

SHOOT STRUCTURE 1 : Shoot Apex and Leaves

Audio file-script



This is the first in a series of audio-visual programs and it is on the Shoot Apex and Leaves.

Before we begin, I hope that you have familiarized yourself with the layout of the lab, where you can find materials and also the use of the computer- if not, then do this now by wandering about to find out where things are, and reading the notes on "Study of Plant Anatomy in the Audio Visual Learning Centre" in the introductory material in your study guide. Ask a member of staff or fellow student if you are unsure. When you have done this return to this sound file.

PAUSE

In the carrel are two microscopes, a box of numbered slides for examination and facilities for preparing and staining fresh sections from plant material on the bench near the window. The carrel also has a computer from which you may access this sound file, high-resolution images referred to as "carrel images", revision modules and other digital resources that may be of interest to you. You will always need your printed study guide while working in the carrel as it contains illustrations and text that I will refer to. The study guide summarizes what's on this sound file, and allows you to listen and look instead of taking notes.

This sound file is not only a source of information, but also a guide, or prompt, to carry out the work contained in each program. If you follow the sound you should complete all the work with reasonable efficiency. At the end of the week's work you may find it useful to run through the sound again, just listening. This will give you an overview and help your understanding of the subject. You can also check by reference to the self-assessment quizzes in the study guide or the revision modules that you have absorbed and understood all the important points.

A high standard of drawing of map diagrams is called for by the end of Semester, so ensure that you get advice and feedback about your drawing from a staff member either in the lab or during the tutorials.

Essential study in the AV lab consists of work in the carrel where you are now sitting, guided by this sound file and viewing the demonstration material labelled "Core". All of this material will be examined in some way be it in the quizzes or the semester examination.

We would like to encourage you to look at the enrichment material provided in the AV lab and the corridor so that you become aware of the diversity of plants in terms of their morphology and anatomy. The enrichment material will not be examined directly but you may use that knowledge in an examination to illustrate a point etc. if you wish. When enrichment materials are present in the audio-visual laboratory they will be clearly labelled as such.

The A-V laboratory is available for your use in all weeks of semester. The slides from the previous weeks work will be available. You may choose to revise work, practice sectioning technique, or explore the anatomy of plant material whenever you wish.

This week we will start by briefly looking at the whole plant, we will then look at the shoot apex and then concentrate on leaves, especially their anatomy. In subsequent programs we will deal with cellular details of tissues and the primary and secondary structure of stems and roots and anatomical adaptations of plants living in extreme environments. Wherever possible the study of anatomy will be followed the next week with study of some aspect of the physiology of that organ for instance this week you study leaves and next week photosynthesis. Towards the end of semester, you will carry out a short project where you can demonstrate your anatomical skills by completing a survey of the anatomy of a plant. For this you will need to prepare sections, draw map diagrams of them and give a talk on your findings. You will be required to submit your microscope slides, text and map diagrams for assessment. So - you had better Start Working towards developing skills in sectioning and drawing from the start of the course and seek help when you need it.

When you are in the AV lab, you will mostly be studying parts of plants. Try to always consider how that bit is related the rest of the plant - build a picture of the Whole plant in terms of structure and function. You should also keep in mind that the plant you are studying in the laboratory is a part of a complex system of interacting organisms in nature, agriculture or horticulture, and that plants from different places may vary morphologically and anatomically in response to their environment.

PAUSE

One last point, the figures and plates in your study guide are preceded with the number of that particular program, for example in Program 2, the first figure is labelled as Figure 2.1, but I will refer to it only as figure 1. High resolution, coloured or moving images on your computer are referred to as carrel image links.

OK – lets get started. As you progress through this course, we want you to develop a clear understanding of the differences and similarities between the two main groups of the Angiosperms (that is the flowering plants), these are the Monocots and Eudicots. A few archaic Angiosperm plants are neither monocots nor eudicots, but in the past were included with eudicots in a group known as dicots. You may still see the term dicot used in place of eudicot. You need to be able to distinguish monocots from eudicots in terms of their morphology and structure of the various parts of the Plant body. Monocotyledons are plants such as grasses, oats, barley etc. and Eudicotyledon plants range from many of the flowers in your gardens to trees such as Eucalypts. Figure 1 in your study guide shows you the morphology often seen in herbaceous plants - in the case of the eudicot it is not greatly different from the seedling stage of shrubs and trees. The carrel image link 1 shows a diagrammatic representation of the anatomy and morphology of a eudicot.

