Measurement of the Effects of Light Quality and Other Factors on the Rate of Photosynthesis

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Light can affect plant growth and development in two distinct ways. The first system for light effects is modification of growth patterns and development through interaction with specific photoreceptors (see Kendrick and Kronenberg, 1986). Relatively small amounts of light with specific spectral quality or spatial direction is responsible for activating these pigments.

The second light dependent system is photosynthesis. Large amounts of light are transduced to chemical energy which is utilized for growth and development (see Gregory, 1977). The photosynthetic transduction of light energy to chemical energy is central to life. It is the ultimate source of chemical energy for almost all organisms. Approximately $2x10^{11}$ tons of carbon are fixed each year. This is more than one-third of a million tons fixed per minute.

Central to the process of photosynthesis is the light harvesting ability of plant pigments. Several types of photosynthetic pigments occur in plants and bacteria (Table 1). Chlorophyll and protein molecules are organized into two complexes, Photosystem I (PSI) and Photosystem II (PSII) (Anderson 1980). PSI is a chlorophyll a/protein complex with a maximum wavelength absorbance of 720 nm (far red light). PSII is a chlorophyll a/chlorophyll b/protein complex with a maximum wavelength absorbance of 690 nm (red light).

In addition to these two Photosystems, the yellow-orange pigment carotene absorbs light in the blue region of the spectrum. Only 20-50% of the light energy absorbed by carotene is transferred to the photosystems. The primary role of carotene appears to be protection of the chlorophyll of the photosystems from excess radiant energy (Krinsky, 1978). Under high levels of radiant energy chlorophyll molecules are excited to the triplet state. A triplet state is an excitation state in which the spin of the excited electron has been reversed. The triplet state of chlorophyll is stable for only several microseconds after which the electron reacts with molecular oxygen to form either singlet oxygen or a superoxide radical.

Table 1. Common types of photosynthetic pigments and their occurrence (adapted from Gregory, 1977).

PIGMENT	OCCURRENCE
Chlorophyll a	All oxygen-evolving organisms
Chlorophyll b	Higher plants and green algae
Chlorophyll c	Diatoms and brown algae
Chlorophyll d	Red algae
α-carotene	Higher plants, most algae
β-carotene	Most plants, some algae
Luteol	Higher plants, green and red algae
Violaxanthol	Higher plants
Fucoxanthol	Brown algae, diatoms
Phycoerythrin	Red algae, some blue-green algae
Phycocyanin	Red algae, some blue-green algae
Allophycocyanin	Blue-green algae, red algae
Bacteriochlorophyll a	Purple and green bacteria
Bacteriochlorophyll b	Some purple bacteria

Both of these forms of oxygen are extremely reactive and lead to irreversible damage to the chlorophyll molecule and other cellular components including membranes and DNA. If a carotene molecule is available, chlorophyll can pass triplet state electrons to the carotene molecule. Carotenoid triplets are harmless because carotene can dissipate the triplet state energy to heat. Thus carotene performs two functions: 1) protection of chlorophyll from oxidation and 2) absorption of light in the 450-500 nm range for utilization by the

photosynthetic processes. Thus, in higher plant species, chlorophyll and carotenoids contribute to the photosynthetic absorption spectra.

The photosynthetic absorption spectra measures the wavelengths of light which are absorbed by photosynthetic tissues. These spectra include absorption of light from primarily photosynthetic pigments. A typical absorption spectra is illustrated in Figure 1. The photosynthetic action or activity spectra measures the utilization of various wavelengths of light by the process of photosynthesis (Figure 2). The activity spectra indicates the photosynthetic yield of various wavelengths of light of differing quantities and energetics which are transferred through the photosystems in the conversion to chemical energy. Without the transfer of energy between pigments, the activity spectrum would consist of two peaks at 720 nm (PSI) and 680 (PSII).

Of primary interest to this laboratory experience is energy transfer through Photosystem II. Energy transfer through PSII performs two important functions. First, ADP is phosphorylated to produce ATP which can be utilized by the Calvin-Benson cycle. Second, energy is utilized in splitting water molecules and generating O_2. The oxygen is liberated as a gas.

