PRACTICAL FOUR

Leaf Structure and an Investigation of the Photochemical Reactions of Photosynthesis

Pre-practical quiz on Moodle

A quiz will be posted on the SLE132 page of Moodle, in the Resources and Assessments section, ONE WEEK before the first practical, and will close the day before the practical class. This quiz will test your knowledge of practical 4 and ensure you have prepared yourself for the class. You will have two opportunities to complete this quiz so please ensure you have thoroughly read the practical notes before you attempt the quiz. Your result from this quiz will contribute 1% of your overall SLE132 mark.

Part 1: The structure and function of the leaf

Photosynthesis is carried out primarily in the **palisade mesophyll** cells of the leaf. The basic arrangement of tissues in the leaf allows for efficient photosynthesis, and the internal structure of leaves varies according to the photosynthetic pathway, C_3, C_4 or CAM, found in the particular species.

Coprosma repens, a native of New Zealand, is a low-lying shrub having **dorsovental** (horizontal) leaves with only one side of the leaf exposed to the sun and therefore only one palisade mesophyll layer near the **upper epidermis**. *Eucalyptus* spp. are sclerophyllous plants which evolved to survive semi-arid conditions, displaying **isobilateral** leaves (vertical). Both sides of the leaf are exposed to the sun at different times of the day, and therefore there are two palisade mesophyll layers on either side of the **spongy mesophyll**.

Transpiration also occurs in the leaf through the **stomata**. The stomata allow carbon dioxide to enter into the leaf, and oxygen out; but they also let water escape through evaporation, and so are located on the leaf where they are least likely to be exposed to the sun. Therefore, dorsoventral leaves will mainly have stomata on the underside of the leaf, away from the sun; isobilateral leaves will have an equal distribution of stomata on both sides of the leaf but, generally, stomata will only be open on the side of the leaf that is not in full sun.

Examination of the structure of the leaf – 1 hour

To examine differences between the internal structure of a leaf from the *C. repens* and the *Eucalyptus sp.*, you will need to make a slide of a cross-section of the leaf for each.

Work in pairs in this section, with one partner preparing the *C. repens* samples while the other prepares the *Eucalyptus sp.* samples. Make sure you have a clear working space enabling forearms and wrists to rest comfortably on the bench.

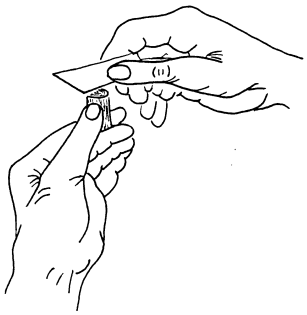
a) Dip your leaf and blade in water and, on the dissecting mat, make a quick cut to produce a flat, horizontal, smooth surface. This surface will be used to obtain your sections.

(**WARNING:** Sharps; risk of cuts; use great care. **RISK:** Low.)

The section of your blade used is now blunt. Do not use it for further sections. For subsequent sections use either a new razor blade or a new section of the blade previously used.

b) Thoroughly wet the blade and leaf with water—they should be kept wet while cutting to reduce cell destruction.

c) Hold the leaf with thumb and first two fingers of the left hand (refer to the diagram below). Make sure that fingers and thumb are below the level of the surface to be cut. Hold the blade with thumb and first finger of right hand (reverse if left-handed). Hold both tissue and blade lightly.



d) You should now make a series of thin sections. Your sections for examination should be made with a single, sliding, circular motion towards the body. It is unnecessary to cut a complete section of the leaf. Thin sections are easier to cut from a small, representative portion of the whole tissue. Often, it is sufficient to begin your cutting just off centre of the surface to be cut, drawing the blade through the centre and toward the edge.

e) Immediately place sections in a petri dish of distilled water.

f) After you have made a series of sections, choose your thinnest sections and transfer them with a paintbrush, or forceps to a drop of toluidine blue on a separate petri dish.

g) Leave the leaf section in stain for only a few seconds ('tap' the leaf section in stain a few times), then place in the petri dish of distilled water to rinse surplus stain away. It is better to under-stain and then re-stain, than over-stain and damage the sample.

h) Finally, mount the stained sections **(at least 4)** on a slide with a drop of distilled water and add a coverslip. Remember to use a paintbrush for all transfers to minimise damage to your section; alternatively make *very* gentle use of the forceps.

