An Investigation into Water Loss from Plants

PLAN

Preliminary Experiment

Diagram

Method

One dicotelydonous leaf was taken and approximately 1cm² of clear nail varnish was painted both on the adaxil (top) and abaxil (bottom) surfaces of the leaf. It was then left for approximately 10 minutes to dry and gently peeled off using a scalpel after the 10 minutes. The peeled nail varnish was then examined first at low power (x40 magnification) and then on high power. It was noticeable that the number of stomata on the top of the leaf was significantly less than the number of stomata on the bottom of the leaf. This provided basic information upon which the plan could be based. It gave foundation for the plan which will be an extension of these findings.

Sketch of Observation

Top Surface Bottom Surface

Conclusion

It can be concluded that there appears to be more stomata on the bottom of the leaf indicating that there may be more chance for transpiration from this area.

Scientific Knowledge

Diagram of Leaf

Each leaf on a plant is comprised of layers as shown above which are not tightly compact and instead have many air spaces necessary for gaseous exchange (to allow exchange of carbon dioxide and oxygen for photosynthesis and respiration). The air in the internal spaces of the leaf has direct contact with the outside air through the stomata. If there is a higher concentration of water vapour within the leaf than there is in the outside air a diffusion gradient will be created and water vapour can diffuse out of the leaf through the stomata, this process is transpiration.
The rate of transpiration will vary in different climatic conditions as this will influence the concentration of water vapour in the air. The higher the humidity of the air the higher the water concentration in it so the concentration gradient will be lower leading to a low rate of transpiration. If the wind speed is high saturated air around the leaf is constantly being replaced by dry air leading to a higher rate of transpiration. If the temperature is high the air has a higher capacity for water vapour and is dryer so the concentration gradient will be steeper leading to more transpiration, also more water in the plant cell walls will be heated turning to water vapour and this will be lost by evaporation. The light intensity will also vary the rate of transpiration as the lighter it is the wider the stoma open to allow more gaseous exchange for photosynthesis. This leads to more water vapour being lost by transpiration.
The opening and closing of stomata varies the contact between the outside air and inner cells of the leaf which determines the rates of transpiration. For photosynthesis

to occur the stomata must open to allow carbon dioxide to enter, which will happen during sunny periods. The guard cells are forced to become turgid and open when the light intensity is high as this is when photosynthesis starts. This means that Carbon Dioxide is used up during photosynthesis increasing the pH of the cells which activates an enzyme which converts starch to glucose. As starch is converted ATP energy is released giving cells energy to activate proton pumps and the cell pumps out protons. Therefore K+ ions diffuse in and make the solute potential more negative so more water moves in and the guard cells become turgid. The guard cells are semi-circular with a thicker cellulose wall on the inside so therefore the inside wall has less elasticity. As they are forced to become turgid the pressure builds up forcing unequal curvature to occur and the outer walls stretch while the inner walls remain relatively unstretched. Therefore the guard cells open creating stoma which inevitably increases the rate of transpiration.

As water leaves the leaf by transpiration it creates a transpiration stream which pulls up other water molecules so water is constantly moving up the xylem. The theory of Dixon Jolly is that due to water molecules being cohesive the water molecules are pulled along each route by the previous one and so columns of water are pulled along.

Water moves through the leaves taking one of three different routes. The apoplast route is the movement of the water (by diffusion) from one cell wall with a high concentration of water through to the next cell wall with a lower concentration of water (down the concentration gradient) eventually leaving by evaporation when a cell wall is in contact with the air at the stomata and the cell wall has a higher concentration of water than the air. Water vapour will leave by diffusion (in the form of evaporation) as the cell wall has no membrane that the water must pass through.

The symplast route is the diffusion of water from the cytoplasm of one cell through the plasmodesmata to the cytoplasm of a neighbouring cell with a lower concentration of water. It will eventually leave when the final cell neighbours an air space (stomata) creating a concentration gradient is created. Water will leave via osmosis as it must travel through the cell membrane to escape into the air.

Water is said to take the vacular route when it moves from the vacuole of one cell, via osmosis through the tonoplast membrane, into the cytoplasm through the plasma membrane, and through the cell wall into the next cell and into the vacuole through the tonoplast membrane of the neighbouring cell travelling down the concentration gradient. It will eventually leave the cell neighbouring an air space as the cell will have a higher concentration than the air causing water to leave through the tonoplast and cell membrane by osmosis.

