LABORATORY

30

Water Movement and Mineral Nutrition in Plants

OVERVIEW

The amount of water needed daily by plants for photosynthesis, cell growth, and maintenance is very small, yet plants require large volumes of water to live. Amazingly, over 90 percent of the water moved throughout plant tissues by the process of **translocation** is lost to the air by **transpiration** (loss of water vapor from the plant surface) or **guttation** (loss of liquid from the ends of vascular tissue at the leaf margins). During this laboratory period you will study some of the factors involved in the movement of water and minerals throughout the plant.

STUDENT PREPARATION

Prepare for this laboratory by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency. Review Laboratory 8, Exercise D, A Look at Osmosis, and be sure to familiarize yourself with the following terms: *water potential, pressure potential,* and *osmosis.*

✓ EXERCISE A Observing Stomata

Stomata are minute openings bordered by **guard cells** in the epidermis of leaves and stems. It is through these openings that gases pass and water evaporates as photosynthesis, respiration, and transpiration occur.

Stomata open and close as a result of changes in turgor pressure within guard cells. Light-activated proton pumps in guard cell membranes actively transport H^+ ions out of the guard cells and, in turn, are responsible for the uptake of potassium ions (K^+) into these cells. An increase in the K^+ concentration inside guard cells causes their water potential (ψ) to become more negative, and water from the surrounding mesophyll cells (which have a more positive water potential) moves into the guard cells. As water accumulates and turgor increases within these cells, they inflate and bulge outward. The stomatal opening increases in size as the guard cells swell (Figure 30A-1).

Stomata usually remain open during the day and close at night, balancing the need for photosynthesis with that for conserving water. When mesophyll cells of the leaf are actively photosynthesizing, the amount of CO_2 present in air spaces between mesophyll cells decreases rapidly, signaling guard cells to open. On hot days, water depletion may cause loss of turgor in the guard cells and the stomata will close. Abscisic acid, produced by mesophyll cells of drought-stressed plants, can also signal guard cells to close during the day. In both cases photosynthetic rates will decrease, but this is a necessary trade-off. C_4 plants and succulent plants that live in hot, dry environments accumulate CO_2 in their leaves in the form of organic acids. Their stomata can remain closed during the day to conserve water while photosynthesis is made possible by release of CO_2 from the organic acids.

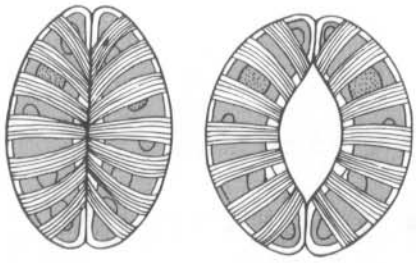


Figure 30A-1 *The mechanism of stomatal movements. (a) A closed stoma. The kidney-shaped guard cells are close together. Note the microfibrils that loop around the guard cells radially. (b) When water enters the guard cells, the microfibrils prevent them from expanding in circumference, so they expand in length and push apart at their attached ends.*

Objectives

- ☐ Describe how water transpires from the surface of a leaf.
- ☐ Diagram a stoma and label its parts.

Procedure

1. Obtain a leaf from the plant provided. Use forceps to remove a small piece of the lower epidermis. Make a wet-mount slide. Alternatively, paint the leaf surface with clear nail polish and, when it is dry, use a piece of clear adhesive tape to remove the "polish peel" from the leaf and mount it directly onto the slide. Examine the peel using the compound microscope at low power (10×). Count the number of stomata visible in one field of view. Move the slide and repeat the count. Record your data in Table 30A-1.
2. Repeat step 1 using a piece of upper epidermis from the same leaf. Record your data in Table 30A-1.

