BIO 311
Plant Structure and Development
Lab Manual

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LABORATORY SCHEDULE

Fall semester, 2012

Lab	Month	Day	Subject
1	Sept.	11, 12	Introduction to the plant body
2		18, 19	Plant cells
3		25, 26	Analysis of plant genes
4	Oct.	2, 3	Meristems, growth and differentiation
5		9, 10	Ground tissues
6		16, 17	Epidermis
7		23, 24	Vascular tissues
8		30, 31	Anatomy of stems
9	Nov.	13, 14	Anatomy of leaves
10		20, 21	Anatomy of roots
11		27, 28	Vascular cambium
12	Dec.	4, 5	Secondary growth

LAB 1 - INTRODUCTION TO THE PLANT BODY

Introduction

Your objectives for this first laboratory are to: 1) learn how to section plant tissue and examine the sections with the light microscope, 2) record your observations as labeled drawings, 3) begin to distinguish the cells that compose the tissues of stems, roots, and leaves, and 4) review the terminology used to describe the structure of seeds, seedlings and growing plants.

It takes practice to make good free-hand sections in which you can distinguish the features of the various cells. Your ability to see these features will also depend on proper adjustment of your microscope, particularly the condenser. Your lab instructor will evaluate your sectioning and microscope technique and offer suggestions.

Prepare your drawings for this and most other labs on 5X8" index cards as explained on pp. 9-11. Preparing the cards will help you organize the information presented in lab. Use the terms in **boldface type** as a guide for deciding which structures to label. The completed cards will help you review for quizzes and exams.

A **tissue** is a group of cells organized into a structural and functional unit. Plant tissues are grouped into three tissue systems. The **dermal tissue system**, which covers the entire plant surface, protects the plant and regulates the flow of materials between the plant and its environment. The **vascular tissue system**, which consists of an arrangement of veins, moves water and nutrients within the plant. The **ground tissue system** fills the space between the dermal and vascular tissue and serve a variety of functions including support, photosynthesis and storage. When you finish this exercise, you should be able to identify dermal, vascular and ground tissue in stems, roots, and leaves. You should also gain a sense of the continuity of these tissues throughout the plant. You will see that the dermal tissue forms a continuous protective covering from roots to leaves. The continuity of the vascular tissue throughout the plant is necessary for transport of water from the roots to the leaves and sugar from the leaves to the roots.

Part 1 – Terminology review

Examine soaked bean seeds, seedlings and mature bean plants. Identify each of the structures listed below. Finally, label each structure on the diagrams on page 5.

simple leaf	**axillary bud**	**tap root**	**plumule**
leaf blade	**compound leaf**	**lateral root**	**hypocotyl**
petiole	**leaflet**	**root apex**	
leaf vein	**node**	**embryo**	
midvein	**internode**	**cotyledon**	
axil	**shoot apex**	**radicle**	

Part 2 – Plant tissues

Refer to the instructions for free-hand sectioning on page 7. Make several cross-sections from the stem of your bean plant, stain with toluidine blue, and prepare a wet mount. Look at your stem sections under the microscope and identify the outermost layer of cells. This is the **epidermis**, a type of the dermal tissue. Just inside the epidermis, **vascular tissues** form a ring of bundles. The **ground tissues** form the **cortex** (between epidermis and vascular bundles) and the **pith** (center of stem).

Card 1-1: Prepare a pie-slice drawing of a bean stem cross section (refer to instructions on page 9-11). Label the tissues.

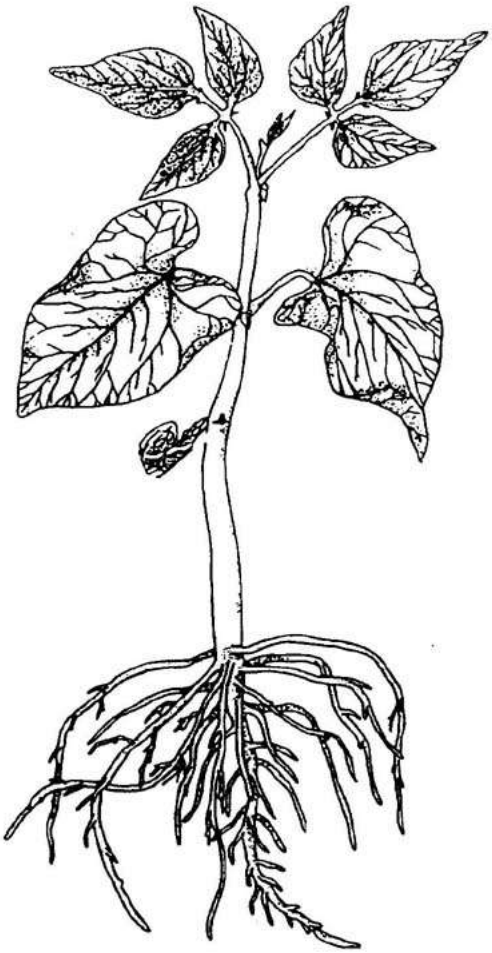
Make free-hand cross sections of the root of your bean plant and stain and mount them. Using the compound microscope, identify the tissues. How does the arrangement of the tissues differ in stems and roots?

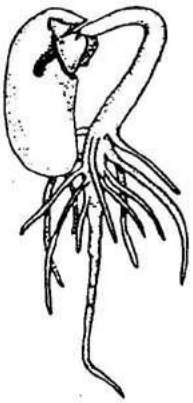
Card 1-2: Prepare a pie-slice drawing of a bean root cross section. Label the tissues.

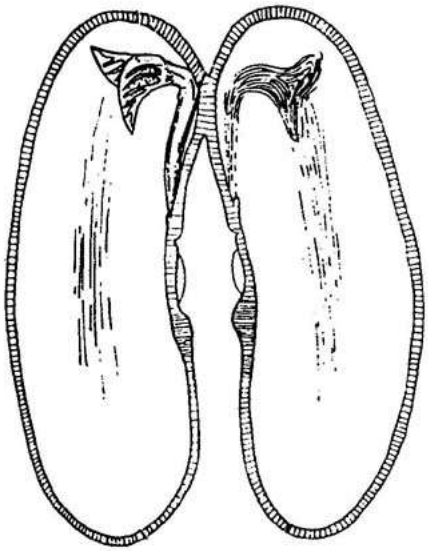
Make free-hand cross sections of a bean leaf by placing a piece of it between two blocks of carrot root. Stain and mount your sections. Using the compound microscope, identify the tissues. Which type of tissue composes the **leaf veins**?

Card 1-3: Prepare a drawing of a bean leaf cross section. Label the tissues.

Plant cell types differ in (1) size, (2) shape, (3) cell wall thickness and composition, and (4) characteristics of the protoplast. How many different cell types can you find in your sections? On the back of each card, list the cell types you find within each tissue system. Use the names of the cell types if you remember them from BIO 102. Otherwise, list the characteristics of each cell type (i.e. 1-4 above) that distinguish it from others.





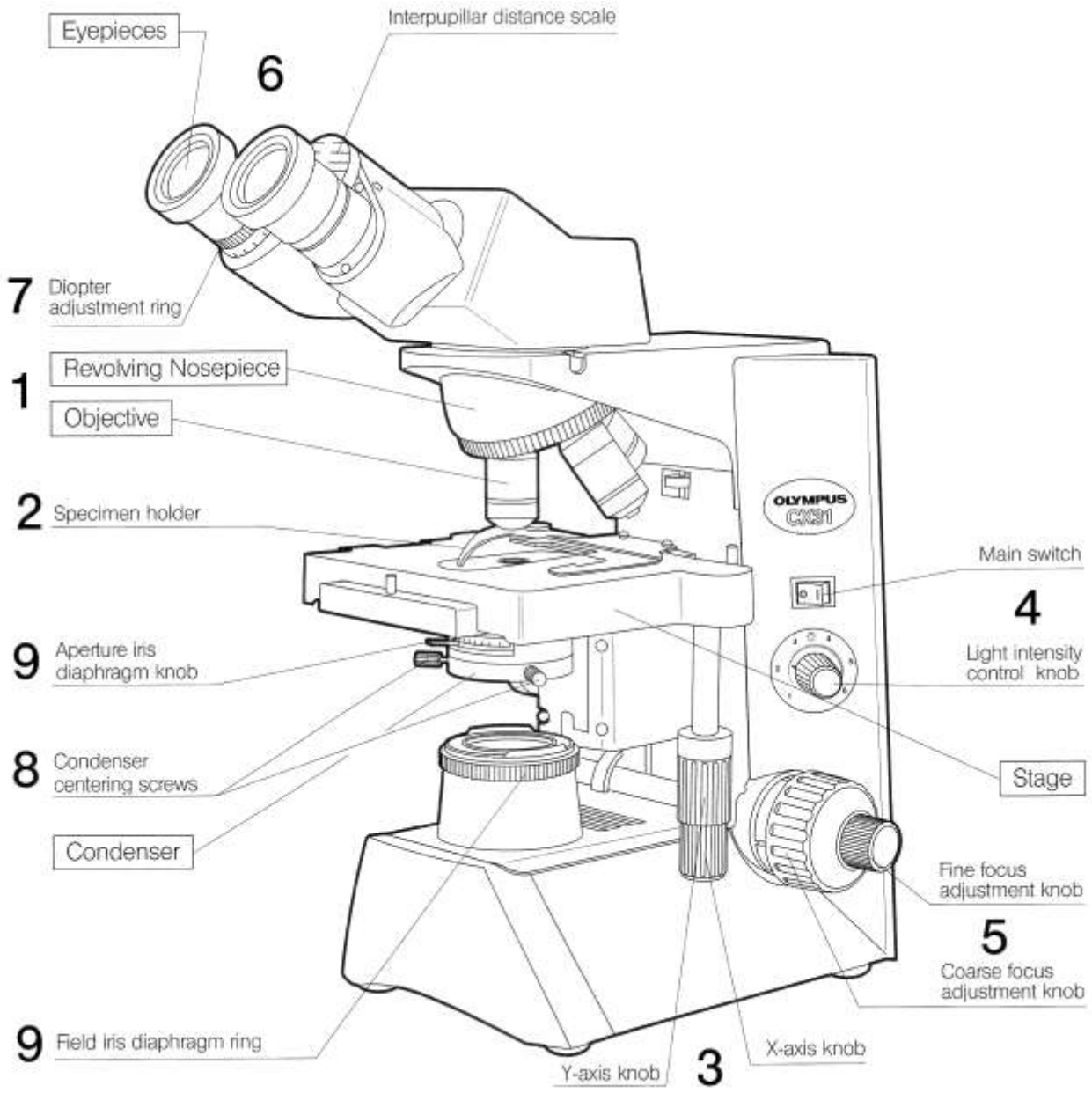


USE OF THE OLYMPUS CX31 MICROSCOPE

Before plugging in/turning on the microscope, make sure that:

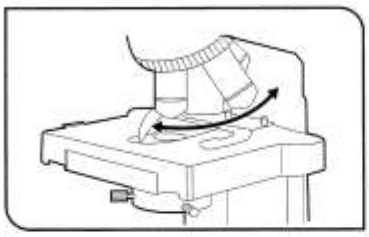
- the **main switch** is turned off
- the **light intensity control** is at the lowest setting
- the **10X objective** is in place

Cleaning lenses. Take care to keep eyepiece and objective lenses clean. If you think the lenses on your microscope need cleaning, **please ask your TA for assistance**.



