PROJECT A: FACTORS WHICH MIGHT AFFECT STOMATAL OPENING (LIGHT)

How light affects the stomatal opening in a leaf

Abstract

My aim of this investigation was to check the affect of environmental factors affecting stomatal opening. My experiment was designed to check the stomata opening in light. Plants move in ways that may not seem obvious. The opening and closing of stomata is one example of this movement.

There are a large amount of growth conditions that can affect a plant. One of the most important of these conditions concerns the type of availability of light present for photosynthesis. By controlling the type of light that a plant receives, its growth can be affected. I chose to measure this growth by observing the number of stomata present on the underside of leaves exposed to the dark and to sunlight. Based on the idea that there are more open stomata present on leaves exposed to the sun, my hypothesis that 'Factors which might affect stomatal opening' (Light) there will be more stomata on the plants exposed to the light.

Hypothesis

I believe the results of my investigations will show that the more the light source the more the stomata will open.

Plan

My hypothesis is to determine factors which might affect the stomatal opening in leaves. A practical experiment can easily be set up to determine these factors. The following procedure should be followed:

Select a plant that has been kept in the light and label the container e.g. "LIGHT." Clip two leaves from this plant. Prepare casts of the leaves surfaces by painting the adaxial (top surface) of one leaf and the abaxial (bottom surface) of the other leaf with clear nail polish. Allow the nail polish to dry for approximately 10 minutes. While the nail polish is drying, label microscope slides as either adaxial (top of the leaf) or abaxial (bottom of the leaf). Cut a piece of sellotape 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. Place the cast on the appropriately labelled slide. Place a cover slip over the cast. Repeat this step for the remaining leaf. Examine the slides under the microscope to determine which leaf surface has stomata.

Once this has been done, the environmental factors which affect stomatal opening (LIGHT) will be discussed.

Before changing any conditions, remove a leaf and paint the appropriate surface with nail polish. Let the nail polish dry. This leaf will be used for initial stomatal conditions and for comparison with stomatal responses to different treatments. Now cut six pieces of aluminium 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. Now do the same to a leaf that has been kept in the dark for 24 hours. *Note:* don't forget to label the container e.g. 'dark'. Place both plants under (or in front of) the light. Record the time that the plants were placed in the light. 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 $30^{o}C$.

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. Prepare microscope slides as before. Be sure to label each slide with the time and the treatment as slides are made. 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. **(1)**

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. We now 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 questions to be answered surrounding stomatal response to environmental conditions.

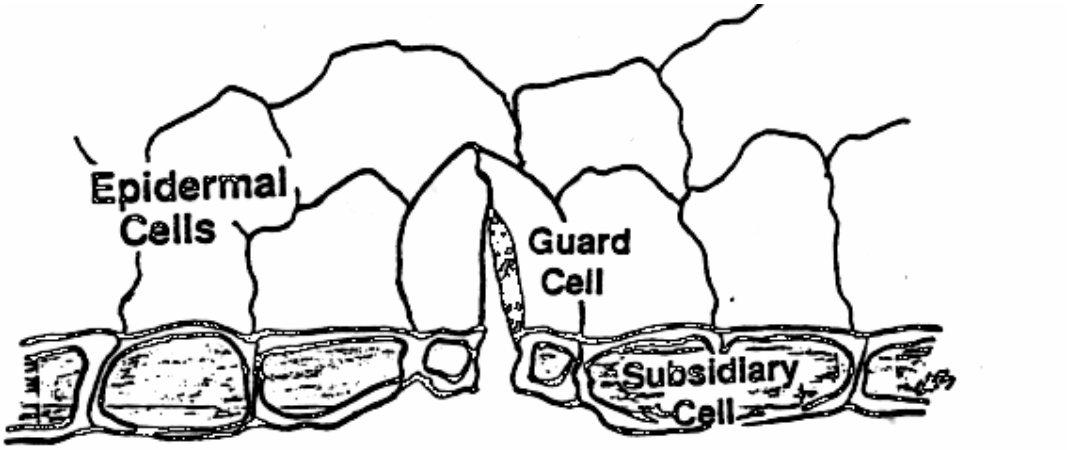

Figure 1. stomatal apparatus including gard cells and subsidiary cells on the lower epidermis of a leaf.

