LABORATORY MANUAL

for

Applied Botany

Estelle Levetin **Karen McMahon**

Robert Reinsvold

Contents

Preface

Since the publication of the first edition of our textbook *Plants and Society* in 1996, many instructors have inquired about the availability of an accompanying laboratory manual that emphasizes the practical aspects of plant science. This *Laboratory Manual for Applied Botany* is the outcome of that interest.

Science education is experiencing a revitalization, as it is recognized that science should be accessible to everyone, not just society's future scientists. One way to make the study of science more substantive to the nonmajor is to require a laboratory component for all science courses. We believe the subject of applied botany, with its emphasis on the practical aspects of plant science, will appeal to the nonmajor as it exemplifies how a basic science can be applied to problem solving. We hope that the *Laboratory Manual for Applied Botany* will make students realize that the study of plants is relevant to their lives and that they can participate in the discovery process of science.

FEATURES

Although the manual includes much of the basic anatomy found in standard botany manuals, it differs by taking a practical approach, examining those plants and plant products that have sustained or affected human society. For example, in Laboratory Topic 1: Cells of Crystal and Color, students are introduced to the components of a plant cell, but they also produce protoplasts for bioengineering, extract plant dyes, and learn how plant crystals are used in forensic science and archaeology. In Laboratory Topic 3: Plant Tissues—The Fabrics of Our Lives, the tissues that make up the plant body are reviewed, but in addition, students see firsthand how plant fibers are utilized to make cloth and paper. The details of wood anatomy are covered in Laboratory Topic 15: The Beauty of Wood, but this topic also differs from the standard treatment in that students examine tree cores for dendrochronological and dendroclimatological data. Other exercises in practical botany include using plant anatomy to study produce (Laboratory Topic 4); monitoring the effect of airborne pollen upon health (Laboratory Topic 6); and the construction of a personal food web (Laboratory Topic 10).

Several laboratory topics are devoted exclusively to economically important crops—11: Leaves of Grass, 12: The Lowdown on Legumes, 13: Food from Underground and Far Away and 14: The Spice of Life. The exercises in these topics introduce students to the important characteristics of the cereals, legumes, starchy staples, and spices. Additionally, students discover through hands-on experimentation the role of gluten in leavened bread, how plant oil can be made into soap, the value of starch grains in identification, and the antibiotic activity of garlic.

Several of the topics are open-ended investigations, such as Laboratory Topic 17: Bioactive Drugs in Action, which uses an animal model to test the effectiveness of several plant-derived drugs, and Laboratory Topic 16: Bioprospecting for Medicinal Plants, which allows students to screen plant extracts for biological activity. In several topics, such as 7: Fleshy Fruits and Flying Seeds, students are encouraged to practice science by designing experiments.

Although the bulk of the exercises deal with the flowering plants, the wood of conifers is discussed in Laboratory Topic 15: The Beauty of Wood, and the diversity and economic impact of the algae and fungi, organisms traditionally viewed as plants, are presented in Laboratory Topic 9: Algae—From Diversity to Dessert and 18: The Fungus Among Us.

DESIGN

Laboratory Manual for Applied Botany has a flexible design. Each of the 18 topics is divided into multiple exercises from which the instructor can pick and choose. The organization of topics follows that of *Plants and Society*, 2d ed.; however, each laboratory topic is complete within itself and instructors may reorder them according to their courses. Most of the laboratory can be completed within a 2–3 hour laboratory period, although a few exercises require students to check back later for results. In Laboratory Topic 2, the tissue culture and cloning experiments, and Laboratory Topic 8, the exercises with Wisconsin Fast Plants are long-term experiments that allow students to practice science beyond the confines of a laboratory period.

Appendix A provides expanded information on the process of science and should prove especially useful in those exercises where students design experiments. Appendices B (Field Trip to a Health Food Store) and C (A Taster's Sampler of Caffeine Beverages and Foods) can be used as additional exercises and worksheets or as substitutes for other laboratory topics. Appendix D lists laboratory protocols, suppliers, suggestions, and hints that should be helpful to the instructors using this manual.

At the end of each laboratory topic, "Questions for Review and Discussion" and "Terms to Know" are useful study aids for the students. Tear-out worksheets are a convenient way for students to record observations and data, to be turned in for review by the instructor.

AUDIENCE

Although this laboratory manual is designed for a plants and society course taken to fulfill a general science requirement, it would also be appropriate in a one semester- or quarter-length introductory botany course in which the instructor chooses to take a nontraditional, applied approach. It may also be the laboratory accompaniment for an upper-level, majors' economic botany course. Since the manual is designed for introductory-level students, even those with a limited scientific background should not encounter any problems with the level of scientific detail in the laboratory topics.

ACKNOWLEDGMENTS

We appreciate the efforts of Marge Kemp (Executive Editor) and Kathy Loewenberg (Developmental Editor) for their strong support of and belief in this project. We also wish to acknowledge the thorough assistance of Mary E. Powers (Project Manager), Lori Hancock (Photo Research Coordinator), and Kennie Harris (copy editor) and the rest of the McGraw-Hill team for their part in the joint effort it takes to create a laboratory manual. Last, we must thank our colleagues, families, friends, and students, who have been a sounding board for ideas, participated in trial runs of the experiments, and in many other ways supported us in this endeavor.

Introduction

With this lab manual, as in most botany laboratories, you will spend the semester looking at a variety of different types of plants. However, this lab manual differs from others in that you will get first-hand experience with plants that are useful to humans as well as some that can be harmful. In so many ways, plants are part of our everyday lives. Many plants and plant products are necessities of life; they provide food, clothing, shelter, and medicines; other plants enhance life by providing beverages and spices; and still others produce compounds that are toxic or allergenic. These applied aspects of botany are the focus of this lab manual.

Laboratories in science courses are designed so that the student can actually experience science. Science facts can be learned from lectures and reading assignments, but science is more than a body of knowledge about the physical and biological world. It is a method of learning through observation and experimentation. You will be using the scientific method during this term, and you will learn techniques, make observations, perform experiments, and draw conclusions. The hands-on experiences will let you see how things work. In addition, you will use other senses in this lab because you will have opportunities to taste and smell some plant products that may be new to you.

The laboratory topics in this manual begin with background material and learning objectives. These should help you focus on the main theme for each week. Also, each laboratory topic consists of several different exercises; your instructor may choose to do one, two, or all of the exercises during a lab period. Individual exercises have introductory material that explains the purpose of the exercise, a list of materials needed, and step-by-step procedures, which should take you through the exercise. Be sure to read the laboratory topic thoroughly before you come to lab each week so that you can be fully prepared to start working. Each lab has review questions or asks you to draw certain structures. Answer the questions from your observations and experiments. In addition, many exercises have worksheets that you will turn in to your instructor. Again, the answers are based on the experiments and observations made in lab. Some experiments run several weeks, and you may need to make arrangements to come in during the week to water plants or observe their growth. Your instructor will provide you with information on access to the lab outside of class time.

What you get out of this lab will reflect your preparation and the effort you put in. Ask questions, compare your findings with those of others, and apply what you learn here to your daily life because plants are all around us.

LABORATORY TOPIC 1

Cells of Crystal and Color

BACKGROUND

In innumerable ways, plants are different from all other forms of life, and many of these differences can be seen by closely inspecting the interior of a plant cell. In this laboratory topic, you will learn about several cellular structures that are unique to plants: cell walls that impart the strength to protect and support; chloroplasts for photosynthesis; chromoplasts and vacuolar dyes that color fruits and flowers to attract animal helpers; and beautiful crystals that provide protection. You will also learn how knowledge of botany has put these cellular assets to practical use—for example, as cell walls become wood for building, plant pigments are extracted for natural fabric dyes, and crystals are used as identification tools in archaeology and forensic science.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Prepare protoplasts and understand how they can be used to advance improvements in agriculture and horticulture.
2. Identify the prominent and distinctive components of a plant cell.
3. Understand how plant crystals have been successfully employed in forensic science and archaeology.
4. Understand the chemistry of plant pigments and extract them for use as textile dyes.

EXERCISE A: Plant Cells Without Walls

One of the most distinctive features of a plant cell is the wall of cellulose encasing it. Although this cell wall provides protection and support to the plant, it is often an impediment when manipulating plant cells through genetic engineering. Removing the cell wall barrier results in a **protoplast,** the entire contents of a plant cell minus the cell wall. To produce protoplasts, isolated plant cells are treated with enzymes that separate cells and break down the cell walls. The introduction of genetically engineered DNA is more readily absorbed by protoplasts than by an intact plant cell with a cell wall. Additionally, protoplasts from desirable species can be fused to create novel hybrid cells. The genetically engineered protoplasts can then be induced through tissue culture techniques (see Laboratory Topic 2) to grow into hybrid plants. These hybrids might include crops with combined nutritional value or disease resistance. In horticulture, a desired flower color might result from protoplast fusion—for example, a truly yellow African violet.

Materials Needed for Exercise A

- Beakers, 50 ml
- Compound light microscope
- Dissecting microscope
- Enzyme powders of cellulase and pectinase
- Ethanol, 70%
- Forceps
- Graduated cylinder or pipet, 10 ml
- Mannitol, 13%
- Marker pen
- Microslide (optional)
- Parafilm
- Petals of geranium
- Petri dishes
- Scalpels

Procedure for Exercise A

1. Microbial contamination can be a problem with the following technique, so wipe the lab bench down with 70% ethanol and allow to air dry before beginning.
2. Wash your hands. Obtain 1–2 petals of geranium. Using a forceps that has been sterilized in 70% ethanol, gently rinse each petal in a petri dish containing 70% ethanol. Next transfer the petal to a petri dish containing 13% mannitol, and rinse.
3. Transfer a sterilized petal to the bottom lid of a sterile petri dish. Using a sterile scalpel, cut the petal into the thinnest strips possible. Cover with the top lid of the petri dish.
4. Measure 10 ml of 13% mannitol into a sterile petri dish. The high concentration of the mannitol solution draws water out of the cell, making the protoplast shrink away from the cell wall. Add the enzyme powders. The enzyme powders consist of a **cellulase** to break down the cell walls of cellulose and a

pectinase to dissolve away the intercellular glue that holds plant cells together. Gently swirl the petri dish to dissolve the powders.

5. Using forceps, transfer the petal strips to the petri dish containing the mannitol/enzyme solution. Seal the petri dish with parafilm. Gently shake the sealed petri dish to completely submerge the strips in the solution.
6. After an hour, you can check the progress of the reaction by viewing the petal cells with a dissecting or a compound light microscope. Do not open the lid of the petri dish. Be careful not to leave the petri dish on the microscope for too long, as the heat of the lamp will interfere with the protoplast release. As the cells lose water, the protoplast pulls back. Draw some representative cells in the following space:

7. Set the petri dish at room temperature, in dim light, overnight. Examine it after 24 hours; approximately 10–20% of the protoplasts will release after that period of time. You may pick up some released protoplasts with a microslide. Place the microslide near an isolated protoplast. The protoplast will flow into the microslide by capillary action. View under the compound microscope, first under scanning (4 × objective) or low (10 × objective) power and then under high (40 × objective) power. In the following space, draw and label visible structures in some representative protoplasts. The membrane that surrounds each protoplast is the **plasma membrane**, which is normally not visible as it adheres to the inner cell wall surface in an intact plant cell.

EXERCISE B: Components of the Plant Cell

In plant cells, structure is partnered to function. In this exercise, you will prepare your own slides from common fruits, vegetables, house and garden plants to see the beauty of form and function within the cells of plants.

Materials Needed for Exercise B

- Aloe plants (*Aloe vera*)
- Avocado
- Bell peppers in yellow, orange, or red varieties
- Carrot
- Compound light microscope
- Coverslips
- Dissecting needles
- Dropper bottle of distilled water
- Dropper bottle of Sudan III
- Glass slides
- Marigold petals
- Tomato

Procedure for Exercise B

1. Obtain a leaf of aloe, the burn plan. Note that the leaves are fleshy, or **succulent**. This is commonly seen in plants in arid environments as an adaptation for water storage. Break open a leaf. Note the gel in the middle of the leaf. This contains **aloin** and **chrysophanic acid**, ingredients that have been known for centuries to heal the skin. Many people keep an aloe plant in the kitchen window and apply the gel to minor burns and cuts. Aloe is the main ingredient in many over-the-counter first-aid creams and in Oil of Olay, a famous cosmetic skin conditioner.
2. Fold the leaf so that the translucent epidermis (it resembles clear plastic wrap) extends over the torn edge. With a forceps, remove the translucent epidermis to reveal a layer of green with splotches of white. This layer is above the gel of the leaf. Using a pair of forceps, take a small piece of this green layer and place it on a slide. Add a drop of distilled water, and cover with a coverslip.
3. Scan under scanning (4 × objective) or low (10 × objective) power for a section of only one or two layers thick. Make sure you focus on the top layer, not the in-between layers. Focus on a small group of cells. Bring to high power (40 × objective). Note that the plant cells have a regular shape. They look like rectangles (fig. 1.1). The outline of the cell is clear because

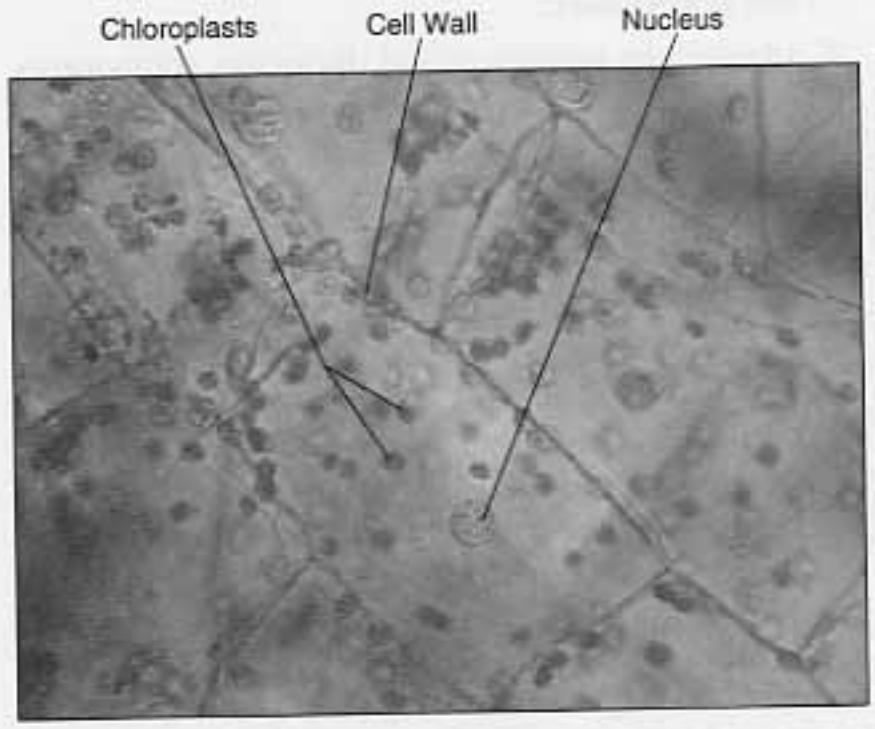


FIGURE 1.1 PLANT CELLS FROM THE MESOPHYLL OF AN ALOE LEAF.

the outermost boundary of a plant cell is the **cell wall**. The cell wall is rigid and gives support to the cell. In these cells, the **primary wall** is composed mainly of the polysaccharide **cellulose**. In some types of plant cells, additional layers are laid down inside the primary wall. This is the **secondary wall**, which is much thicker and contains **lignin** in addition to the cellulose. The strength and character of wood is due to secondary walls. Secondary walls and the cells in which they are found are discussed in both Laboratory Topic 3 and Laboratory Topic 15.

The cohesiveness of plant tissue is due to the **middle lamella**, an intercellular glue composed of pectins, that holds the cell walls of neighboring cells together. Pectinase was used in Exercise A to free individual cells from the middle lamella. The middle lamella is most pronounced when the corners of plant cells adjoin.

4. View the interior of one of these cells. Note the spherical bright green discs that appear to hug the interior side of the cell wall. These are **chloroplasts,** and the green pigment contained within them is **chlorophyll**. Chloroplasts are the sites of **photosynthesis,** in which the energy of the sun drives the joining of water and carbon dioxide to make glucose. (The process of photosynthesis is further discussed in Laboratory Topic 5.) Although not visible, a plasma membrane encases the protoplast. The **plasma membrane** is a lipid bilayer with embedded proteins. It is selectively permeable and regulates what passes into and out of the cell. You could clearly see the plasma membrane in Exercise A.
5. Scan several cells to spot a **nucleus**. The nucleus is a perfect sphere and clearly outlined by the **nuclear envelope**, a double membrane layer. It is larger than a chloroplast, and it may be adjacent to the wall or somewhere in the center of the cell. The interior is granular because of **chromatin**, the form **DNA (deoxyribonucleic acid)** takes in a nondividing cell. DNA is the genetic blueprint of life.

   You may also see one or two smaller circular regions within the nucleus. These are **nucleoli** (sing., **nucleolus**). The nucleolus is rich in **RNA (ribonucleic acid)**, and is involved with protein synthesis in the cell.
6. Although the interior of the cell looks empty, that is really not the case. The center is occupied by the large central **vacuole**. The **tonoplast** is the vacuolar membrane and will not be visible. The vacuole serves as a reservoir for materials, including water, that the cell can draw upon when needed. It may also be a place to sequester waste products. In fact, some products accumulate in such great quantities that they precipitate out as crystals. You may have seen single or bundles of "needles" as you scanned your slide of aloe. These are crystals and are discussed further in Exercise D. Some plant pigments are also stored in the vacuole, giving color to fruits and flowers. These pigments have been used for millennia to dye cloth, much as you will do in Exercise E.
7. Plant color is also imparted by another organelle, the **chromoplast**. Chromoplasts contain the carotenoid pigments of yellow, orange, and red. Chromoplasts are related to chloroplasts; in fact, both originate from a common precursor called a **proplastid** that gives rise to all of the plastids. Plastids include not only chloroplasts and chromoplasts, but **leucoplasts.** Leucoplasts are colorless and include **amyloplasts,** which accumulate starch grains, and **elaioplasts,** which accumulate lipids. Amyloplasts are further discussed in Laboratory Topic 13.

   To see elaioplasts, obtain a small piece of avocado fruit and smear to a thin layer on a slide. Add a drop of distilled water and a drop of Sudan III solution. Sudan III will stain the lipid containing elaioplasts. Focus first under scanning (4 × objective) or low (10 × objective) power and then bring on up to high power (40 × objective). What color do the elaioplasts stain in the presence of Sudan III? Draw a few elaioplasts in the space following:

Chromoplasts come in a variety of shapes and sizes. You will know them by their color and abundance in certain fruits, flowers, and in some cases, roots. To view a chromoplast, take a small piece of the skin of a yellow, orange, or red bell pepper. Place it on a slide, add a drop of distilled water, and cover with a coverslip. Focus first under scanning (4 × objective) or low (10 × objective) power and then bring up to high power (40 × objective). Draw some representative chromoplasts in the following space:

Look closely at the cell wall of the pepper skin. Can you spot the cytoplasmic bridges through the walls? These are the **plasmodesmata** (fig. 1.3) that interconnect plant cells.

Prepare slides as before, and look for chromoplasts in the skin of a tomato, the root of a carrot, or the petals of a marigold. Draw some representative samples in the following space:

EXERCISE C: Plant Ultrastructure

Many of the details of the contents of a plant cell only became discernible with the development of the **transmission electron microscope** (**TEM**) in the 1950s. In microscopy, two properties must be considered: magnification and resolution. **Magnification** tells how much larger the image is compared to the actual size of the specimen. **Resolution** is the ability to discern fine details—in other words, to distinguish two closely spaced objects as two separate points. Resolution is inversely proportional to the wavelength of radiation used in a microscope. Electron microscopes replace the light beam of the compound microscope with an electron beam. Since the wavelength of an electron is much smaller than that of visible light, the electron microscope has at least a thousandfold greater resolution than the light microscope.

The **scanning electron microscope** (**SEM**) scans an electron beam across the surface of specimens that have been coated with a thin layer of gold. This produces a three-dimensional image of plant structures. In this exercise, you will view both scanning and transmission electron micrographs and learn the ultrastructure of a plant cell.

Materials Needed for Exercise C

Scanning electron micrographs of plant structures

Transmission electron micrographs of plant cells

Procedure

1. Examine figure 1.2 as an example of the three-dimensional image possible with a scanning electron microscope. View other scanning electron micrographs that are available in the laboratory.
2. Using table 1.1 and figure 1.3, identify and label plant cell ultrastructure in worksheet 1–2, figure 1.5 at the end of this laboratory topic.

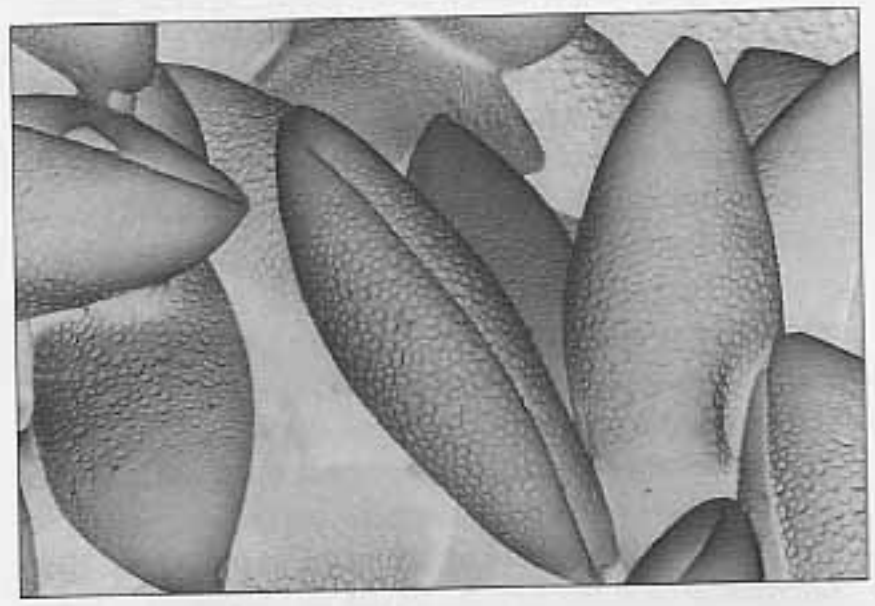


FIGURE 1.2 SCANNING ELECTRON MICROGRAPH OF POLLEN GRAINS OF AMARYLLIS.

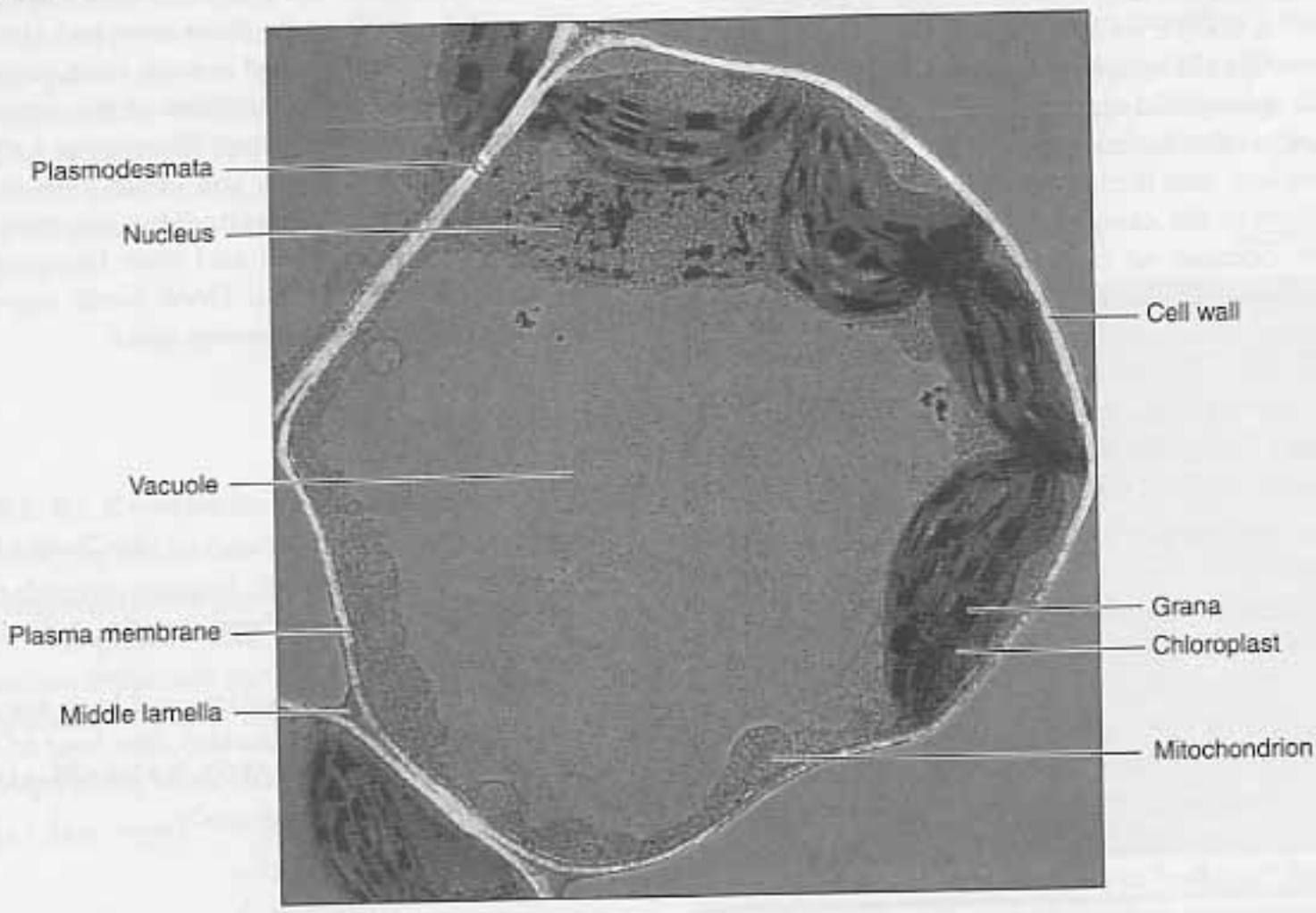


FIGURE 1.3 TRANSMISSION ELECTRON MICROGRAPH OF A PLANT CELL.

TABLE 1.1 PLANT CELL ULTRASTRUCTURE

Cellular Structure	*Description*	*Checklist*
Cell wall	Rigid outer structure that supports and protects primary wall of cellulose; in some plants, a secondary wall reinforced with lignin forms	
Chloroplasts	Double membrane–bounded organelles; site of photosynthesis; chlorophyll (green) plastids, internal organization of grana, membranous stacks, and stroma, protein-rich matrix	
Chromoplasts	Pigmented plastids of yellows, oranges, and reds; impart color to fruits, leaves, and flowers	
Cytoskeleton	Scaffolding of microtubules and microfilaments; gives shape and support	
Cytosol	Matrix of the cell, bounded by the nucleus and plasma membrane	
Endoplasmic reticulum	Network of membranous canals through the cytoplasm; functions in transport; lipid synthesis associated with smooth ER; rough ER studded on its free surface with ribosomes, associated with protein synthesis	
Golgi apparatus	Stack of flattened membranous stacks; site of packaging, modification, and secretion	
Leucoplasts	Colorless plastids; include starch-storing amyloplasts and lipid-storing elaioplasts	
Microbodies	Membrane-bounded organelles; location of certain metabolic reactions	
Middle lamella	Intercellular material that glues adjacent plant cells together; composed of pectins	
Mitochondrion	Double membrane-bounded organelle; site of aerobic respiration; cristae are folds of the inner membrane, matrix is the compartment enclosed by the inner membrane	
Nucleolus	One or more dark-staining areas in the nucleus; RNA-rich regions involved in protein synthesis	
Nucleus	Control center that contains genetic blueprint of DNA in the form of granular chromatin; surrounded by nuclear envelope, a double membrane with small openings, the nuclear pores	
Plasma membrane	Lipid bilayer with embedded proteins; governs the movement of materials into and out of the cell	
Plasmodesmata	Cytoplasmic bridges that cross cell walls and connect neighboring plant cells	
Ribosomes	RNA-rich organelles; site of protein synthesis; may be found free in the cytoplasm or attached to ER	
Vacuole	Very large, centrally located organelle bound by a tonoplast membrane; depository for storage, wastes and pigments (reds and blues)	

EXERCISE D: Crystal Persuasion

Plants do not have an excretory system, and therefore some substances have the potential to accumulate to toxic levels. By forming crystals that precipitate out in the cell vacuoles, such substances are taken out of solution, thereby becoming inert and harmless. In fact, stored crystals are often quite beautiful. They have also been used for identification purposes in forensic science and archaeology. Most contain calcium, either calcium oxalate or less commonly, calcium carbonate. **Phytoliths** are crystals found in the walls of certain families and genera of plants, usually monocots. They are made of silica (monosilicic acid) that is dissolved in the water absorbed by the roots. Plants cannot use silica, so often it is deposited in cell walls. If the plant dies or is cooked, the silica deposits survive intact for millenia, and because specific groups of plants produce uniquely shaped phytoliths, they have been used in archaeology as an identification tool. In this exercise, you will discover the types of crystals found in several plants.

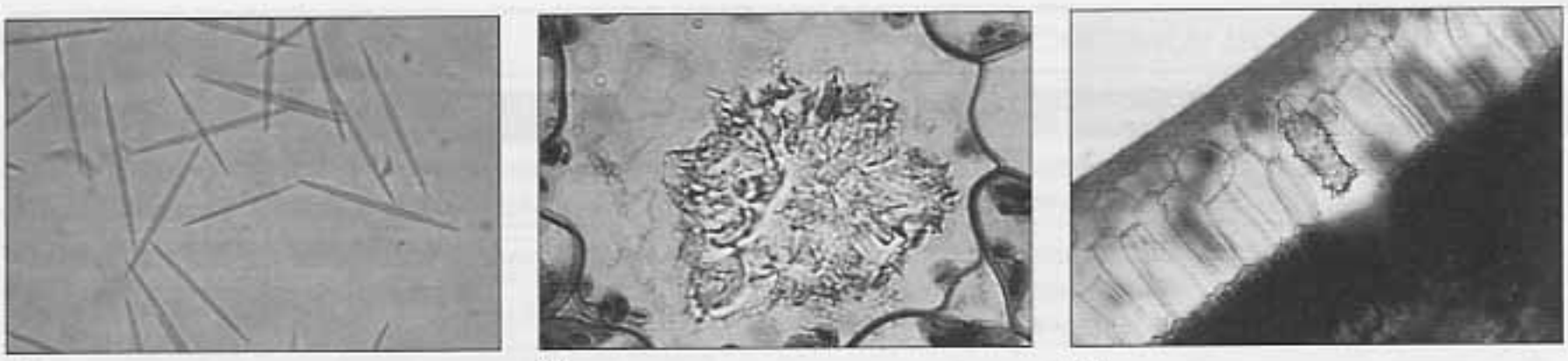


FIGURE 1.4 PLANT CRYSTALS. (*A*) RAPHIDES IN PINEAPPLE. (*B*) DRUSE IN LEAF CELLS OF ELEPHANT EARS. (*C*) CYSTOLITH IN LEAF CELLS OF RUBBER TREE (*FICUS ELASTICA*).

Materials Needed for Exercise D

Compound light microscope
Coverslips
Dissecting needles
Dropper bottles of distilled water
Elephant's ear (*Colocasia ultissima*)
Glass slides
Pineapple, can of crushed
Razor blades, single-edged
Rubber tree (*Ficus elastica*)

Procedure for Exercise D

1. Take a small sample of crushed pineapple and spread it thinly on a slide with a drop of distilled water. Cover with a coverslip. View first under scanning (4 × objective) or low (10 × objective) power. Look for the bunches of needlelike crystals (fig. 1.4*a*). Focus under high power (40 × objective) for a closer look. These are called **raphides**. Composed of calcium oxalate, they are common in many plants. Recall that you saw these crystals in the leaf cells of aloe viewed in Exercise B. You may also find these crystals in the leaves of dumbcane (*Dieffenbachia*). This common houseplant got its nickname because, if the leaves are eaten, the sharp raphide crystals stab the tongue and throat, causing inflammation to such an extent that the person is struck dumb or mute. If dieffenbachia is available, tear up a piece of leaf tissue and prepare a slide for viewing under the microscope. Sketch some representative raphides in the following space:

2. Another crystal of calcium oxalate takes the form of a star or **druse** (fig. 1.4*b*). To view a druse, tear up a piece of elephant's ear. Place it on a slide with a drop of distilled water, cover with a coverslip, and view first under scanning (4 × objective) or low (10 × objective) power to scan for the druses. Bring up to high power (40 × objective) for a closer look. Elephant's ear is taro, the source of poi, the native dish of Hawaii. Poi is prepared from the underground stem of taro, but the leaves are also eaten as foods are wrapped and cooked in taro leaves. In fact, the word *luau*, which has come to mean a Hawaiian-style feast, is the native word for these leaves. Sketch a druse in the following space:

3. A **cystolith** (cell rock) is another type of plant crystal. It is composed of calcium carbonate and is present in a small number of plant families, including that of the rubber tree. Since these leaves are quite stiff, you should be able to cut a small piece of thin cross section of a *Ficus elastica* leaf without too much trouble. Place your thin section on a slide, add a drop of distilled water, and cover with a coverslip. Scan under scanning (4 × objective) or low (10 × objective) power to search for the colorless epidermal cells where the cystoliths are located in **lithocysts** (rock cells). Focus under high power (40 × objective) when you spot what looks like a hanging bunch of grapes (fig. 1.4*c*). That's the cystolith! Sketch a cystolith in a lithocyst in the following space:

EXERCISE E: Plants to Dye For

Flowering plants brought color to the green world. In mosses, ferns, and gymnosperms (e.g., conifers), the predominant color is green, but in the angiosperms, a variety of other colors have been added to the palette. Vibrant or subtle, these colors shimmer from fruits, petals, seeds, and even some leaves. It is no wonder that humanity long ago created methods to extract and enhance these colors from the plant rainbow.

For most of human history, dyes for cloth and cosmetics were obtained from natural sources, either vegetable, animal, or mineral. Most of these natural sources were replaced by the synthetic aniline dyes first obtained from coal tar dyes during the latter half of the nineteenth century. Lately, though, there has been renewed interest in vegetables dyes, which are admired for their subtle and unique hues. In this exercise, you will learn about the chemistry of plant dyes and extract dyes from a variety of plants.

Materials Needed for Exercise E

Beakers, 50 ml	Dropper bottles of vinegar
Beakers, 250 ml	Forceps
Compound light microscope	Glass slides
Coverslips	Hot plate
Dropper bottles of ammonia	Marker pens
Dropper bottles of distilled water	Natural wool yarn, mordanted with alum
	Red cabbage leaves

Procedure for Exercise E

1. Peel off the epidermis of a red cabbage leaf by folding a small section of a leaf in half and grabbing the thin purple layer with a forceps. Place a small piece in a drop of distilled water on a glass slide and cover with a coverslip. Examine first at scanning (4 × objective) or low (10 × objective) power and then bring up to high power (40 × objective).
2. Note that the interior of each cell is purple. The purple color is due to the presence of a pigment called **anthocyanin**, which is contained within the vacuole of the plant cell. Note also the cell walls that define the boundary of each cell. Scan around. Do you see the prominent crystals in the vacuoles of many of the cells? Using figure 1.4 as a guide, identify the type of crystal you see.

Locate another cell structure prominent in these cells. It is spherical and centrally located. Note that it has one or two small, dark-stained circles within it. What is it?

3. Anthocyanins have been used for millennia as a source of natural textile dyes. They also have some unique chemical properties that you will be investigating.
4. Tear up 1 red cabbage leaf, and place it in a 250-ml beaker; add just enough distilled water to cover. Also add to the beaker a length of undyed wool that has been treated with the mordant alum and simmer for 30 minutes on a hot plate. (Alum, or potassium aluminum sulfate, is a **mordant**, a chemical agent that makes the dye adhere to the fabric. It can be found in the spice section of a supermarket.) The color of the dye may change, depending on whether a mordant is used or not, and if the fabric is treated with different mordants.
5. Use the same procedure to set up other dye vats and wool. Some plants to try include strawberries, blackberries, blueberries, cranberries, beets (the pigment here is betacyanin), yellow onion skins, red onion skins, mullein leaves, Indian blanket flowers (*Gaillardia pulchella*), or any other plant of your choosing. Record your findings in worksheet 1–2 at the end of this laboratory.
7. After you have completed dyeing the wool in the red cabbage juice, decant the anthocyanin solution. Pour off equal volumes of this solution into two 50-ml beakers. Label one beaker "A" and the other "B."
8. Add a few drops of ammonia to beaker A. What color change do you see? Add a few drops of vinegar to beaker B. What color change do you see?

Anthocyanins are natural pH indicators in that, in the presence of acid or base, they undergo a chemical change that is visible in color. The **pH scale** is used to measure the **acidity** or **alkalinity** of substances. It ranges from 0 to 14, with 7 being neutral, the lower end of the scale is acid, and the higher end is alkaline or basic. In the presence of acid, anthocyanins give up hydroxy ions (OH^-), resulting in a red color. In the presence of a base like ammonia or baking soda, they pick up hydroxy ions, resulting in a blue or green color. How could you reverse the color changes?

TERMS TO KNOW

acidity 7
alkalinity 7
amyloplast 3
anthocyanin 7
base 7
cellulase 1
cellulose 2
cell wall 3
chlorophyll 3
chloroplast 3
chromatin 3
chromoplast 3
cystolith 6
cytoplasm 5
DNA (deoxyribonucleic acid) 3
druse 6
elaioplast 3
hydroxy ions(OH^-) 7
leucoplast 3
lignin 2
lithocysts 6
magnification 4
middle lamella 3
microbodies 5
mitochondrion 5
mordant 7
nucleus 3
nucleolus 3
nuclear envelope 3
pectinase 1
pH scale 7
phytoliths 5
plasma membrane 3
plasmodesmata 3
plastids 5
primary cell wall 2
proplastid 3
protoplast 1
raphides 6
resolution 4
RNA (ribonucleic acid) 3
scanning electron microscope (SEM) 4
secondary cell wall 2
succulent 2
tonoplast 3
transmission electron microscope (TEM) 4
vacuole 3

QUESTIONS FOR REVIEW AND DISCUSSION

1. What is a protoplast? How are they useful in genetic engineering?
2. Why do crystals form in plants? Why are crystals found most often in the epidermis, the outermost layer of a plant organ? (*Hint:* Think of the dumbcane example.)
3. Apparently the victim of a kidnapping gone wrong, the body of a young child is found concealed in the basement of her home. As the police try to construct a timeline for the kidnapping and murder, they interview the parents. The mother insists that her daughter disappeared before the evening meal, which included a pineapple compote dessert. An analysis of the stomach contents conflicts with the mother's statements. What did the medical examiner's office find in the girl's stomach that causes them to doubt the mother's testimony?
4. What hues are found in the anthocyanins? What pigments and colors are found in chromoplasts?
5. List the plant cell structures that are visible under the light microscope. What cellular structures or details are only visible under the electron microscope?

ADDITIONAL RESOURCES

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Mauseth, J. D. 1998. *Botany: An introduction to plant biology*, 2nd ed. Sudbury (MA): Jones and Bartlett Publishers.

Mauseth, J. D. 1988. *Plant anatomy*. Menlo Park (CA): Benjamin/Cummings.

Moore, R., W. D. Clark, and D. S. Vodopich. 1998. *Botany*. New York: McGraw-Hill Companies, Inc.

Northington, D. K., and E. L. Schneider. 1996. *The botanical world*. New York: McGraw-Hill Companies, Inc.

Raven, P. H., R. F. Evert, and S. Eichhorn. 1999. *Biology of plants*, 6th ed. New York: W. H. Freeman/Worth.

Tobin, A. J., and R. E. Morel. 1997. *Asking about cells*. Fort Worth (TX): Saunders College Publishing.

ON THE WEB

Botany Online: Cells and Tissues—The Structure of a Cells and Cell Division
http://www.botany.uwc.ac.za/sci_ed/pupil/cellinks/index.htm#division

Cellupedia
http://library.thinkquest.org/C004535/introduction.html

Ethnobotanical Leaflets
http://www.siu.edu/~ebl/

Molecular Expression Photo gallery
http://micro.magnet.fsu.edu/micro/gallery.html

Plant Cell
http://www.rrz.uni-hamburg.de/biologie/b_online/e04/04a.htm

OTHER ACTIVITIES

1. Check out scanning and transmission electron micrographs on plant cell structure from web sites or books from the library.
2. Bring in a variety of colorful flowers, fruits, and vegetables to examine for chromoplast appearance.
3. Create a portfolio of yarn samples dyed with plant pigments.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 1-1 EXERCISE A: PLANT CELLS WITHOUT WALLS
EXERCISE D: CRYSTAL PERSUASION

1. What is the purpose of cellulase in the protoplast exercise? Pectinase?

2. Why is it necessary to be gentle with protoplasts?

3. At one time it was thought that vegans, those whose diet is based solely on plants, would not get sufficient quantities of calcium. After completing these laboratory exercises, what do you think of that assumption? Why?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 1-2 EXERCISE C: PLANT ULTRASTRUCTURE

4. Label the structures of a plant cell as seen in a transmission electron micrograph.

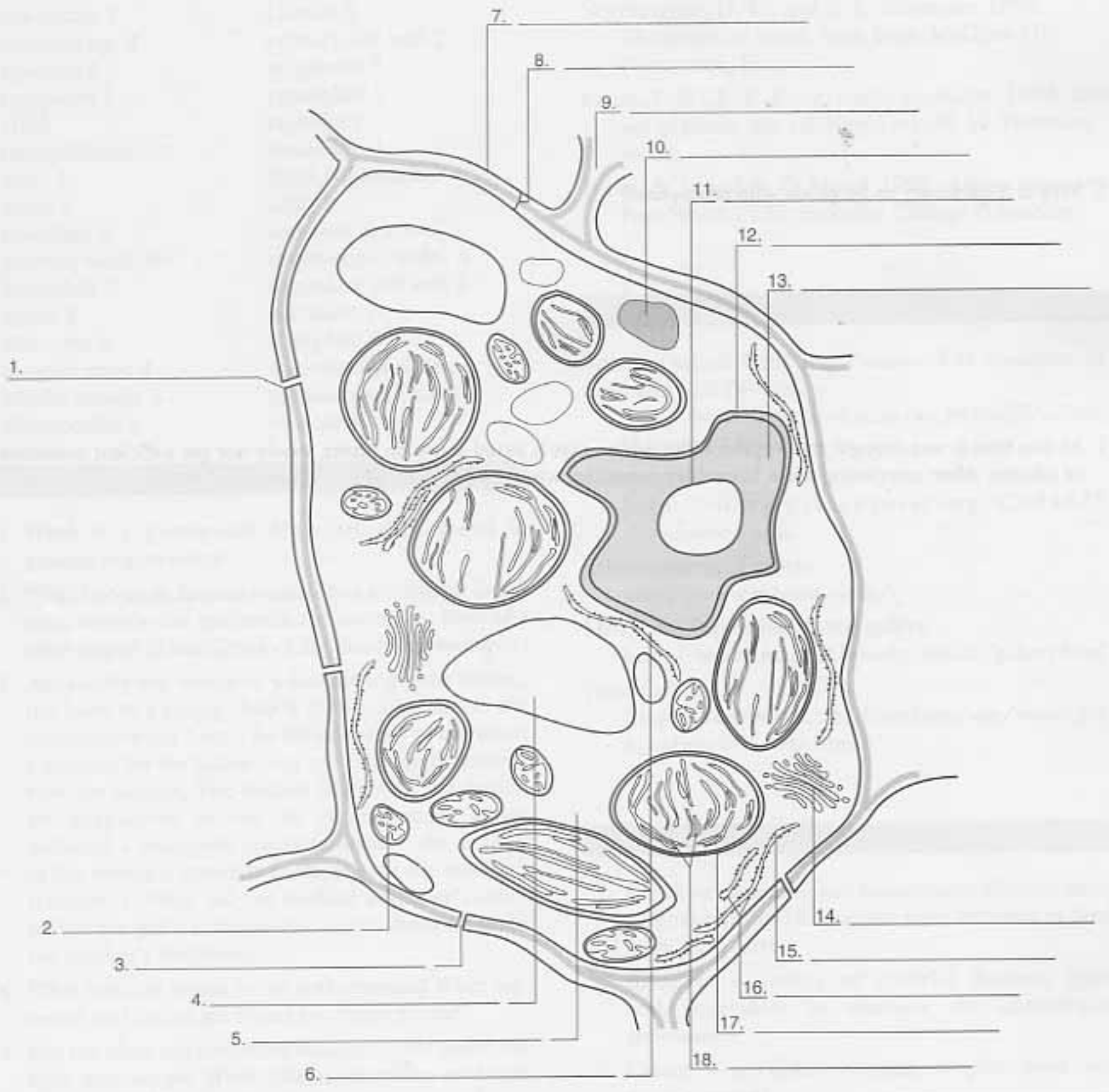


FIGURE 1.5 DIAGRAM OF A TRANSMISSION ELECTRON MICROGRAPH OF A PLANT CELL.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 1-3 EXERCISE E: PLANTS TO DYE FOR

Plant Source	*Part Used*	*Color*

5. From your knowledge of anthocyanins, what factor appears to determine if the same vacuolar pigments are red in one plant (cranberry) but blue in a different plant (blueberry)? Explain.

LABORATORY TOPIC 2

Cell Division and Cloning

BACKGROUND

The process of cell division is the principal method of plant growth, with the majority of growth occurring in stem tips, root tips, and other meristematic areas. **Meristems** are regions of active cell division. Most of the cells produced in meristems differentiate into specialized tissues, while other cells remain meristematic, continually producing more new cells. **Apical meristems**, found at the tip of every stem, branch, and root, contribute to the increase in length of the plant. Tissues that develop from apical meristems contribute to the **primary growth** of the plant; primary tissues include leaves, nonwoody stems, and nonwoody roots. Some plants have **secondary meristems** that give rise to increases in diameter, which constitutes **secondary growth**. The vascular cambium and cork cambium are the two secondary meristems that occur in woody plants.

Cell division is characterized by two events: **mitosis**, which produces two exact copies of the nucleus, and **cytokinesis**, which is the division of the cytoplasm that occurs during the later stages of mitosis. Once mitosis begins, the process continues without stopping until two daughter cells are produced.

The production of new cells through cell division not only results in plant growth and enables plants to repair wounds, but can even lead to the formation of new, genetically identical individuals called **clones**. In this laboratory topic, we will examine the process of cell division. We will also see how this process is used to clone new plants through tissue culture and asexual reproduction.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Describe the events that occur during all the stages of cell division.
2. Recognize the stages of cell division under the microscope.
3. Describe the process of tissue culturing.
4. Understand the need for sterile technique in tissue culturing.
5. Understand how cell division contributes to asexual reproduction.
6. Describe how to make cuttings for plant propagation.

EXERCISE A: Cell Division

Cell division is just a small part of the cell cycle, but it is the only time when **chromosomes** can be seen under the microscope. The rest of the cell cycle, when the cell is not dividing, is called **interphase** (fig. 2.1*a*). During interphase, the genetic material exists as long strands of DNA and protein known as **chromatin**; during cell division, the chromatin becomes organized into chromosomes.

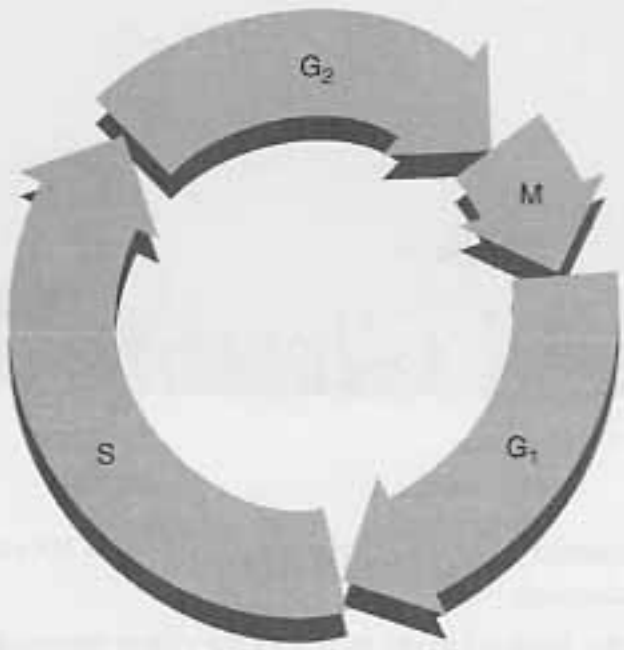
The cell is metabolically active during interphase and is also making all the preparations for the next round of cell division. Three metabolic stages characterize interphase: **G_1, S,** and **G_2**. During G_1 (first gap), the cell is actively growing and rapidly synthesizing proteins and other metabolites. The S (synthesis) stage is when DNA and other components of the chromosomes are duplicated. The final preparations for cell division take place during the G_2 (second gap) stage, and as this stage ends, mitosis begins. The process of mitosis occurs in four continuous phases: **prophase, metaphase, anaphase,** and **telophase**.

Prophase

Numerous events occur during prophase. The threadlike chromatin condenses and coils into recognizable chromosomes. When the chromosomes become visible, they are seen to consist of two identical **chromatids**, which are joined at a constriction known as the **centromere** (fig. 2.1*b*). As prophase continues, the nucleoli and nuclear membrane disperse into the cytoplasm, leaving the chromosomes free in the cytoplasm, and **spindle fibers** begin appearing (fig. 2.2).

Metaphase

During metaphase, the chromosomes rearrange themselves so that the centromeres lie across the center of the cell (metaphase plate). The **spindle**, which consists of numerous spindle fibers, becomes fully developed. Some spindle



(a)

Interphase

Phase	Main Events	*Vicia faba*
G_1	Cells metabolically active; organelles begin to increase in number	4.9 hr
S	Replication of DNA	7.5 hr
G_2	Synthesis of proteins; final preparations for cell division	4.9
M	Mitosis	2.0
	Total	19.3 hr

(b)

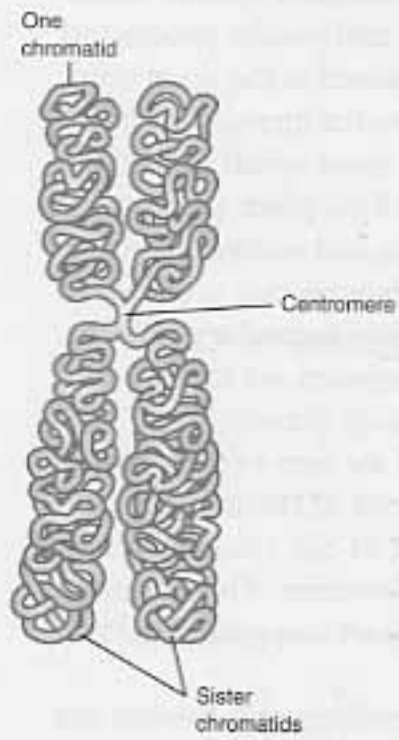


FIGURE 2.1 (*A*) THE CELL CYCLE CONSISTS OF FOUR STAGES (G_1, S, G_2, AND M). THE EVENTS THAT OCCUR IN EACH STAGE ARE DESCRIBED USING THE BROAD BEAN (*VICIA FABA*) AS AN EXAMPLE. (*B*) MICROGRAPH AND DRAWING SHOWING THAT A DUPLICATED CHROMOSOME CONSISTS OF TWO SISTER CHROMATIDS HELD TOGETHER AT THE CENTROMERE (× 9,600).

fibers extend from the centromeres to the poles of the cells, while other fibers extend from pole to pole (fig. 2.2).

Anaphase

The two chromatids that make up each chromosome are separated during anaphase. Spindle fibers pull the chromatids to the opposite poles of the cell. Once they separate, each former chromatid is called a chromosome. The end result of anaphase is that the genetic material is divided into two identical sets, each with the same number of chromosomes. By the end of anaphase, the spindle is no longer apparent (fig. 2.2).

Telophase

At each pole of the cell, the chromosomes unwind and lengthen during telophase. Also, nuclear membranes and nucleoli reappear, so that at the end of telophase, two distinct daughter nuclei are visible (fig. 2.2).

During the later part of anaphase and continuing through telophase, **cytokinesis** takes place. The

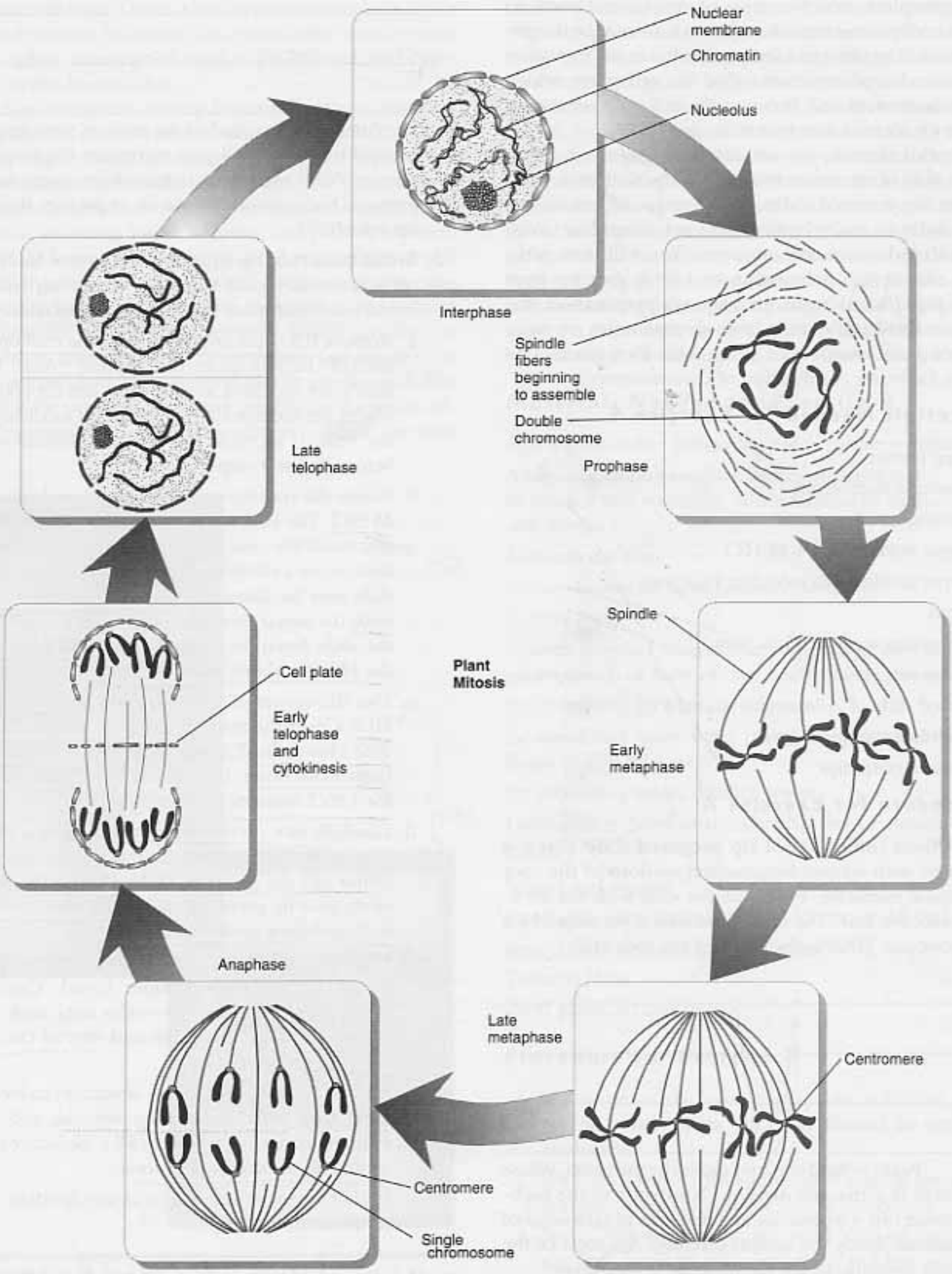


FIGURE 2.2 MITOSIS IN A PLANT CELL.

phragmoplast, which consists of vesicles and portions of the endoplasmic reticulum, forms between the daughter nuclei. The phragmoplast participates in the formation of a disc-shaped structure called the **cell plate**, which expands outward and becomes the cell walls separating the newly formed daughter cells (fig. 2.2).

In this exercise, you will first study mitosis in a prepared slide of the onion root tip. In the sectioned material on the prepared slides, all the stages of mitosis can be found quite easily; however, it is not always easy to distinguish individual chromosomes. You will then make your own squash preparation of a fresh root tip from *Vicia faba* (broad bean). In a **squash preparation**, the cells are flattened, and the large chromosomes are more dispersed and usually seen in the same focal plane. This allows for better examination of chromosomes.

Materials Needed for Exercise A

Bunsen burner
Compound microscope
Dissecting needles
Dropper bottles with 1 M HCl
Dropper bottles with toluidine blue stain
Forceps
Germinating seeds of *Vicia faba*
Paper towels
Prepared slide of *Allium* (onion) root tip
Razor blades, single-edged
Slides and coverslips

Procedure for Exercise A

1. **Allium (onion) root tip prepared slide** This is a slide with stained longitudinal sections of the root apical meristem. First scan the slide with the 10 × objective lens. The end of the root is protected by a root cap. What is the shape of the root cap?

Right behind the root cap is the meristem, where there is active cell division. Now shift to the high-power (40 ×) objective and find cells in each stage of mitosis. Study the section carefully. Are most of the cells dividing, or are the majority in interphase?

MOST ARE IN INTERPHASE.

After you have studied the section, your instructor will test your ability to recognize the stages of mitosis. When you can recognize all the stages on the prepared slide, you can move on to prepare the root tip squash.

2. **Broad bean root tip squash** Using a razor blade, cut off a secondary root from a germinating seed of broad bean, and place the root on a glass slide.
   a. Remove 0.5–1 cm of growth from the root tip and discard the remainder of the root. (*Note:* Your instructor may have already done this for you and placed the roots in Carnoy's solution to kill and fix the cells. If so, rinse the roots in distilled water before the next step.)
   b. Cover the root tip with two or three drops of 1 M HCl. The HCl helps dissolve the middle lamella that holds the cells together. Using a slide holder, forceps, or a clothespin to hold the slide, pass the slide over the flame of a Bunsen burner for 5 seconds (or pass it through the flame five times). Put the slide down on a paper towel, and let it sit in the HCl for about 4 minutes.
   c. Use the corner of a paper towel to blot up the HCl. Cover the root tip with two drops of toluidine blue stain. Pass the slide above the Bunsen burner two times. Place the slide on a paper towel for 1 to 2 minutes.
   d. Carefully blot up the excess stain. Using a single-edge razor blade and a dissecting needle, macerate (chop up) the root tip. Make sure all the pieces of the root tip are on the slide. Add a drop of fresh stain and then apply a coverslip.
   e. Place the slide on a paper towel (coverslip up). Cover with a second paper towel. Carefully apply pressure to the coverslip area with your thumb in order to squash and spread the root tip tissue.
   f. Scan under low power (10 × objective) to look for areas with cells undergoing mitosis, and then examine under high power (40 × objective). Try to find all the stages of mitosis.
   g. If your first root did not yield any dividing cells, try a second root tip.

EXERCISE B: Clones from Tissue Culture

Under suitable conditions, certain cells from a mature plant can resume cell division or even differentiate into

another cell type. This is what happens when plants repair wounds caused by injury. The result is the development of a **callus**, an irregular mass of undifferentiated plant cells, at the injured site.

A callus can also develop from small pieces of plant tissue placed in an appropriate culture medium in the laboratory (fig. 2.3*a*). The tissue removed from a plant and placed into culture is called an **explant**. The tissue culture medium contains both mineral nutrients and organic compounds, including sugar, vitamins, and plant hormones. The plant hormone **auxin** controls cell enlargement as well as cell division in culture. **Kinetin**, another hormone, also induces cell division. Both are included in the culture medium for healthy callus growth. Callus can also be induced to differentiate and develop roots and shoots if the relative proportions of auxin and kinetin are changed. The result is the production of small plantlets in the tissue culture medium (fig. 2.3*b*). These plantlets, which can then be transferred to soil, are genetically identical to the small piece of plant tissue originally placed in the culture medium. Tissue culturing allows botanists and horticulturalists to clone plants with desirable traits, and it is widely used in the nursery business and in plant biotechnology. In this lab, we will use tissue culturing to clone tobacco plants.

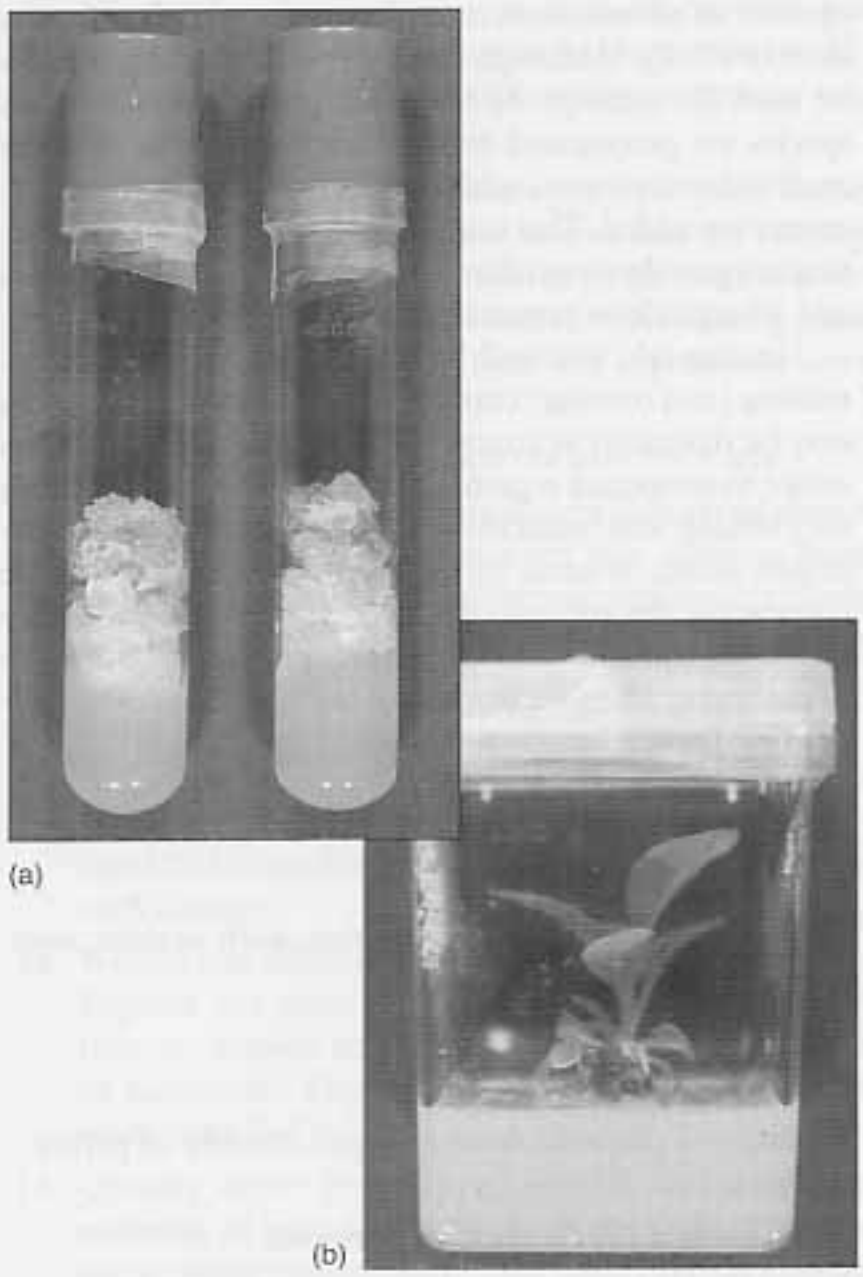


FIGURE 2.3 PLANT TISSUE CULTURE. (A) CALLUS GROWTH FROM EXPLANT. (B) ADDITION OF HORMONES ALLOWS THE CALLUS TO DIFFERENTIATE INTO A PLANTLET.

Tissue culturing requires the use of **sterile techniques** (as described in steps 2 and 3 in the procedures) because the culture medium we are using is rich in nutrients and can easily become contaminated with bacteria and/or fungi. Fungal spores and bacteria are abundant in our surroundings, both in the air and on the surface of objects. On the tissue culture medium, these microorganisms would quickly overgrow the plant tissue or developing callus, so every possible precaution should be taken to minimize contamination.

