Chapter 9

An Introduction to Plant Vascular Systems

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Introduction

This laboratory exercise, designed in the Biology Department at Furman University, is one of two plant components for the freshman biology laboratory. Most of the techniques used in this laboratory are not new; indeed many have been presented at other ABLE meetings (Brewer, 1992; Dickey, 1995). This laboratory exercise studies one of the most important features of plants which make them suited to life on dry land: the vascular system which distributes water, nutrients and sugars throughout the plant from their disparate sources (leaves, roots, atmosphere, soil, etc.). This lab was constructed with two goals in mind. First, we wanted the students to do more than simply looking at microscope slides as they examined plants. Secondly, we wanted this laboratory exercise to have a strong investigative component paired with the use of scientific techniques, as do our other freshmen labs.

Materials

Plant Material Needed for a Laboratory of 32 Students

Part A: Microscopic anatomy of the xylem system in dicot stems

Two to four *Geraniums* with ten or more leaves provide enough material for a class of 32 students. We usually use the petioles, but stem material can also be used. We have found that herbaceous plants that develop woody stems work better than plants with very fleshy stems. For example, *Tradescantia*, *Impatiens*, and *Begonia* stems crush too easily in the microtomes.

Part B: Measuring the rate of xylem flow in clover petioles

Clover (*Trifolium* sp.) plants with 30 leaves will provide enough material for a class of 32 students. Dig up the clover from a lawn the morning before use. Place the clover in trays with water.

Part C: Response of stomata to leaf microenvironmental conditions

Two plants per group. Any small, inexpensive, ornamental houseplants without fuzzy or waxy leaves may be used. *Tradescantia*, *Coleus*, or any sort of ivy work well.

Stock Solutions Needed for a Class of 32 Students:

3% Acridine Orange: (Sigma Chemicals A-6014)

For 50-ml: Add 1.5 grams of acridine orange to 50-ml of water.

10% Acridine Orange: (Sigma Chemicals A-6014)

For 50-ml: Add 5 grams of acridine orange to 50-ml of water.

0.5% Safranin O solution (prepared in 50% ethanol) (Sigma Chemicals S-2255)

For 50-ml: Combine 25-ml of distilled water with 25-ml of 100% ethanol. Add to 0.25 grams of Safranin O.

0.5% Methyl Green (prepared in 70% ethanol) (Sigma Chemical M-8884)
For 50-ml: Combine 15-ml of distilled water with 35-ml of 100% ethanol. Add 0.25 grams of Methyl Green.
70% Ethanol:
For 100-ml: Combine 30-ml of distilled water with 70-ml of 100% ethanol.
95% Ethanol:
For 100-ml: Combine 5-ml of distilled water with 95-ml of 100% ethanol.
1:1 (v:v) Glycerol-Water
For 50-ml: Combine 25-ml of glycerol with 25-ml of distilled water.

Nut and Bolt Microtomes:
Interior diameters of the nuts should be 0.5-inch. The bolt should be 1.25-inches long.

Materials needed per group of four students for Part A:

Pasteur pipets with bulbs	5
Labeling tape	1 roll
Marking pen	1
Razor blade	1
3% Acridine Orange	1.5-ml
Nut and Bolt Microtome	1
Needle probe	1
Microscope Slides	3
Microscope Cover Slides	3
"Microbeaker"	This sounds fancy, but it is the cap of a 1.5-ml microcentrifuge tube. Do not disconnect the cap from the microcentrifuge tube.
0.5% Safranin O solution	1.5-ml
Kimwipes	1 box
70% Ethanol	2-ml
0.5% Methyl Green	1.5-ml
95% Ethanol	2-ml
White sheet of paper	1
1:1 glycerol-water solution	1-ml
Compound Microscope	1
Gloves	4 pair (1 pair per student)

Materials needed per laboratory section for Part A
~100-ml of melted paraffin (type used for histology)
Pasteur pipets with bulbs for the paraffin
Dark room or a "darkened" room
Hand-held UV light source
Dissection microscope
Optional: Fluorescent microscope

Materials needed per group of four students for Part B

Small test tube of water	1
Razor blade	1
10% acridine orange	1.5-ml

Light source	1
Kimwipes	1 box
Cellophane tape	1 roll
Ruler	1
Gloves	1 pair per student

Materials needed per laboratory section for Part B

Hand held UV light source
37 ^{o}C incubator (optional)
Light filters (optional)
Large glass beaker (optional)

Materials needed per group of four students for Part C

Plant kept in dark for 24 hours	1
Plant kept in light for 24 hours	1
Labeling tape	1 roll
Labeling pen	1
Scissors	1
Clear finger nail polish	1 bottle
Cellophane tape	1 roll
Microscope slides	5
Microscope cover slides	5
Microscope with ocular micrometer	1
Aluminum foil	4 pieces ~5" X 5" square

Series of plants held at different environmental conditions for use by students in the investigative portion of this section.

