

**Experiment to investigate the relationship between the number of stomatal pores on the upper and the lower surfaces of the leaves of a mesophyte plant and the rate of transpiration from those surfaces.**

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**The aim of the experiment**

The aim of the experiment is to investigate how the number of stomatal pores is related to the rate at which water is lost from the leaves. A mesophyte plant is chosen and the comparison is between the upper and the lower surfaces of its leaves.

**Experimental hypothesis**

Taking into account the relative background scientific information, it is expected to be proven that the rate of transpiration from a leaf of a plant is proportional to the number of stomatal pores on the surface of that leaf.

**Null hypothesis**

Negative results would be to establish that the transpiration rate is inversely proportional to the number of stomatal pores or is not affected by it whatsoever.

## Introduction

Water is the universal solvent for a huge amount of chemical substances in all living organisms. Plants require water for many different reasons. It is used to uptake inorganic minerals from the ground, to transport nutrients such as amino acids and carbohydrates along their stems and to control their temperature. Water plays a very significant role in the life cycle of plants being a vital assumption for their life.

Plants take up water by the younger parts of the roots. Water then moves across the cortex of the root towards the central tissues via apoplast, symplast or vacuolar pathways. Whichever the root is, it finally enters the xylem tubes and is transported upwards to end up in leaves from where it evaporates.

This movement takes place at various rates at different parts of the day and different seasons of the year. It is maintained by and hence dependent on the difference of the water potential between the soil and the atmosphere. Water evaporates from the leaves creating a water potential difference between the leaves and the stem which draws water upwards. By the same way water is drawn from the soil into the roots and from the roots into the stems.

The above mentioned evaporation of water through the leaves of a plant is referred to as transpiration. It is easy to see that the rate of transpiration is very important in respect to the movement of water inside the plant since it triggers the establishment of the water potential difference. Water is also lost from the lenticles of the stems but in the leaves this takes place to a much greater extent.

The rate of transpiration is affected by several environmental factors. The difference of the water potential between the leaves and the surrounding air is very important, hence humidity is one of those factors. Wind increases the rate of transpiration while temperature might have different effects on it. There are also several factors which affect the transpiration rate in an indirect way, by controlling the stomatal aperture. Although there is some evaporation from the cuticle, water, as mentioned above, is mostly lost from the stomatal pores. Therefore light intensity, carbon dioxide/oxygen ratio, water stress conditions and the biological clock effect are all closely related to the rate at which transpiration occurs. Besides the number of stomatal pores itself is proportional to the rate at which water is lost from the leaves, which is exactly what this experiment aims to prove.

The experiment consists of two major parts

**Part 1 :** Determination of the rate of transpiration on the upper and the lower surfaces of sample leaves of a mesophyte plant.

**Part 2 :** Determination of the number of stomatal pores per unit area on both surfaces

### Equipment

#### **Part 1**

1. *Cobalt chloride paper*, is an easy and straightforward way to compare the rate of transpiration at a specific area of the leaf. It is blue when dry but rapidly turns pink when in contact with moisture.
2. *Microwave*, to dry pieces of the cobalt chloride paper in order to prepare them for attachment on the leaf blade (see method).
3. *Sellotape*, to attach the cobalt chloride paper on the leaf
4. *Forceps*, to prevent contact of cobalt chloride paper with hands (see precautions).
5. *Petridish*, as containers to engulf pieces of cobalt chloride paper when warmed in the microwave.
6. *Stopwatch*, to measure the time taken for the cobalt chloride paper to change colour (see method)

#### **Part 2**

1. *Light microscope*
2. *Transparent nail polish*, to remove a thin layer from the leaf (see method)
3. *Forceps*
4. *Microscope slides*, to examine the samples under the light microscope

### Plan

Prepare small pieces of cobalt chloride paper of appropriate size to fit on the small leaves of the plant (eg. 2cm). Carefully place them in petridishes by means of forceps, to avoid contact with hands. Randomly choose a statistically viable number of leaves on the plant (eg. 10) and number them by attaching small labels on the stems.

Insert a petridish into the microwave and heat for approximately 5 minutes. Again, by means of forceps, quickly attach the blue cobalt chloride paper on the leaf by a sellotape piece and start timing.

Observe the colour change of the cobalt chloride paper as water evaporating from the leaf turns it pink from blue and for more accuracy, compare with a moist sample. Stop timing as soon as the blue colour is lost. Repeat the procedure for five times on each surface of this leaf, then proceed to another leaf until all ten are examined. Record the results into a table.

Using a calculator obtain random coordinates on upper and lower surfaces of the leaves and apply a thin layer of nail polish on those sample areas. Leave to dry for 10 to 15 minutes, then remove the layer by means of forceps and transfer onto microscope slides. Count the number of stomata on a fixed area under light microscope. Record the results into a table.