Jove Lab Bio

Lab 14: Transpiration — Procedure

1. Comparing Transpiration Rates of Leaves

1. To begin the experiment to measure the transpiration rates of leaves from different plant species, you will first create a tool called a potometer, using a piece of plastic tubing and 5 mL calibrated pipette.

2. To assemble the device, simply attach the rubber tubing to the tip of the pipette.

3. Then, submerge the device into a bucket of water and move the pipette around under the water to fully fill the tube, taking care not to create bubbles.

4. Next, cap the open end of the tube with a pipette bulb to keep the water in.

5. Now, taking care not to spill water from the potometer, use a clamp and stand to hold the capped end of the pipette around 1 - 3 inches below the pipette bulb.

6. Then, use a second clamp attached lower on the same stand to secure the open end of the rubber tubing.

7. Next, use a transfer pipette or dropper to remove water from the open end of the tubing until the water level in the pipette portion of the potometer rests at the zero line. HYPOTHESES: In this experiment, the experimental hypothesis might be that leaves from plant species more adapted to hotter or arid environments will have lower transpiration rates than those from humid or wet regions. The null hypothesis would be that leaves of the different plant species will not differ in their transpiration rates.

8. To begin the experiment, carefully use scissors to cut the end of the petiole, or stalk, of the first leaf and then insert the leaf into the tubing.

9. Then, place a layer of lubricant around the area where the plant stem and rubber tubing meet to make a watertight seal.

10. Next, remove the pipette bulb and taking care not to disturb the setup, let the experiment run for the appropriate experimental period, typically 30 - 60 minutes.

11. At the end of the allocated time, record the level of the water in the pipette, noting how much the level dropped in milliliters.

12. Then, refill and reset the potometer water level to zero, and then repeat the procedure for each of the remaining test leaves until the transpiration rates of all four leaves have been measured.

13. Next, to determine the surface area of the leaf samples, lay each leaf on a blank piece of paper and carefully trace the outlines.

14. Then, cut the outlines out and record the mass of each tracing.

15. Finally, cut out and weigh one four by four centimeter square of the same type of paper used to make the leaf tracings. This will act as a reference with known area and weight.

16. Next, you will need to quantify the number of stomata per unit area on each leaf. To do this, first prepare a slide mount by painting at least one square centimeter of the underside of each leaf with clear nail polish. IMPORTANT: Ensure you paint the bottom of the leaf, since the majority of the stomata are located there.

17. When the nail polish is completely dry, carefully press a small piece of cellophane tape directly onto each patch and then gently peel off the tape to remove the painted on polish. NOTE: Use a piece of tape that is smaller than the microscope slide.

18. Then, place each impression and tape onto a separate, clean microscope slide. HYPOTHESES: In this exercise, the alternative hypothesis could be that the leaves of the plants adapted to drier environments will have fewer stomata per unit area than the leaves of plants adapted to wetter environments. The null hypothesis might be that all plants species will have an equal number of stomata per unit area.

19. To observe each impression under the microscope, first use a low magnification to find areas of the leaf impression that contain stomata. These will appear as darker dots or impressions.

20. Then, switch to a higher magnification, keeping the sample viewing window centered on the stomata-containing area.

21. Draw and label your observations, making sure to label each sketch with the plant species.

22. Then, count and record the number of stomata in the field of view. You should perform this count a total of four times in four different areas of the leaf impression, and then determine the average number of stomata per counted area on the leaf.

23. To calculate the number of stomata per mm^2, place a transparent plastic ruler on the microscope stage under the objective and measure the diameter of the field of view.

24. You can then use this number, the diameter or D, to calculate the area of the field of view in mm^2.

2. Results

1. First, calculate the surface area of the 4x4 cm paper square to obtain the number of square centimeters.

2. Then, divide the weight of the paper square by the number of centimeters squared it covers to obtain the weight of 1 cm^2 of paper.

3. Finally, to calculate the surface area of each leaf, divide the weight of the leaf tracing by the weight of 1 cm^2 of paper. This will give you leaf area in centimeters squared.

4. You should now convert this number to meters squared, the standard unit used for calculating transpiration rate.

5. To calculate the transpiration rate for each leaf, divide the total water loss you measured in the potometer in mL by the time the experiment was run for in minutes.

6. Then, divide this by the surface area of the leaf in m^2.

7. Make a bar graph with the four different leaf species on the X axis, and their corresponding transpiration rates on the Y axis. Do these rates appear different?

8. Next, add a second Y axis to your chart and plot the average number of stomata per millimeter squared for each leaf that was observed under the microscope. Do you see a correlation between transpiration rate and number of stomata? If so, does this seem to have any relationship to the native environment the tree would typically inhabit?