Now read the section on “introduction to the whole plant” in your study guide and **look at the demonstration of germinating and developing seedlings**. Note that whilst for eudicots you can see their cotyledons, whether they stay below the ground (e.g. Pea) or are raised above it (e.g. Casuarina or Cucumber), in monocots most of the stored nutrient for seedling development is in endosperm and the cotyledon can only be seen when the embryo is viewed under a microscope. When you have completed these tasks return to this sound file.

PAUSE

In Seedlings the roots take up inorganic nutrients and water and the cotyledons or endosperm provide organic nutrients for seedling development. Eventually the shoot that develops harvests light and uses its energy to fix carbon and manufacture other organic products. In later programs we will consider roots and transport within the plant. But this week we will examine the shoot

apex, introduce you to leaf morphology then concentrate on the structure of the leaf and how it is related to photosynthesis and transpiration.

PAUSE

Look at Slide 1, a longitudinal section of *Vicia faba* shoot apex. This is the French Bean plant and it is a Eudicot. Examine it under the dissecting and then compound microscope and Compare it with Carrel image link 2. You may be able to distinguish cells differentiating into vascular strands - xylem may have small areas of wall thickening visible as oval shapes or spirals. You will also be able to see bulging leaf primordia towards the apex and more mature leaves arising further down and covering over the apex. Thus the apex of this plant is protected by its leaves.

Next examine **Slide 2** which is the apex of the water plant, *Myriophyllum*, also a Eudicot. Compare this with the photograph in Plate 1 and also shown in Carrel Image Link 3. In this species the nodes and internodes are very clear, the internodes increasing in length successively back from the tip. You will also notice that in the stem there are gas spaces present, this is a common feature of water plants and you will see more of this later in semester. Return to the sound when you have the slide in front of you.

PAUSE

Myriophyllum is a convenient plant to examine shoot development because of its regular pattern of development. The apical dome is clearly visible and a succession of leaf primordia appear as bulges on the axis. The mature leaves cover and shield the apex but not to the extent that occurs with terrestrial plants such as *Vicia faba* where the apex needs protection from desiccation.

Leaf primordia originate with periclinal divisions in the outer cortex (that is along the circumference of the stem). As the leaf develops, its outer surface begins anticlinal divisions which increase its diameter. The leaf then takes shape due to the activity of the apical and peripheral meristems.

Shoot elongation between nodes takes place by cell elongation. The mechanism of this will be dealt with in lectures. In *Myriophyllum* the increase in length of the internode is made more obvious by the increase in size of the gas cavities that occupy the periphery of the internodal ground tissues.

Another feature of the shoot apex that is shown in both Slide 1 and Slide 2 is the presence of small axillary buds that under the appropriate conditions will grow out to become lateral branches. Carrel Image link 1 shows two types of these – one is a branch with leaves, the other is a branch with flowers. In the shoot, all laterals, whether leaves or branches, have a superficial origin. That is, their vascular tissues arise from the periphery of the vascular cylinder of the stem. This contrasts with branching in roots which arises from near the centre of the tissues, we will deal with this in the primary roots program.

The shoot tip therefore shows all of the features of the mature shoot but they are compressed and in miniature. To emphasize this point, **look at the mature structure of a cabbage on the demonstration bench**. In cabbage (*Brassica oleracea*) the leaves have matured but they have not unrolled, and neither have the internodes expanded. They expand only at a later stage when the plant goes to seed, that is when it produces a flowering stalk in the second year of growth. The plant hormone gibberellic acid when applied to the plant causes this elongation of the stem as occurs at flowering.

Not all shoots grow exclusively by expansion of cells produced by the apical meristem. In monocots there are frequently continuously dividing meristems at the bases of leaves and the bases of internodes. These are called intercalary meristems and their divisions give rise to the cells above them which expand and differentiate into new leaf and node tissue. It is for this reason that grasses continue to grow after having been eaten by cattle or mowed in the garden lawn even when the leaf apical meristem has been cut off. Figure 2 in the study guide shows the

position of intercalary meristems in monocots. Examine this diagram and then return to the sound.

PAUSE

Before we leave the shoot apex and its growth you may have noticed that the leaf primordia (the little buds) do not arise at random, but in a regular arrangement, and this arrangement determines the form of the shoot. This pattern of leaf primordia frequently follows a spiral. The pattern is known as phyllotaxy.

Shoots are made up of leaves and stems or internodes, as you have seen in the development of the shoot apex. However, just as you might expect, some plants have adapted to a different way of harvesting light and use photosynthetic stems called cladodes or modified petioles called phyllodes. We will study these structures at the end of the primary stems program, in the meantime we will concentrate on the types of plants whose shoot consists of true leaves and stem.