Table 30A-1 Distribution of Stomata

Name of Plant Used: _____		
	Number of Stomata in One Field of View:	
	Lower Epidermis	Upper Epidermis
	_____	_____
	_____	_____
Average	_____	_____

3. Switch to high power (40×) and, in the space provided next to the table above, prepare a drawing of several epidermal cells, guard cells, and stomata. Label these parts on your drawing.

   a. Are stomata more numerous on one surface than the other? _____ *If so, which one?*

   _____

   b. Compare your counts with those of a classmate who used a different plant. Are they the same? _____ *How do you explain this?* _____

   _____

Elodea, a common aquarium plant whose leaves are only two or three cells thick, has no stomata.

c. How does gas exchange occur? _____

4. Mount another piece of epidermis (upper or lower) on a microscope slide, but this time mount it in a 20% sucrose solution. (If you use the nail polish technique, soak your leaf in sucrose for 5 minutes and then blot it dry prior to making the "polish peel.") Add a coverslip and use a compound microscope to make your observations. You may need to use high power (40×).

   d. Do the stomata appear to be different? _____ *e. Are they opened or closed?* _____

   f. Why? _____

   g. What is the shape of the guard cells? _____

5. On the left side of the space provided below, diagram a stoma treated with sucrose.

6. Remove the coverslip from your slide and place the *same* piece of epidermis into a drop of water on a clean slide. Observe your slide immediately.

   h. Have the guard cells changed in shape? _____ *Are they open or closed?* _____

   i. Why? _____

7. In the remaining space above, diagram a stoma as it appears after the change from the sucrose solution to water. Label the guard cells.

✓ EXERCISE B Guttation

Guttation is the loss of water from the ends of veins at the tips and margins of leaves. It will occur only when soil moisture levels are high and the relative humidity is 100 percent. Under these conditions, transpiration, the loss of water vapor, is slow or absent, and a buildup of pressure in the roots (root pressure) forces water up the xylem. The water exudes through special openings called **hydathodes** at the tips and margins of leaves (*not* through stomata). Much of the dew you see on grass in the early morning is not water condensed from the air, but rather water from inside leaves leaving by the process of guttation.

Objectives

☐ Explain how guttation occurs in a leaf.

Procedure

Examine the young grain seedlings on demonstration. Droplets on the leaves are the result of guttation.

a. Where on the leaves do the droplets of the water of guttation appear? _____

✓ EXERCISE C Transpiration

Transpiration, the loss of water vapor by plant parts, occurs mainly through the stomata of the leaves. Water can move up a plant to the leaves by being pushed from the bottom or pulled from the top. **Root pressure** is not sufficient to push water all the way from the bottom to the top of a large plant such as a tree. It is more likely that water is pulled up through the plant body by the **cohesion–tension mechanism.**

As water leaves the intercellular spaces between mesophyll cells in the leaf and evaporates through the stomata, it is replaced by water from within the cells themselves. Since water moves out of these cells freely but solutes do not, the solute concentration within the mesophyll cells increases and the water potential (ψ) of the cells decreases. Water will then move into the mesophyll cells from surrounding cells with higher water potentials—for example, the cells of the xylem. Thus, as a result of transpiration, a gradient of differences in water potential from the xylem to the air outside the leaf is formed and water tends to be "pulled" upward. The **cohesion** of water molecules (one hydrogen-bonding to another) and their **adhesion** to the walls of the xylem cells cause the water to be pulled up as a continuous column.

The upward transpiration pull on the fluid within the xylem causes a **tension** (negative pressure) to form, pulling the walls of the xylem inward (you can actually measure the decrease in stem diameter of a plant on a hot sunny day, when the transpiration rate is very high). Tension, since it is "negative" pressure, causes water potential in the xylem to decrease. The decrease in water potential, transmitted through the column of fluid in the xylem, all the way to the roots, causes water to move from the soil across the cortex of the root and into the xylem of the stele, once again moving from an area of higher water potential to an area of lower water potential.

The opening of stomata, which allows transpiration to occur, is also required for the entry of CO_2 used in photosynthesis. A balance must be maintained between the two processes, transpiration and photosynthesis, by regulating the opening and closing of stomata.

Objectives

- ☐ Determine how environmental conditions affect the rate of transpiration.

✓ PART 1

Procedure

Examine the flasks (covered with beakers) in the demonstration area: flask 1, no *Coleus* shoot; flask 2, *Coleus* shoot; and flask 3, *Coleus* shoot with leaves coated with petroleum jelly.

a. In which beaker or beakers have water droplets formed? _____

b. Which of the beakers serves as a control? _____ *How?* _____

_____

c. Where did the water that has condensed on the inside of the beaker come from? Be specific.

_____

d. What was the purpose of the petroleum jelly treatment?

