a plant from the soil/pot, making sure to retain as much of the root system and keeping soil particles attached to the roots. Wrap the root ball of the plant(s) in a plastic bag and tie the bag around the base so that only the leaves are exposed. (Be sure to remove all flowers and buds.) Do not water your plant any more until you finish your experiment! You can also keep the plant in the plastic pot and place it in the plastic bag.

Step 3 Determine the mass of each plant and then its mass for several days under your environmental condition(s).

Step 4 Record your data in your lab notebook or as instructed by your teacher.

Calculations: Determining Surface Area and Transpiration Rates

Step 1 In the first part of this lab, you were asked to investigate methods to calculate leaf surface area and the surface area of all the leaves on a plant or plant cutting (depending on your experimental setup). Your teacher may suggest a particular method. Determine the total surface area of the leaves in cm^2 and record the value.

Step 2 Calculate the rate of transpiration/surface area. If you are using a gas pressure sensor to collect data, you can express these rate values as $kPa/min/cm^2$, where kPa (kilopascal) is a unit of pressure. Record the rate.

Step 3 After the entire class agrees on an appropriate control, subtract the control rate from the experimental value. Record this adjusted rate.

Step 4 Record the adjusted rate for your experimental test on the board to share with other lab groups. Record the class results for each of the environmental variables investigated.

Step 5 Graph the class results to show the effects of different environmental variables on the rate of transpiration. You may need to convert data to scientific notation with all numbers reported to the same power of 10 for graphing purposes.

Step 6 Your teacher may suggest you perform statistical analysis (e.g., a T-test) of your data, comparing results of experimental variable(s) to controls.

Analyzing Results

1. How was the rate of transpiration affected by your choice of experimental variable as compared to the control?
2. Think of a way you can effectively communicate your results to other lab groups. By comparing results and conclusions, explain how changes or variables in environmental conditions affect transpiration rates.
3. Based on data collected from different lab groups, which environmental variable(s) resulted in the greatest rate of water loss through transpiration? Explain why this factor might increase water loss when compared to other factors.
4. Why did you need to calculate leaf surface area to determine the rate(s) of transpiration?

5. What structural or physiological adaptations enable plants to control water loss? How might each adaptation affect transpiration?

6. Make a prediction about the number of stomata in a leaf and the rate of transpiration. What type(s) of experiments could you conduct to determine the relationship between the number of stomata and the rate of transpiration?

7. Create a diagram with annotation to explain how the TACT (transpiration, adhesion, cohesion, tension) mechanism enables water and nutrients to travel up a 100-ft. tree. Predict how a significant increase in ambient (environmental) temperature might affect the rate of transpiration in this tree. Explain your prediction in terms of TACT and the role of guard cells in regulating the opening and closing of stomata.

Evaluating Results

1. Was your initial hypothesis about the effect of your environmental variable on the rate of transpiration supported by the data you collected? Why or why not?

2. What were some challenges you had in performing your experiment? Did you make any incorrect assumptions about the effect of environmental variables on the rate(s) of transpiration?

3. Were you able to perform without difficulty the mathematical routines required to analyze your data? Which calculations, if any, were challenging or required help from your classmates or teacher?

Where Can You Go from Here?

1. Investigate how guard cells control the opening and closing of stomata, including the role of abscisic acid and K^+.

2. Design an experiment to investigate transpiration in two different types of plants — one that is drought tolerant and one that requires a significant amount of water. What predictions can you make about the rate of transpiration in each?

3. If you had to revise the design of your experiment, what suggestions would you make? Why would you make them?

4. If your investigations generated other questions that you might want to research, ask your teacher if you can conduct other experiments.