A. Introduction
In many plants, development of the primary plant body and tissues is just the beginning. For perennial plants such as trees and shrubs, there also is a growth in girth after elongation and the production of primary tissues due to the activity of two LATERAL MERISTEMS that are the source of all SECONDARY TISSUES. These lateral meristems include the VASCULAR CAMBIUM and the CORK CAMBIUM (PHELLOGEN). The growth in girth produced by these two meristems is called SECONDARY GROWTH. Through periclinal and anticlinal cell divisions, these lateral meristems give rise to a RADIAL pattern in the stems and roots of woody plants.

Some of the world’s most interesting plants undergo this secondary growth. The oldest known trees are those of Bristlecone Pine (*Pinus longaeva*) that exists in the White Mountains of California. Some of these trees have been documented to be over 4700 years old. One reason for their extensive life is the presence of a woody secondary body that over time becomes nonfunctional in the center but continues to serve as a storage site for resins, tars, gums, tannins and other secondary metabolic products inhibitory to pathogen invasion. Generally, the tallest known organisms on Earth are trees of coastal redwood (*Sequoia sempervirens*), commonly reaching over 350-370 ft. Other tall species include the Douglas-fir (*Pseudotsuga menziesii*), sometimes reaching more than 300 ft (one was recorded as 329 ft), and Australian eucalyptus trees (*Eucalyptus regnans*; one of which is now measured at 326 ft, but historical records place some over 400 feet).

In today’s lab, we will be investigating this often overlooked aspect of botany, the secondary plant body. The outline for the lab is as follows:

B. Woody Stem Morphology
C. Wood Morphology
D. Types of Wood Sections
E. Vascular Cambium
F. Cells of Secondary Xylem
G. Growth Rings
H. Rays & Ray Initials
I. Bark Formation: the Cork Cambium
J. Lenticels

_A cork harvest from the cork-oak, Quercus suber._
B. Woody Stem Morphology

1. Select a twig from those available on your bench. **OBSERVE** the twig and **IDENTIFY** all of the structures indicated in the figure. Pay special attention to the position of the bud scale scars.

*How many years of growth are visible on your twig?*

*Compare your twig to your neighbor’s.*

*Can you tell them apart?*

*What structures might distinguish one woody stem from another?*

Illustration showing the morphology of a woody stem.

C. Wood Morphology

**Observe and identify the following classifications using the “tree cookies” on your table:**

1. **Hardwood vs. Softwood.**
   Hardwoods are angiosperm woods, softwoods are gymnosperm (e.g., conifer) woods. Gymnosperm woods lack vessel elements and fibers, so they are generally more uniform and tend to be softer than angiosperm wood. See the dissecting scopes for a recap on the difference of these.

2. **Heartwood vs. Sapwood.**
   Heartwood is the innermost and non-conducting portion of a tree/shrub’s wood. Often darker due to the accumulation of substances such as oils, gums, and tannins. These accumulated substances may make the heartwood aromatic. **Sapwood** = the outermost, generally conducting portion of the wood. Often lighter than heartwood in color. Also contains living parenchyma cells distributed as rays, which may store and transport reserve materials.

3. **Early wood vs. Late Wood.**
   Early wood is the first-formed wood of a growth ring (i.e., a growing season). Its cells are larger and this part of the ring is therefore less dense than that formed towards of the end of the growing season. **Late wood** is the latter-formed wood in a particular growth ring; consists of
smaller diameter cells. The alternation of early and late woods in woody stem is responsible for the growth rings seen in transverse section! See below.

Bark is technically all tissues outside the vascular cambium, and includes the phloem. The inner bark consists of all tissues between the vascular cambium and the cork cambium, made up chiefly of secondary phloem. The outer bark consists of all of the tissues outside the innermost cork cambium plus the cork cambium.

Based on the pictures below, can you see why carving your initials into a tree might be harmful to the tree? What two functions of the tree are most likely negatively affected by carving of initials?

D. Types of Wood Sections
There are three basic types of wood section you need to be familiar with. Each type differs by its orientation with respect to the axis of the stem and can be distinguished by the structures visible.

