Experiment #3

Measurement of Photoinhibition in Sun and Shade Leaves

Note to the Instructor

Soybeans are suitable for this experiment since they grow rapidly and develop broad leaves that are easily accommodated in the leaf chamber. Ideally, the high light plants should be grown at at least half full sunlight (approx. 1000 µmol quanta/m^2/s), which may be achieved by growing them in a greenhouse. A growth chamber with a high light output may also be used. Shade plants should be grown at approximately 200 µmol quanta/m^2/s which can be achieved by using neutral density screens (made from cheesecloth) in the greenhouse or growth chamber. All environmental conditions, except light, should be the same for both populations of plants.

The upper leaves of sun plants, which have not been subjected to shading, should be used in the experiment. The upper leaves of the shade plants should also be selected, so that leaves in the same relative position (and of similar developmental stage) are used from both populations of plants.

If measuring only fluorescence values, the entire experiment may be conducted in a single lab period by one lab group. If measuring CO_2 exchange as well as fluorescence, the experiment may still be completed in one lab period if one lab group collects data using the sun leaf and another lab group collects data using the shade leaf.

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'm – F)/F'm (expressed as ΔF/F'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. However, after re-darkening, quantum yield returns quickly

to its maximum value as the PSII electron acceptors become re-oxidised and the transthylakoid membrane potential collapses.

In this experiment quantum yield will be measured in a leaf from a plant that has been grown in shade conditions and in a leaf that has been grown at a higher irradiance. The first measurements will be made after each leaf has been kept in the dark for 30 minutes, then each leaf will be exposed to saturating light at approx. 2000 µmol quanta/m^2/s. Measurements of maximum quantum yield will then be made at different times after this exposure to high light. It will be shown that exposure of the shade leaf to excessive illumination causes an irreversible decline in quantum yield, while quantum yield in the sun plant recovers to its original level. If CO_2 exchange is measured in these leaves it will be shown that photosynthesis in the shade leaf is inhibited by exposure to the high light level whereas photosynthesis in the sun plant is unaffected. This experiment therefore demonstrates the phenomenon of photoinhibition, in which the photosynthetic apparatus of plants grown in low light conditions is adversely affected by exposure to high light levels. It should act as a cautionary note to those students who attempt to re-vitalize a sorry house-plant by placing it on a sunny porch.

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 plant grown in a shade environment and a plant grown in a sunny environment.

Set Up File Required

Fluores if measuring chlorophyll fluorecence only
FluorCO_2 if measuring chlorophyll Fluorescence and CO_2 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 60 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_2 exchange as well as fluorescence, place a dark-adapted leaf from the shade plant 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_2 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 µmol quanta/m^2/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 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 Fo 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 Fm. Note that the saturating flash disturbs the dark-adapted state of the leaf. Therefore, if you have any difficulty in obtaining a good Fm value and need to adjust the geometry of the system, you must dark adapt the leaf before measuring Fm again.

(10) Having measured Fm, turn on the actinic light source and adjust the "Actinic" potentiometer on the control box by turning it clockwise slowly until a light level of approx. 2000 µmol quanta/m^2/s is attained. This will cause Fluorescence to increase quickly and then decline slowly to a steady value Ft when photosynthetic induction is complete. Maintain the leaf at this light level for 20 minutes. If measuring CO_2 and fluorescence, this period should be long enough to obtain a steady measurement of CO_2 uptake. Press the red Flash button on the actinic at then end of this period to measure F'Fm.

(11) 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.

(12) If using the leaf clamp, cover the leaf with an opaque cloth or other opaque material to keep it in the dark. If using the leaf chamber darken the leaf by placing a card over the chamber window. Keep the leaf in the dark for 5 minutes and then remove the opaque material.

(13) Start data collection by clicking on the START icon and wait until a steady Fo value is attained. Then press the Flash button to obtain a value of Fm.

(14) Darken the leaf for a further 5 minutes and then repeat the Fo and Fm measurements. Repeat this procedure, until you have measurements of Fo and Fm 5, 10, 20, 30, 40 and 50 minutes after exposure to the 2000 µmol quanta/m^2/s irradiance level. Between each measurement keep the leaf in the dark.

(15) Stop data collection by clicking on the STOP icon and save your data under an appropriate file name by selecting "Save As" from the FILE menu.

(16) If you are measuring CO_2 exchange, expose the leaf once more to 2000 µmol quanta/m^2/s and wait until a steady CO_2 uptake value is reached.

