

# Experiment #1

## Measurement of Photochemical Quantum Yield and the Kautsky Effect

### 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:

$$F + D + P = 1$$

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  $(F_m - F_o)/F_m$  (expressed as  $F_v/F_m$ ) in a dark-adapted leaf, or  $(F'_m - F)/F'_m$  (expressed as  $\Delta F/F'_m$ ) in an illuminated leaf (see equations 1 to 10 of the review section).

In this experiment you will be measuring both  $F_v/F_m$  and  $\Delta F/F'_m$  and observing how quantum yield changes after the onset of illumination. If you are measuring  $\text{CO}_2$  exchange at the same time as measuring fluorescence you will observe that  $\text{CO}_2$  fixation does not begin immediately when the actinic light is supplied. Instead, there is an induction period before measurable  $\text{CO}_2$  uptake occurs.

### 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.

### **Set Up File Required**

ChlFluor if measuring chlorophyll fluorescence only

Fluores if measuring chlorophyll fluorescence and CO<sub>2</sub> exchange

### **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) Adjust the time axis on the fluorescence graph to a maximum of 10 minutes. Click on the Collect icon to start collecting data. Obtain a zero fluorescence reading without any leaf in the leaf chamber. 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.
- (2) If measuring CO<sub>2</sub> exchange as well as fluorescence, place a dark-adapted leaf into the leaf chamber and place an opaque card (such as a business card) over the window of the chamber to maintain the dark condition. If not measuring CO<sub>2</sub> exchange keep the sample leaf in a dark condition while you configure the fluorescence apparatus.
- (3) Turn on the light control box by turning the switch to the ‘On’ position and turn the “Intensity” potentiometer clockwise until it clicks. This will maintain the leaf in the dark until the potentiometer is turned clockwise. You may also set the potentiometer to a desired light setting before selecting the ‘On’ position with the switch. This will allow you to expose the leaf immediately to the desired actinic irradiance without moving through a range of lower irradiances.
- (4) Turn on the light control box by turning the “Intensity” potentiometer clockwise until it clicks, but do not activate the actinic light.
- (5) Observe the voltage signal from the fluorescence detector both graphically and numerically in the data box at the bottom of the computer screen. Fluorescence will have a low value because the card contains very little material that fluoresces at the wavelengths detected by the photodetector.

- (6) Press the red “Flash” button on the front of the Actinic Light Control Box. This will produce a flash of light with an intensity in excess of  $5000 \mu\text{mol quanta/m}^2/\text{s}$ . A spike will be seen on the graph showing actinic light level. This is an event marker and the recorded level does not correspond to the light level during the flash. Note that there is little increase in the fluorescence signal during the flash of light.
- (7) If not measuring  $\text{CO}_2$  exchange place the dark adapted leaf in the leaf clamp. Stop data collection by clicking on the STOP icon. There is no need to save the data collected with the card in place.
- (8) Start data collection by clicking on the Collect Icon. Remove the card so that the leaf is exposed to the LED source, and adjust the gain control on the fluorometer, if necessary, to set the fluorescence value at an appropriate value with a low signal noise. The value you obtain at this time is the  $F_0$  value. 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 (and the leaf chamber if it is used) to optimize signal characteristics. If you make significant adjustments to the system it may be necessary to re-measure zero, without the leaf in the chamber, at the end of the experiment.
- (9) Press the Flash button on the light control box and observe the transient increase in the chlorophyll fluorescence signal. The peak value represents  $F_m$ . Note that the saturating flash disturbs the dark-adapted state of the leaf. Therefore, if you have any difficulty in obtaining a good  $F_m$  value, and need to adjust the geometry of the system, you must dark adapt the leaf before measuring  $F_m$  again.
- (10) Having measured  $F_m$ , turn on the actinic light source by turning the “Actinic” potentiometer on the control box clockwise. Adjust this slowly until the light level increases to approximately  $200 \mu\text{mol quanta m}^2/\text{s}$ . This will cause fluorescence to increase transiently to a level close to that of  $F_m$ . When it reaches this level, press the “Flash” button on the actinic light control box to obtain a value of  $F'_m$ .
- (11) Press the flash button every 20 seconds as the fluorescence signal declines from its maximum at  $F'_m$ , or apply saturating pulses automatically by turning the “Auto Flash” control potentiometer on the Actinic Light Control box clockwise to almost its full range.
- (12) When the steady fluorescence signal ( $F_t$ ) has reached a steady value click on the STOP icon to stop data collection.
- (13) Save your data by selecting “Save As” from the FILE menu. Give your data an appropriate name other than the name of the set-up file, and save it in the location allocated by your instructor.

### ***Data Analysis***

- Open the file containing your data. 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 Fm value in the dark adapted leaf. Click and hold on the mouse as you drag the cursor across your data so that a black box appears around the Fo and Fm values collected at the beginning of your experiment.
- 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 in the dark adapted leaf. Record these data in Table 1. Remember to subtract the zero value if this is significant. 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 to the next point in which you applied a saturating flash to the leaf and Zoom In as described above. Record the Ft value attained just before the saturating flash and the F/m value attained during the flash. Record these data in Table 2.
- Calculate the quantum yield of photochemistry ( $F_v/F_m$  and  $\Delta F/F_m$ ) at each stage in your experiment.

### *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 F 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

**Table 1. Dark-Adapted Values**

<b>F<sub>o</sub></b>	<b>F<sub>m</sub></b>	<b>F<sub>v</sub>/F<sub>m</sub></b>

**Table 2. Values in Illuminated Leaf**

<b>Time after Actinic On (seconds)</b>	<b>F<sub>t</sub></b>	<b>F'<sub>m</sub></b>	<b>ΔF/F'<sub>m</sub></b>
Stable Value			

**Explain the change in photochemical quantum yield between the dark-adapted state and the stable value after illumination.**

*In the dark-adapted leaf F<sub>v</sub>/F<sub>m</sub> should approximate to 0.8, whereas the value of ΔF/F'<sub>m</sub> in the illuminated leaf at steady state 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 as irradiance is increased.*

**Describe and explain the changes that occur in fluorescence yield (F<sub>t</sub>) and quantum yield (ΔF/F'<sub>m</sub>) after transition from darkness to light (Kautsky Effect).**

*Within seconds of the actinic light being turned on the fluorescence yield increases. It does not, however, reach the maximum value obtained during the saturating pulse after dark-adaptation (F<sub>m</sub>). This increase in fluorescence yield represents reduction of the electron acceptors associated with photosystem II (e.g. Q<sub>A</sub>) though full reduction of these acceptors may not be attained. Full reduction only occurs with the imposition of saturating light pulses to obtain F'<sub>m</sub> values. The F'<sub>m</sub> values decline rapidly after the saturating pulse because electrons may be passed to PSI as photosynthesis is activated.*

*F<sub>t</sub> declines from its maximum value usually within 1-2 seconds of illumination, and then begins a slower decline over several minutes. A corresponding decline in F'<sub>m</sub> is also seen. The decline in F<sub>t</sub> is due to activation of photosynthesis and the opening of more electron acceptors. The decline in F'<sub>m</sub> results*

*from the development of the transthylakoid proton gradient for ATP generation, and has the effect of increasing heat dissipation from the chloroplasts.*

*After several minutes of continuous illumination  $F'/m$  values begin to increase again. This is linked with Calvin Cycle activity and associated use of the ATP generated by photophosphorylation. The partial relaxation of the transthylakoid potential (due to ATP synthesis) reduces heat loss from the chloroplasts.*

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