How-to-do-it

Photosynthesis

I: An Assay Utilizing Leaf Disks

Guy L. Steucek
Robert J. Hill
Class/Summer 1982[1]
Department of Biology
Millersville University
Millersville, PA 17551

Approximately three billion years ago, a unique process developed on earth: photosynthesis. This biochemical phenomenon markedly altered the nature of the planet and life on it; reduced carbon (CH_2O) which fuels the biosphere is produced by photosynthesis, and atmospheric oxygen (O_2) originated from and is maintained by photosynthetic activity. An earth devoid of atmospheric oxygen could not support respiratory metabolism and would not shield terrestrial life from deleterious ultraviolet radiation. Hence, it appears trite to say that every general course in biology should address this topic. Yet, it is true. Moreover, students should study photosynthesis in the laboratory.

The rather involved metabolism of photosynthesis may be represented by a very simple summary reaction:

Photosynthesis Summary Reaction

$$CO_2 + H_2O \xrightarrow[\text{plant}]{\text{green}} CH_2O + O_2$$

$$+ 112 \text{ Kcal/mole}$$

A myriad of techniques has been utilized to assay photosynthesis; unfortunately many require expensive equipment or take too much time and exceed the limitations of a classroom or laboratory period. As a result, frustrated biology teachers often ask: How can I demonstrate photosynthesis in an effective and understandable way? The intent of this article is to introduce the floating leaf disk assay of photosynthesis and to illustrate how it can be used to study photosynthesis in laboratory exercises.

[1]Class/Summer 1982: Lillian Awad, Roland Brown, Richard Brown, Karen Christine, Kevin Copie, Billie Babe Edkin, Wendy Fisher, Denis Foley III, Jessica Haag, Linda Hamitz, Mark Klingerman, Vicki Ludlow, Judy Martin, Amy Nguyen, Vicki Robinson, Barb Terril-Kettering, Julie Theobald, Tracy Tucker, Susan Warfield, Paul Wilson, Donna Witmer, and Larry Zook.

Procedure

With reference to the summary reaction for photosynthesis presented above, this assay of photosynthesis utilizes the rate at which oxygen is produced as a measure of the whole process. Disks of leaf tissue are vacuum-infiltrated in order to replace intercellular air with liquid; hence, the disks sink in a dilute buffer solution after infiltration. As photosynthesis takes place, the oxygen (O_2) produced accumulates in the leaf disk imparting buoyancy; the leaf disk floats. The rate of photosynthesis in the disks of the leaf tissue is determined by noting the time required for submerged leaf disks to float. While this technique has been introduced by others (Wickliff & Chasson 1964; Witham, Blaydes, & Devlin 1971), our modifications of the assay are significant and the pedagogical approach is unique.

1. *Buffer, Infiltration, and Bicarbonate Solutions:*

Buffer, pH 6.8, is made by adding 182 ml of 0.1 *M* citric acid to 618 ml of 0.2 *M* Na_2HPO_4 and 200 ml of distilled water. To make a 0.1 *M* citric acid solution, dissolve 19.2 gm of anhydrous citric acid ($C_6H_8O_7$ F.W. 192) in water and bring to a volume of one l. To make a 0.2 *M* Na_2HPO_4 solution, dissolve 28.4 gm of anhydrous, dibasic sodium phosphate (Na_2HPO_4 F.W. 142) in water and bring to a volume of one l.

The *infiltration solution* is made by adding one drop of Tween-80 (Fisher Scientific, #T-164) or X-77 (Chevron Corp.), a surfactant, to 100 ml of buffer. X-77 may be obtained from farm supply centers or local farmers who use it as a wetting agent. The surfactant serves as a wetting agent and hastens infiltration. Ordinary clear, liquid dish-detergent works well if Tween-80 and X-77 are not available.

The *bicarbonate solution* is made by adding 0.2 gm of sodium bicarbonate ($NaHCO_3$) to 100 ml of buffer (no detergent present).

