Experiment #4

Inhibition of Photosynthesis by High Temperature

Note to Instructor

In this experiment the students examine the inhibition of photosynthesis by high temperature and determine if the photochemical reactions of photosynthesis, or the dark reactions of the Calvin Cycle, are most adversely affected. This involves measuring quantum yield in dark-adapted leaves, and in leaves maintained under illuminated conditions, a measured period after being heated to a specific temperature. Three replicate leaves are used for each treatment to allow for statistical analysis of the data. The experiment is, therefore, somewhat more involved than experiments 1 – 3, and it is advised that one group of students should analyze the dark-adapted leaves while another group analyzes the illuminated leaves. Also, because of the time constraints of the experiment, it is not easy, during a single lab period, to measure CO_2 *exchange of the leaf samples while measuring fluorescence. Two other groups of students could measure* CO_2 *exchange in the leaf samples after fluorescence characteristics have been measured.*

Introduction

Before beginning this experiment it is important to read the theoretical background in the review section of this manual entitled "Chlorophyll Fluorescence and Photosynthetic Activity".

Chlorophyll Fluorescence measured by the apparatus you will be using arises from the dissipation of energy absorbed by chlorophyll molecules in photosystem II of photosynthesis. Fluorescence (F) is one of three processes by which this energy may be dissipated, the others being heat production (D) and transduction of light energy to chemical energy by photochemistry (P). According to the laws of energy conservation these 3 processes are related as follows:

$$\mathbf{F + D + P = 1}$$

The probability of F, D or P being the predominant process in energy dissipation changes with the condition of the leaf. Usually, plant scientists are most interested in the quantum yield of photosynthesis (P) since this provides a relative measurement of the efficiency with which the leaf converts light energy to chemical energy. This parameter can be measured as (Fm – Fo)/Fm (expressed as Fv/Fm) in a dark-adapted leaf, or $(F^{\prime}m - F)/F^{\prime}m$ (expressed as $\Delta F/F^{\prime}m$) in an illuminated leaf (see equations 1 to 10 of the review section).

Maximum quantum yield is measured by measuring fluorescence just before and just after applying a brief flash of saturating light to a leaf that has previously equilibrated in the dark. In healthy leaves maximum quantum yield in dark-adapted leaves has a value between 0.75 and 0.85. This value declines during illumination, partly because the proportion of oxidized electron acceptors in PSII declines with increasing irradiance, and partly because heat dissipation is stimulated by the establishment of the chloroplast transthylakoid proton gradient.

In this experiment you will be measuring quantum yield in leaves that have been pre-treated at different temperatures. As you will see from the review section, quantum yield (P) is related to photosynthetic activity by the following relationship:

$$\mathbf{ETR = P \times PAR \times 0.42}$$

Where PAR is the irradiance (µmol quanta/m^2/s) incident on the leaf. Therefore, P is directly proportional to ETR in leaves that are maintained at the same irradiance level.

One group of students will use leaves that have been kept in the dark after the heat treatment, and another group will use leaves that are kept under a constant light level after the heat treatment. The former group will therefore investigate the effects of heat solely on the photochemical apparatus (since no Calvin Cycle activity occurs in the dark), while the latter group will investigate effects on the entire photosynthetic apparatus. Other groups of students may measure CO_2 exchange in the leaf samples after fluorescence has been measured.

For most plants photosynthetic activity is inhibited as the temperature increases above 40• C. To characterize the susceptibility of a plant to elevated temperature a T_{50} value is detremined representing the temperature at which 50% of electron transport is suppressed after 5 minutes at that temperature. In this experiment you will determine the T_{50} for the plant material provided by your instructor.

Materials Required

- A chlorophyll fluorometer with a gain potentiometer to control the level of the LED light, a DIN cable to take the Fluorescence signal to the computer interface and a ground cable.

- An actinic light control box with a potentiometer for controlling the level of the actinic light, a timer to control the frequency of saturating light pulses from the actinic light, a manual push switch to provide saturating light pulses as required, and a cable to take the irradiance signal from the light source to the computer interface.

- A pulse-modulated LED chlorophyll excitation light on a cable for attachment to the fluorometer

- A chlorophyll Fluorescence detector on a cable for attachment to the fluorometer

- A laboratory stand

- A filtered 50W actinic halogen light source mounted in a lamp housing and attached to a metal bracket for positioning on the laboratory stand

- An aluminum bracket attached to the actinic lamp holder. This incorporates a leaf clamp for holding the leaf in a stable position with respect to the LED light and Fluorescence detector, and fittings for mounting the LED light and Fluorescence detector above the leaf clamp It also holds a light sensor that is calibrated to measure the PAR incident on the leaf.

