CAROLINA

Student Guide

Name/Group # _____

Date _____

AP® Biology Laboratory 9
Transpiration

Objectives

- Use a simple potometer to study transpiration
- Make sections of a stem and observe the water-conducting tissues

Background to Activity A

Transpiration is the loss of water vapor from a plant. Plant epidermis has microscopic pores called stomates, which allow for gas exchange between the interior of the plant and the external atmosphere. Plants typically have a waterproof layer, the cuticle, which coats the epidermis. For such plants, most (but not all) water lost by transpiration escapes through stomates. Although all aboveground parts of plants commonly have stomates (except woody stems), leaves usually have the greatest total number and thus account for most of the water lost from a plant by transpiration. Recall from Lab 1, *Osmosis and Diffusion*, that water is absorbed from the soil by the roots. From the roots, water moves up the stem to cells within the leaves. Water evaporates from the surfaces of these cells into the spaces between the cells until the spaces are saturated. Unless the air outside the leaves is also saturated, the water potential inside the leaf will be greater than outside the leaf and water vapor will diffuse from the leaf through its stomates to the surrounding air. This lowers the water potential of the leaf spaces to below that of the cells within the leaves and more water diffuses from the cells into the spaces, and so on. This loss of water and lowering of water potential exerts a powerful tension or pull on the water contained within the specialized water-conducting tissues of the stem and helps move water from the roots to the aerial portions of the plant.

Activity A: Measuring Transpiration With a Potometer

Materials

The following materials are needed to construct two potometers: 2 bush bean seedlings, 2 1.0-mL pipets in 0.01 divisions, 2 pieces of flexible tubing, single-edged razor blade, 2 ring stands, 4 extension clamps.

Your group will be assigned to test transpiration under two of the treatment conditions described below. Treatment-specific additional materials and initial setup instructions follow.

A. ***Normal Room Conditions***

Additional material: thermometer. Once your potometer is set up, measure the air temperature near the plant.

B. ***Heat***

Additional materials: lamp with 100-W bulb, thermometer. Once your potometer is set up, position the lamp so that you can feel its warmth at the plant's location. Measure the air temperature near the plant. Compare this temperature with the measurement taken by a group testing Treatment A. The Treatment B temperature should be warmer.

C. *Moving Air*

Additional material: fan. Once your potometer is set up, position the fan so that the plant will be exposed to a gentle breeze. Set the fan on low speed.

D. *High Humidity*

Additional materials: plastic bag, spray bottle. Once your potometer is set up, spray the leaves with water and cover the plant with the plastic bag. Leave the mouth of the bag open.

Introduction

In this activity, you will use a potometer to measure transpiration. Your potometer will consist of a tube attached to a pipet. Both are filled with water, and then the shoot (stem and leaves) of a plant is inserted into the open end of the tube. As the shoot transpires, it absorbs water from the potometer and the water level in the pipet will drop. Notice that you are actually measuring the liquid water absorbed, not the water vapor that is lost. In most cases, the two will be equivalent.

Procedure

Potometer Assembly

Work cooperatively in pairs to assemble the potometers. Read the following instructions carefully and familiarize yourself with the procedure before beginning.

1. Slowly lower the tip (the pointed end) of a pipet into water until all the air has been replaced by water. Do the same with a piece of tubing. Remove all the air from both the pipet and the tubing.
2. While keeping both pipet and tubing underwater, insert the tip of the pipet into the tubing.
3. While keeping the open end of the tubing underwater, close the top of the pipet with your finger and raise the pipet until you are holding the pipet/tubing combination vertically. Check for leaks. If there are any, start over (If you see any air bubbles, tap the pipet several times to dislodge them). Lower the pipet/tubing back into the water.
4. Obtain a bean seedling that has had the soil washed from its roots. Immerse the roots and part of the stem in the water beside the open end of the tubing. Do not allow the leaves to become wet. Also, try to avoid crushing the seedling's stem.
5. Identify a point on the stem where you will make your cut. Select a location where the stem is the same diameter as, or slightly larger than, the inside diameter of the tubing. There should be two or more healthy leaves on the shoot.
6. Cut the stem underwater. Make the cut at an angle rather than straight across. Make a clean cut without crushing the stem. Keep the cut end of the stem underwater at all times.
7. Insert the cut end of the stem into the