Notes for the Instructor

This laboratory exercise is divided into three parts:

PART A: Microscopic Anatomy of the Xylem System in Dicot Stems
PART B: Measuring the Rate of Xylem Flow in Clover Petioles
PART C: Response of Stomata to Leaf Microenvironmental Conditions

In Part A using a simple Nut and Bolt Microtome to section plant tissues has been described in detail by Dicky (1995). What is different about the laboratory presented here is that students compare tissues that are important in the support of the plant with tissues that are important for the transport of water in the plant. By using differential staining techniques, the students see that the supporting lignified tissues of the plant stem correspond to the water transport of the stem. We discuss the importance of maintaining structural integrity of the xylem cells during tension created by transpiration. We use acridine orange as a fluorescent transport dye to help identify the xylem. Acridine orange carries several health and safety hazards and can be substituted with any other dye that contrasts with the red safranin O stain.

In Part B, the flow rate of water through the xylem is investigated. By using a dye dissolved in the water, the students can easily determine the flow rate. Students then design their own experiments to see how changing environmental conditions alters transpiration which in turn

changes the xylem flow rate. Again acridine orange is used in this section, but other dyes may be substituted. For example, Methyl Green works well.

In Part C the students use fingernail polish to make casts of a leaf epidermis to examine the effect of microevironments on stomatal aperture. This is an adaptation of Carol Brewer's (1992) ABLE presentation in that ocular micrometers are used to measure the stomatal aperture. Students then analyze their data using the Student t Test.

All three parts of this laboratory exercise can be done in a 3-hour period if students are divided into teams and share the work. We run this laboratory with students divided into teams of four:

Two students work on Part A: Microscopic anatomy of the xylem system in dicot stems.

One student works on Part B: Measuring the rate of xylem flow in clover petioles.

One student works on Part C: Response of stomata to leaf microenvironmental conditions.

If laboratory time is limited or teamwork is not appropriate, only one or at most two of the parts should be done per laboratory session.

Student Outline

Introduction

Two of the most important developments shown by plants which make them suited to life on dry land are: (1) reproductive systems which do not depend upon standing water for gamete or population dispersal, and (2) vascular systems which distribute water, nutrients and sugars throughout the plant from their disparate sources (leaves, roots, atmosphere, soil, etc.). In today's lab you will investigate various aspects of plant vascular systems.

Organizing your lab group

At the beginning of lab, divide your group into two teams. One team will work on the microscopic anatomy of the dicot stems (Part A), while the other investigates the rate of xylem flow in clover (Part B) and stomata responses (Part C). Although the activities involved will be divided among the group members, all lab group members should view the results of each activity whenever those results are obtained during the lab period. When there is a lull in the pace of the activities assigned to you, review the procedures your other lab group members are performing, so that you will appreciate their results when you see them.

PART A: MICROSCOPIC ANATOMY OF THE XYLEM SYSTEM IN DICOT STEMS

Background: Xylem System

The xylem system of plants is that portion of the vascular system that transports water and nutrients from the roots to the leaves. The functioning of this system is dependent primarily upon the "negative pressure" generated in the xylem tube system by the evaporation of water from the upper parts of the system, including the leaves. Such water loss "pulls" water upward in the xylem system by the cohesive forces between water molecules within the xylem tubes. However, just as a flimsy drinking straw will collapse if subjected to too much negative pressure from the user, the tubes of the xylem system need structural support to keep from collapsing. This support is provided by lignin.

Lignin is, after cellulose, the most abundant organic substance in most plants. It is a highly branched polymer of phenylpropane units that can be covalently bound to cellulose. The subunits of lignin are joined together by the action of the enzyme peroxidase, forming a physically rigid, largely indigestible polymer matrix that branches in three dimensions. Lignin is found in the cell walls of various types of supporting and conducting plant tissue. Lignin is stained by Safranin O, while cellulose is stained by Methyl Green (two stains you will be using on the dicot stem).

