EDVOTEK®

BIG IDEA 4
INVESTIGATION

AP BIOLOGY 11

EDVO-Kit: AP11

Transpiration

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to apply principles of diffusion, osmosis, cohesion and adhesion to movement of water within plants. Students will study water potential transport and the affect of plant environment on transpiration. Sections of fresh plant stem tissue will be observed by microscope and the vascular tissues identified.

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Safety Data Sheets can be found on our website at:
www.edvotek.com/safety-data-sheets

Experiment Components

Store the entire experiment at room temperature.

This experiment is designed for 10 lab groups.

A Bush Bean seeds, *Phaseolus vulgaris*
B 2x Toluidine Blue O stain
C Parawax

- Plastic tubing
- Microtomes (nuts and bolts)
- Petri plates
- 1 mL pipets

Requirements

- 10 mL pipets
- Petroleum jelly
- Light source with 100 Watt bulb
- Fan(s)
- Plant mister (a spray bottle)
- Potting soil
- Large plastic bags
- Ring stands & clamps (or buret holder)
- Microscope slides
- Microscope(s)
- Cover slips
- Slide mounting medium, i.e. 50% glycerol
- 50% ethanol
- New razor or scalpel blades
- Weighing scale or balance
- Small spatulas

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

Background Information

PRINCIPLES OF TRANSPIRATION

Plants can be conceptually organized into groups. Primitive plant groups include green algae, brown and red algae, fungi, and mosses. These groups lack specialized differentiated structures for water transport; therefore, they are highly dependent on water in their immediate environment. The green algae inhabit primarily aquatic or very moist land regions. The red and brown algae inhabit the sea and are more formal names for seaweeds. Fungi and mosses also require moist land regions.

Vascular plants, as a group, possess tissues that are specifically designed for efficient water and solute transport. Hence, these plants are the most predominant land plants. Examples of vascular plants include ferns, gymnosperms, such as the conifers, and angiosperms or flowering plants. Angiosperms are the most common plants. This group is subdivided into monocots, such as grasses and corn, and the dicots, such as oak and maple trees, potatoes, roses and beans. Beans are the subject of this experiment.

In vascular plants, such as beans, the upward movement of water from the roots to the stems and leaves occurs primarily through the xylem tissue. Phloem tissue is responsible for the upward and downward movement of organic solutes, such as sugars, and inorganic solutes, such as calcium, magnesium, and phosphorus. The solvent for these solutes is water. Organic nitrogen compounds, such as amino acids, may move upward from the roots in both the xylem and phloem.

Free standing vascular land plants present an interesting question. Vascular plants lack a physical pump, so how do these plants transport water from their roots to their stems and leaves? In particular, how does a tree, which is 300-400 feet tall, transport water and nutrients upward through the xylem to its extreme height? This problem has been the subject of experimental investigation for several hundred years.

One suggestion was that air pressure simply pushed water up from the roots. However, the pressure of air at sea level is one atmosphere and is only capable of supporting a frictionless column of water approximately 35 feet high, insufficient for a 400 foot tree. Another early suggestion was that the water was simply pushed upward from below by hydrostatic root pressure. There is a tendency for the net inward movement of water to move into the xylem tissues in the roots due to negative pressure or tension in the xylem, and the accumulation of dissolved minerals within the xylem. This results in a lowering of the water potential in the xylem. Therefore, water moves into the xylem by osmosis. In actuality, water may be in higher concentration in the xylem than in the surrounding endodermal cells. If water movement were simply by diffusion, the water would tend to move in the opposite direction, out of the xylem. In addition, a tall column of water would also exert a large downward hydrostatic pressure, again forcing the water out of the xylem. Experimental data suggests that root pressure is created by the activity of living cells and is responsible for the flow of water into the xylem.

Root pressure causes **guttation**, the exudation of droplets of water on the leaf margins of herbaceous dicots seen in early morning. This is the result of both low transpiration rate at night and of root pressure pushing xylem fluid into the plant.

If roots are killed or deprived of oxygen, all root pressure disappears. This suggests water is actively transported by cellular activity into the xylem. However, experimentally measured root pressures have not exceeded 1-2 atmospheres. Again, this pressure is inadequate to account for water movement to a height of 400 feet. It might be sufficient for small, short plants, but root pressure alone is not large enough to move water to extreme heights.

