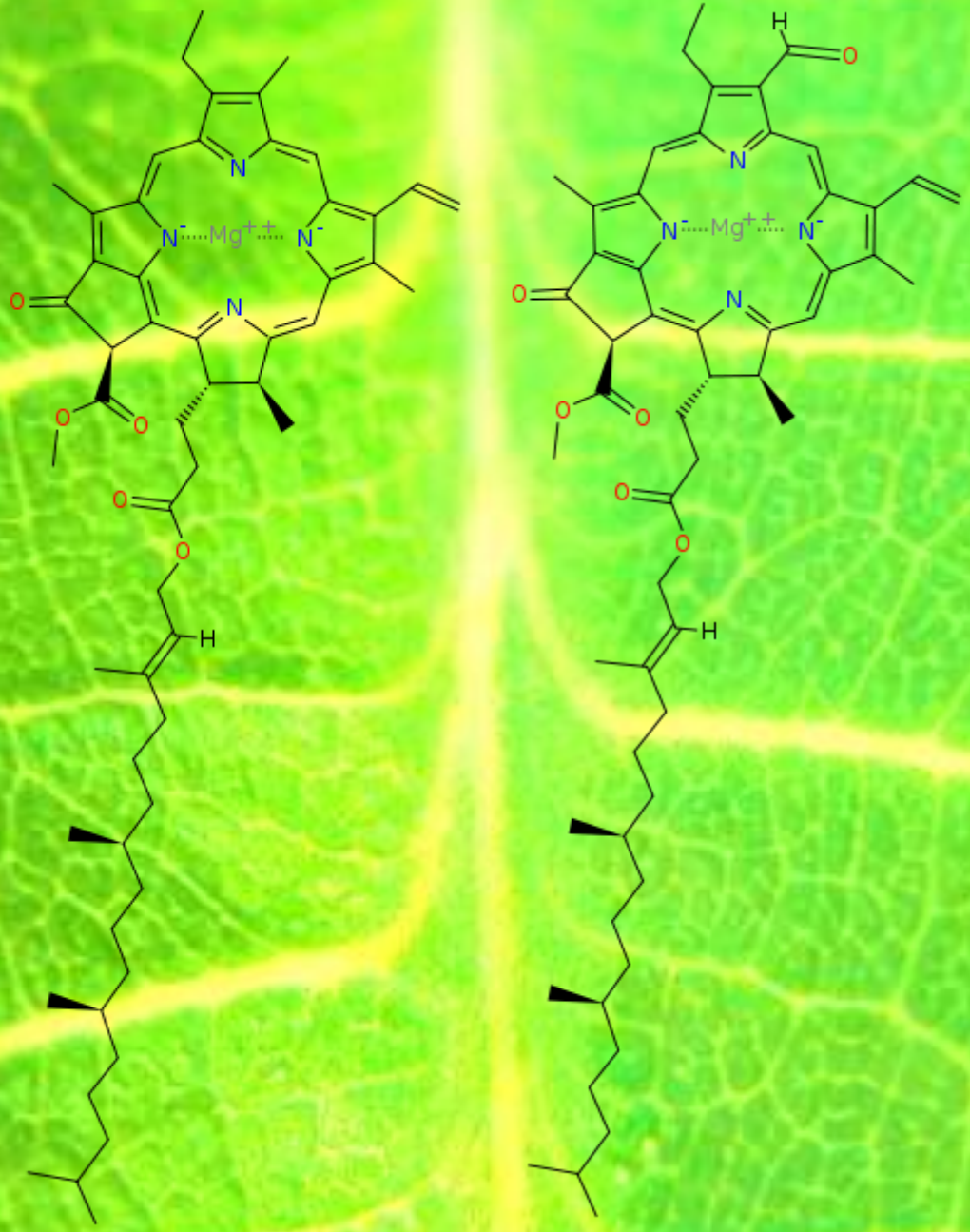
MANCHESTER 1824
The University of Manchester

Measuring Photosynthesis

Activities to measure components of the photosynthetic apparatus in leaves



Title	Measuring Photosynthesis
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Target level	Year 10 and above
Areas of the curriculum covered	Photosynthesis

Introduction

Photosynthesis is the basis of life of earth. It provides the energy that drives virtually all ecosystems. This activity centres on photosynthesis and how plants can be bred to increase their ability to capture light energy.

At present in the world, there is a growing food crisis. The global population is growing and demand for food is rising even faster, as nations become richer. At the same time, global climate change is making for increasing uncertainty in weather. Heat waves, droughts, floods, unseasonal cold weather and cloudy summers have all been blamed for poor crop yields. These conditions can affect all parts of a plant's life cycle but photosynthesis is in front line – it can respond to changes in the environment within seconds and the wrong conditions can lead to photosynthesis being reduced for days, even after the poor conditions have gone away. If we are to optimise the growth of crops to meet the growing demand, we need to understand how plants are affected by their environment and breed new varieties that are better able to tolerate poor environments.

