# BIOLOGY – IT KEY SKILLS

## INTRODUCTION.

The purpose of this assignment is to compare stomatal densities of the upper and lower epidermis of a leaf.
This assignment of stomata is also relevant to my AS biology course as stomata is a key factor in transpiration of plants, and transpiration and the transport of water is a major section of the syllabus. This assignment will therefore help me to understand why transpiration occurs and how the stomata affect it as stomata's activity is related to the rate of transpiration.
It is said that in general, the greater the number of stomata per unit area, the greater the rate stomatal transpiration, however distribution and densities are also important.

Stomata are pores in the epidermis layer, which is found in the mesophyll spongy layer of the leaf. They are found mainly in leaves, but also in stems.
There are two main functions of stomata

1. To allow gaseous exchange of carbon dioxide and oxygen between the inside of a leaf and the surrounding atmosphere - When the stomata are open, carbon dioxide diffuses into the sub-stomata air chambers and then into the intercellular spaces between mesophyll cells. When it comes into contact with the wet surface of a cell it goes into solution and diffuses into the cytoplasm. Oxygen travels via the same route but the opposite way.
2. To permit the escape of water vapour from the leaf – this is the evaporation of water vapour from spaces in the mesophyll cells of the leaf otherwise known as transpiration.

The diagram below shows a vertical section through a stoma.

Each stoma is bordered with two semicircular guard cells whose movements due to changes in water content, control the size of the stomata by changes in their turgidity.
If water is drawn into the guard cells by osmosis the cells expand and their turgidity is increased. . But they do not expand uniformly in all directions. The thick, inelastic inner wall makes them bend. The result is that the inner walls of the two guard cells draw apart from each other and the pore opens creating the stoma. Stomata and changes in turgidity can be seen very clearly under an electron microscope. (A diagram of stomata under an electron microscope is presented later on in the assignment)

As noted before stomata activity affects the rate of transpiration, but now more specifically, it is the turgidity of stomata determines is the main cause of transpiration. It is thought that in normal circumstances when a stoma opens the turgidity of the guard cells is increased by their taking up water from the surrounding epidermal cells but it is also know that the turgidity of the stomata is also affected by external factors of the particular environment, such as light, wind, and humidity.
During the day stomata tend to be open, this is because the guard cells of the stoma become flaccid to light. This is important as it allows gaseous change of carbon dioxide and oxygen to take place for photosynthesis of the plant. This can be investigated by means of a perometer, an instrument for measuring the resistance to the flow of air through a leaf. If you attach a perometer to a leaf and take measurements of its resistance to airflow at intervals, you will find that there is a generally less resistance during daylight hours than at night. This is because the stomata open during the day and close at night.
For wind, in still air, a highly saturated air shield builds up around the stoma. Air movement will sweep this layer away, which decreases the humidity of the stomata therefore increasing transpiration also a xeromorphic feature of some leaves is the presence of sunken stomata, the stomata grooves into the epidermis which then a high humidity can build up inside the stoma and reduce transpiration rates. An increase in temperature also has an effect as the guard cells become more flaccid thus increasing the capacity of the stoma therefore increasing transpiration rates.

A main factor that affects the distribution and densities of stomata is the type of plant. There are two main types of plants, monocotyledonous and dicotyledonous.
Monocotyledonous (monocot) or more modern liliidae, have leaves that have parallel veins and therefore do not grow to a large size. Also their leaves are held vertically rather than horizontally, which affect where the stomata is distributed and thus the densities, leafs of a moncotylic nature therefore have equal stomatal densities on both upper and lower epidermis.
Dicotyledonous (dicot) otherwise known as magnoliidae, has branched veins and therefore can grow very large leafs. Their leaves grow horizontally and as a result most or all of the stomata are found on the lower epidermis. This is because of the cuticle found on the upper epidermis. If there was no cuticle,

stomata wouldn't be necessary as gaseous exchange would be much more efficient, however, then transpiration could not be controlled. This is because a waxy cuticle reduces water loss but further control is exercised by stomata. It is estimated that about 90% of the water absorbed by the roots of the plant is lost by the leaves in transpiration.

An example of a monocot leaf is one of a maize plant. On the table below this monocot plant is compared to a dicot leaf - an oak tree leaf.

| | Upper epidermis | Lower epidermis |
|---|---|---|
| Oak leaf- dicot | 0 | 45000 |
| Maize leaf- monocot | 5200 | 6800 |

You can clearly see that the monocot leaf has similar stomatal densities and the dicot has contrasting results.