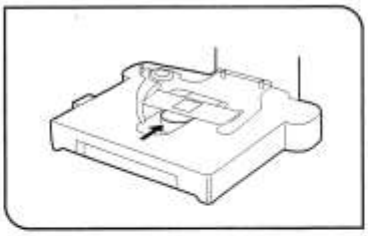
Using the Olympus CX31 microscope (step numbers are indicated on the previous page)**:**

1



• Turn the revolving nosepiece to engage the 10X objective.

2

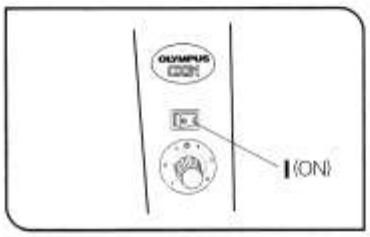


• Place a specimen on the stage.

3

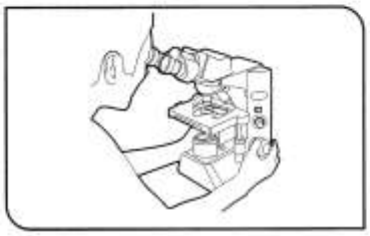
• Turn the X-axis knob and Y-axis knob to move the specimen into the light path.

4



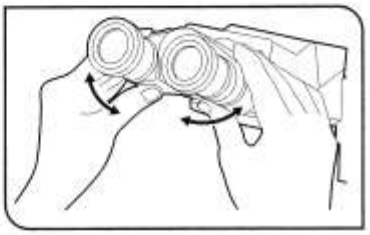
• Set the main switch to " I " (ON) and adjust the brightness with the light intensity knob.

5



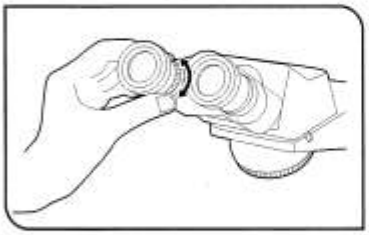
• Turn the coarse and fine adjustment knobs to bring the specimen into focus.

6



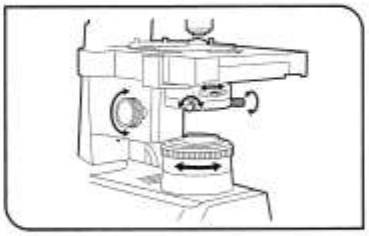
• Adjust the interpupillary distance.

7



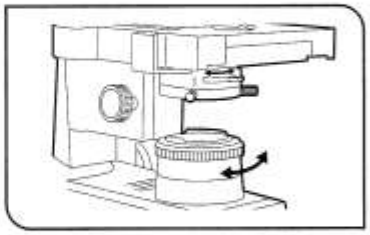
• Adjust the diopter.

8



• Center the field iris diaphragm.

9



• Adjust the aperture iris diaphragm and field iris diaphragm.

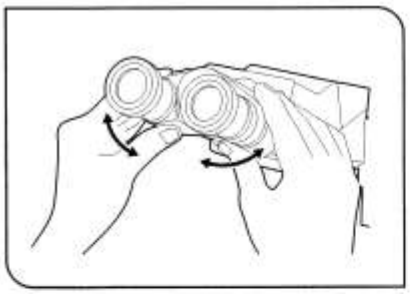
10 Engage the objective to be used for observation in the light path, then readjust the focus.

11 Re-adjust the aperture iris diaphragm, field iris diaphragm and brightness and start observation.

*Detailed instructions for steps 6-9 can be found on the next page.

When you are finished using the microscope, make sure that:

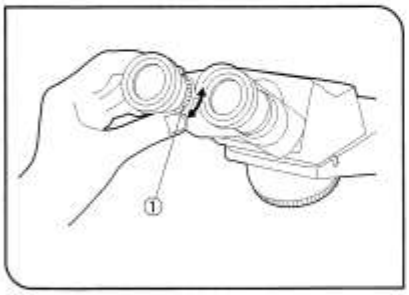
- your **slide** is removed
- the **brightness control** is turned to the lowest setting
- the **main switch** is turned off



6 Adjusting the Interpupillar Distance

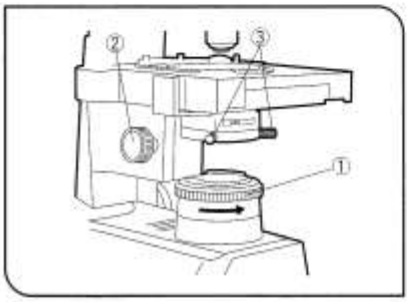
While looking through the eyepieces, adjust for binocular vision until the left and right fields of view coincide completely. The index dot • indicates the interpupillary distance.

※Note your interpupillary distance so that it can be quickly duplicated.



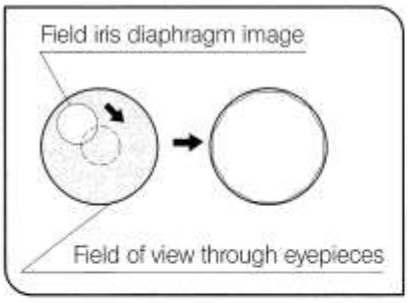
7 Adjusting the Diopter

1. Looking through the right eyepiece with your right eye, rotate the coarse and fine adjustment knobs to bring the specimen into focus.
2. Looking through the left eyepiece with your left eye, turn the diopter adjustment ring ① to focus on the specimen.



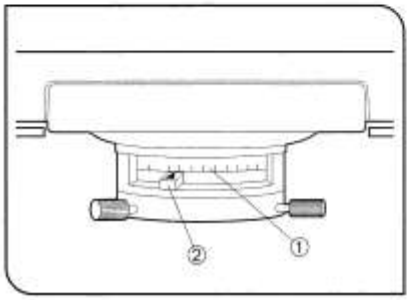
8 Centering the Field Iris Diaphragm

1. With the 10X objective engaged and the specimen brought into focus, turn the field iris diaphragm ring ① counterclockwise to stop down the diaphragm to near its minimum size.
2. Turn the condenser height adjustment knob ② to bring the field iris diaphragm image into focus.
3. Rotate the two condenser centering knobs ③ to adjust so that the field iris diaphragm image is centered in the eyepiece field of view.
4. To check centration, open the field iris diaphragm until its image touches the perimeter of the field of view. If the image is not precisely inscribed in the field of view, center again.
5. When used for actual observation, open the field iris diaphragm until its image is slightly larger than the field of view.



9 Aperture Iris Diaphragm

• The aperture iris diaphragm is used to adjust the aperture of the illumination system, and its adjustment also affects the resolution and contrast. Also note that stopping down the aperture iris diaphragm results in increases the focal depth.



Adjustment method

Since the microscope specimens generally have low contrast, it is recommended to adjust the aperture iris diaphragm to about 70% or 80% of the aperture of the objective.

Adjust the aperture iris lever ② to the aperture scale ① on the condenser. As the aperture iris lever is wide, align its center with the desires scale.

★Do not stop down the aperture iris diaphragm too much, or ghosts may be observed.

SECTIONING PLANT MATERIAL

Note: Dispose of slides in the sharps disposal container. Dispose of leftover stains in the chemical waste bottle.

Materials:
1. Razor blades
2. Forceps and plastic drinking straws cut at an angle to use as spatulas.
3. Spot plate for staining.
4. Clean slides and coverslips.
5. Toluidine blue (0.05% aqueous) and other stains as necessary.
6. Finger bowl with water and paper towels.
7. Dropper bottle containing water.
8. Kimwipes.

Procedure:
1. Sit comfortably with your forearms resting on the bench and your elbows close to your sides. Hold tissue between your thumb and forefinger.

2. Wet razor blade, fingers and tissue with water from the finger bowl. Water should drip from your fingers during sectioning.

3. Cut the tissue quickly and smoothly in the plane desired. Now section slowly by drawing the razor blade toward you in a smooth slicing motion; the razor should rest on the tip of your thumb. Use your thumb to control the thickness and evenness of the sections. **This takes practice**. Concentrate on getting very thin portions of some sections. It is not necessary to obtain complete cross sections.

4. Transfer sections to a water-filled depression in the spot plate **before they dry**. Do not dull the razor blade by touching it to the spot plate; use your spatula.

5. Transfer sections to a depression containing toluidine blue and stain for 15 seconds. Do not use stain that has evaporated and begun to precipitate.

6. Rinse sections in a depression containing water.

7. Mount sections on clean slides in a drop of water. To apply the coverslip, hold it at an angle and touch the water drop with one edge. Lower the coverslip slowly to avoid air bubbles. Semi-permanent mounts can be made by fixing tissue in phosphate-buffered glutaraldehyde, mounting in glycerol jelly, and sealing the coverslip with nail polish.

CYTOCHEMICAL STAINS

General staining
1. Immerse sections in toluidine blue solution (0.05% in water) for about 15 seconds.
2. Transfer to water.
3. Mount in water.

Starch
1. Immerse sections in IKI solution (2% potassium iodide, 0.2% iodine in water) for about 15 seconds.
2. Transfer to water.
3. Mount in water.

Protein
1. Immerse sections in amido black solution (1% in 7% acetic acid) for about 1 minute.
2. Transfer to 7% acetic acid.
3. Transfer to water.
4. Mount in water.

