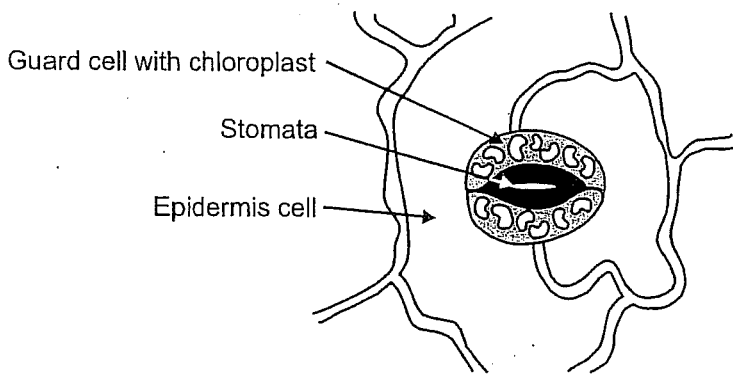


"Hole-y" Moley Examining Stomates

As plants colonized the land, there was a selection pressure for plants to grow and increase in size. This required the development of supportive tissue. An increase in height also meant that some plant parts were away from sources of water. The problem of support and need for water was solved by the development of vascular tissue. Plants also evolved a waxy cuticle as a way to reduce water loss. This waxy cuticle can be found on leaves and herbaceous stems. While this wonderful adaptation prevents water loss, it also prevents the gas exchange in the leaves. Evolution and natural selection has an astonishing way of working. With the need for gas exchange, came the development of stomates. *Stomates* are tiny pores found on leaves. While these pores will allow for gas exchange to occur, some water will also exit the stomates. This process is known as transpiration.

The plant regulates the opening and closing of these stomates with two cells that surround the stomate known as *guard cells*. These cells may be either kidney shaped or dumb-bell shaped. When these cells experience turgor pressure, they swell up and open the stomate. The guard cells, in general, close at night when the plant experiences water stress, or when the leaf has low levels of CO_2 .



The number of stomates a leaf has varies with the plant species. For some species, the stomates are found on both the upper and lower leaf surfaces while on others they are just found on the lower surface. Below is a list of selected plant species with the number of stomates per mm^2 :

Leaves with no stomates on upper surface		
Plant	# stomates per mm^2 on lower surface	# stomates per mm^2 on upper surface
Norway Maple	400	0
Rubber Plant	145	0
Lily	62	0

Leaves with few stomates on upper surface

Pumpkin	269	28
Tomato	130	12
Bean	281	40

Leaves with stomates nearly equal on both surfaces

Oats	23	25
Corn	158	94
Garden Pea	216	101

In this investigation, you will count the number of stomates found on dicot and monocot leaves on both the upper and lower surfaces in a field of view. You will determine the relationship between magnification and the size of the field of view when you look into a microscope. Using this mathematical relationship, you can then determine the number of stomates in a square millimeter of leaf surface.

PURPOSE

The purpose of this investigation is to compare and contrast the stomates found on a monocot versus a dicot. You will also determine the number of stomates per mm^2 on both the upper and lower surfaces of a leaf.

MATERIALS

light microscope

coverslips

Hawaiian schefflera (*Brassaia arbricola*)

leaves of several plant species

TI-83 graphing calculator (optional)

microscope slides

clear nail polish

airplane plant (*Chlorophytum comosom*)

clear metric ruler 10 cm long

Safety Alert

CAUTION: Fingernail polish can be an irritant if it comes in contact with sensitive tissue. Avoid eye contact; do not ingest. Immediately run water in an eye wash for 10 minutes if fingernail polish comes in contact with your eye.

