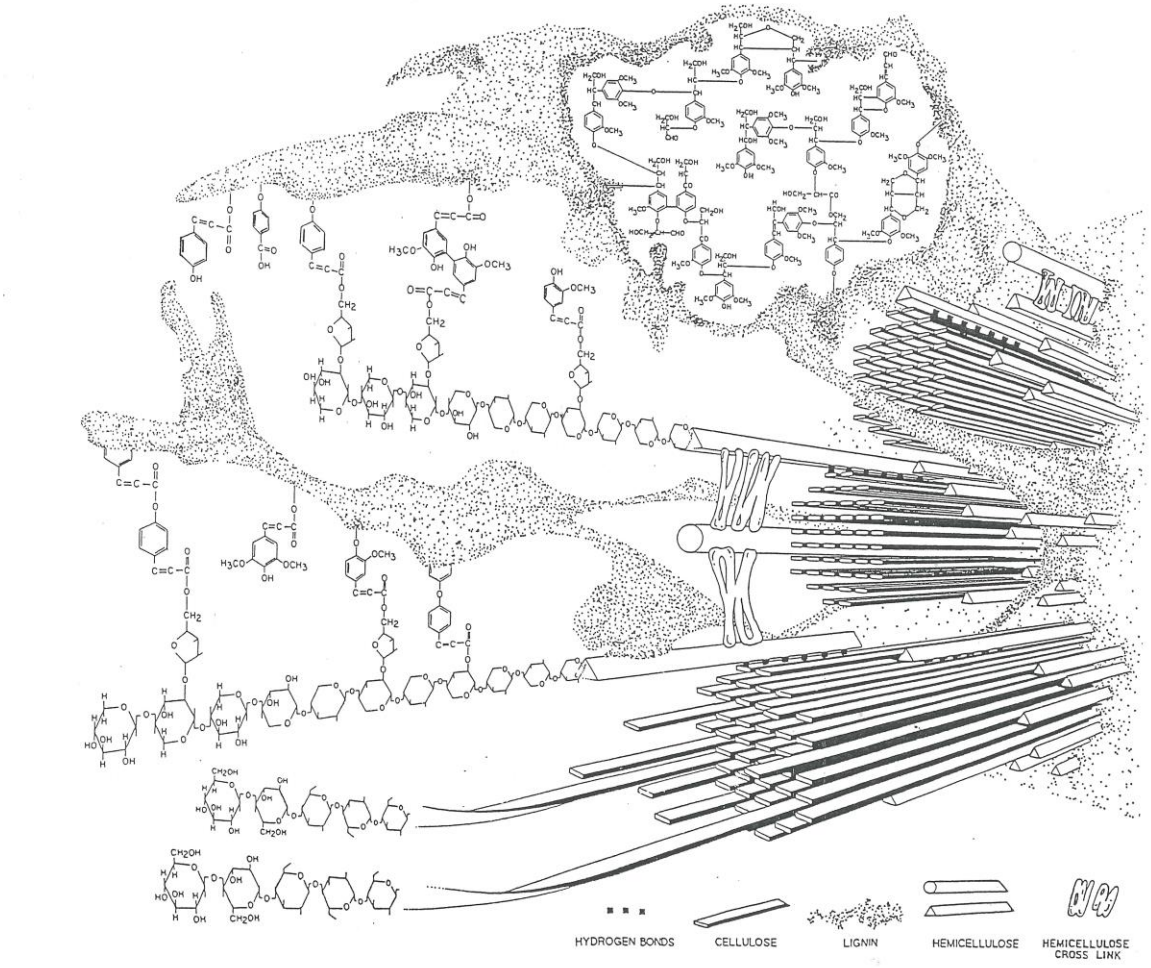
LABORATORY GUIDE TO PLANT ANATOMY

FALL 2022: Nineteenth Edition



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PROPRIETARY STATEMENT

PREFACE

This laboratory manual is designed to accompany a one-semester upper division college course in plant anatomy. Its purpose is to provide detailed descriptive analysis of the internal anatomy of seed plants, with emphasis on the angiosperms.

The exercises are intended to be largely self-guided. The student will study structural elements, often from more than one perspective, and generate labeled drawings in order to conceptualize the three-dimensional arrangement of cells and tissues. Use of colored pencils and color-keying is strongly suggested to enhance the clarity and continuity of drawings.

In these exercises, plant anatomy is approached as both a structural and functional science, and a recurring theme is the relationship between structural elements and their functions. By comparing and contrasting the arrangement of similar elements among major taxonomic groups, the student should also gain insight into patterns of development and differentiation, and come to appreciate the evolutionary significance of structural modifications.

The manual is divided into two sections. Section I (Exercises 1-3), offers an opportunity to review the basic concepts and terminology of plant anatomy, and provides a framework for the more detailed analysis in Section II (Exercises 4-16). A brief section on microscope use is included in Exercise 1 for the convenience of instructors who would like their students to review this essential skill.

An additional exercise (Exercise 17) has been added to this edition of *Laboratory Guide to Plant Anatomy* to provide students with experience in scanning electron microscopy. This investigation utilizes information from previous exercises to prepare and view trichomes using electron microscopy. Funding for this part of the course was provided by the National Science Foundation Instrumentation and Laboratory Improvement Program Project No. DUE-9551202.

The exercises are based largely on commercially available prepared slides, although many units also include live plant materials, models, or herbarium specimens. A complete listing of slides and other materials is provided in Appendix A.

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j.e.b.

ACKNOWLEDGEMENTS

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Many students have also helped to take photographs and import them into this Laboratory Guide. Among those originally involved, the authors acknowledge the work of Veronica Barabash, Michael Carpenter, Sara Cook, Justin Dowe, Camie Fertig, Russell Grafrath, Stacy Hauser, Hui-Ju Huang, Ling-Li Huang, Masaak Ikeda, Shang-Wen Liaw, Linda Luna, Brent Owens, Karen Randell, Malini Rao, George Richardson, Yu-Ying Shih, Parissa Solatpour, Mei-Chen Sung, Amy Tankersley, and Darla Wyatt. Many Plant Anatomy students have helped update this Laboratory Guide over the past decade, and they are truly appreciated for their contributions.

The authors gratefully acknowledge the guidance of Dr. Nels Lersten, whose laboratory exercises at Iowa State University provided both the inspiration and conceptual framework for this manual.

LABORATORY GUIDE TO PLANT ANATOMY

Section I

Section II

Exercise 1--The Microscope and Plant Cell Structure

Introduction

This exercise is devoted to a study of cells, the fundamental structural and functional units of the plant. Simple staining techniques are used to highlight the structure of typical epidermal cells. Careful observation will reveal cell wall configuration, the presence of organelles and inclusions, and the arrangement of cells within epidermal tissue.

Skilled use of the compound light microscope is essential in this exercise and throughout the investigations in this manual. The unit begins with a brief review of microscope technique.

Materials

For Wet Mounts:

Allium (onion) bulb
Elodea (pond weed) leaves
Gynura aurantiaca (Purple Velvet) or *Viola* (violet) leaves
Tradescantia zebrina (Wandering Jew) leaves

Prepared Slide:

Coleus leaf, x-section

The Microscope

Before beginning the exercises in this manual, review the following basic guidelines for microscope use and care.

Care and Handling. The compound microscope is a precision instrument, and should be handled carefully at all times. Always carry the microscope with both hands, one grasping the arm, the other supporting the base. Never force mechanical parts, and report any malfunction immediately to your instructor. When cleaning the ocular and objective lenses or condenser, use only clean dry lens paper. Paper towels or cloth will scratch the lenses. Store the microscope with its low power (4X or 10X) objective in viewing position.

Operation. With the low power objective in position, center the *Coleus* leaf x-section slide over the stage opening and, viewing from the side (**not** through the eyepiece), use the coarse adjustment knob to lower the lens to its lowest position. Then look through the eyepiece and raise the lens using the coarse

adjustment until the field comes into focus. Scan the slide at low power and select an area to be examined at higher magnification. Again viewing from one side, swing the high dry (40X) objective into position. Since most microscopes are parfocal, only minor adjustment with the fine focus should be required. Notice the very short focal length (distance from lens to stage) of the high dry objective lens. To prevent broken slides and damaged lenses, **never** use the coarse adjustment at high magnification.

Depth of focus is relatively shallow at high magnification. When viewing thick sections, repeatedly focus up and down in order to visualize the three-dimensional arrangement of cells. Open and close the iris diaphragm beneath the stage to regulate the amount of light transmitted through the opening. Usually, at higher magnification more light is required for optimum viewing. However, reduced light intensity often produces better contrast on thin or unstained sections.

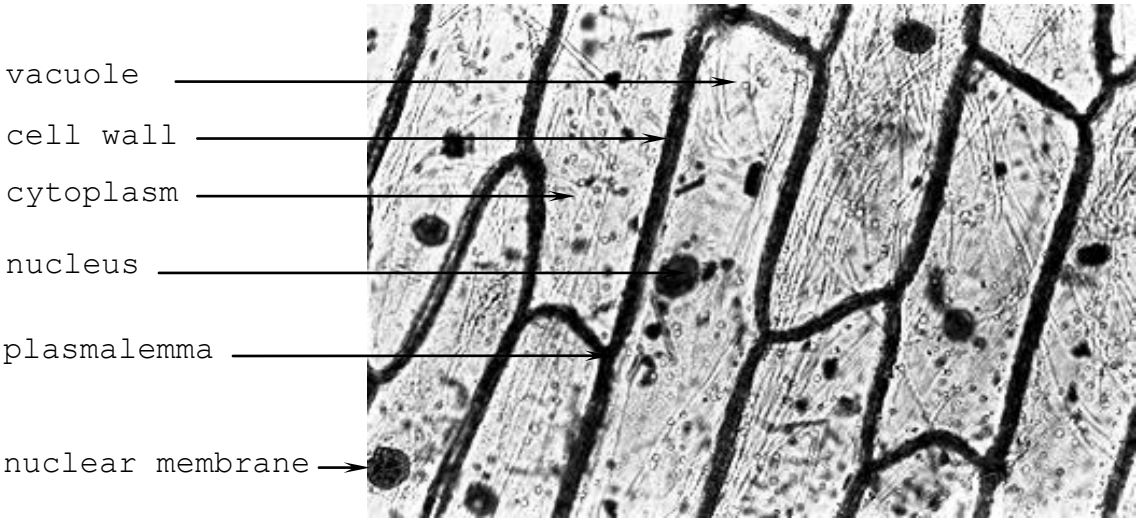
Keep in mind, the compound microscope produces an inverted image (upside down and reversed from left to right). Also, as magnification increases the field of view becomes smaller. Carefully center areas to be examined in detail before switching to the high dry objective. Total magnification is calculated by multiplying the power of the ocular (usually 10X) by that of the objective.

Cell Structure

1. Peel a small piece of filmy epidermis from the inside of a freshly cut onion ring and prepare a wet mount in water. The onion bulb is actually a compact collection of basal leaves adapted for storage. Reduce the light, and examine first under low magnification. Primary cell walls, made up largely of cellulose, appear as oblong "boxes."

Add a few drops of crystal violet and view under high magnification. Crystal violet is a basic stain which bonds well with the acidic contents of the nucleus and produces an even but less intense stain throughout the cytoplasm. Like most cells of higher plants, these epidermal cells are uninucleate. One or more intensely stained nucleoli should be visible within the nucleus. Nucleoli are believed to be the sites of ribosomal RNA synthesis. The cell membrane, or plasmalemma, is held tightly against the inner cell wall surface in fully turgid cells. At this magnification, neither the cell membrane nor nuclear membrane are resolved. A large vacuole occupies most of the cell's volume, as it does in many plant cells. The vacuole contains water and a variety of mineral and organic substances, and is involved in water uptake and growth, storage and digestion, and the maintenance of turgor.

Draw three or four adjoining cells and label to indicate the location of each underlined feature.



2. Mount a whole *Elodea* leaf in water, select an area near the leaf margin, and observe under high magnification. Cell walls appear as glassy lines in this unstained preparation. Again, a large central vacuole fills most of the cell. Cytoplasm is confined to the periphery and is densely packed with bright green disc-shaped chloroplasts. Look for cytoplasmic streaming, or cyclosis, easily observed as chloroplasts are carried along by the cytoplasmic flow. At one point the chloroplasts may appear to pile up, as if held back by an invisible barrier. Careful focusing with reduced light may reveal the nearly transparent nucleus at this location.

The middle lamella, although not well resolved, lies between the primary cell walls of adjacent cells, and is best visualized along corner junctions. It consists largely of cementing pectic substances which bind adjacent cells together.

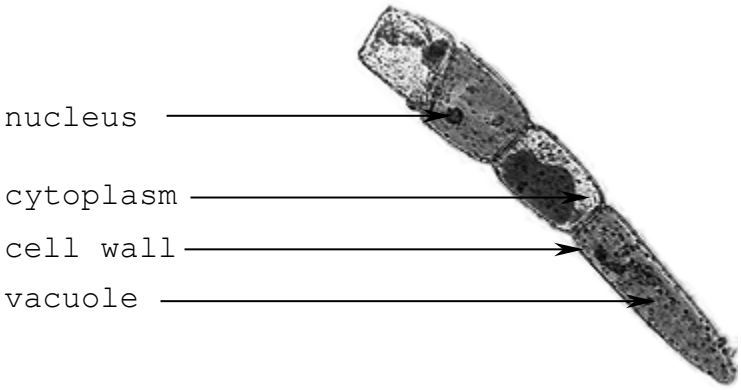
When mounted in tap water (a hypotonic solution), *Elodea* cells are fully turgid. The plasmalemma is therefore in close contact with the cell wall and nearly impossible to detect. Flood the preparation with a 5% NaCl solution (hypertonic), and observe the cells as they undergo plasmolysis. As water is lost, primarily from the vacuole, the plasmalemma shrinks away from the cell wall and the chloroplasts appear to be "rounded up" in the interior of the cell.

Draw two or three cells, and label to show the location of each underlined feature. Draw one cell showing the effects of plasmolysis.

Specialized Epidermal Cells

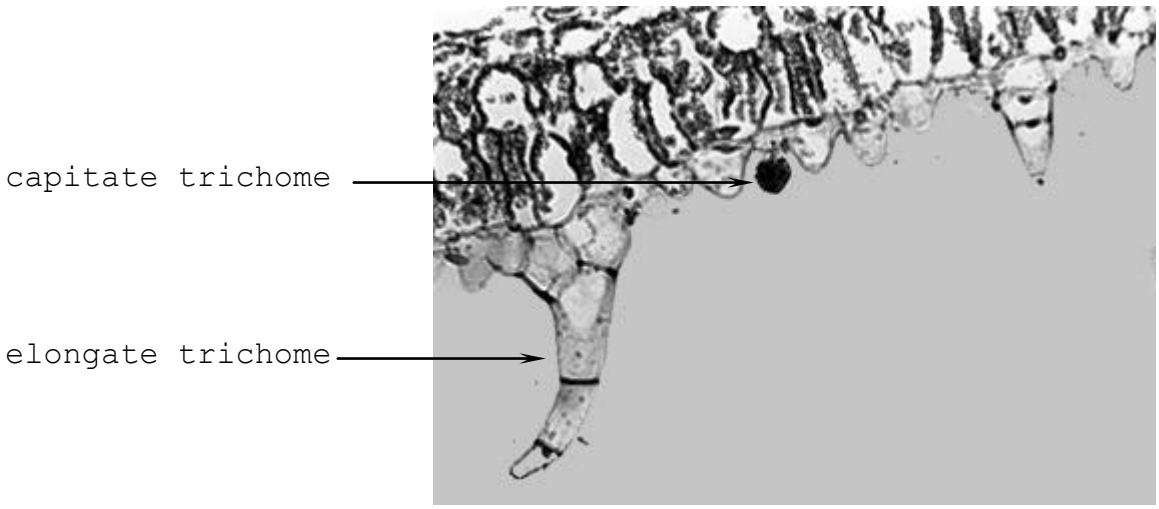
3. Gently scrap leaf hairs (trichomes) from a Purple Velvet or African Violet leaf and prepare a wet mount in water. Each trichome is made up of several modified epidermal cells. At high magnification, the cell wall, vacuole, cytoplasm, nucleus, and nucleolus can be visualized.

Draw one or two trichomes showing the arrangement of cells. To the right of this drawing, draw a single cell enlarged and labeled with the underlined features.



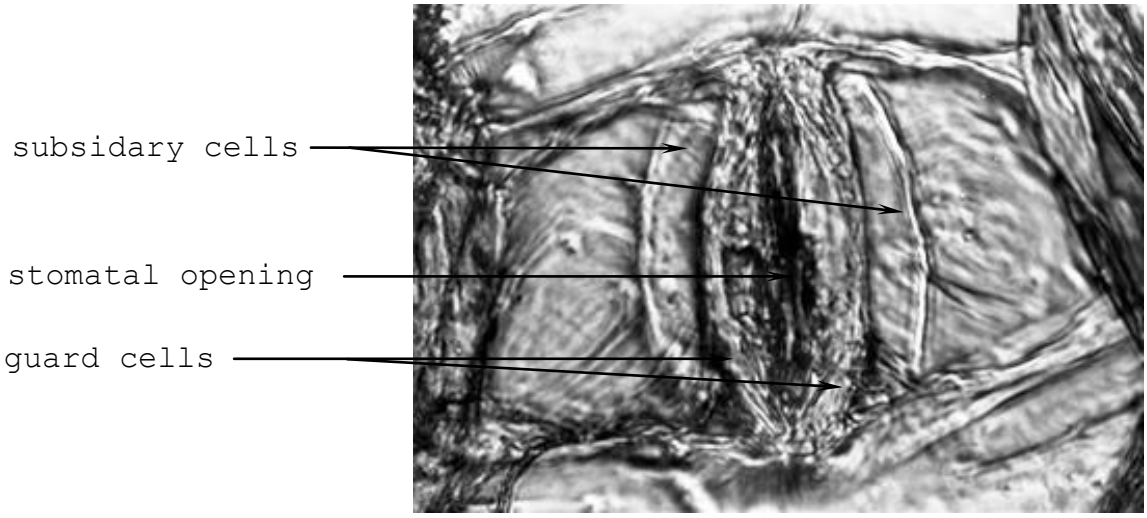
4. Examine the prepared *Coleus* leaf x-section at high magnification, and look for two types of trichomes. One is elongate and similar to the leaf hairs of Purple Velvet. The other is a smaller capitate form that functions in secretion.

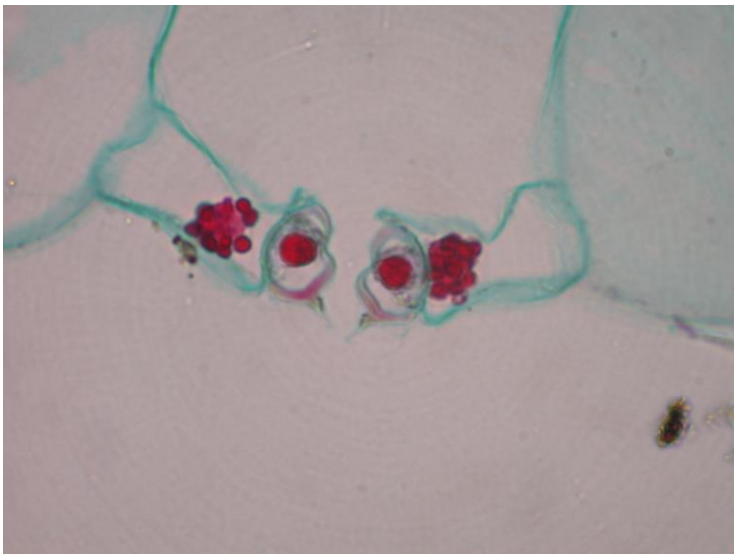
Draw a portion of *Coleus* leaf epidermis and label to show both types of trichomes.



5. Strip away a small portion of lower epidermis from a *Tradescantia zebrina* leaf, mount in water, and add a few drops of safranin stain. Examine at low magnification and observe the irregular contour of these cell walls. Safranin readily highlights these primary cell walls, due largely to the presence of cutin in the epidermis. Scan to locate scattered stomata, easily distinguished by the presence of chloroplasts in guard cells surrounding the stomatal opening. Guard cells are the only epidermal cells that contain chloroplasts. Examine a single stomatal complex at high magnification. In *Tradescantia*, each is composed of two guard cells (immediately enclosing the opening), adjacent subsidiary cells and the stomatal opening itself. Stomatal complexes function in gas exchange. Their structure is examined in more detail in Exercise 12 (Leaves).

Draw a section of leaf as it appears at low magnification, showing the overall arrangement of epidermal cells and stomata. Draw a single stomatal complex at high magnification, and label with the underlined terms.





Exercise 2--General Anatomy and Morphology

Introduction

Roots, stems, leaves and flowers, the major "organs" of higher plants, are remarkably specialized in form and function. Yet the plant body retains a fundamental structural unity. This exercise provides an overview of anatomy and morphology, a review of basic terms, and a framework for more detailed studies in Units 4-16.

Materials

Prepared Slides:

Zea mays (corn) root tip, long. section
Ranunculus (buttercup) root, x-section
Monocot and dicot stems, x-section
Syringa (lilac) leaf, x-section

Herbarium Specimens:

Ginkgo
Zea (corn)
Cercis (redbud)

Models and Live Materials:

Flower model, or fresh blooms if available
Generalized root tip model (optional)
Dormant woody stems

Roots

1. Look at the generalized root tip model, or view the long. section of *Zea mays* root tip. First locate the rootcap, which protects the growing root apex as it penetrates the soil. By convention, three general regions are often identified in the primary root apex. Just behind the root cap is the zone of active cell division, where new root and root cap cells are produced. At high magnification, look for cells in various stages of mitosis. In the region of elongation, cells enlarge and continue to divide.

In the region of maturation, cells begin to develop the form and function of mature root tissue, and epidermal cells produce

outgrowths called root hairs. Although useful for reference, division of the apex into these three regions is somewhat arbitrary, as events are highly intergraded from one region to the next.

Diagram a growing root tip, and label with the underlined terms.

2. Examine the prepared x-section of *Ranunculus* (dicot) root. This section illustrates the basic structural arrangement of mature primary roots. In both monocots and dicots, a cortex surrounds a central vascular cylinder, or stele. Beneath the outer epidermis, a broad layer of cortex functions as food storage tissue. The innermost cortical cells form the endodermis, a single layer of smaller thick-walled cells enclosing the stele. The endodermis controls movement of water and solutes into the vascular cylinder.

Among higher plants, the arrangement of xylem and phloem within the stele is highly variable. *Ranunculus* shows one common pattern for dicots, in which xylem (stained red on most preparations), is at the center of the stele. Bundles of phloem are near the periphery of the stele, between radiating arms of xylem. Between the vascular tissues and endodermis is an irregular region of thin-walled cells called the pericycle.

Detailed analysis of development and structural variation in monocot and dicot roots is found in Exercises 6 (Vascular Architecture), 7 (Primary Roots), and 9 (Primary Root and Stem Development).

Diagram the root x-section and label to show the location of each underlined feature.

Stems

3. Examine the dormant woody stems on display and identify the following external features. Buds and leaves arise along the stem at nodes. The area between nodes is an internode. Terminal buds produce stem elongation and lateral buds give rise to branches. At the base of each lateral bud is a leaf scar, formed at the point of leaf abscission. Each spring, scales covering the terminal bud drop off, creating a terminal bud scar. The distance between two terminal bud scars represents one year's growth. Look also for lenticels, small light or dark disruptions in the bark, which function in gas exchange.

Draw a segment of stem and label to show the underlined features.

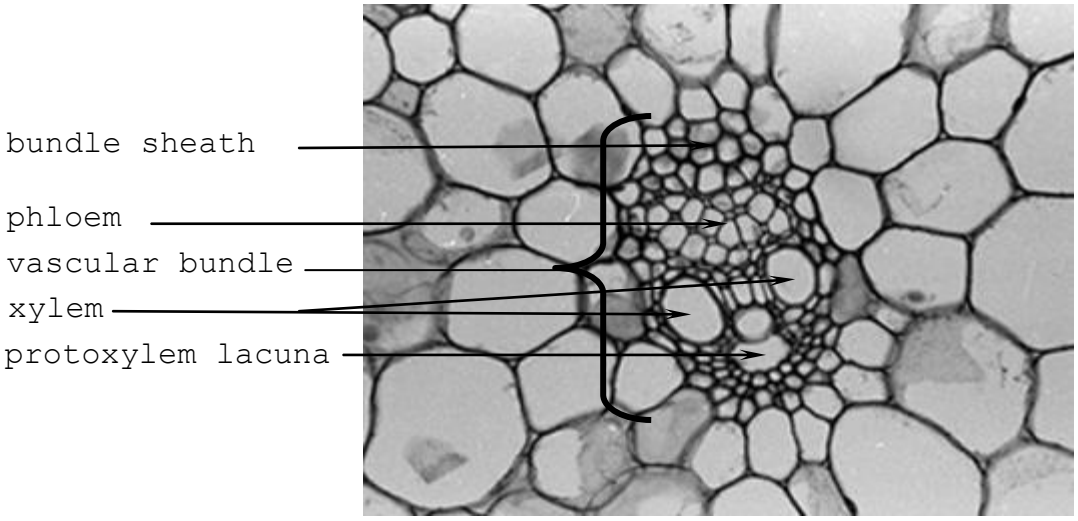
4. Examine the prepared slide showing x-sections of both monocot and dicot stems. These sections illustrate the basic architecture of herbaceous stems in the primary state of growth. At low magnification, locate the dicot stem, distinguished by its

ring-like arrangement of vascular bundles around a large central pith. The cortex lies outside the ring of vascular bundles and beneath the epidermis. Examine one vascular bundle at high magnification and note the arrangement of xylem and phloem, with xylem (large, usually red-stained cells) just interior to phloem (smaller, blue-stained cells)

Scan the monocot stem under low magnification and note the typical complex arrangement of vascular bundles. Within each vascular bundle, two or three large xylem cells and an air space, the protoxylem lacuna, are prominent. Smaller phloem cells are between the xylem and darkly-stained cells of the bundle sheath. Detailed analysis of angiosperm and gymnosperm stem anatomy is found in Exercises 6 (Vascular Architecture), 8 (Primary Stems), 9 (Primary Root and Stem Development), 10 (Secondary Growth) and 11 (Wood Anatomy).

Draw dicot and monocot stem x-sections, showing the arrangement of vascular bundles in each, and label with the appropriate underlined terms.

Monocot:



Leaves

5. Examine leaf venation patterns on *Ginkgo*, *Zea*, and *Cercis* herbarium specimens. *Ginkgo*, a deciduous gymnosperm, has numerous dichotomously branching veins. This open-dichotomous pattern, with no minor veins, is considered more primitive than patterns found in most monocots and dicots. *Zea* shows the parallelodromous pattern typical of monocots, in which veins are nearly parallel and connected by commissural bundles. *Cercis*, a dicot, shows typical craspedodromous reticulate pattern, with vein endings.