2. *Plant Material: Cutting Leaf Disks:*

A great variety of plant material has been used with this assay (Wickliff and Chasson 1964); however, some leaf tissues may be difficult to infiltrate. This is particularly true of hairy leaves, i.e. geraniums. Other tissue samples may have very slow photosynthetic rates, i.e. succulents. For best results, use fresh leaves from rapidly growing plants, although we have successfully used fresh spinach leaves purchased from grocery stores.

After transferring 3.0 ml of *infiltration solution* to a 20 × 175 mm test tube, cut leaf disks with either a #2 cork borer or a single hole paper punch (see Figure 1A). We found the paper punch to be superior to the cork borer; uniform 6 mm disks can be obtained from leaf tissue in a matter of seconds. Paper punches can be purchased from most office supply stores. Disks should be placed in the infiltration solution at once; desiccation of disks must be avoided. For convenience, add ten leaf disks per test tube.

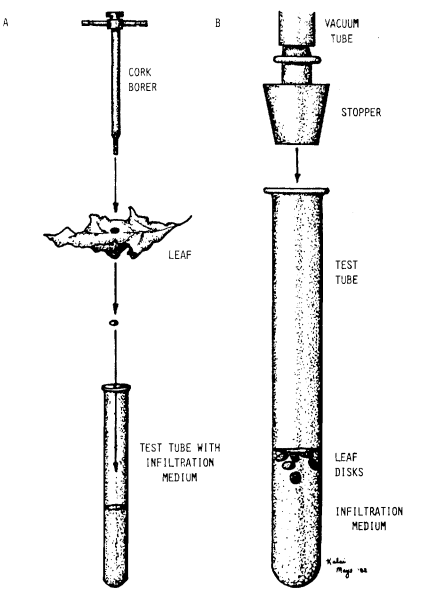

FIGURE 1. A. Leaf disks are cut using a cork borer or single hole paper punch and are immediately placed in infiltration solution. B. To replace the intercellular air with fluid, the test tube and hence leaf disks are evacuated three times; a fresh charge of air is permitted to enter the vessel after each evacuation.

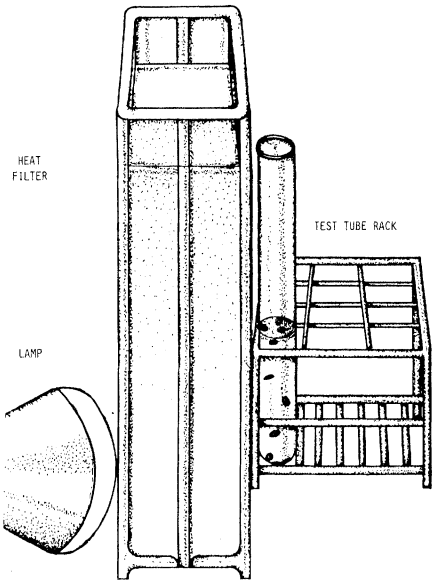

FIGURE 2. Arrangements of the lamp, heat filter, and test tube rack for a photosynthesis assay. The bottom of the test tube should be situated adjacent to the center of the lamp.

3. *Vacuum Infiltration of Leaf Disks:*

Air fills the intercellular space of leaf tissue. In order to replace this air with fluid so the leaf disk will sink, place the leaf disks in the *infiltration solution* (pH 6.8) under vacuum (see Figure 1B). A water aspirator (Fisher Scientific, #09-956) provides a source of vacuum. A rubber stopper attached to the vacuum source is held over the aperture of the test tube. The test tube is evacuated three times, and a fresh charge of air is permitted to enter the vessel after each evacuation. Under vacuum the intercellular air is drawn from the leaf disk; *infiltration solution* enters this space when the vacuum is released.

After infiltration, add 15 ml of *sodium bicarbonate solution* to the test tube and swirl. Infiltrated leaf disks can be stored in the dark until the time of the photosynthesis assay, i.e., stored in a cabinet.

4. *Photosynthesis Assay:*

Figure 2 illustrates the proper arrangement of a lamp, heat filter, and test tube rack for a photosynthesis assay. A 150-watt, incandescent flood lamp provides adequate light. A chromatography tank or a small aquarium filled with tap water makes an excellent heat filter. Test tubes are supported in test tube racks. Prior to conducting a series of assays, a light meter is used to determine if the light intensity is uniform in the area where test tubes containing leaf disks are to be placed.

