Chapter 3

Responses by Stomata on Leaves to Microenvironmental Conditions

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Introduction

Stomatal aperture provides much information about how leaves sense and respond to environmental conditions. Recent evidence suggests many species have nonuniform stomatal opening under both laboratory and field conditions, for example, different areas of the same leaf seem capable of responding independently to microenvironmental conditions (Spence, 1987). This exercise can be conducted in conjunction with discussions of photosynthesis and transpiration, as well as implications of microenvironmental conditions to gas exchange. Introductions to stomatal physiology and guard cell activity can be found in Nonami et al. (1990), Salisbury and Ross (1985), Wu and Sharpe (1979), Zeiger et al. (1987), and Zeiger (1990).

The purpose of this exercise is to demonstrate how stomata respond to microenvironmental conditions by making casts of leaf surfaces and using them to evaluate stomatal opening and closing. This exercise takes approximately 1 day to set up and can be completed in one 3-hour laboratory period. Leaf surface casts also can be made during a field trip for viewing at a later time.

Objectives

1. To learn about stomatal distribution and anatomy on adaxial and abaxial leaf surfaces.
2. To generate and test hypotheses concerning stomatal responses of leaves subjected to different microhabitat conditions.
3. To interpret data on stomatal apertures and suggest explanations for observed patterns.
4. To learn a technique for convenient microscopic study of leaf surfaces.

Notes for the Instructor

Environmental Conditions Affecting Stomatal Aperture

A number of environmental conditions will affect stomatal aperture (Zeiger et al., 1987). These conditions can be manipulated to either open or close stomates. Bright light, leaf temperature less than 30°C, low wind speeds, and wet soil all lead to stomatal opening. Sudden and prolonged darkness, leaf temperatures above 30°C, high wind speeds, and dry soil nearly always ensure stomatal closure.

Problems and Advantages

A variety of surface replica techniques are described in the literature (e.g., Neill et al., 1990; Weyers and Johansen, 1985; Weyers and Travis, 1981). Use of surface replicas to interpret stomatal activity has several inherent problems. Organic solvents in nail polish affect stomatal movements. Interpretation is affected by the thickness of application because thick replicas may distort the image of the stomatal aperture. Large stomatal apertures will be easier to measure than narrow ones and this may also bias interpretation. It is important that nail polish only be applied to dry leaves or the replica will be cloudy and may not dry properly.

However, a surface replica technique has many advantages for teaching about stomates. This technique is fast and inexpensive. It is very good for qualitative assessment of leaf surface features and it can be used effectively both in the laboratory and the field. Furthermore, replicas can be made ahead of time and interpreted later when a microscope is available.

Preparing Plants for Light Experiments

Each team of students will need two plants. If plant materials are grown in the campus greenhouse, cuttings must be started far enough in advance so that they will have adjusted to transplanting at least 1 week prior to use.

Two groups of plants must be pre-treated prior to use by students. Thoroughly water all plants. Place half of the plants needed in a dark closet for at least 24 hours prior to use. Place the remaining plants in bright light.

It is very important that plants to be used in the “light treatments” have been exposed to bright light for at least 4 hours before use. When plants are exposed to sudden darkness (e.g., lights off in a classroom over a lunch period or overnight), most stomates will close. For maximum success, ensure that lights in the classroom will not be turned off during the day when these activities are planned.

Select plants for investigation that have large, easily-viewable stomata. The plants should have at least 15 leaves because several leaves may be needed to practice the technique before reliable results are achieved. Common ivy, Swedish ivy, wandering Jew, inch plants, and *Zebrina* are all suitable for use. Do not use plants that have dry soil or have been subjected to stressful conditions unless you wish to demonstrate conditions causing stomatal closure. Normal stomatal function may be impaired in stressed plants.

Materials

The following materials are recommended for each group of two students: one "dark treated" and one "light treated" plant, one bottle of clear nail polish, microscope slides and cover slips, forceps, scalpel, thermometer, lamp, permanent marker (e.g., Sharpie), aluminum foil, black electrical tape, Scotch tape, and a microscope with an ocular micrometer.

Extensions of this Exercise

Making surface casts is easily accomplished in the field as well as the laboratory. To relate ecology and physiology in a field setting the following activities could be pursued using the techniques outlined in this chapter.

1. Compare the same species or several species in two or more different habitats.
2. Compare sun and shade leaves on the same species to evaluate the effect of canopy position on stomatal response and stomatal density.
3. Compare stomatal response on well-watered plants with plants allowed to dry out. Then monitor the time required for stomata to respond to watering.
4. Monitor the effect of temperature on stomatal opening. Well-watered and dry plants would be an interesting comparison.

