Photosynthesis Experiments Using Leaf Discs

Objectives:
1. Reinforce methods of experimental design and statistical analysis.
2. Test some parameters that might have an effect on photosynthesis
3. Learn how leaf anatomy is adapted to optimize photosynthetic efficiency.

I. Introduction:

Photosynthesis is the process by which light energy is converted into chemical bond energy by autotrophic organisms. During the light dependent reactions of photosynthesis light energy is used to generate ATP and to reduce $NADP^+$ to NADPH. During the light independent reactions of photosynthesis, carbon dioxide is synthesized into glucose using chemical bond energy stored in ATP and the reducing power of NADPH. In eukaryotic autotrophs, both sets of reactions take place in a specialized organelle, the chloroplast. In autotrophic bacteria (cyanobacteria) the light dependent reactions occur on the plasma membrane and the light independent reactions occur in the liquid cytoplasm.

The entire process of photosynthesis can be summarized:

$$6\ CO_2 + 6\ H_2O \xrightarrow{\text{LIGHT}} C_6H_{12}O_6 + 6\ O_2$$

The rate at which photosynthesis occurs might depend upon both the <u>quality</u> (i.e. wavelength or color) and <u>quantity</u> (i.e. brightness) of light, as well as other parameters.

Leaf Anatomy: In most plants, the primary organ in which photosynthesis occurs is the leaf. In your experiment today, you will eventually be using portions of leaves to test hypotheses about photosynthesis. First we will examine leaf anatomy.

With a microscope, examine a prepared slide of a cross-section of a leaf. The prepared slide may be labeled: "Typical dicot leaf" or "privet leaf" or "*Ligustrum* leaf, c.s."

OBSERVE the cross-section on MEDIUM (100x) or HIGH (400x) power.

The cross-section of the leaf is several cell layers thick. Each layer of cells has a different function and a different morphology ('form fits function').

In the cross-section, the uppermost and bottom most layers of cells are the **upper epidermis** and **lower epidermis,** respectively. Each of these epidermal layers is just one cell thick. These cells have relatively thick walls and they secrete a layer of wax called the **cuticle** on the surface of the leaf. What do you think would be a function of the cuticle?

Observe the cells which make up the lower epidermis; you may see gaps in this array of cells. These gaps are called **stomata**; each one (a stoma) is surrounded by two **guard cells**. Each pair of guard cells is able to open and close a stoma, thus regulating the entrance and exit of air, and thus of oxygen , carbon dioxide, and water. For example, the stomata may close if the leaf becomes too hot or dry, in order to stop water loss from the leaf. Air which enters the leaf through the stomata first encounters a layer of cells called the **spongy parenchyma**. The spongy parenchyma is so-named because, like a sponge, it has relatively large air spaces amongst the cells. In the leaf, these spaces allow for the circulation of air throughout the leaf.

Above the spongy parenchyma lies a layer of cells that are rectangular and tightly packed. This layer is called the **palisade parenchyma**. Can you tell which layer of parenchyma has more chloroplasts per cell ?

Draw and label palisade and spongy parenchyma, epidermis, air space, stomata, cuticle.

II. Methods

1. Cut 5 discs from the green part of a leaf of the *Dieffenbachia seguine* plant with a paper punch. Handle them carefully; the discs consist of live cells, and in this condition they can be easily damaged.

2. Put the 5 discs in the syringe, and replace the plunger.

3. Fill the syringe with buffer. The buffer is 0.1 M $NaHCO_3$ (0.1 moles [8.4 g] of sodium bicarbonate, or baking soda, dissolved in 1 liter of water). The buffer has already had most of the dissolved gasses (mostly air) removed from it. Sodium bicarbonate dissociates into a sodium ion and a hydrogen carbonate ion in water, like this:

$$NaHCO_3 \leftrightarrow Na^+ + HCO_3^-$$

Hydrogen carbonate in the presence of hydrogen ions can from carbonic acid, H_2CO_3 which can break down into carbon dioxide and water.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

4. Expel any airspace in the syringe.

5. Seal the tip of the syringe by pressing it hard against a rubber stopper or your finger. Pressing against a rubber stopper gives a better seal. Now back off the plunger, swirl the disks, and immediately expel any new air space. Gas will have expanded and moved out of the leaf discs.

6. Repeat step 5 until no more bubbles form, and the leaf discs all sink. Once all the discs have sunk they can be used in your experiment.

7. The syringe can stand on its plunger. Place it in the light source provided. Watch to see whether the leaf discs rise, and record in seconds how long it takes for each one to rise.

Answer the following questions about what you have just done:

At first, why do the leaf discs float?

When you pull back on the plunger, where have the bubbles come from?

Why do the leaf discs eventually sink in the syringe?

Why do the leaf discs rise when placed in the light?

What is the purpose of the sodium bicarbonate in this experiment?

Now that you have familiarized yourself with the methods, examine these problems:

Problem 1: Is the rate of photosynthesis higher under bright light than under dim light?
Describe an experiment that could be designed to answer this question. After designing and carrying out your experiment, take what you have learned about the methods, and move on to Problem 2.

Problem 2: Is pigmentation necessary for photosynthesis ?
Describe and carry out an experiment to answer this question.

III. Results

Put data in a data table as you collect it. Templates for statistical tests are available in class and at the Biology Department's Statistics web site : (www.radford.edu/~biol-web/stats.html). Study the examples for entering data in the spreadsheet template. Open the template and enter your data as instructed.
Graph the data. Describe your results.

IV. Conclusions.

State whether or not your hypotheses were supported. Tell about how your experiment could be improved. What other factors might affect photosynthesis that could be tested with this method?