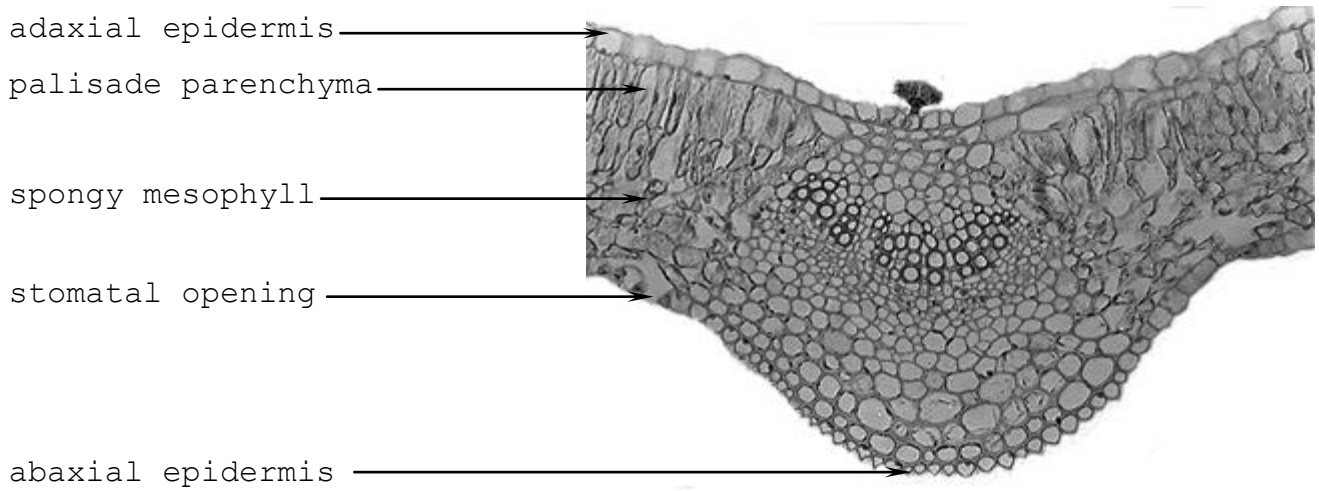
Draw *Ginkgo, Zea*, and *Cercis* leaves and label with the appropriate venation pattern.

6. Examine the prepared slide of *Syringa* (dicot) leaf x-section, and locate the following general features. Between the upper (adaxial) and lower (abaxial) epidermis is mesophyll, the primary site of photosynthesis. In *Syringa* and many other dicots, the mesophyll is differentiated into two layers: elongated densely packed palisade cells, just beneath and perpendicular to the upper epidermis; and irregular, loosely-packed spongy mesophyll. Air spaces within the mesophyll allow for gas exchange via stomatal openings in the epidermis. In *Syringa*, stomata are found on both upper and lower leaf surfaces, but are more numerous in the lower epidermis. Look in the lower epidermis for pairs of smaller, more intensely stained cells. These are guard cells, which surround the stomatal opening.

Locate the large midvein, flanked by several smaller vascular bundles (veins) within the mesophyll. Note that some smaller veins appear in cross-section, others longitudinal or oblique. This is evidence of reticulate venation commonly seen in dicots. In contrast, vascular bundles in monocot leaves usually are more uniform in size and nearly parallel to one another.

A detailed analysis of leaf structure is found in Exercise 12 (Leaves).

Draw a portion of *Syringa* leaf x-section and label the underlined features.



Flowers

7. Examine the flower model or fresh blossoms, if provided, and review the following general terms used to describe external flower anatomy.

The flower arises from a supporting stalk, or pedicel. In complete flowers, four whorls of floral appendages are inserted into a cupped or dome-shaped receptacle. The outermost whorl is made up of sepals, which together comprise the calyx. The corolla, made up of petals, is inserted just inside the calyx. Together, the calyx and corolla are called the perianth. If the perianth is not differentiated into calyx and corolla, the individual members are called tepals. These non-reproductive flower parts provide protection during flower development and later may attract pollinators. (Wind pollinated angiosperms usually do not have petals.)

The male reproductive structures, or stamens, are inserted inside the petals. Usually, each stamen is made up of a slender filament ending in enlarged pollen-producing anthers. One or more female reproductive structures or carpels (pistils) are at the center of the flower. Typically, each carpel is made up of: (1) an enlarged ovary, bearing the ovules; (2) an elongated style, through which the pollen tubes grow toward the ovary; and (3) a broad stigma, where pollen grains adhere and germinate. If live blooms are provided, make a transverse cut through the ovary and examine under the dissection microscope to locate the ovules.

Recall that flower parts of monocots generally occur in threes or multiples of three, and those of dicots in fours or fives or multiples of four or five.

Reproduction in angiosperms and gymnosperms will be examined in Exercise 14 (Reproduction and Life Cycle).

Draw a generalized flower and label to show the location of each underlined term.

Exercise 3--Cell Types and Tissues

Introduction

This unit surveys the basic cell types and tissues that make up the bodies of higher plants. Individual cells are classified as three types--parenchyma, collenchyma or sclerenchyma--according to cell wall structure. These categories also are used to classify **simple tissues**, masses of cells made up of only one cell type. Thus, parenchyma is a simple tissue made up of parenchyma cells. Simple tissues often are organized into **complex tissue**, containing more than one cell type. Xylem, for example, may be thought of as a complex tissue made up of conducting and supporting sclerenchyma and associated parenchyma.

Simple and complex tissues function within three major **tissue systems**. The dermal system (epidermis and periderm), forms the outer protective covering of the plant. The vascular system (xylem and phloem) conducts water and nutrients and provides structural support. The ground system (pith, mesophyll, and cortex) forms a supporting matrix for the vascular tissues and performs a variety of metabolic functions.

Materials

Prepared Slides:

Zea mays (corn) stem, x-section
Syringa (lilac) leaf, x-section
Medicago (alfalfa) stem, x-section
Sambucus (elderberry) leaf, x-section (optional)
Helianthus (sunflower) stem, x-section
Yucca (Spanish Bayonet) leaf, x-section (optional)
Nymphaea (water lily) leaf, x-section

For Wet Mounts:

Solanum (potato), tuber
Pyrus (pear), fruit

Parenchyma

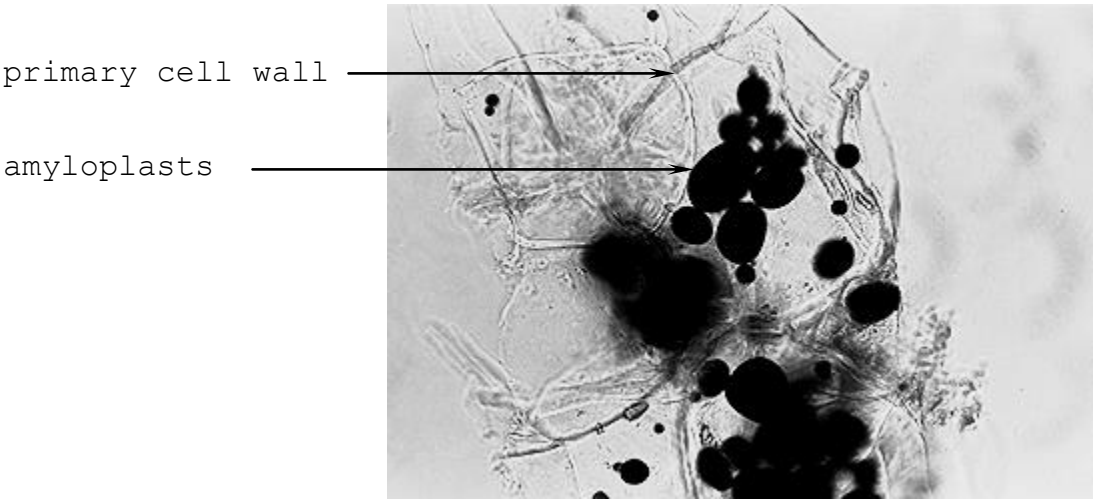
1. Examine the *Zea mays* stem x-section at low magnification, and observe vascular bundles embedded in ground tissue composed of parenchyma. Center a group of parenchyma cells, and switch to high magnification.

The "original" plant cell in evolutionary terms, parenchyma remains the most abundant cell type and most common tissue of the ground system. Like most parenchyma, these cells have only thin primary cell walls, composed of cellulose and hemicellulose. Note also the presence of numerous intercellular spaces, formed during growth by the separation of adjacent primary walls through the middle lamellae (schizogenous origin). Parenchyma cells contain living protoplasts at maturity, and carry out a variety of metabolic functions, including photosynthesis, transport and storage. These structural parenchyma cells in *Zea mays* also function in food storage.

Draw a group of five or six parenchyma cells and label with the underlined features.

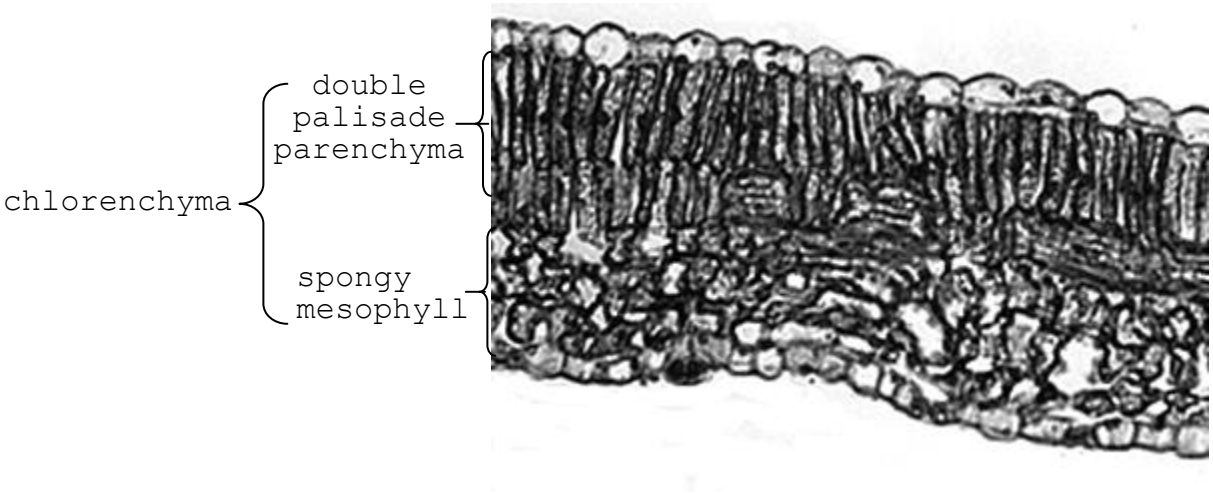
2. Cut a thin sliver of potato tuber and prepare a wet mount using tap water and a few drops of iodine stain. Select a thin area near the edge and observe under high magnification. Primary cell walls of this food storage parenchyma appear as thin, glassy lines. Within the cells, observe amyloplasts (plastids), each containing one or more deeply stained starch grains. (Recall that starch stains blue-black with iodine in potassium iodide.) Careful focusing with reduced light should reveal concentric layers around a single point, the hilum, within each starch grain. This layering results from the alternate addition of amylose and amylopectin.

Draw a group of parenchyma cells and label with the underlined features.



3. View the prepared slide of *Syringa* leaf x-section. Parenchyma cells specialized for photosynthesis make up both the palisade parenchyma, just beneath the upper epidermis, and spongy mesophyll, which fills much of the leaf interior. Parenchyma tissue containing chloroplasts is called chlorenchyma. Large intercellular spaces within this parenchyma facilitate gas exchange with the external environment.

Draw a portion of leaf x-section showing tissue details of parenchyma. Label, using each underlined term.

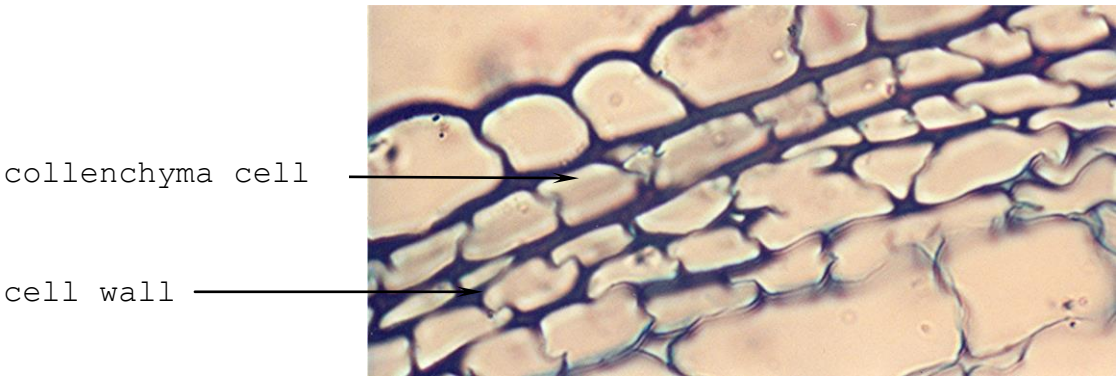


Collenchyma

4. View the prepared x-section of *Medicago* stem or *Zea mays* stem at low magnification. Collenchyma cells, with their unevenly thickened primary cell walls, can be seen in the cortex, just inside the epidermis. Center an area within the collenchyma tissue and examine under high magnification. These cells are typical angular collenchyma, in which primary walls are thickened at the corners, and the protoplasts appear rounded.

In herbaceous stems and leaf petioles, collenchyma typically forms a continuous supporting cylinder beneath the epidermis. Like parenchyma, collenchyma cells usually have only primary (non-lignified) cell walls, remain alive at maturity, and are capable of resuming meristematic activity. Collenchyma's thickened primary walls are strong but plastic, and are capable of stretching to accommodate growth.

Draw a group of five or six collenchyma cells, and label with the underlined terms.



5. In leaves, collenchyma tissue often occurs as strands within large vascular bundles. Scan the *Syringa* or *Sambucus* leaf x-section at low magnification and locate the prominent midvein (midrib). Examine this area at high magnification and observe collenchyma just inside the upper and lower epidermis (*Syringa*), or restricted to the abaxial (lower) portion of the midrib (*Sambucus*).

Outline the leaf midrib, showing tissue details of collenchyma, and label with the underlined terms.

Sclerenchyma

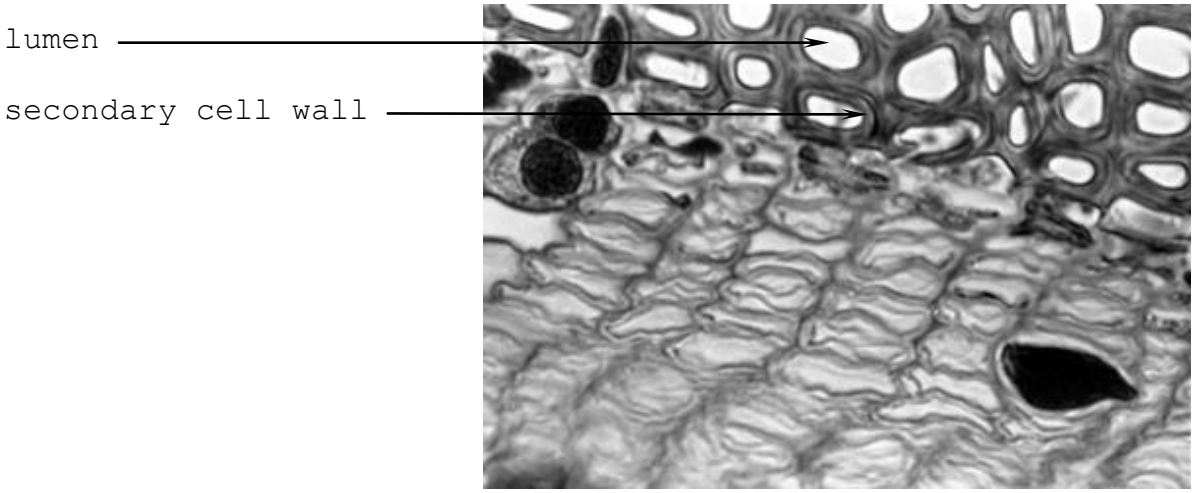
6. Sclerenchyma cells are placed in two categories according to function: mechanical sclerenchyma (fibers and sclereids); and conducting sclerenchyma (xylem tracheary elements). Sclerenchyma cells have rigid, usually lignified secondary walls, deposited inside the primary wall after cell elongation has stopped. In mature tissues, sclerenchyma cells often lack protoplasts.

On the *Helianthus* stem x-section, center one vascular bundle and examine under high magnification. The bundle cap on the exterior side of the bundle is made up of fibers, identified by their darkly stained (red on most preparations) and evenly thickened secondary walls. Fibers are elongated axially and typically occur in strands associated with vascular tissues. They provide support in plant tissues that are no longer elongating. The wood of many flowering plants, for example, contains abundant fibers.

Sketch one vascular bundle, showing the arrangement of fibers within the bundle cap, and label with the underlined terms.

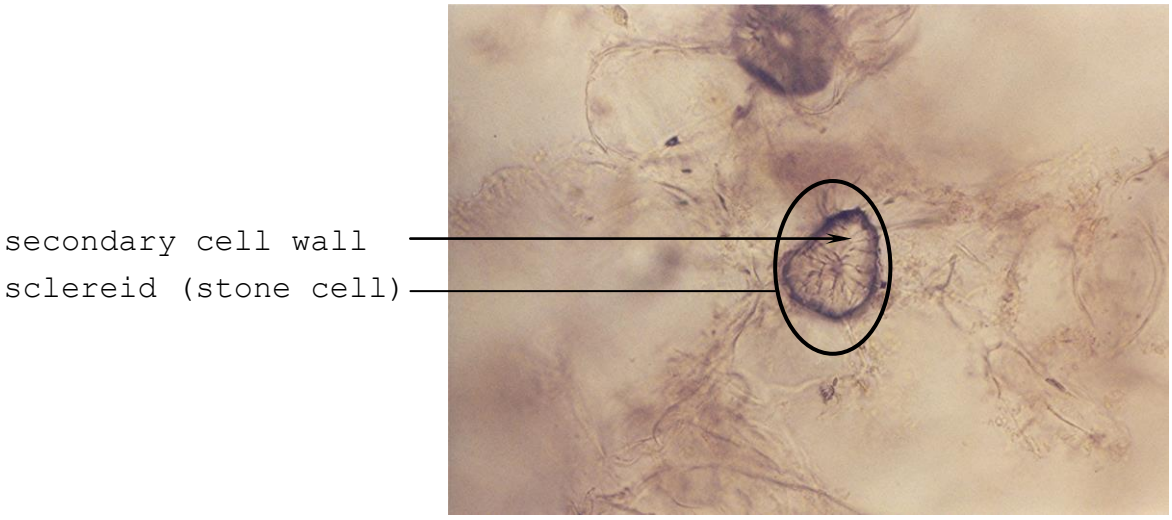
7. Examine the *Yucca* leaf x-section, if available. This rigid leaf contains many large, thick-walled fibers which form enormous bundle caps. Notice the thick secondary cell walls and narrow lumens of these cells.

Outline one segment of this x-section. Draw a group of five or six fibers, and label with the underlined terms.



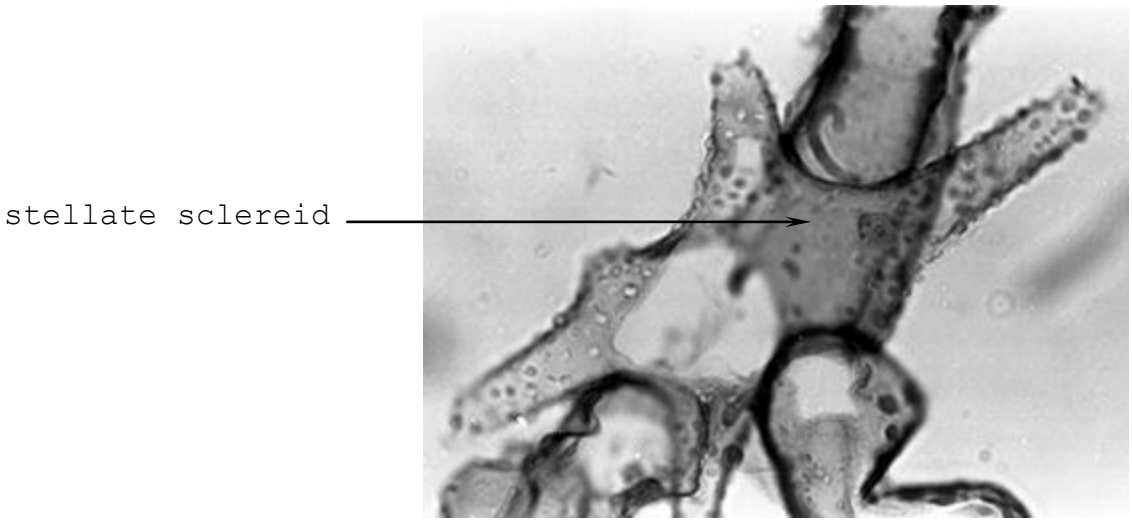
8. Mount a thin sliver of *Pyrus* fruit flesh in tap water, and scan at low magnification to locate scattered sclereids called stone cells. Although varied in shape, sclereids typically are shorter than fibers; often, like these stone cells, they are roughly cuboidal (isodiametric). Thick, strongly lignified secondary cell walls oriented in three dimensions produce hard brittle surfaces. These stone cells give pear flesh its "gritty" feel. Sclereids occur as isolated cells (idioblasts), in clusters, or as continuous sheets in stems, leaves, fruits and seeds. For example. the shells of nuts and the stones or pits of fruit are composed of sclereids.

Draw two or three stone cells and label with the underlined terms.



9. Examine the *Nymphaea* leaf x-section. Stellate (star-shaped or branching) sclereids occur as idioblasts within the mesophyll of this leaf. Note their distribution and varied shape.

Outline the x-section, showing two or three stellate sclereids.



Note: Conducting sclerenchyma (xylem tracheary elements), are examined in Exercise 4 (Xylem).

Exercise 4--Xylem

Introduction

This exercise is the first in a series of detailed studies which build on the overview presented in Exercises 1-3. Comparative study of monocot, dicot and gymnosperm xylem reveals the structure and arrangement of tracheary elements--tracheids and vessel members--and their associated parenchyma and sclerenchyma.

The student will make drawings which integrate stem x-sections with long. views in order to visualize the three-dimensional structure of primary xylem. Long. sections also are used to study the development of secondary cell walls.

Materials

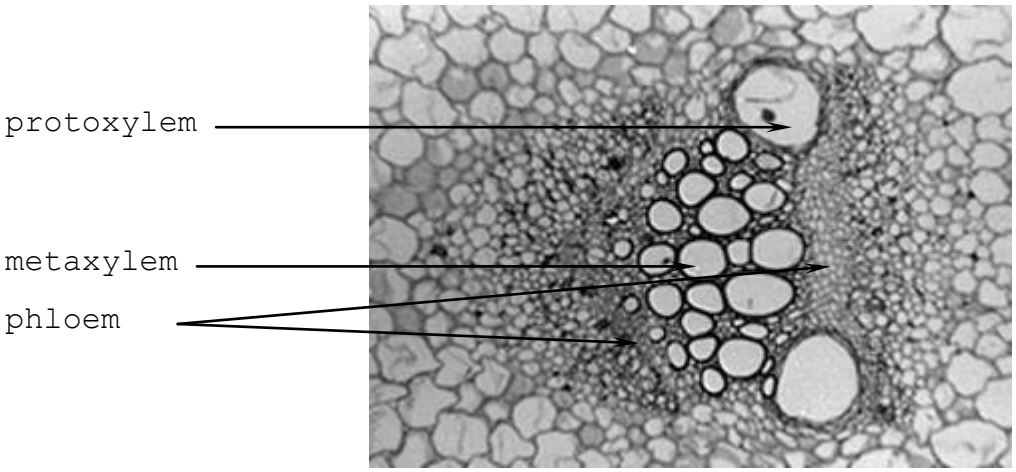
Prepared Slides:

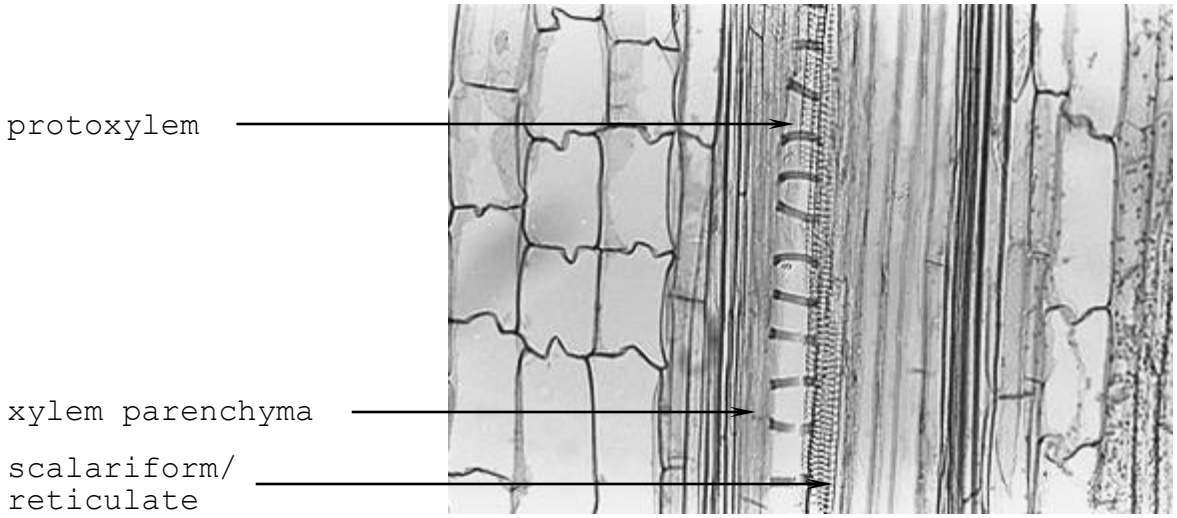
Cucurbita (pumpkin) stem, x-section
Cucurbita stem, long. section
Zea mays (corn) stem, x-section
Zea mays stem, long. section
Pinus (pine) maceration
Pinus, wood planes

Dicot Xylem

1. View the *Cucurbita* stem x-section under low magnification. This ring-like arrangement of vascular bundles is typical of dicots in the primary state of growth. Select one vascular bundle and examine under high magnification. The largest elements, near the center of the bundle, are primary metaxylem vessels. These stain pink or red on most preparations due to the presence of lignin in their secondary cell walls. Smaller metaxylem vessels and protoxylem, the smallest first-formed xylem elements, occur among thin-walled xylem parenchyma cells. Note that *Cucurbita* vascular bundles are bicollateral--a somewhat unusual arrangement in which phloem occurs both interior and exterior to the xylem.

Outline one vascular bundle; draw and label the underlined details of xylem tissue. Place labels on the right and allow space for a second drawing below.