The leaf I will be studying is a dicotyledonous type so based on all of my research I predict that there will be a greater number of stomata on the lower epidermis of my leaf.

The plan:
The aim of this investigation is to try and count the number of stomata on both sides of the leaf and then compare the results, therefore a method has to be devised to try and view the number of stomata. Viewing a leaf under a light microscope does not allow the number of stomata to be counted, as this microscope is not powerful enough. Therefore an alternative would be to get an imprint of the leaf. This can be done by painting the upper and lower leaf with clear nail varnish and then this imprint of the stomata can be seen and counted under the light microscope.

FAIR TEST
To make this experiment a fair test, I will conduct the experiment on different areas on both sides of the leaf to see if this affects the density of stomata. Also four different people will count the number of stomata, so to get an unbiased number and then to calculate an average.
The stomata in the field of view will only be counted, to ensure everyone is counting the same surface area. The same magnification of **x 400** (high power) will be used when viewing under the microscope.

The apparatus used included:
- Nail varnish
- A leaf
- 2 glass slides and cover slips
- A light microscope
- Twisters

METHOD - Using the eyepiece graticule.

To do this, you need to have a scale (graticule) in position in your eyepiece, so that it can be seen when you look down the microscope. The scales are usually on small circular pieces of glass or acetate.

1. To insert the graticule scale in your eyepiece, remove the ey epiece lens from the microscope and carefully unscrew the top lens.
2. If you look down into the lens body, you will see a ledge running round the sides about half way down, drop the scale into the lens body so that it rests on the ledge. Then replace the len s. N.B it doesn't matter if the scale is upside down but if it annoys you then unscrew the lens again and turn the scale over.
3. When you look through the microscope, you should see the scale overlying your specimen.
4. To calibrate the scale, you need to use a stage micrometer. This can be a special slide with a scale engraved on it. It usually consists of a scale 1cm long, which is divided into 100 units, each of which is 0.1mm (100 um) there is an extended line every $10^{th}$ unit.

To calibrate the eyepiece sca le

1. Place the stage micrometer on the microscope stage and hold it down with the clips.
2. Using the eyepiece lens with the scale in, look through the microscope and focus it so you see both scales clearly. This is usually easier if you focus your eye on the eyepiece scale and adjust the microscope so the stage comes into focus as well.
3. Move the stage micrometer carefully so that the starting units of the two scales. Note down the number of divisions along each of the two scales that this represents as these represents 1 division on the eyepiece scale.
4. The equation for this is:

100 um in 1 scale micrometer division.

1 division on the eyepiece scale =

$$\frac{\text{No. Of coincided divisions X No. of divisions on the scale micrometer scale}}{\text{Number of divisions on the eyepiece graticule scale}} \text{ X 10}$$

1000 um in 1 eyepiece graticule division.

At x100 and x400 magnification the lines on the scale will have a definite thickness. It is important to measure from one side of one scale mark to the same side of the next coinciding mark.

All my procedures of stomata count were done on high power therefore 1 division on the eyepiece scale = 3.5 um.

When you measure another specimen, you will already have the calibration figures so all you have to do is count how many eyepiece scale divisions your specimen covers, and multiply that by the calibration factor for tat objective lens.

Method for determining stomatal density

1. Apply nail varnish onto a section of the leaf
2. Allow 2 minutes for nail varnish to dry and gently peel off varnish with twisters, bringing the varnish, which has an imprint of the surface of the leaf. Nail varnish was used instead of cello tape to gather an imprint because as the varnish is liquid it can mould and fill every space around the stomata on the surface, therefore creating an accurate and clear imprint to show. Cello tape might not fill every space therefore missing out stomata, which results in an incorrect perception of the stomata densities.
3. Place the peeled nail varnish onto a slide with the imprint side up, add 1 drop of water and apply cover slip on top.

Then the stomata are ready to be calculated.

**RESULTS**

Eyepiece graticule calibration calculations.