- A DC power supply specific for your local power grid.

- A 4 channel Universal Lab Interface (ULI) and Logger Pro software or 2 channel Serial Box Interface and Data Logger software.

- A water bath that may be regulated at 34• C, 38• C, 42• C, 46• C, 50• C, and 54• C.

- 3 leaves of a suitable plant (e.g. soybean) for each temperature and light treatment. That is, 3 leaves for the 34• C dark-adapted treatment, and 3 leaves for the 34• C illuminated treatment etc. for a total of 36 leaves. As far as is possible, all leaves should be of similar age and from the same part of the plant.

- Aluminum foil for wrapping leaves prior to immersion in the water bath.
- Tissue paper for keeping illuminated leaves moist prior to fluorescence analysis.
- A light bank that can provide even illumination to 18 leaves at the same time.

Set Up File Required

Fluores

System and Software Set-Up

Follow the procedures for setting up the fluorescence system as described in the section above entitled “Configuration of Chlorophyll Fluorescence System.”

Load the Logger Pro Program and the appropriate set-up file by following the instructions in the section above entitled “Running Logger Pro.”

Experimental Procedure

(1) Wrap the detached leaves in aluminum foil and place 3 of them in the water bath at a temperature of 34• C. Leave them in the water bath for exactly 5 minutes, and then remove them to the lab bench. If you are in the group measuring dark adapted leaves do not remove the aluminum foil from the leaves but keep them in the darkened condition for 1 hour before measuring fluorescence. If you are in the group using illuminated leaves, remove the foil from the leaves and place a piece of moist tissue paper over them to keep them hydrated. Place the leaves under a light bank for 1 hour before measuring fluorescence.

(2) Increase the temperature of the water bath to 38• C and repeat step 1 with another 3 leaves. Be careful to time you experiment so that each leaf is subjected to a 5 minute temperature treatment, and 1 hour at room temperature, before fluorescence is measured. Also, if using the illuminated leaves, be sure that the same amount of tissue paper is used to keep the leaves moist so that they receive the same amount of illumination from your light source.

(3) Increase the water bath temperature to 42• C, and then to 46• , 50• C and 54• C, repeating step 1 above each time.

(4) Adjust the time axis on the fluorescence graph to a maximum of 15 minutes. Click on the Collect icon to start collecting data. Obtain a zero fluorescence reading without any leaf on the leaf clamp. If the numerical value for fluorescence on screen reads 0.00, use a small screw-driver to adjust the zero control on the rear of the fluorometer until the value increases to just above zero (e.g.0.05). This is necessary because Logger Pro cannot read negative voltages and the system may have a significant zero offset unless a true zero is measured. Stop data collection but don’t save the data.

(5) After 1 hour at room temperature place the first leaf from the 34• C treatment on the leaf clamp. Turn on the actinic light control box and turn the “Intensity” potentiometer clockwise until it clicks, but do not activate the actinic light.

(6) Click on the Collect icon to start collecting data and observe the voltage signal from the Fluorescence detector both graphically and numerically in the data box at the bottom of the computer screen.

(7) Adjust the gain control on the fluorometer, if necessary, to set the Fluorescence reading at an appropriate value with a low signal noise. The value you obtain at this time is the Fo value (dark-adapted leaf) or Ft (illuminated leaf). A reading between 0.2 and 0.6 is usually optimal. At this point you may adjust the position of the LED and detector housing to optimize signal characteristics.

(8) Press the Flash button on the light control box and observe the transient increase in the chlorophyll Fluorescence signal. The peak value represents Fm (dark-adapted leaf) or $F^{/}m$ (illuminated leaf).

(9) Place the second leaf from your 34• C treatment on the leaf clamp and measure Fo (or Ft) and Fm (or $F^{/}m$) as described above. Repeat with the third leaf from the 34• C treatment.

(10) Stop data collection by clicking on the STOP icon. Save you data under an appropriate name by using the “Save As” option in the File menu.

(11) Repeat the above procedure with the 3 leaves from each temperature treatment, ensuring that in each case the leaves have been maintained for exactly 1 hour at room temperature after the treatment.

Data Analysis

- Open the file containing data from the 34• C treatment. Your data will appear on the screen exactly as it appeared when you saved it at the end of the experiment.

- Click on the graph showing your Fluorescence data and then select VIEW from the main menu. Click on GRAPH LAYOUT, select ONE PANE and then click on OK. The Fluorescence graph will now fill the entire screen making data analysis easier.

- Place the cursor to the left and just above the part of the trace showing your first measured Fm or $F^{/}m$ value. Click and hold on the mouse as you drag the cursor across your data so that a black box appears around the Fo (or Ft) and Fm (or $F^{/}m$) values.

