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12 Biology

***Practical Number 10*:**
Testing the effect of characteristics of leaves on the transpiration rate of * Rubiaceae, Verbenaceae, Oleaceae, and Rutaceae

Introduction

There is significant difference between plants and animals. It is generally a plant that produces its own food while an animal has to consume other organisms whether plants or other animals. Plants thus can be classified as autotrophs and primary producers in trophic levels.

Plants undergo special process called photosynthesis to produce their ow n food and nutrients. In other words, light energy is converted into chemical energy useful for plants. They require carbon dioxide from the air and water from the ground. Additionally, the light from the sun and pigments like chlorophyll in green plants a re also essential for reaction to take place. Then products for photosynthesis are glucose and oxygen.

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

The importance of sunlight is that it is composed of a wide range of visible wavelengths. It is thus white light. White ligh t is the mixture of all colors from short -wavelength red to longer-wavelength violet (Clegg, 2007). The relationship between sunlight and chlorophyll is that the structure of chlorophyll allows it to absorb some colors of the light and reflect the rest. As chlorophyll is green pigment in green plants, it, hit by white light, reflects only green light into human eyes while absorbing the rest of red and blue light (Clegg, 2007). Therefore, green plants look green to humans. Different pigments for different pl ants work the same. They absorb certain colors of light and reflect specific color.

The reflected color of light determines the color of plants. Then what is the role of absorbed light? Some of light energy absorbed by chlorophyll is used to initiate phot osynthesis. This is reason for the need of presence of sunlight during photosynthesis, especially the first step of the process. The first stage is called photolysis. Light energy is to split water into one oxygen and two hydrogen atoms. Oxygen as waste pr oduct leaves a plant and ATP energy is also produced by absorbed light (Clegg, 2007). The second and last step is carbon fixation. In this part, carbon dioxide (CO_2), hydrogen atoms from photolysis, and energy from ATP together form organic molecules of su gar, or glucose (Clegg, 2007).

As mentioned before, oxygen leaves a plant. Not only oxygen but also water leaves the plant. More exactly, water evaporates from the plant mainly at leaves. This process is so - called transpiration. On the underside of leaves , there are a number of structures of stomata, which look similar to a pore and they have what is called guard cells. In fact, stomata is the passage for intake of carbon dioxide and way out of oxygen. During this, water evaporates as well. When there is l ot of water in plant, guard cells are widely open to transpire. If the plant lacks H_2O, it closes guard cells for no more loss of water. Transpired water should be replaced by more water from soil through the roots, stem and xylem (*Transpiration*, 2009).

The significance of transpiration is a plant continues to pulls up water from the soil whenever water leaves the leaf to operate photosynthesis and bring and spread minerals throughout the plant (*Transpiration*, 2009). What's more, evaporation of water takes up heat and cools the leaves.

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This transpiration rate is as influenced by a number of factors as is photosynthesis. The factors involve temperature, light intensity, humidity, soil water and so on. This laboratory would like to focus on different species of plants.

Research question

How does the different species of plants (*Rubiaceae, Verbenaceae, Oleaceae, Rutaceae*) with different characteristics of leaves affect the transpiration rate of the plants by calculating the volume of water uptake per unit are a per unit time?

Variables

Independent: the species of plants *(Rubiaceae, Verbenaceae, Oleaceae, Rutaceae*) with different characteristics of leaves
Dependent: transpiration rate of the plants (the volume of water uptake)
Controlled:

- The strength of wind:
  The stronger and greater wind blows, the faster the transpiration happens by evaporating water from stomata quickly.
- Temperature of air:
  The higher temperature of air causes faster evaporation of water. Moreover, stomata is close when the temperature is too hot. Closed stomata can not transpire water and it is huge influence.
- Light intensity:
  Photosynthesis briskly takes place in sunny days using sun as the source of light energy. The high rate of photosynthesis can lead to the high rate of transpiration.
- Time of measuring:
  If the apparatus remains longer, it means the plant has more chance of transpiring.
- Humidity:
  Within the high humidity, plants would not seem to lose water to their surroundings as there is already much water in the air. This is associat ed with diffusion. The lesser the amount of water molecule in the air, the more water molecules from plants would move into it.
- The amount of water provided:
  Plants with more amount of water would transpire faster and much more than those in low provision of water.
- Surface area
  The bigger the surface area of a plant, the more there are stomata and thus the greater the rate of transpiration is. When keeping the surface area constant, the number of leaves does not matter if the size of leaves is different. Fo r example, five small leaves and five big leaves have certainly different surface area.