This laboratory exercise explores the effect of light energy of various wavelengths on photosynthesis. Oxygen evolution from PSII is used as a measure of photosynthetic activity. This laboratory exercise requires minimal equipment and is presented in a form which can be used in an introductory level course. Modifications are suggested at the end of this exercise which increase the complexity of data analysis and interpretation for use in advanced courses.

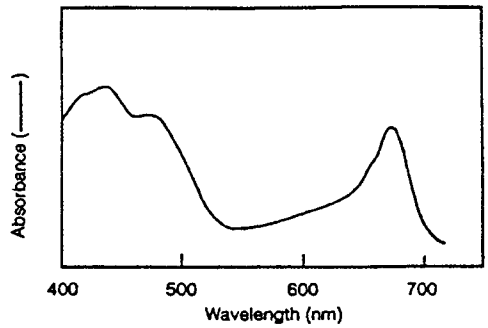

Figure 1. Absorbance spectra for green tissue of higher plants. (Adapted from Withrow, 1959)

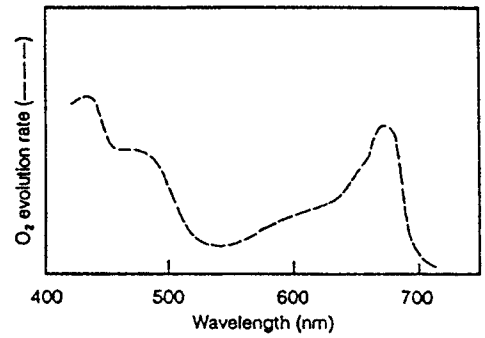

Figure 2. Action spectra of photosynthesis of higher plants. (Adapted from Withrow, 1959)

GOALS OF THE EXPERIMENT

1. Determine the effect of light quality on photosynthetic evolution of oxygen.
2. Determine the effect of light quantity on photosynthetic evolution of oxygen.
3. Observe the results of the transfer of light energy between photosynthetically active pigments.

TIME REQUIREMENTS

0.5 hr approximately 10 days prior to the experiment to plant seed.
1.0 hr prior to the experiment to prepare test solutions and calibrate filters.
1.0 hr to leaf discs and setup the experiment,
1.0 hr to run the experiment and collect data.

MATERIALS AND EQUIPMENT

Seed [radish, turnip, Chinese cabbage, Fast Plant (*Brassica rapa*) or almost any Brassica sp.]
Plastic 35mm film cans
Osmocote fertilizer pellets
Nail punch
Soil mixture (1:1 peat: vermiculite)
Unwaxed cotton cord
Plastic storage box or plastic shoe box
Pellon fabric
Scott brand household paper towel
10 ml disposable syringes
Cellophane or transparent film — yellow, red, blue, green, orange
1 M sodium bicarbonate solution
Fluorescent light strip
0.1 N HCl
0.1 N NaOH
pH meter
Plastic soda straws
Wire test tube racks
Various glassware
Distilled water
Photographic light meter

METHODS

Seedling Preparation. This step of the experiment should be done in advance of the experiment by the students or instructor. Seeds are planted in plastic film cans as illustrated in Figure 3 and 4. Plant 5 seeds per film can. Each student should prepare 4 film cans. Students can prepare the film cans during the laboratory period a week prior to the experiment; the instructor will add water to initiate the germination approximately 10 days before the laboratory period. For optimal growth, place the seeds/seedlings under a fluorescent light bank to maintain constant illumination at < 350 ft. candles and 22° C. Seedlings are used when the first true leaves are fully expanded; this should require 8-10 days, depending on the species which is selected.

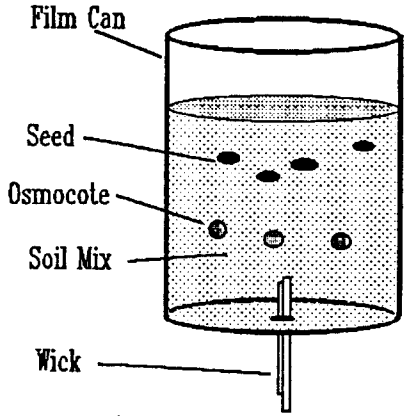

Figure 3. Diagrammatic representation of the use of plastic 35 mm film cans to germinate and grow seedlings and plants. Hole in bottom of can is made using a nail punch. Unwaxed cotton cord is used as a wick. The soil is peat:vermiculite (1:1). Three or four pellets of Osmocote are placed in each can as fertilizer.

