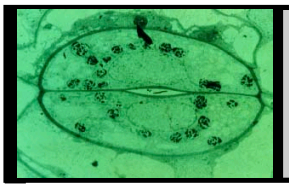

PLANT ANATOMY AND TRANSPIRATION

OVERVIEW

The Big Picture: *The plant kingdom consists of a large diversity of organisms sharing common characteristics. Today we will examine some of these characteristics in a variety of plants. Many plants today are land-dwelling. To adapt to life on land, plants evolved vascular tissue. Plants with vascular tissue are organized into three major organ systems: stems, leaves, and roots, which help them to obtain necessary nutrients and components for photosynthesis. The process of transpiration (water and gas exchange with the environment) is central to the proper functioning and survival of plants. Today in lab, we will explore components of transpiration, including whole-plant water balance.*

Before Lab	• Background Information: o Russell et al., CH 31 and 32
During Lab	• Part A: Plant Form and Function: examine unique characteristics of four different clades of plants. • Part B: Transpiration: Perform a leaf peel (B1-B2) and investigate the effects of environmental conditions on transpiration rate in an angiosperm plant (B3). • Part C: Collect data on corn embryo experiment that was started last week. Add your data to the class pool as your instructor specifies
Assignments (Due at the beginning of next week's lab)	• Worksheet 7A Plants (Individual) • Worksheet 7B Transpiration (Group) *DUE AT THE END OF LAB TODAY*

Thought and Discussion Questions:

- What features are found only in plants which distinguish them from members of other kingdoms?
- Over evolutionary history, what have been trends in
  - Dependency on water for survival? For fertilization?
  - Development and organization of vascular tissue in stems?
- Where on the overall plant body do plants have their greatest surface area?
- Is there something similar to a mouth and an anus in a plant?
- Is water loss through transpiration harmful or beneficial for plants?
- Is there a relationship between transpiration rate and photosynthesis rate?
- If you compare the top and bottom surfaces of a leaf, do you expect the top to have more stomata, fewer stomata, or about the same number of stomata?
- Stomata are found not only on leaves, but also on green stems. Would you expect to find them on woody stems, such as a large tree trunk? Why or why not?

Learning Objectives:

(1) Be able to identify unique characteristics of mosses, ferns, gymnosperms, and angiosperms.

(2) Be able to describe how plant bodies and growth are organized into root and shoot systems.

(3) Be able to explain transpiration to a fellow student

(4) Be able to perform a leaf peel and identify stomata, guard cells, and epidermal cells.

(5) Be able to discuss why stomatal density might differ within a leaf, among leaves from different parts of a large plant, and among leaves found on plant species specialized to different environments.

(6) Be able to generate and test hypotheses about how transpiration rate in plants is influenced by the environment.

(7) Be able to interpret data from low-pressure sensor readouts and explain what it tells us about transpiration rates.

PART A: THE KINGDOM PLANTAE

- Plant evolution and life cycles

PART B: TRANSPIRATION

- Leaf physiology and mechanisms regulating transpiration

PROCEDURE: A1**:** Non-Vascular Plants: Mosses and relatives

- Examine fresh specimens and slides for a typical moss species, answering worksheet questions.

PROCEDURE A2: Seedless Vascular Plants: Ferns and relatives

- Examine the fresh specimens and prepared slides for a typical fern species, answering worksheet questions

PROCEDURE A3: Non-Flowering Seed Plants: the Gymnosperms

- Examine the specimens of typical gymnosperms, answering worksheet questions.

PROCEDURE A4: Flowering Seed Plants: The Angiosperms

- Examine the specimens of typical angiosperms, answering worksheet questions.

PROCEDURE B (1-3): TRANSPIRATION AND WATER TRANSPORT

B1 ESTIMATION OF STOMATAL DENSITY

Today we will learn a method for examining the stomata on the epidermal surfaces of a leaf and use this method to quantify the density of stomata on upper and lower leaf surfaces. We will then use a t-test to examine if there are differences in stomatal density on upper and lower leaf surfaces of a peace lily leaf.

1. **Work in groups of 3-4**. Before you begin collecting data about the number of stomata on the upper and lower surfaces of a *Spathiphyllum* (peace lily) leaf, think about whether and why you expect to find a difference. Record your hypothesis and your rationale for choosing this hypothesis in the appropriate place on Worksheet 7B Transpiration.

2. Obtain two glass slides. Label one slide “upper surface” and the other “lower surface”.

3. With your naked eye, observe the difference between upper and lower leaf surfaces, and then cut a leaf with a long petiole from *Spathiphyllum* and bring it to your lab station.