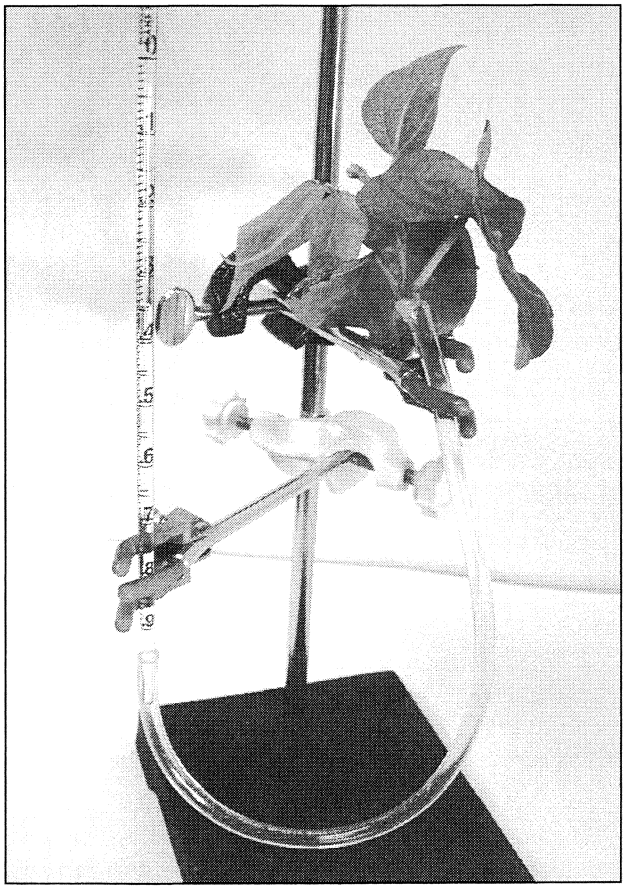


Figure 1. Assembled potometer

tubing while still holding both underwater. (If the cut end of the stem is removed from the water, air may block the water-conducting vessels. If this happens, quickly cut the stem again underwater at least 3–4 cm above the first cut. This will remove the blocked portion. If this is not possible, discard the shoot and obtain a new plant.)

8. Make sure that the end of the stem is immersed in the water within the tubing. Close the top of the pipet with your finger. Lift the entire assembly out of the water. Bend the tubing into a "U" shape until the water level in the pipet is slightly higher than the end of the tubing into which the stem is inserted. Remove your finger from the end of the pipet and check for leaks. If there are none, proceed to the next step. If you detect a leak, use petroleum jelly to make an airtight seal between the tubing and the stem. *The seal must be airtight or the experiment will not work.* Do not put petroleum jelly on the cut end of the stem. If the petroleum jelly does not stop the leak, ask your teacher for assistance.
9. Mount your potometer on a ring stand using extension clamps (see Figure 1). As before, keep the water level in the pipet slightly higher than the opposite end of the tubing.

Taking Readings

1. Let the potometer equilibrate for 10 minutes after it has been fully assembled.
2. After equilibration, record the beginning water level in the pipet under Time 0 in Table 1.
3. Take and record readings every three minutes for a total of 30 minutes. Record the data in Table 1.

Table 1: Potometer Readings

Treatment: _____

Time (min)	0	3	6	9	12	15	18	21	24	27	30
Reading (mL)											
Water Loss (mL)											
Water Loss per m^2											

Determining Leaf Surface Area

1. At the end of your experiment, cut the leaf blades from your bean plant. Do not include the leaf stems.
2. Use one of the following methods to determine the total leaf surface area of your bean plant:

Method 1 (Leaf Trace Method)

- Trace the outline of each leaf on a piece of graph paper. Use the graph paper grid to determine the total leaf area in cm^2.

Total leaf surface area = _____ cm^2

Method 2 (Leaf Mass Method)

- Blot any water from the leaves and then determine their total mass. Do not discard or crush the leaves.

  Total mass of leaves _____ g

- Cut a 1 cm^2 from one bean leaf and determine its mass.

