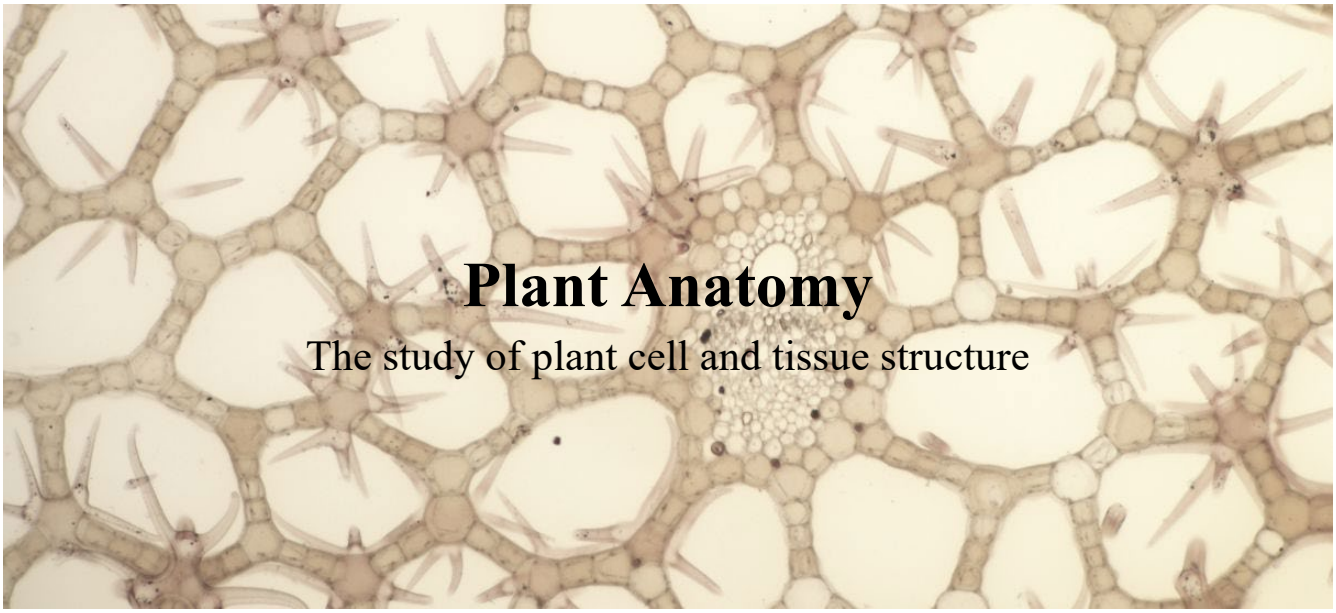

CU-PAC Plant Anatomy Online Lab Manual

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This manual is intended to provide a concise Plant Anatomy laboratory based on the Natile W. Uhl Cornell University Plant Anatomy Collection slides which can be accessed at http://cupac.bh.cornell.edu/. This version also includes "on-hand activities" for which a list of plantings (list of taxa/seeds to be planted, planting schedule, amount of time from planting to when they will be used, medium in which seeds need to be planted, etc.) and other plants needed for successfully accomplishing the labs are provided at the end.

A secondary objective for this manual is to provide sample questions that can help to determine the students' mastery of the material. It is intended to solidify the students' comprehension of the learned concepts. This study guide and manual are built as a way of developing a strong background in basic plant anatomy in relation with organs/structures as the student goes deeply into more complex topics. Therefore, at first a great deal of time should will be invested in building general concepts and developing observational skills.

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DISCLAMER: Although we have tried to comply with ADA standards, many images of slides are colored by plant anatomical stains that highlight certain aspects of the specimen in only certain colors.

Topic Synopses

Covered Topics

TOPIC 1: THE PLANT CELL- Organization of the eukaryotic cell: Introduction to cell wall (extracellular matrix, shape) and cell content (living protoplast). Importance of good descriptions (size, shape, position of different cell types, etc). Protoplast: cytoplasm, organelles (nucleus and plastids), membranes (plasmalemma and tonoplast), vacuoles, ergastic substances (oil droplets, starch grains, crystals, lipids, etc.). Cell wall: function, dynamic structure (exchange of biochemical information). Plasmodesmata: origin and function. The 3 layers of the cell wall (middle lamella, primary wall and secondary wall). Structure and components of the wall. Wall growth. Special structures (primary pit fields, pits, crassulae and trabeculae).

TOPIC 2: THE THREE TISSUE SYSTEM- Observations in plant anatomy, accuracy and completeness based on macro-and microscopic information. Components of a plant: cells, tissues, organs = unity. Planes of sectioning, Three basic tissues: dermal (epidermis), ground tissues (parenchyma, collenchyma, and sclerenchyma) and vascular (xylem and phloem).

TOPIC 3: APICAL MERISTEMS (shoot and root)- Definition, types (apical and axillary). Indeterminate and determinate growth, meristematic activity. Promeristems and initial cells. Apical organization, emphasis on tunica-corpus and mantle-core theories. Differences among seed plants, pteridophytes and bryophytes. Regions of the shoot apical meristem; transitional tissue regions (protoderm, ground meristem or procambium).

TOPIC 4: EPIDERMIS- Definition, origin, function and characteristics. Epidermal cell types: basic epidermal cell, stoma complex cell, and epidermal appendages. Uniseriate and multiseriate epidermis. Cuticle: definition, origin, function and characteristics. Waxes. Special case: graminea (bulliform, silica and cork cells). Stoma complex, basic stomata types and classification. Epidermal appendages: unicellular and multicellular trichomes, function, origin. Non-glandular and glandular trichomes.

TOPIC 5: GROUND TISSUES. PARENCHYMA: Origin, cell types, relationships with other tissues, where do we find it, types (stellate, transfer cells, chlorenchyma, aerenchyma). Schizogenous and lysigenous spaces.**COLLENCHYMA & SCLERENCHYMA:** supporting tissues (stereome), origin, cell types, relationships with other tissues, where do we find them. Angular, lamellar and lacunar collenchyma. Sclerenchyma cell types: fibers, sclereids and fiber-sclereids.

TOPIC 6: PRIMARY VASCULAR TISSUES- XYLEM and PHLOEM. Introduction to vascular tissues. **XYLEM:** cell types (tracheary elements, fibers, vessels), origin, function, characteristics. Primary xylem (protoxylem and metaxylem). Tracheids structure (annular, scalariform, pitted). Vessel elements: perforations and perforation plates, development of the perforations. Secondary xylem. **PHLOEM:** cell types (sieve cells, sieve-tube members, sieve plates, companion and albuminous cells), function, characteristics. Callose plugs. Primary phloem (protophloem and metaphloem).

TOPIC 7: STEM- functions, ontogeny (embryo). **PRIMARY GROWTH:** shoot apical meristem (SAM), origin of primary tissues (epidermis, vascular tissues, rest of tissues). Epidermis (epidermal cells, idioblats, trichomes, etc). Cortex (parenchyma, sclerenchyma and collenchyma). Endodermis (including Casparian bands) and starch sheath. Types of vascular bundles. Stele: concept; type of steles (protosteles and siphonosteles), origin.

TOPIC 8: STEM SECONDARY GROWTH- lateral meristems, vascular cambium (secondary xylem and secondary phloem), phellogen or cork cambium (periderm). Cambium- meristematic cells (fusiform initials and ray initials). Annual growth and perennial plants.

TOPIC 9: WOOD THE VERTICAL SYSTEM (tracheary elements, fibers and wood parenchyma). Classification of annual rings of xylem (diffuse, ring). Timber classification (paratracheal, apotracheal and boundary parenchymas). Tyloses. **THE HORIZONTAL SYSTEM** (xylem rays or vascular rays): rays and cell arrangement, dimensions of the rays: 1- uniseriate, biseriate and multiseriate; 2- homocellular or homogenous rays and heterocellular rays (ray tracheids, parenchyma cells: procumbent and upright cells).

TOPIC 10: SECRETORY CELLS, TISSUES AND STRUCTURES- Classification: nature of secretory product, mechanism of secretion and purpose of secretion. Nectaries (extrafloral and floral). Epidermal appendages, hydathodes, salt glands, osmophores, digestive glands, adhesive cells, resin ducts, mucilages, oils, gums, myrosin cells, laticifers (articulate and non-articulate), and gases.

TOPIC 11: NODAL ANATOMY- Leaf traces and leaf gap; types of nodes in dicotyledons: two-trace unilacunar, one-trace unilacunar, trilacunar, and multilacunar; branch traces and branch gaps.

TOPIC 12: LEAF- Classification, simple and compound leaves, stipules, venation patterns. Phyllotaxis (alternate, opposite, whorled). Leaf architecture history. Structure and anatomy of mature dicot, monocot and gymnosperm leaves. Palisade and spongy mesophylls. Vascular bundles and bundle sheaths. Transfer cells. Anatomical differences between C3 and C4 plants. Plastochron concept. Senescence and abscission: concept, deciduous and evergreen species. Anatomical changes during senescence.

TOPIC 13: ROOT- Functions, types. Tap root and lateral roots. Growth and differentiation (root cap, elongation region, zone of root hairs and lateral roots), root apical meristem (RAM). Apical organization (apical cell, histogen and korper-kappe theories). Patterns of vascular differentiation. Origin of lateral roots (initiation, organization and emergence).

TOPIC 14: FLOWER, FRUITS, AND SEEDS- FLOWERS: Functions, characteristics. Classical view (compressed shoot, sepals, petals, receptacle, adroecium, gynoecium). Development of stamens and pollen. Development of carpels and ovules. **FRUITS:** Definition. Fruit types. Pericarp (exocarp, mesocarp and endocarp). **SEEDS:** definition. Embryo development, embryo/endosperm relationships. Testa and endosperm.

Introduction to Plant Anatomy Lab Manual

This manual has two major goals: 1- to expose the students to a variety of anatomical data and concepts so that they will have an appreciation for the content and organization of plant anatomy, and 2- to allow the students to gain the ability to perform practical diagnoses on anatomical "unknowns".

The first objective is facilitated by providing the student with a guided tour through basic plant anatomy. The subject matter is so extensive that it is necessary to pick and choose among materials, and no two people will do this in precisely the same way. Having this in mind, this Study Guide and Laboratory Manual has been prepared as a tool for introducing basic plant anatomy to a variety of students based on the Cornell University Plant Anatomy Collection that includes slides of the Plant Anatomy slide teaching sub-collection, and those of famous plant anatomists and plant development specialists such as Eames, Bierhorst, Esau, Eggert, and Kaplan

The second goal depends more on the students than it does on the instructors. The instructors can assist the student in the laboratory by answering questions and asking some of their own to see how well the student understand the materials. But the real test of the students' progress is whether or not the observations the student are asked to make prompt the student to become an independent observer. The student should strive for original observations in the laboratory. If the student can observe on their own, they will begin to see how plant anatomy data are accumulated, how concepts are formulated, and how illustrations are chosen to represent what an author or investigator has in mind.

To succeed in completing these laboratory activities the student will have to emphasize original, independent observations.

Making original observations is prerequisite to adequate learning and to attaining the skills required for original research. Observations that repeat what has already been done correctly by others can be entirely original, if after looking at what the material has to offer the students conclude themselves that previous interpretations are correct or understandable. Students must keep an open mind, look only for what is there rather than for what the students are supposed to see, and remain alert for artifacts in the preparations.

Anatomy is an old science, and as such it has accumulated many accepted concepts. However, basic plant anatomy is not without controversy, and our understanding of things that have been studied for years must be continuously improved. Nevertheless, this study guide and laboratory manual will stay quite close to the well-known aspects of basic plant anatomy as it is based on already prepared slides that illustrate examples in detail.

Becoming an independent observer is prerequisite to being able to perform new anatomical studies, and to being able to apply anatomical studies to related fields. No one can become a plant anatomist in one semester, and it is not the goal of this study guide and manual to turn any or all students into plant anatomists. But unless the students have some feeling for the way in which one goes about anatomical studies, how evidence is recognized, and how concepts are formulated, the students will have missed out on the "practical" aspects of basic plant anatomy. Can the students describe what they are seen in the photo? Can the students tell what it means? Do the students comprehend its relationship to the organization of the plant? The students should ask themselves these questions in each laboratory. If answers come back, they are accomplishing the goals. If the answers do not come back, let the teacher and student work together to unlock the powers of observation.

Disclaimer

Some labs in this manual have "hands on" activities, nevertheless the topics that have on-hand activities also have photographs associated to them. You are responsible for what you or your students get out of the manual. You may jump around the topics or skip one or two entirely. It is up to you if you want to add or subtract to anything in this manual.

Additional readings

There is no textbook associated with this manual. The following books are recommended for consultation:

- Beck, C.B. 2010. An Introduction to Plant Structure and Development. 2nd. Edition. Cambridge University Press, New York, New York.
- Esau, K. 1965. Plant Anatomy. 2nd. Edition. John Wiley & Sons, New York New York.
- Fahn, A. 1967 or 1982. Plant Anatomy. Pergamon Press, Oxford.
- Gifford, E.M. and A.S. Foster. 1989. Morphology and Evolution of Vascular Plants. 3rd.Edition. Freeman and Co., New York, New York.
- Mauseth, J.D. 1988. Plant Anatomy. Benjamin Cummings Comp., Inc. Menlo Park, California.
- Romberger, J.A, Z. Hejnowicz, and J.F. Hill. 2004. Plant Structure: Function and Development. 2nd. Edition, The Blackbourn Press, Caldwell, New Jersey.
- Arora, D.K. 1996. Morphology and anatomy of leaf. Anmol Publications.
- Cutler, D.F. 1978. Applied Plant Anatomy. Longman.
- Dickison, W.C. 2000. Integrative Plant Anatomy. Harcourt Academic Press.
- Evert, R. F. and S. E. Eichhorn. 2006. Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. Wiley and Sons.
- Fahn, A 1979. Secretory tissues in Plants. Academic Press.
- Iqbal, M. 1990. The Vascular Cambium. Wiley and Sons.
- Rudall, P. 1992. Anatomy of flowering Plants. Cambridge Univ. Press.

Organization of the study guide and manual

This manual is organized by topics and the students can use it to orient themselves on the role the lab plays in developing each subject, and be sure to read the laboratory exercise before starting the lab to do the work. If the lab time is to be used effectively, the students must understand the organization of each lab exercise to regulate the use of their time in the laboratory. Certainly, the students should never consider a laboratory exercise completed if they do not have a grasp of how each part of that lab contributes to the development of a topic.

Copyright

Plant Anatomy Stains

Common stains

Below are common plant anatomy stains used in this lab manual. **Treat all stains as though they may be hazardous. Avoid contact with skin, eyes, and breathing dust or fumes from solutions. You should wear proper safety equipment when preparing or using anatomical stains (gloves, lab coat, etc.)** Another reason to use gloves and a lab coat is that these stains will stain clothing and YOU, too!

1. **Iodine potassium iodide (IKI, Lugol solution)**- Formulation:
    - 2 g potassium iodide (IK)
    - 100 ml distilled water
    - 0.2 g iodine

    Dissolve the potassium iodide in the water, and then dissolve the iodine in that solution. Starches will easily satin blue to black.

    Caution: Iodine vapors are toxic. Avoid breathing in vapors. Handle with care.

2. **Safranin O (1%)**- Formulation:
    - 50 mg safranin powder
    - 50 ml distilled water

    Dissolve safranin powder in water in a 100ml beaker. Stir constantly until the powder is dissolved. Filter with filter paper. Safanin O will stain xylem and fibers bright red. Nuclei and plastids will stain pink.

3. **Toluidine Blue O (TBO)**- Formulation:
    - 50 mg Toluidine Blue O
    - 100 ml water

    Dissolve Toludine Blue O powder in water. Check pH of solution. A pH of 6.8 is preferable. This is a metachromatic stain: lignin stains blue to blue-green, pectins stain pink, nuclei stain blue to greenish blue.

4. **Aniline blue**- Formulation:
    - 0.5% (w/v) aniline blue stock solution was made by dissolving aniline blue in 0.2 M phosphate buffer pH 6.5 or deionized water, and filtering through Whatman no. 1 paper. Stains callose tissue.

5. **Phloroglucinol HCl**- Formulation:
    - 2 g Phloroglucinol
    - 80 ml of 20% Ethanol

- 20 ml of concentrated HCl (12 M)

Dissolve Phloroglucinol powder into ethanol. Add HCl. Cover with aluminum foil to prevent degradation. Stains lignin.

Caution!: Mix stain in fume hood. Concentrated HCl is very corrosive. Handle with care.

Topic 1: The Plant Cell

Objectives

After studying this topic, you should be able to perform the following:

1. describe "the cell" as the unit of construction of multicellular plants.
2. be able to recognize and describe the parts of a cell.
3. visualize and describe how the parts of a cell are integrated into the organized unit we call a cell.
4. be able to distinguish between generalized and specialized cells on the basis of the condition of the cell parts.

The cell consists of a protoplast surrounded by a wall. The first part of this topic deals with the protoplast, mostly, whereas the second part deals with the wall and cell shape. This unit is designed to develop your skill in recognizing cellular details that will be used to describe cell types.

Part One

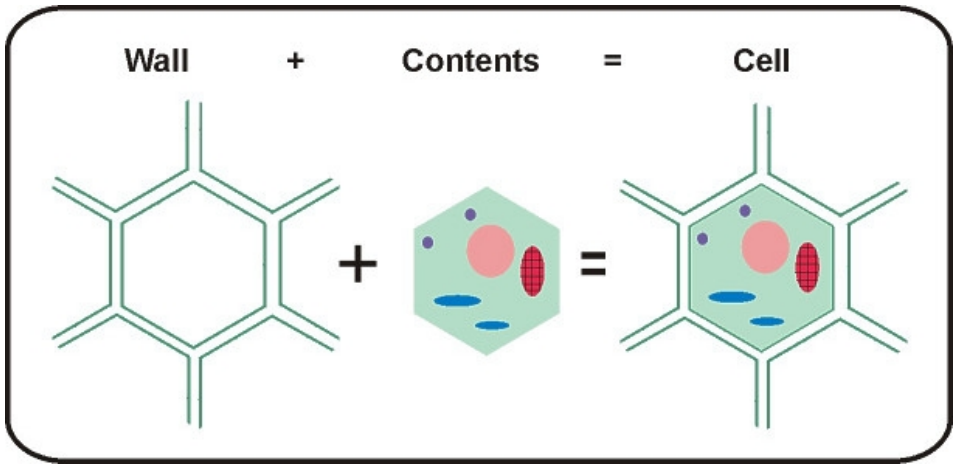
THE PROTOPLAST AND ITS PARTS:

1. Each time we fabricate a classification or invent new terms we should have a definite purpose in mind. Classification of the parts of a cell is useful because it:
    a. helps us to organize our thinking about cell functions
    b. helps us to make specific comparisons to distinguish between different cell types.

    On the first point, we can simply state that just as the cells are specialized compartments within the plant, the parts of the cell are compartments that carry out the various functions of the cell. On the second point we can state that we can make direct comparisons of the various cell types by noting the emphasis or degree of specialization of the various parts of the cell, such as the wall, the plastids, vacuoles, ergastic components, etc.
2. How are the concepts of cell organization recorded in a diagram or any other illustration? First, the cell wall is separable from the protoplast. A definite line represents the boundary or interface between the protoplast and wall. However, the cell wall is secreted by the protoplast (and is often referred to as the extracellular matrix), and while the wall is being deposited, the boundary with the protoplast can become obscure. If a living cell is subjected to plasmolysis (shrinkage due to the application of a hypertonic solution), the protoplast pulls away from the wall and the distinction between the non-living wall and the living protoplast is enhanced. But while the protoplast is in contact with the wall, it is connected to the protoplasts of adjacent cells in the tissue. These connections through the walls are the plasmodesmata. We can assert that the cell is part of a tissue, because it is connected to other cells. The frequency of connections can be greater between particular cells as opposed to others.
3. You should be able to notice also, that there are other indications that the cell is part of a tissue:
    a. The walls are two layers joined together at the middle lamella. Each cell secretes its own wall, thus the partition between any two adjacent cells consists of two walls stuck together.

b. The walls are extended outward from the corners of the diagram, to imply that they continue on around adjacent cells.

c. The polygonal shape of the diagram suggests that the cell is being pressed upon from all sides by other cells. Barring complications, a cell that is not pressed upon from the sides would appear circular in sectional view. Therefore, it is also implied that the cells of the tissue are tightly pressed together because there are no spaces at the corners. As soon as the tissue loosens up, for example with aging, gas-filled spaces would form at the corners.



Cell features are in the wall or in the contents:
Learn the features of cell walls and cell contents.
We will use three features to identify cell types and the kind of tissues.

From this discussion you should conclude that diagrams or drawings can record considerable information because the details imply what the object is like. In a drawing, some details are left out to simplify the illustration. But all the details that are included must be chosen to represent the properties of the object faithfully.

4. It is a simple matter to show that the walls of the cells in a tissue form a continuous three-dimensional network. The protoplasts also form an interconnected mass by way of the plasmodesmata. Biological processes govern the movement of substances from the walls into the protoplasts, for the latter are living and therefore differentially permeable. Plasmodesmata provide direct connections for intercellular transport, an alternative to exchange between the protoplasts and their walls. To emphasize that two phases exist in a tissue the walls are said to comprise the nonliving apoplast while the interconnected protoplasts comprise the living symplast.

5. The recognition of apoplast and symplast starts us on a characterization of non-living vs. living components in a tissue. It is the protoplast that has the attributes of living material, not the cell wall. But within the protoplast one recognizes components that contribute directly to the properties of a "living" cell and others that do not.

6. Should the use of the terms protoplasmic vs. non-protoplasmic be considered the same as living vs. non-living? This is a debatable point, but not an important issue for our purposes. The unit of organization that guarantees perpetuity in the plant is the cell. A cell is alive, because it has (or is) a living protoplast. Whether or not protoplasmic components are alive is an interesting question, but the outcome of debate on this issue has no real impact on the study of plant

anatomy. Moreover, if the question is posed at the molecular level, how can it be answered at all? For example, we claim that ergastic substances are non-protoplasmic and non-living. Yet within seconds, molecules stored in ergastic components could be incorporated into protoplasmic components. In an important way, the balance in metabolism between the creation and utilization of ergastic substances is also part of the life process. It will be important to recognize whether or not cells retain their living protoplasts and to what degree the protoplasts are specialized when the cells are mature. This knowledge will help us distinguish between cells of different types.

7. A young cell (e.g., one newly derived from an apical meristem) or a meristematic (stem) cell shows the general features of cell structure because it lacks any specialization. It has the potential to become any type of cell; thus, it has all the parts required for any cell but in an unelaborated, unspecialized form. A parenchyma cell has the lowest level of specialization of any mature cell type and the most generalized character throughout its life. Therefore, it is quite reasonable to use a young parenchyma cell to illustrate the general features of cell structure. A typical mature parenchyma cell has a large, centrally located vacuole that presses the protoplasm against the wall of the cell. One would also represent some specialization of the plastids in a mature parenchyma cell, e.g. as chloroplasts or as amyloplasts, depending on the location of the cell within the plant.
8. Finally, it is important to remember the following generalizations.
    a. The more or less uniform, generalized cells of an apical meristem become the diverse, mature cell types by changes in cell components. The meristematic (stem) cell is not a cell type but the precursor of all cell types.
    b. The various components of the meristematic (stem) cell may be emphasized, deleted, or altered in more subtle ways to produce the specialized condition in the mature cell type.
    c. Specific cell features must be understood as endpoints in differentiation, which can be traced back to their origins in some part or parts of the meristematic (stem) cell.
    d. Differentiation of cells can occur over short distances (a few μm).

Any control mechanisms proposed for such differentiation must be able to operate over short distances to produce the required discriminations. Hints as to what control mechanisms might be at work are best obtained by reference to the overall organization of the plant and its parts, for the fate of a cell is a function of its location in the plant.

Laboratory: The Protoplast

Objectives -- After practicing these activities you should be able to:

1. Diagnose the organization of whole cells by visualizing the parts with the microscope.
2. Recognize chloroplasts as opposed to other cell parts.
3. Determine the contribution of plastid and vacuolar pigments to the colors of flower petals and variegated leaves of *Coleus*.
4. Locate and identify crystals in plant cells.
5. Diagnose unknowns for the properties dealt with in 1-4.

Activities 1-5 of this lab involve preparing microscope specimens from living materials. These suggestions will help you develop perspective on how to fit the observations into the task of accomplishing each objective.

Activity 1

A. Try to hold down your observations to approximately 10 minutes. Mount the filament of a stamen of *Tradescantia zebrina* in water. Cut off the anther sac. The strands of cells that grow from the surface of the filament are called stamen hairs. Determine the boundaries of the individual cells of a stamen hair. Where is the color contained in these cells? Be certain to see that it is not in the wall and not in the cytoplasm. Homogeneous coloration of this kind is contained in the aqueous vacuole of the cell. The cell wall is rough on the outer surface, not smooth. Try to visualize the protoplasm separately from the wall and from the vacuole by focusing to a median plane in the cell. The cytoplasm has a granular appearance. One large, colorless object in each cell is a nucleus. If chloroplasts are present they should be green. There are not any. (Do not be fooled by a greenish tint introduced by dimming the light with the iris diaphragm of the condenser.) The parts of the cell that can be identified and named are the cell wall, cytoplasm, vacuole and nucleus. Look for continuous, directional movements of granules in the cytoplasm (evidence for cytoplasmic streaming). This movement is accepted as evidence that a cell is alive. Random movements of particles (Brownian movements) are evident in dead cytoplasm, in vacuoles, and suspensions of cell components. The granules are probably diverse but there are no visible criteria to name or identify them with the present technique.