Background Information

The transport of water up the roots in the xylem is controlled by differences in **water potential** which explains water movement from cell to cell over long distances in the plant. Water always moves from an area of high water potential to low water potential. Gravity, pressure, and solute concentration are contributors to water potential, while root pressure, osmosis, and adhesion and cohesion of water molecules contribute to the actual movement.

The **cohesion**, or **transpiration pull theory**, originally postulated in the early 1800's is strongly supported by experimental evidence. This theory suggests that water is pulled up the xylem from the roots and surrounding soil due to transpiration. The "pull" on the water is due to both cohesion of water molecules to each other and adhesion of water molecules to the walls of the xylem cells. Transpiration begins with a large loss of water from the plant by evaporation due to the opening of the stomata on the surface of the leaf during the day to provide CO_2 for photosynthesis. Stomates open into the air spaces surrounding the mesophyll cells of the leaf. The moist air in these spaces has a higher water potential than the outside air and therefore evaporation occurs from the leaf surface. This allows water from the mesophyll cells to move into the air spaces (again from higher water potential to lower water potential). The result is a lowering of the water potential of the mesophyll cells which enable water to move by osmosis from xylem and surrounding cells to the mesophyll cells. The movement of water out of the xylem into the mesophyll cells results in transpirational pull from leaves at the top of tree to the roots, as long as the column of water within the xylem does not break.

This theory rests on the assumption that there is a cohesive molecular force between water molecules allowing the molecules to be pulled upward from the roots. In fact, on a molecular level, water is highly organized. A strong cohesive force between adjacent water molecules does exist. This cohesive force is due to the high degree of hydrogen bonding between the individual water molecules. It is strongly suggested from experimental data, that the evaporative loss of water from the plant by transpiration plays a major motive role in the net flow of water from the surrounding soil, into the roots, and up the xylem to the leaves.

Environmental factors play a major role in the rate of transpiration. This includes those factors which affect the opening of the stomata, and those physical factors which affect the evaporation rate through the open stomata. Some of these factors include temperature, light intensity, air currents Using a **potometer**, this experiment will allow measurement of transpiration from the bush bean under several environmental conditions.

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

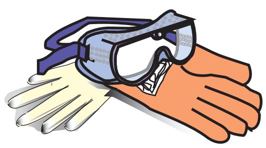
Students will:

1. Apply principles of diffusion, osmosis, cohesion and adhesion to movement of water within plants.
2. Study transpiration in the transport of water within plants.
3. Understand how differences in water potential affect transport throughout the plant.
4. Study the affect of plant environment on transpiration and make sections of fresh plant stem tissue and observe and identify vascular tissue under microscope.

WORKING HYPOTHESIS

If environmental conditions affect the opening and closing of stomates on the leaf surface, then the rate of transpiration will also be affected.

LABORATORY SAFETY GUIDELINES



1. Wear gloves and goggles while working in the laboratory.
2. Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:
- Record your observations.

After the Experiment:
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Investigation I: Transpiration in *Phaseolus vulgaris* (Bush Bean)

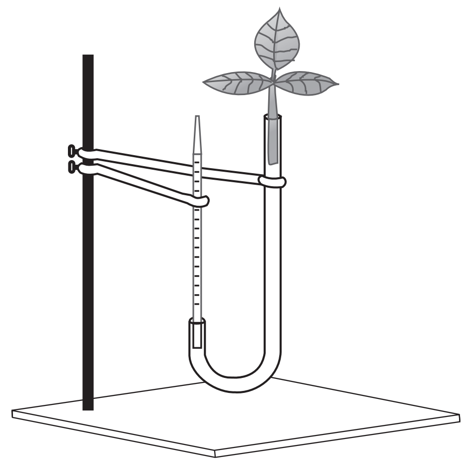


Figure 1

1. Carefully place the non-tapered end of a 1 mL pipet into one end of a 16” piece of vinyl tubing. A small amount of petroleum jelly can be placed on the outside of the pipet at the non-tapered end to help insertion of the pipet.

2. Bend the tube into a U shape and secure onto a ring stand with clamp(s) as shown in Figure 1. The top of the tubing and the top of the pipet must be level in order to fill completely with water.

3. Fill the tubing and 1 mL pipet completely with water from the tubing end, using a 10 mL pipet. No air bubbles should be present in the tubing or 1 mL pipet.