To initiate an assay, simply place a test tube with leaf disks in the test tube rack and note the time. Every minute thereafter, count the number of leaf disks that are floating. For most uniform results, each test tube should be agitated at the end of the minute time interval so that all leaf disks are temporarily suspended in a vortex.

5. *Data Collection and Presentation:*

Printed data forms greatly facilitate data collection; we suggest the following format:

Time		_____
Minutes from Time Zero		_____
Tube 1	NDF	_____
	%	_____
Tube 2	NDF	_____
	%	_____
Tube 3	NDF	_____
	%	_____
Tube 4	NDF	_____
	%	_____
Tube 5	NDF	_____
	%	_____

NDF stands for the number of disks floating and % is determined as follows:

$$\% \text{ Floating} = (\text{NDF}/\text{Total Number of Leaf Disks}) \times 100$$

The time required for a leaf disk to float is an index of the rate of photosynthesis in that leaf disk. Some leaf disks will be "early floaters" and others will be "late floaters." By plotting the percentage of leaf disks floating as a function of time, the time required for 50 percent of the leaf disks to float can be determined (see Figure 3). This is called the ET_{50} or effective time for 50 percent of the leaf disks to float. The reciprocal of ET_{50} or $1/ET_{50}$ is related to the rate of photosynthesis. This time ($1/ET_{50}$) in minutes $^{-1}$ can be used as a simple measure of the rate of photosynthesis.

Experiments

A number of experiments can be performed to illustrate the credibility of the summary reaction for photosynthesis as presented above. Results from student laboratory investigations are presented, utilizing leaf disks from English ivy *(Hedera helix)*.

Light Effects

The data presented in Figure 3 illustrate that leaf disks float in the presence of light and sink in darkness as the oxygen generated by photosynthesis is consumed by respiration;

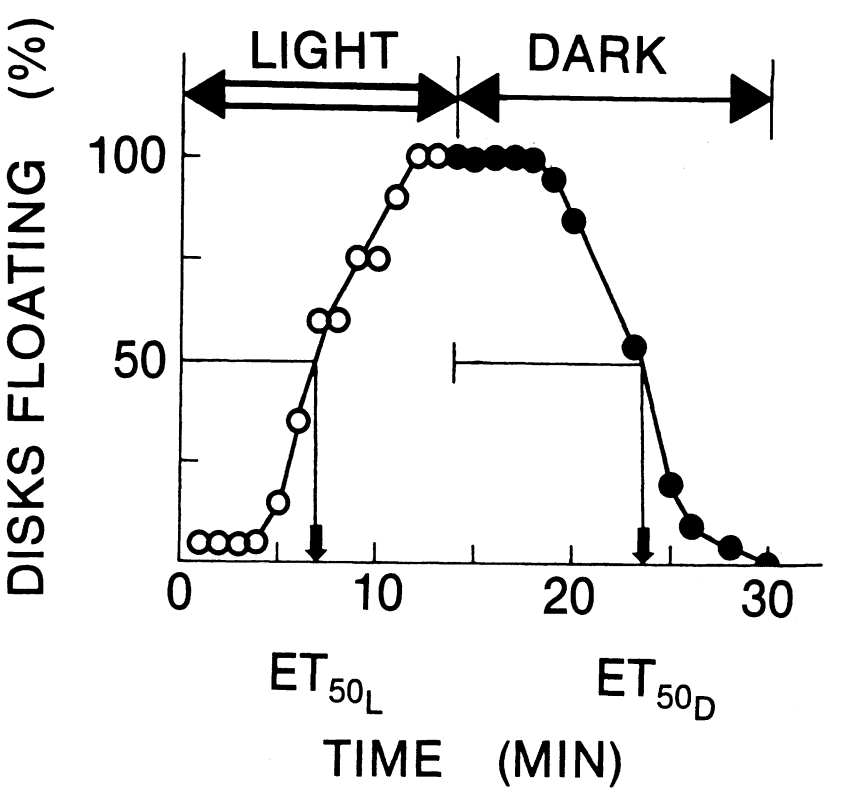

FIGURE 3. Percentage of leaf disks floating as a function of time. The arrows indicate that the lamp was turned on (LIGHT) at time 0 and off (DARK) at time 14 minutes. The light $ET_{50_{Light}}$ is 7.0 minutes and the dark $ET_{50_{Dark}}$ is 9.3 minutes; please see the text for an explanation.

