INS 2007-08 Lab V
An Introduction to Plant Vascular Systems
and Reproductive Systems

Based on: Thompson, L. K. 2000. Introduction to plant vascular systems. Pages 180-194, *in* Tested studies for laboratory teaching, Volume 21 (S. J. Karcher, Editor); and Thompson, L. K. 2000. Plant reproductive systems: An investigative approach. Pages 198-217, *in* Tested studies for laboratory teaching, Volume 22 (S. J. Karcher, Editor).

Introduction

Two of the most important developments shown by plants which make them suited to life on dry land are: (1) reproductive systems which do not depend upon standing water for gamete or population dispersal, and (2) vascular systems which distribute water, nutrients and sugars throughout the plant from their disparate sources (leaves, roots, atmosphere, soil, etc.). In today's lab you will investigate various aspects of plant vascular systems, flower and pollen structures in angiosperms, as well as adaptations that aided the increasing use of wind and animals for gamete and population dispersal.

I. MICROSCOPIC ANATOMY OF THE XYLEM SYSTEM IN DICOT STEMS

Background: Xylem System

The xylem system of plants is that portion of the vascular system that transports water and nutrients from the roots to the leaves. The function of this system is dependent primarily upon the "negative pressure" generated in the xylem tube system by the evaporation of water from the upper parts of the system, including the leaves. Such water loss "pulls" water upward in the xylem system by the cohesive forces between water molecules within the xylem tubes. The tubes of the xylem system need structural support to keep from collapsing and this support is provided by lignin.

Lignin is, after cellulose, the most abundant organic substance in most plants. It is a highly branched polymer of phenylpropane units that can be covalently bound to cellulose. The subunits of lignin are joined together by the action of the enzyme peroxidase, forming a physically rigid, largely indigestible polymer matrix that branches in three dimensions. Lignin is found in the cell walls of various types of supporting and conducting plant tissue. Lignin is stained by Safranin O, while cellulose is stained by Methyl Green (two stains you will be using on the dicot stem).

You will determine which anatomical regions of a dicot stem in cross-section contain cell walls composed of lignin and which regions contain the tubes of the xylem system.

Experimental Protocol:

You will produce hand-made sections (thin slices) through a dicot stem, the xylem system of which you have stained with a fluorescent dye, Acridine Orange (**caution: it is a mutagen**). You will observe one of the sections under ultraviolet light to observe and document the regions of the stem stained with Acridine Orange. You will stain another section with Safranin O and Methyl Green to observe and document the regions of the stem which have walls containing lignin, as opposed to only cellulose. You will then compare the distribution of the xylem system and the distribution of lignified cell walls.

Read the entire procedure (steps 1-16) carefully before you begin. Divide the steps among your group. Be prepared for steps which are timed and must be done immediately following one another. With label tape, mark five Pasteur pipets: "ethanol solutions," "Safranin O," "Methyl Green," "water," and "glycerol-water."

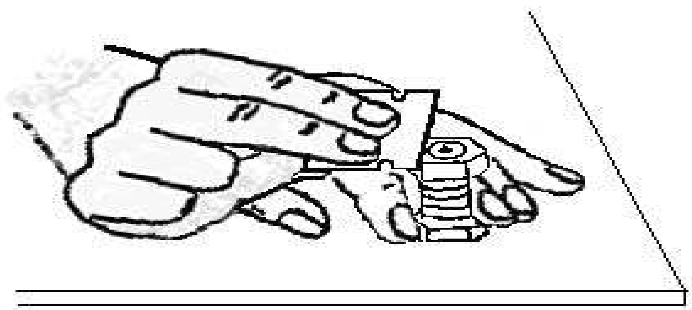
Staining the stem's xylem system with the fluorescent dye:

1. Obtain a fresh 1-cm length of a dicot stem from the instructor. Be careful to note and remember which end is the base of the stem. Carefully cut the base of the stem at a 45^{o} angle with a razor blade. Place the stem in a tube containing a small amount (only about 2-3 mm depth) of 3% Acridine Orange. Let the stem sit in the tube for ten minutes. During this time, the dye-containing solution will be transported up the xylem system of the stem.

Preparing sections of the stem:

2. Loosen the nut from the "nut and bolt microtome" so it is barely secured to the end of the bolt. This makes the "well" formed by the interior of the nut as deep as possible. Stand the bolt up so that its head is on the table. Remove the stem from the Acridine Orange. Add a small amount of melted wax to the bottom of the "well" inside the nut and immediately stand the stem piece up in the melted wax. When the stem can stand up on its own, add more melted wax to fill the well completely and to form a mound of melted wax around the base of the protruding stem. Let the wax harden completely (about ten minutes).