Hypothesis

All four different species of plant * *Rubiaceae, Verbenaceae, Oleaceae,* and *Rutaceae* would transpire at the different rates from each other. Even though ha ving the same surface area, each has its own characteristics of leaves, and therefore the transpiration

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rate would be various. More specifically, the four species have leaves with the different number of stomata, size of stomata, thickness of waxy cuticles , hair, succulence, leaf type and leaf shape. Especially the certain species in *Rubiaceae* family has palmate leaf type, obovate leaf shape, and thick waxy cuticles; *Verbenaceae* has a soft leaf of elliptic shape and small pinnate type and thick waxy cuticle s; the leaves of *Oleaceae* are hard, big elliptic-shaped and palmate type with much waxy cuticles; *Rutaceae* has soft alternate single leaves, ovate leaf shape and thin waxy cuticles. The importance is cuticles. They are waxy layers on the surface of a plant including leaves. Because of their waxiness and thus the hydrophobic nature, cuticles limits the water movement through leaves. The thicker the waxy cuticle layers on a leaf, the slower the transpiration rate would be.

Materials

- a potometer with a rubber tubing, a syringe, capillary tube, and three -way tap
- secateurs
- leafy shoot of plants (**Rubiaceae, Verbenaceae, Oleaceae,* and *Rutaceae*)
- a 50(w) x 30(d) x 25(h) cm tub (big enough for a potometer to be completely put)
- water
- a clamp stand
- 2 x clamps
- stopwatch
- a 30cm ruler (± 0.1 cm)
- sheets of squared paper (1cm grid)
- a calculator

Method

1. Cut four different kinds of branch of plants using secateurs *Make sure the thickness of the branches fits well to the rubbing tube of a potometer
2. Put 35 L of water into a tub (much enough for a potometer to be sunk)
3. Place a potometer into the water completely to allow all water to enter throughout the whole tube
4. Pull plunger to allow water to enter into a syringe
5. Turn a three-way tap (the middle one of a three -way tap for closure) to the side of rubbing tube to close the way for water to enter
6. Push (not completely) the plunger to have all air bubbles go out
7. Then turn the tap again to the side of capillary tube to block the water -passing way
8. Push the plunger to make sure th ere is no air bubble left in the rubbing tube
9. Use secateurs to cut the small end of a leafy shoot of * *Rubiaceae* (this is done in the water tub) with its cut part still kept in water.
10. Insert the branch into the rubber tubing (it is still conducted in the w ater tub) making sure there is no empty space between branch and the tubing.
11. Set up two clamps onto a clamp stand with shorter one to the bottom and longer one to the upper.
12. Have a shorter clamp hold the potometer and longer one catch the branch (refer to the Diagram 1 below)
13. Open the whole passage except the syringe by turning the three -way tap to the side of it.
14. Operate stopwatch for 1minute, measuring from 0mL
15. Measure and record how much distance in *cm* water has been travelled, or reduced (it can be measured via attached ruler -like markings in cm on capillary tube)