All these routes will bring water from the cells to the air spaces in the leaf and only if there is a concentration gradient between the water vapour in the leaf and water vapour in the air, water will leave by transpiration.

There are more stomata on the lower surface or the leaf so this is where most water is lost from as transpiration occurs. More are located on the bottom of the leaf as it is necessary to have them and the bottom surface is the most practical place. This is because if they were located on the top, this is the surface where the sun will beat down upon, heating the water molecules even more causing more evaporation and a steeper concentration gradient so more transpiration. Having the stomata underneath the leaf ensures that they are in the shade therefore while air can still reach it for gaseous exchange the leaf is slightly cooler and so less water will be lost. This reduces the risk of the plant from losing too much water and becoming dehydrated.

Prediction

Based on the preliminary experiment and scientific knowledge above I hypothesise that there will be more transpiration therefore loss of water from the abaxil surface of the leaf than from the adaxil. This is due to the larger number of stomata on the bottom of the leaf therefore a larger surface area of cells is in contact with the outside air and so more water can escape.
Rack1 will have the maximum amount of water loss as no stomata are blocked so water will be free to escape through these via transpiration.
Rack 2 will have a smaller mass loss than tube 1 as stomata on the top layer are blocked therefore the amount of stomata is reduced less cells are exposed to the air at the stomata therefore less transpiration will occur.
Rack 3 will have even less of a mass loss than tube 2 as the bottom will be covered in Vaseline and as the bottom contains the most stomata, this will limit the amount of cells exposed to the air and very little transpiration will occur as there are very few stomata on the top surface of a leaf.
Rack 4 will have minimal water loss therefore the mass should not change dramatically from the original weight. This is because all stomata on both the top and the bottom surfaces of the plant will blocked therefore no water from the plant cells of the leaf will be exposed to the air, therefore no concentration gradient can occur and water cannot escape from the leaf. However, the masses may change slightly as water may be lost by transpiration through the very little number of stomata on the petiole.

Apparatus List

- Vaseline: Used to spread over the leaf and block stomata on one certain surface to monitor water loss from the other surface. It's used as it is easily spread, cheap, non-permeable and it acts as a seal for transpiration.
- Metal Spatula: Used to apply Vaseline in one smooth continuous layer. It is more accurate than cotton wool as it will spread more efficiently and hold less back and makes it easier to apply. More accurate and safer than spreading with finger.
- 20 healthy privet shoots holding 5 leaves each: All must be from top layer of bush to ensure than the numbers of stomata will be similar as they have been exposed to the same amount of sunlight. All leaves must be approximately the same size and all from the same bush to ensure continuity.
- 20 test tubes: To place the shoots in and hold them in water to ensure they have a continuous supply of water. This is better than keeping them dry which means that they could run out of water to lose and so an accurate overview of water lost would not be obtained.
- 5 test tube racks: To hold the test tubes and prevent the water spilling.
- Vegetable Oil: To make a thin layer over water in test tube to prevent water loss by evaporation so that the difference in mass is entirely due to transpiration. Vegetable oil is used as it is the cheapest type of oil, does the same job and it much more practical to use as it will not stain everything black.
- Syringe: To take up and measure accurately 1cm^3 vegetable oil and place it in test tube to settle on top of water. The syringe should measure to 0.1cm^3.

- Distilled water: To keep the shots in a constant supply of water to make sure they do not run out so that all possible transpiration can occur. Allow 25cm³ per test tube.
- Measuring Cylinder: Used to accurately measure 10cm³ distilled water for the shoots in the test tubes. The measuring cylinder should measure to 1cm³.
- Scientific scales: To weigh the shoot before and after time delay to observe how much water is lost during transpiration. They must be scientific so that they are accurate enough to pick up even the slightest difference in mass due to water loss so that the percentage of water lost can be found. The scientific scales can measure to 0.01g, which will make readings very accurate.
- Calculator: To use the original weight and the mass lost to work out a percentage mass lost. This is more accurate than simply using a mass lost and heavy leaves would be holding a lot more water, therefore lose more and this would not provide a true measurement of water lost.
- Light: To ensure that all plants have the same exposure to light so that they all photosynthesise the same amount so that the stomata all open the same amount allowing the same amount of water to loss.
- Thermostatically controlled environment: A room should be selected where the climatic changes will not change the temperature of the room. The shoots should all be placed at the same place in the room to try to maintain a constant temperature for each shoot.
- Thermometer: To monitor the temperature and keep it constant. It should be a wall mounted thermometer so that it can monitor the temperature of the air accurately.
- Clear Nail Varnish: To paint thin layer over leaf to gain an imprint of the leaf.
- Scalpel: To gently peel nail varnish off in one coat so as a clear imprint is obtained.
- Slide: Upon which to place layer of nail varnish on under microscope.
- Microscope: To examine and observe difference in number of stomata.
- Graticule: To measure stomata to obtain difference in surface area of stomata to explain differences in transpiration between top and bottom surfaces. It is accurate as it measures down to 0.1mm (100 micrometres).