3. Hold the head of the bolt flat on the table with one hand. Hold the razor blade with the other hand (Figure 9.1). With the razor blade, carefully shave off the mound of wax and stem from above the top of the nut. Run the blade over the nut's surface to make the wax completely smooth.
4. Turn the bolt clockwise about 1/12th of a turn (see Figure 9.2) to make the wax cylinder protrude slightly. Again holding the microtome and blade as shown in Figure 9.1, slice the blade over the nut's surface to make a shaving of the wax surface through the stem. A smooth, quick slicing motion works best.
5. With the needle probe, gently remove the stem section from the wax shaving. If the section is thin and not torn, give it to your lab partner who will immediately proceed with the fluorescent viewing of the stem section (steps 6-8). (An otherwise good section that is too thick for staining is quite suitable for fluorescent viewing.) Cut the wax block smooth with the nut's surface again and return to step 3 to cut a section you will stain for lignin and cellulose (steps 9-15). This section needs to be as thin as possible. If you leave some stem remaining in the wax block of the microtome for a while, place a drop of water on it to keep it moist in case you want to cut more sections later. If you make so many unsuccessful shavings that the wax block becomes too loose in the ever-shallowing well of the nut, you will need to remove the wax from the well and start again with another piece of stem.



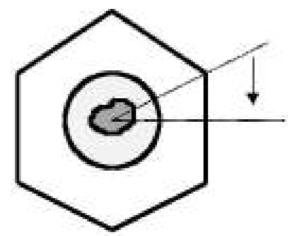


Figure 9.2. Demonstration of a 1/12th turn on the Nut-and-Bolt Microtome

Figure 9.1

Observing the fluorescently stained xylem system:
6. With the needle probe, carefully transfer the stem section to a clean microscope slide. Be careful not to let the section fall off the slide when your transport it.
7. Observe the slide under long-wave ultraviolet illumination with the high power of the dissection microscope. The Acridine Orange will fluoresce a yellow or orange color. Be sure to "go through focus" to focus clearly on the cell walls exposed on the cut surface of the section. Any pale green fluorescence is the natural fluorescence of the plant tissues and should be disregarded.
8. Which area(s) of the stem cross section have fluorescent cell walls? Label these areas on the drawings of stem cross sections. Remember that not all of the xylem tubes remained opened at the 45^{o} cut at the stem's base, so not all of the xylem tubes will have transported the Acridine Orange solution and thus have walls which are stained. Save the slide so your lab partner can study it.

Staining a section to observe cellulose and lignified cell walls:
9. Gently transfer a section into the "microbeaker" with the needle probe and immediately cover the section with Safranin solution using a Pasteur pipette. Let the section sit in this solution for 3 minutes.
10. Remove the Safranin solution by "blotting" it out of the microbeaker with the twisted corner of a Kimwipe. Immediately add 70% ethanol. Let the section sit in 70% ethanol for 2 minutes, replacing the ethanol solution in the microbeaker with fresh 70% ethanol 2-3 times during this 2 minutes. This step removes excess Safranin from the section.
11. Remove the 70% ethanol by blotting and immediately add the Methyl Green solution. Let the section sit in this solution for 1 minute.
12. Remove the Methyl Green solution by blotting. Immediately add 95% ethanol. Let the section sit in 95% ethanol for 2 minutes, while replacing the ethanol solution with fresh 95% ethanol 2-3 times during the 2 minutes. This step removes the excess of both stains. During this procedure, have the microbeaker on a white sheet of paper to observe the appearance of the section. At the end of the 2 minutes, the section should be translucent and blue or purple. A dark section is one which has been cut too thick, and a translucent but red one has not yet been rinsed long enough in the 95% ethanol. However, do not keep the section in 95% ethanol for too long or too much of the stains will be removed from the section.
13. Remove the 95% ethanol with blotting. Gently transfer the section to a clean microscope slide. Blot off any excess ethanol solution. Immediately add a drop of 1:1 glycerol-water. Gently cover with a cover slip, but do not apply pressure on the cover slip since the section is easily crushed. Add additional glycerol-water to the edges of the coverslip, if needed.