2. Examine a vascular bundle on the *Cucurbita* long. section. It may be helpful to begin at one "cut end" of the section, first locating the broad red-stained metaxylem vessels. Smaller protoxylem vessels, the first tracheary elements to mature in the developing shoot, often show a helical (spiral) or annular (ring-like) pattern of secondary cell wall deposition. In these cells, secondary walls may begin to form as the shoot elongates, and the cell walls must accommodate growth. Look for evidence of this--widely spaced and/or tilted annular rings or helices that appear stretched or uncoiled. In spite of this adaptation, protoxylem vessels do not remain functional. As primary growth continues in dicots such as *Cucurbita*, early protoxylem cells are torn and apparently obliterated by the surrounding parenchyma.

As elongation slows, larger metaxylem cells mature and persist as the tube-like water conducting vessels of primary tissue. They typically show helical or more rigid scalariform/reticulate or pitted side walls. Pits in side walls permit lateral movement of water from vessel to vessel, or from vessels into adjacent parenchyma or other tissues.

Attempt to find all four patterns of secondary cell wall thickening--annular, helical, scalariform/reticulate and pitted. It may be necessary to examine more than one slide.

Look also for the remnants of vessel element end walls, which are transverse, or nearly so. Perforations, large holes in the end walls of vessel elements, represent areas from which the primary walls have been completely removed. Perforations facilitate the longitudinal conduction of water through vessels.

Observe relatively thin-walled xylem parenchyma cells (rectangular in long. view) between the vessels. Notice that these cells are longer than parenchyma of the pith and cortex. Look carefully at sclerified parenchyma cells adjacent to large metaxylem vessels and note the simple pits in their walls. Simple pits are defined as narrow, straight-sided channels through the secondary wall.

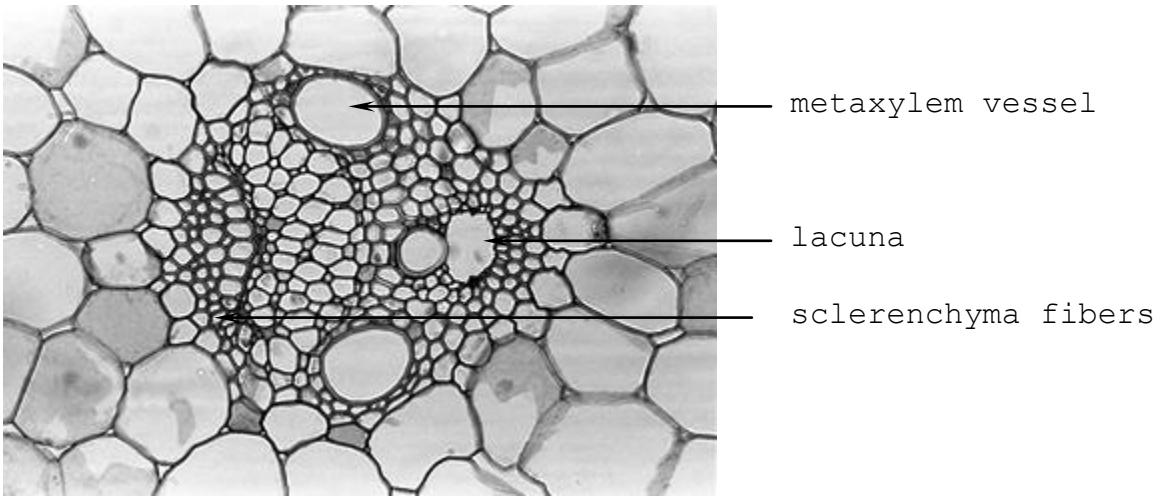
Draw a representative area of xylem and label the underlined cell types and structural features. Using arrows or dashed lines, connect each cell type with a corresponding cell in the x-section above.

Monocot Xylem

3. The *Zea mays* stem, viewed in x-section, shows the complex arrangement of vascular bundles typical of monocots. Select one vascular bundle and examine under high magnification. In monocots, the stretching of protoxylem vessels during stem elongation results in rupture of the cell walls, forming one or more large open canals called protoxylem lacunae. These appear in x-section as open areas, not bounded by cell wall. The lacuna is often the largest feature of the vascular bundle.

Two or three large metaxylem vessels and a number of smaller vessels also are conspicuous. Xylem parenchyma cells can be seen among the metaxylem. Sclerenchyma fibers, identified by their darkly stained and evenly thickened secondary walls, make up the bundle sheath.

Draw one vascular bundle and label the underlined tissue details. Place labels on the right and allow space for a second drawing below.



4. View the long. section of *Zea mays* stem, and locate an area in which the long. plane passes through a vascular bundle. Again, the most conspicuous feature may be the open protoxylem lacuna. At least one of the large metaxylem vessels and two or more smaller vessels should be visible near the lacuna. Look for annular or pitted secondary cell walls in these vessels.

Xylem parenchyma, thinner-walled rectangular cells between or to one side of the vessels, are much more compact than parenchyma of the ground tissue. Very long, narrow sclerenchyma fibers can be seen along one or both sides of the vascular bundle, in the bundle sheath. Both sclerenchyma and parenchyma provide support within the vascular bundle.

Draw one vascular bundle and label the underlined cell types and structures. Using arrows or dashed lines, connect one cell of each type with a corresponding cell in the x-section above.

Gymnosperm Xylem

5. Observe the *Pinus* wood maceration under low and then higher magnification. *Pinus* xylem, and gymnosperm xylem in general, consists almost entirely of tracheids, long narrow cells with tapered ends. Water moves from tracheid to tracheid through large bordered pits in the walls of these cells. Although the details of pit structure are not visible at this magnification, bordered pits are characterized by a border, or thickened margin, around their inner aperture.

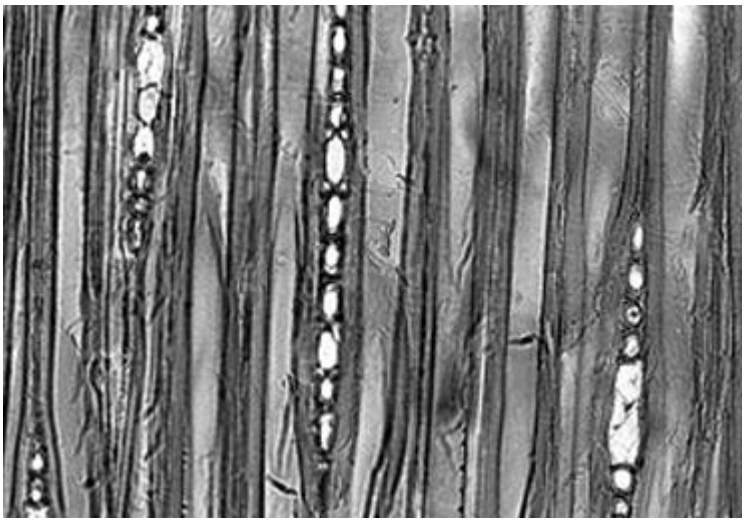
Draw one or two tracheids with bordered pits.

6. On the *Pinus* wood planes slide, first locate the radial view. To identify this plane, scan the long. sections at low magnification, and look for segments of ray running across the field of view.

Observe again that tracheids are narrow and tapered, with overlapping end walls. Water passes through the large bordered pit pairs in these overlapping walls, but the walls of tracheids are not perforated.

In evolutionary terms, tracheids are considered to be more "primitive" than vessel elements. Their tapered shape and overlapping junctions provide structural support. Modification of tracheids--broader cells, more transverse end walls, and perforated end walls--likely gave rise to vessel members, which predominate in monocots and dicots. Broad vessel members with perforated end walls offer less resistance to water movement than tracheids, but provide less structural support. Fibers and sclerified parenchyma have become the principle supporting tissue in xylem made up mostly of vessels. Thus, more fibers usually are found in hardwoods (dicots) than in softwoods (gymnosperms).

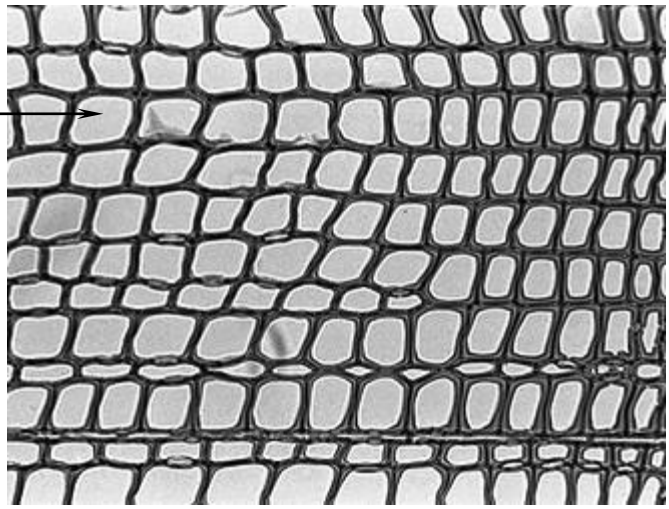
Draw three or four overlapping tracheids with their bordered pits.



7. Locate the x-section on the *Pinus* wood planes slide. Tracheids of the first year's growth surround the central pith. Notice that tracheids near the stem's center, produced during early growth (spring wood), are larger than tracheids produced later in the growing season (summer wood). This feature, which results in "growth rings" of secondary growth, will be observed in greater detail in Exercises 10 (Secondary Growth) and 11 (Wood Anatomy).

Draw a segment of the x-section showing the regular arrangement of tracheids.

tracheid



Exercise 5--Phloem

Introduction

Phloem is a complex tissue specialized for the transport of organic nutrients, especially the products of photosynthesis. In contrast to the relatively passive movement of water in xylem, translocation in phloem is a dynamic process involving the active loading and unloading of photosynthate as it moves throughout the plant. Phloem cells, therefore, must remain alive in order to function.

This unit examines the structure and three-dimensional arrangement of phloem conducting elements--sieve cells and sieve tube members--and their associated cells.

Materials

Prepared Slides:

Cucurbita (pumpkin) stem, x-section
Cucurbita, stem long. section
Zea mays (corn) stem, x-section
Zea mays stem, long. section
Pinus (pine) young stem, x-section
Pinus stem tip, long. section (optional)

Dicot Phloem

1. Examine the *Cucurbita* stem x-section, and note again that these vascular bundles are bicollateral; phloem occurs both interior and exterior to the xylem. Observe one bundle at high magnification and locate the following cell types within the external and internal phloem: (1) large, blue-stained sieve tube members (STMs) of the metaphloem; (2) smaller, more densely stained companion cells in contact with STMs; and (3) phloem parenchyma, relatively undifferentiated non-conducting cells intermediate in size between STMs and companion cells. All three cell types are classified as specialized parenchyma, and have only primary (non-lignified) cell walls. Protophloem has been destroyed during stem elongation and obliterated by the surrounding parenchyma.

On some sections, a zone of small, thin-walled cells arranged in regular rows can be seen between the xylem and external phloem. These cambium cells, remnants of the procambium, may give rise to secondary growth.

Outline one vascular bundle, showing the arrangement of xylem and phloem. Draw tissue details of the phloem, and label using the underlined terms. Place labels on the right and allow space for a second drawing below.

2. Examine the *Cucurbita* stem long. section. Locate the conspicuous red-stained xylem vessel/s and look on both sides for blue-stained internal and external phloem. Sieve tube members (STMs) are easily identified by the presence of reddish P-protein "plugs," wedged at the sieve plate (end walls) between adjoining cells in the column. When phloem is damaged or cut, P-protein and callose are carried to the sieve plate and accumulate, effectively sealing the damaged area. P-protein plugs are not present in live functioning phloem. A vertical column of STMs make up one sieve tube. Recall that in angiosperms, mature STMs nearly always lack nuclei. During differentiation, the tonoplast (vacuolar membrane) also breaks down, and the resulting watery cytoplasm is sometimes called mictoplasm.

Look carefully for narrow, more densely stained companion cells among the STMs. Companion cells contain nuclei, and function in the loading and unloading of photosynthate.

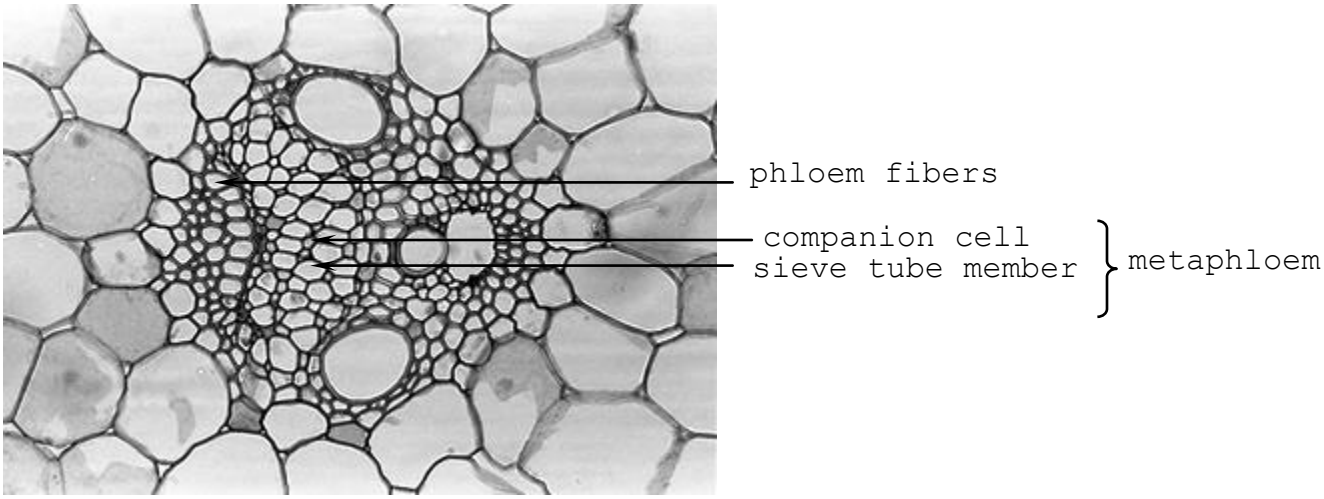
Sieve areas are specialized primary pit fields, with enlarged plasmodesmata called sieve pores connecting the protoplasts of adjoining cells. Look for sieve areas on lateral walls between STMs and between STMs and companion cells. In nearly all angiosperms, sieve areas on end walls contain greatly enlarged sieve pores, and are called sieve plates. Sieve plates maximize longitudinal conduction between STMs.

Draw a small area of phloem and label to show the underlined features. Use arrows or dashed lines to connect one of each cell type with a corresponding cell in the x-section drawing above.

Monocot Phloem

3. Examine one vascular bundle within the *Zea mays* stem x-section. Metaphloem is easily identified by its "checkerboard" pattern, created by the regular arrangement of STMs and smaller, more densely-stained companion cells. Phloem fibers make up the bundle sheath.

Draw one vascular bundle and label the tissue details of phloem, using each underlined term. Place labels on the right and allow space for a second drawing below.



4. Scan the *Zea mays* stem long. section and locate an area in which the long. plane passes through a vascular bundle. This view reveals the shape and vertical arrangement of cells within the metaphloem. Look for blue-stained STMs between xylem vessels and fibers of the bundle sheath. Notice that STM sieve plates are almost perfectly transverse. P-protein does not occur in *Zea mays* and many other monocots. Look carefully alongside the STMs for narrow companion cells, which appear densely cytoplasmic and contain a nucleus. Note also, long darkly-stained phloem fibers at the bundle periphery. These supporting cells, which make up the bundle sheath, are classified as sclerenchyma due to their lignified secondary walls.

Draw a representative area of phloem within one vascular bundle and label with the underlined terms. Use arrows or dashed lines to connect one of each cell type with a corresponding cell in the drawing above.

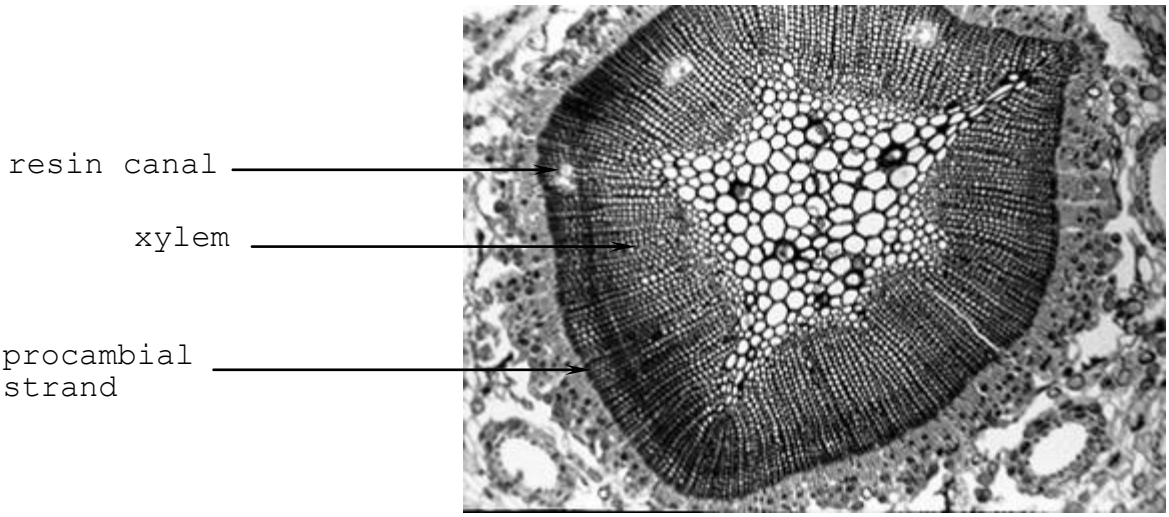
Gymnosperm Phloem

5. View the *Pinus* young stem x-section. The phloem of *Pinus* is made up of sieve cells, seen near the periphery of this section as several layers of large blue or green-stained cells with relatively thick primary walls. Sieve cells, found in lower vascular plants and gymnosperms, are considered to be more "primitive" conducting elements than STMs, seen in nearly all flowering plants.

Albuminous cells, functional counterparts of companion cells in angiosperm phloem, are very small and difficult to identify in *Pinus*.

Small thin-walled cells between the xylem (tracheids) and phloem make up the procambial strand. Circular resin canals, each lined by a single layer of epidermal cells, also are conspicuous in this x-section.

Draw a segment of *Pinus* x-section and label with the underlined features.



6. Examine the *Pinus* stem tip long. section, if available. Near the periphery are several rows of blue-stained, relatively thick-walled sieve cells. Sieve cells are relatively long (compared to STMs), and have rounded or obliquely overlapping end walls. Examine these cells at high magnification and look for sieve areas, visible as circular light spots evenly distributed on lateral and end walls.

Draw several adjoining sieve cells and label the underlined features.

Exercise 6
Vascular Architecture

Introduction

Vascular architecture--the diverse ways in which xylem and phloem are assembled into functional conducting tissue--is the subject of this exercise. Primary roots and shoots may be categorized on the basis of stellar characteristics (overall arrangement of vascular tissues within the stele) and bundle type (arrangement of xylem and phloem within each vascular bundle).

Placing plants into categories based on their vascular architecture is useful in comparative analysis, and reveals much about phylogenetic relationships.

This unit is intended to provide an overview of vascular architecture. Detailed analysis of vascular, ground, and epidermal tissues of primary roots and stems is found in Exercises 7 (Primary Roots), 8 (Primary Stems), and 9 (Primary Root and Stem Development).

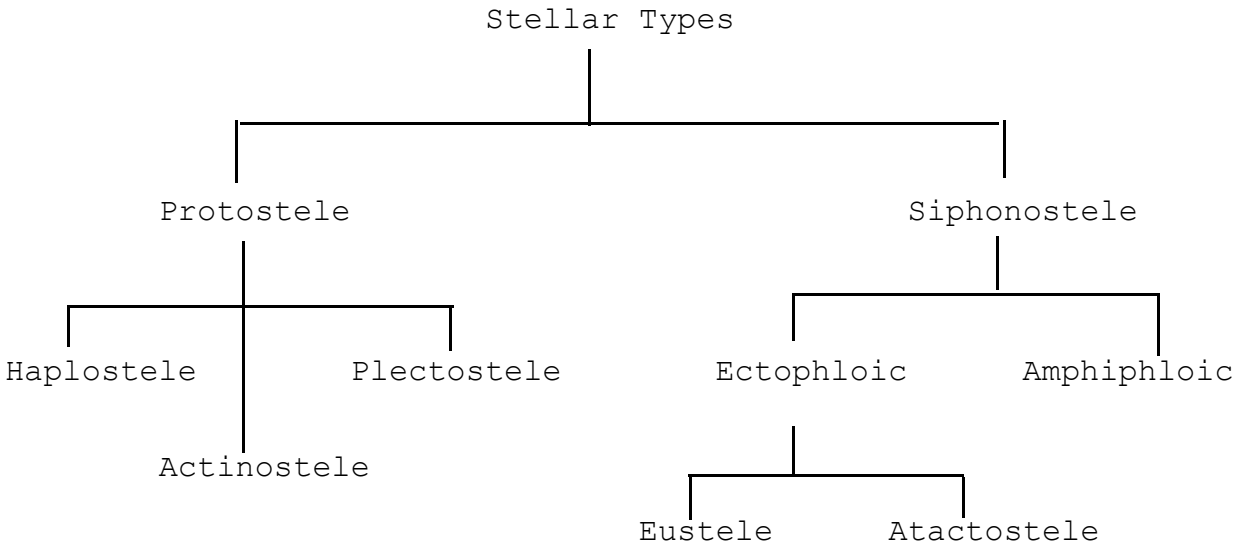
Materials

Prepared Slides:

Ranunculus (buttercup) root, x-section
Osmunda (fern) stem, x-section
Lycopodium (clubmoss) stem, x-section
Medicago (alfalfa) stem, x-section
Zea mays (corn) stem, x-section
Marsilea (fern) stem, x-section
Cucurbita (pumpkin) stem, x-section
Helianthus (sunflower) stem, x-section
Elodea (waterweed) stem, x-section
Acorus (sweet flag) stem, x-section

Stellar Types

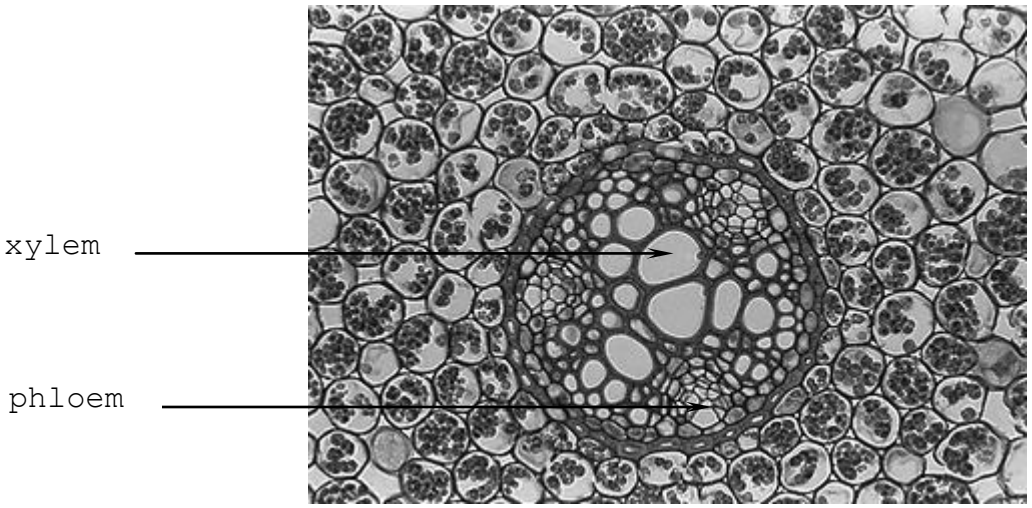
The stele, or "central column," of the primary stem and root is made up of vascular tissues and associated parenchyma and sclerenchyma. Two basic stellar types--protostele and siphonostele--are classified according to the arrangement of xylem and phloem. A number of subcategories are identified within each stellar type.



Make an outline drawing of each x-section described below, showing the arrangement of xylem and phloem, and label with the underlined terms.

1. View the *Ranunculus* (dicot) root x-section. This arrangement is a protostele, characterized by a solid core of xylem (no central pith) and an absence of separate vascular bundles. Phloem surrounds the xylem core. Phylogenetically, the protostele is considered to be the first pattern to appear in vascular plants. It remains the dominant pattern in the shoots of lower vascular plants (cryptogams) and in the roots of seed plants, with the exception of monocots.

The undulating margin of the central xylem further identifies this protostele as an actinostele.



2. The *Osmunda* stem illustrates the simplest and earliest type of protostele, the haplostele, in which the xylem core is more or less circular in cross section.

3. View the x-section of *Lycopodium*, a club moss. This protostele is a plectostele, in which xylem appears not as one mass, but as a series of plates. The tissue between the plates of xylem is phloem.

4. The *Medicago* (alfalfa) stem x-section illustrates a typical siphonostele pattern, identified by the presence of a central pith. Considered more phylogenetically "advanced" than the protostele, the siphonostele is found in the shoots of virtually all seed plants and in the roots of many monocots. Broad stele grasses appear to be at the endpoint of this evolutionary trend.

This siphonostele is further classified as ectophloic, since phloem occurs only to the outside of the xylem. The single ring of vascular bundles also identifies this stem as a eustele.

5. The fern *Marsilea* illustrates a typical amphiphloic siphonostele. A pith is present and phloem occurs on *both* sides of the xylem. Xylem and phloem form a continuous cylinder.

6. The *Cucurbita* stem represents an unusual arrangement of siphonostele. The single ring of vascular bundles identifies it as a eustele; but the pattern is amphiphloic (phloem occurs both interior and exterior to the xylem). This is an atypical arrangement in seed plants. Amphiphloic siphonosteles normally occur only among the cryptogams.

7. The *Zea mays* (monocot) stem is also a siphonostele. A pith is present, even though it cannot be differentiated from the cortex. This stele also would be classified as ectophloic, since phloem occurs exterior to the xylem within each bundle. This complex pattern of bundles, typical of monocots, is called an atactostele.

Bundle Types

Outline each x-section described below and draw tissue details of one vascular bundle, showing the arrangement of xylem and phloem. Label with the underlined terms.

8. Examine several vascular bundles on the *Medicago* stem x-section. These collateral bundles have phloem on only one side of the xylem. Most commonly, as in this section, phloem is external to the xylem. Collateral bundles are the most common type of axial bundle.

9. *Helianthus*, like *Medicago*, has collateral bundles arranged within an ectophloic eustele. An unusual feature of this stem, however, is the presence of isolated phloem strands scattered among the bundles.

10. Vascular bundles of *Cucurbita* are bicollateral; phloem occurs on two sides of the xylem (both internal and external).

11. Vascular bundles of *Elodea* are amphiphloic (amphicribral), an unusual arrangement in which phloem surrounds the xylem. The arrangement here is equivalent to a protostele (haplostele).

12. View the x-section of *Acorus* (sweet flag) rhizome. These amphixylic (amphivasal) bundles, in which xylem completely surrounds the phloem, are an uncommon type restricted to a few monocot genera.