Materials Needed for Exercise B

3 or 4 petri dishes containing callus culture medium

Additional culture medium in magenta jars will be needed in 3 weeks for differentiation of roots and shoots.

Antibacterial soap

Bunsen burner or spirit (alcohol) lamp

Cotton Balls

Growth chamber (if available) set at 28°C with 12-hour photoperiod, or bank of cool white fluorescent lights

Jar containing 15% bleach solution

Jar containing soapy water (two drops of dishwashing liquid in 250 ml water)

Jar containing sterile distilled water

Laminar-flow hood or transfer chamber (if available)

Parafilm

Scalpel and forceps

Small glass plate (approximately 15 cm × 15 cm)

Spray bottle containing 70% ethanol

Tobacco plant

Vinyl gloves (if available)

Procedure for Exercise B

1. If a laminar-flow hood is available in the lab, turn on the blower. This will bring filtered air into the work area.
2. Wash your hands with antibacterial soap. If gloves are available, they will provide added protection.
3. Spray 70% ethanol on the work area of the laminar-flow hood or your lab bench and use a cotton ball to wipe the entire area. Maintain a clean work area throughout the culturing. Sterilize all instruments by dipping each instrument in alcohol and then passing it through the flame of a Bunsen

burner or spirit lamp. Be careful to keep the jar of alcohol away from the flame.

4. Cut a 1 to 2 cm segment from the stem of a tobacco plant. Use a segment from the region between leaves (called the internode) near the top of the plant. *Note*: the tobacco plant **should not** be close to your sterile work area.
5. Use forceps to dip the stem segment in soapy water. This will remove any small insects, loose spores, and bacteria.
6. Transfer the stem segment to the jar of bleach for 3 minutes.
7. Rinse the stem segment for 2 minutes in sterile distilled water.
8. While the stem segment is in the bleach and the sterile water, wipe the glass plate with 70% alcohol and allow it to dry.
9. Transfer the stem segment to the glass plate.
10. Using the scalpel and forceps (remember to dip them in alcohol and flame), trim off and discard the epidermis (the outermost layer). Also trim off and discard the ends of the segment. Cut the remaining segment into three or four slices.
11. Place each section on the surface of the culture medium in a petri dish.
12. Seal each petri dish with a strip of parafilm.
13. Place the dishes in a growth chamber at 25°C under 12-hour photoperiod. If no growth chamber is available, the cultures can be grown under a bank of cool white fluorescent bulbs. If a timer is available, set it for a 12- to 16-hour photoperiod, although the cultures will do well even under continuous illumination.
14. Check the cultures each week. It may take a while before growth is visible. Record your observations on the appearance of the tissue on worksheet 2–1 at the end of this laboratory topic.
15. Once the callus is growing well (about 3 weeks from now), it will be time to transfer it to the differentiation medium.
16. Repeat steps 1, 2, and 3 to sterilize the work area.
17. Obtain three or four jars containing the differentiation culture medium.
18. Open the dishes containing the growing callus. Using sterile technique, transfer the callus to the differentiation medium in the jars. Close the jars. If you are using magenta jars, they will have a good seal. If you are using other jars, you may want to seal the lid with parafilm.
19. Replace the jars in the growth chamber or under the bank of lights.
20. Continue to evaluate your cultures every week for another 6 weeks. Remember to record your observations in worksheet 2–1. At the end of the experiment, you may want to transfer the developing plants to potting soil in a 4-inch pot.

EXERCISE C: Cloning Herbs From Cuttings

In tissue culturing, the addition of an appropriate combination of auxin and kinetin leads to the development of roots and shoots from undifferentiated callus. This allows new plants to develop exclusively through cell division. This type of reproduction is known as **asexual reproduction** or **vegetative propagation**. In addition to tissue culture, there are several other means of asexual reproduction. One technique that is widely applied by both home gardeners and professional nursery owners is the use of stem cuttings. In this method, a section of the stem is cut from the plant and then placed in an environment to promote the development of adventitious roots (roots that develop from a stem or a leaf). Although other parts of the plant can also be used for cuttings, by far the largest number of plant species are propagated by stem cuttings. Some cuttings readily develop roots, while others only do so when hormones are added. This is usually done by dipping the cutting in a powder or gel that contains a mixture of hormones and a fungicide to prevent infection of the cutting.

In this lab, you will be starting an herb garden by making (and rooting) cuttings from several herbs. **Herbs** may be described as aromatic leaves or seeds from plants native to temperate regions. The plants that provide herbs for cooking and medicine are usually nonwoody (herbaceous) plants or small shrubs. For each herb, we will be comparing the success of hormone treatment with the untreated control. Later in this manual, we will use some of the leaves when we work with herbs and spices in Laboratory Topic 17.

Materials Needed for Exercise C

3-inch pots

Actively growing potted herb plants, such as mint, sage, oregano, thyme, and rosemary

Labels for the pots

Permanent markers

Potting soil prepared from an equal mixture of perlite and peat

Rootone or a similar rooting hormone

Razor blades, single–edged

Procedure for Exercise C

1. Select three different herbs for your herb garden.
2. Obtain six pots and six labels, two for each herb.

3. You will be preparing two cuttings from each herb. One cutting of each will be dipped in a rooting hormone, while the second will be the untreated control. Put your initials and the name of the herb on each label. On the label also put a (+) for the cutting dipped in the hormone and a (–) for the untreated control.
4. Fill the pots with the potting soil, and add water. Use a pencil to make a hole in the soil.
5. Cuttings should be made with a fresh razor blade at a 45° angle to the stem.
6. Make cuttings from each herb you have selected. Each cutting should be the top 8 to 10 cm of a different branch.
7. Remove any flowers or flower buds from the cutting. This will help the cutting use all the stored carbohydrates for root formation.
8. Remove the bottom two leaves from each cutting.
9. Dip one cutting of each type in Rootone. This is a hormone that promotes rooting in plants that are difficult to root. Gently tap the cutting to remove any excess Rootone.
10. Insert the cuttings in the holes you have made in the soil. Each cutting should be inserted into the potting mix to a depth one-third to one-half its length. Make sure the cutting is vertical and upright. Gently pack the soil around the stem. Place the appropriate label in each pot.
11. Water again, and then place the pots on a tray.
12. Place the trays in a well-lighted area, but **avoid direct sun**. Keep the soil moist until the cuttings have rooted (they may need to be watered two or three times each week). Your instructor may want you to cover the trays with a clear plastic sheet to minimize water loss.
13. After 2 weeks, gently dig up each cutting and very carefully rinse off any clinging soil. In table 1 of worksheet 2–2, record whether roots are present or not for each cutting.
14. We will also combine the results from the whole class. Express the class results as a percent of cuttings that developed roots for the treated and control of each herb. Enter the class results in table 2 on worksheet 2–2.
15. Quickly repot the cuttings in soil, and continue watering at least once a week. (*Note*: Depending on the growing conditions in your laboratory, it may be necessary to water more frequently. Your instructor will provide guidance on this.) These plants can be used again for Laboratory Topic 17, and at the end of the semester, you will be allowed to take home the remaining plants in your herb garden.

TERMS TO KNOW

anaphase 14
apical meristem 13
asexual reproduction 18
auxin 17
callus 17
cell plate 16
centromere 13
chromatids 13
chromatin 13
chromosomes 13
clones 13
cuttings 18
cytokinesis 13
explant 17
herbs 18
interphase 13
kinetin 17
meristems 13
metaphase 13
mitosis 13
phragmoplast 16
primary growth 13
prophase 13
secondary growth 13
secondary meristem 13
spindle 13
spindle fibers 13
squash preparation 16
sterile techniques 17
telophase 14
tissue culture 16
vegetative propagation 18

QUESTIONS FOR REVIEW AND DISCUSSION

1. Describe the events during the stages of mitosis.
2. Differentiate between mitosis and cytokinesis.
3. What is the role of the spindle fibers?
4. What is the centromere?
5. What do we call the specific regions of a plant where there is active cell division? Where do we find these regions?
6. What is the advantage of asexual reproduction for plant propagation?
7. What might be some of the disadvantages of asexual reproduction?
8. What is an explant?
9. What do we mean by sterile technique? Why is it necessary for tissue culturing?

ADDITIONAL RESOURCES

Druse, K. 2000. *Making more plants: The science, art and joy of propagation*. New York: Clarkson Potter Publishing.

Kyte, L., and J. Kleyn. 1996. *Plants from test tubes: An introduction to micropropagation*, 3d ed. Portland (OR): Timber Press.

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Stern, K. 2000. *Introductory plant biology*, 8th ed. NewYork: McGraw-Hill Companies, Inc.

ON THE WEB

Texas A&M University, Plant Tissue Culture Information Exchange

http://aggie-horticulture.tamu.edu/tisscult/tcintro.html
Access Excellence, Plant Tissue Culture
http://www.accessexcellence.com/LC/ST/st2bgplant.html
About.com, Mitosis
http://biology.about.com/library/weekly/aa060800a.htm?terms=mitosis
Access Excellence, Mitosis
http://www.accessexcellence.org/AB/GG/mitosis.html
Koning at East Connecticut State University, Vegetative Propagation
http://koning.ecsu.ctstateu.edu/vegprop/vegpropa.html

OTHER ACTIVITIES

1. For Exercise A, your instructor may also have onion root tips available for preparing chromosome squashes. Follow the same procedure as for the *Vicia faba* roots.
2. For Exercise C on cloning herbs, try rooting some of the cuttings in water. Some plants readily root in water, while others do not. Plants rooted in water should be transferred to a potting mixture as soon as the roots are visible.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 2-1 EXERCISE B: CLONES FROM TISSUE CULTURE

Medium	*Date*	*Description of Tissue Culture*
Callus culture medium		Explants placed in culture
Callus culture medium		
Callus culture medium		
Callus culture medium		
Differentiation medium		
Differentiation medium		
Differentiation medium		
Differentiation medium		
Differentiation medium		
Differentiation medium		
Differentiation medium		

1. When did callus growth begin?

2. When did roots first appear?

3. When did leaves first appear?

4. How many plantlets developed per culture?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 2-2 EXERCISE C: CLONING HERBS FROM CUTTINGS

TABLE 1 INDICATE WHETHER ROOTS WERE PRESENT OR NOT FOR THE HERBS YOU USED.

Name of Herb Used	*Treated with Rootone*	*Untreated Control*

TABLE 2 PERCENT OF HERB CUTTINGS DEVELOPING ROOTS: SUMMARY OF CLASS RESULTS

	Percent of Cuttings with Roots	
Name of Herb	*Treated with Rootone*	*Untreated Controls*

LABORATORY TOPIC 3

Plant Tissues— The Fabrics of Our Lives

BACKGROUND

Angiosperms are complex multicellular organisms composed of many different types of cells. Groups of cells that are specialized in structure and function are referred to as **tissues**. The basic tissue types found in plants are dermal, ground, and vascular tissues. **Dermal tissues** are the outer layers of the plant, **vascular tissues** are the conducting tissues, and **ground tissues** include everything else. These three tissue types make up the familiar organs of plants: stems, roots, and leaves. In addition to their roles in plant structure, plant tissues have been used by humanity for thousands of years for fabrics, writing materials, and building materials. Until the advent of synthetics, plant tissues really did provide many of the "fabrics of our lives." In this laboratory topic, we will examine the types of cells and tissues found in plants and their use in making fabrics and paper.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Identify the major cell and tissue types in vascular plants and describe their functions.
2. Recognize the types of plant cells that have been used by society.
3. Describe the features of plant cells that make them useful as commercial fibers.
4. Describe the types of plant materials that can be used in making paper.
5. Describe the steps involved in making paper.

EXERCISE A: Plant Tissues

Ground tissues constitute the majority of nonwoody plant organs. They are diverse in size and shape, and they perform a variety of functions. The three ground tissues are parenchyma, collenchyma, and sclerenchyma (fig. 3.1). Of these, parenchyma shows the greatest diversity in form and function.

Parenchyma consists of thin-walled, living cells that occur in different regions of the plant body. Parenchyma cells can be almost any size or shape; however, they are frequently described as a 14-sided polygon. They may

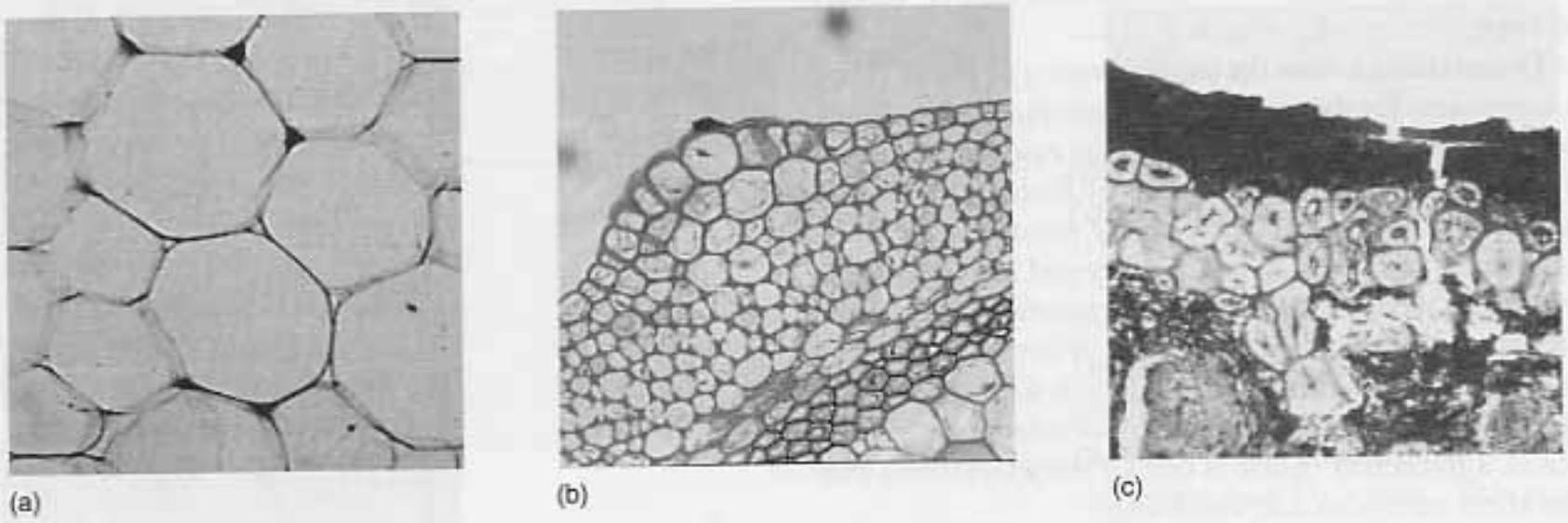


FIGURE 3.1 GROUND TISSUES. (A) PARENCHYMA CELLS, THE MOST COMMON CELL TYPE IN PLANTS, HAVE THIN PRIMARY WALLS. (B) COLLENCHYMA CELLS HAVE UNEVENLY THICKENED PRIMARY WALLS. (C) SCLERENCHYMA CELLS HAVE THICKENED SECONDARY WALLS.

occur singly or as groups of cells, thereby forming a tissue. Parenchyma cells have many diverse functions. In leaves, parenchyma cells are photosynthetic; in underground stems and roots, parenchyma cells are used for storage. In nonwoody plants, parenchyma cells make up the majority of the plant body.

Collenchyma consists of living, elongated cells that develop an unevenly thickened primary wall. These cells occur as filaments or in bands just below the epidermis in leaves, stems, and flowers. The walls, which are flexible and plastic, provide support in young, actively growing plant organs.

Sclerenchyma consists of cells with thick, lignified, secondary walls and variable shapes that usually have no living protoplasm when mature. Lignin, a unique component of cells with secondary walls, is a strong, complex polymer that increases wall strength, water impermeability, and resistance to decay. Sclerenchyma provides support and mechanical protection to mature plant parts. Pits are easily seen in the walls of some sclerenchyma cells. Sclerenchyma is subdivided into two groups: **sclereids**, which are small, irregularly shaped cells, and **fibers,** which are elongated cells. Sclereids may occur in a complete layer or tissue in a seed coat (as a walnut shell), or they may occur as clusters of cells or even individual cells that are randomly distributed. When sclereids form a seed coat, they have a protective function; when they occur in leaves, they provide mechanical support. In other instances, the function is unknown. The shape of sclereids is variable, but they are seldom long and thin. By contrast, fibers are thick-walled and very elongated sclerenchyma cells. The secondary wall is usually highly lignified. Fibers may occur singly, but more commonly they are groups of cells. Fibers may be associated with the vascular bundles in the leaves and stems. They also comprise a significant part of the wood in many plants. Many plant fibers have commercial applications in the manufacture of fabrics and rope.

Dermal tissues form the outer covering of plants. They are responsible for the environmental interactions in plants. The two types of dermal tissues are the epidermis and the periderm. The **epidermis** consists of flattened cells that make up the outermost layer of young plants and the nonwoody parts of older plants. In leaves and stems, the epidermal cells secrete **cutin**, a waxlike material that prevents water loss from the plant. The layer of cutin is known as the **cuticle**. In some leaves, the cuticle is so thick that the leaf has a shiny, waxy appearance. In succulent plants, such as cacti, a thickened cuticle is one of the adaptations that helps them survive in arid conditions.

Trichomes are hairlike structures that occur in the epidermis of many plants. Although individual trichomes are microscopic, they may be abundant enough to give leaves a hairy or fuzzy appearance. Trichomes, which may be branched or unbranched, vary greatly in size and shape. Some trichomes help prevent water loss in a plant, while other trichomes are glandular. Glandular trichomes are able to secrete various chemicals, such as nectar, enzymes, or aromatic compounds, that impart an aroma or scent to certain plants. You may recall the aroma of the herbs we used for asexual propagation in Laboratory Topic 2. The essential oils that provide the aroma and flavor to herbs are found in glandular trichomes.

We find numerous pores in the epidermis of leaves and green stems; these pores are called **stomata** (sing., **stoma**). The pores allow for exchange of gases, such as carbon dioxide, oxygen, and water vapor, between the plant and the environment. Each stomatal pore is regulated by a pair of cells called **guard cells**, which occur on either side of the opening (fig. 3.2). Guard cells, which are sausage-shaped in many plants, are the only epidermal cells that contain chloroplasts.

The **periderm** replaces the epidermis on plants that have secondary growth. For example, periderm that develops from the cork cambium makes up the outer bark of mature trees. We will look at the cells that compose the periderm in Laboratory Topic 15.

Vascular tissues are conducting tissues. The vascular tissues occur in all parts of the plant and are easiest to see as the veins in leaves. The two types of vascular tissue are **xylem**, which conducts water and minerals upward from the roots, and **phloem,** which conducts organic materials throughout the plant. Both xylem and phloem are composed of several different types of cells.

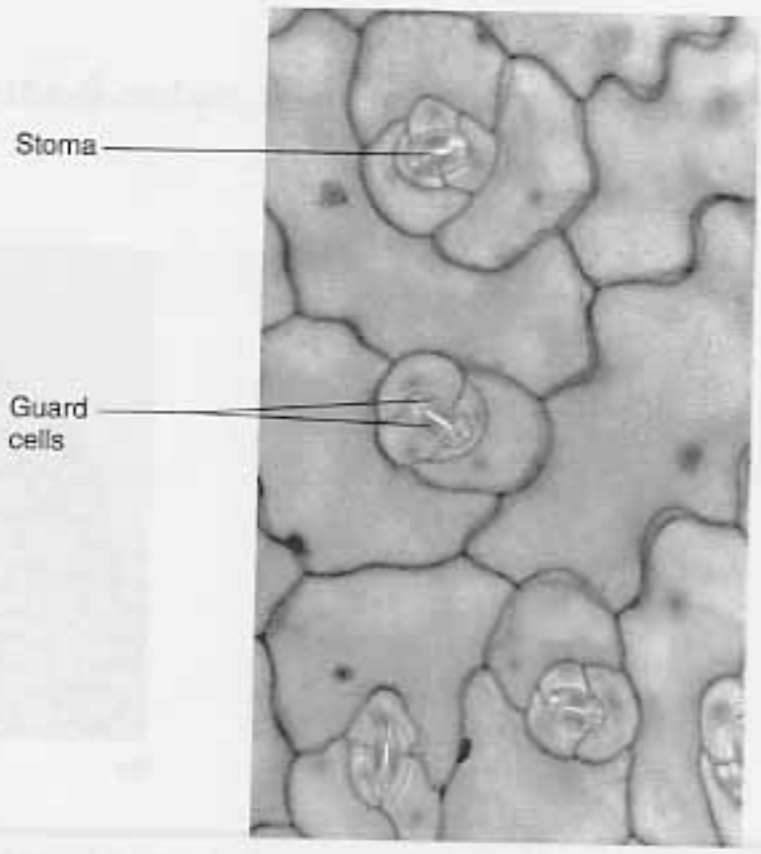


FIGURE 3.2 LEAF EPIDERMIS CONTAINS STOMATA FOR GAS EXCHANGE (×] 270).

The water-conducting cells of the xylem are tracheids and vessel elements (fig. 3.3*a*). At maturity, these cells have secondary walls and lack cytoplasm. **Tracheids** are elongated cells with tapering ends and many prominent pits in the side walls. Within the xylem, tracheids overlap, and water can pass from one tracheid to another through the pits in adjoining walls. Tracheids also function in support. In contrast to the long, thin tracheids, vessel elements are shorter and wider. They often have horizontal end walls with large openings (fig. 3.3*a*). **Vessel elements** are attached end to end at these large openings to form long, pipelike vessels. Water readily moves through the vessels, passing from one vessel element to the next through the large openings. Like the tracheids, the side walls have numerous pits. In addition to the conducting cells, xylem also contains fibers and parenchyma cells. The fibers provide additional support, while the parenchyma cells are involved in storage and other metabolic activities. Primary xylem originates from the apical meristem, and secondary xylem originates from the vascular cambium. In trees, the secondary xylem can become very extensive; it makes up the tissue we call wood. We will look at the cells in wood in today's lab; xylem is examined in more detail in Laboratory Topics 4 and 15.

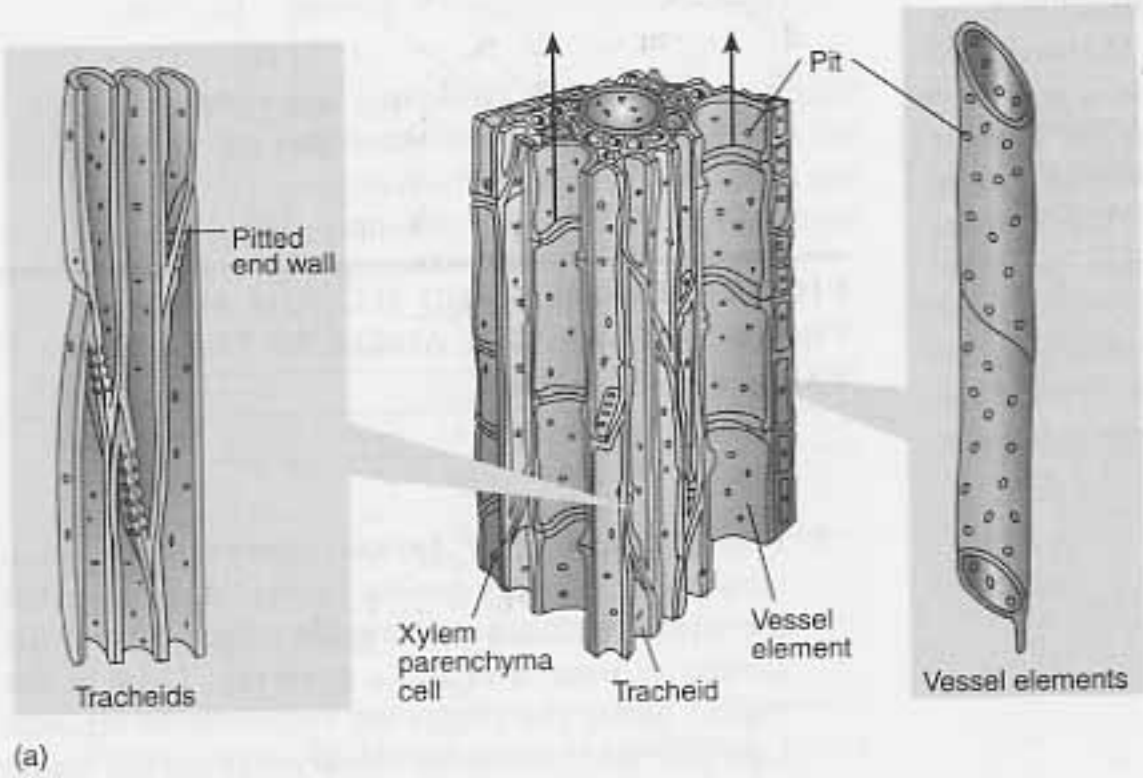


FIGURE 3.3 (*A*) XYLEM. TRACHEIDS AND VESSEL ELEMENTS ARE THE CONDUCTING CELLS. ARROWS INDICATE THE DIRECTION OF WATER FLOW. (*B*) PHLOEM. COMPANION CELLS LOAD SUGARS INTO THE SIEVE-TUBE MEMBERS FOR TRANSPORT.

In the phloem, the cells involved in transporting organic materials are known as **sieve-tube members** (fig. 3.3*b*). Sieve-tube members are elongate, living cells with thin primary walls. The end walls are often horizontal and have several to many large pores. These regions with pores are called sieve plates; cytoplasmic connections occur between adjacent sieve-tube members through the pores in the sieve plate. Organic materials are transported through these cytoplasmic connections from one sieve-tube member to another. Beside each sieve-tube member is a companion cell that is physiologically and developmentally related to the sieve-tube member. The companion cell functions in loading or unloading organic material into the sieve-tube member before and after transport. The phloem also contains fibers and parenchyma cells. Primary phloem is produced by the apical meristem, and secondary phloem by the vascular cambium. In this exercise, we will look at sieve-tube members; phloem is examined in more detail in Laboratory Topic 4.

Materials Needed for Exercise A

Dissecting microscope and compound microscope

Dropper bottle containing distilled water

Dropper bottle containing phloroglucinal-HCl stain. This is a useful stain for studying plant structure because it stains lignified walls red. You must be careful when using this stain because it is prepared with hydrochloric acid.

Dropper bottle containing toluidine blue stain

Glass slides, coverslips, single-edged safety razor, and dissecting needles

Pieces of potato, onion, celery, pear, a geranium leaf, and one other leaf type

Prepared slide of *Cucurbita* stem

Prepared slide of macerated angiosperm wood

Prepared slide of *Sambucus* (elderberry) stem

Prepared slide of *Sedum* epidermis

" " " CORN STEM

Procedure for Exercise A

1. **Freehand section of a piece of potato.** Your instructor will demonstrate how to make freehand sections of plant tissue with a razor blade. Hold a wedge of potato in one hand (fig. 3.4) and a single-edged razor blade in the other hand. Make a thin cut at right angles to the long axis of the plant tissue, and place the section in a drop of distilled water. Add a coverslip. Examine with the compound microscope at low (10 × objective) and high (40 × objective) power. The parenchyma cells are filled with starch grains (leucoplasts or amyloplasts) that almost obscure the shape of the cells. Draw these parenchyma cells in the following space:

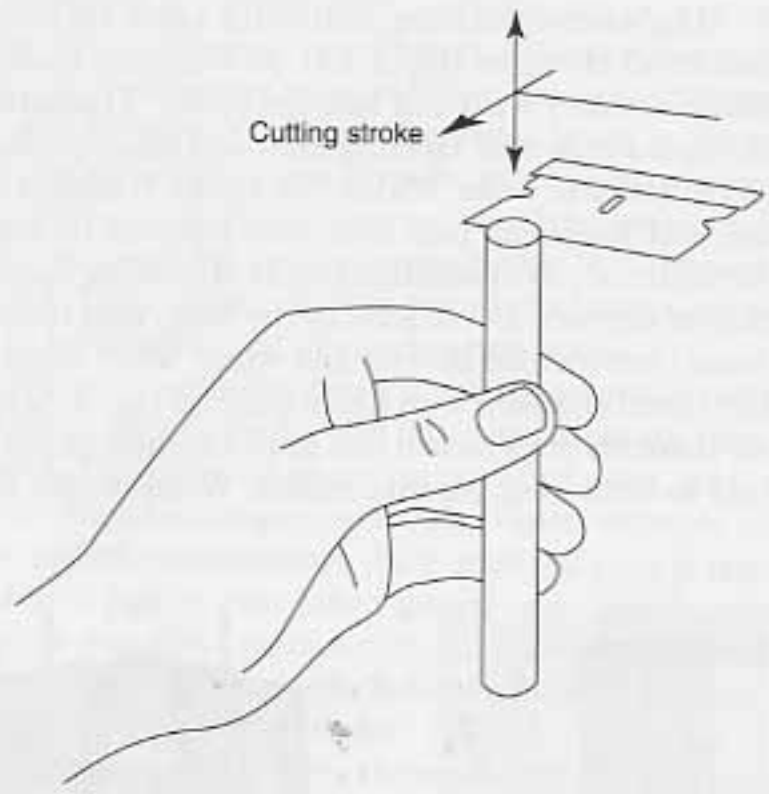


FIGURE 3.4 FREEHAND SECTION. MAKE A THIN CUT AT A RIGHT ANGLE TO THE LONG AXIS OF THE TISSUE.

2. ***Sambucus* (prepared slide).** Examine a slide that shows a cross section of *Sambucus* (elderberry) stem. Parenchyma cells are in the pith (the center of the stem). Are these the same shape as the potato cells? How do they differ? Can you locate any collenchyma cells on this slide? Where are they?

3. **Freehand section of *Apium* (celery).** Make a thin section of the celery petiole (stalk). Make sure that the section includes one or more ridges. Mount the section in water and add a coverslip. Look at the region under the ridges for collenchyma strands. Can you see the uneven thickenings on the walls? Draw the collenchyma cells you see in the following space. You should also see parenchyma cells below the collenchyma.

4. **Mashed section of *Pyrus* (pear).** Obtain a very small section of pear fruit. Using dissecting needles, mash it on the slide. Add a drop of phloroglucinol-HCl stain and then a coverslip. (Be careful when adding this stain because it is prepared using hydrochloric acid.) What is the shape of the sclereids in pear? How

are they arranged? Draw a few of the sclereids in the following space:

5. ***Sedum* epidermis (prepared slide).** Examine a slide showing the epidermis of a sedum leaf. What is the shape of the epidermal cells? Locate the stomata and guard cells. How are the guard cells different from the other epidermal cells?

6. **Fresh section of *Allium* (onion) epidermis.** Take a small portion of onion and carefully remove the inner epidermis from one section. This layer should peel off quite easily. Place the epidermis in a drop of toluidine blue stain. Add a coverslip and examine with the compound microscope. This tissue shows a typical monocot epidermis (rectangular, bricklike cells). Are there any stomata? Guard cells? Draw a portion of the epidermis in the following space:

7. **Fresh specimens of leaf trichomes.** Obtain a geranium leaf and one other leaf as provided by your instructor. Examine the leaves with the dissecting microscope. Take a small part of a leaf and examine its edge under your compound microscope. Can you see the trichomes? Some of the leaves (such as the geranium leaf) may contain glandular trichomes. See if you can locate a glandular trichome. In the following space, draw the trichomes, and indicate whether they are glandular or nonglandular:

8. **Macerated angiosperm wood (prepared slide).** Macerated wood is wood that has been treated with chemicals to dissolve the middle lamella between the cells. This allows the cells of the wood to separate. Examine a prepared slide. Can you find vessels, tracheids, fibers, and parenchyma cells? Draw the types of cells you see in the following space:

9. ***Cucurbita* (squash) stem, cross section (prepared slide).** In squash stems, the sieve-tube members in the phloem have prominent sieve plates. The phloem occurs in the vascular bundles in the stem. Your instructor will explain where to find the phloem. Look for the sieve plates. Companion cells should also be visible beside the sieve-tube members. When you have finished examining the sieve-tube members, look at the cells beneath the epidermis below the ridges. Can you recognize the types of cells you are looking at? What are they?

EXERCISE B: Economic Fibers

Botanically, the term *fiber* describes one type of sclerenchyma cell, but commercially the term has a much broader definition and includes other plant cell types as well as animal and synthetic sources of fabrics. Since prehistoric times, plants have been used as a source of material for textiles and cordage. Archaeological evidence indicates the Swiss Lake dwellers used linen fabric 10,000 years ago. Similar evidence for the use of cotton has been discovered in excavations in coastal Peru.

Commercial fibers are categorized as bast fibers, hard fibers, and surface fibers. **Bast fibers,** or soft fibers, are phloem fibers from a variety of dicot stems. Bast fibers may or may not contain lignin, but they are always soft and flexible. The best-known bast fibers are linen or flax (*Linum usitatissimum*), hemp (*Cannabis sativa*), jute (*Corchorus capsularis*), and ramie (*Boehmeria nivea*).

Hard fibers are derived from certain leaves, where bundles of fibers are associated with the vascular bundles (fig. 3.5). These fibers typically have heavily lignified walls and feel hard and stiff to the touch. Sisal (*Agave sisalina*) and Manila hemp (*Musa textilis*) are widely used hard fibers. Today they are mainly used to produce rope, although in the past these fibers were used to make fabric as well.

Surface fibers are found on the surface of seeds and fruits. The best-known surface fiber is cotton (*Gossypium* spp.). Cotton fibers are seed hairs (trichomes) that cover the surface of a cotton seed. As many as 20,000 trichomes may be present on a single seed. The trichomes are long and composed of approximately 90% cellulose. Other surface fibers include kapok (*Ceiba pentandra*) and coir (*Cocos nucifera*).

One final type of botanical fabric that should be considered is bark cloth, a primitive fabric made in tropical and subtropical countries from the soft inner bark of certain trees. In the Pacific Islands, paper mulberry (*Broussonetia papyrifera*) trees are the source of bark cloth. To prepare this fabric, the inner bark is cut into narrow strips, alternately soaked in water to soften the fibers, and then beaten with a mallet. The result is a well-matted, thin, and flexible material. Bark cloth is known as *tapa* cloth in the South Pacific and *kapa* cloth in Hawaii. Today, it is primarily used for decorative and ceremonial objects. A similar type of bark cloth is made in Mexico and Central America from the bark of *Ficus* trees. This bark cloth is presently used for decorative bark paintings called *amate* (see cover photo), but it was used as paper by the Aztec civilization.

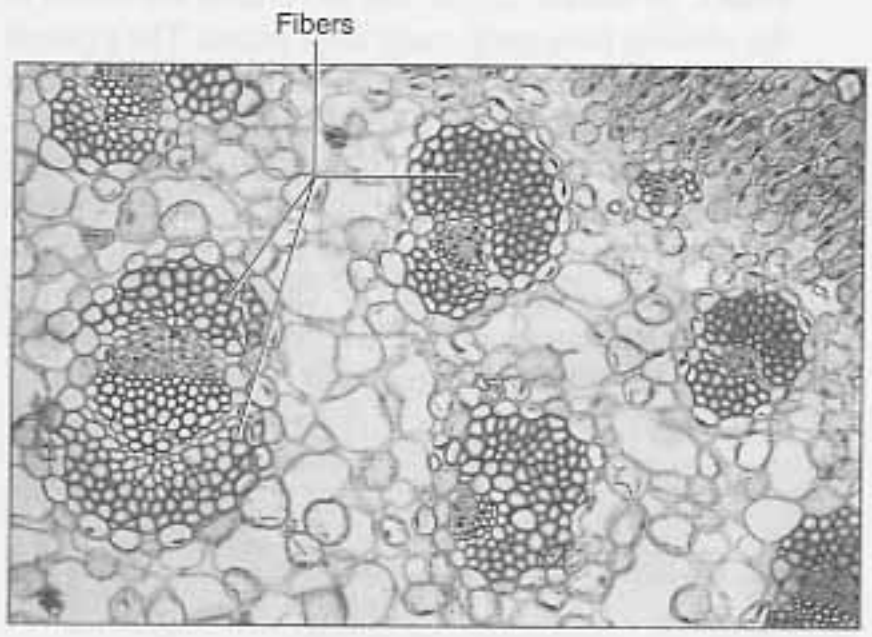


FIGURE 3.5 HARD FIBERS OCCUR IN CERTAIN LEAVES, WHERE THEY ARE ASSOCIATED WITH THE VASCULAR BUNDLES.

Materials Needed for Exercise B

Bulk samples of linen, cotton, jute, sisal, manila hemp, tapa cloth, and other materials (as provided by instructor)
Compound microscope
Dissecting needles
Glass slides and coverslips
Phloroglucinol-HCl stain
Prepared slides of *Agave* leaves
Tiny samples of each of the above fabrics

Procedure for Exercise B

1. Examine the bulk samples of materials made from several plant fibers. Evaluate the texture of these fabrics, and enter a brief description in worksheet 3–1 at the end of this laboratory topic.
2. Obtain a tiny sample of each of these fabrics or rope, and place it on a microscope slide. Add a drop of phloroglucinol-HCl stain. With two dissecting needles, shred the material. Allow the material to sit in the stain for several minutes; then add a coverslip and examine under the microscope. Remember that phloroglucinol is a specific stain for lignin. Evaluate the staining reaction, and enter it in worksheet 3-1.
3. Examine the prepared slide of *Agave* leaves. The intensely stained regions are bundles of fibers surrounding the vascular tissue. These bundles of fibers are the source of the hard fibers used to prepare sisal rope or fabric. Compare the slide with figure 3.5.

EXERCISE C: Papermaking

Historians believe that the Sumerians utilized the earliest writing surfaces, beginning about 5,000 years ago; these were clay tablets. About 4,500 years ago, the Egyptians developed **papyrus** sheets (the word *paper* comes from this), and since that time writing surfaces have remained largely of botanical origin. Papyrus (*Cyperus*

papyrus) is a sedge (grasslike plant growing in wet places) that is native to Egypt and surrounding areas. The writing surface was made from thin slices of pith (from the center of the stem) that were beaten and laid lengthwise; then other strips were laid crosswise on top. The mat was moistened, then pressed, dried, and rubbed smooth. Papyrus continued to be used as a writing surface for about 3,000 years. Today, it is mainly used for decorative pieces (fig. 3.6).

True paper is prepared from pulp, which is a slurry of separate plant cells dispersed in a watery suspension. Papermaking can be traced back to the second century in China, where the inner bark of the paper mulberry tree (along with other plants) was used to prepare pulp. Similar processes were independently developed by the Aztecs and the Mayans in the New World. Many different types of plant material can be used as a source of pulp, including straw, leaves, stems, and even old rags. The cells in the pulp are matted into a thin layer and then compressed; however, the cells must be long enough to form a mat when the water is drained off. Typically, these cells are tracheids, vessels, and fibers, but in papermaking terms, they are all called fibers. Wood pulp was first used to make paper in 1840, and today most paper is prepared from wood pulp. In fact, each year approximately one billion trees are cut down to satisfy our demand for paper, and active research is being conducted to find alternative sources of pulp. In this lab, we will be making paper from pulp produced from a variety of plant materials.

FIGURE 3.6 MODERN PAINTING ON PAPYRUS SHEET.

Materials Needed for Exercise C

Blender

Extra piece of screen

Iron and ironing board (or other surface that will not be harmed by the heat of the iron)

Microwave oven (optional)

Mold and deckle frame for papermaking

Plastic tub (dishwashing tub) containing warm water

Rolling pin

Several pieces of blotting paper

Sponge

Various plant materials, such as leaves, flowers, petals, straw, etc.

Procedure for Exercise C

1. Select the type of plant material you want to use for your paper, and tear or cut it into small pieces (about 2–3 cm in diameter).
2. Read the following instructions completely before you begin.
3. Obtain the papermaking wooden form, called a **mold and deckle** (fig. 3.7). The mold is the bottom half with the mesh screen attached. The deckle is the open frame. Notice that the deckle fits snugly on top of the

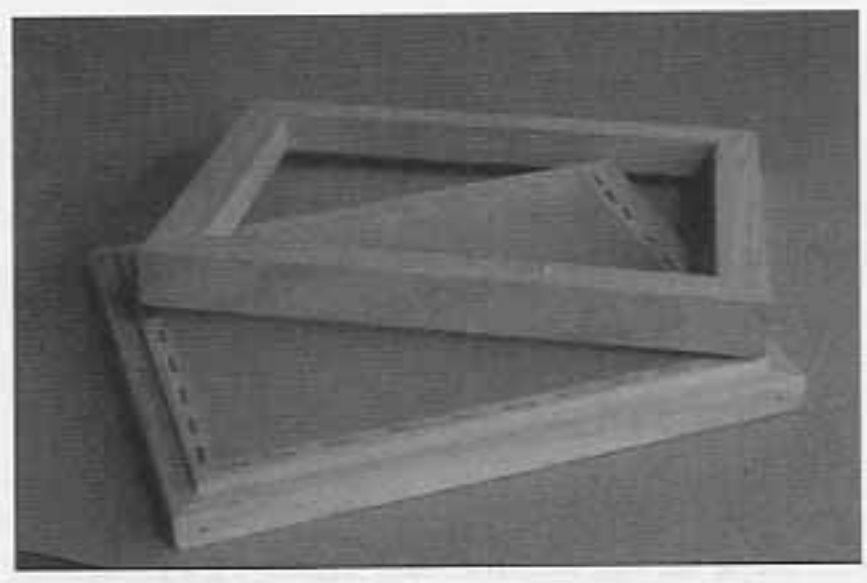


FIGURE 3.7 THE MOLD AND DECKLE IS THE BASIC UTENSIL FOR MAKING PAPER.

mold. This is the basic utensil that has been used to make paper for hundreds of years.

4. Put the mold and deckle together, and place it in the plastic tub. Hold the mold and deckle down, and add enough lukewarm water so that the level of the water is just below the top of the mold and deckle. Leave them in the water.
5. Fill the blender about one-half to two-thirds full with lukewarm water. Add the material you have selected for pulp and let it soak up water for 2 or 3 minutes. Cover the blender and blend on low for about 10 seconds, then on high for about 30 seconds. This should make a nice, smooth slurry. If there are lumps, continue blending for another 10 to 15 seconds.
6. While holding the mold and deckle in the water, slowly pour the pulp onto the screen. If any pours on the deckle, push it onto the mold. Swirl the pulp around to distribute it evenly over the screen. Try not leave any thin spots. If you have too much pulp, save it for a second sheet of paper.
7. While holding the mold and deckle together with both hands, lift straight up out of the water. Hold the form over the tub until it stops dripping. (If the pulp has shifted too much, you can put the mold and deckle back in the water and swirl it around again to redistribute.)
8. Place the mold and deckle on your lab bench (on a dry towel or paper towels). Carefully lift the deckle off the mold. Place the extra piece of screening on the "new paper." Using a damp sponge, gently squeeze down, pressing out more water. Squeeze out water from the sponge, and continue blotting and squeezing. Then, holding the extra screening on the paper, turn the mold over onto a dry area of the towel. Use the sponge to squeeze out more water from the underside of the mold.
9. Very carefully lift the mold away. This will leave your new paper on the separate piece of screen. (*Note:* If you find that the paper is sticking to the mold, peel it off gently, starting at one corner.)
10. Obtain a piece of dry blotting paper and place it on top of your new paper. Turn this sandwich (blotter/paper/screen) over so that the screen is on top and the dry blotter is on the bottom. Use a rolling pin to roll over the screen several times; this will squeeze out more water into the blotter.
11. Carefully remove the screen and replace it with a dry blotter. Flip this over again so that the new dry blotter is on the bottom. Roll again. This process of pressing the sheet to a dry blotter is called "couching." Repeat one or two more times with additional dry blotters.
12. Your new paper is now ready to dry. If a microwave oven is available, place the paper in the microwave for 1 to 2 minutes on high. Alternatively, the paper can be placed in a warm place to dry. (*Note:* the blotting paper can also be dried in the microwave and reused by other students.)
13. If your new paper is wrinkled, iron it between two sheets of clean white paper. Set the iron on "wool" setting with no steam. (*Note:* If you intend to use your paper for writing, you may wish to add sizing, which adds a smooth coating to the paper. It also prevents ink from bleeding through the paper. Starch or gelatin can be used for the sizing, but this is an optional step that can be done at home.)

TERMS TO KNOW

bast fibers 30
collenchyma 26
companion cells 27
cuticle 26
cutin 26
dermal tissues 25
epidermal cells 26
epidermis 26
fibers 26
glandular trichomes 26
ground tissues 25
guard cells 26
hard fibers 30
lignin 26
macerated wood 29
mold and deckle 31
papyrus 30
parenchyma 25
periderm 26
phloem 26
sclereids 26
sclerenchyma 26
sieve plate 27
sieve-tube members 27
stomata 26
surface fibers 30
tracheids 27
trichomes 26
vascular tissues 25
vessel elements 27
xylem 26

QUESTIONS FOR REVIEW AND DISCUSSION

1. What are the three tissue types in vascular plants? What are their primary functions?
2. What is the function of vessel elements and tracheids?
3. What is the function of fibers and sclereids in plants?
4. What are the conducting cells in the phloem? How do they differ from the conducting cells in the xylem?
5. What cells regulate the opening and closing of the stomatal pore? How do these cells differ from other cells in the epidermis?
6. What are trichomes?
7. What parts of the plant supply economic fibers that are used in fabrics and cordage?
8. What can be used as sources of pulp for paper production?
9. Briefly describe the steps in papermaking.

ADDITIONAL RESOURCES

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Luoma, J. R. 1997. The magic of paper. *National Geographic* 191(3): 88–109.

Mauseth, J. D. 1988. *Plant anatomy.* Menlo Park (CA): Benjamin/Cummings Publishing Co.

Simpson, B. B., and M. Conner-Ogorzaly. 1995. *Economic botany. Plants in our world*, 2d ed. New York: McGraw-Hill Companies, Inc.

Thompson, J. 1994. Cotton, king of fibers. *National Geographic* 185(6): 60–87.

ON THE WEB

Plant Tissues in Kimball's Biology Pages, John Kimball
http://www.ultranet.com/~jkimball/BiologyPages/P/PlantTissues.html

Plant Biology at OSU Lima, Plant Tissues and Cell Types
http://www.lima.ohio-state.edu/biology/celltype.html

Institute of Paper Science and Technology, Museum of Papermaking
http://www.ipst.edu/amp/

Wayne's Word, Plant Fibers: Fibers for Paper, Cordage, and Textiles
http://daphne.palomar.edu/wayne/traug99.htm

Economic Botany: Plants for People, J. R. Voos
http://euphrates.wpunj.edu/courses/bio352/week13/WEEK13.HTM

OTHER ACTIVITIES

A scanning electron microscope (SEM) can provide details of leaf surfaces and trichomes not visible with a light microscope. These details can be seen by studying photographs (micrographs) taken with an SEM. Examine micrographs of leaf surfaces. These may be available in lab or your instructor may have you research this topic in the library or on the Internet. (If an SEM is available in your biology department, you may have the opportunity to see the leaves directly at high magnification.) Observe the structure and branching pattern of any trichomes visible. Look for both glandular and nonglandular trichomes. Describe them. Also note the leaf surface. Do the trichomes and leaf surface appear the same as they do when viewed with the dissecting microscope? What differences can you see?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 3-1 EXERCISE B: ECONOMIC FIBERS

Fabric/Fiber	*Texture*	*Phloroglucinol-HCl Reaction*	*Lignified or Nonlignified*
Cotton			
Linen (flax)			
Jute (burlap)			
Manila hemp			
Sisal			
Tapa cloth			
Other			

What can you conclude about the presence of lignin and the texture of the resulting product?

LABORATORY TOPIC 4

Plant Architecture

BACKGROUND

Even the most casual observer of the green world knows that **roots** anchor and absorb water and minerals, **leaves** trap solar energy in the process of photosynthesis, and **stems** support the leaves, conduct water, and transport organic solutes. Roots, stems, and leaves are the vegetative organs that comprise the architecture of plants—and are the targets of investigation for this laboratory. You will see how the construction of these organs reflects their functions and how to recognize the telltale anatomical differences between them. You will learn that the great monocot–dicot divide in the angiosperms is reflected in discernible differences in their vegetative organs.* Some of your conceptions of common vegetables may change as you learn that they are not always what they seem. You will also revisit a Victorian craft to reveal and appreciate the lacy beauty within leaves.

This discussion is limited to the primary or herbaceous plant body: the roots, stems, and leaves that originate from primary meristems (see Laboratory Topic 2). Woody stems and roots from secondary growth are discussed in Laboratory Topic 15.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Understand the functional anatomy of roots, stems, and leaves.
2. Recognize the anatomical differences between the vegetative organs of monocots and dicots.
3. Recognize how environmental selection results in modifications of plant anatomy.
4. Use a leaf key to identify trees.
5. Create a leaf skeleton.
6. Use knowledge of plant anatomy to practice supermarket botany.

*The **angiosperms** or **flowering plants** are the chief sources of food, beverages, medicines, wood, herbs and spices, cloth, perfumes, vegetable oils, and much more. Within the flowering plants, there are two groups: the **monocots** and **dicots**. Some familiar monocots include all grasses, palm trees, orchids, and lilies while dicots are exemplified by oaks, maples, mints and roses.

EXERCISE A: Roots to Anchor and Absorb

The typical plant root is a vertical, underground axis, and as such it provides the plant with a firm footing in the soil. Roots also function as the primary source of water and mineral absorption. Additionally, roots are often storage sites for plants. In arid regions, the root may store water. Roots may also be enlarged for the storage of starch, an energy reserve.

The root systems are organized as either **tap** or **fibrous**. Common to many dicots, a tap root system has a single main root, enlarged for water and/or food storage, and many smaller branch roots. The fibrous root system is typical of monocots in which there are several main roots of equal size.

There are several functional regions at the tips of roots. The **root cap** is the thimble-shaped region of short-lived cells at the very end of a root. It protects underlying tissues as the root grows through the soil. It is also the part of the root that detects direction so that roots grow down, in the direction of gravity. Immediately above the root cap lies the **apical meristem** or **zone of cell division**, a site of actively dividing cells that accounts for the growth of roots and replaces cells in the root cap as they are worn off. Moving upwards, the next region is the **zone of elongation**, an area of rapid root growth as cells elongate. The **zone of maturation** is the final region in which embryonic cells differentiate into specialized tissues that make up the anatomy of a root. It is marked by the appearance of **root hairs**, fine extensions of the epidermis. Root hairs are the sites where water and minerals are first absorbed into the root.

In a cross section, the root tissues are arranged into two distinct regions. A larger outer region, called the **cortex**, surrounds the central core or **vascular cylinder**. The cortex is adapted for storage and the passage of water and minerals to the vascular cylinder. It is in the vascular cylinder that the conducting tissues of xylem and phloem are located.

Materials Needed for Exercise A

Compound light microscope
Dissecting microscope
Dropper bottle of distilled water
Dropper bottle of iodine solution (I_2KI)
Dropper bottle of phloroglucinol
Elodea specimen
Epiphytic orchid specimen (GREEN HOUSE)
Fibrous root example—grass
Filter paper to line petri dishes
Glass slides
Parsnips
Petri dish
Prepared slide of buttercup (*Ranunculus*) root cross section
Prepared slide of *Elodea* root cross section
Prepared slide of orchid root cross section
Radish seedlings
Razor blades, single-edged
Taproot example—dandelion

Procedure for Exercise A

1. Observe the display of the root systems of a grass and a dandelion (fig. 4.1). How would you describe the differences between these two systems? Which has a single main root? In which are there several roots of equal size? Which is an example of a taproot system? Which is a fibrous root system?

_____

_____

_____

_____

Taproots can extend to significant depths in some species; in mesquite, the taproot reaches a depth of 32 m (96 ft). Taproots are often greatly enlarged for food and/or water storage. The fibrous root, by contrast, is shallow but spreading. How can these two different root system designs both achieve the same result of securing a water supply? What are their alternative strategies?

_____

_____

_____

_____

(a)

(b)

FIGURE 4.1 ROOT SYSTEMS. (A) THE FIBROUS ROOT SYSTEM OF A GRASS. (B) THE TAPROOT SYSTEM OF A DANDELION.

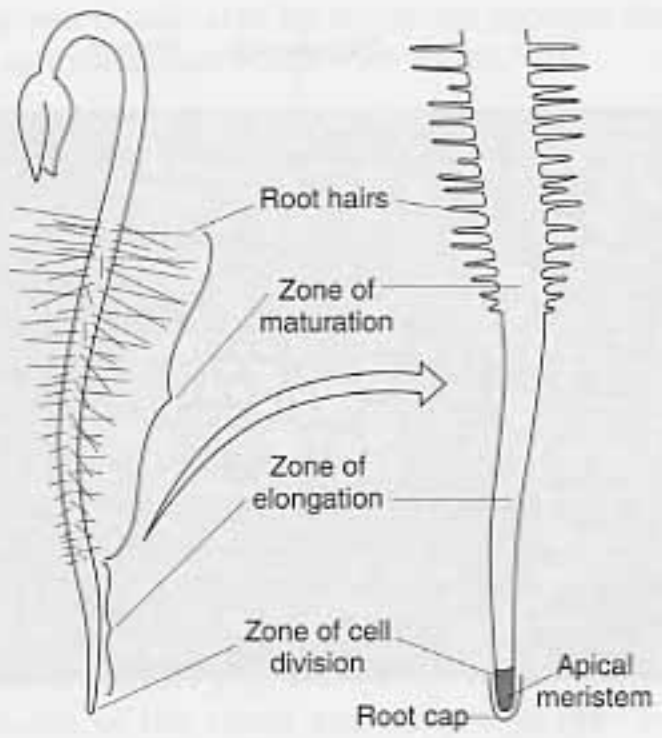


FIGURE 4.2 ROOT HAIRS AND ZONES IN A RADISH SEEDLING.

2. To view the zones of a root tip, obtain a young radish seedling and quickly transfer it to a petri dish kept humid with a lining of moist filter paper. Examine the seedling through the petri dish lid under the dissecting microscope. Locate the thimble-shaped root cap (fig. 4.2) at the very tip of the root. Immediately behind the root cap lies the zone of cell division. Next locate the zone of elongation. The zones of cell division and elongation are the sites of root growth. Still further up, the zone of maturation is indicated by the appearance of root hairs. Each root hair is an extension of a single epidermal cell and extremely fragile.
3. To examine the anatomy of a mature root, obtain a fresh parsnip. Make a thin cross section with a razor blade at the smaller end of the root. Place your thin section on a glass slide and view under scanning (4 × objective) or low (10 × objective) power of the compound light microscope. Do not cover with a coverslip. Observe the large, thin-walled cells of the cortex, the outer region of the root. Add a drop of iodine, the indicator test for starch. What color change do you notice? What type of tissue comprises the root cortex? What is stored here?

THE COLOR CHANGED TO A DARKER SHADE OF ~~YELLOW~~ BLACK + YELLOW.

SCLERENCHYMA TISSUE COMPRISES THE ROOT CORTEX WHERE THE STARCH IS STORED.

The central core is the vascular cylinder or **stele**. Add a few drops of phloroglucinol to the stele of the parsnip section. What color change do you notice? What type of tissue is found in the stele? (Refer to Laboratory Topic 3.)

THE COLOR CHANGED TO AN ORANGISH HUE.

Let's take a more detailed look at the internal anatomy of a root by viewing the prepared slide of the buttercup root.

4. Obtain a compound light microscope and a prepared slide of a cross section of buttercup (*Ranunculus*) root. The prepared slide has been sliced very thin, making it easier to observe the fine details.

   Again, observe the slide first under scanning (4 × objective) or low power (10 × objective) to see the overall organization of the root (fig. 4.3). From the exterior, note the single layer of **epidermis**. Immediately inside the epidermis and extending to the vascular cylinder is the cortex. As noted previously, the cortex consists of parenchyma cells, many of which contain stained starch grains. The innermost layer of cortex is the **endodermis**. The endodermis has been stained red. A waxy material, the **Casparian strip**, encircles each endodermal cell wall. Only the faces of the cell walls adjoining the cortex and vascular cylinder lack a Casparian strip. Because of this strip, water and minerals pass through the endodermal cells, not between them.

   Within the vascular cylinder, note the red-stained cross or star of xylem. The thin-walled phloem is located between the xylem points. This pattern of xylem and phloem within the vascular cylinder is typical of dicot roots.
5. Root anatomy can be modified by the environment. Examine a living specimen of *Elodea*, an aquatic angiosperm. *Elodea* is a **hydrophyte**, a plant adapted to a very wet environment—in this case, ponds and streams. *Elodea* is common in freshwater systems throughout North America, and you may recognize it as a popular aquarium plant. What observations can you make about the roots of the living *Elodea* specimen? How do they differ in appearance from the root system of a typical land plant of grass or dandelion that you examined earlier?

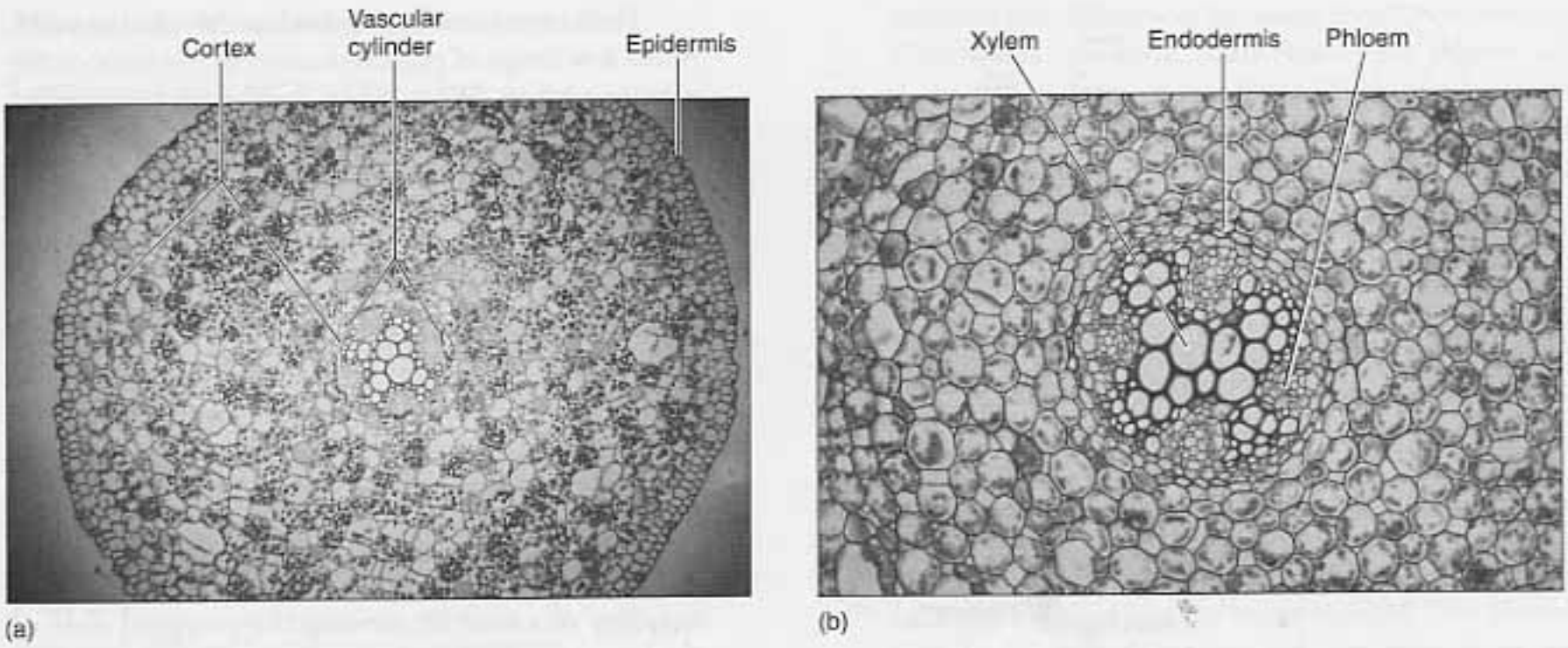


FIGURE 4.3 ROOT ANATOMY. (*A*) DICOT ROOT (BUTTERCUP) IN CROSS SECTION. (*B*) THE VASCULAR CYLINDER TYPICALLY SHOWS A STAR OR CROSS PATTERN.

Now examine the prepared cross section of the *Elodea* root (fig. 4.4*a* and *b*). How does the anatomy of the *Elodea* root differ from that of the buttercup root viewed earlier? Why do you think these modifications in the *Elodea* root evolved? (*Hint:* Examine the vascular cylinder closely!)

6. Many tropical orchids are **epiphytes**, or aerial plants. These are nonparasitic plants that rest upon the branches of other plants. Picture a gigantic tropical tree festooned with hundreds of orchids and vines. Observe the epiphytic orchid on display. Locate the roots. Unlike the roots you may typically think of, these epiphytic orchids have aerial roots, roots growing not in the soil but out into the air. Notice that the tips of the aerial roots are green. What is present that imparts this green color? What

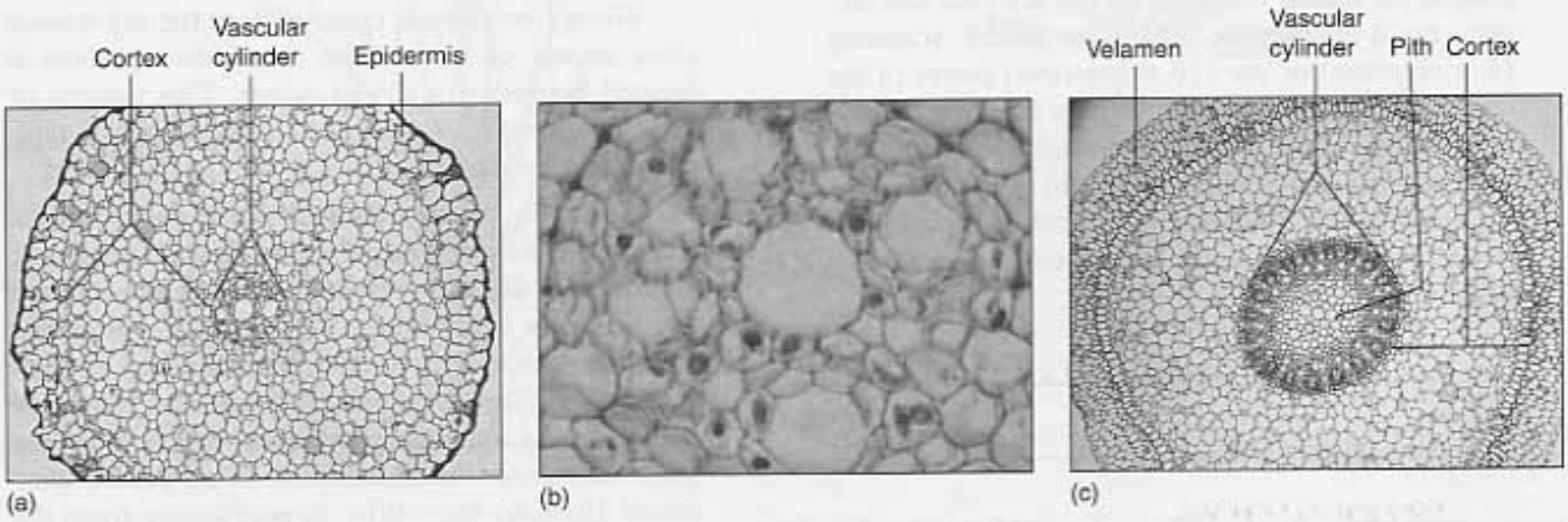


FIGURE 4.4 ROOT ADAPTATIONS. (*A*) THE ANATOMY OF AN *ELODEA* ROOT SHOWS ADAPTATIONS TO AN AQUATIC ENVIRONMENT. (*B*) CLOSE-UP OF VASCULAR CYLINDER OF *ELODEA* ROOT. (*C*) THE VASCULAR CYLINDER OF A MONOCOT ROOT TYPICALLY SURROUNDS A PITH. NOTE THE VELAMEN, A CHARACTERISTIC OF EPIPHYTIC ORCHID ROOTS.

function is indicated by the green pigment that we do not usually associate with roots?

Note that away from the root's growing tip, the root is no longer green, and is covered with a white tissue. This is **velamen**.

Obtain a slide of an orchid root cross section, and locate the epidermis, cortex, endodermis, and vascular cylinder (fig. 4.4*c*). Note the different organization of the xylem and phloem in the vascular cylinder of the monocot root. Unlike the dicot root, monocot roots have a center of parenchyma tissue, or **pith**, in the vascular cylinder. Sketch the stele of a monocot root.

Locate the velamen on the periphery of the cross section. What tissue appears to give rise to velamen? What function do you think velamen serves in the aerial root of an orchid?