4. Evenly place one coat of nail polish on the upper and lower surface of the leaf. Do not let the coated leaf touch the lab table or your fingers.

5. Tape the petiole of the leaf on the edge of the lab bench and allow it to hang until dry, no more than 30-45 minutes.

6. When dry, peel a layer of the nail polish from the upper surface of the leaf and place it on the appropriately labeled glass slide.

7. Do the same for a layer of the nail polish from the lower surface.

8. To fasten the peel to the glass slide, place two very small drops of nail polish on the sides of the coverslip and place it over your peel on the glass slide. Don't use too much or it will spread over your peel and damage your sample.

9. Allow the coverslip polish to dry and then examine your peel at 40X and 100X (total magnification).

10. In a single field of view at **100X**, count the number of stomata on the upper surface of the leaf. Record this information on Worksheet 7. In addition, give your data to your instructor so it can be added to the pooled class data. Since your counts are based on a standard area (one field of view at 100X magnification), this is an estimate of **stomatal density**.

11. In a single field of view at **100X**, count the number of stomata on the lower surface of the leaf. Record this information on Worksheet 7B, and also add your data to the pooled class data as your instructor directs.

12. Record the class data in the appropriate place on Worksheet 7B Transpiration.
13. Follow the directions hanging above the computers in lab to use SPSS to perform a t-test.

B3: MEASURING TRANSPIRATION RATE

In this part of the lab, we will quantify transpiration rate in an angiosperm leaf using a micropressure sensor, and investigate whether plants adjust their transpiration rate in response to various environmental factors, such as light.

1. In groups of 3-4 (same groups as you worked in for the leaf peel experiment), choose a variable which you think will have an effect on plant transpiration (e.g. light exposure). Record the variable on Worksheet 7B Transpiration.
2. Develop a hypothesis about how this variable will affect the rate of plant transpiration. Record your hypothesis on Worksheet 7B Transpiration.
3. Find a work station which is attached to a low-pressure sensor by the computers.

4. On the computer, open the program named "DataStudio" by double-clicking the icon with the same name.
5. Fill a beaker with tap water. Bring your beaker, plastic tubing, and scalpel to the potted *Spathiphyllum* plant. Locate one leaf with petioles ("stems") that will fit inside the plastic tubing. Ask your instructor for advice about which leaf to use – not all leaves will be appropriate for this experiment, and it is critical to the success of this experiment that you use the correct sized leaf. Using your scalpel carefully cut the petiole at least 3 inches from the leaf and quickly place it in the beaker of water.
6. Bring your tube to the front bench to fill this tube with water by following the directions from your lab instructor. Make sure that NO air bubbles or air pockets are present in the tube. Leave a space of air (~3-5 cm) in the tube by the white connect sensor piece. Once the proper amount of water is in the tube, hold your finger tightly over the end lacking the white connect sensor. Do not let go until the tube is attached to the sensor (see below).
7. Take your water-filled tube (carefully sealing the end lacking the white connector with your finger) to the workstation. Make sure that the white connector is dry by wiping it with a paper towel or Kimwipe. Continue to hold on to the end without the white connector, and have someone else attach the white connector to the sensor. Twist the white connector to the right and press it together with the plastic end on the sensor until the two connectors 'click' in place. Now connect the other end of the tube to the clamp. Place cotton around the end of the tube and tighten it in the clamp until it is secure (do not over-tighten the clamp or water will not be able to flow through the tube.)
8. Remove your leaf from the beaker and quickly but carefully insert it into the tube. Gently push the petiole of the leaf at least one inch into the tube. Do not push too hard or the petiole will bend and be damaged. You will need to obtain a new leaf if this happens. Placing the leaf in the tube may displace a few drops of water.
9. Find a jar of Vaseline and scoop out a small wad of Vaseline from the jar and swath it around the leaf/tube connection. Smooth the Vaseline. It is essential to have a good seal between the leaf and tube opening.
10. Check your set-up and get approval from your instructor. Once your set-up has been checked by your instructor, you are ready to being your experiment.

11. Record transpiration at baseline conditions (room temperature in the absence of whatever variable you are testing). To do this, click 'start' in the computer program Data Studio. Your experiment should start at 2kPA or above, and produce a continuous pressure decrease after 10 seconds. If the pressure starts below 2kPA or a pressure decrease is not occurring, then there is a problem with equipment setup. Notify your instructor immediately – she can help you troubleshoot.

12. After recording transpiration at room conditions for 300 seconds (5 min), click 'stop". Use the computer program to find the transpiration rate (slope of the line of the graph). Ask your instructor how to do this. Record your transpiration rate (slope) on Worksheet 7B Transpiration.