  Mass of 1 cm^2 of leaf = _____ g/cm^2

- Total leaf surface area in cm^2 = $\frac{\text{Total mass of leaves}}{\text{Mass/cm}^2 \text{ of leaf}}$ = _____ cm^2

3. After completing Method 1 or Method 2, convert the leaf surface area into m^2.

   Leaf surface area in m^2 = $\frac{\text{Total leaf surface area cm}^2}{10{,}000\ \text{cm}^2/\text{m}^2}$

   Total leaf surface area = _____ m^2

Analysis of Results, Activity A: Measuring Transpiration With a Potometer

1. Fill in the Water Loss row of Table 1 for each time interval. For example: reading at 0 minutes = 0.10 mL and the reading at 6 minutes = 0.05 mL, then the water loss is 0.05 mL.
2. Calculate the water loss per m^2 for each time interval by dividing the water loss by the total leaf surface area. Record your results in Table 1.
3. Determine class averages and complete Table 2 for all treatments.

Table 2: Class Averages for Cumulative Water Loss in mL/m^2

	Time (Minutes)										
Treatment	0	3	6	9	12	15	18	21	24	27	30
A. Normal Room Conditions	0										
B. Heat	0										
C. Moving Air	0										
D. High Humidity	0										

4. Graph the class averages from Table 2. Title the graph and supply the following information:

   a. The independent variable is _____.

   b. The dependent variable is _____.

   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

5. What is the purpose of converting the data to mL/m^2?

_____

_____

_____

_____

6. Using your graph or data from Table 2, calculate the average rate of water loss per minute per m^2 for each treatment.

   A. Normal Room Conditions: _____

   B. Heat: _____

   C. Moving Air: _____

   D. High Humidity: _____

7. Write a hypothesis that this experiment is designed to test.

   _____

   _____

   _____

8. Which of the four treatments best represents a control for this experiment?

   _____

   _____

   _____

9. Summarize the results of the experiment, comparing the results of each variable tested to the control you identified in Question 8. Include a brief explanation of how the treatment may have produced the observed effect.

   Treatment ___. Condition _____: _____

   _____

   _____

   _____

   _____

   Treatment ___. Condition _____: _____

   _____

   _____

   _____

   _____

   Treatment ___. Condition _____: _____

   _____

   _____

   _____

   _____

10. Suppose that you set up a potometer with a bean seedling that is badly wilted. Its leaves are wrinkled and drooping. Over the course of 30 minutes you observe that the leaves expand and return to an erect state. You also record a drop of 0.54 mL in the pipet of the potometer. Is this an accurate measure of the amount of water transpired by the seedling? Explain your answer.

11. A 1-hectare field contains 2500 bean plants having a total leaf surface area of 557 m^2. Ambient temperature is 21°C, winds are calm, and humidity is 45%. If each plant is transpiring at the average rate determined for Treatment A in 6 above, how much water is lost from the field each hour due to transpiration? Show your calculation in the space below.

12. How would you expect the following conditions to affect the rate at which water is transpired from the 1-hectare field described in 11 above?

Ambient temperature of 35°C: _____

Winds of 15 to 25 km/h: _____

Humidity 100%: _____

Ambient temperature of 35°C, winds of 15 to 25 km/h: _____

Background to Activity B

Plants, like animals, are composed of different organs, tissues, and cells. Parenchyma cells are the basic plant cell type and other, more specialized cells originate from parenchyma cells. Parenchyma cells seldom form secondary cell walls and thus usually have primary cell walls only. These are thin and composed of cellulose and pectins. Parenchyma cells are found in all plant organs. They form the pith and cortex of stems.

Epidermal cells cover the outer, nonwoody surfaces of the plant.

Fibers are elongated cells with thickened secondary cell walls. They add structural strength to stems and leaves.

Xylem and phloem are the water-conducting tissues of plants. In general, xylem transports water and dissolved minerals upward from the roots to the stem, leaves, and other aerial parts of the plant. It is the tissue that replenishes the water lost from leaves through transpiration.

Phloem transports water containing dissolved carbohydrates throughout the plant. For example, sugars produced by photosynthesis in the leaves can be transported down to the roots for storage as starch in parenchyma cells. Potatoes are largely composed of parenchyma cells filled with starch. (The next time you eat French fries, remind yourself that you are eating cooked parenchyma cells.) Maple trees also store starch in their roots. In the spring, the starch is converted back into sugar. The sugar is transported upward to the buds where it is metabolized to provide the energy and raw materials needed for new growth. Humans have learned to cut through the bark to the phloem and draw off the sugary sap to produce maple syrup.