Record your findings about root structure of monocots and dicots in worksheet 4-1 at the end of this laboratory topic.

EXERCISE B: The Nuts and Bolts of Stem Anatomy

When you think of stems, chances are you picture an upright axis of green that supports leaves. But in addition to support, the stem is the conduit for water and minerals up from the soil and roots as well as for transporting the organic products of photosynthesis (sugars) to the nonphotosynthetic, growing, and storage regions of the plant. Some stems are enlarged for storage of either water or starch. In this exercise, you will use a nut-and-bolt microtome to thin-section monocot and dicot stems in order to get a close-up look at their anatomy.

Materials Needed for Exercise B

Asparagus stem
Beakers, 30 ml
Compound light microscope
Coverslips
Creeping charlie (*Plectranthus australis*)
Dissecting needles
Dropper bottle of distilled water
Dropper bottle of methylene blue
Glass slides
Nut-and-bolt microtomes
Paraffin wax, melted
Petri dish lid
Razor blade, single-edged

Procedure for Exercise B

1. Obtain a small beaker and add a small volume (about 0.5 cm depth) of methylene blue. Using a razor blade, cut off a 5 cm section of the stem of creeping charlie and immediately place in the beaker, making sure the base of the stem is in contact with the dye. Allow the stem to set in the dye for about 10 minutes to give enough time for the methylene blue to be transported up the xylem.

   As you wait, prepare as above a section of asparagus stem to place in methylene blue.
2. Obtain two nut-and-bolt microtomes. A **microtome** is a device that is used to cut very thin sections for viewing under a microscope. Screw the nut so that it is on the last few rungs of the bolt. This should make a deep well.
3. Cut off about a 3 cm section of creeping charlie, a member of the dicot mint family. Trim the stem so that it fits into the well created by the nut and bolt and extends slightly above it. Obtain the melted paraffin from the warming tray. Position the stem section in the center of the well and fill the well with paraffin. Allow a cap of paraffin to top the stem. Set the nut and bolt in an undisturbed place to allow the paraffin to harden. This will take 10–15 minutes. As you wait, prepare the stem of asparagus, a member of the monocot lily family, in the same way.
4. When the paraffin has completely hardened, use a razor blade to trim off the top by cutting level with

FIGURE 4.5 NUT-AND-BOLT MICROTOME READY FOR SECITONING.

the top of the nut (fig. 4.5). Then twist the bolt so that the paraffin-embedded stem section is just a few millimeters above the nut. Shave off a section, and place it in the lid of a petri dish. Repeat the same procedure with the asparagus stem. Try to make your stem sections as thin as possible.

5. Place sections in a petri dish lid. Use dissection needles to gently remove the wax from the stem section.
6. Place a stem section on a glass slide, add a drop or two of distilled water, and cover it with a coverslip. Observe under the compound light microscope.
7. Examine the creeping charlie stem first under scanning (4 × objective) or low (10 × objective) power. Note the outermost single layer of cells, the epidermis (fig. 4.6). Note the large center of parenchyma tissue. As in roots, a center of parenchyma tissue is a pith. What would the region around the pith be called?

Observe the interrupted ring of **vascular bundles,** the xylem stained by methylene blue. In stems, the xylem and phloem are organized into bundles rather than a vascular cylinder. A ring of vascular bundles is characteristic of the herbaceous dicot stem.

7. Now examine the asparagus cross section you made. Again, what tissue comprises the outermost single layer in this herbaceous stem?

Locate the methylene blue-stained vascular bundles in asparagus. Do they form a ring as they did in the creeping charlie? Is a pith observable? How would you describe the pattern of vascular bundles in the asparagus, a monocot stem (fig. 4.7*a*)?

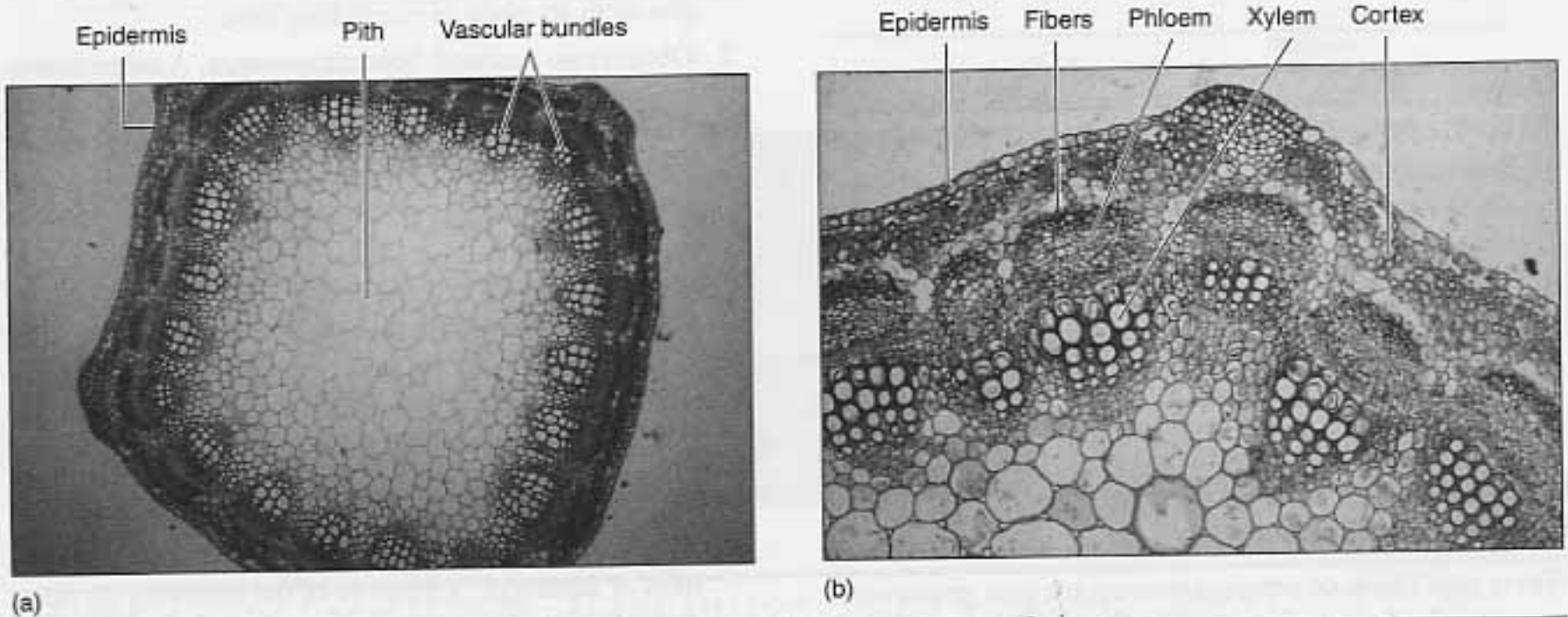


FIGURE 4.6 (A) DICOT STEMS HAVE VASCULAR BUNDLES IN A RING. (B) CLOSE-UP OF VASCULAR BUNDLES IN A DICOT STEM.

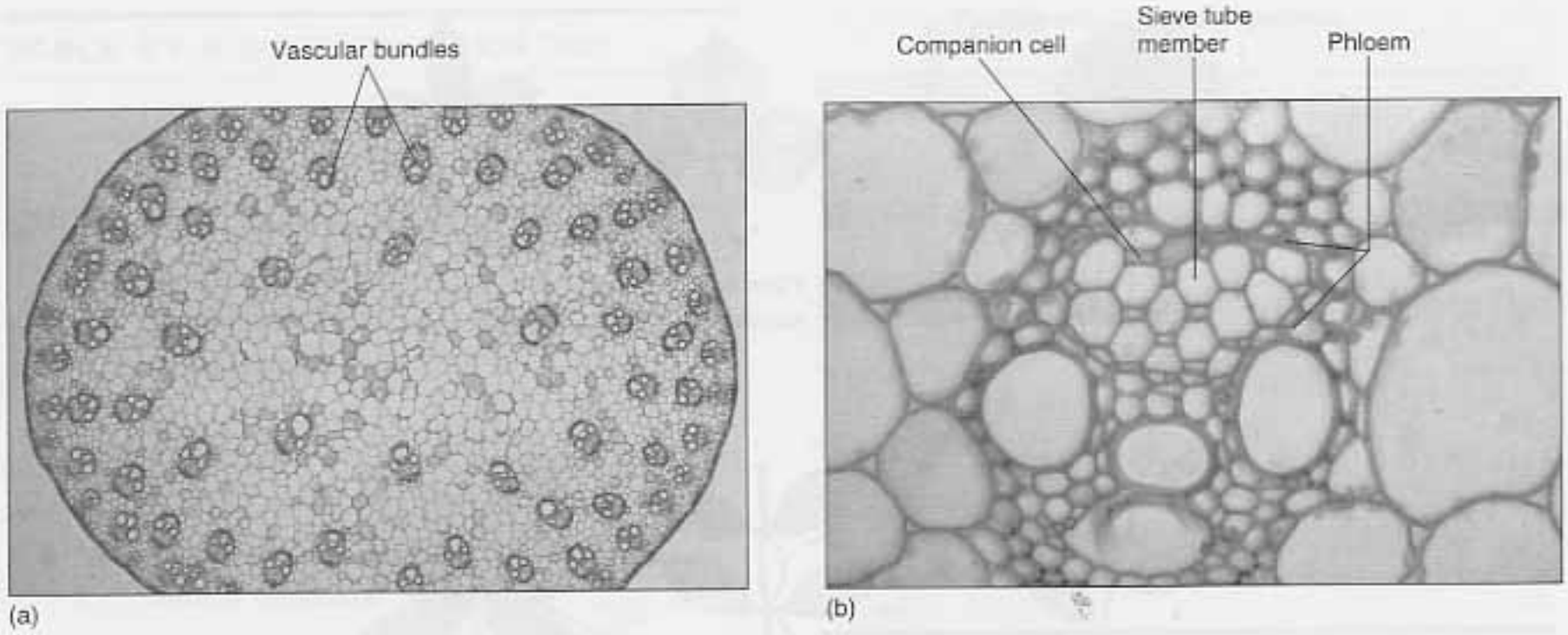


FIGURE 4.7 (*A*) IN MONOCOT STEMS, THE VASCULAR BUNDLES ARE SCATTERED. (*B*) CLOSE-UP OF A VASCULAR BUNDLE IN CORN, A MONOCOT STEM.

8. To see fine details, obtain a prepared slide of a cross section of a corn stem corn (*Zea mays*). Zoom in with 40 × high power on one of the vascular bundles. The entire bundle has been compared to a monkey face in appearance (fig. 4.7*b*). What type of cells or tissues would the two large, red "eyes" be?

_____

_____

The "forehead" of the "monkey face" is phloem tissue, and you may actually see a pattern of small square **companion cells** alternating with the larger **sieve-tube members**. The "mouth" is an air space. The entire "face," or vascular bundle, is surrounded by thick-walled fibers.

Record your findings about stem structure in monocots and dicots in worksheet 4-1 at the end of this laboratory topic.

EXERCISE C: Leaves for Identification

Leaves are the primary photosynthetic organs for most plants. As with stems and roots, the leaves of some species have been adapted to store water. Think of succulent plants like the jade plant or agave. In other species, leaves may be starch-storing. Leaves also are important players in gas exchange and in the water stream that flows upwards from the soil and transpires from the leaves. These functions of leaves are also discussed in Laboratory Topic 5.

Leaves are composed of three parts: a blade, a petiole, and a pair of stipules (fig. 4.8*a*). The **blade** is the flat, expanded portion of the leaf. The **petiole** is the stalk that supports the blade; in some plants, the petiole is absent. When this happens, the leaf is said to be sessile. **Stipules** are paired structures that may be found at the base of leaves. Their presence in many species is temporary since they are often shed early in the growing season. Stipules vary greatly in appearance. Some are like the spines, commonly called thorns, at the base of a rose leaf. Others, such as those of the sycamore tree, look like miniature versions of the leaves.

Leaves are further classified on the basis of composition, arrangement, and venation (fig. 4.8*a*). The appearance of the blade is important in determining **leaf composition**. If a blade is undivided, the leaf is classified as **simple**. However, if the blade is divided into separate pieces or **leaflets**, the composition of the leaf is said to be **compound**. In some leaves, even the leaflets are subdivided!

Often it may be difficult to tell whether you are looking at a simple leaf or a leaflet. Luckily, the position of the **axillary bud** (fig. 4.8*a*) is a good clue as to whether the leaf is simple or compound. The upper angle that forms between the top surface of a leaf and the stem is called the **axile**, and it is here that the axillary bud can be found. Axillary buds are only found in the axile of a leaf. Leaflets do not have axillary buds.

There are two types of compound leaves. When the leaflets occur in a featherlike pattern, it is a **pinnately compound** leaf. If all the leaflets arise from a single point, it is a **palmately compound** leaf (fig. 4.8*a*).

Arrangement pertains to how leaves are ordered on a stem. **Nodes** are areas on a stem that give rise to leaves or branches. The areas of stem between nodes are called **internodes**. If there is only one leaf at a node, the leaf arrangement is called **alternate**. If the number of leaves

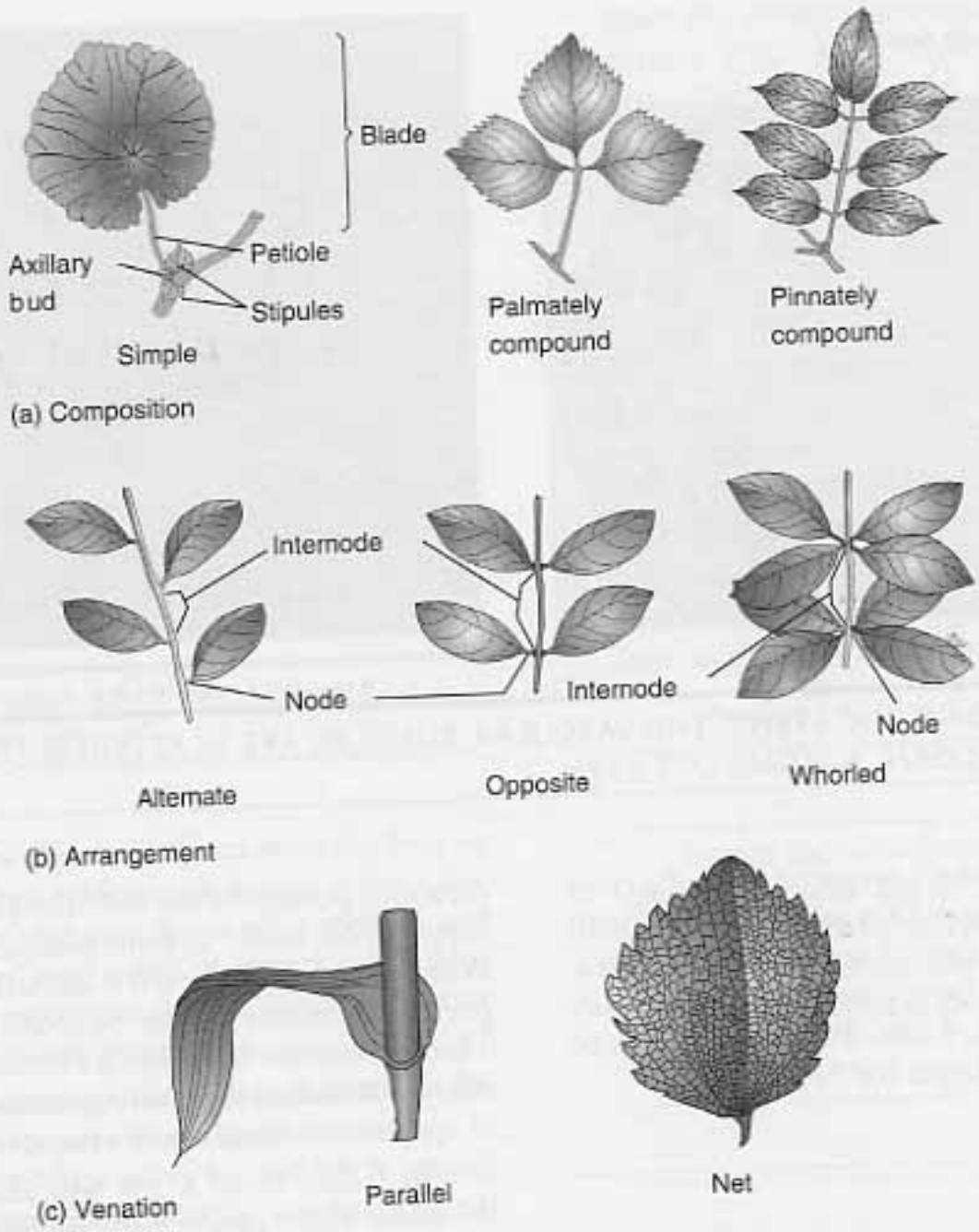


FIGURE 4.8 LEAF MORPHOLOGY. (A) A LEAF HAS A BLADE, A PETIOLE, AND A STIPULE PAIR. AXILLARY BUDS ARE FOUND IN THE AXILE. IN LEAF COMPOSITION, LEAVES MAY BE SIMPLE, CONSISTING OF A SINGLE BLADE, OR COMPOUND, IN WHICH THE BLADE IS SUBDIVIDED INTO LEAFLETS. (B) LEAF ARRANGEMENT. ALTERNATE, OPPOSITE, OR WHORLED INDICATES THE NUMBER OF LEAVES COMING OFF A NODE. (C) LEAF VENATION. THE VENATION PATTERN IS COMMONLY PARALLEL IN MONOCOT LEAVES AND NET IN DICOT LEAVES.

at a node is upped to two, the arrangement is **opposite**. With more than two leaves at a node, the arrangement is **whorled** (fig. 4.8*b*).

Leaf venation is another easily recognizable characteristic. Leaf veins are continuations of the vascular bundles, you saw earlier in the stem. Venation refers to the pattern of the veins, and once again, it divides along monocot and dicot class lines. In most monocot leaves, all of the major veins are **parallel**. In dicot leaves, the veins branch into ever smaller veins to forming an overall lacy appearance. This type of vein arrangement is called **net** (fig. 4.8*c*).

In this exercise, you will learn how a diagnostic key based on the characteristics of leaves can be helpful in tree identification. You will also learn the components of leaf anatomy and how the anatomical design of leaves can be modified by environmental selection.

Materials Needed for Exercise C

Branch of living privet
Compound light microscope
Leafy branches for tree leaf key
Leaves to demonstrate composition, venation, and arrangement
Living *Aloe vera* plant
Prepared slide of aloe (*Aloe*) leaf cross section
Prepared slide of privet (*Ligustrum*) leaf cross section

TABLE 4.1 A LEAF KEY TO COMMON TREES

a. Leaves simple . . . . . b
  b. Margin entire c
    c. Leaves opposite or whorled . . . d
      d. Leaves opposite . . . dogwood
      d. Leaves whorled . . . .catalpa
    c. Leaves alternate . . . . redbud
  b. Margin lobed . . . . e
    e. Leaves alternate . . . . . oak
    e. Leaves opposite . . . . . maple
a. Leaves compound . . . f
  f. Pinnately compound . . . . . g
    g. Leaves alternate . . . . black walnut
    g. Leaves opposite . . . . ash
  f. Palmately compound . . . . . buckeye

Procedure for Exercise C

1. Examine the leaves from several species on display and identify for each the blade, petiole, and stipules.
2. Examine the leaves on display in the lab and determine for each whether it is simple or compound, and if compound, whether the leaflets are arranged pinnately or palmately. Also determine leaf arrangement (alternate, opposite, or whorled) and venation patterns (net or parallel).
3. With just a basic understanding of leaf morphology, you can use a key to identify some common trees. A key is a tool that through a series of paired questions leads to the identification of an unknown, in this case, the identify of a tree. Practice your botanical know-how and identify some common trees using the leaf key in table 4.1.
4. To study leaf anatomy, obtain a compound light microscope and a prepared slide of a privet leaf cross section. The privet is an evergreen hedge used frequently in landscaping.

   At the top surface of the cross section, locate the **upper epidermis** (fig. 4.9). Note that the epidermal cells are translucent and do not contain chloroplasts. Observe the noncellular layer, or **cuticle,** that covers the upper epidermis. It is composed primarily of **cutin,** a waxlike substance that reduces evaporative water loss.

   The middle layers of the leaf are called appropriately the **mesophyll** (middle leaf). It is here that the photosynthetic cells are located. Examine the upper layer of the mesophyll. These are the **palisade cells**. Sketch their shape in the following space:

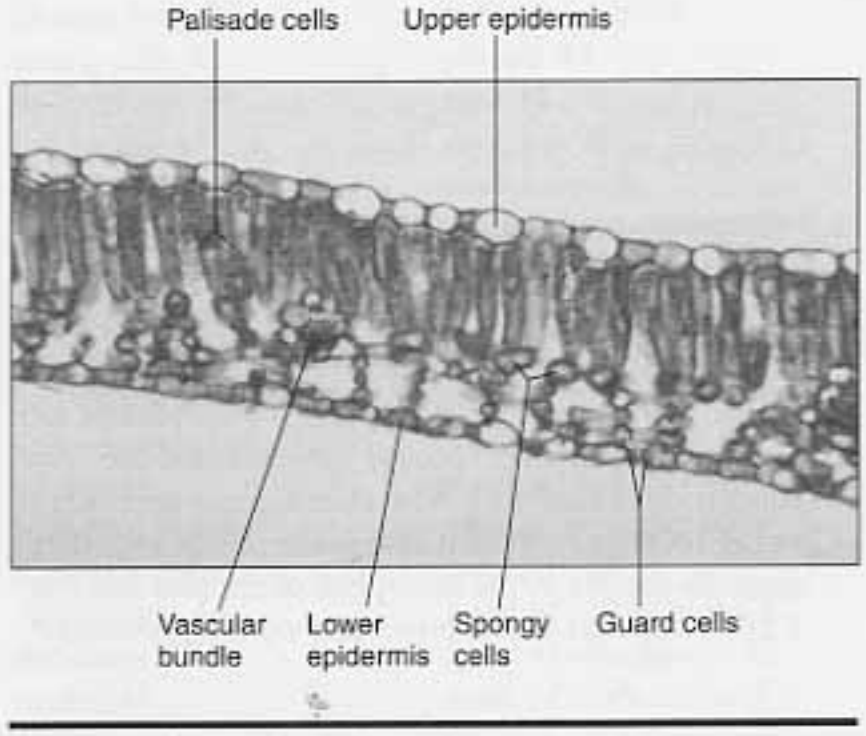


FIGURE 4.9 CROSS SECTION SHOWING THE LEAF ANATOMY OF PRIVET (*LIGUSTRUM VULGARIS*).

Less regular in arrangement and shape are the underlying **spongy cells**. Sketch a sample of these cells in the following space:

The intercellular spaces in the spongy mesophyll allow a continuous pathway through which gases can diffuse from the atmosphere into the leaf and vice versa. Locate the **vascular bundles** that can be seen in the mesophyll. Vascular bundles conduct water and organic solutes throughout the leaf and are, in fact, the veins now seen in cross section that you saw when viewing the leaf surfaces. Identify the xylem (red-stained) and phloem in a vascular bundle and the surrounding **bundle sheath** of fibers (also red-stained).

The undersurface of the leaf is again covered with epidermis sealed with a much thinner cuticle layer. **Guard cells** can be seen in the **lower epidermis**. In cross section, guard cells are paired, green, and smaller than the epidermal cells. Often the **stoma,** (pl. **stomata**), appears as a space between the guard cell pair. The presence of what organelles would account for the green color of guard cells? Draw a pair of guard cells in the following space:

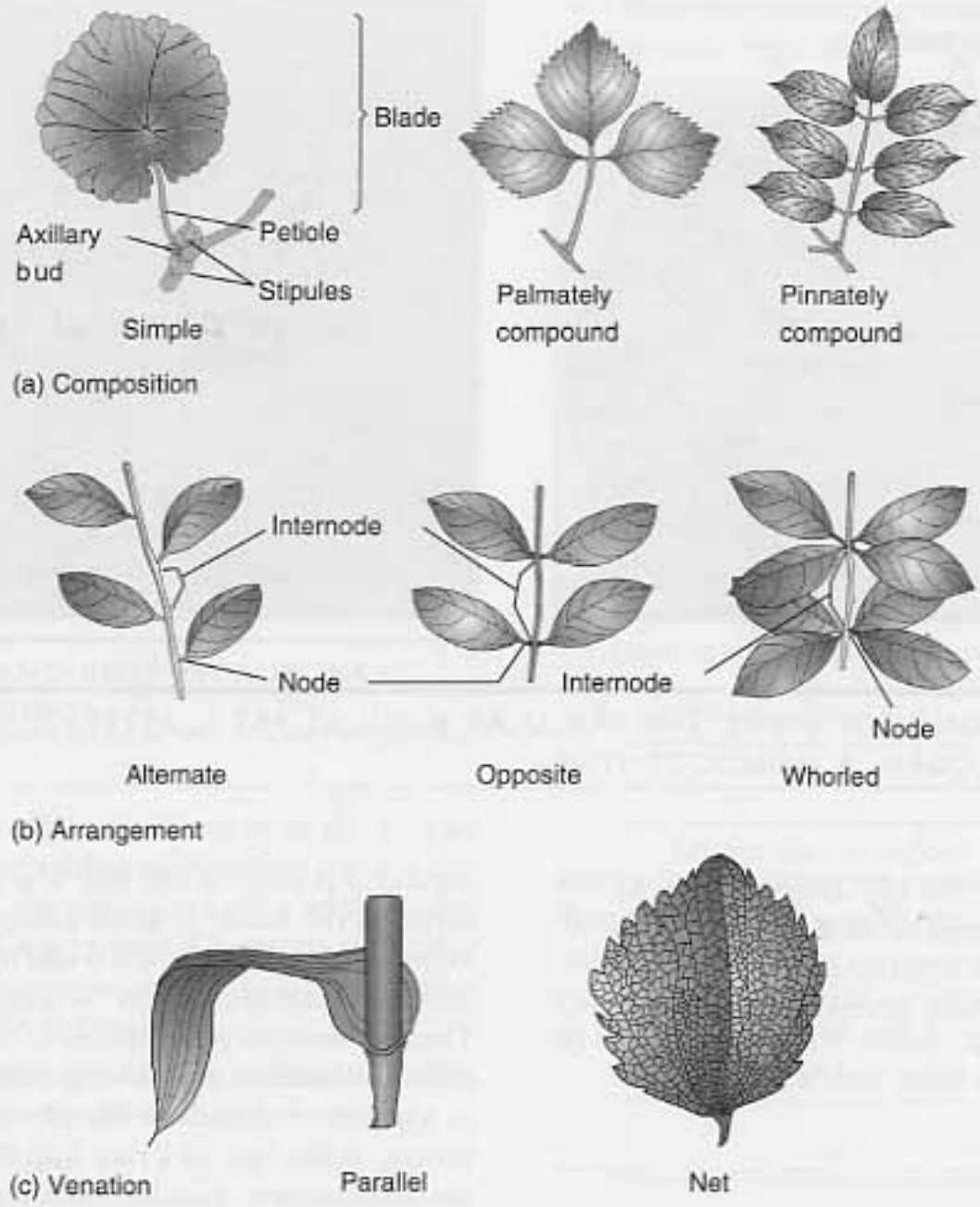


FIGURE 4.8 LEAF MORPHOLOGY. (*A*) A LEAF HAS A BLADE, A PETIOLE, AND A STIPULE PAIR. AXILLARY BUDS ARE FOUND IN THE AXILE. IN LEAF COMPOSITION, LEAVES MAY BE SIMPLE, CONSISTING OF A SINGLE BLADE, OR COMPOUND, IN WHICH THE BLADE IS SUBDIVIDED INTO LEAFLETS. (*B*) LEAF ARRANGEMENT. ALTERNATE, OPPOSITE, OR WHORLED INDICATES THE NUMBER OF LEAVES COMING OFF A NODE. (*C*) LEAF VENATION. THE VENATION PATTERN IS COMMONLY PARALLEL IN MONOCOT LEAVES AND NET IN DICOT LEAVES.

at a node is upped to two, the arrangement is **opposite**. With more than two leaves at a node, the arrangement is **whorled** (fig. 4.8*b*).

Leaf venation is another easily recognizable characteristic. Leaf veins are continuations of the vascular bundles, you saw earlier in the stem. Venation refers to the pattern of the veins, and once again, it divides along monocot and dicot class lines. In most monocot leaves, all of the major veins are **parallel**. In dicot leaves, the veins branch into ever smaller veins to forming an overall lacy appearance. This type of vein arrangement is called **net** (fig. 4.8*c*).

In this exercise, you will learn how a diagnostic key based on the characteristics of leaves can be helpful in tree identification. You will also learn the components of leaf anatomy and how the anatomical design of leaves can be modified by environmental selection.

Materials Needed for Exercise C

Branch of living privet
Compound light microscope
Leafy branches for tree leaf key
Leaves to demonstrate composition, venation, and arrangement
Living *Aloe vera* plant
Prepared slide of aloe (*Aloe*) leaf cross section
Prepared slide of privet (*Ligustrum*) leaf cross section

from both sides of the leaf. Now that you know leaf anatomy, what soft tissues specifically are you scraping off?

_____

_____

_____

_____

4. You should now have a leaf skeleton that shows the venation pattern of the leaf.
5. If desired, stain with dyes by immersing the leaf in a dye solution.
6. Place the leaf between two pieces of newspaper, add a heavy book or board on top to press the leaf, and allow to dry.

EXERCISE E: Supermarket Botany

Examining some familiar vegetables is a good way to test your knowledge of plant anatomy. Don't rely on common but often erroneous assumptions when assessing the vegetative status of a vegetable. Look for solid anatomical evidence before drawing a conclusion. Remember that many vegetables have been greatly modified from their wild ancestors by selective breeding and can appear much different from what is considered typical for a root, stem, or leaf.

Materials Needed for Exercise E

Assorted vegetable produce. (See list in worksheet 4-2 at the end of this laboratory topic.)

Knife

Procedure for Exercise E

1. Take a close look at some common and uncommon vegetables, and using your knowledge of plant anatomy, identify them in terms of plant organs. In some cases, it will be necessary to cut a cross section of the vegetable in order to discern important anatomical details.
2. Record your findings in worksheet 4-2.

TERMS TO KNOW

angiosperms 37
apical meristem 37
axile 43
axillary bud 43
blade 43
bundle sheath 45
Casparian strip 39
companion cells 43
cortex 37
cuticle 45
dicot 37
endodermis 39
epidermis 39
epiphytes 40
fibrous root 37
guard cells 45
hydrophyte 39
internode 43
leaf arrangement: alternate, opposite, whorled 43
leaf composition: simple or compound—pinnately or palmately 43
leaf venation: parallel or net 44
leaflets 43
mesophyll 45
microtome 41
monocot 37
nodes 43
palisade cells 45
petiole 43
pith 41
root cap 37
root hairs 37
sieve-tube members 43
spongy cells 45
stele 37
stipules 43
stoma (pl., stomata) 45
taproot 37
vascular bundles 42
vascular cylinder 37
velamen 41
zone of cell division 37
zone of elongation 37
zone of maturation 37

QUESTIONS FOR REVIEW AND DISCUSSION

1. What are some differences between monocot and dicot roots?
2. How does the organization of a monocot stem differ from that of a dicot stem?
3. Can you tell from a cross section of a leaf whether the leaf is a monocot or a dicot? Explain.
4. You saw how aloe leaf anatomy has been modified to store water in an arid environment. Can you think of other leaf modifications that would conserve water?
5. How can you distinguish a leaf from a leaflet?
6. You find a fossil plant organ. Your general knowledge that roots are underground and stems are aerial won't help you identify this fossil's vegetative status. What anatomical details would you look for in a stem? In a root?

ADDITIONAL RESOURCES

Dickey, J. 1995. *Laboratory investigations for general biology.* Redwood City (CA): Benjamin/Cummings.

Hirasuna, D. 1985. *Vegetables.* San Francisco (CA): Chronicle Books.

Levetin, E., and K. McMahon. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Mauseth, J. D. 1998. *Botany: An introduction to plant biology,* 2d ed. Sudbury (MA): Jones and Bartlett Publishers.

Mauseth, J. D. 1988. *Plant anatomy.* Menlo Park (CA): Benjamin/Cummings.

Moore, R., W. D. Clark, and D. S. Vodopich. 1998. *Botany.* New York: McGraw-Hill Companies, Inc.

Northington, D. K., and E. L. Schneider. 1996. *The botanical world.* New York: McGraw-Hill Companies, Inc.

Raven, P. H., R. F. Evert, and S. Eichhorn. 1999. *Biology of plants,* 6th ed. New York: W. H. Freeman/Worth.

Rupp, R. 1987. *Blue corn and square tomatoes.* Pownal (VT): Garden Way Publishing.

Simpson, B. B., and M. Conner-Ogorzaly. 2000. *Economic botany: Plants in our world,* 3d ed. New York: McGraw-Hill Companies, Inc.

ON THE WEB

Angiosperm Anatomy
http://www.uwc.ac.za/sci_ed/std8/anatomy/index.htm

Ethnobotanical Leaflets
http://www.siu.edu/~ebl/

Wayne's Word: A Newsletter of Natural History Trivia
http://daphne.palomar.edu/wayne/wayne.htm

OTHER ACTIVITIES

1. Purchase carnivorous plants to view a less typical function of leaves.
2. Construct a leaf key to the trees in your area based on characteristics of leaves.
3. Examine the differences between sun and shade leaves. Trace the shape of sun versus shade leaves on graph paper and calculate the difference in surface area between the two types. Speculate as to why these size differences occur.
4. Investigate the history and folklore of some common vegetables. Find medicinal uses, unusual cultivation

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 4-1 EXERCISES A, B, AND C: PLANT ARCHITECTURE

List the anatomical details that distinguish roots, stems, and leaves and those that separate monocots from dicots.

Plant Organ	*Unique Anatomical Features*	*Monocot*	*Dicot*
Root			
Stem			
Leaf			

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 4-2 EXERCISE E: SUPERMARKET BOTANY

Examine vegetables from the produce aisle, and use your knowledge of plant anatomy to determine what plant organ they are. Give reasons for your answer.

Name of Vegetable	*Scientific Name*	*Anatomy of Edible Part*	*How Do You Know?*
Artichoke	*Cynara scolymus*		
Asparagus	*Asparagus officinale*		
Beet	*Beta vulgaris*		
Belgian endive	*Chicorium intybus*		
Brussels Sprout	*Brassica oleracea*		
Cauliflower	*Brassica oleracea*		
Cabbage	*Brassica oleracea*		
Carrot	*Daucus carota*		
Celery	*Apium graveolens*		
Daikon	*Raphanus sativus*		
Kale	*Brassica oleracea*		
Kohlrabi	*Brassica oleracea*		
Lettuce	*Lactuca sativa*		
Radish	*Raphanus sativus*		
Rhubarb	*Rheum raponticum*		
Spinach	*Spinacea oleracea*		
Swiss chard	*Beta vulgaris*		
Turnip	*Brassica rapa*		

LABORATORY TOPIC 5

Plants Do It All—Photosynthesis, Respiration, and Transpiration

BACKGROUND

Physiology is the branch of biology that focuses on the function of structures and the variety of dynamic processes that allow an organism to live. Understanding all the processes, such as photosynthesis or protein synthesis, can help us understand the organism. This understanding can also help us adjust conditions for practical purposes, such as to increase crop yields or control pests. This laboratory topic introduces three important processes in the lives of plants—photosynthesis, respiration, and transpiration. Unfortunately, this is only a glimpse into the fascinating inner workings of a live plant.

This lab is divided into four exercises. The first explores the flow of carbon dioxide related to photosynthesis and respiration. The second investigates factors affecting the production of starch via photosynthesis. The third and fourth exercises explore the flow of water out of plants via transpiration; Exercise C examines transpiration for individual leaves and plants, and Exercise D demonstrates what happens when plants act together in a community.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Better understand the factors that can affect the physiology of plants.
2. Relate the processes of photosynthesis and respiration to plant growth.
3. Better understand the process of transpiration in plants.
4. Explore the factors that affect the rate of transpiration in plants, including anatomical features such as stomata and environmental conditions such as temperature.
5. Understand the processes of scientific inquiry by posing questions, designing investigations, collecting empirical data, testing hypotheses, and communicating the results.

EXERCISE A: The Ins and Outs of CO_2

The common statement *"Animals respire, plants photosynthesize"* is only partially correct. Although it is true that plants are capable of producing their own organic compounds through the process of photosynthesis, plants are so versatile that they can also respire just like animals.

Photosynthesis is a complex series of reactions involving the capture of light energy, conversion to chemical energy, and finally the synthesis of carbohydrates. It is one of the main biosynthetic processes by which energy and carbon enter the network of living organisms. A summary of the ultimate reactants and products of photosynthesis can be stated as:

Equation 1: $\text{Light} + \underset{\text{carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O} \rightarrow \underset{\text{Sugar}}{(CH_2O)_n} + \underset{\text{Oxygen}}{O_2}$

This equation is deceptive in its simplicity because photosynthesis involves numerous intermediate steps. Photosynthesis can be divided into two series of reactions. The first series, **light harvesting**, results in the capturing of light energy and the temporary storage of this energy in ATP and NADPH. The second series of reactions, **carbon fixation**, uses the energy in the ATP and NADPH to synthesize sugars from CO_2.

Light harvesting starts with the absorption of light energy by the pigment **chlorophyll** embedded in the membranes of the chloroplasts within plant cells. The energy excites electrons within the chlorophyll molecule to a higher energy state. At this energy state, the electrons are easily transferred to other chemicals that accept the electrons. The electrons are transferred from one chemical to the next much like the way buckets of water are passed from one person to the next in a bucket brigade. Eventually, the electrons are transferred to an electron acceptor called $NADP^+$ (nicotinamide adenine dinucleotide phosphate). To replace the lost electrons of the chlorophyll molecules, water (H_2O) is split to produce electrons, protons (H^+), and oxygen gas (O_2). In addition, a proton gradient is generated that is used to produce **ATP** (**adenosine triphosphate**) from ADP

(adenosine diphosphate) and P_i (inorganic phosphate). ATP is a temporary energy carrier. The $NADP^+$ is converted to **NADPH** by the adding of two electrons and one proton (H^+). At the end of the light-harvesting phase, NADPH, ATP, and O_2 are produced. A summary of the light-harvesting phase can be stated as:

EQUATION 2: $\text{Light} + H_2O + ADP + P_i + NADP^+ \rightarrow ATP + NADPH + H^+ + \frac{1}{2}O_2$

In the **carbon fixation** phase of photosynthesis, the energy and electrons of ATP and NADPH are used to form carbon–carbon bonds. Carbon dioxide (CO_2) enters a cyclic series of reactions (the Calvin cycle), and eventually sugars $(CH_2O)_n$ are produced. The name of the enzyme that catalyzes the fusion of CO_2 to the first chemical in the cycle (ribulose bisphosphate) is **rubisco**. This is worth mentioning since rubisco is the most abundant protein on our globe. The first sugar to exit the cycle is a three-carbon sugar, G3P (glyceraldehyde 3-phosphate). G3P can be used to generate other sugars, such as glucose ($C_6H_{12}O_6$). The summary of the carbon-fixation phase of photosynthesis can be stated as:

EQUATION 3: $ATP + NADPH + CO_2 \rightarrow ADP + P_i + NADP^+ + (CH_2O)_n$

Take some time to examine the three equations. Equation 1 is the summary of equations 2 and 3. What information is lost by only looking at equation 1? Which phase requires light? Which phase is not directly dependent on light, yet needs the products of the light-dependent phase?

The two phases of photosynthesis actually occur in two different locations within the chloroplasts. The light-harvesting phase occurs on the membranes of the thylakoids, whereas the carbon-fixation phase occurs in the stroma, the space between the thylakoids. Given this, what compounds cycle back and forth between the two phases? Does carbon enter the system in both phases?

All animals, including humans, ultimately depend on plants to produce the oxygen gas (O_2) they need. O_2 is actually a by-product of photosynthesis. We are lucky that plants produce oxygen. For example, an average hectare of corn produces enough oxygen per day to support 325 people. Where do the atoms that make up the O_2 come from? If you remove a plant from the light, what happens to its oxygen production?

What is the fate of the products of photosynthesis? G3P can easily be converted into various six-carbon sugars, such as glucose or fructose, or stored as starch, a polysaccharide formed as a chain of glucose molecules. When these basic sugars are combined with other elements, such as nitrogen or phosphorus, all the other organic compounds in a plant, such as proteins, nucleic acids, lipids, or alkaloids, can be formed. In this way, plants make all their basic building blocks. And since animals are incapable of carbon fixation themselves, plants make the basic building blocks for animals as well. Animal food has to come from other organisms, and ultimately most of it comes from plants. In Laboratory Topic 10, you will relate your own needs for energy to production of chemical forms of energy by plants. Of course, the plants could probably care less about the welfare of animals.

Respiration is another fundamental process of living organisms. Before we proceed, it is important to compare two definitions of respiration. Many of you may consider respiration the process of breathing air in and out of your body. Mammals like yourself pull air into their lungs by contraction of the diaphragm and exhale the air as the diaphragm relaxes. The inhalation and exhalation of air is one valid definition of respiration. Humans even use breathing as a sign of life itself.

Biologists often use another definition of respiration to describe what happens to some of the components of the air, particularly CO_2 and O_2, at the cellular level. **Cellular respiration** is defined as the process by which cells release energy from organic compounds to generate ATP through a series of chemical reactions involving the transfer of electrons. In **aerobic respiration**, oxygen (O_2) is the final electron acceptor. In **anaerobic respiration**, or fermentation, some other chemical is the final electron acceptor. The main results of cellular respiration are organic compounds broken down to simpler compounds, with some energy becoming available for use in other metabolic steps.

The overall process of aerobic cellular respiration can be stated as:

EQUATION 4: $(CH_2O)_n + O_2 \rightarrow CO_2 + H_2O +$ energy (ATP and heat)

Compare equation 4 with equation 1. On the surface, respiration appears to be merely the reverse of photosynthesis. But in reality, aerobic respiration is another complex series of reactions that can be divided into three phases: glycolysis, the Krebs cycle, and the electron transport chain.

In **glycolysis**, a molecule of the six-carbon sugar glucose is oxidized to two molecules of the three-carbon pyruvate, and some of the energy is recaptured in the production of ATP. The **Krebs cycle** completes the oxidation of pyruvate to produce carbon dioxide (CO_2) and reduced electron carriers. In the **electron transport chain**, a proton (H^+) gradient drives the production of even more ATP and is coupled with the transfer of electrons to oxygen (O_2), producing water (H_2O). After the entire process of respiration is complete, much of the energy released from the glucose is recaptured in the production of ATP. Since no conversion of energy is 100% efficient, some of the energy is lost as entropy and is no longer available to the organism. The ATP, however, can be used for all the other normal processes of life, such as synthesis of new tissue, response to external stimuli, or movement of materials throughout the body.

Just like animals, plants use aerobic respiration to recapture the energy held in the sugar molecules produced during photosynthesis. Just like animals, plants use glycolysis, the Krebs cycle, and the electron transport chain to produce ATP. Plants, however, do not need to consume preformed organic compounds as animals must. They produce their own organic compounds via photosynthesis. For this reason, some people say, "Plants make their own food." Again we emphasize, plants photosynthesize *and* respire. Animals *only* respire.

As long as the net production of new material via photosynthesis exceeds the breakdown of molecules to produce ATP via respiration (P > R), a plant will grow and increase in biomass. But sometimes, plants need to rely heavily on the energy stored in sugars, and respiration can exceed net gain from photosynthesis (R > P). This is common when photosynthetic tissues are not yet available, such as in a germinating seed or regrowth of buds in the tips of branches each spring. What do you suppose happens when a plant is kept in the dark for a long time or at night?

Take a look at equations 1 and 4 again. Notice how on the surface, carbon dioxide is consumed by photosynthesis, yet produced by respiration (fig. 5.1). If you had an experimental system with only plants, the balance between photosynthesis and respiration could be determined by monitoring the levels of carbon dioxide around a plant. In this activity, we will use a CO_2 sensor to measure the concentration of CO_2 in the air surrounding plants in various conditions and see what happens. Relate what you observe to what could be happening biologically.

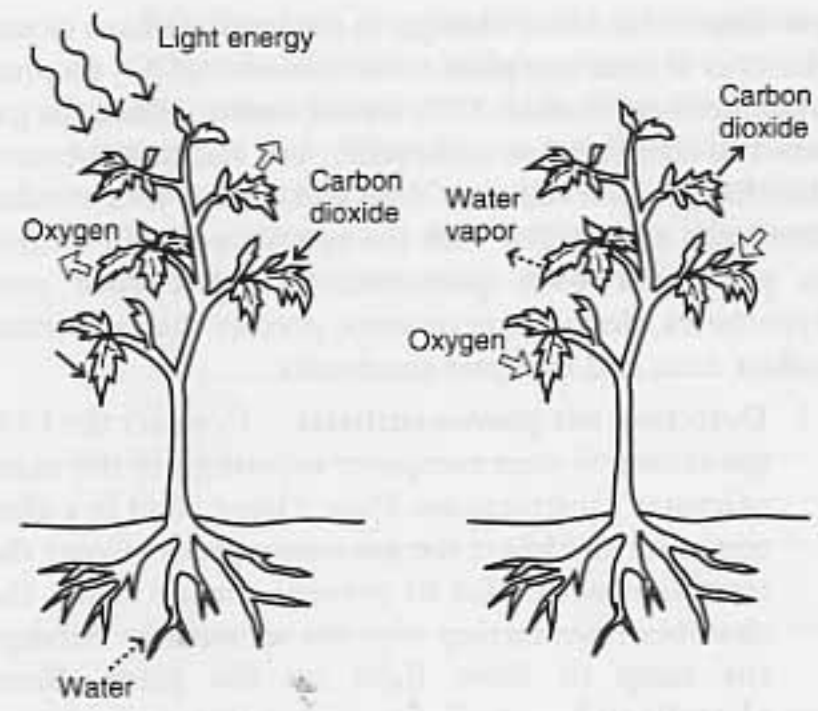


FIGURE 5.1 COMPARISON OF PHOTOSYNTHESIS AND RESPIRATION IN PLANTS.

Materials Needed for Exercise A

Bell jar

Cellulose acetate or diluted clear nail polish (see Instructors' Notes at the end of this manual for preparation)

Coleus (coleus) plants

Cotton balls

Dry peas, beans, or other seeds

Gas sampling bottle, 250 ml

Glass plate

Glass terrarium or jar with lid

Logger Pro software

Microwave oven

PC or Macintosh computer

Peas, beans, or other seeds soaked for 24 hours

Petroleum jelly (Vaseline)

Photoflood lamp

Potato, apple, or other plant organ void of chlorophyll

Ring stand

Sodium pyrogallate (see Instructors' Notes for preparation)

Split rubber stopper

Universal lab interface

Various plants, such as *Pelargonium* (Geranium) or *Zebrina*

Vernier CO_2 gas sensor

Water-filled heat shield to control temperature generated from lamp

Procedure for Exercise A

The Vernier CO_2 gas sensor monitors the concentration levels of CO_2 gas in air. In this exercise, you will use the

gas sensor to monitor changes in the levels within a closed chamber as your test plant either consumes CO_2 via photosynthesis or produces CO_2 via respiration. When the gas sensor is connected to a computer, you can easily observe the changes in levels over time and record your results. Once you are familiar with the system, you will be able to pose your own questions, formulate your own hypotheses, design experiments, predict the outcomes, collect data, and interpret the results.

1. **Detecting net photosynthesis.** Connect the CO_2 gas sensor to your computer according to the manufacturer's instructions. Place a large plant in a glass terrarium and insert the gas sensor probe. Cover the terrarium with a lid to prevent the air inside the chamber from mixing with the air outside. Arrange the lamp to shine light on the plant. Since photoflood lamps often generate intense heat, you may need to place a water-filled heat shield between the lamp and the terrarium. Monitor the levels of CO_2 for 10–60 minutes. Note the change in the levels and the rate of change. What is happening? What does this tell you about what is happening biologically? Record your results in worksheet 5-1 at the end of this laboratory topic.

   Try changing the light intensity by moving the lamp further from the plant or inserting filters. What happens to the rate of change? What happens if you turn off the light entirely? Record your results in worksheet 5-1.

   Try other plants, or change the conditions, such as temperature. You may try to start with a higher initial CO_2 level by exhaling into the sample chamber. Pose your own questions and design your own experiments. What affects photosynthesis? When is the consumption of CO_2 the greatest?

2. **Detecting respiration in plants.** Cut a fresh potato into 1 cm^3 cubes and place approximately ten of them in a 250-ml sampling bottle. Connect the CO_2 gas sensor with the split rubber stopper. Monitor the change in the levels of CO_2. What do you observe? Is the potato respiring? Is the potato alive?

   Cut another fresh potato into 1 cm^3 cubes. This time, microwave the cubes just long enough to kill any living tissue (approximate 30–40 seconds). Place the cubes in the sampling bottle and monitor the CO_2 levels as before. Now what do you observe? How do these results compare with those of the fresh potato cubes?

   Now place dry peas, beans, or other seeds in the sampling bottle. Monitor the CO_2 levels. Compare these results with peas, beans, or seeds soaked in water overnight. Is there any difference? What is happening within the seed?

   Try other tissues, such as cut pieces of an apple or a carrot. What do you observe?

   Record your results in worksheet 5-1.

3. **Do plants need oxygen?** We mentioned that plants are aerobic, but is this really true? What would happen if plants were deprived of oxygen? Do seeds need oxygen to germinate, grow, and survive?

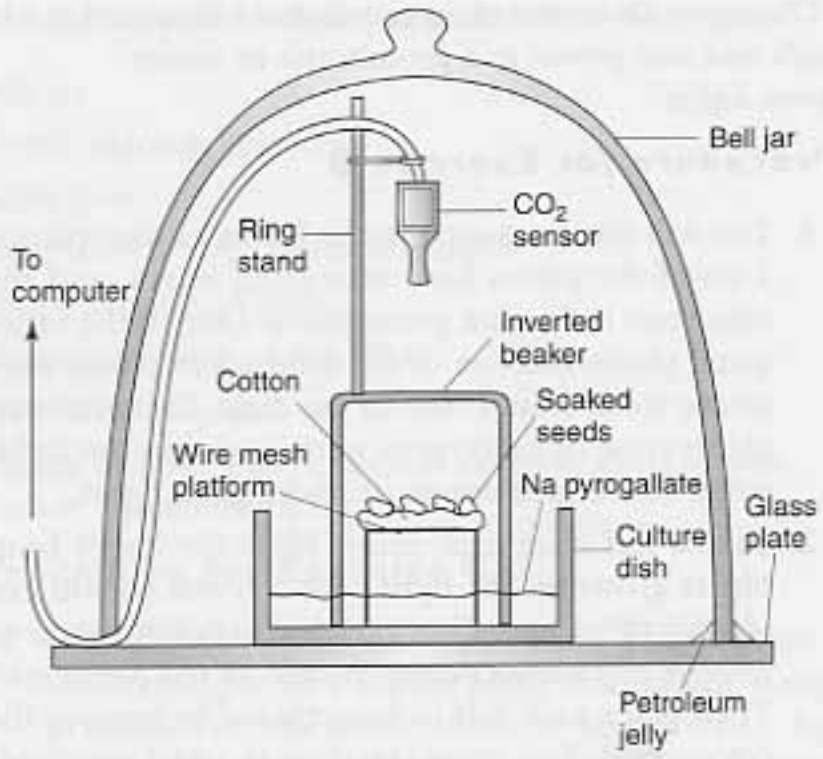


FIGURE 5.2 APPARATUS TO DETERMINE RELATIONSHIP OF OXYGEN TO RESPIRATION IN GERMINATING SEEDS.

For this activity, we will use sodium pyrogallate to remove oxygen from our test chamber. The setup is illustrated in figure 5.2. Make a platform out of wire mesh, and place it in a 15-cm-diameter culture dish. Cover the platform with moist cotton. Place seeds (peas or beans) that have been soaked in water overnight on the moist cotton. Add 200 ml of freshly prepared sodium pyrogallate in the bottom of the culture dish below the platform. Suspend the CO_2 sensor over the seeds with a ring stand. Cover the whole setup with a bell jar sealed to a bottom glass plate with petroleum jelly. The cord to the sensor must extend under the lip of the bell jar. Monitor the CO_2 levels over time. As a control, establish a similar setup with another set of soaked seeds on moist cotton. Instead of sodium pyrogallate, fill the bottom of the culture dish with water. Again monitor the CO_2 levels.

What do you observe? What is the correlation between CO_2 production and the availability of O_2? From what you observe, do you think the seeds need O_2 for respiration?

Remove the CO_2 sensors, and place each setup in a warm location in the lab. Cover the jars with paper, or place them in the dark. Observe the seeds after several days. What do you see? What is the effect of O_2 on germination? Speculate on the correlation between germination and respiration. Why was it important to keep the seeds in the dark?

Record your results in worksheet 5-1.

4. **How does CO_2 enter leaves?** Where does CO_2 enter a leaf? Does it enter through the epidermal cells, or does it enter through the stomata?

   a. Remove a healthy leaf from a *Coleus* plant. Make an epidermal peel of the lower surface of the leaf by barely scoring the surface with a razor blade and peeling the outer layer of cells off. Mount with a drop of water on a microscope slide and examine under the microscope. Look for **stomata**, openings in the surface surrounded by a pair of cells called guard cells (see fig. 3.2). Count the number of stomata per cm^2. Make another epidermal peel of the upper surface of a leaf. Count the number of stomata per cm^2 for this surface and compare. Which surface has more stomata? (Usually in *Coleus* one surface has nearly all of the stomata. Is this the case for your plant? Which surface, upper or lower, has more?)

   b. An alternative way to view the stomata on the surface of a leaf is to make an impression of the surface with cellulose acetate. Paint the surface of the leaf with cellulose acetate, and let dry. Peel off the impression and observe with the microscope. If you have difficulty removing the impression, try wilting the leaf in a warm (not hot) oven first and then remove the peel.

c. Set up four *Coleus* plants for this experiment. For the first plant, cover the upper surfaces of all the leaves with petroleum jelly. Place the plant in the test chamber, insert the CO_2 sensor, and monitor the levels of CO_2 for approximately 10–120 minutes. For the second plant, cover the lower surfaces of all the leaves and monitor the CO_2 in the test chamber. For the third plant, cover both the upper and lower surfaces and monitor the CO_2. Finally, for the fourth plant, leave all the leaves uncovered and monitor the CO_2. This is your control. Place all four plants under high light intensity to stimulate photosynthesis.

What do you observe? What is the relationship between the relative rates of CO_2 uptake and the number of stomata on the open surface? Record your observations in worksheet 5-1. From your experiment, do you conclude that most of the CO_2 enters the leaf through the epidermis or the stomata?

EXERCISE B: Saving for Another Day—Storing Starch

Some of the first carbohydrates produced via photosynthesis are simple sugars such as glucose. Glucose is highly soluble in water, so many plants convert it to starch, the insoluble storage polysaccharide. Laboratory Topic 13 explores starch in a variety of food crops. In this activity, we will examine where starch is stored in a leaf after photosynthesis.

Materials Needed for Exercise B

Beaker of boiling water

Beaker of hot ethanol in a double boiler (*Caution*: ethanol ignites easily.)

Beaker of Lugol's iodine solution

Plants with solid green leaves, such as wandering jew (*Tradescantia*), geranium (*Pelargonium*), or ivy (*Hedera*)—one plant grown for 4 days in the dark and one grown in a greenhouse or under grow lights

Plants with variegated leaves, such as *Coleus*, variegated geranium (*Pelargonium*), or spider plant (*Chlorophytum*)—one plant grown for 4 days in the dark and one grown in a greenhouse or under grow lights

Procedure for Exercise B

1. You will test for starch in the leaves of four plants. Two of the plants have variegated leaves, and the other two have solid green leaves. One of the variegated plants and one of the solid green plants were grown for at least 4 days in the dark. The other two plants (one of each type) were grown in the light, either in the greenhouse or under grow lights.
2. Take a leaf from each plant. Mark the leaves from plants grown in the light with a notch so you can identify them later. Place each leaf in boiling water to remove any anthocyanins (purple or red pigments). Then place each leaf in hot ethanol to remove the chlorophyll. This should be done in a well-ventilated area. Finally, soak each leaf in iodine solution to detect starch.
3. Draw an outline of each leaf on worksheet 5-2 at the end of this laboratory topic. Indicate where starch is detected. What do you conclude is the effect of light on the production of starch? What is the relationship between the pattern of variegation and the detection of starch? Why do you suppose this relationship exists? If a leaf has little to no starch, how does the plant stay alive?

EXERCISE C: Transpiration

Another important process in plants is the movement of water from the roots to the leaves. **Transpiration** of water from the surface of the leaves is an important part of this movement. Do plants transpire water at the same rates under the same conditions? What conditions increase the transpiration of water? When should you be more concerned about watering your houseplants or garden plants? When should you water more, or less?

In this exercise, we will detect water vapor as it evaporates from leaves. Then we will compare plants under different conditions to see what variables affect the transpiration of water. Finally, we will look at the relative number of stomata in a given area of leaf and relate this factor to transpiration rates.

Materials Needed for Exercise C

Bell jar

Cobalt chloride test strips

Glass plate

Paper clips, string, or rubber bands

Petroleum jelly (Vaseline)

Plastic wrap (e.g., Saran Wrap)

Potted *Coleus* plant

Variety of other plants, such as *Zebrina*, *Pelargonium*, *Sedum*, *Tradescantia*, etc.

Procedure for Exercise C

1. **Comparing transpiration and evaporation.** Cover the pot of a *Coleus* plant with plastic wrap to expose only the shoot (stem and leaves). Place the potted plant under a bell jar. Seal the bottom edge of the bell jar to a glass plate with petroleum jelly. Set up two controls for comparison. Place a wet sponge under the second bell jar sealed to a glass plate. Seal the third bell jar to a glass plate and keep it empty. After 2–3 hours, observe the inner surface of the bell jars. What do you see? Record your observations in worksheet 5-3. From your experiment, what can you conclude about the role of water evaporation in plants?

2. **Detecting water loss (transpiration).** This activity uses cobalt chloride test strips to detect the presence of water vapor. The strips are blue when dry and turn pink as they become moist. Normally they must be stored in sealed bottles or jars over anhydrous calcium chloride so they stay dry until needed. If the strips are pink, dry them in a warm oven until they return to blue.

   Using forceps, carefully remove two cobalt chloride test strips from the bottle and quickly reseal the bottle. Do not touch the strips with your fingers. Also, do not ingest the cobalt chloride.

   Attach the strips to a leaf with a paper clip so the blue region is in contact with the surface of the leaf. Attach one strip to the upper surface and one to the lower surface. Time how long it takes for each strip to turn pink and record the times in worksheet 5-3.

   Repeat this procedure for a variety of other plants. If the time required for the strips to turn color exceeds 30 minutes, record the water loss as negligible.

   As a control, remove one strip and suspend it in the air about 20 cm above the table top with a ring stand. Time how long it takes for this strip to turn pink. This becomes your rough measure of the relative humidity of the air in your room.

   What do you observe? What features of the plant seem to affect the rate of transpiration? Which surface of the leaves on each plant has the greater transpiration rate?

3. **Counting stomata.** After measuring the water loss from a plant from step 2, remove the leaf. Paint the upper and lower surfaces of the leaf with cellulose acetate, and let dry. Peel the impression off the surface and examine with a microscope under low-power magnification. Calculate the number of stomata per cm^2. Record the numbers in worksheet 5-3.

   How does the number of stomata on each surface of the leaf correlate with the relative rate of transpiration? Based on that observation, where do you speculate water leaves the leaf?

4. **Design your own experiment.** Now that you have become familiar with cobalt chloride strips as a tool, it's time to use them to discover more about transpiration. Pose a question, formulate a hypothesis, and design an experiment. Be sure to include appropriate controls, collect the data, and interpret the results. Some possible questions include:

   - Do leaves on the south side of a tree (the sunny side) transpire more than leaves on the north side of the same tree (the shady side)? Is this correlated with the relative abundance of stomata?

- Do desert plants transpire more water from the leaves or from the stem?
- Does the time of day affect transpiration through leaves?
- Do wind or other environmental conditions affect the rate of transpiration?

Work in groups of three or four. Choose a new and unique question. Formulate your hypothesis and design your experiment on worksheet 5-4. Get the approval of your instructor before you proceed.

Collect your data and interpret your results. Prepare a short report to share with your fellow students in other groups.

EXERCISE D: Corn Clouds

When plants grow as a community, their combined transpiration is an important component of the water cycle. Because of this, plants can significantly affect weather patterns. To observe this phenomenon, visit a field of corn.

Materials Needed for Exercise D

Sling psychrometer or digital hygrometer for measuring relative humidity

Procedure for Exercise D

A field of actively growing corn is a great demonstration of the dramatic effect plants can have on our world. The best time for this activity is when the corn is at least chest height in late summer or early fall, depending on the region of the country. If they do not grow corn in your region, other crops can also show the phenomenon. This demonstration works best when the overall relative humidity is 60% or less.

First, get permission from the landowner before entering a cornfield. Measure the relative humidity in the center of the field and compare it with the relative humidity outside the field. (**Relative humidity** is a measure of the relative saturation of water in the air.) What do you notice? Where did that water vapor come from?

TERMS TO KNOW

aerobic respiration 54
anaerobic respiration 54
ATP (adenosine triphosphate) 53
carbon fixation 53
cellular respiration 54
chlorophyll 53
electron transport chain 55
glycolysis 55
Krebs cycle 55
light harvesting 53
NADPH 54
photosynthesis 53
relative humidity 60
respiration 54
rubisco 54
stomata 57
transpiration 58

QUESTIONS FOR REVIEW AND DISCUSSION

1. How does your knowledge of photosynthesis and the growth of plants help explain when and where certain crops are grown? In North America, what seasons would you expect to be the most productive in terms of photosynthetic rates?
2. Why do you think starch is a better storage carbohydrate for plants than a simple sugar like glucose?
3. In a log of wood, where did all the mass come from?
4. How is the primary production of organic chemicals by plants important to you personally?
5. One of the reasons we eat food is to get energy. Trace the energy of your food back to sunlight.
6. If plants were kept in the dark for long periods of time in a closed system, what would happen to the levels of CO_2? To the levels of O_2?
7. If plants can photosynthesize, what is the importance of respiration to plants?
8. You demonstrated that fresh potatoes respired and were alive. If the potatoes were stored in a root cellar and never attacked by fungi or bacteria, what do you suppose would eventually happen? Why?
9. Describe some of the adaptations of leaves to minimize or maximize transpiration.
10. Why do plants lose so much water via transpiration if water is critical to their survival?

ADDITIONAL RESOURCES

Attenborough, D. 1995. *The private life of plants,* Vol. 2: *Putting down roots.* [Video] British Broadcasting Corp.

Hall, D., and K. Rao. 1994. *Photosynthesis,* 5th ed. Port Chester (NY): Cambridge University Press.

Jones, H. 1992. *Plants and microclimate: A quantitative approach to environmental plant physiology,* 2d ed. Port Chester (NY): Cambridge University Press.

Levetin, E., and K. McMahon. 1999. *Plants and society,* 2d ed. New York: McGraw-Hill Companies, Inc.

Simpson, B., and M. Connor-Ogorzaly. 1986. *Economic botany: Plants in our world,* 2d ed. New York: McGraw-Hill Companies, Inc.

Taiz, L., and E. Zeiger. 1998. *Plant physiology,* 2d ed. Sunderland (MA): Sinauer Associates, Inc.

Walker, D. 1999. *A leaf in time.* London: Portland Press Ltd.

ON THE WEB

American Society of Plant Physiologists
http://www.aspp.org
National Gardening Association
http://www.garden.org
What Is Photosynthesis? Site prepared by the Arizona State University Center for the Study of Early Events in Photosynthesis.
http://photoscience.la.asu.edu/photosyn/education/learn.html
Introduction to Photosynthesis and Its Applications. Site prepared by Wim Vermaas of Arizona State University.
http://photoscience.la.asu.edu/photosyn/education/photointro.html
Photosynthesis and the World Wide Web. Article by L. Orr and Govindjee for the XIth International Photosynthesis Congress (Aug. 1998).
http://photoscience.la.asu.edu/photosyn/photoweb/default.html#introduction

OTHER ACTIVITIES

1. Clip a high-contrast, black-and-white film negative onto a leaf, and place it in the light for at least 4 days. After 4 days, detect the presence of starch. What is the pattern?
2. Extract sugar from sugar beets or sugarcane.
3. Use cobalt chloride strips to measure transpiration in your favorite houseplants in sunny or shady locations, and plan a watering regime based on what you detect.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 5-1 EXERCISE A: THE INS AND OUTS OF CO_2

1. DETECTING NET PHOTOSYNTHESIS

Attach graphs of the change in CO_2 over time for your plants (print out the results monitored by your computer). Record the plant species, environmental conditions, light intensity, and any other factors of importance.