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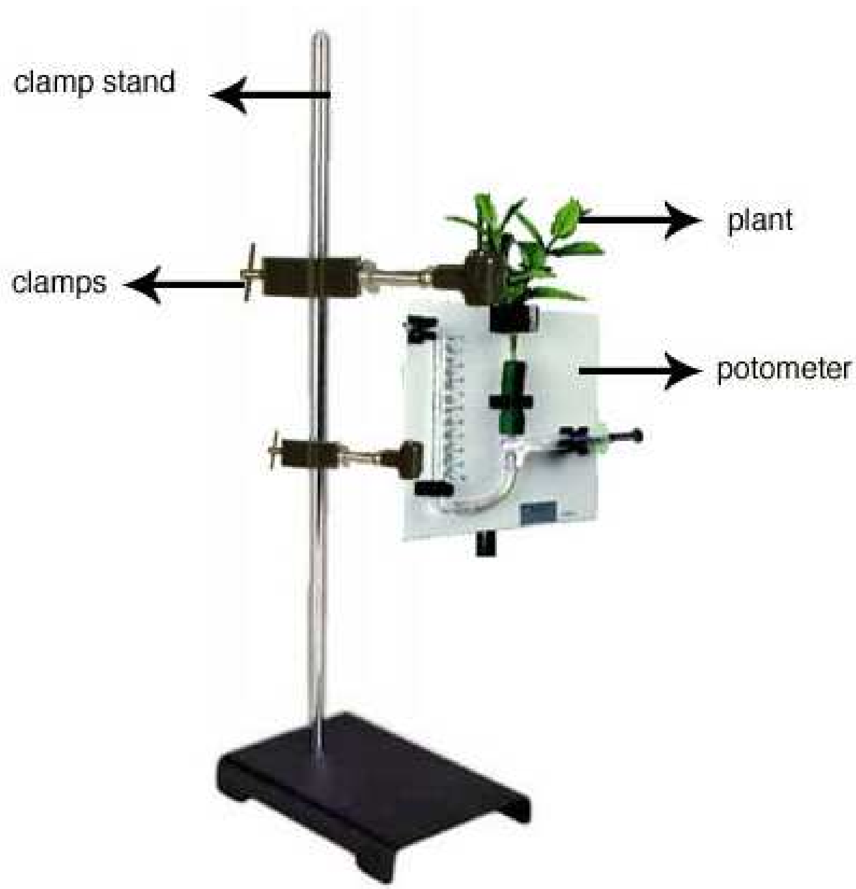
16. Measure the radius of the capillary tube by using a ruler
17. Figure out the area of all detached leaves by putting them on 1c m squared grid and counting 1cm^2 squares.
18. Calculate the volume of water uptake per unit time per unit area
19. Repeat step 1-18 with the rest of other species.
20. Repeat step 1-19 four times more.

* In this experiment, there cannot be control of no treatment.

Diagram 1: a potometer set up on a clamp stand to measure th e transpiration rate



The image of a potometer (*Philip Harris Potometer*, n.d.)

Result

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Table 1: raw data for the distance (± 0.1 cm) that water uptake by four different plants (Rubiaceae, Verbenaceae, Oleaceae,* and *Rutaceae*) has travelled during one minute**

Note: the measurement of potometer is the same as a ruler. Thus uncertainties are based on ± half of the place value of the last measured value, provided there is limit for the precision at both ends of the ruler.

Plant species	**The distance of water uptake (± 0.1 cm)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
Rubiaceae	1.6	0.6	0.5	0.4	0.4
Verbenaceae	0.4	0.5	0.5	0.5	0.5
Oleaceae	0.5	1.8	1.1	0.8	1.6
Rutaceae	0.4	0.4	0.4	0.4	0.5

Table 2: processed data with the average distance that different plant species' water uptake travelled and standard deviation (± 0.1 cm)

Note: 1.6 is circled because it is an outlier. However, the calculation for standard deviation requires at least five samples, so the outlier is included in the calculations for both mean and standard deviation.

Plant species	**The distance of water uptake (± 0.1 cm)**					**Mean (± 0.1 cm)**	**Standard deviation (± 0.1 cm)**
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**		
Rubiaceae	1.6	0.6	0.5	0.4	0.4	0.7	0.5
Verbenaceae	0.4	0.5	0.5	0.5	0.5	0.5	0.0
Oleaceae	0.5	1.8	1.1	0.8	1.6	1.2	0.5
Rutaceae	0.4	0.4	0.4	0.4	0.5	0.4	0.0