Xylem and phloem occur together in vascular bundles, with the phloem facing the outside of the stem and the xylem facing inward. The "strings" of a celery stalk are vascular bundles. A cap of thick-walled fibers often develops to the outside of the phloem. Vascular bundles with fibers are sometimes called fibrovascular bundles.

The four basic stem types are categorized according to their structure: herbaceous dicot, woody dicot, herbaceous monocot, and woody monocot. The bush bean is an example of a herbaceous dicot.

Activity B: Structure of a Herbaceous Dicot Stem

Materials

Single-edged razor blade, bolt, nut, bush bean seedling, paraffin wax, forceps, glycerin, 50% ethanol, 3 petri dishes, toluidine blue O, distilled water, slide, coverslip, microscope.

Introduction

A microtome is an instrument for cutting thin sections of tissue that can be viewed under a microscope. Typically, a section of tissue is stained before viewing. A good stain will selectively react with or be absorbed by certain cellular structures more than others. For example, potato cells contain organelles called leucoplasts. If a section of potato is treated with a stain containing iodine, the leucoplasts will stain dark blue-black, indicating that they contain starch. In this activity, you will use a simple microtome consisting of a nut, bolt, and single-edged razor blade to make cross sections of a herbaceous stem. You will stain the cross sections with toluidine blue O. This will stain primary cell walls purple. Thickened secondary cell walls will stain blue. You will then observe the stained cross section under a microscope and identify tissues within the stem.

Procedure

1. Place a nut on a bolt and turn it just enough to secure it to the bolt. This will leave a well about 5–7 mm deep that is formed by the hole in the nut and the end of the bolt. This nut-and-bolt combination is your microtome.
2. Using a single-edged razor blade cut a section of bean stem (or other herbaceous dicot) that is about the same length as the depth of the well. Make both cuts straight across the stem, not at an angle.
3. Stand the cut section of stem in the well of your microtome. Pour melted paraffin (**Caution:** *Hot liquid!*) around the stem until the well is filled. It may be necessary to reposition the stem after pouring the paraffin, but do not move the stem while the paraffin is solidifying.
4. Allow the paraffin to solidify completely before proceeding.
5. Hold the microtome so that the outer surface of the nut is at eye level. Twist the nut clockwise until you see the paraffin and stem rise just above the surface of the nut.
6. **Caution:** *During this step, be aware of the position of your fingers and the razor blade. Make certain that, if the razor should slip, you will not cut yourself or anyone else.* Place the blade of a single-edged razor on the surface of the nut at a sharp angle (10–15°). Push the razor blade forward and at the same time slide it to the side; try to cut off the thinnest slice possible of paraffin and stem.
7. Remove the paraffin from the section and use forceps to transfer the section to a petri dish containing 50% ethanol.
8. Repeat steps 5–7 until you have produced 8 to 10 sections.
9. Allow the sections to remain in the ethanol for five minutes or more before proceeding.
10. Transfer the sections from the ethanol to a dish of toluidine blue O stain. Leave them in the stain for 5 to 10 minutes.
11. Transfer the sections into a dish of distilled water to remove the excess stain.
12. Place one or two drops of glycerin on a microscope slide. Select your best cross section (thin is better than round) and transfer it into the glycerin. If you get a lot of air bubbles, work them away from the section with a teasing needle. Add a coverslip and observe with a microscope.
13. Use the simplified diagram (Figure 2) to help you identify the vascular bundles and other tissues of the stem. Once you have located the tissues, create and label your own more accurate and detailed drawing of the stem cross section.