I found that 3.5 micrometers coincided with every graticule division to give 1 division of the eyepiece graticule. To calculate one division on the eyepiece scale I followed to following equation:

$$\text{1 division on the eyepiece scale (in } \mu\text{m)} = \frac{\text{The number of micrometers found coincided X Number of stage micrometer in a micrometer}}{\text{Number of divisions on the graticule in a micrometer}} \times 10$$

Therefore if I followed the equation:

$$\text{1 division on the eyepiece scale (in } \mu\text{m)} = \frac{3.5 \times 100}{1000} \times 10$$

$$= 3.5\ \mu\text{m}$$

RESULTS OF STOMATA DENSITY COUNTS. My group's results.

| Field or area | Stomata count of 4 people | Average |
|---|---|---|
| 1 | 17, 17, 20, 18 | 17 + 17 + 20 + 18 / 4 = **18** |
| 2 | 11, 11, 11, 12 | **11** |
| 3 | 20, 19, 19, 20 | **20** |
| Total average stomata count | **18, 11, 20 / 3** | 16 |

Lower epidermis stomata count.

| Field or area | Stomata count of 4 people | Average |
|---|---|---|
| 1 | 0, 0, 0, 0 | 0 + 0 + 0 + 0 / 4 = **0** |
| 2 | 0, 0, 0, 0 | **0** |
| 3 | 0, 0, 0, 0 | **0** |
| Total average stomata count | **0, 0, 0,** / 3 | 0 |

Upper epidermis stomata count.

Now the average stomata count of both sides of the leaf have been calculated, I can now calculate the stomata density as numbers of stomata per cm2.
To do this I first needed to calculate the area of the field view in cm2.
This answer will then be used in the calculation of the radii of the field of view to calculate stomata per cm2.

<u>CALCULATIONS OF STOMATAL DENSITY AS NUMBERS OF STOMATA / $CM^2$</u>

The formulae to calculating the area of a circle is

$$\pi r^2$$

$r = 45 \times 3.5$

To calculate the radius you have to times the 45 graticule divisions in field view and the 3.5 μm that coincide with 1 graticule together. This will give the radius in centimetres.

$r = \frac{157.5}{1000}$

To find out the radius in millimetres, you divide the answer by 1000 as 1000 micrometers go into 1 mm.

$r = \frac{0.1575}{10}$

To find the radius in centimetres, you divide the answer by 10 as 10 millimetres go into 1 cm.

$= 0.01575$

This is the radius of field view in cm on high power

$\pi \times (0.01575)^2$

To get the area of the field view circle incorporate the information into the formula

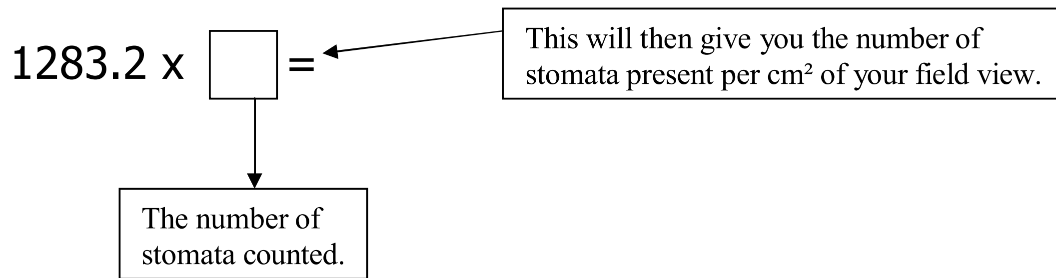
$= 0.0007793$

This is the area of my field of view

$\frac{1}{0.0007793}$

To find out how many of these areas will fit into 1 cm, you divide the answer by 1.

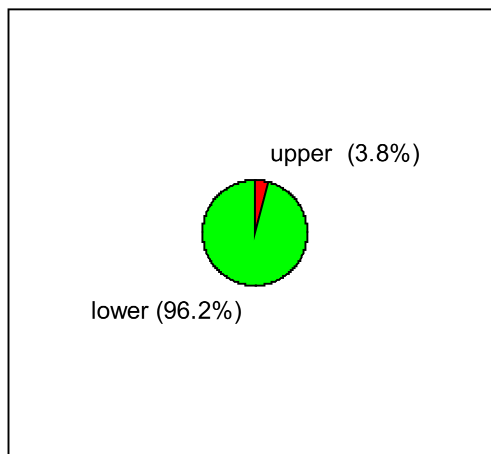
$= 1283.2$

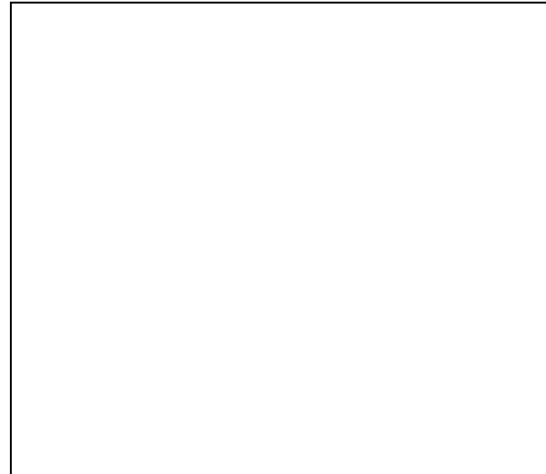



After I calculated how many stomata per cm² where in my field of view, I gathered the calculated results from the other groups & constructed a table.