4. Insert a freshly cut stem from a 2 week old bush bean seedling into the end of the tubing and seal with petroleum jelly. Do not get petroleum jelly onto the cut end of the stem or the experiment will not work! No bubbles should be present in the tubing or 1 mL pipet (Figure 1).

5. Expose the stem to one of the following conditions assigned by your teacher:

   - Room conditions
   - 100 watt light source placed 1 meter from the stem
   - Fan 1 meter from stem on low to create a breeze
   - Mist of water from plant mister (cover the potometer immediately with a plastic bag after misting)

6. After a 10 minute equilibration period, read the water level in the pipet and record below as time 0. You may want to use a marker to draw a line at the water level for each time point.

7. Obtain a reading once every 5 minutes for 30 minutes.

TABLE 1: Potometer Readings

Time (min)	0	5	10	15	20	25	30
Reading (mL)							

8. After 30 minutes, cut off the leaves of the bush bean seedling and blot off any excess water gently. Weigh the leaves. Weight of leaves __________grams.

Investigation I: Transpiration in *Phaseolus vulgaris* (Bush Bean)

9. Estimate the **total leaf surface area** for the plant.

- Cut a 1 cm^2 section out of a leaf
- Mass the cut section = ________grams.
- Multiply cut section mass by 10,000 to obtain the mass per square meter of the leaf = ________g/m^2
- Using the formula below obtain the total leaf surface area (m^2):

$$\frac{\textbf{Total Weight of Leaves (grams)}}{\textbf{grams/m}^2} = \textbf{Total Leaf Surface Area (m}^2\textbf{)}$$

- Leaf Surface Area (m2) = ___________

10. Using the formula below calculate water loss in mL/m^2 at each reading (Table 1) by dividing the reading by the calculated leaf surface area:

$$\frac{\textbf{(water level at each T in mL) - (water level at Time 0 in mL)}}{\textbf{total leaf surface area in m}^2}$$

TABLE 2: Individual Water Loss in mL/m^2

Time (min)	0	5	10	15	20	25	30
Water Loss (mL)							
Water Loss per m^2							

Record the class averages in Table 3, below.

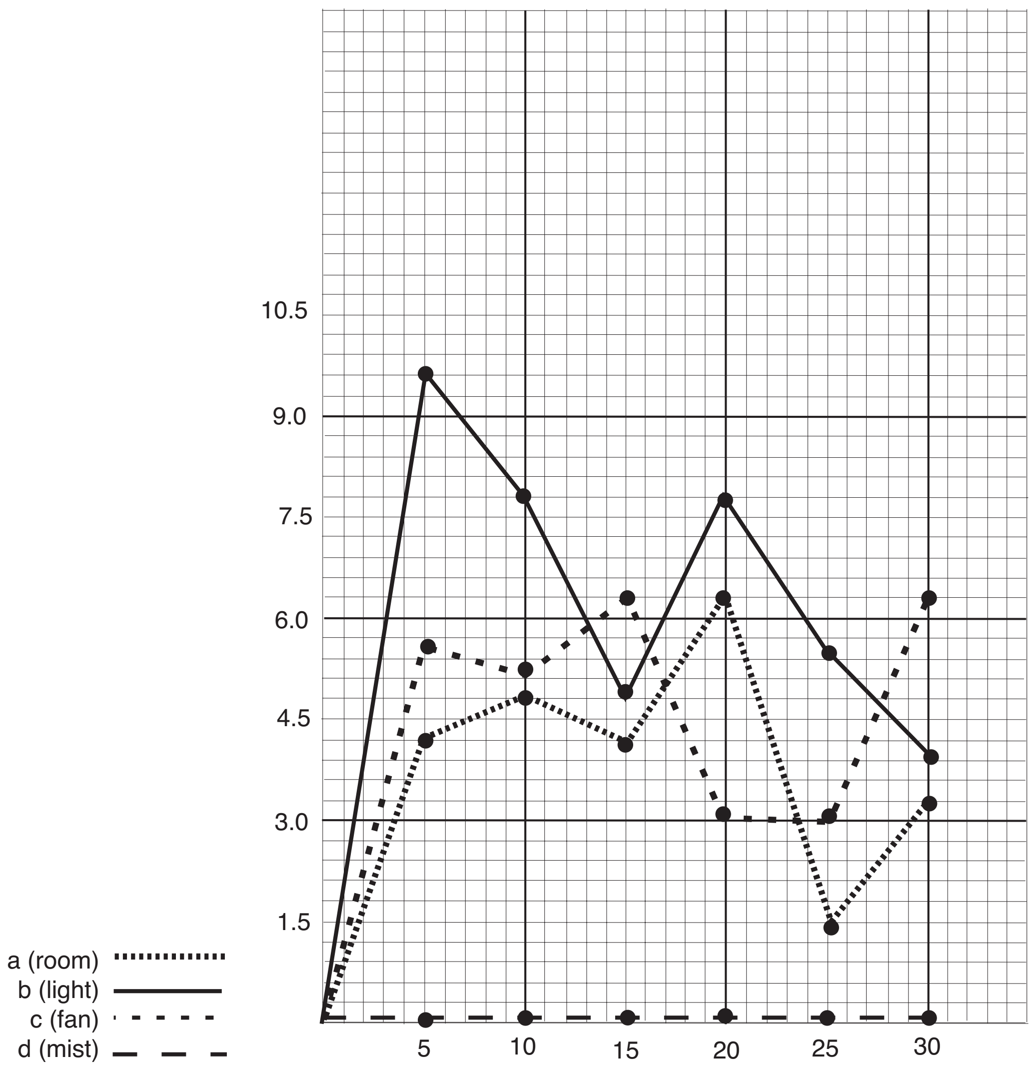
TABLE 3: Class Average Water Loss in mL/m^2