thus the rate of respiration in leaf disks can also be assayed. The rate of photosynthesis is inversely related to the ET_{50}, which is found by noting the time at which half of the disks are floating. The light intensity can be changed by altering the distance between the heat filter and the lamp. Figure 4 presents the results of such a study. Clearly, the rate of photosynthesis is directly related to light intensity.

Bicarbonate Effects

Bicarbonate provides carbon dioxide (CO_2) for the photosynthetic reaction. Figure 5 shows the effect of carbonate concentration on photosynthesis.

Plant Tissue Effect

The fact that green plant tissue is required for photosynthesis was first demonstrated by Jan Ingenhausz in 1773; this observation may be confirmed using leaf disks from variegated leaves. Disks devoid of chlorophyll can be compared with green disks. *Coleus* plants are an excellent source of this tissue and can be obtained from local growers and/or distributors. Non-green tissue from storage organs, such as potato tubers, will not float when infiltrated and subsequently illuminated.

Photosynthesis changes as leaves mature. By excising disks from leaves varying in maturity, students have found that the rate of photosynthesis is greatest in leaves just prior to full expansion; very immature leaves and very old leaves have lower rates of photosynthesis. To relate photosynthetic rate to leaf maturity, simply sample leaves at varying distances from the shoot apex; count the number of leaves from the apex to the sampled leaf. After assaying photosynthetic activity, one can plot the rate of photosynthesis ($1/ET_{50}$) as a function of leaf number or number of leaves from the apex.

Action Spectrum

A rather crude action spectrum can be developed using different colored flood lamps to illustrate the wavelengths of light used in photosynthesis. The rate of photosynthesis is plotted as a function of light quality or wavelength of light. Photosynthetic activity is highest with blue and red light and lowest with green light, thereby implicating chlorophyll as the photoreceptor for photosynthesis.

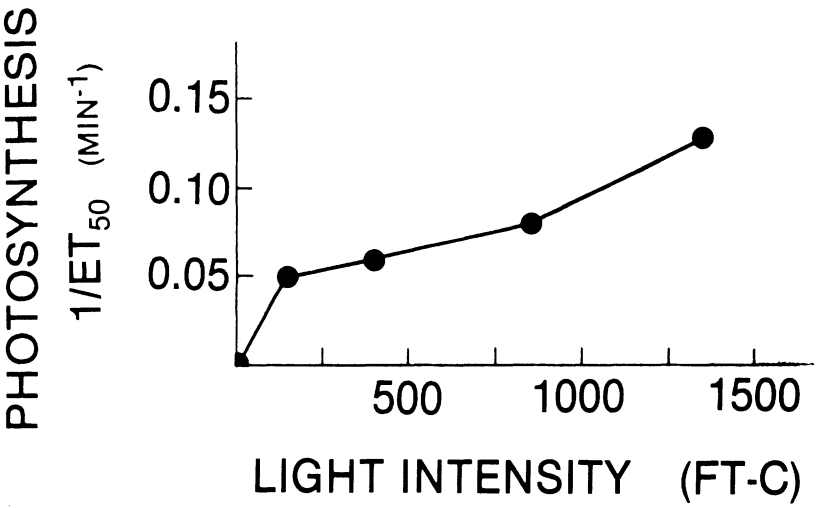

FIGURE 4. Photosynthesis as a function of light intensity. $1/ET_{50}$ is an index of the photosynthetic rate of the leaf disks. Each point on this graph was obtained by plotting data for a given light intensity as in Figure 3 to obtain the $1/ET_{50}$ value.