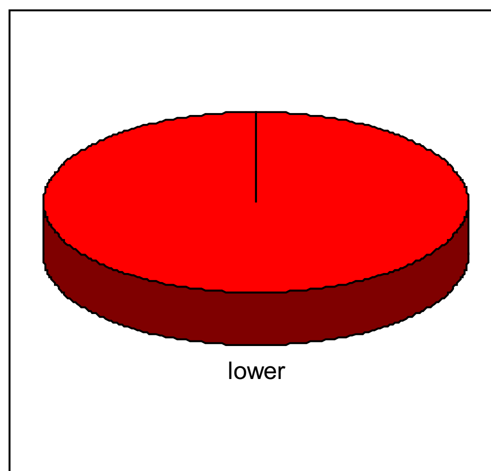
| Group | No. Of stomata per cm² on the upper epidermis | No. Of stomata per cm² on the lower epidermis |
|---|---|---|
| 1 | 1353 | 7958 |
| Mine | 0 | 20, 531 |
| 3 | 0 | 10, 266 |
| 4 | 428 | 11, 549 |
| 5 | 0 | 21, 173 |
| 6 | 0 | 5405 |
| 7 | 2458 | 15, 641 |
| 8 | 0 | 7699 |
| 9 | 216 | 5656 |
| 10 | 532 | 21, 814 |
| AVERAGE | 499 | 12, 769 |

The graph below shows the comparison of the average upper and lower epidermis data for the whole 10 groups.



The graphs below show my results for the comparison of stomata per cm2.



## **The Chi – squared test.**

This is a simple statistical test, which looks at the difference between observed and expected values as data and then relates them to a probability level. This makes it possible to identify how likely it is that the values are significantly different or similar.

The formula for the chi – squared test is as follows:

The $\chi$ is the Greek letter Chi.

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

O is the observed value
E is the expected value

The top part of the formula for $\chi^2$ considers the size of the difference between the observed and expected values. This difference could be either positive or negative. To avoid the mathematical problems associated with negative values, the difference is squared.
The bottom part of the formula relates the size of the difference to the magnitude of the number involved. The sigma – $\Sigma$ 'sum' symbol is required because there is not just one pair of observed and expected values but several (in this case 2)

From background research, I found out that the expected values of percentage stomata densities for the upper and lower epidermis on a monocot leaf is:
**Upper – 7%**
**Lower – 93%**

My observed values were

**Upper** – **4%** ⟶ 499/ (total) 13268 x 100 = 3.8% to nearest integer **4%**
**Lower** – **96%** ⟶ 12769/ 13268 x 100 = 96.2% = **96%**

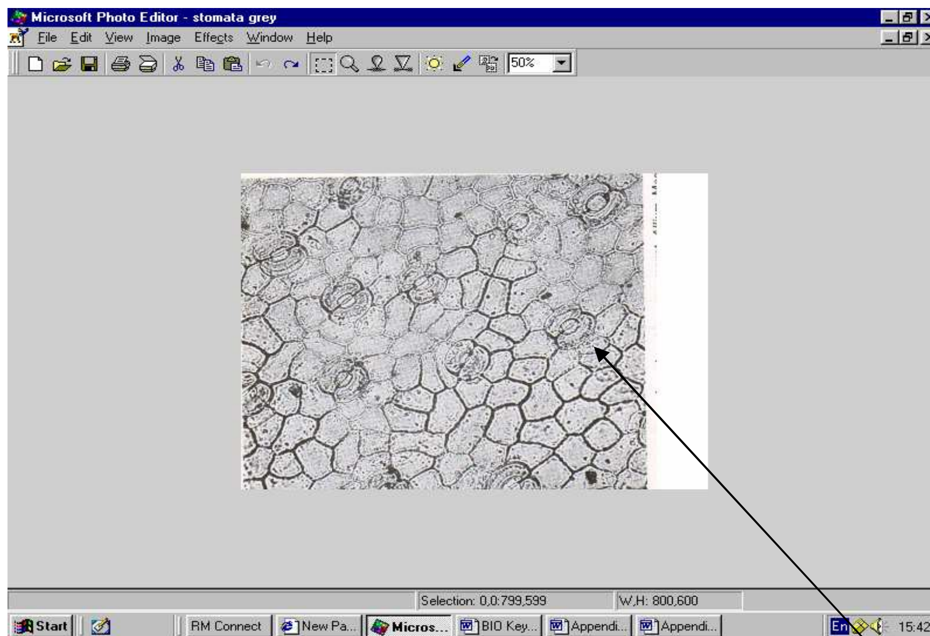
| Upper | Lower |
|---|---|
| **E** = 700<br>**O** = 499 | **E** = 9300<br>**O** = 12769 |
| **O – E** = -201 | **O – E** = 3469 |
| **(O – E)** $^2$ = 40401 | **(O – E)** $^2$ =12033961 |
| $\frac{(O-E)^2}{E} = \frac{40401}{700}$<br>= 57.71 | $\frac{(O-E)^2}{E} = \frac{12033961}{9300}$<br>= 1293.97 |
| $\Sigma$ = 57.71 + 1293.97 = 1351.69<br>$\chi^2$ = 1351.69 | |