	Time (min)						
Treatment	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Room							
Light							
Fan							
Mist							

11. Plot the class average data of water loss (mL/m^2) versus time (minutes). Using liner graph paper, plot water loss on the y-axis vs. time on the x-axis.

Investigation I: Analysis of Results

1. Using class averages, graph the results from each treatment.
2. Label the independent variable (horizontal x-axis).
3. Label the dependent variable (vertical y-axis).
4. Title the Graph:



5. What hypothesis is being tested in the experiment?

Investigation II: Visualization of Plant Stem Cell Structure

1. Obtain and assemble the nut and bolt microtome. Adjust the bolt so that there is a small cup at the end.
2. Cut a fresh slice of bush bean stem about 5-6 mm in length. It should be slightly longer than the cup you have formed in the microtome.
3. Place the cut stem into the microtome so that it stands up. Using a Pasteur pipet, fill the cup with melted parawax provided by your instructor. Work quickly as the parawax will cool and solidify in the pipet.
4. Wait 5-10 minutes for the parawax to completely solidify. When the wax has solidified, lay the microtome on its side and with a new single-edged razor or scalpel blade, carefully slice away the excess wax (Figure 2).
5. Twist the bolt slightly to expose a thin piece of wax. Carefully slice off a thin section of wax/stem. With this simple apparatus, you are preparing thin cross sections of the bush bean stem. Using a spatula, place the sections in the petri plate containing 50% ethanol. Prepare 10 -12 sections. Allow the sections to soak for 5 minutes.
6. Transfer the sections to the Toluidine blue O stain solution contained in another petri plate. Stain for 5-10 minutes.
7. Transfer the stained sections to the petri plate containing distilled water.
8. Place the sections onto a microscope slide and add a drop of mounting medium or 50% glycerol, and cover with a cover slip.
9. Observe the sections under a compound light microscope.
10. Make drawings of the structures you observe. Try to see the plant stem cell types discussed below.

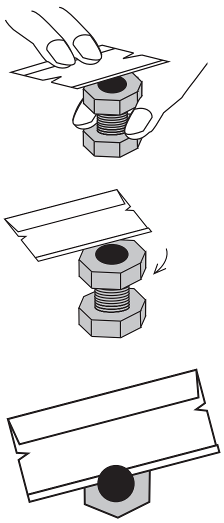


Figure 2

FUNDAMENTAL TISSUE

Parenchyma tissues are found in roots, stems and leaves. They are relatively unspecialized cells with thin primary cell walls and no secondary cell walls. In leaves, however, they have the highest concentration of chloroplasts and are main sites for photosynthesis. Parenchyma cells in the roots and stems are storage sites for nutrients, such as starch, and water. Starch grains can be seen within these cells. When turgid (full of water), the parenchyma are responsible for the support and shape of the plant.

