Experiment #2

Effect of Irradiance Level on Photochemical Quantum Yield and Electron Transport Rate 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 leaves should be grown in approximately 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 leaf chamber. 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.

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 $\Delta F/F'm$) in an illuminated leaf (see equations 1 to 10 of the review section).

The relative quantum yield of photochemistry (P) can be used to estimate the rate of photosynthetic electron transport in the leaf provided that the photon flux incident on the leaf is known. Since 1 µmol of photons causes the excitation of 1 µmol of electrons from chlorophyll, and P represents the proportion of these electrons that are used in photochemistry, the electron transport rate (ETR) is related to product of P and the photon flux density of photosynthetically active radiation at the leaf surface (PAR). However, not all the light incident on a leaf is absorbed by chlorophyll molecules, since some is transmitted through the leaf and some is reflected. About 84% of incident light is absorbed by chlorophyll molecules in the average leaf, with 50% of the photons activating chlorophyll molecules associated with PSII and 50% activating PSI. Therefore, an estimate of electron transport rate can be derived from the following:

$$ETR = P \times PAR \times 0.42$$

In this experiment you will be measuring quantum yield in a dark adapted leaf and then in the same leaf at different light levels. You will also determine how electron transport rate changes at the different irradiances. If you are measuring CO_2 exchange at the same time as measuring fluorescence you will be able to construct a light response curve for photosynthetic CO_2 fixation from which you can determine the light compensation point, photochemical efficiency and the light saturation point of photosynthesis. Photochemical quantum yield and photochemical efficiency (measured as the slope of the light response curve for CO_2 fixation) should show similar responses to increasing irradiance. Photosynthetic rate and electron transport rate should also show a similar response to increasing irradiance up to the point of light saturation..

The experiment will be conducted with a leaf from a plant that has been grown in shade conditions and then repeated with a leaf that has been grown at a higher irradiance. The photosynthetic apparatus of each leaf will have adapted to its growth condition, and you should be able to identify differences between the leaves in the parameters that you measure.

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 30 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 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. 500 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. If measuring CO_2 and fluorescence, wait until the CO_2 value reaches a steady state before proceeding to step 9. This may take several minutes due to photosynthetic induction and the slow opening of stomata after illumination.

(11) Reduce the actinic light level to approx. 50 µmol quanta/m^2/s, wait until a steady Ft value (and CO_2 value) is attained and then press the Flash button to measure $F^{/}m$ at the new light level. Record the irradiance in Table 2 but do not attempt to record Ft or $F^{/}m$ until the experiment is complete.

(12) Repeat the procedure at 100, 200, 300, 400, 600, 800, 1200, 1600 and 2000 µmol quanta/m^2/s.

(13) Save your data by selecting "Save As" from the FILE menu. Give your data an appropriate name other then the name of the set-up file, and save it in the location allocated by your instructor.

(14) Repeat the experiment using a leaf from the Sun plant and enter your data in Tables 3 and 4.

Data Analysis

- Open the file containing data from 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. 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 the first saturating flash to the illuminated 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.

- Repeat the procedure at each irradiance used in your experiment. At the highest light intensities it may be more difficult to determine accurate values of Ft and Fm, since these values are closer together. In these cases, use the statistics function of Logger Pro, as described below, to obtain these values precisely.

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

- For each leaf calculate the quantum yield of photochemistry at each irradiance used in your experiment and plot this against irradiance.

- For each leaf calculate electron transport rate at each irradiance and plot this against irradiance.

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 "Analyse" 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. Dark-Adapted Values

Fo	Fm	Fv/Fm

Table 2. Values in Illuminated Leaf

Irradiance μmol quanta /m^2/s	Ft	$F^{/}m$	$\Delta F/F^{/}m$	e^- Transport Rate (μmol/m^2/s)

Sun Leaf

Table 3 Dark-Adapted Values

Fo	Fm	Fv/Fm

Table 4. Values in Illuminated Leaf

Irradiance μmol quanta /m^2/s	Ft	$F^{/}m$	$\Delta F/F^{/}m$	e^- Transport Rate (μmol/m^2/s)

For each leaf explain the change in photochemical quantum yield between the dark-adapted state and the first stable value after illumination.

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 and explain the relationship between quantum yield ($\Delta F/F'm$) and irradiance.

In each leaf quantum yield declines as irradiance increases. This is because quantum yield is an index of the efficiency of light energy conversion into chemical energy. This efficiency will be greatest when PSII electron acceptors are fully oxidized and are therefore able to accept electrons from excited chlorophyll molecules. The proportion of oxidized acceptors is greatest under low light conditions because as irradiance increases, and electron release from chlorophyll molecules increases in parallel, the number of PSII electron acceptors that are reduced will increase as a consequence. At the highest light intensities, the rate of reductant supply from photochemistry exceeds reductant use in the Calvin Cycle. As a result, the level of $NADPH_2$ increases and the availability of $NADP^+$ for reduction declines. Since there is insufficient NADP+ to accept electrons from the photosystems, the electron acceptors become fully reduced and quantum yield falls to its minimum value.

Shade plants may be adapted for greatercapture of photons at low light levels, but do they utilize these photons in photosynthesis more effectively than sun plants? Use your data to corroborate your answer.

If shade plants were more efficient than sun plants at transducing light energy, they should have a greater photochemical efficiency, as indicated by higher values of quantum yield at the lower light levels. If there were no difference in efficiency between the two plants, the quantum yields would be similar. If efficiency were lower in the shade plant values of quantum yield at low irradiance would be less.

For each leaf describe and explain the relationship between electron transport rate and irradiance. Explain any differences that occur in this relationship between the sun and shade leaves .

In each leaf electron transport rate increases in an almost linear fashion with increasing irradiance because more chlorophyll molecules are excited by the greater number of photons impinging on the leaf and provide more electrons to the electron carriers in the photosystems. At the highest light levels the relationship curves off to reach a steady maximum rate. This occurs when the chlorophyll molecules are light-saturated and any further increase in photon flux cannot increase the rate of electron release from the chlorophyll molecules.

Typically, electron transport rate will reach its maximum value at a higher irradiance in the sun plant than in the shade plant. This is because sun plants have less chlorophyll per reaction center than shade plants, but a greater concentration of reaction centers, and a greater capacity for electron transport. Also, the chloroplasts of sun plants tend to have a greater stromal volume than those of shade plants and a greater content of stromal proteins including the key carboxylating enzyme ribulose bisphosphate carboxylase-oxygenase. For these reasons, sun plants photosynthesize more efficiently in high light and attain greater maximum rates of photosynthesis than shade plants. Sun plants therefore have a greater ***photosynthetic capacity****. Although the chloroplasts of shade leaves have more grana than those of sun leaves, they have fewer reaction centers and these centers become saturated at lower light levels. Therefore, they have a lower light saturation point for photosynthesis. At the light saturation point, photosynthetic rate is limited by the ability of the leaf to transduce light energy into chemical energy, so that the reduced electron transport activity, and reduced content of stromal proteins, in shade leaves contributes to their reduced photosynthetic capacity.*

Often, electron transport rate may increase with irradiance to a maximum level and then decline somewhat as irradiance is increased further. This occurs more commonly, and to a greater degree, in shade leaves, and is caused by photoinhibition. This process is imperfectly understood but it is thought that excess excitation of chlorophyll leads to a stimulation of heat dissipation processes in the chloroplast so that energy transduction by both photochemsitry and fluorescence declines.

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.

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