(17) Repeat the entire procedure using the sun leaf.

Data Analysis

- Open the file containing data from the first part of the experiment with the shade plant. 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 and calculate Fv/Fm. 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 point in which you applied the saturating flash to the leaf after illumination at 2000 μmol quanta/m^2/s. 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 1 and calculate $\Delta F/F^{/}m$.

- Open the file containing the rest of your fluorescence data for the shade leaf and repeat the procedure for measuring Fo and Fm as described above. Record these data in Table 2 and then plot the Fv/Fm values against time after exposure to 2000 μmol quanta/m^2/s.

- Open the files containing data collected with the Sun Plant and repeat the data analysis procedure. Record your data in Tables 3 and 4.

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

Shade Leaf

Table 1. Initial Values

Condition	Fo or Ft	Fm or F′m	Quantum Yield
Dark Adapted Leaf Leaf at High Light Level			

Table 2. Values after Illumination

Time After Exposure to High Light Level (min)	Fo	Fm	Fv/Fm
5			
10			
20			
30			
40			
50			

Table 3. Initial Values

Condition	Fo or Ft	Fm or F′m	Quantum Yield
Dark Adapted Leaf Leaf at High Light Level			

Table 4. Values in Illumination

Time After Exposure to High Light Level (min)	Fo	Fm	Fv/Fm
5			
10			
20			
30			
40			
50			

For each leaf explain the change in photochemical quantum yield between the dark-adapted state and after illumination at high light level.

In each dark-adapted leaf Fv/Fm should approximate to 0.8, whereas the value of $\Delta F/F'm$ in each 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.

For each leaf, describe the relationship between quantum yield and time after illumination. Explain any differences you observe.

It is likely that both the sun and shade leaves will show some reduction of quantum yield within the first 20 minutes after exposure to 2000 µmol quanta/m^2/s. This reduction will likely be significantly greater in the shade leaf. Twenty minutes after high light exposure the sun leaf should show a significant recovery of quantum yield to approximately the pre-illumination level. The shade leaf, however, is not adapted to high light conditions and quantum yield will not recover to pre-illumination levels.

The extent of the reduction of quantum yield in the shade leaf is indicative of its degree of photoinhibition. That this reduction occurs in the Fv/Fm value (i.e. the quantum yield in the dark-adapted leaf) indicates that the inhibitory mechanism occurring in high light affects the photochemical apparatus directly, and is not associated primarily with inhibition of Calvin Cycle components. However, photoinhibition does lead to an inhibition in carbon fixation. If CO_2 exchange was measured at 2000 µmol quanta/m^2/s in the two leaves, photoinhibition may have been observed as a decline in CO_2 fixation rate between the two measurement periods. The degree of inhibition would likely be significantly greater in the shade plant.

What environmental conditions, in addition to high light levels, would likely exacerbate photoinhibition? Why should this occur?

Any environmental condition that inhibits Calvin Cycle activity will exacerbate photoinhibition under high light levels. Such conditions include low temperature and water stress.

Photoinhibition occurs when the photon flux impinging on the leaf saturates the capacity of the leaf to process light energy. This capacity is lower in shade plants, relative to sun plants, because they have fewer reaction centers and a lower content of Calvin Cycle enzymes. However, photoinhibition becomes a problem in all plants when the activity of the Calvin Cycle is inhibited under high light conditions. This is because the Calvin Cycle must consume photochemically generated reductant to ensure the continued generation of this reductant. If the Calvin Cycle is inhibited, electron acceptors in PSII and PSI become fully reduced and cannot accept high energy electrons from excited chlorophyll molecules. Under these conditions the electrons may reduce molecular O2 to form oxygen free radicals that can damage chloroplast membranes.

Can you identify any non-physiological adaptations of shade leaves to low light environments?

Adaptations of shade leaves to low light include the development of larger, thinner leaves. Ideally, students should section leaves and examine them under the microscope where it will be seen that sun leaves are thicker because they develop longer palisade parenchyma cells, or an additional layer of these cells. Sun leaves also have fewer air spaces and a greater surface area of cells per leaf than in shade plants. In shade leaves, the chloroplasts are more numerous in the upper mesophyll cells, moving

there by phototaxis to maximize the capture of light supplied from above. Lower mesophyll cells usually have very few chloroplasts.

Copyright 2000
Qubit Systems Inc.
All Rights Reserved

Disclaimer: Documents from commercial sources are not to be taken as an endorsement of the company or its products.