What factors had the greatest effect on photosynthesis?

2. DETECTING RESPIRATION IN PLANTS

Attach graphs of the change in CO_2 over time for your plants (print out the results monitored by your computer). Record the plant species, environmental conditions, light intensity, and any other factors of importance.

What factors had the greatest effect on respiration?

3. DO PLANTS NEED OXYGEN?

What is the effect of O_2 on germination?

What is the correlation between germination and respiration as monitored by changes in CO_2 levels?

4. HOW DOES CO_2 ENTER LEAVES?

What did you observe from this experiment?

What is the correlation between stomata abundance and movement of CO_2?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 5-2 EXERCISE B: SAVING FOR ANOTHER DAY—STORING STARCH

Sketch the outline of the leaves and indicate the variegation pattern. Use a colored pencil to indicate where you detected starch. If no starch was detected, report "no starch."

Variegated leaf grown in light	Variegated leaf grown in dark
Solid leaf grown in light	Solid leaf grown in dark

What is the effect of light on starch production?

What is the correlation of the pattern of variegation with starch production?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 5-3 EXERCISE C: TRANSPIRATION

1. COMPARING TRANSPIRATION AND EVAPORATION

What did you observe from the demonstration of the plant and sponge under bell jars?

What can you conclude about the role of water evaporation and transpiration?

2. DETECTING WATER LOSS (TRANSPIRATION)

Record the time required to turn the cobalt chloride strip from blue to pink.

Upper surface of leaf_____

Lower surface of leaf_____

Which surface of the leaf had the fastest transpiration rate, upper or lower?

How long did it take your control strip suspended in the air to turn pink?

3. COUNTING STOMATA

Record the number of stomata per cm^2.

Upper surface of leaf_____

Lower surface of leaf_____

What is the correlation between the abundance of stomata and transpiration rates?

NAME _____

DATE _____

LAB SECTION NUMBER _____

WORKSHEET 5-4 EXERCISE C: TRANSPIRATION (continued)

DESIGN YOUR OWN EXPERIMENT

Pose a question:

State your hypothesis:

Rationale:

Describe your experimental design. (What are your experimental units? What are your controls? How many replicates of both experimental variables and controls are you planning to get reasonable results?)

Instructor approval of design: _____
(Signature)

Report your results. (You should create your own summary tables or graphs.)

Interpret your results. (What does it all mean? What additional questions do your results suggest?)

LABORATORY TOPIC 6

Say It with Flowers

BACKGROUND

Flowers are sexual reproductive structures, and as such, they play a pivotal role in the life cycle of angiosperms. However, flowers have many connections to human life as well. The beauty of flowers enhances the surroundings in our yards and our parks, as well as inside our homes. Flowers have also been used as an enduring expression of love and remembrance between people. The essential oils from petals have furnished the fragrances of flowers to the production of perfumes, incense, and aromatherapy oils. However, of all the flower parts, pollen is probably the one with the greatest number of uses and applications.

Pollen has symbolic meaning to several Native American tribes. Health food advocates believe that bee pollen (a mixture of pollen plus some bee enzymes) is the perfect food with curative properties. The study of fossil pollen has helped geologists find deposits of oil and reconstruct past environments. Pollen is used to produce extracts for testing and treating allergy patients; the familiar "allergy shots" used to desensitize patients are very dilute pollen extracts. In this laboratory topic, we will examine flower structure as well as some of the uses of pollen.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Identify the parts of a flower and know the function of each part.
2. Describe the features of a flower that would indicate whether the method of pollination is by animals or by wind.
3. Describe the features of pollen grains that are useful in identification.
4. Understand the use of pollen for reconstructing past environments.
5. Understand the use of pollen counts for studying the relationship between exposure and hay fever symptoms.

EXERCISE A: Flower Structure

Flowers are modified branches bearing four sets of floral organs. The floral organs are sepals, petals, stamens, and carpels. These four flower parts are in whorls on the receptacle, the expanded top of the flower stalk, or pedicel (fig. 6.1).

The **sepals** are the outermost floral organs. They are leaflike structures that cover the unopened flower bud. In most flowers, the sepals are green and photosynthetic. The **petals** make up the next whorl of flower parts. Petals are often brightly colored and conspicuous; their function is to attract animal pollinators.

The male and female structures are usually located in the center of the flower. The **stamens** are the male structures, and each stamen consists of an **anther** supported on a stalk, called the **filament**. The anther consists of four chambers, where meiosis occurs and where **pollen** develops. Each pollen grain is a male gametophyte and is capable of producing sperm during the growth of the pollen tube just prior to fertilization.

The female structures are **carpels**, which are located in the middle of the flower. Flowers can have from one to many carpels. When there is only one carpel present, it is called a **simple pistil**. When a flower has many carpels, they may either be fused together to form one **compound pistil** or remain as many separate simple pistils. Carpels, whether single or fused, consist of three parts: the **stigma**, the **style**, and the **ovary**. The stigma, which is at the tip of the carpel, receives pollen on its sticky, feathery, or hairy surface. One to many **ovules** develop within the ovary at the base of the carpel. The style connects the stigma to the ovary.

The ovule includes the female gametophyte, and when mature it contains an egg that can be fertilized by sperm from the pollen. Following fertilization, the ovary becomes the **fruit** and each fertilized ovule becomes a **seed** (fig. 6.2). An ovary can have one ovule (as in a peach or plum) or thousands of ovules (as in a watermelon or pumpkin). The resulting fruits can have one to thousands of seeds.

Monocots generally have floral organs in 3s or multiples of 3, whereas dicots have floral organs in 4s or 5s or

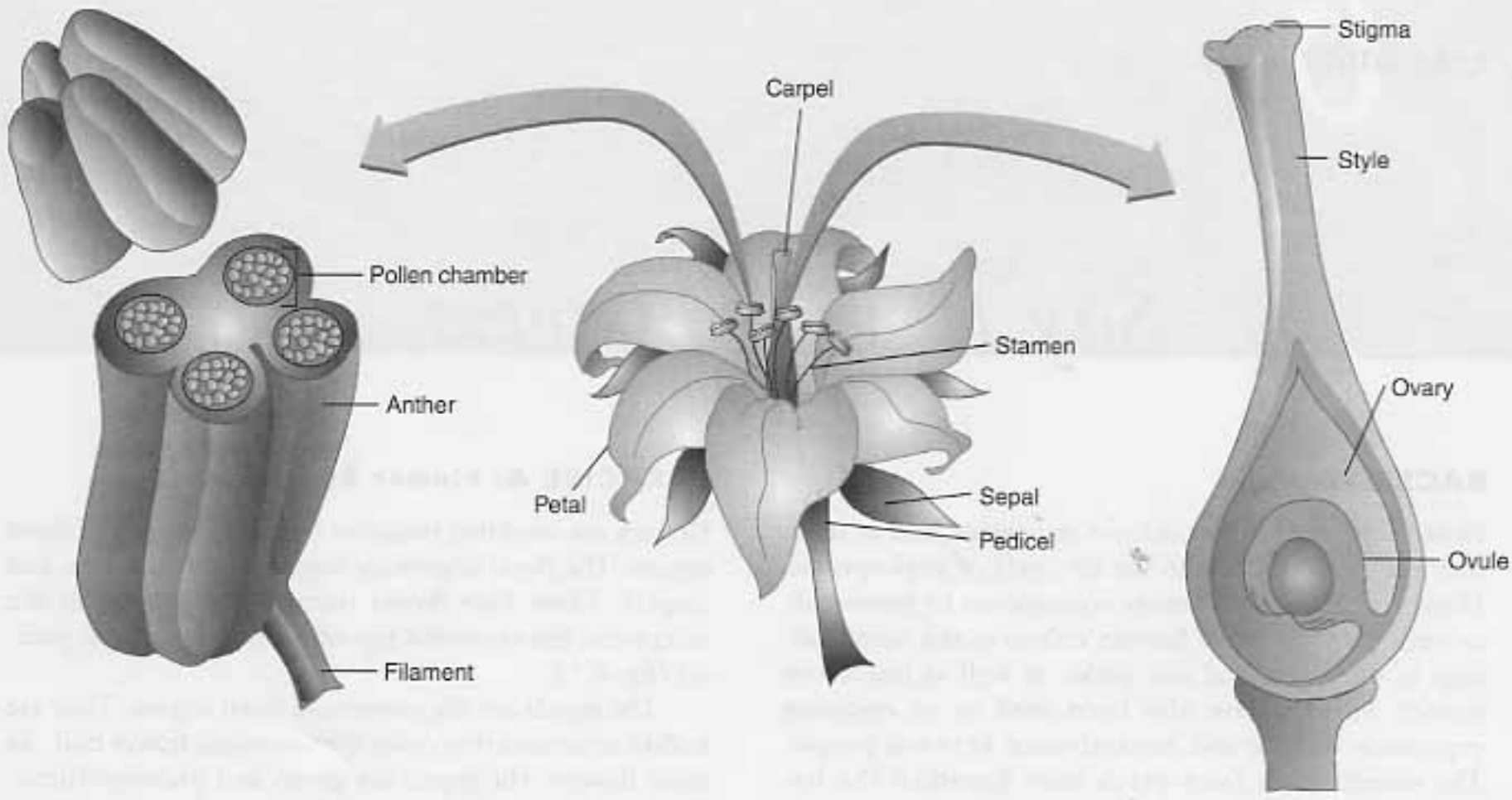


FIGURE 6.1 PARTS OF A FLOWER. (*LEFT*) CROSS SECTION OF ANANTHER. (*RIGHT*) LONGITUDINAL SECTION OF A PISTIL.

multiples of 4 or 5. For example, a lily, which is a monocot, has 3 sepals, 3 petals, 6 stamens, and a 3-part ovary formed from the fusion of 3 carpels. The flower of a wild geranium, a dicot, consists of 5 sepals, 5 petals, 10 stamens, and 5 fused carpels with separate stigmas.

Flowers that contain all four floral organs are known as **complete** and **perfect** flowers; however, the basic flower structure is frequently modified. In some flowers, one or more flower parts are missing; such flowers are **incomplete**. Incomplete flowers that lack either carpels or stamens are also called **imperfect**. If carpels are missing, the flower is **staminate** (male); and if stamens are lacking, the flower is **carpellate** or **pistillate** (female).

Although many flowers develop individually on a stalk, other flowers are grouped into a cluster called an **inflorescence**. Many times what looks like a single flower at first glance, such as a sunflower or a daisy, is actually an inflorescence. Another example is dogwood; the small flowers are in a cluster and surrounded by showy pink or white **bracts** (leaflike structures that often look like petals).

Pollination is the transfer of pollen from the anther to the stigma. In a flower that is self-pollinated, the transfer occurs within a single flower. By contrast, cross-pollination involves the transfer of pollen from one plant to another. A variety of animals, including insects, birds, and even mammals, serve as agents to transfer pollen for many flowers; wind carries the pollen for other plants. Animal-pollinated plants usually have large, showy flowers. The petals are brightly colored, and essential oils impart scents to attract the pollinator. These flowers often produce nectar, which is a reward for the animal. Pollen in the flowers is usually large, heavy, and sticky. By contrast, wind-pollinated flowers tend to be small and inconspicuous. They are often formed in an inflorescence and lack petals. Wind-pollinated flowers produce copious amounts of small, lightweight pollen.

Materials Needed for Exercise A

Dissecting microscope and compound microscope

Four different flowers. Select from those provided by the instructor.

Prepared slide of *Lilium* (lily) anther

Prepared slide of *Lilium* (lily) ovary

Razor blades, single-edged or scalpel and dissecting needles

Procedure for Exercise A

1. Examine the flowers available in lab. You should be able to determine whether you are looking at a single flower or an inflorescence. You should also be able to identify sepals, petals, stamens, and carpels. Which flowers are incomplete? Which are imperfect? Based

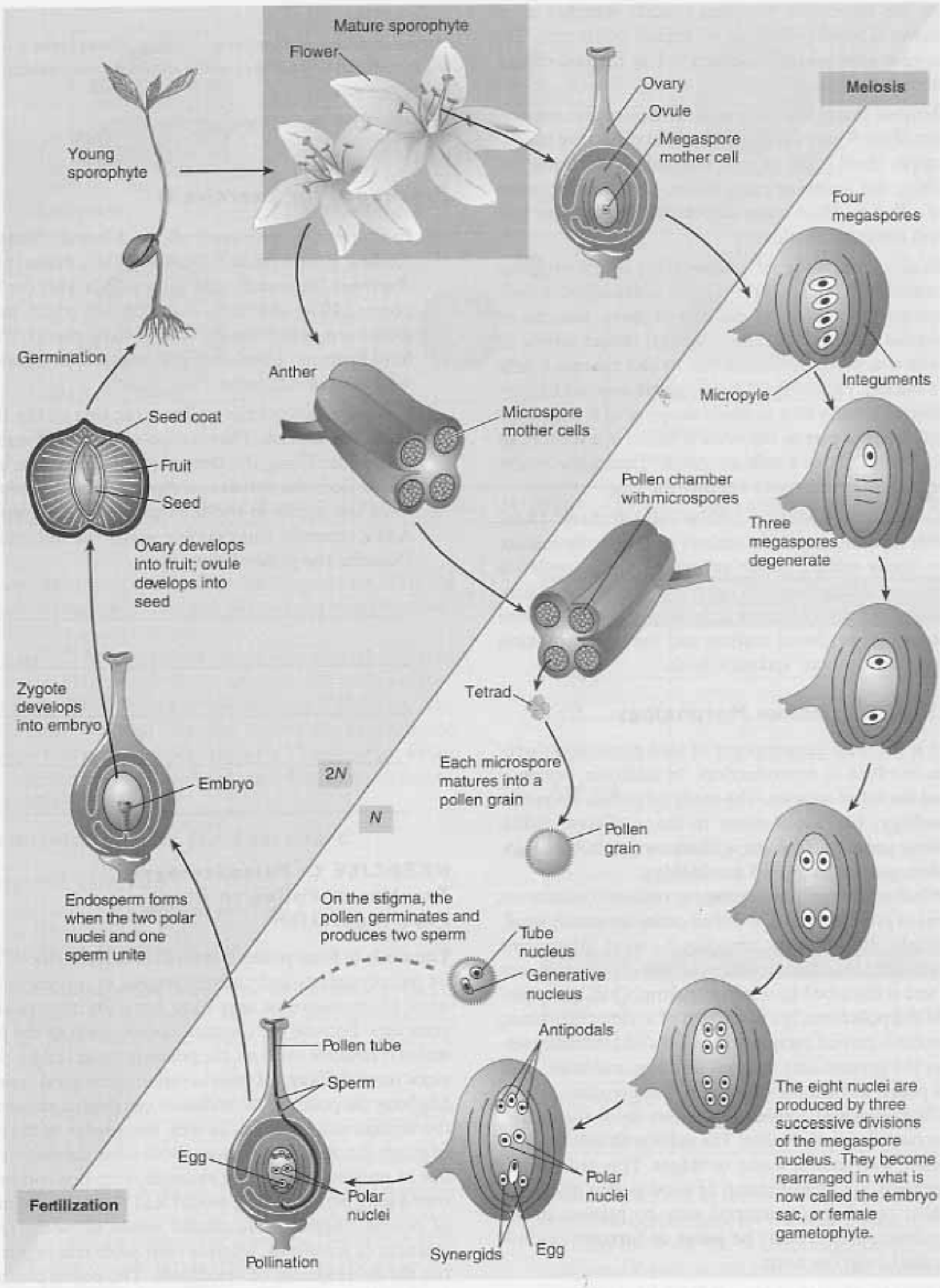


FIGURE 6.2 REPRODUCTION IN AN ANGIOSPERM.

on the structures you see, predict whether each flower is wind pollinated or animal pollinated. Fill in your answers in worksheet 6-1 at the end of this laboratory topic.

2. Remove the sepals and petals, and place the remainder of the flower on the stage of the dissecting microscope. How is the stigma adapted to catch pollen? Using the scalpel or razor blade, cut open the ovary of a flower. How many carpels are visible? Can you find the ovules inside?
3. Examine the slide of *Lilium* (lily) ovary with the compound microscope. These slides show nearly mature ovules. There are two or three sections of ovaries on each slide, with several ovules visible in each ovary. When mature, the ovules contain 8 cells (8 nuclei). One of the 8 is the egg. It may not be possible to actually find an ovule showing all 8 cells. Typically, only a part of the ovule is found in a section, so that only 2, 3, or 4 cells are visible. Locate the ovules on the slide and try to identify the egg.
4. Examine the slides of *Lilium* (lily) anthers. These slides are cut across the anthers and show the mature or nearly mature pollen grains. When the pollen is mature, the anthers split open and release the pollen. There are two different slides available in lab—one showing the closed anthers and the other showing the open anthers. Examine both.

EXERCISE B: Pollen Morphology

Pollen is the male gametophyte of seed plants and therefore is involved in reproduction. In addition, pollen is studied for other reasons. The study of pollen, known as **palynology**, has applications in many diverse fields, including geology, ecology, anthropology, archaeology, criminology, medicine, and aerobiology.

Wind-pollinated plants produce millions (sometimes billions) of pollen grains. The pollen grains are usually small, lightweight, dry, and easily carried by the wind. When some wind-pollinated trees are in flower, clouds of pollen are seen if the tree is disturbed by wind or shaking. Only a tiny percent of this pollen reaches the stigma of a compatible plant. Most of it is carried away by the wind and eventually settles on the ground and in lakes, streams, and bogs. This excess pollen is the focus of study by palynologists.

The pollen wall consists of an inner layer, the **intine**, and an outer layer, the **exine**. The exine wall may be quite elaborate, with spines, warts, or ridges. This ornamentation permits the identification of most pollen grains. In addition, openings (apertures) may be present in the exine; these openings may be **pores** or **furrows** (technically called colpi) or both.

Materials Needed for Exercise B

Compound microscope

Dissecting needles and forceps

Pollen stain

Prepared slides of *Juniperus* (cedar), *Pinus* (pine), *Betula* (birch), *Quercus* (oak), *Ambrosia* (ragweed), and grass pollen

Stamens from flowers available in lab

Procedure for Exercise B

1. Examine the prepared slides labeled *Juniperus* (cedar), *Betula* (birch), *Quercus* (oak), *Pinus* (pine), *Ambrosia* (ragweed), and grass pollen with the high power (40×) objective. Can you tell which pollen grains are ornamented? Which have pores? Which have furrows? Label each pollen photo in figure 6.3 with the correct name.
2. Prepare a slide of the pollen from one of the flowers available in lab. Place a drop of the pollen stain on your slide. Using the dissecting needle, scrape some pollen from the anther and dip it in the drop of stain. Swirl the needle in the stain to dislodge the pollen. Add a coverslip and examine under the microscope. Describe the pollen below.

_____

_____

_____

_____

_____

EXERCISE C: Paleoecology—The Use of Pollen to Study Past Vegetation

The study of fossil pollen is one of the main methods used by paleobotanists and paleoecologists in reconstructing what an environment may have been like thousands of years ago. Because the chemical composition of the exine makes it resistant to decay, the pollen is preserved for thousands (even millions) of years in various geological deposits. Studying the pollen in the sediment can help us understand the vegetational history of an area. Knowledge of the types of plants can also give us ideas about what the climate was like in previous times. For example, very dry conditions over a long period of time would lead to the development of deserts. Slightly more rainfall would allow the establishment of grasslands, whereas even more rain might permit the development of woodlands. The pollen preserved from each of these communities would be quite different. Changes in the pollen record from different layers in the sediment may indicate climate change occurring over a span of thousands of years in a given area. Fossil pollen

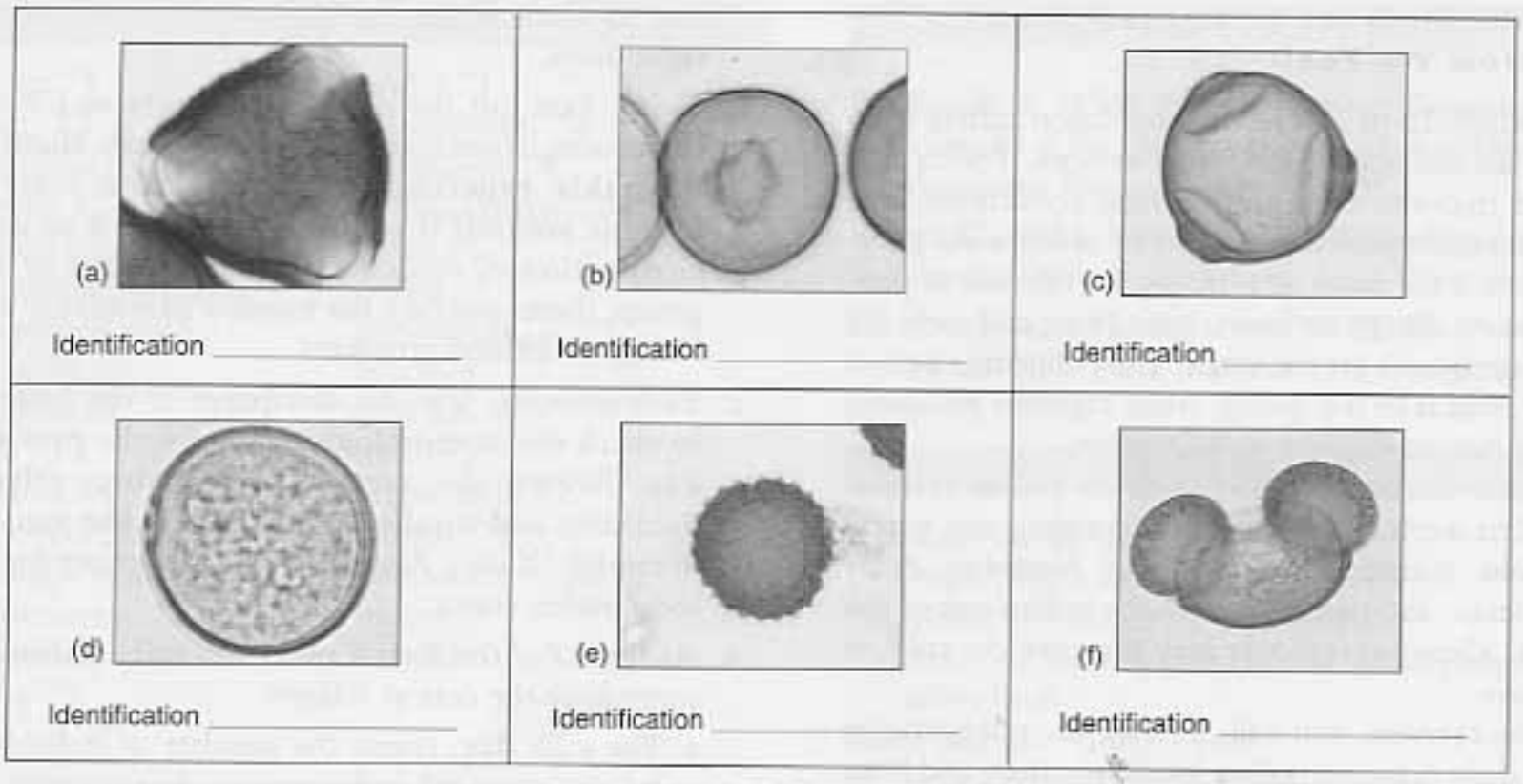


FIGURE 6.3 POLLEN GRAINS HAVE VARIOUS TYPES OF APERTURES AND SURFACE ORNAMENTATION.

has also been analyzed from archaeological sites. This has helped us determine the types of food and building materials used by ancient peoples.

You will be examining slides with several different types of pollen. These slides simulate the pollen recoveries from Ferndale Bog in southeastern Oklahoma. The pollen analyses from that site record the vegetational changes that occurred over the past 12,000 years. From our examination of these slides, we will attempt to reconstruct these changes.

Materials Needed for Exercise C

Compound microscope

Prepared slides of simulated geological site

Procedure for Exercise C

1. You will be given one of the slides that represents a certain time. Record the time period indicated on your slide and then place the slide on your microscope.

   My slide is from _____ years before present.

2. Focus the slide with the 10 × objective and then the 40 × objective. Move the slide so that the objective lens is near the edge of the coverslip. Count each type of pollen on one single traverse across the slide (the area under the coverslip).
3. Record your data in the following table. Determine the percent of each type of pollen.

POLLEN TYPE	NUMBER OF POLLEN GRAINS COUNTED	PERCENT OF TOTAL
Pine		
Oak		
Grass		
Ragweed		

4. We will be combining all the data from class. Write your data on the board. In the following table, calculate the average percentage from the class for each of the time periods.

TIME	PINE	OAK	GRASS	RAGWEED
700 yrs ago				
2,000 yrs ago				
5,000 yrs ago				
8,000 yrs ago				
12,000 yrs ago				

5. On worksheet 6-2 at the end of this laboratory topic, graph the percentages for the four pollen types that the class counted. For these pollen graphs, the vertical axis (Y-axis) is the time with the present time at the top and 12,000 years ago on the bottom. Each horizontal axis (X-axis) represents the average percentages you have calculated.

EXERCISE D: Does Airborne Pollen Affect How We Feel?

Approximately 20 to 25% of the population suffers from allergies, including hay fever and asthma. Pollen and spores are important triggers of these conditions. The small lightweight pollen produced by many wind-pollinated plants is the cause of suffering for millions of people. For many allergy sufferers, springtime and early fall are when symptoms are the worst. The pollination period for many trees is in the spring, while ragweed pollinates from mid-August through mid-October.

Weather has significant effects on pollen release. Most pollen is released on warm, dry, sunny, and windy days. Cold temperature and high humidity delay pollen release, and rainfall washes the pollen out of the air. Thus, allergy symptoms may improve on cold or humid days.

In this exercise, you will describe the relationships between daily ragweed pollen levels, weather, and your own (and your friends') allergy symptoms.

Materials Needed for Exercise D

Access to daily pollen counts and weather data from news media

Symptom diaries

Procedure for Exercise D

1. Obtain 5 to 10 copies of the symptom diary (see table 6.1). Your instructor will tell you how many to take. Add dates to the 5 days on diary so that everyone in class is conducting this exercise on the same dates.
2. *Today*: Pass out the daily diary sheets to friends, classmates, roommates, etc. **Please ask them to take this experiment seriously.** You may fill out one yourself if you wish. The aim is to get a crosssection of students on campus. Out of this group, there may be a fair number of students with allergy or asthma problems.
3. *Each morning*: Use the newspaper or the Internet to check the meteorological data for the **previous day.** Record the average temperature, relative humidity, and wind speed, as well as the amount of rainfall (if any). Also check the newspaper for the local pollen count.
4. *At the end of five days*: Collect the daily diaries and summarize the data as follows:
   a. For each **day**, count the number of individuals who experienced each symptom. For example, if 3 out of 5 experienced "sneezing" on Day 1, Place a "3" on the blank diary form (table 6.1).
   b. On what days did the greatest number of people have symptoms?
   c. What were the most common symptoms?
   d. What day had the highest pollen counts?
   e. In lab next week, your instructor will collate all the data from everyone in class and then correlate these with the weather and pollen counts.

TABLE 6.1 SYMPTOM DIARY—DOES AIRBORNE POLLEN AFFECT HOW WE FEEL?

Summarize the results of your survey on this form.
For each symptom experienced, place a "Y" in the box for each day.

Symptom	Day 1	Day 2	Day 3	Day 4	Day 5
Sneezing					
Runny nose					
Watery, itchy, or red eyes					
Coughing					
Shortness of breath					
Wheezing					
Sore throat					
Congestion					
Took extra medication for allergy problems					
Missed class due to allergy problems					

TERMS TO KNOW

anther 71
bracts 72
carpellate (pistillate) flowers 72
carpels 71
complete flowers 72
compound pistil 71
exine 74
filament 71
fruit 71
furrows 74
imperfect flowers 72
incomplete flowers 72
inflorescence 72
intine 74
ovary 71
ovules 71
paleoecology 74
palynology 74
perfect flowers 72
petals 71
pollen 71
pollination 72
pores 74
seed 71
sepals 71
simple pistil 71
stamens 71
staminate flowers 72
stigma 71
style 71

QUESTIONS FOR REVIEW AND DISCUSSION

1. What is a complete flower? What floral organs are present?
2. What is the difference between a flower and an inflorescence?
3. Plants have evolved a number of mechanisms to attract animal pollinators. What are some of these characteristics? What is the benefit to the animals?
4. What were some of the differences in the pollen types you examined under the microscope?
5. Which pollen type had air sacs? What do you think would be the advantage of these air sacs?
6. Based on the simulated pollen recoveries, describe how the vegetation around Ferndale Bog changed over the last 12,000 years.
7. What are the characteristics of a flower that could cause hay fever?
8. Based on the total number of participants for the hay fever study, what percent exhibited allergy symptoms? How does this compare to the national average?

ADDITIONAL RESOURCES

Bradley, R. S. 1999. *Paleoclimatology: Reconstructing climates of the Quartenary*, 2d ed. San Diego: Academic Press.

Faegri, K., and J. Iversen. 1989. *Textbook of pollen analysis*, 4th ed. New York: John Wiley & Sons.

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

ON THE WEB

National Allergy Bureau
http://www.aaaai.org/nab/

University of Arizona Palynology Labs
http://www.geo.arizona.edu/palynology/index.html

Royal Botanic Gardens Kew
http://www.rbgkew.org.uk/ksheets

OTHER ACTIVITIES

A scanning electron microscope (SEM) can provide details of pollen grains not visible with a light microscope. These details can be seen by studying photographs (micrographs) taken with an SEM. Examine micrographs of various types of pollen. These may be available in lab, or your instructor may have you research this topic in the library or on the Internet. (If an SEM is available in your biology department, you may have the opportunity to see pollen grains directly at high magnification.) Observe the surface of the pollen grain. Describe the aperture and any ornamentation present on the grain. You may want to compare the SEM image with the appearance of the pollen under the light microscope. What features appear different in the micrographs?

NAME TREVOR

DATE 10/25/04

LAB SECTION NUMBER

WORKSHEET 6-1 EXERCISE A: FLOWER STRUCTURE

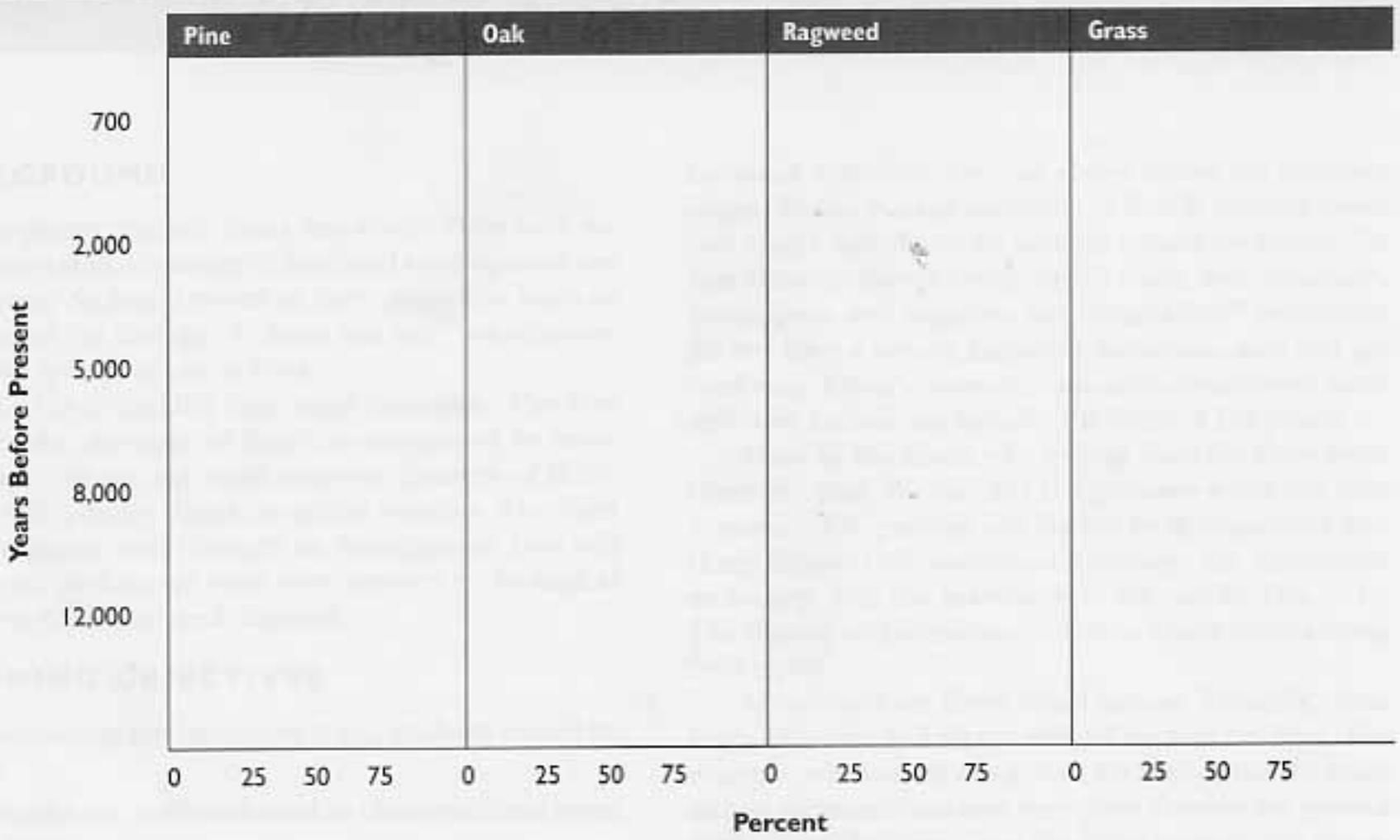
	Flower 1	Flower 2	Flower 3	Flower 4
Name of the flower	SNAPDRAGON	LILY	AFRICAN DAISY	
Single flower or an inflorescence?	SINGLE	SINGLE	SINGLE	
How many sepals?	FIVE	SIX TEPALS	EIGHTEEN	
How many petals?	ONE	SIX TEPALS	EIGHTEEN	
How many stamens?	FOUR	SIX	~~MANY~~ EIGHTEEN	
Is there a fragrance or aroma?	NO	NO	YES	
How many carpels?	ONE	ONE	EIGHTEEN	
How is the stigma adapted to trap pollen?	ELONGATED PRETTY NORMAL	STICKY	STICKY	
Do you think the flower is wind or animal pollinated?	ANIMAL	ANIMAL	ANIMAL	
OVARY SUPERIOR OR INFERIOR	SUPERIOR	SUPERIOR	INFERIOR	
PLACENTATION (AXILE, PARIETAL, FREE CENTRAL, BASAL)	AXILE	FREE CENTRAL ~~BASAL~~	FREE CENTRAL	

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 6-2 EXERCISE C: PALEOECOLOGY



LABORATORY TOPIC

7

Fleshy Fruits and Flying Seeds

BACKGROUND

Why do plants produce fruits and seeds? How have we as humans taken advantage of fruits and seeds to meet our own needs? Seeking answers to these questions helps us understand the biology of plants and our own relationship with specific plants as food.

This lab is divided into three exercises. The first explores the diversity of fruits as recognized by most botanists. The second emphasizes the diversity of fleshy fruits, with primary focus on edible varieties. The third exercise guides you through an investigation that will allow you to discover your own answers to biological questions related to seed dispersal.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Describe the attributes used to distinguish the types of fruits.
2. Recognize a diversity of fruit types.
3. Describe the origin, history, and use of common edible fruits.
4. Understand the effects of variables such as seed and fruit morphology or wind speed on dispersal distances.
5. Conduct a scientific inquiry by posing questions, designing investigations, collecting empirical data, testing hypotheses, and communicating the results.
6. Recognize specific plant adaptations as successful reproductive strategies.

EXERCISE A: Diversity of Fleshy Fruits

Botanically, a **fruit** is a ripened ovary and its contents. Within the fruit are seeds. The embryo of the next generation lies within the seed. Most botanists restrict the term "fruit" to the flowering plants and do not refer to the mature female reproductive structures of gymnosperms (e.g., pine cones) as fruits. However, the botanical definition does not always follow the common usage. To the average consumer, a fruit is typically sweet and would most likely be eaten as a snack or dessert. To that same consumer, some "true" fruits, such as squash, beans, peas, and eggplant, are "vegetables." Vegetables do not have a strictly botanical definition, so it can get confusing. Other common foods, such as sunflower seeds and corn kernels, are actually the fruits of the plants.

Most of the tissue of a typical fruit develops from the ovary wall. We call this the **pericarp** when the fruit is mature. The pericarp can further be distinguished into three layers: the outermost **exocarp**, the innermost **endocarp**, and the **mesocarp** in the middle (fig. 7.1). The texture and consistency of these layers varies among fruit types.

Many fruits are fleshy when mature. Typically, these fruits are sweet and attract animals to feed on them. For example, when a bird eats a fruit, the bird carries the seeds to new locations (and may even plant them in the ground with natural fertilizer). Just like other animals, our ancestors ate many fruits from the wild. As time progressed,

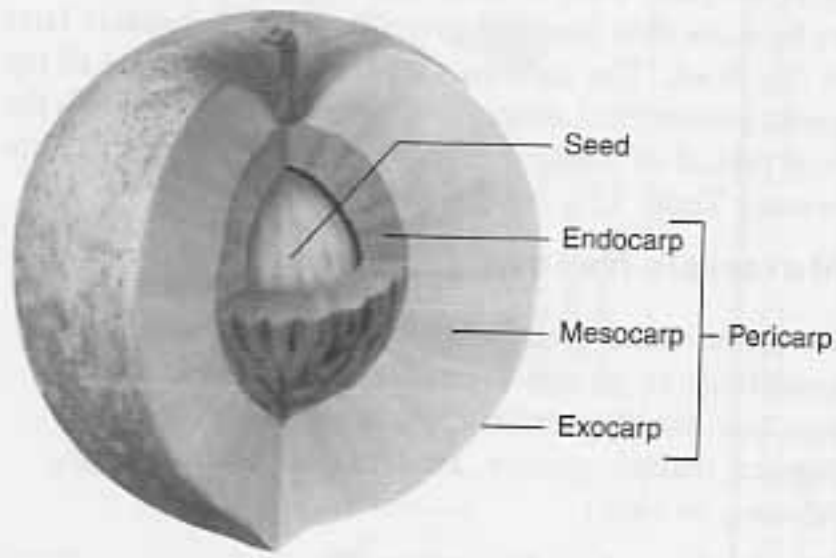


FIGURE 7.1 REGIONS OF THE MATURE OVARY WALL (PERICARP) OF A PEACH. AS A DRUPE, A PEACH HAS A THIN EXOCARP, FLESHY MESOCARP, AND STONY ENDOCARP.

they learned how to grow bigger, sweeter, and more abundant fruits. Nowadays, some of these plants, such as apples, oranges, bananas, and mangoes, resemble their wild counterparts only remotely. Many "domesticated plants" are major commodities in international commerce.

Other fruits are dry when mature. Some mature dry fruits split open and release their seeds. When a dry fruit splits, it is called **dehiscent**. Other dry fruits stay intact, often with the fruit wall surrounding the seed(s). These fruits are **indehiscent**. Each plant has evolved its own type of fruit adapted to the reproduction of the species.

Materials Needed for Exercise A

Variety of plant fruits (e.g., maple, elm, ash, milkweed, poppy, sunflower, apple, strawberry, grape, olive, cucumber, pineapple, green pepper, orange, dill, acorn, maize, or pea pod)

Procedure for Exercise A

Fruits are classified based on their fleshiness, dehiscence, position of ovary, and number of carpels, as well as other characteristics. Examine the samples of fruits provided in the lab. Use the flowchart in figure 7.2 to identify the type of fruit. Record the characteristics of each fruit on worksheet 7-1 at the end of this laboratory topic. Remember that one of the main functions of each fruit is to move seeds to a suitable location for ensuring the next generation. As you examine each fruit type, consider specific morphological adaptations that may make the fruit fulfill its function of seed dispersal.

EXERCISE B: Edible Fleshy Fruits of Commerce

In this exercise, we will emphasize fleshy fruits of commercial value. Two of these fruit types are so important to humans that they are given their own chapters later in this book. The caryopsis type of fruit is typical of the grains covered in Laboratory Topic 11. The legume is the fruit typical of the bean family and is introduced in Laboratory Topic 12.

Materials Needed for Exercise B

Common fruits used as human food, depending on availability (e.g., apple, strawberry, grape, olive, walnut, cucumber, pumpkin, tomato, pineapple, green pepper, mango, papaya, avocado, coconut, orange, banana, or okra)

Procedure for Exercise B

A variety of fruits are available in lab for you to explore. For each fruit, take note of its shape, texture, taste, type, probable origin, and any other interesting facts. Record your findings in worksheet 7-2. Be sure to enjoy a fruit salad when you are through examining the fruits.

1. **Green or bell pepper (*Capsicum annuum*).** Green peppers are examples of a simple fruit type called a **berry**. A berry is fleshy when mature, derived from a single ovary, and multiseeded. Take a green pepper and cut it in half. What do you see? The inside is divided into chambers. In the flower, each chamber was a carpel. The carpels of peppers are fused into a single ovary.

   Can you see the green bulges on the inner wall of the pepper? Each bulge is an individual cell. Normally, it takes a microscope to see cells, yet these are large enough to see with the unaided eye.

   Where are the seeds attached to the pepper? The tissue on the wall where the seeds are attached is called the **placenta**. If you enjoy the taste of "hot" peppers, the highest concentration of capsaicin is in the placenta. Laboratory Topic 14 provides an opportunity to test for yourself the "hotness" of different chili peppers, relatives of green peppers.

   All members of *Capsicum* are native to the New World. In fact, they were unknown to Europeans before Columbus. In just the last five centuries, their popularity has spread to Asia, Africa, and Europe. In fact, China grows ten times more chiles than the United States.

2. **Tomato (*Solanum esculentum*, formerly *Lycopersicon esculentum*).** Tomatoes are also native to the New World. When the Europeans first encountered tomatoes, they thought they were poisonous or hallucinogenic. There is some truth to this belief since the leaves of the plant are toxic to livestock, and many other members of the same plant family are deadly. The former scientific name means "juicy wolf peach" and refers to the German belief that tomatoes could be used to evoke werewolves. Think of all the European cuisines that now use tomatoes as the primary base. What would Italian pasta be without tomato sauce?

   Like peppers, tomatoes are sometimes not considered fruits. To the botanist, a tomato is like a bell pepper and therefore a typical example of a berry. Like the pepper, a tomato is fleshy when mature, derived from a single ovary, and multiseeded. Typically, we eat tomatoes when the seeds are not quite ripe, but if you get a sun-ripened tomato, the seeds are mature.

   Cut a tomato into sections and notice where the seeds are attached.

3. **Apple (*Malus domestica*).** Although wild apples probably originated in western Asia, apple trees spread across North America before humans arrived. Nevertheless, the edible apples of today come from Old World varieties. The phrase "as American as apple pie" may say more about our multicultural heritage than about the land we call home.

I. Fruit derived primarily from the ovary of a flower

Simple fruit: Fruit derived from one ovary of a single flower

Pericarps fleshy at maturity

Pericarps fleshy throughout

Hesperidium: Partitions evident in cross section; leathery and glandular exocarp separable from inner layers — Lemon

Pepo: No partitions evident in cross section; leathery to woody exocarp inseparable from inner layers — Watermelon

Berry: More or less fleshy throughout and not as pepo or hesperidium — Tomato

Pericarps divided into thin, skin-like exocarp; thick, fleshy mesocarp; and hard, stony endocarp

Drupe: Pericarp with fleshy mesocarp and stony or hard endocarp; usually single seed — Peach

Aggregate fruit: Fruit derived from several ovaries of a single flower

Multiple fruit: Fruit derived from a cluster of several ovaries from several flowers clustered together on one stem — Pineapple

Pericarps dry at maturity

Dehiscent: Fruit splits open when ripe; contains numerous seeds

Legume: Fruit of one carpel; splits along *two* seams; member of Fabaceae — Snow pea

Follicle: Fruit of one carpel; splits along *one* seam — Milkweed

Capsule: Fruit of 2 or more fused carpels; splits in a variety of ways — Poppy

Silique: Fruit of 2 fused carpels that separate, leaving a persistent partition between; member of Brassicaceae — Silique

Indehiscent: Fruit does not split open when ripe; usually contains only 1 or 2 seeds

Achene: Bears 1 seed that is attached to inside of pericarp at only one point — Sunflower

Caryopsis (grain): Bears 1 seed that is completely fused with pericarp — Corn

Samara: An achenelike fruit with a flat, winglike outgrowth — Maple

Nut: Hard exocarp and single seed; usually attached to extra tissue (e.g., cap of acorn) — Bur oak

II. Fruit derived mostly from floral parts other than the ovary

Accessory fruit: Calyx or receptacle fused with ovary wall; can be simple, aggregate, or multiple

Pome: Simple fleshy fruit derived from inferior ovary surrounded by a fleshy hypanthium (receptacle) — Apple

Accessory aggregate: Most of fruit derived from receptacle; individual ovaries form berries, drupes, or achenes (e.g., strawberry or blackberry) — Raspberry

FIGURE 7.2 FLOWCHART OF THE CLASSIFICATION OF FRUIT TYPES.

The apple has been a part of history and mythology. The generic name, *Malus*, means "bad" in Latin, a reference to Eve's tragic nibbling in the Garden of Eden. The apple is also central to the legends of William Tell, Johnny Chapman (Johnny Appleseed), and Isaac Newton. And who doesn't associate apples with a great teacher?

An apple is a type of fruit called a **pome**. Examine an apple cut longitudinally and another cut transversely. The central star or "core" is derived from the original ovary in the apple blossom. The fleshy part of a pome is actually derived from the stem that supported the flower (called the **receptacle**). Pears and quinces are also pomes.

4. **Grape (*Vitis vinifera* and *Vitis lambrusca*).** Grapes grew wild in both the Old and New Worlds before Columbus sailed across the Atlantic. *Vitis vinifera*, the most widely cultivated grape, is used for making wine. It is native to the Mediterranean. The practice of making wine from grapes goes back to ancient times, possibly from 10,000 years ago. *Vitis lambrusca* is native to North America and is the source of many varieties of table grapes, such as the Thompson seedless.

   Each grape is a berry. But while most true berries are multiseeded, many grapes have been bred to have very few seeds or none at all.

   Cut a grape into sections and notice where the seeds are attached.

5. **Orange (*Citris sinensis*).** The orange and other citrus fruits, such as lemons, grapefruits, and limes, are all hesperidia. A **hesperidium** is a special type of berry with a leathery, glandular rind and juice-filled cells inside. The rind is filled with aromatic oils. Have one person stand on one side of the room. Have another person take an orange peel and squeeze it. How long does it take the person across the room to smell the orange? The aromatic or essential oils are volatile compounds that we can detect in very low concentrations. Essential oils are used throughout the spice and perfume industries to evoke pleasant emotions and senses. You can explore essential oils more in Laboratory Topic 14.

   The orange is one of the most widely grown fruits in the world. It is restricted to frost-free zones since it does not handle cold weather well. In the United States, primary production areas are California, Texas, and Florida.

   Cut an orange in half. What is it about a hesperidium that still qualifies it as a type of berry?

6. **Pumpkin (*Cucurbita pepo*).** Pumpkins, squashes, gourds, and melons are other types of special berries. The fruit develops from a single ovary and is multiseeded. The fruit wall has a thick or leathery rind. This type of special berry is called a **pepo**. Usually, we only use the term pepo for members of the squash or melon plant family.

   In the United States, pumpkins are seasonal fruits associated with Halloween jack-o-lanterns and Thanksgiving pies. Pumpkins and squashes were more important in the past and were one of the first plants to be domesticated in the New World.

   The seeds of pumpkins are highly nutritious and could replace sunflower seeds if marketed well.

7. **Olive (*Olea europaea*).** Olives are good examples of the fruit type called a **drupe**. If you try cutting into a drupe, you will cut through the fleshy outer layers until you reach a hard center. The outermost layer, the exocarp, is like a skin. The middle layer, the mesocarp, is fleshy. The innermost layer, the endocarp, is very bony, like a stone. Within the endocarp a drupe has a single seed. Peaches, cherries, and plums are other examples of drupes.

   Olives are pickled or used to extract olive oil. Unlike most fleshy fruits, olives contain more lipids than carbohydrates. Fresh olives also contain oleuropein, which is very bitter. Before olives are edible, they need to be processed with a strong alkali to degrade the oleuropein. Most olives, however, are used to extract olive oil. Because olive oil is unsaturated and has a pleasant taste, it has been a favorite of health-conscious consumers and gourmet chefs.

   Try cutting a green olive in half. What do you notice?

8. **Coconut (*Cocos nucifera*).** The coconut palm tree is one of the most versatile plants of the tropical region. Not only does it provide food and drink, but it is also a source of oil, fiber, and building material. The fruit is formed from a flower with three carpels, yet only one carpel develops to maturity. The three "eyes" on the end of a coconut represent these three carpels. The mature fruit is about 30 cm (1 foot) in length and has a fibrous mesocarp. In grocery stores in the continental United States, most of the mesocarp has been removed down to the hard endocarp. Inside there is only one seed (the largest known). The embryo is relatively small, and most of the space is filled with nutritious endosperm. When a coconut is green and immature, this endosperm is liquid and is called coconut milk. As it matures, cell walls are laid down, and the endosperm develops into the oil-rich coconut "meat." This solidified "meat," called copra, is the form we use in baking.

   Coir fibers in the coconut's mesocarp are longer than cotton fibers and spun into yarns used for ropes and matting. The fibrous mesocarp helps the fruit float in seawater. Although native to the Indo-Pacific region, coconuts may have been carried by the currents to the coasts of South America, Southeast Asia, and Polynesia.

Examine the outside of a coconut. If it was bought at the store, you are probably only looking at the brown, hard endocarp. Because of this hard, stony endocarp, a coconut is considered a drupe like the olive or the peach. Do you see the three "eyes"? Use a hammer to crack open the coconut and see the inside. Be sure to taste the coconut meat.

9. **Strawberry (*Fragaria*).** The red, juicy, fleshy structure we call a strawberry is not truly a berry. In fact, the red part is actually the expanded receptacle (or stem) of the flower. On the surface of the strawberry are little structures that resemble small seeds. These are the "true" fruits of the strawberry plant because they are derived from the ovaries. The true fruit type is an **achene**. Because the complete red strawberry is actually a cluster of achenes, the strawberry is considered an **aggregate fruit**. Because the red part is not derived from an ovary, it is also an **accessory fruit**. So the strawberry is an **accessory aggregate fruit**.

   Strawberries are native to both the New World and the Old World. Several species are cultivated, and many grow wild, providing a favorite treat for the summer hiker. Some of the cultivated varieties are actually hybrids between naturally occurring species. The taste of strawberries is one of the most distinctive and is used to flavor many foods.

10. **Pineapple (*Ananas comosus*).** A pineapple is a **multiple fruit** composed of 100 to 200 ovaries of separate flowers fused together. The "fruit" represents the whole inflorescence, including flowers and pedicels. Most of the cultivated varieties of pineapple are parthenocarpic and seedless.

    Pineapples are indigenous to the New World and were used by native peoples before the Europeans arrived. Columbus described the pineapple in his journal from his second voyage. He also noted that the fruit resembled a pine cone, a characteristic that is still reflected in the common English name for the plant.

    Fresh pineapple has a naturally occurring protease called bromelain, which can cause skin irritations in people who work with pineapples. This enzyme also acts as a meat tenderizer because it degrades protein. What happens when you add fresh pineapple to Jello gelatin? Why can you add cooked pineapple to gelatin salad without problems?

11. **Avocado (*Persea americana*).** The avocado is also called the alligator pear. When you look at the texture of its exocarp, you can see why. The avocado is native to Central America, and its use can be traced to 7000 B.C. in southern Mexico. The word *avocado* comes from the Aztec word *ahaucacuahatl*, meaning testicle tree. This name is based on the appearance of the fruit, which is commonly found hanging in pairs.

    Avocados are different from most fleshy fruits. While most fleshy fruits are sweet, with high concentrations of simple carbohydrates, avocados are high in lipids. In fact, 30% of the green flesh can be oil. For this reason, eating too much guacamole can be very fattening!

12. **Papaya (*Carica papaya*).** The papaya is a smooth-textured fruit native to Central America. The fruits are born on the main stems of the papaya trees, rather than on the branches as with many other fruits. Like the pineapple, papayas produce an effective protease that can be used as a meat tenderizer. This enzyme, called papain, can be extracted from the milky latex that exudes from cuts into the flesh of unripe papayas. Technically, the fruit type is a berry, since it is derived from a single ovary.

13. **Mango (*Mangifera indica*).** The mango is native to southwestern Asia but grown in many regions, including South America. The flowers of a mango tree are small, and they grow in long clusters. Usually only one flower per cluster matures into a fruit. The mangos grow on long, bare stalks that make the tree look like it's decorated with hanging ornaments.

    The mango is in the same family as poison ivy and poison oak. Often, workers in the orchards get skin rashes similar to poison ivy. If you are particularly sensitive, you may want to avoid too much contact with mangos.

14. **Kiwi (*Actinidia chinensis*).** The kiwi is a newcomer to American markets. It is native to Asia and had limited distribution up until a few decades ago. Originally, kiwi was known as Chinese gooseberry until the New Zealand growers held a contest for a new name. With the new name came increased marketing and greater popularity. The kiwi is still grown in orchards of dioecious vines in New Zealand, but it is also grown in California and other regions of the world. The kiwi is another example of a berry.

    Finish your exploration of the fruits by enjoying a fruit salad. The tastes are as important as the shapes and textures, so take note of each.

EXERCISE C: Dispersal of Flying Seeds

Dispersal is the movement of organisms away from their place of birth or from centers of population density. For plants, dispersal is a very important concept related to their long-term success in nature. Consider a tree. If all the seeds were to fall at the base of the tree and germinate, the offspring would compete with the parent tree. In most cases, this would not be advantageous; for example, the parent would be able to extract more water and minerals due to its established root system. Can you think of an instance when it might be advantageous?

The survival of a species over time is like an obstacle course. At each stage of an organism's life, it must contend with the hazards of its surroundings, such as environmental stress, competition, predation, and disease. At each hurdle, only the best-adapted individuals can survive to pass on their genes. The ultimate survivors are often only the tiny minority that natural selection has failed to eliminate. Dispersal is but one of the hurdles a new individual must face, yet it is an important one.

Dispersal of plants involves the movement of diaspores away from the parent plant. A **diaspore** is a generic term for the specific plant part that is dispersed. In lower plants, such as mosses and ferns, diaspores are gametes and spores. In higher plants, diaspores are primarily the pollen and seeds. A successful dispersal mechanism is necessary to maintain the number of individuals within a given population as well as to allow the range of the species to expand. Effective pollen dispersal requires the transport of pollen to receptive stigmas and subsequent fertilization. Similarly, the success of seed dispersal depends on seeds reaching a site that has favorable conditions for germination and seedling establishment.

Although most animals are self-mobile, plants are sedentary organisms. The dispersal of seeds and pollen is thus dependent on external agents, such as wind, water, and animals (especially birds and insects). Any adaptation or modification that allows a diaspore to reach a "safe" site and survive will have a selective advantage. Complex interrelationships between the plant and its dispersal agent have developed over the course of time. Examples include nocturnal pollination by bats, elaborate floral displays that attract specific insects, the parachutelike achenes of dandelions, and the coevolution of selected fruits and frugivorous birds.

Seeds that depend on the wind for dispersal are engineering marvels (fig. 7.3). The distance covered by the seed depends on three factors: (1) the height of seed drop, (2) the terminal velocity of the seed, and (3) the wind velocity. **Terminal velocity** is the limiting speed of a free falling body when the downward force of gravity equals the upward force due to air friction. Terminal velocity is proportional to the surface area and density (mass/volume). Any modification that increases the surface area relative to the mass increases air resistance and decreases the terminal velocity. Any environmental condition (such as humidity) that increases the mass relative to the volume (i.e., increased density of the seed) increases the terminal velocity. The smaller the terminal velocity, the longer it takes for the seed to settle to the ground under calm conditions. The height at which the seed is released also affects this flight. **Wind velocity** affects the horizontal path of the seed. Microclimatic conditions such as topography, temperature, and plant density interact to determine the dispersal of wind-dispersed species.

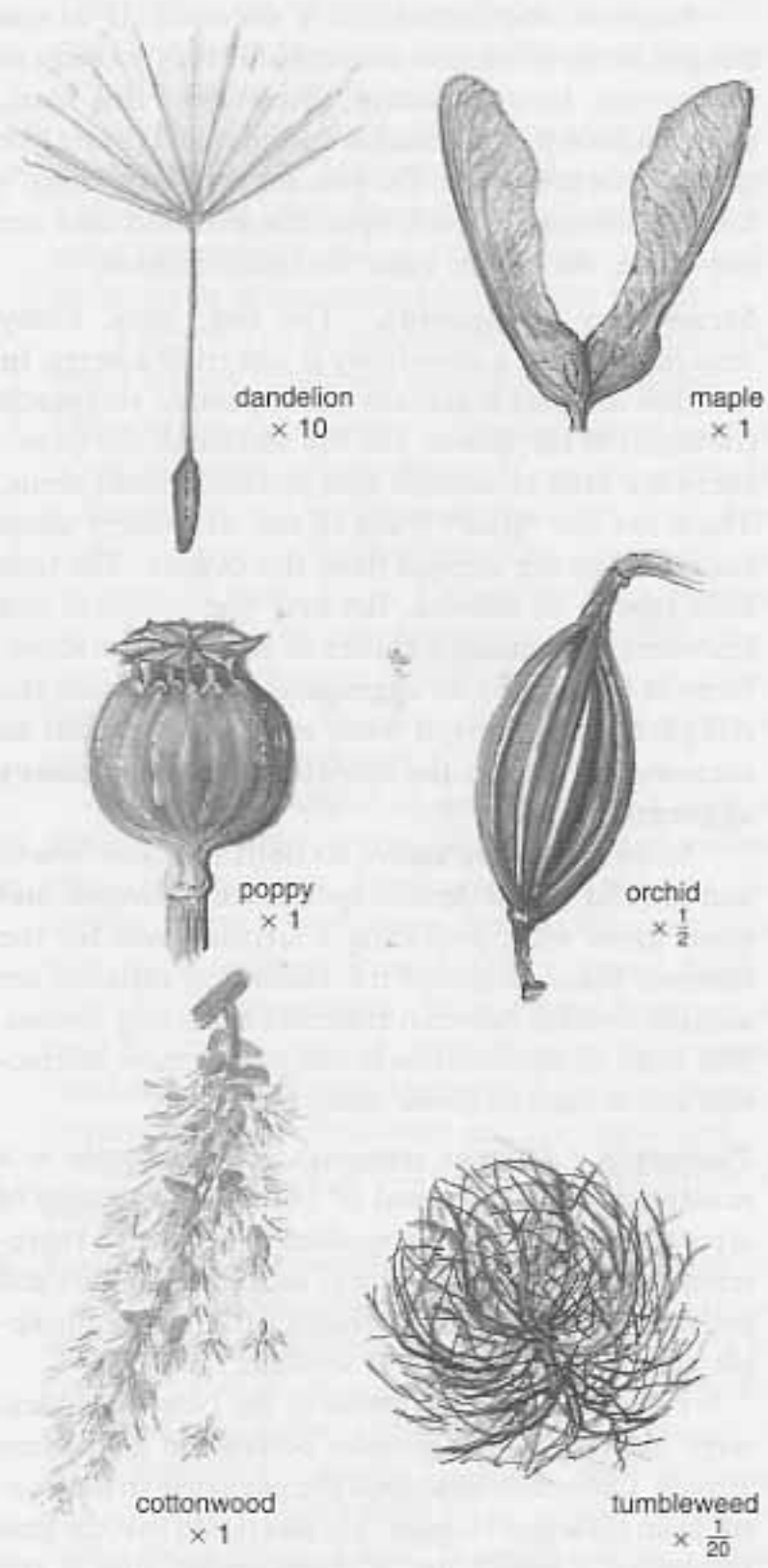


FIGURE 7.3 TYPES OF SEEDS AND FRUITS DISPERSED BY WIND.

The reproductive success of wind-dispersed seeds is further influenced by the ability of seeds to land in suitable habitats and to germinate there. Larger seeds often have greater energy reserves for seed survival and germination. However, their increased mass often decreases the dispersal distance. Reproductive success thus depends on the interaction of all the physical and biological factors, and numerous successful strategies have evolved.

Materials Needed for Exercise C

Measurement devices (e.g., metersticks, stopwatch, balance, anemometer, etc.)

Other aids (e.g., ladders, fans, staircase, etc.)

Variety of wind-dispersed seeds and fruits (e.g., milkweed, ash, elm, tree-of-heaven, maple, dandelion, and clematis)

Procedure for Exercise C

1. If the season and weather permit, take a short trip around campus and examine the diversity of seeds and fruits produced by plants. Take notice of all types of plants, from tall trees to common weeds. Try to "think like a plant." If the function of the fruit and seed is to ensure successful reproduction of the next generation of plants, what strategies are the various plants employing?
2. Record some of your observations in the space provided here:

   Observation 1: _____

   _____

   Observation 2: _____

   _____

   Observation 3: _____

   _____

   Observation 4: _____

   _____

   Observation 5: _____

   _____

   Observation 6: _____

   _____

3. Hopefully, your observations will spark your curiosity and help you pose new questions. Capture as many of these questions as you can, and record them in the following spaces. The questions can be the links between what you already know and what you still need (and want) to discover. Questions represent problems in need of solutions. Choose a question that you can investigate further and circle it.

   Question 1: _____

   _____

   Question 2: _____

   _____

   Question 3: _____

   _____

   Question 4: _____

   _____

   Question 5: _____

   _____

   Question 6: _____

   _____

4. Write a hypothesis for the question you choose. A **hypothesis** is a possible explanation for a problem. In many ways, it is an educated guess, often based on prior knowledge and insight into the problem.

   State your hypothesis: _____

   _____

5. Once your hypothesis is stated, you can design an experiment to test it. If the question is too broad, a complicated experiment requiring sophisticated equipment may be needed. In that case, redefine your question so you can design a simple experiment. For example, you may ask, What is the effect of surface area on the dispersal distance of wind-dispersed seeds? Explain the overall design of the experiment you will conduct to test your hypothesis. Be sure to describe the type of data you will collect and how you will collect that data. Also include the nature of the control or comparison group used to evaluate your hypothesis and experimental approach. Refer to Appendix A for more information on experimental design and the nature of data.

   Describe your proposed experiment: _____

   _____

   _____

   _____

   _____

   _____

6. State your **prediction** for each hypothesis. The easiest way to do this is to rewrite your hypothesis as an "If"—"then" statement.

   State your prediction: _____

   _____

   _____

7. Have your instructor check your experimental design before you begin your experiment.
8. Perform your experiment and record your data. Design your own data tables in advance and use them to record your data.
9. Analyze your data. Compile your raw data into a summarized table or graph. Bar graphs are best for comparing the means of discrete groups, such as two or more plant species. A line graph or scatterplot is best to show trends when the independent variable is continuous rather than discrete. Remember that the role of the table or graph is to help summarize and interpret the data.
10. Interpret the results of your experiment. Do you accept or reject your hypotheses? Why? Sometimes the experiment suggests new insights into the relationships behind the problem. You may generate new questions that can lead you in new directions.

    Conclusions: _____

    _____

    _____

    _____

    _____

11. Prepare a convincing argument on what you learned from your experiment. Be sure to base your conclusions on your data and not speculate too far beyond what you observed.

    Discussion: _____

    _____

    _____

    _____

    _____

12. Communicate your findings to your classmates. Present your original question, experimental design, summarized data, and conclusions. What did you learn? What do you still want to know?

