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.

In general, animals possess one or more mechanisms, such as those involved in excretion, that let them ingest solutions of nutrients and transport and/or eliminate any excess ions or water. However, plants take a different approach; they 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 (the evaporation of water from the plant surface). 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 transpiration. 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, and these differences account for water movement from cell to cell or over long distances in the plant. Several factors, including environmental pressure and solute concentration, contribute to water potential, with water always moving from an area of high water potential (higher free energy, more water) to lower potential (less free energy, less water). The process 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, as modeled in Figure 1.

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

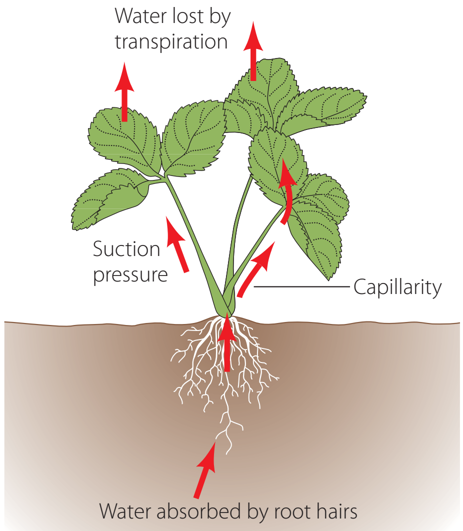

Figure 1. Transpiration Model

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. To maintain homeostasis, plants must adjust their rates of transpiration in response to environmental conditions.

This investigation encourages independent student thinking and ultimately provides the student with opportunities for more open-ended experimentation. In the first part of this investigation (stomatal peel), you guide students through an investigation, ask them focused questions, and give them suggestions for further study. However, the expected outcome is unknown. This type of inquiry is referred to as structured inquiry and is suitable for introducing groups of scientifically naïve students to inquiry. In a guided inquiry investigation, the procedure for conducting the investigation is developed by the students. The second part of this lab, in which students select an environmental factor and explore its effect on transpiration, is an example of this type of inquiry. With experience, students will be able to investigate questions about transpiration that they themselves have formulated and use procedures of their own design to investigate answers. Such open inquiry is the ultimate goal of any biology program. (For more information about the different types of inquiry-based investigations, please refer to Chapter 4 in this manual.)

In this investigation, students begin by exploring methods to calculate leaf surface area and then determine the average number of stomata per square millimeter in a particular kind of plant. From their data, several questions about the process of transpiration in plants should emerge. Students can explore these questions in their own investigations.

PREPARATION

Materials and Equipment

- Representative plant species that are available in a particular region or season, such as *Impatiens* (a moisture-loving plant), *Coleus*, oleander (more drought tolerant), *Phaseolus vulgaris* (bean seedlings), pea plants, varieties of *Lycopersicon* (tomato), peppers, ferns, or even Wisconsin Fast Plants (If students plan to investigate transpiration in several different species of plants, you will have to purchase a variety of plants, or students can use cuttings from plants found on campus. Note that the plants can be used to study other biological concepts, such as plant evolution, natural selection, genetics, adaptation, and plant reproduction.)
- Safety goggles, calculator, microscope, microscope slides, clear cellophane tape, clear nail polish, scissors
- Graph paper and metric ruler as needed to determine leaf surface area
- Potometer, which students 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 (It is recommended that you have available clear plastic tubing of different sizes to accommodate stems from different plants.) A syringe *without needle* can be used for filling the tubing with water, or the tubing can be filled using a water bottle. Students should be able to make the observations that air bubbles in the tubing could interfere with transpiration and that when assembling the potometer, as shown in Figure 2, the end of the stem must be immersed in the water. If students are using a gas pressure sensor, the tubing is inserted directly into the device; no pipette is required.

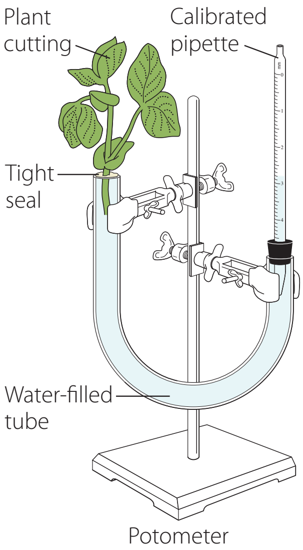

Figure 2. Potometer Assembly

- For whole plant transpiration, small potted plants with many green leaves (e.g., *Impatiens*, tomato seedlings), the plastic container they come in, one-gallon size plastic food storage bags, and string (If using this method, students place the entire potted plant or root ball with dirt in the plastic bag.)