B. Try to do this part in 10 minutes or less. Mount a whole leaf of *Elodea*. In a part of the leaf that is one or two layers thick, determine the boundaries of the individual cells. The parts that can be easily identified are cell walls and chloroplasts. It is not possible to overlook the chloroplasts. Do these all have the same shape? As cytoplasmic streaming moves the plastids, see if the outlines you see are all consistent with each other as different views of the same object. A chloroplast is somewhat discoidal (an oblate spheroid) in *Elodea*. Perhaps you can see grana, and locate starch grains by staining with IKI (iodine solution or Lugol solution). Check for nucleus and vacuole before and after staining with IKI. These parts are not easily seen in the living cell. Try looking at cells along the edge of the leaf to locate nuclei. The vacuole has no color in the living *Elodea* cell. But if there were no vacuole the cytoplasm (and the plastids) would probably occupy the whole volume of the protoplast. Are the plastids only at the periphery of the cell?

C. Keep this part to 5 - 10 minutes or less. Choose a plant stem or petiole that sections easily (e.g. *Begonia* or *Impatiens)*. Make hand sections and determine if there are any intact cells by focusing up and down to see all sides of the cell. Characterize the organization of these cells by running down a checklist of cell components that are identifiable with the light microscope. Is cytoplasmic streaming evident? The likely answer is no in this case. At different locations in the section, cells will have plastids that are lighter or darker green. But all the green cells are organized more or less the same. Try to find a nucleus with plastids clustered around it, in an intact cell. Look in a "pithy" portion of the section (viz., a region of uniform parenchyma near the center of the section). Epidermal cells generally have poorly developed, nearly colorless plastids (except in guard cells) compared to mesophyll cells, and plastids may be small and few in number.

D. Spend at least 10 -15 minutes here. Look at images of transverse and paradermal sections of *Syringa* leaf. Colors are from dyes, not natural. Try to locate cells that show cell wall, nucleus, plastids and evidence for vacuole all at once. In a transverse section, this is most likely in the palisade mesophyll in the ground tissue system. It is less likely in the dermal tissue system.

What does cell size and shape have to do with this? What might cell differentiation have to do with this? Nuclei are present in epidermal cells, but will not be present in each cell in a section because the nucleus is in only one part of a relatively large cell. (Use the alternative planes of sectioning to prove this point. Why does the paradermal section offer a better sampling of nuclei in the epidermis than the transverse section?) Thus, you will need to develop criteria for knowing, reliably, whether a part of a cell is absent from the cell versus only not represented in the particular thin section of the cell that you saw. The vascular bundles present special problems. Here, certain cells are actually enucleate while others lack all cell contents (only walls are present). For now, at least, you should recognize the latter, conducting cells of the xylem.

Activity 2: Determining that plastids contribute to the coloration of plant parts.

A. Spend 10-15 minutes here. Make a section of a green tomato fruit (*Solanum lycopersicum*) and determine what cellular components contribute the green color. Try to section a ripe (red) tomato fruit. Alternatively, tease apart (macerate) part of the fruit into a drop of water with a needle. What cellular components contribute the red color? Plastids can be wholly or partially responsible for the color of an object. When green, yellow, orange and red colors are localized in protoplasmic particles, they are almost certainly located in plastids. Alternatively, similar colors can be located in vacuoles or in cell walls, but the distribution of the color as seen in each cell will then be characteristically different. Peel off the epidermis by itself from a red tomato fruit. Look for color in the walls. This color is not red. It should appear yellow or amber instead. Recall Activity 1A for the appearance of vacuolar pigments. How does the present case differ? You can look for partially ripened fruits and see if they have "partially ripened plastids". Would this supply evidence that the red plastids (chromoplasts) are an altered form of the green ones (chloroplasts)?

B. Literally, this takes only few minutes. Take a section of each of three colors of peppers (*Capsicum*). Crush part of the fleshy tissue into a small drop of water. How is the red, green, yellow and orange colors distributed? If it remains in "particles" it must not be water soluble, and it is probably in plastids (chromoplasts). Do not expect to see much detail in these preparations. Investigate these for chloroplasts and intermediate plastids, using sections. Do the plastids of fruits always become colored brightly? When a fruit is red or yellow, is this because of color in the plastids? Don't jump to conclusions. Approach the problem empirically when you set about to do a diagnosis of an unknown.

Activity 3: Spend 10 -15 minutes on this activity. Determining that the colors of plastids and vacuoles combine to produce variegated leaves (This applies to petals, also.).
Vacuolar pigments are homogeneous at the cell level, as in the *Tradescantia* stamen hair cells. Variegated leaves of *Coleus* show a variety of color patterns. What accounts for these colors and how is their distribution related to the organization of cells and of the leaf tissues? Try to analyze the color patterns of leaves by using transverse hand sections of fresh materials. Choose the location of sampling so that your sections overlap regions of different colors. You already know how to recognize plastid pigments, by their localization in organelles. By carefully focusing your microscope you should be able to see when vacuolar pigments and plastid pigments are in the same cell. If there is no vacuolar pigment, how can you tell whether the cell was made less colorful by injury? Remember that if a cell is broken, vacuolar pigments leak out because they are water soluble. Therefore, the thickness of your section must enter into a consideration of this problem. Analyze the colors of the *Coleus* leaf to determine which colors are in vacuoles and which are in plastids, and which color(s) are in the mesophyll (ground tissue system) and which are in the epidermis.

Activity 4: Spend about 10 minutes here. Looking for crystals.

Section petioles and midribs and blades of *Hedera*, *Parthenocissus*, *Ficus*, *Musa*, etc. Look for crystals and identify the kinds of crystals that are present. If transverse sections prove to be inadequate to demonstrate crystals in situ, try longitudinal sections. How specialized are the crystal-containing cells in each case? Are they scattered among other cells? If so, they can be called idioblasts. Sometimes the word idioblast is applied to crystal cells only when they differ in form from the surrounding cells. Try to get a similar result with paradermal sections. Crystals form in vacuoles. Most often they have no evident function apart from the accumulation of wastes in a biologically inactive form.

Activity 5: 15 minutes to an hour.

A. *Begonia.* Section stem or petiole, and name all components that can be diagnostically identified, using cells from all regions of your sections.

B. Petals and Fruits: Determine the sources of coloration, diagnosing the pigmentation of vacuoles and plastids in each tissue layer: upper epidermis (adaxial), ground tissue, lower epidermis (abaxial), or in "skin vs. pulp" in the case of a fruit.

Questions

1. Name and characterize the interface between:
    a. Protoplasm and wall
    b. Protoplasm and vacuole
    c. Cytoplasm and plastids
    d. Cytoplasm and nucleus

    as you considered the following: Are membranes present? How many in each case?
2. What is the biological significance of compartmentalization at intracellular level?
3. What cell components are easily seen with a compound microscope?
4. How does the concept that cells are compartmentalized help us to organize our information about cell structure and function?
5. How are the living and nonliving parts of a cell fitted together in an organized unit?
6. How do you recognize the following in a plant cell
    a. a chloroplast
    b. a vacuole
    c. a raphid
    d. a cystolith

Part Two: The Cell Wall

1. How useful is the concept of primary wall vs. secondary wall? If one is interested in growth, it is useful to have the concept that the primary wall is that part of the wall that is produced during the increase in area of the cell surface. But surely at the ultrastructural level there must be some fibrils that are transitional between the obviously stretched primary wall vs. totally unstretched layers of the secondary wall. This appears to be the case. As a further distinguishing feature,

one could point out that the microfibrils of secondary wall layers are parallel, within each layer, while the fibrils in the primary wall layers appear to be in a more random, netlike arrangement. However, some workers believe that growth of the cell can pull the primary wall fibrils into a nearly parallel arrangement if they are not parallel to begin with. Some evidence from electron micrographs indicates that wall fibrils are actually parallel in the primary wall when it is produced. The situation appears to be very complicated. In general, we will stay with the idea that the primary wall is the wall built during cell expansion, whereas the secondary wall comes afterwards. But if you have only one slide or one stage of development at your disposal, then it is not possible to derive from direct observation whether the walls are primary or secondary. In such cases we will simply inform you of which walls are considered secondary and which are not.

2. Pits and pitting. Note that the terminology that is applied is related to the concept of primary vs. secondary walls. We will refer to the occurrence of thin areas in walls as pitting whether the walls are primary or secondary. A pit is a discontinuity in the secondary cell wall. The concept that a pit is a distinct entity added to a primary pit field (which exists only in the primary wall) is supported by the fact that the pitting in the primary wall does not always coincide with pitting in the secondary wall.

Laboratory: The Cell Wall and Shape

Objectives: After performing the activities of this lab, you should be able to:

1. Recognize that physical and biological factors regulate cell shape.
2. Detect cells with irregular shapes and speculate on how these shapes came about.
3. Recognize that cells of irregular shape are especially significant in aeration of plant tissues.
4. Detect and characterize pitting in the walls of various cells.
5. Apply direct and indirect evidence to the interpretation of wall structure.

The activities in this lab consist of examining non-biological and biological materials for obtaining information on cell shape **(Activities 1, 2)**, and examining dead and living cells for the properties of their walls **(Activity 3)**.

Activity 1: Showing that isodiametric soap bubbles resemble in shape the cells of *Sambucus* (elderberry) pith.

One common shape for cells in compact tissues is the many sided solid of approximately equal dimensions in all directions. An angular appearance in isodiametric cells is maintained as long as the cells are pressed together. You will recall that the cells in ripened fruits tend to be more rounded than angular because they continue to enlarge when they are no longer completely cemented together. A free cell should attain the shape of a sphere, unless energetic forces are supplied to prevent the cell from attaining this shape, which is said to require the least energy for maintenance.

Using a straw, blow soap bubbles in a test tube and with the stereomicroscope observe the shapes of bubbles at the interior and at the periphery. Start with a few isolated bubbles and build up the mass after observations on the shapes of the first few aggregates that are derived. To obtain a "solid" mass of small bubbles, blow with the tip of the straw pressed gently against the bottom of the tube. Use the stereomicroscope to examine the bubbles and compare with freehand sections of *Sambucu*s pith viewed with the compound microscope (also, save these sections for Activity 3). Look for parallels in shape between the bubbles and the cells. Where do curved surfaces occur in the bubbles and in the cells? Is

there anything analogous to an intercellular space in the soap bubble system? Try to find tiny bubbles at the corners formed by large bubbles, to answer this question.

Activity 2: Detecting armed parenchyma and irregular cell shapes.
If cells elongate uniformly, they become cylinders or polygonal prisms. If the ends of the cells overlap, the cells taper at their ends, but their shape is still fairly regular. Many tissues contain cells of irregular shape with large intercellular spaces. If the cell has conspicuous tubular projections the cell is said to be "armed", and this is a common shape among parenchyma cells.

A. Obtain a piece of "reed" (stem of *Scirpus validis*) and cut it transversely and longitudinally to see that it is constructed of chambers, bounded by longitudinal walls (or vertical partitions) and transverse septae (or diaphragms). Use a stereomicroscope to see that the chambers are not entirely empty. Make hand sections to observe the plant part at the cellular level. You should see that the chambers are filled by a web of cells with irregular shapes. Look at images of *Scirpus* stem in transverse section to complete this activity. First note that the images confirm that the chambers are separated by partitions that are one layer of cells thick. Identify vascular bundles. How are these situated in the stem? (*Scirpu*s is a monocot.). Now look for a diaphragm (a sheet of cells parallel to the plane of sectioning) that fully or partially closes one of the chambers. The diaphragms are thin, so any curvature causes them to be "incomplete" in the sectional view. Diagnose the shape of the cells in a diaphragm, and be sure to distinguish between cells and intercellular spaces. The next observation calls for patience and skill. Look in the chambers for evidence of cells. They are lightly stained but the cells are there: as the stereomicroscope showed, the chambers are not empty. Diagnose the shapes of the cells in the chambers.

B. Tease apart a flower petal of *Aster* (daisy family), and look for elongated, armed parenchyma in the ground tissue system. You have to tear the tissue apart thoroughly or this exercise will not work! Look at the fragments of the petal for air trapped in the intercellular spaces and for the cells that are revealed at the torn edges of fragments. You should see elongated cells with short arms sticking out in all directions. This result will not be obtained unless you are really vigorous when you pull the petal apart with your needles!

C. Make a transverse section of a leaf of pineapple (*Ananas*), and look for armed parenchyma. Survey the whole section at low power to locate the armed parenchyma as small "spots" in the mesophyll. What other shapes do parenchyma cells have in this leaf?

Activity 3: Looking at cell walls, especially for pits.

A. Resume study of the sections you made of *Sambucus* pith. Mature pith here consists of dead cells, and we may say, for simplicity, that only walls are present. Unless your section is from relatively old pith, this simplification may not be warranted. If your sections are thick enough, a gas bubble may be present in any cell that is whole. These bubbles are formed from the gas present in the empty cells of the intact tissue. They are not introduced by cutting the section. The partition between two adjacent cell cavities consists of two walls. Look for pitting in the wall. Pitting in these walls is easiest to see in face views of the walls. Look for small areas that seem to differ slightly in color or density.

B. More favorable material for seeing pitting is obtained by cutting cells with thicker walls. Cut transverse sections of *Ficus* leaf blade, near and in the midrib and orient yourself to find the multiple upper epidermis (adaxial) . Find pitting in face views of walls and in sectional view.

   Demonstrate that the wall is continuous, although thinner, in the region where pitting occurs. The pitting in the epidermis is all simple. Note that the shape of a pit in a secondary wall need not be any different from that of a primary pit field in a primary wall. After you have done the

above you can see what problems arise with prepared slides, Look at the image of *Ficus* leaf. Is pitting visible? Perhaps easily in face view but not in sectional view. Also, try transverse and paradermal sections of green tomato skins for pitting in the thick anticlinal walls of the epidermis. The pitting in parenchyma cells shows up best in fresh materials, but you should be able to find pitting in given images. When we look at tracheary elements in the xylem, you will have no trouble seeing pits in images. Spend a minute or two looking for pits in the images of ground tissue system of *Lobelia* stems.

C. In hard and thick-walled cells the pitting is prominent. In images of *Yucca*, coconut, date, pecan, etc. locate thick-walled cells and look for branched and straight pits. Note that "ramiform" is the same as "branched", but a somewhat better term to use. Considering what brings about the shape of the canal, branched is not a good description of what happens. Remember that the wall is built from the outside toward the inside of the cell! Thus, the pits converge with time; they do not branch.

D. In sections of *Diospyros* (persimmon) endosperm, look for thick walls and plasmodesmata. Are the plasmodesmata in pitted areas of the wall? Very likely some of the lines you see are clumps of plasmodesmata or their size is enhanced by staining. Single plasmodesmata should be beyond visibility of a light microscope. However, no pitting is present. Now, try to prepare a slide with pear pulp. Dye the pulp with a drop of Safranine. What do you see?

E. In sections of *Sequoia* (redwood) wood, find face views of pits. Face views of pits can be seen in the two types of longitudinal sections, radial and tangential. Reconstruct or visualize the three-dimensional shape of a circular bordered pit with circular apertures. What changes are required in your reconstruction to accommodate the elongated inner aperture?

Questions

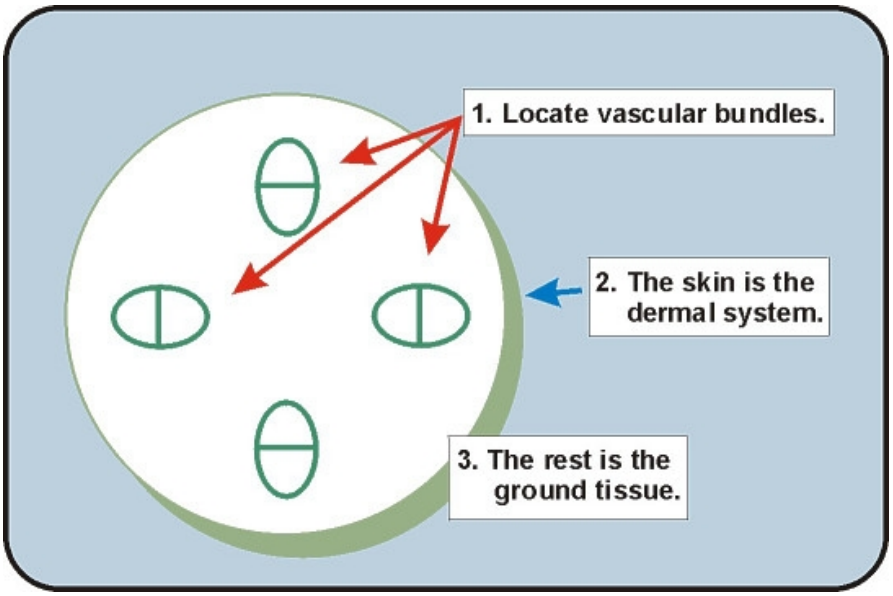
1. Why is the cell wall important in plant design?
2. Criticize or clarify the following statements:
    a. “Pitting is the occurrence of holes in the wall”
    b. “Two cells are separated by one wall”
3. What are plasmodesmata? Where do you find them?
4. Is there a necessary concurrence on the location of pasmodesmata and pitting? Justify your answer.
5. Evaluate the following statement: “A pit is an area in the wall where secondary wall is not deposit”. Justify your answer.
6. There are many different type of pitting, construct a table of the different pitting types and explain the differences.

Topic 2: Three Tissue System

Objective

After studying this topic you should be able to describe the concept of three tissue systems and apply this concept in making comparisons of typical monocotyledonous and dicotyledonous plants.

In the lab activities it will be necessary for you to be able to recognize the three basic tissues: dermal, vascular and ground.



How to get started: First make a map, emphasizing the locations of vascular bundles. Then look for cellular features like thick walls and variations in the colors.

Commentaries:

1. Our preoccupation with vascular plants leads us to emphasize the arrangement of the vascular tissue in our description of organization. The vascular tissue is continuous from root, to stem, to leaf and flower, and the arrangement of this tissue system is an important anatomical feature in each of the plant parts. There is a "typical" arrangement of vascular bundles for each part of the plant, and the arrangement of xylem and phloem is correlated with the identity of the part in many instances.

   In addition to the vascular tissue system, one can quickly recognize the dermal tissue system, the superficial layer (or layers) of the plant part. The tissue filling the space between the vascular tissue system and dermal tissue system is simply called the ground tissue system.

2. One might be inclined to speculate that this approach must be too simple to be significant. For example, even in hand sections one sees more than three types of cells. The so-called ground tissue system is not uniform, and the cells in the dermal tissue system sometimes resemble those in the ground tissue system directly beneath it. How does one cope with these (and other) details while still recognizing only three tissue systems?

   The answer is that the recognition of the three tissue systems is only the beginning of a description. It is the first level or hierarchy that we will recognize in the anatomical analysis or diagnosis of a plant and its parts. However, for some purposes, a description of the arrangement of the vascular tissue system is sufficient to answer the question one has stated: e.g. Distinguish

the anatomy of the stem in a typical monocot from that in a typical dicot. (The emphasis on typical will become clear later.)

The "scattered" bundles of the monocot contrast in arrangement with the "ring of bundles", as seen in a transverse section of the stem of a dicot. We then realize that in the dicot the ground tissue system is divided into two **tissue regions**, the **pith** at the center and the **cortex** at the periphery. This means that recognizing the three tissue systems leads to noticing other details and provides a way of recording them.

3. If one wishes to describe any tissue system completely, **cell types** and their arrangements relative to each other have to be accounted for. As an example, we say that vascular tissues are **complex tissues** because they consist of more than one cell type. Tissue regions can be complex, also. Cortex and pith can contain more than one type of cell.

   As details accumulate, the description becomes more and more complete. We eventually acknowledge that the same cell type can occur in more than one tissue system. But the recognition of the three tissue systems provides a convenient organizational background, against which we can begin to catalog all the levels of detail that we will have to cope with. It is not intended that the recognition of the tissue systems should imply all the cell types that are to be found there. We are not trying to cope with all levels of detail simultaneously. It is much more orderly to approach our descriptions by introducing details in successive stages of the analysis. For example, suppose you are comparing the anatomy of two monocot stems. Both have the typical arrangement of scattered vascular bundles. Now you must look for another level of detail to tell the stems apart or you must verify by careful observations whether the stems are alike in every regard.

4. Now if the concept of three tissue systems is to be taken seriously, a few more requirements must be met. The concept must be useful in thinking about the changes that take place as plants grow. For example, **mature tissues** come from immature cells continuously as the plant grows by **apical meristems**. Growth continues with **lateral meristems** forming the **secondary tissues** of the plant body. Moreover, we know that when a plant forms seeds, a single cell (the **zygote**) produces a multicellular **embryo**, and the complex plant is organized anew. It is important for you to keep in mind the following:

   a. Mature tissues developed from immature tissues originated from apical meristems.

   b. The transition to secondary growth.

   c. The origin of a highly organized plant from an embryo.

      In addition, by the end of this topic you should see these additional advantages to the concept of three tissue systems, which we already covered:

   d. Allows comparisons among species.

   e. Allows comparisons of plant parts.

   f. Provides an organizational framework for coping with details.

LABORATORY

This lab accomplishes the objective of applying the concept of three tissue systems in three ways:

1. Among species. **(Activity 1)**
2. Among plant parts. **(Activity 2)**

3. With developmental changes. **(Activity 3)**

Remember that your study of plant structure should lead to thoughtful and original observations. Moreover, you have the ability to prepare your own materials for about half the observations you will be asked to make. A little skill and a little patience couple together and make it possible for you to put fresh materials to good use for anatomical studies. Since all prepared slides are derived from fresh collections in some way, manipulation of fresh materials also improves the appreciation and understanding of the use of prepared slides. We must also begin to develop the important concept that simple observations are prerequisite to the more complex observations and for ultimate explanations.

Although this lab emphasizes organization look for cellular details as well. Try to anticipate the need for developing the various topics by looking for all the levels of detail that must be coped with, eventually. **Recognizing** that there are things to name is very important to your success in the laboratory.

REMEMBER: Work out the details! Seeing that there are things you need to describe will help you prepare for new learning in this course. But it also shows that your own powers of observation will guide you toward a complete knowledge of the subject.

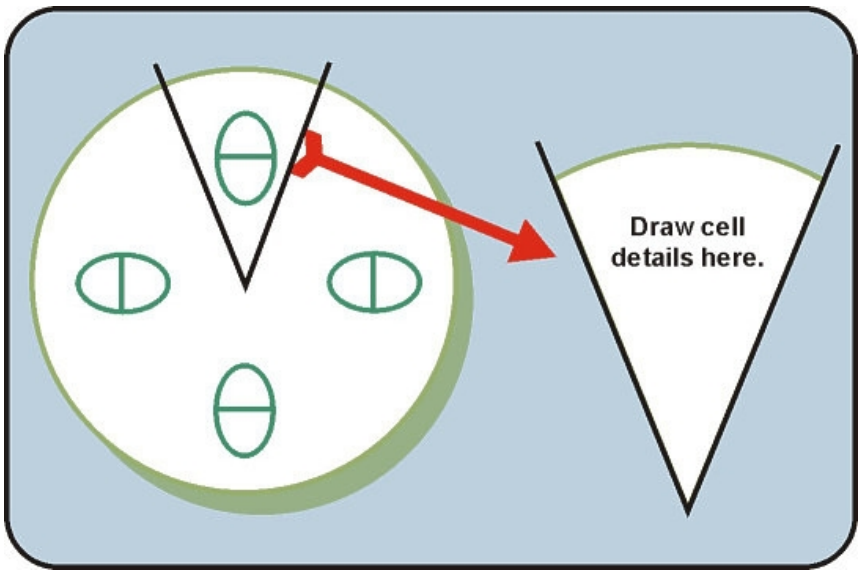
Activity l

Select four plants (two monocots and two dicots) for intensive study, and the instructor will discuss sampling procedures with you at the beginning of the lab. We are not going to emphasize completeness of the sample in this lab, but you must become aware of how a sample has to be chosen to answer a question, and what limitations a given sample imposes on your conclusions. For example, will a section through a node furnish a different answer to a question than will be furnished by a section through an internode? This situation varies with the question! Always try to formulate a question (objective) **before** you decide how or where to take a sample.

If you can **establish the organization of stems**, you will have a basis for comparing different plant parts and different plants. Use hand sections, mostly transverse, and observe them without staining. Use a stereomicroscope and compound microscope for observational tools. Determine the heterogeneity of cells in sectional view. Apply the accepted terminology to whatever parts you identify.

Suggested procedure for summarizing observations: Sketch segments of the transverse sections of the stems of the four different plants, and annotate your drawings. Look for and record the similarities and differences in organization. Be particular about the relative locations of cell types. Look for cells with thickened walls and note their distributions.

Review the "commentaries" numbered 1, 2 and 3, at the start of this topic in light of your drawings. Using the scheme below, to guide your records of what you see.



Recording details in context: On the map (diagram of section) details like the location of thick-walled cells under the surface can be shown by shading. Reserve details of cell size and shape for enlarged drawings.
Notes and legends complete the drawings

Distinguish between monocots vs. dicots with a description of the arrangement of the three tissue systems. Expand or modify the description to distinguish between the two dicots, and between the two monocots.

Consider sampling problems -- How does one go about sampling structural materials from a whole plant? For example, if you compare a node of the monocot stems with the adjacent internodes, **some** aspects of the tissue systems may be totally different, but not in all cases. Also, if you compare a seedling of one dicot and an old plant of another, the amount of secondary growth could enter into apparent differences between species. Secondary growth in the dicots could add to the differences between the dicots and the monocots in our sample.