    _____

    _____

    _____

    _____

TERMS TO KNOW

accessory fruit 87
achene 87
aggregate fruit 87
berry 84
dehiscent 84
diaspore 88
dispersal 87
drupe 86
endocarp 83
exocarp 83
fruit 83
hesperidium 86
hypothesis 89
indehiscent 84
mesocarp 83
multiple fruit 87
pepo 86
pericarp 83
placenta 84
pome 86
prediction 90
receptacle 86
terminal velocity 88
wind velocity 88

QUESTIONS FOR REVIEW AND DISCUSSION

1. Fruits that we use as food have been genetically modified over years of breeding. After examining several common fruits that are used as food, what trends do you see? What features have been enhanced?
2. One of the characteristics of a domesticated crop is an inability to reproduce naturally in the wild. Which of the fruits presented in lab appear to have lost this ability? Why would these plants have difficulty in the wild?
3. If fleshy fruits are dispersed by animals, what do the animals get for their efforts?
4. What features of seed morphology had the most influence on seed dispersal in your experiments?
5. How would you design your experiment on seed dispersal differently if you were to repeat the experiment?

ADDITIONAL RESOURCES

Janick, J., and J. Moore, eds. 1975. *Advances in fruit breeding*. West Lafayette (IN): Purdue University Press.

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Minorsky, P. V., and R. P. Willing. 1999. Samara dispersal in boxelder: An exercise in hypothesis testing. *The American Biology Teacher* 51:482–86.

Monselise, S. 1986. *CRC handbook of fruit set and development*. Boca Raton, (FL): CRC Press.

Morton, J. 1987. *Fruits of warm climate*. Miami (FL): Morton.

Samson, J. 1986. *Tropical fruits*, 2d ed. New York: Longman.

Simpson, B., and M. Conner-Ogorzaly. 1986. *Economic botany: Plants in our world*, 2d ed. New York: McGraw-Hill Companies, Inc.

Thomson, J. D., and P. R. Neal. 1989. Wind dispersal of tree seeds and fruits. *The American Biology Teacher* 51:482–86.

ON THE WEB

USDA, ARS Fruit Laboratory Home Page. Site dedicated to current research in fruit breeding, production, and disease.
http://www.barc.usda.gov/psi/fl/fl.html

Fruit Online. Site dedicated exclusively to the international fruit business.
http://www.fruitonline.com/

The Fruit Pages. Commercial site dedicated to everything you want to know about fruit.
http://www.thefruitpages.com

OTHER ACTIVITIES

1. Compare the effect of fresh and cooked pineapple and fresh and cooked papaya on gelatin.
2. Try a blindfolded taste test of the edible fruits you studied in this lab. Record your observations of texture, taste, and smells and then identify the fruits.
3. Conduct a competition to design a seed dispersal strategy that would improve the reproductive success of a real seed, such as milkweed. For the competition, reproductive success can be assessed as the product of the mass of the embryo (new individual in the seed) and the distance traveled.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 7-1 EXERCISE A: DIVERSITY OF FLESHY FRUITS

Name of Plant	Dry or Fleshy	If Dry, Dehiscent or Indehiscent	Structures other than Ovary Involved in Fruit Development	Fruit Type	Comments

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 7-2 EXERCISE B: EDIBLE FLESHY FRUITS OF COMMERCE

Name of Plant	Fruit Type	Texture	Taste	Probable Origin	Comments

LABORATORY TOPIC 8

Genetic Diversity of Our Food

BACKGROUND

Consider a cabbage. Why does it look the way it does? Why does it have the shape of a ball? Why are some cabbages red and others light green? What determines that a cabbage seed becomes a cabbage and not a rosebush? In a field of cabbage, why does each plant vary somewhat from the others even though the seeds were purchased from the same seed company? Why are the cabbages grown in my garden never as big as those shown in catalogs? What factors influence the final appearance of any specific cabbage plant?

Consider your own family. What traits do you share with your brothers and sisters? What traits are different? If you all had the same parents, why don't you all look exactly alike? Maybe you are an "identical" twin. Are you really completely identical, or are there differences that your friends and parents have no problem recognizing?

The answers to these questions are fundamental to understanding the underlying mechanisms that influence the growth, development, survival, and reproduction of all living organisms. The answers also explain how organisms that seem so similar can be so different and also why some that seem so different are actually very similar.

We know that an organism's appearance and growth pattern depend on both internal and external factors. Internal factors include the genetic coding stored in DNA, how the DNA is decoded to produce specific enzymes or proteins, and how these enzymes and other gene products affect metabolism. A structure, such as a leaf, is a product of many genes being activated and many metabolic steps occurring in specific sequences. The final product is influenced by external factors such as the amount of light, the available water, and the mineral nutrients in the soil. Laboratory Topic 5 explores some of the factors affecting photosynthesis, respiration, and transpiration. An organism's appearance, what we often call the **phenotype**, is a result of the interaction between the genetic potential of the individual and the physical and chemical influences of the environment.

This lab explores the variation existing within a group of economically important plants called the brassicas. Some members of this group still grow wild, some even as weeds. Other members have been bred and cultivated by humans for millennia as important foods for humans and livestock. Still another member of this group has been exploited as a research tool because it grows to maturity so fast. These plants are called rapid-cycling brassicas (Rbr) or Fast Plants. We will use several of these diverse yet related plants to explore which features are due to genes and which are due to the environment.

This lab is divided into three exercises. Exercise A explores the diversity of brassicas cultivated as food. In Exercise B, you will determine whether turnips and Chinese cabbage are members of the same species by cross-pollinating the vegetables with a rapid-cycling brassica (Rbr, Fast Plant). In Exercise C, you will follow in the footsteps of generations of plant breeders and select a trait to change in a population of Rbr plants. You will see if your population can evolve by human-guided selection, one of the basic mechanisms for crop improvement.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Better understand the genetic component of plant growth and development.
2. Recognize the genetic diversity of plants, in particular edible members of *Brassica*.
3. Relate the concept of a biological species to crop varieties, ability to interbreed, and range of heritable traits.
4. Understand the life cycle of a fast-growing plant from seed to seed.
5. Relate the process of human-guided selection to changes in populations of *Brassica rapa* over several generations.
6. Connect human-guided selection to plant domestication and crop improvement.

EXERCISE A: Genetic Diversity of Crops

The produce section of a supermarket displays a vast diversity of fresh fruits and vegetables—more, in fact, than most of us have personally sampled. But "fruits" and "vegetables" are only one way of classifying these plant products. What other ways can we make some sense out of all the variety observed? A produce manager might prefer to group the types according to the care they require—for example, fleshy varieties may need to be sprayed occasionally with water to keep them from wilting, while dry varieties such as potatoes, onions, and nuts can easily be placed in bins. On the other hand, you as a consumer may find it more convenient if all the sweet fruit is grouped together.

Now think like a biologist. How might a biologist divide the plants products? Scientists usually try to group organisms that are related. The most basic of these groups is the species. A **species** is a population of organisms that have many characteristics in common and produce fertile offspring through the exchange of genetic information. In seed plants, genetic exchange and reproduction are accomplished through pollination and fertilization.

In this exercise, you will look carefully at the variation among common vegetables and try to group these organisms into one or more biological species. We will then test your groupings by determining whether the plants within them can produce fertile offspring from controlled crosses.

Materials Needed for Exercise A

Edible plant products purchased from a local market, depending on availability (e.g., turnip, rutabaga, red cabbage, green cabbage, kohlrabi, broccoli, cauliflower, collards, bok choy (pak choi), brussel sprouts, chinese cabbage, rapini, mustard greens, root mustard, kale, savoy cabbage)

Wisconsin Fast Plants (Rbr) (grown from seed at least 1 week)

Procedure for Exercise A

1. **Diversity of brassicas.** Examine all the vegetables available in the lab. Their names should be provided in case you do not recognize them. First concentrate on how each one differs from the others. How can you tell them apart? List as many distinctive features as you can on worksheet 8-1 at the end of this laboratory topic.

Now switch your attention to the similarities among all the vegetables. What characteristics do they all share or at least some of them share?

By comparing the similarities with the differences, classify the plants into groups that you think reflect their biological relatedness. Try to assign each vegetable to a group that you think best represents a species.

Now compare your groups with the classification in table 8.1. These are the species recognized by most experts on the brassicas. How does your classification compare with theirs? Were you surprised? What experiment could you conduct to test whether you or the experts are more correct?

All plants represented in this activity are members of the genus *Brassica*. Brassicas are members of a large family of plants called crucifers. The name refers to the characteristic cross-shaped pattern of the four petals of the flower. The brassicas include many important food and oil crops (fig. 8.1).

Six of the most important *Brassica* species are closely interrelated. Each species has a different number of chromosomes. The number of chromosomes and the ability of each species to interbreed with other species in the group help botanists justify the boundaries of each species. The relationship between the six important *Brassica* species can be represented as a triangle, with the three diploid species forming the points of the triangle and the amphidiploid species (crosses between the first three) on the sides (fig. 8.2). The diploid species have the following characteristics:

- *Brassica nigra* is a common weed. It often grows in disturbed habitats, such as plowed fields. If the haploid (N) set of chromosomes for this species is designated as a, then the diploid can be designated as aa (or $2N = 16$).
- *Brassica oleracea* is the species of most of the vegetables displayed in this lab. The haploid (N) set of chromosomes has 9 chromosomes and can be designated as b. The diploid is thus bb (or $2N = 18$). Included in this species are cabbage, kale, broccoli, cauliflower, collards, brussels sprouts, and kohlrabi. Why do you think botanists classify all these crops in the same species?
- *Brassica rapa* ($2N = 20$) includes turnips, Chinese cabbage, and Rbr. Chinese cabbage is consumed by millions of people around the world, especially throughout Asia. Since it can be stored during the winter, it provides a rich source of vitamins, especially vitamin C, even in times when most fields are dormant. There are 10 chromosomes in the haploid set, designated as c. The diploid (cc) has 20 chromosomes.

The other three species form from crosses between pairs of the above diploid species. The resultant hybrid species are tetraploids and have four sets of chromosomes (two sets from each parent).

- *Brassica carinata* (genome = bbcc, $4N = 34$) results from a cross between *B. nigra* and *B. oleracea*. It is a tall, leafy plant found in Ethiopia. The seeds are pressed as a source of edible oil.

TABLE 8.1 ECONOMICALLY IMPORTANT BRASSICAS.

*Species (Genome)**	*Subspecies or Variety*	*Cultivar Group or Common Name*
Brassica nigra (genome = bb, 2N = 16)	—	Black mustard
Brassica oleracea (genome = cc, 2N = 18)	*acephala*	Kales
	alboglabra	Chinese kale, kailan
	botrytis	Cauliflower, heading broccoli
	capitata	Cabbage
	costata	Portuguese cabbage, tronchuda
	gemmifera	Brussels sprouts
	gongylodes	Kohlrabi
	italica	Broccoli, calabrese
	medullosa	Marrow stem kale
	palmifolia	Tree cabbage, Jersey kale
	ramosa	Thousand-head kale
	sabauda	Savoy cabbage
	sabellica	Collards
	selensia	Borecole
Brassica rapa (genome = aa, 2N=20)	*chinensis*	Pak choi, bok choy
	narinosa	Tsatsai
	nipponsinica	Mizuna, mibuna
	oleifera	Turnip rape, toria
	parachinensis	Saichin, choy sum
	pekinensis	Chinese cabbage
	perviridis	Tendergreen, komatsuna
	rapifera	Turnip
	trilocularia	Yellow sarson
	utilis	Broccoletto, broccoli raab
Brassica carinata (genome = bbcc, 4N = 34)	—	Ethiopian mustard
Brassica juncea (genome = aabb, 4N = 36)	*capitata*	Head mustard
	crispifolia	Cut leaf mustard
	faciliflora	Broccoli mustard
	lapitata	Large petiole mustard
	multiceps	Multishoot mustard
	oleifera	Indian mustard, raya
	rapifera	Root mustard
	rugosa	Leaf mustard
	spicea	Mustard
	tsa-tsai	Big stem mustard
Brassica napus (genome = aacc, 4N = 38)	—	Fodder rape
	oleifera	Oil rape
	rapifera	Swede, rutabaga

*The haploid complement of chromosomes is a = 10, b = 8, and c = 9.

Data taken from Wisconsin Fast Plants Program. 1987. Around the world with brassicas WFP021097. University of Wisconsin—Madison

- Rutabagas and rape are members of *Brassica napus* (genome = aacc, $4N = 38$), a cross between *B. oleracea* and *B. rapa*. Because of the high carbohydrate content of rutabaga roots, they are grown more as food for livestock than for human consumption. The seeds of rape are pressed to produce canola oil. This vegetable oil is third behind soybean and peanut oil in production.
- *Brassica juncea* (genome = aabb, $4N = 36$), or mustard, is a cross between *B. rapa* and *B. nigra*.

All brassicas produce glucosinolates, the distinctive flavor of mustard, with *B. juncea* producing the highest levels.

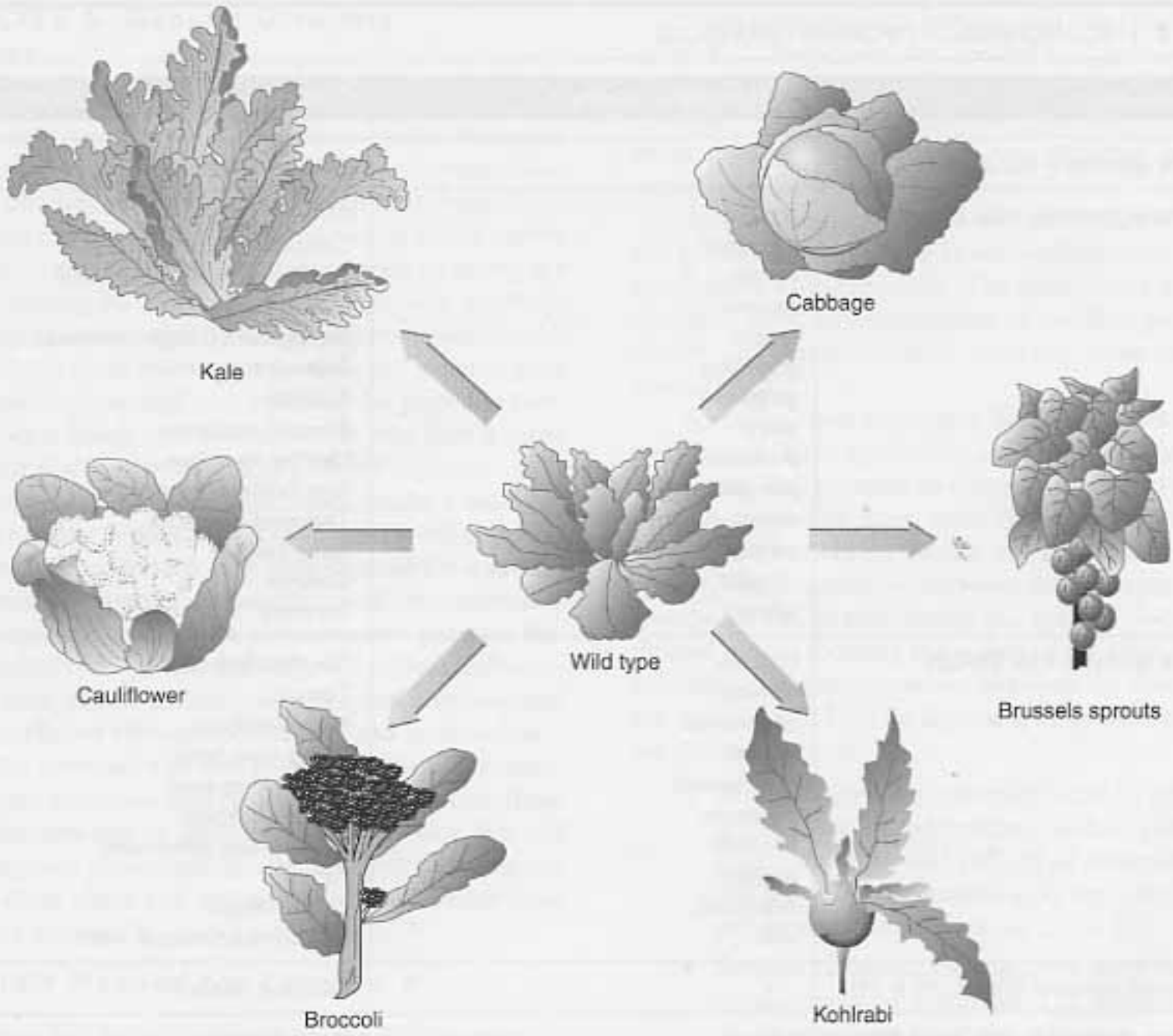


FIGURE 8.1 GENETIC DIVERSITY OF BRASSICAS.

EXERCISE B: They Look So Different; Do They Really Belong to the Same Species

As stated in the introduction to this laboratory topic, members within a species often share characteristics and can interbreed. In Exercise A, you learned that Chinese cabbage, turnips, and Rbr are all members of the same species even though the mature vegetative stages of these cultivars appear to have little in common. If, however, you plant a few seeds of turnip or Chinese cabbage along with the Rbr, you will find that the seedlings and first few leaves of all the young plants look similar. They even taste similar. Go ahead and try some!

Only when the vegetative parts grow further do the plants become different. These differences are a result of centuries of **domestication**. Over 3,000 years ago, farmers in the Mediterranean region selected for plants with large roots that could be stored and fed to animals. At about the same time in China, farmers selected for plants with leaves that curled into a ball-shaped head. The farmers carried out this selection process by saving the seeds from the best plants in every generation. Over time, this process resulted in two distinct forms—the turnip and the Chinese cabbage. Was the divergence great enough to create two new species? The only sure way to test this is to see if they can still interbreed and produce fertile offspring.

Materials Needed for Exercise B

2-liter soda bottles or plastic pots

Beesticks

Chinese cabbage

Soil mixture (equal parts peat moss and vermiculite [or sand])

Turnip

Wisconsin Fast Plants (Rbr) seeds and kit supplies

9.2. Leave it blank if it is less than 50 per ml. If the genus is not listed in table 9.2, it has a value of 0. Add up all the index values to get the total score.

11. Totals above 20 indicate water with high levels of organic wastes, 15 to 19 indicate moderate levels, and below 15 indicate low levels, or organic pollution. However, the Palmer score only evaluates organic pollution, and totals below 15 may also indicate the presence of other pollutants. You should also examine the diversity of algae in the water sample. Clean water tends to have high levels of diversity (many different genera present), while polluted waters have fewer taxa. Does your water sample show evidence of pollution?

12. Compare your results with those of others in the class for the same water sample. Were the results the same? If there were differences, speculate on the cause. Also compare the results of the other water samples used in class. Were all the samples polluted?

EXERCISE D: Effects of Nitrates and Phosphates on Algal Growth

In the natural environment, algal blooms occur periodically due to upwelling of nutrients or changing climatic conditions; however, in recent years, blooms have increased in frequency, distribution, and intensity around the world. Algal blooms can be found in most groups of algae, and they have devastating impacts on both marine and freshwater ecosystems. When dense blooms occur, oxygen stress can develop. This chain of events often begins with nutrient-rich runoff that produces the algal bloom, similar to the polluted waters described in Exercise C. When the bloom organisms die, oxygen is used up by the bacteria that cause their decay. Depletion of oxygen can even lead to the death of the ecosystem. Blooms can also contribute to taste and odor problems when they occur in reservoirs supplying water to cities and towns.

Algal blooms are especially hazardous when the algae are capable of producing toxins. Although only a small percentage of algae are toxin forming, the result of blooms involving toxins can be dramatic. These blooms, called **harmful algal blooms**, can sometimes directly harm people; at other times, they can cause massive fish kills or accumulate in shellfish. The shellfish are generally not affected, but they become poisonous to humans and other animals. Toxin-producing species are found in various groups of algae, including the cyanobacteria, dinoflagellates, and diatoms. The dinoflagellates are probably the best-known toxin producers, and blooms of these organisms have been called **red tides**.

The increased incidence of algal blooms is related to nutrient pollution, especially from agricultural runoff, human sewage, and animal wastes. The enhanced availability of nitrogen and phosphorus is believed to trigger bloom conditions. In this exercise, we will examine the effects of increasing levels of these nutrients on the growth of algae. We will be culturing a common freshwater alga (*Chlorella*) on a basal growth medium as well as on the same medium with four times and ten times the level of nitrate and phosphate. At the end of 2 weeks, we will evaluate the difference in the cultures.

Materials Needed for Exercise D

3 sterile test tubes, 15 ml

Bold's basal algae growth medium without nitrogen and phosphorus

Culture of *Chlorella*

Growth chamber or bank of cool white fluorescent bulbs

Nitrate-phosphate (N-P) solution

Pipets and pipetor

Procedure for Exercise D

1. Obtain 3 sterile test tubes; label them A, B, and C, and add your initials. Add 8.0 ml of Bold's basal growth medium to each tube. This medium has all the nutrients needed for algal growth except nitrogen and phosphorus. These will be added separately.
2. Add 1 ml of the *Chlorella* culture to each tube.
3. Add the remaining ingredients to each tube using the following chart. The control culture will have the lev-

els of nitrogen and phosphorus recommended in Bold's basic medium.

TUBE NUMBER	EXPERIMENTAL CONDITION	AMOUNT OF N-P SOLUTION TO ADD	AMOUNT OF DISTILLED WATER TO ADD
A	Control—basic level of N and P	0.1 ml	0.9 ml
B	4X basic level of N and P	0.4 ml	0.6 ml
C	10x basic level of N and P	1.0 ml	None

4. Once you have added the N-P solution, place the lid on the tube and gently shake the solution to make sure everything is distributed.
5. Place the tubes under a bank of cool white fluorescent lights or in a growth chamber.
6. The cultures will need 10 to 14 days to grow. Check the cultures during the next 2 weeks. At the end of the time, evaluate the density of the cultures in the three tubes. Your instructor will indicate what method of evaluation to use.
7. Which algae were most abundant in the polluted water you analyzed?
8. How are algal blooms related to oxygen stress in aquatic environments?
9. Describe the industrial use of algal polysaccharides.

TERMS TO KNOW

agar 116
alginic acid 116
carrageenan 116
centric 115
Chlorophyta 115
Chrysophyta 114
cyanobacteria 113
diatoms 114
diatomaceous earth 117
dinoflagellate 114
Euglenophyta 115
frustule 114
harmful algal blooms 120
heterocysts 115
hydrocolloids 116
Kingdom Monera 113
Kingdom Protista 114
pennate 115
Phaeophyta 115
Pyrrophyta 114
red tides 120
Rhodophyta 115

QUESTIONS FOR REVIEW AND DISCUSSION

1. Which algae have rigid, glasslike walls?
2. The term "seaweed" refers to which groups of algae?
3. How can you distinguish the various groups of algae?
4. Which group of algae has prokaryotic cells?
5. Which type of algae is the source of agar?
6. Describe the cell wall of dinoflagellates.

ADDITIONAL RESOURCES

James, D. E. 1975. Algae: Pollution indicators. *Carolina Tips*, 38(15):57–58.

Levetin, E. and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Palmer, Mervin L. 1969. *Algae in water supplies. An illustrated manual on the identification, significance and control of algae in water supplies.* Washington, D.C.: U.S. Department of Health, Education and Welfare.

Simpson, B., and M. Conner-Ogorzaly. 1995. *Economic botany: Plants in our world*, 2d ed., New York: McGraw-Hill Companies, Inc.

Sze, P. 1998. *A biology of the algae*, 3d ed. New York: McGraw-Hill Companies, Inc.

ON THE WEB

Chartiers Creek Study, Washington and Jefferson College
http://www.washjeff.edu/Chartiers/Chartier/biot_algae.html

Pfiesteria piscicida, U.S. EPA
http://www.epa.gov/OWOW/estuaries/pfiesteria/

Phychology, Mining Company
http://botany.about.com/cs/phycology/index.htm

Harmful Algae, Woods Hole Oceanographic Institute
http://www.redtide.whoi.edu/hab/

Introduction to the Green Algae, University of California—Berkeley
http://www.ucmp.berkeley.edu/greenalgae/greenalgae.html

Algae, An Introduction, Smithsonian Institution
http://www.nmnh.si.edu/botany/projects/algae/Algintro.htm

OTHER ACTIVITIES

Take a trip to a local water treatment plant. Guided tours are often available. In most areas, active measures are taken to control algae in drinking water, and these will be described during the tour.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 9-1 EXERCISE C: ALGAE AS INDICATORS OF WATER POLLUTION

Palmer Algal Pollution Score for Water Sample from _____

Algal Genera	Number of Individuals		Total	Algal Density in Sample*	Correction if Sample Was Concentrated	Palmer Index Value
	Traverse 1	Traverse 2				
					Palmer Score for Environment	

* Calculate the density (number of individuals per ml) from Equation 1 in the procedure for Exercise C.

LABORATORY TOPIC 10

Your Piece of the Sun

BACKGROUND

As food, plants provide us with energy, chemical building blocks, minerals, and vitamins. Worldwide, humans get 80% of their calories from food of plant origin. In addition, plants are nutritious. We can meet our needs for all eight essential amino acids by eating a diversity of plants. Plants are higher in dietary fiber and lower in saturated fats than animal products. Many of our necessary vitamins are readily available in fruits and vegetables. Finally, consumption of plants makes good ecological sense since less energy is lost through the food web.

This laboratory topic is divided into two exercises. Exercise A explores the flow of energy from the sun through your personal food web. You will have an opportunity to determine your "piece of the sun." Exercise B allows you to compare your food consumption against the Recommended Daily Allowances prescribed by the United States Department of Agriculture (USDA). You will be able to see which foods are more nutritious in various ways and to assess how balanced your diet actually is. You will then determine if a carefully planned vegetarian diet can meet a person's nutritional needs.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Relate their own food consumption to energy flow through the food web.
2. Describe the additional energy supplements needed to support the agricultural and distribution systems related to food production.
3. Compare energy consumption from food of plant origin to energy consumption from food of animal origin.
4. Understand the macronutrients and micronutrients required for proper human health.
5. Compare their daily dietary consumption to USDA Recommended Daily Allowances.
6. Relate the nutritional quality of food to the sources of those foods.
7. Compare different types of diets on a nutritional basis.

EXERCISE A: Your Piece of the Sun

All organisms function by using and processing energy. Energy enters the web of life via photosynthesis, is stored in **biomass**, and is released by cellular respiration (see Laboratory Topic 5). As a living being, you are dependent on a continual inflow of energy. Since humans are not capable of photosynthesis, we must get all our energy from the food we eat. Where did the energy in our food come from? Ultimately, it came from the sun.

The objective of this exercise is for you to get a sense for "your piece of the sun." In other words, we will calculate the amount of energy needed to support you as an organism, in particular, a normal consumer of the early twenty-first century. By keeping track of your food consumption, you will estimate how much energy you consume in an average year. Then, based on what you typically eat, we will build your personal **food web** (fig. 10.1). You will be able to account for the energy as it flows from the sun, through other organisms, and finally to you. You will see the connections between you and the other plants and animals needed to provide your food. If you lived like other animals, this food web would be adequate to describe all the energy inputs needed to meet your metabolic needs. However, modern humans are different from other organisms. We use additional sources of energy for cooking, processing, transporting, refrigerating, fertilizing, and cultivating our food. You will estimate some of these additional subsidies and add them to your total energy consumption. Since most of this added energy comes from fossil fuels, it can be considered "your piece of the past."

It is so easy to go to the grocery store or visit your favorite fast-food restaurant that many of us have lost touch with the agricultural system that helps provide our food. In this exercise, you will connect the food you eat with the bigger picture.

Total Solar Energy
(needed to support you)
(22) _____ 10^6 kcal yr^{-1}

Solar energy reflected or reradiated
(23) _____ 10^6 kcal yr^{-1}

Plant crops: Gross production
(8) _____ 10^6 kcal yr^{-1}

Plant respiration
(9) _____ 10^6 kcal yr^{-1}

Waste, etc.
(10) _____ 10^6 kcal yr^{-1}

Your consumption of plants
(5) _____ 10^6 kcal yr^{-1}

Crops fed to livestock Gross production
(14) _____ 10^6 kcal yr^{-1}

Livestock: Gross energy intake
(11) _____ 10^6 kcal yr^{-1}

Your consumption of meat and dairy products
(6) _____ 10^6 kcal yr^{-1}

Plant respiration
(15) _____ 10^6 kcal yr^{-1}

Waste, etc.
(16) _____ 10^6 kcal yr^{-1}

Animal respiration
(12) _____ 10^6 kcal yr^{-1}

Waste, etc.
(13) _____ 10^6 kcal yr^{-1}

Phytoplankton: Gross production
(20) _____ 10^6 kcal yr^{-1}

Fish & shellfish: Gross energy intake
(19) _____ 10^6 kcal yr^{-1}

Your consumption of fish & shellfish
(7) _____ 10^6 kcal yr^{-1}

You
Your gross energy intake
(1) _____ 10^6 kcal yr^{-1}

Respiration and other losses
(21) _____ 10^6 kcal yr^{-1}

Fish respiration
(18) _____ 10^6 kcal yr^{-1}

Waste, etc.
(17) _____ 10^6 kcal yr^{-1}

Your BMR
(2) _____ 10^6 kcal yr^{-1}

Energy activity energy
(3) _____ 10^6 kcal yr^{-1}

Digestion and waste
(4) _____ 10^6 kcal yr^{-1}

FIGURE 10.1 FLOW OF ENERGY THROUGH THE HUMAN FOOD WEB.

Materials Needed for Exercise A

USDA Nutrient Database or nutritional reference guides

Calculator

Procedure for Exercise A

1. **Food diary** For seven days, keep a complete record of all the food and drink you consume (except water). Record the amount and types of items you eat on worksheet 10-1 at the end of this laboratory topic. You will use this data for both Exercise A and Exercise B.
2. **"Your Piece of the Planet:" Estimate the land area needed to produce your food** At the end of the week, assign the food you ate to the subcategories (column 1) on worksheet 10-2. Convert all your data to kilocalories. *Note*: The dietary Calorie as given in the diet books and on package labels is actually a kilocalorie to the scientist. The USDA Nutrient Database for Standard Reference, Release 13, is an excellent source for caloric values. This is available free-of-charge on the Internet (see Additional References at the end of this laboratory topic or on CD-ROM.) Alternatively, you can examine packaging labels or other nutritional reference guides.

   Some foods, such as a slice of pizza, should be assigned to more than one category. To sort the kilocalories among the categories, use the labels on the food packages and your own judgment. For example, the kilocalories in most kinds of commercial salad dressings can be divided about half and half between vegetable oil and sugar. If some food that you eat is not listed, include it in the most closely related category.

   Total the kilocalories for each food subcategory (column 2 of worksheet 10-2). Multiply the weekly values by 52 to estimate your annual intake per food subcategory (column 3).

   Not all crops are equal in their production of edible food. For a plant such as an orange tree, we eat only a small portion of the plant. For other crops, such as sugar produced from sugar beets, the yield per acre is much greater. In column 4, approximate yields are given for each food subcategory. The units are given in kcal m^{-2} yr^{-1}. To determine the cropland needed to produce each type of food (column 5), divide the annual energy consumption per subcategory (column 3) by the yield for each subcategory (column 4). The values for seafood correspond to the square meters of ocean on the continental shelf needed to support the fish and shellfish in your diet.

   Calculate the subtotal of annual consumption (kcal yr^{-1}) and land area (m^2) for all the food you ate that came from plants. Also calculate the subtotal of annual consumption (kcal yr^{-1}) and land area (m^2) for all the food you ate that came from animals. The animal land area is actually the cropland (e.g., corn or hay) needed to support the animals you ate. Keep the values for seafood separate from the subtotal for animals.

   To determine the overall area needed to support you ("your piece of the planet"), add the subtotals for plant cropland, animal land, and ocean surface.

   Total area needed to support you = _____ m^2

   For comparison, convert the area from square meters to acres and hectares (1 acre = 4,047 m^2, 1 hectare = 10,000 m^2).

   Total area needed to support you = _____ acres = _____ hectares

   This is just the land used to produce the food. It does not include the land occupied by roads, barns, the feedlot where the cattle are fed, bakeries, mills, fertilizer factories, etc.
3. **"Food as energy:" Calculating your energy intake** To determine your average daily energy intake, add the kilocalories you consumed for the entire week (column 2) and divide by 7. How hungry were you?

   Average daily energy intake = _____ kcal

   For most people, the daily energy intake is in the range between 1,500 and 3,000 kcal. How do you compare?

   Now calculate your annual energy intake by adding all the kilocalories of the subcategory.

   Annual energy intake = _____ × 10^6 kcal yr^{-1}
4. **"Your energy needs:" Estimating your energy requirements** Convert your body weight from pounds to kilograms. (1 lb = 0.454 kg).

   Body weight = _____ kg

   Determine the energy needed to maintain your **basal metabolic rate** (BMR). Multiply your weight times the conversion factor below:

   Women: 21.6 kcal kg^{-1} day^{-1}

   Men: 24.0 kcal kg^{-1} day^{-1}

   BMR = _____ kcal

   Add the energy cost associated with a general activity level as a percentage of BMR. Multiply your BMR times the factor related to your level of activity from the following scale:

   Sedentary = 20 % of BMR

   Light = 30 % of BMR

   Moderate = 40 % of BMR

   Heavy = 50 % of BMR

   Daily activity energy = _____ kcal

   Add the energy cost associated with eating and digesting the food you consume. In part, this also

includes the indigestible portion of food that passes through your body and exits in the feces. For most Americans, the energy cost associated with eating and digesting is 10% of average daily energy intake (see step 3). For vegetarians who eat significant levels of plant fiber, this value may be as high as 20% of daily energy intake.

Daily eating and digesting energy = _____ kcal

Add your BMR, energy for activity, and energy for digesting to determine your daily energy requirement.

Daily energy requirement = _____ kcal

Determine the difference between your daily energy requirement and your average daily consumption.

Daily energy difference = _____ kcal (surplus or deficit?)

If, on any given day, you eat food containing slightly fewer kilocalories than you use in your energy-requiring activities, you will lose weight as you draw on energy in your fat stores. A kilogram of fat contains about 7,500 kcal, so a total deficit of this amount over a period of time will cause you to lose a pound of body weight. In contrast, if you eat food containing more kilocalories than you use, you will gain weight at the same rate. It is true that people vary in their metabolic rates; yet activity levels determine the bulk of this difference, so it pays to exercise. Are you likely to gain or lose weight if you continue at the same rates of activity and consumption?

_____

_____

_____

_____

_____

5. **Building your food web** Now you can trace the energy back through your personal food web. The goal is to account for all the transfers and losses of energy in your food web (fig. 10.1). Each empty line on the food web has a number that corresponds to the specific instructions on the following list. All the values are given on an annual basis.

   Since you are now working with rather large quantities of kilocalories, it will be more convenient to express all annual values in millions (10^6) of kilocalories. In addition, to calculate the annual energy intake, we assume that your eating habits during the last week were typical of the entire year. We know this is not completely true, but the data are still useful for making a reasonable estimate. (*Note*: Since we made these assumptions, you should not report more than 3 significant digits of precision for your numbers, even though your calculator may give you many more.)

   Line 1: Enter your annual energy intake.

   Line 2: Multiply your BMR times 365 days.

   Line 3: Multiply your daily activity energy times 365 days.

   Line 4: Multiply your daily eating and digesting energy times 365 days.

   Line 5: Enter the annual consumption of plant products (worksheet 10-2).

   Line 6: Enter the annual consumption of meat and dairy products (worksheet 10-2).

   Line 7: Enter the annual consumption of fish and shellfish (worksheet 10-2).

   Line 8: The gross primary production of cropland that supplies you with food can be calculated by multiplying the area of the cropland by 12,000 kcal m^2. This is an oversimplication, since primary productivity varies with season, region, crop, weather, etc. For our mathematical model, it is a reasonable average value. Enter on line 8 the value of 12,000 times the area in square meters devoted to plant cropland (worksheet 10-2).

   Line 9: Remember that plants also respire to meet their own metabolic needs. The amount of energy lost from the food web due to the cellular respiration of plants can be calculated as approximately 60% of their gross production of energy. Of course, this varies with the crop as well. Some plants, such as maize (a C4 plant), are more efficient than other plants, such as wheat (a C3 plant), so the loss from respiration is less. Enter on line 9 the value of 0.6 times the value on line 8.

   Line 10: Enter the remainder of the value of line 8 after subtracting line 5 and line 9. This value represents plant material that is not edible and left in the field, such as wheat stems.

   Line 11: For the pathway through the livestock, you can assume you consume 10% of the energy taken in by the animals themselves. In other words, 10% of the calories fed to the livestock made it to you as food. This again is a simplification, since chickens and hogs are slightly higher than cattle in feed conversion to human food. The value on line 11 (animal energy intake) is 10 times the value of line 6.

   Line 12: Animals use more of their energy for respiration than plants, so for line 12 enter the value of 0.78 times line 11. (Seventy-eight percent of the energy goes to meet metabolic needs.)

Line 13: The waste from animals includes bone, hides, and trimmings. That energy is lost from your food web. It is the difference between the energy used for respiration and the energy consumed by you from animal products. Enter 0.12 times line 11.

Line 14: To determine the gross production of the plants used to support the livestock, make the same assumptions as you did for line 8. Enter the value of 12,000 times the land area in square meters of land needed to support animals (worksheet 10-2).

Line 15: For plant respiration, enter on line 15 the value of 0.6 times the value on line 14.

Line 16: For plant waste, enter the remainder of the value of line 14 after subtracting line 11 and line 15.

Line 17: If you consumed fish or other seafood, the weight of the meat often equals the weight of the indigestible materials (e.g., scales, fins, and bones) that you had to discard. Based on this assumption, you can enter on line 17 the value equal to line 7.

Line 18: Fish are fairly efficient in converting their energy into new biomass, so you can assume that fish respiration is also equal to the value of line 7.

Line 19: To determine the energy intake for fish, add lines 7, 17, and 18, and enter the total.

Line 20: To determine the gross production of the ocean, multiply the square meters of the ocean surface (worksheet 10.2) by 2,000 kcal m^{-2} yr^{-1}. This is not as productive as the terrestrial systems, yet it is a reasonable estimate for the ocean waters over the continental shelf. The primary production in the ocean is via phytoplankton. Many of the food chains of fish used as human food are also quite long already and there are intermediate losses in each trophic level, so this assumption is quite reasonable.

Line 21: Respiration and other losses of the phytoplankton can be calculated as the difference of line 20 minus line 19.

Line 22: Most of the light energy from the sun that reaches the earth is either reflected back into space or absorbed by the ground and then reradiated as heat. Only a very small fraction is absorbed by photosynthetic plants and plankton to make new biomass. In fact, only approximately 0.5% of the total solar radiation enters the food web. Of course, this also varies from plant to plant. To calculate the total solar energy needed to support you, multiply 200 times the sum of line 8, line 14, and line 20.

Line 23: The amount of solar energy reflected or reradiated is line 22 minus the total of line 8, line 14, and line 20.

This completes your food web. It should resemble the energy flow diagrams used to describe other organisms, but it is designed specifically around you. Line 22 represents the amount of solar energy needed to support your needs for energy from food. It is "your piece of the sun." To get another comparison that may make more sense, you can convert the energy in kcal on line 22 to gallons of gasoline by dividing by 34,800. There are 34,800 kcal per gallon of gasoline.

"Your piece of the sun" is equivalent to _____ gallons of gasoline

6. **"Your Piece of the Past:" Estimating Your Energy Subsidy** If you were a hunter or forager, the energy flow diagram you prepared in figure 10.1 would be adequate to explain nearly all of your energy needs (within the limits of the assumptions and approximations). But modern humans get their food from intensively managed agricultural systems. Then we demand even more energy for harvesting, cooking, processing, storing, packaging, transporting, distributing, selling, fertilizing, irrigating, cultivating, and controlling pests. Figure 10.2 illustrates some of the added energy that goes into your food web. By relating each of these inputs in terms of kcal, we can attempt to estimate the true energetic costs of supporting someone like you.

A rough estimate for the additional energy subsidy to your support system depends on several factors, including (1) the proportion of highly processed foods in your diet (e.g., prepared frozen pizza or other convenience foods), (2) store-bought versus home-grown, (3) the costs of shopping and preparing (e.g., number of trips and distance to the grocery store), (4) energy for cooking and storage (e.g., freezing uses more energy than dry storage), and (5) energy costs of fertilizers, pesticides, and other farming practices. Select the level of energy subsidy you think best fits the type of food you eat on a regular basis from the following list. Multiply your annual energy intake by the conversion factor.

Low processing (primarily grow your own food and eat it raw)	2
Medium processing (rely on store-bought food, cook, refrigerate)	15
High processing (mostly convenience foods, highly processed, heavy packaging)	30

Energy subsidy = _____ kcal

Since most of the energy added to your food diagram comes directly or indirectly from fossil fuels, the added energy can be considered "your piece of the past." We are taking energy stored by plants millions of years ago to produce and distribute today's food. As before, you can convert the kilocalorie to gallons of gasoline to illustrate the point.

"Your piece of the sun" is equivalent to _____ gallons of gasoline

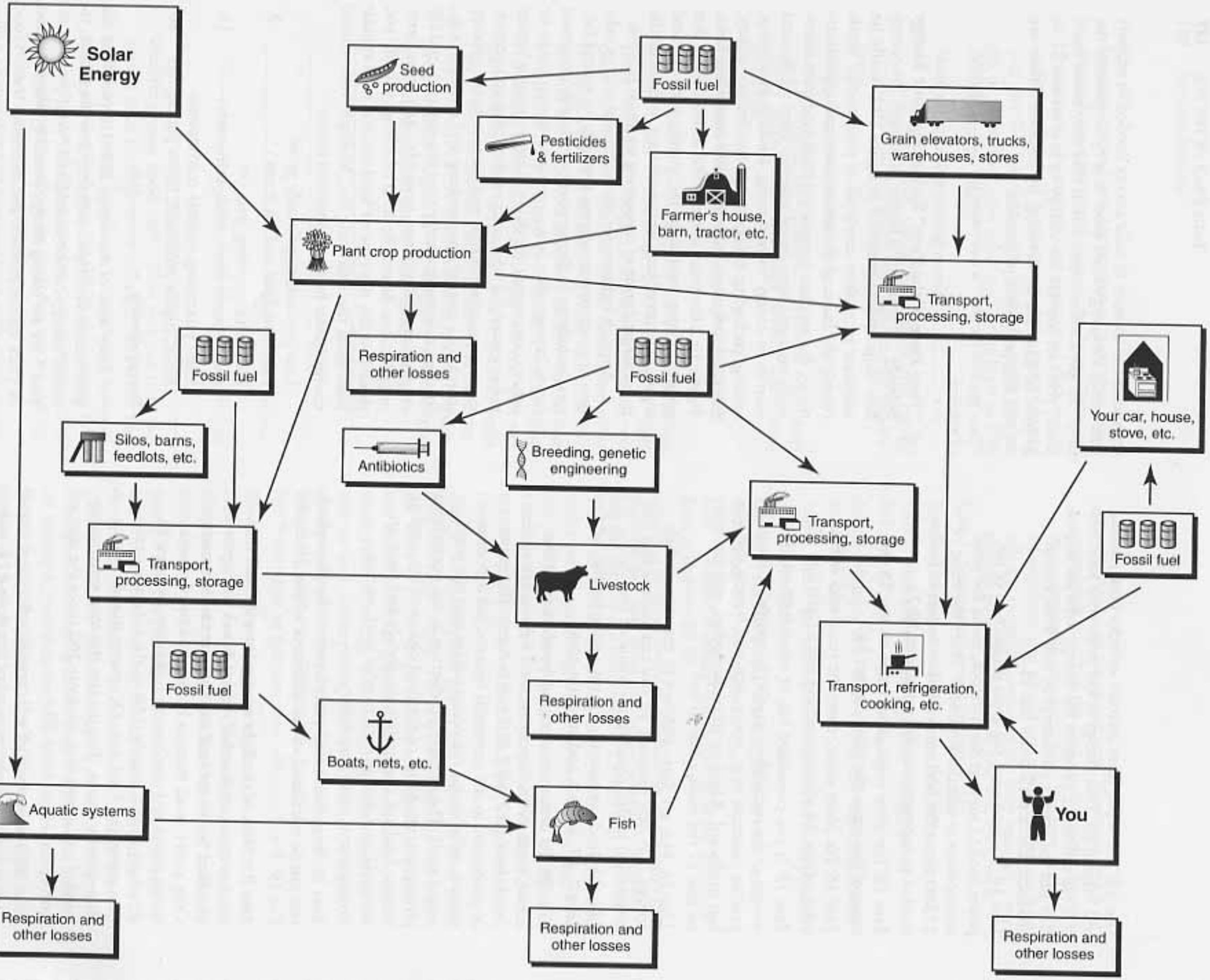


FIGURE 10.2 THE MODERN AGRICULTURAL SYSTEM REQUIRES ADDITIONAL ENERGY INPUTS TO PRODUCE FOOD.

Remember, these added uses of energy only support your food needs. Your use of energy to warm your house or go to the movies in your car are not even touched on.

Using what you learned from this exercise, what solutions can you offer to help solve the problem of feeding a hungry planet? If everyone on the planet had the same energy demands as you do, would there be enough land and food to go around? What if the population doubles? What if it doubles again?

EXERCISE B: How Balanced is Your Diet

The food we eat provides us with energy, water, and molecules for growth, maintenance, and repair. The role of food as a source of energy was explored in Exercise A. In this exercise, we will examine the molecules needed for proper health. These can be classified as **macronutrients**, if needed in large amounts, or **micronutrients**, if needed in small yet essential quantities. The macronutrients are organic chemicals that include **carbohydrates**, **lipids**, and **proteins**. These compounds, especially the carbohydrates and lipids, supply most of our energy. The macronutrients are our source of building materials to make more living material molecules, cells, and tissues in our bodies.

Carbohydrates can be grouped as **simple sugars** (monosaccharides and disaccharides) and **complex carbohydrates** (polysaccharides). Simple sugars, such as glucose, fructose, or sucrose, are cheap sources of energy. In our bodies, these can easily be oxidized by respiration to produce ATP. They are also building blocks for more complex carbohydrates such as glycogen. When consumed in excess, our bodies usually convert sugars to fat. Simple sugars are naturally found in many plant foods, such as fruits. Often, however, simple sugars are added to foods to sweeten and enhance the flavor. Most Americans consume much more sugar than they need. The simple sugars only provide energy and are not required for other nutritional needs.

Complex carbohydrates are larger chemicals composed of long chains of simple sugars linked together. When we eat complex carbohydrates instead of simple sugars, the energy is released over a longer period of time, so we avoid sudden peaks or valleys in blood sugar levels. Complex carbohydrates include starch and glycogen, which are both polymers of glucose units.

When plants photosynthesize, they store the carbohydrates they produce as **starch**. Because of this, many foods of plant origin are high in starch. In Laboratory Topics 11 and 13 we will explore starch more in specific classes of foods. **Glycogen** differs from starch only in the degree of branching in the glucose chains. Although it is not found in foods of plant origin, our bodies temporarily store high levels of glucose as glycogen. Another complex carbohydrate is cellulose. It is also a polymer of glucose, but the bonds between the glucose units are different from those in starch or glycogen. This difference prevents our bodies from digesting cellulose. However, even though we do not digest it, cellulose and other indigestible polymers, such as lignin and pectins, play an important nutritional role as **dietary fiber**. The fiber absorbs water in the intestines and helps move waste material through the colon. High-fiber diets are recommended to help reduce the risk of colon cancer.

Most of the lipids in our diet are fats. Like carbohydrates, lipids primarily provide energy to our bodies. Fats provide even more energy per gram than carbohydrates. Fat consumption in the United States increased from 34% of the diet in the 1930s to 42% in the late 1950s and 1960s. The current levels have dropped off somewhat, but they are still higher than recommended levels. Animal products are often higher in lipid content than many plant products. However, as explored in Laboratory Topics 12 and 13, some foods of plant origin are also relatively high in fat, such as avocados (so watch the guacamole).

Diets high in fat, especially fats composed of **saturated fatty acids**, are associated with many health problems, including certain forms of cancer and cardiovascular diseases. Saturated fatty acids occur more commonly in fats of animal origin, but some plant lipids, such as coconut oil and palm oil, are also high in saturated fatty acids.

Usually it is recommended that a person's total daily fat intake be less than 65 g for a 2,000 kcal diet (with less than 20 g as saturated fat). In most diets, especially if animal products are consumed, the level is often higher. However, two fatty acids, linolinic acid and linoleic acid, are essential in your diet because you cannot synthesize them. Both are found in many seeds, nuts, and animal products.

Proteins are composed of various combinations of 20 amino acids. We use proteins in our bodies as enzymes, contractile molecules in muscles, and structural components of bone, cartilage, skin, and blood. Although we consume proteins in our food, it is actually the amino acids we are after. If we have the amino acids, we can form new proteins. A typical adult needs 50 to 60 g of protein per day. In addition, we need to consume adequate amounts of eight **essential amino acids** (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) since our bodies cannot synthesize them. A balance of proteins from grains and legumes can provide all the eight amino acids.

Micronutrients are required in smaller quantities, yet are often needed for key metabolic reactions. Micronutrients are either vitamins or minerals. **Vitamins** are organic compounds that often act as coenzymes in metabolic reactions. When left out of a diet, the deficiencies often lead to debilitating diseases and even death. Vitamins are either water-soluble or fat-soluble. The **water-soluble vitamins** are not stored in the body and tend to be

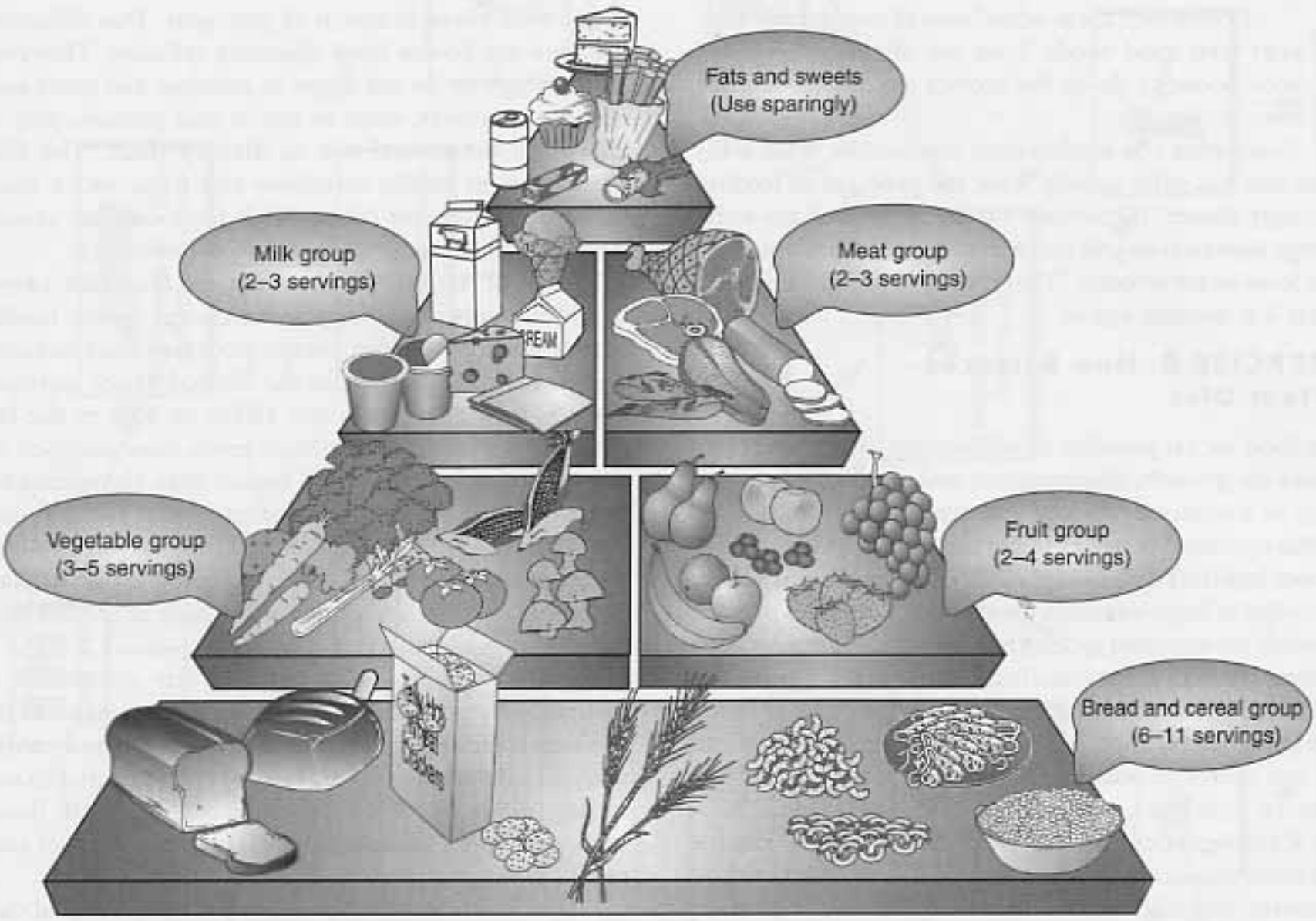


FIGURE 10.3 THE FOOD PYRAMID ILLUSTRATES THE RECOMMENDED DIETARY GUIDELINES SET FORTH BY THE U.S. DEPARTMENT OF AGRICULTURE.

removed by the kidneys. They must be added to the diet on a regular basis. The **fat-soluble vitamins** can be stored by the body and used as needed. High levels of water-soluble vitamins are often washed away in urine, whereas high levels of fat-soluble vitamins can actually cause toxicity.

Minerals are elements also needed in the diet to help ensure proper health and to aid metabolism. People in different stages of their lives, such as infants or pregnant women, often have different mineral requirements, so general recommendations need to be adjusted according to life phase.

The USDA has advanced the food guide pyramid (fig. 10.3) as a simple guide to eating. In addition, nutritional labels that provide caloric and nutritional data to the consumer are required on all processed foods in the United States.

Materials Needed for Exercise B

USDA Nutrient Database for Standard Reference, Release 13

Personal food consumption data (worksheet 10-1)

Procedure for Exercise B

1. **Assessing the nutritional balance of your diet .** You collected data on all the food you consumed for seven days. If you are a typical student, you probably eat better on some days than others. Select one day that you think is typical for your lifestyle and eating habits. In Exercise A, we looked at the caloric value of food. Here we examine the other nutritional aspects of food in greater detail.

   For the day you chose, use the USDA Nutrient Database for Standard Release, Release 13 (see Additional Resources) to complete worksheet 10-3. Look up each food item. Often you can match the brand name directly and choose the serving size appropriate for you. Occasionally you will need to pick a food item closest to what you actually ate. Make adjustments for the serving size. In other words, if you only ate half of a regular hamburger with condiments and vegetables, you can get the data for a full sandwich and divide by 2. Total the nutrients from all your food and drinks.

2. **Compare your food consumption with RDA** For the past 50 years, the USDA has produced a set of nutrient and energy standards known as the **Recommended Daily Allowances (RDA)**. These recommendations provide a nutrition reference for people of different life phases (e.g., adults versus children). Currently these standards are being revised through a collaborative effort of experts in both the United States and Canada. The new recommendations are being called **Dietary Reference Intakes (DRI)**. For some nutrients, DRI values are available. Unfortunately, revised recommendations are not yet complete for all the nutrients. Therefore, both RDA and DRI values are presented in table 10.1.

   Compare your data with that on table 10.1 and with nutritional facts labels on food packages. List the nutrients in which you were deficient, at least for the day you selected as typical of your food consumption. For each of these deficient nutrients, identify at least one food item, preferably of plant origin, that could supply your nutritional needs. You should find a food rather than merely suggesting vitamin pills or other nutritional supplements.
3. **The vegetarian choice.** For reasons of culture, belief, or health, some individuals choose to omit

TABLE 10.1 RECOMMENDED DAILY ALLOWANCES (RDA) AND DIETARY REFERENCE INTAKES (DRI)*

NUTRIENT	UNITS/DAY	ADULT MALES		ADULT FEMALES		CHILDREN	PREGNANT WOMEN
		19 to 30	31 to 50	19 to 30	31 to 50	4 to 8	
Energy	Kcal	2,900	2,900	2,200	2,200	1,800	2,500
Protein	g	58	63	46	50	28	60
Vitamin A	RE	1,000	1,000	800	800	700	800
Vitamin D	μg	5	5	5	5	5	5
Vitamin E	mg alpha TE	10	10	8	8	7	10
Vitamin K	μg	70	80	60	65	30	65
Vitamin C	mg	60	60	60	60	45	70
Folate	μg	400	400	400	400	200	400
Thiamin (B_1)	mg	1.5	1.2	1.1	1.1	0.6	1.5
Riboflavin (B_2)	mg	1.7	1.7	1.3	1.3	0.6	1.6
Niacin	mg	16	16	14	14	8	17
Pyridoxine (B_6)	mg	2	2	1.6	1.6	0.6	2.2
Cyanocobalamine (B_{12})	μg	2.4	2.4	2.4	2.4	1.2	2.2
Calcium (Ca)	mg	1,000	1,000	1,000	1,000	800	1,000
Phosphorus (P)	mg	700	700	700	700	500	700
Iodine (I)	μg	150	150	150	150	120	200
Iron (Fe)	mg	10	10	15	15	10	30
Magnesium (Mg)	mg	420	420	310	320	130	320
Zinc (Zn)	mg	15	15	12	12	10	19
Selenium (Se)	μg	70	70	55	55	30	75
Fluoride (F)	mg	3.8	3.8	3.1	3.1	1.1	3.1

Data extracted from:

National Academy of Sciences, 1989, *Recommended Daily Allowances*, National Academy Press.

National Academy of Sciences, 1997, *Dietary Reference Intakes*, National Academy Press.

National Academy of Sciences, 1998, *Dietary Reference Intakes*, National Academy Press.

*As recommended by the U.S. Department of Agriculture.

meat from their diets. At least 7 million Americans call themselves **vegetarians**. Their range of food selection is quite diverse. Red meat abstainers reduce or eliminate red meat (beef, pork, or lamb) from their diets. Pollovegetarians eliminate red meat entirely, but may eat poultry. Pescovegetarians eat fish, but no red meat or poultry. Lactoovovegetarians consume dairy products (milk and cheese) and eggs, but eat no meat. Vegans eat a wide variety of plant products, but no animal products of any kind. Fruitarians eat only fresh or dried fruits and nuts. Proponents of Zen macrobiotics restrict themselves to brown rice and herb tea (and sometimes whole grains and vegetables). Within each category, there are variations—for example, ovovegetarians who only eat eggs and not meat or dairy.

Many vegetarian diets are diverse enough to easily meet the USDA's Recommended Daily Allowances. Although meat is often a major source of protein, most vegetarian diets allow an individual to meet his or her overall protein needs and also consume adequate amounts of the eight essential amino acids as long as they eat a variety of plants such as grains, nuts, and legumes. Meat, fish, and poultry are major contributors of iron, zinc, and the B vitamins (especially B_{12}), so some vegetarians need to find alternative sources or supplements.

Look again at your food diary. Consider the three vegetarian diet choices on worksheet 10-4. What foods that you ate would you have to remove if you accepted each of the vegetarian diets?

Now use the USDA Nutrient Database to examine carefully what that food item (maybe a hamburger) provided in terms of nutrients and energy. Does removing the specific animal product create any potential deficiencies? What are the deficiencies? Suggest alternative foods that would be acceptable within the bounds of that vegetarian diet to meet these deficiencies. Remember to consider the loss of calories as well.

TERMS TO KNOW

basal metabolic rate (BMR) 127
biomass 125
carbohydrate 131
complex carbohydrates 131
dietary fiber 131
Dietary Reference Intakes (DRI) 133
energy intake 127
fat-soluble vitamins 132
food web 125
lipids 131
macronutrients 131
micronutrients 131
minerals 132
proteins 131
Recommended Daily Allowances (RDA) 133
saturated fatty acids 131
simple sugars 131
vegetarians 134
vitamins 131
water-soluble vitamins 131

QUESTIONS FOR REVIEW AND DISCUSSION

1. Modern humans require enormous amounts of energy to maintain their lifestyles. We know that many energy-related factors, such as available land and fossil fuels, are limited. How can you personally reduce your overall energy consumption without affecting your annual energy intake?
2. Why do you think starch is a better storage carbohydrate for the plant than a simple sugar like glucose?
3. From an energy perspective, does it make more sense to eat animals or plants? How do the two food sources compare from a nutritional perspective?
4. What are some ways to reduce the use of fossil fuels in the U. S. food system? Include changes in both culture and technology. Where are the biggest savings possible?
5. How does the U. S. food system compare with early agricultural societies in terms of energy subsidy, food choices, and nutritional considerations? How does it compare with hunter-gatherer societies?
6. What food items would you list as key foods that contain at least 75% of some of the major human nutrients?
7. What food items in your diet have little to no nutritional value?

ADDITIONAL RESOURCES

Committee on Dietary Reference Intakes. 1997. *Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride.* Washington, D.C.: National Academy Press.

Committee on Dietary Reference Intakes. 1998. *Dietary reference intakes for thiamine, riboflavin, niacin, B_6, folate, B_{12}, pantothenic acid, biotin, and choline.* Washington, D.C.: National Academy Press.

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Morholt, E., and P. Brandwein. 1986. *A sourcebook for the biological sciences.* 3d ed. San Diego: Harcourt Brace Jovanovich Publishers.

National Academy of Science. 1989. *Recommended daily allowances.* 10th ed. Washington, D.C.: National Academy Press.

Simpson, B., and M. Conner-Ogorzaly. 1986. *Economic botany: Plants in our world*, 2d ed. New York: McGraw-Hill Companies, Inc.

U.S. Department of Agriculture, Agricultural Research Service. 1999. *USDA Nutrient Database for Stan-*

dard Release, Release 13. CD-ROM, Government Printing Office.

U.S. Department of Agriculture, U. S. Department of Health and Human Services. 2000. *Dietary guidelines for Americans*, 5th ed. Home and Garden Bulletin No. 232, Government Printing Office.

ON THE WEB

USDA Nutrient Database for Standard Release, Release 13. Nutrient Data Laboratory Home Page for the Food and Nutrition Information Center
http://www.nal.usda.gov/fnic/foodcomp

Food and Drug Administration
http://www.fda.gov

Center for Nutrition Policy and Promotion, USDA
http://www.usda.gov/cnpp

The Northeast Regional Food Guide. Guidelines for vegetarians from National Research Council, USDA, and American Dietetic Association.
http://www.nutrition.cornell.edu/foodguide/guidelin.html

USDA Food Guide Pyramid. A guide to daily food choices.
http://www.nal.usda.gov:8001/py/pmap.htm.

OTHER ACTIVITIES

1. Plan a diet that is nutritionally balanced and allows you to lose weight.
2. Use common chemical tests to determine the macronutrients in different types of food. Benedict's solution can be used to detect simple sugars. Lugol's iodine solution can detect starch. Lipids are detected with Sudan III or Sudan IV. Proteins are detected with the biuret test.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 10-1 EXERCISE A: YOUR PIECE OF THE SUN

Keep an accurate record of the food and drink items you consume for 7 days, including every entré and snack. Record the food item and the amount consumed; you will convert to kcal later in lab. Labels on food packages may be an additional source of nutritional information. Be sure to include all beverages except for water.

Day 1

Day 2

Day 3

Day 4

Day 5

Day 6

Day 7

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 10-2 EXERCISE A: YOUR PIECE OF THE SUN

Instructions for computing energy intake by food types:

Use the data reported on worksheet 10-1 to complete the chart on the following page.

Column 1: Assign each food item to a food subcategory. The subcategories relate to the relative amount of energy in edible food produced per square meter of cropland or ocean surface (yield). You may need to split the energy (kcal) for a specific food into more than one subcategory. For example, most salad dressing can be divided about half and half between vegetable oil and sugar. If a food is not listed, assign the energy from the most closely related food type.

Column 2: Total energy (kcal) for the week for each food category. The total for this column divided by 7 is your **average daily energy intake.**

Column 3: Multiply the weekly values by 52 to estimate your annual energy consumption for each subcategory. The total for this column is your **annual energy intake**. Add all the plant food subcategories for your **plant consumption subtotal**. Add all the animal subcategories for your **animal consumption subtotal**.

Column 4: These amounts represent the approximate yield in edible food per square meter of land surface per year (kcal m^{-2} yr^{-1}).

Column 5: To determine area of cropland (or ocean surface) for each subcategory, divide the value in column 3 by the value in column 4.

