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Rate of Transpiration

AP* Biology Big Idea 4, Investigation 11

An Advanced Inquiry Lab

Introduction

All living things acquire nutrients, ions, and water from the environment. Plants absorb these materials from the surrounding soil and air via the processes of osmosis, diffusion, and active transport. The water, nutrients, and ions are then transported throughout the plant within xylem because of differences in water potential.

Concepts

- Adhesion
- Biotic vs. abiotic factors
- Cohesion
- Osmotic potential
- Stomata
- Transpiration
- Turgor potential
- Water potential

Background

Water must follow the laws of thermodynamics. Consequently, water always moves from regions of high energy to regions of lower energy. In a plant this means that water flows from regions of high water potential to regions of lower water potential. This occurs through the processes of osmosis, root pressure, and adhesion and cohesion of water molecules. In plants, water potential is equal to the sum of the osmotic potential (ψ_s) and the turgor or pressure potential (ψ_p).

Osmotic potential is a measure of the amount of solutes (dissolved minerals and other nutrients) in the water within the plant. The root cells expend energy to actively transport dissolved minerals into the root. An increased solute concentration in the roots causes a lower amount of free energy and therefore a negative water potential in the root tips. Water then flows by osmosis from the region of high energy in the soil to the region of lower energy in between the root cells. Osmotic potential is always negative in a plant.

Turgor potential occurs when water molecules enter a cell and apply pressure to the cell walls. Living plant cells have positive turgor potential. The cells in wilted leaves have zero turgor potential. Specialized water transport cells called xylem have a negative turgor pressure because water is removed from xylem by the adjacent cells due to osmosis. Plants require a consistent supply of water around their roots because they constantly lose water through their leaves via transpiration. *Transpiration* is the loss of water by evaporation from the leaves and is the main method for pulling water from the roots to the leaves.

Transpiration begins with evaporation of water through the *stomata* (singular: stoma or stomate). Stomata are tiny openings (pores) used for the absorption of carbon dioxide (CO_2) for photosynthesis and oxygen (O_2) for cell respiration (see Figure 1). Thousands of stomata occur on the underside of a typical dicot or on the upper surface of a plant whose leaves float on water. Each stoma is formed by a pair of specialized cells known as *guard cells* that are responsible for regulating the size of the pore's opening. By adjusting the size of the opening, the guard cells control the rate of CO_2 and O_2 uptake and the loss of water by the leaf. In this way, by regulating the diffusion of CO_2 into the cells, the guard cells also control the rate of photosynthesis in the leaf. The guard cells swell when they are full of water, opening the stoma into air spaces that surround the middle layer of leaf cells. This middle layer of cells is called the mesophyll (*meso*=middle, *phyll*=leaf). The mesophyll cells are covered with a thin layer of water from the xylem. The water coating the cells evaporates due to the lower water potential in the outside air. New water molecules then move onto the mesophyll cells by osmosis from the xylem.

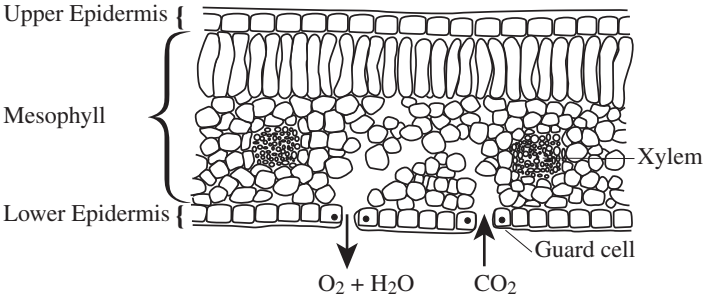

Figure 1.

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As each water molecule moves onto a mesophyll cell, it exerts a pull on the column of water molecules in the xylem, from the leaves to the roots (see Figure 2). This transpirational pull is caused by the cohesion of water molecules to one another due to hydrogen bond formation, and by the adhesion of water molecules to the walls of the xylem cells. The upward transpirational pull on the fluid in the xylem causes negative pressure to form in the xylem, pulling the xylem walls inward and creating decreased water potential inside the xylem. This decrease in water potential is transmitted all the way from the leaves to the roots. Water then moves inward from areas of higher water potential in the soil, through the root hairs, and into the xylem.