13. Allow your leaf sufficient recovery time (~5 min). Reset the pressure by disconnecting the white connector, and re-connecting it until it "clicks" in place. Then, subject your leaf to the experimental condition you are investigating. Repeat the above steps to record transpiration rate in this new condition. Again, make sure that the pressure starts at 2kPa or above. When you have recorded for 300 seconds (5 min), click 'stop". Again, find the transpiration rate and record this slope (transpiration rate) on Worksheet 7B Transpiration.

14. As a group, answer the questions on Worksheet 7B Transpiration. **Turn in this completed worksheet before you leave lab**.

PROCEDURE C: COLLECT CORN EMBRYO DATA

Today you will collect data on the corn embryo germination experiment that you began last week. You will measure (in mm) the length of shoots and roots that have formed over the past week.

1. In the same group of four that you worked in last week, obtain the eight media plates that your group set up last week. Also obtain a ruler.

2. For each plate (A-H), measure the length of the shoots formed for each embryo (there should be four data points per plate – one for each embryo). Record this data on the Corn Embryo Data Sheet table located on the next page. **Make sure that you record the length in mm**. Calculate the mean shoot length of the embryos on each plate (A-H) and record that in the appropriate location on the Corn Embryo Data Sheet.

3. Repeat step 2, but measure and record the length of the roots formed for each embryo on each plate (A-H).

4. Follow the directions from your lab instructor to add your data to the class pool. **Do not leave lab without adding your data to the pool**. Next week, you will analyze the pooled class data and use these results to write a lab report. Your lab report will be due during Lab 10 (week of 11/16-11/20).

5. Dispose of your corn embryo plates as directed by your instructor.

CORN EMBRYO DATA TABLES

Record your group's data on the two data tables below. To record your class data, follow the directions from your instructor.

Table 1. Shoot Length (mm)

Embryo cultivated on medium:

Embryo #	A ABA absent GA absent	B ABA absent GA present	C ABA present GA absent	D ABA present GA present	E gluc absent GA absent	F gluc absent GA present	G gluc present GA absent	H gluc present GA present
1								
2								
3								
4								
Mean:								

Table 2. Root Length (mm)

Embryo cultivated on medium:

Embryo #	A ABA absent GA absent	B ABA absent GA present	C ABA present GA absent	D ABA present GA present	E gluc absent GA absent	F gluc absent GA present	G gluc present GA absent	H gluc present GA present
1								
2								
3								
4								
Mean:								

*Group Names*_____

Day/Time/Instructor _____

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Worksheet 7B: Transpiration (GROUP) (10pts)

As a group, answer these questions and **turn in this completed worksheet before you leave lab today** (one worksheet per group).

1. (1 pt) What is your group's hypothesis regarding stomatal density on the upper and lower surface of the peace lily leaf?

Enter your Pooled Class data in the Table below:

	Upper surface	Lower surface
Group 1		
Group 2		
Group 3		
Group 4		
Mean		
Std Deviation		

2. (1 pt) Use SPSS to perform a t-test to determine if there is a difference between the mean density of stomata on the upper and lower surface of peace lily leaves (use the class data). Report the p-value below. Based on the p-value found above, do peace lily leaves have a different number of stomata on the upper and lower surfaces? Briefly explain.

Worksheet 7B: Transpiration (Group) (10 pts)

3. (1 pt) Suggest a biological rationale for your finding regarding stomatal density.

TRANSPIRATION RATE IN *SPATHIPHYLLUM* LEAVES:

4. (1 pt) What is the environmental condition you will test to determine if it affects transpiration rate in the *Spathiphyllum* leaf? Provide hypotheses to predict how this condition will affect the transpiration rate in the *Spathiphyllum* leaf. Provide a biological rationale for your hypothesis.

5. (1 pt) Fill in the table below with the data from your experiment.

	Condition (treatment)	**Transpiration rate (kPa/sec)**
Trial 1	Baseline	
Trial 2	Experimental (Describe)	

6. (1 pt) How did the environmental condition affect the transpiration rate? Did this agree with your hypothesis? Explain.

Worksheet 7B: Transpiration (Group) (10 pts)

7. (1 pt) If you were to perform a statistical analysis test to determine if the mean transpiration rate was affected by your environmental condition, which type of test would you perform? Explain.

8. (1 pt) Explain why you cannot perform the test you described above with <u>your</u> data set.

9. (1 pt) In this experiment, what is the independent variable and what is the dependent variable?

10. (1 pt) Explain how the two experiments you performed (leaf peel and transpiration rate) relate to each other.