<u>Method</u>

- Cut 20 healthy shoots from the same privet bush, all from the top layer and prune them so that they each have 5 of approximately the same size leaves on them. Give them each a number from 1 to 20.
- Leave all leaves on 5 shoots as they are and simply place in test tubes in a test tube rack. Label this rack 1.
- Using a metal spatula spread an even layer of Vaseline across the top layer of all leaves on another 5 shoots and place them each in a test tube and put the test tubes in a test tube rack labelled 2.
- Using the metal spatula spread an even layer of Vaseline across the bottom layer of all leaves on another 5 shoots and place each one in a test tube and put tubes in a test tube rack labelled 3.
- Using the metal spatula spread an even layer of Vaseline across both the top and bottom surface of all leaves on the remaining 5 shoots and place in test tubes and put them in a test tube rack and label it 4.
- There should now be 4 test tube racks each containing 5 test tubes.

- Using a syringe measure out 10cm³ distilled water and add it to the first test tube. Repeat this for all 20 test tubes.
- Add 10 drops vegetable oil to the first test tube. It will float on the water to stop evaporation. Repeat this for all 20 test tubes.
- Place the extra empty test tube rack on the scientific scales and turn on at 0. Then place each test tube in the rack one at a time and weigh them and record the reading. Replace all test tubes in their original rack.
- Place all test tubes along the same bench, under an electric light bulb. Make sure the leaves of each shoot are not touching to prevent blocking of the stomata or spreading of vaseline.
- Note the time and leave the leaves to transpire. Return and take a measurement in the original way at the same time the next day (24hours later) and the same way 48 hours later.
- Record all results in results table as shown below.
- Take the new mass from the original mass to establish a change in weight, therefore amount of water lost through transpiration. Use these figures to calculate a percentage mass change.
- To further the investigation into number of stomata paint nail varnish over top and bottom of four randomly selected leaves. Leave to dry for 10 minutes.
- Carefully peel off using scalpel and place on slide.
- Place slide under microscope and place graticule on top of it.
- Use graticule to measure the area of view and then count the number of stomata in this area. Use the microscope on x 100 magnification.

Justification Of Method

The above method has been chosen as it is the best way to generate valid results.

- The percentage mass change ensures that the actual amounts are not being compared but instead the relative percentages which is a much better sign of water loss. This is because leaves weighing different amounts will have different amounts of water to lose. Using the percentage change eliminates this from the results.
- A graticule is used to measure the stomata, which is much more accurate than simply counting them as the actual amount of stomata in a certain area could be found.
- 4 leaves are examined under the microscope to find an average number of stomata.
- 5 repeats are performed for each condition so an average can be taken to may the results more accurate and representative of the all privet leaves.
- A control in the form of untreated shoots (all in rack 1) is set up so that the results can be compared to a ‘normal’ shoot so the effects of treatment can be seen.
- All shoots are from the same part of the same bush to ensure continuity and eliminate unnecessary room for error. This may happen if completely different leaves are used as they may have a different number of stoma or the stoma on a different surface. Shoots from the bottom of a bush may have a different amount of stomata than those from the top which would leave room for error in results. Taking all shoots from the same place eliminates this variation.

- Test tubes are organised and labelled and placed in labelled racks to avoid confusion and maintain accuracy of the results.
- The environment in which the experiment is carried out is constant and the same for all shoots. This eliminates other factors which may cause variation in the rate and amount of transpiration.
- Statistics (T test) will be used to test the results accuracy to further ensure the reliability of the results.

