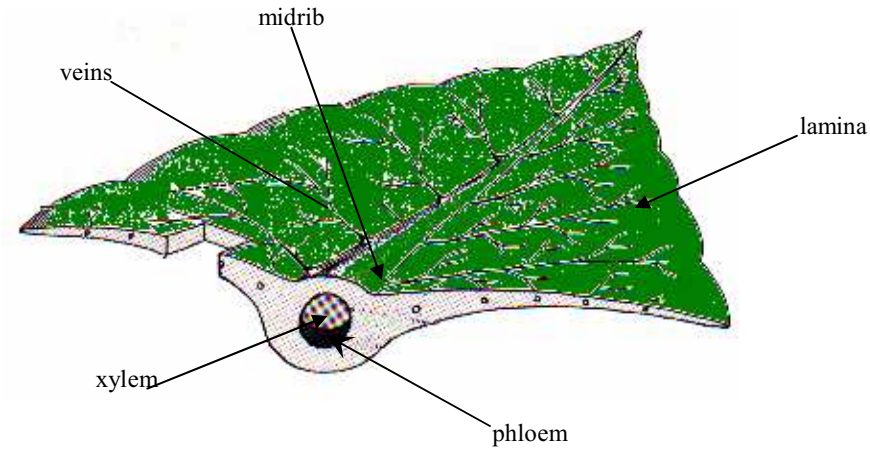
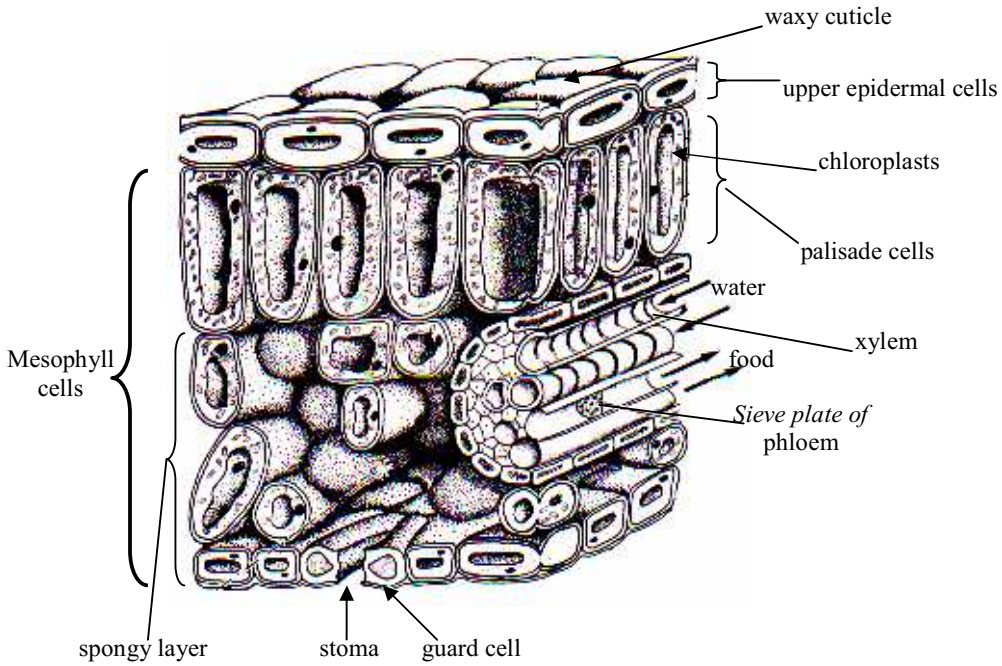
DENSITY OF STOMATA AND DESICCATION RATES

Since the investigation is to study and experiment with one of the factors that effect the amount of water lost from a leaf, it is important to describe the structure of the leaf.

Enlarged portion of leaf



The structure of a leaf



The function of the leaf is pertinent to its structure. A leaf usually consists of the following parts: -
Petiole - the narrow stalk of the leaf attaching it to the stem.
Lamina - the photosynthetic part of the leaf.
Mid-rib and veins - consist of many tiny tubes which convey water into the leaf and carry food from it
Epidermis - the outermost layer of cells of a plant. This layer is one cell thick.
Waxy cuticle - covers the epidermis and reduces water loss.

The lower epidermis consists mainly of stomata. Stomata are pores, which lead to an extensive system of air spaces between the cells of the leaf. The spaces allow diffusion of gases in and out of the cell.

Before deciding on which variable to vary it is essential to establish all the factors both environmental and structural influencing water loss from leaves. This is to ensure that during experimentation the other factors are constant or are controlled.