PROCEDURE

1. Write a hypothesis concerning the number of stomates that will be found on the upper surface of a leaf versus the bottom surface of a leaf. Keep in mind the structure and function of stomates.
2. Obtain a leaf from both plants above. Apply a coat of clear nail polish approximately 1 cm x 1 cm to the upper and lower sides of each leaf. Be sure to do this on different areas of the leaf. Do not make the layer of polish extremely thick. On the airplane plant, coat the green area of the leaf. Make the area approximately 1 cm².
3. While the polish is drying, determine the area of the field of view. Lay a clear metric ruler on the stage of the microscope. Using the low power objective (10x), estimate the diameter of the field of view to the nearest .25 mm.

a. Diameter _____ mm

4. Determine the diameter of the field of view on high objective (43X) by doing the following calculations:

$$\frac{\text{magnification number of high-power objective}}{\text{magnification number of low-power objective}} = X \qquad \frac{\text{diameter of low-power field of view}}{X} = \text{diameter of high-power field of view}$$

5. Determine the radius of your field of view on high power.
 - a. _____ radius mm
6. Determine the area of a circle for your field of view. Remember the area of a circle = πr^2 .
7. Peel off the nail polish from the back of the airplane plant leaf. Be sure you make note of which plant you are using and whether it is the upper surface or the lower surface. Place the peeled nail polish on a microscope slide, cover with a cover slip, and examine it under low power.
8. Observe the slide under the (43x) objective or (430x) for the total magnification. On your student answer page draw the stomates as they appear in the field of view. Count the number of stomates observed. Do this for 5 different areas of the slide. Record in the data table.
9. Repeat steps 7 and 8 for the upper surface of the airplane plant and both the lower and upper surfaces of the Hawaiian schefflera.
10. For each surface examined, calculate the average number stomates per field of view, then determine the average number of stomates per mm² of leaf surface area. Show your work on the student answer sheet.
11. Write a conclusion for this experiment. It should include information about the location of stomates, the number of stomates found in a certain area, and comparison of dicot stomates with monocot stomates.

** Optional – These calculations can be done on a TI-83 graphing calculator by doing the following:

12. To enter the number of stomates in a list on the calculator. Turn on your calculator **ON** and press **CLEAR** to clear the screen.
13. Clear all lists by pressing **2nd**, **+** [mem]. Move cursor to ClrAllLists (by pressing the **↓** key OR press **4**) and then press **ENTER** twice. Done should appear on the screen.
14. Press **STAT**, then select EDIT and press **ENTER**.
15. Now enter the data (number of stomates) collected for lower surface of the airplane plant in L1, the data for the upper surface of the airplane plant in L2, the data for the lower surface of the Hawaiian schefflera in L3 and the data for its upper surface in L4 (Figure 1).

Figure 1

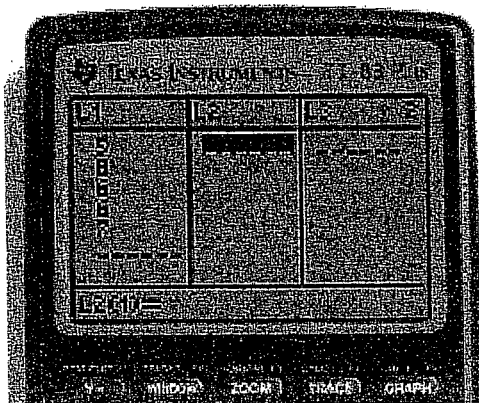
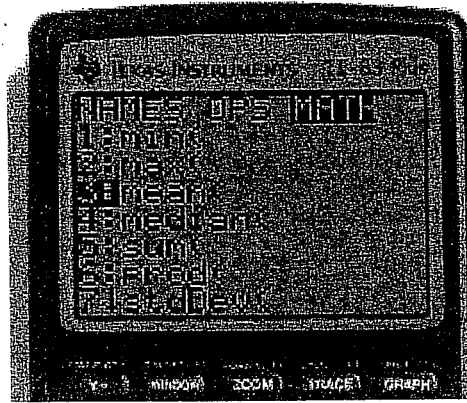