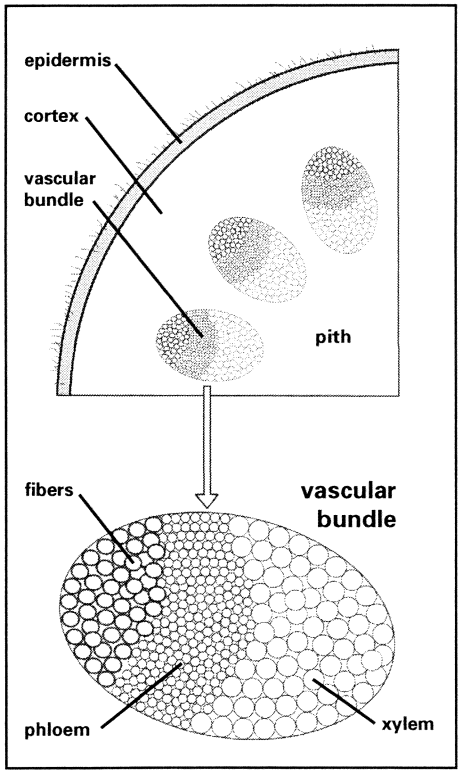
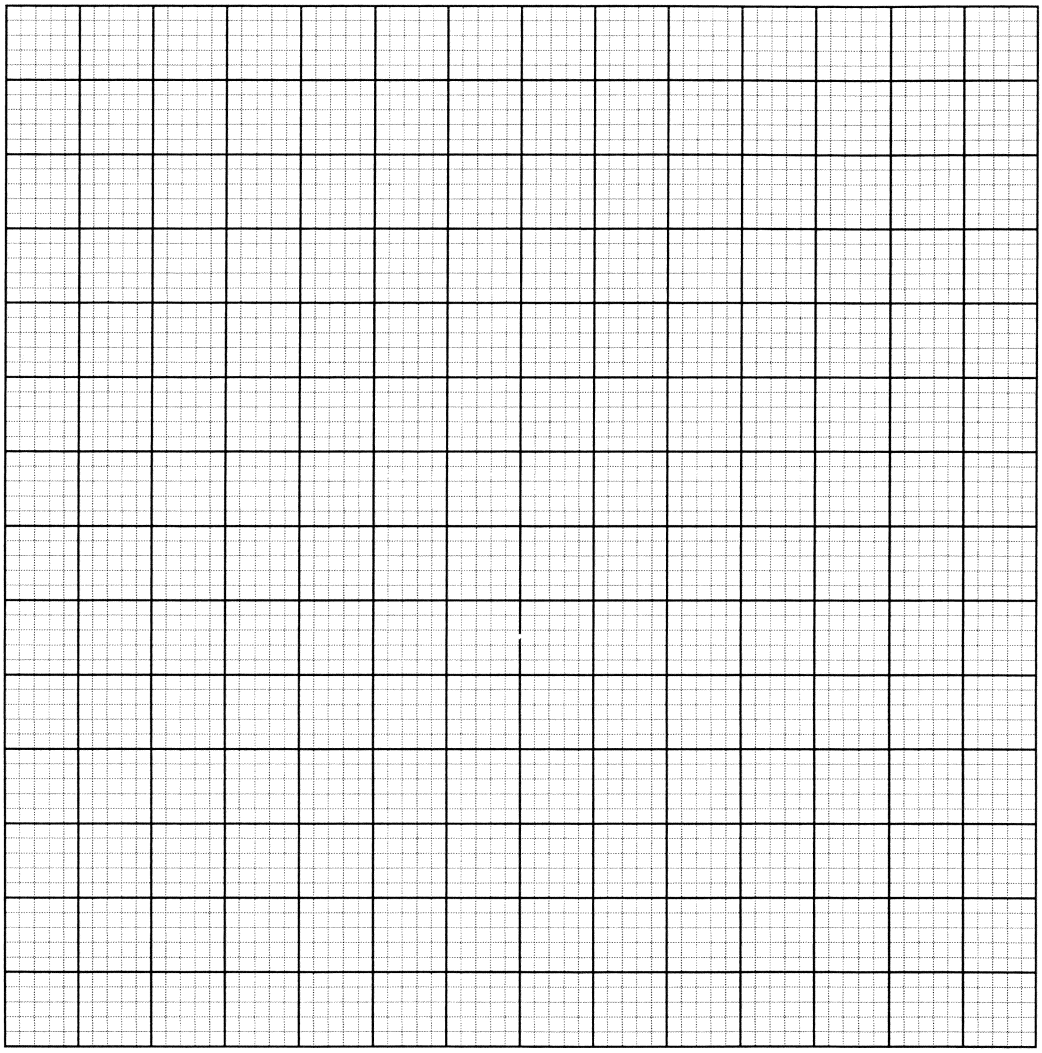


Figure 2. Herbaceous dicot stem

Title: _____



Laboratory 9. Transpiration

Overview

This lab consists of two parts. Activity A is the primary investigative activity and should be the focus of student efforts. In **Activity A (Measuring Transpiration With a Potometer)**, students measure water loss by transpiration. In **Activity B (Structure of a Herbaceous Dicot Stem)**, students observe the tissues in a herbaceous stem.