You must always ask: Am I dealing with characteristics that are a function of location, age, and stage of development as well as species diversity? (Or any other possible factor of interest.) This situation leads one to adopt, eventually, a systematic approach to identifying and appraising sources of variability that affect our objectives.

Answer this "minimal" question: In the two monocots, does the node differ from the internode in mature stems?

Activity 2

Choose at least one plant and compare the stem and leaf as seen in hand sections. Try to characterize the differences, using the concept that there are three tissue systems to deal with. Do not exhaust your time in the lab with this activity. You can return to this part of the lab if you have time later. NOTE: corn (*Zea*) seedlings work well for this activity.

Activity 3

The materials available should permit some insight into the usefulness of the concept of the three tissue systems during three types of developmental changes:

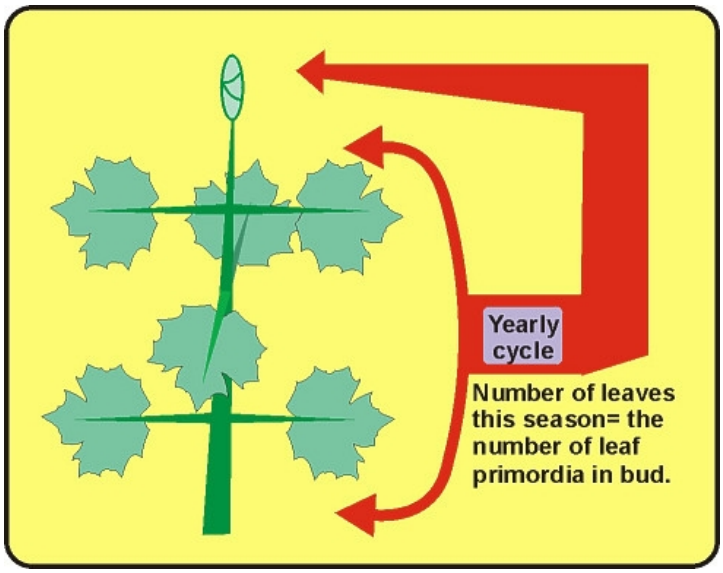
a. The onset of secondary growth.

b. The development of mature tissues from an apical meristem.

c. The differentiation of parts in an embryo.

A. Does secondary growth lend itself to the recognition of three tissue systems? Examine images of stems for details of changes with the onset of secondary growth. Epitomize the changes (or lack of changes) in each of the tissue systems.

Does the *Zea* stem undergo similar changes caused by secondary growth? You can use your hand sections to look for evidence on this point. A relevant comparison would be the stem of a young plant (seedling) vs. that of an old plant. The vascular tissue system of old and young plants consists of scattered bundles. There is no secondary growth from a cambium in the corn stem. We will return to this topic later in future labs. Compare images with your hand sections to determine whether the images bring out any features not seen in hand sections, and **vice versa**.



How to get started: This season's mature primary tissue has much more bulk than the tiny, meristematic shoot for next year, concealed in the bud. Typically, parts of the mersitem earmarked for maturation grow faster than the part reserved for proliferation.

B. Are there three tissue systems in the apical meristem at the tip of a shoot? What parts of a bud account for the shoot that is formed each year? Obtain a twig of maple (*Acer*) and make the following observations:

Determine the length of this years growth (axis with leaves attached, note changes in surface color and texture) and the number of leaf pairs expanded during this growing season (= number of leaves attached to the stem). Verify that the shoot terminates in a bud, covered by bud scales.

Anticipate the number of leaf primordia and young leaves that are inside the bud. This number could be zero, on one extreme, or it could be as high as the usual number of leaves expanded in a season. If it is the latter, it would mean that a tiny, **subapical** portion of the bud is responsible for most of the visible shoot formed during the **next** season (viz. next spring). You should find that the *Acer* bud approximates this situation. Begin to dissect the bud under a stereomicroscope.

With a needle or scalpel dissect away each pair of bud scales. This is easily accomplished by bending back the scales from the tip. The last pair of bud scales is hairy and there is an abrupt transition to the vegetative leaves. What are the finger-like projections on the young leaves? Comparing young and mature leaves should help you realize that these are the lobes of a leaf in the meristematic condition.

In relative and/or absolute terms: How long is the region of the axis that bears next years leaves?

Find the **shoot apex**, which is a dome nestled between the youngest leaves. This dome continues to grow throughout the life of the shoot, making room for the formation of more leaves and bud scales with the progression of the seasons. The dome contains the region of **proliferation** in the shoot tip. Look at the longitudinal section of the apical meristem of a maple (*Acer*) shoot. Confirm that the apical dome (shoot apex) **does not contain mature cell types**.

Try to find the protoderm, procambium and ground meristem in the **region below the dome**. The formation and growth of leaves is associated with the formation of vascular tissue. Thus, the base of the dome, where leaves form, is the **region of organogenesis and histogenesis** in the shoot. The part of the *Acer* bud containing leaf primordia is rather tiny compared to what it becomes as a mature shoot! Therefore, its tissues must be meristematic, and we find there the **meristematic precursors of the three tissue systems**.

Optional activity

If you are good at dissections, try to find the shoot apex in one of the corn (*Zea*) seedlings. The apex may be far down, at soil level, if the seedling is young enough.
How does the development of the embryo relate to the usefulness of recognizing three tissue systems?

Reconnoiter: You may feel that it is hard to visualize what you are responsible for in the first few lab sessions. **We must build observational skills.** You must be able to say -- "I can find things that should be described." Formulate the descriptions as time passes, but remember that you are not just learning facts. You are also getting organized and developing observational skills.

CONCLUDING ACTIVITIES

Questions 1-4 are for background, not the type of content for questions to appear on exams.

1. How many levels (hierarchies) of organization can be detected in plants? To answer this question you will have to deal with these terms, among others: plant parts; tissues, tissue regions, tissue systems; cells, cell types, cell parts, organelles. Is it clear how these terms fit the question?
2. Try to decide for yourself what constitutes a description of a cell type, viz., what aspects or features of cells must be dealt with in such a description?
3. How are the sections that you see in the images treated to enhance the demonstrability of certain cell types? To answer this question, speculate on how the features that already differentiate cells in unstained sections could contribute to differential staining.
4. What is the basis for differential staining? Make the best guess you can and bring the question up for discussion if you want to. Consider chemical and physical properties of cell constituents, but do not try for a complete or complicated answer.

Questions 5-12 have the content, but not the form of exam questions. Could be easily rewritten for exams. Be able to **verbalize** the answers.

5. If you designate a tissue **system** properly, to what extent does this designate the type(s) of cells found there? Answer this question, also, for tissue regions (cortex and pith).

6. Is the concept of three tissue systems applicable to a plant that has secondary tissues? Describe the changes (or lack thereof) in the three tissue systems during the transition to secondary growth.
7. Epitomize (give a brief answer covering crucial points) the differences between typical dicot and typical monocot stems with respect to arrangement of the three tissue systems. Could the location of your sample within each plant have an effect on the appearance of the three tissue systems? How could this affect your comparison of two dicots? of two monocots?
8. Now that you have looked at two monocotyledonous stems,
    a. Could you tell the plants apart from a single section of each? (This is like asking, "Can plant anatomy be useful in plant identification?") You are allowed to choose the locations of the sections.
    b. Can you describe a hierarchy of similarities and differences between the two? (This is like asking, "Is there a systematic way to describe anatomical variation?")
    c. Would your answers to *a* and *b* be necessarily the same if you were working with two other (new to you, "unknown") monocots?
    d. Can you use your answer in *b* to describe two dicotyledonous stems?
9. List the information that one must know **and criteria that must be applied** to answer this question: In the two monocots studied in the lab, do the nodes differ from the internodes in mature stems?
10. When do the three tissue systems first appear during the growth of an embryo? (You can answer in terms of stage, size, shape, number of cells or cell layers present.)
11. How close to the apical meristem of the shoot can three tissue systems be recognized?
12. A bud is a highly condensed shoot. Compare the distance between leaf pairs within the bud vs. that found on the fully elongated twig of a maple, and answer this question: What grows faster, the region near the shoot apex, or the region further behind it? Clarify any difficulties that arise in answering this question.

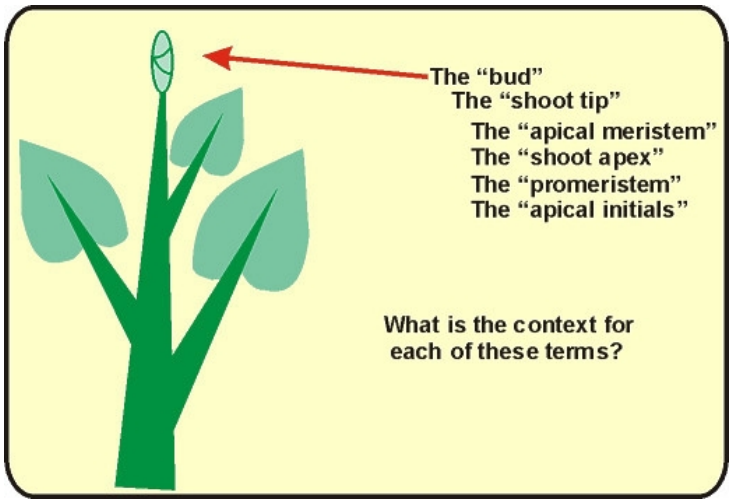
Topic 3: Meristems

Objectives for the Shoot Apical Meristem (SAM)

1. List the characteristics of the apical meristem of the shoot that are basic to its organization and be able to recognize these features.
2. Judge the validity of the concept of apical initials and supply reasonable alternatives to this concept in describing proliferation of the shoot apex.
3. List the cytological characteristics of procambium and be able to recognize procambium.

An embryo is at first **generally meristematic** and that the meristems are "set aside" for continued growth at relatively late stages in embryogeny.

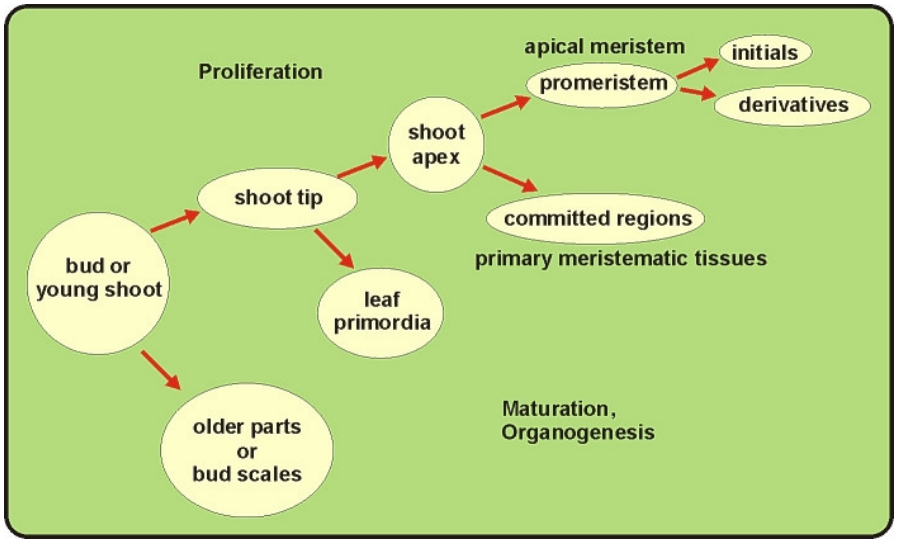
Another important concept relates to the presence of **initial cells** (meristematic, stem) in a meristem. Such cells are said to have a definite position, and divide to produce **derivatives** while maintaining their own original position.



Terms have meaning in context:
Always try to identify or create a context for terminology.
Your learning will be more meaningful this way!

A meristem at the tip of a shoot is considered an apical meristem. The meristem is an organized region of the plant and some authors have tried to relate that organization to how the meristem operates.

Words like **apical meristem, shoot tip**, or **growing point** do not have a constant meaning among all writers; e. g., sometimes the word **shoot apex** is used as a synonym for **apical meristem**. Other times, apical meristem is intended to mean **promeristem**, the initials and their recent derivatives. Let us agree that the **promeristem** has no histological differentiation, and is concerned with proliferation, only. Somewhere below the promeristem we will find the upper limits of the primary meristematic tissues, and this region is concerned with tissue and organ (leaf) formation. Let us also agree that the portion of the shoot tip that is above the youngest leaf should be referred to as the shoot apex. If the shoot apex is larger than the promeristem, it will contain a region that is transitional between the region of proliferation and the region of histo- and organogenesis.



The stem proliferates from the tip: a meristem, part of the region is reserved for growth (proliferation) and part is committed to maturation. In theory, this distinction can be applied at the cellular level, within the meristem itself.

The young tissues produced by the apical meristem are **generally meristematic** (most all cells divide). By examining the shoot tip of an *Acer* twig you learned to appreciate the fact that much of the growth of a stem comes from below the shoot apex, when the internodes elongate. This is commonly referred to as subapical growth. **Subapical growth** fades away as the internode matures. In certain kinds of plants, there is **residual meristematic activity** at the base of each internode.

In summary, we recognize the presence of meristems because the function of cell proliferation becomes restricted to particular parts of a plant. If a plant part is generally meristematic its growth is **diffuse** and no meristem can be recognized. If growth is sustained in one region while it eventually stops in others, then a **meristem** is present to be recognized. These concepts hold true for primary growth **and** secondary growth.

A comment on the concept of differentiation:
The meaning of the word **differentiate**, and any of its derivative forms, depends on the context or application of the term. To add significance and precision to the use of the word, it is wise to designate **what kind** of differentiation one has in mind. For example, the apical meristem has a **functional** differentiation into a region of proliferation and a region of histogenesis and organogenesis. In the former there is no differentiation with respect to histology, but there may be **cytological** differentiation among the cells. In the region of histogenesis and organogenesis there is **histological** differentiation (because the primary meristematic tissues of the three tissue systems can be recognized) and there is **differentiation of plant parts** (stem vs. leaves).

The late changes in cytological differentiation are referred to as cell **maturation**, because a stable condition or endpoint is being approached. Let us avoid the argument that alleges "the only mature cell is a dead cell". This tongue-in-cheek allegation relates to the implication that a mature cell **should not be able to change**. This is not a useful concept in the study of plant anatomy. Much of a mature plant part can consist of parenchyma cells. These are capable of remarkable changes under the right conditions, for each parenchyma cell has the potential to reproduce the whole plant. This potential is not manifested under normal circumstances, and it is therefore permissible to consider a parenchyma cell as a mature cell type.

Certainly, there is more than one approach to studying cellular differentiation. In this course we will be concerned with the descriptive aspects of differentiation -- the changes in the structural characteristics of cells -- rather than in "causal" aspects of differentiation.

The concept of stem (initials) cell:

a. are in a position to perpetuate a plant or plant part,

b. are mitotically active,

c. maintain themselves in their original position.

Concepts of apical organization:

a. Apical cells do exist, but they are not present in all meristems.

b. Analysis of organization by planes of cell division is the basis for the tunica-corpus concept.

c. Cytohistological zonation can be recognized regardless of how the cells are arranged in the apex.

d. The concept of a totally inactive region at the tip of the apical meristem has been refuted as a general model of apical organization of the vegetative shoot.

There are several major points students should remember-

a. Although types of apical meristems can be recognized, the simplest classifications are the best, and one can always resort to the recognition of a region of proliferation and a region of histo- and organogenesis, regardless of other organizational features.

b. Apical cells are recognized by their large size and strategic location in the apex. Among vascular plants, adult apices with "unique apical cells" are restricted to the vascular cryptogams (pteridophytes).

c. Most conifers have periclinally dividing apical initials in the surface layer. These divisions are antithetic to a tunica-corpus organization but not to the presence of protoderm.

d. A tunica layer has no periclinal divisions. The number of tunica layers is judged subjectively.

e. Gymnosperms have a mantle-core organization in the shoot apex. Most angiosperms have a tunica-corpus organization.

f. The apex may change during a plastochron, with respect to size, shape, number of tunica layers, and cytohistological zonation.

g. The position of new leaves is a function of existing leaves, interacting with the shoot apex.

h. Leaf primordia are important in regulating the arrangement of the vascular tissue system in the shoot.

Objectives for the Root Apical Meristem (RAM)

1. List the characteristics of the apical meristem of the root that are basic to its organization and be able to recognize these features.

2. Judge the validity of the concept of apical initials and supply reasonable alternatives to this concept in describing proliferation of the root apex.

3. List the cytological characteristics of procambium and be able to recognize procambium.

When we discuss the root apex, look for these points:

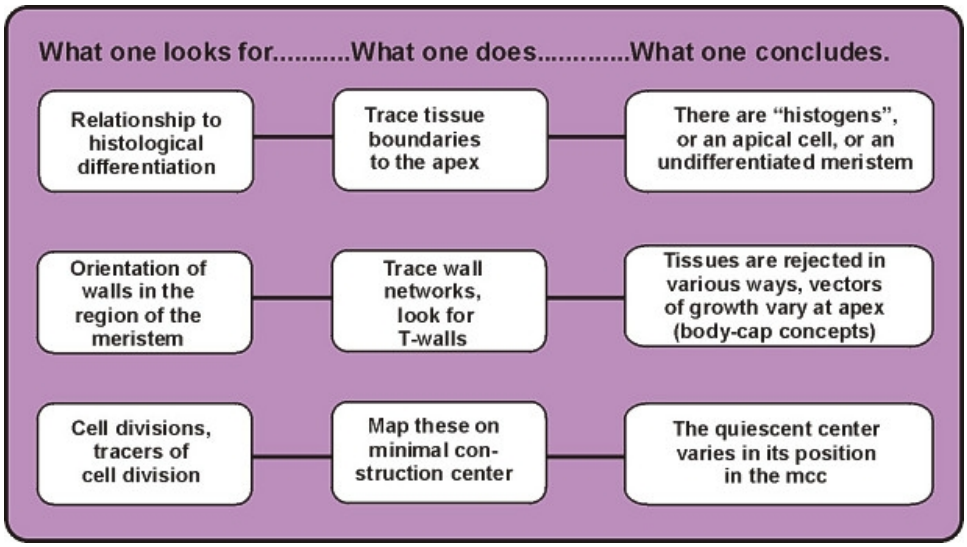
a. The first approximation of how the root apex operates is obtained by looking for a relationship between the mature parts of the root and the meristematic region.

b. This leads to looking for regions within the meristem that generate the root cap, epidermis, cortex, and central cylinder. Such regions seem to exist within some meristems, and they have been called histogens. The cells of a histogen are called initials.

c. In some roots (viz. grasses) a conscientious analysis of geometry leads to contradictory predictions on the growth of cells within the meristem, where one would expect to find initials. (Recall the properties of initials: they have a critical position with respect to geometry; they are mitotically active; they maintain their position in perpetuity.)

d. The histogens, taken together, comprise the region of initials in the classical sense. Experimental evidence indicates that of all these cells, only the ones generating the root cap are mitotically active.

e. Therefore, the geometry of the apex tells us how it is organized but not how it operates. A neutral term is needed to describe the organized region once called the region of initials. We can use the term "minimal construction center". This is physically equivalent to the classical histogens, but does not imply, in name, that the region contains initials.



Three approaches to root apical meristems: 3 approaches that have contributed importantly to the literature on the root apical meristem. We should borrow freely form these and recombine their elements to obtain a contemporary view of how the root meristem works.

LABORATORY

Objectives

1. Recognize and describe the organization of the shoot apical meristem.
2. Recognize and enumerate variables that affect the details of apical organization.
3. Recognize procambium and enumerate and evaluate criteria for identifying procambium.

Activity 1: Relating the details of apical organization to gross morphology.

A. Obtain a shoot of *Coleus* (live) and the images of the median *Coleus* tip. Look at the images and note the outline of the section and the size and density of the cells in each of the "bumps" and

projections along the sides. You should now determine what it is in the whole shoot that accounts for each of these bumps.

Note the arrangement of leaves. Could a single longitudinal section contain the median plane of every pair of leaves or only every other pair?

B. Run the tip of your finger along the stem and you should be able to detect a small ridge or swelling **between** the bases of the pair of leaves at any node. This ridge appears as a bump on either side of a section but the cells in this particular kind of bump are large and vacuolated.

C. Dissect the leaves from the shoot, looking for smaller and smaller leaves, the younger of which are referred to as primordia. From the shape of the leaf you should be able to predict that even young leaves would appear as relatively large outgrowths on either side of the section. Only the smallest leaf primordia would appear as simple bumps along the side of the section, and these should be right at the top of a section, next to the shoot apex.

The only other objects that contribute directly to the outline of the section are branch meristem. From the branching pattern of the whole plant you should realize that in *Coleus* these are always in the axils of the leaves. If you are careful, you should be able to remove the leaves from the shoot until you can see the shoot apex under the stereomicroscope. Visualize how the section on the prepared slide can be a view of the median plane of the axis you are now examining.

Optional activity: Try to make a thick "median section" of the shoot tip by hand by trimming away peripheral tissue so that the shoot tip lies flat on a slide. If you succeed, the fresh preparation will have a striking resemblance to the images when you focus your microscope properly.

Activity 2: Recognizing a median section within a series of sections.

You have used median sections so far. In general, the median (or middle) section reveals the most about the organization of an apical meristem. The median section was chosen from within a series of consecutive sections. You should now consider what criteria are involved in determining which section in a series is median.

Look at the series of sections of *Coleus* shoot tip. Quickly scan the series from left to right and back again to determine which of the successive sections most resembles the section you worked with on the previous activity. The apex of *Coleus* is a low dome. Note how the clarity of the cell arrangement and the shape of the outline of the section change as you look at each section of the series. The median section is at the center of symmetry, i.e. symmetrical changes take place in both directions away from the median. It is not always the middle section of the series but that is the median section.

Activity 3: A survey of apical organization.

Starting with *Coleus*, make a survey of the organization of different apices. *Coleus* has an apex that is a low dome, and there are numerous cells of equal size near the tip of the apex (numerous apical "initials"). The cells are in layers, all the way across the tip of the apex (tunica-corpus organization). To add other apices to the survey, proceed by asking the following questions:

a. Is the apex probably a dome, as in *Coleus*?

b. Are there numerous apical "initials" or a few large cells at the tip of the apex, or even **one** (unique) apical cell?

c. If there are numerous "initials", are they layered into one (or more) tunica layers and a corpus?

d. To what extent is it possible to recognize zones or regions of differing cytological properties within the shoot apex (i.e., distal to the youngest leaf)?

A. Begin with *Coleus* and bean (*Phaseolus*). Compare the apices of these dicotyledonous angiosperms with the apices of *Pinus* and/or *Pseudotsuga* (Douglas fur). The differences are typical of the contrast between angiosperms and gymnosperms. For a marked contrast in shape of the apical meristem, compare the above with *Elodea* (water weed).

   Coleus, with regard to the arrangement and number of primordia seen in a section.

B. *Equisetum* (a vascular cryptogam, horsetail). For comparison, see the apex of *Marsilea* (a water fern). These apices each have an "apical cell" of unusually large size at the center of symmetry.

Activity 4: Procambium and primary vascular differentiation

The primary vascular tissue system in a shoot is typically a complex network of strands that interconnect within the stem and extend into the leaves.

Obviously, no single section can reveal all aspects of the arrangement of the vascular tissue system, and serial sections are required to confirm how far the procambium extends into the apical meristem. Longitudinal sections can show the extent of the strands quite dramatically, but "**gaps**" or discontinuities in a single section are to be expected, because the sinuous path of the strands through the shoot will bring them into and out of the plane of a section.
Important consideration: What do you look for when deciding whether or not procambium is present in a section you are examining?

Use serial sections of *Coleus* to locate the extension of the vascular system into leaves. Do this for older and younger leaves alike. Find the smallest leaves and the highest location in the stem into which extensions of procambium can be recognized. Note that in general, when a strand can be followed directly outward into a leaf, there seems to be a discontinuity of the procambium, directly above that location in the stem. This interruption is known as the leaf gap.

Activity 5: Looking at young roots.

Objective: To gain a sense of proportion and perspective on the size and spatial relationships of young roots, their branches, root hairs, root cap, etc.

1. Note the branching pattern of the roots and designate the axes as primary, secondary, tertiary, etc. Look for root hairs. You can use these plants for hand sectioning to examine roots for their internal anatomy. Venturesome souls can also attempt serial hand sections to evaluate the anatomy of the transition region between root and stem. These are optional activities.
2. Looking at the longitudinal sections of the RAM, identify:
    a. columella
    b. the quiescent center and all the tiers
    c. epidermis
    d. cortex
    e. endodermis
    f. stele
    g. lateral root cap

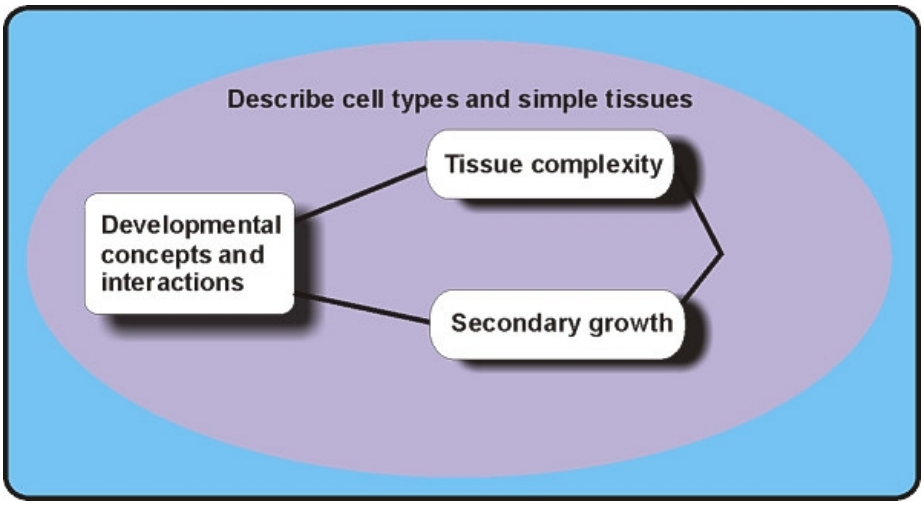
Questions

1. Describe in a succinct and meaningful way the relationship between growth in the apical meristem of the shoot and the growth of the shoot as a whole.