WORKSHEET 10-2—(Continued) NAME DATE

FOOD SUBCATEGORY	(1) KILOCALORIE TALLY (KCAL)	(2) WEEKLY CONSUMPTION (KCAL WK^{-1})	(3) ANNUAL CONSUMPTION (KCAL YR^{-1})	(4) YIELD (KCAL M^{-2} YR^{-1})	(5) CROPLAND AREA (M^2)
PLANT PRODUCTS					
High-yield plant foods Sugar from sugar beets or sugarcane High-sugar products: soft drinks, candy Sports drinks, Gatorade				3500	
Medium-high-yield plant foods Corn cereal, cornmeal, corn chips Rice or rice cereal Potatoes, potato chips, fries, mashed apples				1500	
Medium-low-yield plant foods Wheat cereal, flour, bread, pasta Beans, peas, peanut butter, peanuts Carrots, broccoli Pears, citrus fruits, other tree fruits Tomatoes, tomato sauce				850	
Low-yield plant foods Orange or other fruit juices Lettuce, onion, and specialty vegetables Vegetable oil or margarine Wine or beer Sweet corn or popcorn				250	
		PLANT SUBTOTAL			
ANIMAL PRODUCTS					
High-yield animal foods Milk				400	
Medium-yield animal foods Beef, pork, chicken, eggs				200	
Low-yield animal foods Cheese, yogurt, butter				40	
		ANIMAL SUBTOTAL			
SEAFOOD					
Tuna, perch, shrimp, shellfish, etc.				2	
		TOTAL			

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 10-3 EXERCISE B: HOW BALANCED IS YOUR DIET?

Select one day from your week of reported food consumption (worksheet 10-1 food diary) that you think is typical of your normal eating habits. Analyze each food item with data from the USDA Nutrient Database. Complete the following table.

NUTRIENT	UNITS/DAY	YOUR TOTALS	DEFICITS OR EXCESSES
Energy	Kcal		
Protein	g		
Selected essential amino acids:			
Methionine	g		
Lysine	g		
Tryptophan	g		
Total lipid (Recommended levels <65 g, 2,000 kcal; <80 g, 2,500 kcal)	g		
Saturated fatty acids (Recommended levels <20g, 2,000 kcal; <25 g, 2,500 kcal)	g		
Monounsaturated fatty acids	g		
Polyunsaturated fatty acids	g		
Cholesterol (Recommended levels <300 mg.)	mg		
Total carbohydrate (Recommended levels <300g, 2,000 kcal; <375 g, 2,500 kcal)	g		
Dietary fiber (Recommended levels <25g, 2,000 kcal; <30 g, 2,500 kcal)	g		
Simple sugars	g		
Vitamin A	RE*		
Vitamin D	μg		
Vitamin E	mg α TE		
Vitamin K	μg		
Vitamin C	mg		
Folate	μg		
Thiamin (B_1)	mg		
Riboflavin (B_2)	mg		
Niacin	mg		
Pyridoxine (B_6)	mg		
Cyanocobalamine (B_{12})	μg		
Sodium (Na) (Recommended levels <300 mg.)	mg		
Calcium (Ca)	mg		
Phosphorus (P)	mg		
Iodine (I)	μg		
Iron (Fe)	mg		
Zinc (Zn)	mg		

*RE = retinol equivalents, αTE = d-alpha-tocopherol equivalents.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 10-3 ANALYSIS OF ENERGY INTAKE BY FOOD TYPES. (continued)

1. What nutrients are low or missing from your diet?

2. Identify some food items that you could include in your diet to meet these deficiencies.

3. If you have a vitamin deficiency, find out how that vitamin affects health, and state the disease that develops if the deficiency is severe enough.

4. How balanced is your intake of protein, carbohydrates, and lipids? Are you consuming too much fat? Are you getting enough complex carbohydrates?

5. How adequate is your consumption of dietary fiber? How does it compare with recommended levels?

6. A proper diet needs to be combined with adequate physical activity. What are you going to do to exercise at least 30 minutes per day?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 10-4 EXERCISE B: HOW BALANCED IS YOUR DIET?

Compare the data you collected in worksheet 10-3 with other dietary choices. What food items would be eliminated from your diet if you choose to abstain from red meat, became a lactoovovegetarian, or became a vegan? Complete the following table using the food items that would be left.

NUTRIENT	UNITS/ DAY	YOUR TOTALS	RED MEAT ABSTAINER	LACTOOVOVEGETARIAN	VEGAN
Energy	kcal				
Protein	g				
Essential amino acids					
Methionine	g				
Lysine	g				
Tryptophan	g				
Total lipid	g				
Saturated fatty acids	g				
Monounsaturated fatty acids	g				
Polyunsaturated fatty acids	g				
Cholesterol	mg				
Total carbohydrate	g				
Dietary fiber	g				
Simple sugars	g				
Vitamin A	RE*				
Vitamin D	μg				
Vitamin E	mg α TE*				
Vitamin K	μg				
Vitamin C	mg				
Folate	μg				
Thiamin (B_1)	mg				
Riboflavin (B_2)	mg				
Niacin	mg				
Pyridoxine (B_6)	mg				
Cyanocobalamine (B_{12})	μg				
Sodium (Na)	mg				
Calcium (Ca)	mg				
Phosphorus (P)	mg				
Iodine (I)	μg				
Iron (Fe)	mg				
Zinc (Zn)	mg				

*RE = retinol equivalents; αTE = d-alpha tocopherol equivalents.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 10-4 — COMPARISON OF YOUR DIET TO OTHER CHOICES. (Continued)

1. If you chose each of the vegetarian diets, what, if any, food would you need to eliminate from your current diet?

Red meat abstainer:

Lactoovovegetarian:

Vegan:

2. If you did eliminate these foods from your diet, what nutrients should you be most concerned about lacking?

3. What foods could you substitute for the animal products to replace the lost nutrients?

LABORATORY TOPIC 11

Leaves of Grass

BACKGROUND

In many ways, the grass family, Poaceae, is the most important family affecting human affairs. We depend on grasses for food, fiber, and fuel. Cereal grains provide the primary source of carbohydrates and protein for most of the world's population (fig. 11.1). According to the United Nations Food and Agricultural Organization (FAO), the top four crops produced globally in the years 1997–2000 were grasses—namely, sugarcane, maize, wheat, and rice. Half of the calories consumed by humans worldwide are from cereals, compared to only 16% from animal products. In addition, 44% of total protein consumed per day comes from cereals, compared to only 37% from animal products (FAO, 1998). Grains and forage grasses constitute the majority of feed for livestock, so even meat can be considered in large part as "processed grains."

Many alcoholic beverages, including beers, ales, and sake, are produced by fermentation of grains. When some of the initial mashes are further distilled, we are able to make many types of whiskey, including scotch and bourbon.

Sugar, vegetable oil, and starch are extracted from grains. These chemicals are used in such an array of products that it is safe to say that every person is affected daily by grasses. Sugarcane is the primary producer of sugar for processed foods. Cornstarch is used in foods, as well as in glues, lubricants, fillers for medicines, plastics, and sizing for paper.

Grasses provide humans with many other products. Ethanol from corn fermentation is being added to gasoline as an alternative fuel. Bamboo and other grasses are used in tropical lands to construct water pipes, furniture, and buildings. Grass thatch is used for roofing in many areas of the world. Settlers of the Great Plains used the tough mats of the roots of prairie grasses to build their sod houses. The pliable stems are used in basket making and weaving.

Horticulturally, grasses are used extensively for turf and lawns. The effort, time, and money devoted to maintaining suburban lawns, golf courses, and soccer fields attests to our fascination with this plant family.

Grasses are the dominant species in various ecosystems, including prairies, tropical savannas, and bamboo forests. Herbivores that depend on grasses range from the endangered giant pandas of China to common field mice, from elephants to pronghorn antelope, and from grasshoppers to American bison.

On the negative side, because grasses release their pollen to the wind, the pollen is a major cause of hay fever (see Laboratory Topic 6). Also, some of the most aggressive and hard-to-control weeds are grasses. In regions such as California, exotic grasses have replaced indigenous species and changed the landscape significantly.

This lab will focus on only a few of the grasses, emphasizing those that have influenced the development of human civilizations and continue to affect our lives and our health. One important grass is wheat, which has often been called the "staff of life." We will investigate how the protein chemistry of wheat flour affects the taste, texture, and digestibility of raised bread. Another important grass is maize (corn), which formed the basis of the Mayan, Incan, and Aztec civilizations in the New World. We will explore how native Americans may have improved the nutritional properties of corn.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Recognize the unique features of a typical grass plant and understand how these vegetative and reproductive features are exploited by humans.
2. Relate the botanical source with the chemical properties of flour.
3. Relate the evolution of wheat with the chemical properties of quality bread.
4. Understand how gluten proteins affect the elasticity of bread dough and the ability of bread to rise.
5. Relate pigments of blue corn with nutrients and applied chemistry.
6. Describe the steps of making bread, beginning with grinding grain into flour.

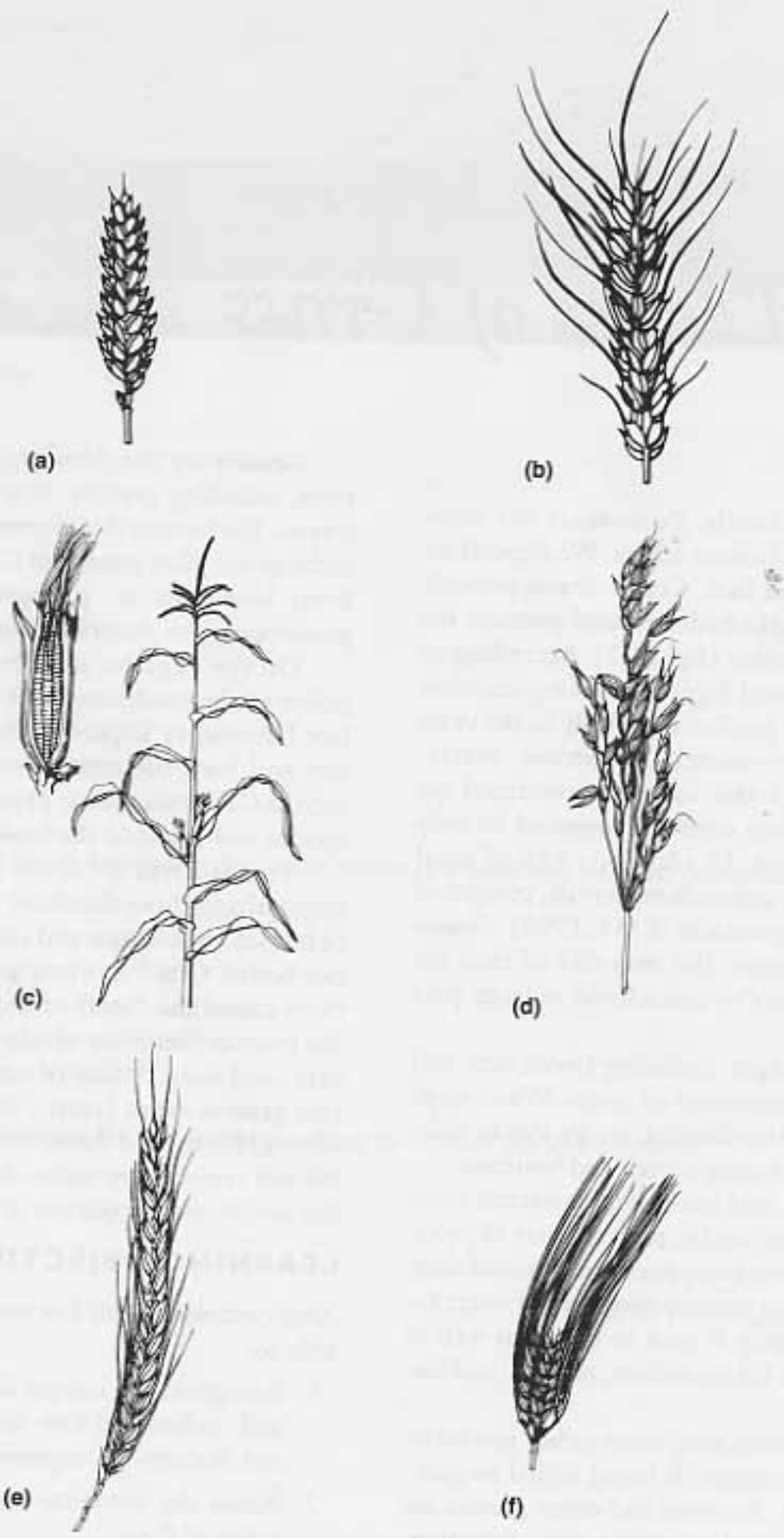


FIGURE 11.1 IMPORTANT COMMERCIAL GRASSES: (A) CLUB WHEAT; (B) DURUM WHEAT; (C) MAIZE; (D) OATS; (E) TWO-ROW BARLEY; (F) SIX-ROW BARLEY; (ILLUSTRATION BY MOLLY OGORZALY). (PLANTS IN OUR WORLD 3/ED., BY SIMPSON AND OGORZALY.)

EXERCISE A: A Typical Grass Plant

Anthropologists often suggest that the single most important step in the development of human culture was the shift from nomadic hunter-gatherers to farmers. This shift probably occurred independently in more than one region of the world, but each time, a common grass played an important role. In the Middle East, humans

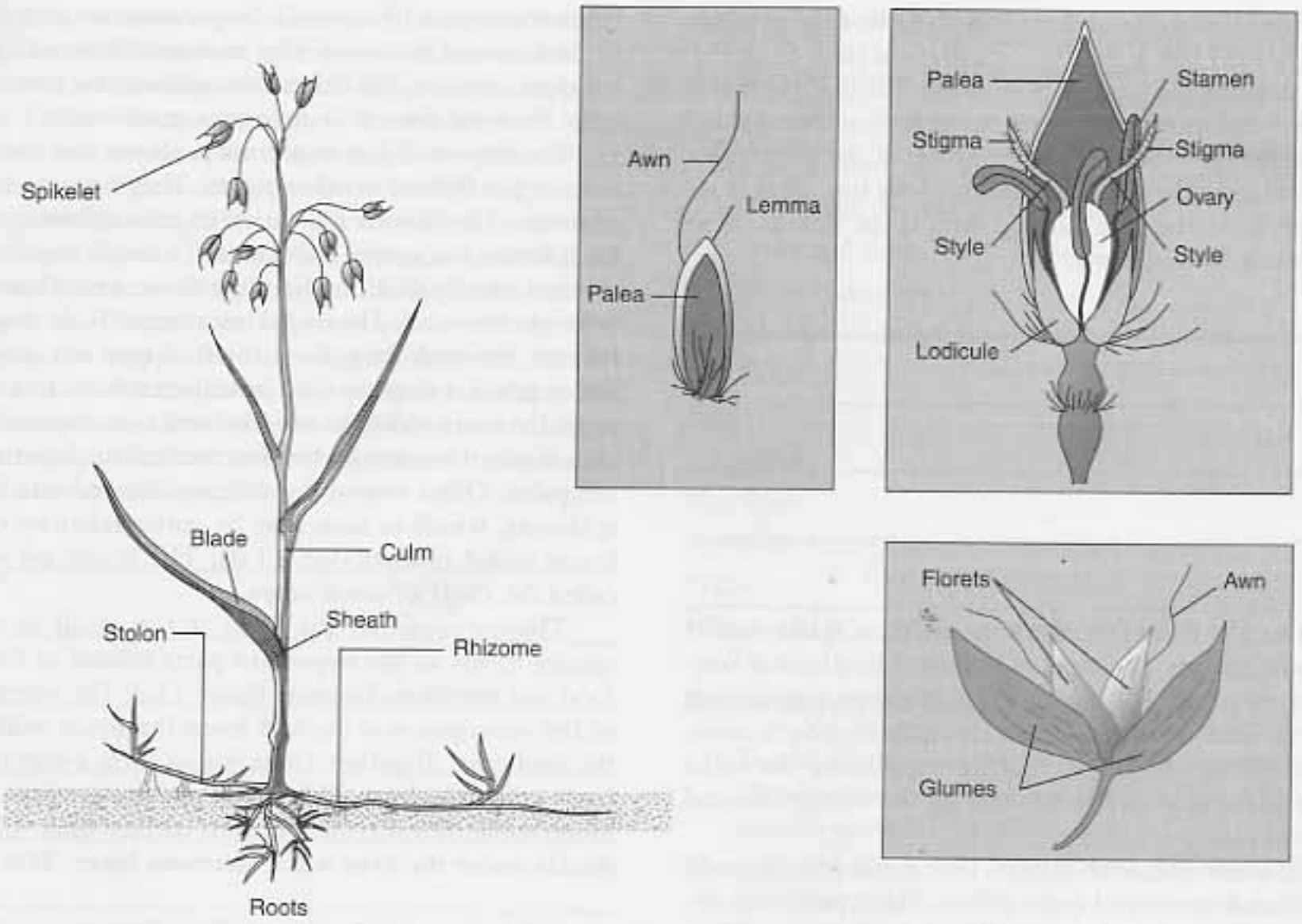


FIGURE 11.2 TYPICAL GRASS PLANT. (A) WHOLE PLANT. SOME GRASSES CAN REPRODUCE VEGETATIVELY THROUGH STOLONS OR RHIZOMES; HOWEVER, THE MAJOR CEREAL CROPS LACK THESE STRUCTURES. (B) EACH FLOWER OR FLORET IS SURROUNDED BY A LEMMA AND PALEA. (C) THE GRASS FLOWER CONSISTS OF THREE STAMENS AND ONE CARPEL WITH TWO SEPARATE STYLES AND STIGMAS. (D) GLUMES SUBTEND EACH SPIKELET THAT BEARS ONE OR MORE FLORETS.

learned to cultivate wheat and barley. In Mesoamerica, the grass was maize. In Asia, the domestication of rice allowed for a stable food supply. In sub-Saharan Africa, grasses such as sorghum and millets were exploited because they could tolerate low soil nutrients and drought. Why did grasses play such a significant role in each location? In this exercise, we will examine some of the unique features of a typical grass plant that may answer this question.

Materials Needed for Exercise A

2 week-old corn (*Zea mays*) plants

Horticultural tray of any lawn grass, 2–4 weeks old (12" × 12" × 2")

Variety of other grass plants, live or pressed.

Procedure for Exercise A

1. **Typical grass plant** Examine a plant of maize (*Zea mays*) that is at least 2 weeks old. Carefully remove the plant from the soil and wash the roots. Compare your plant to the typical grass plant illustrated in figure 11.2.

What is the shape of the leaves? How does this shape compare to the leaves of many other plants? Look at the center of the leaf. Can you see the strong yet flexible **midrib**? What is the function of the midrib? How does it hold the **blade** of the leaf so it can absorb light and photosynthesize? Can you see lines running parallel to the midrib? These are **veins** of vascular tissue (xylem and phloem). You may wish to consult Laboratory Topics 3 and 4 for assistance in identifying plant tissues and structures.

Grass leaves often have a base that closes around the stem (**culm**) like a sheath. Peel the leaf back from the culm to reveal the hard, swollen **node**. The space between nodes is the **internode**. Grass stems have an **intercalary meristem** (growing area) at the base of the internodes in addition to the apical meristems of the tips. How does growth from the base allow grasses to be well-adapted to grazing by herbivores?

Cut a culm at the internode. What is it like inside? In most grasses, the culm is hollow. The physical construction of the hollow stem is actually an engineering marvel. The stem has a high strength-to-weight ratio, making it an efficient design for supporting the aerial parts of the plant while minimizing the energy diverted to stem tissue.

Examine the root system. The roots are all nearly the same diameter and quite diffuse. This type of root system is a **fibrous root system**. Think again about an herbivore biting the grass plant. How could a fibrous root system with many roots be well adapted to herbivory?

Sketch your grass on worksheet 11-1. Use your drawing to convey all the unique features you have observed.

2. **Sod-forming grasses** Examine a tray of lawn grass. What parts of the plants are similar to the maize plant? Carefully remove the plants from the tray. How easy is it to separate individual plants? Can you see how the plants are intertwined in a mat? These are some of the growth habits we want in a lawn or turf.

   Take a small clump of the sod from the tray and try washing the soil from the roots. You may notice that the roots are great at holding the soil. This feature of grasses helps them hold the soil and prevent erosion when they are used in landscaping.

   Try to separate a single grass plant. Notice the horizontal stems on the surface (**stolons**) or immediately below the surface (**rhizomes**). New shoots can grow from the stolons and rhizomes. The stolons of Bermuda grass are quite obvious. We desire this attribute for lawns, yet curse the same feature in aggressive grass weeds.

   The flowers of grasses are not as showy and conspicuous as the flowers in other plants. They have no sepals or petals. The flowers are clustered into inflorescences. Each flower has several stamens and a single carpel. The stamens usually stick out from the flowers to release the pollen to the wind. The carpel has a single ovule that will become the seed. In grasses, the fruit type is a **caryopsis,** or **grain**, a single-seeded indehiscent fruit. In a caryopsis, the ovary wall fuses with the seed coat. Surrounding each flower there may be two bracts called the **lemma** and the **palea**. Often several flowers are clustered into short **spikelets,** which in turn may be surrounded by other bracts called **glumes** (fig. 11.2). The bracts are often called the **chaff** of cereal crops.

   The caryopsis of the grass is too small in most species to see all the important parts related to human food and nutrition. Examine figure 11.3. The outer wall of the caryopsis was derived from the ovary wall and the seed coat. Together, these tissues form a very dense layer called the **bran**. When eaten, the bran is usually indigestible, but it contributes to dietary fiber. Immediately under the bran is the **aleurone layer**. This layer

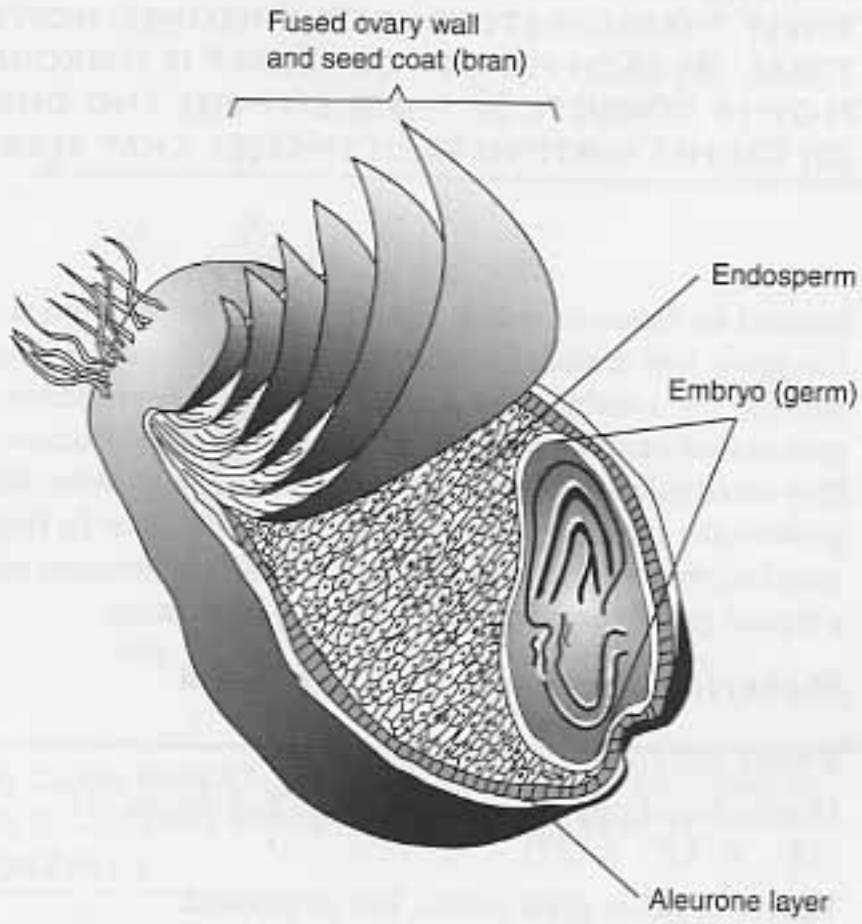


FIGURE 11.3 A CARYOPSIS (GRAIN), THE TYPICAL FRUIT OF GRASSES.

is high in proteins, especially some of enzymes that are necessary when the seed germinates. At one end of the caryopsis is the **embryo**. Surrounding the embryo is a layer filled with starch, called the **endosperm**. The embryo of the caryopsis is also called the **germ**. Since it is a small, intact plant, the germ is high in proteins, lipids, and vitamins. If the seed is allowed to germinate, the enzymes in the aleurone layer become activated and turn the starch into simple sugars for the embryo to use for growth. The embryo will grow into a seedling, consuming the supply of stored carbohydrates in the endosperm until the leaves start photosynthesizing and producing more carbohydrates. By what process do germinating seeds release the energy stored in the starch (see Laboratory Topic 5)?

To produce flour from grain, the grain needs to be ground either by stones or rollers. To produce flour with a long shelf life, the bran, aleurone layer, and germ are removed. If kept dry, starch lasts almost indefinitely. Unfortunately, producing flour that lasts longer also reduces its nutritional value. Whole-grain flours are more nutritious than refined white flours. However, the lipids in the germ go rancid after several weeks unless stored in a refrigerator.

EXERCISE B: The Botany of Baking

In this exercise, you will make small balls of bread from different types of flour to see how flour affects bread quality. Breads made of different types of flour have different properties, depending on the chemistry of the flour. In particular, the elasticity of the dough and its ability to rise are directly related to certain types of protein in the flour.

Yeast (*Saccharomyces cerevisiae*) is a single-celled fungus that reproduces by budding new cells. Yeast is added to dough to produce CO_2 bubbles. The yeast contains amylases that break down the starch to simple sugars. The yeast uses the sugars in respiration, releasing the stored energy and producing CO_2 as a by-product. Baking soda, which produces CO_2 bubbles chemically without yeast, is often used for fast-rising muffins or cookies.

Materials Needed for Exercise B

All-purpose wheat flour
Baking pans
Buckwheat flour (not a true grain)
Butter, honey, cinnamon (optional)
Dry activated yeast
Granulated cane sugar (sucrose)
Mixing bowls and mixing spoons
Oat flour
Oven
Rice flour
Rye flour
Semolina flour (from durum wheat)
Water
Whole-wheat, stone-ground wheat flour

Procedure for Exercise B

1. To activate the yeast, mix 5 ml of active dry yeast with 30 ml warm water and a pinch of sugar. Allow the yeast to grow for 15–30 minutes.
2. Add the yeast to 300 ml of flour, and mix thoroughly in a bowl. Add 5 ml of sugar (sucrose). Add 90 ml of water. Mix, and allow to stand for 2–3 minutes. Repeat for each type of flour.
3. On a floured surface, knead the dough until smooth (at least 5–10 minutes). Then form it into balls of 1 cm diameter. Record your observations on the texture and appearance of the dough in worksheet 11-2.
4. Let the dough rise for 20 minutes in a warm (not hot) location of the room.
5. Measure the new diameter of each of the balls to the nearest millimeter. Calculate the mean diameter of all the balls.
6. Place the balls of dough on baking pans and bake at 190–232°C for 20–30 minutes or until brown.
7. After removing the baked dough balls from the oven, allow them to cool, and then taste one of the bread balls from each of the flour types. Record your observations regarding taste, texture, mouth feel, and any other impressions on worksheet 11-2. (You may find that adding a little butter or honey to the bread enhances flavor. If you add butter or honey, make your observations on taste and texture both before and after.) *Caution*: Some people are allergic to the gluten in wheat. If you have an allergy to wheat or wheat products, do not sample the bread yourself. Instead, rely on the subjective observations of your classmates to complete the worksheet.

EXERCISE C: Crude Gluten Content

The evolution and domestication of wheat began about 11,000 years ago in a region of today's Mideast known as the Fertile Crescent. The first cultivated species was **einkorn wheat,** *Triticum monococcum*. One of the first genetic changes in this species was the characteristic of **nonshattering**. Normally, when grains in grasses are mature, they shake loose from the florets. In einkorn wheat, the grains stayed attached to the florets until after harvest. This trait enabled the farmer to collect more of the grain rather than lose some of it as it scattered to the ground. A change in genetic composition that results from the interaction of humans and a crop is the process of **domestication**.

About 8,000 years ago, a hybrid formed between einkorn wheat and a goat grass (*Aegilops* sp.). The new wheat species (*Triticum turgidum*) had twice as many chromosomes (28) as either of the original parents (both 14). *T. turgidum* had 4 sets of chromosomes and was **tetraploid**, whereas each of the parents had 2 sets of chromosomes and was **diploid**. The caryopsis of this wheat was plumper than in einkorn wheat, so the harvest was increased. **Emmer wheat** is an early variety of *T. turgidum*. Like einkorn, emmer had nonshattering grains, and these grains were covered with additional bracts. The later varieties of *T. turgidum* known as **durum wheat** had a mutation that allowed the grains to be easily removed from the bracts, a character known as **free-threshing**. It eliminated much of the labor needed to remove the grains from the chaff after harvest. Durum wheats are currently the basis of **semolina flour,** which is used for making pasta.

Another hybridization between emmer wheat and another goat grass *Aegilops squarrosa* produced a wheat with 6 sets of chromosomes (a **hexaploid**). This species (*Triticum aestivum*) is the bread wheat of today. The changes in this species included more protein in the endosperm—in particular, a special class of proteins called **glutens**, which gave the flour elastic properties. The earlier types of wheat (einkorn and emmer) had less gluten, and other grains, such as rice, corn, and oats, have very little gluten content. When mixed with water and the starch of flour, gluten gives dough the ability to stretch and expand. As CO_2 bubbles are formed from the respiration of the yeast as they consume some of the starch, the gluten molecules stretch like many balloons. Baking the dough kills the yeast and fixes the bubbles. The resultant bread is fluffier, lighter, easier to eat, and possibly more digestible than bread made from grains containing little or no gluten.

Gluten is actually a complex collection of proteins. Generally, gluten is divided into monomeric proteins (**gliadins**) and polymeric proteins (**glutenins**). The ratio of these proteins is often considered to contribute to a flour's properties. When these two types of proteins are mixed with water, they form the gluten. In this exercise, you will measure the amount of gluten in some of the flours used in Exercise B and compare the rising to the gluten levels. The method you will use is a common method for detecting the crude gluten content. Alternative methods include HPLC, gel electrophoresis, or a new technique described by H. Wieser (2000) using turbidimetry (see Other Activities).

Materials Needed for Exercise C

A cup or porcelain dish
All-purpose wheat flour
Balance
Drying oven
Gluten-free flour
Rye flour
Semolina flour

Procedure for Exercise C

1. Weigh 25 g of flour and place it in a cup or porcelain dish. Add sufficient water (approximately 15 ml) to make a firm dough ball. With a spatula or small spoon, work the flour and water mixture into a ball. Try to keep the dough from adhering to the spatula or the inside of the cup.
2. Cover the dough ball with water, and allow it to stand at 22–25°C (room temperature) for 1 hour.
3. Gently knead the dough ball in a stream of tap water. Continue until all the starch and other water-soluble matter is removed. The washing requires approximately 15 minutes. To test whether the remaining gluten is starch free, squeeze a few drops of the wash water from the gluten ball into a beaker of clear water. If starch is present, the water will become cloudy.
4. Cover the washed gluten with water, and allow it to stand at room temperature for another hour.
5. Remove the gluten from the water, and press as much water from the ball as possible.
6. Dry for 24 hours at 100°C in a drying oven. Cool, and then weigh the dry gluten.
7. Calculate the percentage of dry gluten present in the flour.
8. Repeat for each type of flour, and record your results in worksheet 11-3.

EXERCISE D: Blue Corn

Maize or corn (*Zea mays*) originated in the Tehuacan Valley of Mexico at least 7,000 years ago. Throughout North

and South America, it was cultivated and revered as a gift from the gods. When the Europeans came to the West, maize was quickly spread to Europe, Africa, and Asia. Currently, it is one of the top crops produced globally.

Blue corn is an ancient variety of maize that is characterized by blue-and-white-colored kernels. It was grown by the Hopi Native Americans, who sustained an agriculturally based culture for centuries in the arid Southwest. They are still probably some of the best dryland farmers in the world. Maize, with its adaptability as a plant, played a key role in the food and ritual of the Hopi. The ritual use has kept Hopi traditions alive even when outside influences have affected their normal lives.

The blue and white color of the kernels is caused by pigment in the aleurone layer. These pigments were used by the Hopi to unlock nutrients in the corn endosperm, making the flour more nutritious. The pigments themselves do not add nutrients, but they indicate when niacin is released.

Niacin, once called vitamin B_3, is part of NAD, the electron transport molecule so necessary in cellular respiration (see Laboratory Topic 5). Niacin is relatively abundant in meat, but many cultures rely mostly on grain, rather than meat, for their nutrition. Niacin can also be synthesized from tryptophan, one of the essential amino acids. Like most grains, maize has only a limited amount of tryptophan, but it does contain niacin tightly bound within the endosperm. If people's diets lack both niacin and a sufficient amount of tryptophan to synthesize niacin, they develop the deficiency disease **pellagra.** Pellagra affects the skin, digestive system, and central nervous system. It is characterized by the four D's—dermatitis, diarrhea, depression, and death. In regions where maize became the primary food, pellagra was common, but the pigments in blue corn played a role in preventing this disease. In this exercise, you will follow in the footsteps of the Hopi and uncover the essential vitamin in blue corn.

Materials Needed for Exercise D

10% by volume calcium oxide (limewater)

Blue corn kernels or blue corn meal

Mano and metate (or grinder)

pH meter

Procedure for Exercise D

1. Grind the corn kernels into a powder. If available, use a **mano** and **metate**, the traditional millstones of Native Americans. Alternatively, use a hand grinder or clean coffee grinder. Make a fine corn meal or flour.
2. Make a moist, doughy paste by adding a few ml of water to the flour.
3. Now you are ready to release the niacin. Add limewater to the paste a millimeter at a time, mixing into the flour. Watch the color of the paste. The flour will turn a darker shade of blue when the pH is equal to 8. At this pH, niacin is released. When you think you have reached the critical pH based on the color, check the pH with a pH meter to see how close you are. It takes practice to perceive the exact point. The Hopi women were masters of the chemistry, even though they did not know its significance.

The Hopi produced their limewater by mixing ash from a fire with water. We know that this produces an alkaline solution that can be used to adjust the alkalinity of the flour. How the Hopi correlated the blue color with niacin is unknown. The blue pigment in the aleurone layer works as a pH indicator and is not connected in any other way with the niacin. However, the ancient customs of the Hopi unleashed the essential vitamin, and the flour paste was used to make a flat bread similar to a modern tortilla, but even thinner.

The Hopi probably also prevented pellagra by diversifying their diet, since the combination of beans and maize gives a better balance of the essential amino acids and essential vitamins.

Blue corn is growing in popularity, especially in the Southwest. The markets include Mexican restaurants and health food stores, where blue corn is an ingredient in tortillas, pancake mixes, cornbread mixes, and chips. Studies at Colorado State University indicate that the protein content of commercial blue corn is consistently 30% higher than that of dent corns in adjacent fields (Johnson and Croissant, 1990). The future of corn may be singing the blues.

TERMS TO KNOW

aleurone layer 149
blade 147
blue corn 151
bran 149
caryopsis (grain) 148
chaff 148
culm 148
diploid 150
domestication 150
durum wheat 150
einkorn wheat 150
embryo 149
emmer wheat 150
endosperm 149
fibrous root system 148
free-threshing 150
germ 149
gliadins 150
glumes 148
gluten 150
glutenins 150
hexaploid 150
intercalary
meristem 148
internode 148
lemma 148
mano 151
metate 151
midrib 147
niacin 151
node 148
nonshattering 150
palea 148
pellagra 151
rhizomes 148
semolina flour 150
spikelets 148
stolons 148
tetraploid 150
veins 147

QUESTIONS FOR REVIEW AND DISCUSSION

1. It has been stated that humans didn't domesticate the grasses, the grasses domesticated humans. Explain this statement.
2. Describe the characteristics of a typical grass plant, both vegetative and reproductive.
3. Grasses have characteristics that often allow them to survive in disturbed habitats. Why is that so advantageous since the advent of humans?
4. Half of the plant calories consumed by humans come from the endosperm. This tissue is only found in flowering plants from a second fertilization of a sperm nucleus combining with 2 polar nuclei in the ovule. The resultant tissue is triploid (3*N*). What is the function of the endosperm to the plant? How do we as humans use the endosperm as food? In what common food product do we consume endosperm?
5. How does the evolution of wheat connect with the change in characteristics that made modern wheat a "super food"?
6. The evolution of wheat, maize, and rice are examples of genetic modifications induced by humans over time. How does this type of genetic manipulation compare with modern methods to genetically modify organisms?
7. What are some of the chemical properties of wheat flour that make it superior to other types of flour for making bread?

ADDITIONAL RESOURCES

Beadle, J. 1980. The ancestry of corn. *Scientific American* 242(1):112–19.

Chapman, G. 1996. *The biology of grasses.* Wallingford, UK: CAB International.

Doebley, J. 1992. Mapping the genes that made maize. *Trends in Genetics* 8:302–7.

Hancock, J. 1992. *Plant evolution and the origin of crop species.* Englewood Cliffs (NJ): Prentice-Hall, Inc.

Iltis, H. 1983. From teosinte to maize: The catastrophic sexual transmutation. *Science* 222:886–94.

Johnson, D., and R. Croissant. 1990. Alternate crop production and marketing in Colorado. *Colorado Expt. Sta. Coop. Ext. Serv. and Dept. of Agron. Tech. Bull.* LBT 90–3.

Levetin, E. and K. McMahon. 1999. *Plants and society,* 2d ed. New York: McGraw-Hill Companies, Inc.

MacRitchie, F. 1999. Wheat proteins: Characterization and role in flour functionality. *Cereal Foods World* 44:188–93.

Mangelsdorf, P., and R. Reeves. 1938. The origin of maize. *Proceedings of the National Academy of Science* 24:303–12.

Richardson, W., and T. Stubbs. 1978. *Plants, agriculture, and human society.* Reading (MA): Benjamin/Cummings Publishing Co.

Salunkhe, D., and S. Deshpande, eds. 1991. *Foods of plant origin: Production, technology, and human nutrition.* New York: Van Nostrand Reinhold.

Simpson, B., and M. Conner-Ogorzaly. 1986. *Economic botany: Plants in our world,* 2d ed. New York: McGraw-Hill Companies, Inc.

Smith, J. P. Jr. 1981. *A key to the genera of grasses of the conterminous United States,* 6th ed. Eureka (CA): Mad River Press.

Wieser, H. 2000. Simple determination of gluten protein types in wheat flour by turbidimetry. *Cereal Chemistry* 77:48–52.

ON THE WEB

Food and Agriculture Organization of the United Nations (FAO). Statistics for numerous years on yields, nutrition, and economic value of all the primary and secondary crops of the world.

http://www.fao.org

Grass Genera of the World: Descriptions, Illustrations, Identification, Information Retrieval; L. Watson and M. J. Dallwitz. An interactive key to all the grasses of the world, including commercially important species.

http://www.biodiversity.uno.edu/delta/grass/

Purdue University Cooperative Extension Service. Articles and information on numerous crops, including cereals and turf grasses.

http://www.agcom.purdue.edu/

OTHER ACTIVITIES

1. Examine and learn the characteristics of other commercially grown grains, besides wheat and corn.
2. Determine gluten protein content using alternative methods such as Weiser, H. 2000. *Cereal Chem.* 77 (1):48–52.
3. Visit a commercial bakery or grain elevator for a tour.
4. Extract and measure the niacin concentration of the blue corn flour before and after adjusting the pH to 8. Standard methods include HPLC, yet microbiological assays have been described.
5. Compare the nutritional qualities of brown rice with white, polished rice.
6. Build a structure with bamboo.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 11-1 EXERCISE A: A TYPICAL GRASS PLANT

1. Sketch the grass plant here to illustrate all its unique vegetative and reproductive features.

2. What characteristics of the grass plant allow for support and flexibility?

3. What characteristics of a grass help the plant respond to herbivory?

4. What characteristics of a grass are well suited to plants living in disturbed habitats, such as plowed fields or urban settings?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 11-2 EXERCISE B: THE BOTANY OF BAKING

DOUGH CHARACTERISTICS

FLOUR TYPE	CONSISTENCY	ELASTICITY	COLOR	STICKINESS	MEAN INCREASE IN DIAMETER (mm)	OTHER COMMENTS

BAKED BREAD CHARACTERISTICS | *YOUR OVERALL RATING*

FLOUR TYPE	TASTE	TEXTURE	COLOR	OTHER COMMENTS	(1 BEING THE BEST)

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 11-3 EXERCISE C: CRUDE GLUTEN CONTENT

FLOUR TYPE	DRY WEIGHT GLUTEN (G)	% GLUTEN IN FLOUR (DRYWEIGHT GLUTEN) ÷ (DRY WEIGHT FLOUR) × 100%	MEAN INCREASE IN DIAMETER (mm)

1. What was the correlation between the ability to rise and the relative amount of gluten in the flour?

2. How did you think the overall quality of the bread compares with its gluten content?

3. How did the gluten content in wheat species evolve over time?

LABORATORY TOPIC 12

The Lowdown on Legumes

BACKGROUND

The **legumes** are members of the bean family, the Fabaceae. This family is a source of many edible seeds, including the various types of peas, beans, lentils, and peanuts. It is also well represented by a number of important forage crops—for instance, clover, vetch, and alfalfa—as well as by trees such as black and honey locusts which are valued in landscaping and for lumber.

Legumes are important food sources because their seeds are often rich in both protein and oil. Legumes have the highest protein content of any plant, and the quality of the protein approximates that of meat and other animal products. For this reason, edible legumes are often nicknamed "poor man's meat" because they afford the impoverished a source of inexpensive and readily abundant protein of a quantity and quality that is usually beyond their means.

Leguminous seeds are often excellent sources of oil as well. These oils may be used for cooking or in the production of soap, paints and varnishes, plastics, biofuels, and a multitude of other commodities.

Legumes are often planted to improve soil fertility. Many have an intimate relationship with nitrogen-fixing bacteria and thus play a significant role in the biogeochemical cycling of nitrogen. Significantly, the nitrogen-fixing bacteria housed in nodules within the roots of a legume can alter nonusable nitrogen gas into a form that the legume and other plants can absorb. Since nitrogen atoms are essential to the manufacture of amino acids and protein, the high protein content of legumes and their soil enrichment properties can be explained by the presence of these bacteria within their roots.

In this lab, you will use a hands-on approach to learn the properties and applications of legumes.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Recognize the characteristics of flower, fruit, and seed that define the family of legumes.
2. Understand the food value of edible legumes.
3. Describe the nitrogen biogeochemical cycle and how legumes and the nitrogen-fixing bacteria housed within their roots play a significant role in this cycle.
4. Recognize the properties of fats and oils and the biological and industrial value of saturated and unsaturated triglycerides.
5. Describe the components of soap, make an all-vegetable soap, and explain the soap-making process.

EXERCISE A: Shipshape Flowers, Splitting Fruits, and Nutritious Seeds

Flowering plants are sorted into families based on the shared characteristics of their flower, fruit, and vegetative body. The legume flower is five-parted; if viewed from the side or front with a bit of imagination, it resembles a sailing ship, and the showy petals have been named to reflect this resemblance (fig. 12.1*a*). The large, topmost petal is the standard or banner (the mainsail). The two bottommost petals form the keel (the hull of the ship) and may be fused. The two side petals are known as wings (side sails).

Legume fruits are **dry fruits,** so named because the cells of the fruit wall are dead at maturity (see Laboratory Topic 7). Dry fruits are further classified into those that split open to release seeds (**dehiscent**) and those whose fruit wall remains intact (**indehiscent**). **Seeds** are mobile packages composed of a protective outer covering, an embryonic plant, and a supply of nutrients.

Materials Needed for Exercise A

Forceps

Hand lens or magnifying glass

Legume flowers

Legume seed mix

Mung bean sprouts

Peanuts in the shell

Pods of green beans, snowpeas, and others

Soy-based foods

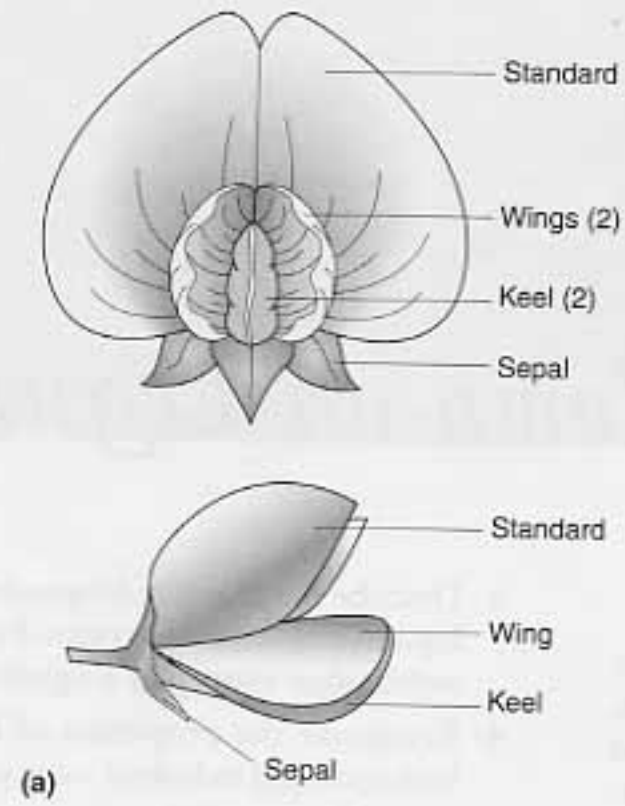


FIGURE 12.1 LEGUME MORPHOLOGY: (A) FLOWER; (B) FRUIT; (C) SEED; (D) SEEDLING.

Procedure for Exercise A

1. Obtain a flower of black locust, wisteria, Texas bluebonnet, garden pea, bean or other legume flower. (You may want to refer to Laboratory Topic 6 to review flower structure.) Is the legume flower regular (radial symmetry) or irregular (bilateral symmetry)?

What is the number of sepals and petals?

Identify the largest and topmost petal, the standard. Find the two side petals, or wings. Is the standard outside or inside the wings? Are the petals of the keel fused together or separate?

Remove the petals and locate the stamens. What is the number of stamens? Are the stamens separate all the way to the base of the filaments, or is there some fusion? If the filaments are fused, are all the stamens fused? How many? Are there any solitary stamens? How many?

_____

_____

_____

What is the number of carpels?

_____

In the following space, draw the shape of a carpel.

Examine two other legume flowers and record your observations in table 1 of worksheet 12-1 at the end of this laboratory topic.

2. The shape of the legume carpel that you drew in step 1 hints at the shape of the fruit to come. Obtain several peanuts (*Arachis hypogea*) in their shells. Observe the shell of a peanut. Carefully apply pressure to the shell. Does the peanut split lengthwise along two seams? Splitting along two seams is typical of a **pod** (also called a **legume**), the characteristic fruit of the Fabaceae (fig. 12.1*b*). Although the peanut matures underground where soil microbes decompose the fruit wall, the "ancestral seams" of the pod are still retained.

Within the shell, find 2–3 peanuts. The peanuts are actually seeds (fig. 12.1*c*). Note the brown papery material surrounding each seed. This is the protective **seed coat,** or **testa**. The two large edible structures per seed are **cotyledons.** Legumes belong to the class of angiosperms called **dicots** (or more formally, **dicotyledons**) in which each seed is typically equipped with two of these seed leaves. The cotyledons are rich in protein and oil, and they supply the young growing plant with nutrients.

Obtain one peanut seed and carefully pull it apart. Observe the miniature peanut plant, and note the shoot with two foliage leaves and the large **radicle** or embryonic root. Examine green beans, snow peas, and other examples of legume pods from the produce aisle and the great outdoors.

3. For a better view of a seedling, obtain a sprout of the mung bean (fig. 12.1*d*). Find the two cotyledons, which may be in the process of shedding the brown seed coat. The embryonic axis above the cotyledons is called the **epicotyl**. It develops into the stem and leaves as the seedling grows. You should also be able to find the first set of **true leaves**, the first photosynthetic organs of the plant. Identify the **hypocotyl,** the area of the stem below the cotyledons. Also find the radicle, the embryonic root.
4. In table 2 of worksheet 12-1, you will find descriptions of several common types of edible beans and peas. Obtain a handful of legume seed mix, and as you sort through it, try to match each bean and pea with the descriptions in the table. Put a check mark by the name of those you have correctly identified. Note also the prominent **hilum** on many of the seeds. The hilum is the scar that marks the attachment point of the seed to the pod. Also look for a small pore; that is the **micropyle**, the opening through which the pollen tube enters the ovule in the ovary of the flower during fertilization.

   Some legume seeds are processed to make prepared foods. One common example is peanut butter, which in its purest form is simply a paste made from ground-up peanuts. Peanut butter was invented by an American physician from St. Louis in 1888 as a nutritious supplement for his patients.
5. The soybean (*Glycine max*) cannot be consumed raw because it contains an inhibitor that interferes with the normal breakdown of protein in the human digestive tract. Despite this drawback, the soybean has been valued for centuries in Asia, where it is the starting ingredient for many processed foods. To make soy sauce, soybeans are fermented in brine. Soy milk is prepared by soaking soybeans in water and then pureeing the mixture. After heating, the liquid that is poured off is soy milk. The remaining solids are soybean curds, or tofu. Although tofu has little taste on its own, it can readily pick up the flavors and colors of added spices. Soy cheeses, ice cream, and many other products attest to the versatility of tofu. Sample the soy products available in the lab.

EXERCISE B: The Nitrogen Goes Round and Round

Nitrogen is an essential element for living organisms. It is a necessary ingredient in the making of nucleic acids (such as DNA), proteins and amino acids. All organisms must be able to obtain a usable source of nitrogen. And that's the dilemma. We live in an atmosphere that is rich in **nitrogen gas (N_2),** a form of nitrogen that cannot be uti-

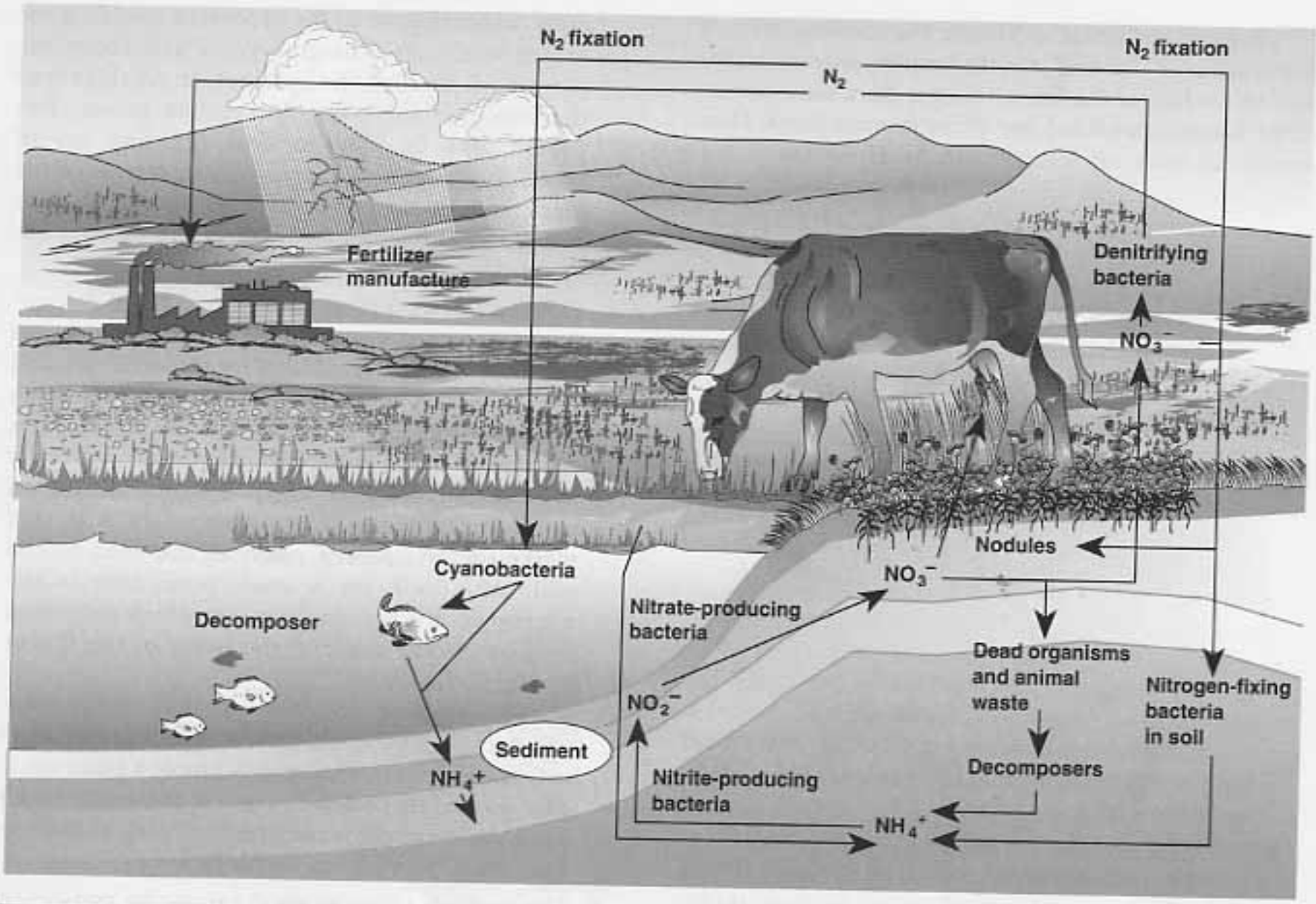


FIGURE 12.2 THE NITROGEN CYCLE. NITROGEN-FIXING BACTERIA LIVING WITHIN NODULES IN THE ROOTS OF LEGUMES CONVERT NITROGEN GAS (N^2E) INTO AMMONIUM (NH_4+).

lized by most forms of life. Fortunately, several species of bacteria and fungi are capable of utilizing atmospheric nitrogen gas and converting it into **ammonium** (NH_4^-), a form that can be readily absorbed by plants (fig. 12.2). This process is called **nitrogen fixation**. Luckily for legumes (and humans), many legumes are in close association with *Rhizobium*, one of the **nitrogen-fixing** bacteria that can convert (or "fix") nitrogen gas to ammonium. *Rhizobium* lives in the roots of its leguminous host, where it can be seen as visible swellings of the roots called **nodules**.

Materials Needed for Exercise B

Clover, bean, or other leguminous plant with root nodules

Coverslips

Dropper bottle of crystal violet stain

Dropper bottle of distilled water

Glass slides

Razor blades, single-edged

Procedure for Exercise B

1. Obtain a clover, bean, or other leguminous plant. Wash the soil away from the roots and look for nodules.
2. Remove a nodule from the root and place it on a glass slide. Chop up the nodule completely with the razor blade.
3. Add a drop of distilled water and a drop of crystal violet stain. Cover with a coverslip.
4. Look for a bacterium that resembles a letter of the alphabet. This is *Rhizobium*, the nitrogen-fixing bacterium.
5. Sketch *Rhizobium* in the following space.

EXERCISE C: Saturated or Unsaturated? You be the Judge

Fats and oils belong to the class of macronutrients called **lipids**, which provide us with more than twice the energy content of carbohydrates or proteins—9 Kcal/g. It is no wonder that animals typically build up energy reserves as fat. While plants with their lower metabolic demands and stationary habit generally store excess energy in the form of starch, an exception is seen in many seeds and some fruits. Oils are often the dominant energy store in the mobile packages of plants, their seeds.

Lipids are a diverse group of organic compounds that share one important characteristic; they are insoluble in water. Chemically, fats and oils belong to the class of lipids known as **triglycerides**. A triglyceride is composed of a molecule of glycerol with three side chains of fatty acids (fig. 12.3*a*). The nature of the fatty acid chain—that is, the number of carbon atoms in the chain and the number of carbon–carbon double bonds—determines the nature of the triglyceride.

Generally, **fats** have longer side chains than oils, and the carbons in the fatty acid side chains are joined to neighboring atoms by single bonds. Fats are solid at room temperature and obtained from animal sources. Tallow (beef fat), lard (pig fat), and butter are all examples of fats. **Oils** have shorter side chains, and the fatty acids contain some carbon–carbon double bonds, characteristics that lead to a lower melting point, which means that oils are liquid at room temperature. Oils are generally obtained from plants.

If there is only one carbon double bond, a fatty acid is said to be **monounsaturated**. If the triglyceride has mainly monounsaturated fatty acids, the oil is also said to be monounsaturated. If the fatty acid has more than one carbon double bond, and if the oil likewise is mostly composed of these kinds of fatty acids, both fatty acid and oil are said to be **polyunsaturated**. Fats on the other hand are described as **saturated** because they consist of fatty acids, the majority of which lack any double carbon bonds. Figure 12.3*b* shows the chemical structures of saturated, monounsaturated, and polyunsaturated fatty acids, and lists some common foods belonging to each group.

Unsaturated fats can be converted to saturated fats by the process of **hydrogenation**. Hydrogenation reduces the number of carbon double bonds as the hydrogen is picked up at these sites and the bonds are converted into single carbon bonds. As a consequence, the unsaturated fat becomes less unsaturated or more saturated and solid at room temperature. Margarine is a good example of a liquid vegetable oil that has been converted into a solid, spreadable product.

Whether a triglyceride is saturated or unsaturated has important implications in both health and industry. Eating a diet high in saturated fats has been linked to cardiovascular disease. In commercial applications, the degree of saturation determines whether an oil is drying, semidrying, or nondrying. **Drying oils** are highly unsaturated, and as their name implies, they dry relatively rapidly as atmospheric oxygen reacts with their carbon double bonds. The reaction breaks up the triglycerides and converts them into foul-smelling compounds. This is what happens when an oil spoils or becomes rancid. If the oil is mixed with paint or varnish, however, the triglycerides are joined to each other by oxygen, forming a protective coating. For this reason drying oils are valued in for making waterproof coatings. **Nondrying oils**, which have a smaller percentage of unsaturated fatty acids, remain wet for a relatively longer time. **Semidrying oils** are intermediate in properties and drying times.

The **Sudan IV test** is one standard for determining the presence of lipids. The red Sudan dye is soluble in lipids but not in nonlipids. Another chemical test uses iodine to determine the amount of saturation in oils. If iodine is added to an oil, it attaches to the carbon–carbon double bond and the reddish brown color of the iodine disappears as it is absorbed. The more unsaturated an oil

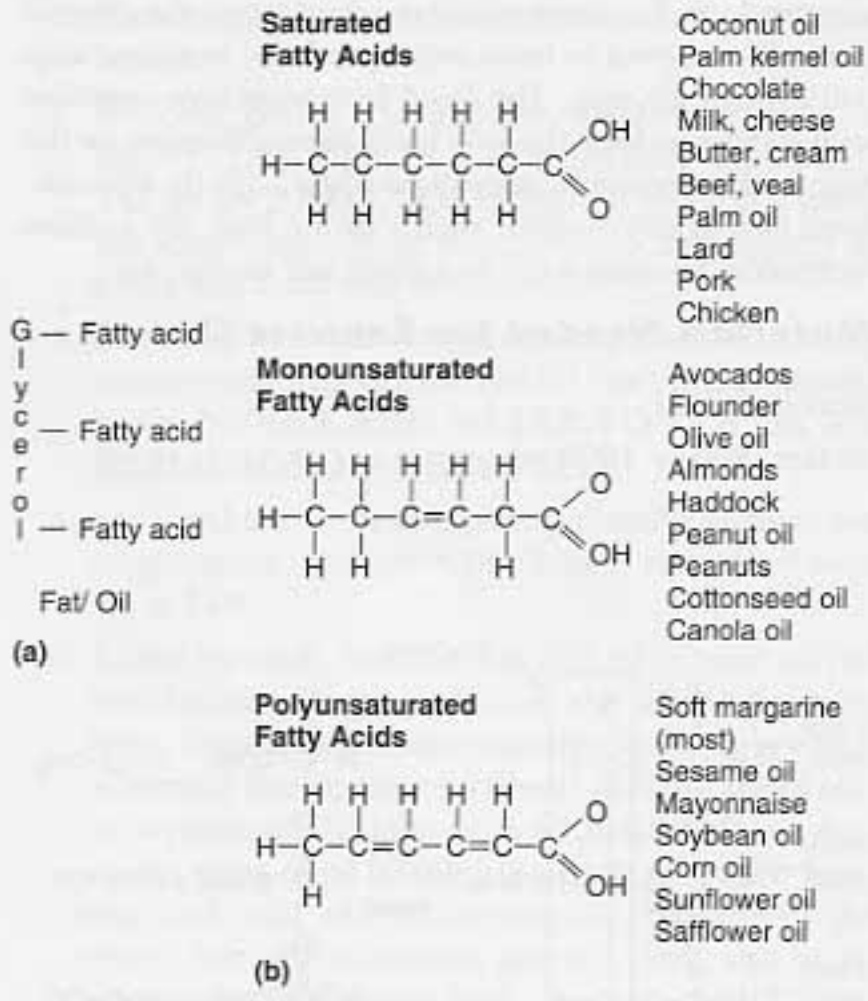


FIGURE 12.3 (A) THE STRUCTURE OF A TRIGLYCERIDE. (B) THE STRUCTURES OF SATURATED, MONOUNSATURATED, AND POLYUNSATURATED FATTY ACIDS, AND EXAMPLES OF THE FOODS CONTAINING THEM.

is, the more iodine it will absorb. The result is expressed in terms of an iodine number; the number is higher for less saturated oils and lower for more saturated oils.

Materials Needed for Exercise C

Avocado extract
Dropper bottle of apple juice
Dropper bottle of distilled water
Dropper bottle of iodine solution (I_2KI)
Dropper bottle of soybean oil or other plant oils
Dropper bottle of vinegar
Dropper bottle of white grape juice or grape juice
Graduated cylinders or pipets
Liquid margarine
Marker pens
Paintbrushes, small
Parafilm
Plastic petri dishes
Soy milk
Sudan IV vial
Test tubes
Test tube rack
Toothpicks
Variety of plant oils

Procedure for Exercise C

1. Obtain a set of 8 test tubes and a test tube rack. Label the test tubes as follows: avocado extract, apple juice, grape juice, margarine, plant oil, soy milk, water, and vinegar. Add 20 drops of the appropriate solution to each of the labeled test tubes. Using a toothpick, add a scoop of Sudan IV crystals to each test tube. Gently shake. Record any color change and your conclusions in table 1 of worksheet 12-2.
2. Obtain two plastic petri dishes, 4 paintbrushes, and 4 different vegetable oils.
3. Label the outer surface of each lid with the name of one of the plant oils and the date and time.
4. With a paintbrush, paint a thin layer of the oil on the inner surface of the lid. Place the lid, with its painted surface exposed to the air, in an undisturbed place.
5. Check periodically for drying, and record your results in table 2 of worksheet 12-2. From these results, conclude if the oil type is drying, semidrying, or nondrying.
6. Measure out 5 ml of oil with a graduated cylinder or pipet, and pour it into a labeled test tube. Add 10 drops of iodine solution one drop at a time, to the oil in the test tube. Cover with a piece of parafilm and shake to disperse the color evenly throughout the test tube. Repeat with the second oil. Record the start time and record the time for each oil to clear the iodine color in table 3 of worksheet 12-2.

EXERCISE D: Lather Up

Soaps can be made from a variety of animal and plant fats, and most soaps are blends of both. The fat or oil selected depends on the characteristics desired in the soap. For example, soaps that contain coconut oil lather up even with seawater. Olive oil also produces a soap that lathers well and is known to be good for the skin. (It should be noted that lathering ability is not necessary for a soap to clean.) Most animal fats make a hard soap, which consequently lasts longer.

In this exercise, you will make an all-vegetable soap according to the **cold process method**, so called because no boiling is required. In this method, a strong alkaline substance, sodium hydroxide (NaOH), also known as lye, is broken down into its component parts, sodium (Na^+) and hydroxy (OH^-) ions, by the addition of water (fig. 12.4). The lye solution is then added to the fats/oil blend chosen, splitting the triglyceride into its component parts—glycerol (an alcohol) and three fatty acids. The glycerol then picks up hydroxy ions (OH^-) and becomes glycerin, which is used to soften skin and is found in many hand and skin products. In commercially prepared soaps, the glycerin is usually removed to make other products, but your soap will contain glycerin. The freed fatty acids now combine with sodium to form the soap itself. **Saponification**, or the soap-making reaction, takes place when acids (in this case, freed fatty acids) combine with a strong base, the sodium hydroxide, to make a salt (a sodium salt in this case).