Lipids
1. Immerse sections in Sudan III or Sudan IV solution (saturated solution in 70% ethanol--about 0.1%) for about 1 minute.
2. Transfer to 70% ethanol.
3. Transfer to water.
4. Mount in water.

Lignin
1. Immerse sections in phloroglucinol solution (saturated solution in 20% HCl--about 0.1%) for 1-2 minutes.
2. Transfer to water.
3. Mount in water.

Callose (sieve plates)
1. Immerse sections in IKI solution (see starch above) for 2 minutes.
2. Transfer to water.
3. Transfer to aniline blue solution (0.1% in water) for five minutes.
4. Transfer to water.
5. Mount in water.

ANATOMICAL DRAWINGS

"Why do I have to do all of these drawings?" This question has entered the mind of every plant anatomy student. This section explains the purpose of anatomical drawings and helps you prepare drawings that record your observations effectively.

So, why drawings? When you make anatomical drawings, you develop several skills including the ability to:

A. interpret complex information,
B. identify diagnostic features that distinguish among similar structures, and
C. represent and communicate this information in visual form.

These skills have applications in many fields. Your employer will probably never ask you about the difference between a tracheid and a vessel member, but he or she may well ask you to examine a complex problem, identify the important points among a confusing array of details, and present your analysis to coworkers. Sound familiar?

Easy steps to better drawings. The purpose of a drawing is to convey information, first to your lab instructor who will evaluate whether you understood the specimen you were asked to draw, and then to yourself as a record of what you will need to recognize when you take exams. A useful drawing includes just the right amount of detail. You can accomplish this by using the following steps to plan your drawings.

1. Select the magnification and field of your drawing according to what you are asked to illustrate. Given the very same prepared slide, you might be asked to illustrate:

A. a cell type,
B. an arrangement of cells within a tissue, or
C. an arrangement of tissues within a structure.

The resulting drawings should be very different.

2. Include details that distinguish the subject from other similar structures. Given the assignments in A-C above, your drawings might be designed as follows:

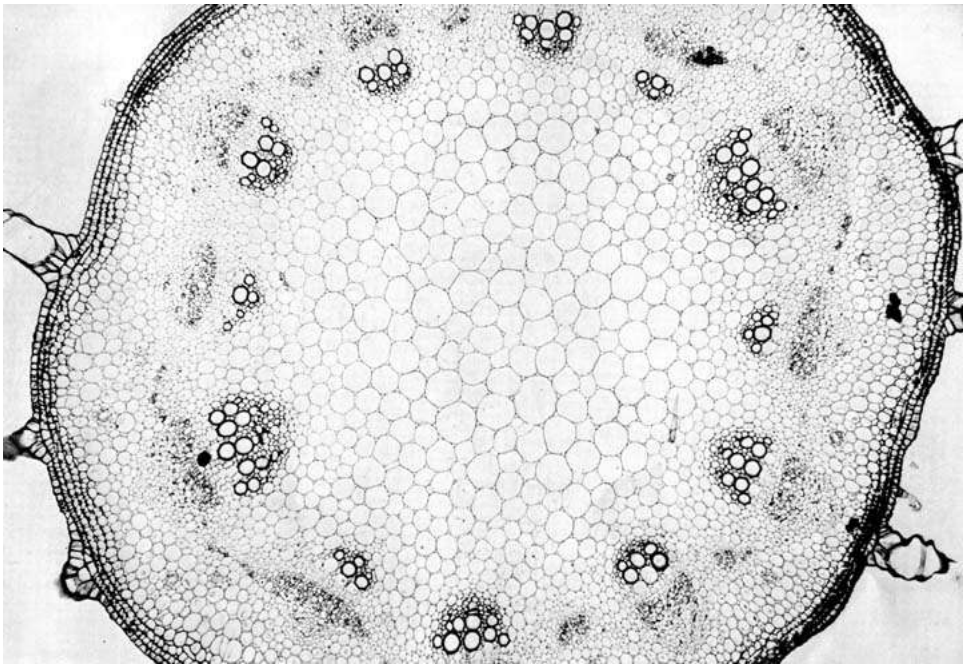
A. Include details of individual cells (a brachysclereid should look different from an astrosclereid).
B. Draw outlines of individual cells with enough detail to distinguish among cell types.
C. You may not need to draw individual cells at all. If the point is to show how vascular bundles are arranged in a stem, you need only outline boundaries of vascular bundles.

3. Represent form, proportion, and spatial relationships accurately.

4. Use insets when information at more than one level of organization must be conveyed.

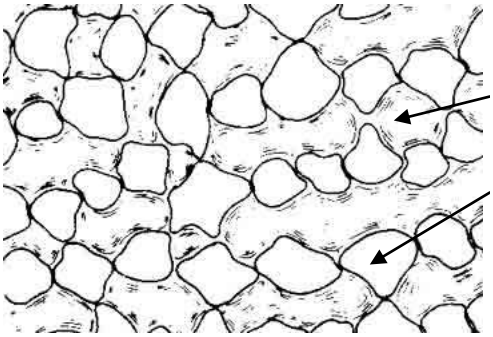
5. Label distinguishing features.

Examples: Three drawings based on a cross-section of *Helianthus* stem.

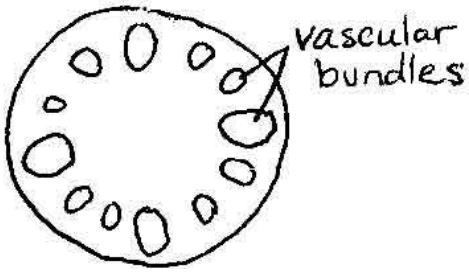


Photograph of a cross section of *Helianthus* stem (c.s.). Mag. 50X.

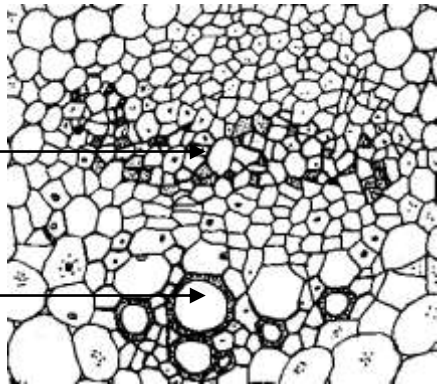
1. Draw a diagram to illustrate the structure of collenchyma cells in *Helianthus* stem.



2. Draw a diagram to illustrate the arrangement of vascular bundles in *Helianthus* stem



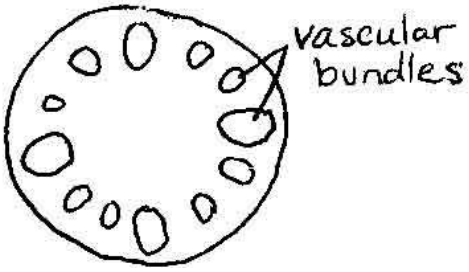
3. Draw a diagram to illustrate the cell types found in the vascular bundes of *Helianthus* stem.



Lab assignments:

Prepare drawings for 311 labs on 5X8" note cards using the following format:

Card number: 1-2 Name: Botany Bob
Date: 9/12/09 Lab section: 02



Vascular bundles in *Helianthus* stem

Sometimes you will be asked to answer questions on the back of the card.

Cards will be graded using the following criteria:

1. Is the subject shown at an appropriate magnification?
2. Is the context clear?
3. Is the level of detail appropriate?
4. Are the appropriate structures labeled?
5. Are questions answered correctly?

Cards will be returned promptly so that you can use them for studying.

LAB 2 - PLANT CELLS

Introduction

To understand the structure and function of plant tissues, you must first understand the structure and function of the cells that compose them. Much of what we know about cells has been learned using biochemical techniques and electron microscopy. These topics will be covered in lecture. This lab focuses on the aspects of plant cell structure that can be observed with a light microscope. Light microscopy has a very important advantage over electron microscopy, namely the light microscope can be used to observe *living* cells. The disadvantage of the light microscope is that the resolution can never be better than 0.2 μm. After completing this lab you should have a better understanding of the dynamic nature of cells. You will also be introduced to some of the variation in structure found among plant cells.

Part 1 - Plant cells in motion

Remove a stamen from a flower of *Tradescantia* or *Rhoeo* (onion epidermis may be used if flowers are not available). Notice the fine white hairs on the filament. Remove the anther and make a wet mount of the filament. Examine the stamen hairs using the compound microscope. Each stamen hair is composed of a file of cells. Focus carefully on a single cell and notice the large **nucleus**. If you watch the cytoplasm carefully you should see small particles moving in a process called **cytoplasmic streaming**. Cytoplasmic streaming is driven by the same proteins that are responsible for muscle movement, **actin** and **myosin**. The moving particles are coated with myosin, which moves them along cables of actin using energy derived from ATP. Try to remember the movement that occurs in living plant cells as you look at prepared slides throughout the semester.

Card 2-1: Draw a stamen hair cell and label the nucleus, cytoplasm, and vacuole.

Part 2 - Plastids

Plastids are derived from **proplastids**, the self-replicating organelles found in meristematic cells. The most familiar plastid is the chlorophyll-containing photosynthetic organelle the **chloroplast**. Other types of plastids include **chromoplasts**, which contain red, orange or yellow carotenoid pigments and **amyloplasts**, which store large amounts of starch. The leaf cells of plants that have germinated in the dark contain **etioplasts**, which develop into chloroplasts as soon as the leaves are exposed to light.

Chloroplasts: Make a wet mount of isolated *Zinnia* mesophyll cells and examine it with the compound microscope. The green, disk-shaped bodies are chloroplasts.

Amyloplasts: Embryos often contain large amounts of stored starch. Why? Examine a prepared slide of *Sagittaria* mature embryo and identify starch grains. Each amyloplast contains several starch grains, but the boundaries of the organelles are usually not visible with the light microscope.

Card 2-2: Draw and label comparative diagrams of chloroplasts (*Zinnia*), and amyloplasts (*Sagittaria*).

Chromoplasts: Make wet mounts of slices of green and red peppers. The plastids in the green pepper are chloroplasts, but the chloroplasts have differentiated into chromoplasts in the red pepper. The red-orange **carotenoid** pigments in these plastids are **hydrophobic** (lipid soluble).

Card 2-3: Draw and label diagrams of cells containing chloroplasts and chromoplasts in green and red peppers, respectively.