2. List the geometrical, cytological, histological and organographic aspects of an apex that can be used to characterized its organization.
3. Criticize and/or support the following statement: “Generally speaking, the organization of the shoot apex of a plant can be predicted accurately from a knowledge of the systematic or evolutionary position of the plant, e.g. a lycopod, fern, gymnosperm, angiosperm”.
4. What is a “meristem” and how does one recognize when a meristem is present?
5. Formulate an analytical statement devoted to presenting the strengths or weaknesses of the following ideas. Emphasize whether or not you agree with each statement:
    a. The cells of a meristem are “eumeristematic” (The prefix eu- means true).
    b. The fate of a cell is a function of its position in the plant body.
    c. Procambium and ground meristem come from specific histogens in the shoot apex.
    d. Whenever there is a protoderm there is a tunica.
6. Formulate a working definition of protoderm and lost a set of criteria that can be applied for the recognition of procambium.
7. Consider the implications of this description for how we use and restrict the use of our terminology: “The first indication that a leaf is forming on the apex of certain plants can be a periclinal division in the tunica layer.”

Topic 4: Epidermis

With this topic we begin the second segment of our study of plant anatomy, cell types and tissues. The topics now will become more concrete and less abstract, more specific and less generalized. A knowledge of the generalizations is prerequisite to knowing where the cell types and tissues come from, and how their specializations can be described and appreciated. This topic deals with the primary dermal tissue system of the plant. After doing this unit, you should be able to characterize the levels and sources of diversity and complexity in the epidermis.



<u>This segment of the course</u>: The "nitty gritty" of the course requires descriptions of the cell types and careful attention to how cells are assembled into tissues. We learn to deal with developmental changes at the whole plant level, including "secondary" growth.

Commentaries: Things to take notice of during the lab.

1. Concentrate on epidermis in Poaceae (grasses) and try to make a list of the sources of complexity in the epidermis of grass leaves: note **kinds of cells** recognized and the **patterns** in which they are arranged.
2. Is the outer wall of the epidermis complex? In what ways?
3. Stomata: What are they and what do they do? How are they inserted in the epidermis and what aspects of their structure can be described that relate to function? Now for something a little more difficult to digest: stomata develop according to a variety of patterns. If the subsidiary cells are directly related ontogenetically to the guard cells, the development is **mesogenous**. If not, the development is **perigenous**. The outcome of development is the mature stomatal apparatus, which can be described according to the **number and arrangement** of subsidiary cells.
   Avoid troublesome errors by noting that (a) different terminologies have been applied to different groups (with near synonymy between certain terms) and (b) it is not true that looking at the mature pattern **always** tells about development.
4. Multiple epidermis: What developmental criterion would you use to recognize a multiple epidermis? What cytological-histological characteristics do the cells within a multiple epidermis have in common?
5. Points to be emphasized:
   a. The epidermis may consist of a single kind of cell or of an assortment of cells.

b. The epidermis can include more than one cell layer (multiple epidermis).

c. The outer wall of the epidermis is especially complex.

d. In part, the patterns of stomatal development can be viewed as differing degrees of activity of the specialized protodermal cells called meristemoids, or of the cells directly under the influence of the meristemoid or guard cell mother cell.

e. Patterns of cell arrangement in grass leaves can be analyzed as the outcome of correlations between the structure of the whole leaf and alternative developments of short cells into various cell types.

LABORATORY

Objectives:

1. Recognize and characterize the several levels of complexity in the epidermis.
2. Interpret and describe a variety of epidermal appendages from aerial parts of plants.

Activity 1: Recognizing complexity in the epidermis.

In some instances the epidermis consists entirely of one specialized cell type, e.g. sclerenchyma. Examples of this situation are found in seeds and fruits, and will be encountered in later labs. For now, we will examine the epidermis as it appears on many leaves and stems. We will examine the epidermis at several levels of complexity to show that the epidermis can be either homogeneous or heterogeneous, sometimes according to a pattern that involves the whole leaf.
Remember to apply what you have already learned about cell shape, contents and walls to characterize the various specializations of epidermal cells. Use hand sections and epidermal peels, and do not neglect the use of the stereomicroscope.

A. *Tradescantia zebrina* (spiderwort). Perform a cross and a paradermal section. The simplest case we could imagine for an epidermis would be like the upper (adaxial) epidermis on the *Tradescantia* leaf. Describe what is found there. Examine the lower (abaxial) epidermis. How does it differ from the upper epidermis in complexity? What is the source of that complexity? You should be willing to recognize three kinds of cells: guard cells, subsidiary cells and "neighboring" cells (ordinary epidermal cells around the stomates).
Make a sketch of guard cells and adjacent subsidiary cells. Later compare this sketch with what you will see in the sedge and the grasses. Consider all the features of these cells that you can see with your microscope. Describe how guard cells differ from subsidiary cells, and how the latter differ from neighboring cells. Pay attention to cell color, as one aspect of cell contents. Estimate an average, or typical, number of chloroplasts per guard cell. This number should be quite uniform in this material. Tetraploid plants of the same species have ca. 2X the number of plastids per guard cell compared to diploids. Are there also chloroplasts in subsidiary cells and neighboring cells? Be a careful observer! At very low magnification subsidiary cells can appear to be bright green. This is related to their transparency and lack of red coloration. Thus the green color is in the mesophyll.

B. *Ornithogalum* (star of Bethlehem). Paradermal section. Sometimes subsidiary cells are absent. This means that there are no cells of special morphology adjacent to guard cells. Confirm this situation in *Ornithogalum* and note that the shape of the guard cells resembles that found in *Tradescantia*. Are the guard cells raised above or sunken below the leaf surface?

C. *Ficus*. Using a cross section of *Ficus*, compare insertion of guard cells, number of layers of epidermis, etc. with *Thevetia* (yellow oleander). Look for cells that are devoted to crystal formation. These contain a stalk, or outgrowth from the cell wall, upon which the crystalline materials are deposited. The ones you see in your preparation should be gorgeous. Compare *Ficus* and *Thevetia* for complexity of epidermis. Identify the sources of complexity. Now we are dealing with multiple vs. simple epidermis, the presence of specialized (crystal) cells, and details such as sunken stomates. It will not be possible to determine if subsidiary cells are present in *Ficus*, due to the sunken stomates.
Is a cuticle distinguishable in these two species? Compare the thickness of cuticle on the leaves you have seen with that on an apple, *Malus*. What is the function of a cuticle, and how does its visible structure relate to that function.

D. Grasses. *Zea*- cl and x.s. and *Triticum* (wheat)-x.s. Be sure you can recognize guard cells and subsidiary cells. The characteristics of the epidermis are useful in grass taxonomy and systematics if sufficient care is exercised in sampling procedures. Note the diversity of cell types and shapes. Look for these variations in the grasses available in the lab. Determine whether sampling "errors" can be important by comparing (in at least one grass):

    1. adaxial vs. abaxial surface.
    2. sheathing base of leaf vs. free portion of blade.
    3. midrib vs. the rest of the blade.

    Does the pattern of cell arrangement in the epidermis correspond in any way to the vasculature of the leaf?

Activity 2: Diversity: look at as many examples (images) as you wish and observe the different epidermis types.

Activity 3: Epidermal appendages.

Some plants have epidermal appendages. Look for matted hairs, scales, branched hairs, multicellular hairs, glandular hairs, etc. Note if more than one type of appendage occurs on any single surface. Are there any patterns to the distribution of the appendages?

Questions

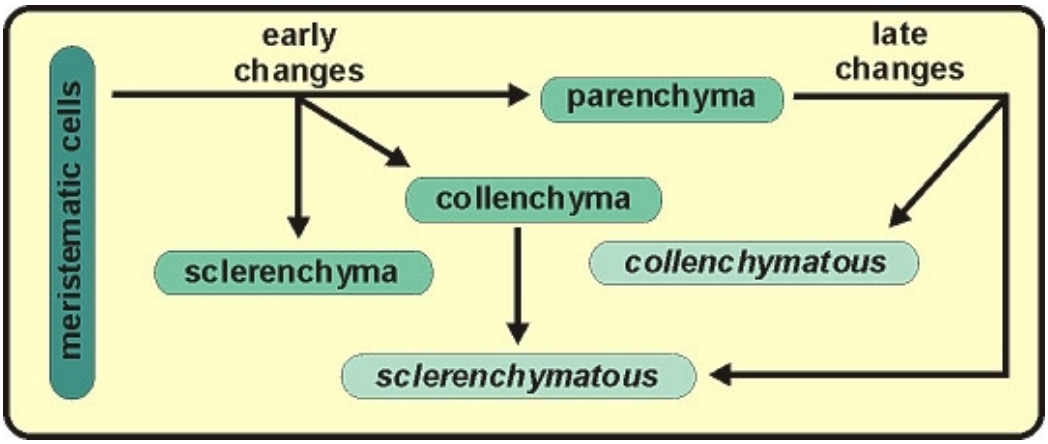
1. When the epidermis consists of a single type cell. What may that cell type be?
2. Described several different situations in which more than one kind of cell is presented in the epidermis.
3. Use a situation in which definite patterns of arrangement are present for the various kinds of cells in an epidermis to illustrate the meaning of the following statement, “The fate of a cell is a function of its location on the plant”.
4. What aspects of stomates are described with words like anomocytic, paracytic, diacytic, etc. (hint: check the common denominator).
5. How should epidermal anatomy used in plant systematics?
6. What are the relationships between structure and function in epidermal cells of various kinds?
7. The rose on “The Little Prince” had her thorns to protect her against the tigers and other large animals. How do epidermal appendages protect against predators with more vegetarian tendencies?

Topic 5: Ground Tissue

PARENCHYMA

Objective: To visualize the cell type called parenchyma, as an assemblage of various living, mature cells. Parenchyma cells are diverse. The sizes, shapes, contents, walls, and functions of parenchyma cells vary.

One factor to consider when addressing "parenchyma" is its developmental changes. The particular name (e.g., collenchyma versus collenchymatous) placed on a cell type can be affected by our understanding of how cells change with aging.



Changes affect cell names: The epithets "collenchymatous" can be used to name cells that closely resemble typical collenchyma and sclerenchyma in final form but not in developmental history or location.

PARENCHYMA LABORATORY

Activity: - Observing different types of parenchyma: Satined with TBO, the primary wall of the parenchyma cells should stain pink-purple.

A. *Apium* (celery): perform cross and longitudinal section of the petiole to observe parenchyma cells.

B. *Potamogeton* (large pondweed) petiole: perform cross section of the petiole to observe aerenchyma.

C. *Ananas* (pineapple) leaf: perform cross and longitudinal section of a leaf. How many types of parenchyma do you observe? What are their differences? How do they differ from *Potamogeton*?

COLLENCHYMA AND SCLERENCHYMA

Objective: To compare the distribution, development and mature properties of the support tissues, collenchyma and sclerenchyma.

This objective will be facilitated if you can prepare a table with specific comparisons to be made between collenchyma and fibers. Sclereids should be added to round out the comparison, but sclereids can be more directly compared to fibers rather than to collenchyma.

Suggested comparisons for collenchyma vs. fibers:

1. origin from primary meristematic tissue(s): which one(s)?
2. timing of maturation relative to elongation in primary plant body.
3. location and distribution relative to dermal and vascular tissue systems, cortex and pith, etc.
4. occurrence in secondary plant body.
5. length and shape of cells.
6. even vs. uneven thickening of cell wall; bearing on types of collenchyma.
7. primary vs. secondary walls.
8. hydration of fresh wall; presence or absence of lignin.
9. reversibility of thickening; changes with aging after "maturity" in whole cells.
10. degree of specialization relative to parenchyma.
11. plasticity vs. elasticity of walls.

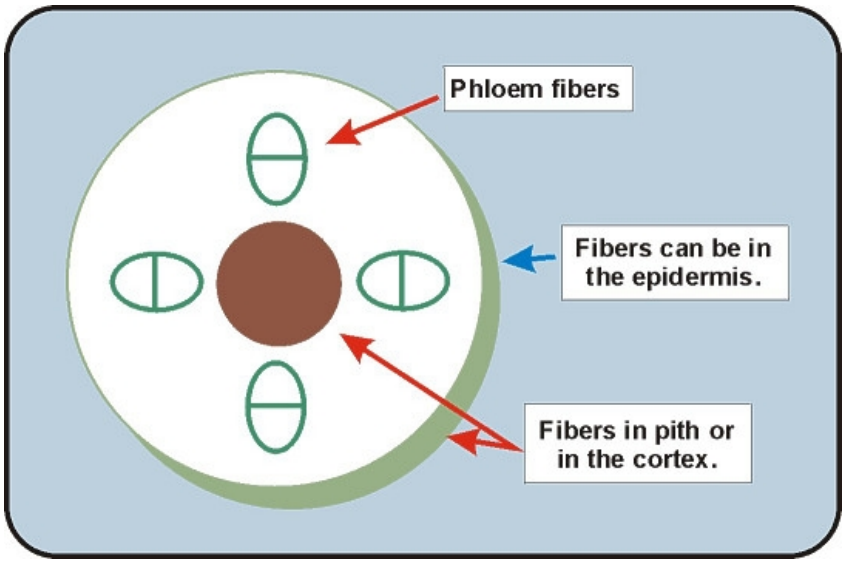
Now go through the list and annotate, where appropriate, indicating similarities and differences between fibers and sclereids.

LABORATORY

After this lab you should be able to: (1) recognize, (2) identify, and (3) characterize (describe) the kinds of collenchyma and sclerenchyma and their distributions in plant parts.

Transverse sections are best for comparisons of leaf vs. stem and for determination of cell types and distributions. The longitudinal sections are best for confirming cell shape. Remember to take note of the orientation of longitudinal sections to determine what is expected to appear in the section. You must know the distribution of the cells you want to see if you are going to make a useful longitudinal section.

NOTE: Sections that are oblique cannot be used to diagnose collenchyma adequately.



Locations for extraxylary fibers:
Only the fibers in the xylem are called xylary.

Those that are elsewhere are “extraxylary”, and can be named according to their locations: phloem fibers, pith fibers, etc.

Activity 1: Distributions and types of collenchyma: Stem and petioles of *Alstromeria* (lily of the Incas) are excellent for observing the 3 types of collenchyma. Can you see the differences?

Look at transverse sections of fresh materials in the expected locations for collenchyma. A square stem is good to start with. Find cells that have glistening white walls (not stained). Start at low magnification, and see if these don’t stand out surprisingly well. Confirm the nature of the wall thickenings with higher magnification. To classify the types of collenchyma one must determine how the wall thickenings are distributed, look for intermediates between types. Note any correlations with location, when you identify each type. For example, where do you find the angular collenchyma, the best lamellar collenchyma, with reference to external shapes of plant parts?

How can one tell the difference between a cell lumen and an intercellular space? This point is essential in identifying lacunar collenchyma. If the middle lamella can be seen in your sections, the verification of lacunar collenchyma should be possible.

Activity 2: Finding primary phloem fibers and secondary phloem fibers in the same section in *Cannabis* (hemp) and *Tilia* (basswood).

Cannabis (hemp) is a woody plant that provides useful commercial fibers. The fibers are lignified, there is a large quantity of secondary phloem accumulated in an aging stem of hemp. Use a transverse section. In *Tilia* the primary and secondary phloem fibers are of the same diameter. But in hemp the primary phloem fibers have a much larger diameter than the secondary phloem fibers. In *Tilia* and hemp the phloem fibers are all lignified. Therefore, they stain dark red to pink in the images.

Viewing the transverse sections should inform you that the primary phloem fibers may not appear in every longitudinal section. When present, their location outside the functional phloem and their thick walls allow identification. FYI: Primary phloem fibers in hemp are longer and wider than the secondary phloem fibers.

Activity 3: Identifying and characterizing sclereids.

NOTE: As you work with the several samples that follow, you will become aware of the fact that somewhat different aspects of the sclereids are revealed in hand sections, clearings, microtome sections and macerations. Try to formulate a concept of how each kind of preparation might be used to best advantage in the study of sclereids.

1. Flesh of the *Pyrus* (pear) fruit contains sclerenchyma, often referred to as "grit" because of the texture the clusters of hard cells contribute to the fruit.
2. Look into the air chambers of a piece of *Nymphaea* (a "water lily") petiole. Arms of branched sclereids project into the air chambers. Examine the individual cells for variety of shape. These sclereids are “idioblasts” because of the way they are inserted among ordinary parenchyma cells. How are they situated in the tissue?
3. Clearings of *Monstera* (Swiss cheese plant) leaves were purposely broken during preparation to release elongated branched sclerenchyma cells. Examine the individual cells and the whole tissue system in which they exist.
4. Leaves of *Pseudotsuga*, a coniferous tree (Douglas fir). Compare cleared leaves and sections. The clearing should tell you that sclereids need not appear in every section of a leaf. It is easier to see the relationship of sclereids to other cells in the sections. However, the shape of whole sclereids, and the number of sclereids present is better seen in clearings.

Activity 4: Survey of the distribution of sclerenchyma in different images in CUPAC. Study the distribution of sclerenchyma as seen in the sectioned materials represented in different samples. In each case speculate how the sclerenchyma or sclerenchymatous cells contribute to support in the plant part that contains them.

Look for sclereids and fibers in the sections. Realize that the absence of longitudinal sections leads to limitations on your interpretations, but the frequency of transverse walls in the sections is a guide to cell length.

In addition to their occurrence in separate strands, bundle caps, etc., fibers are commonly integrated into the xylem and phloem. Fibers that are mixed with the other vascular elements will be studied in a later topic.

Questions

1. Criticize and/or support the following statement: "Parenchyma represents the ground or fundamental tissue of the plant body". Support your idea explaining why.
2. Can parenchyma be considered as a "meristematic tissue"?
3. Describe the basic characters of Chlorenchyma (photosynthetic parenchyma).
4. Discuss the following statement: "Parenchyma is a tissue with only one type of cell, but those cells can have different shapes."
5. The term aerenchyma refers to parenchyma tissue that is composed by cells that are organized in a particular pattern with large amounts of air space between them. What would be the functional advantage of this type of tissue?
6. Many metabolites are stored or accumulated as reserves in the plant organs and therefore in plant tissues. Briefly discuss where do the parenchyma cells "store" metabolites and water.
7. How do you think that parenchyma cells can have such extreme morphological variations?
8. Will it be correct to call collenchyma "a specialized form of parenchyma"?
9. How does the distribution (position) of collenchyma and sclerenchyma make sense in relation to their role as strengthening tissues?
10. List 3 differences among parenchyma, collenchyma and sclerenchyma.
11. Why do you think that collenchyma does not occur in roots (mostly, there are always exceptions to the rule such as the roots of *Dianthera americana* within the family Acanthaceae, which when its roots are exposed to the sun, they produce a noticeable collenchyma cylinder and also chlorenchyma.
12. Collenchyma is mostly found underneath the epidermis as a complete cylinder or in discrete strands. Can you think a reason for this particular position?
13. Discuss the following statement "Sclerenchyma serves a supportive or protective function throughout the plant body and offers mechanical stability to the plant".
14. What are the major differences between sclereids and fibers?
15. Little is known about the sclereid functions, can you think some possible functions based on your knowledge on sclereid shape and position.
16. Describe the two basic types of fibers based on their origin. Do you think you can use the differences to identify them in sections? Why?

Topic 6: Primary Vascular Tissues

Objectives

1. Identify and characterize the cellular elements of the xylem and phloem.
2. Enumerate, evaluate, and describe the sources of the parallels between xylem and phloem on a developmental basis.

Basic facts on Primary Vascular Tissues:

1. Xylem and phloem are both **complex tissues** (more than one cell type is involved in each case).
2. There are evident differences between xylem and phloem, but there are also important similarities.
3. They have to be studied for their development in the primary plant body and in the secondary plant body.

The study of Secondary Vascular Tissues will lead us to a consideration of cambial activity (soon to come). In both primary and secondary vascular tissues it is important to understand development from meristematic cells to comprehend the sources of the similarities between xylem and phloem.

Objectives for this lab:

1. To relate the range of wall patterns in tracheary elements to the maturation of tracheary elements relative to the axis that contains them.
2. To apply practical criteria for visualizing the distinctions between primary and secondary vascular tissues as seen in sectional views.
3. To utilize the experience obtained studying xylem to appreciate that similar relationships exist in the phloem between maturation of conducting elements and maturation of the plant axis.

Try to emphasize the relationships between elongation of the plant axis and the maturation of xylem and phloem, and the cell types for each tissue. Pay careful attention to the wall thickenings of tracheary elements because this information is required background, and strive for an understanding of the **temporal** relationships between axis elongation and xylem maturation.

The parallel treatment of xylem and phloem is purposeful and not accidental. Xylem is emphasized over phloem at this point to develop the concepts of proto- and meta- vascular elements, because xylem is easier for you to study. What you learn about stretching of cells in the xylem applies to the phloem but there is no diagnostic wall sculpturing to be followed in the phloem.

Additionally, the distortions of secondary walls in tracheary elements leave a clear record that some of the tracheary elements mature before elongation stops in the plant part that contains them; and the slowing and cessation of elongation correlates with the type of secondary thickening, suggesting at least a feedback mechanism or interplay between the growth of the axis and the details of maturation that occur in tracheary elements.

LABORATORY

This lab deals with primary vascular tissues and the transition to secondary vascular tissues. Some of your sections will show both xylem and phloem to good advantage simultaneously. **If this happens, take advantage of the situation,** but do not hesitate to concentrate on xylem first and phloem later.

Objectives:

1. Identify a wide range of patterns in secondary wall thickenings in the tracheary elements of a plant axis.
2. Recognize which of these patterns are subject to distortion by axis elongation.
3. Determine to what extent a record of elongation is left behind when wall fragments are embedded in the maturing xylem.
4. Identify sieve tube members in one or more plants.

Activity 1: A study of primary xylem.

A. Start with a 10-minute study of *Melilotus* (sweet clover) stem. In the transverse section, identify the vascular bundles and study the portion of the xylem that is closest to the pith, looking for fragments of walls that are embedded among the cells of the region. One of the primary objectives will be to obtain a concept of what happened to produce these wall fragments. They are part of the **protoxylem** of the primary xylem. The late maturing primary xylem is called **metaxylem**.

   On the radius from the center of the section through a vascular bundle, the protoxylem is toward the pith and protophloem is toward the epidermis. This pattern of xylem maturation is **endarch**. If the first-matured xylem were toward the middle or at the centrifugal locations, with respect to the xylem strand, the xylem would be characterized as **mesarch**, and **exarch**, respectively.

   After orienting yourself with the transverse section, study the longitudinal section. Determine the types of wall thickenings in the primary xylem. See if you can detect wall fragments in the protoxylem region. Make sure you fully comprehend the orientation of the longitudinal section before making final determinations of what is present or absent in the primary xylem.

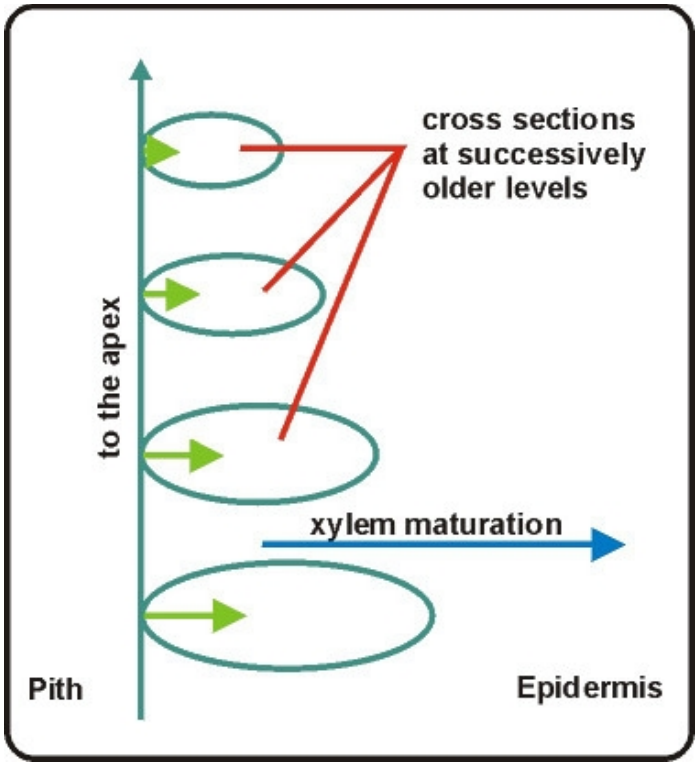
B. The attenuation of the protoxylem is a matter of timing of maturation of the secondary walls in the protoxylem vs. the elongation of the plant axis after that maturation. A stretched helical thickening attenuates into a line, conspicuous because of its thickness and wavy shape. Produce a mechanical analogue of this attenuation from a piece of fresh *Musa* (banana) leaf, as follows:

   If you have the chance of getting a *Musa* leaf, you can use your thumb and first finger of each hand, break the leaf gently across the veins, and slowly pull the fragments apart. The fine threads that emerge are helical secondary wall thickenings. The walls are deforming, not merely slipping out of the leaf. You can prove this because, if you are careful, you can stretch the threads to a dimension longer than the tissue they came from; and if you repeat the breaking of one of the fragments, then you can pull the threads as long the second time as the first.