14. Look at the slide under low, medium, and high powers of your microscope. Cell walls composed mostly of cellulose will appear thin and blue or blue-green (stained with Methyl Green), while those containing lignin will appear thicker and red or orange-red (stained with Safranin). Be sure to "go through focus" to visualize the cell walls best.
15. Sketch the images you see. Which area(s) of the stem cross section have cell walls containing lignin? Do the areas of fluorescent staining and lignin staining of the stem cross section exactly match? Is one a subset of the other? What might be the purpose of the lignified cell walls in areas of the stem that are clearly not associated with the xylem system? Save the slide so your lab partner can study it.

II. COMPARISON OF FLOWER STRUCTURE AND POLLEN IN INSECT VS WIND-POLLINATED PLANTS

Background

This part of the laboratory deals with the reproductive structures of the angiosperms (flowering plants). There are two main objectives in this exercise. First, you should learn to recognize the main structures in flowers by handling and dissecting flowers from a variety of different plants. Second, you will observe how floral structure differs in relation to the two most important agents of pollination, wind and insects.

The flowering plants represent by far the most abundant, diverse, and successful phylum of plants in existence today, with over 235,000 species known. The characteristic feature of this group is the flower, a set of reproductive structures typically comprising sepals, petals, stamens, and carpels. Sepals are typically green structures that enclose the developing flower bud. Petals lie within the sepals. They are also enveloping structures, but are often large and colorful. Stamens are the male reproductive organs and produce pollen grains. Carpels are the female reproductive organs, and enclose ovules. After fertilization by a pollen grain, an ovule within its enclosing carpel will develop into a seed within an enclosing fruit. A flower does not necessarily contain all four types of structures; for example, it is quite possible that petals can be missing.

In this laboratory we will concentrate on gross floral anatomy that can be seen with the naked eye or a dissecting microscope. As any professional or amateur botanist can tell you, identification of plants in the field or garden often requires being able to interpret the structures in a flower.

Experimental Protocol: Observation of floral structures

You are provided with flowers of *Chrysanthemum,Chaenomeles,*and *Poa annua* (annual bluegrass). The first two of these are insect-pollinated plants, while the grass is wind-pollinated. For each plant, there is a 1-page "Dissection Guide" (below). You should examine and dissect each specimen, using the dissecting microscope where appropriate. Try to identify and understand all the structures indicated on the dissection guide. As you make these observations, try to form some generalizations about the typical structure of wind-pollinated versus insect-pollinated plants.

Measurement of pollen diameters

Obtain one of the prepared slides of mixed pollen grains, and the key card that goes with it (which should allow you to recognize the pollen types based on shape and color). Your goal is to measure the diameter of a typical pollen grain from each species. Use 400X magnification on the compound microscope, and determine the conversion factor from ocular units to microns. Each person at your lab table should measure the diameter of 3 or 4 species; the group as a whole should attempt to complete the listing in the table below. If you cannot find one or two of the species, you may skip them.

In addition to the prepared slides, you should also measure the diameter of *Poa* pollen. To do so, place a drop of water on a clean slide. Remove a stamen or two from a flower, and crush the anther in the water drop using a dissecting needle. Cover the drop with a cover slip and observe the slide on the microscope. Finally, calculate the average diameter for each pollination mechanism.

Pollen Diameters in Microns

Pollen Diameters in Microns				
Insect-pollinated				**Wind-pollinated**
Chrysanthemum		*Nicotiana*		*Ambrosia*
Dahlia		*Rosa*		*Atriplex*
Hibiscus		*Saintpaulia*		*Fraxinus*
Lilium (either kind)		*Salix*		*Poa**
Liriodendron		*Scrophularia*		

*from fresh specimens, not prepared slide

Examination of "Unknowns"

You will be given specimens of *Lonicera* and *Alnus*. A dissection guide accompanies each specimen, but you will not be told the mechanism of pollination. Examine these specimens, noting dimensions of floral structures as you did above. Measure the pollen grain diameter. Attempt to make conclusions about pollination mechanisms, and answer the following questions:

1. Based on your dissections of *Chrysanthemum,Chaenomeles,*and *Poa*, what generalizations can you make about the overall structure of the flower (especially the petals) in insect-pollinated versus wind-pollinated plants? What hypothesis can you propose that would explain why this pattern exists?

2. Based on your observations of pollen from both the fresh specimens and the prepared slide, what generalizations can you make about the pollen of insect-pollinated versus wind-pollinated plants? What hypothesis can you propose that would explain why this pattern exists?

3. You were not told what pollination mechanism is used by *Lonicera* and *Alnus.* State what mechanism you think is characteristic of each species, and justify your answers.

Appendix A: Flower Diagrams for dissections.

