Plant Growth: Transpiration

SC Academic Standards: 4.L.5B; 5.L.4; 6.L.4A; 6.L.5B; 7.L.3A; 7.L.3B; 7.EC.5A; H.B.2B; H.B.2C; H.B.6B

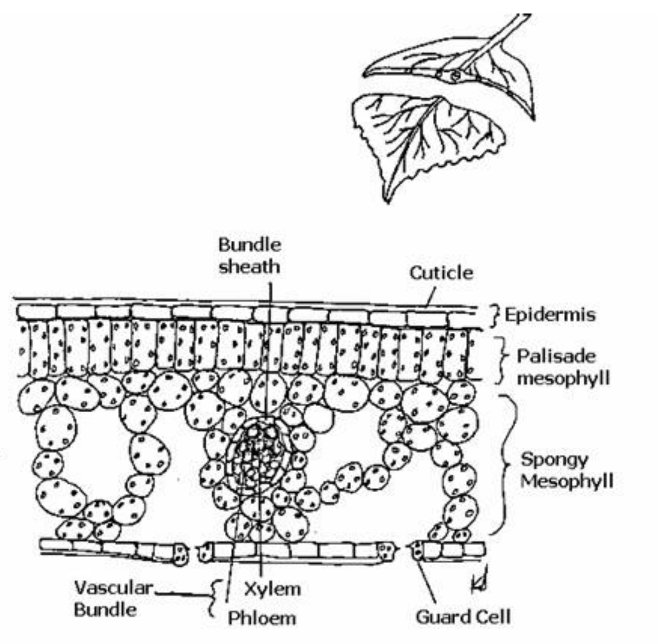
NGSS DCI: 4-LS1.A; 5-LS1.C; 5-LS2.A; 5-ESS3.C; MS-LS1-C; MS-PS3.D; MS-LS2.A; MS-LS2.C; HS-LS1.B-C; HS-LS2 A-C

Science and Engineering Practices: S.1A.1; S.1A.2; S.1A.3; S.1A.4; S.1A.5; S.1A.6; S.1A.7; S.1A.8

Crosscutting Concepts: Cause and Effect: Mechanism and Explanation; Structure and Function; Energy and Matter: Flows, Cycles, and Conservation; and Systems Models.

Focus Question(s): How and when do plants lose water?

Background: The roots of a plant are generally belowground (and thus protected from evaporative forces of the sun) and so do not have a cuticle, or waxy covering. **Roots** are mainly used to absorb water and dissolved nutrients from the soil, and to anchor the plant in place / lend to stability. The **shoots** (stems and leaves) are used for photosynthesis and are covered by a waxy **cuticle** which helps to prevent excessive water loss via evaporation, which, in a plant, is called **transpiration**. However, this protective cuticle can limit gas exchange (and plants need to take in CO_2 from the air and respire O_2 out. So, the cuticle has tiny chlorophyll containing cells called **guard cells** that together in pairs can create an opening between them – and when open, gases can be exchanged (CO_2 in, O_2 out). The opening is called a stomate (**stomata**, pl.) and is generally found on the underside of the leaf, where humidity is generally greater than the topside of the leaf, and so transpiration slower (the smaller the gradient between inside leaf humidity (near 100%) and outside leaf (air) humidity, the slower the rate of transpiration. And hot sun (top of the leaf) increases that gradient, while the shady humid underside decreases the gradient). It is a fine line



between needing CO_2 for photosynthesis and needing to conserve water.

The other cells you will find on the leaf's surface are the **epidermal** cells, which secrete the waxy cuticle. The epidermal cells are clear (they do not contain chloroplasts or chlorophyll) and act to focus light towards the mesophyll cells. Cells between the top and bottom epidermal layers are mesophyll cells, and contain chlorophyll and do photosynthesis.

