Pre-lab Homework: Photosynthesis and Plant Physiology

Read the lab carefully and complete the questions below before coming to lab!

1. This week's lab covers photosynthesis and basic plant physiology. Read the lab, find the summary equation for photosynthesis and write it below.

   Photosynthesis summary equation:

2. During the first part of the lab you will be testing for starch in plant leaves. Why are we testing for starch?

3. In our first experiment, we have placed black paper on some of the leaves. Why would we put on paper that we think blocks all of the light?

4. In the second experiment we are separating pigments by paper chromatography. Other than chlorophyll, what pigments might we find in the spinach leaves?

5. In the third experiment we are examining gas exchange in plant leaves. What differences do you predict you'll see between the plant kept in the light versus the plant kept in the dark?

Photosynthesis and Plant Physiology

GOALS: After successfully completing this lab, a student will be able to:

- List some of the factors that affect photosynthetic rate.
- Explain the connection between light and energy storage in plants.
- Examine gas exchange in plant leaves
- Use the scientific method to investigate biological processes.
- Calculate the Student's t-test to compare two data sets

OVERVIEW:

During this lab you will perform experiments to help you understand two of the basic processes that convert energy from one form to another in biological systems. Your investigations will begin with experiments on photosynthesis. **Photosynthesis** is the process that plants use to convert the energy of light into the stored chemical energy of sugars. Photosynthesis can be summarized with an equation that looks like this:

$$6CO_2 + 6H_2O \xrightarrow[\text{Light}]{} C_6H_{12}O_6 + 6O_2$$

Looking at this summary equation, you can probably guess that many factors affect the rate of photosynthesis. As with any reaction, changing the concentration of the **reactants** (carbon dioxide and water) or the **products** (glucose and oxygen) can alter the rate of the reactions. In this lab, we will investigate the role of light in controlling the amount of photosynthesis. As in any scientific experiment, we will try to isolate one variable, in this case the wavelengths of light, and see what effect these have on the amounts of photosynthesis in leaves. The wavelengths of light correspond to different colors.

EXERCISE 1: Photosynthesis and light

Plant cells convert carbon dioxide into carbohydrates in a process called **photosynthesis**. Recall that the equation looks like this:

$$6CO_2 + 6H_2O \xrightarrow[\text{Light}]{} C_6H_{12}O_6 + 6O_2$$

To investigate this process we need to have some way to measure the amount of the sugar glucose produced by a plant. We will use a closely related compound, **starch**, which is produced by plant leaves as an energy storage product when they have excess sugars. To see the starch, we will use an indicator called Lugol's solution. This solution, which contains potassium and iodine, stains starch a dark

purple/black color, but does not stain sugars such as glucose. To look for the presence of photosynthesis, we will test for the presence of starch using Lugol's solution. Any part of the leaf that stains a dark color indicates the presence of starch and therefore that photosynthesis has been happening at such a rate that excess sugars are produced.

Using the Lugol's solution starch test, we will examine the effects of different wavelengths of light on the ability of plants to perform photosynthesis. A week ago, four filters of different colors were placed on the leaves of geranium plants. One filter is just black construction paper and should block all of the light to the leaf underneath it. The other three filters are colored translucent plastic films that let through only certain colors of light. For example, **the red filters let only red light through to the leaf, the blue only blue light, and the green only green light**. By using these filters we are able to alter the independent variable, in this case the color (or wavelengths) of the light, and see how it affects the dependent variable, in this case the amount of starch produced.

The black construction paper **absorbs** all of the light and so appears black. Your jeans appear blue because they absorb all light except for blue. Your jeans **reflect** blue light. The plastic filters reflect and **transmit** the colors they appear and they absorb all the rest. These wavelengths do have energy, which is why the filters breakdown and dyed fabrics fade over time.

Questions:

What is your hypothesis regarding the effect of different wavelengths on photosynthesis?