Environmental factors

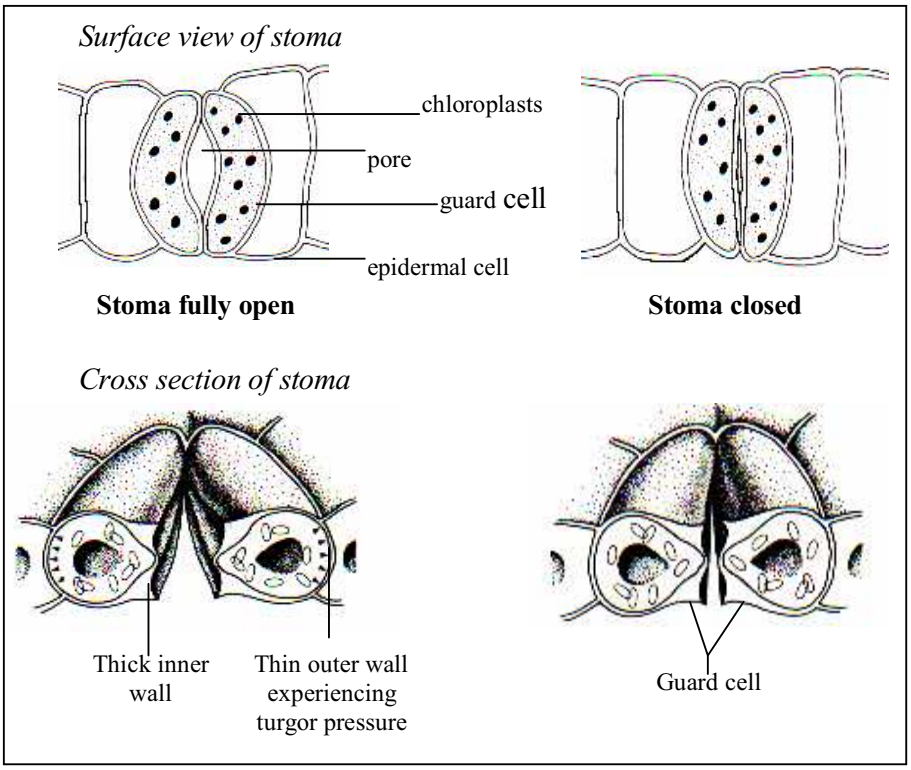
1. *Wind velocity*
   Movement of the air surrounding the leaf removes the surface of moist air formed around the leaf. This increases the diffusion gradient so desiccation occurs faster.
2. *Humidity*
   The more humid the air around the plant, the lower the desiccation rate. This is due to the intercellular spaces of a turgid leaf being saturated with water thereby decreasing the concentration gradient. The rate of desiccation would therefore be lower in wetter air.
3. *Intensity of light*
   Light stimulates the opening of the stomata, water loss occurs through stomata so desiccation will occur faster in light.
4. *Temperature*
   Increased temperature increases the rate of evaporation by providing the latent heat of vaporisation to the water. Warm air holds more water vapour than cold air.

Structural factors

1. *Cuticle*
   The thicker the waxy cuticle on the surface of the leaf, the less water lost by desiccation
2. *Sunken stomata*
   Stomata in some leaves can be sunken in stomatal pits thereby retaining water vapour and cooling the leaf.
3. *Distribution/ number of stomata*
   Stomata are found mostly on the surface of the leaf away from the sunlight and wind (abaxial). On the surface facing the sunlight and wind (adaxial) plants often have few or no stomata present. Stomata primary function is gas exchange, however when open water is prone to pass through.
4. *Leaf surface area*
   The smaller the surface area of the leaf the more efficient the plant is at water retention, desiccation is reduced as surface area is decreased.

It is intended that the factor to be varied is stomatal density, however it is impossible to control the stomatal density of a leaf therefore leaves of different plants/trees will be collected. It is known that stomatal density differs in different plants/trees and in plants growing in different climatic conditions.

STOMATA



The number of stomata in any one leaf, range anywhere between 100 and 100 000 per cm^2. Each stomatal apparatus consists of two guard cells and an adjacent epidermal cell. All of which surround a pore called a stoma. The main function of the stomata is to enable gaseous exchange. Water vapour can therefore be lost through the stomata by diffusion. This is a process called transpiration (desiccation). When water on the wall of the mesophyll evaporates and diffuses out of the leaf, water is lost via the cuticle.

It is known that plants adapt to their environmental conditions and one of the major adaptations is to vary the stomatal densities on their leaves. Plants can be split into two main categories called xerophytes and mesophytes. Xerophytes are plants that have adapted to environments that experience water deficits therefore should have fewer stomata. Mesophytes are those adapted to environments experiencing plentiful water so have more stomata to maximise gas exchange.

In order to keep the investigation fair it is required that all other factors affecting desiccation rate are kept constant. The factors shall be kept constant in the following ways: -

- Wind velocity - the experiment will be carried out in a lab with all windows closed at the rear of the building, so no direct draughts are experienced.
- Humidity - the experiment is carried out inside so humidity is usually constant at all times.

- Intensity of light - All the leaves will be put near the same source or absence of light; the lab has fluorescent tube lights, which are all switched off at the same time.
- Temperature - the experiment is carried out indoors where thermostatic heating is present. A thermometer will also be placed near the experiment to ensure constancy. The experiment will not be carried out near any direct heat or cold.
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The structural factors are impossible to control however relevant observations will be taken into account.