Toluidine blue stains cell walls and cytoplasm in different ways. This aids in identification of tissues, although it is still possible to recognise cell and tissue types without the use of stains.

A blue–green colour indicates phenolic groups of lignin in the cell walls. A purple colour indicates acidic polysaccharides. Table 1 below shows the staining reactions of cell walls with toluidine blue.

Table 1: Staining reactions of cell walls with toluidine blue

Wall colour	**Tissue type**	**Cell type**
Blue to blue green (walls thick and lignified)	xylem sclerenchyma	tracheids (usually narrow) vessels (usually wide) fibres or sclereids
Purple (walls usually thin)	cortex pith phloem cambium	parenchyma cells (peripheral) parenchyma cells (central) sieve tubes and companion cells
Purple (walls usually thick)	collenchyma epidermis	

Biological drawing 1

Make a diagram (from appropriate magnification) of each of your sections and label the **cuticle**, the **upper** and **lower epidermis** (where appropriate), **palisade mesophyll**, **spongy mesophyll** and, if you can see them, the **intercellular spaces, vascular tissues** and **somata** or **stomatal openings**.

Remember that in a diagram you draw layers (with a few representative cells in each layer) - not each individual cell within the sample. Although your diagram is simple, you will still need to provide a full legend in the top left-hand section of your page.

Draw 5 -10 cells in each layer and then continue the layer with a line. Repeat this from the top layer to the bottom of the leaf. If you cannot observe a particular structure, indicate that with a star at the bottom of the page. (10 marks)

Question 1: In which tissue layer of the leaves are the chloroplasts located? (2 marks)

Question 2: Explain the difference in distribution of chloroplasts within the leaves between the two samples. (3 marks)

Part 2: Photochemical reactions of photosynthesis - 2 hours

The overall reaction of photosynthesis can be understood as two closely linked processes. Light-driven **electron transport** produces ATP and NADPH, which feed into the assembly line of the Calvin cycle where enzymes carry out **carbon fixation** by converting CO_2 to glucose.

The first step in photosynthesis is **light absorption** by pigments. This drives the flow of electrons from pigments to $NADP^+$ via a chain of carriers. The pigments then have a deficiency of electrons. These electrons are replaced by a process in which electrons are pulled out of water molecules, splitting the water molecules in the process.

$$H_2O \rightarrow 2H^+ + \tfrac{1}{2}O_2 + 2e^-$$

The $NADP^+$ accepts the electrons and as well picks up the H^+ ions to form NADPH + H^+.
The energy of the NADPH is then utilised, together with ATP, in the final part of photosynthesis, **carbon fixation**, to drive the Calvin cycle in which CO_2 is converted to glucose.