What are some other variables that you can think of that could affect the amount of photosynthesis in these plants? Brainstorm as many as you can think of!

Pick two of these variables from the previous question and explain how we controlled for them.

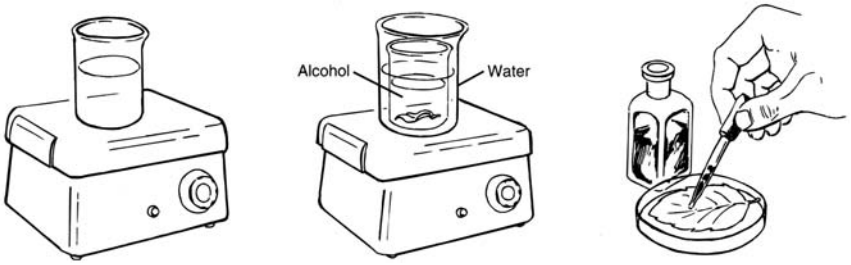
This procedure involves heating a highly flammable liquid. You need to be very careful and follow instructions carefully!

PROCEDURE FOR STAINING STARCH IN LEAVES:

1. Set up a beaker of boiling water for your alcohol bath. To do this, add 200 ml of tap water to a 600 ml beaker and place it on your hot plate as shown in Diagram A. Turn the hot plate to high. **The hot plate will get HOT - please be careful!**

2. Now place approximately 100 ml of 80% ethanol in a 250-ml beaker and carefully set this beaker into the water beaker before the water boils (see Diagram B). When the water begins to boil, turn down the hot plate until the water maintains a slight boil.

3. While the alcohol is warming up, remove the leaf (or leaves) assigned to your group and take them back to your desk. Do not remove the filters!



A B C

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4. **Before you remove any of the filters**, sketch your leaf face up so that after you boil the leaf, you will know where the filters were.

Leaf Sketch **with Filters in Place** (Note colors!)	Leaf Sketch **After Boiling and Staining** (Step 10)

5. After sketching your leaf, carefully remove the filters and place the leaf in the boiling ethanol.

6. After the leaf is a very pale green or white color, remove it from the ethanol bath and place it in a Petri dish. Make sure you **turn off your HOT plate!** Keep a beaker on the hot plate to prevent someone from accidentally leaning on the hot plate while it is cooling down.

7. Rinse the leaf with tap water and spread it out flat, face up, in the Petri dish. Now, add 4-5 droppers full of Lugol's iodine solution to cover the leaf as shown in Diagram C on the previous page.

8. After your leaf has sat in the iodine for 5-10 minutes, the leaf should darken where starch is present. [Note: Sometimes the Lugol's iodine solution is not good, since iodine is light sensitive. If your leaf does not darken, you may want to repeat steps 7-8 using a new bottle of Lugol's iodine solution.]

9. After the leaf is fully "developed," remove the leaf from the stain and place it in a clean Petri dish. Dispose of the iodine in the proper waste bottle. You may need to add a little water to get your leaf to spread out fully!

10. Now sketch your stained leaf in the table above, next to your sketch of the leaf before it was boiled for comparison.

11. After your double boiler has cooled, examine your ethanol solution in normal room lighting and while shining blue light on it in a darkened room/

<u>After completing your experiment on photosynthesis, answer the following questions:</u>

Was your hypothesis supported or rejected? Explain which wavelengths of light were most effective in photosynthesis.

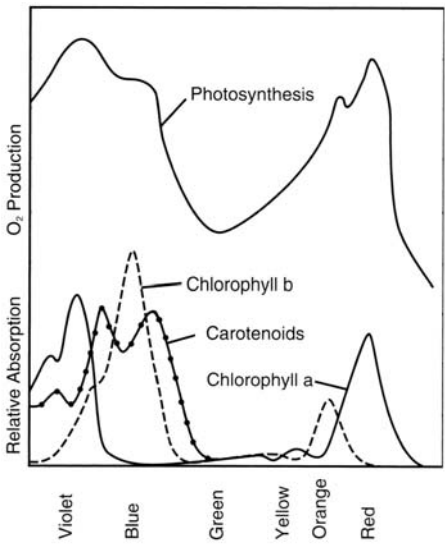
Land plants primarily use a **pigment** called **chlorophyll** to absorb light energy to power photosynthesis. What is the color of the pigment chlorophyll? Why does it appear this color?