Part 3 - Vacuoles:

Vacuolar pigments: In contrast to chromoplasts, **vacuoles** contain **anthocyanin** pigments, which are **hydrophilic** (water soluble). Peel a piece of epidermis off of a red onion bulb or a *Tradescantia* leaf and prepare a wet mount. The red-violet pigments you see are contained within the vacuole. In addition to containing pigments and other materials, vacuoles function similarly to the lysosomes of animal cells.

Tannins: The vacuoles of some cells accumulate phenolic substances called **tannins**. These compounds complex with proteins (the basis for their ability to tan leather) and help protect plants against insects and pathogens. Examine a prepared slide of pine leaf an identify the red-staining tannin cells

Card 2-4: Draw and label comparative drawings of cells with pigmented vacuoles (*Tradescantia*) and vacuoles containing tannins (pine leaf).

Crystals: Some cells accumulate crystalline calcium oxalate in their vacuoles. Calcium oxalate crystals inhibit predation and may serve a calcium storage reservoirs. Crystalline materials are best viewed with polarized light. Use polarizing filters to examine a wet mount of *Sanseveria* stem. The needle-like crystals or **raphides** are responsible for this plant's common name "dumb-cane." If the plant is eaten, the crystals lodge in the tongue causing it to swell. **Druse** crystals can be identified in sections of *Begonia* petiole.

Card 2-5: Draw and label comparative drawings of cells with raphide crystals (*Sanseveria*) and druse crystals (*Begonia*).

Part 4 - Cell walls

Growing plant cells produce **primary cell walls** composed predominantly of **polysaccharides**. Some cell types produce a **secondary cell wall** that may become impregnated with an aromatic polymer called **lignin**. Cells with primary cell walls and those with lignified secondary cell walls can be observed in the flesh of pear fruit. Obtain a small piece of pear flesh and place it on a slide with a drop of **phloroglucinol** (Caution: the phloroglucinol is dissolved in 20% HCl). Place a coverslip over the material and press gently to spread the cells. Examine the slide with the compound microscope. The phloroglucinol reacts with lignin to produce a red color, staining

only cells with secondary cell walls. The cells that appear unstained have only primary cell walls. Save this slide! You will be asked to look at it again in the next section.

Card 2-6: Draw a diagram that distinguishes cells with primary cell walls only from those with secondary cell walls.

Part 5 - Intercellular connections

Plasmodesmata: Cells with primary cell walls are connected to one another by channels called **plasmodesmata**, which perforate the primary cell wall. These channels are lined with plasma membrane and they contain cytoplasm. Plasmodesmata are often clustered in thin regions of the cell wall known as **primary pit fields**. Plasmodesmata are not visible with the light microscope, but they can be seen in electron micrographs.

Simple pits: Connections between cells with secondary cell walls are called **pits**. These are areas where secondary cell wall material is not deposited so only the primary cell wall or **pit membrane** separates the cells. Unlike the plasmodesmata described above, pits do not contain plasma membrane or cytoplasm. **Simple pits** are visible in the sclereids of pear. Take another look at the slide that you stained with phloroglucinol and note that the channels are continuous from one cell to another. Now examine a prepared slide of pear fruit in which simple pits are visible in face view and in side view.

Card 2-7: Draw diagrams to illustrate simple pits in face view and in side view. Label the secondary cell wall, cell lumen and simple pits.

More complex pits called **circular bordered pits** can be examined in sections of pine wood (see radial section for face view, tangential section for side view).

Card 2-8: Draw labeled diagrams to illustrate circular bordered pits in face view and in side view.

LAB 3 – ANALYSIS OF PLANT GENES

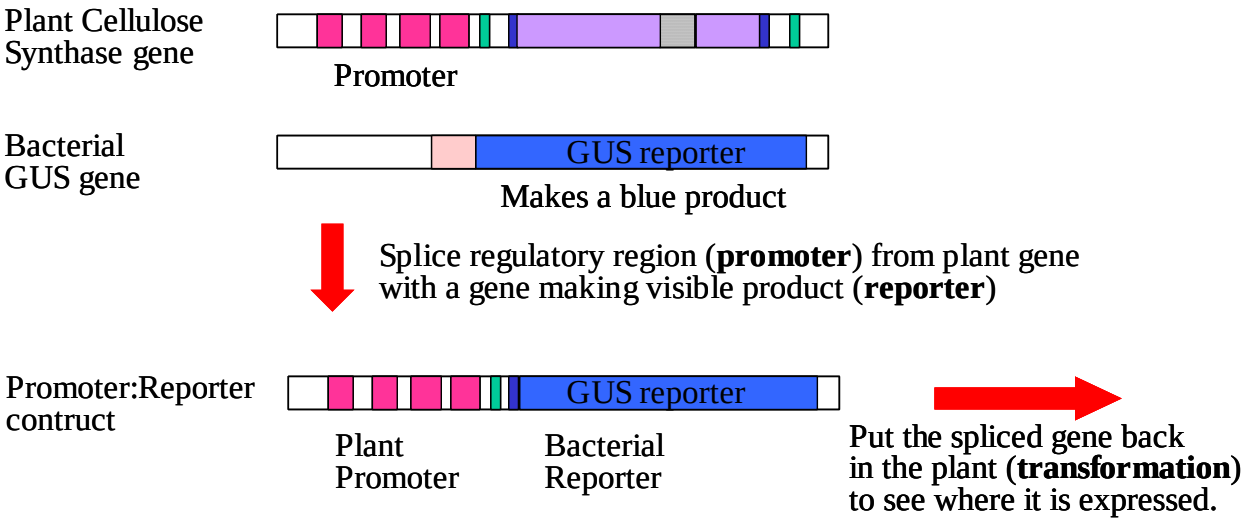
Introduction

When trying to determine the functions of individual genes, biologists often analyze gene sequences and the patterns of gene expression. In this lab, you will be introduced to a few of the methods biologists use to study gene function.

Part 1 – Analysis of Gene expression

The DNA of every cell in a plant contains all of the genes required for the development and function of the entire plant. However, individual cells **express** (i.e. transcribe and translate) only a subset of these genes. In this exercise, you will examine gene expression in two lines of *Arabidopsis* plants that have been transformed with the GUS **reporter** gene (see below). In each line, the GUS gene is linked to the **promoter** for a different cellulose synthase genes (*CesA*3 or *CesA*7) to make a **promoter::reporter construct**. In each transformed plant, the promoter attached to the GUS gene is activated along with the promoter for the corresponding cellulose synthase gene. When incubated with the substrate X-gluc, the β-glucuronidase enzyme encoded by the GUS gene produces a blue color so you can see where the promoter is active.

Information on the expression of *CesA*3 and *CesA*7 has been important for understanding the function of these two genes. The *CesA* genes encode the enzymes that make cellulose, a major component of cell walls. During this lab exercise, you will investigate whether the cell walls of different types of cells are made by the same or different cellulose synthases.



Procedure

Working with a lab partner, do the following:

1. Obtains 3 vials containing the GUS reation mixture and label them "WT" for wild-type (a control) and "*CesA*3::GUS" and "*CesA*7::GUS" for plants transformed with the

promoter::reporter constructs. The GUS reaction mixture contains the x-gluc sbustrate, a buffer, and an oxidative catalyst. The follow reaction takes place in cells that express the GUS gene:

	GUS		Oxidation	
X-gluc	**→**	**X-**	**→**	**X-X**
(colorless)		**(colorless)**		**(blue)**

2. Add tissues from the different plant lines (WT, *CesA*3::GUS, *CesA*7::GUS) to labeled vials. These may include: (1) two seedlings, (2) a mature leaf, and (3) a young leaf. Incubate at 37^{o}C for 1 hour.

While you are waiting, examine the Arabidopsis plants and seedlings and quiz your lab partner on the terminology you reviewed during the first lab. How does *Arabidopsis* differ from bean?

3. Use a pipette to remove the GUS reaction mixture and discard in the appropriate waste bottle. Replace with 70% ethanol. The ethanol removes chlorophyll to allow you to see the blue reaction product.

4. Examine the leaves and seedlings from each line. Describe the location of the blue reaction product. Is it found in leaves? Stems? Roots? Is it found in dermal tissue? Vascular tissue? Other tissues? Is it found in mature parts of the plant? Immature parts of the plant? Is it found in the wild-type plant?

5. Based on your observations, describe potential functions of the *CesA*3 and *CesA*7 genes.

Part 2 – Gene sequence analysis

One way to obtain the DNA sequence of a gene involved in a particular process is by mutation analysis. After selecting mutant individuals with interesting phenotypes, biologists map the mutant gene and determine its DNA sequence using a automated sequence analyzer. The output of the sequence analyzer is a text file containing a nucleotide sequence. To obtain information on the function of the gene, the nucleotide sequence is compared to the sequences of genes of known function that have been deposited in a database. In this exercise you will compare an unknown DNA sequence to sequences in the GenBank database to see what you can learn about potential functions of the gene represented by the unknown sequence.

Procedure

1. You will receive an unknown DNA sequence as a text file.

2. Access the GenBank database from the following URL: http://www.ncbi.nlm.nih.gov/. There's lots of information here, so take a look around!

3. Your TA will show you how to navigate the database and run a BLASTX search.

4. Explain what you learned about potential functions of your unknown gene.

LAB 4 - MERISTEMS, GROWTH, AND DIFFERENTIATION

Introduction

In this lab you will learn about the organization and function of the apical meristems of roots and shoots. Before you get started, some clarification of terminology is in order. The terms **shoot apex** or **root apex** refer simply to a tip of a shoot or root and imply no discrete boundary. An **apical meristem** is a discrete group of cells that divide in an organized manner, thus establishing the pattern of the apex and supplying cells to the rest of the meristematic region. Finally, **initial cells** are cells that divide to produce (1) a cell that stays in the meristem and (2) a cell that is added to the plant body.

Part 1 - Dissection of the shoot apex

Use modeling clay to mount an *Elodea* shoot apex under the dissecting microscope. While observing with the microscope, remove the leaves from the shoot apex. Eventually you will uncover the **apical meristem** surrounded by tiny **leaf primordia**. Notice the orderly arrangement of leaf primordia around the apical meristem. The shoot apical meristem forms cells basipetally to increase the length of the stem and laterally to produce leaves. Compare the three-dimensional shoot apex with a prepared slide of a longitudinal section of the *Elodea* shoot apex. As you examine prepared slides, remember that the shoot apex is three-dimensional and constantly changing. An apical meristem retains a similar *organization* over time, but the *population of cells* of which it is composed changes constantly.