1. cross or “transverse” sections (cut perpendicular to length of stem)

2. radial (a long section cut along one radius of the stem)

3. tangential (a longitudinal section cut at a tangent to the vascular cylinder, and usually through the vascular cambium itself, such that we can see the two types of initials that make up the cambium.)
1. Study the woody stem segment or plastic model labeled as D1. This section contains the sections described above. Identify each section represented by your piece of woody stem. Keep the orientation in your mind when looking at the rest of the laboratory sections.

2. Observe one of the following prepared slides:
   - *Celtis occidentalis*
   - *Abies grandis*

Both slides contain all of three types of wood sections described above. In the space below, draw enough of each region in order to be able to distinguish it from the other regions. Focus on using major structural features from each. The rest of the lab will go into further detail regarding what these structures are.

*Transverse Section:*

*Tangential Section:*

*Radial Section:*
Relate your observations of the anatomy back to the wood section D1.

**E. Vascular Cambium**
Vascular Cambium (VC) = lateral meristem producing secondary xylem and secondary phloem.

The Origin of VC in stems.
VC arises from the procambial (undifferentiated) portion of 1° vascular bundles AND from parenchyma cells between the bundles. Region of VC within bundles is the FASCICULAR CAMBIUM and region between is INTERFASCICULAR CAMBIUM. Once united, we simply call it the VASCULAR CAMBIUM.

![Diagram of stem growth](image)

1. Observe the prepared *Medicago* slide. This slide has three stages of stem growth in transverse sections. Alternatively a *Medicago* slide depicting younger & older stems in cross section may be available.

   Carefully observe the sections and DETERMINE which section represents the youngest stem. If you are not certain of which stem is the youngest and which is the oldest, ask your instructor to clarify. Often the youngest stem is the smallest but that is not always a guarantee.

   **Draw the youngest stem:**

   **Draw the middle aged stem:**

   **Draw the oldest stem:**

   For each, indicate the presence of the interfascicular cambium and fascicular cambium where appropriate.
Try to identify the first PERICLINAL divisions in the interfascicular cambium. What changes in the vascular cambium can you detect?

What changes are apparent in the vascular bundles over time?

Can you find a section where the vascular bundles have been united by the formation of the VC?

At which age did the vascular tissue close?

**F. Cells of secondary xylem**

Secondary xylem consists of the same type of cells as in primary xylem.

- **TRACHEIDS**—The primary conducting elements in gymnosperms (e.g., pine). Cell with pitted walls and dead at maturity. They also function in support.

- **VESSEL ELEMENTS**—The primary conducting elements of angiosperms. Broad empty, tube-like cells connected end-to-end to form VESSELS.

- **Xylem FIBERS**—Very long cells with very thick walls. Vessels function in transport and fibers provide support; both are dead and empty cells at maturity.

- **Xylem PARENCHYMA**—relatively unspecialized, thin-walled cells rather more abundant in the ground tissue.

Various forms and views of vessel elements. (A) a light micrograph of a vessel element from a wood maceration. (B) two adjacent vessels in cross-section, surrounded by tracheids. (C) SEM micrograph of a pitted vessel element, note the open perforation plate. (D) SEM micrograph of a perforation plate at the top of a pitted vessel element.
1. **Angiosperm Wood.**

Observe the prepared Macerated Oak (*Quercus*) secondary xylem slide. This slide depicts wood that has been digested so that the individual cells separate from one another. Then the suspension of cells is mounted and the result is known as a maceration in which we can determine the types of cells present. Keep in mind that this sample is secondary xylem only so only those cells described above for xylem will apply to your observations.

Initially the cells will not be readily distinguishable and you will need to make careful observations in order to distinguish their characteristics. Use your microscopy skills to bring the cells in and out of focus in order to see detail at the upper and lower surfaces. In addition, systematically move around on the slide in order to maximize your ability to find new cells.