If the moisture content in the mesophyll layer of the leaf equals or is less than the moisture level of the outside air, the guard cells will lose their water, and the cells will become flaccid and close. Many environmental conditions influence the opening and closing of the stomata and thus affect the rate of both transpiration and photosynthesis. Temperature, light intensity, air currents, humidity, and the nature of the plant all influence the guard cells to open or close.

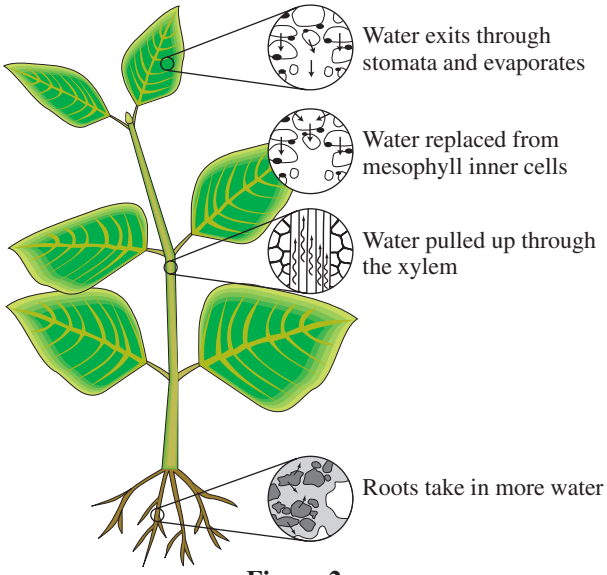

Figure 2.

Experiment Overview

In the *Baseline Activity,* the stomata, which regulate transpiration, will be observed, counted and quantified. A method will be developed to determine the approximate leaf area and therefore the approximate number of stomata for each plant. The study of stomata and leaf area provide a foundation for the development of an open-inquiry, student-designed experiment—see the *Opportunities for Inquiry* section. Biotic, abiotic, and altering experimental techniques are all variables that may affect the rate of transpiration. The rate of transpiration of a control will then be mathematically compared to that of the treatment.

Pre-Lab Questions

1. Very few plants can tolerate salty conditions such as found on the salt flats or along a sidewalk that has been treated with de-icer over the winter. Why do salts affect plants this way? Create a well-labeled diagram to explain.
2. In the *Background* section the location of stomata in a land plant and a plant with leaves that float on water was discussed. What about plants that stay submerged in water? Do they have stomata at all and if so where are they located on the plant?

Materials

- Calculator
- Clear cellophane tape (clear package sealing tape)
- Clear fingernail polish
- Microscope
- Microscope slides
- Plant leaves
- Ruler, clear
- Scissors

Safety Precautions

Nail polish is toxic by ingestion and inhalation. Avoid eye contact. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Baseline Activity

1. Paint a thick patch of clear nail polish on the leaf surface being studied. Make the patch at least one square centimeter.
2. Allow the nail polish to dry completely.
3. Place a piece of clear cellophane (packing) tape onto the dried nail polish patch.
4. Gently peel the nail polish patch from the leaf by pulling on a corner of the tape and "peeling" the fingernail polish off the leaf.

5. Tape the peeled impression to a clean microscope slide.
6. Count all the stomata in one microscopic field and repeat for at least four leaf areas.
7. Use a clear ruler to determine the size of one field of view under the microscope.
8. Calculate the number of stomata per square millimeter.
9. Pool the class data and determine the average number of stomata per square millimeter of the plant species being tested. The total number of stomata varies by species. Since transpiration occurs via the stomata, this value is an important consideration when determining the transpiration rate.
10. The rate of transpiration for a plant is generally reported as volume of water (in milliliters) or the mass of water (in grams) per square meter of leaf area. Develop a procedure to determine the total leaf area of a plant.
    a. The plants may not be destroyed until after testing is complete. However, the remainder of the plant used for the stomata counting activity may be used to help determine the leaf area.
    b. Each group should develop at least one method to estimate the total leaf area of the plants.
    c. Decide, as a class, the procedure that will be used to determine leaf area for the remainder of this laboratory.
    d. The total leaf area and the approximate number of stomata on each plant will need to be calculated as part of the data analysis section of the inquiry portion of this lab.