In a dicot stem segment, you will determine using a microscope which anatomical regions of the stem in cross-section contain cell walls composed of lignin and which regions contain the tubes of the xylem system. In clover petioles, you will determine the rate of xylem flow in the petiole and devise an experiment to study influences on that rate.

Experimental Protocol:

You will produce hand-made sections (thin slices) through a dicot stem, the xylem system of which you have stained with a fluorescent dye, Acridine Orange. You will observe one of the sections under ultraviolet light to observe and document the regions of the stem stained with Acridine Orange. You will stain another section with Safranin O and Methyl Green to observe and document the regions of the stem which have walls containing lignin, as opposed to only cellulose. You will then compare the distribution of the xylem system and the distribution of lignified cell walls.

Read the entire procedure (steps 1-16) carefully before you begin. Divide the steps among your group. Be prepared for steps which are timed and must be done immediately following one another. With label tape, mark five Pasteur pipets: "ethanol solutions," "Safranin O," "Methyl Green," "water," and "glycerol-water."

Staining the stem's xylem system with the fluorescent dye:

1. Obtain a fresh 1-cm length of a dicot stem from the instructor. Be careful to note and remember which end is the base of the stem. Carefully cut the base of the stem at a 45^{o} angle with a razor blade. Place the stem in a tube containing a small amount (only about 2-3 mm depth) of 3% Acridine Orange. Let the stem sit in the tube for about ten minutes. During this time, the dye-containing solution will be transported up the xylem system of the stem.

Preparing sections of the stem:

2. Loosen the nut from the "nut and bolt microtome" so it is barely secured to the end of the bolt. This makes the "well" formed by the interior of the nut as deep as possible. Stand the bolt up so that its head is on the table. Remove the stem from the Acridine Orange. Add a small amount of melted wax to the bottom of the "well" inside the nut and immediately stand the stem piece up in the melted wax. When the stem can stand up on its own, add more melted wax to fill the well completely and to form a mound of melted wax around the base of the protruding stem. Let the wax harden completely (about ten minutes).
3. Hold the head of the bolt flat on the table with one hand. Hold the razor blade with the other hand (Figure 9.1). With the razor blade, carefully shave off the mound of wax and stem from above the top of the nut. Run the blade over the nut's surface to make the wax completely smooth.
4. Turn the bolt clockwise about 1/12th of a turn (see Figure 9.2) to make the wax cylinder protrude slightly. Again holding the microtome and blade as shown in Figure 9.1, slice the blade over the nut's surface to make a shaving of the wax surface through the stem. A smooth, quick slicing motion works best. It is not necessary to cut through the entire wax block to cut the stem.

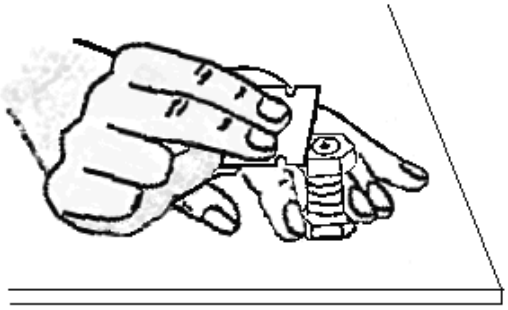

Figure 9.1. Correct hand position for use of the Nut-and-Bolt Microtome

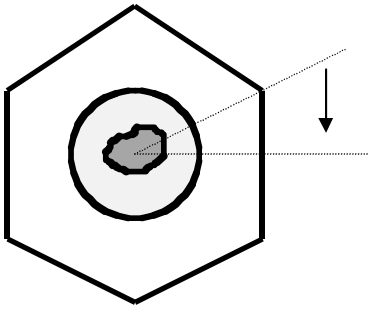

Figure 9.2. Demonstration of a $1/12^{th}$ turn on the Nut-and-Bolt Microtome

5. With the needle probe, gently remove the stem section from the wax shaving. If the section is thin and not torn, give it to your lab partner who will immediately proceed with the fluorescent viewing of the stem section (steps 6-8). (An otherwise good section that is too thick for staining is quite suitable for fluorescent viewing.) Cut the wax block smooth with the nut's surface again and return to step 3 to cut a section you will stain for lignin and cellulose (steps 9-16). This section needs to be as thin as possible. If you leave some stem remaining in the wax block of the microtome for a while, place a drop of water on it to keep it moist in case you want to cut more sections later. If you make so many unsuccessful shavings that the wax block becomes too loose in the ever-shallowing well of the nut, you will need to remove the wax from the well and start again with another piece of stem at step 1.