Stomata are small pores in the surface of a leaf (Figure 1). The core function of stomata is to open and close so that the levels of water loss and carbon dioxide uptake are regulated. Stomata impose a resistance to the diffusion of water vapour and carbon dioxide. When stomata are closed, the resistance to gas exchange is infinitely great. Therefore, stomata provide an effective barrier to the movement of water vapour and carbon dioxide into and out of the leaf. When stomata are open, gas exchange of both water vapour and carbon dioxide proceed.

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 shows us the 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. **(1)**

The usual response of stomata to environmental factors is shown in Fig. 1.2. Closed stomata begin to open in a few minutes after exposure to light and they start to close when returned to the dark. When plants are put in CO_2-free air, stomata tend to open even in the dark. Conversely, an increase in CO_2 concentration above the normal level (330-340 ppm) causes stomata to close in the light. Within range of about 5-25 ° C the effect of temperature is mainly on the rate of opening and closing reactions rather than on aperture size. Temperature above about 25 ° C cause a closure in a number of plants. If a plant is losing more water through transpiration than it is absorbing by the roots a water deficit develops and this usually causes stomatal closure irrespective of light, temperature and carbon dioxide. (Sutcliffe, 1979). **(2)**

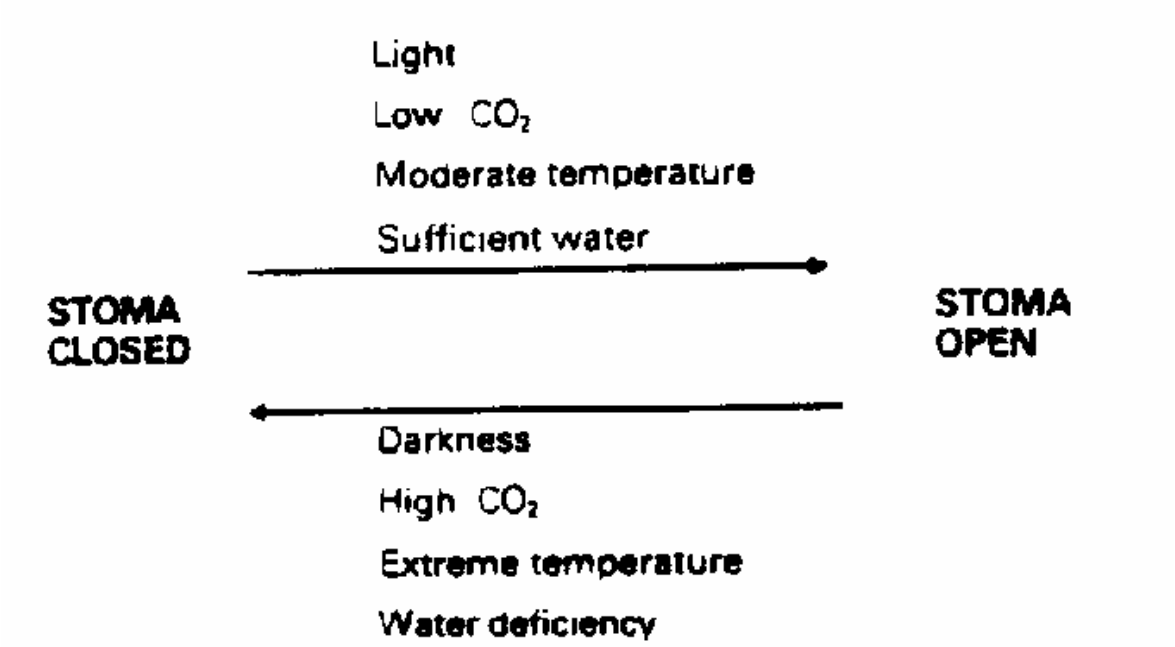

Figure 1.2. Usual responces of stomata to enviormentl factors

Materials and Methods

There are two parts of my experiment the first part was to determine weather the leaves had a stomata or not. The second part was to determine the environmental factors affecting the stomatal opening. I selected different leaves from my local park which is ten minutes away from my house. My aim was to do experiments as safely as possible to get a reasonable range of accurate results. I was able to do this by follow the plan closely as possible.

To follow the plan I first had to follow the safety procedures for safety purposes. For my experiments the following apparatus were needed i.e.

Apparatus

- Microscope slides
- Microscope
- Clear Scotch tape (shiny kind)
- Clear nail polish (not strengthened)

- Various leaf specimens

Safety notes

1. Avoid using plants that may cause skin irritations.
2. The leaf needs to be as dry as possible so the nail polish will adhere.
3. A pubescent leaf will generate a cast of the hairs. One possible way to solve the problem is to remove some of the hairs by placing tape onto the surface and pulling some of the hairs off.