Sample calculation

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-Mean

$$\text{Mean} = \frac{\text{all the values}}{\text{the number of the values}}$$

$$= \frac{(1.6 + 0.6 + 0.5 + 0.4 + 0.4)}{5}$$

$$= 0.7 \text{ cm}$$

-Standard Deviation

$$\text{Standard Deviation} = \frac{\sqrt{\sum(x\text{-Mean})^2}}{\sqrt{n-1}}$$

$$= \frac{\sqrt{\{(1.6-0.7)^2 + (0.6-0.7)^2 + (0.5-0.7)^2 + (0.4-0.7)^2 + (0.4-0.7)^2\}}}{\sqrt{4}}$$

$$= 0.5 \text{ cm}$$

Table 3: data required for the calculation of volume of water uptake (cm^3) per minute per m^2 of four different species (**Rubiaceae, Verbenaceae, Oleaceae,*** **and** ***Rutaceae*****)**

Note: the uncertainty of ± 0.0001m² for total surface area is from 1 cm² grid paper which was used for counting the surface area of plant species. When 1cm² converted to m², it is 0.0001m².

Plant species	total surface area (± $0.0001m^2$)	volume of water uptake (± $0.0001cm^3$)	volume (cm^3) of water uptake per unit time per unit area (m^2)
Rubiaceae	0.0072	0.0055	0.7639
Verbenaceae	0.0041	0.0039	0.9512
Oleaceae	0.0351	0.0094	0.2678
Rutaceae	0.0239	0.0031	0.1297

Note: the radius of the capillary tube is 0.5 mm. From this, the uncertainty is ± 0.1mm. Then when 0.1mm³ is converted into cm³, it is 0.0001cm³.

- *Volume of water uptake*

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Volume = $\pi r^2 h$
$= \pi \times (0.05)^2 \times (0.7)$
$= 0.0055\ cm^3$

r (radius of capillary tube) = 0.5 mm = 0.05 cm
h = (mean of) distance of water uptake

- *Volume of water uptake per minute per m^2*

Volume of water uptake per minute per m^2 = volume of water uptake ÷ total surface area
= 0.0055 ÷ 0.0072
= 0.7639 cm^3 per minute per m^2

Observations (qualitative data)
This experiment was done throughout two days. In one day with more humid condition, the rate of water uptake was slower. When there was sometimes wind blown, it affected the speed of water uptake: faster.
What is more, wet branch slowed down transpiration rate. In other words, the first trial with dry branch had the fastest transpiration rate in a minute, compared to the rest of four trials.
The insertion of branch into a rubber tubing was not always perfect. Thus, some water leaked from the crack between two. Though for the solution to that, vaseline was used, it was not that helpful.
**Rubiaceae* had spider nest on its surface of leaf. This somehow would have effect on transpiration rate because the process happens at the surface of leaves. The surface hidden under the nest would not have transpired as much.
In fact, as time and trial goes, the skill of removing air bubble out improved. For the **Oleaceae*, it was the first time to do experiment. It took much more time and had so various values than the last experiment was conducted. The last experiment was for **Rubiaceae*. Except for the outlier 1.6 due to only dry branch, all the trials were precise and close to each other.

Graph 1: the volume of water uptake (cm^3) per minute per m^2 by four different species of plant (Rubiaceae, Verbenaceae, Oleaceae,* and *Rutaceae*)**
Note: Error bars- data range