   Obtain a piece of leaf and draw out the threads over a drop of water, to a distance of about 1.5 cm. Set the fragments down on a slide on either side of the drop, so that the threads are in the water. Now add a coverslip and examine the mount. Look for coils, in the stretched and the relaxed position. Is there a resemblance between the stretched helix and what you saw in the longitudinal section of sweet clover? Are any of the helices double? Triple? For multiple helices, are the components tied together by small links? These are not easy to see! But if you find them, you have confirmed that the element in question was from metaxylem.

C. Using *Impatiens* or *Coleus* make a first hand investigation of primary xylem in a fresh stem. You should strive to detect and identify various patterns of wall thickenings in each internode and to see how these patterns change (or are added to), as xylem matures across the bundles with aging of the internodes. A conceptual framework for what is happening in the internodes of

real plants is given in figure below. You can use Toluidine Blue O (TBO) for staining your sections.



Gradients of xylem maturation: As a vascular bundle expands with age the xylem matures further and further across the bundle. In the shoots of seed plants, xylem maturation is from the pith side of the bundle toward the epidermis.

One convenient way to approach the problem is to look at successively older internodes on the same plant. We then assume that a younger internode represents an earlier stage of the older internode below it.

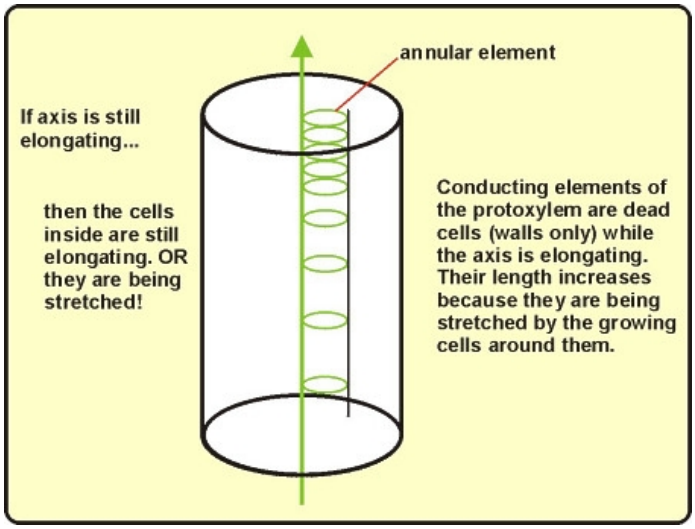
This is not always a safe assumption; for example, flowering and aging affect shoot growth. A better way to approach the problem is to compare what appear to be the "same" internodes at different stages of growth, viz., to compare (for example) the fifth internode formed in seedlings of two different ages. In the older plant the internode will be older by virtue of the difference in planting date between old and young plant. Of course the whole series of internodes on the young plant can be examined in an older state by virtue of the analogous series of internodes in the older plant. Likewise, you could test for changes in the condition of the currently youngest internodes as a function of age of the plant, provided growth conditions have been uniform.

Proceed as follows with seedlings of different ages. As you make sections of older internodes they will be useful for Activity 2, so keep them!

1. Arbitrarily designate the lowest internode on the younger plant as #1. Count upward to the smallest internode you can sample. Count upward on the older plant the same way to find this "same" internode. Now you have a series of paired internodes. The difference between members of a pair in the series gives the first possibility for comparison. Measure the lengths of "same" internodes (e.g., 1-5) on the two plants, and record the data in a table. If the plants are well suited to the comparison, a fully elongated internode on the younger plant should be about as long a fully elongated internode on the older plant, in a comparable position

2. Start with the youngest internode that is convenient for handling (the younger, the better). With the shorter internodes, you may want to section the internode without detaching it from the axis. You don't have to cut the internode off the plant to make these sections. With a transverse section for orientation, section the same piece of tissue longitudinally to show the primary xylem. Be sure to obtain sections in the proper radial orientation and passing through the bundles rather than between them. Keep the rest of the shoot moist while you work with each internode. Save the first slide for comparison with the others you make and diagnose the changes that may have taken place.
3. Compare hand sections of the youngest internode with the "same" internode on the older plants.
4. Work with successive pairs of internodes to extend the sample. This will develop a comparison up and down each plant as well as between plants. Then you can decide whether or not both comparisons lead to the same conclusion. To develop this point you can (optionally) sample right up to the top of the older plant, but save this for later if you feel that you still have time further on in the lab. Determine where each of these features occurs and how it fits into the aging (elongation) pattern:
    a. mature xylem elements present,
    b. stretched protoxylem elements present,
    c. annular, helical, reticulate patterns of secondary wall.

Strive for a concept of correlation between wall patterns and stretching of elements, and look for the details that contribute to the concept. The conviction that a certain element, say one with helical secondary walls, will or will not be stretched depends on the circumstances in which you find it. For a simple case, if pitted elements are present next to helical ones, then the helical ones would not be stretched any longer. On the other hand, if the last element to mature before you section the plant is helical, you would have no direct anatomical evidence on further stretching. Now you would have to predict whether the internode containing the element should undergo further elongation, at the level where you found the element. This is why measurements of internode length (and changes in length) would have to be a part of this kind of developmental study.



Elongation stretches protoxylem: Distortion of primary xylem walls leaves a record of previous elongation. Destroyed elements are replaced by newly matured

cells. As elongation tapers off, metaxylem is formed, with the coordinated changes in wall patterns.

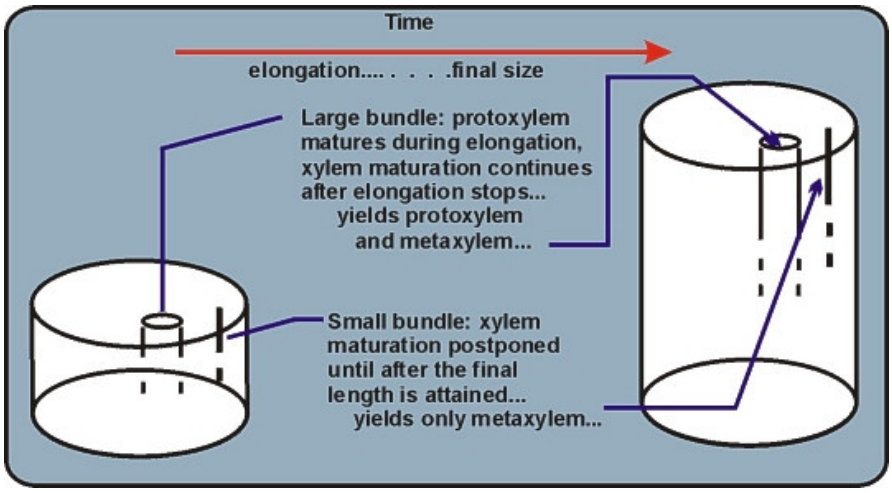
NOTE: The next part of the study of primary xylem is based on prepared slides. You can come back to Activity 1C later if you wish to keep on working with your freehand sections. At any rate, do not discard your hand sections, yet, and do not let them dry out.

D. Protoxylem lacunae and bundles without protoxylem. Use a *Zea* stem cross section. Destruction of the protoxylem elements in corn does not lead to the same morphological situation as seen after the destruction of the protoxylem conducting elements in sweet clover. Instead a **protoxylem lacuna** is formed.

Examine the bundles near and away from the surface of the stem. Are they the same with respect to the development of a protoxylem lacuna? The largest bundles all have lacunae. The presence of the lacuna is positive evidence for protoxylem. Somewhat smaller bundles have seemingly intact protoxylem in the position occupied by the lacunae of larger bundles. In the smallest, most peripheral bundles, no conducting cells are found in the position where protoxylem elements are present in the larger bundles.

If bundles mature their first elements after elongation in the axis is over, protoxylem will be absent in those bundles. The smallest bundles are still procambium when elongation stops in any given part of the corn stem and do not elongate after maturation. In a system of bundles, as in a stem, protoxylem may be present in some bundles and absent in others at any level of sectioning.

For another situation where protoxylem may be lacking, look for bundles that offer little or no indication of stretching in clearings of *Begonia* leaf. These will be the tiniest veins and bundle endings. Compare these bundles with the major veins, which show ample record of stretched (proto-) xylem.



Protoxylem matures during elongation: Vascular bundles can lack protoxylem if their maturation is prevented during elongation of the plant part they are within. The spatial pattern of arrangement of bundles with and without protoxylem is a manifestation of coordination in development of plant parts and plant tissues.

Realize from the beginning that these distinctions are a matter of definition for the sake of coherent description. Subjective judgments are involved in dealing with individual cases, as in the matter of recognizing procambium.

<u>Activity 2</u>: Identifying sieve plates as a diagnostic feature of phloem.

A. Use unstained freehand sections of *Impatiens*, the sections you already have made, or check images. Your thinnest, most nearly radial longitudinal sections should be examined for phloem, which should be toward the exterior of the stem from the xylem. Look for a relatively colorless (white appearing) strip of tissue next to the xylem. The cells will be narrow compared to tracheary elements and relatively nondescript because they are so narrow. The primary phloem can be divided into proto- and metaphloem, and if your section shows secondary xylem, it probably also contains secondary phloem.

   Sieve plates are specialized end walls. Try to find sieve plates in your material or images.

B. Phloem in other species.

   1. Freehand sections can be made from *Cucurbita* (squash) to see extraordinarily wide sieve tubes with large pores in the sieve plates.
   2. *Zea* stems are useful for seeing the arrangement of sieve tubes and companion cells. Sieve plates can be identified in longitudinal sections.

C. Sometimes the staining of **callose** or of sieve tube proteins (slime) can help identify phloem, but these substances are also studied to determine their effect on phloem transport. Wounding increases callose content in sieve elements and causes slime to surge up against the sieve plates. You will examine prepared slides stained for callose during your study of secondary phloem and you can also use one of your sections and stain it with Aniline blue.

D. Wood Macerations: pick two already prepared slides and look for vessels and fibers. Observe the pitting and the perforation plates.

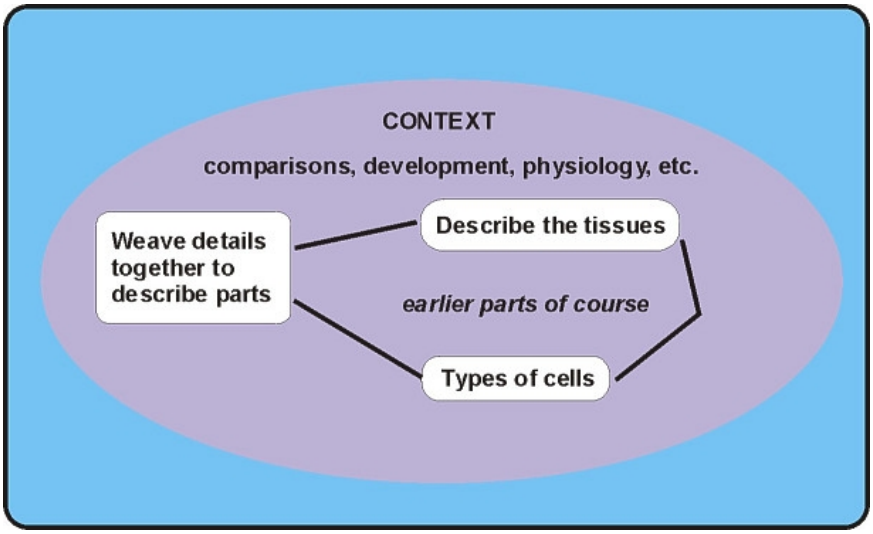
Questions

1. Draw parallels and contrasts between primary xylem and primary phloem with regards to origin, development, maturation, cell types, etc.
2. Outline the ontogeny of a vessel element and sieve tube member in the SAME plant. Contrast the end walls of these two kinds of conducting elements at maturation.
3. What are the “peculiar” aspects of the protoplast of a sieve tube member? Do they relate to de function of the cell? Are similar peculiarities found on functional vessels elements? (Why or why not?)
4. Describe a sieve plate (and any other special cytological features of sieve tube elements).
5. What is the presumed role of the callose in cell walls and cell to cell interactions?
6. What is a companion cell? What is its possible role on the phloem?
7. Criticize and/or support the following statement: “Phloem is composed of several cell types, among them fibers which are associated to the periphery of the phloem. These fibers are known as phloem fibers.” (Hint: xylary fibers are only associated with the xylem, those fibers that are elsewhere are “extraxylary”.)
8. Criticize and/or support the following statement: “Xylem and phloem are intimate related and one cannot survive without the other”. Support your idea explaining why?
9. What is the full range of types of wall thickenings in primary xylem?
10. Design an experiment to study changes in primary xylem- One paragraph will be sufficient.

11. Elongation of the axis somehow influences wall thickenings in primary xylem. What is the outcome of early cessation of elongation with regard to the assortment of wall thickenings in primary xylem?
12. Suggest a model that would describe how the rate of elongation could determine or affect the kinds of wall thickenings in primary xylem.
13. Criticize and/or support the following statement: “It cannot be secondary xylem if there is not first primary xylem”.
14. Criticize and/or support the following statement: “Protoxylem differentiates before they complete elongation. As a result, they are small-diameter cells with extensible wall thickening patterns (annular and helical) which can continue to elongate. Metaxylem differentiate after additional growth and elongation has occurred, therefore metaxylem tracheary elements are larger diameter cells with mostly reticulate, scalariform, and pitted wall patterns”.

Topic 7: Stems

With this topic we begin the final phase of the course: the plant parts. We will be drawing attention to correlations and combinations of anatomical features, taking for granted the information covered in the first two parts of the course.



The third segment of the course: Use what we have already covered in the course to described the anatomy of plant parts, in the context of descriptions that involve the biology of the whole plant.

Objectives: After this topic you should be able to:

1. Enumerate and characterize the sources of variation in the structure of stems during primary and secondary growth.
2. Describe a concept of normal vs. anomalous structure.
3. Argue that the leaves and stem are integrated; that the shoot is the "unit" of development.
4. Demonstrate that the relationship of the stem to the leaves is reflected in the arrangement of vascular tissue in the stem.

To accomplish these objectives some information about leaves will be introduced. The anatomy of the leaf is a separate topic from the relationship of the leaf to the stem.

So how do we start? Look back at your notes from “the three tissue system”, where you were first applying the concept of three tissue systems to the stems of dicots and monocots. These comparisons will now prove themselves useful for extrapolations. Your evaluation of anatomical differences between dicots and monocots depended on recognizing the arrangement of the vascular tissue system. Now we can add that only the so-called "typical" arrangements, those most often encountered, were dealt with at that time. We tend to think of these typical situations as "normal". However, to avoid pathological connotations we avoid the use of the word "abnormal" when describing departures from typical. Instead, we call these variations anomalous anatomical features.

Phase I: Picking out the sections relevant to objectives 1 and 2.
Typically, the anatomy of the stem is described for the internode, with nodal anatomy treated as an additional topic.

1. For primary tissues, one can evaluate
    a. the arrangement of vascular tissue in total,

b. the arrangement of xylem and phloem within bundles,

c. the occurrence and arrangement of separate xylem and phloem bundles.

2. For secondary growth, we compare cambial activity with the "norm", viz., the typical woody dicot. Cambia can be:

   a. unidirectional (unifacial) or bidirectional (bifacial),

   b. single or multiple,

   c. can vary in location and relative activity.

   Anomalous secondary growth assorts and combines these features so that the outcome of cambial activity differs from that in the typical dicot.

3. The differentiation pattern in cambial derivatives can also vary from the norm we see in typical woody dicots.

Phase II:

1. Leaves are arranged in a regular pattern on the stem
2. The development of vascular tissue in the shoot is closely coordinated with leaf development. Thus, part of the vascular tissue in the mature stem is described as leaf traces, and we must deal with interfascicular regions and the so-called leaf gaps.
3. While all parts of the vascular tissue system in the stem can have positional relationships to the leaves, this is not the same as saying that all the vascular strands in the stem are leaf traces. Typically, only the vascular strands connecting the leaf to other vascular strands are called traces.
4. Leaf gap is a term useful in anatomical descriptions. In an evolutionary sense the term is misleading.

LABORATORY

The function of this laboratory is to provide specific, concrete examples to support objectives l and 2 for the study of stems. Thus, after this lab you should be able to:

1. enumerate and characterize the sources of variation in the structure of stems during primary and secondary growth.
2. state a concept of normal vs. anomalous anatomical structure. More detailed objectives are spelled out in each of the lab activities.

Activity 1:
Objective: Obtaining a reference for comparisons of stem anatomy. Below are examples of typical dicot and typical monocot stems. Observe the images but do not spend a lot of time with these.
Dicots: *Helianthus* (sunflower) before secondary growth; *Tilia* during secondary growth.
Monocots: *Smilax* (zarzaparilla) and *Zea*, both herbaceous.

Activity 2:
Objective: to realize that a typical arrangement of bundles can be associated with an unusual arrangement of vascular tissue within each of the bundles.

In the typical dicot stem the phloem is on the outside of the xylem (collateral arrangement of xylem and phloem). This is easily established, but in some stems the phloem is regularly on the outside and the

inside (side toward pith) of the xylem. *Solanum* (tomato) shows this feature (bicollateral arrangement of xylem and phloem), but so do a variety of other plants. In *Solanum* identify the internal phloem. There is no indication that secondary growth is to occur between the xylem and the internal phloem in *Solanum*. This means that secondary growth can be normal (one cambium of the usual kind in the usual location) even though there is a departure from the usual arrangement in the primary tissues. Although there are two "ideal" locations for the formation of cambium between xylem and phloem, *Solanum* uses only one of the locations. *Tecoma* (Yellow trumpet bush, another plant with bicollateral vascular bundles) uses both!

Activity 3:
Objective: To see an anomalous arrangement of vascular bundles in a dicot.

What anomaly would be most likely here? Look at the *Digera* stem. The bundles are "scattered" rather than in one ring. Cambial activity is starting up at the periphery of the stele. Note how the "ring" of bundles that is connected by the cambial region gives the impression of "normal" secondary growth in its early stages. However, instead of a pith inside the ring we have scattered vascular bundles. There is no secondary growth among these bundles, so again (as in *Solanum*) secondary growth starts off in a normal fashion in spite of an anomaly in the primary tissues.

Activity 4:
Objective: To see several examples of various degrees of anomaly in secondary growth.

1. *Boerhavia* (4 o'clocks): Shows a condition that would be attained by *Digera* (false amaranth) in more advanced growth. One cambium has operated for some time and a second cambium is forming outside the maturing products of the first!
2. *Chenopodium* (lamb's quarters): Interpret *Chenopodium* that shows stems of two ages. Look for some of the patterns, e.g, alternating xylem and phloem, which is interpreted as indicative of multiple vascular cambia. Determine how multiple cambia, each producing xylem toward the inside and phloem toward the outside, could result in the formation of the larger stem. Note that the activity of each cambium is typical, and that it is the occurrence of multiple cambia and their formation outside the phloem that is atypical.
3. *Tecoma*: Younger stems of *Tecoma* show it has internal phloem, like *Solanum*. But in *Tecoma* there is cambial activity at each interface between xylem and phloem. Thus, there are two vascular cambia, the inner of these being anomalous because of its location.

   The younger stems show the beginning of cambial activity between the internal phloem and the protoxylem regions. The older stem shows that the inner cambium is bifacial and the differentiation of derivatives gives secondary xylem facing the primary xylem (note change in diameter of vessels from protoxylem to secondary xylem) and secondary phloem facing the internal primary phloem.
   Tecoma is anomalous in primary and secondary growth, but you should see how the primary anomaly sets up the anomaly in secondary growth. Of course only a limited quantity of secondary growth will be accomplished by the inner cambium.
4. Woody vines. Some variations on stem anatomy begin with the onset of secondary growth. Primary tissues are arranged in the normal way, but variations occur in the pattern of secondary growth that result in a more or less anomalous secondary plant body, or distinctive type of stem.
   a. *Clematis*: Only slightly anomalous. Compare with "b" and "c" below.
   b. *Aristolochia* (dutchman's pipe): Opposite the interfascicular regions the cambium organizes large rays. Thus it appears that the pattern of separate bundles is perpetuated. Look for the origin of new rays in the xylem.

c. *Tinospora* (moonseed): Like *Aristolochia* differing only in detail. Look for origin of "new bundles" as well as new rays.

d. Various lianas (=vines) attain unusual shapes because secondary growth does not proceed uniformly in all directions. Try to account for shape on the basis of differential cambial growth. The products of multiple cambia may also be present. These extreme modifications may be hard to understand. You can read more about this in any plant anatomy book.

Activity 5:
Objective: to determine the sources of "woodiness" in monocot stems and to see if this woodiness is related to secondary growth.

1. Secondary growth is less common in monocotyledonous plants than in dicotyledonous plants. Nonetheless, some monocots are "woody". Structural bamboo comes from a stem more than 50 feet tall. These stems, 10-12 cm in diameter, are strong enough for use in construction. Bamboo stems are are also used for "semidecorative" construction and for such light duty uses as fishing poles. Examine bamboo and determine that it resembles other grasses in anatomy. The strength of bamboo is in the sclerenchyma that matures, and this explains the elasticity of a bamboo fishing pole, for example.
2. When a cambium becomes active in a monocot it is not a typical differentiation of secondary vascular tissues that follows. Instead, the inner derivatives differentiate as vascular bundles and ground tissue. Examine images of *Dracaena* (Madagascar dragon tree) or similar stem showing this anomalous secondary growth.

Activity 6: Typical stems, vines, multiple cambia and anomalous monocot cambia.

1. *Helianthus* (sunflower). Typical dicot in primary growth; a ring of collateral bundles. Several ducts in pith and in cortex probably held resinous substances in living plant. These are not part of vascular tissue system.
2. *Tilia* (basswood, linden tree). Typical dicot in secondary growth. Protoxylem regions are against pith. Note progression of small to large elements in primary xylem. Phloem is primary (fibers survive) and secondary (fibers, parenchyma, sieve elements). Early stages in periderm formation not shown in any detail. Cortex present, phloem rays dilated by tangential stress in expanding surface of stem.
3. *Zea* (corn). Typical monocot with scattered bundles of various sizes in an internode. No secondary growth occurs.
4. *Ranunculus* (buttercup). Stem with cortex photosynthetic, as revealed by plastids in aerated tissue. Find stomates and compare to fresh material to confirm chloroplasts. Pith is hollow. Note fibrous cap outside phloem, layer of sclerified cells forming sheath, also. Note some signs of crushed elements in protoxylem region and progression of cell sizes in xylem elements. No secondary growth.
5. *Cucurbita* (squash). Uncomplicated by fiber development, admittedly, but note resemblance of external to internal phloem. Sieve plates can be seen only occasionally in transverse sections, but longitudinal section would confirm that this internal phloem has typical sieve tube members. Note only one protoxylem pole (endarchy) so bundle is truly symmetrical: internal phloem is "added" to ordinary collateral bundle to get a bicollateral bundle.
6. *Solanum* (same genus as potato). Here secondary growth has occurred, along one locus (one cambium) formed by the interface of the xylem and external phloem. Strands of internal phloem

are found between protoxylem and pith. Note any fibers associated with internal phloem, and persistence of internal phloem conducting elements.

7. *Tecoma* (yellow trumpet bush). Here two vascular cambia have been active. Only clear indication of this is in the very large vessel elements, "out of sequence". Pith is at center and the large vessel elements intervene between internal phloem and protoxylem points.
8. *Clematis*, a vine. A ring of vascular bundles is present, and secondary growth maintains this pattern: i.e., there is no vascular differentiation of the conventional kind in the regions between the original bundles.
9. *Aristolochia* (Dutchman's pipe), another vine, at a young stage, gives the aspect of a rather typical dicot stem. But in the older stage the aspect of vascular bundles is maintained because massive rays are perpetuated opposite the original interfascicular regions. Because not all the rays go to the center of the plant we can reason that new massive rays start up within the "bundles" as they broaden.

   Vines are not immune to added anomalies: this "liana" shows internal phloem. The results of having multiple cambia, operating along only part of the circumference of the stem. This activity would distort the stem out of round. The "evidence" for multiple cambia is actually the repeated pattern of xylem - phloem along the radius. One reasons that a cambium would operate at the interface of xylem and phloem for each repeat of the pattern. (Is there an alternative?) The "next" cambium would originate outside the phloem with each cycle.
10. *Chenopodium* (lambs quarters) is supposed to show multiple cambia, also. Xylem exceeds phloem in each repeat of the pattern. Again, the "next" cambium has to originate outside the existing phloem.
11. *Dracaena* (Madagascar dragon tree) superficially resembles *Chenopodium* with regard to secondary growth. But notice that current cambium is the only cambium. Furthermore alignment of vascular conducting elements circumferentially is lacking (no rings present) even though secondary vascular bundles are close together. Thus, cambium is unidirectional with respect to vascular differentiation.

Activity 7:
Objective: to locate and recognize endodermis in a stem.

Revise the concept of endodermis. You should see your "first" endodermis in a stem to prevent you from obtaining the erroneous concept that endodermis with casparian strips is found only in the roots.