Adapting to the environment

Although all plants are sensitive to their environment, we say they are "stressed" when conditions are poor, there is a lot of variation in what individual plants can and can't tolerate. For example, plants from the Arctic can grow as fast as plants in the tropics, even though the temperatures they experience are barely above freezing. Desert plants can survive months without water and plants from Death Valley, the hottest place on Earth, can survive temperatures that would destroy a normal plant. To survive in these different environments, plants have evolved adaptations that allow them to survive. Scientists are trying to understand these adaptations, to identify traits that maybe transferred to crops.

In this activity, we will look at two aspects of photosynthesis. 1) We will use a "Fluorpen" to measure the heat tolerance of different plants. 2) We will use a spectrophotometer to measure the pigment content of some different plants, to examine how plants vary these in response to the environment.

The Fluorpen chlorophyll fluorimeter with a leaf clipped into the measuring position

Activity 1: The Fluorpen

Background theory – pigments are coloured because they absorb specific wavelengths of light and reflect others. Chlorophyll, the main pigment in plants, absorbs blue and red light and reflects green, hence most plants appear green. When chlorophyll absorbs light, the energy is captured through a process called charge separation, converting light energy into chemical energy. At its best, a leaf can capture approx. 80% of light in this way. The capture is never perfect however, some energy is always lost. This can be in the form of heat or as light – fluorescence. Fluorescence can be measured easily using devices such as the Fluorpen because it is a slightly different colour to the absorbed light (longer wavelength). Fluorescence and photosynthesis compete with one another: if photosynthesis becomes less efficient, fluorescence increases. So, by measuring the fluorescence given off by a leaf we can estimate how efficient photosynthesis is.

Hypothesis – Photosynthesis is one of the first things affected by high temperature. As the leaf is damaged, the efficiency of photosynthesis drops (and fluorescence increases). However, plants vary in their sensitivity to heat. For example, we would predict that plants from hot climates would be less sensitive than those that grow in colder places. The aim of this experiment is to test the heat tolerance of a range of different leaves.

Experimental Protocol

For this experiment, you will need some leaves from different plants, a Fluorpen and some water baths which can be set to temperatures up to 80ºC (if you do not have water baths, the experiment can be performed by slowly heating water and removing leaves as different temperatures are reached). You will also need tongs (to remove leaves from the water), thermometers (ideally digital – to check water temperature) and timers (available on most phones).

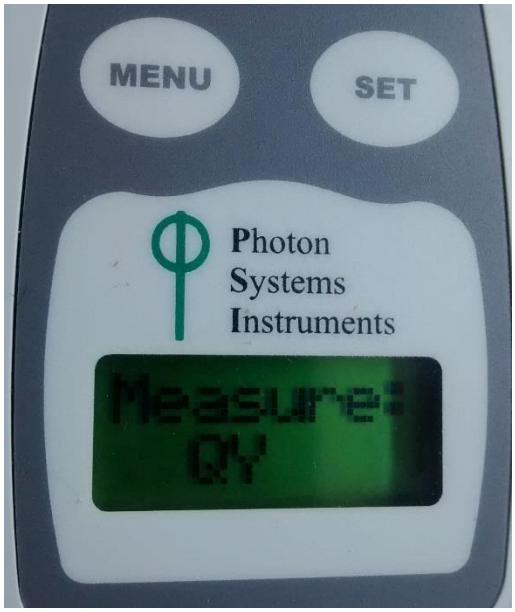
Risk assessment – this protocol involves working with hot water. There is a risk therefore or burns. Water baths should be placed well away from the edge of benches. Tongs should be used to take leaves in and out of the water.

1) *Collect some leaves* – Leaves from a number of different plants should be collected. Try to find species that vary in their leaf structure, e.g. soft leaves from thicker woody leaves. If possible, it is good to compare plants with different origins – Mediterranean plants such as olive, which have tough leaves that have to survive hot summers are good to compare with annual weed species which die off when things get too hot. To keep leaves as healty as possible following collection, wrap them in damp tissue paper and place ina sealed bag. Measurements are never perfect, so it is useful to collect several leaves of each plant. Normally, results would be presented as the mean of at least 3 measurements, with standard error bars shown.
2) *Heat treat leaves* – leaves should be placed in water baths at temperatures of 40, 50, 60, 70, 80 ºC. Leave the leaves for 5 minutes at the specified temperature, then remove them from the water. Place them on tissue paper and carefully pat dry.