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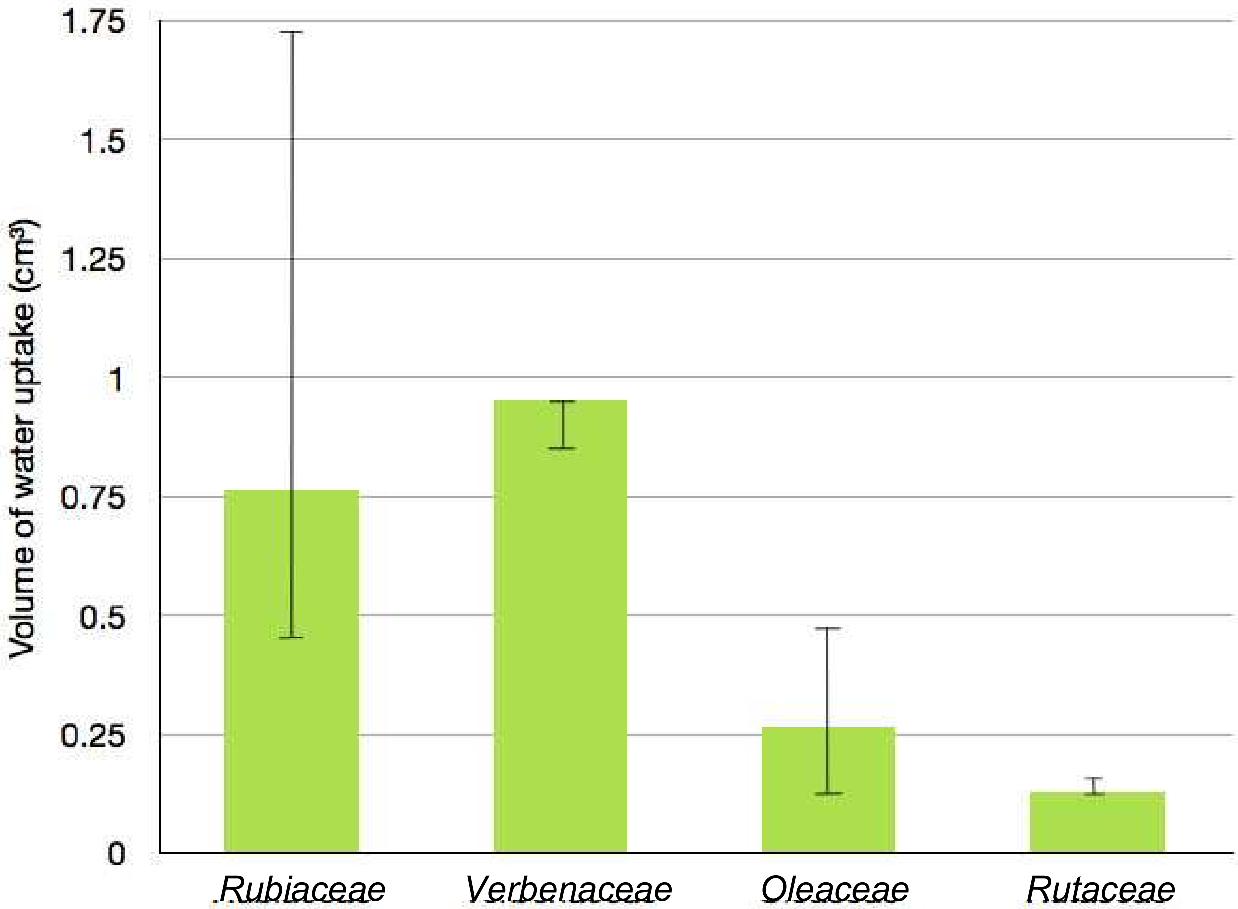
Each mean is drawn in bar graphs, and the highest and lowest transpiration rate result for each plant is shown around it.



Conclusion

As mentioned in hypothesis, different species have different transpiration rate from each other. This can be proven by the data above clearly in Graph 1. The plant from *Rubiaceae* family took 0.7639 mL of water per minute per m² while 0.9 512 mL was absorbed for *Verbenaceae*, 0.2678 mL for *Oleaceae*, and 0.1297 mL for *Rutaceae* according to Table 3. This is because all four species have different leaves. Of all features of leaves having effect on transpiration, the thickness of cuticles and stomatal complex are essential.

Cuticles are hydrophobic waxy layers on the surface of plant and thus make water to move not easily through leaves (*Transpiration*, n.d.). The thicker waxy cuticles can indicate the increasing hydrophobic. Then the transpiration rate increasingly slows d own.

About stomatal complex, it involves stomatal density, stomatal size, and stomatal index. All three aspects of it are different from species to species and thus have impact on transpiration rate of each species. For example, Saadu et al. (2009) found that *Euphorbiaceae* with 16.45a mm^{-2} of stomatal density, 52.30a μm of stomatal size, and 3.03a % of stomatal index has 1.47 x 10^{-4}a mol per m² per sec of transpiration rate (the method for measuring transpiration rate was a cobalt chloride paper method). The family had different transpiration from *Cyperaceae* with 28.95b mm^{-2} of stomatal density, 104.77b

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μm of stomatal size, and 17.16b % of stomatal index has 2.68 x 10 $^{-4}$b mol per m² per sec of transpiration rate. To extend further, Saadu et al. (2009) also discovered that especially high stomatal density is effective in water uptake. *Convolvulaceae* was the family species with the highest stomatal density of 35.75b mm^{-2} and thus had the greatest transpiration rate of 3.07 x 10-4b per m² per sec. Whether the size of stomata and leaf is large or not, it is more important that there are many ways out for water molecule on the surface of leaf for transpiration to happen fast.