Plants need water - they uptake water from the soil, using their roots. Water is transported from the roots to the leaves through the vascular tissue, particularly the xylem. Water exits the leaf when stomata are open, thus the stomata act as pumps, which pull water and nutrients from the roots through the rest of the plant to the leaves in a phenomenon known as transpirational pull. The moist, humid, air in the spaces within the leaf has a higher water potential than the outside (drier) air, and so water tends to evaporate from the leaf surface following a **decreasing water potential gradient.** This transpiration is unavoidable as the plants must open the stomata to take in the CO_2 needed for photosynthesis. About 10 percent of the earth's atmospheric moisture can be attributed to plant transpiration. Many plants have adaptations to reduce or control water loss though, as too much water lost will result in plant death. The rate of transpiration from the leaf depends on the **water potential gradient** between the leaf and the outside air (the more humid the air is, the closer its water potential is to the leaf (so a shallower gradient) and the slower the rate of transpiration). Water always moves from an area of high water potential (in leaf) to an area of low water potential (in air) – and it will move faster the larger the water potential difference is between the leaf and air.

This makes transpiration a major part of the water cycle. Plants absorb liquid water from the soil and then the water travels throughout the plant's body in veins (vascular tissue) and then finally exits the plant (as a gas) at the leaves, through the stomata. This is possible because of the **Hydrogen Bonds** which hold the water molecules together - so when one water molecule **evaporates** from the leaf's surface, another molecule is pulled up from behind (**Cohesion Tension** Theory of Water Transport). Once evaporated, the water is called water vapor, which is part of the atmosphere. When the atmosphere is saturated with water, **precipitation** occurs (rain or snow or fog etc.). Thus, water again enters the soil, is available for plant uptake, and the **water cycle** can start over again. Water, like all nutrients, cycles and recycles throughout the ecosystem. It becomes part of the food chain and is passed from plants to herbivores to carnivores, and is released in a variety of ways including transpiration, sweating, decomposition, and excretion.

<u>Materials</u>: a **potometer** set up (glass jar with tight fitting two holed stopper, one hole has a bent glass tube in it), plant stem with leaves (diameter of stem such that it fits tightly in hole of stopper), fan, Vaseline, clear plastic garbage bag, water sprayer, water, marker pen, ruler, clear nail polish, transparent (not opaque) tape, microscope.

Previous Knowledge: (plant biology): Some species of plant have stomata on both sides of the leaf, and others have stomata only on the lower epidermis. Aquatic plants, with one side submerged in the water, such as a water lily, will have stomata on the topside of the leaf instead of the bottom. The shape of stomata (and the mechanisms for controlling the size of the aperture) differ between monocotyledonous plants (such as grasses) and dicotyledonous plants. Some species seem to respond to prolonged ambient levels of light intensity and carbon dioxide by developing leaves with an altered density of stomata. For example, at high CO_2 levels, a lower density of stomata will not limit the rate of photosynthesis, but will reduce water loss. At higher light intensities, a higher density of stomata will maximize the rate of photosynthesis, but with the risk of enhanced water loss. Studies of stomatal density of plant samples up to 300 years old in botanical libraries have been used alongside evidence of recent changes in carbon dioxide levels in the atmosphere. Studies of stomatal density in fossils has been correlated with information from ice cores to give evidence of how carbon dioxide levels in the atmosphere have affected plants in the past.

Procedures: (* teacher note: **For part 1**, using an aquatic plant such as a water lily gives a good chance to check for understanding: water lilies have stomata on the surface of the leaf, not the underside which is submerged in water. Try to have a variety of leaves to study. Keep them in a water filled vase in a humid location.

For part 2, transpiration, use shoots from a shrub or tree with leaves that have thin waxy cuticles, such as beech, lilac, *Spirea*. Leaves with thicker waxy cuticles (very glossy leaves, like holly) do not work as well. Assemble the potometer before the students enter, and make sure it is filled with water so that the stems are submerged.

Also – for both parts - note that many plants close their stomata when the light intensity is insufficient for photosynthesis. Stems harvested in the autumn or winter may not transpire very much, but you can still see the (closed) guard cells / stomata. You can use a high-intensity light to promote photosynthesis and get transpiration going again. Fluorescent strip lights or halogen lamps are better than ordinary bench lamps) Making the classroom more humid may also result in more open guard cells/ stomata.

SAFETY: TAKE CARE when working with the glass rod as they are breakable (don't let students try to put it into rubber stopper). Have first aid ready for cuts.

Part 1: Stomatal density on leaves

Question: On a leaf, where will most stomata be located (topside or underneath)?

Hypothesis: There will be more stomata on the underside of the leaf because it is more humid there, and transpiration show be slower.
Null Hypothesis: Side of leaf makes no difference in stomatal density, either side is likely to have many stomata.