Sclerenchyma tissues function primarily as support. At maturity, most of these cells are dead, thickened and have secondary cell walls. The two types are **fibers** and **sclereids**. The fibers are long, tapered cells. Common examples are flax and hemp, which are used in ropes. Sclereids are irregularly shaped cells often found in the hard parts of seeds and nut shells. In your stained slides, they are seen as bright blue structures in the cross section immediately outside the vascular tissues.

Investigation II: Visualization of Plant Stem Cell Structure

Collenchyma is a fundamental nonspecialized plant tissue which remains alive during their functional life. Collenchyma functions to provide support to young plants, in the stems of older plants, and in leaves. They are structurally similar to parenchyma except their walls have an irregular thickening. These "thickened" areas can be seen at the edges/corners when viewing a cross section.

VASCULAR TISSUE

Xylem is a vascular tissue which functions as the main conduit for water transport from the roots, through the stems, and out to the leaves. In flowering plants, it is commonly composed of **tracheids** and **vessel elements**. It also includes **parenchyma** and **sclerenchyma cells.** The parenchyma are the only living cells in the xylem tissue. Water is carried in the tracheids and vessels. In addition to water transport, xylem functions to provide aerial support to plants. Label the xylem in your cross section drawings.

Phloem is a vascular tissue which is responsible for the upward and downward movement of materials within plants. It is especially important for the movement of dissolved organic and inorganic solutes. It is composed of **parenchyma** and **sclerenchyma** cells. In addition, it also contains both **sieve cells** and **companion cells**. The elongated sieve cells form the sieve tube and are the cells responsible for the vertical transport of material within the phloem. At the very end of the sieve cells is the **sieve plate**, which serves to pass material from one cell to the next in a vertical fashion. When mature, the sieve cells are simply cytoplasm and lack a nucleus. The companion cells are associated with the sieve cells. They are derived from the same tissues as the sieve cells. They are in direct contact with the sieve cells and have a thin cell wall at the point of contact. They may function in supplying materials to the sieve cells which lack a nucleus. In your cross section of the bean stem, the phloem is located outside of the xylem. Both are arranged together in a structure termed the **vascular bundle**. Identify both the xylem and phloem in your cross sections.

SURFACE TISSUE

The **epidermis** is the principle outer covering of roots, stems, and leaves. Its major role is to function as a barrier to water loss and protection against injury. Depending on the environment, it may be one layer, or several layers thick, especially in dry environments. The epidermal cells are flat, with a large vacuole and a thin cytoplasm. On the aerial parts of plants, they secrete a waxy, water resistant cuticle composed of **cutin**. The epidermal cells interlock as well to form an effective barrier to water loss. Locate and observe the epidermis on your cross section.

Study Questions

1. What is the total leaf surface area for the plant?
2. Determine the average amount of water loss per milliliter per square meter for each treatment:
    a. room ____
    b. light____
    c. fan____
    d. mist____
3. Explain how each condition affects water loss.
4. How did these conditions affect the stem to leaf water gradient?
5. What is the importance of water potential in controlling the transport of water?
6. Explain several mechanisms by which plants try to minimize water loss.

Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

OVERVIEW OF LABORATORY INVESTIGATIONS

The "hands-on" laboratory experience is a very important component of science courses. Laboratory experiment activities allow students to identify assumptions, use critical and logical thinking, and consider alternative explanations, as well as help apply themes and concepts to biological processes.

EDVOTEK experiments have been designed to provide students the opportunity to learn very important concepts and techniques used by scientists in laboratories conducting biotechnology research. Some of the experimental procedures may have been modified or adapted to minimize equipment requirements and to emphasize safety in the classroom, but do not compromise the educational experience for the student. The experiments have been tested repeatedly to maximize a successful transition from the laboratory to the classroom setting. Furthermore, the experiments allow teachers and students the flexibility to further modify and adapt procedures for laboratory extensions or alternative inquiry-based investigations.

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site.