Card 3-1: Draw diagrams of living and sectioned shoot tips of *Elodea*. Label apical meristem and leaf primordia.

Part 2 - Organization of the shoot apical meristem

The shoot apical meristems of different plants vary in size, shape, and organization. You will examine two distinct types today, but there are many other variations.

The apical meristems of the seedless vascular plants (e.g. ferns) feature a single **initial cell**, which provides the precursors for all other cells of the meristem. Examine the prepared slide of the shoot apex of *Equisetum* and identify the initial cell. Refer to your lecture notes to review how initial cells function.

Card 3-2: Draw a labeled diagram of an *Equisetum* shoot apical meristem.

The shoot apical meristems of angiosperms are more complex in structure and function than those of seedless vascular plants. Examine a prepared slide of *Coleus* stem tip and notice the layered structure of the apical meristem. The outermost layers (**L1** and **L2**) consist of cells that divide in an **anticlinal orientation** (perpendicular to the surface). The cells of **L3** can divide in any orientation. Each layer includes its own **initial cells**. Now distinguish the zones (**central zone**, **peripheral zone** and **rib zone**) within the *Coleus* shoot apical meristem. Which zone contains the cells that function as initial cells?

Card 3-3: Draw two diagrams of *Coleus* shoot apical meristems, one to illustrate L1, L2 and L3 and another to illustrate the zones. On the back of the card, describe the functions of each of the layers and zones.

The pattern of initiation of leaf primordia determines the pattern of leaf arrangement (**phyllotaxy**) in the mature plant. Compare the shoot apex of *Coleus* (which has opposite leaves) with that of *Ginkgo* (which has alternate leaves).

Card 3-4: Draw comparative drawings of shoot tips from a plant with opposite leaves (*Coleus*) and a plant with alternate leaves (*Ginkgo*).

Part 3 - The root apex

Examine the root apex of water hyacinth or *Zebrina* and note the prominent **root cap**. In contrast to the shoot apical meristem, the root apical meristem forms cells apically as well as basipetally. This type of organization is necessary to supply cells to the root cap, which constantly sloughs-off cells as the root penetrates the soil. Another difference is that the root apical meristem does not produce lateral organs. Instead, lateral roots arise from deep within mature regions of the root. Note that there are no lateral roots in the region just above the root cap.

Card 3-5: Draw a diagram of a root apex and label the root cap and lateral roots.

Part 4 - Organization of the root apical meristem

As with shoot apical meristems, the organization of root apical meristems varies among taxa. You will examine two examples.

The root apical meristems of seedless vascular plants have a single **initial cell** as does the shoot apical meristem. In addition to several basipetal cutting faces, the root **initial cell** has one cutting face directed distally that contributes cells to the root cap. Study a prepared slide of *Botrychium* root.

Card 3-6: Draw a diagram of a *Botrychium* root tip. Label the initial cell and root cap.

Like shoot apical meristems, the root apical meristems of flowering plants are more complex than those of seedless vascular plants. Examine the prepared slide of *Zea* root tip and note the boundary between the root cap and the rest of the root. The outermost layer of the root tip itself will become the epidermis of the root. Now identify the developing vascular tissue in the center of the root. Where the vascular tissue meets the boundary between the root and the root cap, there is a small group of cells call the **quiescent center**. Surrounding the quiescent center are the initial cells that produce the cells of the root cap, epidermis, vascular tissue and cortex.

Card 3-7: Draw a diagram of a *Zea* root tip and label the root cap, vascular tissue, epidermis, cortex, quiescent center and initial cells.

Part 5 – Tissue differentiation

As cells derived from the apical meristems begin to mature they become specialized for particular functions; that is they **differentiate**. Differentiation leads to the formation of **tissues**. The **meristematic cells** within the root and shoot apical meristems look similar to each other. As the cells produced by the apical meristems differentiate, they become distinct in appearance. Cells in the process of becoming vascular tissue are more elongated than surrounding cells and are called **procambium**. The surface cells covering immature parts of the plant will give rise to the dermal tissue and are called **protoderm**. Examine the prepared slides of *Zea* root apex, *Coleus* shoot apex and *Capsella* embryo and identify procambium and protoderm.

Card 3-8: Draw diagrams and label the differentiating tissues in *Zea* root apex, *Coleus* shoot apex, and *Capsella* embryo.

LAB 5 – GROUND TISSUES

Introduction

This lab focuses on the **ground tissues** that fill the space between the epidermis and the vascular bundles. These are **simple tissues** consisting of a one cell type. Their functions are diverse and include support, storage and photosynthesis.

Part 1 - Classification of ground tissues

The ground tissues are classified in three groups on the basis of cell wall thickness and composition.

Parenchyma tissues are composed of cells that have thin primary cell walls.

Collenchyma tissues are composed of cells with unevenly thickened primary cell walls.

Sclerenchyma tissues are composed of cells with thick secondary cell walls, often containing lignin. The protoplasts often die as sclerenchyma cells mature.

Cell wall thickness can be determined easily with the microscope, but stains are needed to determine the cell wall composition. In lab 2 you used **phloroglucinol** to stain lignified cells. As a review, make a section of pear flesh, stain with phloroglucinol, and note the position of sclerenchyma cells. Phloroglucinol stains only lignified cells, so it is helpful to see how sclerenchyma stains with the more generalized stain **toluidine blue**. Stain some pear flesh with toluidine blue and note the color of the sclerenchyma cells that you identified with phloroglucinol. Prepared slides have been stained with a combination of **saffranin and fast green**. What color does lignin stain with this combination?

Card 4-1: Sketch color-coded drawings of pear flesh stained with phloroglucinol, toluidine blue, and saffranin/fast green and identify sclerenchyma cells and parenchyma cells.

Part 2 - Parenchyma

Among the types of ground tissues, parenchyma includes the greatest diversity of structure and function. Whereas collenchyma and sclerenchyma function in support, parenchyma may function in photosynthesis, storage, secretion or a variety of more specialized tasks. In this section you will examine several types of parenchyma tissues.

Chlorenchyma, parenchyma tissue specialized for photosynthesis, is rich in chloroplasts. Examine the prepared slide of *Ligustrum* leaf. The closely packed cells below the upper epidermis are a type of chlorenchyma called **palisade mesophyll**. Chlorenchyma also occurs in green stems, unripe fruits (like the peppers you saw in lab 2) and some aerial roots.

Aerenchyma, parenchyma tissue specialized for gas exchange, is characterized by large intercellular air spaces. Examine the prepared slide of *Ligustrum* leaf once more, this time

concentrating on the **spongy mesophyll** located between the palisade mesophyll and the lower epidermis.

Card 4-2: Draw a diagram of a *Ligustrum* leaf and label chlorenchyma and aerenchyma.

Aerenchyma is especially well developed in aquatic plants, where it functions in both floatation and gas exchange. Examine aerenchyma in the following plants: 1) *Nymphaea* (water lily) leaf, 2) *Juncus* (bullrush) leaf , 3) Water hyacinth petiole, and 4) *Myriophyllum* stem.

Card 4-3: Draw diagrams to illustrate different arrangements of aerenchyma. What features do all aerenchyma tissues share? What features distinguish different types of aerenchyma?

Storage parenchyma may store starch (in amyloplasts), oil (in oil bodies or plastids), protein (in protein bodies or cytoplasmic granules), hemicellulose (in cell walls), or water (in vacuoles). Prepare specimens as described in the table below.

Card 4-4: After examining each specimen, complete the table by identifying the storage product (e.g. starch, protein, etc.), the storage compartment (e.g. vacuole, cell wall etc.), and the appearance of the stored material (e.g. color and form).

Tissue	Preparation	Storage product	Storage compartment	Appearance
Bean cotyledons	Stain with IKI			
Bean cotyledons	Stain with naphthol blue black			
Avocado fruit	stain with Sudan IV			
Jade plant leaves	Free-hand section			
Persimmon endosperm	Prepared slide			

The **endodermis** is the innermost layer of the root cortex and consists of cells characterized by a **Casparian strip**. This band of suberin blocks the apoplastic flow of water into the vascular tissue of the root. Identify the endodermis in the prepared slide of *Pyrus* (pear) root.

Card 4-5: Draw a diagram of the endodermis from *Pyrus* root. Label the features that distinguish this tissue from others.

Part 3 - Collenchyma

Collenchyma tissue consists of elongated cells with unevenly thickened primary cell walls. The walls of collenchyma cells are rich in hemicellulose and pectin, but contain no lignin. Layers of cellulose microfibrils in the walls alternate between longitudinal and transverse orientations. The resulting plasticity of collenchyma cell walls provides flexible support. Thus, collenchyma usually occurs in bundles in regions that are growing, such as young stems, or that must remain flexible after growth ceases, such as petioles.

Card 4-6: Examine collenchyma from *Cucurbita* (squash) stems and celery petioles and draw examples of each.

Part 4 - Sclerenchyma

Lignified secondary cell walls and an empty **lumen** (resulting from death of the protoplast) characterize sclerenchyma cells. Since lignified secondary cell walls are rigid, sclerenchyma is suited for support in mature non-growing tissues and may function in protection as well. Types of sclerenchyma cells include **fibers** and **sclereids**. Fibers are typically long and thin, and occur in bundles. These cells are the source of commercial plant fibers such as those derived from *Cannabis* (hemp). Sclereids occur in a variety of shapes and sizes. Examine the following types of sclerenchyma: 1) **brachysclereids** (*Hoya* stem), 2) **astrosclereids** (*Nymphaea* leaf), and 3) **fibers** (*Cannabis* stem).

Card 4-7: Draw diagrams to illustrate the differences between brachysclereids, astrosclereids and fibers (cross and longitudinal sections). Label the secondary cell wall, lumen and pits.

Seed coats often consist of several layers of sclereids. Look at the prepared slide of *Phaseolus* (bean) seed and see how many different types of sclereids you can recognize in the seed coat. Also place a drop of macerated bean seed coat on a slide and examine it to see separated sclereids. The rectangular sclereids are **macrosclereids** and the sclereids that are shaped like dog bones are **osteosclereids.**

Card 4-8: Draw a diagram of bean seed coat and label macrosclereids and osteosclereids.