Even though different family species were researched, from this, it still can be known that each species has different variations on the features of its leaf. These variations result in different transpiration rate.

Though in Table 3, Oleaceae absorbed the most volume of water, the surface area for transpiring that much amount of water was as large. More accurate data is volume per unit time per unit leaf area, and this is shown apparently in Graph 1. In Graph 1, *Verbenaceae* absorbed the most amount of water. However, considering the error bar especially of *Rubiaceae*, it is not reliable whether *Verbenaceae* with thick waxy cuticles and elliptic pinnate leaves has the fastest transpiration rate. The matter of reliability of this data will be discussed in evaluation.

Evaluation

In this experiment, there are several errors presented. The first source of errors is random including human errors. What was uncontrolled variable was the weather condition. This was also discussed in observations. The weather cannot be controlled to desired or constant condition for two days even for a few hours. Especially when doing experiment for *Oleaceae*, the condition was so various. The excessively high value of 1.80 cm for the trial 2, as seen in Table 2, was because of wind blown. Blowing wind had the plant transpire faster. As seen in Graph 1, the error bar ranges so widely due to this factor; it was huge impact on the result. Other than wind, the conditions of light, humidity, and temperature would be very different from day 1 to day 2. The experiment on day 1 was in the noon when the temperature was high and humidity was low. On the other hand, the day 2 experiment was in the morning when the temperature was relatively low and the humidity at that time was high. This seems a big issue, affecting enormously the results. *Verbenaceae, Oleaceae,* and *Rutaceae* was done in day 1, so they were likely to transpire faster than when they would be in the condition of day 2. With this, the comparison among plants is not reliable much.

There are always human errors. This often involves incorrectly reading the capillary tube on potometer in this case. Thus, the uncertainties of the equipment can reflect this kind of error, not to mention the uncertainties of the measurement itself. There was another random human error. Whenever the experimenter moves, the amount of either sunlight or fluorescent light to which the plants was exposed varied. This human error is random and different each time. Though it is hard to reduce it to zero, it does not seem to have much impact on the result as the variation in the amount of light was actually very short-term.

Other errors contain air bubble, wet leaves and branches, spider nest or dust, and finally the insertion of rubber tubing. Firstly, the removal of air bubble was the most problematic issue. Quite large air bubbles were stuck in the passage, and it was hard and thus time-consuming to take those out despite a number of adjustments of three -way tab and a syringe. Every trial for each of four plants would have different amount of air bubbles. As mentioned in observations, transpiration test for *Oleaceae* was the very first time trials and therefore, massive air bubbles were stuck in the passage. Seen in the row of *Oleaceae* in Table 2, the lowest value is 0.5 cm where a large air bubble blocked the passage.

Secondly, all wet leaves and branch were the issues. At the dry state of leaves and branch, i.e. the first trial of each plant, the water uptake was much and thus transpiration

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rate was very fast. The outlier 1.60 cm in Table 2 was the evidence. Comparing to the rest of four trials, the first trial had much higher value. The wet state of leaves and branch can be considered as the high humidity in the air, so it slows down the diffusion, loss, or transpiration of water molecules into air with already much water. This error could result in much impact on the transpiration rate and thus the result.

The third source of error is the spider nest or dust on the surface of plants. Though they are not that influential enough, they could have slight effect on transpiration of *Rubiaceae*, blocking some of the stomata. Perhaps the values of distance of water uptake and thus the volume of water uptake per unit time per unit area would be lower than what is expected or supposed. Still this error is small and trivial.

