stomata investigation

Aim:
The aim of this science investigation is to find out where the stomata are located, on the upper or lower epidermis of a leaf.

Prediction:
My prediction is that most of the stomata are found on the lower epidermis of a leaf. I have based this prediction on the function of stomata; to let gases in and out of the leaf i.e. to allow exchange of CO2 and O2 between the inside of the leaf and the surrounding atmosphere and to allow the escape of water vapour from the leaf.
To reduce water loss the leaf has a waxy cuticle on the upper epidermis, which is waterproof, so the leaf uses the lower epidermis for gas exchange.
Science about Stomata:
Stomata are pores perforating the epidermis of the leaves and stem. They are usually most numerous in the lower epidermis of the leaf where there may be as many as 400 per mm, there are generally fewer in the upper epidermis and fewer in the stem. Their functions are:
(1) To allow exchange of carbon dioxide and oxygen between the inside of a leaf and the surrounding atmosphere.
(2) To permit the escape of water vapor from the leaf.
Stomata are important in several physiological processes, not just photosynthesis, but they also represent a hazard in that they may permit excessive evaporation from the leaf. In a sense a plant aces a conflict. If it opens its stomata it runs the risk of losing excessive water, particularly if it lives in a dry habitat. On the other hand if it closes them it may run short of carbon dioxide or oxygen. Plants resolve this problem by not opening them for longer than is necessary. This does not mean that a plant never loses more water than it can replace from the soil. The observation that plants frequently wilt in hot weather bears witness to the fact that they often do lose excessive water. However wilting is not disastrous so long as the plant is given an opportunity to recover later. The controlled opening and closing of the stomata resolve the conflicting needs of the plant.
In order to appreciate how the stomata are controlled we must first understand the mechanism by which they open and close, and this necessitates looking at their structure.
A pair of guard cells borders the stomata pore. These are sausage-shaped and, unlike other epidermal cells, contain chloroplasts. There is a sap vacuole and, a point of great importance; the inner cellulose wall (i.e. the wall lining the pore itself) is thicker and less elastic than the thinner outer wall.
Stomata opening and closure depends on changes in turgor of the guard cells. 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. The same effect can be achieved by blowing up a sausage- shaped balloon to which cellotape has been stuck down one side. As it is blown up it will bend over towards the cellotaped side. 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. Isolated stomata will open when immersed in water, but if placed in a hypertonic solution, they close.

When do the stomata open and close? 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.

The mechanism of stomata opening and closure

At first glance the mechanism causing this diurnal opening and closing might seem obvious. Unlike other epidermal cells, the guard cells have chloroplasts and at daybreak they start photosynthesizing; this leads to an accumulation of sugar in the guard cells whose osmotic pressure increases. This in turn causes water to be drawn into them from surrounding epidermal cells resulting in the opening of a pore. However this theory is unsatisfactory. It is true that in the light sugar (sucrose mainly) accumulates in the guard cells, but the stomata response is too rapid for it to be explained merely by a resumption of photosynthesis.

So we must look for an alternative explanation. One possible hypothesis depends on the fact that the enzymatic conversion of starch to sugar proceeds more readily when comparatively little acid is present (i.e., at a high pH). The conversion to sugar to starch on the other hand is favored by a comparatively high concentration of acid (low pH). During the night carbon dioxide accumulates in the intercellular spaces of the leaf, and this raises the concentration of carbonic acid. The resulting drop in pH favors the conversion of sugar to starch in the guard cells, thereby decreasing their osmotic pressure and causing the stomata to close. In the morning the resumption of photosynthesis lowers the concentration of carbon dioxide. As a result the level of carbonic acid falls, the pH rises, starch is converted to sugar, the osmotic pressure of the guard cells increases, and the stoma opens.

This theory leaves a number of facts unexplained. For example, starch is absent from the guard cells of certain plants; some guard cells lack chloroplasts but still open and close; and the stomata movements of some plants may not necessarily be related to the time of day; in fact in some plants they open at night and close by day. One possibility is that opening is achieved by ions being actively transported into the guard cells from neighboring epidermal cells, thereby building up the necessary solute concentration for drawing in water by osmosis. There is evidence that in tobacco leaves potassium ions can be actively pumped into guard cells. Alternatively water itself may be pumped into or out of the guard cells.

When the stomata are open carbon dioxide diffuses into the sub-stomata air chambers and thence 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. The fixation of carbon dioxide in the dark reactions of photosynthesis creates a concentration gradient that carbon dioxide continues to diffuse into the leaf.

Plan:

The aim of this investigation is to try and count the number of stomata, therefore a method has to be devised to try and view the number of stomata. Viewing a leaf under a microscope does not allow the number of stomata to be counted, as the microscope is not powerful enough. Therefore an alternative would be to get an imprint of the leaf. This can be achieved by painting the upper and lower leaf with nail varnish, and when dry to remove the nail varnish and stick it on to some sticky tape and then viewing under a microscope and recording the number of stomata on each side of the leaf.