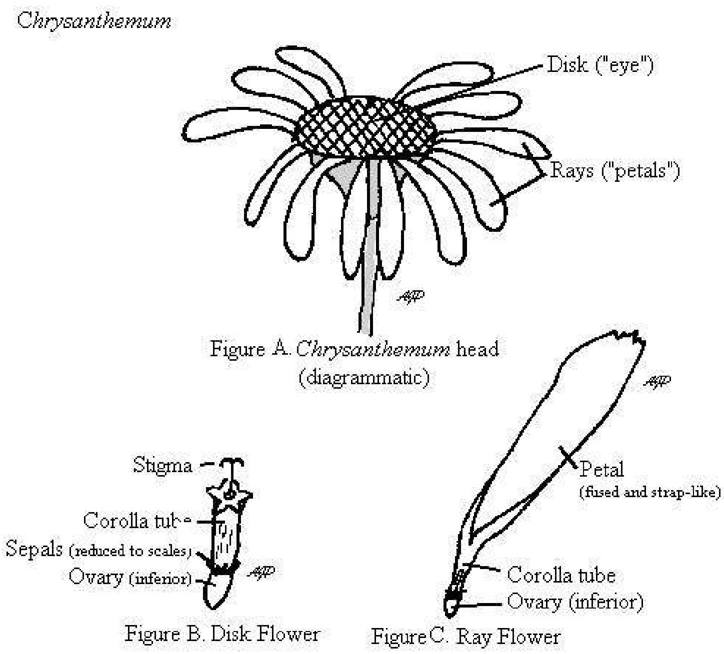
Fall, Winter, Spring Season: *Chrysanthemum* (insect-pollinated)

Chrysanthemum is a member of the sunflower family, Asteraceae. With over 20,000 species, the sunflower family is one of the largest and most successful families of flowering plants in the world. Characteristically, the flowers themselves are very small, but they are grouped together into a compound blossom called a head, that functions in many ways as if it were a single flower. But it is important to remember that anatomically a head is not one flower, but a grouping of dozens or hundreds of flowers.

Look at the overall structure of a *Chrysanthemum* head (Figure 11.6A); then use your fingers to pull it or a razor blade to cut it into quarters. Ignore for a moment the outer row of "petals", and concentrate on the central "eye" of the head, which is technically called the disk. The disk is made up of many small flowers. Pull out a single, open, disk flower (Figure 11.6B). The disk flower has 5 petals, partially fused into a corolla tube. It has an inferior ovary. The sepals are modified into small scales in other species of this family, the sepals may be silky hairs, like the "parachutes" of a dandelion. Looking at the mouth of the petals, you should be able to see the stigma, where pollen grains would land and begin to grow down the style toward the ovary. Inside the corolla tube, you would find the stamens; however, you need not look for them, given the small size of these flowers.

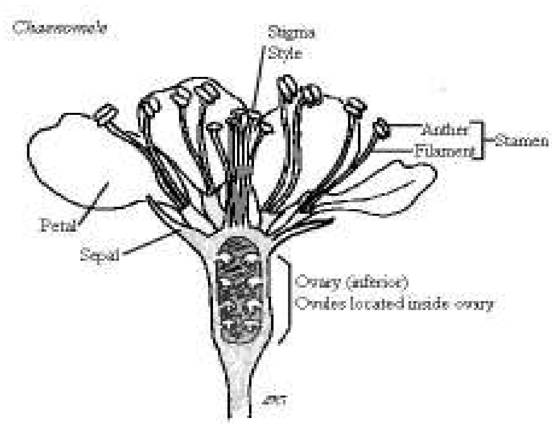
Now turn your attention to the outer "petals" of the blossom that we ignored before. Examine one of these. You will find that at its base there is an inferior ovary, identical to the one you saw in the disk flower. What you hold in your hand is not merely one petal, but an entire flower, called a ray flower (Figure 11.6C). It differs from the disk flower only in that its petals are fused into a long strap, instead of a symmetrical trumpet-shaped corolla tube.

Figure 11.6. Dissection guide for *Chrysanthemum*, a fall, winter, and spring season, insect-pollinated plant.