Exercise 7--Primary Roots

Introduction

The primary roots of seed plants may function in absorption, anchorage, storage, transport, propagation, and growth regulation. In this exercise, a detailed study of primary roots in x-section reveals the characteristic features seen in monocots and dicots. Also examined are specializations related to environment (air and water roots) and symbioses (mycorrhizae, root nodules, and haustoria).

Materials

Prepared Slides:

Ranunculus (buttercup) mature root, x-section
Ranunculus young root, mature protoxylem, x-section (optional)
Smilax (greenbrier) mature primary root, x-section
Zea mays (corn) root, x-section with root hairs (optional)
Triticum (wheat) root, x-section (optional)
Air, water and soil roots, composite x-section
Glycine max (soybean) root nodule, x-section
Pinus root with ectotrophic mycorrhiza, x-section.
Corallorhiza (Coral root) rhizome with endotrophic mycorrhiza, x-section, (optional)
Cuscuta (dodder) haustorium, host stem x-section

Primary Root Anatomy

1. Examine the x-section of *Ranunculus* (dicot) mature primary root. This stele is a protostele (See Exercise 6), seen almost universally in primary roots of gymnosperms and dicots.

In mature *Ranunculus* roots, the arrangement of xylem usually is tetrarch, with four protoxylem poles radiating from a central metaxylem core. Triarch and pentarch steles also occur in Ranunculus. The number of poles present is directly related to the diameter and vigor of the root.

Note that primary xylem in roots is exarch (protoxylem exterior to metaxylem). This arrangement results from centripetal differentiation. Small protoxylem cells near the periphery of the developing vascular cylinder mature first, and maturation progresses **inward**. Centripetal maturation of xylem in roots contrasts with the centrifugal pattern always seen in the xylem of stems.

Primary phloem can be seen between the protoxylem poles, and like xylem, differentiates centripetally. Metaphloem is interior (nearest the xylem) and protophloem is exterior. Smaller

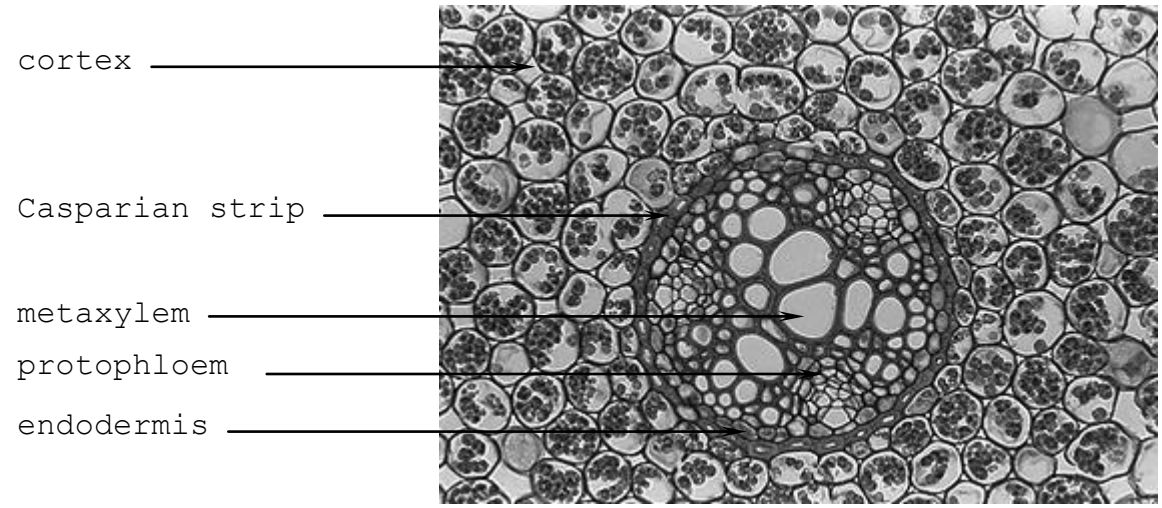
companion cells may be visible among the metaphloem sieve elements. A thin layer of parenchyma can be seen between xylem and phloem.

The pericycle is the outermost layer of the stele. In this x-section, the pericycle is a circular, uniseriate layer of parenchyma cells between the vascular tissues and more intensely-stained endodermis. Resumption of meristematic activity within the pericycle may give rise to lateral roots and, in plants with secondary growth, contributes to the vascular cambium. (Secondary root development is included in Exercise 10).

The darkly-stained endodermis represents the innermost layer of cortical cells. In this x-section, the endodermis is distinct due to staining of the Casparian strip, an impermeable band of suberin deposited within radial and transverse cell walls. By blocking apoplastic movement of water and solutes, the endodermis selectively controls movement of ions into the stele. In x-sections from older portions of root, endodermal cell walls are thicker and more intensely stained, evidence of additional suberin deposition. Eventually, suberin completely covers the endodermal cell walls. Although the endodermis continues to allow active uptake of ions from the cortical symplast (via plasmodesmata), it forms a barrier against leakage of water from the stele.

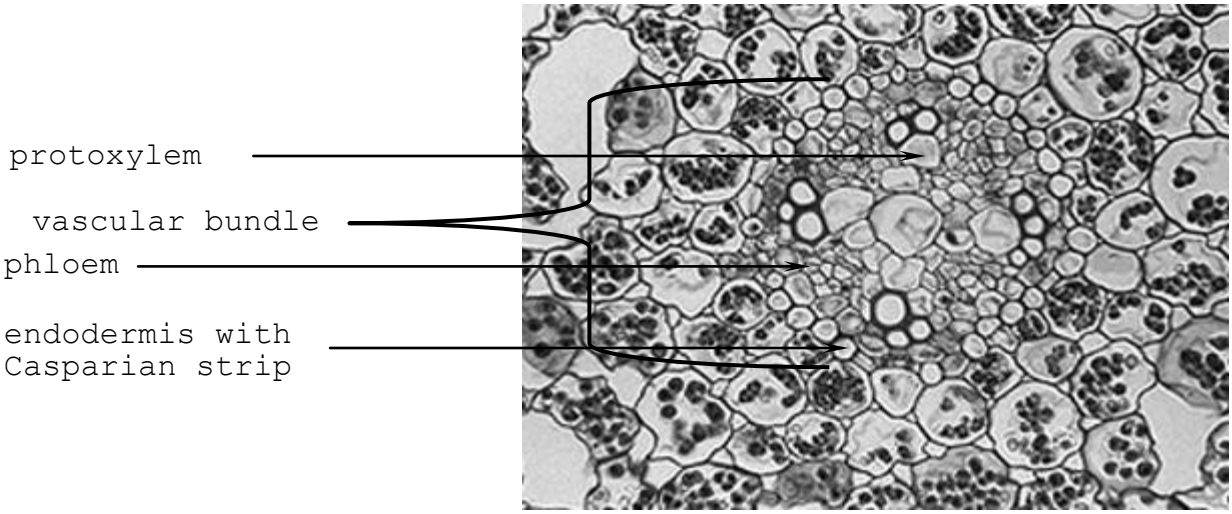
The cortex surrounding the stele is a relatively uniform mass of parenchyma, with abundant intercellular spaces. The epidermis, uniseriate in *Ranunculus*, permits absorption at all levels of the root. However, uptake is most active where root hairs (not present on this section) are abundant.

Draw the x-section of *Ranunculus*, showing tissue details, and label with the underlined terms.



2. Examine the *Ranunculus* young root x-section, if provided. In this immature root, protoxylem has differentiated near the periphery of the vascular cylinder. Metaxylem cells, at the center of the stele, are functionally immature and unlignified. Phloem is visible between the developing protoxylem poles. The pericycle, which differentiates early (near the root apex), and the endodermis, with its Casparian strip, also are distinct.

Outline this section, and label with the underlined terms.



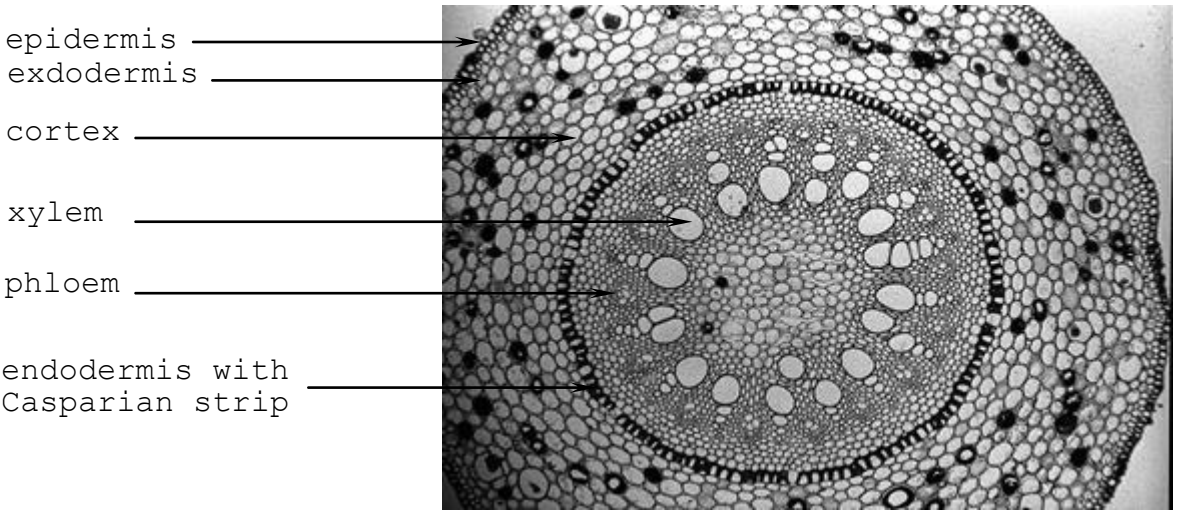
3. The *Smilax* mature root x-section illustrates typical monocot architecture. This stele is interpreted as a siphonostele (See Exercise 6).

Xylem is polyarch (many poles), but broken into distinct bundles. Very large metaxylem elements, seen here and in many other monocot roots, offer little resistance to water movement. This feature compensates in part for a lack of secondary growth in monocot roots and stems. Adventitious roots also bypass the "bottleneck" created by lack of secondary xylem and phloem.

Phloem appears as bundles between the protoxylem poles. Again, due to centripetal development, protophloem is exterior to larger metaphloem elements. In *Smilax* and many other monocots, the pericycle is multiseriate and sclerified. The endodermis is prominent, due to heavily suberized (sometimes lignified) cell walls. Notice that endodermal cells appear asymmetrical due to a greater degree of thickening in radial and inner tangential walls.

Also prominent in this x-section is a distinct uniseriate exodermis immediately beneath the epidermis. The exodermis represents the outermost layer of cortex. Its suberized cell walls form a barrier to apoplastic movement which protects cortical cells from toxins in the soil. Final control of ion entry, however, remains in the endodermis. The exodermis is an evolutionary "refinement" seen in the roots of only a few seed plants.

Draw the x-section of *Smilax* root, showing tissue details, and label with the underlined terms.



4. In the *Zea mays* young root x-section, trichoblasts in the epidermis have given rise to root hairs, tubular extensions of epidermal cells. Note also the large pith at the center of this monocot root.

Outline the arrangement of stele and cortex in this section and draw tissue details of the epidermis.

5. Examine the *Triticum* root x-section, if available. This monocot root has no pith, a relatively common occurrence. A single, large metaxylem vessel occupies the central portion of the stele.

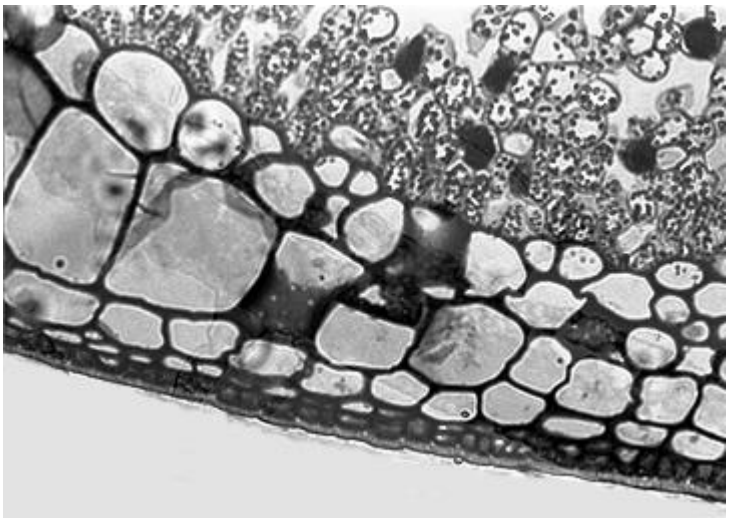
Outline this section, showing the arrangement of xylem and phloem.

Root Specializations

6. On the composite slide of soil, water and air roots, locate the x-section of water root, identified by the presence of an aerenchyma (cortex containing large air spaces). In submerged roots, aerenchyma provides for rapid diffusion of gases and serves as an oxygen reservoir for tissues having no direct access to the air.

Identify the air root by the presence of a multi-layered epidermis, the velamen, made up of large, thick-walled cells which die at maturity. The velamen provides mechanical protection, and may function in water uptake and storage.

Outline water and air root x-sections, showing tissue details of aerenchyma and velamen.



7. The *Glycine max* x-section shows a root nodule formed in response to infection by symbiotic nitrogen-fixing bacteria of the genus *Rhizobium*. Initially, bacteria enter the root via root hairs. A sheathed infection thread grows inward, penetrates the cortex, and triggers the proliferation of inner cortical cells. The resulting nodule seen in this section typically grows outward on one side of the parent root, and may continue to elongate by means of uninfected meristematic parenchyma on the nodule's abaxial side. Branching vascular bundles within the nodule are connected to the parent stele.

Under high magnification, examine parenchyma cells in the interior of the nodule (nodule cortex). Cells harboring the symbiotic bacteria are identified by the dense appearance of their cytoplasm and enlarged nuclei.

Outline the parent root and nodule; draw a small portion of the nodule cortex, showing tissue details, and label the underlined features.

8. The roots of nearly all terrestrial plants form symbiotic mycorrhizal associations with soil fungi. Mycorrhizae are classified as ectomycorrhizae or endomycorrhizae according to the relationship established between cortical cells and the fungal hyphae.

The *Pinus* root x-section has been stained to highlight an ectomycorrhizal association. Fungal mycelium covers the root with a thick sheath, but filamentous hyphae penetrate only the intercellular spaces between cortical cells. Fungal hyphae form a branching network, the Hartig net, and make close contact with cortical cell walls.

Outline the root x-section and label to show the location of underlined features.

9. The *Corallorhiza* rhizome x-section demonstrates an endomycorrhizal association, in which the fungal hyphae actually penetrate the root's cortical cell walls (but apparently, not the plasmalemma itself). At high magnification, locate the hyphae, clearly visible within infected cells or passing between cells.

Outline the section and indicate infected areas of the cortex by shading. Draw two or three infected cortical cells, and label with the underlined terms.

10. *Cuscuta* is a holoparasitic (nonphotosynthetic) dicot. Like other holoparasites, *Cuscuta* obtains both water and organic nutrients by means of haustoria, highly modified roots which penetrate the host plant stem and make connection with both xylem and phloem. In this x-section of host stem, the *Cuscuta* haustorium is in longitudinal section. Look closely at the broad column of parenchyma within the haustorium. A number of cells may have differentiated into xylem vessels. If so, they may be identified by their helical or scalariform secondary cell walls. On some sections it is possible to trace vessels in the haustorium to their connection with vascular tissues of the host.

Outline the section, showing tissue details of the haustorium, and label with the underlined terms.

Exercise 8--Primary Stems

Introduction

This exercise deals with the anatomy of primary stems The vascular structure of stems may be highly complex, due to the production of leaves, lateral branches, and reproductive structures. Adaptation has produced a staggering variety of morphologies and specialized functions. Yet the stems of all seed plants, viewed in x-section, show the same basic pattern of epidermis, cortex, and stele. Nearly all are siphonosteles, with vascular bundles arranged in a single ring (dicots and gymnosperms) or in a more complex pattern (monocots).

Materials

Prepared Slides:

Helianthus (sunflower) stem, x-section
Trifolium (clover) early stem, x-section (optional)
Salicornia (glasswort) stem, x-section
Zea mays (corn) stem, x-section (optional)
Triticum (wheat) mature stem, x-section
Potamogeton (pond weed) stem, x-section

Dicot Stem Anatomy

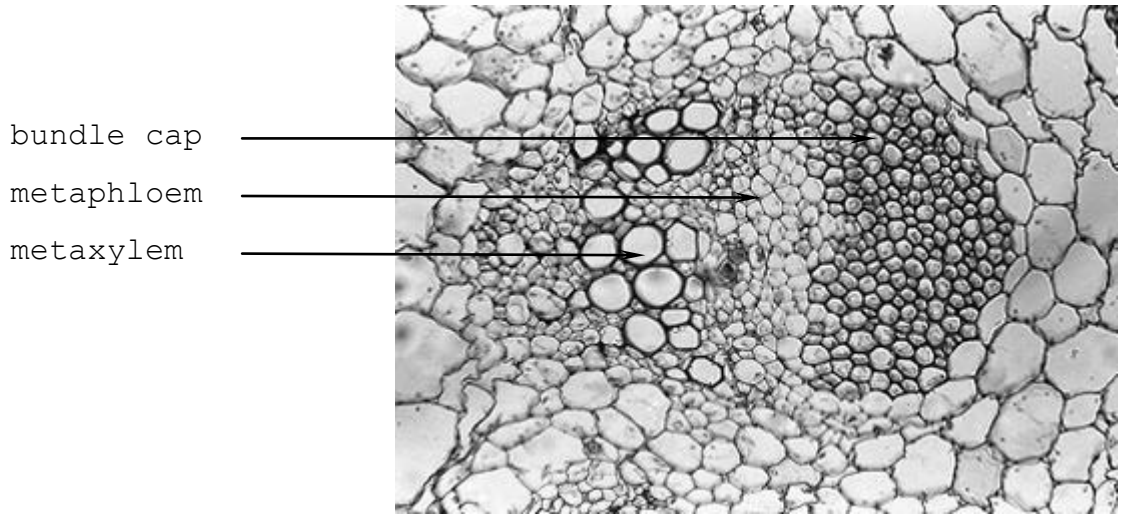
1. Examine the *Helianthus* young stem x-section at low magnification, and note the large central pith surrounded by a single ring of vascular bundles. This arrangement is typical for herbaceous dicots. No secondary growth has occurred at this level in the stem, and interfascicular regions can be seen between the vascular bundles. As in most dicots, the cortex is relatively narrow.

Observe one vascular bundle at high magnification. In this collateral bundle, remnants of small protoxylem cells may be visible among parenchyma cells on the interior side of the bundle (nearest the stem axis). Development of xylem in stems is centrifugal. This means that larger metaxylem elements differentiate exterior to the protoxylem after stem elongation stops, and persist as the water conducting cells of primary growth.

Exterior to the metaxylem is a well-defined area of metaphloem, in which angular sieve tube members (STMs) and smaller, more darkly-stained companion cells are arranged irregularly among non-conducting parenchyma. Development of

phloem is centripetal, with protophloem differentiating exterior to metaphloem. However, protophloem collapses as primary growth continues, and is nearly impossible to identify in x-section. Exterior to the phloem is a prominent bundle cap made up of phloem fibers.

Outline the x-section, draw tissue details of one vascular bundle, and label with the underlined terms.



2. Examine the stem x-section of *Trifolium*, an herbaceous dicot, if available. The arrangement of collateral bundles in this stem is similar to that in *Helianthus*. Although a true endodermis is rare in the stems of seed plants, the innermost layer of cortical cells in this young stem is prominent due to an accumulation of starch, and is identified as a starch sheath. This uniseriate layer is most easily seen just exterior to the darkly-stained bundle caps.

Within each bundle, metaxylem is arranged in distinct radial files, with vessels of each row in direct contact with one another along their tangential walls. This arrangement is common in dicots, and likely facilitates the centripetal (inward) transfer of water as it moves upward in the stem through progressively younger xylem elements. Note also that radial files of metaxylem are separated by parenchyma, with only occasional contact between vessels of adjacent files.

Outline the x-section, draw tissue details of one vascular bundle, and label with the underlined features.

3. On the *Salicornia* stem x-section, look carefully at the boundary between cortex and vascular tissues. The fleshy stem of this dicot has a true endodermis, an uncommon feature. The uniseriate cylinder of cells is distinct due to staining of the suberized Casparian strip. As in roots, the endodermis forms a barrier to apoplastic movement of water and prevents leakage from vascular tissues into the cortex. Lacking in gymnosperms and

most angiosperms, an endodermis is found in the stems of only a few dicots, usually those with rhizomes or other root-like stems.

Outline the x-section, and label to show the location of each underlined feature.

Monocot Stem Anatomy

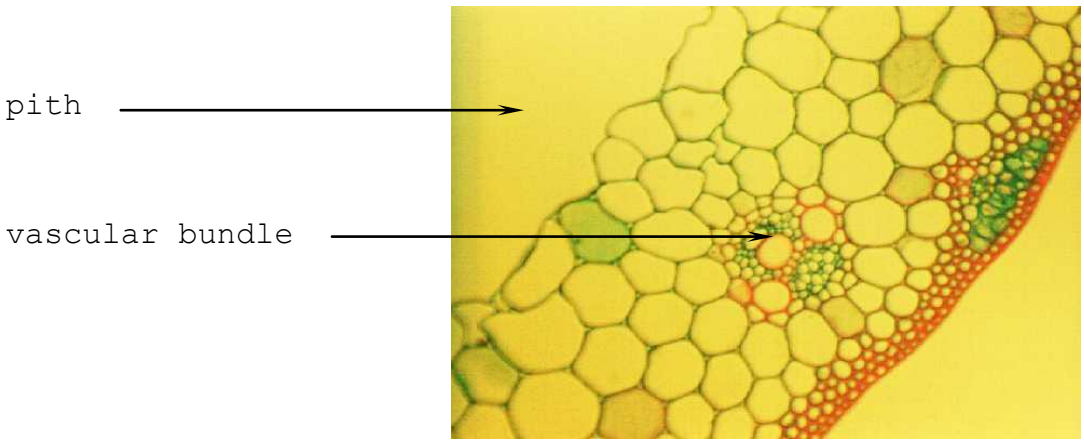
4. You may wish to review the *Zea mays* stem x-section. This species demonstrates typical monocot anatomy, in which vascular bundles are distributed throughout the parenchyma rather than in a single ring. While the pattern of vascular bundles may appear random in x-section, it actually represents a highly ordered arrangement involving both axial bundles and leaf traces.

Descriptions of xylem and phloem within *Zea mays* vascular bundles are found in Exercise 4 (No. 3) and Exercise 5 (No. 3).

Outline the x-section, showing the arrangement of vascular bundles.

5. Examine the x-section of Triticum (monocot) stem. In many grasses, vascular bundles are restricted to the periphery, often appearing in two rings. In Triticum and other grasses with this circular arrangement of bundles, a continuous cylinder of supporting sclerenchyma forms beneath the epidermis. Vascular bundles of the outer ring are embedded in this sclerenchyma. Often, the interior pith breaks down, except at the nodes. On most preparations, a leaf sheath encircles the stem. If the section was taken near a node, massive strands of collenchyma can be seen associated with vascular bundles of the leaf sheath.

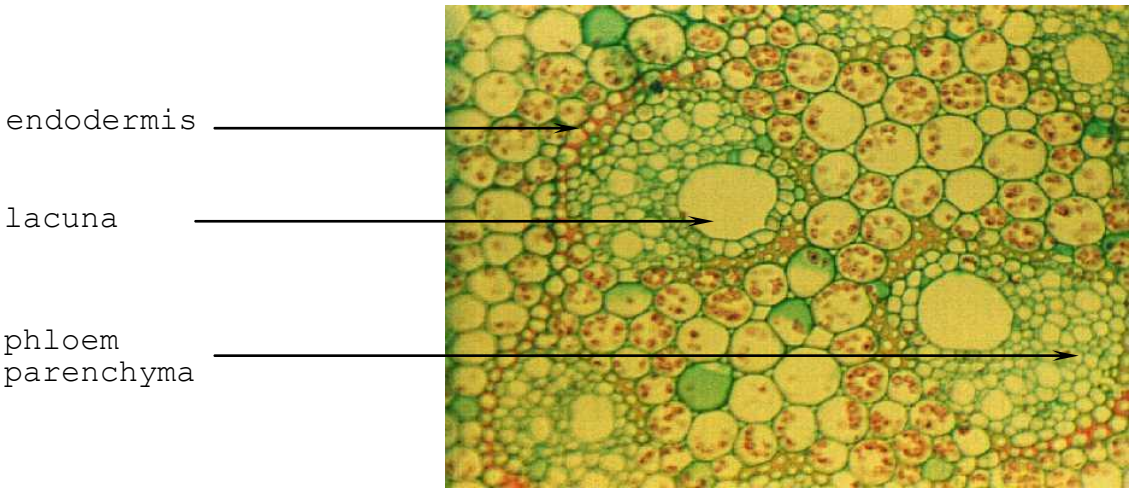
Outline the x-section and label to show the location of each underlined feature.



6. The stem of *Potamogeton* shows structural specialization

related to its aquatic habitat. A wide cortex composed of aerenchyma encloses the compact vascular cylinder. At high magnification, locate the uniseriate, small-celled endodermis, which encircles the vascular tissues. Well-defined sieve elements, companion cells, and phloem parenchyma can be seen within axial bundles and leaf traces. Xylem, however, consists only of broad lacunae, the remnants of tracheary elements torn during stem elongation.

Outline the x-section, showing the arrangement of aerenchyma and vascular bundles. Label with the underlined terms.



Exercise 9--Primary Root and Stem Development

Introduction

The anatomy of apical meristems has been studied extensively in both roots and shoots in order to trace the origin of major tissue systems. In seed plants, distinct regions or "zones" of cells which establish a basic structural pattern can be seen within all apical meristems. Traditionally however, different terminology has been used to describe the meristems of roots and shoots.

Materials

Prepared Slides:

Zea mays (corn) root tip, long. section
Pisum (pea) root tip, long. section
Lateral root development, x-section **or**
Salix (willow) lateral root series, x-section
Hedera (ivy) stem with adventitious root, x-section **or**
Lycopersicum (tomato) stem x-section with adventitious root
Equisetum (horsetail) shoot apex, long. section (optional)
Pinus (pine) shoot apex, long. section
Coleus shoot apex, long. section
Zea mays (corn) shoot tip, long. section
Elodea shoot tip, longitudinal section

Primary Root Development

1. View the long. section of *Zea mays* root tip. This monocot root develops by means of a closed system, in which the vascular cylinder, cortex, and root cap can be readily traced to distinct zones within the meristem. First observe that, in closed systems, initials which produce the root cap appear separate from the rest of the meristem. This is best seen by following the epidermis from older portions of the root toward the apex. The boundary between epidermis (protoderm) and root cap remains distinct. At the apex, the zone of meristem cells just exterior to the protoderm are columella mother cells which give rise to the central column of the root cap, or columella.