*All four cell types will be present!*

*Label and Draw each type of xylem cell.*

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*Xylem vessel elements will be distinguishable by their large size and the presence of perforation plates at the ends. Label the perforation plates.*

*Do the vessel elements have pores on the sides?*

*How can you distinguish the fibers from the tracheids?*
Do the tracheids have perforation plates at their ends?

2. Gymnosperm Wood.
Observe the prepared Pine secondary xylem maceration slide. This slide has been prepared in the same manner as the Oak slide observed previously. It too only has xylem cells present.

Label and draw the types of cells found:

How many did you find of the four possible types?

Were any of the angiosperm cell types missing?

What are the structures on the sides of some of the cells in this maceration?

Remember, a Fiber does not have these structures on it.

How would a cross-section of pine xylem be distinguishable from an angiosperm cross-section?

3. Test your observations with the prepared “Wood Cells” maceration. This maceration is from either a conifer (Pine) or an Angiosperm tree secondary xylem.

Is this Conifer or Angiosperm tissue?

How do you know?
G. Growth Rings

Growth rings are really just rings of xylem from successive years. Below are two woody stem cross-sections: the one on the left is from a 2 yr-old stem, the right from 3 yr-old.

In this section you will become familiar with the anatomy of the rings and the appearance of the cells in an angiosperm. Keep in mind what type of tissue you are looking at in these slides.

1. Observe the prepared slide of a *Quercus* (oak) wood cross-section. If the slide available contains the three types of sections, choose the cross-section for observations.

In the previous exercise you looked at individual cells using wood macerations. Here the cells are in place as they naturally occur. All of the cells types are present here as well.

*Draw a portion of an oak growth ring.*

*Include one entire ring with partial rings at either side. (In essence you will have one ring and two half rings).*

*On your drawing indicate the following, if present.*

- Vessels
- Tracheids
- Fibers
- Parenchyma
- The ring boundaries
- Secondary xylem
The Parenchyma may not be obvious at first but it is there! At higher magnifications, you will notice that spaced somewhat sporadically inbetween all of the vessels, tracheids, and fibers there are regions that seem to be oriented along a different axis. These regions may resemble strings or slivers when you consider the big picture. Some are so large they look like highways moving through the wood tissue. These are RAYS. Rays are the conduction pathways from the outside to the inside of the secondary tissue.

*Look closely at the rays (If not obvious ask the instructor)*

*Are they one or multiple cells wide?*

*These cells are the parenchyma that we saw in the macerations.*

*Indicate a ray on your drawing.*

**KEEP THIS IN MIND FOR SECTION “H” WHEN WE CONTINUE OUR ANALYSIS OF THE WOOD TISSUE.**

2. **RING POROUS vs. DIFFUSE POROUS angiosperm woods.**

The Oak wood you observed is known to be “ring porous”, referring to large vessels formed in the spring (EARLY WOOD) and the not-so-wide-diameter vessels formed in the summer and fall (LATE WOOD).

*On your drawing above, indicate the region of one growth ring that represents the EARLY or spring wood and the region of growth that represents the LATE or summer wood.*

Other woods may be “diffuse porous”, referring to the more even or “diffuse” distribution of large-diameter vessels throughout the growing season. Nevertheless, definite growth rings are discernible in both types of wood.

3. **NONPOROUS wood in gymnosperms, exemplified by the transverse section from *Pinus strobus* (white pine).** This is an example of a conifer wood. As seen previously in the maceration, this secondary xylem does not have a wide variety of vascular cell types. In addition this type of wood is easily distinguished from Angiosperm wood due to the lack of large vessels and the apparent NONPOROUS (no vessels) organization of the rings.

*Draw one growth ring and a portion of the touching rings on either side.*

*Label the following:*

- *Tracheids*
- *Early Wood*
- *Late Wood*
- *Resin Canals (the resin canals are not actually cells but spaces surrounded by cells. The parenchyma cells that line these canals secrete the resin).*
Considering both the conifer and angiosperm wood transverse sections, explain why we see rings in woody stems.

4. Determine if growth rings are present in roots also. Observe the prepared *Quercus* (oak) secondary root cross-section slide.