Describe what happens when you shine blue light on your chlorophyll solution in a darkened room.

Based on your experiment, what color(s) of light do you think chlorophyll can absorb? What color(s) can't chlorophyll absorb?

EXERCISE 2: Investigating plant pigments using paper chromatography

Now that you have figured out which wavelengths (colors) of light are used by plants for photosynthesis, let's look at the pigments that plants use to capture light. **Chlorophyll a** is the most common pigment, and is the main pigment responsible for photosynthesis. However, several other accessory pigments are found in plants, including **chlorophyll b**, and many different kinds of **carotenoids** and **xanthophylls**. These different pigment molecules capture different wavelengths of light and function to extend the range of wavelengths of light that can be used in photosynthesis



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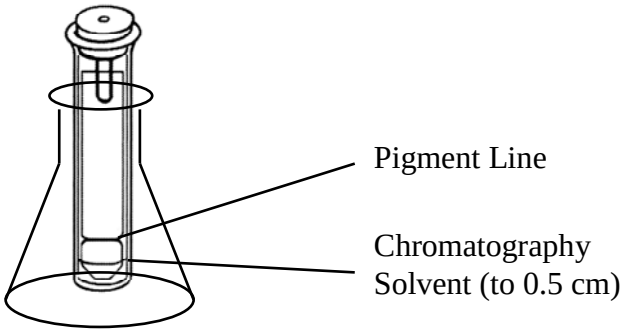
We will use a technique called paper chromatography to separate these plant pigment molecules and investigate their chemical properties. Plant pigments will be neatly placed onto a precise spot on a thin sheet of absorbent paper and dried. Then, one edge of the paper is dipped into a solvent. The paper wicks up the solvent, which migrates through the paper by capillary action. If the pigments present on the paper can dissolve in the solvent, they will be picked up and moved along with the solvent. The pigments can be separated and where they end up depends on the each pigment's size and/or solubility.

PROCEDURE FOR SEPARATING PIGMENTS USING PAPER CHROMATOGRAPHY:

1. Obtain a chromatography set up as shown below, except yours won't have the paper with the pigment line in it yet. The flask will hold your tube upright while your chromatography experiment runs, so your opposable thumbs and other digits will be free for other tasks! ☺



2. Handle the chromatography paper carefully by the edges, because oils from your fingers will interfere with your experiment. Measure a length of chromatography paper that will hang about 0.5 cm from the bottom of the chromatography tube. Hold the chromatography paper next to the chromatography set up. The paper will need to hang from the paper clip and just touch the solvent. Cut it this length and cut one end into a V-shaped tip.

3. To apply your plant pigments, take a leaf of spinach (or other plant of your instructor's choosing), and lay it on top of your chromatography paper above the V. Using the lip of a beaker or a coin, press the leaf into the chromatography paper to form a line of pigment about 2 cm up from the tip of the V. Allow the pigments to dry for two minutes, and then repeat the pigment application with a fresh part of the leaf. Let the second application dry. (you may repeat a third or fourth time if you wish.)

The chromatography solvent is a non-polar mixture of petroleum ether and acetone. **CAUTION: THE CHROMATOGRAPHY SOLVENT IS EXTREMELY VOLATILE AND FLAMMABLE!!** Don't dally, the chromatography solvent will diffuse into the lab and isn't good for us to breathe!