Just interior to the protoderm, is the zone of central mother cells. This region serves as a promeristem, contributing cells to two adjoining zones in the meristem: cortex mother cells (periblem) give rise to the cortex and epidermis; and central cylinder mother cells (pleurome) produce the vascular tissues.

The rate of cell division may be greatly reduced in and immediately around the central mother cell zone. This "quiescent center" is nearly always present in roots, and is thought to serve as a reserve pool of genetically sound cells. If the surrounding meristem is damaged, the central mother cells increase their rate of mitotic activity and reestablish the structural pattern.

Pericycle cells are the first to mature within the vascular cylinder, and the pericycle appears very near the apex as a distinct line of demarcation between vascular cylinder and cortex (best visualized at low magnification).

Outline the root tip, draw tissue details of the meristem, and label to show the location of each underlined feature.

2. Examine the *Pisum* root tip long. section. This meristem shows open development, in which zonation is not distinct and cortex, stele and rootcap appear to be derived from one group of initials. From an evolutionary standpoint, open systems probably are intermediate between closed systems and the primitive meristems of cryptogams, in which all tissues can be traced to a single apical cell.

Outline the root tip, draw tissue details within the meristem, and indicate the location of each underlined feature.

Lateral and Adventitious Roots

3. Examine the *Salix* lateral root series or other x-section showing lateral root development. In both gymnosperms and angiosperms, branch roots arise within the pericycle. The earliest stages of development involve periclinal divisions of cells within the pericycle, causing it to bulge outward into the cortex. The root primordium continues to enlarge by both anticlinal and periclinal divisions.

Initially, the endodermis keeps pace with the primordium (by anticlinal divisions only), and in some species contributes to formation of the root cap. However, the endodermis usually ruptures before the lateral root emerges. As the primordium lengthens, it pushes aside or crushes cortical cells, and eventually ruptures the epidermis. Notice that the vascular cylinder, cortex and rootcap of the lateral root already are well-defined at the time of emergence. Parenchyma cells between the primordium and vascular tissues of the parent stele differentiate into vascular elements and establish connections between old and new vascular tissues.

In triarch and tetrarch roots, lateral roots arise opposite (just exterior to) the protoxylem poles, in diarch roots between xylem and phloem, and in polyarch roots opposite the phloem.

Draw a x-section of parent root with emerging lateral roots. If the *Salix* lateral root series is available, outline each stage in the progression. Label with the appropriate underlined terms.

4. By definition, adventitious roots arise on plant organs other than the root. Emerging adventitious roots may be observed on stem x-sections of *Hedera* or *Lycopersicum*. In young dicot stems, adventitious roots usually arise from the interfascicular parenchyma, but may originate in epidermis, cortex, or immature vascular tissue. Although adventitious roots may be produced anywhere along the stem, they develop most commonly at lower nodes. Note the attachment of vascular tissues to vascular bundles of the stem.

Outline the x-section of *Hedera* or *Lycopersicum* stem, showing the adventitious root in long. section.

Primary Stem Development

In shoots, the apical meristem is defined anatomically as the distal portion of apex, including all cells down to initiation of the first leaf primordium. As with roots, initials within the meristem establish the basic structural pattern of epidermal, ground and vascular tissues. Zonation within the meristem typically is more distinct in angiosperms than in lower vascular plants and gymnosperms.

Patterns established in the meristem continue to develop by active cell division, growth, and differentiation. The transition from meristem to mature stem occurs along a gradient, whose length varies among taxonomic groups and even among tissues of a given plant.

Outline each of the long. sections described below and draw tissue details within the meristems. Label the underlined features.

5. The shoot apex of *Equisetum* typifies the structurally simple meristems of lower vascular plants. If this section is provided, examine the meristem at high magnification. A single large initial, the apical cell, dominates the meristem. The apical cell divides asymmetrically along its downward-directed faces, cutting off a series of daughter cells, and these divide rapidly to produce the shoot.

6. *Pinus* shoot apex shows the distinct zonation typical of seed plant meristems. *Pinus* and many other gymnosperms do not have a stable surface layer. Instead, the outermost layer of apical initials gives rise to both lateral and subadjacent derivatives. At high magnification, attempt to find apical initials undergoing periclinal division (cell plates parallel to outer surface). Meristems in which periclinal divisions occur within the outermost layer are sometimes called mantle-core meristems to distinguish them from true tunica-corpus meristems.

Beneath the apical initials is a zone of central mother cells, identified by their large size and cuboidal shape. These cells divide along the lower periphery of the mother cell zone to

produce the pith-rib meristem. Transverse divisions within the pith-rib meristem typically produce vertical files of cells.

Cells of the peripheral zone, intensely stained on most preparations, originate partly from lateral derivatives of apical initials and partly from the central mother cell zone. The peripheral zone is highly active mitotically, giving rise to leaf primordia, and contributing to increases in both length and width of the primary shoot.

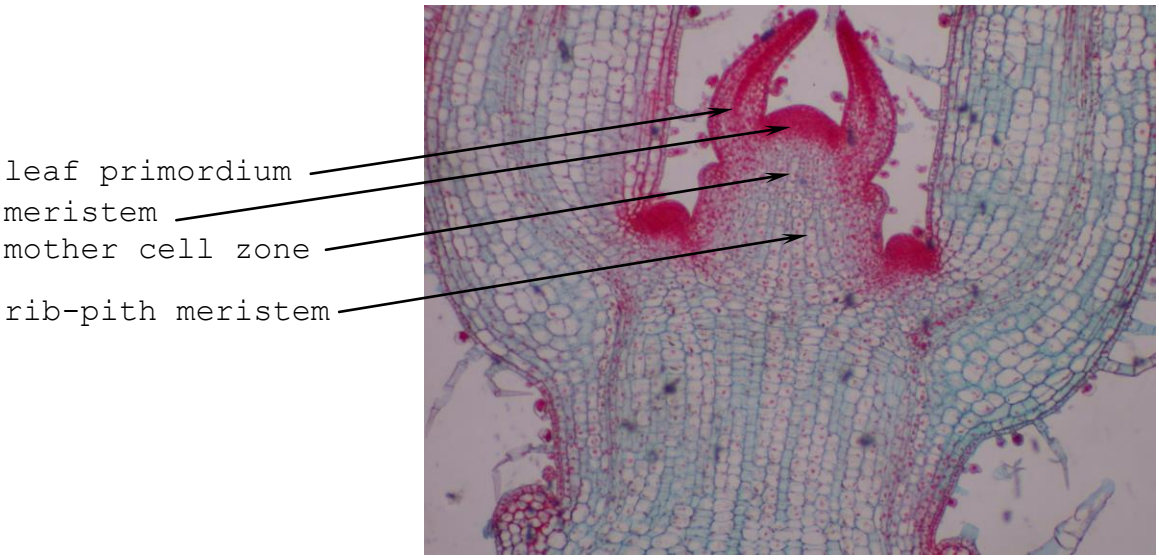
7. On the long. section of *Coleus* shoot apex, scan at low magnification to observe the overall arrangement of meristem, nodes and leaf primordia. The pattern of leaf production in *Coleus* is decussate (opposite). Procambial strands should be distinct within the youngest leaf primordia, and axial buds can be seen, beginning with the second or third leaf axils.

Observe the meristem at high magnification. *Coleus*, a dicot, shows typical angiosperm tunica-corpus organization. This tunica has two layers, a common number among dicots. Cells of the tunica divide only anticlinally (cell plates perpendicular to the surface).

Three zones may be identified within the corpus. The mother cell zone is just beneath the tunica. Divisions within the mother cell zone produce a flow of cells into the rib-pith meristem below, and into the peripheral zone.

On some sections, the meristem shows a slight protrusion on each side, referred to as the leaf buttress. This is the earliest stage of leaf initiation. Mitotic activity increases below the surface, and periclinal divisions produce the lateral protrusions. Anticlinal divisions in the surface layer keep pace with the developing primordium.

In many monocots, leaf primordia arise by periclinal divisions in the surface layer. If the tunica has multiple layers, the entire primordium may by derived from tunica. Commonly, however, the leaf is produced from derivatives of both tunica and corpus.



8. The *Zea mays* shoot apex shows tunica-corpus organization with a single layer of tunica. Note that leaf primordia encircle the apex, forming a closed, humid chamber which protects the meristem. This closed cone is common in monocots. The apex of many monocots remains below ground level, an arrangement of obvious adaptive value in forage grasses.

9. The shoot tip of *Elodea*, an aquatic monocot, is highly elongated, and the meristem is exposed rather than enclosed by developing leaves Note the great height of the meristem above the first leaf primordia.

Observe the pattern of differentiation below the meristem. Air passages and nodal plates gradually become distinct, and leaf traces diverge at a 90° angle from the stele. Few axillary buds form. Look instead for developing mucilage glands in the leaf axils.

Exercise 10--Secondary Growth

Introduction

In both roots and stems, secondary growth is produced by the activity of vascular cambium and phellogen (cork cambium). Secondary stem growth occurs in gymnosperms and woody dicots, and also in many herbaceous dicots. In ordinary secondary stem growth, the vascular cambium develops from procambium between xylem and phloem of vascular bundles, and from interfascicular parenchyma. Vascular cambium produces secondary xylem (wood) to the interior and secondary phloem to the exterior. One or a series of phellogens may develop in the epidermis, cortex, or outer phloem, and give rise to protective periderm.

Secondary growth also occurs in gymnosperm roots, and to some degree in the roots of most dicots. Both procambium and pericycle typically contribute to the vascular cambium. After initiation of the vascular cambium, the root phellogen arises by periclinal division of the outer pericycle. As root diameter increases, the endodermis, cortex, and epidermis usually are ruptured and cast off. This exercise also includes anomalous secondary root growth associated with specialized storage functions.

Materials

Prepared Slides:

Tilia (basswood) three or four year stem, x-section
Medicago (alfalfa) old stem, x-section
Aristolochia (Dutchman's Pipe) young and old stems, x-section
Medicago root, x-section
Pinus (pine) root, x-section
Beta, (beet) root, x-section
Raphanus (radish) root, x-section

Stem Secondary Growth

1. *Tilia*, a woody shrub, shows secondary growth features found in most woody dicots. Examine the section first at low magnification. Primary xylem forms an irregular boundary with the central parenchymous pith, and the primary elements merge imperceptibly with secondary xylem.

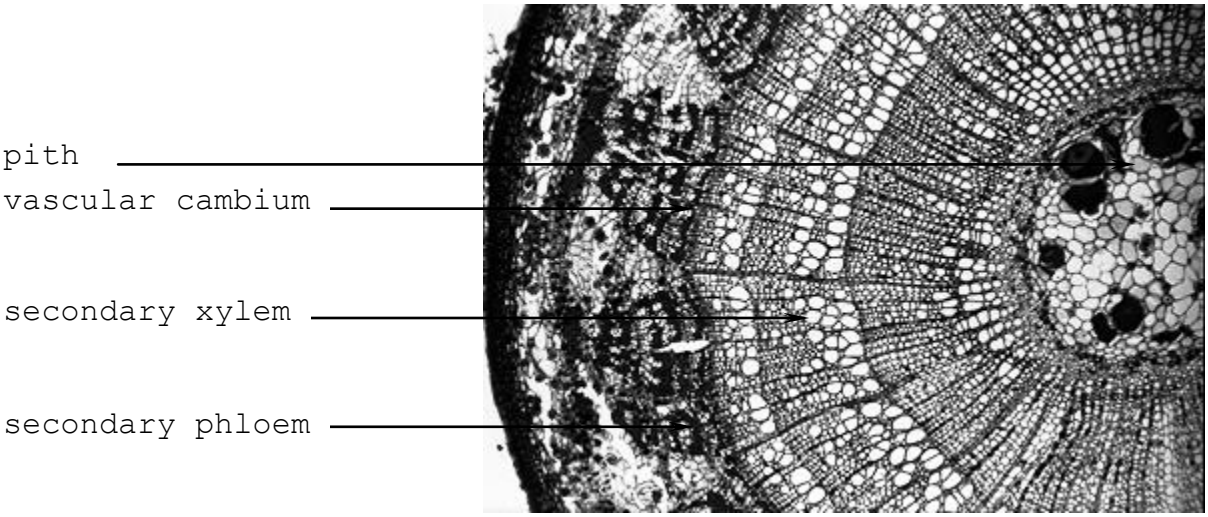
In this stem, the vascular cambium has produced a continuous cylinder of secondary xylem and phloem. Three or four distinct increments of secondary xylem (growth rings) can be seen, delineated by an abrupt change in the size of xylem elements at the boundary between summer wood and spring wood. Examine one of

these boundaries at high magnification. The cambium produces narrow, thick-walled elements (primarily tracheids) near the end of the growing season. When growth resumes the next spring, the cambium produces wider, thinner-walled elements (mostly vessels). Typically, dicot wood also contains fibers and xylem parenchyma, present here in banded paratracheal arrangement. Both wide and narrow rays are present.

Within the secondary phloem, bands of fibers alternate with areas composed of STMs and associated parenchyma. Look near the center of each wide phloem ray for evidence of recent tangential divisions in the ray parenchyma.

A narrow periderm is present, derived from epidermis. Beneath the periderm, the cortex is retained and may be distinguished from the outer primary phloem, which contains protophloem fibers. Fibers develop as the protophloem cells become nonfunctional.

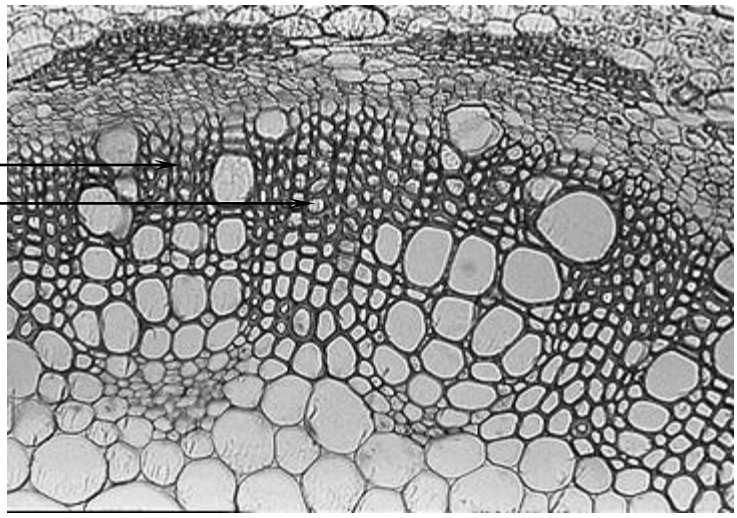
Outline this section, showing the arrangement of secondary xylem and phloem, and label with the underlined terms.



2. In many herbaceous stems, production of secondary xylem and phloem is limited largely to the vascular bundles. *Medicago* illustrates a common pattern. Cambium appears between primary xylem and phloem after elongation of the internode has stopped. In sections taken from the oldest portion of stem, fascicular and interfascicular cambia have fused to form a continuous circle (cylinder) of vascular cambium. Notice however, that the interfascicular portion of cambium has produced mostly parenchyma on the phloem (exterior) side and only sclerenchyma on the xylem (interior) side.

Draw two vascular bundles and intervening tissues. Label the underlined features.

fascicular cambium
interfascicular cambium



3. *Aristolochia* illustrates typical secondary growth in woody vines. The younger (smaller diameter) x-section is in the primary or early secondary state of growth. Vascular bundles surround a large parenchymous pith. Bundle caps of these widely-spaced collateral bundles have fused into a continuous ring of perivascular fibers. The cortex is made up of parenchyma and collenchyma, and in young stems includes a starch sheath, seen just exterior to the perivascular fibers.

On the older stem x-section, secondary growth has resulted in wedge-shaped segments of secondary xylem and phloem separated by wide rays. Ray parenchyma is produced by the interfascicular cambium. Expansion of the vascular tissues has ruptured the cylinder of perivascular fibers, and parenchyma cells have filled the gaps by intrusive growth. This arrangement provides strength, while maintaining flexibility in older woody stems. A prominent periderm has developed exterior to the cortex.

Outline this section and label with the appropriate underlined terms.

Root Secondary Growth

4. Examine the x-section of *Medicago* root, which illustrates the common type of secondary growth in herbaceous dicot roots. The vascular cambium is seen in x-section as a uniform circle, (it is actually a cylinder), of cells exterior to the secondary xylem. Vascular cambium usually is interpreted as a uniseriate, bifacial layer, which produces xylem and phloem mother cells by periclinal divisions. Within the secondary xylem, large vessels are arranged irregularly among fibers and parenchyma cells.

As secondary xylem matures, the cambium is pushed outward, and cambial cells keep pace with the expansion by anticlinal (multiplicative) divisions.

Wide rays composed of parenchyma divide the xylem into sectors and continue through the cambium and secondary phloem. In *Medicago*, rays are produced by cambium which originated in the portion of pericycle opposite (just exterior to) the protoxylem poles. Follow one ray inward to its origin opposite a protoxylem pole, and examine this area under high magnification. Primary xylem has been distorted and partially crushed by enlargement of secondary xylem elements.

Examine the secondary phloem (exterior to the vascular cambium), and identify STMs and companion cells. Primary phloem has been obliterated, and the outer secondary phloem is made up only of fibers and storage parenchyma.

Endodermis, cortex, and epidermis have been ruptured and cast off. Phellogen, derived from the outer pericycle, has produced a distinct protective periderm made up of several layers of cork cells.

Outline the x-section and show tissue details within one triangular sector (from interior to exterior). Label with the appropriate terms.

5. The roots of gymnosperms and woody dicots show similar patterns of secondary growth, but only tracheids are produced in gymnosperm xylem. Examine the x-section of *Pinus* root at low magnification. Note that the first year's xylem is square in x-section. Vascular cambium originates between primary xylem and phloem and forms a continuous cylinder, which initially conforms to the undulating outline of primary vascular tissues. During the first year, more xylem cells are produced in the depressions between xylem poles, and the cambium is pushed outward more rapidly in these areas. Observe that, by the end of the first year, the cambium is square, and by the second year has become circular.

Outline the section, showing the arrangement of secondary xylem, and label the underlined features.

6. The storage roots of *Beta* show an anomalous type of secondary growth in which a series of cambia develop outside the normal vascular cylinder. First, note the diarch primary stele surrounded by ordinary secondary growth. The first cambium stops dividing and differentiates completely. A new cambium then forms within the outer phloem and begins producing xylem, phloem and parenchyma. This pattern is repeated several times, as a series of new cambia develop, each functioning for a limited time. The result is the series of concentric layers of vascular tissue seen here, each made up of storage parenchyma, xylem and phloem. Secondary phloem found interior to secondary xylem is called included phloem.

Outline this section, showing the arrangement of vascular and storage tissues, and label with the underlined terms.

7. Another type of modified secondary growth may be observed in the fleshy taproot of *Raphanus*, in which abundant storage parenchyma is produced in close proximity to conducting tissues. An ordinary vascular cambium, near the periphery, has produced secondary phloem to the exterior and a broad region of secondary xylem. Look carefully within the secondary xylem for small areas of anomalous cambium, which have given rise to isolated strands of vascular tissue.

Outline the section, showing the location of anomalous secondary growth, and label the underlined features.

Exercise 11--Wood Anatomy

Introduction

Wood anatomy, the cellular composition and structure of secondary xylem, is useful in identification, and has become an important tool in phylogenetic analysis.

In both gymnosperms and woody dicots, secondary xylem is made up of an axial system, which functions primarily in longitudinal conduction, and a ray system, specialized for nutrient storage and transverse (radial) conduction. Elements of the axial system are derived from fusiform initials of the vascular cambium. Shorter ray initials in the cambium give rise to cells of the ray system.

Materials

Prepared Slides:

Pinus wood planes
Salix (willow) wood planes **or**
Acer (maple) stem, x-section
Quercus (oak) wood planes
Carya (pecan) wood, tang. section (optional)
Diospyros virginiana (persimmon) wood, tang. section
Sambucus (elderberry) lenticel, x-section

For Display:

Wood blocks cut in transverse, radial, and tangential planes

Gymnosperm and Dicot Wood

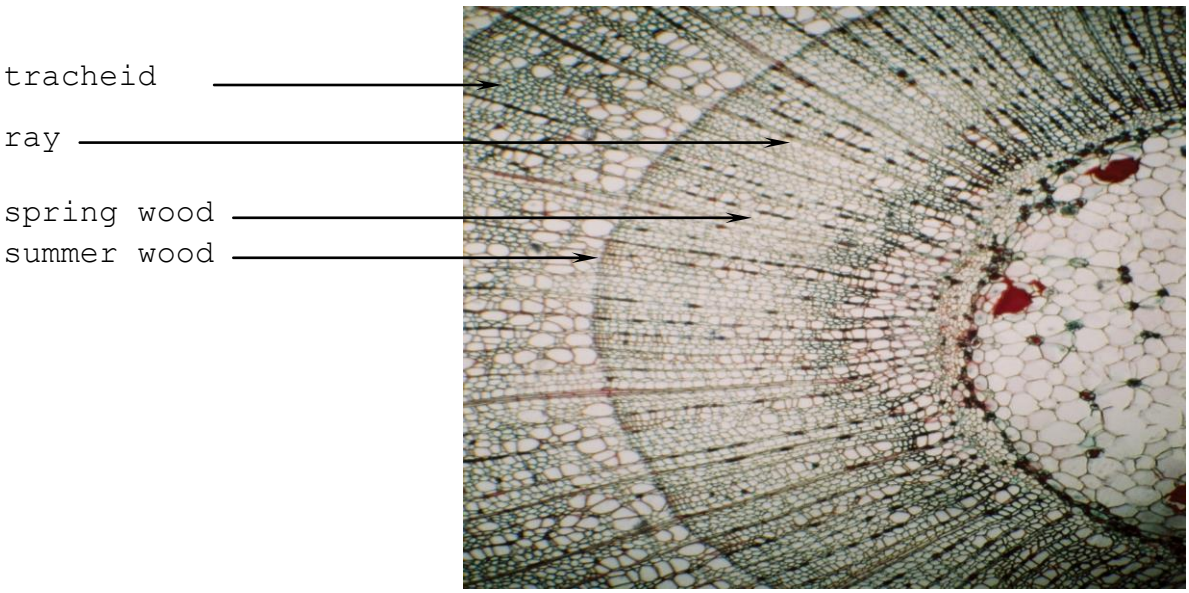
1. The three dimensional structure of wood is best understood when seen from more than one perspective. Look at the wood blocks on display to become familiar with transverse, radial and tangential planes. Also observe general features of wood anatomy, including growth rings, rays, heartwood, sapwood, and bark.

No drawing required.

2. *Pinus* illustrates the relatively simple wood structure of gymnosperms. On the x-section, observe the regular arrangement of tracheids within the axial system. In *Pinus* and most other gymnosperms, the axial system is made up entirely of tracheids. Uniseriate rays appear as dark radial bands in x-section.

Prominent resin ducts (circular in x-section) are lined with a single layer of epithelial cells.

At high magnification, observe the distinct boundary between narrow, thick-walled summer wood tracheids and broader, thinner-walled spring wood tracheids. Look closely for bordered pit pairs in the radial walls between tracheids. Note that bordered pits are not found in tangential walls, except in cells at the boundary between summer wood and spring wood. These pit pairs, which allow radial conduction between growth rings, are called growth ring bridges.



The tang. section reveals the long, tapered shape and overlapping end walls of axial tracheids. At high magnification, look for bordered pit pairs on the radial sides of overlapping walls. These permit longitudinal and circumferential conduction between tracheids.

Move up and down in the tang. section at low magnification and note that each tracheid is in contact with at least one ray. These uniseriate rays, three to seven cells tall, are typical of gymnosperm wood. Examine one ray at high magnification. In *Pinus*, rays are heterocellular (composed of both parenchyma cells and ray tracheids). Ray tracheids usually are found at the upper and lower margins of the ray. However, they are difficult to distinguish from ray parenchyma, which develop lignified walls.

On the radial section, locate areas of cross-field pitting, where axial tracheids are in contact with ray parenchyma and ray tracheids. Examine one of these areas at high magnification. In this plane, circular bordered pits between axial and ray tracheids appear in face view. These very large, oval pits are called fenestriform pits.

Draw a small area from each of the three planes, and label with the appropriate underlined features.

3. Dicot wood usually is more complex than that of conifers. Vessels, tracheids, fibers, and parenchyma are found in varying proportions and arrangements within axial systems. Rays contain only parenchyma, but may be structurally diverse, even within a single plant. Traditionally, dicot woods have been categorized according to the distribution of vessels (pores), as viewed in x-section.

View the *Salix* or *Acer* wood in x-section, and observe that vessels are relatively uniform in distribution, although their size decreases somewhat near the end of the growing season. This wood is classified as diffuse-porous. Vessels are solitary or in small radially-oriented clusters called pore multiples. Rays are uniseriate.

The tang. and radial sections reveal that rays in *Salix* are varied in height and heterocellular (contain both procumbent and upright parenchyma). Upright cells, seen at the margins of these rays, are specialized for transport between the radial and axial systems. On the tangential section, note also that *Salix* wood is non-storied. In non-storied wood, axial elements overlap unevenly and do not form uniform horizontal tiers.

Draw representative segments from each plane and label with the appropriate underlined terms.

tangential section

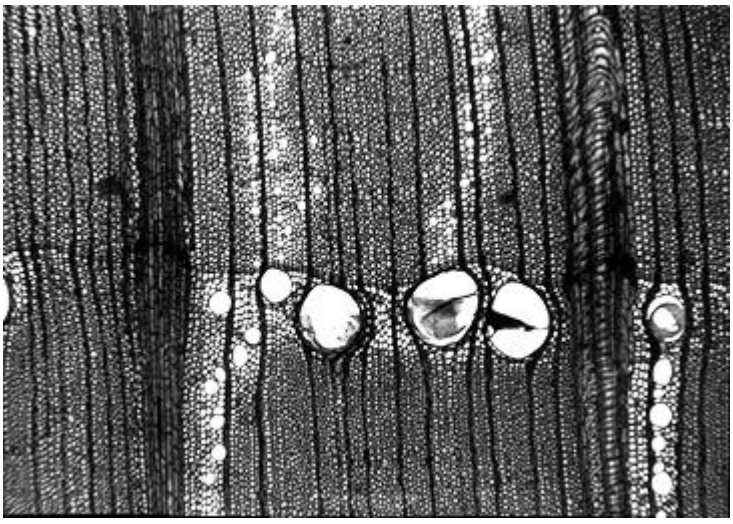
4. On the *Quercus* x-section, note that vessels are much larger and more numerous in spring wood than in summer wood. This wood is therefore classified as ring-porous. Broad spring wood vessels are extremely efficient conduits, offering far less resistance to water movement than the smaller diameter vessels of diffuse porous wood. However, wide vessels cavitate readily with increased water stress, and conduction in ring-porous wood may be limited to the outermost annual ring. Ring-porous wood is relatively rare, found mainly in northern temperate regions. It is considered to be more specialized than diffuse porous wood. Also on the x-section, locate axial parenchyma cells scattered among fibers and tracheids, Parenchyma cells may be identified by the presence of cytoplasmic contents.