Observing the fluorescently stained xylem system:

6. With the needle probe, carefully transfer the stem section to a clean microscope slide. Be careful not to let the section fall off the slide when your transport it.
7. Observe the slide under long-wave ultraviolet illumination with the high power of the dissection microscope. (Have a "dark room" near by.) The Acridine Orange will fluoresce a yellow or orange color. Be sure to "go through focus" to focus clearly on the cell walls exposed on the cut surface of the section. Any pale green fluorescence is the natural fluorescence of the plant tissues and should be disregarded.
8. Which area(s) of the stem cross section have fluorescent cell walls? Label these areas on the drawings of stem cross sections. Remember that not all of the xylem tubes remained opened at the 45^{o} cut at the stem's base, so not all of the xylem tubes will have transported the Acridine Orange solution and thus have walls which are stained. Save the slide so the other members of your lab group can study it also.

Staining a section to observe cellulose and lignified cell walls:

9. Gently transfer a section into the "microbeaker" with the needle probe and immediately cover the section with Safranin solution using a Pasteur pipette. Let the section sit in this solution for 3 minutes.
10. Remove the Safranin solution by "blotting" it out of the microbeaker with the twisted corner of a Kimwipe. Immediately add 70% ethanol. Let the section sit in 70% ethanol for 2 minutes, replacing the ethanol solution in the microbeaker with fresh 70% ethanol 2-3 times during this 2 minutes. This step removes excess Safranin from the section.

11. Remove the 70% ethanol by blotting and immediately add the Methyl Green solution. Let the section sit in this solution for 1 minute.
12. Remove the Methyl Green solution by blotting. Immediately add 95% ethanol. Let the section sit in 95% ethanol for 2 minutes, while replacing the ethanol solution with fresh 95% ethanol 2-3 times during this 2 minutes. This step removes the excess of both stains. During this procedure, have the microbeaker on a white sheet of paper to observe the appearance of the section. At the end of this 2 minutes, the section should be translucent and blue or purple. A dark section is one which has been cut too thick, and a translucent but red one has not yet been rinsed long enough in the 95% ethanol. However, do not keep the section in 95% ethanol for too long, or too much of the stains will be removed from the section.
13. Remove the 95% ethanol with blotting. Gently transfer the section to a clean microscope slide. Blot off any excess ethanol solution. Immediately add a drop of 1:1 glycerol-water. Gently cover with a cover slip, but do not apply pressure on the cover slip since the section is easily crushed. Add additional glycerol-water to the edges of the coverslip, if needed.
14. Look at the slide under low, medium, and high powers of your microscope. Cell walls composed mostly of cellulose will appear thin and blue or blue-green (stained with Methyl Green), while those containing lignin will appear thicker and red or orange-red (stained with Safranin). Be sure to "go through focus" to visualize the cell walls best.
15. Which area(s) of the stem cross section have cell walls containing lignin? Save the slide so the other members of your lab group can study it also.
16. Do the areas of fluorescent staining and lignin staining of the stem cross section exactly match? Is one a subset of the other? What might be the purpose of the lignified cell walls in areas of the stem that are clearly not associated with the xylem system?

PART B: MEASURING THE RATE OF XYLEM FLOW IN CLOVER PETIOLES

In this study you will measure the rate of xylem flow in the clover petiole. To do this, you will measure the distance up the petiole a solution of the fluorescent dye Acridine Orange reaches in a given period of time (steps 17-19). Then, you will devise an experiment to determine the effect of some environmental factor on that rate (step 20).

Measuring the rate of xylem flow:

17. Obtain a clover leaf with the complete length of a single petiole with its associated leaves attached. Keep the petiole's base in a tube of water until it is used. Make a fresh cut at the base of the petiole. Place the stem in the tube of 10% Acridine Orange solution. Put it under the illumination of your bench lamp, and note the time. Allow exactly 2 minutes to elapse.
18. Without delay, take the petiole out of the solution, blot any remaining dye from its cut end. Quickly cut off the leaves, and press the petiole onto the sticky side of a piece of cellophane tape. At 5-mm intervals, make a cut through the petiole with a razor blade, but leave enough of the tape intact to hold the cut pieces together. Next, transfer the pieces to another strip of tape, but line them up right next to each other (like the pipes of a pipe organ) so that the cut ends can be easily compared (be sure to orient them 'top up'). Refer to figure 9.3.