Figure 2



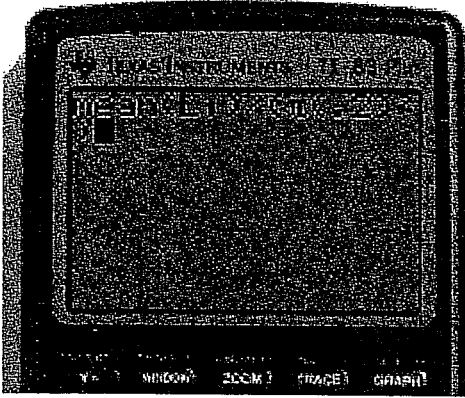
16. Return to the home screen by pressing **2nd**, **MODE** [quit].
17. The following will allow you to determine the average number of stomates per mm^2 for L1 or the lower surface of the airplane plane by doing the following:
 - a. Press **2nd**, **STAT** [list].
 - b. Move to cursor to the right to highlight MATH (this allows to manipulate lists).
 - c. Move the cursor down to highlight MEAN (Figure 2).
 - d. Press **ENTER** (the calculator is ready to average a list).
 - e. Press **2nd**, **1** [L1].
 - f. Press **)** to close parenthesis.

g. Now you want to divide by the equation for the area of a circle.

Press \div , $($, 2nd, \wedge , $($.

Input your value for the radius to the closest mm.

Press $)$, \times^2 , $)$, ENTER (Figure 3).



18. Now you have the average number of stomates/mm² on the lower surface of the airplane plant. Repeat the above steps for L2, L3, and L4. This will give the average number of stomates/mm² of the upper surface of the airplane plant and the lower and upper surfaces of the Hawaiian schefflera, respectively.

Name _____

Period _____

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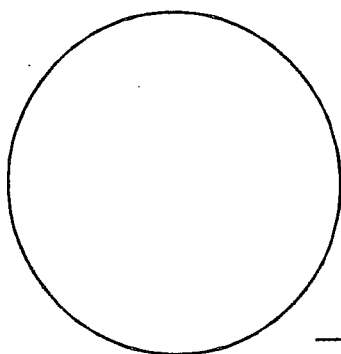
HYPOTHESIS

DATA AND OBSERVATIONS

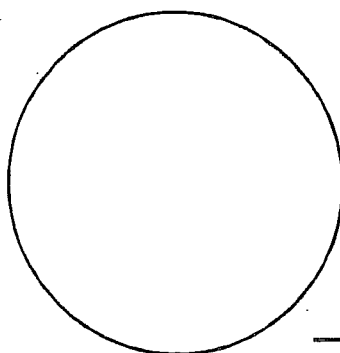
Airplane plant

Upper Surface

Lower Surface



_____x

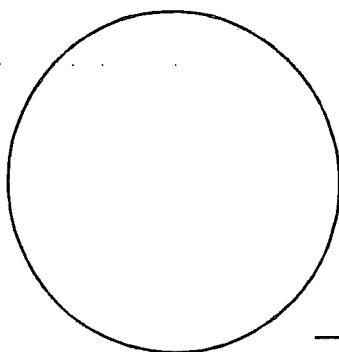


_____x

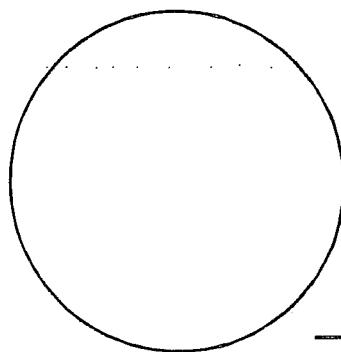
Hawaiian schefflera

Upper Surface

Lower Surface



_____x



_____x

Airplane Plant Upper Surface

Field of View	# of Stomates
# 1	13
# 2	14
# 3	10
# 4	15
# 5	14
Average	13

Airplane Plant Lower Surface

Field of View	# of Stomates
# 1	0
# 2	0
# 3	0
# 4	0
# 5	0
Average	0

Hawaiian Schefflera Upper Surface

Field of View	# of Stomates
# 1	
# 2	
# 3	
# 4	
# 5	
Average	

Hawaiian Schefflera Lower Surface

Field of View	# of Stomates
# 1	
# 2	
# 3	
# 4	
# 5	
Average	

ANALYSIS

This area is provided for your conclusion.

