Are there more stomata per mm^2 on old leaves or young leaves?

Planning

Equipment

Leaves (old and young)
Clear nail-varnish
Microscope
Lamp
Forceps
Slide
Graticule (1cm)

Method

I will take some old leaves and some young leaves. I aim to use three of each. I think this is a realistic amount to use because it is a slow process. The leaves I will use will be from a cherry laurel bush (prunus laurocerasus). I will know which leaves are young and which are old by their colour and size. Young leaves are small and a lighter green. Old leaves are larger, and a darker green. First, I will clean the leaf, to remove dust and dirt. Then I will paint $1cm^2$ of clear nail-varnish on the underside of the leaf, because that is where the stomata are. I will paint the nail-varnish to one side of the mid-rib, but not too near the edge. I will then leave it to dry, while I measure the size of the microscope's field of view, on medium power. To do this, I will use the graticule to measure its diameter, then divide this in half to find the radius. Then I will use the radius measurement in the formula πr^2 to work out the area of the field of view. When the nail-varnish is fully dry, I will carefully peel it off with a pair of forceps, and place it on a slide the same way up as it was on the leaf. Then I will look at it under the microscope. It will be a perfect imprint of the underside of the leaf. I will count the number of stomata I can see in the field of view, and use the area of the field of view to work out how many stomata there are per mm^2. I will repeat this process with all of the leaves.

Controls and variables

The only variable in my experiment will be the age of the leaf. I will try to ensure that all the other conditions remain the same.

- I will make sure that all the young leaves are the same size, and all the old leaves are the same size. This will mean that there will not be a range of ages.
- I will put the nail varnish on the same part of the leaf, as the number of stomata may vary, according to where they are on the leaf.
- I will keep the same nail varnish at the same thickness, so they all peel off in the same way.
- I will always use the same magnification - medium power, so that the field of view will remain constant.
- I will choose leaves from the same side of the same bush, so that all the leaves will have developed in the same conditions.
- I will not distort the nail-varnish peel by pressing down on it, changing the results.

Prediction

I think that age does not affect the amount of stomata on a leaf because of the way in which leaves grow. New leaf cells develop around the edges of the leaf in meristem tissues, the cells of which are able to divide infinitely. After division is complete, genetic programming determines what sort of cell it will become. As a result, whether a cell becomes the one of a stomata's guard cells or not, and the frequency at which they develop is already fixed by the plant's genetic programming, which does not change. Therefore, the number of stomata a leaf has per mm^2 will remain constant throughout its existence, regardless of its age.

Obtaining Results

When I chose my three young leaves, I made sure that they were the smallest leaves I could find. They were all of a very similar size – on average 5cm long, and of a light green colour. The three older leaves were all much larger – on average 15cm long and of a darker green colour. I collected all the leaves from the same side of the same bush, and at the same height to ensure that they had all grown in the identical conditions. When I took them back to the laboratory, I painted them all with the same brand of nail-varnish, of an equal thickness and area, and in the same position, on the underside of the leaf, where the stomata are. I left them all to dry for 24 hours. While they were drying, I measured my field of view. I set the microscope to medium power, with the x10 eyepiece, then noted which microscope I was using, and the settings, so that they would always be the same. Then using a graticule, I measured the diameter of the field of view. It was 0.6mm long. Then I divided this by 2 to

find the radius, and used it in the formula πr^2, to calculate the area: $\pi \times 0.3^2 = 0.283 mm^2$
When the nail varnish was dry, I used a pair of forceps to peel the nail varnish off the leaves. Each time, I peeled a section off, I placed it on a clean slide, and labelled the slide to show whether it was a young leaf or an old leaf. When all the nail-varnish had been peeled off and put on slides, I started to make my observations. I placed a slide under the microscope, focused on the image and counted the stomata I could see. As well as counting the full ones, I also counted the ones that were only partially in the field of view. I counted them slowly and accurately, then wrote down my findings. Then I worked out how many there would be per mm^2 for each result. To do this I worked out how much smaller my field of view was in proportion to a mm^2:

$1 \div 0.283 = 3.53$

This meant that I had to multiply each of my results by 3.53 to obtain the number of stomata per mm^2. I put these results into a table:

	Old Leaves	Young Leaves
Number of stomata per mm^2	204.9	129.7
	190.8	188.7
	130.7	159.6