Sample results table:

Number of Shoot	Original Mass (g)	Mass after 24 hours (g)	Mass after 48 hours (g)	Difference in mass (g)	Percentage mass lost (%)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Safety Precautions

- Goggles: To protect eyes from Vaseline and blunt instrument (spatula).
- Care: When carrying spatula and scalpel care should be taken, equally when carrying fragile test tubes or measuring cylinder.
- Clean up spillages: Water or oil could be spilt, ensure this is cleared up quickly and precisely to stop people slipping on it.
- Overalls: These should be worn to protect clothing from chlorophyll stains, water, oil or Vaseline.
- Latex Gloves: Should be worn to avoid contact of skin with leaves in-case of sweat or grease blocking stomata or bacteria being transferred. Also worn in case of plant being poisonous or an irritant to skin.
- Care: When handling leaves care should be taken to make sure that leaves to not contact skin, eyes or mouth as some may be irritants or poisonous.

Variables

- Which surface of the leaf Vaseline is applied to.
- Number of stomata.
- Mass of shoots.
- Amount of Vaseline: Attempt to keep equal by spreading even thin layer.
- Amount of Water: Control by using measuring cylinder to measure 25cm³ into each test tube
- Amount of Oil: Control be using syringe to measure 10cm³ into each test tube.
- Size of leaves: Attempt to keep all leaves approximately same size.
- Type of shoots: Control by taking every shoot from the same plant.
- Number of leaves: Control by pruning the shoots down to 5 leaves.
- Temperature of room: Kept constant by electric heater.
- Humidity of room: Kept constant by electric heater.
- Condition of leaves: Attempt to pick healthy shoots and leaves.
- Amount of light leaves are exposed to: Kept constant by being placed under light bulb.

Results:

Number of Shoot	Original Mass (g)	Mass after 24 hours (g)	Mass after 48 hours (g)	Difference in mass (g)	Percentage mass lost (%)
1	28.68	27.79	27.10	1.58	5.51
2	28.88	27.91	26.98	1.9	6.57
3	29.85	28.98	28.16	1.69	5.66
4	32.75	31.31	29.93	2.82	8.6
5	31.28	30.72	30.16	1.12	3.58
6	31.61	30.99	30.20	1.41	4.46
7	30.22	29.49	28.56	1.66	5.49
8	31.23	30.36	29.83	1.4	4.48
9	30.76	30.11	29.61	1.15	3.73
10	29.71	29.16	28.76	0.95	3.19
11	29.94	29.44	29.02	0.92	3.07
12	28.16	27.7	27.27	0.89	3.16
13	30.12	29.71	29.35	0.77	2.55
14	31.36	31.16	30.98	0.38	1.21
15	28.43	28.15	27.89	0.54	1.89
16	30.94	30.59	30.32	0.62	2.00
17	31.45	31.22	31.03	0.42	1.33
18	34.01	33.8	33.62	0.39	1.14
19	33.13	32.52	31.38	1.75	5.28
20	33.11	32.90	32.79	0.32	0.97

Average Percentage mass lost: No Vaseline – 5.984%
Vaseline on top – 4.27%
Vaseline on bottom – 2.376%
Vaseline on both – 2.144%

Microscope Findings

Under a microscope at x100 magnification it was found that the view had a diameter of 0.57mm therefore the area of the view was 0.255mm². The diameter was measured using a graticule and the cross-sectional area obtained using the formula:
The area was calculated to obtain a constant area that the stomata were counted in as all leaves were different sizes so would have had different total amounts of stomata. This eliminated the variable: size of leaf for this particular experiment.

	Number of Stomata				
Surface	Leaf 1	Leaf 2	Leaf 3	Leaf 3	Average
Top	0	0	0	0	0
Bottom	33	18	26	25	25.5

This table displays that the average number of stomata top of the leaf was 0, giving an average of 0 stomata /mm². The average number of stomata on the bottom was 25.5 in 0.255mm² giving an average of 113.3 stoma /mm².

Analysis

The average percentage mass losses for each condition clearly show, as expected, that the highest loss (5.984%) was in the leaves with no Vaseline on either surface. This was expected as all stomata were exposed for transpiration. This is a very high loss when compared to that of the leaves where both surfaces were covered in Vaseline so no stomata on the leaves could transpire (2.144%). However, it was expected that the mass wouldn't change significantly and therefore the percentage mass loss is higher than expected. A certain amount of transpiration would have occurred from the uncovered stems which would account for the water loss even when both leaf surfaces were covered in Vaseline.