Objectives

- Use a simple potometer to study transpiration
- Make sections of a stem and observe the water-conducting tissues

Content Standards

This kit is appropriate for Advanced Placement® high school students and addresses the following National Science Content Standards:

Unifying Concepts and Processes

- Systems, order, and organization
- Evidence, models, and explanation
- Constancy, change, and measurement

Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Life Science

- Matter, energy, and organization in living systems

Time Requirements

Activity A: One 45-minute laboratory period

Activity B: One 45-minute laboratory period

Safety

- Use this kit only in accordance with prudent laboratory safety precautions, including approved safety goggles, lab aprons or coats, and gloves. Know and follow all school district guidelines for lab safety and for disposal of laboratory wastes.
- Cutting plant stems requires the use of sharp instruments.
- Hot wax can cause burns.
- Toluidine blue O can irritate skin, mouth, and eyes, and can stain skin and clothing.

Station Setup

Following is a list of the materials needed for one group of students to perform the activities in this lab. Prepare as many setups as needed for your class.

	Activity A	Activity B
two-week old bush bean seedlings	2	1
1.0-mL pipets in 0.01 divisions	2	
16″ pieces of tubing	2	
petroleum jelly (shared)	1	
spray bottle (Treatment D, shared)	1	
plant bag, 12 × 24″ (Treatment D)	1	
*shallow tray or sink with tap water	1	
single-edged razor blade	1	1
nut-and-bolt microtome		1
melted paraffin wax		6 mL
glycerin		10 mL
50% ethanol		10 mL
small petri dishes		3
toluidine blue O		10 mL
*distilled water		10 mL
slide and coverslip		1
forceps (shared by group)		1
*ring stands	2	
*extension clamps	4	
*electric fan (Treatment C)	1	
*lamp with 100-W bulb (Treatment B)	1	
*thermometer (Treatment A, B, shared)	1	
*microscope		1
*stopwatch or clock with second hand	1	

*Not supplied.

Troubleshooting

If necessary, pad the extension clamps to avoid damaging the stem inside the tubing. Most problems with this activity result from leaks around the stem where it is inserted into the tubing. If coating this junction with petroleum jelly does not solve the problem, wrap the stem with Parafilm® or a similar product and reinsert it. Sometimes a plant will wilt during the activity. This indicates that the plant is not absorbing water from the potometer tube. Causes include petroleum jelly covering the cut end of the stem, air entering the stem and blocking the conducting vessels, and crushing of the stem during insertion into the tubing. It may be possible to salvage the plant by recutting the stem but in most cases it will be necessary to begin anew with a fresh plant.

Rarely, you may find that plants receiving treatments B and C will show reduced transpiration. This occurs when the stimulus (heat or air movement) is too strong and results in closure of the stomata.

Sample Answers to Questions in the Student Guide

Activity A: Measuring Transpiration With a Potometer

Treatment: A, *ambient conditions*

Sample Table 1: Potometer Readings*

Time (min)	0	3	6	9	12	15	18	21	24	27	30
Reading (mL)	0.16	0.19	0.23	0.26	0.30	0.33	0.36	0.40	0.43	0.47	0.50
Water Loss (mL)		0.03	0.07	0.10	0.14	0.17	0.20	0.24	0.27	0.31	0.34
Water Loss per m^2		11.07	25.83	36.90	51.66	62.73	73.80	88.56	99.63	114.39	125.46

Total leaf surface area = 0.00271 m^2

Sample Table 2: Class Averages* for Cumulative Water Loss (mL/m^2)

Treatment	30 min
A. Normal Room Conditions	125.46
B. Heat	310.81
C. Moving Air	298.12
D. High Humidity	61.77

* *Student results for Table 1 and Table 2 will vary according to the data collected. These results were obtained using cuttings from geranium seedlings.*

