Plant Water Relations

We will examine the effects of various environmental stresses on water relations. During this lab you will:

a. Learn how to measure the water potential of herbaceous and woody plants using the J 14-leaf press and the pressure bomb, respectively (the use of the pressure bomb is a demo).

b. Determine the water potential of potato tissue using Chardakov's method

c. Learn how an osmometer is used to determine osmotic potential (vapour pressure depression - a hygrometric method). This is a demo.

The maintenance of an optimal physiological state in individual cells, tissues and whole plants is dependent upon a favorable water balance. When, as a part of the normal course of development or because of an inadequate supply of water, plants have reduced water content, development and the rates of all vital functions proceed at reduced rates. Plants adjust their physiology so as to cope with a period of water deficit (drought); however, prolonged drought will lead to the death of most, although not all, plants.

The potential energy (chemical potential) status of water in a plant is described by the term water potential (Ψ_w), which is measured in units of pressure – the megapascal (MPa). In plants the main components that contribute to Ψ_w are hydrostatic pressure (turgor pressure, Ψ_p), and osmotic potential (Ψ_s). These terms generally have negative values – for Ψ_s the negative value arises because dissolved solutes lower the free energy of water. The magnitude of Ψ_p varies depending on where it is measured in the plant e.g. in the xylem and cell walls water is under tension and the values are negative whereas inside plant cells the values are positive due to the constraints of the rigid cell wall on the protoplast. Water movement from the soil to the leaves is a passive process driven by the difference in Ψ_w that exists between the soil and the leaves. From the leaves water diffuses via the stomatal pore into the atmosphere moving down a very steep Ψ_w gradient (the inside of the leaf is moist or humid whereas the atmosphere beyond the leaf is dry). The soil-plant-atmosphere path for water movement is viewed as a continuum and the magnitude of the Ψ_w gradient from the soil to the atmosphere is very large and is the ultimate driving force for water movement. Water moves from an area of less negative (or higher) Ψ_w to an area of more negative (or lower) Ψ_w. Leaf Ψ_w is an indicator of the magnitude of the driving force for water movement that exists between the plant and the soil and is used as an index of plant water content (Barrs, 1968). Measuring leaf Ψ_w is akin to taking our own temperature to gauge our health. It is measured widely and often as a measure of plant health in the broadest sense because of their dependence on water for growth, photosynthesis and overall productivity. The importance of Ψ_w measurements is attested to by the numerous techniques devised to measure it. In this lab we will consider some of these techniques. However, many of the available techniques

to measure Ψ_w are difficult, time consuming and require expensive equipment so we will not be able to use all techniques.

A. Measurement of Water potential

Scholander's pressure bomb (Scholander et al, 1965) will be demonstrated in this lab, it is relatively simple to operate, and gives Ψ_w measurements that agree well with *in situ* measurements (Campbell and Campbell, 1974). In some instances the pressure bomb can give inaccurate results, particularly for herbaceous plants when the soft stem may be crushed when placed in the rubber gasket of the pressure chamber. The pressure bomb operates on the principle that, when a twig or shoot is cut from a plant, the water in the xylem retreats from the cut surface due to the fact that the water column is held under tension. After being placed in the pressure chamber with the cut end of the shoot or twig extending outside the chamber pressure is applied to force the water back to the cut surface. The amount of pressure that must be applied to achieve this is equal (although opposite in sign) to the tension that existed in the xylem before the twig or shoot was cut and is a good estimate of Ψ_p, which approximates whole plant Ψ_w.

The hydraulic press (Campbell and Brewster, 1975) will also be used in this lab and operates on the same principal as the pressure bomb. Hydraulic pressure is applied to a leaf through a thin rubber membrane, squeezing the leaf between the membrane and a plexiglass plate. When the applied pressure equals the leaf Ψ_w, the cell walls and intercellular spaces saturate and water may be forced through the stomates. The saturation of the cell wall is observed through the Plexiglass plate and is taken as the end point of determination. The hydraulic pressure is supplied by a modified 1.5 ton hydraulic jack, and read with a standard pressure gauge.

In this lab, you will be shown how to use a pressure bomb to measure water potential of a woody dicot. You will be using the leaf press to measure water potential of bean and corn plants.

Other quick methods to determine plant tissue Ψ_w is the Chardakov method and to measure the relative water content (RWC). You will use both these techniques in this lab. Chardakov's method relies on the change in density in a solution that occurs after a piece of plant tissue is immersed in it. The solution will gain or lose water depending on the Ψ_w of the plant tissue. To gauge this you will assess density changes by watching whether a drop of the original solution floats or sinks in the test solution after the tissue has been incubated in it. Relative water content is assessed using excised leaf pieces that are quickly weighed to obtain the fresh weight (FW). The leaf pieces are then floated on a solution of pure water to allow them to re-hydrate. After this step the leaf pieces are weighed again (to obtain the turgid weight, TW) and are then dried and the dry weight (DW) is recorded. RWC is calculated as (FW-DW)/(TW-DW).