The data above shows that the percentage mass loss when the bottom surface of leaves was covered in Vaseline (2.376%) is very similar to that when both surfaces are covered (2.144%) showing that the bottom surface is where the most transpiration occurs from. This can also seen when comparing the percentage mass loss when the bottom surface was covered (2.376%) and when the top was covered (4.27%). There is little difference between the percentage mass loss from the bottom (i.e. when the top surface is covered in Vaseline) and from both surfaces (when Vaseline is on neither surface) this shows that most transpiration occurs from the bottom surface and very little from the top. The bottom loses on average 4.27% of the mass while the top only loses 2.376% which is a significant difference, enough to show that there are very few if and stomata on top of the leaf and many on the bottom.

The data collected supports the prediction in that the leaves in rack 1 with no surfaces blocked with Vaseline has the maximum water loss, rack 2 has a smaller percentage mass loss than rack 1, rack 3 loses less mass than either rack 1 or 2 and finally rack 4 loses the least in percentage mass loss.

The microscope findings showed that on all four of the leaves examined no stomata were found in 25.52mm² of the top surface of the leaf. This explains the dramatic difference in water loss from the top and the bottom as the top surface had no or very little stomata which is why the 2.276% lost from the top was so similar in value to the 2.144% lost when no surfaces were exposed. The bottoms of all leaves had quite a high number of stomata therefore more chance for transpiration so a

greater percentage of the mass was lost. The scientific knowledge has been certified by the experiment which has shown that the percentage mass loss is proportional to the number of stomata on the surface of the leaf., It has also proved that there are more stomata on the bottom of the leaf than on the top surface and therefore more water is lost through the bottom surface (measured by percentage mass loss of the leaf).

T-Test

T Test will be carried out to examine the reliability of the results and the likelihood of them being down to chance rather than scientific laws of nature. If the results show that t is greater than or equal to the critical value then it is more likely down to scientific laws than chance. If it is less than the critical angle it is most likely down to chance and so the hypothesis is disregarded and therefore the null hypothesis accepted.

Percentage mass lost through bottom surface

% mass lost	$(x - x)^2$
4.46	0.0361
5.49	1.488
4.48	0.044
3.73	0.291
3.19	1.117

Mean = 4.27

Standard Deviation = 3.0255 / 4
S = 0.8697

Percentage mass lost through top surface

% mass lost	$(x - x)^2$
3.07	0.481
3.16	0.615
2.55	0.030
1.21	1.259
1.89	0.236

Mean = 2.376

Standard Deviation = 2.721 / 4
S = 0.824

Conclusion

The results from this experiment show that the highest percentage mass lost is from the bottom of the leaves indicating that there are more stomata on the bottom. This is proved by the findings of the microscope experiment that show that there is 113.3 stoma / mm² on the bottom surface, therefore lots of transpiration can occur, explaining the higher percentage mass lost, while there is 0 stomata / mm² on the top of the leaf.

The backs up the earlier prediction stating that 'there will be more transpiration therefore loss of water from the bottom surface than from the top' and also stating that 'due to a larger number of stomata on the bottom of the leaf'. The experiments carried out have confirmed this. The results show that the change in mass is proportional to the number of stomata as with 113.3 stoma /mm² on the abaxil surface an average of 4.27% of mass was lost, however with 0 stomata / mm² an average of 2.376% of the mass was lost from the adaxil surface. According to the prediction no water should be lost through the top surface of the leaf but the experiment shows a 2.376% mass loss through this surface, whilst the microscope findings showed that there were no stomata on the top surface therefore the loss should have been prevented by the waxy cuticle. The microscope found 0 stomata in 25.52mm², however although this has been taken to represent the whole leaf there may be other stomata in unexamined areas of the top surface allowing water to be lost, or the mass loss could be due to various inaccuracies in the experiment to be explained later in the evaluation.

However, the reliability is questioned as there were three anomalies, test tube 4, test tube 14 and test tube 19, this is illustrated by a red ring around them in the results table. The T Test displayed a value of T that is greater than that at 8 degrees of freedom at 1% significance on the table. Therefore the null hypothesis is rejected and the original hypothesis accepted. The results are over 99% likely to be down to scientific laws rather than chance.