Student Outline

Introduction

A great deal has been written on the opening and closure of the stomata produced by light and darkness, but much remains to be done. (Francis Darwin, 1898)

Nearly 100 years ago, Francis Darwin showed that stomata on leaves respond to environmental stimuli. While we have a much better idea of the mechanism of stomatal opening and closing as well as information on the responses of stomata to certain environmental conditions (e.g., Zeiger et al. 1987), there are still mysteries surrounding stomatal response to environmental conditions to unravel.

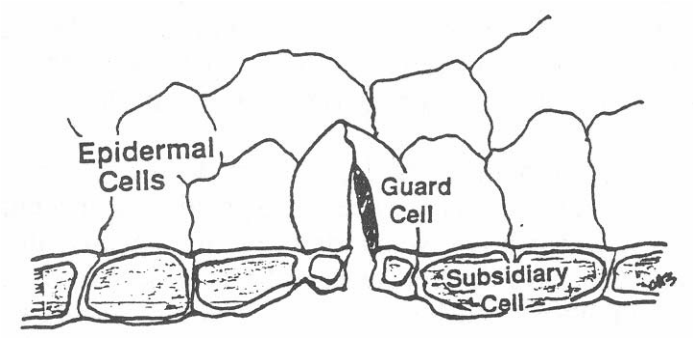

Figure 3.1. Stomatal apparatus including guard cells and subsidiary cells on the lower epidermis of a leaf.

Stomata are small pores in the surface of a leaf (Figure 3.1). The fundamental function of stomata is to open and close so that the rates of water loss and carbon dioxide uptake are regulated. Stomata impose a resistance to the diffusion of water vapor and carbon dioxide (Figure 3.2). When stomata are closed, the resistance to gas exchange is infinitely great. In other words, stomata provide an effective barricade to the movement of water vapor and carbon dioxide into and out of the leaf. When stomata are open, gas exchange of both water vapor and carbon dioxide proceed.

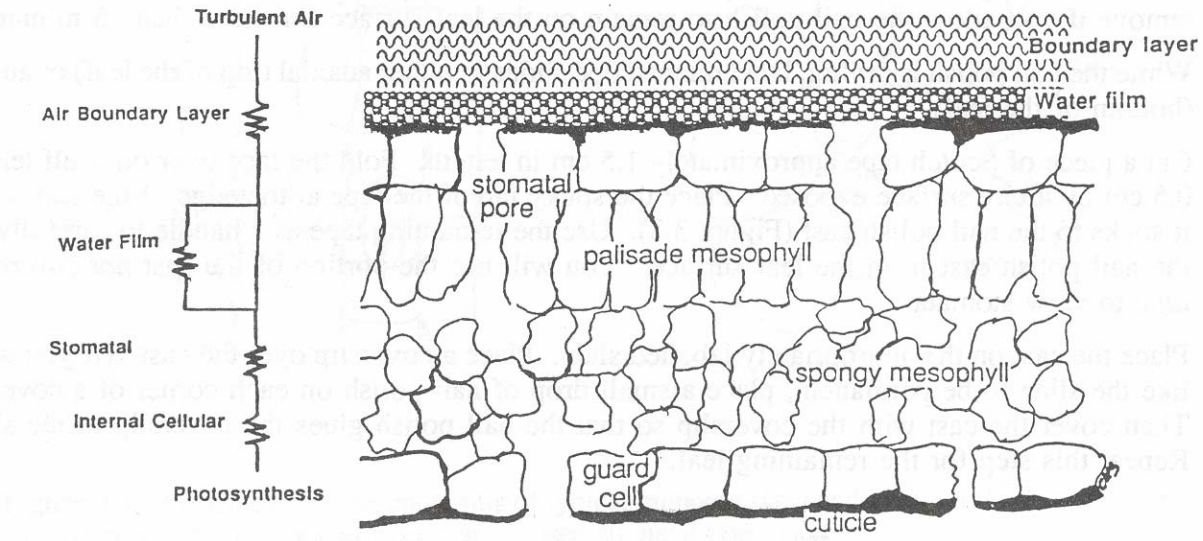

Figure 3.2. Leaf cross-section showing major resistances to carbon dioxide uptake and water loss.

Changes in the degree of stomatal opening reflect the cumulative effect of many physiological responses by a leaf to its environment. Measurements of the degree of stomatal opening on a leaf surface provide a convenient visual indication of stomatal response to environmental conditions. The dimensions of stomatal pores have a big effect on the rate of gas exchange. The rate of gas exchange for the entire leaf is determined by the responses of all the stomatal pores on a leaf to ambient environmental conditions.