Winter Season: Chaenomeles (insect-pollinated)

Chaenomeles (flowering quince) has flowers very much like the "typical" flower seen in diagrams in many textbooks. It differs in only a few details. Instead of a single style and stigma, the style branches into five parts, each of which is topped by a stigma. Like the picture in the book, these flowers have what is called an inferior ovary. The ovary of a flower is the portion that contains the developing seeds; later, the ovary will ripen into the fruit of the plant. An inferior ovary, as in *Chaenomeles,* is located below the point where the petals and sepals are attached. A superior ovary would be located within the flower, surrounded by the petals. For purposes of simplicity, Figure 11.5 shows 8 ovules (unfertilized seeds) inside the ovary of the carpel. When you dissect *Chaenomeles,* you will see many ovules in the ovary.



Take one flower from the plant. Examine it first, then use a razor blade or scalpel to cut it in half down the vertical axis of the flower, as seen in the drawing below. Look for the parts that are indicated.

Winter and Spring Season: *Poa annua* (wind-pollinated)

Poa annua (annual bluegrass) is a member of the grass family, Poaceae. This very large family (about 12,000 species) is perhaps the most important in the world, because many important human foods, including wheat, rice, and corn, are made from the seeds of grasses.

All members of the grass family are wind-pollinated. Since they do not attract pollinating animals, their flowers are small and inconspicuous. Perhaps you did not even realize that grasses are "flowering plants" but they do indeed have small, reduced flowers. There are no petals. Sepals are reduced to vestigial structures. The main parts of the flower are the sexual structures: the stamens and carpel. On the other hand, there are often small leaf-like structures called bracts, which sandwich the flower. The bracts of grasses have specialized names such as glume, lemma, and palea; however, we will not worry about the exact definition of these terms in this lab. A cluster of one or more flowers with their enclosing bracts makes up a unit called a spikelet. To see the tiny flowers of a grass, you will need to use a stereoscopic microscope, and tools such as dissecting needles and forceps to peel away the surrounding bracts.

Examine the branched bluegrass inflorescence (Figure 11.9A). Select a spikelet with whitish stamens or stigmas protruding. Pluck off the spikelet and examine it using the dissecting microscope. The pollen-producing anthers hang out of the spikelet on slender thread-like filaments (anther + filament = stamen). The pollen receiving stigmas are brush-like, to trap pollen from the air. In this species, there are usually 3 or 4 flowers per spikelet.
Using your dissecting tools, peel back the surrounding bracts, to reveal a flower within (Figures 11.9B - 11.9D). It requires patience and manual dexterity, and you may end up breaking the flower and needing to reconstruct it. To measure pollen diameter, place some anthers in a drop of water on a slide, and crush the anthers with your needle. Then cover with a cover slip and look for pollen using the compound microscope.