LAB 6 – EPIDERMIS

Introduction

The **epidermis**, a **complex tissue** composed of several types of cells, forms a barrier between a plant and its external environment. The epidermis is usually just one cell layer thick and forms when protoderm cells derived from the apical meristems differentiate. Its functions are diverse including desiccation resistance, gas exchange, and protection against herbivores and pathogens.

Part 1 - Cell types of the epidermis

Pavement cells fit tightly together and secrete a water-repellent cuticle that reduces water loss and pathogen invasion. **Stomatal pores** are required for uptake of carbon dioxide in photosynthetic tissues and their apertures are regulated by **guard cells**. Other cells of the epidermis may be specialized as hairs or **trichomes**. Prepare an epidermal peel of a leaf from jade plant and identify the cell types.

Card 5-1: Draw a diagram of the peel as viewed under the compound microscope and label the cell types.

Part 2 - Pavement cells and cuticle

Pavement cells cover the surface of a plant and were named for their resemblance to paving stones used for garden paths. Pavement cells lack chloroplasts and are covered by a **cuticle** that may be very thick in xeric-adapted plant. Examine the prepared slide of *Yucca* leaf and identify pavement cells and cuticle.

Card 5-2: Draw a diagram of pavement cells with cuticle in *Yucca* leaf.

Part 3 - Stomatal complexes

Guard cells control the point of entry of carbon dioxide and the point of exit of water vapor in leaves and stems. In most plants, a pair of kidney-shaped guard cells surrounds the stomatal pore. In grasses the guard cells are dumbbell-shaped. In some cases the epidermal cells adjacent to the guard cells are distinct from ordinary epidermal cells and are termed **subsidiary cells**. A **stomatal complex** includes guard cells, stomatal pore and subsidiary cells. Prepare epidermal peels of jade plant and corn, mount them in water, and examine them with the compound microscope.

Card 5-3: Draw diagrams of stomatal complexes from jade plant and corn and label the component structures.

Part 4 – Trichomes and root hairs

Outgrowths of the epidermis called **trichomes** vary greatly in size and complexity. **Root hairs** are epidermal cells with simple outgrowths that absorb water and minerals from the soil. Corn

seedlings have been germinated in water to demonstrate root hairs. Make a wet mount and examine them with dissecting and compound microscopes.

Card 5-4: Draw a diagram of a corn seedling with root hairs.

Plants have a wide variety of specialized trichomes. Examine the leaf surfaces of the plants provided with the dissecting microscope. Then make epidermal peels to examine with the compound microscope. Trichomes on leaves and stems may reduce water loss and/or reduce herbivory. The unbranched trichomes of geranium are straight and those of bean are hooked. Compare the velvety feel of geranium leaf with the sticky feel of bean leaf. Some plants, such as *Eleagnus* (Russian olive), have elaborate branched trichomes.

Card 5-5: Draw diagrams to distinguish between the following types of trichomes: straight, hooked, or branched.

Trichomes may also secrete a variety of compounds. Examples of plants with **secretory trichomes** include tobacco, *Drosera* (sundew), *Dionea* (Venus fly trap), and *Limonium* (sea lavender). Can you identify the functions of these trichomes?

Card 5-6: Draw diagrams to illustrate the trichomes of tobacco, sundew, Venus fly trap and sea lavendar. On the back of the card, describe the functions of each structure.

Nectaries secrete sugary solution that attract and reward pollinators.

Card 5-7: Draw a diagram to illustrate the nectaries of honeysuckle flower.

LAB 7 – VASCULAR TISSUES

Introduction

The **vascular tissues** (**xylem** and **phloem**) are complex tissues that may contain parenchyma cells and fibers in addition to conducting cells. Both tissues occur together in bundles that are continuous throughout the plant. This exercise focuses on the cell-types and organization of the xylem and phloem.

Part 1 - Cell types of the xylem

The water conducting cells of the xylem are know collectively as **tracheary elements**.
In addition to tracheary elements, the xylem of most plants contains **parenchyma cells** and **fibers**. Examine a prepared slide of *Sambucus* (elderberry) stem. Identify the xylem tissue and its component cell types. What characteristics distinguish these cell types?

Card 6-1: Draw a diagram of *Sambucus* xylem and label the cell types.

Part 2 - Development of primary xylem

Tracheary elements are characterized by intricately patterned lignified secondary cell walls that are necessary to prevent collapse of the conducting tubes. Remember that lignified secondary cell walls are rigid. As a result the tracheary elements that mature in elongating tissues (**protoxylem**) deposit secondary cell walls in patterns that still allow the cells to stretch. Tracheary elements that mature after elongation has ceased (**metaxylem**) deposit secondary cell walls in less extensible patterns.

Separate a few vascular bundles from boiled petioles of celery, stain with toluidine blue, and *squash* them between a slide and cover slip. Identify tracheary elements with **annular**, **spiral**, **reticulate** and **pitted** secondary cell wall patterns.

Card 6-2: Draw tracheary elements representing each type of secondary cell wall pattern. Distinguish between protoxylem and metaxylem.

The relationship between tissue expansion and the pattern of secondary cell wall thickenings is also evident in leaves. Examine a prepared slide of a cleared leaf. Note that tracheary elements in leaves have annular and spiral thickenings and that some tracheary elements show evidence of having stretched. What does this say about the relationship between tissue expansion and xylem differentiation?

As a model for the effect of tissue elongation on xylem vessels, you can stretch the vascular bundles of banana or dogwood leaf. Break a piece of leaf perpendicular to a vein and gently pull the pieces apart. Mount the resulting *threads* in water and examine with the compound microscope.

Card 6-3: Draw labeled diagrams of protoxylem tracheary elements before and after they have been stretched.

Part 3 – Functional specialization of xylem cell types

Tracheary elements can be further subdivided into **vessel members,** which are interconnected by open **perforation plates** and **tracheids**, which are connected by **circular bordered pits**. The open perforations of vessel members allow for free flow of water. However, the circular bordered pits of tracheids prevent the spread of embolisms, which can develop due to water stress. As a result, the xylem of many plants contains both vessel members and tracheids.

Fibers in the xylem resemble the fibers in sclerenchyma tissue examined in lab 6 in that they are long, thin, and have a thick, lignified secondary cell wall. However, they differ in pit structure and evolutionary origin. The fibers in the xylem evolved from tracheids and their slit-shaped pits are modified circular bordered pits. **Fiber-tracheids**, also found in the xylem, can be distinguished from fibers and tracheids by the structure of their pits, which is intermediate between a slit pit and a circular bordered pit.

You can gain an appreciation for the diversity of xylem cell types by examining macerations of wood from *Quercus* (oak).

Card 6-4: Draw diagrams of the following cell types: **tracheids**, **vessel members**, **fibers** and **fiber tracheids**, and label the following structures: **simple perforation plates**, **circular bordered pits** and **slit pits**. What are the adaptive advantages of the different types of pits and perforation plates?

Part 4 - Cell types of the phloem

Sieve tube members are the sugar-conducting cells of the phloem in angiosperms. Sap flows between sieve tube members through **sieve plate pores** (modified plasmodesmata). Sieve tube members occur in files, have terminal **sieve plates**, lack nuclei, and are closely associated with **companion cells**, which play an important role in phloem loading. Parenchyma cells and fibers are also common in phloem tissue. The materials carried by the phloem are precious, and plants have evolved elaborate mechanisms to prevent leakage resulting from injury. The **callose** and **p-protein** that you will see plugging the sieve plate pores formed when the plants were prepared for sectioning.

Sieve tube members and companion cells are relatively easy to recognize in *Zea mays* (corn) stem. After locating a vascular bundle in the cross section, find the cluster of thin-walled unlignified cells. The sieve tube members are large and look empty. The companion cells are smaller with prominent nuclei. The surrounding lignified cells are fibers. Can you find sieve tube members and companion cells in the longitudinal section?

Card 6-5: Draw diagrams from cross and longitudinal sections to illustrate the types of cells found in *Zea mays* phloem. Label each cell type and the characteristics that distinguish them.

The stems of *Cucurbita* (squash) contain large sieve tube members in phloem bundles that occur both on the outside *and* on the inside of the xylem (this relatively unusual arrangement is called a bicollateral bundle). Examine cross sections of *Cucurbita* stem and identify sieve plates and red-staining **p-protein**, which blocks the sieve plates following injury. Examine longitudinal sections of *Cucurbita* phloem. Sieve tube members can be recognized by their sieve plates and p-protein plugs. Also identify companion cells.

Card 6-6: Draw diagrams of *Cucurbita* sieve tube members in cross section and in longitudinal section. Label sieve plates, sieve-plate pores and p-protein.

Part 2 - Callose

Like p-protein, callose prevents leakage of phloem contents when a plant is injured by insects or grazing animals. Callose can be detected using aniline blue and fluorescence microscopy or aniline blue/IKI and bright-field microscopy. Stain sections of squash stem as directed and examine for callose using the microscopes available.

Card 6-7: Draw a diagram of squash sieve tube members stained with aniline blue/IKI. Label sieve plates, sieve plate pores and callose.

LAB 8 - ANATOMY OF STEMS

Introduction

This begins a series of three labs in which you will explore the structure of stems, leaves and roots. In addition to learning about the general organization of tissues, you will explore how this organization varies among different plants. For example, plants representing the two major evolutionary lines of flowering plants, monocots and eudicots, differ markedly in the organization of tissues in stems, leaves and roots. Review our lecture notes for a summary of these differences.

Stems support photosynthetic leaves and reproductive structures above the substrate, thus increasing photosynthetic and reproductive efficiency. In addition, stems supply water and minerals to shoots via the xylem and photosynthate to roots via the phloem. In this lab exercise you will examine the general structure of stems in a variety of different types of plants.

Part 1 - Organization of the dicot stem

Examine the prepared slide of *Helianthus* (dicot) stem x.s. and identify the following tissues and regions: epidermis, ground tissue (parenchyma, collenchyma, sclerenchyma, cortex, pith), vascular tissue (xylem, phloem).

Card 7-1: Draw a diagram of *Helianthus* stem and label the tissues.

Part 2 - Vascular bundles

Vascular bundles contain both xylem and phloem, but these tissues are arranged differently within the stem bundles of different plants.