- Select VIEW and ZOOM IN. The data in the black box will now fill the entire screen.

- Select ANALYZE and then EXAMINE. A vertical line will appear on your graph that can be moved along the data points on the graph by moving the mouse. A box will also appear on each graph showing data values and time values. As you move the vertical line on the graph, the numerical display in the box will change to show you the exact data values and time value at the point on the graph where the line is situated. If the box obscures any part of the trace click on it and hold, then drag with the mouse to place the box in a convenient location.

- Scroll the cursor across your Fluorescence data and identify the values of Fo and Fm, or Ft and $F^{/}m$. Record these data in Table 1 or Table 2 and calculate quantum yield. If you have difficulty identifying peak data refer to the **Statistics** section below.

- Select VIEW and then UNDO ZOOM. Your graph will return to its original configuration. Move the cursor the point where you measured fluorescence from your next leaf sample and repeat the above procedure.

- Open the files containing data collected with leaf samples from the other temperature treatments and record the data in Table 1 (dark-adapted leaves) or Table 2 (illuminated leaves).

- Plot mean maximum quantum yield (Fv/Fm) against temperature for the dark-adapted leaves, and mean quantum yield ($\Delta F/F^{/}m$) against temperature for the illuminated leaves.

Using the Statistics Function to Obtain Values

If there is noise on the Fluorescence signal, or the $F^{/}m$ value is only slightly greater than the Ft value, you may use the Statistics function of Logger Pro to obtain the best values for your Fluorescence parameters.

Zoom In on your data as described above and then highlight the data you wish to examine by clicking and dragging over the data with the mouse. A black box will appear around the selected data, which will remain when you unclick on the mouse.

Select “Analyze” from the main menu and then “Statistics”. A box will appear on the screen showing the mean, maximum and minimum values of all the data in the box, as well as other statistical parameters. From this data you can obtain, for example, the mean value of Ft or Fo, before a saturating light pulse was applied. If you use the statistics function to analyze the data following a saturating pulse, the maximum value shown in the statistics box identifies the maximum Fluorescence value (Fm or $F^{/}m$). You may delete the statistics boxes from the screen by clicking on the icon in the top right hand corner of the box.

Results and Discussion

Dark-Adapted Leaves

Table 1.

Temp	Fo	Fm	Fv/Fm	Mean Fv/Fm
34	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
38	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
42	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
46	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
50	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
54	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	

Illuminated Leaves

Table 2.

Temp	**Ft**	**$F'm$**	**ΔF/Fm**	**Mean ΔF/Fm**
34	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
38	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
42	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
46	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
50	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	
54	(a) (b) (c)	(a) (b) (c)	(a) (b) (c)	

Compare the mean quantum yield in dark-adapted leaves at 34• C with mean quantum yield in illuminated leaves at the same temperature and explain any differences you observe.

In the dark-adapted leaves Fv/Fm should approximate to 0.8, whereas the value of $\Delta F/F'm$ *in the illuminated leaves should be significantly lower. After dark adaptation, all photosynthetic electron acceptors are fully oxidized and available for photochemical energy transduction so quantum yield is maximized. After illumination, a proportion of the electron acceptors will be reduced at any one time and therefore not available to accept electrons from chlorophyll. Also, development of the transthylakoid proton gradient during illumination increases the amount of energy that is dissipated as heat from the chloroplasts. Both of these factors contribute to a decline in quantum yield during illumination.*

Compare the graphs showing the relationship between quantum yield and temperature treatment in the dark-adapted and illuminated leaves. Explain any differences that you observe, and measure the value of T_{50} from each graph.

Quantum yield should be lower in the illuminated leaves at all temperature for the reason given in the answer to the question above. Quantum yield in both graphs should remain fairly constant at 34• C and 38• C since these temperatures are not usually inhibitory to photosynthesis. At higher temperatures quantum yield should decline rapidly in the illuminated leaves and less rapidly in the dark-adapted leaves. Therefore, the T_{50} *in the illuminated leaves should be lower than that in the dark-adapted leaves.*

The decline in quantum yield is due to inactivation of photosynthetic mechanisms by high temperature. Since the dark-adapted leaves have no Calvin Cycle activity, the quantum yield measurements represent activity of PSII only. The Calvin Cycle is active in the illuminated leaves, so changes in their quantum yield are indicative of changes in the overall process of photosynthesis. In fact, since these leaves were maintained under the same irradiance (same PAR), their quantum yield is directly indicative of electron transport rate. Because the illuminated leaves show a greater decline in quantum yield than the dark-adapted leaves, the data indicate that the enzymes of the Calvin cycle are more susceptible to heat-inactivation than the photochemical processes involved in PSII.