Saururus (lizard's tail) stem. Note that both cortex and pith are aerated. However, evaluate the boundary layer at the inner edge of the cortex and the perimeter of the vascular bundles. Find the endodermis with casparian strips.

CONCLUDING ACTIVITIES

All good candidates for exams and/or essays. If any of the conceptual questions (e.g., 4-7) give problems, be sure to ask for help!

1. Anomalous growth is widespread and sometimes is characteristic of whole families of plants. With this in mind, give a working definition of normal (typical) and anomalous.
2. Characterize the similarities and differences in the *Solanum*, and *Tecoma* stems with regard to anomalous growth.

3. Diagram the various possible arrangements of xylem and phloem within a vascular bundle, label the diagrams, and name the arrangements.
4. Write an orderly, well organized but brief statement that indicates how the arrangement of primary vascular tissues enters into our concept of normal (typical) vs. anomalous stems. Do the same for cambial growth and patterns of differentiation in secondary vascular tissues.
5. Criticize and/or support the statement that the vascular tissues of the stem is a sympodium of leaf traces, at least in flowering plants.
6. Offer direct and indirect evidence that leaves control the course of vascular differentiation in plants.
7. Distinguish interfascicular region from ray. Can this always be done for a single, transverse section?
8. Compare and contrast the multiple cambia of *Chenopodium* stem and the anomalous cambium of a *Dracaena* stem.
9. Compare and contrast corn stems and palm stems.

Questions

1. Describe in a succinct and meaningful way the relationship between shoot apical meristem and the primary growth of the stem.
2. Draw diagrams of the various possible arrangements of the xylem and phloem within a vascular bundle, label the diagrams, and name the arrangements.
3. Support or attack the following argument: “The stem is unit of construction in the aerial part of a plant”. Please support your answer.
4. Compare and contrast the stem of a palm (a monocot) and the stem of an oak (a dicot). A scheme is sufficient.

Topic 8: Stem Secondary Growth

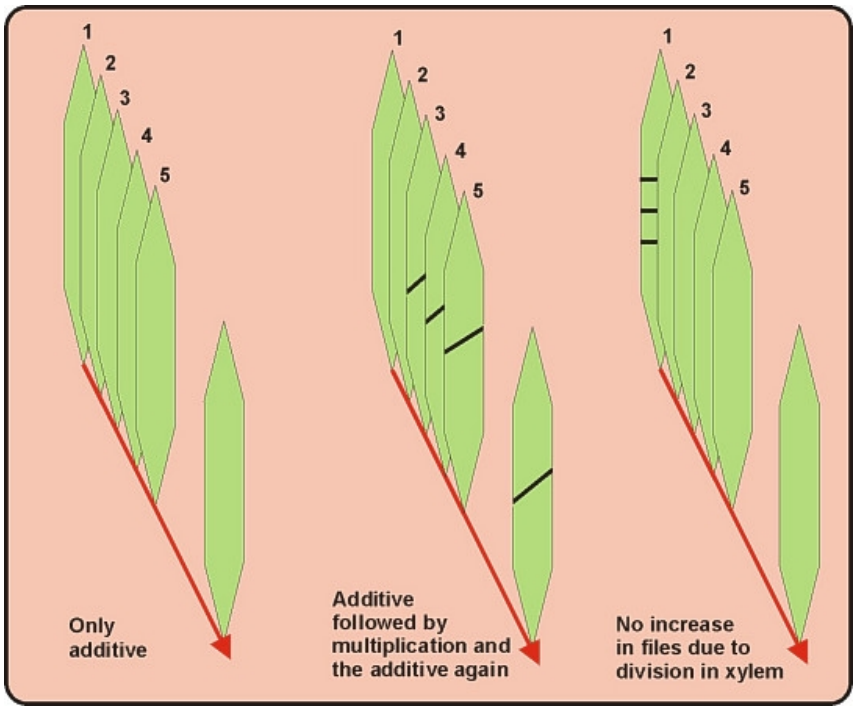
Some Aspects of Cambial Activity

In this topic try to:

1. Emphasize that the organization of secondary vascular tissues is a result of the organization of the vascular cambium.
2. Show that the vascular cambium is a dynamic, changing meristem, seasonally and with aging.
3. Show how the pattern of secondary xylem can be read as a record of changes in the vascular cambium.
4. Outline the origin and activity of the cork cambium and the accumulation of phellogen.

Facts:

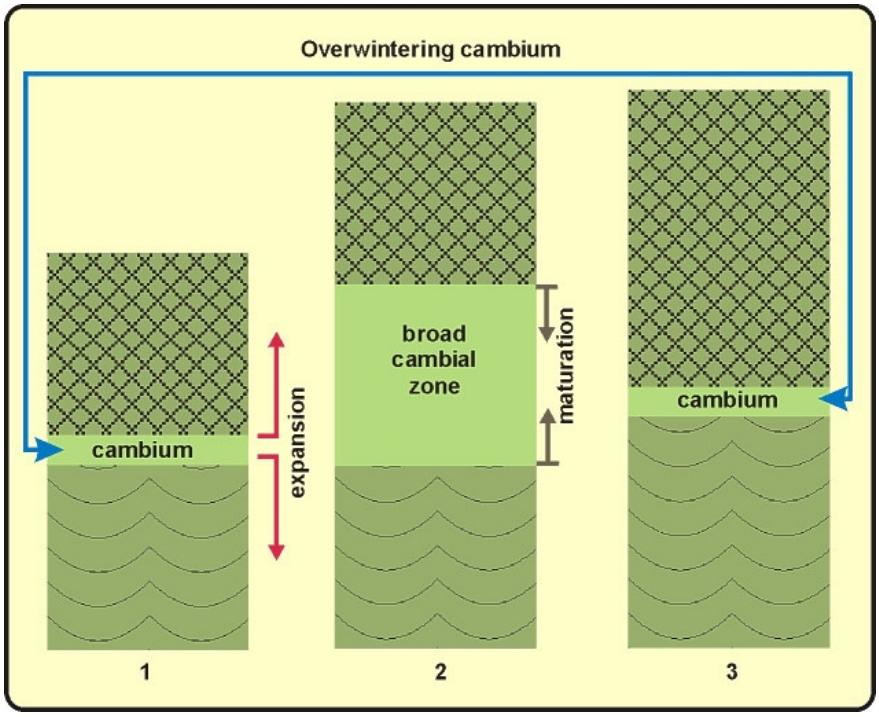
1. The vascular cambium is involved in additive and multiplicative cell division.
2. Both of these are "recorded" in their effect on adding more xylem (and phloem) cells radially and tangentially, respectively.



Cell files record history of cell divisions: Serial tangential views outward in the xylem, along a time line, provide a record of cell divisions. Only radially oriented walls (which are typically oblique) in the cambial initial change the number of files.

3. Multiplicative divisions increase the number of cells in the vascular cambium, in the tangential dimension. However, apical intrusive growth and lateral cell expansion play the dominant role in causing the cambium to increase its area.
4. Many or all of the increased number of initials (due to multiplicative divisions) can be "lost" almost directly after their formation because they are squeezed out of the cambial layer.
5. The conversion of fusiform initials to ray initials accounts for the origin of many new rays in the wood.
6. The cambium is kept "organized" by the physical pressure it experiences.

7. The role of the cambium is to divide and produce derivatives. Xylem and phloem are produced by maturation of these derivatives. The vascular cambium does not produce xylem and phloem directly.



Derivates of cambium become xylem and phloem: In the spring, the vascular cambium is reactivated and produce a broad region of derivatives by cell division and expansion. Cell differentiation and maturation encroach on this region from both sides, so that before the season ends, only one layer of cells remains to start the process over the next year.

Now we need to focus on the periderm. Strive to get the following points:

1. The periderm is secondary tissue derived from a cambium, the phellogen.
2. Typically the phellogen first arises in or near the epidermis in a stem.
3. Subsequent phellogens are formed as discontinuous, overlapping meristems, eventually in secondary phloem, but some exceptions to this generalization are known.
4. The accumulation of periderm and included secondary phloem leads to our recognizing the rhytidome.

LABORATORY

Objectives: After completing this laboratory you should be able to:

1. relate the organization of secondary vascular tissues to the organization of the vascular cambium.
2. secure an understanding of how secondary growth in the vascular system is coordinated with the progressive relocation of the cork cambium.

Activity 1: Cambium

1. ***Robinia*** (black locust):

A. Xylem and Phloem evident. Staining reaction tends to separate xylem (pink) from cambium and phloem. Vessel elements are present (see also, later) and sieve tube members are also clearly seen.
B. Xylem. Vessel elements showing simple perforations in oblique end walls. Helical wall thickening (additional secondary wall) shows in the vessel elements. Xylem parenchyma. Axial elements are once or twice transversely divided in their development as parenchyma, making the cells into "strands". Rays have typical form (try and identify uniseriate rays and large, multiseriate rays).
C. Phloem. Sieve tube members identified by large size, sieve plates with slime plugs, otherwise "open" appearance. Ray cells are typical of ray system.
D. Xylem and cambium. Tendency to stain pink, and transverse subdivision of axial parenchyma are evident in the xylem.
E. Phloem and cambium. Sieve tubes and transversely divided axial elements, but not the staining reaction, distinguish the phloem. **NOTE**: conducting elements of xylem and phloem need not be adjacent to cambium to identify xylem and phloem. Last matured elements can be principally parenchyma.

F. *Robinia*. Callose forms on sieve plates due to trauma. Note phloem axial parenchyma is typical, stranded form and rays are evident. Cambium adjacent to blue (lignified) cells of xylem. This is an outcome of the orientation of the section. Nuclei that appear to be in blue-walled cells are probably in the cambium, i.e., on the "other side" of the blue wall. Note storied character of xylem, cambium, and phloem. They can be evaluated only in tangential section.
G. At higher magnifications, one can see the "pitted" character of cambium cell walls.

2. ***Thuja*** (cedar):
   A. *Thuja*. Transverse section. Using the late wood as a "dividing line" note that not all files of cells continue on both sides of the dividing line, but many of the files do. Derivatives of the cambium occur in rows or files in the radial direction. These rows are a record of cambial activity and a change in number of files suggests a change in the number of initials.
   B. *Thuja*. Radial sections show a file of cells from one initial as a "stockade" of cells, but a single section in this plane offers little help in visualizing changes in number of files across a growth layer. For example, it is hard to see endwalls in the late wood. Actually the only "correct" way to view the entire record, year after year, is with serial tangential sections, not a practical method for use in this lab.

Activity 2: Recognizing vascular cambium in tangential sections.
Note that the *Robinia* vascular cambium is storied, as are the xylem and phloem, to a somewhat lesser degree. Storied vascular tissue comes only from a storied cambium, but non- storied vascular tissues can arise from storied or non-storied cambia.

Activity 3: Seeing some evidence for cambial changes in secondary xylem.

Thuja: observe similar configurations of changing numbers of tiers in secondary xylem in the transverse section. Find two rays that are not very far apart. Follow outward through successive years growth between these rays. This direction coincides with the passage of time. Look for changes in numbers of files, especially in summer wood. You do this by counting the number of (transverse sections of) axial tracheids between the two rays. This number may rise and fall from year to year.

Activity 4: Periderm

1. Barks. The first point that we must accept is that the bark of a tree is not a specific tissue, nor does it have a specific character, except when we take one plant at a time. Trees differ greatly in their ability to retain bark, the tendency to have the bark peel and fissure, the clarity with which the lenticels show through, etc. Some barks are fibrous and others papery. Some are rough, others are smooth. The lenticels give the orientation on the trunk of the tree, and note also that corks are cut for stoppers so that the lenticels will not cause the stopper to leak.
2. The next point we want to establish is that the usual situation in stems is that the first periderm forms near the surface completely around the stem. This leaves most of the cortex intact, at first. Look at a *Tilia* stem for early stages in cork formation. Also look for dilation of interfascicular parenchyma, due to tangential stresses.
   Examples of *Castanea* (chestnut) and *Sambucus* show additional stages, and show lenticel formation as well.
3. *Salix* (willow) rhytidome. Follow from the xylem outward and note the effect of cork development on tissue arrangement. Note that there are multiple layers of cork in the specimen.
4. *Prunus* (plums) or *Betula* (birch) show bark that is perfectly smooth at this stage of development. Note that the appearance of the stem is like an advanced stage of first cork formation. If from a large stem, then the "cortex" must be maintained by diffuse secondary growth (cell enlargement and division, throughout).

Activity 5: Review of Rhytidome.

1. *Sambucus*: Origin of first cork cambium was from subdermal layer. Remains of lamellar collenchyma show that the cork cambium relates to cortex rather than phloem.
2. *Castanea*: Well established cork layer outside old cortex. Again the first occurrence of secondary dermal tissue is superficial or nearly superficial in origin. This is typical of stems.
3. *Salix* (willow) bark shows the cork cambium typically forms within secondary phloem in an older tree. The oldest intact, nonconducting phloem is bounded by a corky layer. Inclusion of the old phloem in the outer bark (rhytidome) coincides with proliferation that disrupts the organization of phloem, but the axial bands of fibers survive.
4. *Salix* in transverse section. Intact phloem near xylem. Bands of fibers survive in rhytidome, beyond layer of cork. Older cork layer appears at outside, older because successive cambia form from outside to inside in rhytidome.

CONCLUDING ACTIVITIES

These questions may seem to call for opinions, but give factual answers!

1. How does the vascular cambium increase in girth?
2. Offer support for the argument that the seemingly independent axial and ray systems of secondary vascular tissue are developmentally related via the changes in the organization of the cambium.
3. Expand on your answer in 2, to show how the changes in the cambium tend to keep a uniform distribution of rays in the xylem as a tree grows in diameter.
4. How do we know that the vascular cambium "loses initials"? Why are gymnosperms and diffuse porous angiosperms preferred to ring porous angiosperms for studies of cambial activity?
5. Why does one need a tangential section of a stem to determine if the cambium is storied?
6. What is rhytidome, and how does it originate?
7. Distinguish between outer bark and inner bark as found on an aging tree.

8. What is the degree of continuity between the primary and secondary dermal tissue systems?
9. What is the degree of continuity between the primary and secondary vascular tissue systems?

Topic 9: Wood

Objectives

By the end of this topic you should have:

1. Obtained a thorough knowledge of the organizational features of secondary xylem and phloem.
2. Extended your awareness of parallels and contrasts between xylem and phloem.

Although you may think that it is impossible, you will (eventually) comprehend "wood" as an entity with three dimensions. It is crucial to understand the degree of integration between axial and ray systems, the features of typical gymnosperm vs. angiosperm wood, etc.

LABORATORY

Part 1: Secondary xylem

Objectives: This part of the lab should help you to:

a. Detect and enumerate anatomical differences among different kinds of wood, for example in the occurrence and distribution of cell types, in the organization of the ray system, etc.
b. Make comparisons among the vessel elements in different species, especially with regard to the kind of pitting and the kind of end walls.
c. Gain the ability to picture the anatomy of secondary xylem in three dimensions by integrating three planes of sectioning.
d. Characterize the seasonal changes in wood during the growing season.

Activity 1: Microscopic Examination of Wood

Sections taken from three principal planes of examination: transverse, radial longitudinal, tangential longitudinal. When macerations are available, they are mentioned in the lab outline. Macerations allow you to visualize whole cells or groups of cells but not the overall organization of the wood. Sections in the principal planes of observation reveal the spatial arrangements of cells if you integrate them in three dimensions. In the exercises that follow, the outline guides your observations for one conifer and one dicot wood, using block diagrams.

A. Conifers

The wood of conifers contains no vessels; tends towards homogeneity.

1. *Thuja*

a. **Growth layers**. Growth layers are revealed by periodic changes in density. The sections stain darker in areas of late wood where more of the area is covered by cell walls. Here the walls are thicker and the cells have smaller diameters.

   The transverse section: dark lines run across. These represent the late wood of each year. The radial longitudinal sections show vertical lines that represent the late wood. Intermittent, horizontal bands represent, in part, the rays. In tangential longitudinal sections, growth layers

are poorly represented. The section may appear uniform or the presence of one or a few dark wavy lines indicates the presence of late wood vs. early wood.

Sections of wood, more than one growth layer is represented, but typically the tangential section is a sample of cells from one or two growth layers as opposed to a larger number of layers represented in transverse section and radial section.

Growth layers are seen in transverse section partly because there is a periodic change in diameters of the axial cells. From early wood to late wood, diameters tend to decrease gradually. If your eye follows a line (file) of cells across the late wood, diameters increase abruptly on the other side. This is the early wood of the next-younger growth layer, and the direction of travel is toward the outside of the tree (toward the cambium), parallel to the rays. As you look for these changes in diameter, you will notice that the wider cells tend to have thinner walls.

The radial section also shows growth layers and the diameters of the cells of different ages. It would seem that a tangential section should show cells that are of only one age, i.e. cells that came from the cambium at the same time, because a tangential section would be (approximately) parallel to the cambium. In practice, a growth layer shows some curvature because the stem is of finite diameter, and the slice made to obtain the section may not be parallel with the growth layer in the longitudinal direction. Thus, growth layers can appear in tangential sections, if a section is large and the growth layer relatively narrow. Look for dark areas in tangential section. Because the other sections tell us that there is a high wall to lumen ratio in the late wood, we should expect a darkly stained area in the section where the late wood appears in tangential section.

In summary, transverse and radial sections show growth layers reliably and one should learn to use these sections to diagnose seasonal changes in wood and a variety of cell properties as well. In tangential sections support is found for what is seen in the other sections and opportunities for describing height and width of rays are present. Always try to predict what the other sections should show for anything you see in each of the sections, and look for that corroboration!

b. **Cell types**. The transverse section cuts all axial cells cross-wise. Almost every axial cell appears to have an empty lumen and thick wall with large pits that are bordered. Thus, virtually all axial cells are tracheids. The bordered pits are on the radial walls of early wood cells, and on the radial and tangential walls of late wood cells.

   Radial section confirms that axial cells have tapering end walls that bear circular bordered pits. These vary in size and in shape of aperture with the season.

   In the tangential section, the pits are seen in face view on the tangential walls only where the late wood is present.

   One can confirm this feature of pit distribution in transverse section, where a large sample of early wood and late wood cells can be seen at a glance --with the sectional views of pits in radial and tangential walls seen at the same time. But the face views of pits must be visualized in the longitudinal sections. Always determine the type of pitting on conducting elements from longitudinal sections, using transverse sections for supporting evidence on the distribution of the pits on radial versus tangential walls.

   Axial cells with contents and simple pits in the walls are very hard to find. Thus, axial parenchyma is scanty to absent. Rays are seen to consist of procumbent parenchyma cells (simple pits in these cells) in the radial section. Rays appear as narrow stripes of parenchyma cells in the transverse section. In the tangential section the ray cells have to be cut cross-wise,

considering their dimensions in the other two sections. Here we see that the rays are only one cell wide. Thus, they are called uniseriate rays. The tangential section also effectively samples the heights of the rays. Neither heights nor widths of the rays can be judged accurately in the other sections. But ray cell shape and cell type are usually characterized in the radial section, with supporting evidence for the description from the other sections.

2. *Pinus*

Another typical conifer wood. Axial system lacks vessels. Compare two species: *Pinus ponderosa* and *P. strobus.*

Answers the following questions.

a. Circular bordered pits: Do they change size with season? Do the pit apertures change shape? Do the pits reside on the radial wall and tangential wall in early wood, in late wood? Check both species!
b. Resin canals: Are they present in axial system, in ray system?
c. Rays: Are there uniseriate rays? Do the multiseriate rays all contain resin canals? Are ray tracheids present? Yes. There will be circular bordered pits and thicker walls in the ray tracheids. Although the cell shapes are not much different between the two species look for differences in the smoothness vs. roughness of the walls in the ray tracheids.

Macerations of *Tsuga* (hemlock), *Larix* (larch), and *Ginkgo*. You will find them helpful in constructing a concept of the cross field, or areas of contact between the axial and ray elements. Macerations are also excellent for picking up indications of apical intrusive growth by the tracheary elements. Look for branched tips on the tracheids.

B. DICOTYLEDONOUS WOODS

1. *Liriodendron*

Vessels are present. Wood is diffuse porous. The end walls of vessel elements (perforation plates) are best viewed in the radial section, where they are seen to be scalariform (ladder-like). Pitting between vessel elements is bordered and mostly "opposite", as seen in radial and tangential sections. Axial parenchyma appears in transverse section as cells with contents, and simple pits, at the end of the late wood (terminal position). Parenchyma seen in radial section as shorter than other axial cells, and is only at end of each growth layer. Simple pits in these cells. Look for late wood in tangential sections, and again try to identify parenchyma, on the basis of cell length. Use the radial section to see that the cells along the margins of the rays stand "upright". Not every ray shows this because not every ray is cut to show its margins. All cells in the rays are parenchyma cells. Note indications that cells have two shapes (heterocellular rays) occur also in tangential section. In this section, note uniseriate to multiseriate condition (one to many cells wide) for rays.

All sections show thick-walled, elongate cells called fibers. Pits on these cells are tiny. These cells can be called fiber-tracheids due to this type of pitting.

In summary, it is seen from these observations that *Liriodendron* wood is more complex than a conifer wood due to diversification of cell types. As the diameters of vessel elements decrease during the season, there is more room for fibers (fiber-tracheids) in the late wood. The distribution of parenchyma betrays a seasonal variation in the occurrence of this cell type, also.

2. *Magnolia*

a. Type of perforation plate?
b. Type of pitting between vessel elements?
c. Location of axial parenchyma?
d. Rays homo- or heterocellular?
e. Width of rays?
f. Other types of cells in axial systems?

Other items: - Can you see a helical wall layer (tertiary thickening) inside the thick, uniform wall layer of a vessel element? (See, also *Tilia* for this feature.)

3. *Tilia*

Diffuse porous. Simple perforation plates, alternate pitting on tracheary elements. Wall of vessel shows so-called tertiary thickening, a helical thickening that is the innermost layer of the secondary wall (therefore differing from the helical thickening of the primary xylem elements, which is the entire secondary wall). This condition exists to a lesser degree in our sample of *Magnolia*. Axial parenchyma banded but not only terminal. Rays: all cells procumbent, rays uni- and multiseriate. Macerations are available for study.

Make a quick comparison here with *Taxus*, where the helical tertiary wall layer combines with large circular bordered pits, in tracheids.

4. *Quercus*

(Oak). Ring porous. Ring porosity is visible to the naked eye in the transverse section. You can account for this feature in terms of arrangement of cell types. Note variation in diameters of vessels. Selective placement of the large pores is the essence of ring porous wood.

The simple perforation plates are transverse. What do you see of a perforation plate in radial sections? Only the rim can be detected, and that with difficulty, especially if tyloses get in the way! (Vessel elements may be filled with ingrowths from adjacent parenchyma.)

Rays uni- and multiseriate. Small and large. Compare pitting in fibers with that in tracheary elements. Axial parenchyma is diffuse and difficult to identify in our sample except where the cells have dark contents.

Sometimes disruptive growth is recorded. Find disoriented cells in the radial sections, on the periphery of the large vessel elements. How do you account for these areas of disorder? See how much wider these vessel elements are (in transverse section) than the unexpanded cells in the same area.

5. *Fraxinus*

(Ash). Ring porous. Make observations like those for *Quercus*. Here the axial parenchyma is around each vessel. Confirm this with the longitudinal sections. There are macerations of *Fraxinus*. The shortness of the vessel elements and the minute size of the pits can be appreciated best in the macerations.

Part 2. Secondary Phloem

Objectives

a. To obtain an insight into the parallels in organization in secondary xylem and secondary phloem.

b. To obtain a clear understanding of the organization of a sieve plate.

Note: Where it can be determined, the outside of the stem will be opposite from xylem.

Activity 1: Phloem

1. *Liriodendron*

1. (Tulip tree). Transverse section. Staining reaction distinguishes xylem from cambium and phloem. Rays run through xylem, cambium, and phloem. Growth layers represented in full in xylem as revealed by pattern of changing cell size and composition of wood (terminal parenchyma present). In phloem, banding of fibers alternating with axial parenchyma (including companion cells) and sieve elements is not an annual growth pattern but is repeated during growing season, instead.
2. Radial section. Sample shows how "suture" occurs in cambial region of ray due to lack of cell expansion at end of season.
3. Radial section with sieve plate consisting of several sieve areas. Lateral sieve areas in cell below sieve plate are much smaller and their pores are smaller, also. Sieve plate is scalariform, and analogous to perforation plate in xylem.
4. Sieve plate (scalariform), has tiny connecting strands in original preparation. Would appear as dots in white rings of callose. Look for these.

2. *Carya*

1. (Hickory). In tangential view (note rays). Scalariform pattern is cell wall. Shorter scalariform pattern at left is part of sieve plate.
2. The cell wall shows hardly any detail in our preps. Each lighter region is a sieve area on lateral wall, with relatively small protoplasmic strands connecting adjacent cells. Darker regions are thickened wall.
3. A sieve plate, on the other hand, shows: (1) purple network of cellulose, (2) colorless callose rings, (3) red staining where connecting strand enters callose ring. This view from radial section maximizes area seen in face view for sieve areas on sieve plates.
4. A sieve plate from older phloem in same section does not show protoplasmic contents. Conducting elements become crushed, also, in old phloem.
5. Sieve plate in sectional view. Number of views like this one will be maximized in tangential section. Large purple spots are part of heavy cellulose bars that make sieve plate scalariform. These lie between adjacent sieve areas and are seen in this view to be divided by compound middle lamella. Lighter cellulose network seen within sieve plate in face view appears as dots in this view, "connecting" the compound middle lamella from bar to bar. Callose is unstained but contents of pore are red, and are seen to penetrate sieve plate, depending on level of focus within section.
6. Same as above, refocused to show other connecting strands.