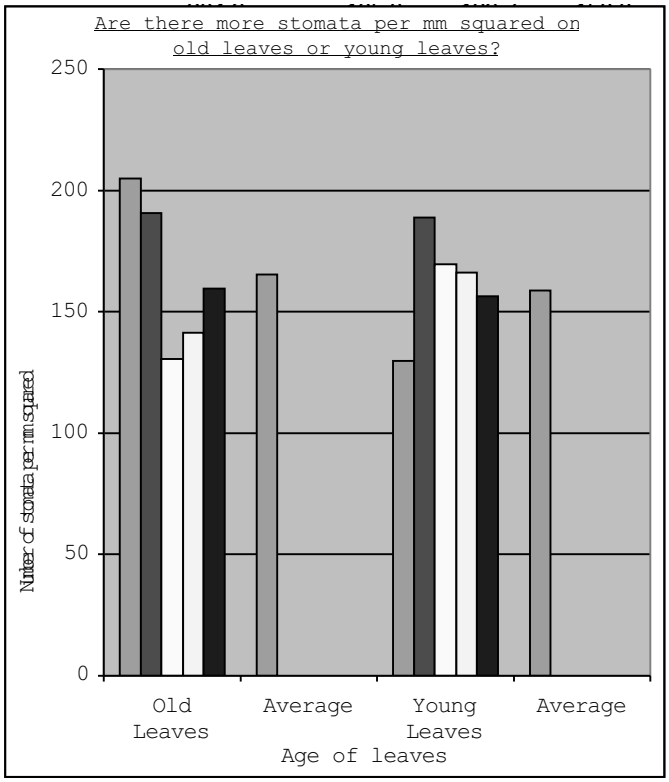
I thought these results looked quite random, so I decided to look at some more leaves. I prepared them in exactly the same way as I had done before: The leaves were of the same size, colour, and position on the same bush. I used the same thickness, area and brand of nail-varnish in the same position on the underside of the leaf, and left them for the same amount of time. When I made observations, I used the same microscope, power and eyepiece as I had done the first time. I counted the stomata in the same way as I had done before, and worked out the calculations in the same way. However, I only had time to do two more young and old leaves. I added these results to the table:

	Old Leaves	Young Leaves
Number of stomata per mm^2	204.9	129.7
	190.8	188.7
	130.7	159.6
	141.3	166.7
	159.0	156.1
Average	165.3	160.2

<u>Analysing</u>

My results indicate that the number of stomata varies from leaf to leaf, but when the mean is compared between old and young leaves the results are very similar - they only differ by 3%, or 5 stomata. This suggests

that the age does not affect the number of stomata per mm^2 on a leaf. This is a graph of my results:



My findings support my prediction because they prove that age does not affect on the amount of stomata per mm^2 that a plant has. I think this is due to the way in which leaves grow. New leaf cells develop around the edges of the leaf in meristem tissues, the cells of which are able to divide infinitely. After division is complete, genetic programming determines what sort of cell it will become. As a result, whether a cell becomes the one of a stomata's guard cells or not, and the frequency at which they develop is already fixed by the plant's genetic programming, which does not change. Therefore, the number of stomata a leaf has per mm^2 will remain constant throughout its existence, regardless of its age. The number of stomata may vary from leaf to leaf due to differences in the stomatas' positioning on the leaf.

Evaluating

I think that the evidence I obtained is as accurate as I could make it. The observations I made are quite accurate, because I counted the stomata as well as I could, but, due to slight imperfections on the surface of the nail-varnish, some small parts were not in focus. This meant that in these parts the stomata were difficult to count. However, I do not think that I made any major mistakes. I do not think that there are any anomalous

results. The procedure was very suitable, because using a nail-varnish peel is the only way to count the stomata with the equipment that I had access to. The microscope I used relied on light shining through the specimen, and it is impossible to look at leaves in this way due to their thickness. The reliability if my evidence could have been improved by obtaining more results. If I had looked at more leaves, then I would have been able to calculate more accurate averages. Also, I should have ensured that I always looked at exactly the same part of the leaf, as precisely as possible, in case the number of stomata per mm^2 varied greatly depending on where it was on the leaf. I always painted my nail-varnish in about the same place, over an area of 1cm, but my microscope was only able to look at $0.283mm^2$. This means that the particular place on the leaf that I looked at could have varied enormously from leaf to leaf. I think my evidence is sufficient to support a firm conclusion, because, despite the fact that I only did 5 of each different age, they still showed that age does not seem to have an effect on the number of stomata per mm^2 a leaf has. However, I think that there is a way that I could have provided additional evidence for a conclusion. The best way to discover whether age affects the number of stomata per mm^2 that a leaf has would be to look at the same leaf as it matures. I could have left the leaf growing on the tree, and painted the nail-varnish on without taking the leaf off the tree, then taken the dry nail-varnish peel back to the laboratory to observe. I could have marked where I had painted the nail-varnish the last time, and each month, I could have repeated the experiment, comparing it to the last result I had got. This way I would know for sure how age affects the stomata, because I would be looking at the same leaf as it grew in the same conditions, rather that different leaves, growing in slightly different conditions. Also, when I drew a graph of my results, the data would be continuous, allowing me plot a line graph and discover the true relationship between the age of a leaf and the amount of stomata it has.