Are growth rings present?

5. Continue your observations of the differences between DIFFUSE POROUS, RING POROUS, and NONPOREUS types of wood. Angiosperms have both diffuse and ring porous since they alone have vessel elements! Conifers have nonporous wood.

Locate the DISSECTING MICROSCOPE stations already set up. Stations 1-6 have examples of the different types of wood growth rings. Study these closely. Use your observations to determine what type of growth rings are present and indicate if you are looking at an Angiosperm or a conifer.

<table>
<thead>
<tr>
<th>Ring Type</th>
<th>Angiosperm or Conifer</th>
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<tbody>
<tr>
<td>Station A</td>
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<tr>
<td>Station B</td>
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<tr>
<td>Station C</td>
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**H. Horizontal Transport through Secondary Tissues: Rays & Ray Initials**

The vascular cambium (VC) is made up of "fusiform initials" and "ray initials". These initials in turn give rise, respectively, to the axial-oriented secondary tissue and the radial or horizontally oriented conduction structures called RAYS.

1. **Fusiform initials** = meristematic cells of the VC, arranged longitudinally, and form the axial (vertical-conducting) system of 2° vascular tissues.

2. **Ray initials** = horizontally oriented, meristematic cells that form the "vascular rays" or "radial system". This system allows transport of food from 2° phloem to 2° xylem, and transport of water and ions from 2° xylem to 2° phloem. Very important, indeed.
1. Go back to either the *Celtis occidentalis* OR the *Abies grandis* slide with the three different sections present. Observe the Tangential section and sketch an example of a ray below.

*What is the function of rays?*

*How many cells thick does a typical ray appear?*

*You should be able to see multiple sizes of rays.*

*Be able to relate the position of the rays in the tangential section to the rays that are present in the radial and the transverse sections. Rays are visible in all sections.*

*Sketch what the rays look like in your radial section*
I. Bark Formation: the Cork Cambium

The CORK CAMBIUM (abbreviated here as CC) is a lateral meristem producing PHELLODERM (living parenchyma cells) to the inside, and PHELLEM (“cork”, dead and suberized at maturity) to the outside. Appears in both secondary growth roots and stems.

Together, these three (cork cambium, phelloderm, and phellem) comprise the PERIDERM, which functionally replaces the epidermis in secondary growth stems and roots.

1. Stems: The CC usually appears during first year of growth, but after VC. Most commonly originating in a layer of cortical cells just below the epidermis (although sometimes in epidermis!). Repeated divisions of the CC result in the formation of radial rows of cells (most of which are cork cells).

**PREPARED SLIDE:** *Pelargonium* (geranium) stem x-section (cork).

*Notice the vascular cambium in these sections.*

*Look for the newly formed cork cambium.*

*Where is it with regard to the vascular cambium?*

2. Observe the pieces of BARK on your lab bench. Technically these fragments do not constitute the entire bark since we cannot be for certain that secondary phloem is also present. However, they do provide us with an excellent opportunity to see the multiple PERIDERMS that are present. Just like with growth rings in the secondary xylem, we can see alternating layers of periderm in the bark.

*How many periderms can you count.*

*Are the outermost periderms intact?*

*What is the purpose of the periderm?
J. Bark Breathing: Lenticels
The suberized cork cells of periderm are fairly impermeable to gases and water (remember the cork in wine bottles). The 2° plant body still must breath. **Lenticels** are portions of the periderm with numerous intercellular spaces, allowing for gas exchange.

1. **PREPARED SLIDE:** *Sambucus* lenticel (cross-section).

A single lenticel has been sectioned (as in the above Figure).

First, a bit of orientation: find the **vascular cambium** and the **outer bit of secondary xylem** and the **secondary phloem** (which is externally bound by the fiber (red-staining) caps of the primary phloem).

Second, find the young cork cambium: the cells it leaves behind are the short-lived and inconsequential phelloderm. The derivative cells yielded in advance of it (to the outside) become phellem (cork).

*Relate your understanding of the Periderm to the cell layers present in the section.*