4. Graph the class averages from Table 2. Title the graph and supply the following information:

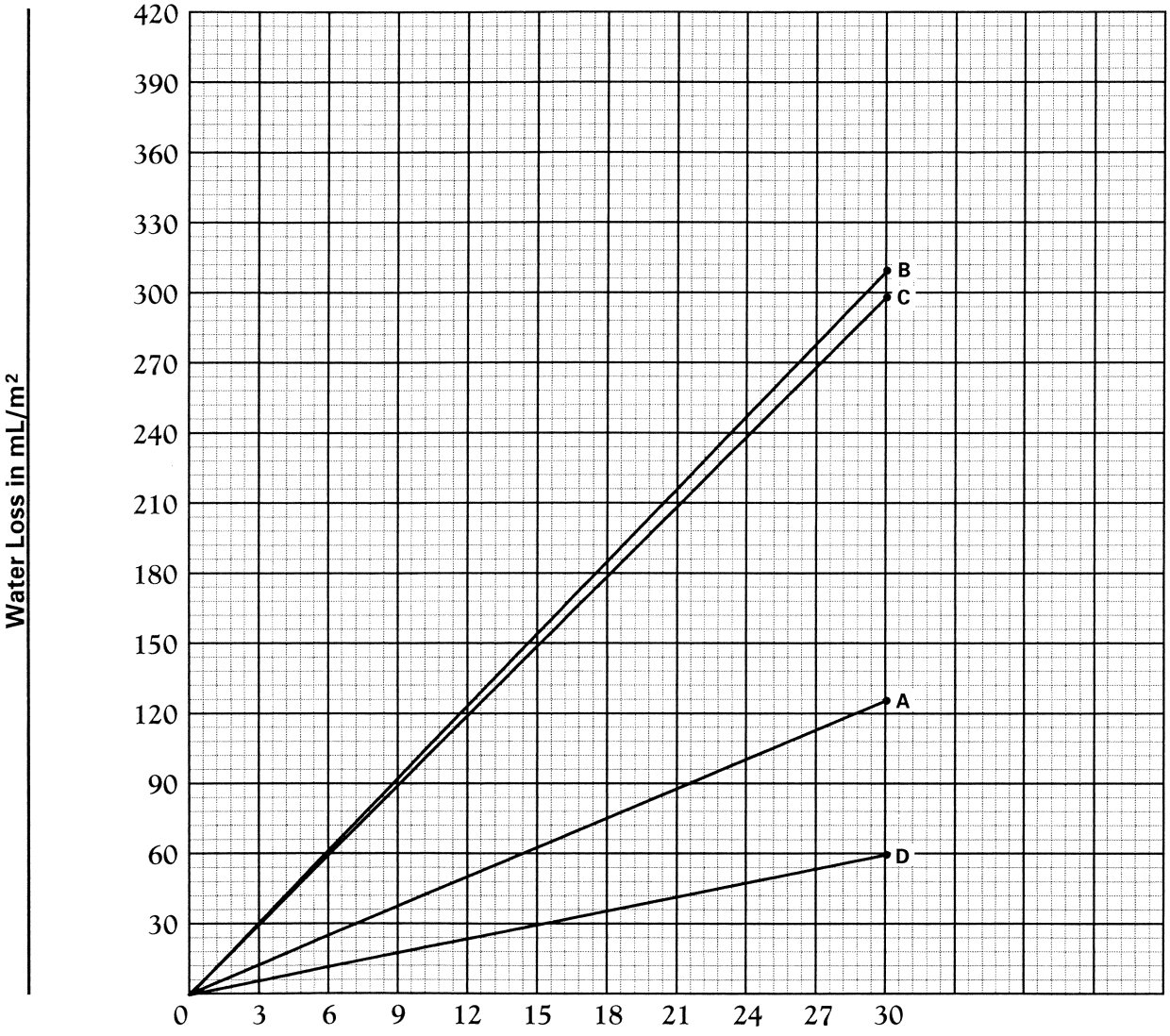
   a. The independent variable is *time*.

   b. The dependent variable is *water loss in mL/min/m^2*.

   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

Sample Graph

Title: Transpiration Water Loss Under Four Conditions



Key:

A = Normal Room Conditions

B = Heat

C = Moving Air

D = High Humidity