In radial and tang. views, parenchyma appears as strands of relatively short, thin-walled cells lying between masses of long, overlapping fibers and tracheids. Abundant fibers make this wood extremely hard and durable.

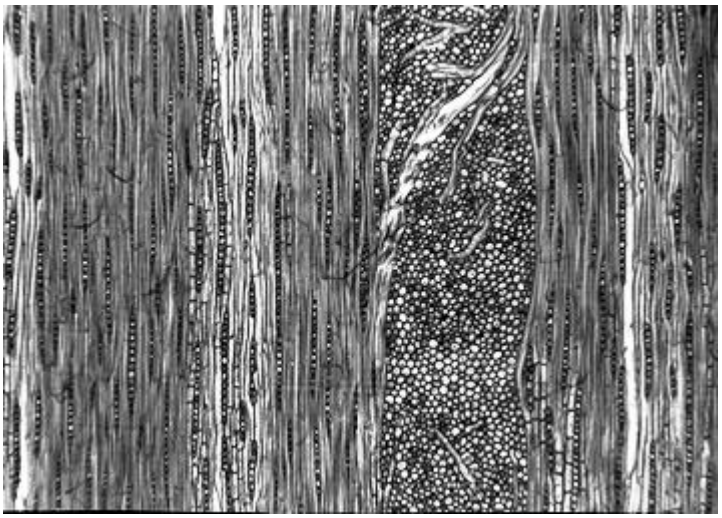
Vessels containing tyloses may be seen in all three planes. The tangential and radial views show multiple tyloses occluding the vessel lumen. The radial and tangential planes also reveal that *Quercus* wood is nonstoried. The ray system contains both low uniseriate and tall multiseriate rays.

Outline a sector of *Quercus* wood in x-section through two annual rings. Draw a representative area in tang. view, including one vessel with tyloses. Label both drawings with the appropriate underlined terms.

cross section



tangential section



5. If provided, examine the tang. section of *Carya*, another commercially important hardwood. This non-storied wood is ring-porous and contains a high proportion of fiber tracheids and long fibers, particularly in the summer wood.

No drawing required.

6. The tangential section of *Diospyros virginiana* shows storied structure in which both rays and axial elements are aligned in horizontal tiers. Vessel elements of this ring-porous wood have unusually thick walls.

Draw a small area in tangential view, showing the storied arrangement of vessels and rays, and label with the underlined terms.

7. Examine the *Sambucus* stem x-section and locate one or more lenticels on the outer surface. Within these modified areas of periderm, phellogen produces a mass of loose tissue, called filling tissue, to the exterior. Unlike normal cork cells, filling tissue contains intercellular spaces which permit gas exchange through the periderm. In *Sambucus*, the filling tissue is non-suberized and relatively unspecialized in structure.

Draw one lenticel, and label with the underlined features.

Exercise 12--Leaves

Introduction

As the plant's principle photosynthetic organs, foliage leaves function as the site of organic synthesis, a process essential for life on earth. Variations in leaf morphology and anatomy provide for maximum photosynthetic efficiency. Genetics and environment interact to dictate the nature of structural adaptations. Leaf venation pattern and stomatal configuration, in particular, have become increasingly important in the construction of natural phylogenetic groups.

In this exercise, leaf peridermal and x-sections illustrate features typically associated with monocot, dicot, and gymnosperm leaves, and also provide examples of structural variations related to environmental and physiological specialization.

Materials

Prepared Slides:

Syringa (lilac) leaf, x-section
Syringa leaf, peridermal section with venation
Nymphaea (water lily) leaf, x-section (optional)
Nerium (oleander) leaf, x-section (optional)
Syringa leaf abscission, long. section

Zea mays, (corn) leaf, x-section
Bouteloua (side-oats gramma) leaf, x-section
Iris leaf epidermis, whole mount
Iris leaf, x-section
Yucca (Spanish bayonet) leaf, x-section (optional)
Typha (cattail) leaf, x-section (optional)
Pinus leaf, x-section

Optional Fresh Materials:

Kalanchoe or other fleshy dicot leaves
Tradescentia zebrina (Wandering Jew) leaves
Zea mays leaves

Dicot Leaf Structure

1. *Syringa*, a woody shrub, displays typical bifacial (dorsoventral) mesomorphic leaf structure. A well differentiated single layer of palisade parenchyma lies below the adaxial (upper) epidermis. These columnar cells, oriented perpendicular to the surface, are the primary sites of photosynthesis.

Although they appear to be tightly packed under the light microscope, their elongated lateral walls actually are separated by a few micrometers, allowing free circulation of CO_2.

A chlorenchymous spongy mesophyll is present below the palisade layer. Throughout the mesophyll, intercellular spaces arise schizogenously during leaf growth, as palisade and spongy parenchyma cells are pulled apart by continued expansion of the epidermis and vascular bundles. Abundant intercellular spaces provide flexibility and facilitate CO_2 diffusion.

Note that stomatal complexes (stomata) are found on both upper and lower (abaxial) leaf surfaces (amphistomatic leaf), but are more numerous in the abaxial epidermis. This arrangement is typical for horizontally oriented mesophytic leaves.

Locate the large central vein (midrib) and note that it projects below rather than above the lamina (leaf blade), a nearly universal feature. The midrib contains a single collateral vascular bundle, with typical arrangement of adaxial xylem and abaxial phloem. Examine the midvein region at high magnification. Fibers are associated with both xylem and phloem, and abundant angular collenchyma are found just beneath both epidermal surfaces. Although the metaxylem is arranged in regular files similar to those produced by a vascular cambium, no secondary growth has occurred in this vein.

A number of first and second order lateral veins are visible within the mesophyll. Select a vein appearing in x-section and examine under high magnification. A bundle sheath made up of parenchyma surrounds the vascular tissues and extends to the upper and lower epidermises as bundle sheath extensions. Bundle sheath extensions normally accompany first, second, and third order veins, but not minor veins. They are believed to play a role in the transfer of water from xylem to mesophyll and epidermis. When sclerenchyma is present, the bundle sheath or sheath extensions also may help protect vascular tissues from attack by sucking or chewing insects.

Draw a complete adaxial to abaxial segment, including the midvein and one smaller vein in x-section, and one or two stomata. Label with the underlined terms.

2. Examine the prepared peridermal section (cut parallel to the surface) of *Syringa* leaf, which shows typical dicot craspedodromous reticulate venation. The smallest minor veins surround small areas of mesophyll called areoles; Observe that many minor veins terminate blindly at vein endings within the areoles. Minor veins distribute water into the mesophyll and function in the loading and translocation of photosynthate. At high magnification, look closely for xylem tracheids of the vein endings, identified by their annular or helical wall thickenings. In *Syringa*, conducting phloem is present in the minor veins, and is made up of STMs and relatively large companion cells. Note also the close association between bundle sheath parenchyma cells and minor veins. Both companion cells and bundle sheath parenchyma are believed to function in the loading of photosynthate and other solutes into sieve elements.

Diagram the venation pattern, and draw tissue details within two areoles. Include one vein ending showing terminal tracheids and one showing terminal phloem elements. Label the underlined features.

3. Prepare a whole mount of lower leaf epidermis from *Kalanchoe* or other fleshy dicot leaf, if provided. An irregular arrangement of ordinary epidermal cells, with undulating anticlinal walls, is typical in dicots. In *Kalanchoe*, two crescent-shaped guard cells surround each stomatal pore. The arrangement of subsidiary cells is defined as paracytic, a common configuration in which the subsidiary cells are in parallel alignment with the guard cells.

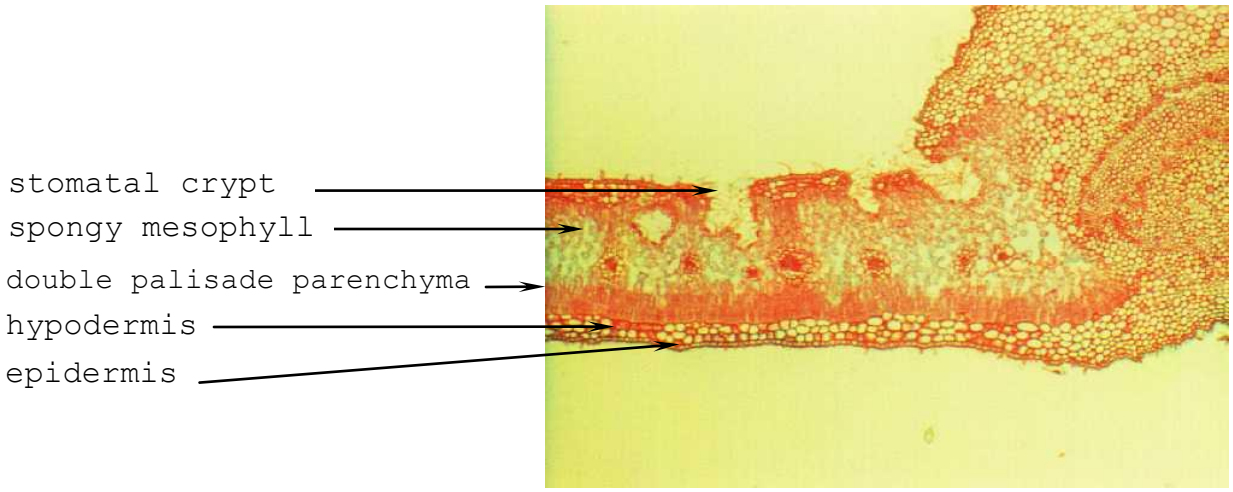
Draw a small area of epidermis, showing several ordinary epidermal cells and one stomatal complex.

4. The floating leaves of *Nymphaea* show a number of hydromorphic and other adaptations related to their habitat. The palisade parenchyma is several cells thick, a common arrangement in leaves exposed to strong sunlight. An extensive aerenchyma with huge air spaces within the spongy mesophyll provides flotation. Look within the mesophyll for large trichosclereids (stellate) with branches protruding into the air spaces. In Nymphaea and other plants with floating leaves, stomata occur only on the adaxial surface. Vascular bundles are stacked vertically within the midrib, an unusual structural variation.

Draw a small segment of the x-section, and label the underlined features.

5. *Nerium*, a xerophyte, shows several adaptations to a sunny, hot, and dry environment. A dense waxy cuticle and thick-walled epidermis and hypodermis (just beneath the epidermis) promote water retention and protect against excessive solar radiation. The mesophyll is compact, composed of a well-developed double palisade parenchyma and a reduced spongy mesophyll with small intercellular spaces. The resulting low internal surface to volume ratio conserves water but reduces CO_2 absorption. Stomata are restricted to trichome-lined stomatal crypts on the abaxial surface.

Outline a segment of the lamina from adaxial to abaxial surface. Draw a portion of abaxial epidermis, showing tissue details of one stomatal crypt. Label the underlined features.



6. Structural preparations for leaf abscission can be seen in the long. section of *Syringa* petiole. The abscission zone at the base of the petiole contains two layers. The abscission or separation layer, through which detachment occurs, appears as a band of thin-walled cells. Abscission involves dissolution of middle lamellae, enzymatic degradation of cell walls, and rupture of tracheary elements. Although not visible here, tylose formation and callose deposition often occur in vascular tissues before separation. Just proximal to the abscission layer is a protective layer (cicatrice), which seals the exposed surface after leaf fall. The protective layer is formed by deposition of suberin and wound gum within cell walls and intercellular spaces. In *Syringa* and other woody species, the protective layer is eventually replaced by periderm.

Outline the section and label to show the location of underlined features.

Monocot Leaf Structure

In the leaves of *Zea mays*, a panicoid grass, the mesophyll is not strongly differentiated into palisade and spongy parenchyma. This species is one of many grasses showing Kranz anatomy, a term used to describe anatomical features associated with C_4 metabolism. Within the mesophyll, prominent bundle sheaths are made up of large, relatively thick-walled parenchyma cells. Abundant chloroplasts within the sheath parenchyma are concentrated along cell walls nearest the surrounding mesophyll. A close spatial association between bundle sheath cells and mesophyll and a high organelle content (microbodies, chloroplasts and mitochondria) within sheath cells are characteristic of C_4 grasses. In contrast, bundle sheath cells of C_3 plants have few organelles and appear clear against the background of chlorenchyma.

Draw a narrow sector of the x-section, including a small vascular bundle, and label with the underlined terms.

8. C_4 anatomy is even more distinct in *Bouteloua*, a chloridoid grass. Examine one vascular bundle at high magnification. A double sheath made up of an inner mestome layer with thickened, suberized walls, and an outer sheath of thin-walled parenchyma surround the vascular tissues. The outer sheath is surrounded by mesophyll cells, which in x-section appear to radiate from the bundle. Kranz, a term originally used to describe this wreath-like arrangement of mesophyll, now includes both mesophyll and bundle sheath; and Kranz syndrome refers collectively to the structural and metabolic characteristics of C_4 plants.

Also conspicuous in the *Bouteloua* x-section are bulliform cells, very large epidermal cells with thin walls, found on the upper leaf surface. Bulliform cells, alone or in conjunction with adjacent hinge cells in the mesophyll, become flaccid during water stress and contribute to rolling or folding of the leaf.

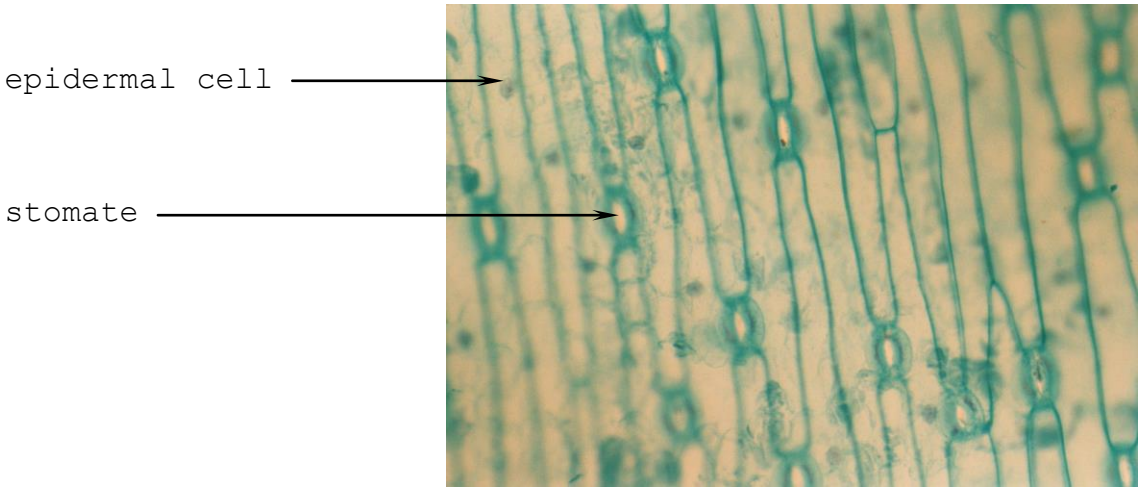
Outline the leaf x-section, showing the overall arrangement of vascular bundles and bulliform cells. Draw details of one vascular bundle and surrounding mesophyll as seen at high magnification, and label with the underlined features.

9. If fresh *Tradescantia* leaves are provided, prepare a whole mount of abaxial epidermis. Typically, in both monocots and dicots, stomatal complexes are scattered irregularly among ordinary epidermal cells. In *Tradescantia*, the crescent-shaped guard cells are surrounded by four subsidiary cells. The polygonal epidermal cells seen here have relatively straight anticlinal walls; however, sinuous epidermal cell wall contours also occur among monocots. An unusual feature of this epidermis is the presence of plastids containing anthocyanin pigments. These contribute to the purple-red coloration of *Tradescantia zebrina*.

Draw a small section of epidermis and label each underlined feature.

10. Observe the prepared whole mount of *Iris* epidermis. On the long blades of monocot leaves, epidermal cells may be arranged in a regular pattern, and elongated parallel with the leaf axis. Note that stomata are arranged in longitudinal rows which follow the overall pattern. Look for narrow bands of epidermis which do not contain stomata. Stomata usually are not found in epidermis which lies over vascular bundles, sclerenchyma, or other compact tissues with few intercellular spaces.

Draw a small section of epidermis, showing the arrangement of epidermal cells and stomata.

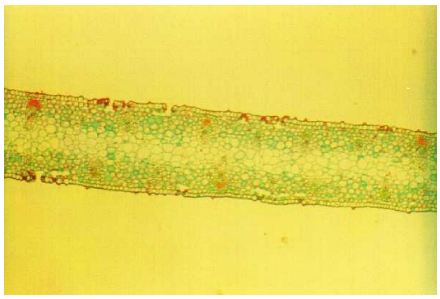


11. If fresh *Zea mays* leaves are available, prepare a whole mount of epidermis. Scan the preparation at low magnification and note that stomata are aligned in rows parallel with the leaf axis. Examine one stomatal complex at high magnification. These "dumbbell-shaped" guard cells are characteristic of grasses. Two subsidiary cells are oriented parallel with the guard cells (paracytic).

Diagram the arrangement of stomata and ordinary epidermal cells, and draw details of one stomatal complex. Label the underlined structures.

12. Examine the prepared Iris leaf x-section. Iris leaves, like those of many other monocots, are unifacial. Both sides are identical, and the leaf has the appearance of a single panel folded upon itself. Look closely at the vascular bundles, and note that xylem is always interior and phloem exterior. This type of unifacial leaf is believed to develop by exaggerated radial expansion of the adaxial meristem, with little or no enlargement of lateral meristems.

Outline the section and label the underlined features.



13. *Yucca* leaves are isobilateral, a xeromorphic feature also related to their vertical orientation. However, these leaves are not truly unifacial. The mesophyll is uniform and appears identical on both sides, but adaxial and abaxial surfaces are present, as evidenced by the orientation of vascular bundles. In each bundle, xylem is adaxial and phloem abaxial.

These leathery, perennial leaves have strongly developed sclerenchyma. Vascular bundles near the periphery have massive fibrous bundle caps which extend outward to the thick-walled hypodermis. Interior bundles are sheathed by fibers, and some bundles contain only fibers (fiber bundles).

Outline a portion of the x-section, showing arrangement of the vascular bundles, and label with the underlined terms.

14. *Typha* leaves have a complex isobilateral structure. Adaxial and abaxial mesophylls, each with a set of vascular bundles, are separated by a central region of large air passages. Vascular bundles also occur in the sheets of cells (septa) between air passages.

Outline a portion of the x-section and label to show the underlined features.

Gymnosperm Leaf Structure

15. The *Pinus* needle is representative of conifer leaf anatomy. A thick layer of cuticle covers the thick-walled epidermis, and a sclerified hypodermis lies just beneath the epidermis. Stomata, seen at regular intervals around the periphery of the x-section, are actually arranged in longitudinal rows parallel with the leaf axis. Guard cells of the stomata are deeply sunken, with overarching subsidiary cells.

Two vascular bundles are present in the needle, each with adaxial xylem and abaxial phloem. Transfusion tissue, made up of short tracheids and parenchyma, surrounds the vascular bundles. Look closely for circular bordered pits in the tracheid walls. Transfusion tissue is characteristic in conifer leaves, and probably functions in the movement of water and nutrients between the vascular bundles and mesophyll. An endodermis, with heavily suberized and lignified cell walls, surrounds the vascular and transfusion tissues.

The mesophyll in *Pinus* is uniform, but some gymnosperm leaves have well developed palisade parenchyma. Plicate (rosette) mesophyll cells, with prominent wall invaginations, are common in *Pinus* species, but not among conifers in general. Look also for resin canals within the mesophyll.

Outline the x-section. Draw and label tissue details of the underlined features.

Exercise 13--Trichomes, Secretory Structures, and Idioblasts

Introduction

Specialization of epidermal and ground tissue cells produces unique structures that enhance the plant's responsiveness to its environment. Many specialized structures are secretory (glandular), producing a wide variety of secondary plant products, including oils, digestive enzymes, mucilage, resin, gum, and latex. Secretory structures may be internal or external.

Trichomes (epidermal outgrowths) may be secretory, and often produce aromatic oils or toxic substances that discourage herbivory. Non-secretory trichomes, or plant hairs, protect surfaces from excessive sunlight, promote water retention, and mechanically discourage insect attack. Root hairs also are classified as trichomes.

Any cell which differs significantly from the surrounding tissue is referred to as an idioblast. These include isolated sclereids, lithocysts, and other cells that produce crystals or unusual inclusions.

Materials

Prepared Slides:

Elaeagnus angustifolia (Russian Olive) leaf, x-section
Verbascum thapsus (verbascum) leaf, x-section
Ricinus communis (castor bean) extra-floral nectary, x-section
Brassica oleracea (cabbage) hydathode, median long. section
Dionaea muscipula (Venus Flytrap) leaf, x-section
Drosera rotundifolia (sundew) leaf, x-section
Asclepias syriaca (common milkweed) leaf, x-section
Aloe stem, x-section
Ficus elastica (Rubber Tree) leaf, x-section

Optional Fresh Materials:

Aloe stems

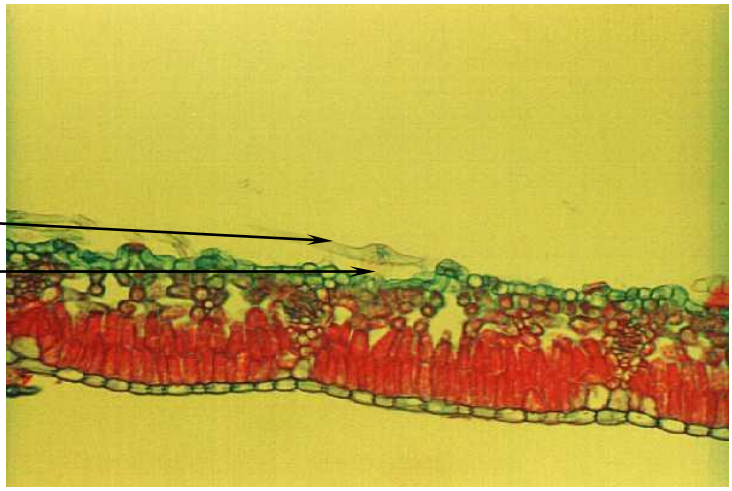
Trichomes

1. On the *Elaeagnus* leaf x-section, observe non-glandular trichomes (plant hairs), found primarily on the abaxial surface. These peltate trichomes are composed of branched cells joined along their lateral walls to form a disk-shaped structure. These trichomes differ from scales and squamiform hairs because they are supported by a stalk.

Draw and label one peltate trichome.

peltate trichome

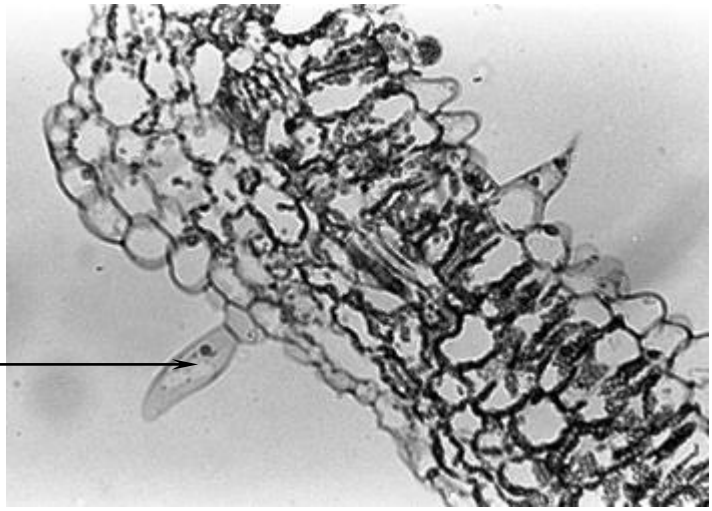
stalk



2. The *Verbascum* leaf x-section demonstrates both glandular and non-glandular trichomes. The non-glandular trichomes are dendroid, tree-like structures in which branch cells radiate from each "node." The glandular trichomes are distinctly capitate. The head is made up of one of more cap cells, each with a prominent nucleus. Close examination of the head should reveal the cell wall and cuticle, between which an oil secretion is found. Below the head is a supporting stalk, and a basal cell.

Draw both types of trichomes and label with the appropriate underlined terms.

dendroid trichome



Secretory Structures

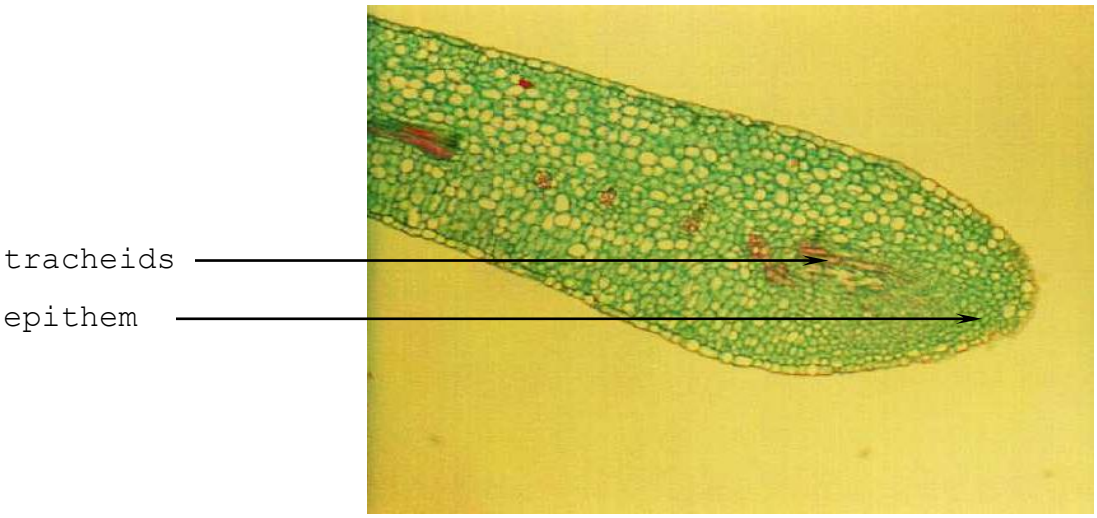
In addition to secretory trichomes, plants produce a variety of glandular structures, including nectaries, hydathodes, salt glands, osmophores, digestive glands, adhesive cells, resin canals, and laticifers.

3. Nectaries, like the extra-floral nectary of *Ricinus communis*, produce a sugary liquid that attracts pollinators. Locate a nectary on this section by following branches of vascular tissue, containing both xylem and phloem, that end in one or more layers of modified epidermal cells. These secretory cells have dense cytoplasm, small vacuoles, and often are covered externally by a cuticle.

Draw a x-section of the nectary and adjacent tissues. Label with the underlined terms.

4. Hydathodes, usually found at leaf margins or tips, release water from the leaf in a process called guttation. These structures are believed to function passively in the release of excess water, but some may also "pump" water actively to facilitate the transport of mineral nutrients. Inspection of the *Brassica* hydathode reveals small strands of tracheids ending in an area of mesophyll, called the epithem. Water passes from the tracheids, through intercellular spaces in the epithem, and out of the leaf through adjacent stomata. In *Brassica* hydathodes, the stomata cannot close, and are called water pores.