Procedures

1. I selected a plant that has been kept in the light and labelled the container of the plant "LIGHT." I Clipped two leaves from this plant then casts of the leaves surfaces were prepared by painting the top surface adaxial of one leaf and the bottom surface abaxial of the other leaf with clear nail polish. It is important that nail polish only be applied to dry leaves or the replica will be cloudy and may not dry properly. I allowed the 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 was drying, I labelled microscope slides as either adaxial (top of the leaf) or abaxial (bottom of the leaf).

3. I Cut a piece of Sellotape approximately 1.5 cm in length. Tape was folded over on itself leaving 0.5 cm of sticky surface exposed. Sticky tab of the tape was placed at the edge of the leaf so that it sticks to the nail polish cast (Figure 1.3). remaining tape was used as a handle to carefully pulled the nail polish cast from the leaf surface. For viewing stomata I used the portion of the cast.

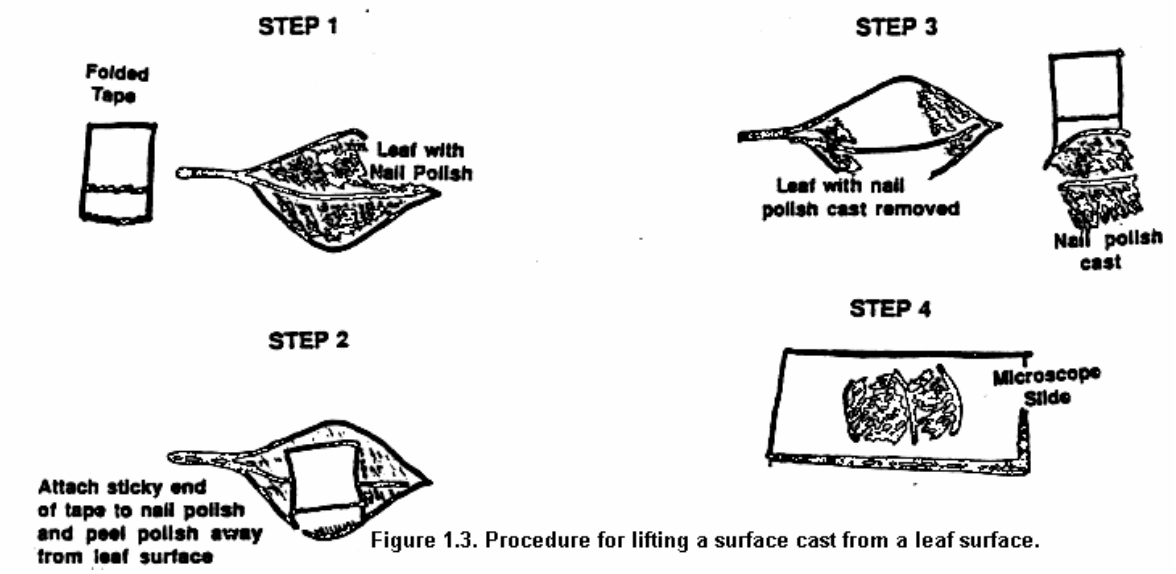

Figure 1.3. Procedure for lifting a surface cast from a leaf surface.

4. The cast was placed on the appropriately labelled slide along with the cover slip. I made the slides permanent by placing a small drop of nail polish on each corner of a cover slip. The cast

was covered with the cover slip so that nail polish glues the cover slip to the slide. This step was repeated with the remaining leaves.

5. Slides were examined under microscope to determine which leaf surface has stomata. Entire leaf cast was surveyed. The leaf surface with stomata looked similar to one of the illustrations in Figure 1.4.