In addition, Technical Service is available from 8:00 am to 5:30 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Pre-Lab Preparations

INVESTIGATION I: TRANSPIRATION IN *PHASEOLUS VULGARIS* (BUSH BEAN)

1. Plant the bush bean seeds (A) in well drained potting soil, 3 weeks before the lab.

2. The seedlings should be about 2-3 weeks old for use in this lab.

3. Place directly under a fluorescent plant growth light and water as needed. (Optional: Place in a bright window.)

INVESTIGATION I - On day of lab, each lab group will need:

- 16" piece of clear plastic tubing
- 1 mL graduated pipet
- Ring stand and 2 clamps or buret holder
- 1 bush bean plant, 2 week old seedling
- Petroleum jelly
- Fan for 2 groups
- 100 watt light source for 2 groups
- Mister and plastic bag for 2 groups
- None of the above for 2-4 groups
- Access to scale or balance

INVESTIGATION II: VISUALIZATION OF PLANT STEM CELL STRUCTURES

Prior to Lab:

1. Prepare 120 mL of 50% ethanol. Dilute 63.2 mL of 95% ethanol with 56.8 mL of distilled water.

2. Prepare Toluidine Blue O stain. Dilute 60 mL of Toluidine Blue O stock solution (B) with 60 mL of distilled water.

On the day of the lab:

3. Melt 50 grams of the parawax (C) in a 200 mL beaker which has been placed in a water bath at 75°C.

INVESTIGATION II - Each lab group will need:

- 5 mL melted parawax
- 3 petri dishes, top or bottom as follows:
  1 dish containing 10 mL 50% ethanol
  1 dish containing 10 mL diluted Toluidine Blue O stain
  1 dish containing 10 mL distilled water
- 1 nut and bolt microtome
- Plant stem
- Razor or scalpel blade to cut microtome sections
- Small spatula to handle microtome sections

Experiment Results and Analysis

INVESTIGATION I: TRANSPIRATION IN *PHASEOLUS VULGARIS* (BUSH BEAN)*

In this experiment, under **ideal** results, one would expect that a higher rate of water evaporation from the leaves occurred during the light treatment (as the leaf temperature increased) and in the fan treatment. If this experiment had been continued for a longer period of time, the stoma would close to prevent excessive water loss and one would expect to see a decrease in the cumulative water loss.

*Sample data (ideal results)

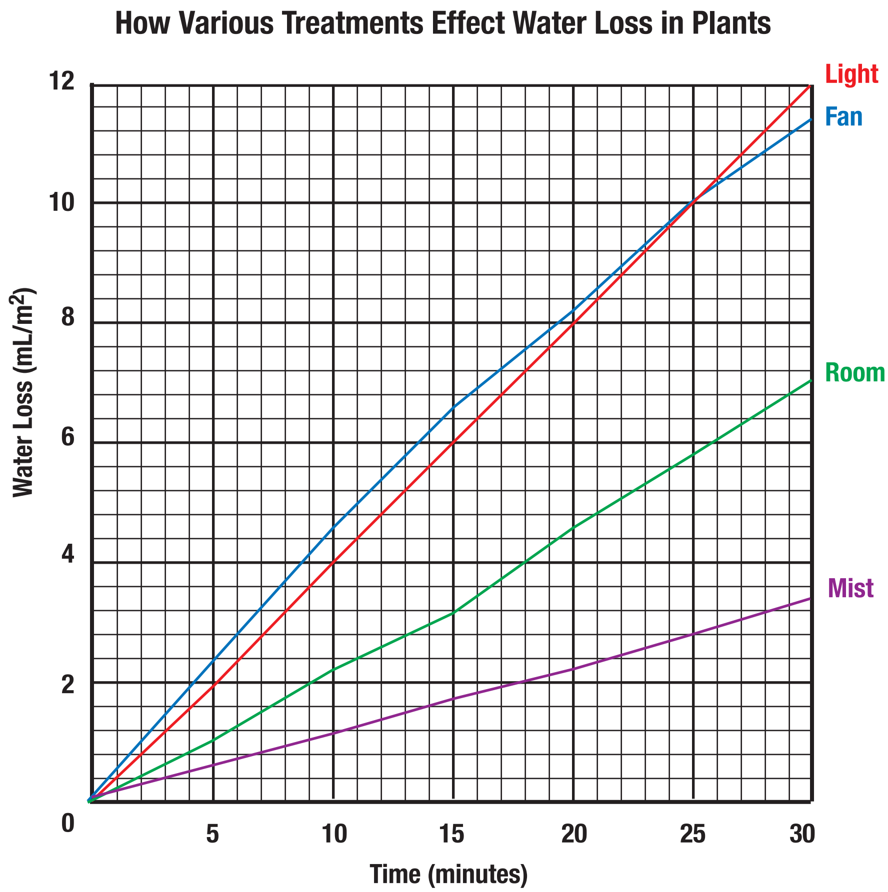
	TIME (minutes)						
Treatment	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Room	0	1.1	2.3	3.4	4.7	5.8	7.0
Light	0	2.0	4.1	6.1	8.0	10.1	12.0
Fan	0	2.3	4.5	6.6	8.2	10.0	11.4
Mist	0	0.6	1.2	1.7	2.2	2.8	3.4

However, results similar to those below may have occurred. This is due to excessive water loss which will cause leaf stoma to close, resulting in little cumulative water loss. This would account for large dips on the graph. Large increases on the graph are due to leaf stoma opening.