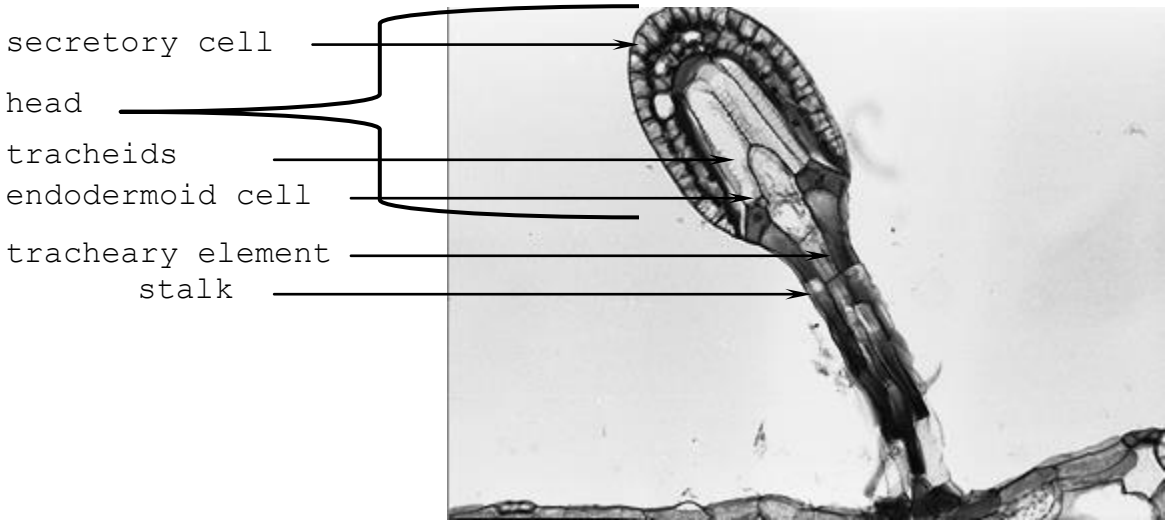
Draw the hydathode, label with underlined terms, and indicate the direction of water flow.



5. The specialized leaves of insectivorous plants produce external digestive glands. In *Drosera*, these glands are relatively complex. A narrow strand of tracheary elements (tracheids) branches from the vascular system and enters the gland through its stalk. Within the head, the tracheids are greatly enlarged. At the base of the head, the tracheids are surrounded by transfer cells, although these are difficult to distinguish. A thin layer of endodermoid cells completely encloses tracheids and transfer cells. Two layers of secretory cells cover the head. These large cells produce proteolytic enzymes and absorb digested nutrients.

In *Dionaea muscipula*, the digestive gland is a short, stalked structure not connected to the vascular system of the plant.

Draw both digestive glands and label the tissue details of *Drosera*.

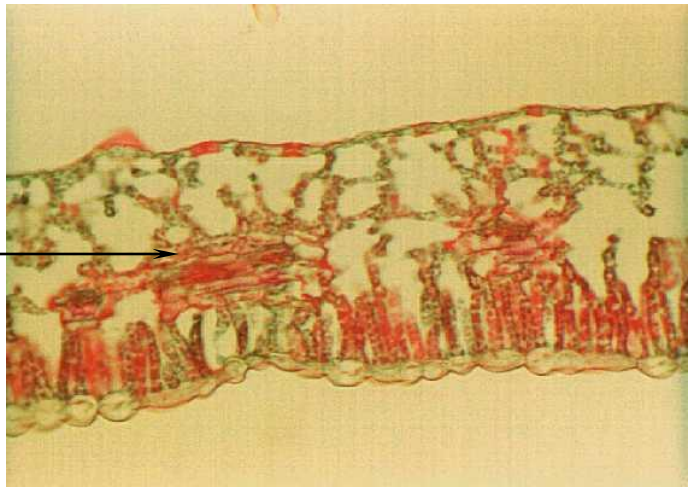


6. Laticifers are internal secretory structures which produce latex, a chemically complex, usually milky-white fluid. The function of latex is unknown, although it likely deters herbivory. Often called latex tubes, laticifers may be single-celled (non-articulated) or multicellular structures (articulated). Both forms may be long and highly branched. They occur most often in soft tissues, but may be found in wood.

Branching, non-articulated laticifers may be observed within the spongy mesophyll of *Asclepias syriaca* leaf. Non-articulated laticifers originate within the embryo and, as root and shoot elongate, invade newly formed tissues by intrusive growth. In contrast, articulated laticifers grow by adding new parenchyma cells, which differentiate and fuse with the existing structure.

Draw a representative portion of x-section showing vascular tissues and laticifers.

non-articulated laticifer



Idioblasts

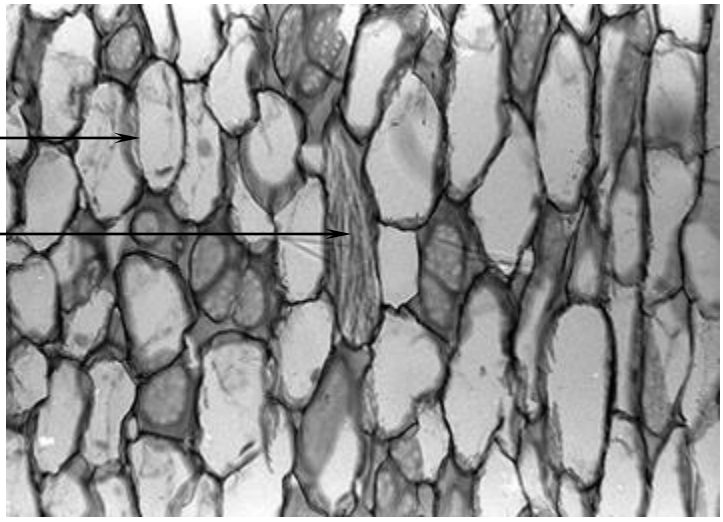
7. Crystals and cells containing crystals are classic idioblasts, differing markedly from neighboring cells. Crystals usually are composed of calcium oxalate. They may be classified as druses (square, star-shaped, or prismatic), raphides (needle-shaped, occurring in bundles), and styloids (long and rod-like, occurring singly or in pairs).

Raphides can be seen in fresh preparations or the prepared x-section of *Aloe* stem. Many pith parenchyma cells contain large bundles of these needle-like crystals. Since calcium oxalate crystals are colorless, they are best observed at low to medium light intensity. In fresh preparations, parenchyma cells may be disrupted, releasing the crystals.

Draw several parenchyma cells containing raphide bundles.

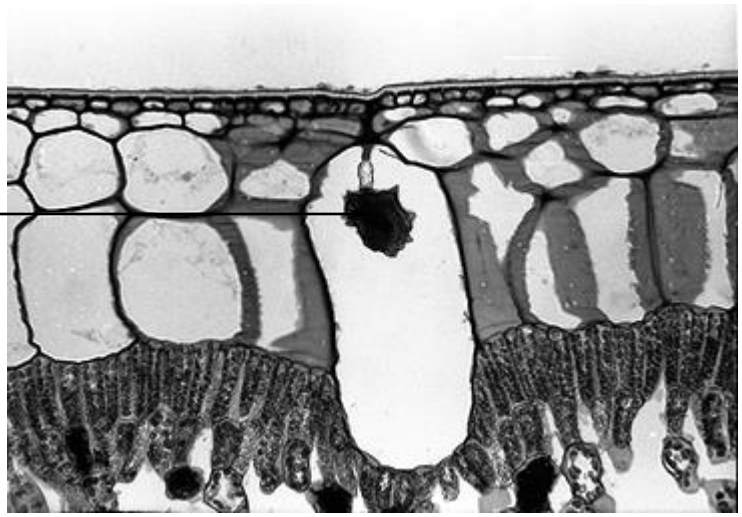
pith parenchyma cell

raphides



8. The prepared x-section of *Ficus elastica* leaf demonstrates an unusual type of crystal cell called a lithocyst. Look within the upper epidermis for large cells containing a single crystalline inclusion called a cystolith. The cystolith appears to "hang" from a stalk connected to the cell wall. Cystoliths typically are found in epidermis, and are composed of calcium carbonate.

Draw a portion of leaf epidermis, including one lithocyst, and label with the underlined terms.



Exercise 14--Reproduction and Life Cycle

Introduction

The life cycle of all plants involves an alternation of generations between haploid gametophytes and diploid sporophytes. The evolutionary trend has been from a dominant gametophyte generation in lower plants toward increasing dominance of the sporophyte in higher plants. The ultimate expression of this trend is found among angiosperms, in which gametophytes are borne within, and nutritionally dependent upon, floral structures of the "parent" sporophyte. Within the flower, cellular events that produce embryo and endosperm are unique to the angiosperms.

In this unit, the student will study the life cycle of *Lilium* (lily). Although polygonum embryo sac development is the simplest and most common among angiosperms, fritillaria development in *Lilium* was selected for study because cellular events are easy to observe in the large ovules of this species.

This exercise also includes examination of reproductive structures in *Pinus*, a representative gymnosperm.

Materials

Prepared Slides:

Lilium (lily) anther pollen tetrads, x-section
Lilium anther mature pollen, x-section

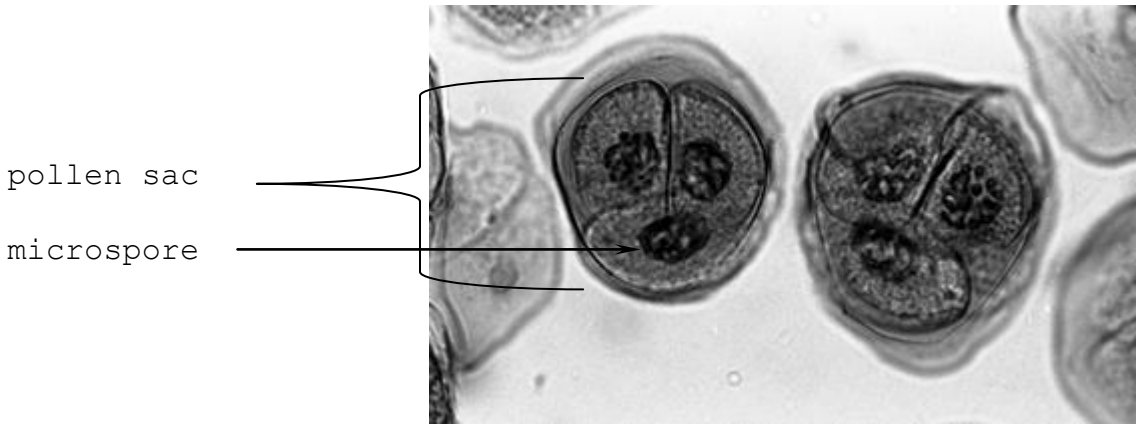
Lilium ovary megaspore mother cell, x-section
Lilium ovule 2-nucleate stage (end of meiosis I), x-section
Lilium ovule megaspores (lst 4-nucleate stage), x-section
Lilium ovule 2nd 4-nucleate stage, x-section
Lilium ovule, mature embryo sac (8-nucleate stage), x-section

Pinus (pine) mature male strobilus, long. section
Pinus female strobilus with megaspore mother cell, long. section
Pinus mature female strobilus, with megagametophytes (optional)

Lily Life Cycle: Stamen

1. Microsporogenesis and the development of pollen grains (male gametophytes) take place within the anther. Examine the slide labeled *Lilium* anther pollen tetrads. This x-section reveals four microsporangia, often called pollen sacs, within the anther. Masses of microspore mother cells (microsporocytes) lining the pollen sac undergo meiosis, each yielding a tetrad of four haploid microspores.

Outline the x-section and label to show the location of each underlined feature. Enlarge and label one tetrad.



2. Look next at the *Lilium* slide labeled anther mature pollen. Microspores of the tetrad separate, and each undergoes one division to form a pollen grain (male gametophyte). Each mature pollen grain contains two cells: a large vegetative cell (tube cell) and a smaller generative cell. The nuclei of these two cells are clearly visible within each pollen grain, but the plasmalemmas are not resolved. When the pollen grain germinates on the stigma, the tube cell develops into a pollen tube, which grows downward through the style and enters an ovule within the ovary. The generative cell will divide to produce two sperm (male gametes).

Outline the x-section. Enlarge and label one pollen grain.

Lily Life Cycle: Pistil

Megasporogenesis and development of the embryo sac (female gametophyte) take place in the ovule, within the ovary. Each diploid megasporocyte undergoes meiotic division to form four haploid megaspores. In polygonum development, the most common type, three of the megaspores degenerate and only one divides mitotically to produce the eight-celled embryo sac. This scheme is termed monosporic. *Lilium*, however, follows the tetrasporic fritillaria scheme. Cell walls do not form between the megaspore nuclei, and all four participate in formation of the embryo sac.

Study the five *Lilium* ovule x-sections in the order listed to visualize the series of divisions that produce the megaspores and embryo sac. Pay particular attention to the size of nuclei, an indication of ploidy. Outline each section, showing tissue details of the ovule, and label with underlined terms.

3. Ovary megaspore mother cell. The ovary contains three chambers, or locules, each with two vertical rows of ovules. Examine a single ovule very carefully. The stalk which connects the ovule to the ovary wall is called the funiculus. Note that the ovule bends back 180° toward the ovary wall, and lies alongside the funiculus. This type of ovule, termed anatropous, is the most common. In contrast, atropous ovules are borne upright on a straight funiculus.

The nucellus is the central mass of cells in which the embryo sac will develop. It includes the large megasporocyte, or megaspore mother cell. The basal portion of the nucellus, nearest its attachment to the funiculus, is called the chalaza, and that end of the ovule is the chalazal end. In *Lilium*, inner and outer integuments arise from the funiculus to enclose the nucellus. The integuments do not close completely around the nucellus, leaving a small opening, the micropyle, at the far end. Pollen often enters the ovule through the micropyle. In this anatropous ovule, the micropylar end is nearest the ovary wall.

4. Ovule 2-nucleate stage. In this ovule, the megasporocyte has completed its first meiotic division (meiosis I). Two haploid nuclei are visible within the embryo sac.

5. Ovule megaspores first 4-nucleate stage. The second division of meiosis (meiosis II) has resulted in 4 haploid megaspores. Recall that in the fritillaria scheme, cell walls do not form between the megaspores. One megaspore nucleus remains at the micropylar end. The other three migrate to the chalazal end.

6. Ovule second 4-nucleate stage. The nucleus at the micropylar end divides by mitosis, resulting in two haploid nuclei. As the three nuclei at the chalazal end begin to divide, fusion occurs, producing a single large, triploid nucleus. Mitosis continues, resulting in two triploid nuclei.

7. Ovule mature embryo sac 8-nucleate stage. All four nuclei divide again to form the 8-nucleate mature embryo sac. One triploid nucleus at the chalazal end and one haploid nucleus at the micropylar end migrate to the center of the embryo sac. These are the polar nuclei. The remaining three triploid cells at the chalazal end are called antipodals. One of the three remaining haploid nuclei at the micropylar end becomes the egg (female gamete). The other two are synergids.

At fertilization, two sperm enter the embryo sac. One unites with the egg to produce the zygote. The other fuses with the polar nuclei, to produce the endosperm (pentaploid in *Lilium*). This double fertilization occurs only in angiosperms.

After fertilization the mature embryo sac develops into a seed, containing the embryo and nutritive endosperm. Seed anatomy and embryogenesis in angiosperms are included in Exercise 15 (Seeds and Seedlings)

Gymnosperm: Strobili

9. In gymnosperms, microspores and pollen are produced within microsporangia on microsporophylls (scales) of the male strobilus (cone). Examine the prepared slide of *Pinus* mature male strobilus long. section. The main axis of the cone bears a series of microsporophylls, structurally related to primitive leaves. Each bears one or more microsporangia, in which microspores are produced. On most prepared slides, microspores already have developed into mature pollen. Examine pollen within a microsporangium at high magnification and note that each grain has two rounded wing-like expansions which enhance windborne pollination.

Draw several microsporophylls, showing their arrangement on the male strobilus, and label with the underlined terms.

10. Female (megasporangiate) strobili, or seed cones, are more complex and usually larger than male cones. Examine a prepared slide of *Pinus* female cone. The main axis bears heavily sclerified leaf-like structures (bracts). At the base of each bract is a highly modified axillary bud. Each bud axis bears a single ovuliferous scale In *Pinus*, each ovuliferous scale is fused to the bract and bears two ovules on its upper surface. Usually, only one ovule is visible on each scale in long. sections.

Within immature ovules, the large megasporocyte (megaspore mother cell), is visible within the megasporangium (nucellus). This cell will undergo meiosis, usually yielding only one viable megaspore. A single integument protects the developing ovule, which otherwise is completely exposed when the strobilus opens.

In mature female ovules, the megagametophyte is much larger than the eight-celled embryo sac of angiosperms. In *Pinus*, it contains two eggs and thousands of cells.

Draw a bract, ovuliferous scale, and ovule. Label the underlined features.

Exercise 15--Seeds and Seedlings

Introduction

Fertilization stimulates embryogenesis and development of the ovule into a mature seed. Nearly all seeds contain the same basic elements. Each is composed of a dormant sporophyte embryo, food reserves stored in special nutritive tissues or within the embryo, and a protective seed coat (testa), derived from one or both integuments. The seed coat protects the embryo and maintains dormancy until environmental conditions are favorable for germination and development. Food reserves ensure growth of the seedling until photosynthesis is established. Because seeds increase the likelihood of successful reproduction and dispersal, they have been a major factor in the spectacular evolutionary success of higher plants, especially the angiosperms.

This investigation focuses on seeds, seedlings, and embryogenesis in angiosperms.

Materials

Prepared Slides:

Cucurbita (pumpkin) seed, x-section
Zea mays (corn) embryo, near-median long. section
Capsella (shepherd's purse) embryo series, long. sections

Fresh Materials:

Soaked *Phaseolus* (lima or similar bean) seed
Soaked *Zea mays* kernel
Phaseolus, *Pisum* (pea), and *Zea mays* seedlings

Dicot Seeds and Seedlings

1. Examine a soaked *Phaseolus* seed. On the concave surface, locate the micropyle, a small pore where the pollen tube entered the ovule, and the hilum, an oval scar left by abscission of the funiculus from the ovary wall. The thin seed coat, or testa, is impervious to water and air in the dormant seed, but is easily peeled away after the seed has begun to imbibe.

After the testa is removed, all remaining tissues in this seed are part of the embryonic plant. Gently separate the two fleshy cotyledons, and examine the embryo with a hand lens or dissection microscope. The cotyledons, sometimes called seed leaves, contain food reserves which will supply the emerging seedling until it becomes photosynthetically independent. Inspection will reveal well-developed primordia of the first foliage leaves (plumule). The epicotyl is the portion of

embryonic axis between the plumule and the point where cotyledons are attached (cotyledonary node). The hypocotyl is defined as the portion of axis below attachment of the cotyledons. The embryonic root, or radicle, is well developed in this embryo.

Most dicot seeds, including those of *Phaseolus*, contain little or no endosperm at maturity. Endosperm produced following double fertilization is digested and absorbed by the developing embryo, and food reserves are stored primarily in the cotyledons. Seeds of this type are classified as exalbuminous.

Draw external and internal features and label with the underlined terms.

2. Observe the prepared x-section of *Cucurbita* seed. At high magnification, several layers should be visible within the testa (seed coat), including a prominent uniseriate layer of sclerenchyma, made up of heavily lignified sclereids. Interior to the sclerenchyma is a multiseriate layer of irregular parenchyma cells. A narrow, darkly-stained band containing nucellar and endosperm cells lies just exterior to the cotyledons, which fill the seed's interior. Two vascular bundles are usually visible within the testa, one on each side of the seed.

Outline the x-section and label to show the location of each underlined feature.

3. Examine the dicot seedlings on display. Bean seedlings demonstrate epigeal emergence, in which the hypocotyl elongates first and raises the cotyledons above ground level. Fleshy cotyledons, like those of *Phaseolus*, continue to supply nutrients until reserves are depleted, then wither and are shed. Compare the first foliar leaves, epicotyl, cotyledons, hypocotyl, and taproot with the corresponding embryonic structures.

The *Pisum* seedling shows hypogeal emergence, in which the epicotyl elongates, and the cotyledons remain below ground level.

Sketch both seedlings and label with underlined terms and structures.

Monocot Seeds and Seedlings

4. Observe the external features of a soaked *Zea mays* kernel, The corn kernel is actually a dry, indehiscent fruit called a caryopsis, which contains a single seed fused to the ovary wall. The outer covering of the kernel is therefore a pericarp derived from ovary wall (see Exercise 16), rather than a seed coat derived from integuments. Carefully cut the kernel longitudinally into two equal halves. A few drops of iodine, dropped onto the cut faces, will highlight the starchy endosperm, which fills more than half the seed. Seeds which contain a large endosperm at maturity are classified as albuminous, the most common type in monocots. Under the dissecting microscope, also

attempt to identify the single large cotyledon and the embryo.

Sketch the kernel in long. section as it appears under the dissection microscope. Label, using the underlined terms and structures.

5. Study structural details of the *Zea mays* embryo on a prepared near-median long. section. Grass embryos usually are well differentiated, and their morphology is complex.

The embryo is pressed against the endosperm by its single large cotyledon, the scutellum. The scutellum is attached to the embryo axis at the mesocotyl (scutellar node), often interpreted as the first node of the embryonic axis. After germination, the scutellum digests and absorbs the endosperm.

The epicotyl of the embryonic axis bears several leaf-like primordia. The first of these is the coleoptile, a protective sheath which encloses the shoot axis. During germination, the coleoptile elongates, emerges from the kernel, and pushes upward to the soil surface. Above ground, the foliage leaves emerge through a slit near the apex of the coleoptile. One or more leaf primordia also encircle the apex, forming a closed, humid cone. In grasses, the embryonic root or radicle also is protected by a sheath of tissue, the coleorrhiza. Some anatomists suggest that the coleorrhiza represents the true radicle, and the enclosed meristem is that of a lateral root primordium.

Outline the embryo and label to show the location of each underlined structure.

6. Observe the *Zea mays* seedlings on display. Most monocots, including the grasses, show hypogeal emergence. The cotyledon remains below ground, within the seed, where it continues to absorb nutrients from the endosperm.

Gently remove a seedling from the soil and examine both the root and shoot. The primary root, derived from the radicle, emerges from the kernel. Adventitious roots also should be present, the first whorl emerging from the first (scutellar) node within the kernel, and the second whorl from the second (coleoptilar) node. The portion of shoot between node 2 and the first leaf represents the coleoptile.

Sketch the seedling and label with the underlined terms and features.

Embryogenesis

Make an outline drawing of each stage described below and label to show the location of underlined features.

7. Long. sections of *Capsella* embryo show the typical progression of embryogenesis and seed maturation in dicots. Examine the slide labeled "embryo before cotyledons," and first locate the stalk-like funiculus, by which the ovule was attached

to the placenta. This ovule, like that of *Lilium*, is anatropous (inverted). At this early stage, the nuclear endosperm (light area in which no cell walls are visible), nearly fills the embryo sac. Free nuclei are displaced to the periphery of this coenocytic endosperm by a large central vacuole. In the ovule's micropylar region, the filamentous suspensor, with its very large, highly vacuolated basal cell, is clearly visible. Nucellar tissue not yet digested by the endosperm can be seen in the chalazal region.

Axial polarity within the embryo begins in the zygote, which divides unequally to establish chalazal and micropylar poles. The embryo proper develops from cells of the chalazal pole. Cells of the micropylar pole form the suspensor, which anchors the embryo and pushes it into the endosperm. The embryo proper, seen here at an early globular stage, appears as a small sphere of cells on top of the suspensor. At high magnification, protoderm and the beginning of procambium may be visible within the globular embryo.

Capsella ovules have both an inner and outer integument (bitegmic). The single layer of intensely-stained cells in contact with the embryo sac is the endothelium, or integumentary tapetum. This layer is derived from the epidermis of the inner integument, and is believed to be nutritive tissue which plays a role in absorption of nutrients by the embryo.

8. The second slide, labeled "embryo with cotyledons," is at the early cotyledon stage. The two cotyledons have begun to expand, giving the embryo a heart-shaped appearance. The endosperm remains free-nuclear.

9. The "embryo with bending cotyledons," is sometimes described as torpedo-shaped. The hypocotyl is elongated. The radicle and root cap have begun to differentiate, and the enlarging cotyledons have begun to bend toward the chalazal region. Strands of procambial tissue are clearly visible in the hypocotyl, and extend into both cotyledons. Although an epicotyl does not develop in *Capsella* embryos, the apical meristem is organized and can be seen as a small raised area between the cotyledons. At this stage, the endosperm has become cellular.

10. At the "mature embryo" stage, all the nucellar tissue has been digested. The endosperm, in turn, has largely been absorbed by the embryo. This type of exalbuminous seed is common in dicots. Except for its basal cell, the suspensor has been crushed by the enlarging embryo, Note also that the integuments have been modified into a highly sclerified seed coat.

Exercise 16--Fruits

Introduction

The fruits of flowering plants represent a major evolutionary innovation, offering the developing seeds greater protection and more efficient means of dispersal. "True" fruit is defined anatomically as the ripened ovary of a flower, produced by enlargement and modification of the ovary wall. Many fruits, however, incorporate other floral parts and/or portions of receptacle or pedicel. These are defined as accessory fruits.

The transformation from flower to fruit is a complex process, and the derivation of specific tissues within the mature fruit is often difficult to trace. A number of classification schemes have been devised. The flow chart used in this exercise follows one commonly-used scheme based on the number of ovaries represented in the mature fruit (simple or compound), the nature of the fruit wall (fleshy or dry), and the method of seed dispersal (dehiscent or indehiscent).