Evaluation

The method used in this experiment was a basic and simple one. There are several of the experimental procedures that could be considered unsuitable and may be improved. There were some inconsistencies in the results that indicated modifications were needed. There were three anomalies in the results in test tubes 4, 14 and 19. Test tube 4 had a significantly higher percentage mass loss than that of the other shoots which were all untreated. This could be due to errors in weighing or simply that the leaf contained a particularly high amount of excess water available to be lost. The waxy cuticle preventing much transpiration from the adaxil surface may have been damaged allowing more water vapour to be lost. It could have had an unusually high number of stomata due to natural mutation or a large number of lenticels on the stem of the shoot allowing much loss to occur from the petiole or stem. To improve accuracy the petiole and stem should have been covered to make sure all mass was lost through the stomata of the leaf rather than through lenticels.

Test tube 14 has a much lower percentage water loss than the other similarly treated test tubes. This can be explained by faults in the implementing of the procedure. During the period of leaving the leaves for the first 24 hours to transpire the shoot lost one of its leaves, significantly reducing the amount of stomata available for transpiration to occur through. The reason for the loss of leaf is unexplained as the shoot was healthy but it would be more accurate if this result was dismissed as it brings down the average of all of the rest of the percentage of water lost from shoots under the same treatment.

Test tube 19 had a much higher percentage water loss than any of the other tubes under the same treatment. This is particularly significant as very little loss was expected as both sides of the leaf were covered in Vaseline. This may be due to experimental errors such as not covering the whole of both surfaces with Vaseline if parts are accidentally missed. It could also be due to a high amount of transpiration from the lenticels on the stem of the shoot. Once again accuracy would be improved if Vaseline was spread over the whole shoot as well as selected areas of the leaves.

Although a conscious attempt was made to keep all leaves around the same size, this was not always practical or possible. With such a high number of leaves required a wider range was selected so size varied. This was the main error in the experiment as a great range in size would mean wide variation in the number of stomata meaning that each leaf had different potential for water vapour loss as smaller leaves with fewer stomata would not have the ability for mass transpiration so they would loose less water vapour and their percentage mass losses would be lower. With more time and wider availability of the leaves there would be more room for selection for equal sizes. Another limitation was the spread of Vaseline which was carried out by the human eye. A more accurate method would be to use a pre-measured amount of Vaseline e.g. 1g on each leaf to ensure they all had the same amount and therefore eliminate this variable as a source of error.

The shoots were all leaf for 48 hours in a room where the central heating was not in operation day and night and the guard cells would close due to a lack of light for photosynthesis (as explained in the scientific explanation) so there would be a limited demand for gaseous exchange. This would have an effect on the results as transpiration would cease overnight a smaller amount of water would be lost An improvement to this would be to limit the amount of climatic fluctuations by keeping all shoots in a constant enclosed environment with constant humidity, wind speed, temperature and light intensity. This could possibly be achieved by performing the experiment in an incubator.

The scientific scales were a very accurate way to measure the weight loss but it would be better to leaves the shoots for a longer period of time to allow all possible transpiration to occur. 7 days would be much more accurate than just 2. Repeats of all readings would also improve the accuracy as an average could be taken providing a more reliable result.

The graticule method of finding the view area and how many stomata in that area is quite inaccurate as human error in counting large numbers of stomata could easily occur. To improve this many different areas of the leaves should be examined under the microscope and the number of stomata counted and an average per mm^2 examined found. This would make the number more accurate and representative of the whole leaf.

The prediction, scientific knowledge and microscope findings all indicated that there would be no, or an insignificant amount of, water lost from the top surface of the leaf. However the percentage mass lost showed that an average of 2.376% of the leaf's mass was lost through the top surface. This could be due to areas on the bottom surface being missed when the leaf was being covered with Vaseline, meaning that stomata on the bottom were exposed and water could have been lost from the bottom surface. It is likely that although the microscope found none there are a few stomata on the top surface of the leaf allowing water to be lost from the top surface. It is also likely that the uncovered lenticels on the petiole accounted for a certain amount of the mass lost, these should have been blocked using Vaseline.

Although there were many limitations the results were not extremely distorted as all leaves had been under the same conditions so most limitations affected all of them. The only shoots that can be considered affected were shoots 4, 14 and 19 which gave anomalous results. The T Test showed that it was more than 5% likely that the results were down to chance rather than the result of scientific occurrences. With the suggested improvements the accuracy may be improved and the results may become significant.