Now let us examine the morphology - the external appearance - of monocotyledonous and eudicotyledonous leaves. **You are supplied with shoots of several plants on the bench near the window.** Go to the side bench and collect samples of *Tradescantia*, *Ligustrum*, and *Acalypha*. Tissue and then return to the sound. First take the shoot of *Tradescantia* and look at Figure 3 in the study guide. Note the long linear leaf blade and the sheathing leaf base clasping the stem. If you look at *Zea mays* on the demonstration bench, you will see some similarity. These two plants are monocotyledons.

Now look at leaves of *Ligustrum* and *Acalypha*. Here the leaf base is small and extends as a petiole to the broad leaf lamina, which has a distinct upper and lower side, that is, it is dorsiventral. In the axil of the leaf is an axillary bud which, if it grows, will become a leafy branch or a floral shoot. Figures 1 & 3 in the study guide and carrel image links 1 and 3 indicate these clearly.

The point where the leaf joins the stem is a node. The stem between nodes is called an Internode, and in many monocotyledons the internode is almost wholly covered by the sheathing leaf base.

PAUSE

Now examine the pattern of leaf veins or venation, firstly in *Acalypha* where it can be seen, particularly clearly because the cells sheathing the veins or vascular bundles are coloured red with anthocyanin, a plant pigment. Hold the leaves against the light and note the main vein or midrib as a continuation of the petiole. Other large veins depart at intervals, and between these is a network or reticulum of smaller veins. Reticulate venation is typical of Eudicots.

Now look at the monocot leaves and you will see the parallel venation typical of monocots.

The veins are vascular bundles containing xylem and phloem tissues that transport water inorganic and organic substances about the plant

As an interesting sideline, some leaf shapes may be phenotypic responses to a given set of environmental conditions. In some plants such as the Australian natives *Eucalyptus* and *Acacia*, there are distinct leaf forms for juvenile and adult leaves. If you are interested, have a look at the demonstration of these different forms in the demonstration material. A selection of leaf shapes and venation patterns are shown in Figure 4 for your reference but we will not examine you on the variety of leaf shapes in this course

We will now concentrate on leaf structure.

There are three tissue systems in plants: the dermal, ground and vascular tissues. Read the introduction to leaf structure in your notes and look at Figure 5 which describes the tissue and cell types present then return to the sound.

PAUSE

Division of an apical or peripheral meristem leads to three tissue meristems. The protoderm gives rise to the dermal tissue, namely the epidermis which consists of epidermal cells, stomata and trichomes (hairs).

The ground meristem leads to the development of the mesophyll tissue that may consist of chlorenchyma, parenchyma Collenchyma and/or sclerenchyma.

The provascular meristem leads to the tissues and cells of the vascular strands, or veins.

You will study vascular tissues to greater depth in the Ground and Vascular Tissues program. In this program we will concentrate on Dermal and Ground Tissues. PAUSE

Now lets turn to the internal structure, or anatomy of the leaf blade to see how these tissue systems fit together. First we will look at the dermal tissue: the epidermis and stomata which control gas exchange and then at the ground tissue: the mesophyll where the organelles that carry out the biochemical reactions of photosynthesis are found.

The outermost layer of cells in all aerial plant parts is slightly different from cells inside it and is called the epidermis. Following the directions in your Study Guide prepare a small piece of *Acalypha* leaf and using Plate 2 in the Study Guide or Carrell image link 4, identify the non-chlorophyllous epidermal cells and stomatal guard cells. When you have identified the cells return to the sound.

PAUSE

The outer surface of all epidermal cells is covered with cuticle, which is a waxy waterproof substance based on cutin. You will be familiar with the fact that leaves cannot be wetted. This is due to the Cuticle. It is almost impermeable to both water and air and we must regard its presence as one of the major differences between algae and terrestrial plants. Without it terrestrial plants could not exist as they would dry out as fast as a wet piece of blotting paper hung in the Open air.

Cuticle covers the whole surface except for the stomatal pores and may have a complicated and characteristic structure. The loss of water and uptake of CO₂ by leaves therefore takes place through stomatal pores whose aperture can be controlled by the plant.

You will have observed that a single stoma in *Acalypha* consists of 2 crescent shaped guard cells with an opening between them - the stomatal pore. Look at figure 6 in the Study Guide. This shows the uneven thickening of the guard cell walls on which stomatal function depends. The walls of the guard cells around the pore are thicker and less extensible than the outer walls. When the turgor of the cells increases due to accumulation of solutes the guard cell volume increases and the cells curve outwards. Physical models confirm that this picture accounts for opening stomatal pores. Conversely, when water is lost the guard cell volume is reduced, their inner walls become less bent and the pore aperture decreases and can close completely.