Opportunities for Inquiry

1. Consider the following questions while reflecting upon your knowledge about transpiration in a flowering dicot.
    a. How does the weather and environment affect transpiration?
    b. How does the number of stomata per square meter of leaf area affect the transpiration rate?
    c. Do different waters, liquids, or ions affect transpiration rate?
    d. Does one testing method have a lower experimental error than another?
    e. How does coating the leaves or otherwise compromising the plant affect transpiration rate?
    f. Are there ways to increase transpiration rate?
2. Plan, discuss, execute, evaluate, and justify an experiment to test a question regarding transpiration.
    a. Decide upon one question that your group would like to explore.
    b. Develop a testable hypothesis.
    c. Discuss and design a controlled procedure to test the hypothesis.
    d. List any safety concerns and the precautions that will be implemented to keep yourself, your classmates, and your instructor safe during the experimental phase of this laboratory.
    e. Determine what and how you will collect and record the raw data.
    f. How will you analyze the raw data to test your hypothesis?
    g. Share your hypothesis, safety precautions, procedure, data tables, and proposed analysis with your instructor prior to beginning the experiment.
    h. Once the experiment and analysis are complete, evaluate your hypothesis and justify why or why not the hypothesis was supported by your data.
    i. Present and defend your findings to the class.
    j. Make suggestions for a new or revised experiment to modify or retest your hypothesis.

Teacher's Notes
Rate of Transpiration

Materials Included in Kit (for 8 groups of students)

Fingernail polish, clear, 2
Petroleum jelly, 5 g, 8*
Pipets, serological, 1-mL, 8*
Polyethylene bags, 12″ × 22″, pkg. of 3, 3
Rubber bands, 30*
Syringes, 12-mL, 8*
Tubing, latex, amber, 3/16″, 2″, 16*
Tubing, plastic, clear, 1/8″, 132″*

**Used for Option 1 only.*

Additional Materials Needed (for each lab group)

Balance, 0.001-g precision (shared)
Calculator
Clamps, test tube, 2*
Pan of tap water*
Ruler, clear metric
Scalpel*
Support stand*
Whole plants (optional see *Lab Hints* for suggestions)

**Used for Option 1 only*

Materials Included in Kit (for *Pre-Lab Preparation*)

Seeds, red bean, 4 oz
Greenhouse cover, 11″ × 22″
Planting tray insert, 72-cells
Planting tray, 11″ × 22″
Potting soil

Additional Materials Needed (for *Pre-Lab Preparation*)

Dissecting needle or small paper clip*
Scissors*
Water, tap

**Used for Option 1 only*

Pre-Lab Preparation

Start bean seeds at least two weeks in advance.

1. Soak the red bean seeds in warm water for one hour prior to planting
2. Fill the cells 2/3 full of potting soil and place into the planting tray.
3. Sow 2 seeds per cell.
4. Cover the seeds with a thin layer of soil.
5. Gently mist or sprinkle water onto the soil. Do not overwater.
6. Cover the tray with the greenhouse cover and place the tray in a warm location. *Note:* The optimal soil temperature for the germination of bean seeds is 65–85 °F. If the seeds start to mold, remove the cover for a day to allow better air circulation around the seeds. If the mold continues, remove the seeds and begin new seedlings.
7. When the seeds begin to sprout, remove the cover and place the tray in a sunny location or under a grow light.
8. Rotate the tray ¼ turn daily and water when the soil just below the surface feels dry to the touch.

Teacher's Notes *continued*

Preparation for Option 1—Using a Potometer

1. Use scissors to cut the clear plastic tubing into 16″ lengths.
2. Use a dissecting needle or a small unbent paper clip with a hook bent into the end to remove the cotton plugs from the pipets.

Preparation for Option 2—Whole Plant Method

1. Saturate the plant with water the day before starting the inquiry activity.

Safety Precautions

Scalpels are sharp instruments; use caution when cutting, always cut away from your body and away from others. Avoid eye and skin contact with the nail polish. Avoid prolonged respiration of nail polish. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Scalpels may be disposed of according to Flinn Biological Waste Disposal Method V, sharps and broken glass. All other materials in this laboratory may be disposed of using Flinn Biological Waste Disposal Method VI, in the regular trash.