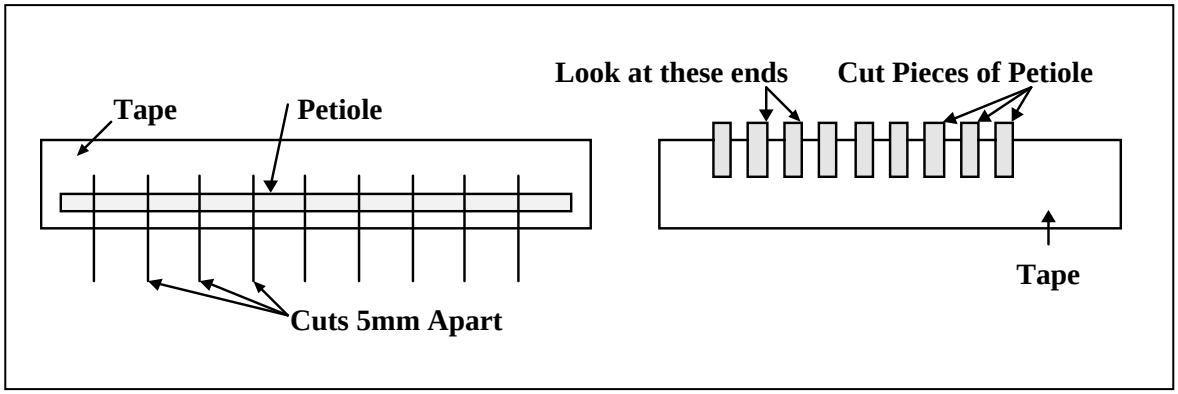

Figure 9.3. Cutting the petiole (left) and aligning the pieces for viewing (right).

19. Take the tape to the long-wave ultraviolet cabinet and under UV illumination, observe the cut surfaces of the petiole for a reddish or orange fluorescence, indicating the presence of Acridine Orange. Any greenish fluorescence is the natural fluorescence of the plant tissues, and should be disregarded. Find the first segment that lacks Acridine Orange fluorescence at its top. The dye reached somewhere within this segment. Estimate the rate of xylem flow in mm/minute by multiplying the number of fully labeled segments by five, adding 2.5, and dividing by 2. Thus, if the dye had stopped somewhere in the seventh segment, the rate would be about [(5 X 6 mm) + 2.5 mm]/2 minute = 16.25 mm/minute. (The value of 2 is the number of minutes allowed for the dye to travel. If you modify this, you must change the value to correspond to your change in method.) Show this petiole preparation to others in your group.

Devising an experiment to measure the effect of an environmental factor on the rate of xylem flow:

20. Test one of the following influences to see if it alters the xylem flow rate. Consult with your instructor before carrying out the experiment. Write your plan and results in the "Part B: experimental" section.

    a. Effect of Temperature: Perform the flow rate determination on a petiole kept in a 37^{o}C incubator during the Acridine Orange labeling with the same light intensity as the petiole in step 17. Compare this to the rate you determined in step 19.
    b. Effect of removing leaves: Remove the leaves from a petiole and then determine its xylem flow rate. Compare this to the rate you determined in step 19.
    c. Forward vs. reverse flow rates: Cut a petiole near its top, and then cut it in half. Place the bottom end of the top half in the dye and determine its xylem flow rate ("forward flow"), while at the same time placing the top end of the bottom half in the dye and determining its rate ("reverse flow").
    d. Flow rate at different petiole levels: Cut off the bottom half of a petiole, and determine the flow rate in the top half (with the leaves still attached). Compare this to the value you determined in step 19.
    e. Effect of light exposure: Determine the rate in a petiole kept in the dark during the labeling and compare it to one you determined in step 19.
    f. Effect of relative humidity: Determine the flow rate in a petiole kept under a large glass beaker along with a large wad of water-soaked filter paper to saturate the beaker with water vapor. Compare this to the value you determined in step 19.

PART C: RESPONSE OF STOMATA TO LEAF MICROENVIRONMENTAL CONDITIONS

Background

Gas exchange (uptake of carbon dioxide and release of oxygen and water vapor) in plants occurs through microscopic openings in the surface of a leaf called stomata (Figure 9.4). As the stomata open and close they regulate the rate of water loss and carbon dioxide uptake. When stomata open, the plant is able to take up carbon dioxide needed for the light independent stage of photosynthesis. At the same time both water vapor and oxygen can leave the plant leaf. This loss of water vapor from the plant leaf is called transpiration. It is the driving force for movement of water through the xylem. When stomata close, gas exchange is greatly reduced so that the movement of water vapor and carbon dioxide into and out of the leaf is halted. Ultimately the rate of photosynthesis and the rate of water movement in the plant will also be affected.