_____

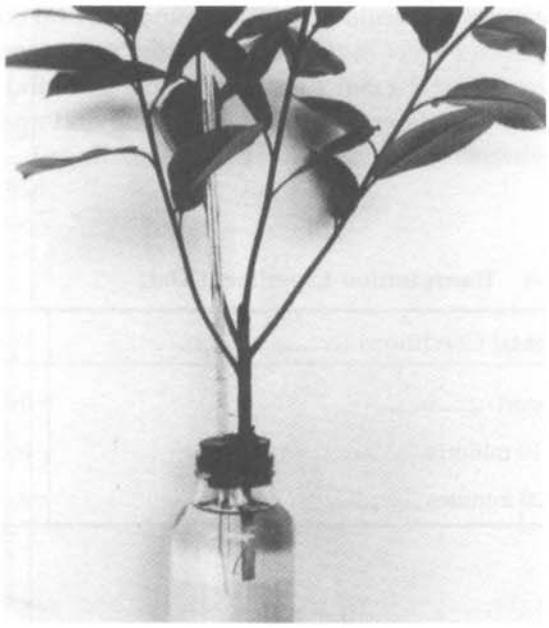
PART 2

Any environmental condition that increases evaporation will increase transpiration. Your laboratory instructor will demonstrate the method for assembly and use of a simple **potometer** (Figure 30C-1), an apparatus used to measure water transpiration in a plant shoot.

Procedure

1. Work in pairs. Each pair of students will determine the rate of transpiration using one of four treatments (to be assigned by your laboratory instructor):
   - A. Establish a control by running the experiment under room conditions.
   - B. Simulate wind by placing the plant about 2 meters from a fan. Use a low or medium setting (too much wind will cause stomata to close).

Figure 30C-1 *A sample potometer.*



C. Increase the humidity (vapor pressure) by spraying the plant with water and covering it with a plastic bag.

D. Increase the temperature of both the leaf and the air and increase the light intensity by placing the plant a prescribed distance from a flood lamp. (Ask your instructor for assistance—this distance will vary with different types of plants; the usual distance is 1.25 to 1.5 meters.)

Formulate a hypothesis on how the rate of transpiration is affected by the altered environmental conditions assigned to you.

HYPOTHESIS:

NULL HYPOTHESIS:

What do you **predict** will happen to the transpiration rate in your experiment compared with the rates for other treatments?

What is the **independent variable**?

What is the **dependent variable**?

2. Now determine transpiration rate. Set up your potometer as demonstrated. Choose the plant you will use and push the stem into the hole in the rubber stopper of the potometer top. Put petroleum jelly (Vaseline) around the base of the plant on the top side of the rubber stopper. Fill the potometer bottle *all the way to the top* with water. Make a fresh cut on the bottom of the stem (do this under water if possible) and immediately push the stopper (including plant and pipette) into the potometer bottle. Make sure to push hard to get a complete seal around the rubber stopper. The water should have risen up the pipette, past the last mark, and the level should not be dropping too quickly.

3. Allow the plant to equilibrate to the experimental conditions for 10 minutes. In Table 30C-1, record the water level in the pipette of the potometer at the end of 10 minutes as "(a) ml at start." After an additional 10 minutes, take a second reading and record your data in Table 30C-1; 10 minutes later, take a third reading and again record your data. (Your instructor will indicate whether you should take more readings.)

Table 30C-1 Transpiration Experiment Data

Experimental Conditions _____	Water Loss per 10-minute Interval
(a) ml at start _____	(b) − (a) _____
(b) ml at 10 minutes _____	(c) − (b) _____
(c) ml at 20 minutes _____	Average _____

4. After completing your last reading, remove all of the leaves from the plant that you used for your experiment. Using a balance, determine the combined mass (in grams, g) of all the leaves.
5. Determine the transpiration rate as average milliliters (ml) of water loss per 10 minutes.

   Convert this to water loss per hour (ml/hr). _____ ml/10 minutes; _____ ml/hr
6. Express the transpiration rate as the average amount of water loss in milliliters per hour per gram of leaf material.*

   $$\text{Transpiration rate per gram of leaf tissue} = \frac{\text{transpiration rate (ml/hr)}}{\text{mass of leaf tissue (g)}}$$

   Transpiration rate: _____ ml/hr per gram
7. Pool the class data and determine the average transpiration rate for each of the four treatments.
8. Using Figure 30C-2, make a bar graph to represent the pooled class data. Choose an appropriate scale for the vertical axis.