Alignment with AP Biology Concepts and Curriculum Framework

Big Idea 2: Biological systems utilize free energy and molecular building blocks to grow, to reproduce and to maintain dynamic homeostasis.

Enduring Understandings

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 exchange of matter and free energy.

Big Idea 4: Biological systems interact, and these systems and their interactions possess complex properties.

Enduring Understandings

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 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 4.2, SP 4.3, SP 6.4).

Teacher's Notes *continued*

- 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, SP 5.2).
- 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).

Science Practices

1.1 The student can create representations and models of natural or man-made phenomena and systems in the domain.

1.4 The student can use representations and models to analyze situations or solve problems qualitatively and quantitatively.

2.2 The student can apply mathematical routines to quantities that describe natural phenomena.

4.1 The student can justify the selection of the kind of data needed to answer a particular scientific question.

4.2 The student can design a plan for collecting data to answer a particular scientific question.

4.3 The student can collect data to answer a particular scientific question.

5.2 The student can refine observations and measurements based on data analysis.

5.3 The student can evaluate the evidence provided by data sets in relation to a particular scientific question.

6.2 The student can construct explanations of phenomena based on evidence produced through scientific practices.

6.4 The student can make claims and predictions about natural phenomena based on scientific theories and models

Lab Hints

- Enough materials are provided in this kit for 8 groups of students. This laboratory activity can reasonably be completed in two 50-minute class periods if using Option 1. Option 2 will need five or six full and partial class periods. The inquiry assignments may be completed before coming to lab, and the data compilation, calculations, and the summative assessment should be completed after the lab.
- Two optional procedures for the transpiration activity are attached. Depending upon your class you may want to allow the students to research potential methods online—both of these methods are easily found online. Or, you may decide to give one of these two optimized procedures.
- The benefit of Option 1 is that the entire experiment is conducted over the course of a single lab period but the protocol can be tricky to set up. Many teachers have found it helpful to have students add a drop of food coloring onto the top of the water in the potometer. Use a blunt syringe to do this. The dye provides enough contrast to make the change in the water level visible.
- The benefit of Option 2 is that the setup is easily performed but the experiment must be run over the course of a week. The treatment must be left on 24 hours a day which may or may not be a problem at your school.
- Helpful hints to determine leaf surface area:

  a. Recall that leaf area is typically reported in square meters.

  b. Trace leaves onto graph paper and count the numbers of whole squares covered and estimate the amount that are partially covered. Determine the total leaf area in m^2. Since the leaves remain attached to the plant, the tracing will have significant error involved.

  c. Mass a known size of one leaf. For example, a two by two centimeter area. The larger the area the better. After completing the inquiry portion of the lab allow the students to strip off all of the leaves then mass to determine the total leaf mass. From this they can determine the total leaf area.
- Although red bean seeds and plant supplies are included, any small plant or twig may be used. Impatiens, pansies, coleus, oleander, peas, tomatoes, peppers, ferns, and ivy will all work.

Sample Data

There are four well-documented experimental conditions to test along with the room conditions (control). Flinn Scientific has provided these to you as a guide. Obviously your students will discover many more treatments. Ensure that each experimental condition is situated in your room such that it does not influence any of the other treatments. The reason for the typical results is given for each treatment.

1. Gentle breeze—Place a fan at least one meter from the plant, on low speed. Higher speeds cause the stomata to close. An increase in wind speed results in an increase in the rate of leaf water loss because the water evaporates more rapidly off the leaf, creating lower water potential in surrounding air and therefore a greater rate of transpiration.
2. High humidity—Mist leaves with water and cover with a transparent plastic bag, leaving the bottom of the bag open. The increased humidity in the air surrounding the leaf decreases the water potential gradient between the saturated air in the leaf air spaces and the air surrounding the leaf, resulting in a decreased rate of leaf water loss.
3. Strong light—Floodlight with heat sink or overhead projector light. The heat sink ensures the leaves do not become heated. It is simply a 1-L beaker filled with tap water placed between the floodlight and the plant. Absorption of light results in an increase in leaf temperature since the rate of water evaporation increases as the temperature increases, the increase in leaf temperature results in an increased rate of water loss.
4. Dark—Place the plant under a box with a flap to allow a small flashlight to illuminate the potometer to take readings. There are multiple factors leading to a decreased transpiration rate. The contained area gradually develops a higher humidity plus, without light, the rate of photosynthesis decreases. This treatment will only show valuable results when using the whole plant method.