- Fan, heat lamp, water, small plastic bag, spray bottle with water, salt, and other chemicals provided by you
- Petroleum jelly to make an airtight seal between the cut end of the stem and tubing filled with water (You can also use small clamps to seal without the "goop.")

If students choose to consider an environmental variable for which you don't have materials available, they will ask for advice.

Timing and Length of Lab

This investigation requires approximately four lab periods of about 45 minutes each. This includes one period for students to calculate leaf surface area and the number of stomata, one period for students to design an experiment(s), a *minimum* of one period to conduct an experiment(s), and one period for students to discuss and share their results and conclusions with the class. (If students have prepared and examined a stomatal peel in a prerequisite biology course, they might be able to skip this part of the investigation.)

If students are using a potometer method to determine transpiration rate(s), data collected over a 24-hour period provide more quantifiable results; if using the whole plant method, students need to determine the mass of their plant(s) for several days. If time is an issue, the prelab and summative assessments can be assigned for homework.

Students can work in pairs or small groups to accommodate different class sizes.

Safety and Housekeeping

- Remind students to be careful when assembling their equipment and when using a razor blade or scalpel to cut the stem of their plant cutting to a 45° angle.
- Students should wear safety goggles while conducting their experiments.
- Nail polish is toxic by ingestion and inhalation; students should also avoid eye contact with it.
- Plant cuttings can be disposed of in the trash, and any paper waste should be recycled.
- Plastic tubing and pipettes can be reused.
- If a syringe is used to assemble the potometer, make sure the needle is removed.
- Students should always be supervised while working in the lab.

ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to cellular processes (big idea 2) or interactions (big idea 4). In addition, some questions raised can connect to evolution and natural selection (big idea 1). As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

Enduring Understandings

- 1A2: Natural selection acts on phenotypic variations in populations.
- 2A3: Organisms must exchange matter with the environment to grow, reproduce, and maintain organization.
- 2B1: Cell membranes are selectively permeable due to their structure.
- 2B2: Growth and dynamic homeostasis are maintained by the constant movement of molecules across membranes.
- 2D1: All biological systems from cells and organisms to populations, communities, and ecosystems are affected by complex biotic and abiotic interactions involving the exchange of matter and free energy.
- 4A4: Organisms exhibit complex properties due to interactions between their constituent parts.
- 4A6: Interactions among living systems and with their environment result in the movement of matter and energy.

Learning Objectives

- The student is able to connect evolutionary changes in a population over time to a change in the environment (1A2 & SP 7.1).
- The student is able to use calculated surface area-to-volume ratios to predict which cell(s) might eliminate wastes or procure nutrients faster by diffusion (2A3 & SP 2.2).
- The student is able to justify the selection of data regarding the type of molecules that an animal, plant, or bacterium will take up as necessary building blocks and excrete as waste products (2A3 & SP 4.1).
- The student is able to represent graphically or model quantitatively the exchange of molecules between an organism and its environment, and the subsequent use of these molecules to build new molecules that facilitate dynamic homeostasis, growth, and reproduction (2A3 & SP 1.1, SP 1.4).
- The student is able to predict the effects of change in a component(s) of a biological system on the functionality of an organism(s) (4A4 & SP 6.4).
- The student is able to apply mathematical routines to quantities that describe interactions among living systems and their environment that result in the movement of matter and energy (4A6 & SP 2.2).
- The student is able to use visual representation to analyze situations or solve problems qualitatively to illustrate how interactions among living systems and with their environment result in the movement of matter and energy (4A6 & SP 1.4).

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

Before students tackle this investigation, they should be able to demonstrate understanding of the following concepts. The concepts may be scaffolded according to level of skills and conceptual understanding.

- The relationship between cell structure and function
- The physical and chemical properties of water
- The movement of molecules and ions across cell membranes by the processes of osmosis, diffusion, and active transport
- Photosynthesis, particularly the transport and roles of CO_2, O_2, and H_2O
- The exchange of matter between biological systems and the environment

This investigation reinforces the following skills:

- Measuring distance, volume, and/or mass using the metric system
- Estimating leaf surface area
- Using a microscope to examine cell structure
- Constructing data tables and graphs
- Communicating results and conclusions

If students have not acquired these skills previously, the procedures in this lab will help them develop them.