Do your results support your hypothesis? _____ *Your null hypothesis?* _____

Was your prediction correct? _____

What can you ***conclude*** *about the rate of transpiration under your assigned conditions?*

a. Under which condition was the rate of transpiration greatest? _____

b. Consider all four conditions and explain how each condition caused an increase or decrease in transpiration.

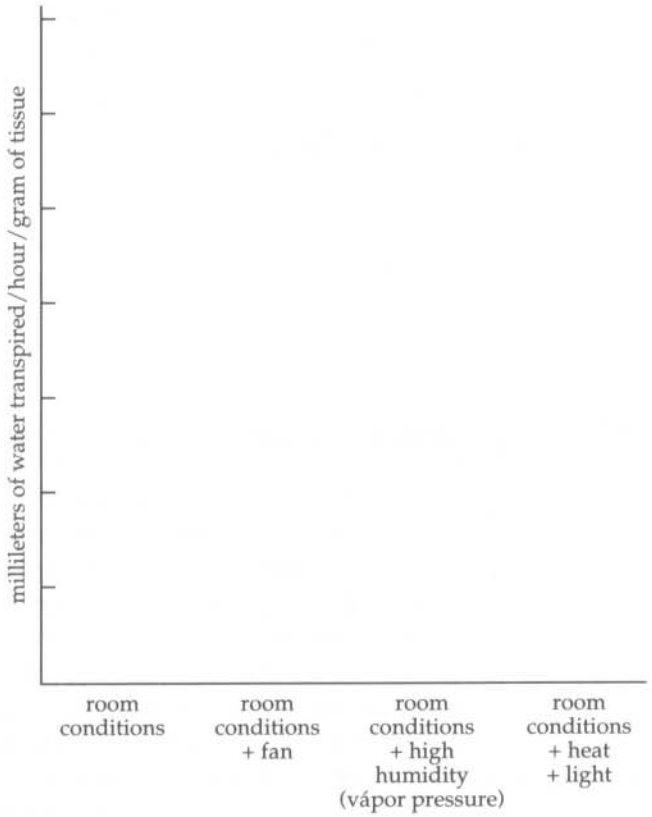
_____

_____

_____

**You may want to determine the transpiration as a function of surface area (rate per square meter of leaf tissue). Estimate the total leaf surface area in square meters (m^2). To do this, divide the total mass (g) of all leaves stripped from the plant (step 4) by the mass (g) of 1 m^2 of leaf tissue. (Your instructor will give you the latter value for the type of plant being used.) For total surface area, multiply by 2 to account for both surfaces of a leaf. Calculate transpiration rate per square meter by dividing the rate (ml/hr) by the surface area (m^2) of leaf tissue of your plant.*

Figure 30C-2 *Class data for effects of environmental conditions on transpiration rate.*



c. Is it possible that one of the test plants might transpire more than another of equal size under the same conditions? _____ *Explain.* _____

_____

d. Do you think that the number or size of the leaves might affect the transpiration rate? _____

Why? _____

_____

e. How would the number and distribution of stomata affect the transpiration rate? _____

_____

✓ EXERCISE D The Pathway of Water Movement Through a Plant

Water moves from the roots to the stem and then into leaves and flower parts through the xylem of the vascular bundles. Thus there is a continuous flow of water from the lower parts of plants to the upper parts.

Objectives

☐ Determine the pathway of water movement in a plant.

Procedure

1. Examine the carnation and celery plants that have been placed in one or several containers of food coloring. Note the cut surface of the celery petiole.

a. Does the dye appear only in certain tissues? _____

2. Use your dissecting microscope to examine a cross section of the celery. In the space below, make a sketch showing the location of the water-conducting tissue.

   b. Recall your study of root, stem, and leaf anatomy. Certain tissues are continuous through these three organs. Through which tissue does water move? _____

EXERCISE E Plant Mineral Nutrition

In addition to carbon dioxide and water, plants need a variety of minerals to form the organic molecules needed for life. These minerals are usually obtained from the soil and enter the plant with the water absorbed through the roots. The major mineral nutrients (**macronutrients**) required are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). In addition to these, plants also require smaller amounts of **micronutrients** such as iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn). The general effects of the lack of any of these nutrients are reviewed in Table 30E-1.