Prediction: I will count more stomata on the underside of the leaf.

Procedure (Part 1):

1. Each group should obtain a study leaf. Different groups should have different species.

2. Paint a thick patch of clear nail polish on the leaf surface being studied

   Do this with the a) top side of the leaf
   b) underside of the leaf

3. Allow the nail polish to dry completely

4. Tape a piece of clear (transparent) cellophane tape (the tape must be clear – do not use Scotch tape or any other opaque tape. Clear packing tape works.

5. Gently peel the nail polish back so that the nail polish stays stuck to the tape and peels off of the leaf.

6. Tape the peeled leaf impression to a very clean microscope slide. Trim away excess tape with scissors.

7. Scan the slide using low power until you find a good area where you can see stomata. Now that you've found the stomata, put the microscope into High Power (400x). Each stoma is bordered by two sausage shaped cells that are usually smaller than the surrounding epidermal cells (these are the guard cells).

- Where on the leaf did you see stomata?
- Topside ?________ Bottomside ? _______ Both ? _______
- If you noticed stomata on both top and bottom, are there more stomata on any one side?

Leaf type 1 =	Group 1	Group 2	Group 3	Average stomatal density
Topside of Leaf				
Bottomside of leaf				

Table 1. Stomatal Density on leaves of species _____________________________.

8. **Sketch** and **Label** the Stoma, Guard Cells, and Epidermal Cells.

Part 2: Environmental Factors Affecting Rate of Transpiration.

Transpiration is the loss of water by evaporation from the surface of a plant. Most of the loss is from the stomata, but the plant can lose water vapor from any surface that is not well-protected by the cuticle or bark. There are many environmental factors which can affect the rate of transpiration. In the laboratory we can test the affect of heat, wind, humidity, and number of leaves on transpiration rate.

Question: How does **wind** affect the rate of transpiration in plants?

Hypothesis:

Null Hypothesis:

Question: How does **humidity** affect the rate of transpiration in plants?

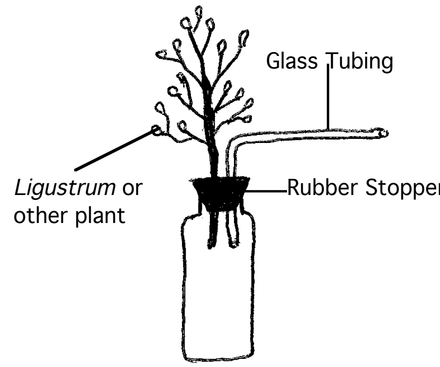
Hypothesis:

Null Hypothesis:

Environment	Hypothesized Effect on Transpiration
Wind	
High Humidity	

In order to carry out these experiments you need to get into groups of two to four students and choose which experiment you would like to execute. For each experiment you will need the following materials:

1. a **potometer**, which consists of
   a) a glass jar with a tight fitting two-hole rubber stopper and
   b) a glass tube bent at a right angle with the short end all the way through one of the holes in the stopper



A simple potometer

2. a woody branch which will fit snugly into the second hole of the stopper.
3. a wax pencil or marker
4. a millimeter ruler

In addition, you will need the following equipment, depending on which environmental factor you are testing—either a fan, or a spray jar and a large clear plastic garbage bag.

Procedure (Part 2):

1. Fill the jars completely full of water. The instructor should already have the bent glass tube inserted into one hole of the stopper, and a tree branch in the other hole. *The branch must fit snugly enough so that no water can evaporate out of the jar through the hole!*

2. Wipe some Vaseline around the hole in the stopper where the glass rod and the branch enter – this will help seal any space that remains.

3. With a small amount of pressure, twist and press the stopper into the jar. Water will squirt out of the tubing, but it should then stabilize about 2.5 cm from the tip of the glass tube.

4. After the water has stabilized a few minutes, begin the experiment under the environmental conditions present in the lab. (This is the base line or control for the experiment). Make **ONE MARK** on the tube at the starting water line. Make note of the distance (in mm) of the water line from the starting point after three (3), six (6), nine (9), twelve (12) and fifteen (15) minutes; record your data on the next page:

5. Remove stopper, refill potometer and replace cork, as in step 3. With the **same** plant do the same procedure again, using something to change the environment (fan or sprayer and bag). Record the data in the table and on the graph. Put the graph on the blackboard.