3) *Measure fluorescence* – measurements are performed with the Fluorpen:
    a. *Switch on the Fluorpen* – press and hold the "Set button" until you here a noise.
    b. *Place in "measurement" mode* – Press the "Menu" button until you see the line ">Measure Ft, QY & …." scroll across the top line of the screen. Press "Set"
    c. *Select correct measurement* – Press "Menu" until you see ">QY" on the top line of the screen. Press "Set".
    d. *Place a leaf in the leaf clip* – at the top of the device, there is a simple clip. Press and hold to open, place the leaf you wish to measure in the clip, with the top side of the leaf facing the glass window on the device.
    e. *Make a measurement* – Press "Set" to make a measurement. A number will be displayed on the lower line of the screen. This is a measure of the "Quantum yield" (QY) of the leaf, i.e. what proportion of light is the leaf able to use in photosynthesis. The maximum normally seen is about 0.85. In stressed leaves, values can fall to near zero. If you forget to put a leaf in, you will get a value of zero.

If leaves are stressed by heat, the value of QY is expected to fall. It is predicted that, in heat tolerant leaves, the value will be maintained at higher temperatures.

Alternative experiments – the Fluorpen is easy and quick to use and difficult to break. There is therefore no reason why students should not just play with it. It is often referred to as a "plant stress meter" because the values you get are a good indicator of overall plant health. You could use it to look at low temperature (can a leaf tolerate freezing), drought (how long without watering a plant before the values drop) or light (compare north and south facing leaves on a sunny day). Plants that are nutrient starved are expected to give lower values than fertilized ones. Students can be encouraged to come up with their own hypotheses to test.



Close up of the Fluorpen, showing the menu ready to measure. A measurement is taken by pressing "Set"

Activity 2 – Measuring Plant Pigments

Background Theory – in order to drive photosynthesis, plants use chlorophyll to absorb light. Plants contain 2 different forms of chlorophyll – chlorophyll *a* and chlorophyll *b*. These absorb slightly different wavelengths of light. In nature, plants have to compete with each other to capture light. Plants that grow in the shade will often elongate their stems, growing towards the light to try position themselves more in the sun. Some plants however adopt a different strategy. Plants that are adapted to the shade will not try to outgrow their neighbours. Instead, they will optimise their growth to suit the conditions they see. In particular, while plants in the sun will tend to make more chlorophyll *a*, those in the shade will make more chlorophyll *b*. This allows them to optimally absorb the wavelengths "missed" by the sun leaves. The same effect can be seen within individual plants, where leaves that are more exposed to the sun will contain more chlorophyll *a* and those in the shade more chlorophyll *b*. A lot of research is examining how the shape of plant canopies and the structure and composition of leaves can be optimised to maximise the photosynthesis from crops.

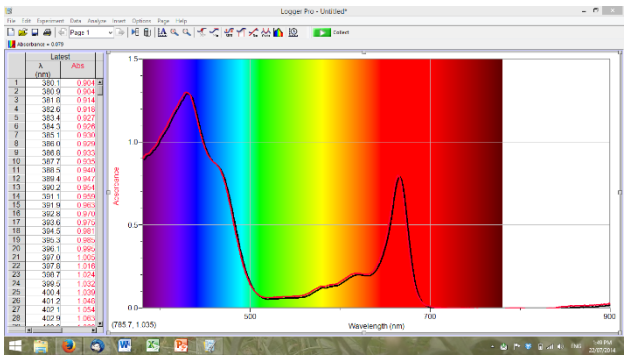
Hypothesis – Leaves from the more shaded parts of plants will contain more chlorophyll *b*, relative to chlorophyll *a* (i.e. the Chl *a:b* ratio will be *lower*).

Experimental protocol

To measure chlorophyll, we need to first extract it from leaves. This is done by grinding the leaves in a solvent (e.g. 96% ethanol). The 2 forms of chlorophyll have overlapping absorbance, so we need to separate the contributions of these. We will measure the absorbance of our chlorophyll solution at 2 wavelengths. Knowing the relative absorbance of each chlorophyll at each wavelength, we can use simultaneous equations to calculate the concentration of each. Fortunately, these are already derived for us, so a simple spreadsheet can be set up to make the calculations.

For this experiment, you will need plant material, 96% ethanol, pestles and mortars, 10 ml measuring cylinders, filter funnels and papers (or a centrifuge), plastic cuvettes and a SpectroVis spectrophotometer.

Risk assessment – this experiment involves working with 96% ethanol. Students should wear gloves and eye protection. Avoid inhaling fumes. Work in a well-ventilated area. In case of ethanol coming into contact with skin or eyes, wash with copious amounts of water.



Screen shot of the Logger Pro programme showing a spectrum of chlorophyll.