The stomatal pore opens into a relatively large sub-stomatal cavity which in turn is connected to all the intercellular spaces in the leaf through which gases diffuse, see carrel image link 5.

Prepare an epidermal peel of *Tradescantia* and tear a section of *Ligustrum* (that is you rip the leaf) as described in your notes. It often helps to leave the epidermis or the tear sections in dilute teepool for a while as this reduces the surface tension of gas bubbles and thus the number present

in the section when viewed down the microscope. You can also mount the sections in teepol if you wish.

When you have done this and are ready to view the preparations of *Ligustrum* and *Tradescantia* return to the audio file.

PAUSE

Stomata in Eudicotyledons can vary greatly in size and density, they can also vary with the type of leaf - dorsiventral leaves such as *Acalypha* or *Ligustrum* have most or all stomata on the lower surface. In pendant leaves there may be many more on one or other surface, or there may be a similar number on both surfaces. Some Examples of stomatal density are given in Table 1. Compare your preparation of *Ligustrum* to the micrographs produced under the scanning electron microscope shown in plate 3 before returning to the sound.

PAUSE

Now examine the epidermis of *Tradescantia* which is a monocot. Compare your preparation to the permanent mount provided in Slide 3, the return to the sound.

Were the pores of your preparation of *Tradescantia* open or closed? The photo in Plate 4 shows a pore slightly open as does the carrel image link 6. Frequently, stripping the epidermis has the effect of closing the stomatal pore, but these often open if left in water or, even better, a dilute KCl solution under the light of the microscope lamp. Also shown in Plate 4 is the stomata of *Commelia cyanea*, this is an Australian native species which is related to *Tradescantia*.

The stomata of Grasses (Graminae or Poaceae) and sedges (Cyperaceae) (but not of all monocots) -are quite different to those of *Tradescantia* and *Commeliana*. Examine figure 7. The guard cells are long and narrow with very thick walls in the middle and bulbous ends. However, opening and closing follows the same principles as the other kind of stoma -the difference is just in the type of asymmetric thickening. Now read the description in figure.7. Also have a look at the demonstration slides of the epidermis with stomata of *Zea Mays* and other grass stomata.

When your have completed this read the notes on cuticle, look at the demonstration slides (if you have time) and return to the audio file.

As we noted earlier, the epidermis is covered by a cuticle made of a waxy substance. Look at carrel image link 7 showing the leaves of the of *Nymphaea*, the water lily and *Banksia serrata* with Sudan staining which partitions into waxes and oils. You can see that the cuticle thickness can vary greatly. In *Nymphaea*, the cuticle is very thin, the plant lives in water and presumably would rarely be water stressed. *Banksia* on the other hand lives in dry soils with poor water retaining capacity and must minimise water loss. These environmental responses will be investigated further later in semester.

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Now lets turn to trichomes. Trichomes may be simple hair-like structures of one or more cells or complex multicellular gland-like structures. The role of simple non-glandular trichomes may be to protect the leaf in some way or to increase the humidity immediately around the stomata. Glandular trichomes on different species tend to be characteristic of particular species and are used in taxonomy. They may expel very different substances, for example the digestive enzymes of carnivorous plants, toxins such as strychnine or salt from a mangrove leaf.

If you have time look at the demonstration of thrichomes, then answer self quiz 1 return to the sound.

Pause

Check your answers to the quiz with those at the end of this weeks program. If you don't agree with the answers given discuss yours with a staff member in the lab or the tutorial. Then return to the sound.

PAUSE

We now turn to the ground tissue - the mesophyll. Read the notes on *Ligustrum* and look at slide 4. Use Figure 8, Plate 5 and the carrel images link 9 to help you identify the tissues present. Then return to the sound.

PAUSE

In *Ligustrum*, as in most Dorsiventral leaves of eudicots, the upper mesophyll is elongated perpendicular to the surface and closely packed. This is called palisade mesophyll. The Lower mesophyll is less closely packed and is called spongy mesophyll. Gas spaces are much larger in proportion in spongy mesophyll. They run up between palisade mesophyll providing a continuous gas phase from external atmosphere through the stomatal pore and the gas space of the leaf to the most remote mesophyll cells.

Note that in order to build up a more realistic three dimensional picture (like that in Fig. 8) we need to look at the leaf parallel to the leaf surface also. Use your tear preparation of *Ligustrum* and compare them to Plate 6 and the carrel images link 10.

Now look at slide 5 which is of a pendant leaf of *Eucalyptus*. These leaves have isobilateral organisation with palisade cells on both sides. Then do self-quiz 2 and check your answers. Then return to the sound.