Materials Needed for Exercise D

Beaker, 400ml
Beaker, 800 or 1000 ml

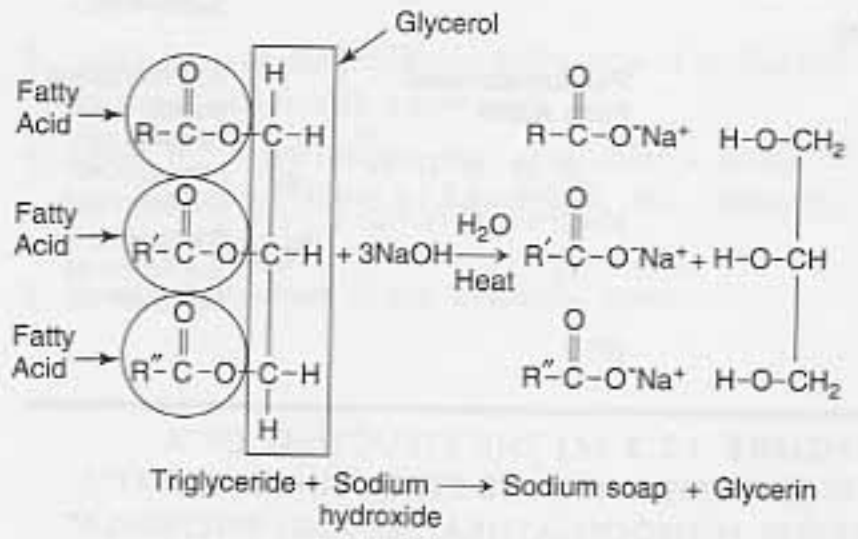


FIGURE 12.4 THE SOAP-MAKING REACTION.

Coconut oil
Distilled water
Essential oil for fragrance (optional)
Graduated cylinders
NaOH or lye
Oatmeal powder (optional)
Olive oil
Palm oil (substitute for vegetable shortening)
pH paper
Rubber gloves
Safety goggles
Soap molds
Soybean oil (substitute for palm oil)
Spices for color (optional)
Thermometers
Vegetable shortening (substitute for palm oil)
Wooden or plastic spoons

Procedure for Exercise D

1. All of the plant oils should be warmed and melted beforehand in an oven at 65°C.
2. Measure 240 ml of distilled water into a 400-ml beaker.
3. Wearing gloves and goggles, weigh out 85 g of NaOH (lye). **LYE IS EXTREMELY CAUSTIC TO SKIN AND CLOTHING**. If you feel a burning sensation on your skin, proceed immediately to a sink and flush well with water.
4. In a hood or a well-ventilated area, slowly add, while stirring with a spoon, the NaOH (lye) to the distilled water. **NEVER ADD WATER TO LYE OR AN EXPLOSION MAY RESULT!**
5. Stir until the lye is completely dissolved. Note the temperature, and place the beaker in a water bath set at 35°C.
6. As the lye cools, measure out 225 ml of palm oil (oil that has been expressed from the seeds of a palm tree). You may substitute vegetable shortening, which is partially hydrogenated soybean and cottonseed oils, or soybean oil. Add the oil to a 1,000 ml beaker. Add to the palm oil, 210 ml of coconut oil (taken from the seed) and 180 ml of olive oil (taken from the fruit). Stir with a wooden spoon to mix, and set to cool in the 35°C water bath.
7. When the temperatures of both the fats and the lye are about 35°C, **SLOWLY** add the lye solution to the oils while stirring constantly with a wooden spoon. It is important that you continue to stir constantly and vigorously while saponification takes place. Share the stirring duties with your lab partner so that the mixture is constantly stirred during the soap-making process. The most common reason a soap fails to form is failure to stir vigorously and continuously.
8. Observe how the mixture becomes cloudy and starts to thicken almost immediately. During the saponification process, the lye solution pulls off the fatty acid chains one by one and splits them from the glycerol. The glycerol is now available to react with the hydroxy ion, and the fatty acids react with the sodium ions. The saponification process is nearly completed when the "soap traces." A **trace** is the temporary impression left when you dribble some of the mixture from the spoon onto the surface. For this recipe, it should take approximately 10–15 minutes.
9. After the soap traces, colorants and fragrances can be added. Use at about 15 ml of an essential oil for fragrance, and mix thoroughly. If a different color is desired, add a spice such as cinnamon, turmeric, or paprika until you have the desired color. Mix thoroughly. For texture and a mild abrasive, add oatmeal flakes that have been reduced to a powder by a blender.
10. Your soap is now ready to pour into molds. Let the poured soap set for 24–72 hrs.
11. After 24–72 hours, remove the soap from the mold. If you have difficulty removing the soap, place the mold in the freezing compartment of a refrigerator for 10 minutes or so. The soap should then pop out easily.
12. Wrap the individual soap bars in plastic wrap, or place them in a sealable plastic bag.
13. Allow the soap to cure for 2–4 weeks. During the curing, the pH of the soap changes from alkaline to near neutral (pH 7). To test the pH, add a few drops of distilled water to the surface of the soap and touch it with pH paper. You could also perform the time-honored test of soap-makers by gingerly touching the tip of your tongue to the soap. If it burns the tongue, the soap is still too alkaline. If there is no burning sensation, suds up!

TERMS TO KNOW

ammonium (NH_4^-) 162
cotyledon 161
dicotyledons 161
drying oils 163
epicotyl 161
fats 163
hilum 161
hypocotyl 161
legume 161
micropyle 161
monounstaturated oils/fatty acids 163
nitrogen fixation 162
nitrogen gas (N_2) 161
nondrying oils 163
oils 163
pod 161
polyunsaturated oils/fatty acids 163
radicle 161
saponification 164

saturated fats/
fatty acids 163
seeds 159
seed coat (testa) 161
semidrying oils 163
triglycerides 163
unsaturated oils/
fatty acids 163

QUESTIONS FOR REVIEW AND DISCUSSION

1. Describe the features of the typical legume flower and fruit.
2. Identify the parts of a peanut in a shell.
3. Identify the key markings of a seed and seedling.
4. What is the role of legumes in the nitrogen cycle?
5. What is the diagnostic test for the presence of lipids?
6. What makes a triglyceride a fat? An oil?
7. What property of oils is important in determining a good drying oil?
8. What is the iodine number? How does the iodine number reflect the degree of unsaturation in an oil?
9. What is saponification? Chemically, what is a soap?

ADDITIONAL RESOURCES

Bloomfield, L. A. 2000. Cleaning agents. *Scientific American* 282(40):108–9.

Cavitch, S. M. 1997. *The soapmaker's companion: A comprehensive guide with recipes, techniques & know-how.* Pownal (VT): Storey Books.

Levetin, E., and K. McMahon. 1999. *Plants and society,* 2d ed. New York: McGraw-Hill Companies, Inc.

Snyder, C. H. 1998. *The extraordinary chemistry of ordinary things,* 3d ed. New York: John Wiley & Sons.

ON THE WEB

Cal Photos, Berkeley Digital Library Project
http://dlp.CS.Berkeley.EDU/photos/
International Legume Database & Information Service
http://www.ildis.org
Wayne's word: A Newsletter of Natural History Trivia
http://daphne.palomar.edu/wayne/wayne.htm

OTHER ACTIVITIES

Now that you know the characteristics of the legume flower, fruit, and seed, make a list of legumes you find in a nearby park, garden center, or supermarket.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 12-1 EXERCISE A: SHIPSHAPE FLOWERS, SPLITTING FRUITS, AND NUTRITIOUS SEEDS

TABLE 1 LEGUME FLOWERS

For each legume flower, count the number of stamens. Also note if there is any fusion of the stamens at the base, and, if so, in what pattern.

COMMON NAME	SCIENTIFIC NAME	NUMBER OF STAMENS	FUSION OF STAMENS, PATTERN

TABLE 2 EDIBLE LEGUMINOUS SEEDS

Check off the types of edible beans and peas as you find them in the seed mix.

COMMON NAME	DESCRIPTION	CHECKLIST
Adzuki beans	Small, red	
Anasazi beans	Medium, mottled red and white	
Black (turtle) beans	Small, black oval	
Black-eyed peas	Medium, oval white with bull's-eye black spot	
Broad (fava) beans	Large, flat, oval	
Chick-peas (garbanzos)	Yellowish, medium, round	
Great northern	Large, oval	
Kidney beans	Red or white, oval	
Lentils	Small, lens-shaped, green, yellow, orange, or red	
Lima beans	Large–medium, white, oval	
Mung beans	Small, green	
Navy beans	Medium oval, white	
Pinto beans	Small, oval, mottled pink and brown	
Split peas	Small, yellow and green, split	
White beans	Large, medium, and small sizes	

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 12-2 EXERCISE C: SATURATED OR UNSATURATED?

TABLE 1 SUDAN IV TEST FOR THE PRESENCE OF LIPIDS

TEST SUBSTANCE	*OBSERVATION*	*PRESENCE OF LIPIDS*

TABLE 2 DRYING TIMES FOR VARIOUS PLANT OILS

OIL	*DRYING TIME*	*OIL CLASSIFICATION*

1. Which of the oils tested is a drying oil? Semidrying? Nondrying?

2. Which of these oils is the most unsaturated?

3. What in the air do you think is reacting with the carbon double bonds of an unsaturated triglyceride to bring about drying?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 12-2 EXERCISE C: SATURATED OR UNSATURATED? *(continued)*

TABLE 3 IODINE VALUES AND OILS

OIL	*TIME TO CLEAR IODINE COLOR*

1. Which of these oils has the highest degree of saturation? Why?

2. Would an oil with a high iodine value most likely be a drying or nondrying oil? Why?

LABORATORY TOPIC 13

Food from Underground and Far Away

BACKGROUND

Starch, as a complex carbohydrate, is an important source of calories in the human diet. Most nutritionists suggest that approximately 60% of the daily diet consist of complex carbohydrates. Starch is the primary energy storage product of plants. Thus, starchy staples, those crop plants that are proficient starch producers and savers, are important to the human food supply. In this laboratory, you will learn about the chemical makeup of starch and use a simple test to identify its presence. You will also see firsthand the **starch grains** within the **amyloplasts** of plant cells and come to recognize their usefulness as a "fingerprint" identification tool. Last, you will learn to recognize a variety of underground storage organs and acquaint your palate with some starchy staples and other crops that are not commonly eaten in most parts of the United States.

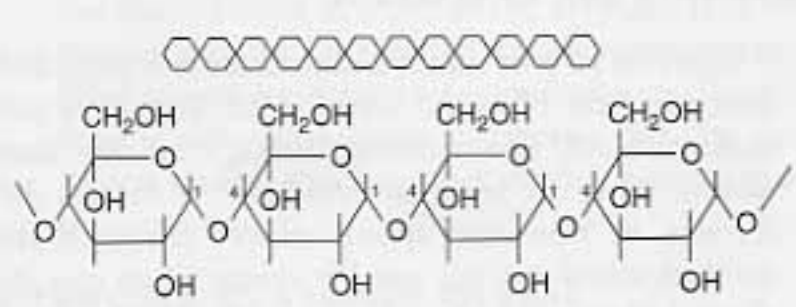
LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

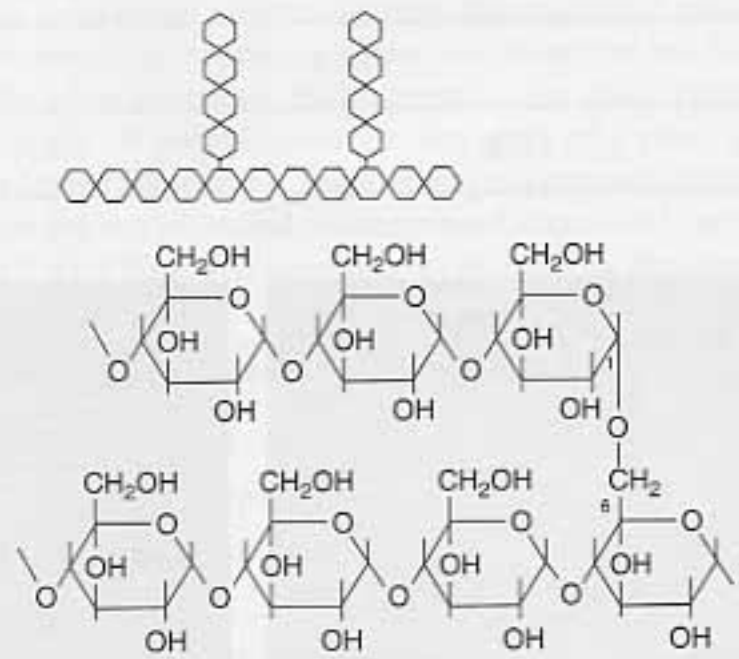
1. Name the chemical constituents of starch.
2. Apply the diagnostic test for the identification of starch and identify starch grains from a variety of plants.
3. Recognize the variety of plant organs that have been modified as underground storage organs.
4. Name the characteristics of the major starchy staples: white potato, sweet potato, cassava, yam, and taro.
5. Appreciate the potential nutritional and commercial value of crops from other regions of the world.

EXERCISE A: Making Plastic

Starch is classified as a **polymer**, a large molecular compound composed of many smaller units, or **monomers**. In the case of starch, the monomer is glucose, which forms a ring on itself. Enzymes link the glucose molecules together through a **condensation reaction** in which a molecule of water is created from the atoms given off as the glucose molecules bond. How the glucose is put together determines the particular type of starch. **Amylose** is composed of unbranched chains of glucose rings, numbering from several hundred to a few thousand units. Picture a ring of glucose as a paper clip, and then join the paper clips one to another in a straight chain. That's amylose (fig. 13.1*a*). Add side branches to the paper clip chain and you've created **amylopectin**. Amylopectin is larger than amylose—several hundred thousand glucose rings long, with branches coming off every twenty-fifth glucose unit (fig. 13.1*b*). Vegetable starch is typically 80%



(a) Unbranched chain of amylose



(b) Branched chain of amylopectin

FIGURE 13.1 THE STRUCTURE OF STARCH: (A) AMYLOSE; (B) AMYLOPECTIN.

amylopectin and 20% amylose, although these percentages may change depending on the plant source.

One of the greatest technological advancements of the twentieth century was the invention of plastics. Plastics are also made from **polymerization** of monomers. Although industrial plastics are man-made and synthesized in the laboratory under conditions of high temperature and pressure, natural polymers like starch can be the base for homemade plastics.

Materials Needed for Exercise A

Dropper bottle of vegetable oil
Food coloring
Graduated cylinders, 10 ml and 50 ml
Microwave oven
Petri dish bottoms
Plastic spoon
Polarizing filters
Sealable plastic bag
Starch
Water

Procedure for Exercise A

1. Weigh out 10 g of starch and transfer to a petri dish bottom. Add 10 ml of water. Mix. You have just make oobleck! Stir it slowly, then stir quickly, then slowly. What are your observations? Is there a difference in how the spoon moves through the oobleck when you stir quickly versus when you stir slowly? How so?

_____

_____

_____

_____

_____

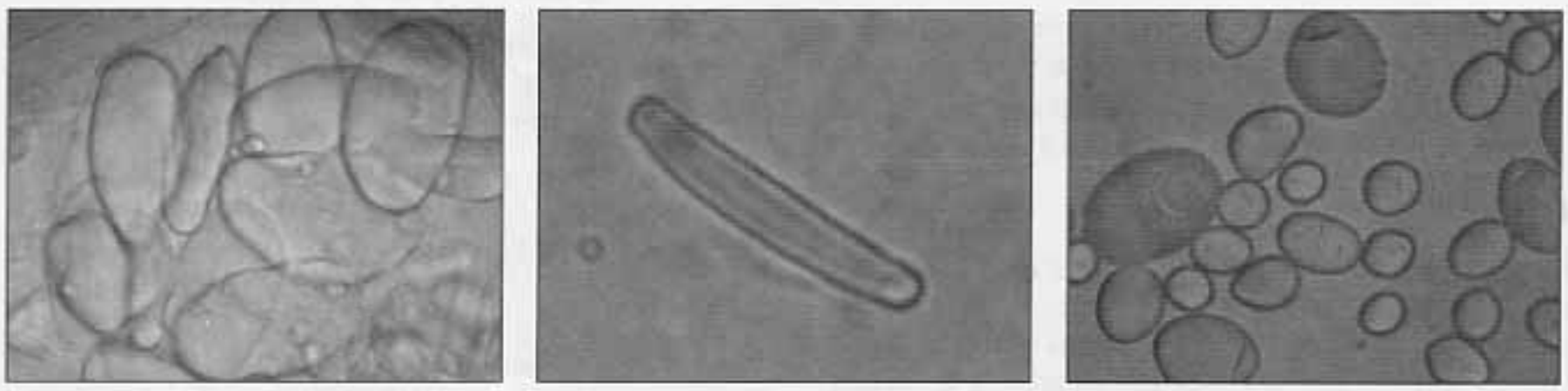
2. Weigh out 20 g of starch and place it in a sealable plastic bag. Add 30 ml of water. Then add 4–5 drops of vegetable oil and 2–3 drops of the food coloring of your choice.
3. Seal the bag closed and knead it to mix the contents. Unseal a small opening as a vent and place the bag in a microwave oven on high for 30–40 seconds.
4. Remove the bag. When it is cool to the touch, open it.
5. Take the mixture out of the bag and roll it into a ball. You've created homemade plastic.

EXERCISE B: Starch Grains

Plant cells have several unique organelles (see Laboratory Topic 1). Within plant cells are the plastids, a family of membrane-bounded organelles. Two classes of this group are chloroplasts and chromoplasts. Chloroplasts are the green, chlorophyll-containing organelles of photosynthesis; chromoplasts store the pigments of red, yellow and orange that color may flowers and fruits. Another type of plastid is the **leucoplast**, which is colorless. A leucoplast that stores starch is an **amyloplast**. The **starch grains** within an amyloplast may have recognizable shapes and other distinguishing properties that can prove useful as identification tools in archaeology and forensics (fig. 13.2). One useful characteristic of starch grains is their appearance in polarizing light. A Maltese cross is seen in each starch grain; its appearance varies somewhat with species.

Materials Needed for Exercise B

Compound microscope
Coverslips
Dissecting needles
Dropper bottle of distilled water



(a) (b) (c)

FIGURE 13.2 STARCH GRAINS OF (A) BANANA; (B) CROWN OF THORNS; AND (C) POTATO.

Dropper bottle of iodine solution (I_2KI)

Glass slides

Plants to examine for leucoplasts and starch grains (e.g., white potato, crown of thorns, banana)

Polarizing filters

Starch of arrowroot, potato, corn, etc.

Procedure for Exercise B

1. Place a drop of distilled water on the center of a glass slide. Using a dissecting needle, obtain a very small sample of the starchy flesh of a banana and put this sample in the drop of water. Carefully cover with a coverslip, pressing firmly to spread a thin film of banana flesh on the slide. If the coverslip rocks, you have too much banana flesh. Remove some and try again.
2. Examine the slide under the low power (10 × objective) of a compound microscope. Look for wide elongated ovals some of which resemble the shape of a banana. In the table provided in worksheet 13-1 at the end of this laboratory topic, record the name of the plant you are testing and draw a few of the starch grains you see.
3. Add a drop of iodine solution to the edge of one side of the coverslip. Place a bit of paper towel on the other side of the coverslip to draw the iodine solution across the slide. Observe through the microscope as the iodine solution diffuses across the slide. What happens when the iodine solution comes in contact with the starch grains of banana? Do you notice a color change? Describe.

_____

_____

_____

_____

The iodine test is the standard test for the presence of starch.

4. Now try to find the starch grains of crown of thorns (*Euphorbia splendens*). In this case, just pull a leaf from the stem. Note the milky exudate. Squeeze a few drops of this exudate onto a glass slide. Add a drop of water and a drop of iodine solution. Cover with a coverslip.
5. Observe the slide under the low power (10 × objective) of a compound microscope. Look for the structures stained blue-black. These are the starch grains. Bring up to high power (40 × objective) to see more clearly. Draw the shape in worksheet 13-1.
6. Now take a look at the starch grains in a potato. Using a razor blade, slice a small piece from the fleshy part of a white potato.
7. On a glass slide, cut this slice into tiny pieces. Add a drop of water and a drop of iodine solution. Carefully cover with a coverslip. If the coverslip rocks, you have too much potato flesh. Remove some and try again.
8. Observe under the low power (10 × objective) of a compound microscope. Look for the dark-stained structures. Some should still be in the cells while others were released when you cut the slice. Bring up to high power (40 × objective) to see more clearly. Draw the shape in worksheet 13-1.
9. Repeat this process for any other plants provided for this purpose in the laboratory. Record the shapes of the starch grains in worksheet 13-1.
10. Add a bit of arrowroot starch to a glass slide. Add a drop of water and cover with a coverslip. Observe under the low power (10 × objective) and of the compound microscope to see the starch grains of arrowroot. Bring up to high power (40 × objective) to see more clearly. Obtain two polarizing filters. Place one over the light source below the stage. Hold the second filter in front of the eyepiece lens while looking into the microscope. Slowly rotate this upper filter as you continue to examine the starch grains. Stop when you see the distinctive Maltese cross. Repeat making slides of other starch grains using corn or potato starch.

EXERCISE C: Storage Organs

At the start of the growing season, stored starch serves as the energy source for new aboveground growth. In stressed environments when conditions are not favorable for photosynthesis, starch provides the plant with a ready supply of organic solutes. Any part of a plant may be adapted for storage. Some storage organs are roots, while others are modified underground stems and leaves (fig. 13.3). (*Note*: For assistance in identifying the plant parts in the following exercise, refer to Laboratory Topic 4.)

Materials Needed for Exercise C

Bulb of daffodil

Compound microscope

Corm of crocus

Coverslips

Dissecting needles

Dropper bottle of iodine solution (I_2 KI)

Dropper bottle of distilled water

Glass slides

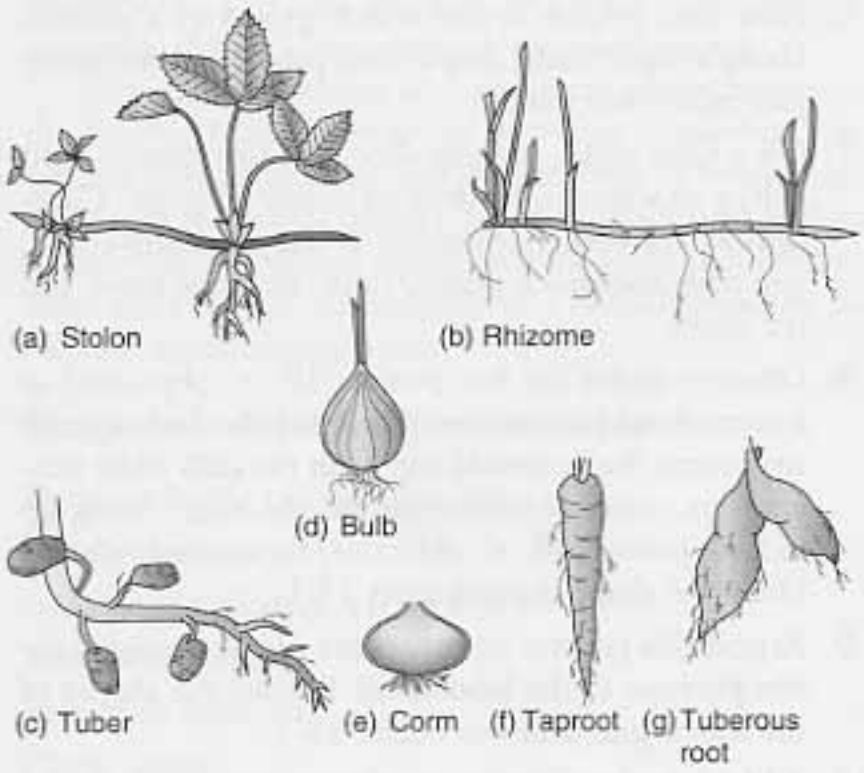


FIGURE 13.3 STORAGE STEMS AND ROOTS.

Razor blades, single-edged or knives
Rhizome of ginger
Storage organs from other plants
Taproot of carrot
Tuberous root of spider plant
Tuber of caladium

Procedure for Exercise C

1. Obtain a daffodil bulb and observe the papery brown coverings on the outside of the bulb. These are modified leaves that act as a protective barrier and deterrent to discourage microbes and other soil organisms. Note the veins running through these leaves. What is the venation pattern? Is this a monocot or dicot plant?

Now slice the bulb longitudinally. What does this resemble?

The **bulb** is a modified stem with storage leaves. These white storage leaves make up the bulk of the bulb. The stem at the base of the bulb gives rise not only to leaves but also to roots. Pick off one white leaf and note its thin, translucent covering (epidermis). Take a small sample from the fleshy middle or mesophyll of a leaf and place the sample on a glass slide. Add a drop of water and iodine solution. Cover with a coverslip. Using a compound microscope, view the slide under the low (10 × objective) power first, and then switch to high (40 × objective) power. Observe the numerous starch grains within each parenchyma cell of the leaf.

If your bulb has sprouted, it is easy to recognize a third type of leaf. These are the green foliage leaves, the ones that appear aboveground when the bulb is planted. What would their main function be?

2. Next obtain and examine a crocus corm. As with the bulb, papery leaves on the outside form a protective barrier. Again, it is easy to see the venation pattern of these leaves. What is the pattern? Is the crocus a monocot or dicot plant?

Now slice the corm longitudinally. Note that the **corm** is a solid stem, unlike the stem and storage leaves in the bulb. Take a small piece of the corm flesh and place it on a glass slide. Add a drop of water and a drop of iodine solution. Cover with a coverslip, and look for starch grains first under low (10 × objective) power and then under high (40 × objective) power. Record in worksheet 13-1.

3. The rhizome of ginger is the next storage organ to examine. Unlike the bulb and corm, which are ver-

tically oriented, a **rhizome** shows a distinctive horizontal orientation. The rhizome is an underground horizontal stem. You may also find adventitious roots arising from the rhizome.

You can verify the starch storing capacity of the rhizome by taking a small sample of it and preparing a slide to observe the starch grains. Record in worksheet 13-1

4. Examine the tuber of a caladium. **Tubers** are enlargements found at the ends of some rhizomes. Examine closely the "eyes" of the tuber. Botanically speaking, the eyes are collections of buds.

   Would a tuber be a root or a stem? How do you know?

   _____

   _____

   _____

   _____

   _____

   Sample a piece of the inner storage region of the caladium tuber, and examine it under the microscope for starch grains. Record in worksheet 13-1.

5. Obtain a carrot and cut it horizontally. Note the central core of tissue encircled by a large outer region. Have you seen this arrangement before? In what vegetative organ? What is this core region called? Would a carrot be a root or a stem? How do you know?

   _____

   _____

   _____

   _____

   _____

   Sample a piece of the inner storage region of the **taproot** of the carrot and examine for starch grains under the microscope. Record in worksheet 13-1.

6. Examine the many enlarged underground structures of the spider plant, or airplane plant (*Chlorophytum comosum*). These are **tuberous roots**, a type of root that develops in many monocots when fibrous roots enlarge for storage.

7. Examine the other examples of underground storage organs available in the lab. List the ones you examine, and determine whether the storage organ is a bulb, corm, tuber, rhizome, taproot, or tuberous root. Record your findings in worksheet 13-2.

EXERCISE D: The Starchy Staples

The crops called starchy staples are equal in food value to the cereals and legumes. But, unlike the cereals and legumes, the starchy staples are not exclusive to a single family. This vegetable group is so named because of the high quantities of starch sequestered within the underground storage organs. Although most of these crops are tropical in origin, several are adapted to grow under temperate conditions.

Materials Needed for Exercise D

Cassava
Dropper bottle of distilled water
Dropper bottle of iodine solution (I_2 KI)
Dropper bottle of Sudan III
Potato chips
Sweet potato
Taro
True yam
White potato varieties: round white, russet, long white, round red

Procedure for Exercise D

1. White potatoes (*Solanum tuberosum*) originated in the Andean highlands of South America. Although tropical, the growth at the cooler temperatures of higher altitudes allows its widespread cultivation in temperate climates. Obtain and examine a white potato. Note the "eyes," which as discussed in Exercise C, are collections of buds at nodes.

   Cut the potato in half and note the ring of tissue just inside the skin. This ring of tissue can also be seen in a potato chip. What is it called? What is the large center region called? What vegetative organ (root, stem, or leaf) of plants has this organization?

   _____

   _____

   _____

   _____

   _____

What conclusion can you draw about the identity of the storage organ of the potato? Record your conclusion by continuing to fill out worksheet 13-2 (begun in Exercise C).

The most familiar varieties of the white potato belong to just four groups: round white, russet, long white, and round red. The round white is a multipurpose variety that works for all preparations—boiling, baking, or converting into chips, fries or flakes. The russet type is the classic baking potato, and its oblong shape is also ideal for processing into French fries. Long whites and round reds are sold as new potatoes because they are harvested early in the growing season while the skins are still thin. New potatoes are used for roasting, steaming, or boiling. View each type on display in the lab.

2. The sweet potato (*Ipomea batatas*) is the tuberous root of a vine in the morning glory family. The sweet potato is native to tropical South America, where Christopher Columbus encountered it on his first voyage to the New World. The Arawak people of the Caribbean called it *batatas*, which became "potato" in English; and the same name was bestowed upon *Solanum tuberosum* because it too was an underground crop from the New World. The sweet potato is also often confused with the true yam, another tropical underground crop. In the United States, sweet potatoes and yams are simply varieties of *Ipomea batatas*. The sweet potato has a yellower, drier, and starchier flesh than the yam, whose flesh is sweeter, moister, and more orange in color.

   Obtain and examine a sweet potato. Note the orange color of its flesh. What orange plant pigment would account for this color? What vitamin would you expect to find in abundance in the sweet potato ?

   _____

   _____

   _____

   _____

   _____

   Cut the sweet potato horizontally. Note the inner circle. What inner ring of tissue can be found in roots?

   _____

   _____

   _____

   _____

   _____

   Take a small piece of the sweet potato flesh and prepare a slide to examine its starch grains. Add sweet potato to the chart in worksheet 13-1 (begun in Exercise B), and draw a few of the starch grains.

3. Cassava, *Manihot esculenta*, is known by many common names, including manioc, yucca, mandioca, and tapioca. A tropical member of the spurge family, cassava is actually a very large tuberous root from a small tree or bush. Sweet and bitter varieties are classified according to the concentration of cyanogenic glycosides. Cyanogenic glycosides release deadly hydrocyanic acid. Sweet varieties have low levels of these glycosides, while bitter varieties have much higher levels that must be removed through extensive preparations to make the cassava safe to eat. Traditional methods of detoxifying bitter varieties vary among cultures, but may include one or more of the following processes: drying, grating, boiling, fermenting, and soaking. Most people are familiar with tapioca, in which the moistened starch of cassava is gently heated to form gelatinized beads. The tapioca pearls are then cooked with milk, eggs, and sugar to make pudding.

   Take a sample of cassava or its starch to view the starch grains. Because the starch grains are some of the smallest in the plant world, cassava starch and its products are easily digestible and a valuable food for infants and invalids. Record and draw cassava starch grains in worksheet 13-1.

4. True yam, one of several *Dioscorea* species and a pantropical native, is an important staple in many tropical countries. The tubers of a vine may reach lengths of 2–3 m (6–9 ft) at the time of harvest. The tuber can be prepared and eaten in ways similar to the white potato. Additionally, the tubers are a source of saponins, or vegetable steroids. Historically, the early research that led to the development of the birth control pill was dependent on extracting inexpensive steroidal precursors from the yam. If available, examine the starch grains of yam.

5. Taro (*Colocasia esculenta*) grows best in flooded conditions. Native to the tropics of southeast Asia, it was brought along in the canoes of the early migrants who eventually settled Polynesia and the Hawaiian Islands. Poi is the fermented dough of taro, a traditional staple of the Polynesian and Hawaiian peoples. Examine the corm of the close relative of taro, elephant ears, *C. ultissima*. Take a sample of the corm or its starch to view the starch grains, and draw their shape in worksheet 13-1.

EXERCISE E: What are You Eating?!

Although the estimated number of edible plants approaches 50,000 species, less than 300 have been widely cultivated. Of these, wheat, rice, and corn con-

tribute nearly two-thirds of the plant-derived calories in the human diet. This means there are literally thousands of potentially useful plants that most of humanity has yet to discover. Government organizations and researchers are constantly on the lookout for plants that are little-known but potentially useful to bring to the greater attention of consumers in the world's marketplace.

Materials Needed for Exercise E

A variety of little-known plant foods (amaranth, arrowroot, cherimoya, Jerusalem artichoke (sunchoke), jicama, malanga, quinoa, sunchoke and tomatillo)

Procedure for Exercise E

Examine and sample each of the little-known food plants available in the lab. Using your knowledge of the anatomy of plants, decide what plant part is represented by the following plants, and record your observations in worksheet 13-3. If necessary, refer to Laboratory Topics 4 and 7 to ascertain what part of the plant is eaten. Draw starch grains in worksheet 13-1.

1. Arrowroot (*Maranta arumdinacea*) is the source of an easily digested starch because of its tiny starch grains. To the Arawak people, natives of the Caribbean Islands, arrowroot was the dietary staple they called *aru-aru* or "meal of meals." They also valued it medicinally and used it to draw arrow poisons from wounds. Europeans named it arrowroot after this practice. Examine either the starch or the plant part. Make a slide to view the starch grains.
2. The Jerusalem artichoke is neither an artichoke nor from Jersualem. It is actually the underground storage organ of a sunflower (*Helianthus tuberosus*), which is native to North America and was prized by early Native Americans. Once the plant came to the attention of the early colonists, it was exported to Europe. There, the Italians called the plant *girasole* (turning to the sun) because its flowers follow the path of the sun during the course of the day. English speakers soon corrupted *girasole* to *Jerusalem*. The name artichoke was bestowed by Samuel Champlain, who thought the taste of the vegetable was reminiscent of the globe artichoke. The misnomer Jerusalem artichoke stood until the 1960s, when a campaign to introduce North American consumers to the crop came up with the name "sunchoke." A versatile vegetable, the sunchoke can be eaten raw, baked, boiled, or fried. Its primary carbohydrate is inulin, a polymer of fructose that, unlike glucose polymers, can be safely consumed by diabetics. Sample the sunchoke, and make a slide to examine its starch grains.
3. Jicama is a starchy rootstock that is also known as yam bean and Mexican turnip. It is the storage organ of a leguminous vine (*Pachyrrhizus erosus*), and can be eaten raw or cooked. In Mexico, jicama is usually served raw with a little lime juice, chili pepper, and salt. Native to Mexico, the plant was introduced to China in the seventeenth century, where it is commonly used in stir-fry dishes. Sample jicama, and make a slide to examine its starch grains.
4. Malanga, also called cocoyam, is a relative of taro. It is the underground storage organ of one of several species of *Xanthosoma* from Central and South America. Like taro and arrowroot, malanga is valued for its tiny starch grains and easily digestible starch.
5. Two noncereal grains that have high nutritional potential are quinoa and amaranth. The Incas coined the name *quinoa* which means "mother grain." High in carbohydrates and quality protein, the "grain" (*Chenopodium quinoa*) is cooked like rice or ground into a flour that can be mixed with wheat flour to make bread and other baked goods.

   Another noncereal grain is amaranth, one of several species within the genus *Amaranthus*. These nongrass grains can be toasted, boiled, popped, or ground into a flour that is mixed with wheat to make a variety of baked goods. Its protein content rivals that of the cereal grains.

   Examine quinoa and amaranth. Botanically, what parts of these plants are actually eaten or ground for flour? Sample foods made from quinoa and amaranth.
6. Two fruits that have been making inroads in North American supermarkets are the tomatillo and the cherimoya. Tomatillo, or the husk tomato, is a staple of *salsa verde*, the green salsa served with Mexican dishes. It can also be sliced and eaten raw. The husk tomato (*Physalis ixocarpa*) is so called because of its resemblance to a tiny tomato in a paperlike husk. Sample the tomatillo and determine its fruit type. Cherimoya, or custard apple, was known by the Incans. The fruit grows on a tree (*Annona cherimola*) that is native to the uplands of Peru and Ecuador. The fruits are chilled, and then the custardlike flesh is scooped out and eaten alone or with cream. Sample the delicious cherimoya, and determine its fruit type.

TERMS TO KNOW

amylopectin 171
amyloplast 172
amylose 171
bulb 174
condensation reaction 171
corm 174
leucoplast 172
monomers 171
polymer 171
polymerization 172
rhizome 175
starch grains 172
taproot 175
tubers 175
tuberous roots 175

QUESTIONS FOR REVIEW AND DISCUSSION

1. What is a polymer? What monomer makes up starch?
2. How does amylose differ from amylopectin?
3. What is the function of amyloplasts in plant cells?
4. How can starch grains be used to identify plants?
5. List the distinguishing characteristics of bulbs, corms, rhizomes, taproots, tubers, and tuberous roots and give examples.
6. Name the characteristics of the five major starchy staples: white potato, sweet potato, cassava, yam, and taro.
7. What characteristics of alternative crops mark them as having high potential for further development?

ADDITIONAL RESOURCES

Epstein, H. 1996. Crippling harvest. *Natural History* 105(7):12–15.

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

ON THE WEB

Cal Photos, Berkeley Digital Library Project
http://dlp.CS.Berkeley.EDU/photos

Ethnobotanical Leaflets Starch Research Page
http://www.siu.edu/~ebl/amylose.htm

Wayne's word: A Newsletter of Natural History Trivia
http://daphne.palomar.edu/wayne/wayne.htm

OTHER ACTIVITIES

1. Starch can be puffed and made into packing pellets that are 100% biodegradable. These pellets can be used in a variety of projects and experiments. For example, a homemade glue can be made by making a mixture of water and pellets. Wettened pellets can be used like papier-mâché, as a sculpturing material. You could also subject the pellets to different environmental conditions (temperature, light, precipitation) to test biodegradability.
2. Search your local supermarket or health food store for exotic produce. Research the nutritional value, method of preparation, country of origin and other interesting facts for each novel plant. Be adventurous and prepare an entrée, salad, or dessert with these plants that are new to you. Enjoy!

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 13-1 EXERCISES B, C, AND D: STARCH GRAINS, STORAGE ORGANS AND STARCHY STAPLES

Draw a few representative starch grains for each plant examined in lab.

COMMON NAME	STARCH GRAINS

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 13-2 EXERCISES C AND D: STORAGE ORGANS AND STARCHY STAPLES

Identify the storage organ (bulb, corm, rhizome, tuber, taproot, tuberous root) for each plant examined in lab.

COMMON NAME	STORAGE ORGAN
Begonia	
Canna	
Dahlia	
Garlic	
Ginger	
Iris	
Onion	
Parsnip	
Shamrock	
Sweet potato	
Tulip	
Turnip	
Water chestnut	
White potato	
Yam	

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 13-3 EXERCISE E: WHAT ARE YOU EATING?!

COMMON NAME	SCIENTIFIC NAME	PART USED AND USAGE	COMMENTS (TASTE, NUTRITIONAL VALUE, SHAPE OF STARCH GRAINS, ETC.)
Amaranth			
Cherimoya			
Jerusalem artichoke/sunchoke			
Jicama			
Malanga/cocoyam			
Quinoa			
Tomatillo			

LABORATORY TOPIC 14

The Spice of Life

BACKGROUND

The distinctive aromas and tastes of many ethnic foods are due largely to the presence of herbs and spices. However, for thousands of years, herbs and spices have also been used in medicine, perfumes, cosmetics, and as dyes. The **secondary products** in herbs and spices are the components that have made these plants important to society. Secondary products are compounds synthesized by plants but not involved in major metabolic pathways. At one time, these compounds were considered merely waste products of metabolism; however, they are now believed to have diverse functions, including attracting pollinators, discouraging grazing animals, and inhibiting bacterial or fungal pathogens. Secondary compounds are usually categorized by their chemical structure as **alkaloids**, **glycosides**, **essential oils**, or other classes of compounds. In this lab, we will examine herbs and spices and also investigate the properties of the secondary compounds in these plants.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Recognize the major herbs and spices.
2. Identify the parts of herb and spice plants that contain essential oils.
3. Describe the glandular trichomes that contain essential oils.
4. Understand the Scoville scale for rating the "heat" of chili peppers.
5. Describe how compounds are tested for antibiotic properties.

EXERCISE A: Herbs and Spices

There is no real botanical distinction between herbs and spices. Herbs are usually described as aromatic leaves or seeds from plants native to temperate regions, while spices are various parts of plants that are native to tropical areas. Thus, the distinction between herbs and spices is only a geographic one. In common usage, the terms are often interchangeable. We may speak of "spicy" mustard, yet mustard plants are technically herbs native to temperate areas.

We can only speculate as to how and when early societies first began using spices. Historical records from the time of the ancient Egyptian civilization describe many familiar herbs and spices. From the time of the ancient civilizations through the Renaissance, spices were important commodities. Fortunes rose and fell as one country after another tried to dominate the spice-rich islands in the East Indies. The search for quicker routes to these islands drove the Age of Exploration in the fifteenth and sixteenth centuries. Today, spices are still prominently marketed throughout the world.

Many plant families contain herbs and spices, but four families stand out because they contain a large number of herbs. These are the mint family (with spearmint, peppermint, marjoram, oregano, rosemary, sage, basil, and thyme), the parsley family (with parsley, caraway, dill, fennel, celery, anise, coriander, cilantro, cumin, and chervil), the lily family (with onions, garlic, leeks, shallots, and chives), and the mustard family (with black and white mustard and horseradish). In this lab, we will examine many of the herbs and spices known to the ancient world as well as some New World spices.

Materials Needed for Exercise A

Whole spices (e.g., cinnamon, cloves, peppercorns, allspice, nutmeg, mace, saffron, or ginger)

Whole herbs (e.g., oregano, basil, sage, rosemary, parsley, thyme, mustard, cilantro, garlic, onion, horseradish, cumin, or mint)

Procedure for Exercise A

1. Your instructor will have a variety of herbs and spices available in lab. Although you may be familiar with many of these items in powdered form, what is available in the lab is the whole spice or herb. As a result, you may not recognize them right away.
2. Crush a small portion of each herb or spice to smell the aroma. For some of the larger herbs or spices,

your instructor may provide the crushed (or powdered) form along with the whole product. Your instructor will indicate whether tasting is allowed.

3. For each of the spices available, record the name of the herb or spice and the part of the plant it comes from in table 1 on worksheet 14-1 at the end of this laboratory topic.

EXERCISE B: Essential Oils

The characteristic properties of many herbs and spices are largely due to the presence of essential oils, which are volatile substances that contribute to the aroma (or the essence) of certain plants. Chemically, these oils are classified as terpenes, which are unsaturated hydrocarbons with the common building block of $C_{10}H_{16}$. Essential oils impart flavor to foods; in addition, many have medicinal properties. Some essential oils reportedly function as antibiotics, decongestants, stimulants, or pain relievers. Essential oils can be found in various plant organs, but they typically occur in leaves, flowers, or fruits. For commercial applications, the essential oils are extracted by several methods, including steam distillation, expression, and solvent extraction. They may then be used in processed foods and in manufacturing cosmetics, soaps, and candles. The recent interest in **aromatherapy** has greatly increased the use of essential oils, and they can now easily be purchased in many stores. In this lab, we will examine several essential oils and observe the plant structures where they occur.

Materials Needed for Exercise B

5 essential oils, labeled A through E

Blotting paper

Dissecting microscopes

Dropping pipets

Leaves from herbs grown by students (see Laboratory Topic 2) or those available in lab

Procedure for Exercise B

1. For many of the leafy herbs, the essential oils are concentrated in glandular trichomes on the leaf surface. Select leaves from the herbs being grown by the class and examine them with the dissecting microscope. You should be able to see the glandular trichomes. Which surface (upper or lower) has the most trichomes? In the following space, draw several of these trichomes.
2. Rub your fingers over the leaf. Can you smell the essential oil on your fingers?
3. Several essential oils are available in class. These are in small bottles labeled A through E. Place a drop of each essential oil on a separate piece of blotting paper. Smell the aroma. Identify the source of each essential oil. You may need to go back and reexamine the herbs and spices available in lab. Record your answer in table 2 of worksheet 14-1.

EXERCISE C: How Hot Is That Jalapeno?

Probably the most important contribution from the Western Hemisphere to the world's spice racks are the capsicum peppers. These peppers (also called chili peppers) are the fruits of plants in the genus *Capsicum*, which includes several cultivated species and hundreds of varieties. These peppers are unrelated to black pepper (*Piper nigrum*). Among the varieties of capsicum peppers are the mild bell peppers and the fiery habaneros, with dozens of varieties in between. The fiery taste of chilies is due to the presence of a group of alkaloids known as capsaicinoids. The most important of these is **capsaicin**. The capsaicin content of bell peppers is negligible, but it is quite high in jalapenos, cayennes, and habaneros. In 1912, Wilbur Scoville came up with a method of measuring the biting taste of these peppers. This method uses a panel of five tasters who sample extracts of the peppers diluted in sugar water. The greatest dilution, which can still be detected by 3 of the 5 tasters, is considered the heat level. The units used are **Scoville Heat Units**. These range from 0 for bell peppers to 1,000–5,000 for jalapenos to 100,000–300,000 for the hottest habaneros. In modern commerce, the Scoville Heat Units are measured more precisely with HPLC (high pressure liquid chromatography). For this exercise, we will use a modification of Scoville's taste test to measure the "heat" of several varieties of capsicum peppers.

Materials Needed for Exercise C

10 disposable dropping pipets

10 ml of 95% ethanol

10 test tubes with lids

100 ml of 5% sugar solution

2 test tube racks

Chili peppers (e.g., jalapeno, habanero or others)

Mortar and pestle

Vinyl gloves

Procedure for Exercise C

1. Obtain 5 test tubes. Label these 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000. Place them in a test tube rack, and fill each tube with 9 ml of the 5% sugar solution.

2. Select two peppers from those available in lab. Using vinyl gloves, cut off and weigh out 5 g of one of the peppers. Place it in a mortar and add 5 ml of 95% ethanol. Using the pestle, grind for several minutes. Transfer 1 ml of the solution into the tube marked 1:10. Cover and shake well. Transfer 1 ml into the tube marked 1:100. Shake well. Repeat with the remaining tubes. This is a **dilution series**. The solutions in the tube now range from 1:10 to 1:100,000 (fig. 14.1).
3. Place a disposable pipet into each tube.
4. Thoroughly wash the mortar and pestle.
5. Repeat this procedure for the other pepper.
6. Five volunteers from the class are needed to rate the peppers. They should wash their hands before they begin.
7. Place one drop of each solution at the end of each volunteer's index finger. Begin with the 1:100,000 solution. The volunteers now taste the drop. Is the capsaicin distinguishable? Repeat with the 1:10,000 solution, etc.
8. In worksheet 14-2, record the dilution level at which each volunteer first tastes the pepper. The Scoville test requires that 3 out of 5 taste the capsaicin at a particular dilution. If the capsaicin is tasted at the 1:10,000 dilution, the Scoville units for the pepper will be 10,000.
9. The tasters now need to drink some water (or milk), eat an unsalted cracker, and rinse their mouths before proceeding to the taste test for the next pepper. They should also wash their hands to remove any traces of the first pepper.

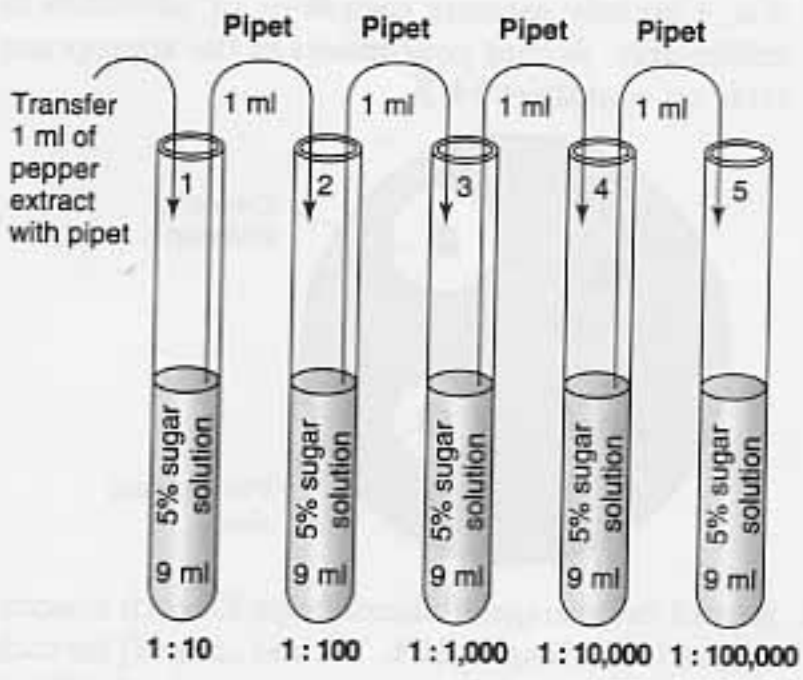


FIGURE 14.1 DILUTION SERIES.

10. Repeat the extraction and test for your second pepper. Be sure to wash the mortar and pestle thoroughly before you test the second pepper.

EXERCISE D: Antibiotic Activity of Secondary Products in Garlic

Many herbs and spices have long been used as medicinal compounds. Garlic may have one of the oldest histories. The *Ebers Papyrus*, a 3,500-year-old Egyptian medical document, contains 22 medicinal uses of garlic. The active molecule in garlic is a sulfur-containing volatile compound known as **allicin**. This compound is not active in the intact clove of garlic; it is released only when garlic is cut. In this exercise, we will examine the reported antibiotic properties of garlic by testing garlic extracts against two widely distributed bacteria. Three concentrations of garlic will be tested to see their effects on bacterial growth. We will also compare the effects of fresh or raw garlic (F) with cooked or roasted garlic (R).

We will be using the filter-paper-disc agar diffusion method. This method will let us determine the effectiveness of the garlic extracts by measuring the diameter of the **zone of inhibition** that results from the diffusion of the extract in the agar medium around the disc. We will begin by soaking small filter paper discs in specific concentrations of garlic and then transferring them to the surface of an agar plate that has been heavily inoculated with the microorganism to be tested. Following incubation, the plates will be examined for the presence of growth inhibition, which will be evident by a clear zone surrounding each disc. The zones of inhibition will be measured and compared to the control.

Materials Needed for Exercise D

12 petri dishes containing nutrient agar
250-ml flask of sterile distilled water
3 or 4 cloves of garlic
6 empty, sterile petri dishes
Beaker containing distilled water
Bottle of sterile filter paper discs
Cultures of *Bacillus subtilis* and *Escherichia coli*
Jar containing 100 ml of 15% bleach
Scalpel, forceps
Sterile mortar and pestle
Sterile pipets and pipetors

Procedure for Exercise D

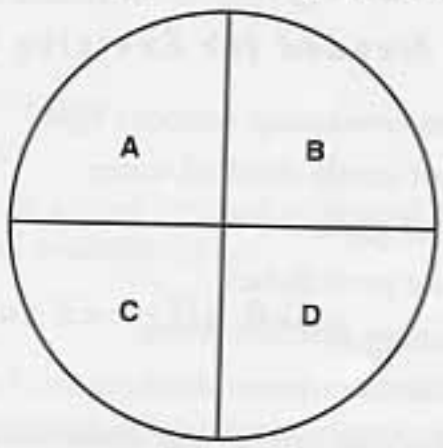
Preparation of garlic extracts

1. Peel 3 or 4 cloves of **fresh garlic** (F). Cut off the attachment scar on the bottom.
2. Weigh out 10 g of garlic. Surface sterilize by dipping in 15 % bleach for 5 seconds and rinsing in distilled water.

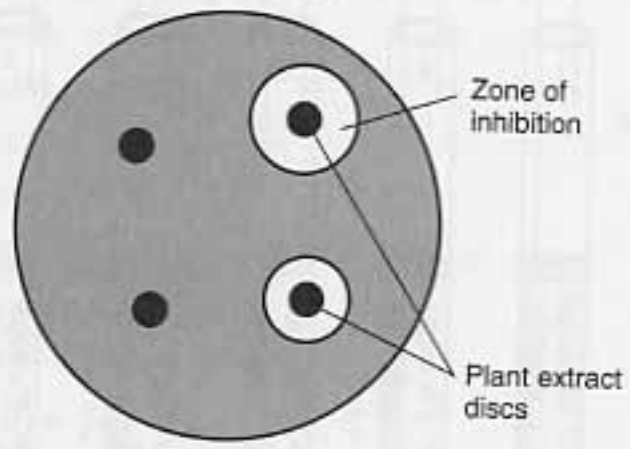
3. Using a sterile mortar and pestle, thoroughly grind the garlic in 10 ml of sterile distilled water. The results should be a pulpy slurry with *no* chunks. Transfer the slurry to an empty petri dish. Label the petri dish **A.** This will be your full-strength sample.
4. Weigh out 1 g of the full-strength slurry, and transfer it to a sterile test tube, and add 9 ml of sterile distilled water. Mix well. Pour this into an empty petri dish. This will be your 10% sample. Label the petri dish **B.**
5. Transfer 1 ml of the 10% sample into a sterile falcon tube and add 9 ml of sterile distilled water. Mix well. Pour this into an empty petri dish. This will be your 1% sample. Label the petri dish **C.**
6. Place 10 ml of sterile distilled water into an empty petri dish. This will be your control. Label the petri dish **D.**
7. Place 6 sterile filter discs in each petri dish. Allow the discs to incubate in the solution for at least 5 minutes before transferring the discs to the culture plates.
8. Repeat steps 1–7 above with **roasted garlic (R)**, but skip step (2)—that is, **DO NOT** dip in bleach or water.

Bacterial cultures

1. Once you have prepared your garlic extract, begin preparing your culture plates.
2. Obtain 12 petri dishes containing nutrient agar. Turn the plates over. With a permanent marker, mark the bottom into quadrants as shown in the following diagram. Label the quadrants **A, B, C,** and **D.**



3. On the lid of the petri dish, label the plates with the names of the bacteria as follows:
   a. Write near the edge of the dish, and also label each dish with your initials.
   b. On 6 plates, write *E. coli (Escherichia coli)*. Then label 3 of these plates **F** and the other 3 **R** (for fresh and roasted).
   c. On the other 6 plates, write *B. subtilis (Bacillus subtilis)*. Then label 3 of these plates **F** and the other 3 **R.**
4. Using sterile technique, inoculate all agar plates with their respective test organism. Watch as your instructor demonstrates the method of inoculation. It is also described here:
   a. Dip a sterile cotton swab into a thoroughly mixed culture. Remove excess inoculum by lightly pressing the swab against the side of the flask.
   b. Using this swab, streak the entire agar surface horizontally, vertically, and around the outer edge of the plate to provide a heavy growth over the entire agar surface.
   c. Allow the culture plate to dry for about 5 minutes *with the lid on.*
   d. Repeat until all plates have been inoculated with the cultures.
   e. Be sure to use a different sterile swab for each organism.
5. Using sterile forceps, apply the filter discs that are soaking in your garlic extracts to the agar surface. Carefully and gently press each disc with the forceps to make sure the disc sticks to the agar surface. Be careful—do not press the discs into the agar.
   a. Apply discs soaking in the full-strength solution to all the quadrants labeled **A.**
   b. Apply discs soaking in 10% solutions to all the quadrants labeled **B.**
   c. Apply discs soaking in 1% solutions to all the quadrants labeled **C.**
   d. Apply discs soaking in control solutions to all the quadrants labeled **D.**
6. Incubate all bacterial plates at 37°C for 20 to 24 hours. Invert these cultures. The cultures should be checked tomorrow morning in case growth is extremely rapid.
7. Following incubation, examine all cultures for the presence or absence of inhibition surrounding each disc. Carefully measure each zone of inhibition in millimeters. Record your results in the appropriate table on worksheet 14-3.



8. Record the average inhibition zone for each concentration (full strength, 10%, 1%, and control) for each organism in the tables on worksheet 14-3. Which concentration was the best? Which were not effective

in inhibiting the bacteria? How did the roasted garlic compare to the fresh?

TERMS TO KNOW

alkaloids 185
allicin 187
aromatherapy 186
capsaicin 186
dilution series 187
essential oils 185
glycosides 185
Scoville Heat Units 186
secondary products 185
zone of inhibition 187

QUESTIONS FOR REVIEW AND DISCUSSION

1. The aromatic flavors of herbs and spices are due to secondary compounds. What is the function of these secondary products in plants?
2. What secondary products are important components of herbs and spices? Where are they found in plants?
3. Distinguish between herbs and spices?
4. What plant families are known to contain many herbs?
5. Speculate on how early societies discovered spices.
6. How can we utilize the antibiotic properties of garlic?

ADDITIONAL RESOURCES

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Swahn, J. O. 1997. *The lore of spices: Their history, nature and uses around the world*, reprint ed. New York: Barnes & Noble Books.

ON THE WEB

The Chile Pepper Institute, New Mexico State University
http://www.chilepepperinstitute.org/
Web page offering information on the history and uses of spices
http://www.theepicentre.com/index.html
Mayo Clinic Web Site article on the health benefits of garlic
http://www.mayohealth.org/mayo/9802/htm/garlic.htm

OTHER ACTIVITIES

1. Repeat the antibiotic activity test with other herbs or spices. Develop a hypothesis about which other herbs or spices might have antibiotic activity.
2. Essential oils can be extracted from herbs with a simple distillation apparatus. Use about 20 to 25 herb leaves with a small amount of water. When heated, the essential oil will evaporate along with water vapor. Because the oil is lighter than water, it will float on the water, and it may be possible to separate the two layers.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 14-1 EXERCISES A AND B: HERBS AND SPICES AND ESSENTIAL OILS

TABLE 1 HERBS AND SPICES

HERB/ SPICE	*PLANT PART*

TABLE 2 ESSENTIAL OILS

ESSENTIAL OIL	*IDENTITY*
A	
B	
C	
D	
E	

NAME _____

DATE _____

LAB SECTION NUMBER _____

WORKSHEET 14-2 EXERCISE C: HOW HOT IS THAT JALAPENO?

Pepper 1: _____

Mark the box with a **Y** if the volunteers taste the capsaicin at the various dilutions.

	1:10	1:100	1:1,000	1:10,000	1:100,000
Volunteer 1					
Volunteer 2					
Volunteer 3					
Volunteer 4					
Volunteer 5					

Scoville units for pepper 1: _____

Pepper 2: _____

Mark the box with a **Y** if the volunteers taste the capsaicin at the various dilutions.

	1:10	1:100	1:1,000	1:10,000	1:100,000
Volunteer 1					
Volunteer 2					
Volunteer 3					
Volunteer 4					
Volunteer 5					

Scoville units for pepper 2: _____

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 14-3 EXERCISE D: ANTIBIOTIC ACTIVITY OF SECONDARY PRODUCTS IN GARLIC

TABLE 1 EFFECTS OF FRESH GARLIC ON *E. COLI*

	Zone of inhibition (in mm)			
	Culture 1	*Culture 2*	*Culture 3*	*Average*
Full strength				
10%				
1%				
Control				

TABLE 2 EFFECTS OF ROASTED GARLIC ON *E. COLI*

	Zone of inhibition (in mm)			
	Culture 1	*Culture 2*	*Culture 3*	*Average*
Full strength				
10%				
1%				
Control				

TABLE 3 EFFECTS OF ROASTED GARLIC ON *B. SUBTILIS*

	Zone of inhibition (in mm)			
	Culture 1	*Culture 2*	*Culture 3*	*Average*
Full strength				
10%				
1%				
Control				

TABLE 4 EFFECTS OF ROASTED GARLIC ON *B. SUBTILIS*

	Zone of inhibition (in mm)			
	Culture 1	*Culture 2*	*Culture 3*	*Average*
Full strength				
10%				
1%				
Control				

LABORATORY TOPIC 15

The Beauty of Wood

BACKGROUND

The forests of the world hold some of our richest treasures. Throughout human history, civilizations have depended on forests as a source of building material, fuel, food, and medicine. Even today, the most commonly used domestic fuel is not petroleum but wood. Globally, 80% of the domestic fuel used for cooking or heating is firewood or charcoal. Unfortunately, our heavy use of wood and fossil fuels may contribute to the greenhouse effect by releasing more carbon dioxide into our atmosphere. At the same time, living, growing forests may help moderate potential global warming by extracting carbon dioxide via photosynthesis and storing the carbon in the wood of intact trees. The balance of carbon in the environment pivots on our management of forests and our use of wood and wood products.

Why has wood played such a significant role in the affairs of humans over the ages? What is it about the composition of wood that makes it such a choice material for constructing buildings or making quality pieces of furniture? What is the function of this special tissue in the life of a tree? Finally, how can we use our knowledge of the growth pattern of trees to precisely date wooden objects from our prehistoric past?

The exercises in this laboratory topic explore wood and the plants that produce wood. First you will examine the cellular structure of wood, and then apply this knowledge to different types of wood. Exercise C introduces the growth pattern of a typical twig or branch, and Exercise D gives you an opportunity to identify many of the common trees on your campus or in your community. The final exercise introduces dendrochronology, a method that uses the annual growth pattern of wood to understand tree biology and to date events and artifacts.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Identify the types of cells found in wood.
2. Understand wood anatomy well enough to identify types of woods.
3. Describe how the growth of a twig is related to cell and tissue types.
4. Identify common trees and connect the specific tree characteristics with tissue types.
5. Use dendrochronological techniques to connect patterns of growth to meteorological data.

EXERCISE A: The Composition of Wood

The growth of plants is **indeterminate**—that is, plants continue to grow throughout their lives. Indeterminate growth has allowed some plants to reach enormous sizes and very old ages. For example, General Sherman, a giant sequoia (*Sequoiadendron giganteum*) in California, is the largest living tree, with close to 1.8 million kilograms (2,000 tons) just in its aboveground parts. The oldest recorded tree, called the Eon Tree, a coastal redwood (*Sequoia sempervirens*), was at least 6,200 years old when it fell in 1977. In contrast, most animals exhibit **determinate growth**. In other words, once an animal reaches maturity, it no longer grows. Animals reach a fixed size that is determined by their genes interacting with environmental conditions. The largest known animal was a female blue whale (*Balaenoptera musculus*), which tipped the scales at 170,000 kilograms (190 tons).

Plants grow and add new cells to their tissues in regions called **meristems**. Growth in length is controlled by **primary meristems** located in the tips of stems and roots. The tissues derived from the primary meristems were covered in Laboratory Topics 3 and 4. The growth in the girth or diameter of stems and roots is controlled by **secondary meristems**. Secondary meristems are formed later in the development of a stem or root, and they occur along the entire axis of the stem or root, rather than just in the tips. In a cross section of a stem, the two secondary meristems, the **vascular cambium** and the **cork cambium**, appear as circles of dividing cells parallel to the surface. In the first year of secondary growth, the vascular cambium starts as a ring between the primary xylem and primary phloem of the vascular bundles and the adjoining cortical cells (fig. 15.1). When the cells of

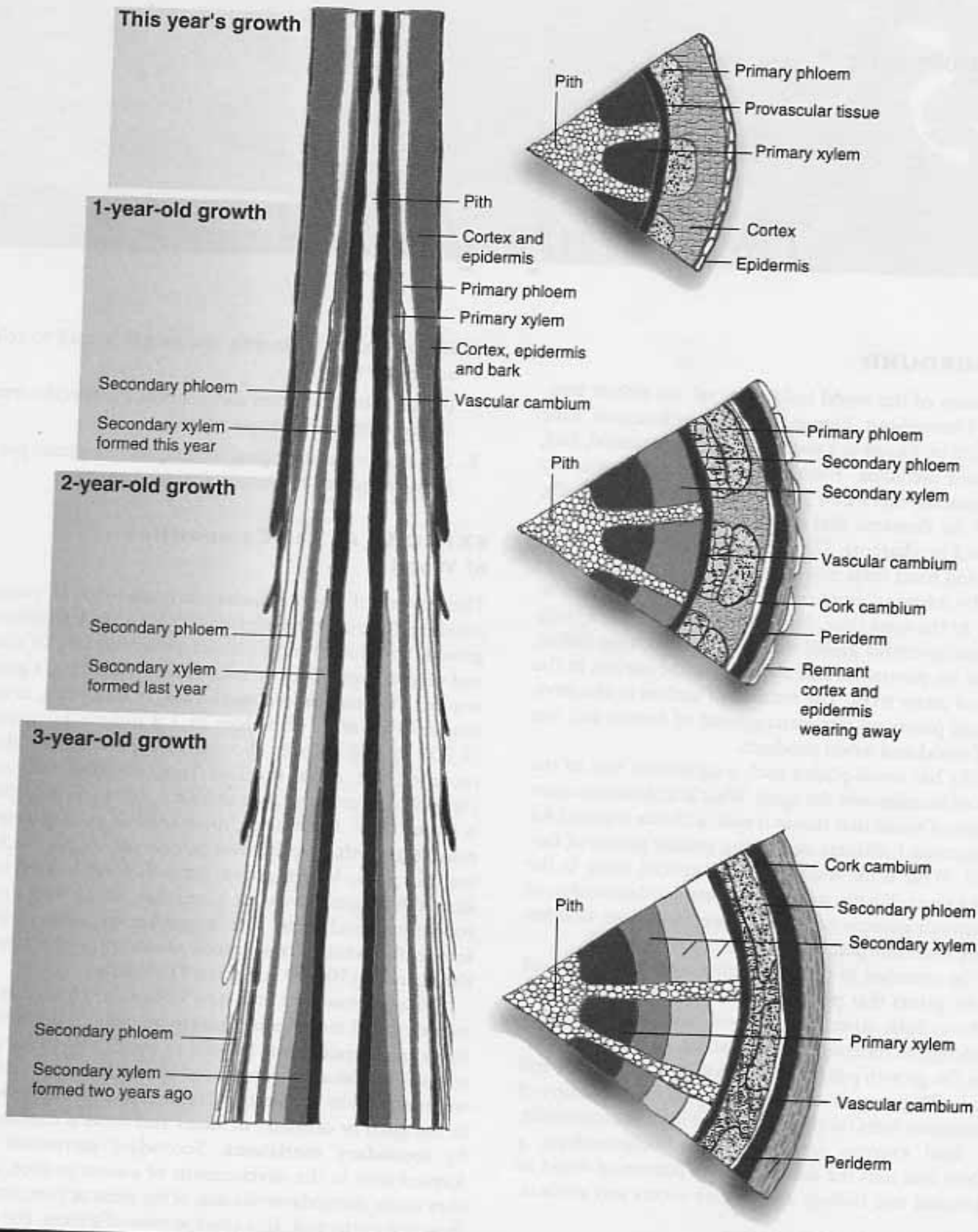


FIGURE 15.1 SECONDARY GROWTH OF A STEM.

the vascular cambium divide, **secondary xylem** cells are produced to the inside and **secondary phloem** to the outside. Each year, a ring of new secondary xylem and secondary phloem is produced. The cork cambium forms outside the vascular cambium and likewise produces new tissue to the inside (**phelloderm**) and new tissue to the outside (**cork**). In many trees, it is difficult to differentiate phelloderm, cork cambium, and cork, so all three tissues are collectively called **periderm**. The periderm replaces the epidermis in the secondary plant body as the outer protective layer that restricts the loss of water and controls the exchange of gases. **Bark** is composed of the periderm and all the layers of secondary phloem—or all the tissue outside the vascular cambium.