Table 1: Data from transpiration experiments comparing control, humid environments, and windy environments.

Time (minutes)	Distance H_2O moved - Control (mm)			AVG control	Distance H_2O moved - Humid (mm)			AVG humid	Distance H_2O moved - Wind (mm)			AVG wind
0												
3												
6												
9												
12												
15												

6. **Sketch a line graph** showing the average distance the water line moved versus time for each of the conditions using the graph paper below (use averages). Remember the difference between a control experiment and a controlled variable? The control is your comparison group – it is how you can tell if your independent variable is causing your data to do something different than normal.

Hunidity versus Control

What is the independent variable?

What is the dependent variable?

What are some controlled variables?

Wind versus Control

What is the independent variable?

What is the dependent variable?

What are some controlled variables?

What **conclusions** can you draw by comparing the control (baseline) data with the experimental data?

<u>Data Analysis</u>: In the data table notice there are smaller boxes to put individual group data, then you can take an average of those individual data (your replicates). It is always good to stress replication in science. Now you can sketch a line graph showing the average distance the water line moved versus time for each of the conditions (use the average). Also stress the difference between a control experiment and a controlled variable. The **control** is your comparison group – it is how you can tell if your independent variable is causing your data to do something different than normal. **Controlled** variables are the all the variables except your independent variable – the variables that should be the same in both control and experimental treatments.

<u>Extensions:</u> You could give students guidance on investigating a particular factor affecting the rate of transpiration, such as

- **Light intensity**. Place the plant shoot and potometer at different positions round the laboratory to alter the light intensity. Or you could use a lamp set at different distances from the plant. Check the intensity of light reaching the plant by placing a light meter by the leaves of the shoot.
- **Temperature**. It may be possible to find places which are at different temperatures; but it may not be easy to ensure that all other variables are controlled.
- **Surface area**. There are at least two ways of estimating the effect of surface area. An added extra dimension would be to try to establish if the upper or lower surface is more important in transpiration. Stomata are usually more abundant on the lower surface of leaves.

    Method 1: Measure the water uptake by the shoot. Remove a number of leaves from the shoot and measure the rate of water uptake again. Keep removing leaves until all the leaves are off the plant shoot. Label the leaves as you remove them, then estimate their area by placing on squared paper and tracing their outlines. Add together the areas of the leaves to find the total area and multiply by two to get the total surface area, as each leaf has an upper and a lower side. Add the leaf areas together in the reverse order that they were removed to get figures for the change in the surface area of leaves attached to the plant shoot. (or just repeat the control after removing approximately 50% of the leaves – carefully, so you don't rip large holes in stem epidermis).

    Method 2: Measure the water uptake by the shoot. Use vaseline to cover one (bottom) or both sides of a leaf. Test again. Calculate the surface area of the leaves as above. This could get messy! (Generally there are more stomata on the bottom of a leaf versus the topside).

Reflection Questions:

- **Where are the stomata usually located on a leaf: topside or bottom? Why?** Usually more on the bottom because the bottom is in the shade, and protected from wind, both of which help increase humidity. Higher humidity of the air means a smaller concentration difference between the 100% humidity inside leaf, and so rate of diffusion/ evaporation / transpiration is slower. Some aquatic plants like water lilies have stomata on top, because the underside of leaf is submerged, but these plants generally don't have issues with drying out or losing too much water anyway.

- **Why do terrestrial plants need a waxy cuticle?** To prevent water loss.

- **How is water loss controlled in terrestrial plants?** 1) cuticle 2) placement of stomata (usually on more humid underside) 3) opening / closing of stomata - closing at night when photosynthesis isn't occuring, closing during drought stress, etc. Hormones signal guard cells to uptake or release water, an uptake of water makes guard cells turgid, and they spread apart creating the hole or pore (the stomata is open!).

- **What environmental factors increase the rate of transpiration**? (wind, sun)

- **When are rates of transpiration likely to be highest: day or night? Why?** Daytime because it is usually less humid and windier.

- **For photosynthesis to occur, when must the stomata be open: day or night? Why?** Daytime, when there is sunlight.

- **When do plants usually have their stomata open?** Daytime.