*Sample data

	TIME (minutes)						
Treatment	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Room	0	4.2	4.7	4.2	6.3	1.6	3.7
Light	0	9.6	8.0	4.8	8.0	5.6	4.0
Fan	0	5.6	5.3	6.3	3.1	3.1	6.3
Mist	0	0	0	0	0	0	0

Idealized Results



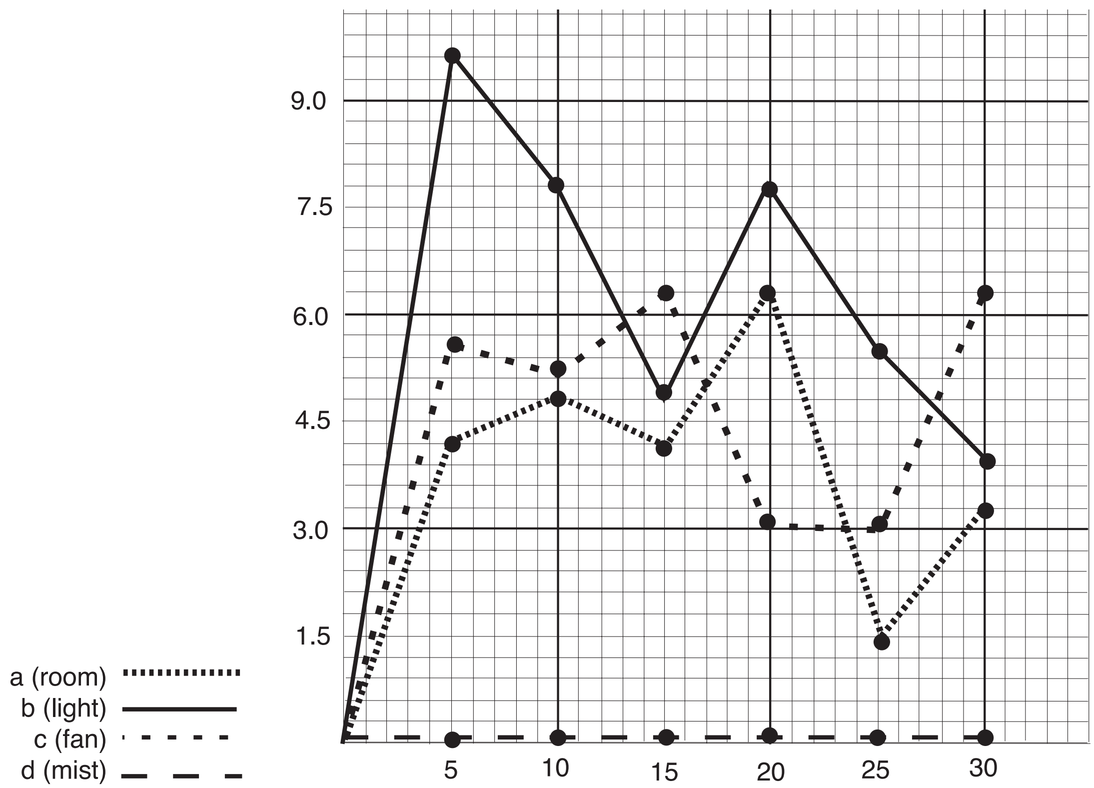
Label the independent variable (horizontal x-axis):
Time (minutes)

Label the dependent variable (vertical y-axis):
Water Loss (mL/m^2)

Title the Graph:
How Various Treatments Effect Water Loss in Plants

What hypothesis is being tested in this experiment?
If environmental factors effect the opening and closing of stomates on the leaf surface, then the rate of transpiration will also be affected.

Expected Results and Analysis



INVESTIGATION II: VISUALIZATION OF PLANT STEM CELL STRUCTURE

Refer to text references for photos of plant stem sections.

Please refer to the kit insert for the Answers to Study Questions