All images to this point as seen with hematoxylin and safranin.

3. *Vitis*

1. (Grapevine). Look at parts of 2 scalariform sieve plates. Stained for callose (lacmoid, or resorcin blue) gives blue rings. Cellulose network (brown to grey) can also show blue cast within sieve areas due to covering of callose over entire sieve area. Rings of callose go right through the sieve plate from cell to cell. Pore contents (connecting strands) go through centers of the callose rings.
2. Lateral sieve areas show much fainter development of pattern of rings.

3. Contrast what is seen when sieve areas on cell wall are compared to sieve areas within sieve plate.
4. Staining of callose on both sides of sieve areas seen in sectional view. Helps emphasize these points: Sieve areas here are between two sieve tube members, thus analogous to pitting on side walls between two vessel elements. Sieve areas are analogs of bordered pits in xylem. They are sieve tube members' versions of what should be done to specialize primary pit field of recent derivative of cambium. As in xylem pitting, what we see of sieve areas in mature cells is a function of which two cell types are cooperating to make the sieve areas: e.g., sieve tube members only vs. sieve tube members paired with parenchyma (such as companion cell).

4. *Pinus*

Radial section. Lignified walls of xylem stain blue. Patches of blue dots on light grey cells are callose on sieve areas. Here, sieve areas of sieve cells are analogs of circular bordered pits of tracheids. Lack of more specialized sieve areas at ends of sieve cells means these cells cannot be called sieve tube members. One generalizes from occurrence of tracheids with sieve cells and vessels with sieve tube members that the level of specialization in the phloem should be comparable to that in the xylem of the same plant. Some exceptions to this generalization are to be expected.

Activity 2: Secondary phloem as a tissue.

A. *Liriodendron*: Notice that both phloem and xylem are present.

   Phloem and xylem are similarly organized. Find rays that extend from xylem to phloem, continuously. In the phloem, are there cell arrangements analogous to the growth layers of the xylem? The banding of fibers in the phloem does not correspond to annual rings. Callose is not stained. Look for connecting strands (=pore contents). The radial section shows face views of sieve plates, and also of perforation plates. Compare the end walls of conducting elements in xylem and phloem -- how are they similar and how do they differ? What are the side walls of sieve tubes like? How do they compare with the side walls of vessel elements?

B. *Vitis*: Here it is stained so that callose is dark blue. Lignified walls are lighter blue. Compare the structure of sieve areas on end walls vs. that of sieve areas on cell walls, in conducting cells of the phloem. The difference in size of pores is the key feature that allows the designation "sieve tube". This slide can also be used to demonstrate septate fibers (fibers that are subdivided, internally) in xylem and phloem.

C. At this point you should have the following concept of a sieve plate: Specialized wall (usually end wall). Has perforated cellulose area (sieve area) or areas. These openings are called pores and have contents (pore contents, connecting strands). These connections go through wall like plasmodesmata but are much larger. Sieve areas of sieve plate more specialized than lateral sieve areas, which in turn are more specialized than pit fields or pits by virtue of pore size.

   You can here add a study of *Carya* for first-hand experience with some rather large connecting strands.

D. *Pinus*: has no sieve tubes, nor vessels. Compare the size of the sieve areas (in a sieve cell) and circular bordered pits (in a tracheid) in the radial section. The blue staining on the sieve areas is due to callose. The blue staining of the xylem walls is due to lignin. Single connecting strands are probably not resolvable. Are phloem and xylem similarly organized in *Pinus*?

CONCLUDING ACTIVITIES

All more or less factual questions!

1. List parallels and contrasts between xylem and phloem with regard to cell types present, organization of the tissues, functions of the tissues, destruction with continued growth, etc.
2. Diagram and describe the end wall of a sieve tube member at various stages of development and senescence. Note the relationship of cytoplasmic structures to the wall, and the placement of cellulose and callose when the element is immature, mature (transporting), and past functioning.
3. Define sieve area and sieve plate in such a way that you are able to distinguish between a sieve area on a lateral wall and one in a compound sieve plate. What does it mean to say that a simple sieve plate consists of one sieve area?
4. Which cells in the vascular tissues seem to have the greatest capacity for apical intrusive growth? Which have the greatest tendency to increase their diameter during development?
5. What is a cross field in secondary xylem? Describe (or sketch) all the kinds of pits (and pit pairs) found in the cross fields of a conifer with ray tracheids and ray parenchyma.
6. Define, describe, diagram, etc. to show the meaning of:
    a. heterocellular ray
    b. homogeneous ray system
    c. uniseriate ray
    d. terminal parenchyma
    e. banded parenchyma
7. What are the most diagnostic differences between primary and secondary vascular tissues in a woody dicot?
8. Describe the appearance of spring wood and summer wood as seen in a gymnosperm for:
    a. tangential surface of a board, sanded smooth
    b. tangential section in a prepared slide, as seen with the naked eye
    c. transverse section in a prepared slide as seen with the microscope
9. What anatomical feature(s) would account for a, b, c in 8?
10. Repeat 8 and 9, for a ring porous dicot like oak.
11. Diagram or describe the anatomical details that would allow you to recognize tyloses when you see them.
12. Are macerations of wood useful in the study of wood anatomy? If so, how?
13. Diagram the face view of a circular bordered pit and a sieve area as seen in radial section of a pine stem in secondary growth. What differentiated regions of the cell walls of the cambial derivatives could give rise to circular bordered pits (xylem side) and lateral sieve areas (phloem side)?

Topic 10: Secretory Tissues

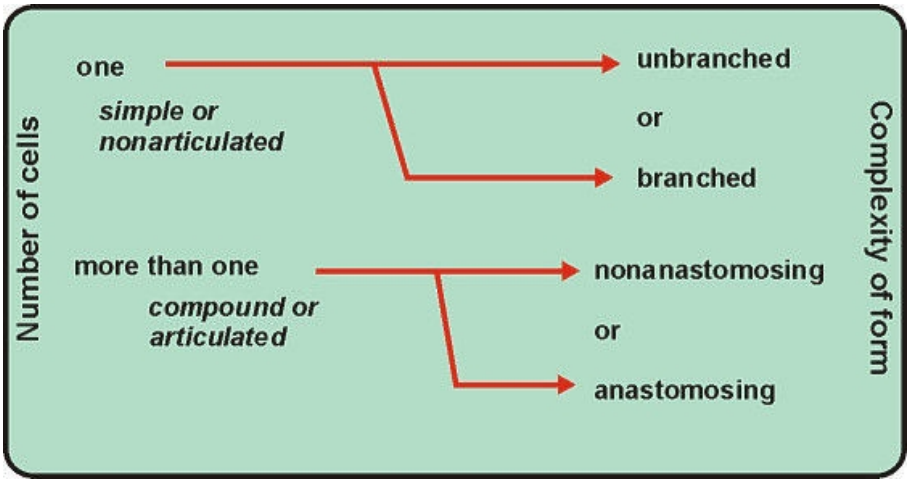
Secretory Cells, Tissues and Structures

Objectives: By the end of this topic you should:

1. Develop an appreciation for the "specialized structures" associated with the secretion process.
    a. Nectaries
    b. Hydathodes- exude excess water
    c. Glandular trichomes
    d. Salt glands
    e. Osmophores- fragrance producing
    f. Digestive glands
    g. Adhesive glands
    h. Resin ducts
    i. Mucilage cells
    j. Oils glands- internal and external
    k. Gum ducts
    l. Myrosin cells
    m. Gases
    n. Laticifers (latex)
2. Realize that laticifers combine properties found in other cells, but in a "unique" way. (Laticifers intergrade with parenchyma, may have indistinct vacuoles and much callose like sieve elements, positive turgor pressure like sieve elements, remove their walls like vessel elements, grow apically like phloem fibers, and branch like sclereids.)

Remember that:

1. Laticifers contain latex (what is latex?).
2. Laticifers can be simple (one cell that may branch), or compound (several cells that fuse, possibly including anastomoses).



<u>Classification of laticifers</u>: The terms used to described laticifers call to the number of cells involved with the formation of each latex tube and the complexity of the pattern formed by the tubes in the plant

3. The end walls of the cells can disappear.

4. Laticifers differentiate throughout the plant by apical intrusive growth or via differentiation within the ground or vascular tissue systems.
5. Laticifers can be multinucleate (via two ways; what are they?).

LABORATORY

External secretory structures

Activity 1: Glandular (secretory) trichomes

1. Prepare thin sections of petioles, stems, or leaf of *Pelargonium* (geranium), *Coleus* , *Salvia*, *Begonia*, *Chrysanthemum*, etc. Mount sections under water and observe the trichomes.
2. Look under a stereoscope at the glandular hairs of a *Drosera* (Venus flytrap).
3. Observe hydathodes on the leaf margin and epidermis of *Amborella*.

Internal secretory structures

Activity 2: Secretory ducts and cavities

1. Prepare a CS of a leaf of *Pinus* (pine) and stain it with TBO or Sudan dye, the resin ducts will look orange.
2. Prepare a CS of a *Helianthus* (sunflower) stem and stain with TBO, the secretory ducts are in the cortex.
3. Prepare a CS of a *Musa* (banana) fruit stalk, the secretory ducts are full of oil droplets.
4. Prepare a paradermal section of a *Citrus sinensis* fruit (orange), stain with Sudan, the oil ducts will be clearly stained.
5. Prepare a CS of a petiole of *Apium graveolens* (celery), the petioles have very narrow secretory ducts that will stain with TBO, they are located in the cortex. Note the epithelial cells that lined the secretory duct.

Activity 3: Making the slides from a *Ficus* or *Euphorbia* plant.

1. Set up the samples of latex for microscopic examination. Look for starch grains.
2. Using the plant part make longitudinal and transverse sections to demonstrate laticifers in two ways:
    a. Look for latex in the cells of unstained sections, and thereby ascertain the location and character of laticifers.
    b. Dye some of your sections with TBO and others with IKI (for starch). In cross section, laticifers stained with TBO will look as thick walled (pink-purple) cells in the cortex and pith. In longitudinal section they appear as elongated structures (sometimes ramify) through the tissues, the starch grains are shaped as a dumbbell or “dog bone”.

Questions

1. Criticize or defend the following statement “The cell is a form of secretion”. Justify your answer.
2. Why do you think the plant anatomists use artificial classifications for the process of secretion? Justify your answer.
3. What are laticifers? What are the principal contents of laticifers?
4. Select four types of secreting structures and compare them.

Topic 11: Nodal Anatomy

Objectives

By the end of this topic you should be able to:

1. Visualize, in a three-dimensional perspective, the vascular system in the shoots of several plants.
2. Evaluate the shortcomings of the term leaf gap as applied to certain interfascicular regions of the stem and to the nodes of certain plants.

Remember that the three-dimensional aspects of the vascular system with regard to leaf-stem relationships will lead us directly to a consideration of nodal anatomy. It only remains for you to make three- dimensional reconstructions in lab to wind up the objective.

Here is the problem: the term seems to imply that the leaf causes the gap. Ontogenetically this may be accurate, and the slower closing of the leaf gap during secondary growth, as compared with other interfascicular regions allows us to say that leaf gaps are special among interfascicular regions. Evolutionarily speaking, the leaf did not cause leaf gaps. Thus, morphologists are no longer comfortable with the term.

LABORATORY

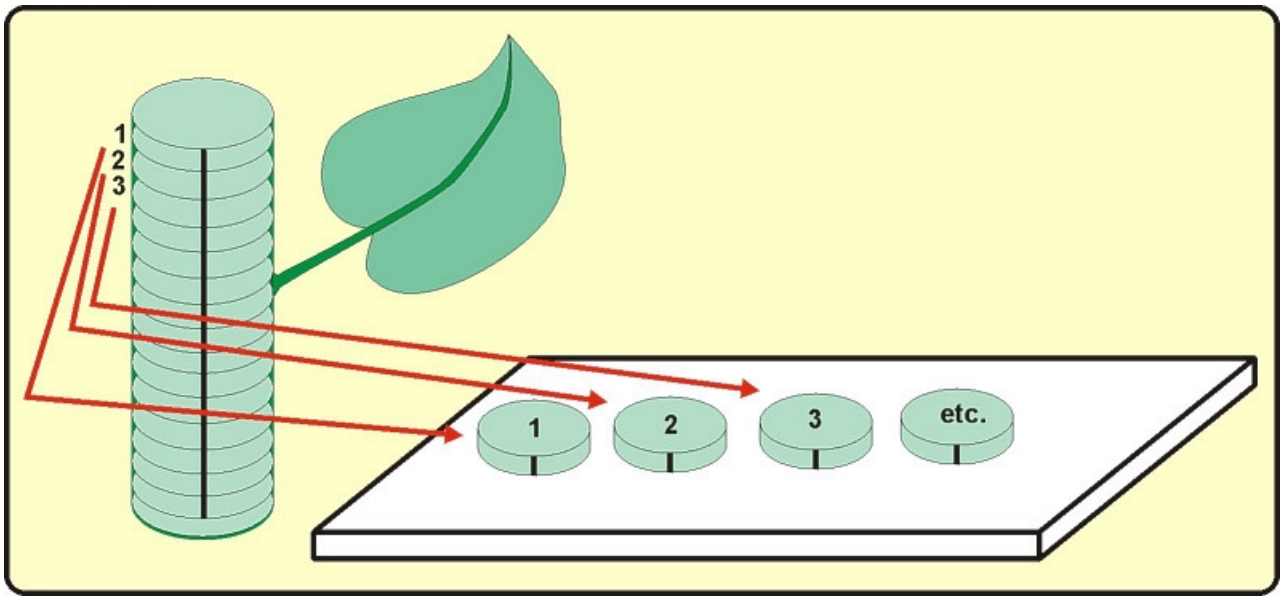
The objective of this lab is to build a three- dimensional concept of nodal anatomy using information from serial hand sections.

What to do: For each plant studied (for example: *Impatiens*, *Pelargonium*, *Coleus*, etc.), remove a shoot with a number of leaves attached. Keep the shoot in a bowl of water when you are not handling it. Work in the tender portions of the shoot, not in parts that have become woody. Make a transverse section in the internode to get started. If secondary growth has obscured the individuality of vascular bundles you are working too far downward in the plant. In plants like geranium, you may have to work with internodes that have some secondary growth. This is quite acceptable, because the leaf traces will still be recognizable.

Now with a sharp razor, make a shallow incision longitudinally on the stem in a specific relationship to the nearest leaf (e.g., at right angles to leaf insertion). This incision will serve as an orienting reference point that shows up in each section you make. The incision should run from one internode to the middle of the next internode. When you remove leaves, leave a small piece of the petiole attached for reference, also.

How to proceed: Starting about 5 to l0 mm above the leaf insertion (node) make serial sections transverse to the axis. Alternatively, you can start below the node and work your way up if this seems more natural. Lay out each section on a microscope slide before making the next section. Do not make the sections more than 0.5 mm thick, but they do not have to be as thin as you use for cytological investigations.

KEEP THE SECTIONS IN ORDER AND PLACE EACH SECTION ON THE SLIDE WITH THE NEWLY CUT SURFACE DOWNWARD



Serial reconstructions: to reconstruct an object with serial sections it is required that you know the absolute relationship of the sections to each other. Think of a sliced loaf of bread: the slices must be in their original positions to give the loaf the right shape, but each slice could be examined individually if we so desire

Arrange the sections from left to right and keep your slides in order if you make more than one. With the sections on a slide under the stereomicroscope, a dissecting needle can be used to move the sections around. You will have to use the correct amount of water to keep the sections moist without letting them float here and there. You can use coverslips, also.

Using the compound microscope, identify and follow the vascular strands from section to section, making as many sketches as are necessary to record the important changes in position that take place from level to level. For orientation, scan the series from end to end before any sketches are made. Then identify and count the bundles that connect the base of the leaf to the stem.

Reconstruct the three-dimensional arrangement of bundles from one internode to the next. If possible, construct a composite diagram based on your transverse sections so that the major relationships can be reported in one drawing.

IMPORTANT ADVICE: The estimate is that it will take approximately l to l-l/4 hours to complete this lab, so it is not wise to expect that you will be able to repeat your observations in tandem, any replicates have to be started pretty much together. Also, do not waste time on a bad set of sections, but start over again **QUICKLY** if the crucial part of the node is not observable. Learn from the frustrations of a first try; identifying the crucial interval on the stem is the major part of the battle. You may wish to make an exploratory set of sections deliberately, to refine your final observations and learn early in the game what you are up against.

Other observations: Take a few moments to spot check the petiole of the leaf you are working with on the plant that is your primary responsibility. Can the number of bundles at the base of the petiole be

accounted for by the number of traces that enter the leaf? Does the number of bundles change from the base of the petiole to the distal end? These changes can be followed with serial sections if you have time.

Questions

1. Attack or support the statement that “The vascular tissues of the stem is a sympodium of leaf traces, at least in flowering plants”.
2. Offer direct or indirect evidence that leaves control the course of vascular differentiation in plants”.
3. Discuss the differences of “leaf trace” and “leaf gap”.

Topic 12: Leaves

There are two points you need to remember when working on this topic:

1. Remember that what you may think of as the upper surface of a leaf can usually be identified as the **adaxial** surface (toward the axis) while the opposite side (back, underside) is the **abaxial surface** (away from the axis).
2. Leaves are the most homoplastic organ of a plant, so variations in leaf anatomy relate to leaf shape (centric vs. flat leaves), pattern of venation, complexity of epidermis, number of palisade layers, compactness of ground tissue, presence of crystals, glands, environments, etc.

LABORATORY

Objectives: After this lab the student should be able to elaborate the following statements, with details and examples.

1. The flat, broadleaf is a three-dimensional object with cytologically and anatomically differentiated layers and regions.
2. Leaf anatomy varies due to species, environment, ecological adaptations, etc.

Activity 1: Typical leaves as three-dimensional structures.
Leaves are three-dimensional objects, even though they are recognizably flat in the majority of common temperate plants. To assure that a reasonably sound three-dimensional concept of leaf structure is attained, we will utilize leaf clearings as well as sections.

A. Clearings of *Gleditsia* (locust) and *Acer* (maple) . Use either one or both of these leaves for study. Remember that the protoplasmic contents are removed when a clearing is prepared. This makes it possible to mount the leaf segment whole, and one can focus up and down to see how the leaf is put together.

   At different focuses the student can easily distinguish the several layers that are present. The abaxial epidermis has plentiful stomates. These might be hard to see. Also, sometimes pieces of epidermis fall out of the leaf when a clearing is made. Do the epidermal cells differ in shape on the two faces of the leaf? The palisade cells are essentially cylinders. How many of the palisade cells are attached to one cell of the epidermis? Note the side of the patches of mesophyll between veins. Estimate how far (number of cells away) the middle of such a patch is from a vascular bundle. Is the xylem in direct contact with the mesophyll? Look for a sheath of cells that covers the vascular bundle. Near the bundle endings look for indications that these cells intergrade with mesophyll cells. Where two small veins are close together, look for indications that they share a common sheath. Also, near the bundle endings look for tracheary elements that are not particularly elongate, and that may have squat, angular shapes.

B. Paradermal section of *Zea* (corn). This section will allow the student to appreciate the differences among a grass (a monocot) and the two dicots from the previous activity. In this section, the student can clearly observe the parallel venation pattern typical of the monocots, in addition to the position of the stomata and type of epidermal cells (long and short). Check carefully the vascular bundles. Can you differentiate the xylem and phloem? The bundle sheath?

C. Transverse and paradermal sections of *Syringa* (lilac). These sections should allow the student to continue to observe a three-dimensional study of the leaf with a cytological study. Images illustrate regions of the paradermal section that correspond to each layer seen to transverse the section.

Do you see similarities between the paradermal sections and the clearings used earlier? Can you identify plastids, nuclei, etc? Are all the cells cytologically equal? Determine the distribution of stomates, and the location of xylem and phloem in transverse section. How do these correspond? Specifically, use the xylem (adaxial) and phloem (abaxial) to tell which side of the leaf is which. Then determine whether the ad- or abaxial side has more stomates. Is a bundle sheath present? If so, what kind of cell comprises the sheath? Especially for the larger veins, look for evidence of specialization of the ground tissue above and below the bundle, forming an extension from the sheath to the epidermis.

Activity 2: Variations in leaf anatomy due to species differences.

A. Transverse sections of *Prunus* (peach) and *Citrus* (grapefruit) leaves, compare these with *Syringa* for differences in details. Here we wish to make the point that considerable variation occurs among leaves of the same basic structure. For example, if two kinds of leaves contain crystals, the crystals may be of different types. The grapefruit leaf contains oil glands. Of course these features cause local disturbances in the leaf structure. But comparing the leaves elsewhere, one comes to the conclusion that they are not remarkably different.
Is the positional correlation between stomates and the xylem-phloem arrangement the same as in *Syringa*?

B. Grass leaves. You have handled grass leaves while studying their epidermis. Their internal anatomy is both variable and interesting.

1. *Zea* as seen in transverse sections of the leaf. Where are the guard cells found -- only on one surface of the leaf, or on both surfaces? What kind of bundle sheath is present? Look for modifications of the ground tissue that resemble bundle sheath extensions.

   It is not quite consistent to call these "bundle sheath extensions" in *Zea* because the sheath is a somewhat different type of cell from that found in the extension. Just a little more information: Additional aspects of the bundle sheath can be visualized, and the generally parallel course of the venation is evident. Note however, that the veins are cross linked so that in the grass leaf, as in most dicots, a reticulum of vascular bundles exists in the leaf. This reticulum facilitates the transport of water to the leaves and of photosynthate out of the leaves, by placing all mesophyll cells in close proximity to vascular tissue. However, physiological evidence indicates that bundles of different sizes have different functions. Only the smaller-diameter bundles are specialized for the loading of photosynthate from the mesophyll. Larger-diameter bundles seem to specialize in systemic transport.

2. Compare and *Echinochloa* (millet) and *Triticum* with *Zea*.
   Zea is a C4 grass, with respect to photosynthesis and there are strong correlations between this alternate pathway of photosynthesis and certain anatomical features. The tropical grasses are especially well represented among the C4 plants. Interpreting the correlations is not straightforward. Is it the physiology, or the environment, or the genetic group that is determining the correlation? Is it photosynthesis or some other process that is at the basis of the selection pressure for anatomy?

Activity 3:

Variations probably due to environment (often confounded with species differences):

A. An aquatic leaf. *Potamogeton*. Air chambers and a very simple mesophyll.
Adaptive in aquatic environment where carbon dioxide exchange is from water to leaf, not from

air to leaf. Internal gas space is in large chambers that make the leaf float or stand upright and which act as internal gas reservoirs. These would be enriched in oxygen during the day and enriched in carbon dioxide during the night.

B. Xeromorphic leaves, or leaves with xeromorphic features.

1. Fleshy xeromorphic leaf. *Dasylirion* (desert candle) has a fleshy xeromorphic leaf. Does the leaf have a well aerated ground tissue throughout? Is there potential for significant water storage in such a leaf?
2. Needle leaves. Needle leaves of conifers show some xeromorphic features, presumably advantageous under the desiccating effects of winter which require adaptations to a "dry" environment. These features can be demonstrated in pine needles. Note the transfusion tissue, which is a common feature in gymnospermous leaves. See herbarium specimens of various plants with needle leaves. Not all needle leaves look alike anatomically. The delicate needles of *Taxodium distichum* (bald cypress) do not show markedly xeromorphic features, but neither do they overwinter. See a transverse section. Cleared leaf images are available for study. These are especially good for the distribution of transfusion tissue, distribution of stomates, and detection of elongated fiber-like idioblasts in the mesophyll. These features can be noted in a transverse section, but they are more fully comprehensible in the clearings. Note that the clearings are suitable for detection of small numbers of stomates on the upper surface.
3. Centric leaves. Examine *Hakea lorea* (bootlace oak) as one example from the group. Here we see several variations that are said to have adaptive advantages in a dry environment. The epidermis is covered with a thick cuticle that seems appropriate to dry habitats, as do the sunken stomates (reduced exposure to wind movement of gases). The leaf is centric (subcylindrical) rather than flat, reducing surface to volume ratio, and has some aspects of stem-like structure, with the center "resembling" a pith. The leaf is tough and ruggedly constructed. Sclerenchyma is present both as fibers and as idioblasts that reside in palisade. While you have the image in view, correlate the arrangement of xylem and phloem in the bundles relative to the surface of the leaf. Does the centric leaf resemble a stem in this feature?
4. A xeromorphic grass leaf: *Ammophila* (marram grass). This leaf is adapted to roll when it dries. Rely on the arrangement of xylem and phloem to tell you which surface is abaxial. Are there stomates on this surface? No. It is composed entirely of tightly arranged cells.

   Look carefully on the convoluted adaxial surface for stomates. They are there. You should be able to appreciate how rolling of the leaf protects the leaf from water loss. Note the distribution of sclerenchyma in this leaf. One side of the leaf is "tender" compared to the other side. It is the "tough" side that is outside when the leaf rolls. The distribution of stomates and sclerenchyma is consistent with the overall adaptation of the leaf. In other words, anatomical correlations are to be expected when certain adaptive mechanisms are present.

Comment:

For additional photos of leaves go to home page and type “leaf” in Keywords.