Skills Development

Students will develop the following skills:

- Preparing a stomatal peel using nail polish
- Making a wet mount of leaf epidermal tissue for microscopy
- Calculating leaf surface area and number of stomata/surface area
- Assembling a potometer
- Calculating transpiration rates
- If equipment is available, learning how to use a gas pressure sensor and computer interface

Potential Challenges

If students have a solid understanding of the aforementioned concepts, they should be able to select an environmental variable and design an experiment around the effect of the variable on the rate of transpiration. The skills and concepts may be taught through a variety of methods in an open-inquiry investigation. Transpiration rates may be measured by several means, including the use of a potometer with or without a gas pressure sensor and computer interface or the use of the whole plant method. Only two methods are detailed, and any alternative procedure may be equally and successfully substituted.

The equipment is simple, the materials are few, and the cost is low (with the exception of the initial purchase of probes and computer interfaces, which can be used for myriad investigations). Staining of stomatal peels is not necessary; if contrast is low, direct students to adjust (close) the condenser aperture diaphragm. A typical stomatal peel prepared for observation without staining looks something like Figure 3.

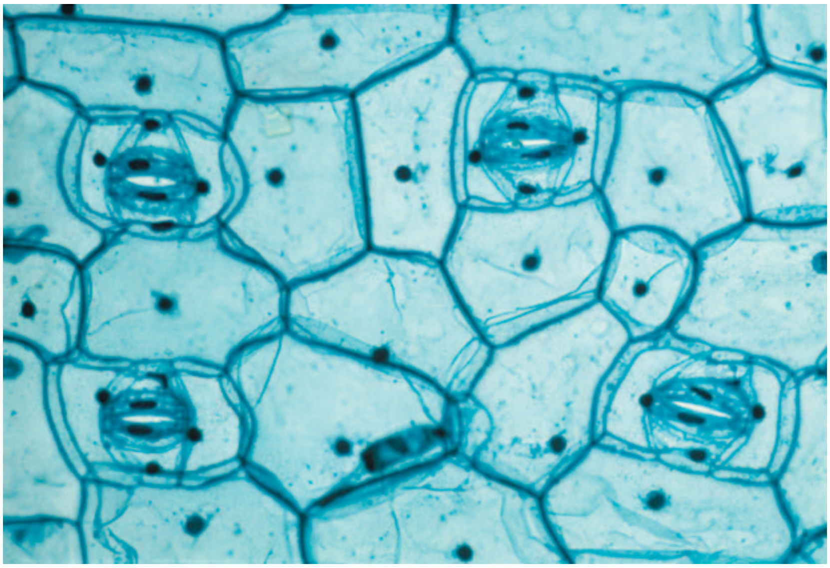

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Figure 3. Micrograph of Stomatal Peel

The potometer is easy to assemble from common materials found in a biology or physical science laboratory, and the experiments can be performed using a 0.1-mL or 1.0-mL pipette in place of a gas pressure sensor. (The size of the pipette depends on the diameter of the stem from the plant used for investigation. Some plants naturally have larger stems!)

If students are unfamiliar with the use of the gas pressure sensor and computer interface, they may have to review their use prior to collecting data. The applications of mathematics are straightforward, and it's your choice whether you provide students with formulas, data tables, and graph paper. However, it is suggested that students develop their own visuals to record their data. It also is recommended that students help each other analyze data and present their individual/group data and conclusions to the class as a means to develop both written and verbal communication skills. Refer to Chapter 6 in this guide for suggestions for student presentations.

If using potometers, when inserting the plant cutting into the plastic tubing, students often leave a small gap between the end of the stem and the top of the column of water; the column of water must make direct contact with the xylem in the stem for transpiration to occur. Students also struggle with filling the plastic tubing with water without forming air bubbles. One trick is to attach a small plastic syringe (without a needle) to the end of the pipette with a piece of rubber tubing and use the syringe to pull water up into the potometer, leaving the syringe attached to keep the water under negative tension.

Once the plant cutting is in place and the tubing/pipette completely filled with water, the syringe is carefully removed. In place of using petroleum jelly to prevent the apparatus from leaking, small, inexpensive clamps work well. If using a 0.1-mL pipette to record water loss, students can have difficulty reading the small increments on the pipette. Assembly of potometers can be challenging; as a result, you might opt to suggest that students use the whole plant method to determine transpiration rates. However, keep in mind that although students might struggle as they assemble equipment, in the process they are learning new lab skills and will experience satisfying "ah-ha" moments when they are finally successful.