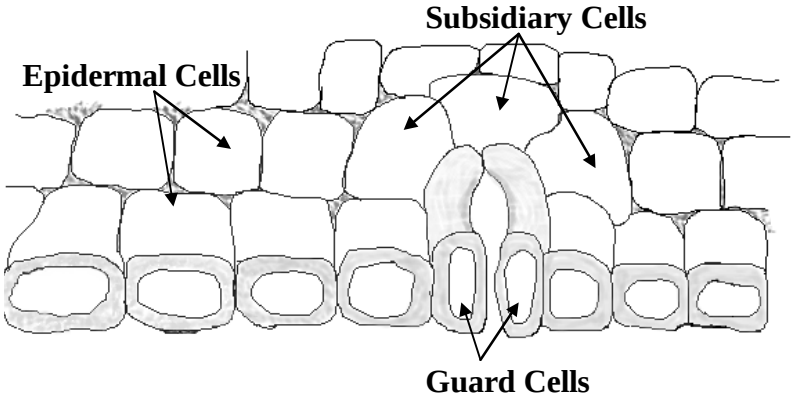

Figure 9.4. Plant leaf epidermis.

The degree to which a stoma is open or closed is a reflection of the environmental stresses acting on the leaf. The plant leaf can respond to the need for carbon dioxide during photosynthesis and thus respond by opening stomata, or the plant leaf can respond to the need to conserve water by closing the stomata. The plant leaf is always in a delicate balance between opening and closing the stomata. Measuring the degree of stomatal opening provides a visual indication of stomatal response to environmental conditions. The degree of stomatal opening has a large influence on the rate of gas exchange. Although the stomata are microscopic, the great number of them in the leaf surface can determine the entire leaf's response to ambient environmental conditions.

To study stomatal activity, you will first examine leaves from plants that have been kept in the light or dark. This will be followed by alternative experiments that each lab group will design on their own. You will evaluate how stomata respond to these different conditions by using casts of leaves viewed in a microscope to observe stomatal response under the different conditions.

Experimental Protocol:

Determining which leaf surface has stomata.

21. The plants made available to you for this first activity will have been watered thoroughly. Half of the plants were placed in darkness 24 hours ago. The "darkened" plants should be kept in darkness until they are needed.

22. Select a plant that has been kept in the light and label the container of the plant "LIGHT". Clip two leaves from this plant. Prepare casts of the leaves surfaces by painting the top surface of one leaf and the bottom surface of the other leaf with clear finger nail polish. Allow the finger nail polish to dry (about 10 minutes).
23. While the nail polish is drying, label microscope slides as either "top" or "bottom."
24. Cut a piece of Scotch tape approximately 1.5 cm long. Fold the tape over on itself leaving 0.5 cm of sticky surface exposed (step 1 of Figure 9.5). Place the sticky tab of the tape at an edge of the leaf so that it sticks to the nail polish cast (step 2). Use the remaining tape as a handle to pull the nail polish cast from the leaf surface (step 3) carefully. Place the cast on the appropriately labeled slide (step 4). Place a coverslip over the cast. Repeat this step for the remaining leaf.

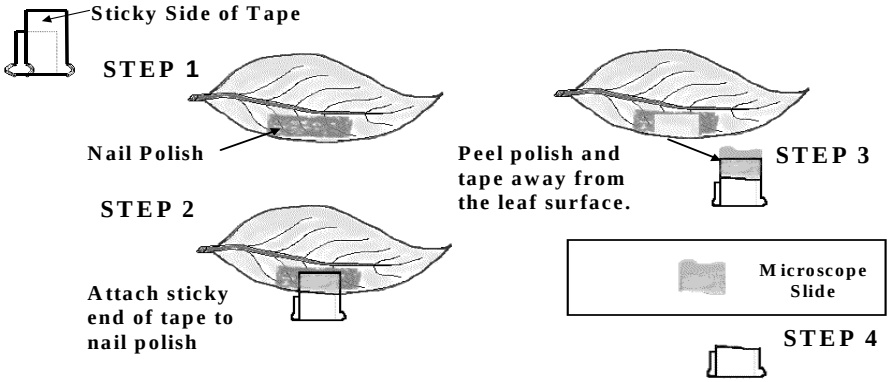

Figure 9.5. Procedure for stomatal casts in nail polish.