Option 1—Using a Potometer

Table 1. Water Loss (mL)

Time (min)	0	3	6	9	12	15	18	21	24	27	30
Room Conditions	0.0	0.002	0.005	0.005	0.012	0.017	0.022	0.028	0.032	0.036	0.042
Gentle Breeze	0.0	0.025	0.054	0.088	0.112	0.142	0.175	0.208	0.246	0.283	0.325
High Humidity	0.0	0.002	0.004	0.006	0.008	0.011	0.014	0.018	0.019	0.021	0.024
Strong Light	0.0	0.021	0.042	0.070	0.091	0.112	0.141	0.158	0.183	0.218	0.239

Table 2. Leaf Surface Area (m^2)

Mass of all leaves (g)	1.200
Mass of 1 cm^2 of leaf (g)	0.010
Mass per m^2 of leaf (g/m^2) (above × 10,000)	100
Leaf surface area (m^2) (line 1 ÷ line 3)	0.012

Table 3. Water Loss (mL/m^2)

Time (min)	0	3	6	9	12	15	18	21	24	27	30
Room Conditions	0.0	0.167	0.416	0.667	1.00	1.42	1.83	2.33	2.67	3.00	3.50
Gentle Breeze	0.0	2.08	4.52	7.30	9.38	11.81	14.59	17.37	20.49	23.62	27.09
High Humidity	0.0	0.147	0.369	0.517	0.664	0.886	1.18	1.48	1.62	1.77	1.99
Strong Light	0.0	1.76	3.52	5.86	7.62	9.38	11.73	13.19	15.24	18.18	19.93

Table 4. Rate of Water Loss

	Rate of Water Loss ($mL/min/m^2$)
Room Conditions	0.102
Gentle Breeze	0.889
High Humidity	0.081
Strong Light	0.664

Option 2—Whole Plant Method

Table 4. Mass of Plant (g)

Day	1	2	3	4	Water Loss (g)
Room Conditions	93.33	87.82	78.79	69.76	23.54
Dark	89.02	85.01	80.77	76.46	12.56
0.5% Salt Water	98.20	90.55	78.63	68.07	30.13

Table 5. Leaf Surface Area (m^2)

	Room Conditions	Dark	0.5% Salt Water
Mass of all leaves (g)	2.02	2.26	3.04
Mass of 1 cm^2 of leaf (g)	0.023	0.025	0.030
Mass per m^2 of leaf (g/m^2) (1 cm^2 × 10,000)	230	250	300
Leaf surface area (m^2) (line 1 ÷ line 3)	0.0088	0.0090	0.0101

Table 6. Rate of Water Loss

	Rate of Water Loss (g/m^2)
Room Conditions	2675
Dark	1396
0.5% Salt Water	2983

Acknowledgment

Special thanks to Kathy Van Hoeck, York Community High School, Elmhurst, IL for providing the instructions for the whole plant method to Flinn Scientific.

References

AP Biology Investigative Labs: An Inquiry-Based Approach. College Entrance Examination Board: New York, 2012.

Biology: Lab Manual. College Entrance Examination Board: New York, 2001.

Rate of Transpiration and supporting supplies are available from Flinn Scientific, Inc.