MATERIAL:

1. You are provided with 14- to 21-day-old bean and corn plants, also provided are potted geranium plants. All plants were subjected to:
    a. water deficit stress (by withholding water for 4 - 7 days),
    b. well-watered conditions. These are controls
2. Corn and poplar plants
3. Potatoes
4. Scholander's Pressure bomb with nitrogen cylinder
5. J-14 leaf press
6. Balance
7. Oven at 65^{o}C
8. Cork borer
9. Containers, water and sucrose solutions with and without methylene blue for determining Ψ_w using Chardakov's method
10. Petri dishes.
11. Saran-wrap
12. Paper towels
13. Vortex mixer
14. Aluminum foil

PROCEDURES:

A. Measurement of water potential using the leaf press

1. Use three leaves from each of two plants that were subjected to the same treatment for determining Ψ_w using the J-14 leaf press. Read the instructions in the hand out for the J-14 leaf press carefully.

2. Excise the leaf blade from the petiole. If the leaf is too large to fit in the press, cut an approximately 2 x 2 cm^2 piece. Place leaf sample upside down on a piece of filter paper on the membrane and attach the top plate. Close the valve and apply pressure gently using the hydraulic jack.

3. The end point is represented when water saturates the cell walls. This is recognized as the first darkening of the tissue and is often accompanied by an expulsion of water from some of the stomata. Record the pressure at which the end-point is attained. Additional pressure will force more water from the leaf but Campbell and Brewster (1975) have reported that darkening of the leaf best agrees with pressure bomb and leaf hygrometer measurements of Ψ_w. Darkening of the xylem tissue and expulsion of water from the cut edge of the tissue often occurs well ahead of the true endpoint. If one is aware of this, it is usually not difficult to distinguish between the expulsion of xylem water and the saturation of the cell walls. Campbell and Brewster 1(975) reported that the best results are obtained with a rapid pressure increase (2-5 bars/sec). If you apply too much pressure it can be released and additional readings taken on the same sample. Water on the plexiglas from the first reading may make

determination of subsequent end points more difficult. Calculate the mean Ψ_w and SD for a given treatment.

4. A demo will be given on the use of the Pressure bomb.

B. Measurement of water potential using Chardakov's Method

1. Dispense 10 mL water or sucrose solution (0.05, 0.1, 0.15, 0.2, 0.25, 0.3 0.5 molal) into each of eight LABELLED tubes

2. Cut a 3 cm portion from a potato. Use a 4 mm diameter cork borer to prepare from this at least 24 uniform tissue samples. Work quickly to minimize evaporation. Keep the tissue pieces wrapped in moist towels.

3. Put three pieces of potato into each solution. It may be necessary to top up your solution to ensure that the potato pieces are completely submerged but you must keep the final volume in EACH tube/container the same.

4. Incubate the potato pieces for 1.5 h with periodic mixing. After the mixing time is up pour off the solutions into a set of empty LABELLED tubes. Mix using a vortex mixer.

5. Using a Pasteur pipette remove a small amount of the appropriate methylene blue solution. There is one methylene blue solution for each of the sucrose concentrations being tested. Being very careful immerse the pipette in the solution that previously held the tissue pieces until the tip is at the centre of the tube and slowly release one drop of the methylene blue solution. Note whether the drop of dye sinks, disperses or floats to the surface and note too whether it moves rapidly or slowly.

6. Repeat for each sucrose solution and for the water. Be sure to use a different pipette for each solution!

7. Record your results in the table below.

Table 1. Response of drop (float, disperse, sink)

Sucrose (molality)	Drop response
0	
0.1	
0.2	
0.3	
0.4	
0.5	
0.6	
0.7	

Determine the sucrose concentration at which the drop did not sink or rise – this is the concentration at which the drop dispersed. This sucrose concentration matches the Ψ_w of the potato piece (Ψ_w tissue = Ψ_w sucrose solution).

Calculate Ψ_s using the van't Hoff equation, using the value for the "RT" term given below. This is equivalent to Ψ_w in an open system such as the beakers you used.

$\Psi_s = -miRT$ where:

m = molality (moles per 1000g)
i = ionization constant (1 for sucrose)
R = gas constant (0.00831 liter MPa mol^{-1} K^{-1})
T = temperature (K)
*RT = 2.436 (L MPa mol^{-1} for a temperature of 20^oC)

Units are MPa

C. Measurement of Relative Water Content

1. Collect 10 leaf pieces from a leaf using a cork borer. Place the leaf on a firm surface before you excise the leaf pieces using the cork borer. Use leaves from the plants provided. For this exercise ensure that you select leaves from well-watered and drought-stressed plants.

2. Immediately wrap your leaves in saran-wrap and place in a dark cupboard or drawer. The reason for this is to minimize evaporation.

3. Weigh the leaf pieces on the lab balance. You must complete this step quickly to minimize water loss from the leaf pieces.

4. Dispense 20 mL water into a Petri dish. You will need one Petri dish per 10 leaf pieces. Place the leaf disks so that they are immersed in the water in the Petri dish. Place the Petri dish in a fridge overnight.

5. The next day carefully blot the leaf pieces dry using tissues. Quickly re-weigh to obtain the turgid weight.

6. Wrap the leaf pieces carefully in a piece of aluminum foil and place in an oven set at 65^{o}C. Leave overnight

7. The next day weigh the leaf pieces to obtain the dry weight.

8. Calculate the RWC for all the leaf pieces you harvested.