Card 7-2: Draw a single stem vascular bundle from each of the following plants and label protoxylem, metaxylem, phloem, fibers and procambium (if present): *Helianthus*, collateral bundles), *Zea mays* (corn, collateral bundles), *Cucurbita* (pumpkin, bicollateral bundles),

Part 3 - Steles

The arrangement of vascular bundles in a stem or root is know as the **stele**. Examine stem cross sections from the following species as examples of stelar types. If possible, note the position of the protoxylem (internal, medial or external). Then, construct a DICOTOMOUS KEY that could be used to classify the different types of steles.

Protostele - *Psilotum* (primitive vascular plant)
Siphonostele - *Adiantum* (a fern)
Dissected siphonostele - *Polypodium* (a fern)
Eustele - *Helianthus* (a dicot)
Atactostele - *Zea mays* (a monocot)

Card 7-3: KEY

A.

AA

B.

BB.

C.

CC.

D.

DD.

Part 3 - Nodal anatomy

Nodes are where vascular bundles leave the stem to enter leaves. Study the nodal anatomy of *Pelargonium* and *Perilla* by mounting a portion of a stem containing a node upside-down in clay under the dissecting microscope. Serial-section the stem with a razor blade and note changes in the stele. How do differences in the two plants relate to the observation that *Pelargonium* has large stipules? Identify **leaf gaps**, **leaf traces, axial bundles** and the **phyllotaxy** for each.

Card 7-4: Choose *Pelargonium* or *Perilla* and draw a diagram to illustrate a three dimensional reconstruction of your serial sections. Label your drawing.

Examine a prepared slide of *Adiantum* stem and identify leaf gaps and leaf traces. In what way does the structure of leaf gaps in a siphonostele differ from the leaf gaps in a eustele? Examine a prepared slide of a *Zea* node and note the numerous leaf traces that enter the sheathing leaves.

Part 4 - Environmental adaptation of stems

Plants that are adapted to dry or xeric environments are called **xerophytes**, those adapted to aquatic environments are called **hydrophytes,** and those adapted to more moderate conditions are **mesophytes**. **Halophytes** are adapted to saline conditions and share many characteristic with xerophytes and also tend to be succulent. Adaptive variations include: stomatal location, amount of intercellular air space (i.e. aerenchyma) and amount of sclerenchyma. Some xerophytes have very small leaves and photosynthetic stems.

Card 7-5: Identify the adaptive features associated with the anatomy of the following stems and prepare labeled drawings: *Ephedra* (**xerophyte**), *Salicornia* (**halophyte**) *Myriophyllum* (**hydrophyte**).

LAB 9 - ANATOMY OF LEAVES

Introduction

Leaves are the primary photosynthetic structures in most plants. In this lab you will learn about the structure of leaves and their development following formation of leaf primordia at the shoot apical meristem.

Part 1 - Leaf anatomy in eudicots, monocots, and conifers

Eudicot leaves typically have **netted venation** and may be simple or compound. In addition, most are **bifacial**. Examine the examples provided and identify the **base**, **stipules**, **petiole** and **lamina**. Examine the cross section of *Ligustrum* (privet) leaf and identify: **midvein**, **upper** and **lower epidermis**, **palisade** and **spongy mesophyll**, **vascular tissue** (**xylem**, **phloem**), and **bundle sheath**. How can you tell the **adaxial** from the **abaxial** surface? Now look at the **paradermal section** and identify the same tissues. Pay particular attention to the vascular bundles, which you can now see in longitudinal section, and the difference in packing of spongy and palisade mesophyll. Which type of mesophyll has the greatest volume of intercellular spaces per total volume? Which has the greatest free surface area per total volume?

Card 8-1: Draw diagrams of *Ligustrum* leaf in cross and paradermal sections. Label the tissues and structures in boldface type above. On the back of the card, describe the differences between palisade and spongy mesophyll.

Monocot leaves are characterized by **parallel venation** and sheathing leaf bases. Examine the examples provided and identify the **sheath**, **ligules** and **lamina**. Examine the prepared slide of *Zea* (corn) leaf and identify the different tissues as you did for the eudicot leaf. Is the distinction between palisade and spongy mesophyll obvious? How does this relate to the orientation of these leaves on the plant? What is the function of the enlarged bundles sheath cells in this plant? Compare leaf anatomy of *Zea* (C4) with that of *Triticum* (wheat, C3). How do the bundle sheath cells differ? What functional difference is related to this structural difference?

Card 8-2: Draw diagrams of *Triticum* leaf and *Zea* leaf in cross section. Label tissues and structures as you did for *Ligustrum* leaf. On the back of the card, describe the structure and function of bundle sheath cells in C3 and C4 grasses.

The leaves of most conifers (gymnosperms) are non-deciduous and therefore tough and leathery. Examine the examples of conifer leaves. Now examine the prepared slide of pine leaf cross section. Identify as many tissues as you can, including the single **vein**. Pine leaves exhibit a variety of adaptations such as: (1) **sunken stomata**, (2) **isobilateral symmetry**, (3) an **endodermis** and (4**) resin ducts**. Identify these adaptations and think about the functions of each. Compare the anatomy of *Pinus* leaf with that of *Taxus*. How do differences in anatomy relate to the orientation of these leaves on the plant?

Card 8-3: Draw diagrams of *Pinus* leaf and *Taxus* leaf in cross section. Label tissues and structures, as well as special adaptations in boldface type above. On the back of the card, describe how these specializations help protect the leaves against winter conditions.

Varying stomatal position is one way that plants have adapted to increase water use efficiency. Many leaves like those of *Ligustrum* (card 8-1) are **hypostomatic** with all or most stomata on the shaded lower surface. **Sunken stomata**, as seen in *Pinus* (card 8-3) and **stomatal crypts**, as seen in the prepared slide of *Nerium* (oleander) leaf, further reduce water loss. Examine the prepared slide of *Zea* (corn) leaf to see an example of **amphistomatic** position, in which stomata occur on both surfaces of the leaf. Floating leaves, such as those of water lily (*Nymphaea*) are **epistomatic** with stomata on the upper surface.

Card 8-4: Draw diagrams to illustrate differences in stomatal position in *Ligustrum*, *Pinus*, *Nerium*, *Zea* and *Nymphaea* leaves. On the back of the card, describe the relationship between stomatal arrangement and leaf orientation in *Ligustrum*, *Zea* and *Nymphaea* leaves.

Part 2 - Leaf development

The development of a typical dicot leaf is illustrated in the slides of *Syringa* (lilac) leaf buds, in which several stages in the development of leaf primordia and young leaves can be viewed in cross section. Can you tell at which stage vascularization occurs? What changes can you note as you look at progressively older leaves? Compare the prepared slides of young *Syringa* leaf with that of mature *Syringa* leaf. Pay particular attention to the amount of air space in each tissue. What developmental scenario could lead to the arrangement of air spaces seen in mature leaves?

Card 8-5: Draw diagrams of several stages in the development of *Syringa* leaf. Label the tissues as they become distinct. On the back of the card, describe the process through which air spaces form in the spongy mesophyll.

Monocot leaves have a prolonged period of development during which they elongate from a **basal meristem**. Examine the prepared slide of *Zea* node. Can you identify the basal meristem in the leaves? How does vascular tissue in the leaf become connected to that in the stem? Can you see how the basal meristem forms parallel veins?

Card 8-6: Draw a diagram of a *Zea* node. Label the basal meristem and mature and developing vascular tissue.

The final stage in the development of deciduous leaves is **abscission**. This process is controlled in such a way as to minimize the vulnerability of the plant to pathogens and xylem cavitation. Examine the prepared slide of an abscission zone. Identify the **protective layers** and the **separation layer**. What other characteristics of the abscission zone have evolved to minimize injury?

Card 8-7: Draw a diagram of an abscission zone and label the protective and separation layers.

LAB 10 - ANATOMY OF ROOTS

Introduction:

Roots anchor plants in the soil and absorb and conduct water and nutrients. Lateral roots do not arise in a predetermined pattern as do lateral shoots. The site of lateral root formation is influenced by heterogeneity in the soil microenvironment, so root systems are highly variable. The anatomical structure of roots, however, is quite uniform. In addition to the absence of apically-derived lateral appendages, roots are distinguished from stems by the presence of a root cap and vascular organization. In this lab you will examine the general anatomy of roots and the origin of lateral and adventitious roots.

Part 1 - Generalized root anatomy and development

To get an idea of how the morphology of the root system differs in monocots and dicots, examine the seedlings of radish and barley that have been germinated in Petri dishes. **Tap root systems** are common in dicots. Identify the taproot on the radish seedling. **Fibrous root systems** are characteristic of monocots. Note the absence of a dominant taproot on the barley seedling. Also note where the roots on the barley seedling originate. Roots originating from stem tissue are **adventitious roots** (a.k.a. nodal roots, crown roots, prop roots). For both seedlings identify: **root cap**, **root hairs**, **embryonic root**, **lateral roots**. You may also detect the slimy **mucigel** secreted by the roots.

Card 9-1: Draw labeled diagrams to illustrate the differences between a tap root system and a fibrous root system.

Examine the prepared slides of mature and immature *Ranunculus* root cross section. Identify the following in the immature root: **epidermis**, **cortical parenchyma**, **endodermis**, **pericycle**, **phloem**, **protoxylem**. Now examine the mature root and identify the same tissues plus **metaxylem**. The differences between the mature and immature roots are most obvious in the xylem, endodermis and cortex. What changes occur in the endodermis and what is the functional significance of these changes? What type of stele does this root have?

Card 9-2: Draw diagrams of immature and mature *Ranunculus* roots. Label the tissues. On the back of the card, describe the changes that occur as the root matures.

The following examples illustrate some of the common anatomical variations among angiosperm primary roots:

Zea mays - **Polyarch** stele with a parenchymatous pith. Note the alternating protoxylem and phloem and the large metaxylem elements. This type of stele is interpreted as a **protostele** in which the central xylem differentiated as parenchyma. Unlike stem pith, which differentiates from ground meristem, this root pith differentiates from procambium. This is a common pattern in monocots, especially those with large diameter roots.

Smilax (monocot) - Polyarch stele with central xylem. Roots of this plant have highly sclerified endodermis. Note the crystal cells in the outer cortex.

Psilotum stem - just to remind you of the similarity between the stem anatomy of this primitive, rootless plant and that of angiosperm roots. Can you identify the endodermis?