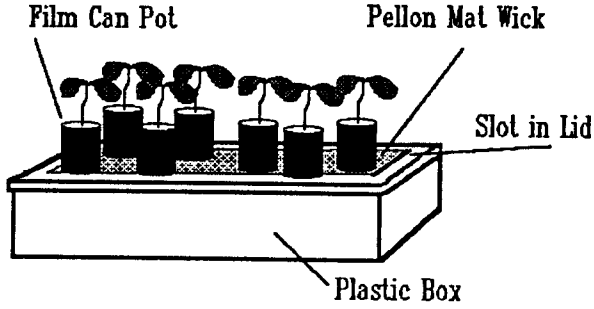

Figure 4. Planting trays for use with the film can system. Plastic boxes are used as water reservoirs. A slot is cut into the lid of the box. A rectangle of bleached Pellon covers the top of the box and extends through the slot into the reservoir. The Pellon mat serves as a wick.

Solution Preparation. A solution of 1 M sodium bicarbonate should be prepared. The pH of the bicarbonate solution should be adjusted to 8.0. Each student will need approximately 70 ml of solution.

Filter Calibration. Cellophane or transparent plastic is used as filters to provide light of different wavelengths for this experiment. Cellophane can be purchased from many scientific supply companies, floral supply companies or office supply companies (in the form of overhead transparency material or report covers). For optimal results similar quantities of light should be transmitted through the filters. To calibrate the quantity of light that is transmitted by the filters you can use a hand held photographic light meter. Cut small test squares of the filter material and place them between the light source (a small fluorescent light stick usually works best) and the light meter. Adjust the distance from the light source and the number of layers of filter material to obtain similar light meter readings. The spectral quality of the light can be measured by placing a strip of the filter material in a spectrophotometer and measuring the absorbance spectra of the material. If a larger budget is available, Wratten filters can be purchased from any photography store. Information concerning the spectral characteristics of these filters can be found in the CRC Handbook of Chemistry and Physics.

Leaf Disc Preparation. Excise the pairs of first true leaves from the seedling using a single edge razor blade. Place the excised leaves in pairs from the same seedling on a moist paper towel. The leaves should be fully expanded and bright green at this stage. Uniform discs can be cut from the leaves using a plastic straw. Care should be taken to avoid major veins within the leaf; the vascular tissue is not photosynthetic and has greater density which can bias the results.

Photosynthesis Chambers. Each student should prepare a minimum of six chambers (1 replicate). Each chamber consists of a 10 ml syringe. Wrap each chamber with the appropriate color of cellophane or plastic filter material. When wrapping the chambers, use the number of layers of cellophane/plastic which was determined in filter calibration. Remove the syringe plunger from each of the chambers. Place 5 leaf disks in each chamber. Add 8 ml of the bicarbonate solution to each chamber. Replace the plunger in each chamber and remove the air from the chambers. The volume of bicarbonate solution in the chambers after

air removal should be 7.5 ml. The leaf discs will float until the discs are degassed and the intercellular spaces within the discs are filled with the bicarbonate solution. It is critical that each chamber is degassed in the same manner. To degas the leaf discs, place your finger over the end of the syringe. Next, slowly pull the syringe plunger to the 10 ml/cc mark on the syringe. Hold the plunger at the 10 ml/cc mark for 20 sec. before releasing the plunger. You should shake the syringe to swirl the leaf discs. If the leaf disks do not sink, you should repeat the procedure. You should place the chamber/syringe in the dark (a desk drawer is fine) after the discs sink. The procedure is repeated for each syringe/chamber until all are degassed.

EXPERIMENTAL PROCEDURE

1. Place the degassed photosynthesis chambers in test tube racks. The distance which the test tube racks are placed from the light source was determined in the METHODS section under Filter Calibration.
2. Turn on the light source and record the time.
3. Observe the chambers and record the time at which each disc floats in each treatment. If any discs do not float or require times of greater than 125% of the period required for the initial disc(s), examine the discs at the conclusion of the experiment.
4. Average the time periods required for the discs to float for each treatment.