Students are asked to investigate methods for calculating leaf surface area. However, it is recommended that students use the leaf tracing method described in the following paragraph. Using a common alternate method, leaf mass, presents several inherent problems. The method is dependent upon the part of the leaf from which a section is cut because leaves usually have variation in thickness; thus, the calculated surface area of a 1-cm^2 section could vary significantly from one group to another. A modified leaf tracing method can be done *without* removing leaves from the plant, thus rendering the purchase of more plants unnecessary.

Leaf Trace Method to Calculate Leaf Surface Area

Leaf surface area may be calculated with the leaves still attached to the plant. Alternatively, students may cut off several leaves, arrange them on a piece of graph paper or grid (constructed so that a square of 4 blocks equals 1 cm^2), and trace the edge pattern directly onto the graph paper. Count all the grids (squares) that are completely within the tracing and estimate the number of grids that lie partially within the tracing. The total surface area can then be calculated by dividing the total number of blocks covered by 4. Leaf surface area is recorded in cm^2. Students are then asked to calculate leaf surface area in mm^2.

THE INVESTIGATIONS

Getting Started: Prelab Assessment

You may assign the following for homework; as a think, pair/group, share activity, in which pairs or small groups of students brainstorm ideas and then share them with other groups; or as a whole-class discussion to assess students' understanding of key concepts pertaining to transpiration in plants:

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 annotation.

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 then 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.

Data Tables and Charts

If using the gas pressure sensor to measure water loss, students might find the following tables useful for recording their data/results. However, it is recommended that students construct their own visuals, including tables and graphs, for reporting data. Although the computer interface will generate graphs on the screen, you may elect to have students draw, label, and annotate their own graph.

You may need to explain kPa. The pascal (Pa) is a unit of pressure, and there are 1,000 pascals in 1 kilopascal (kPa). One kPa is approximately the pressure exerted by a 10-g mass resting on a 1-cm^2 area. If students have studied chemistry and/or physical science or physics, they may recognize that 101.3 kPa = 1 atm of pressure.

Table 1. Individual/Group Data

Test	Rate (kPa/min)	Surface Area (cm^2)	Rate/Area (kPa/min/cm^2)	Adjusted Rate (kPa/min/cm^2)
Experimental				
(Experimental 2)				
Control				

Table 2. Class Data

Test Variable	Adjusted Rate (kPa/min/cm^2)

Designing and Conducting Independent Investigations

Students begin by investigating methods to calculate leaf surface area and then determine the average number of stomata per square millimeter in a particular kind of plant. Several questions about the process of transpiration in plants should emerge from the data, including the following:

- Do all plants have stomata? Is there a relationship between the number of stomata and the environment in which the plant 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 to 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?

Students are then asked to design an experiment to investigate one or more questions, and their exploration will likely generate more questions about transpiration. For a supplemental activity, students can make 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 lab also provides an opportunity for students to apply, review, and/or scaffold concepts they have studied previously, including the relationship between cell structure and function, evolution of plant structures, the movement of molecules and ions across cell membranes, the physical and chemical properties of water, the forces provided by differences in water potential, photosynthesis, and the exchange of matter between biological systems and the environment.

Summative Assessment

The following are suggested as guidelines to assess students' understanding of the concepts presented in the investigation, but you are encouraged to develop your own methods of postlab assessment. Some of the tasks can be assigned for homework following completion of the investigation.

1. Have the students record their experimental design, data, results, and conclusions in a lab notebook, formal lab report, or mini-poster. Students can prepare a class graph reflecting their conclusions about the effects of environmental variables on the rate of transpiration in plants. Based on the students' product, do you think students have met the learning objectives of the investigation?

2. Were the students able to construct a graph from a data table? Did they correctly label the *X* and *Y* axes and appropriately title the graph?

3. Have the students prepare a lesson on transpiration for younger students at the school, following the adage that "you can't really learn something until you have to teach it."

4. Have the students come up with a list of common misconceptions they had about the process of transpiration before conducting their investigations.

5. Did the students have an adequate (i.e., basic) understanding of water potential and the movement of water and nutrients across cell membranes before designing their experiment to investigate transpiration? Did the students have an adequate understanding of the physical and chemical properties of water before investigating transpiration?

6. Were the students able to determine leaf surface by using appropriate mathematical skills? Which applications of mathematical skills were challenging for the students?

7. If you used the suggested gas pressure sensor protocol, were the students able to navigate through the computer interface to the lab investigation without much difficulty? Why or why not? If the students had difficulty, ask them to teach other students how to use the equipment.