1) *Collect some leaf material* – collect leaves from either the top of a plant or leaves growing in the shade of others, inside a canopy. Leaves once collected should be wrapped in damp tissue and placed in a plastic bag.
2) *Measure out pieces of leaf (optional)* – if you wish to quantify the total amount of leaf you are using, you can draw round them on graph paper and count the squares to measure the area. Alternatively, if you have a leaf cutter (a cork borer) you can reproducibly cut pieces of known size. This is only necessary if you want to estimate the total chlorophyll in the leaf. If you only want to know the relative amounts of *a* and *b*, this is not needed. Typically, a 2 cm^2 leaf piece is about the right size.
3) *Extract the chlorophyll* – place your leaf piece in a mortar. Add 2-3 drops of 96% ethanol. Use a pestle to grind the leaf thoroughly to produce a thick paste. This works best with soft tissues. For tougher leaves, it helps to add a small amount of acid washed sand. Once the sample is thoroughly ground, add further drops of ethanol, stirring with the pestle. Once you have added about 3-5 ml of ethanol, use a Pasteur pipette to transfer the liquid to a 10 ml measuring cylinder. Wash the mortar with further ethanol and transfer this to the measuring cylinder. Keep doing this until you have 10 ml in the measuring cylinder. Your pestle and mortar should be clean at the end, with all chlorophyll transferred to the measuring cylinder.
4) *Filter the sample* – the 10 ml of extract should be filtered through a clean filter to remove the leaf debris. Alternatively, you can transfer the extract to a centrifuge tube and centrifuge at approx. 2000 x g for 10 minutes.
5) *Set up the spectrophotometer* – the SpectroVis spectrophotometer is a self-contained instrument, capable of measuring a full spectrum from approx. 300-800 nm in a few seconds. It contains a built in white light which is shone through a cuvette containing your sample. To ensure accurate measurements, it is essential to ensure that the spectrophotometer is set up carefully in advance.
    a. *Attach the spectrophotometer to the computer, start the software* – the spectrophotometer is controlled using a program called Logger Pro
    b. *Blank the spectrophotometer*
        i. Place a plastic cuvette containing clean 96% ethanol in the cuvette holder. Make sure that the clear sides of the cuvette are facing the arrow and the white bulb symbols.
        ii. Check that the software is set to read absorbance (Y-axis labelled "Absorbance") – if it is not, go to Experiment > Change Units > Spectrophotometer 1 > Absorbance in the menus.
        iii. Zero the spectrophotometer. Select Experiment > Calibrate > Spectrophotometer 1. (n.b. this will involve waiting for some time, while the spectrophotometer warms up).
    c. Place the spectrophotometer into measurement mode – select the green arrow labelled "Collect" at the top of the screen
6) *Measure absorbance* – place your filtered green extract into a cuvette and place this in the spectrophotometer. Record the absorbance value at the following 3 wavelengths: 652 nm, 665 nm, 750 nm. This can be done by finding the corresponding wavelength from the table on the left hand side of the screen. Values should either be recorded on paper, or transferred directly into a spreadsheet.

7) *Calculate the chlorophyll content* –Chlorophylls absorb at 652 and 665 nm but not at 750 nm. By recording at 750 nm, we can correct for errors such as having a scratched and dirty cuvette. Therefore the absorbance reading at 750 nm should be subtracted from the other two readings. The chlorophyll content in the chlorophyll extract can be calculated using the following equations:

$$\text{Chlorophyll a} = 18.22 \times A_{(665-750)} - 9.55 \times A_{(652-750)}$$
$$\text{Chlorophyll b} = 33.78 \times A_{(652-750)} - 14.96 \times A_{(665-750)}$$

Typically, the chlorophyll *a:b* ratio of a leaf will be in the range 2.5-5, with plants from shade having a lower ratio (i.e. more chlorophyll *b*).

Top view of the SpectroVis with a cuvette in the cuvette holder. The direction of the cuvette should be with the clear sides facing the white bulb symbol and the arrow.

Alternative experiments – with a spectrophotometer, it is possible to look at pigments in other tissues. If you perform the same procedure with different coloured leaves (e.g. purple leaves) you can show that the sample still contains chlorophyll, but that the green colour is masked by other pigments. The purple or red colours often seen in leaves are due to anthocyanins. These are water soluble pigments so can be separated from the chlorophyll using a 2-phase separation. Chloroform is commonly used for this, but it also works using baby oil. Extract pigments in ethanol, mix 1 part ethanol with 1 part baby oil and mix thoroughly in a sealed tube. Leave to settle. The chlorophyll will partition into the baby oil, leaving the anthocyanins in the ethanol. You can then measure the absorbance spectrum of each fraction.