PAUSE

Another type of leaf is shown by *Bromus*, a grass. Examine slide 6 and compare with plate 7 and carrel image link 11 then return to the sound.

In *Bromus* and in most grasses, but not in all monocots, the leaf has a distinct Upper and lower surface, but the mesophyll tissue is not differentiated into palisade and Spongy mesophyll –it is homogenous. Gas spaces run through the leaf between the cells as In the other leaf types.

Bromus also has an indented upper margin which may allow leaf rolling, depending on the volume of motor cells.

Another feature of *Bromus* that distinguishes it from *Ligustrum* is the supporting tissue above and below the veins. Note that this is sclerenchyma – lignified, thick walled cells stained red with safranin. In *Ligustrum* the thick walls tissue near the midrib did not stain red with safranin - it was collenchyma. However, this difference is not typical of some Australian natives which may have lignified tissue supporting the midrib.

Note the i-beam pattern of support in *Bromus*. Do self quiz and check your answers before proceeding.

Now examine the demonstration slides of leaf structures if you have time to see what variations on the basic pattern they exhibit. Look for palisade and spongy mesophyll for mechanical supporting tissues - collenchyma or sclerenchyma – and for grooves etc related to rolling up.

Then return to the sound.

Now before examining photosynthesis let us briefly consider a totally different question - that of the mechanical support of the leaf. Normally the majority of leaves are hanging but are held with

their plane closer to the horizontal. The support needed comes from two sources - one is the mechanical support we have already considered and the other is turgor. The relative contribution of the two can be gauged from seeing the difference between a wilted and a normal leaf. In a wilted leaf the cells are not turgid and only the mechanical tissue supports the leaf. In the normal leaf the cells are turgid, i.e. have a hydrostatic pressure inside the cells, (the same pressure that blows up a car tyre or balloon). The pressure makes the cells, and consequently the leaf, more rigid.

We shall deal with turgor and tissue rigidity in lectures and the water relations in Lab. classes.

So far we have examined the mesophyll in some detail, the supporting mechanical tissue briefly and the bundle sheath briefly. But now it is necessary to emphasise the title of the section - the mesophyll tissue of leaves and plants with C3 photosynthesis, -because the next type of leaf to examine is that with C4 photosynthesis.

Before looking at C4 species let us in anticipation summarise the chief characteristics of C3 and C4 species.

C3 species we have already examined. The important point is that in these species photosynthesis takes place in mesophyll cells and not in the bundle sheath. You have already observed for yourself that there are few chloroplasts in bundle sheaths of the C3 plants you have examined. Biochemically C3 species have the well known carbon reduction cycle (sometimes called the "Calvin" cycle after its chief Discoverer). This operates in all their chloroplasts producing, as the first stable product of CO₂ fixation, a 3 carbon compound.

In contrast to this, there are a number of species in which the first stable product of CO₂ fixation is a 4-carbon compound. These are called C4 species for short. C4 species also have the C3 carbon reduction cycle, fixing CO₂ by photosynthesis, but this occurs in bundle sheath cells, which are large and packed with chloroplasts while the mesophyll cells convert CO₂ to a labile 4 carbon compound which flows through to the bundle sheath cells and breaks down to give CO₂ there which is photosynthesised.

The main feature of C4 species is that bundle sheath cells are packed with chloroplasts, while ordinary Mesophyll cells may have fewer, structurally simpler chloroplasts. This wreath-like structure around the vascular bundles in leaves of some species was observed by Haberlandt in the 1880's. He called this Kranz (German for 'wreath') structure, and speculated correctly about a division of labour between bundle sheath and mesophyll, but it was not until the mid 1960's that Hatch and Slack, working in the David North Res. Lab. of CSR in Brisbane discovered and clarified the biochemical side of the C4 syndrome.

Read the notes on Mesophyll tissue of leaves of plants with C4 photosynthesis. Look at Plate 8 and Carrel image link 13. In grasses the lateral cell count between the bundle sheaths (which does not include the bundle sheath cells) is 4 or less in C4 species and greater than 4 in C3 species. Do a lateral cell count on your slide of *Bromus* - what do you conclude? Now look at Carrel image link 12 of *Zea Mays* - what would you conclude here?

OK now do the exercise in identification using both Kranz Anatomy and cell count methods. Then return to the audio file.

PAUSE

Do your identifications using the two criteria agree? If Not see if you can reconcile them and finally check your answers with the correct answers on webCT. When you have done that you are finished. Please clear up your Carrel and leave it ready for the next person to use.

Goodbye until next time