The macronutrients N, P, and K are used in large amounts by growing plants and can therefore be rapidly depleted from the soil. Consequently, these are the three most common components of fertilizer. In fact, types of fertilizer are usually designated according to their percentage content of N, P, and K. For example, a common garden fertilizer, 5-10-10, contains 5% nitrogen, 10% phosphorus, 10% potassium, and 75% filler.

The purpose of this exercise is to determine how deficiencies in macronutrients and micronutrients affect plant growth. For this experiment, you will grow sunflower seedlings hydroponically. **Hydroponics** is a method of growing plants in which soil is not used. Instead, plants are placed in a liquid environment (medium), and growth occurs through the uptake of nutrients from the liquid via the roots.

Objectives

☐ Describe how deficiencies in nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and iron affect plant growth.

Procedure

Work in pairs. As many as eight treatments may be used in this experiment. Your instructor will assign you to the treatment you will be using. Each represents a different mixture of macronutrients and micronutrients in a hydroponic growth medium:

Treatment 1 Control (contains all macronutrients and micronutrients)

Treatment 2 Lacks potassium (K)

Treatment 3 Lacks nitrogen (N)

Treatment 4 Lacks iron (Fe)

Treatment 5 Lacks phosphorus (P)

Treatment 6 Lacks calcium (Ca)

Treatment 7 Lacks magnesium (Mg)

Treatment 8 Lacks sulfur (S)

An aquarium is assigned to each treatment. It contains the hydroponic medium for that treatment.

After reviewing the information in Table 30E-1, formulate a hypothesis about the role of macronutrients and micronutrients in promoting plant growth. (Consider all treatments.)

Table 30E-1 Dichotomous Key to Mineral Deficiencies in Plants

A. Effects localized on older leaves or generalized to the whole plant.	
B. Local, occurring as mottling or chlorosis (loss of chlorophyll) with or without necrotic spotting (brown spotting) of the lower (older) leaves; little or no drying of lower leaves.	
C. Lower leaves curved or cupped under with yellowish mottling or necrotic spots at tops and margins; leaf drop.	**Potassium**—Maintains osmotic balance of cells; responsible for osmotic changes in guard cells and regulation of the opening and closing of stomata; required in protein synthesis and in some enzymatic reactions.
C. Lower leaves chlorotic between the principal veins at leaf tips; leaf margins of a light green to white color; typically, no necrotic spots.	**Magnesium**—Constituent of chlorophyll; involved in numerous enzymatic reactions; stabilizes ribosomes.
B. General, also yellowing and drying or "firing" of lower leaves.	
C. Plant light green, lower leaves yellow, drying to light brown color; plants dwarfed.	**Nitrogen**—Constituent of amino acids, proteins, nucleic acids, chlorophyll, and hormones.
C. Plant dark green or may have purple tint; leaves narrow in proportion to length; plants dwarfed.	**Phosphorus**—Important as a constituent of nucleic acids, phospholipids, ATP, coenzymes, and some proteins; also involved in energy metabolism.
A. Effects localized on terminal growth, young leaves, and buds.	
B. Dieback involving the terminal bud is preceded by peculiar distortions and necrosis at the tips or bases of young leaves making up the terminal growth; root meristems also develop abnormal growth; growth stunted.	**Calcium**—Constituent of cell walls; constituent (as calcium pectate) of the middle lamella.
B. Terminal bud remains alive; chlorosis of upper or bud leaves, with or without necrotic spots; veins either light or dark green.	
C. Young leaves with necrotic spots scattered over chlorotic leaf; smallest veins tend to remain green, producing a checkered effect.	**Manganese**—Constituent and activator of enzymes involved in photosynthesis, respiration, and nitrogen metabolism.
C. Young leaves without necrotic spots; veins either light or dark green.	
D. Young leaves are light green, never white or yellow; leaves do not dry up; veins are light green or of the same shade as interveinal tissue.	**Sulfur**—Constituent of proteins and coenzyme A.
D. Young leaves or all leaves become yellowish; principal veins characteristically darker green than tissue between the veins.	**Iron**—Needed in energy transfer molecules in respiration and photosynthesis; also involved in chlorophyll synthesis.

HYPOTHESIS:

NULL HYPOTHESIS:

What do you **predict** will happen to the growth of plants deprived of certain macronutrients or micronutrients?