25. Examine the slides under high power to determine which leaf surface has stomata. Carefully survey the entire leaf cast. The leaf surface with stomata should look similar to one of the illustrations in Figure 9.6. For future observations, it will only be necessary to make nail polish casts from the leaf surface with stomata.

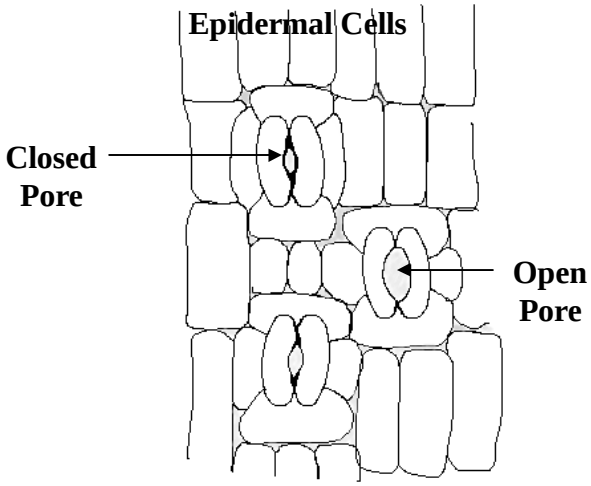

Figure 9.6. Epidermal cells with open and closed stomata.

Stomatal response to light exposure

26. Select a plant that has been kept in the dark for 24 hours. Label the container of this plant "DARK." While the plant is still in the dark, remove a leaf and paint the appropriate surface with nail polish. This leaf will be used to establish the initial conditions of the stomata, before any manipulation of the plant (the zero time point in the "Light exposure" section below).
27. Cut four pieces of aluminum foil so that they will each be large enough to entirely cover both sides of one leaf. Gently fold one piece of the foil over a leaf of your "DARK" plant. Tape the edges of the foil together so that no light can reach the leaf surface. Repeat this step for two additional leaves on this plant.
28. Place the plant under (or in front of) the light. Record the time that the plant was placed in the light. Monitor the temperature next to the uncovered leaves. DO NOT LET THE TEMPERATURE OF THE PLANT RISE ABOVE 30^{o}C.
29. At the time points indicated below, remove a covered leaf and uncovered leaf from the plant. Immediately paint the appropriate surface of the two leaves with nail polish. Do not let the newly uncovered leaf be exposed to light any more than is necessary before adding the polish. Let the polish dry.
30. Prepare microscope slides as before. Be sure to label each slide with the time and the treatment as slides are made. For the easiest comparison, place both casts from each time point side by side on a separate slide.
31. View each slide under the microscope. Using the ocular micrometer, measure the size of the stomatal opening for eight randomly selected stomata from each cast and record the data below.
32. At what time point was the average stomatal aperture(s) different for leaves covered with foil compared to those not covered by foil? Statistically support your conclusion by applying the Student's t test to the two groups of values at the time point you chose. Refer to "The Researcher's Toolkit" at the back of this lab. What do your results imply?

Additional Experiments:

Now that you have seen and measured the influence of one factor (light exposure) on stomatal aperture, we want you to design an experiment of your own that would test the effect another biologically significant factor may have on stomatal aperture. On the front table of the lab will be found a number of different plants and apparatus that you can use for your experiment. These will include, but not be limited to the following: well watered plants, plants that have been desiccated for 48 hr, different species of plants, plants held at different temperatures for 48 hr, and a variety of lights and filters. Check with your instructor for further details on what is available. As you design your experiment, keep the following points in mind. First, form your hypotheses. Try to ensure that you are testing something of biological relevance. Make sure that you can actually test the effect with the available supplies and in the time allotted. What do you need to use as a control? What methods do you need to use to answer your hypotheses? How will you analyze the data and draw conclusions from it? These are just a few of the things you should be considering.

Completely describe and report on the results of your experiment. Put into practice all you have learned from previous labs about making testable hypotheses, good tables, and good graphs and drawings (if needed). Consider the following questions as well.

Compare your results with the hypotheses you originally proposed. How do your results compare with what you predicted would happen?

If your results are different from what you expected, suggest some possible reasons for the differences.

What are some other environmental stimuli that would affect stomatal opening and closing? How would each of these conditions affect stomatal response?

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Appendix A

Comparing the Means of Two Groups Using the Student's t Test

Whenever you gather measured values from two groups of experimental subjects or observations, their means (averages) are likely to be different. To a researcher, the important question is whether the difference is over and above the minor differences in means you would expect simply from experimental error and biological variation alone. To help arrive at a conclusion, the researcher can employ the Student's t test, which analyzes the difference between two means in light of the error associated with each of those two means.