Fair Test:
To make this investigation a fair test, the test will be carried out on different types of leaves to see if this will affect the number and location of the stomata.
Also three different people will count the number of stomata, so to get an unbiased number and then an average will be taken. The stomata in the field of view will only be counted, to ensure everyone is counting the same surface area. The same magnification will be used when viewing under the microscope.

Safety:
Safety goggles were worn when looking down the microscope, to prevent serious accidents in case someone is pushed.

Apparatus:
Nail Varnish
Sticky tape
Leaves
2 Glass slides
Microscope

Method:
The method was carried out on the following leaves :
Grape Ivy: This plant originates from South Africa and is a climbing plant. It is often found on walls of homes in the country. This plant can cope in moderate to warm conditions.
Crassula: This plant originates from South Africa and is a succulent plant (holds water). This plant tends to grow low on the ground in rocky surroundings.
Spider plant: This plant also originates from South Africa and grows better in dry conditions. It has slim green leaves which then grows clumps of small plants of it. This plant has six fleshy roots which are used to store food. The spider plant can be found in homes in sunny positions.
Begonia: This plant originates from China/Japan and is found in warm to hot conditions. In Britain they are found in greenhouses. The Begonia cannot cope with frosty conditions and therefore cannot grow outside in this country.
Geranium: This is a common plant found in Britain today, however it originates from Iran/Central Asia. It grows better in sunny, warm conditions but can also cope with frosty conditions outdoors.
After looking at different plants you would expect most of then to be well adapted to there habitat in which the live, most of the plants are from hot and dry places like Asia and Africa where for a plant it is essential to prevent water loss as water is scarce in these kinds of places and the loss of excessive water could lead to problems for the plant. From the list of plants above which we have used in our experiment Geranium is a very common plant in our houses and can live in cold and frosty conditions like Britain where water is plentiful.
Method:
A leaf was taken and clear nail varnish was painted on the upper and lower surface of the leaf. This was allowed to dry (ca. 1 minute) and then sticky tape was placed over the nail varnish, the sticky tape was peeled off, bringing with it the varnish which had an imprint of the surface of the leaf. This was repeated both for the upper and lower surface of the leaf.
The sticky tape was then placed on a slide and observed under a microscope and was

viewed under 100x magnification. The stomata were counted in the field of view. Three different people counted the number of stomata in the same field of view, an agreement was reached and an average was taken.
View under the Microscope: Stomata

Results:
* Groups which carried out the experiment out once
PLANT GROUPS UPPER
EPI-DERMIS AVERAGE LOWER
EPI-DERMIS AVERAGE
GRAPE IVY
1 0 1 0 2.50
73 46 44 62.0
2 9 * * 85 * *
CRUSSULA
1 26 17 12 18.33 37 31 24 30.67
ONLY ONE GROUP
SPIDER PLANT 1 0 0 0 0.00 66 77 75 71.33
2 0 0 0 66 70 74
BEGONIA
1 0 0 0 0.00 53 48 45 53.33
2 0 0 0 66 55 53
GERRANIUM 1 29 * * 29.00 32 * * 32.00
ONLY ONE GROUP
Graph:

A Graph to Show the number of Stomata on the Upper and Lower Epidermis of Different types of Leaves.

Results and Discussion
From the graph it can be seen very clearly that most of the stomata will be found on the lower epidermis. Although there is slight variation depending on the type of leaf for e.g. Crassula has an average of 18 stomata on the upper epidermis, but it has nearly double the amount on the lower epidermis. The geranium was the only leaf that did not show a great variation but a clear conclusion can not be drawn from this as the number of stomata were only counted once on both the upper and lower epidermis. The spider plant and begonia had no stomata on the upper epidermis and although the grape ivy showed an average of 2.5 stomata, group 1 only counted 1 stomata but group 2 counted 9, a clear conclusion cannot really be drawn from this because group 2 only carried out the test once.

Evaluation:
The sources of error in my investigation could have been; different people counted the number of stomata, error could have occurred if someone did not know what a stomata looked like or they did not look in the same field of view. To try and overcome this error everyone was given a picture of the stomata 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 and there 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 blue, 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 clock started you would measure the time it takes for the cobalt thiocyanate to go pink as this indicates that water has escaped out of the leaf. The time varies in which the colour change takes place depending on the temperature and humidity. Generally the pink colour develops more rapidly on the lower epidermis of the leaf than upper surface, the reason already being discussed in the investigation.

Conclusion:

My prediction that the greatest number of stomata will be found on the lower epidermis was proved correct, as was seen by all the leaves inspected. Other than the Crassula all the other leaves proved without doubt that the greatest number of stomata are found on the lower epidermis. The grape ivy, spider plant, begonia and the geranium were all thin leaves without a visible waxy 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 they are relatively thin leaves so the exchange of CO_2 and O_2 can occur relatively quickly and easily through the stomata of the lower epidermis.

Further Work:

If this investigation was to be carried out again I would use a greater variation of leaves, different shapes, sizes, thickness and leaves from different habitats to see what affect this would have. Also when peeling off the nail varnish the area would be calculated so that everyone was counting in the same area also make sure that everyone repeated the test. Attempts should be made to carry out similar investigations