OBSERVATIONS AND QUESTIONS

Plot a graph of the time versus color. If the spectral absorbance maxima for the filters are available, plot the graph of time verses the maxima. What changes occur in the color of the cotyledons during the experiment? Is degreening delayed or promoted by any of the treatments?

Compare your activity spectra to the standard activity spectra in Figure 2. How do the spectra correspond? How do the spectra differ? How do you account for differences?

Why should you average the times required for the discs to float?

Examine the leaf discs which did not float or required excessive periods of time to float. Do you see any differences in these discs? Develop a hypothesis to explain why these discs did not float. How would you test your hypothesis?

The filters were calibrated to provide similar quantities of light, but is all light equal? (Hint—Does a unit of blue light contain the same amount of energy as a unit of red light?) If all light is not equal, how would this fact alter the interpretation of your results?

The solutions in the photosynthetic chambers were degassed at the beginning of the experiment. Photosynthesis requires the gas carbon dioxide. What was the source of carbon dioxide for the leaf discs during the experiment?

SUGGESTIONS FOR MODIFICATIONS AND ADDITIONAL EXPERIMENTS

1. Compare the effect of different quantities of light on photosynthesis by changing the light source. A light meter which provides light intensity in foot-candles, Einsteins, or other units is required to calibrate the light sources. Syringes without filters can be used. Plot the intensity of the light source verses the time required for the disc to float. Interpolate the line to the X-axis (light intensity). The point where the line crosses the X-axis is the light compensation point. At high light intensities, the plot will plateau. The plateau indicates the light saturation point.
2. Compare the floatation rates of leaf discs removed from C-3 and C-4 plants. Students can observe the differences which exist for the two types of plants in photosynthetic rates at the same light intensity, different light compensation points, and different light saturation points (Chollet and Ogren, 1975). Corn and soybean are C-4 and C-3 plants which most students recognize and are ideal for this experiment.
3. The action of several herbicides can be examined. The herbicide DCMU is a specific poison for Photosystem II. The herbicide fluoridon inhibits carotenoid biosynthesis. Thus the protective role of the carotenoids is negated. Tissues can be pretreated with fluoridon and moved into dim light or placed in the dark approximately 24 hr prior to the experiment. During the experiment the exposure to bright light destroys the chlorophyll present within the discs.

POTENTIAL PROBLEMS AND CONSIDERATIONS

Two potential problems exist. First, all of the photosynthesis chambers must be degassed with consistent vacuum. If excessive vacuum pressure is placed on any single sample, the sample will give aberrant results. Thus the plungers of

syringes should be drawn back to the same position to degas uniformly.

Secondly leaf discs should contain no major veins. The density of the tissue must be as consistent as possible. If the students avoid cutting discs which contain the midrib, high consistency can usually be achieved. Similarly necrotic or yellowing regions of the leaves should be avoided.

Literature Cited

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Editor
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Dear Editor;

I detect a slight interruption in the early morning caffeine infusion ritual as the perforated ceiling tiles announce "We apologize for the delay, now that the fog has lifted we will begin boarding USAIR flight 228 to Pittsburgh." At 10,000 feet, dilated pupils contract in response to the overwhelming brilliance of the cloudscape. Is it airspace or cyberspace? Once again I return home satiated from the Annual AMCBT Meeting.

Approximately 90 AMCBT members and prospective members made the trek to Henderson, KY to share ideas and visions for teaching college biology. Unfortunately, only 12 attendees returned the 1995 program planning sheets before leaving. Others have been promised, and I know I will hold them soon. However, for those who were unable to attend, a copy of the planning form is printed in this issue. Please consider attending and making a presentation at the 1995 AMCBT Annual Meeting at Alverno College, Milwaukee, WI , to be held September 28-30. The theme for this meeting is "Breaking Through Technological Barriers." We are not just seeking presentations from techno-junkies and cyberpunks, but also presentations from neophytes who have no intention of metamorphosing into hackers. Please plan to share your ideas and experiences (rewarding and/or frustrating) as we enter the fascinating world of cyberspace.

Thanks.

Buzz Hoagland, 1995 Program Chair
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