The last error is whether the branch fits into the rubber tubing, as briefly mentioned in observations. When there was room between the tubing and the branch, water leaked there. As soon as the three-way tap was manipulated to open all the passages, the water uptake for few seconds was so great. The problem here is that the water was not absorbed by the plant but moved to the crack. Therefore, sooner or later, the water uptake suddenly stopped or greatly slowed down. The difference is so great that this would have very large effect on the results and their error bars.

There two limitations to consider about. One is whether the removal of root affects the ability of water uptake. The water uptake by roots would not be the same as by branch or stem. The other is whether water uptake can necessarily mean the water loss or evaporation, which is what really transpiration means. Even if two measurements are different, it can be inferred that the large amount of water uptake leads to that much amount of water evaporation.

Time management was also the source of error. Since it was poor, the divided experiment was conducted for two days which had so different condition of weather that the effect on the result was great. All this was discussed in earlier part of evaluation.

To comment on precision and accuracy of this experiment and result, precision only for *Verbenaceae* and *Rutaceae* is high and accuracy is high as long as only general difference between species is concerned. In terms of high precision, the standard deviation for those two species was very close to 0 (standard deviation 0.0 in Table 2 was rounded off). This indicates small scatter around the mean and further implies few random errors for them. In fact, most of many random errors took place when conducing the trials of *Rutaceae* and *Oleaceae*. Therefore the standard deviation of two species is 0.5, meaning wider scatter around the means and finally the lower precision.

Accuracy is high as long as only the general conclusion is compared. In other words, the general conclusion that different species transpire at different rates also applies for known data or other research paper. However, when it comes to 'how' different species result in different transpiration rate, there are so many other aspects of leaves to compare such as waxy cuticles, stomatal complex, succulence, the side of occurrence of transpiration, etc. The result is not that reliable when taking all those account. Whether the family *Rutaceae* transpire the slowest is not sure. However, the fact of different transpiration rate for different species is quite reliable with the help of high precision of result for *Verbenaceae* and *Rutaceae.*

For better experiments, it is the fist thing to correct errors mentioned above. Keeping the weather controlled is nearly impossible. However, the best way can be to complete an experiment as quickly as possible in one day. The weather condition such as the amount of light and temperature is so various even during few hours.

As mentioned briefly, human errors are random so they can not be fully removed. The error of fluctuating amount of light due to moving experimenter can be improved by placing the set up of a potometer in a dark closed system with one source of light, not open space with sunlight and fluorescent light. This further can prevent the change in temperature, wind, humidity, etc.

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To solve the air bubble problem, the sufficient amount of actual practice is needed. As experienced during the experiment, as trials went the skill improved. Another practical way is first of all to remove air bubbles in a syringe. The air bubbles in the syringe caused bigger and more bubbles in the passage. Since the syringe can be detach from the potometer, it is possible to pull water up to the end and push the plunger back all the way to the other end to remove all air bubbles. Then fill the syringe up with water again and connect it to the three-way tap. Now water from the syringe would get rid of all air bubbles stuck in the passage when the tap is adjusted properly.

In regards to the error of wet and dry state of branch and leaves, it seems the best way to just wait until the wet branch dries. The paper towel to dry those would be helpful for reducing the waiting time.

Similar to the method right above, it is important to eliminate any water, dust , or even spider nest out of the surface of plant. These blocked stomata and prevented transpiration from occurring at the spot.

The matter of suitable insertion of branch into a rubber tubing could be resolved by having different size of rubber tubings, cutting the branch at the same thickness as the rubber tubing. As temporary expedient, vaseline can be used. This will prevent the leaking of water from the crack between the branch and rubber tubing.

The next step for improved laboratory is to repeat as much as possible. As long as biology is concerned, variations are inevitable. Just to decrease the variations, size of error bars, and standard deviation, many repetitions are required. The more there are repetitions, the greater possibility there is that the results get closer to the expected outcome.

For future research, more than four families of plant species can be data range, which would give more information. Since this laboratory experiment is quite superficial in that it only identifies the differences in transpiration rate, deeper consideration is required on how the differences come from. It would be better not only to check the appearance of leaves but also the quantitative data for example like the thickness of waxy cuticles and stomatal size as in other experiments done by others.

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Appendix

Rubiaceae **Verbenaceae**

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Oleaceae

Rutaceae

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