8. Have the students write one or two questions based on the concepts in this investigation that could appear on an AP Exam.

Where Can Students Go from Here?

The following are possible extension activities for students:

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?

SUPPLEMENTAL RESOURCES

Prelab Activities

http://www.cjhs.org/teacherssites/taylor/accbio/PLANTS/STOMATE%20 LAB.pdf This resource, Flinn Scientific, *Bio Fax!*, "Lasting Impressions: Counting Stomata," Publication #10226, provides a quick lesson and protocol on preparing a thin section of leaf epidermis (stomatal peel) to view cell structure and stomata.

http://www.phschool.com/science/biology_place/labbench/lab1/intro.html This resource provides an interactive review of the processes of osmosis, diffusion, and active transport, including the concept of water potential. This would be a great way to introduce students to the concept of transpiration.

http://www.mhhe.com/biosci/genbio/virtual_labs/BL_10/BL_10.html In this virtual investigation, students study the process of transpiration in vascular plants and compare the rates of transpiration for several species under varying environmental conditions. This is a simple review of major concepts involved in the process of transpiration.

http://www.ucopenaccess.org/course/view.php?id=15 In these AP-level virtual investigations, students explore the major concepts of osmosis, diffusion, and transpiration. They can work through the diffusion lab as a prelab review of the principles of movement of water, ions, and molecules across cell membranes by the processes of osmosis and diffusion.

http://www.visionlearning.com/library/module_viewer.php?mid=57&l This resource provides a simple explanation of the structure of the water molecule, hydrogen bonding between water molecules, and the ways in which the molecular structure of water leads to unique properties, including adhesion and cohesion.

Procedural Resources

http://www.pbs.org/teachers/connect/resources/4423/preview/ This resource provides an alternative method for calculating leaf surface area using a geometric model. An advantage of the method is that a leaf does not have to be removed from the plant.

http://local.brookings.k12.sd.us/krscience/open/plants/Whole%20Plant%20Transpirationteacherguide.doc Using the whole plant method, this resource presents an alternative procedure to using potometers to determine transpiration rates.

Resources for Extensions of Investigation

http://www.accessexcellence.org/AE/AEC/AEF/1994/case_leaf.php This resource provides supplemental activities for students to investigate stomata and their role in transpiration in plants.

http://biology.arizona.edu/sciconn/lessons2/Loredo/Overview.htm In this resource, students initially describe differences between plants that seem healthy and those that appear unhealthy. Based on their observations, the students propose hypotheses and design and conduct experiments. The resource contains a thorough teacher's guide, student material, an assessment piece, and student evaluation.

http://www.nature.com/nature/journal/v455/n7210/abs/nature07226.html This article, *The transpiration of water at negative pressures in a synthetic tree*, is a resource for teachers, and perhaps students, who want to learn more about real-world applications of transpiration, including using the principles behind transpiration for technological uses of water under tension.

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TRANSPIRATION*

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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.

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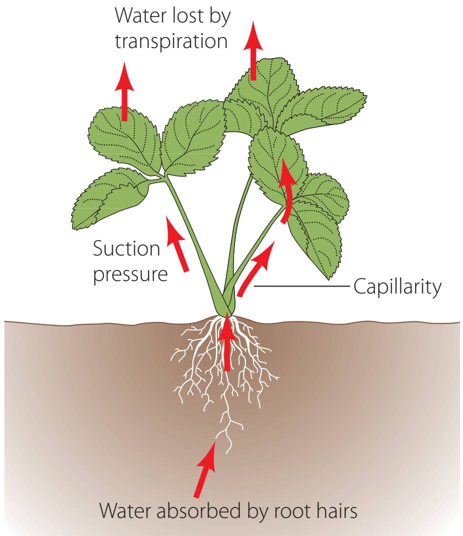

Figure 1. Transpiration Model

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.")

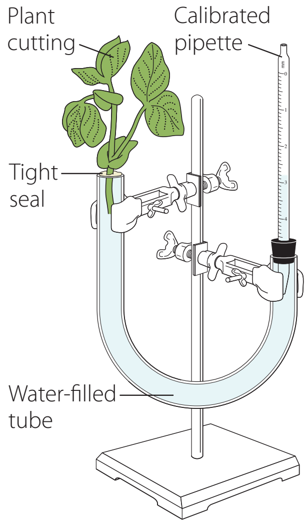

Figure 2. Potometer Assembly

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!

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.