4. Remove the cork briefly and poke the paper clip in the cork through **the flat end** of the chromatography paper. Put the cork and the paper into the tube such that the V just dips into the solvent, but your pigment line does not. If needed, slide the paper clip in the cork to adjust the height of the dangling chromatography paper.

The solvent should wick up the paper, picking up some of the pigments along the way.

5. When the solvent has come within 2 cm of the top, remove your chromatography paper from the tube and let it dry. Recork your tube and put the chromatography set up back on the counter.

¡¡DO NOT POUR OUT THE CHROMATOGRAPHY SOLVENT. LEAVE THE SOLVENT IN THE CHROMATOGRAPHY SET UP WITH THE CORK IN PLACE!!

After completing your experiment on paper chromatography, answer the following questions:

How many pigment bands do you see on your chromatography paper, besides the original pigment line?

Using the information sheet provided, which pigment corresponds to each of these bands?

Which pigment molecule is least polar (and goes the farthest in the non-polar solvent)?

Which pigment molecule is most polar (and 'sticks' to the polar chromatography paper, and hardly moves from the pigment mixture line)?

Staple or sketch your pigment strip below here.

EXERCISE 3: EXAMINATION OF LEAF STOMATA

Background

Gas exchange (uptake of carbon dioxide and release of oxygen and water vapor) in plants occurs through microscopic openings in the surface of a leaf called stomata (Figure 9.4). As the stomata open and close they regulate the rate of water loss and carbon dioxide uptake. When stomata open, the plant is able to take up carbon dioxide needed for the light independent stage of photosynthesis. At the same time both water vapor and oxygen can leave the plant leaf. This loss of water vapor from the plant leaf is called transpiration. It is the driving force for movement of water through the xylem. When stomata close, gas exchange is greatly reduced so that the movement of water vapor and carbon dioxide into and out of the leaf is halted. Ultimately the rate of photosynthesis and the rate of water movement in the plant will also be affected.

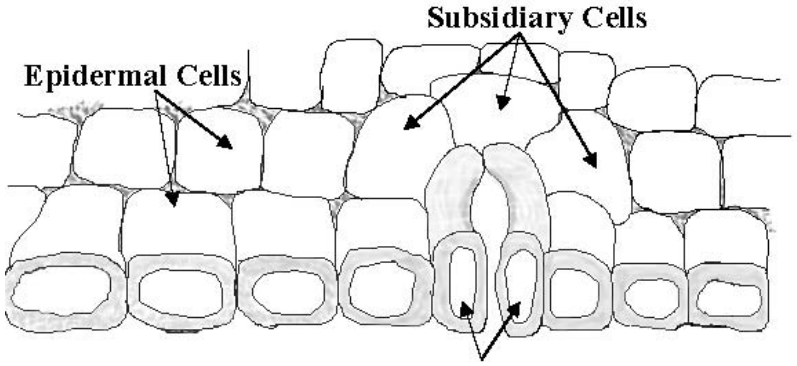


Figure 9.4. Plant leaf epidermis.

The degree to which a stoma is open or closed is a reflection of the environmental stresses acting on the leaf. The plant leaf can respond to the need for carbon dioxide during photosynthesis and thus respond by opening stomata, or the plant leaf can respond to the need to conserve water by closing the stomata. The plant leaf is always in a delicate balance between opening and closing the stomata. Measuring the degree of stomatal opening provides a visual indication of stomatal response to environmental conditions. The degree of stomatal opening has a large influence on the rate of gas exchange. Although the stomata are microscopic, the great number of them in the leaf surface can determine the entire leaf's response to ambient environmental conditions.

To study stomatal activity, you will examine leaves from plants that have been kept in the light or dark. You will evaluate how stomata respond to these different conditions by using casts of leaves viewed in a microscope to observe stomatal response under the different conditions.

Experimental Protocol: Determining which leaf surface has stomata.

1 The plants made available to you for this activity will have been watered thoroughly. Half of the plants were placed in darkness 24 hours ago. The "darkened" plants should be kept in darkness until they are needed.