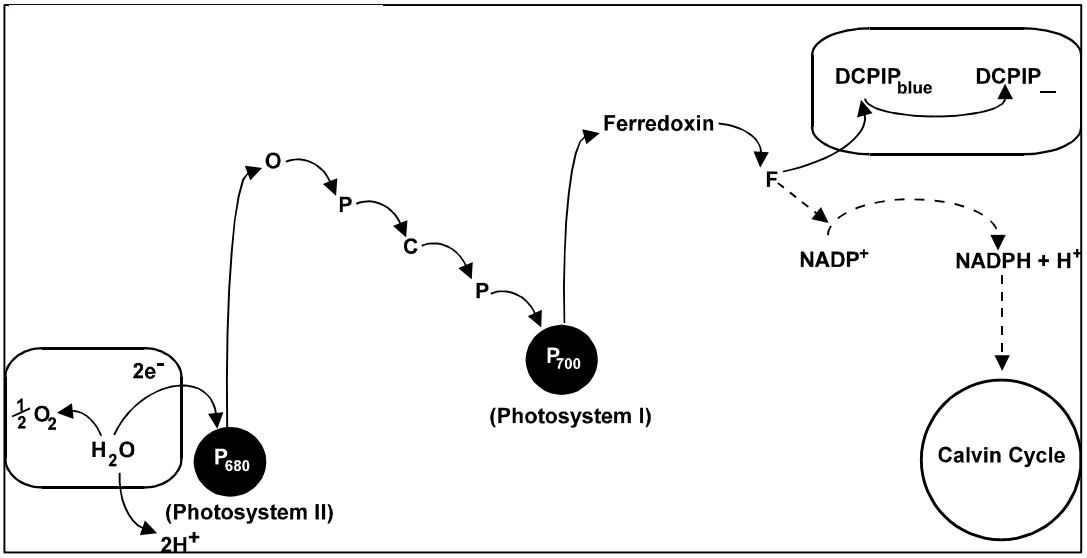


Figure 2

In the 1930s, Robert (Robin) Hill isolated chloroplasts from leaves and showed that they would continue to carry out electron flow and oxygen production in the absence of CO_2 as long as they were provided with an electron acceptor.

Hill used a dye that loses colour when it gains electrons, so that the rate of photosynthesis could be measured by observing the rate at which the dye changed colour.

In this experiment, we will be substituting a blue dye DCPIP (2,6-dichlorophenolindophenol), for $NADP^+$ and measuring the rate at which the photochemical reactions occur by noting the rate at which DCPIP, which is blue, becomes colourless after it has accepted electrons.

Another way of saying this is that DCPIP is blue when oxidised and colourless when reduced.

$$DCPIP_{blue} + 2e^- + 2H^+ \rightarrow DCPIPH_{2\ colourless}$$

Some questions to consider during the experiment

- Is oxygen produced as a result of carbon fixation or of electron transport?
- Can electron transport continue to produce NADPH if there is no CO_2? Why or why not?

For your information - Chloroplast extraction

NOTE: The extraction will be carried out for you. As it involves the extraction of active enzymes, it needs to be carried out as quickly as possible and, ideally, on ice.

1. For your information, the method for obtaining the chloroplast extract is as follows:
2. All beakers and test tubes are first cooled down in an ice bath to cool down.
3. Stalks are removed from five silverbeet leaves and the leaves ground in 200 mL of chilled **buffer solution A** for a few two-second pulses in a Waring blender.
4. ***Buffer solution A:*** *potassium di-hydrogen orthophosphate 10 mM, di-sodium hydrogen orthophosphate 10 mM, magnesium chloride 5 mM (pH 6.5).*
5. The extract is then poured through the two layers of muslin into one beaker (squeezing the muslin to allow more extract through).
6. Suspension is poured through the eight layers of muslin into the other beaker (not squeezed this time—extract allowed to drain through).
7. The suspension is then spun at full speed in the centrifuge for one minute.
8. The supernatant is poured off and the green chloroplast sediment suspended in a total of 15 mL of buffer solution C (the same as Buffer solution A but without magnesium chloride; pH 7.6). Gently agitate.
9. The suspension is then stored on ice until ready for use.

Re-zeroing the spectrophotometer

Your demonstrator will do the initial zero reading, you should not have to do this again.

Take a clean tube and add 6.7 mL of buffer solution B (same as buffer solution A but 1/10 the concentration; pH 7.6) and 0.2 mL of the chloroplast suspension (note the chloroplasts will slowly sink to the bottom of the container they are in so it is advisable to mix the stock suspension before you remove the 0.2 mL). Mix by gentle agitation. Adjust the spectrophotometer so that this pale green suspension gives a **zero absorbance reading**.