What is the **independent** variable?

What is the **dependent** variable?

1. Record your treatment type: _____

2. Remove five sunflower seedlings from the stock container. Plant the five sunflower seedlings in the aquarium assigned to your treatment.

   Each aquarium contains a styrofoam float that has been divided into a grid system. Your instructor will assign you five locations within this grid to plant your seedlings. Record these locations:

   _____ _____ _____ _____ _____

   To plant a seedling, remove the cotton plug from the grid location. Carefully insert the seedling through the hole so that its roots extend beneath the foam and its stem is standing above the float. (Do not lift the float out of the tank or push it beneath the solution!) Cotton should be repacked or twisted loosely around the stem to keep the plant from slipping through the hole.

3. Collect day 1 ("time zero") biomass data for the sunflower seedlings. To do this, remove two seedlings from the stock container. Using a balance, determine the mass of each plant and record these values in Table 30E-2.

Table 30E-2 Data on Seedling Mass

Week	Seedling	Mass (g)
Time zero (day 1)	1	
	2	
1 Week growth	1	
	2	
2 Weeks growth	1	
	2	

4. Collect day 1 ("time zero") chlorophyll content data for the sunflower seedlings. To determine the chlorophyll content of the two plants (used in step 3), they must be crushed. Cut one plant into tiny pieces (use the entire plant, including roots!) and put these into 30 ml of 90% acetone in a mortar. Grind the plant pieces with a pestle 50 times, then filter the acetone through a double layer of cheesecloth into a 50-ml beaker. (Be sure to use only 50 strokes—this will standardize your procedure.) Pour this solution into a clinical centrifuge tube, leaving approximately 2 cm of the tube unfilled. Repeat this procedure with the other plant. Label the centrifuge tubes "1" and "2," to correspond to the seedling they contain, and place both centrifuge tubes into a clinical centrifuge opposite one another. **Be sure to record the locations of each of your tubes.** Once other students have loaded their tubes into the centrifuge, spin the samples using setting 6 for 20 minutes. After centrifugation is complete, decant the supernatant into a Spectronic 20 tube or cuvette until it is filled to within approximately 2 cm of the top.

For measuring absorbance, use a blank containing 7.5 ml of 90% acetone to adjust your Spectronic 20 to 100 percent transmittance and 0 percent absorbance. Measure the absorbance of both chlorophyll samples at 663 nm. Record all data in Table 30E-3.

Table 30E-3 Data on Chlorophyll Content

Week	Seedling	Absorbance (663 nm)	Chlorophyll Content (mg/l)	Standardized Chlorophyll (mg/g)
Time zero (day 1)	1			
	2			
1 Week growth	1			
	2			
2 Weeks growth	1			
	2			

5. Determine chlorophyll content in milligrams per liter (mg/l) using the following formula:*

   Chlorophyll content (mg/l) = absorbance at 663 nm × 13.4

6. Record the chlorophyll content for each of your seedings in Table 30E-3. Standardize your chlorophyll measurements for each seedling by factoring in the biomass of the plants. Use the following formula:

$$\text{Standardized chlorophyll content (mg/g)} = \frac{\text{chlorophyll content (mg/l)} \times 0.030 \text{ (l)}}{\text{seedling biomass (g)}}$$

7. Record the standardized chlorophyll content values in Table 30E-3 and give your data, along with the biomass measurements in Table 30E-2, to your laboratory instructor to be added to a cumulative class database. Be sure your names and treatment group are included with your data.
8. During the next two weeks, at the beginning of each laboratory period, make observations (height, color, leaf side, wilting) on the plants in each treatment tank. Record these observations in Table 30E-4 and give copies of this information to your laboratory instructor. (Again, be sure your names and treatment group are included with your data.)
9. Repeat steps 3–7 for a pair of your seedlings in week 1 and for another pair in week 2. Record the biomass data in Table 30E-2 and the chlorophyll content data in Table 30E-3 for each week. Give all of your data to your instructor (include your names and treatment group).
10. Data for each treatment will be collected for all laboratory periods in week 1 and again in week 2. At the ends of weeks 1 and 2, your instructor will perform a chi-square median statistical analysis of differences in biomass and chlorophyll measurements for all treatments. Results for each week will be distributed to you. Be sure to familiarize yourself with the chi-square median analysis (Appendix I) so that you will understand how to interpret your data and come to a conclusion.