Prior to conducting photosynthetic assays, the distance between the lamp and the heat filter should be adjusted such that all light sources provide light of the same intensity. One should be aware that the sensitivity of light meters varies with light quality or color.

Relative Rates of Photosynthesis and Respiration in Leaf Tissues

While photosynthesis utilizes light energy to reduce carbon dioxide, respiration is the process taking place in all plant cells whereby reduced carbon compounds are oxidized to CO_2; the energy released is used to do biological work. The simple summary reaction for respiration follows:

Respiration Summary Reaction

$$CH_2O + O_2 \longrightarrow CO_2 + H_2O - 112Kcal/mole$$

The ratio of the rates of photosynthesis to respiration in leaf tissue can be determined using this assay procedure by noting the effective time for half of the leaf disks to float in the

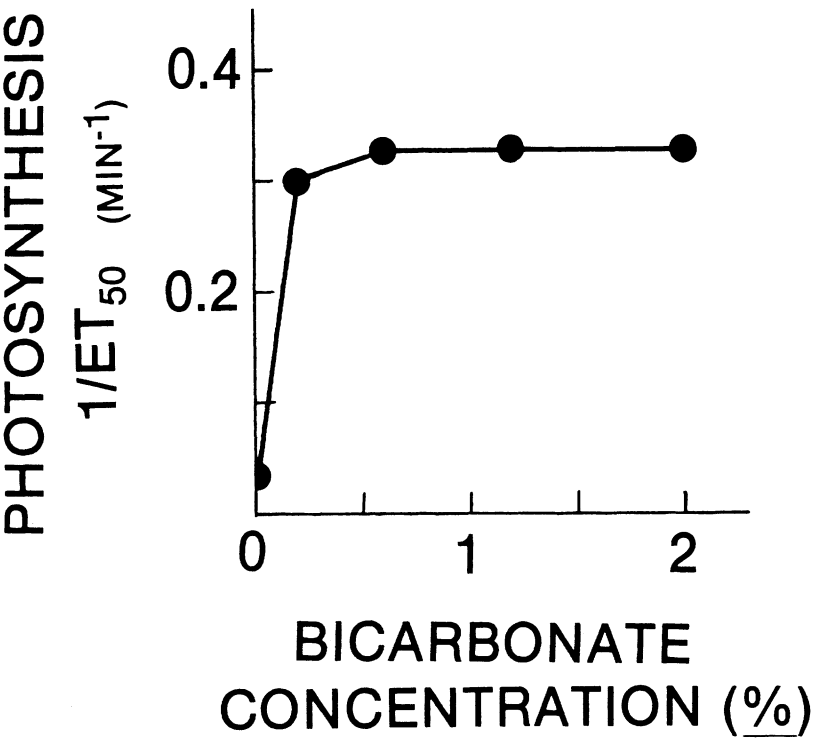

FIGURE 5. Photosynthesis as a function of bicarbonate concentration.

light $ET_{50\,Light}$ and the effective time for half the leaf disks to sink in the dark $ET_{50\,Dark}$ (see Figure 3).

In the dark, $1/ET_{50\,Dark}$ is proportional to the rate of respiration:

$$1/ET_{50\,Dark} = \text{rate of respiration} = 1/ET_{50RS}$$

for the example presented in Figure 3:

$$1/ET_{50RS} = 1/9.3 \text{ min} = 0.11 \text{ min}^{-1}$$

In the light, $1/ET_{50\,Light}$ is proportional to the rate of photosynthesis minus the rate of respiration since both processes occur in the light:

$$1/ET_{50\,Light} = \text{rate of photosynthesis} - \text{rate of respiration}$$

therefore,

$$\text{rate of photosynthesis} = 1/ET_{50\,Light} + 1/ET_{50\,Dark} = 1/ET_{50PS}$$

for the example presented in Figure 3,

$$1/ET_{50PS} = 1/7 \text{ min} + 1/9.3 \text{ min} = 0.25 \text{ min}^{-1}$$

The ratio of the rates of photosynthesis to respiration is:

$$\text{Photosynthesis/Respiration Ratio} = \frac{1/ET_{50PS}}{1/ET_{50RS}}$$

for the example presented in Figure 3:

$$\text{Photosynthesis/Respiration Ratio} = \frac{0.25}{0.11} = 2.5$$

Students may wish to study the influence of leaf maturity on the photosynthesis to respiration ratio.