Assay optimisation

An assay optimisation was carried out to determine the length of time needed to run your experiment. This is important as parameters can change that mean the time required to see a change in the experiment can vary, such as the concentration of chloroplasts in the solution or the concentration of DCPIP solution. This step has been carried out by technical staff.

Procedure

Do not touch the spectrophotometer dials/buttons for the remainder of the experiment. Work in groups of about four

Each group will be given a beaker with chloroplast extract that will need to be kept on ice. **Consider:** Why is it so important to keep the extract on ice?

In this part of the prac you are going to examine how the amount of light is related to the progress of the light reactions.

Before you begin

1. Start by discussing the aim and hypothesis for the experiment and how you are going to test this using the equipment provided. What are your expected results?

Some lights are <u>HOT</u> – DO NOT HOLD THEM or hold the test tubes too close to them

2. You need to allocate a person to each task, so one person will be the record keeper, another the time keeper, another will measure the solutions…
    a. Point lamps toward the window side of the room and NOT into other people's experiments,
    b. have a ruler set up,
    c. get timers ready,
    d. have a test tube holder in a cupboard nearby to hold negative first control sample
3. Try not to put fingerprints on the test tube and cuvettes
4. Make a mark on the test tube and try to insert it into the spectrophotometer the same way each time to ensure the most accurate results

Your experimental assay

You will be setting up three assay conditions, the control, the dark control and the experimental assays, 26 cm from a light source and measuring the rate of photosynthesis by reading the absorbance at 590 nm (or how blue the assay is). Each assay condition will contain the following volumes of buffer, DCPIP and chloroplast solution.

Assay	Buffer (µL)	DCPIP (µL)	Chloroplast solution (µL)
Control **C**	1100	0	50
Dark control **DC**	950	150	50
Experimental **E**	950	150	50

*You may be given different volumes by the demonstrators depending on the setup on the day.

Follow the next steps carefully to prepare and run your experiment:

Setup:

1. On your bench, set up a ruler with a lamp (**off**) at one end.
2. At the top of the cuvette/tube (so as not to interfere with the spec reading), label the tubes/cuvettes C, DC and E for control, dark control and experimental respectively.
3. Put blu tack on the bench 26 cm from the lamp (this will hold the cuvettes/tubes in position).
4. Add buffer and DCPIP to cuvettes as per the table below:

Assay	Buffer (µL)	DCPIP (µL)
Control **C**	1100	0
Dark control **DC**	950	150
Experimental **E**	950	150

Experiment:

5. To start the experiment, add 50 µL of chloroplast solution to each cuvette (C, DC and E).
6. Cover with parafilm (or gloved finger) and gently invert to mix thoroughly mix the assays.
7. Quickly read the absorbance (590 nm) for each cuvette and record on the table provided.
8. After reading, place cuvettes on blue tack on the bench (26 cm from the lamp – keep the lamp off). Cover the dark control with foil tent.
9. Turn **on** lamp and timer.
10. At regular intervals (eg. 2 min - provided by demonstrator) read absorbance each cuvette, control, dark control and experimental.
11. Return to blu tack after reading absorbance – **remember to cover the dark control with foil.**

Time (min)	**0**									
Control (C)										
Dark control (DC)										
Experimental (E)										

Assessment (14%)

Complete the pre-prac quiz on Moodle: **1%** final assessment

Part 1 (3%): Submit to your demonstrator before you leave the practical class:

- Diagrams of TS sections through a *Coprosma repens* and a *Eucalyptus sp.* leaf.
- Answers to questions 1 and 2 about leaf structure and distribution of chloroplasts.

Part 2: Assignment (10%): An Assignment Template for the presentation of your data will be available on Moodle in Resources and Assessments, and is to be completed by each individual student (this is not a group assessment) and submitted on-line to the Practical 4 Assignment Turnitin Dropbox on Moodle and is due two weeks of the practical class.