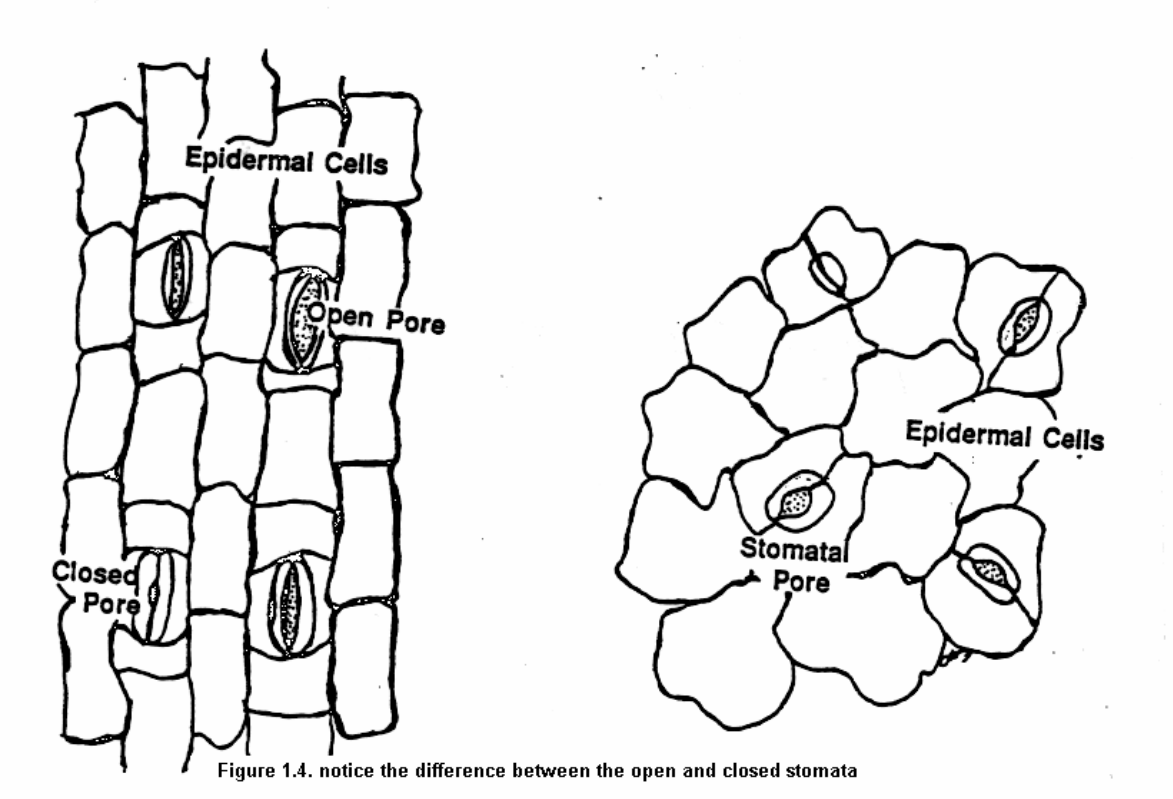

Figure 1.4. notice the difference between the open and closed stomata

After finding the leaves which contain stomata I performed another experiment to find the environmental factors (LIGHT) which affect stomatal opening. For this I have performed the following steps i.e.

1. By kept the condition same selected leaf was painted with the nail polish. The leaf was placed aside temporarily so that the nail polish can dry. This leaf was used to see initial stomatal conditions for comparison with stomatal responses to different treatments.

2. While the leaf was drying I cut six pieces of aluminium foil so that they will each be large enough to entirely cover both sides of one leaf. One piece of the foil was folded gently over a leaf. The edges of the foil was taped together so that no light can reach the leaf surface.

3. Steps 1 and 2 were repeated for a plant that had been kept in the dark for 24 hours. The container was labelled with this plant "DARK."

4. Both plants were placed in front of the light. Time was recorded that the plants were placed in the light on the data sheet (Table 1). Temperature was monitored 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, one covered leaf was removed and one uncovered leaf from each plant. The appropriate surface was painted with nail polish. The nail polish was left to dry, then cast was remove.

6. Microscope slides were prepared as before. For the easiest comparison, both casts were placed from the "LIGHT" plant side by side on one slide; both casts were placed from leaves off the "DARK" plant side by side on a second slide.

Results

1. According to my observations and calculations abaxial surface of the leaf has more stomata than the adaxial surface of the leaf. This is to aid in preventing dehydration.

2. The leaves which were placed in the dark there stomata were closed while the leaves which were placed in the light there stomata were opened.

3. Bright light, leaf temperature less than 30oC, low wind speeds, and wet soil all lead to stomatal opening. Sudden and prolonged darkness, leaf temperatures above 30oC, high wind speeds, and dry soil nearly always ensure stomatal closure

References

1. http://www.zoo.utoronto.ca/able/volumes/vol-13/3-brewer/3-brewer.htm#fig3-2

2. http://www.water.hut.fi/wr/kurssit/Yhd-12.135/kirja/stomata.htm

3. http://www.mercer.edu/camps/message/summer2000/summer2000-stomata.htm

4. Glenn and Susan Toole biology for advanced level (4th edition)