There is one more thing that needs to be added to the chi – squared test, which is the degree of freedom.

This relates a critical value to your chi – squared values to show if there is a significant difference in the densities of stomata on the upper and lower epidermis or if it was just by chance.
The degree of freedom is calculated = the number of categories minus one
In this case it is 2 – 1 = 1.
The critical value at one degree of freedom according to a s tandard calculated table of chi – squared results is 3.84.

If your chi $\chi^2$ value is greater than or equal to the critical value then there is a significant difference between the observed and expected values.
As you can see my chi $\chi^2$ value is way above the critical value so there is a significant difference between my observed and expected values.

The diagram below shows an image of stomata.




From the book of where I received this image I measured the stomata to be 11 mm long. To calculate the magnification of this image you
Size of image / actual size
= X100

**DISCUSSION**

The conclusions I can gather from my results are that there is a higher percentage density of stomata on the lower epidermis and on the higher epidermis. You can see from my graphs that the lower epidermis had a density of 12769 stomata/$cm^2$, which contained 96% of the stomata counted and the upper had a density of 499 stomata/$cm^2$ , which contained 4% of all the stomata counted. This therefore proves my prediction correct.

These results have also proved biological significance based on my re search in the introduction. My results have proven that with a monocot leaf there is a higher stomata density percentage on the lower epidermis as these leaves are horizontally held and not vertically, a cuticle would have formed on the upper epidermis. This would affect the stomata density, as the main function of stomata - gaseous exchange, would be disturbed by the cuticle, so the stomata are located on the lower epidermis to prevent excessive water loss, as they have no waxy cuticle to protect them, also monocot leaves are relatively thin so the exchange of $CO_2$ and $O_2$ can occur relatively quickly and easily through the stomata of the lower epidermis.

The sources of error in my investigation could have been:
The micrometer scale took a lot of time to focus on the eye, and kept on disappearing so it was hard to take an accurate measurement of anything underneath the microscope, also the scales can be badly and easily scratched making taking accurate measurements difficult.
I would perform tests on leaves of which I know the name of so I can do background research of the leaf so I can make a precise prediction of stomata densities.
Different people counted the number of stomata – an error could have occurred if someone did not know what stomata looked like or became subjective on if or they did not look in the same field of view. To try and overcome this error everyone was shown what stomata looked like before the investigation.
Although magnification was kept constant someone may have adjusted it.
When peeling the nail varnish, it was difficult to peel it off completely with the twisters and was a chance of mixing up slides.
Another similar experiment, which could be carried out, is using cobalt thiocyanate. In the anhydrous state cobalt thiocyanate is blu e, but when hydrated it turns pink. A piece of cobalt thiocyanate paper is placed on each side of a leaf and sandwiched between two glass slides clamped together, and then a stop watch is to measure the time it takes for the cobalt thiocyanate to go pink as this indicates that water has escaped out of the leaf which would be through the stomata. The time varies in which the colour change takes place depending on the temperature and humidity. Generally at room temperature the pink colour develops more rapidl y on the lower epidermis of the leaf than upper surface, the reason being there are more stomata placed on the lower epidermis.
Further investigations to do with the topic are to carry out tests on a greater variation of leaves, of different shapes, sizes, thickness and leaves (maybe dicot leaves) from different habitats to see what affect this would have on the densities of stomata on a leaf.