2 Select a plant that has been kept in the light and clip two leaves from this plant. Prepare casts of the leaves surfaces by painting the top surface of one leaf and the bottom surface of the other leaf with clear finger nail polish. Allow the finger nail polish to dry (about 10 minutes).

3 While the nail polish is drying, label microscope slides as either "top" or "bottom."

4 Cut a piece of Scotch tape approximately 1.5 cm long. Fold the tape over on itself leaving 0.5 cm of sticky surface exposed (step 1 of Figure 9.5). Place the sticky tab of the tape at an edge of the leaf so that it sticks to the nail polish cast (step 2 of Figure 9.5). Use the remaining tape as a handle to pull the nail polish cast from the leaf surface (step 3 of Figure 9.5) carefully. Place the cast on the appropriately labeled slide (step 4 of Figure 9.5). Place a coverslip over the cast. Repeat this step for the remaining leaf.

5 Examine the slides under high power to determine which leaf surface has stomata. Carefully survey the entire leaf cast. The leaf surface with stomata should look similar to one of the illustrations in Figure 9.6. For future observations, it will only be necessary to make nail polish casts from the leaf surface with stomata.

6 Select a plant that has been kept in the dark for 24 hours. While the plant is still in the dark, remove a leaf and paint the appropriate surface with nail polish.

7 Prepare microscope slides as before.

8 View each slide under the microscope. Using the ocular micrometer, measure the size of the stomatal opening for eight randomly selected stomata from each cast and record the data in a table on the next page.

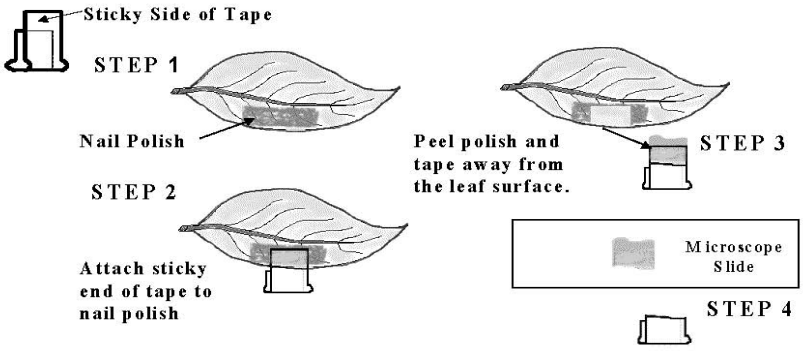


Figure 9.5. Procedure for stomatal casts in nail polish.

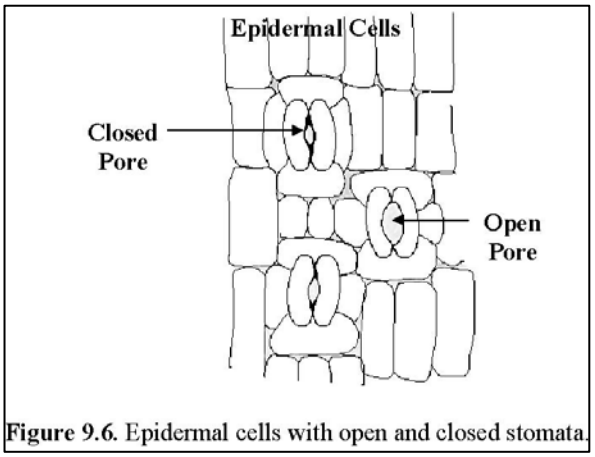


Figure 9.6. Epidermal cells with open and closed stomata.

Part B: Examination Of Leaf Stomata

Which side(s) of the leaf has (have) stomatal pores:

Were the pores open or closed in the light? What about the in the dark?:

Sketch of the epidermal surface with stomata, guard cells, and epidermal cells labeled.

Compare the mean stomatal opening size using the Student's t Test