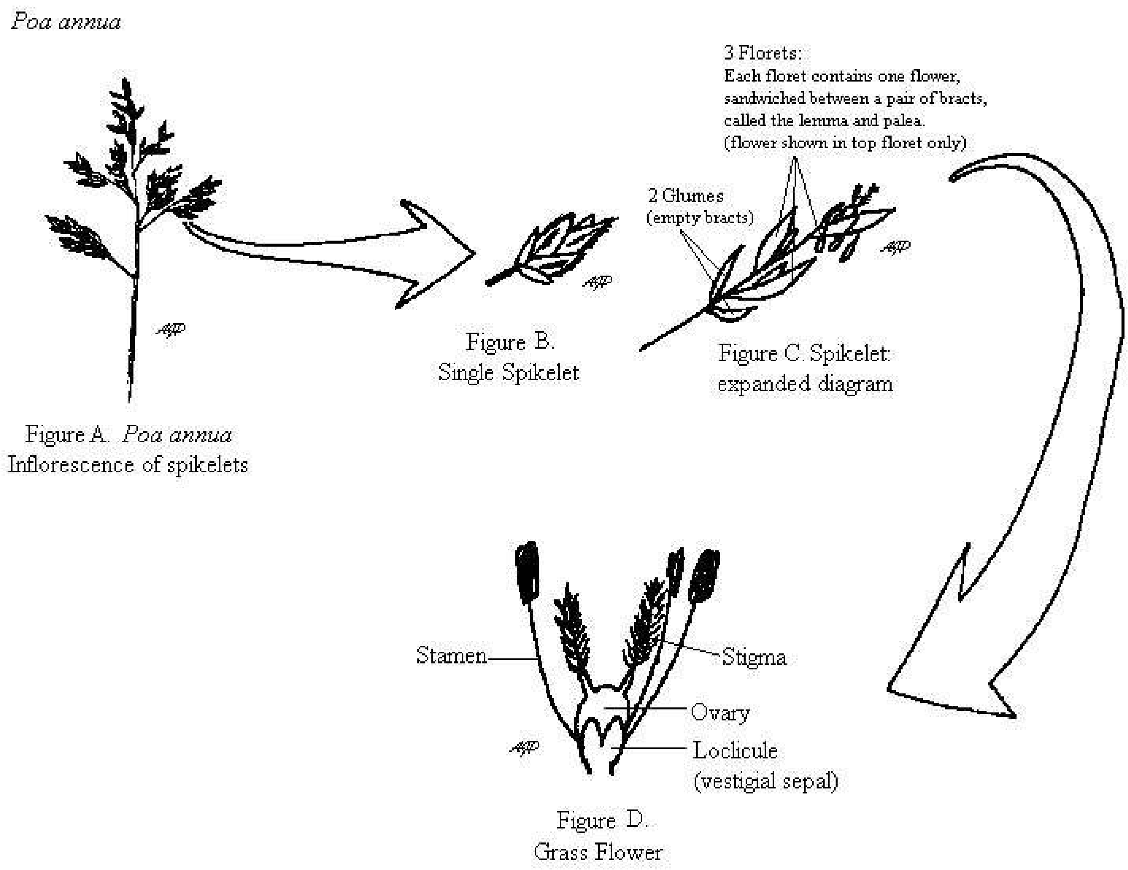
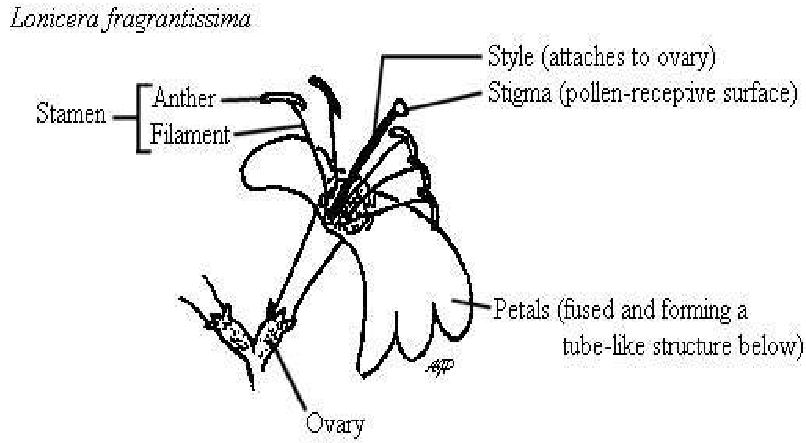


Figure 11.9. Dissection guide for *Poa annua* (annual bluegrass), a winter and spring season, wind-pollinated plant.

Winter and Spring Season: *Lonicera fragrantissima*

Lonicera fragrantissima (bush honeysuckle) flowers differ from the "typical" flower diagrammed in your book in only a few details. The petals are partially fused into a funnel-shaped structure called a corolla tube. The mouth of the funnel does not have perfect radial symmetry (star shape) but is bilaterally symmetrical. Also, these flowers have an inferior ovary, which you saw previously in *Chaenomeles.*

Find the indicated structures on your *Lonicera* flower, cutting or tearing into the corolla tube as necessary. You will probably see nectar, a sweet liquid, inside the corolla tube. You also may notice the pleasant fragrance of these flowers (reflected in their Latin name). Measure the pollen diameter by dusting an anther in a drop of water on a microscope slide, then covering the drop with a cover slip.



Winter Season: *Alnus serrulata*

Alnus serrulata (alder) is a small tree found along streams in our region. The flowers differ in many respects from "common" flowers. Each flower is either all male or all female. The male flowers are found in dangling inflorescences called catkins. The female flowers are in upright catkins nearby. Because both male and female flowers occur on the same plant, this species is described as monoecious. If there were separate male trees and female trees, as in both willow and persimmon, the species would be described as dioecious.

The diagram (Figure 11.4) below shows you how to see the flowers. Use a dissecting microscope. In both male and female catkins, the flowers are partially hidden behind small scales called bracts. The male flowers are fairly small; each flower consists of simply four sepals and four stamens. The female flowers are even smaller. Each female flower is basically a carpel, consisting of an ovary and two styles; both sepals and petals are absent in the female flowers.

Collecting pollen is easy, as the tabletop around the plants is probably covered with it. Place some pollen in a drop of water on a slide, apply a cover slip, and measure the diameter at 400X. You are not told the pollination mechanism. Decide the mechanism based on the characters

To measure pollen diameter, dust a few anthers in a drop of water on a slide. Apply a cover slip and examine the slide under the compound microscope at 400X total magnification.