Card 9-3: Draw diagrams of *Zea* root and *Smilax* root and label the tissues. On the back of the card, explain variations in the organization of protosteles as seen in *Ranunculus* root, *Zea* root, *Smilax* root, and *Psilotum* stem.

Part 2 - Development of root systems

Examine a prepared slide of *Pistia* or *Salix* root with developing lateral (branch) roots. Where do the meristematic cells appear to be located? Identify the vascular connection between the lateral root and the stele of the primary root. Can you see a root cap? What happens to epidermal, cortical and endodermal tissue during this process?

Card 9-4: Draw a labeled diagram of a developing lateral root. On the back of the card, explain the process of lateral root development.

Make a free-hand section of a *Zebrina* node such that you section an **adventitious root** longitudinally. From what tissue was the adventitious root derived. How do vascular connections become established? Adventitious root development can be seen in cross sections of *Lycopersicon* (tomato) stem. How does the origin of adventitious roots compare with that of branch roots?

Card 9-5: Draw a labeled diagram of a developing adventitious root. On the back of the card, explain the process of adventitious root development and how it differs from lateral root development.

Part 3 – Specialized roots

Card 9-6: Describe the functional adaptations associated with the anatomy of the following roots. Which tissues have become altered as a result of the adaptations?

Aerial roots – Section the aerial roots of orchid. The multiple-layered epidermis (velamen) has peculiarly thickened walls.

Storage roots – Section the storage root of *Daucus* (carrot). Which tissues function in storage?

Nodules - The symbiotic relationship between the bacterium *Rhizobium* and plants in the legume family form nodules that function in nitrogen fixation. Section the nodules and examine with the compound microscope.

Mycorrhizae - Examine the examples of symbiotic associations. Can you tell which are **ectomycorrhizae** and which are **endomycorrhizae**?

LAB 11 - VASCULAR CAMBIUM

Introduction

Many small, herbaceous annuals have no secondary growth at all. However, plants that grow large or persist for several years require a larger stem diameter for support, increased amounts of vascular tissue to supply greater numbers of branches and leaves, and a means to replace vascular tissues that cavitate due to winter freezing. Some monocots are large and perennial, even without secondary growth. These plants have adapted specialized strategies for increasing their girth and vascular supply. However, dicots and gymnosperms undergo secondary growth through activation of a secondary meristem called the **vascular cambium**.

The most obvious function of the vascular cambium is to produce **secondary xylem** and **secondary phloem**. The vascular cambium arises through division of cells of the **residual procambium** and ground tissues and forms a cylinder that separates **primary xylem** from **primary phloem**. Cells of the vascular cambium divide **periclinally** to produce the secondary vascular tissues and **anticlinally** as required to keep up with the increasing girth of the stem or root. At the end of the growing season the vascular cambium may go dormant only to be reactivated in following seasons. In this lab you will see how vascular cambium is initiated in stems and roots and examine the organization of cambial initials.

Part 1 - Initiation of the vascular cambium in stems

First examine a prepared slide of a mature stem of *Ranunculus*, an annual eudicot with no secondary growth. Can you identify undifferentiated tissues between the xylem and phloem? Notice also the fibers that completely surround the vascular bundles.

Next look at the prepared slide of *Helianthus* (sunflower) stem with separate bundles. Can you identify radially layered cells between the xylem and phloem that appear to have divided recently? Can you identify similar cells among the parenchyma between the vascular bundles? This is where the **fascicular cambium** and **interfascicular cambium** are initiated.

Card 10-1: Prepare a drawing that illustrates the differences in stem vascular bundles between a plant without secondary growth (*Ranunculus*) and a plant with secondary growth (*Helianthus*). Label fascicular cambium and interfascicular cambium.

Now examine a prepared slide of an old *Helianthus* stem and notice the changes that have taken place. Identify the cylindrical **vascular cambium**, which formed as the fascicular and interfascicular cambia merged. Also identify **pith**, **primary xylem**, **secondary xylem**, **secondary phloem** and **primary phloem**.

The slide containing sections of *Sambucus* stem shows more extensive development of secondary vascular tissues. Identify **pith**, **primary xylem**, **primary phloem**, **xylem rays**, **phloem rays**, **axial elements** of the **secondary xylem** and **secondary phloem**, and **vascular cambium**.

Card 10-2: Draw a diagram of *Sambucus* stem with secondary growth and label the tissues shown in boldface type above.

Part 2 - Initiation of the vascular cambium in roots

Examine the prepared slides of two stages in the development of *Pyrus* (pear) roots. First note that *Pyrus* is unusual in have two endodermal layers, on of which accumulates phenolics. Can you identify where the vascular cambium arises? Label **secondary xylem**, **secondary phloem** and **vascular cambium**.

Card 10-3: Draw diagrams of *Pyrus* root with early secondary growth and mature *Pyrus* root. Label the tissues and show where the vascular cambium originates.

Part 3 - Structure of cambial initials

Examine prepared slides of vascular cambium from *Robinia* (black locust) and *Juglans* (walnut). These are planar sections of a cylindrical cambium, so each section contains differentiating secondary xylem and phloem as well as cambial initials. Look for patches of cells that lack the features of tracheary elements or sieve tube members. Identify **fusiform initials** and **ray initials**. What cells do each type of initials give rise to? What differences do you note in the arrangement of cambial initials in the different species?

Card 10-4: Draw diagrams of the cambium of *Robinia* and *Juglans*.

Part 4 - Woody stems and roots

Although cambium initiation differs in roots and stems, the resulting secondary vascular tissues are strikingly similar. Examine prepared slides of wood stems and roots of *Tilia*.

Card 10-5: Draw diagrams of a woody root and a woody stem of *Tilia*. Label similarities and differences.

LAB 12 - SECONDARY GROWTH

Introduction

What most people call "wood", plant anatomists know as **secondary xylem**. Secondary xylem has the same cell types and the same functions as primary xylem. However, secondary xylem develops from the vascular cambium and its organization is different from that of primary xylem. Specifically, secondary xylem consists of an axial system that develops from fusiform initials and a radial system that develops from ray initials.

The outer part of a woody stem or root is commonly known as **bark**, but actually consists of two distinct tissues, the **secondary phloem** and the **periderm**. As the diameter of a woody stem increases, the bark must expand radially to accommodate the enlarged circumference of the wood. The developmental processes that accomplish this radial expansion are revealed in the cellular organization of the secondary phloem Secondary phloem, like the secondary xylem, arises from the vascular cambium and consists of an axial system and a ray system. The periderm arises from a meristem known as the **cork cambium**.

Part 1 - Structure of woody stems

Examine the wood blocks provided and identify: **axial elements**, **ray elements**, **annual rings** and **bark**. You cannot see the vascular cambium without a microscope, but you should be able to tell where it is.

You should be familiar with **cross sections** by now. A longitudinal section of wood cut on a radius is called a **radial section** and contains rays sectioned longitudinally. A longitudinal section of wood cut on a tangent is a **tangential section** and shows rays in cross-section. Using wood blocks and the diagram on page 38, make sure you understand the different types of sections.

Part 2 - Secondary xylem

Examine the prepared slide of pine wood that includes cross, radial and tangential sections.

Card 11-1: Draw diagrams of pine wood in cross, radial and tangential section. Label axial elements, ray elements and annual rings.

Phylogeny, environmental pressures, and its origin in the vascular cambium influence the structure of wood. Keep these factors in mind as you examine the anatomy of different types of wood. The following is a list of general characteristics to look for when examining wood anatomy:

Axial system:
1. Cell types present - tracheids, vessel members, fibers, parenchyma.
2. Characteristics of vessels (if present) - perforation plate type, diameter, length.
3. Arrangement of vessels -**ring porous** or **diffuse porous**

Ray system:
1. Structure
    Uniseriate (consisting of a single file of cells)
    Multiseriate (consisting of two or more files of cells)
2. Cellular composition
    Homocellular (consisting of one cell type)
    Heterocellular (consisting of two or more cell types, e.g. tracheids, parenchyma, upright cells, procumbent cells)

Thin sections of wood provide different kinds of information depending on the plane in which they are cut. Examine cross, radial and tangential (XRT) sections and macerations of the following woods. Copy the chart on page 39 and fill in the characteristics of each on the included chart.

Pinus (gymnosperm)
Magnolia or *Liriodendron* (angiosperms)
Quercus (angiosperms)

If you have time to look at others, choose from: *Tilia, Chamaecyparus, Acer,* or *Juglans.*

Part 3 - Secondary phloem

The secondary phloem functions in both transport and protection and this dual function is reflected in the cell types present. Examine a cross section of *Tilia* stem and identify the following cell types: phloem fibers, sieve tube members, companion cells, axial parenchyma, and ray parenchyma. Now look carefully at the phloem.

Card 11-2: Draw a diagram of the secondary phloem of *Tilia* and label the cell types. One the back of the card explain: 1) the relationship between xylem rays and phloem rays, and 2) the role of phloem rays in radial expansion of the phloem.

Part 4 - Cork cambium and periderm

The **cork cambium** can develop from cells of the epidermis, cortex, primary phloem or secondary phloem. This meristem produces **cork**, which replace the function of the epidermis in the growing stem. Examine the development of cork cambium by comparing prepared slides of *Sambucus* stem with and without cork. Look for dividing cells just below the epidermis. Notice that the developing cork cells are arranged in radial files that do not coincide with the epidermal cells.

Card 11-3: Draw labeled diagrams to illustrate the development of cork cambium and cork in *Sambucus*.

Gases must be able to penetrate the cork to reach growing cells below. This is accomplished by **lenticels**, which are clusters of loosely-packed cells produced by the cork cambium. Look at the bark samples on display to see different types of lenticels. Now, examine the prepared slide of a *Sambucus* lenticel.

Card 11-4: Prepare a labeled drawing of a *Sambucus* lenticel.

The texture of bark (e.g. smooth, scaly, fibrous) depends on the structure of the cork cambium. Examine the bark samples on display and try to envision the organization of the cork cambium that produced each type.

WOOD CHARACTERISTICS CHART

	Pinus	*Magnolia*	*Quercus*
Axial cell types			
Vessel types			
Vessel arrangement			
Ray structure			
Ray cellular composition			

PLANES OF SECTION IN WOOD

Illustrations of cross (X), radial (R) and tangential (T) sections of wood.
(From: R. H. Holman and W. W. Robbins, **A Textbook of General Botany**, Wiley and Sons, Inc., New York, 1924.