Materials

Prepared Slides:

Citrus, young fruit, x-section.
Cucumis sativus (cucumber) young fruit, x-section
Asclepias syriaca (common milkweed) fruit, x-section
Capsella (Shepherd's Purse) fruit, x-section

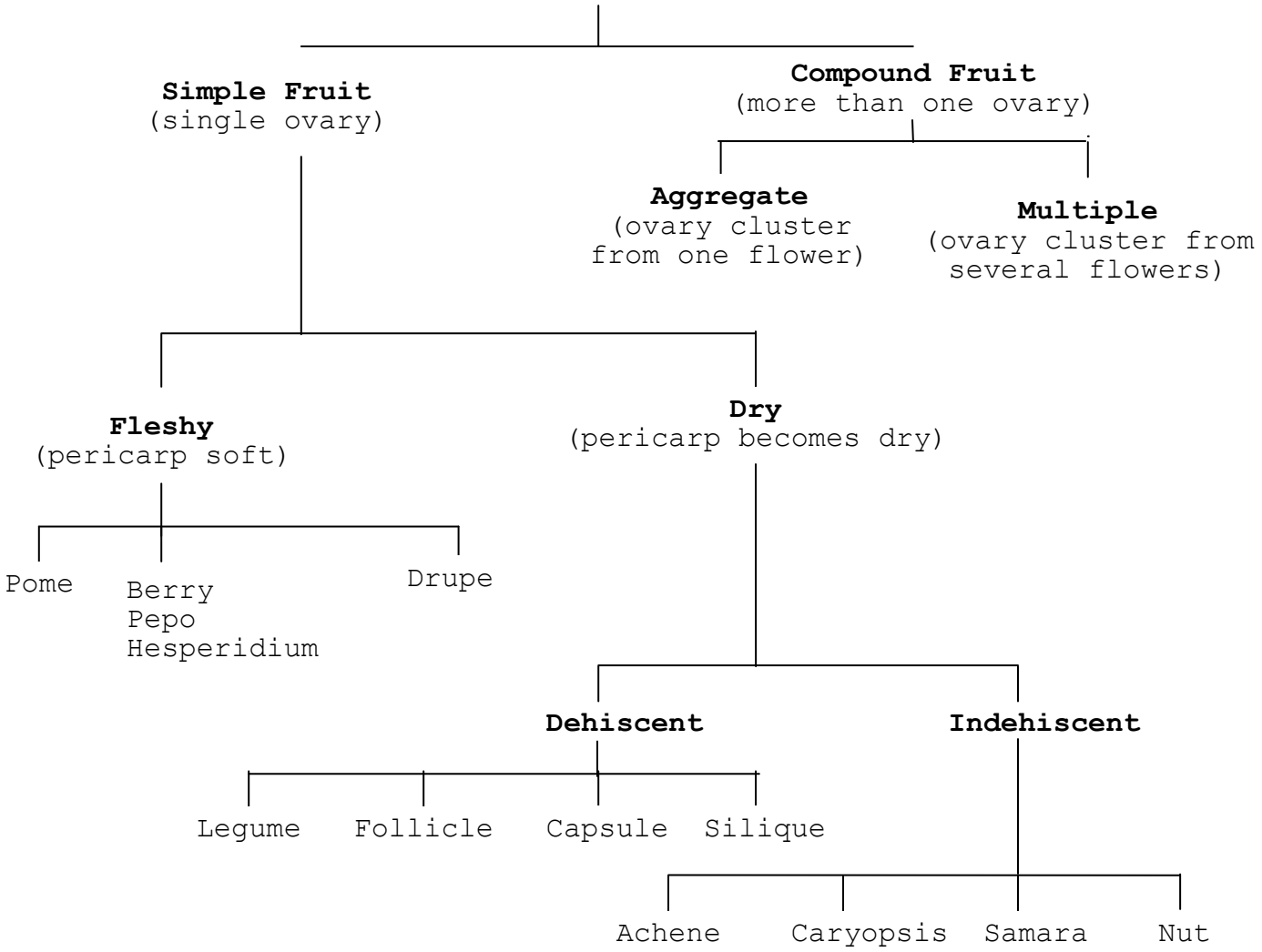
Fresh Fruits For Dissection:

Lycopersicon (tomato)
Prunus (peach)
Citrus sinensis (orange)
Cucumis (cucumber)
Pyrus malus (apple)
Phaseolus (bean) pod
Asclepias syriaca (common milkweed)
Papaver (poppy)
Zea (corn)
Helianthus (sunflower)
Acer (maple)
Juglans (walnut)
Frageria (strawberry)
Ananas comosus (pineapple)
Musa acuminata (banana)

Classification of Fruits

The classification scheme presented below is based primarily on characteristics of the fruit wall. Simple fruits develop from a single ovary, made up of one carpel or a number of fused carpels. A compound fruit is derived from a cluster of ovaries produced by one or several flowers. Both simple and compound fruits are also defined as **accessory** if they contain extracarpellary tissue.

The general term "fruit wall" is used with reference to all fruit walls, whether or not they contain accessory tissue. Pericarp is a more specialized term, usually applied to fruit walls derived only from ovary. Simple fruits may be soft and fleshy, or hard and dry. Dry fruits may be dehiscent (fruit splits open while still on the plant) or indehiscent (fruit does not open spontaneously).



Simple Fruits: Fleshy

Examine and draw each of the fruits provided, including x-sections where appropriate. Label the underlined terms.

1. **Berry**. Make a transverse cut through a tomato and observe the fruit wall, in this case a true pericarp. Three layers can be easily seen. The thin outer "skin" is the exocarp. Inside the exocarp is the fleshy mesocarp, and closest to the seeds, the endocarp. The presence of several locules (seed-bearing chambers) indicates a fusion of carpels; and this fruit is described as multicarpellate. Much of the fleshy portion is composed of the large central placenta, the region of ovary wall to which the seeds are attached. Placentation is axile (seeds attached at or near the center of fruit). During development of this fruit, placental tissue fills the locules. As the fruit ripens, tissue in the locule is degraded to a gelatinous mass. The tomato is classified as a berry because it is derived from a single ovary, is fleshy throughout, and contains more than a few seeds.

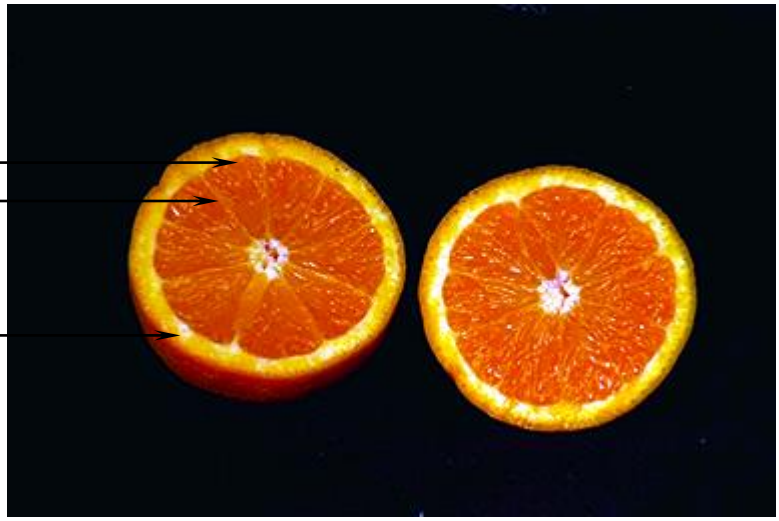
2. **Pepo**. Fruits of the Cucurbitaceae, which include cucumbers, squash and pumpkins, are called pepos. These fruits are somewhat similar to the tomato, but have a leathery exocarp. Examine the prepared x-section of *Cucumis*, which shows the fruit at an immature stage. The developing seeds are embedded in parenchyma and individual locules are difficult to distinguish. As a result, interpretation of the placental arrangement is difficult. Pepos are derived from an inferior ovary, but no boundary between ovary and extracarpellary tissues can be seen.

3. **Drupe**. Cut a peach in half longitudinally and note the exocarp (skin) and fleshy mesocarp. The endocarp forms a darkened sclerenchymous pit or "stone," that protects the single seed. The groove which encircles the fruit represents a suture between fused carpels. A drupe is similar to a berry, except for its hard endocarp.

4. **Hesperidium** (citrus). Members of this group are berries with leathery exocarps. The fruit is derived from a superior ovary (perigynous flower), and usually contains 10 carpels. Examine the prepared x-section of citrus ovary or very young fruit. Two rings of vascular bundles are visible, one within the ovary wall and one within tissues of the central placenta. Look also for oil glands around the periphery of the section, within the exocarp.

Compare this immature stage with a ripened orange. In the fully ripe fruit, the pigmented outer pericarp, or exocarp, is made up of epidermis and a relatively compact parenchyma, which contains oil glands and crystals. Under the dissection microscope, the oil glands should be visible, in a ring around the periphery just inside the epidermis. The white portion of the fruit wall is the mesocarp, or albedo (white tissue). Partitions between the carpels are composed of endocarp, derived from inner epidermis of the ovary, and from mesocarp. In some

citrus fruits the carpels, or segments, separate easily through parenchyma in the partitions. In the mature fruit, the carpels are packed with engorged juice sacs, which form as outgrowths of the endocarp.



5. **Pome.** Cut an apple in transverse section to reveal the star-shaped arrangement of five locules, each containing two seeds. Pome fruits develop from an inferior ovary (epigynous flower). All fruits derived from inferior ovaries contain accessory tissue, and thus are also classified as accessory. In the apple, only the central "core" of the fruit is derived from ovary. The fleshy edible portion often is interpreted as a derivative of the floral tube (hypanthium), made up of the bases of sepals, petals and stamens. The boundary between ovary and accessory tissues is difficult to define. Look carefully under the dissection microscope for a layer of compact parenchyma, which forms an irregular ring just inside the prominent petal bundles. This dense layer is the presumed boundary. In pome fruits, the carpels are lined by a papery endocarp.

6. The **banana** is classified with tomatoes, cucumbers, and other fleshy fruits derived from a single ovary. Transection reveals three carpels and axile placentation. This fruit usually develops parthenocarpically (without maturation of seeds). When this occurs, the ovules degenerate, appearing in the mature fruit only as black "dots," and the locules are filled with pulp. The banana also develops from an inferior ovary, and thus is an accessory fruit. The peel is derived from sepals.

Simple Fruits: Dry

Examine and make an outline drawing of each fruit on display.

Dehiscent fruits open spontaneously to release their seeds during the course of development. Usually, dehiscent fruits contain many seeds, and are considered more primitive than indehiscent fruits. They may develop from a single carpel (legume and follicle), or from one or more fused carpels (capsule and silique).

7. **Legume.** Bean "pods" are actually legumes, simple fruits derived from a single ovary in which the folded margins of a single carpel are joined along the ventral margin (ventral suture). Legumes split open along both the ventral suture and dorsal median vein. The sclerified median bundle is the "string" in string beans. Look carefully at the pericarp. Exocarp, mesocarp, and endocarp can be distinguished. In beans and other dehiscent fruits that open by splitting, sclerenchyma cells within these layers are oriented in different planes. As the fruit dries, the cells of each layer shrink along their longitudinal axes, creating shear forces which cause the pericarp to twist and finally split along lines of weakness.

8. **Follicle.** Examine the prepared slide or mature fruit of milkweed. This fruit is similar to a legume. However, the single carpel splits open only along the ventral side.

9. **Capsule.** Capsules are dry, dehiscent fruits made up of two or more fused carpels. In many capsules, the fruit splits along outer sutures formed by the union of carpels. The poppy capsule undergoes poricidal dehiscense, in which the ovary wall breaks down between vascular bundles, forming pores through which the seeds are dispersed.

10. **Silique.** Examine the prepared x-section of *Capsella* fruit. The silique is a specialized capsule made up of two fused carpels divided by a "false" partition. The fruit splits open along both margins, but seeds remain attached to the partition.

Indehiscent fruits commonly have only one seed, and are considered phylogenetically advanced. Indehiscent fruits often excise and fall to the ground when mature, and the seeds are freed by decomposition of the fruit wall. Many indehiscent fruits are eaten and dispersed by animals. Achenes, caryopses, and nuts contain only one seed. A samara may be two seeded.

11. **Achene.** Sunflower seeds are typical achenes, one-seeded fruits in which the seed may be separated from the pericarp. The edible portion is the seed, attached at one end of the sclerified pericarp.

12. **Caryopsis.** The corn kernel is a caryopsis. In this single-seeded fruit, the pericarp and seed coat are fused.

13. **Samara**. The fruit of maple is a two-seeded samara. In this type of fruit, the pericarp is modified into wing-like structures adapted for wind dispersal.

14. **Nut**. Walnuts, pecans, acorns, etc. are one seeded fruits similar to achenes, but covered by a thick, extremely hard pericarp. In the walnut, several carpels are originally present in the ovary; however, all but one degenerate during maturation of the fruit.

Compound Fruits

Compound fruits develop from more than one ovary. Aggregate fruits represent a cluster of ovaries from a single flower. Multiple fruits develop from a cluster of ovaries formed on separate flowers.

15. **Aggregate**. The strawberry is an aggregate fruit in which a cluster of individual ovaries from the same flower are embedded within a large, fleshy receptacle. Thus the strawberry is both aggregate and accessory. The individual fruits are achenes. Cut the fruit longitudinally and notice that vascular bundles are visible, leading from the pith of the receptacle to individual achenes.

16. **Multiple**. The pineapple is a multiple fruit. This type of compound fruit develops from the ovaries of many different flowers. The fused and ripened ovaries are berries. Since the pineapple fruit also incorporates the scales of inflorescences, it is both multiple and accessory.

Exercise 17--Scanning Electron Microscopy

A Special Laboratory Investigation Written by Amy Bivin

Introduction

Scanning electron microscopy is a versatile and widely used tool for modern research. The scanning electron microscope (SEM) uses a focused beam of high energy electrons that systematically scan across the surface of a specimen. The interaction that occurs between the specimen and the electron beam produces electron signals at or near the specimen surface. The electron signal is converted into an electronic signal which is seen on a cathode ray tube (TV screen). An important advantage of using the SEM is its greatly improved resolution compared to that of the light microscope. Resolution is the ability to distinguish between two points, on the same object, as separate and distinct points. The resolution of the SEM averages 7 nm (0.2 μm with a light microscope).

Several steps are necessary in order to prepare a specimen for viewing with the microscope. Preparation of a specimen can involve surface preparation, fixation, dehydration, critical point drying, mounting and coating. Because each specimen has differing composition many methods of specimen preparation can be utilized. Careful selection of preparation techniques as well as specimen selection are vital for viewing success.

The process of fixation involves stabilizing the ultrastructure of a cell by interacting with proteins using aldehydes. The degree of stabilization is based on the type of fixative employed as well as the pH, duration, and molarity of the fixative.

In this experiment a post fixation step was included in specimen preparation. The process of post fixation will stabilize unsaturated lipids and help reduce shrinkage or distortion of fixed tissue.

Following post fixation is dehydration. Dehydration reduces surface tension which will reduce overall stress to the tissue. An organic solvent is used in a graded series to promote a gradual displacement of water with solvent. Dehydration is a preliminary step to critical point drying.

Mounting of the dehydrated specimen is done using aluminum stubs. The purpose of mounting is to securely attach the specimen so it can withstand manipulations inside the microscope chamber. When mounting, it is important to consider stub type, stub size, specimen orientation and type of adhesive to be used.

Critical point drying completely eliminates the effects of surface tension forces on the biological material. This step is performed with an instrument called a Critical Point Dryer.

The following procedure briefly describes how botanical specimens were prepared for this experiment. Although, you will be viewing previously prepared specimen, it is important to be familiar with the procedures involved in reaching the viewing point.

PART I: Materials

Instruments:

Fume Hood
pH Meter
Analytical Balance
Critical Point Dryer
Sputter Coater
Scanning Electron Microscope

Chemicals and Supplies:

Fresh Coleus
Razor Blade
Glass Petri Dish
Latex Gloves
3% Gluteraldehyde
Phosphate Buffer (pH 7.2 to 7.4)
1% Osmium Tetroxide
Ethanol (10,20,50,75,80,90,100%)

PART I: Specimen Preparation

Fixation: Remove leaves from the plant stem. Dissect leaf tissue in a 3% Gluteraldehyde solution buffered to pH 7.2 to 7.4. Transfer the dissected pieces to a small vial containing fresh gluteraldehyde solution. Allow tissue to fix at 4 °C for 12-16 hours. The gluteraldehyde solution should be replaced after 2-3 hours. Use a fume hood when working with gluteraldehyde.

After primary fixation, remove tissue from gluteraldehyde solution and wash in cold phosphate buffer (pH 7.2). This washing cycle should be performed for 1 hour. Old buffer should be replaced with fresh buffer every 15-30 minutes.

Post Fixation: Place tissue that has been washed in a 1% osmium tetroxide solution that has been buffered for 1-2 hours at 4 °C. This procedure should be performed under a fume hood.

Dehydration: Leaf tissue should be dehydrated in a graded ethanol series using concentrations of 10,20,50,75,80,90,100%. Dehydration at each concentration should occur for at least 10 minutes.

Critical Point Drying: Critical point drying was performed following the instructions listed in the Critical Point Dryer manual.

Sputter Coating: Specimens were mounted on aluminum stubs using double sided adhesive tape. Parameters were set at 7nm for 180 seconds. The instruction manual was followed to complete this procedure.

PART II: Materials

Prepared Coleus Stubs
Scanning Electron Microscope

PART II: SEM Examination

View coleus leaves with the SEM. You are searching for the presence of trichomes. Use previous knowledge to identify structures seen on the leaf surface.

1. Look inside chamber.
2. Notice where stubs are placed in the chamber.
3. Close chamber and bring the microscope up to appropriate vacuum.
4. Locate leaf and bring into focus.
5. Use the video imager to make a micrograph (permanent record) of the screen image.

Examine micrographs and determine the type(s) of trichomes present. Look for other structures seen on the micrograph.

APPENDIX A

Index of Prepared Slides for Plant Anatomy
Listing of Slides in Custom Slide Boxes at UCO

INDEX OF PREPARED SLIDES FOR PLANT ANATOMY

STEM

Acorus stem c.s.
Aloe stem c.s.
Aristolochia young & old stems c.s
Carya CRT
Coleus shoot apex l.s.
Cucurbita stem c.s.
Cucurbita stem l.s.
Cuscuta haustorium host stem c.s.
Diospyros CRT
Elodea shoot tip l.s.
Elodea stem c.s.
Equisetum shoot apex l.s.
Hedera stem w/adventitious root c.s.
Helianthus stem c.s.
Lycopersium stem w/adventitious root c.s.
Lycopodium stem c.s.
Marsilia stem c.s.
Medicago stem c.s.
Medicago old stem c.s.
Monocot and Dicot stems c.s.
Osmunda stem c.s.
Pinus shoot apex l.s.
Pinus stem tip l.s.
Pinus young stem c.s.
Pinus maceration
Pinus CRT
Potamogeton stem c.s.
Salicornia stem c.s.
Salix CRT
Sambucus lenticel c.s.
Tilia 3 or 4 year stem c.s.
Trifolium early stem c.s.
Triticum mature stem c.s.
Zea mays shoot tip l.s.
Zea mays stem c.s.
Zea mays stem l.s.
Quercus (Oak) CRT

EMBRYO

Capsella embryo before cotyledons n.m. l.s.
Capsella embryo w/early cotyledons n.m. l.s.
Capsella embryo w/bending cotyledons n.m. l.s.
Capsella mature embryo n.m. l.s.
Lilium anther mature pollen c.s.
Lilium anther pollen tetrads c.s.
Lilium ovary MS.M.C. c.s.
Lilium ovule 2-nucleate stage c.s.
Lilium ovule 2nd 4-nucleate stage c.s.
Lilium ovule mature embryo sac c.s.
Lilium ovule megaspores c.s.
Pinus female strobilus w/MS.M.C. l.s.
Pinus mature male strobilus l.s.
Zea mays embryo l.s.

INDEX OF PREPARED SLIDES FOR PLANT ANATOMY (continued)

ROOT
Beta root c.s.
Corallorhiza rhizone c.s.
Glycine root nodule c.s.
Medicago root c.s.
Pinus root c.s.
Pinus root w/ectotrophic mycorrhiza c.s.
Pisum root tip l.s.
Ranunculus young & mature root c.s.
Ranunculus mature root c.s.
Ranunculus young root w/mature protoxylem c.s.
Raphanus root c.s.
Salix root c.s., lateral root series (5)
Smilax mature 1° root c.s.
Triticum root c.s.
Zea mays root tip l.s.
Zea mays root c.s. w/root hairs

LEAF
Asclepas syriaca lead c.s.
Bouteloua leaf c.s.
Brassica oleraeca hydathode l.s.
Coleus leaf c.s.
Dionaea muscipula leaf c.s.
Drosera rotundifolia leaf c.s.
Elaeagnus angustifolla leaf c.s.
Ficus elastica leaf c.s.
Iris leaf c.s.
Iris leaf epidermis w.m.
Nerium leaf c.s.
Nymphaea leaf c.s.
Pinus leaf c.s.
Sambucus leaf c.s.
Ricinus communis extra-floral nectary c.s.
Syringa leaf abcission l.s.
Syringa leaf c.s.
Syringa leaf para.s.
Typha leaf c.s.
Verbascum Thapsus leaf c.s.
Yucca leaf c.s.
Zea mays leaf c.s.

FRUIT
Asclepas syriaca fruit c.s.
Capsella fruit c.s.
Citrus young fruit c.s.
Cucumis sativus young fruit c.s.
Cucurbita seed c.s.

NOT TRIARCH
Air, water and soil roots, composite c.s.
Hibiscus older stem c.s.
Lateral root development c.s.

PLANT ANATOMY SLIDES

Acer (Maple) stem c.s.	1	Elaeagnus angustifolla leaf c.s.
Acorus (sweet flag) stem c.s.	2	Elodea (Waterweed) shoot tip l.s.
Air, Water and Soil	3	Elodea (Waterweed) stem c.s.
Allium root tip c.s.	4	Euphorbia stem l.s.
Aloe (Liliaceae) stem	5	Equisetum (Horsetail) shoot apex l.s.
Annual & Perennial stems c.s.	6	
Aristolochia 1 yr. stem c.s.	7	
Aristolochia young and old stems	8	Ficus elastica (Rubber Tree) leaf c.s.
Asclepas syriaca leaf c.s.	9	Forsythia stem tip l.s.
Asclepas syriaca fruit c.s.	10	
Asclepas syriaca stem l.s.	11	
	12	Ginkgo maceration
	13	Glycine (Soy Bean) root nodule c.s.
	14	
Bacillus radicicola	15	
	16	Hedera stem w/adventitious root c.s.
Beta vulgaris (Beet) root c.s.	17	Helianthus (Sunflower)root c.s.
Bouteloua (Mesquite-grass) leaf c.s.	18	Helianthus (Sunflower) stem c.s.
Brassica oleraeca hydathode	19	Helianthus (Sunflower) stem l.s.
	20	Hibiscus older stem c.s.
	21	
	22	
Capsella embryo series l.s.	23	Iris (Iridaceae) leaf c.s.
Capsella embryo before cotyledons	24	Iris leaf epidermis whole mount
Capsella embryo w/early cotyl. l.s.	25	
Capsella embryo w/bending cotyl.	26	
Capsella floral bud l.s.	27	Lactuca stem c.s.
Capsella fruit c.s.	28	Lateral root development c.s.
Capsella mature embryo n.m. l.s.	29	Ligustrum (Privet) c.s.
Carya (Pecan) CRT	30	Lilium (Lily) anther mature pollen c.s.
Citrus (Lemon) young fruit c.s.	31	Lilium anther pollen tetrads c.s.
Clematis stem c.s.	32	Lilium ovary megaspore mothercell c.s.
Coleus (Labiatae) leaf c.s.	33	Lilium ovule 2-nucleate stage c.s.
Coleus (Labiatae) stem c.s.	34	Lilium ovule 2nd 4-nucleate stage c.s.
Coleus (Labiatae) shoot apex l.s.	35	Lilium ovule mature embryo sac c.s.
Corallorhiza (Coral-root) rhizome c.s.	36	Lilium ovule megaspores c.s.
Cucumis sativus young fruit c.s.	37	Lilium (Lily) root tip l.s.
Cucurbita (Pumpkin) fruit c.s.	38	Lycopersicum (Tomato) floral bud l.s.
Cucurbita (Pumpkin) root c.s.	39	Lycopersicum stem w/advent. root c.s.
Cucurbita (Pumpkin) seed l.s.	40	Lycopodium (Clubmoss) stem c.s.
Cucurbita (Pumpkin) stem c.s.	41	
Cucurbita (Pumpkin) stem l.s.	42	
Cuscuta haustorium host stem c.s.	43	Marsilea (Fern) stem c.s.
	44	Medicago (Alfalfa) old stem c.s.
	45	Medicago (Alfalfa) root c.s.
Dionaea muscipula leaf c.s.	46	Monocot and Dicot floral bud l.s.
Diospyros virginiana CRT	47	Monocot and Dicot roots c.s.
Drosera rotundifolia leaf c.s.	48	Monocot and Dicot stems c.s.
	49	
	50	

PLANT ANATOMY SLIDES (continued)

Nerium (Oleander) leaf c.s.	1	Salicornia (Glasswort) stem c.s.
Nymphaea (Water Lily) leaf c.s.	2	Salix (Willow) CRT
Nymphaceaceae leaf c.s.	3	Salix root c.s., lateral root series (t)
	4	" "
	5	" "
Orchid root c.s.	6	" "
Osmunda (Fern) stem c.s.	7	" "
	8	Sambucus (Elderberry) leaf c.s.
Pelargonium old stem c.s.	9	Sambucus lenticel c.s.
Pelargonium young stem c.s.	10	Sambucus stem c.s.
Phaseolus (Bean) root c.s.	11	Smilax (Greenbriar) mature root c.s.
Phaseolus root tip l.s.	12	Smilax root c.s.
Bean root nodule c.s.	13	Solanum stem c.s.
Phaseolus seed l.s.	14	Syringa (Lilac) leaf abcission l.s.
Pinus (Pine) CRT	15	Syringa leaf c.s.
Pinus fem. strobilus w/megaspore	16	Syringa leaf paradermal w/venation
Pinus mature male strobilus l.s.	17	Syringa sun & shade leaf c.s.
Pinus leaf c.s.	18	
Pinus maceration	19	
Pinus shoot apex l.s.	20	
Pinus stm tip l.s.	21	Tilia (Basswood) CRT
Pinus young stem c.s.	22	Tilia 1,2, 3 year stem c.s.
Pinus root c.s.	23	Trifolium (Red Clover) early stem c.s.
Pinus root w/ecto. mycorrhiza c.s.	24	Triticum (Wheat) mature stem c.s.
	25	Triticum root c.s.
Pisum sativum (Pea) root tip l.s.	26	Typha (Cat-tail) leaf c.s.
Poa pratense leaf c.s.	27	
Potamogeton (Pond Weed) stem	28	
Prunus leaf abs.	29	
Pteridum rhizome	30	Ulmus seed l.s.
	31	
	32	Verbascum Thapsus (Mullein) leaf c.s.
	33	
Quercus alba (Oak) maceration	34	Yucca (Spanish Bayonet) leaf c.s.
Quercus (Oak) CRT	35	
Quercus (Oak) stem c.s.	36	
	37	Zea mays (Corn) embryo n.m. l.s.
	38	Zea mays (Corn) leaf c.s.
	39	Zea mays (Corn) root tip l.s.
Ranunculus young and mature root	40	Zea mays (Corn) root c.s. w/root hairs
Ranunculus mature root c.s.	41	Zea mays (Corn) shoot tip l.s.
Ranunculus root w/protoxylem c.s.	42	Zea mays (Corn) stem c.s.
Raphanus root c.s.	43	Zea mays (Corn) mature stem c.s.
Raphanus (Radish) root tip l.s.	44	Zea mays (Corn) stem l.s.
Ricinus communis e.f. nectary c.s.	45	
Rose stem tip l.s.	46	Zebrina leaf c.s.
	47	
	48	
	49	
	50	