Questions

1. Give an overall appraisal of the structure of a typical dicot leaf and relate each feature to the function of a leaf as a photosynthetic plant part.
2. Submerged leaves tend to be simpler than those in an aerial environment. We discuss the characters of these leaves when we learned about tissues, so think about that carefully for answering the following question: If you submerged a maple leaf, would you reduce its rate of photosynthesis?
3. To what extent we can generalized about the distribution of stomates on the surface of a leaf? Write a brief statement summarizing all the variations you can see.
4. You have been perusing through a box of slides that have no labels. You are so lucky that you find a cross section that is circular. The bundles are scattered, but the phloem is always toward the nearest surface. Can you conclude that this is a section of a stem?
5. What is a midrib [principal(s) vein(s)]? How does it differ from the veins that irrigate the thinner parts of the leaf blade?
6. What size of vascular bundle should you look for in a section of a leaf if you are looking for good examples of Bundle sheaths? For bundle sheaths extensions? Explain your reasoning?
7. Give an overall appraisal of how meristems are involved in leaf development.
8. Why are hormones important in leaf development? How are they related to meristematic activity?
9. In your own words explain why it is important to understand how the SAM functions in relationship with the origin of leaves?
10. How is parallel venation different from reticulate? How is it similar? How is parallel venation related to the way a grass leaf grows in length?
11. Outline (briefly) leaf abscission in deciduous trees. Are the details the same in all plants?
12. How are cell division and cell expansion coordinated or integrated during growth of a leaf?

Topic 13: Roots

Objectives

By the end of this topic you should be able to:

1. List the characteristics of roots, including those that set them apart from shoots.
2. Describe the organization of the root apex and evaluate the concept of apical initials in this context.
3. Evaluate the concept of a quiescent center in roots.

REMEMBER THAT:

1. The root apex is covered with a rootcap.
2. There is a quiescent region in the apical meristem of the root.
3. Root primary xylem is exarch (except very rarely, in lower plants).
4. Protoxylem is sometimes little- or unstretched.
5. Xylem and phloem are radially arranged.
6. Endodermis is (always) present.
7. Branching originates endogenously.
8. Vascular cambium arises between xylem and phloem and in the pericycle.
9. Cork cambium arises in pericycle.
10. Mycorrhizal roots are common.
11. Roots can have normal or anomalous secondary growth.

LABORATORY

After this laboratory you should be able to enumerate the characteristics of roots. The detailed objectives are listed with each activity.

Activity 1: Primary tissues.

Objective: To learn the histological features of a variety of roots in primary growth.

1. Analyzing a root for certain histological changes that take place during primary growth.
    a. *Ranunculus*, two stages. A comparison of the two stages gives some information on the developmental changes. Look for these major features: What is the arrangement of xylem and phloem? Pith is absent, but younger stage shows incomplete maturation of the metaxylem. Two stages in development of secondary wall on the endodermis (look for casparian strips in both stages). Look for differences in the endodermis opposite the phloem vs. the xylem. In which of these two locations does thickening of walls begin first?
    If the two stages have different numbers of protoxylem poles, this is not a developmental change related to maturation, directly. Instead it relates to the relative vigor of the root. Roots with larger meristems have more protoxylem poles. It is true that a root can change its vigor, reflected in the size of its meristem, and thus the number of protoxylem

poles, as it grows through the soil. But for any segment of the root, the number of protoxylem poles is determined directly behind the meristem and does not change as that part of the root matures. Do not confuse the slight amount of secondary xylem with a change in the number or extent of the protoxylem poles.

b. *Smilax herbacea*. Two stages of primary growth. *Ranunculus* is a typical dicot with respect to root structure. In its general features, *Smilax* can be used to typify root structure in the monocotyledonous plants. Note the numerous protoxylem poles, the lack of conducting elements at the center (pith may be said to be present), and the stages of thickening of the endodermis. Look for differences in the endodermis opposite xylem vs. phloem. How does this endodermis differ from that in the *Ranunculus* root? Look near the surface of the root for an additional specialized layer (exodermis). Compare it with that seen in the endodermis for similarities and differences. Exodermis and root hypodermis can be used interchangeably for our purposes. Characterize the ground tissue throughout the cross section as thin walled or thick walled. Do the "pith" and cortex contrast in this regard?

2. Extending your experience with the histological variations of roots in primary growth. We have two orchid roots for you to examine. One is simply marked "Orchid root"; the other is marked "*Vanilla* root".

   a. Orchid root. One stage of primary growth. Look again for endodermis and exodermis. Note these differences from previous observations and special features: How far under the surface of the root is the exodermis? The intervening tissue (velamen) is a multiple epidermis. Look at the endodermis as a complete ring. Does it show differences outside the xylem vs. outside the phloem? You have been asked to consider this feature in *Ranunculus* and *Smilax*. Is there uniformity on this point?

   b. *Vanilla* root. One stage of primary growth. The cell layers with specialized walls are the endodermis and epidermis. Characterize the condition of the cells in these two locations. Is there anything unusual about the habit of certain orchids that might prompt you to look for specializations in their roots, or that might lead you in retrospect to see some relationship between anatomical and ecological specializations? See a whole orchid plant when you consider this question.

Summarize, for your own use, the locations of specialized layers within the root, i.e., in which layers might one find cells with especially thickened, chemically altered walls? What anatomical specializations of epidermis have you encountered today in lab?

Activity 2: Secondary growth.

Objectives:

1. To see that roots, like stems, undergo secondary growth in the vascular and dermal tissue systems.
2. To develop the skill to recognize protoxylem poles in roots after secondary growth is well advanced.
3. To establish that the cork cambium arises in the pericycle.

A. *Abies* (fir, a conifer). There is a resin canal present. Do not be misled by this into thinking there is a vessel element at the center of the axis. Two stages of secondary growth are represented. Where does the periderm arise? The endodermis was already crushed and emptied of cell contents. Look for early periderm formation (dark cell layer). Confirm that the root is diarch on

the basis of looking for two patches of protoxylem elements with small diameters. The root was exarch in its maturation of primary xylem. This should help you figure out which elements are metaxylem. Where is the primary phloem?

B. *Populus* (poplar). Two stages of secondary growth. How many protoxylem groups can you find? Compare with *Abies* and, further, make a comparison with secondary growth in stems. Is the root remarkable for its differences from the stem in the later stages of secondary growth? How could you confirm that this is a section of a root and not of a stem?

Activity 3: Lateral branching in roots.

Objective: To establish that branch roots arise endogenously, with a positional relationship to the xylem and phloem of the parent axis.

A. *Salix nigra* (willow) . Longitudinal and transverse sections. Confirm that the lateral roots form endogenously. Typically, the origin is just inside the endodermis. Using a transverse section, describe the position of lateral roots as a function of stele anatomy: are the roots in a definite position with relation to xylem or phloem?. How does a root emerge from the parent axis when the branch root is formed endogenously?

B. *Brassica* (mustard): Determine whether this root was in primary or secondary growth when it was collected. How many protoxylem poles does the root have at this level of sectioning? Is there any correlation between position of laterals and the location of xylem poles in the parent axis? Has this had any effect on the outline of the parent axis?

Activity 4: Mycorrhizal roots.

Objective: To determine, in one example, the extent to which fungal hyphae penetrate a mycorrhizal root.

Sections of *Monotropa*: examine for hyphal growth (fungus) in the root of a higher plant. Such fungus-root tissues are called mycorrhizae. The presence of the fungus can change the gross morphology, branching pattern, and anatomy of a root system. Sometimes it can be established that the host plant is more productive when the fungus invades the root system, exists in a wider variety of habitats, or derives other benefits. The fungal layer also affects ion uptake and other host- environment relationships. Mycorrhizae are important in many crop plants, and it is often said that in field plants fungus-free roots are exceptional, rather than the rule.

Questions

1. What is a quiescent center, and does it make the classical concept of apical initials obsolete in the roots? If so, why? If not, why not?
2. Speculate on the role of the quiescent center in the root apex.
3. Criticize the statement “The geometry of the root apex is best described in the context of a minimal construction of growth, because geometry itself does not reveal the place of growth, we have no obligation to relate geometrical pattern to the growth of the apical meristem of a root”.
4. Why would it be said that the Kopper-Kappe theory is the “tunica-corpus” theory of the root?
5. What are the positional relationships between branch roots and the xylem and phloem of the parent axis?
6. Describe briefly the stages of lateral root formation.
7. Describe (a diagram will do!) the anatomy of the root, the stem and a leaf of a typical dicot.

Topic 14: Flowers, Fruits, and Seeds

Objectives

After this topic is completed you should be able to:

1. Enumerate the differences between a vegetative shoot apex and one that has started to flower.
2. Discern similarities and differences in the anatomy of floral parts and leaves.
3. Comprehend some of the range of variability in which parts of a flower develop into a fruit.
4. Appreciate the difficulties in obtaining a useful classification of fruits that faithfully represents botanical similarities and differences between fruits.
5. Defend the usefulness of the distinction between fleshy and dry fruits for anatomical purposes.
6. Apply a "trivial" classification to fruits, emphasizing the development of the layers of the fruit wall, for a number of familiar fruits.

This may appear to be a long list of objectives, but really, it is necessary to be more specific with objectives in this topic than in others of similar length. Many feel rushed at this stage and this feeling will be aggravated if you fail to recognize exactly what is expected of you to get out of this topic. So...read the chapters on flower, fruit and seed from any textbook. Why is it that all three are to be covered in one topic? This is because the essence of what you want you to take away from that large packet of information can be distilled into a relatively small container. So concentrate on the main points as outlined below and take recommendations on the reading seriously.

Focus on the idea that the rate of biological activity is altered in the apex as flowering begins. Some information suggests that there is a speeding up of the production of primordia with flowering. Remember that:

1. Sepals are often quite leaflike, petals less so.
2. Stamens typically do not resemble leaves.
3. Carpels are leaflike in their development and in their vascularization -- not in their mature anatomy.

So one must consider development as well as mature anatomy, and vasculature as well as overall histology in making these comparisons.

For Activity 3 it is necessary to develop the idea that a fruit wall may consist only of carpel(s) or of the carpels plus floral tube and even receptacle.

Our attack in the lab will be to take a number of common fruits and look at the anatomy of the fruit wall and seed coats to gain a notion of how these assist in protection and dispersal of the seeds.

LABORATORY

Objectives: By the end of this lab you should be able to:

1. Diagnose the anatomy of a fruit wall and seed coats to determine the types of cells present.
2. Make a one to one correspondence between the texture of a fruit and the predominance of parenchyma vs. sclerenchyma in the fruit wall.

3. Recite the "trivial" names attached to certain familiar fruits when they are classified, and make a direct anatomical interpretation of what these "types" of fruits are.

Activity l:

1. Comprehending where fruits come from. Median sections of flowers are available through CUPAC. This activity is to help you establish the continuity between flower and fruit. Special attention should be paid to the interpretation of superior vs. inferior ovaries, because this distinction carries over into our designating the fruit wall as consisting of carpels vs. carpels plus floral tube, etc. Spend time surveying these materials and try to relate them to the rest of the lab where possible.
2. Dissect a flower to expose the stamens and gynoecium. Take a stamen and dissect the anthers within a drop of water on a slide. Observe under microscope.
3. Section the gynoecium, observe the locules, position of ovules, and the placentae.

Activity 2: Analyzing the fruit wall and seed coats. The descriptions that follow are based on prepared slides. Examine the fresh materials that are available for the purpose of correlating texture, hardness, etc., and microscopic features. Feel free to make macerations and hand sections to add to your observations.

It is profitable to study fruits and seeds together. The "responsibility" for protecting the embryo may reside in the fruit or seed or both, and the anatomy of the fruit can be related to mechanisms of seed dispersal. There is no such thing as a typical fruit, and it is therefore impossible to set out a norm from which variations can be studied. Instead we will take the approach of analysis, determining what cell types are responsible for the characteristic quality of a variety of fruits.

1. Typical berries from superior ovaries.
    a. *Epigaea* (Plymouth mayflower): A convenient place to start, since we have an image of the flower for comparison (Activity l).

       Hard seeds are embedded in a mass of pulp. Look for seeds that appear to be intact and examine the seed coats for sclerenchyma. What is the unusual aspect of the sclerenchyma in the seed coat? Compare these cells with the macrosclereids you saw in an earlier lab.

    b. *Solanum lycopersicum* (tomato): As in *Epigaea*, the fruit is divided into locules and the seeds are attached to a placenta. As the tomato ripens, the seed coat becomes mucilaginous.
2. Hesperidium. A "berry" with a rind, from a superior ovary. Seeds with firm coats.
    a. Orange rind: An orange, *Citrus* x *sinensis*, is a hesperidium. Note the oil glands and the looseness of the tissue away from the surface.

    b. The whole system of fruit and seeds can be seen in the young stages of the lime, or any other citrus fruit while the fruit is still small enough to section whole. Note the division of the fruit into locules. Note the difference between ovules and juice sacs in the images.
3. Berries from inferior ovaries. (See *Ribes* (currant) flower section from Activity l for comparison.). Images of *Vaccinium* (blueberry) represents blueberry, cranberry, etc. Same family as *Epigaea*. Hard seeds are embedded in a mass of soft pulp. In the grape (*Vitus*), we spit out the seeds (unless your time is too valuable) with the blueberry we just let them go "crunch" between our teeth because they are so tiny. The seed coats also crunch against the microtome

blade and cause some damage to the knife while they fracture as they are sectioned. Compare the seed coat of *Vaccinium* with that in *Epigaea*.

4. Pomes. (See quince (*Cydonia*) flower section from Activity 1 for comparison.) Inferior ovary plus floral tube gives rise to fleshy fruit. Outer layer of wall consists of epidermis. Fleshy middle layer may have stone cells. Inner layer is leathery or stony. Seeds have hard coats.
    a. Small pome of crab apple (*Crataegus*). The whole system of fruit and seed can be seen in one section in a small pome. The fleshy part of the fruit has numerous stone cells. This pome approaches a drupe, in the kinds of layers present in the fruit wall but not in the number of stony locules.
    b. Images of the fleshy layer of pear (*Pyrus*) fruit. You looked at stone cells in the pear fruit during the lab on sclerenchyma. Take a minute or two to refresh your memory on what these look like, for comparison with the stone cells in the crab apple.
    c. Apple (*Malus*) epidermis. The apple is a pome. The outer fruit wall is not highly specialized. A thick cuticle overlies several layers of cells with somewhat thickened walls. How thick is the cuticle, relative to the size of the epidermal cells? Look for locations where the cuticle is inserted between anticlinal walls.
    d. Images of apple seeds. Look for sclereids in the seed coat. Are they the epidermis or a subepidermal layer? The sclereids contribute to the indigestibility of the seeds and would aid in their survival should the pome be eaten by an animal. Hence, the seeds are dispersed with droppings. Recall that the inner layer of the fruit wall in an apple is leathery. This offers some additional protection for the seeds but not as much as the stony layer in the crab apple that you have examined.
5. *Pepo*. A berry-like fruit with a tough rind. From a flower with an inferior ovary. Seeds hard.
    a. *Citrullus* rind (watermelon). Look for the thick cuticle, and for pegs or wedges of cuticle between anticlinal walls of the epidermis. Is there additional specialization in the rind? Look for a layer of developing sclerenchyma. Locate vascular bundles. Why would you expect to find a fruit to be highly vascularized? The vascular bundles in the apple are spaced out and individually identifiable. Those in the watermelon are crowded together. The boundaries of the floral tube cannot be defined precisely in this fruit derived from a flower with an inferior ovary.
    b. Squash (*Cucurbita*) fruits, young stages. Note that the section from the younger fruit shows epidermal appendages, but the older does not. However, the older fruit does have stomata. Although the squash is a pepo, as one might expect, it resembles watermelon in general rather than in detail. Likewise, one could find some differences within the squashes as a group because they are diverse and even within different lines of the same type of squash. Hence, there is opportunity to select anatomical characteristics as they affect marketability, because of shipping and storage properties. Our melons are pepos. Cucumbers, gourds and pumpkins are pepos also. Think of all the melons there are and how their rinds differ (muskmelon, honeydew, crenshaw, etc.). There are many varieties of cucumbers. Some correlations exist between the anatomy of the rind and the ability of the cucumber to become a pickle! These correlations are less direct than the differences in the anatomy of "summer" and "winter" squash, but they are there to measure, nonetheless. With gourds the tough, elastic rind may be the source of a variety of musical instruments (including those for Latin rhythms, and the Indian sitar). Of course the glory of the pumpkin is the pie.

Activity 3: Hard fruits. An inferior or superior ovary can be involved. The wall of the fruit is mostly hard throughout (much sclerenchyma). Seeds tend to have papery coats if the fruits are indehiscent.

1. **Nuts**. The fruit does not open. A single seed matures.
    a. *Quercus* fruit, (Oak). The acorn is a nut. In the sections, determine the locations of sclerenchyma and determine whether more than one type is present. Note that the seed coats are crushed. The seed does not escape from the fruit wall during seed dispersal. The fruit wall is part of the "seed coat" in a practical sense (see also, grass fruits and achenes).
    b. *Tilia* fruit. Another nut, but with a seed that has a protective coat as well. In the fruit wall, see how many types of cells you can identify. Note the arrangement of the elongated cells. In the seed coat, what type of sclereids comprise the epidermis? This type of cell in this arrangement is essentially diagnostic for a seed coat! Look for differential staining in the walls of these cells. The vacancy at the center of the ovule should be occupied by the embryo, which was unfortunately lost during sectioning.
2. **Achenes**. The fruit wall does not open. A single seed matures. Achenes are defined differently from nuts in some classifications, so they have been kept separate here.
    a. *Aster* (daisy) fruit. Where are the protective layers in that fruit-seed system?
    b. *Angelica* fruit. Like an achene, but receiving the special name schizocarp. A schizocarp breaks into two mericarps, each one resembling an achene. A similar production of more than one achene per pistil occurs in *Coleus*. But, the achenes of a strawberry, *Fragaria* (strawberry), (the gritty little seed-like things) each come from a separate pistil on a fleshy receptacle, all in one flower. In the edible fig there are numerous flowers attached to one inverted receptacle. Here, each flower makes only one achene.
    c. Edible fig (*Ficus*). The flowers are “inside” because the receptacle is inverted; male flowers are near the opening, female below. The flowers are wasp pollinated. Can you see indications of developing hard tissue in the achenes?
3. **Drupes vs. Nuts**. The peach (*Prunus*) is a typical drupe: derived from a superior ovary; the fruit is fleshy except for a stone which protects the seed. A number of fruits that we think of as nuts grow from flowers with inferior ovaries with the fruit wall developing a leathery or fleshy layer and a stony layer inside. The outer layer or layers are shed cleanly by hickory and pecan , they rot off or are rubbed off the walnut, but persist as shreds or strings on almonds. Even a coconut fits in this grouping based on specialization of wall layers, and is described as a drupe in some books and as a nut in others.
   Carya, the pecan: Examples are in two parts. Examine first the part. This represents the pecan "nut" that is marketed. Note the anatomical similarities with the acorn. Now examine the second part, which is the fleshy young fruit wall, taken at this age because it tends to break down later on. Like the walnut, to which it is related, the pecan tree furnishes us with fruits that have hardened endocarps. Black walnuts are notorious for the mesocarp, which furnishes a source of natural dye, that can be applied to your clothes, either intentionally or unintentionally.
4. **Capsule**. Dehiscent. Often hard or leathery. *Hamamelis* is the witch hazel plant. Just note that the wall is fully as hard as a nut, it is classified as a capsule because it breaks open at maturity. The classification of this fruit is related to "behavior" of the fruit rather than to its anatomy.
5. **Caryopsis**: Grass fruits. One seed. Fruit wall and seed coats fused. Called a caryopsis or grain.

a. *Zea* (corn). A representative of a diverse group of fruits, whose anatomical parts are so distorted during development as to make them virtually unidentifiable without developmental studies. Example shows a near median section of the embryo. Note the close contact between cotyledon and endosperm.
   Corn grains have a relatively thin fruit wall, and they lack the additional protective parts that are present in the wild grains that may have been the ancestral stock. This is no accident, for corn is an example of a highly selected crop plant. What is the mechanism of seed dispersal in cultivated corn?

b. *Triticum*: Study the images of *Triticum* (wheat) grains.

Comments

For additional photos of fruits go to home page and type "fruit" in Keywords or type the specific genus in the Genus search.

Questions

1. Compare and contrast vegetative and flowers apices with regard to cytological zonation.
2. How would you determine the length of a plastochron during vegetative growth? During flowering?
3. To what degree do carpels resemble leaves? At what stages of development are the various similarities evident?
4. Define superior and inferior ovary. Does the distinction between the two necessarily lead to easily described differences?
5. What characters would you use to differentiate flowers?
6. There are flowers that are unisexual, explain how this can happen from the developmental point of view. Uphold your answer.
7. What factors do you think induce flowering?
8. What are the differences in development between an inflorescence and a flower? Explain.
9. Microsporogenesis is the process of producing microspores by meiosis while microgametogenesis refers to the development of the multicellular (2-celled) pollen grain. Explain the differences between these two processes and defend you answer.
10. Does the pollen wall develop concurrently with microsporogenesis and microgametogenesis? Explain.
11. Describe in detail a microsporangium in very early stages of development, during miosis, and when pollen grains are already mature.
12. Criticize or defend the following statement: "A mature pollen grain is 2-celled (2-nucleate) so by definition a pollen grain at this stage is a multicellular macrogametophyte (one cell inside another cell). One nucleus is the tube nucleus and the other known as the "generative nucleus" will further divide to produce two sperms".
13. Define "apocarpy" and "syncarpy".
14. Why do you think "incompatibility" is necessary in some cases?
15. Briefly describe the process of megasporogenesis.

16. What type of tissues are you expected to find in an ovule?
17. How many types of ovules there are? Diagrams will do!
18. Defend or criticize the following statement “Fruit wall and seed coat fuse during their development”.
19. Briefly described the process from fertilization to a full embryo.
20. Can you predict the type of fruit a plant will produce based on characteristics of the flower. Defend your answer.
21. What are parthenocarpic fruits?
22. Compare “dry” and “fleshy” fruits. (Similarities and differences)
23. What is the origin of the testa? And of the endosperm? What are their functions? What type of cell are characteristics of each one?
24. What is inside the seed?

Appendix 1

Disclaimer

1. **The dates for planting depend on when you will be using the plants in the labs, therefore you can modify the dates accordingly. The presented planting list starts in August.**
2. **The amount depends also on the number of students. The amounts suggested here are enough for 10-14 students.**

Plant date	Seed types	Final # plants needed	Comments
8/2	Flax	4, 6" pots	use supports
	Sunflower	1 x 18	grows for 8 wks
	Marigold	2 x 18	grows for 8 wks
	Borage	1 x 12	grows for 8-10 wks
	Corn	2 x18	grows for 4-6 wks
	Beet	1 x 12	grows for 6 wks
	Fava bean	1 x 18	grows for 8 wks
	Dahlia	1 x 18	grows for 8 wks
	4-o'clocks	1 x 18	grows for 8-10 wks
	Oats	1 flat	grows for 6 wks
8/9	*Zinnia*	1 x 18	grows for 8 wks
	Impatiens	1 x 18	grows for 8 wks
	Sunflower	1 x 18	grows for 8 wks
8/23	Corn	1 x 18	grows for 3 wks
	Hollyhock	1 x 12	grows for 8 wks
	Dahlia	1 x 18	grows for 8 wks
8/29	Sunflower	1 x 18	grows for 4 wks
	Marigold	1 x 18	grows for 4 wks
	Fava bean	1 x 18	grows for 4 wks
	4 o'clocks	1 x 18	grows for 4 wks
9/6	*Zinnia*	1 x 18	grows for 4 wks
	Impatiens	1 x 18	grows for 4 wks
	Sunflower	1 x 18	grows for 4 wks
	Squash	1 x 12	grows for 4 wks
	4 o-clocks	1 x 18	grows for 8 wks
10/4	Beet	4, 6" pots	grows for 8 wks
	4 o'clocks	1 x 18	grows for 4 wks

10/11	*Zinnia*	1 x 18	grows for 7 wks
	Impatiens	1 x 18	grows for 7 wks
	Marigold	1 x 18	grows for 7 wks
	Bachelor Buttons	1 x 18	grows for 7 wks
11/8	Corn	1 x 18	grows for 10 days
	Fava Bean	1 x 18	grows for 10 days
	Oat	1 x 18	grows for 10 days
	Pea	1 x 18	grow for 10 days

ALL THESE (11/8) SHOULD BE PLANTED IN VERMICULITE ONLY, WILL USE THE PLANTS FOR ROOTS.

OTHER PLANTS NEEDED

A. Plants to be consumed by heavy pruning (November 10):
    1. THREE (3) potted Begonias use ONLY the one with SMALL PINK FLOWERS.
    2. THREE (3) potted Geraniums (any kind)
    3. THREE (3) Impatiens (common variety)
    4. THREE (3) Coleus
B. Small quantities to be collected from permanent stocks (greenhouse plants) Approximate Date:

9/6	6 *Tradescantia* flowers 2 *Diffenbachia* leaves (with petioles, small plant) 2 *Ficus* leaves (with petioles)
9/13	2 medium to large, well-branched *Coleus* plants-Will harvest 18-20 lateral branches
9/15	18 *Tradescantia* leaves 2 large *Ornithogalum* leaves 2 *Ficus* leaves with petioles 3 shoots of Spanish moss
10/4	10” of a banana leaf 2 well-branched *Impatiens* plants to chop up for sections of stems
11/1	2 Fig leaves with petioles

	1 plant (small to medium) pencil tree for the classroom and sample (return plant mostly intact) 1 *Poinsettia* 2 Papaya leaves with petioles
11/3	*Dracaena* plant (for the classroom example, return plant intact)
11/8	1 *Citrus* leaf 1 oleander leaf