- **Some plants, called CAM plants, live in very arid places like deserts. What adaptive strategy has evolved among these plants in order to minimize daytime water loss?** Crassulacean acid metabolism, also known as CAM photosynthesis, is an elaborate carbon fixation pathway in some plants. These plants take in and 'fix' carbon dioxide (CO_2) during the night, storing it as the four carbon acid malate. The CO_2 is released during the day, where it is concentrated around the enzyme RuBisCO, increasing the efficiency of photosynthesis. The CAM pathway allows stomata to remain shut during the day; therefore it is especially common in plants adapted to arid conditions.

Models and Explanations: In this lab we explored transpiration in plants. **A student who demonstrates understanding** of this concept can explain 1) that plants photosynthesize during the day and do cell respiration during the day and night. 2) when photosynthesizing CO2 is taken in through the stomata (openings in the waxy cuticle). 3) water vapor is unavoidably lost when the stomata are open for gas exchange. 4) plants that want to limit water loss can close their stomata (during droughts, or when not photosynthesizing , i.e. nighttime). 5) the rate of transpiration is faster when the concentration of water vapor in the air is low compared to the concentration in the leaf / stomata. The greater the concentration difference, the faster the rate of transpiration. Thus 6) things that dry the air surrounding the leaf will increase the rate of transpiration – things such as wind or sun – but things that decrease the concentration difference (like humidity, which is greater in the shade) will decrease the rate of transpiration.

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Student Worksheet:

Transpiration is the loss of water by evaporation from the surface of a plant. Most of the loss is from the stomata, but the plant can lose water vapor from any surface that is not well-protected by the cuticle or bark. Plants may control loss of water by closing / opening their stomata. Generally, it is advantageous to the plant to have the stomata open when the rates of transpiration are lowest, so water loss is not excessive, however, when stomata are closed most plants can't photosynthesize. There are many environmental factors which can affect the rate of transpiration. In this laboratory we can 1) use a microscope to look at and count stomata on different leaves, and top versus bottom sides of leaves and 2) perform a controlled experiment to test the effect of wind and humidity on rate of transpiration.

Part 1: Stomatal Density

Question: On a leaf, where will most stomata be located (topside or underneath)?

Hypothesis:

Null Hypothesis:

Prediction:

Results:

Leaf type =	Group 1	Group 2	Group 3	Average stomatal density
Topside of Leaf				
Bottomside of leaf				

Table 1. Stomatal density on top and bottom of leaf species ____________________

Compare your leaf stomatal density with students / groups who looked at different species of leaves.

- **Which leaf species had the most stomata?**
- **Which species had the least?**
- **Where were the stomata located (top or bottom)?**

Conclusion:

Sketch and **Label** the Stoma, Guard Cells, and Epidermal Cells.

Part 2: Measuring the Rate of Transpiration

Question 1: How does **wind** affect the rate of transpiration in plants?

Hypothesis:

Null Hypothesis:

Question 2: How does **humidity** affect the rate of transpiration in plants?

Hypothesis:

Null Hypothesis:

Time (minutes)	Distance H_2O moved - Control (mm)			AVG control	Distance H_2O moved - Humid (mm)			AVG humid	Distance H_2O moved - Wind (mm)			AVG wind
0												
3												
6												
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15												

Table 2: Data from transpiration experiments comparing control, humid environments, and windy environments.

Sketch a line graph showing the average distance the water line moved versus time for each of the conditions using the graph paper below (use averages). Remember the difference between a control experiment and a controlled variable? The control is your comparison group – it is how you can tell if your independent variable is causing your data to do something different than normal.

Humidity versus Control

What is the independent variable?

What is the dependent variable?

What are some controlled variables?
variables?

Wind versus Control

What is the independent variable?

What is the dependent variable?

What are some controlled

The **rate of transpiration** is calculated like this:
Distance water moved /Time to move that distance. Units are mm/min.

Rate of Transpiration in Humid Conditions: _________________ mm/min

In the Control: ______________________ mm/min

In Windy conditions: ____________________ mm/min

Conclusions & Reflection Questions:

- When are rates of transpiration likely to be highest: day or night? Why?
- For photosynthesis to occur, when must the stomata be open: day or night? Why?
- When do plants usually have their stomata open?
- How can plants control water loss in times of drought?
- Some plants, called CAM plants, live in very arid places like deserts. What adaptive strategy has evolved among these plants in order to minimize daytime water loss?