Use the constant 13.4 as determined by O. T. Lind, Handbook of Common Methods in Linnology, *p. 132, C. V. Mosby, St. Louis, MO, 1979.*

Table 30E-4 Appearance of Plants after 1 and 2 Weeks

Week	Treatment	Observations
1 Week growth	Control	
	− K	
	− N	
	− Fe	
	− P	
	− Ca	
	− Mg	
	− S	
2 Weeks growth	Control	
	− K	
	− N	
	− Fe	
	− P	
	− Ca	
	− Mg	
	− S	

a. *Which plants demonstrated the greatest amount of growth (increase in biomass)?* _____ *Why?* _____

b. *Did you see a color change in any of the plants?* _____ *Describe any changes.* _____

c. *Why did these changes occur?* _____

d. *In those plants with color changes, was their growth also affected?* _____ *If so, how?* _____

e. *Which plants demonstrated the greatest chlorophyll content?* _____

Why? _____

f. *Were there differences in biomass and chlorophyll content between control and experimental plants for all experimental treatment groups?* _____ *For your treatment?* _____

g. *If there was a difference, was it statistically significant according to the results from the chi-square median test? For all experimental plants?* _____ *For your treatment?* _____

For your treatment? _____ *Was your prediction correct?* _____

Do your results support your hypothesis? _____ *Your null hypothesis?* _____

What can you ***conclude*** *about the role of macronutrients and micronutrients in controlling plant growth? For all treatments?* _____

Laboratory Review Questions and Problems

1. You cut some flowers and leave them beside your sink for several hours before putting them into a vase. When you finally arrange the flowers, some of the stems are too long and you cut them and immediately place them in the vase. The next day you notice that many of the flowers are wilted, but those that you cut a second time are fine. How could you explain this given what you know about the mechanism of transpiration?

2. Leaves of plants growing in shady forests tend to be large and luxuriant, while those of plants growing in sunny grasslands tend to be narrow with little surface area. Explain in terms of transpiration.

3. One factor that causes stomata to open is the depletion of CO_2 in the air spaces of the leaf. How does this fact relate to the process of photosynthesis?

4. On a hot, dry day with temperatures above 30 to 35°C, cellular respiration in leaf cells increases. In these conditions, what would happen to the stomata? Why?

5. Some plants, including cacti and succulents, open their stomata at night and close them during the day. Why? How would they fix CO_2 for photosynthetic processes? [*Hint:* These plants use crassulacean acid metabolism (CAM).]

6. Suggest how each of the following leaf modifications would be of advantage to a plant in the designated type of environment.

Modification	Environment	Advantage
Sunken stomata	Arid, windy	
Leaves modified as spines	Sunny, arid	
Stomata only on upper epidermis	Leaves float on water surface	

7. Both magnesium and iron deficiencies result in a failure to form chlorophyll. How would you expect plants deficient in these nutrients to look? If you completed the mineral nutrition experiment (Exercise E), did it support this expectation?

8. Leguminous plants obtain nitrogen in the form of NH_4^+. What is the name of this process of reducing N_2 to NH_4^+? What is responsible for this process? Why is it beneficial to rotate plantings of leguminous and nonleguminous crops?

9. Mycorrhizal fungi associated with the roots of certain plants help to transfer phosphorus and other relatively immobile nutrients such as zinc, manganese, and copper into the plant roots. What is responsible for this increased transfer?

Seedling

Seedling is a simulation of plant competition and plant physiology. **Seedling** simulates the growth of a crop plant in diverse outdoor environments or in a growth chamber with constant, user-controlled conditions.

- **Seedling** allows you to manage a growing plant by taking the sugar it synthesizes each day and allocating it either to add leaves, increase stem height or stem diameter, or grow more roots. Your choices can create a vigorous and competitive plant or one that cannot survive dehydration, shading from other plants, or windstorms. However, beware: Even well-grown plants can be cropped and trampled by cows that sometimes visit this simulation!

- **Seedling** can also simulate three growth-chamber experiments: the effects of interplant distance on competition, plant responses to differing temperature and light conditions, and the influence of temperature and humidity on transpiration.

This is your opportunity to experience the challenges that plants must face to grow, compete, or die!