Many researchers have noticed that stomatal response to seemingly identical treatments can vary considerably. Stomata, then, seem to function as separate entities which respond individually to the same environmental stimuli. The ecological implications of this "patchy stomatal response" are the focus of a great deal of current research. Knowledge of stomatal response increases our understanding of carbon dioxide assimilation and transpiration rates, as well as the nature of ecophysiological adaptations of plants to their environments.

To study stomatal activity, leaves will be subjected to light and dark treatments. We will evaluate how stomates respond to these different conditions by preparing casts of leaf surfaces for microscopic evaluation.

Based on the information provided above, state hypotheses describing the effect you expect the conditions listed below to have on stomatal aperture:

1. Light-treated leaves:
2. Dark-treated leaves:
3. Will the entire leaf surface respond in the same way to environmental conditions?

Procedure 1: Determining Which Leaf Surface Has Stomata

1. 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 (adaxial) of one leaf and the bottom surface (abaxial) of the other leaf with clear fingernail polish. It is important that nail polish only be applied to dry leaves or the replica will be cloudy and may not dry properly.

   • Allow the finger nail polish to dry for about 10 minutes. *Note:* Casts will be very difficult to remove if you allow the nail polish to remain on the leaf surface for more than 15 minutes.

2. While the nail polish is drying, label microscope slides as either adaxial (top of the leaf) or abaxial (bottom of the leaf).

3. Cut a piece of Scotch tape approximately 1.5 cm in length. Fold the tape over on itself leaving 0.5 cm of sticky surface exposed. Place the sticky tab of the tape at the edge of the leaf so that it sticks to the nail polish cast (Figure 3.3). Use the remaining tape as a handle to carefully pull the nail polish cast from the leaf surface. You will use the portion of the cast *not covered by tape* to view stomata.

4. Place the cast on the appropriately labelled slide. Place a coverslip over the cast. (If you would like the slide to be permanent, place a small drop of nail polish on each corner of a coverslip. Then cover the cast with the coverslip so that the nail polish glues the coverslip to the slide.) Repeat this step for the remaining leaf.

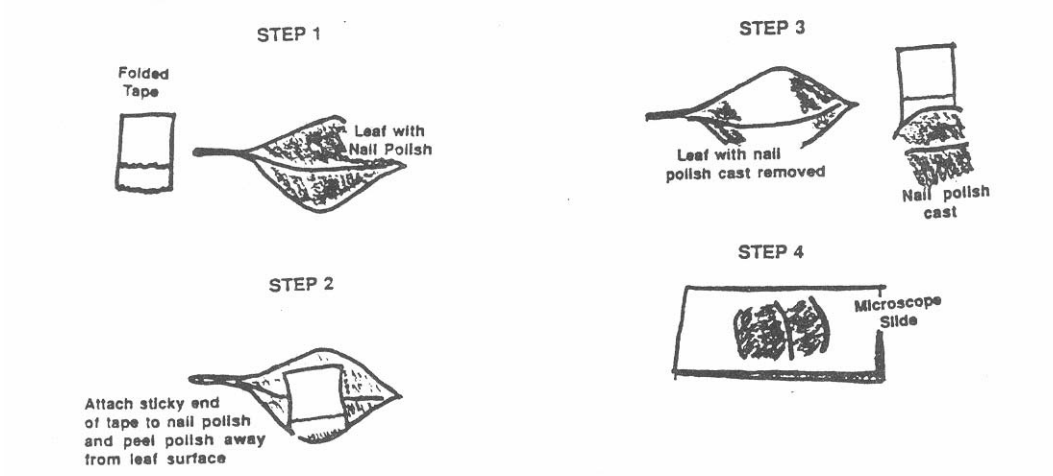

Figure 3.3. Procedure for lifting a surface cast from a leaf surface.

5. 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 3.4. For future observations, it will only be necessary to make nail polish casts from the leaf surface with stomata.