Catalog No.	Description
FB2038	Rate of Transpiration
FB1469	Grow Lab II—Compact Indoor Garden
FB0494	Jewel 74″ Plantmobile
FB1275	Leaf Structure Model
FB1512	Potometer
AP6421	Lamp Bulbs, Clear, 150-W

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Option 1. Using a Potometer

Materials

Clamps, test tube, 2
Pan of tap water
Paper towels
Petroleum jelly, 5 g
Pipet, serological, 1-mL
Plant stem
Rubber bands, 3
Ruler
Scalpel
Support stand
Syringe, 12-mL
Tubing, amber, latex, 3/16″, 2″, 2
Tubing, plastic, clear, 1/8″, 16″

Safety Precautions

Scalpels are sharp instruments; use caution when cutting, always cut away from your body and away from others. Although the materials in this lab activity are nonhazardous, follow normal safety precautions. Wash hands thoroughly with soap and water before leaving the laboratory.

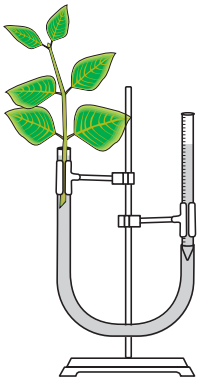

Figure 3.

Procedure

1. Place two test tube clamps on opposite sides of the support stand (see Figure 3).
2. Place one end of the latex tubing onto one end of the clear plastic tubing. Secure tightly with a rubber band.
3. Place the second piece of latex tubing onto the other end of the clear plastic tubing. Secure tightly with a rubber band.
4. Place the tapered tip of the 1-mL pipet into the free end of the latex tubing. Secure tightly with a rubber band.
5. Submerge the tubing and the pipet in a pan of water. Use the syringe to draw water through the tubing until all the air bubbles are eliminated. Leave it submerged in the water. *Note:* Air bubbles will prevent water from entering the stem of the plant.
6. Remove all flowers and buds from the plant.
7. Place the plant into a small plastic bag, leaving the stem sticking outside the bag.
8. Complete the next three steps quickly.
   a. Use a scalpel to cut the plant stem from the roots just above the surface of the soil.
   b. Wrap a rubber band around the bag, creating a moderately tight seal to keep water off of the leaves of the plant in steps 7 through 10. The rubber band will be used later to create a seal around the plant stem.
   c. Place the plant stem into the pan of water.
9. Have one group member hold the plant leaves out of the water while a second member completes the following steps. *Note:* Do not allow any air bubbles into the potometer. If an air bubble appears, quickly immerse the pipet, tubing, and part of the stem (but not the leaves) into the pan of water. Use the syringe to flush the air bubble from the potometer. If an air bubble touches the cut end of the stem, then the stem must be cut again under water.
   a. Use the scalpel to create a new cut on the plant stem while it is under water.
   b. While the plant stem and tubing are submerged, insert the freshly cut stem into the open end of the latex tubing.
   c. Secure the rubber band around the tubing with the stem inside.
   d. Place a generous amount of petroleum jelly around the top of the tubing and the stem junction to seal the opening. Note: Do not put petroleum jelly on the cut end of the stem because it will interfere with osmosis.
10. Bend the tubing upward into a "U." Use the clamps on the support stand to hold both of the stoppers (see Figure 3).
11. If needed, wrap paper towels around the tubing to secure the tubing within the clamps.
12. Remove the bag from the leaves and let the potometer equilibrate for 10 minutes.
13. Expose the plant in the tubing to the experimental conditions.

Option 2. Whole Plant Method

Materials

Balance, 0.001-g precision

Small potted plants, 2

Bags, plastic 11″ × 22″, 2

Rubber bands, 2

Water, tap

Safety Precautions

Although the materials in this lab activity are nonhazardous, follow normal safety precautions. Wash hands thoroughly with soap and water before leaving the laboratory.

Procedure

1. Saturate the plant with water the day before starting the inquiry activity.
2. Remove a plant from the pot. Keep the roots and as much soil as possible.
3. Place the root ball and soil into the plastic bag and secure with a rubber band (see Figure 4).
4. Remove all flowers and buds.
5. Determine the mass of each plant over the course of a week as the plant is exposed to the experimental conditions.

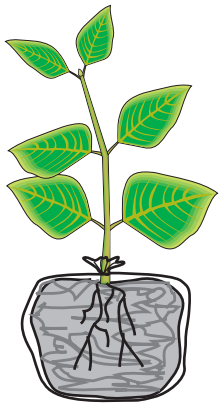

Figure 4.