An error associated with the mean of a group of values is called the standard error of the mean, or SEM for short. It is an estimate of the "reliability" of the mean, that is how close it is likely to be to the "true" mean of all values from all the subjects you could possibly have included in your group. The more values in your group, the more reliable your mean is likely to be, and hence smaller the SEM. In addition, the less experimental or biological variability there is in your measurements, the smaller the SEM. The formula for SEM includes both of these factors:

$$SEM = \sqrt{\frac{\Sigma\,(\bar{x} - x)^2}{n\,(n-1)}}$$

n = group size

x = a value

$\bar{x}$ = group mean

The numerator of the fraction compiles the variability of the values from the mean ("intrinsic variability"), while the denominator includes the groups size effect on the SEM.

To employ the Student's t test, you must first calculate the mean and SEM for each of the two groups of values you are comparing. For example, suppose you measured the body mass of five male students, and the values were: 70, 90, 50, 55, and 75 kg. The mean would be 68 kg. The first value (70) differs from the mean by 2, and the square of that difference is 4. The second value (90) differs from the mean by 22, and the square of that difference is 484. This is done for each value in the group, and all these squares are added together. This yields a sum of 1030. In this case, n(n-1) is 5(4) or 20. The SEM is thus the square root of (1030/20), or 7.2 kg. When reporting such values, it is customary to write them as "68 ± 7.7 kg (SEM) (n=5)."

Suppose you then measured the body mass of six female students and found the mean to be 46 kg, with a SEM of 5.3. Of course, the means are different, (68 vs 49), but perhaps that difference is simply due to "sampling error", that is you just happened to pull five heavy males and five light females from the campus, whereas in fact there is no difference between the means of all males and all females on campus. This is where the Student's t test can help.

Calculate the t value by the following formula:

$$t = \frac{\left| \overline{x}_a - \overline{x}_b \right|}{\sqrt{SEM_a^2 + SEM_b^2}}$$

Subscripts a & b refer to the two groups

In the case of our male-female comparison, the t would be:

$$\frac{\left| (68-46) \right|}{\sqrt{7.2^2 + 5.3^2}}$$

or 2.46

The larger the t, the more likely it is that the difference between the two means is "statistically significant." This t is then compared to tabulated "critical t values." Some of these values are given below.

Sum of both group sizes:	7	8	9	10	11	12	13	14	15	20
critical t:	2.57	2.45	2.37	2.31	2.26	2.23	2.20	2.18	2.16	2.10

Thus, in our case the critical t is thus 2.26, since we analyzed 11 total students. If the calculated t is greater than or equal to the critical t, then the probability that the difference between the two means is due to chance sampling error alone is less than 5%. In our case, we can thus conclude (with this level of certainty) that the means of the body weights of males and female students are indeed significantly different.

There are a couple things to keep in mind about the Student's t test.

Only two groups can be compared. It is not valid to compare numerous groups to each other by repeating the Student's t test for each comparison. Each group can be included in only one Student's t test. For example, if you were comparing the body weights of freshmen, sophomore, junior, and senior men (i.e., four groups), you could not use Student's t tests to see which groups were significantly different from which other groups. That would require six separate Student's t test comparisons, using each group's data in more than one t test. You could, however, compare freshmen with sophomores with one test, and then juniors and seniors with a separate test, and stop there.

Also, if the calculated t is less than the critical t, that does not mean that there is in fact no difference between the means. It only signifies that your experiment, with your measurements, failed to show any signiticant difference. A further study using more subjects or more precise measurements may indeed find a significant difference.

Appendix B - Guidelines for Student Notebooks

Part A: Microscopic Anatomy of the Xylem System in Dicot Stems

Illustrate the staining pattern for transported acridine orange and the staining pattern for lignin in the cross section of the plant stem/petiole
Compare the stain patterns.

Part B: Measuring the Rate of Xylem Flow in Clover Petioles

Control
Calculations for the xylem flow rate in the clover plants

Experimental:
Hypothesis for the influence of some factor on the xylem flow rate:
Calculations:
Revised hypothesis in light of actual results:

Part C: Response of Stomata to Leaf Micronenvironmental Conditions

Initial observations of stomata on leaf surface:
Side(s) of the leaf has (have) stomatal pores:
Open or closed state of pores:
Sketch of the epidermal surface with stomata, guard cells, and epidermal cells labeled.