Additional Studies

This laboratory exercise lends itself nicely to an open-ended investigatory approach. Often after students complete one of the exercises above, they are asked to design their own experiments to be conducted in subsequent laboratory periods. A great variety of topics can be explored; some suggestions follow:

1. Leaf disk size and geometry may influence the assay procedure. Using different cork-borers, one can vary the diameter of the disk. Some students have studied the effect of leaf thickness on the assay. A variety of paper punches which yield "cutouts" of varying geometry can be studied. Is one geometry more reliable than another with this assay procedure?
2. By placing the test tube rack in a water bath, the influence of temperature on photosynthesis can be investigated. The effect of a 10°C increase in temperature on the rate of a process is called Q_{10}. If a Q_{10} (Salisbury and Ross 1978) of 1.0 is found, the rate limiting factor for photosynthesis would be the photochemical portion of the reaction. Whereas, a Q_{10} of 2.0-3.0 would imply a biochemical rate limiting step.
3. Using buffers varying in pH permits students to study the influence of acidity and alkalinity on photosynthesis. Perhaps they could relate their findings to the acid rain problem.
4. In addition to placing bicarbonate in the assay solution, glucose may also be added. This permits students to study the influence of glucose on the process of photosynthesis. An osmotic agent which is not metabolized such as mannitol or sorbitol should be used as a control.
5. Inhibitors of respiration can be studied using floating leaf disks which are buoyed with O_2 produced by photosynthesis. In the dark, $1 \times 10^{-3}M$ potassium azide will greatly retard the sinking of leaf disks (South and Sung 1980); controls without azide will sink within 30 minutes.

Not only does the floating leaf disk assay for photosynthesis permit students to study the process of photosynthesis in the laboratory, it encourages them to design their own experiments and "do science." In the following article, we describe how this assay procedure may be employed to identify herbicide resistance in weeds when photosynthesis is the site of action of the agent. The evolution of ecotypes resistant to weed killers represents an international problem that could be resolved with the aid of studies by students.

Acknowledgments

We thank Betty Garman, Scott Kriner, Kalai Mays, Melvin Norbeck and Gail Twiford for their technical assistance.

References

Salisbury, F.B. & Ross, C.W. (1978). *Plant physiology*. Belmont, CA: Wadsworth.

South, D.B. & Sung, S.S. (1980). A new method for screening herbicides for use in pine nurseries. *Canadian Journal of For. Res., 10*, 164-168.

Wickliff, J.L. & Chasson, R.M. (1964). Measurement of photosynthesis in plant tissues using bicarbonate solutions. *Bioscience, 14*, 32-33.

Witham, F.H., Blaydes, D.F. & Devlin, R.M. (1971). *Experiments in plant physiology*. New York: Van Nostrand Reinhold.

Photosynthesis

II. An Assay for Herbicide Resistance in Weeds

Robert J. Hill
Guy L. Steucek

During the last fifty years, the environment has been assaulted with a variety of chemical agents. Some of these have grown to be relatively innocuous, while others are potentially dangerous. Questions arise whether chemical agents in the environment act directly *(in situ)* as mutagens or indirectly as an artificial selective pressure. Yet, there now exists an impressive list of laboratory-confirmed physical and chemical mutagens entering the biosphere. Whether these are responsible for germinal mutations is still in dispute. Spontaneous somatic mutations, however, often are associated with environmental factors (Gardener 1972). When dominant mutant genes in germ cells are expressed, the progeny are immediately affected and obviously display the mutation, unlike somatic mutations which are not heritable. Selective pressures, on the other hand, would merely cause a shift (change) in the frequency of certain genes in the population and be reflected in the survival of tolerant individuals.

Pesticides represent only one class of chemical agents. With intensive and extensive use, some organisms formerly controlled by pesticides are now resistant. It will be helpful to define briefly some of the responses an