Most of the water is lost through stomata therefore transpiration rate is related to the stomatal density.

The hypothesis to be tested is:

AS STOMATAL DENSITY INCREASES THE RATE OF DESICCATION WILL INCREASE.

APPARATUS AND MATERIALS

Justification for apparatus use and method

The apparatus used for such an experiment is basic. However the main piece apparatus used to measure the rate of desiccation has to be verified.

There are two main methods:

- using a potometer which measures the rate of water absorption by the leaf
- using a top pan balance which measures the loss in weight as the loss in water

The weight loss method is the preferred method because it is easier to administer.

Rather than use one leaf to measure water loss several should be used so a significant water loss is measured.

It has been suggested that stomata are only present on the abaxial side of the leaf it has however been found that they are present on both side, just not in equal numbers. Stomata will therefore be counted on both sides and added together to calculate the density per cm^2.

Safety precautions and ethical implications

- Take care when picking leaves of nettles and other harmful plants.
- Any visible organism on leaves should be left and not picked for both safety precautions and ethical implications.

Other major ethical implications do not exist in such a small-scale experiment.

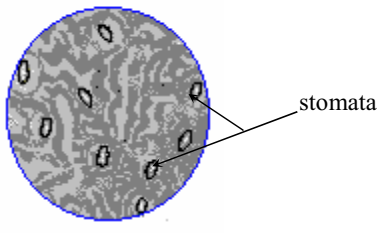
APPARATUS

10 leaves of 6 different plants		scissors	string	balance
microscope	nail varnish	slides	cover slips	scaled slide
clear cellotape	grid paper	calculator	pencil	labels
stop watch				

METHOD

1. Collect ten leaves (about the same size) from six *different* plants/trees.
2. Ensure they are handled carefully and kept separately.
3. Take the first type of plant leaves and paint both sides of the leaf with nail polish.
4. Leave the polish to dry for around ten minutes.
5. Cut two pieces of clear cellotape
6. Stick one of the pieces of tape, to the corner of abaxial side of the leaf.
7. Using it as a handle pull the surface of polish off the leaf.
8. Using the scissors remove the tape and place the cast on a slide labelled abaxial.
9. Place the cover slip on the cast using water if necessary. (use the correct procedure for slide preparation)
10. Repeat steps *6 – 9* only now for the adaxial side.
    Dispose of the leaves.
11. Place the abaxial slide under the microscope and count the number of stomata in the field of view.

This should be the view through the eyepiece.



12. Move the slide around and recount. Repeat this three more times. So in total five counts have been made.
13. Repeat steps *11 & 12* for the adaxial slide.

14. Repeat steps *3 – 13* for the remaining five types of plant leaves. Fill the results in the table below.

TYPE	1	2	3	4	5	6
COUNTS						
MEAN						

15. Determine the area of the microscope field of view using the scaled slide.
    Then use the formula below:

$$\pi r^2$$

Where *r* is the radius of the field of view

16. Calculate the density of stomata per leaf using this formula:

$$(B + D)/ A$$

Where,
B is Number of stomata on abaxial side.
D is Number of stomata on adaxial side.
A is Area of field of view in micrometers.
Divide by 1000 to get the density in mm.

17. Weigh a piece of grid paper record the result.
    The paper used should have no margins and be whole.

The area of the paper should be known and be exact.

18. Draw around one of the nine leaves of the first type of plant.

19. Cut out the leaf trace and reweigh the paper. (not the leaf shape paper)

20. Use the mass ratios to calculate the surface area of the leaf.

R= (C / W) * 100

Surface area of leaf = 2(R% of area of original paper)

Where

R is ratio

C is weight of cut paper

W is weight of whole paper

21. Repeat steps *17 – 20* for the remaining fifty-three leaves.

22. Record the results in the table below.

	SURFACE AREA (cm^2)					
TYPE	**1**	**2**	**3**	**4**	**5**	**6**
1						
2						
3						
4						
5						
6						
7						
8						
9						
TOTAL						

23. Cut a piece of string about 10cm long and weight it.
24. Use the string to tie eight of the nine leaves together using the petioles and reweigh.
25. Deduct the result to step *23* from step *24*.
26. Repeat steps *23 –25* for the other 5 types of leaves.
27. Hang the leaf lines up in the conditions described.
28. Reweigh every 2 hours. For the whole day.

The control

1. The ninth leaf of each type should be weighed individually.
2. Then covered in nail polish and left to dry
3. Then reweigh
4. Reweigh every 2 hours.

Because it is the control no weight loss should be experienced.

The total leaf surface area of the eight leaves should then be used to calculate the water loss per m^2. This can be done using the below calculation:

(10000/TOTAL SURFACE AREA) * AVERAGE WATER LOSS.

Having done this, quantitative results have to be calculated by calculating the average % mass loss. Graphs will be plotted and the gradients calculated.

The results can be tabulated below:

STOMATAL DENSITY	WATER LOSS

The Rank Spearman test will be undertaken to test the results to see if they match the hypothesis.