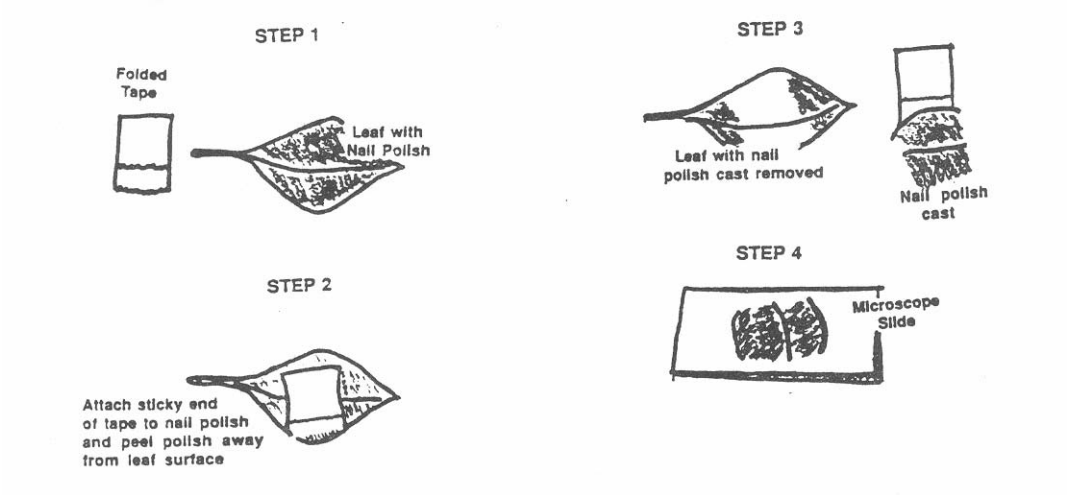

Figure 3.4. Appearance of the epidermis of typical monocot (*left*) and dicot (*right*) leaves. Notice the difference in appearance of open and closed stomatal pores.

Questions

1. Which surface(s) of the leaf has (have) stomatal pores?
2. Were the pores open or closed?
3. Sketch the epidermal surface and include stomata, guard cells, and epidermal cells. Be sure to label your diagram.

Procedure 2:
Stomatal Response to Micro-environmental Conditions—Making Leaf Surface Casts

1. Before changing any conditions, remove a leaf and paint the appropriate surface with nail polish. Put this leaf aside temporarily so that the nail polish can dry. This leaf will be used to document initial stomatal conditions for comparison with stomatal responses to different treatments. While this leaf is drying, complete step 2.
2. Cut six (6) 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. Tape the edges of the foil together so that no light can reach the leaf surface.
3. Repeat steps 1 and 2 above for a plant that has been kept in the dark for 24 hours. Label the container with this plant "DARK."
4. Place both plants under (or in front of) the light. Record the time that the plants were placed in the light on the data sheet (Table 3.1). Monitor the temperature next to the uncovered leaves by placing a thermometer on the leaf surface. *Do not let the temperature of the plant rise above 30°C.*

5. Every 15 minutes for 90 minutes, remove one covered leaf and one uncovered leaf from each plant. Immediately paint the appropriate surface with nail polish. Let the nail polish dry, then remove the cast.
6. 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 the "LIGHT" plant side by side on one slide; place both casts from leaves off the "DARK" plant side by side on a second slide. During the 15 minute intervals between removal of leaves, you should have time to prepare slides for the casts you have just made.

Table 3.1. Stomatal data sheet.

Record measurements of stomatal aperture or the number of open and closed stomates in the appropriate columns below. Then use the recorded data to compute averages for each treatment and time period.

Time	Stomatal aperture (or number of open and closed stomates)			
	Plant in dark for 24 hours		Plant in light continuously	
	Uncovered leaf	Covered leaf	Uncovered leaf	Covered leaf
T_0		–		–
T_{15}				
T_{30}				
T_{45}				
T_{60}				
T_{85}				
T_{90}				

Procedure 3: Evaluation of Leaf Surface Casts

1. View each slide under high power. If ocular micrometers are available, measure the size of the stomatal opening for 10 randomly-selected stomates. If micrometers are not available, count the number of opened and closed stomates in 10 randomly-selected fields.
2. Refer to Figure 3.4 to review the appearance of opened and closed stomata. Record your measurements or counts in Table 3.1.
3. Compute the average stomatal aperture or number of opened and closed stomata for each time period. Record the averages in Table 3.1.

Questions

1. Initially (at T_0), was stomatal opening different between plants kept in the dark for 24 hour versus those which were left in the light?
2. When was the average stomatal aperture (or number of stomata open versus closed) different for leaves covered with foil when plants kept in the dark for 24 hour are compared with plants kept in the light? Explain your results.
3. Was the average stomatal aperture (or number of stomata open versus closed) different for leaves left uncovered when plants kept in the dark for 24 hour are compared with plants kept in the light? Explain your results.
4. Compare your results with the hypotheses you originally proposed. How do your results compare with what you expected to happen?
6. If your results are different from what you expected, suggest some possible reasons for the differences.
7. What are some other environmental stimuli that would affect stomatal opening and closing? How would each of these conditions affect stomatal response?

Acknowledgments

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