Materials Needed for Exercise A

Macerated tissue of hardwood

Prepared slides of 3-year-old *Tilia* stems, transverse section

Prepared slides of 3-year-old *Pinus* stems, transverse section

Prepared slides of oak (*Quercus*) wood, transverse section, radial section, and tangential section

Procedure for Exercise A

Only trees and shrubs exhibit secondary growth and produce wood. Coniferous trees, often called **softwoods,** are native to temperate regions of the world. **Hardwoods** are members of the Class Dicotyledones and reproduce with flowers instead of cones. In temperate regions, hardwoods are often deciduous trees that lose their leaves for winter. The flowering trees of the tropics are also hardwoods, but they tend to keep their leaves all year round like the conifers. The terms "softwood" and "hardwood" imply a difference in the density of the wood. Although most coniferous woods are "softer" than many "hardwoods," this is not always the case. Despite the overlap, it is worth examining the two types of wood because they have anatomical differences between the wood types.

1. **Cell types in wood (prepared slide).** Examine a slide of macerated hardwood tissue with a microscope. To prepare this slide, wood was treated to separate the cells and then stained. Look at the diversity of cells on the slide. What do you notice? What cells are similar? What cells are distinct?

_____

_____

_____

_____

Many of the cells are long, slender, and tapered at both ends. Most of these are **fiber** cells. Fibers consist primarily of cell wall. The cell wall is composed mainly of **cellulose** interwoven with **lignin**. Fibers provide structural support for the stem and are found not only in wood but in other plant tissues. They act much as steel rods do to reinforce concrete. When functional, these cells are dead and have lost all their inner contents.

As noted in Laboratory Topic 3, most of the cells in paper pulp are fibers. You may have noticed some of the cells were broken or frayed at the edges. When we recycle paper, we reuse the fibers. In the processing, many of the fibers break and become shorter, so there is a practical limit to how many times paper can be recycled. Usually some new virgin pulp is added with recycled fibers to make the final product.

Another long, narrow plant cell is a **tracheid**. These are similar to fibers but not quite as long, and they usually do not break in the maceration process. The tracheids have pits in the side walls that allow water to pass from one cell to the next. Like fibers, they are dead when functional and essentially composed entirely of cell wall.

Vessel elements are shorter, wider, and shaped like a barrel. The top and the bottom have large openings so when vessel elements are connected end to end in the stem, they form long tubes called vessels. These cells are the primary water-conducting cells in flowering plants. In contrast, conifers have only tracheids and no vessel elements.

The final cell type you should find is a thin-walled, short cell. This is a **parenchyma cell** of a **xylem ray**. Usually you can observe contents within the cell since when functional these cells are alive and retain their organelles and cytoplasm.

Identify each type of cell and sketch the cells in the following spaces.

Fiber cell	Tracheid
Vessel element	Ray parenchyma

2. **Transverse section of basswood (*Tilia*).** With a microscope, examine a prepared slide of a transverse section of a 3-year-old stem of *Tilia* and compare it with figure 15.2. In the very center of the stem, you

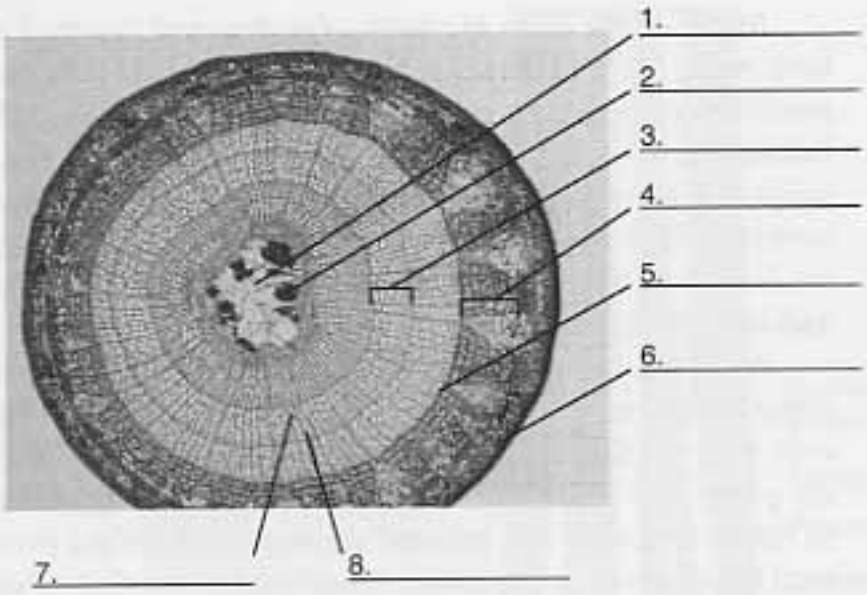


FIGURE 15.2 CROSS SECTION OF A WOODY, 3-YEAR-OLD BASSWOOD (*TILIA*) STEM.

will see some of the remnants of the primary tissue. The thin-walled, large cells in the center are parenchyma of the **pith**. The pink-staining cells clustered near the outer boundary of the pith are part of the **primary xylem**. Three or more concentric layers of **secondary xylem** lie just outside the pith and primary xylem. Secondary xylem is **wood**. In temperate regions such as the United States, one ring of tissue is formed each year. Which ring do you think is the oldest—the innermost or the outermost? The cells in the annual rings have different diameters at different seasons. In spring, the cells are relatively large because they have plenty of nutrients and are growing fast. The tissue in this region of the ring is called **earlywood** or **springwood**. Toward the end of the growing season, late summer or fall, the cells are not growing as fast, and more material is deposited in the cell walls. The cells have smaller lumens, or openings. The resulting tissue is denser and called **latewood** or **summerwood**. In *Tilia*, you can easily see when all cell division stopped for one year and started again the following spring; it is a clear, circular ring.

In many types of wood, including *Tilia*, rays of parenchyma cells form lines along the radii of the stem. These xylem rays appear as stripes running perpendicular to the concentric growth rings. The cells in the rays are thin-walled parenchyma cells. Since they are alive, carbohydrates and minerals must be transferred laterally from the phloem. The nutrients are transferred from cell to cell. In older wood, the innermost parenchyma cells of rays balloon into adjacent vessel elements, forming structures called **tyloses**. The tyloses help block the vessels of the older, inner growth rings and thus prevent many fungi from easily growing up through the open vessels. As the cells become tyloses, they release many phenolic compounds. The phenolics have antifungal properties that can provide another means of containing and controlling fungal infections.

At the outer edge of the secondary xylem (wood) lies the **vascular cambium**, a ring one to two cells in thickness. The vascular cambium is the meristem that divides to produce secondary xylem to the inside and secondary phloem to the outside. The cells of the vascular cambium are usually thin-walled and relatively small in diameter in cross section. Sometimes they are hard to see, but look for them at the outer edge of the xylem.

Immediately outside the vascular cambium is the secondary phloem. This tissue conducts carbohydrates and minerals throughout a tree. In summer and fall, the carbohydrates are moving from the photosynthesizing leaves to all the growing portions of the plant, including the roots. In spring, some of the stored carbohydrates in the roots move up to the growing buds of the stems.

The secondary phloem is identified by the alternating triangular-shaped sections of darker staining cells. The triangles that point toward the center of the stem are the phloem rays. Like the rays in the xylem, the phloem rays consist primarily of parenchyma cells. Alternating with these rays are wedges pointing outward. This region of the phloem contains the **sieve-tube elements**, which are the primary carbohydrate-conducting cells.

Outside the phloem lie the remaining tissues. Although there are several tissues in this outer region, it is difficult to distinguish them, especially in *Tilia*. Collectively, we can refer to all these tissues as the periderm. The bark is everything outside the vascular cambium, so it includes both the periderm and the secondary phloem.

Find all the tissues in the slide, and label them on figure 15.2.

3. **Transverse section of pine (*Pinus*).** Examine a prepared slide of a transverse section of a 3-year-old stem of *Pinus*. Before we go into depth, what do you notice that is different from the *Tilia*?

_____

_____

_____

_____

Pinus is typical of many conifers. If you look closely, *Pinus* does not have any vessel elements, only tracheids in the secondary xylem (wood). You should see some large cavities scattered within the secondary xylem. These cavities are **resin ducts,** or resin canals. The resin ducts are lined with parenchyma cells that secrete an oleoresin. We make turpentine and resin

from this oleoresin. For the tree, it probably works as an insect repellent or antifungal agent.

You can see the difference between earlywood and latewood by comparing the average diameters of the tracheids. In *Pinus*, the gradation between earlywood and latewood is more gradual throughout the growing season. Annual growth rings become evident, however, when growth stops. Although we typically see only one growth ring per year, occasionally a double ring may occur. Many trees of the tropics that grow all year long show little or no growth-ring pattern.

Pinus also produces rays in the xylem and phloem. How is *Pinus* similar to and different from *Tilia*?

Like *Tilia*, *Pinus* has a vascular cambium, secondary phloem, and periderm, and may still have a visible pith. Find all these tissues in the slide, label them on figure 15.3.

4. **Three-dimensional structure of oak (*Quercus*).** Examine a prepared slide of oak (*Quercus*) wood showing three faces of a three-dimensional block of the wood: transverse section, radial section, and tangential section (fig. 15.4). The transverse section is the same plane you observed in *Tilia* and *Pinus*. If the tree were standing, it would correspond to a horizontal cut. The radial surface would be a vertical cut parallel to the radius of the tree. The tangential surface would also be vertical, yet perpendicular to the radius and almost parallel to the outer surface of the stem. All three thin sections were made within the secondary xylem, so you are only looking at cells within wood (usually no bark or pith).

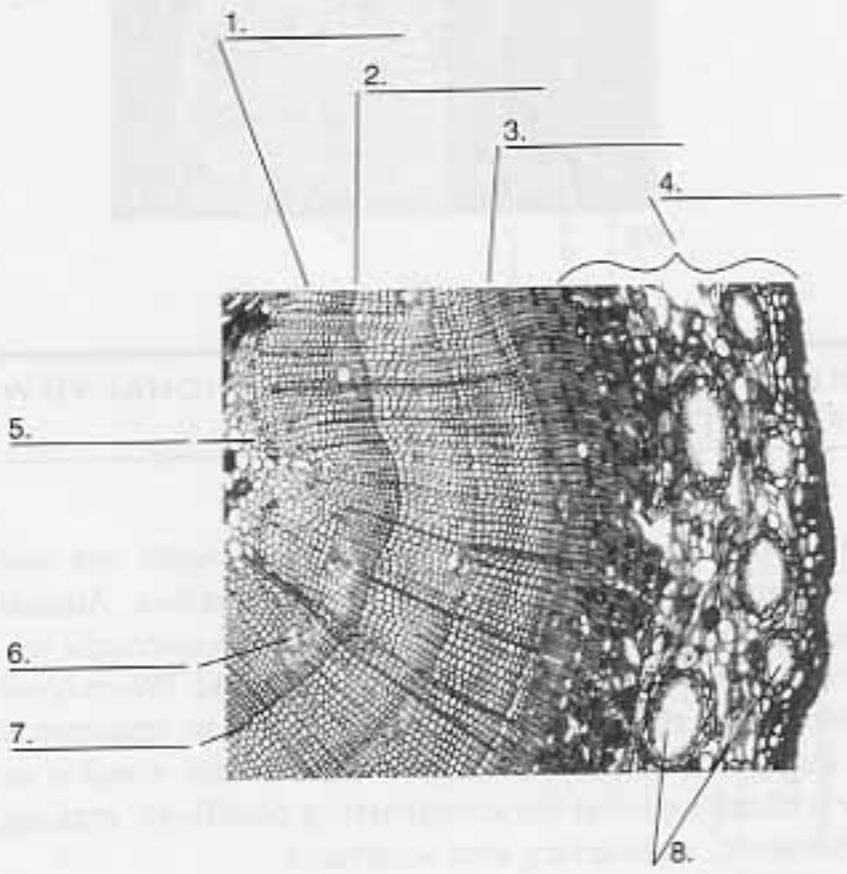


FIGURE 15.3 CROSS SECTION OF A WOODY, 3-YEAR-OLD PINE (PINUS) STEM.

If you start with the **transverse section**, you should recognize similarities with both *Tilia* and *Pinus*. You should see at least a portion of a growth ring and be able to distinguish earlywood from latewood. In *Quercus*, the vessels are more abundant in the earlywood, and the latewood is primarily all fibers. The high number of fibers helps make oak a relatively dense or "harder" wood. The vessels give the wood a porous appearance. When vessels are arranged as in oak (i.e., all the vessels clustered in the earlywood), the wood is referred to as **ring porous**. When vessels are scattered across the entire growth ring, both earlywood and latewood, the wood is referred to as **diffuse porous**. Do you think *Tilia* is classified as ring porous or diffuse porous? Why?

In the **radial section**, the vessels appear wide and open. The stem, and thus the vessels, are cut lengthwise. The cut was made down the radius of the stem, so you should see both the earlywood and the latewood of at least one growth ring. What are the long, narrow cells tapered at both ends? Where are the vessels more abundant? Where are the fibers more abundant?

While still looking at the radial view, what do you suppose are the bands of cells running across the vessels and fibers? How can you be more certain? What is the shape of these cells?

The **tangential section** is also cut lengthwise, so the vessels and fibers look similar to those seen in the radial view. The xylem rays are cut perpendicular, so they appear

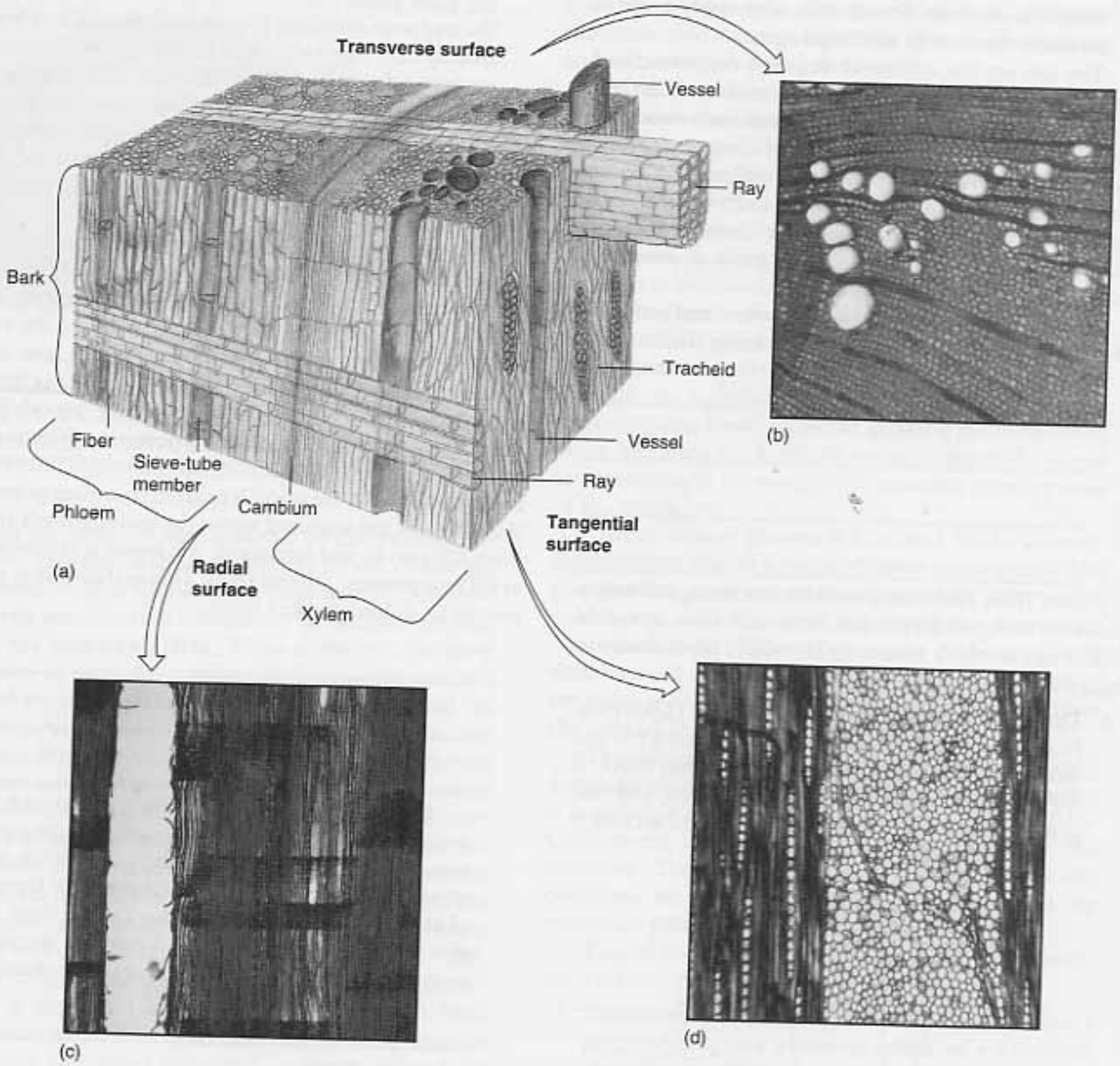


FIGURE 15.4 THREE DIMENSIONS OF WHITE OAK (QUERCUS) WOOD. (A) THREE-DIMENSIONAL VIEW. (B) TRANSVERSE SECTION. (C) RADIAL SECTION. (D) TANGENTIAL SECTION.

as a band of cells that collectively have a cigar shape. Some rays may be several cells thick, while others are narrow. Xylem rays are one of the diagnostic features used to distinguish types of wood.

When you can recognize vessels, fibers and parenchyma ray cells, you can use your knowledge to distinguish different types of wood as explained in the next exercise.

EXERCISE B: Common Types of Wood

The beauty and strength of wood lies in the orientation of its cells and tissues. The vessels, tracheids, and fibers are oriented along the long axis, while the rays of parenchyma are oriented along the radius. Annual growth rings are composed of alternating concentric layers of softer earlywood and denser latewood. When combined, all these cells help make wood an immensely strong material for its weight. That is why wood is an excellent material for constructing buildings, making furniture, and carving into sculptures.

Woods vary in texture, color, growth pattern, smell, feel, and even taste. The appearance of a cut surface can be described as rough, smooth, dull, lustrous,

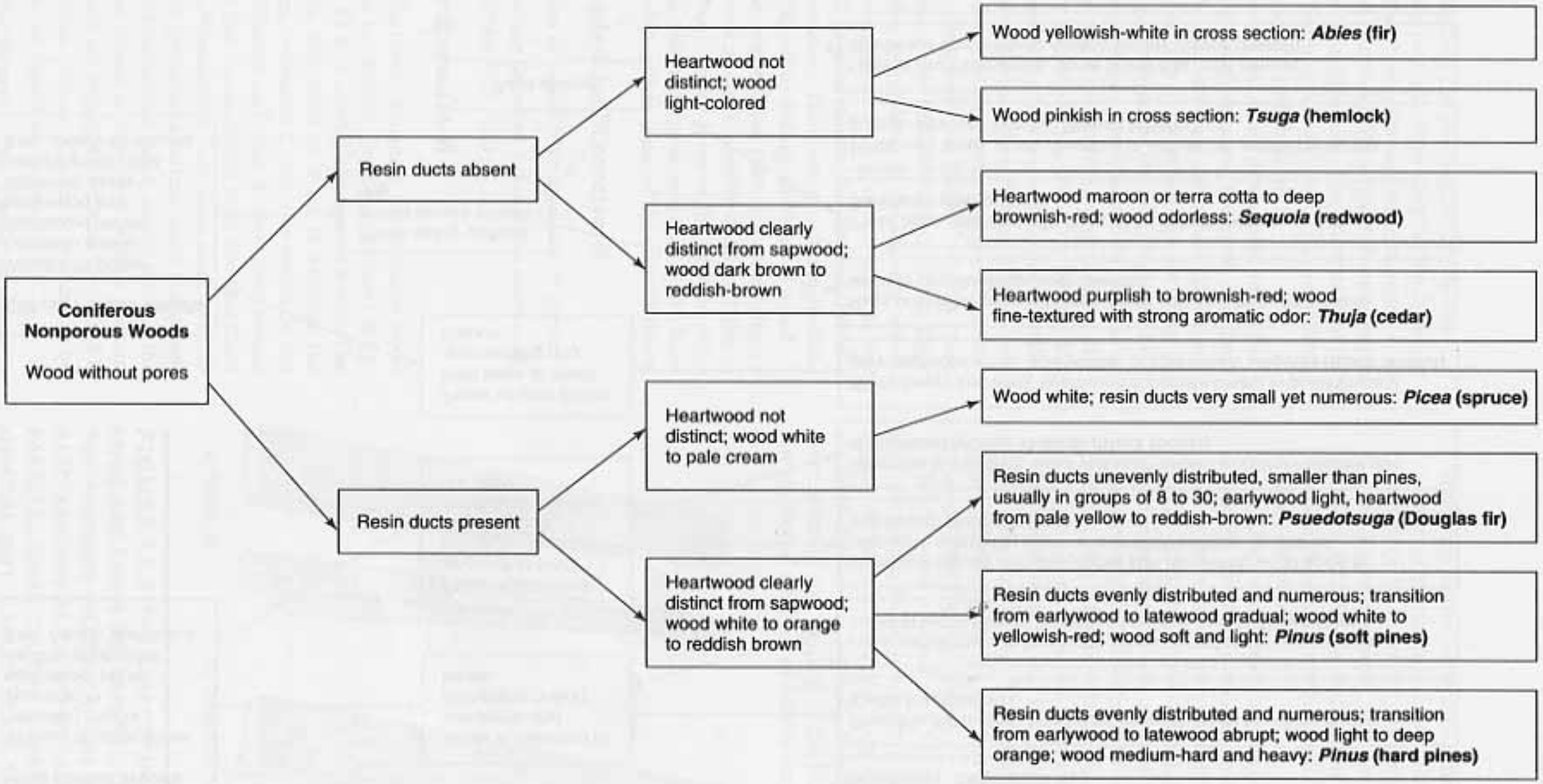


FIGURE 15.5 FLOWCHART FOR CONIFEROUS WOODS.

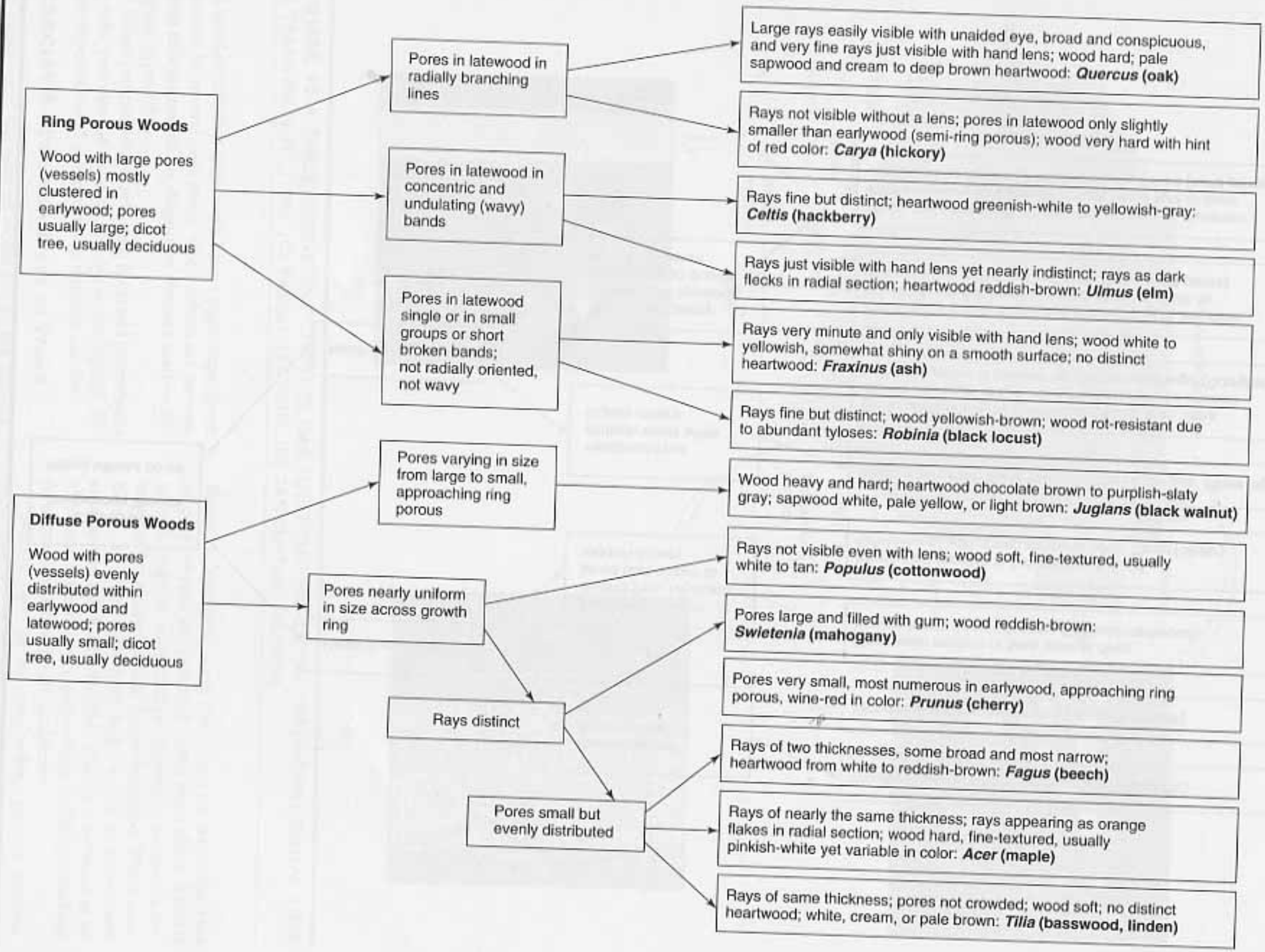


FIGURE 15.6 FLOWCHART FOR DICOT WOODS.

or greasy. When milled, the orientation of the saw cut yields different grain patterns depending on the direction of the fibers and the three-dimensional configuration of the earlywood and latewood. We exploit these patterns when selecting wood for furniture. The orientation of the saw cut can also affect how a board responds to the forces of compression, tension, and shearing.

Each year a tree grows, it lays down more wood (secondary xylem). The vessel elements, tracheids, and fibers are dead during the first year they are produced. In contrast, the parenchyma cells in the xylem rays are living. As the years pass, the parenchyma in the inner regions of the wood die. This inner core, the **heartwood**, and is no longer living tissue, yet it continues to give the tree support against gravity and wind. The wood on the outside, the **sapwood**, maintains the function of water conduction. As sapwood becomes heartwood, darkening often occurs. The dark color is due to deposition of various chemicals, including tannins, dyes, and oils. These chemicals come from the dead parenchyma cells of the rays. Often these parenchyma cells also form tyloses in the adjacent vessels. The change from sapwood to heartwood adds to the beauty of the wood. In some species, the sapwood does not transform into heartwood. Thus, we can use the presence or absence of heartwood to distinguish types of wood.

In this exercise, you will use your new knowledge of the cells and tissues of wood to identify different types of wood. You will be able to see most of the characteristics without any magnification. At most, you may need a hand lens or dissecting scope to see some of the characteristics.

Materials Needed for Exercise B

Small blocks or discs of wood from a variety of species

At least one cut surface showing a transverse section of stem (cut across the growth rings)

Procedure for Exercise B

1. **Wood identification.** Examine the blocks of wood provided in the laboratory. Use the flowcharts in figures 15.5 and 15.6 to identify the type of wood. The first distinction you need to make is whether the wood has **pores,** or **vessels**. Coniferous woods do not have vessel elements and thus do not have vessels (pores). They can be identified with the flowchart in figure 15.5. Woods from flowering, dicot trees do have vessels. As mentioned earlier, when the vessels are arranged in a concentric band, usually more in the earlywood, the wood is classified as ring porous. When the vessels are evenly distributed, the wood is classified as diffuse porous. The dicot woods can be identified with the flowchart in figure 15.6.
2. **The attractive figure.** The **figure** of wood is defined as any feature or pattern in wood that enhances its beauty. It includes the ornamental markings on the surface of timber produced by the relative arrangement of the different elements of the timber or by inherent coloring. Sometimes figure is used synonymously with the term "grain," but grain is actually the orientation of the tracheids and vessels. Grain, however, contributes to the overall figure of wood.

   Examine different cuts of wood or blocks of wood showing the three planes of cut as in fig. 15.7. When the wood is sawed with a radial cut, the plane of the cut is along the radius of the log. The growth rings appear as more or less continuous vertical lines and fairly evenly spaced if the tree had regular growth. In reality, only a few boards can be cut along the radius. To get the most boards from a log, the mill cuts the log tangentially so that the plane is at right angles to the radius of the log. The cutting plane intersects the growth rings in such a way that they look like parabolas or portions of parabolas.

   When wood is sawed with a transverse cut, the plane is perpendicular to the long axis of the log. Growth rings appear as concentric circles. Rays appear as lines radiating from the center. Typically, this type of cut promotes more splitting and warping of the board, so mills do not saw the broad surfaces of boards transversely.

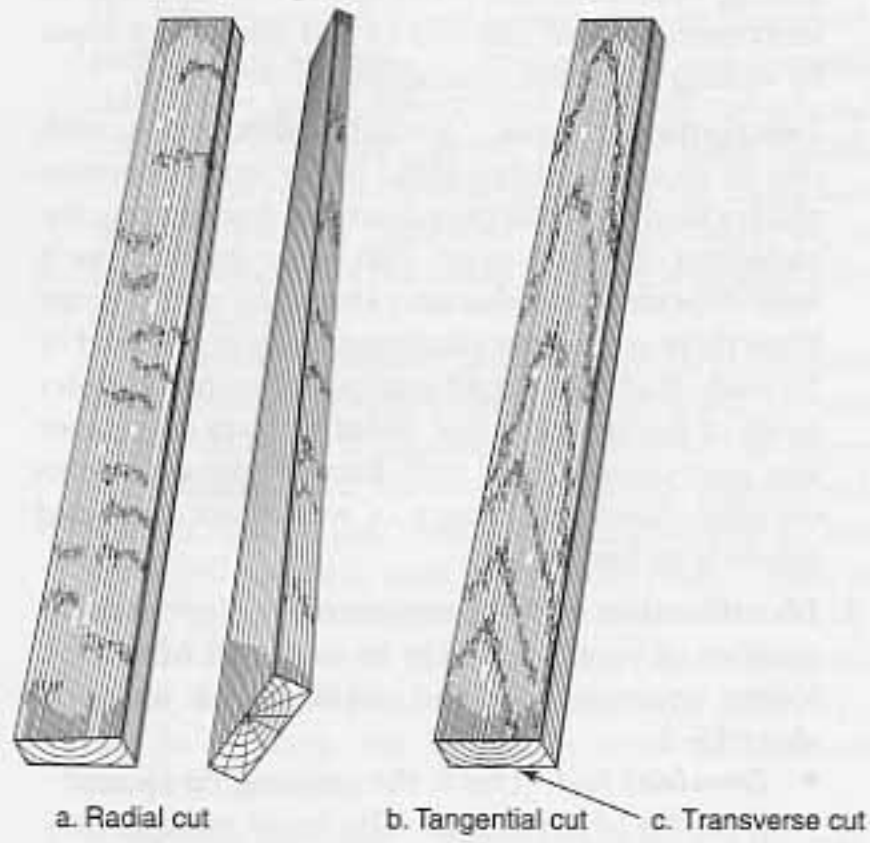


FIGURE 15.7 THE DIRECTION IN WHICH THE LOGS ARE SAWN DETERMINES THE PATTERNS ON THE FINISHED WOOD. (A) THE RADIAL CUT, ALSO KNOWN AS QUARTER-SAWN, PRODUCES PARALLEL LINES ON THE BOARD. (B) THE TANGENTIAL CUT, CALLED PLAIN-SAWN, PRODUCES WAVY BANDS. THE TRANSVERSE CUT (C) IS THE END OF EACH BOARD.

EXERCISE C: Twig Morphology

The objective of this exercise is to learn the external characteristics of woody twigs. Most of the characters can be seen with the unaided eye, but you may want to use a hand lens or dissecting scope to view the smaller features.

Materials Needed for Exercise C

Hand clippers

Hand lens or dissecting scope

Sharp knife or razor blade

Woody twigs from trees

Procedure for Exercise C

1. **Twig description.** Take a walk outside, and collect a sample of twigs from various deciduous trees. After you have 5 or 6 twigs from different species, lay them out and observe their characteristics. What features do they have in common? What distinctive characters are found on some of the twigs but not others? Select one of the twigs and describe it as thoroughly as you can. At this point, use your own words and don't worry about the precise terminology. Your job is to describe the twig so accurately and precisely that someone else can identify it from among your collection. Complete the first half of worksheet 15-1 at the end of this laboratory topic by writing a detailed description of your twig.
2. **Description critique.** Exchange descriptions with one of your classmates. Read his or her description and try to identify the twig described from among the collection of 5 or 6 twigs. Did he or she describe it well? Was there one character that easily gave it away? Were there any unique characters, such as a wound or blemish, that you would not expect to be on other twigs of the same species? What features do you see that your classmate did not? Record your comments on your classmate's copy of worksheet 15-1 and return it to him or her.
3. **Identification of twig structures.** Now examine another of your twigs. Try to locate all of the following structures then find and label them on worksheet 15-2.
   - ***Terminal bud:*** This is the growing tip located at the tip of the branch. The apical meristem is in the center of the bud. Normally when this bud grows, the entire branch is extended. What type of growth would you expect, primary growth or secondary growth?
   - ***Axillary bud:*** At the axis of each leaf is another bud. If no leaves are present, the axillary bud is immediately above the scar left on the twig when the leaf dropped. In many trees, the growth of an axillary bud is inhibited by hormones produced in the terminal bud. What do you suppose would happen if the terminal buds on a live branch were removed?
   - ***Bud scales:*** Covering either of the buds there may be one to many scale-like leaves that protect the bud. Note the texture and color of the bud scales.
   - ***Leaf scar:*** If you collected your twig in late autumn, winter, or before new growth in the spring, you will notice scars left on the twig where a leaf was once attached. If you have a twig with leaves, carefully remove a leaf. What is the shape of the scar? How big is the scar compared to the diameter of the twig? How are the scars (or leaves) arranged on the twigs? Are the scars opposite each other, whorled, or alternating down the twig?
   - ***Vascular bundle scars:*** Within the leaf scar you may see small "spots." These spots correspond to the vascular bundles (veins) that ran from the stem into the leaves. What is the pattern of the vascular bundle scars? How many do you see?
   - ***Bud scale scars:*** These are thin scars left behind from the bud scales of terminal buds of the previous years' growth. Usually they form a cluster encircling the stem. Many trees produce a terminal bud at the end of the growing season that remains dormant through the winter. When the tree expands in spring, the terminal bud scales are dropped, leaving the scars behind. You can determine how far the twig grew during the last year by measuring the distance between clusters of bud scales.
   - ***Lenticels:*** On the bark of the twig, you may see raised, rounded or linear slitlike markings. These are lenticels, raised areas of loose cork that provide regions for gas exchange. They function the same as stomates in younger tissue. Does your twig have lenticels? What is the shape of the lenticels?
   - ***Nodes and internodes:*** The region of the twig where leaves are attached is a **node**. The region of the twig between two nodes is called the **internode**. If we were to make a thin cross section of the stem at the node and compare it with the internode, we would notice that the internal arrangement of tissues, especially vascular bundles, is different. This is because in the node some of the vascular bundles travel out to the leaves. Compare the lengths of the internodes along one growing season. Was growth greater at the beginning, middle, or end of the growing season? Does it look consistent from year to year?

- ***Thorns, spines, and prickles:*** All these structures are sharp projections attached to the twigs. Thorns are modified stems. Spines are either modified leaves or stipules (extensions at the base of leaves). Prickles are merely epidermal or cortical outgrowths, as in roses. Does your twig have any of these structures?

EXERCISE D: What Is The Name Of That Tree

The objective of this exercise is to learn the characteristics of trees while using a dichotomous key. Laboratory Topic 4 provides a dichotomous key using the leaves of trees. In this exercise, we will concentrate on the characteristics of trees during the winter. You should use both keys to identify the trees on your campus and in your community.

Materials Needed for Exercise D

Trees around campus

Dichotomous keys in this laboratory and in Laboratory Topic 4

Procedure for Exercise D

Take another walk around campus or around your community. Now that you know what features to look for and the terms used by botanists, use the dichotomous key in table 15.1 to identify the trees you see.

The key consists of pairs of choices (each pair has the same line number). Compare your plant specimen with the description at step 1, and choose which direction to go. After you make a choice, your next decision step is at the end of the dotted line to the right. If you reach a step that identifies your specimen, the name of the tree is given. For some trees, this name is the species. For others, it is the name of the genus that combines related species. If you want to know more about these trees, consult additional resources.

The key concentrates on some of the common coniferous and deciduous trees of North America. Keep in mind that you may encounter horticultural varieties or non–native trees that are not included in this key.

EXERCISE E: Dendrochronology–Reading The Record Of Time

The objective of this exercise is to use the pattern of growth rings to understand the history of a tree. The process of **dendrochronology**, or tree-ring dating, uses the perennial growth of woody plants as integrators of all the factors (such as rainfall, fire, and competition) that affect growth. You will use the same techniques used by researchers to accurately date archaeological buildings or to understand prehistoric cycles of drought.

For this exercise, you will compare the growth of a stand of trees with meteorological data. Each member of the class will analyze the tree rings of separate trees. Then the data of the class will be pooled to look for overall trends in growth for a region or grove of trees. Finally, you will connect the tree-ring analysis with independent meteorological records to detect possible explanations for the growth patterns.

Materials Needed for Exercise E

Graph paper (square mm)

Hand lens or dissecting microscopes

Increment tree borer

Meteorological data

Metric rulers (at least mm rule)

Plastic drinking straws

Razor blades

Rubber bands

Slotted supporting blocks for mounting cores

Trees

Wood glue

Procedure for Exercise E

1. **Coring a tree.** Use an increment borer to extract a core from the center of a tree. Avoid any areas of the tree where the growth rings may be distorted, such as near branches or on the uphill and downhill sides of the trunk. Direct the borer level and toward the center of the tree. Firmly press the borer against the bark at right angles to the axis of the trunk and turn the handles clockwise. Once the borer tip is firmly anchored in the wood, pressure is needed only on the handles.

   You may encounter pockets of pitch or decayed areas. If the borer enters a pitch pocket, it will become harder to turn. If this happens, remove the borer immediately or else the borer will become clogged and hard to clean. If you encounter a decayed region, the borer will suddenly turn much more easily. Again, it is best to remove the borer.

   Be careful with the tip of the borer. It needs to be kept sharp to work effectively. Also, try not to damage the tip, since it is difficult to sharpen correctly.

2. **Removing the core.** Turn the borer into the tree to a depth of penetration sufficient to include the pith, or as far as reasonable given the size of your borer. Insert the extractor spoon that comes with the borer into the borer from the handle end. When the extractor is inserted to its full length, give the borer a full turn counterclockwise to break the core free. The extractor spoon can be removed, hopefully with the core intact. Remove the borer by turning counterclockwise.

3. **Handling the core.** The core is fragile and needs to be handled carefully. Place the core in a plastic drinking straw. Sometimes it helps to slit the straw along one side in advance and then fold the straw around the core. Corrugated cardboard can also be used to secure the core until you return to the lab.

   Be sure to jot down notes about the tree sampled, location, slope, altitude, soil, associated species, and any physical characteristics of the tree worth noting.

4. **Mounting the core.** If the wood is wet, let it dry at room temperature for a few days. Once dry, carefully mount the core to a supporting block of wood with a groove notched down the center. Orient the core so you can look into the cells. Glue the core to the slotted block. Wrap rubber bands around the core and block while the glue dries. Put the most recent growth rings on the right (this is the end with the bark), and mark the area with a marker. Common convention in tree-ring analysis is to place the most recent wood on the right.

5. **Surfacing the core.** Once the glue is set, remove the rubber bands. Cut a smooth surface on the core with a razor blade held at a low angle. You want to shave off the top surface so it is easier to see the growth rings. If the core was mounted at the correct orientation, you should now be cutting the vessels and tracheids in cross section. If the core is too difficult to cut, you can use sandpaper to prepare a smooth surface.

6. **Tree ring measurement and recording data—Skeleton Plot Method.** The simplest and most common method for recording patterns in the growth rings is called the **skeleton plot**. It has the advantage of being relatively fast, but it does require a little practice to be consistent in recording the results.

   Take a 2 × 10-inch strip of graph paper (1 square mm grid). Place the strip of paper lengthwise in the horizontal position. Write zero on the extreme left end of the rule. Place a hash mark every 10 squares to the right. Each vertical line corresponds to one year, or one growth ring. Every hash mark is a decade. The innermost ring on the core is plotted at zero, and the plotting progresses from this point to the right (or to the outside of the core).

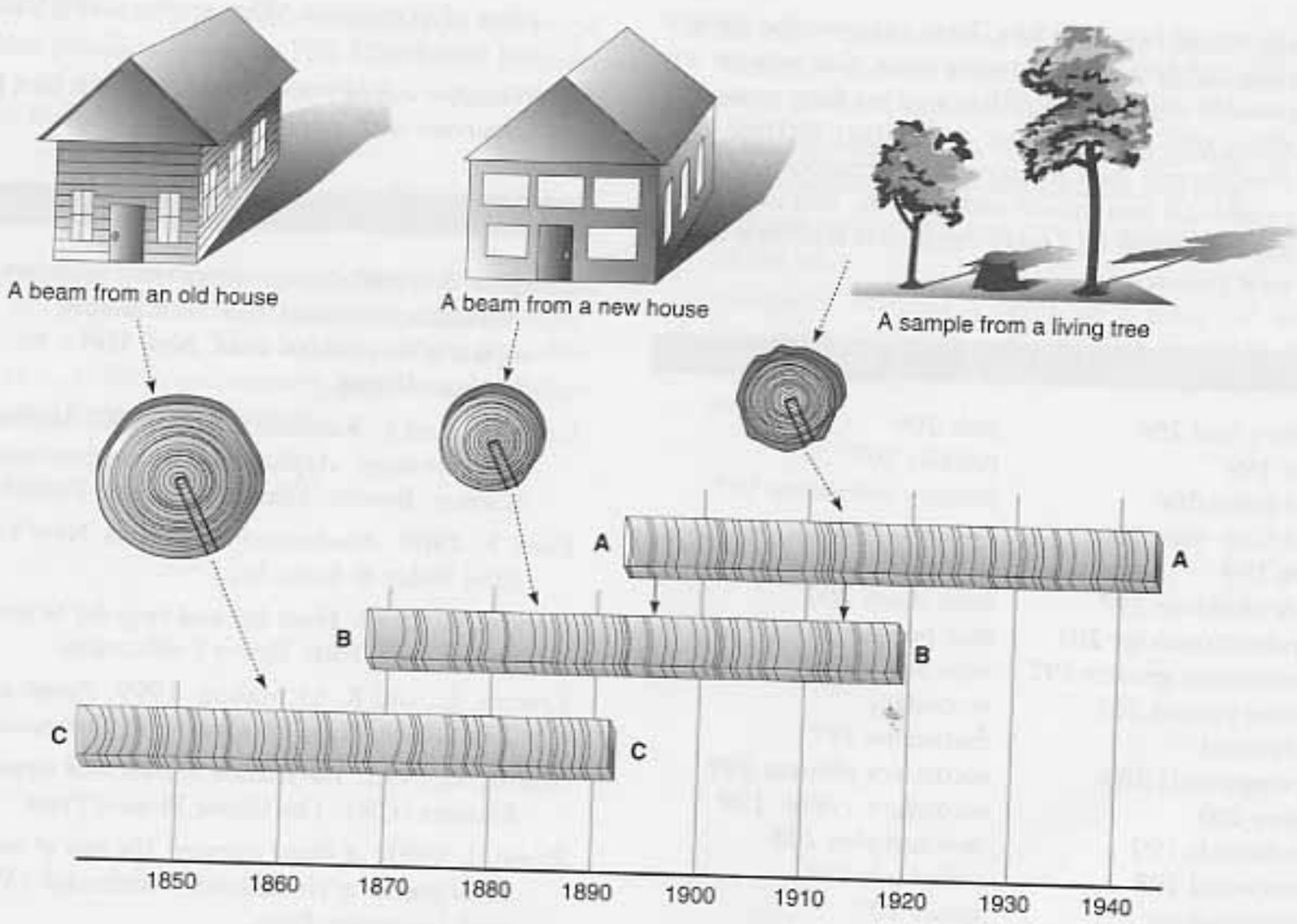
   For the skeleton plot, the narrow rings are of most interest. Mark a line on your plot corresponding to the narrow years. The height of your line should correspond to the narrowness of the growth ring compared to its neighbors (fig. 15.8). In other words, the narrowest growth ring gets the highest line on your skeleton plot. By convention, the narrowest ring gets a line 2 cm in height. Rings of average width are not marked. Rings with widths between the narrowest and the average are given a height relative to their degree of narrowness. Wide rings are marked by a "B."

7. **Creating a composite skeleton plot.** After the skeleton plots of each of the samples in a group are completed, compare all the plots at one time. Since all the cores in this experiment were taken from live trees, the most recent year (on the right) is the same for all the samples. Line up all the plots vertically on the same date. If you were using cores from deadwood, you would not know the date and would need to look for similar trends to identify the likely correspondence of dates. Once the plots are lined up, place another strip of graph paper under all the plots and graph a composite of all the skeleton plots. Since the heights of the lines were made using a degree of judgment, the composite plot helps to even out differences between individuals. The composite skeleton plot also helps identify anomalies such as missing rings or double rings. Try to match as many of the rings as possible to review common trends or patterns.

   After the composite skeleton plot is completed, you can date the core. Since you took your core from a live tree, the growth ring to the right is the current year. You can work toward the left to see when each specimen was a sapling. Remember that even if you were lucky to pass through the pith of the stem, the age of the innermost ring corresponds only to the year the tree reached the height at which you bored your hole. It is not the year the seed germinated, because it takes any plant some time to reach a given height. What would you expect if you took your core from a higher position on the trunk? How would the core of a major branch compare with a core from the trunk?

   If you have a specimen core taken from deadwood, such as a log from an archaeological site, you can date your specimen by comparing your composite skeleton plot with a master chronology. Several master chronologies are available via the Internet or tree-ring analysis laboratories (see Additional Resources at the end of this laboratory topic). Master chronologies were created by combining data from several samples and overlapping the results. Dendrochronologists have created master chronologies that go back nearly 9,000 years in some locations. Tree-ring analysis is more accurate than using ^{14}C for dating wooden archaeological specimens, such as a beam used in the cliff dwellings of Mesa Verde.

8. **Connecting tree-ring data to weather patterns.** Get access to meteorological data for your region for the past century. Most colleges or cities maintain accurate records for temperature and precipitation. For this comparison, you are mostly interested in the values for the entire year rather than daily or monthly values. Examine your composite skeleton plot and look for clusters of narrow rings or



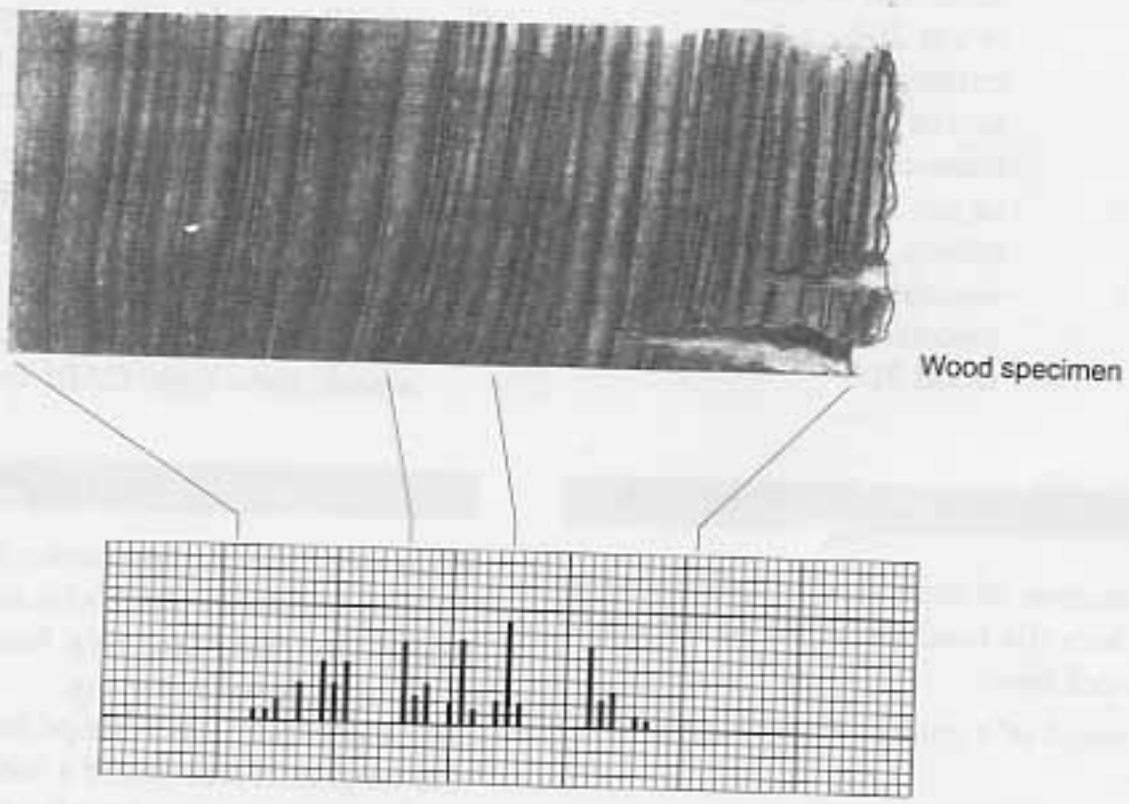


FIGURE 15.8 SKELETON PLOT FOR TREE-RING ANALYSIS. EACH VERTICAL LINE ON THE GRAPH PAPER CORRESPONDS TO ONE GROWTH RING. THE HEIGHT OF THE LINE CORRESPONDS TO THE NARROWNESS OF THE RING COMPARED TO OTHERS. (THE INNERMOST RING IS ON THE LEFT.)

clusters of broad widths. Now examine the meteorological data for those same years. Can you see any possible correlations? What weather factors seem to affect tree growth more than other factors? For now, merely look for possible interactions of environmental conditions and growth. You could test any hypothesis by a more exhaustive statistical analysis if you desire.

TERMS TO KNOW

axillary bud 206
bark 199
bud scales 206
bud scale scars 206
cork 199
cork cambium 197
dendrochonology 207
determinate growth 197
diffuse porous 201
earlywood (springwood) 200
figure 205
hardwoods 199
heartwood 205
indeterminate growth 197
internode 206
latewood (summerwood) 200
leaf scar 206
lenticels 206
meristems 197
node 206
periderm 199
phelloderm 199
pith 200
prickles 207
primary meristems 197
radial section or cut 201
resin ducts 200
ring porous 201
sapwood 205
secondary meristems 197
secondary phloem 199
secondary xylem 199
skeleton plot 208
softwoods 199
spines 207
tangential section or cut 201
terminal bud 206
thorns 207
transverse section or cut 201
tyloses 200
vascular bundle scars 206
vascular cambium 197
wood 200

QUESTIONS FOR REVIEW AND DISCUSSION

1. What is the function of each type of cell found in wood? How does the function relate to the shape or form of the cell type?
2. Compare the wood of a conifer with the wood of a flowering tree.
3. The oleoresin in the resin ducts of conifers has some of the same biological functions as the phenolics released in the heartwood. What is the adaptive significance of these chemical compounds to a long-living organism? How can we possibly exploit the compounds found in the oleoresin or phenolics for our own use?
4. Relate the cellular construction of wood to its strength properties. Look at wood from the viewpoint of an engineer. What are the vector forces wood can experience?
5. What saw cut of wood would make the best plank for a staircase with significant foot traffic?

ADDITIONAL RESOURCES

Carlquist, S. 1988. *Comparative wood anatomy: Systematic, ecological, and evolutionary aspects of dicotyledon wood.* New York: Springer-Verlag.

Cook, E., and L. Kairiukstis, eds. 1990. *Methods of dendrochronology: Applications in the Environmental Sciences.* Boston: Kluwer Academic Publishers.

Esau, K. 1960. *Anatomy of seed plants.* New York: John Wiley & Sons, Inc.

Harlow, W. 1959. *Fruit key and twig key to trees and shrubs.* New York: Dover Publications.

Levetin, E., and K. McMahon. 1999. *Plants and society,* 2d ed. New York: McGraw-Hill Companies, Inc.

Martin, L. 1992. *The folklore of trees and shrubs.* Chester (CN): The Globe Pequot Press.

Perlin, J. 1991. *A forest journey: The role of wood in the development of civilization.* Cambridge (MA): Harvard University Press.

Simpson, B., and M. Conner-Ogorzaly. 1986. *Economic botany: Plants in our world.* 2d ed. New York: McGraw-Hill Companies, Inc.

Stokes, M., and T. Smiley. 1996. *An introduction to tree-ring dating.* Tucson: The University of Arizona Press.

Wimmer, R., and R. Vetter, eds. 1999. *Tree-ring analysis: Biological, methodological, and environmental aspects.* New York: CABI Publishing.

ON THE WEB

What tree is it? An interactive key to common trees.
http://www.oplin.lib.oh.us/products/tree/

Rocky Mountain Tree-ring Research, Inc.
http://www.rmtrr.org

Tree Collections Microscope images of transverse, radial, and tangential sections of a variety of common woods.
http://micro.magnet.fsu.edu/trees/

The Ultimate Tree-Ring Web Pages. Links, databases, and references related to dendrochronology.
http://web.utk.edu/~grissino/

An Educator's Guide To Dendrochonology. Produced by the Laboratory of Tree-Ring Research, The University of Arizona, Tucson.
http://www.plantbio.ohiou.edu/epb/instruct/ecology/dendro.htm

Woods of the World. Detailed information on 910 wood species and products, covering 95% of all wood in trade.
http://www.forestworld.com/wow/wow_home.html

OTHER ACTIVITIES

1. Inventory all the trees on your campus. Present the data as a map, brochure, or interactive database accessible on a Web Site. This could be useful for other students, alumni, community members, landscape staff, and long-range planners.
2. Identify the wood used to produce specific pieces of furniture or other wooden objects. Relate the wood to its practical application or beauty.
3. Use tree-ring analysis to date wooden objects from an archaeological site or historical dwelling.
4. Use rounds cut out of a tree to reconstruct the tree in the lab.
5. Carefully examine a knot in a piece of wood. Use colored clay to reconstruct a model to show how the tree grows new tissue around a branch or wound.

NAME _____

DATE _____

LAB SECTION NUMBER _____

WORKSHEET 15-1 EXERCISE C: TWIG MORPHOLOGY

1. Write a description of one of your twigs in as much detail as possible.

2. Critique of above description. Classmate's name _____

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 15-2 EXERCISE C: TWIG MORPHOLOGY

Use your twig specimens to locate and label all the structures on this diagram.

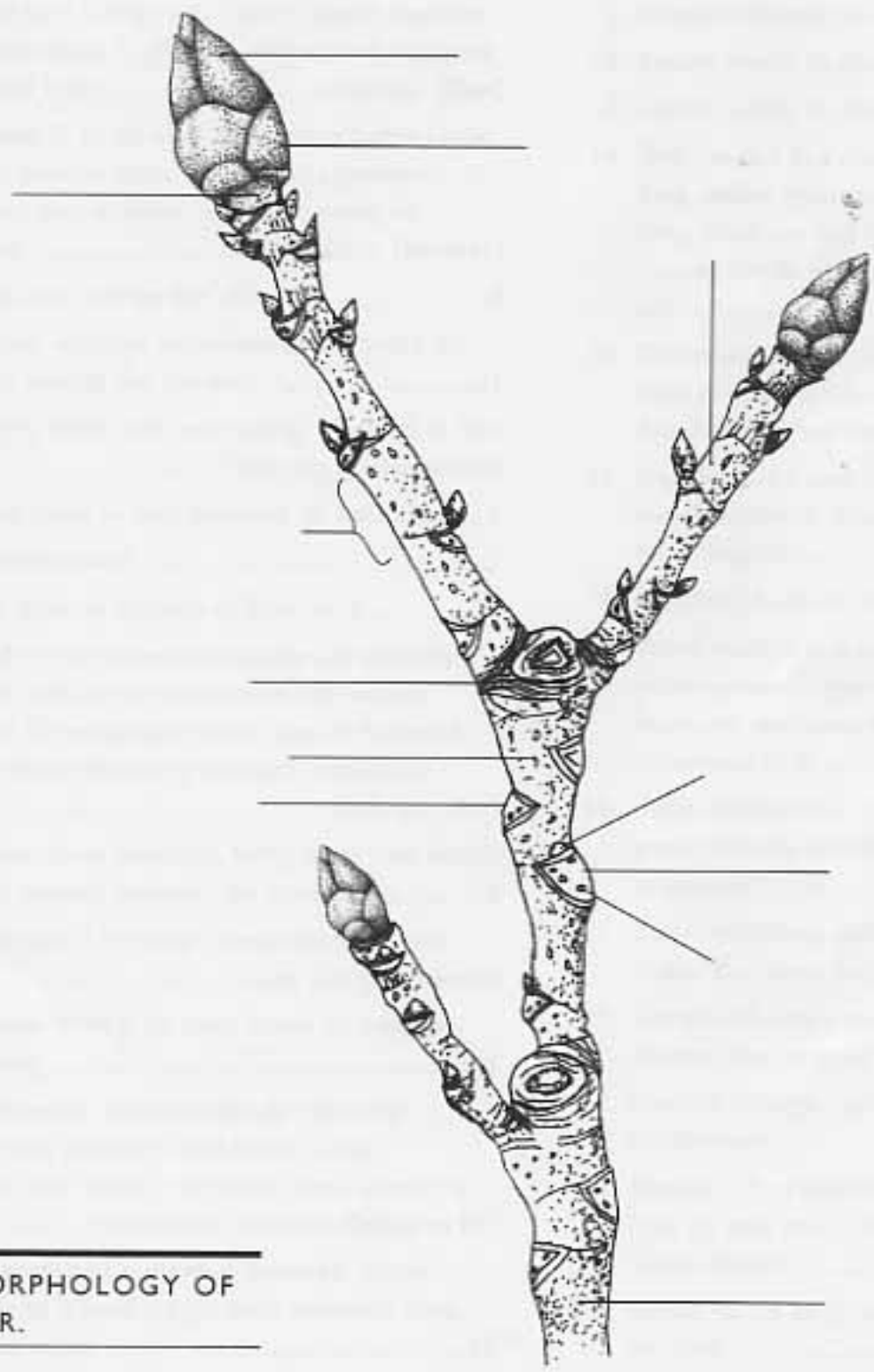


FIGURE 15.9 MORPHOLOGY OF A TWIG IN WINTER.

How is your specimen different from this diagram?

TABLE 15.1 WINTER KEY TO SOME COMMON TREES

1. Evergreen tree producing seeds in cones; narrow, needle-shaped leaves 2
1. Deciduous tree that loses its leaves in autumn or evergreen tree with broad leaves, seeds produced in fruits from flowers 24
2. Seeds produced in a cone that appears "berrylike," either red, blue, or black 3
2. Seeds produced in a woody cone 4
3. Seeds surrounded by a scarlet aril, 1 cm in length (appears like a berry); leaves about 3 cm long, narrow, and flattened; bark dark, thin, and scaly ..... *Taxus* spp. (**Yew**)
3. Seeds within a small (5 to 20 mm) fleshy cone, starting pale green and turning blue or black, with frosty appearance to surface; leaves very tiny, scalelike, and overlapping on branches; aromatic ..... *Juniperus* spp. (**Juniper**)
4. Cones with scales that overlap like shingles 5
4. Cones whose scales, with the exception of the broad flattened tips, do not overlap like shingles 21
5. Leaves scalelike, very small, and overlapping; branches in flattened sprays ..... *Thuja* spp. (**Arborvitae**)
5. Leaves narrow and more or less flattened, or needlelike 6
6. Leaves narrow and flattened 7
6. Leaves needlelike, often in clusters of 2 or more 11
7. Cones borne erect on branches, falling apart at maturity leaving the central stalk of the cone erect; thin woody bracts not present between cone scales; tips of flattened leaves often rounded or shallowly notched (friendly to a hand grasp) ..... *Abies* spp. (**Fir**)
7. Cones hanging down from branches, falling from tree intact; thin woody bracts present between the cone scales 8
8. Cones usually less than 2 cm long; leaves appearing two-ranked ..... *Tsuga* spp. (**Hemlock**)
8. Cones usually greater than 2 cm long; leaves arranged spirally around the twig 9
9. Leaves definitely flattened with rounded tips (friendly to a hand grasp); buds pointed; cone bracts longer than the scales and with three prominent lobes, appearing forked ..... *Pseudotsuga menziesii* (**Douglas fir**)
9. Leaves stiff and 4-angled to somewhat flattened, sharp-pointed (unfriendly to a hand grasp); buds rounded; cone bracts shorter than scales 10
15. Cones 7–9 cm long; leaves spine-tipped, sharp, extending at right angles to the branch, when chewed have a sharp, acid, pungent taste; twigs smooth; branch arrangement of mature trees gives crown a layered appearance ..... *Picea pungens* (**Colorado blue spruce**)
10. Cones about 5 cm long; leaves acute to blunt, usually pointing forward on stem; lacking sharp acid taste; twigs more or less pubescent; crown not layered ..... *Picea engelmannii* (**Engelmann spruce**)
11. Leaves in tight spirals toward tips of short, spurlike branches; leaves deciduous in winter ..... *Larix* spp. (**Larch**)
11. Leaves in clusters, usually 2–5 needles per cluster 12
12. Leaves in clusters of 2–3 13
12. Leaves in clusters of 5 17
13. Leaves mostly in clusters of only 2 (rarely 3) 14
13. Leaves mostly in clusters of 3 15
14. Two needles in a cluster, each needle less than 7.5 cm long, yellow green to green, often twisted; cones 3–5 cm long, often remaining closed for many years, open with heat of fire or gap opening in forest, common in western U.S. ..... *Pinus contorta* (**Lodgepole pine**)
14. Two needles in a cluster, each needle 8–14 cm long, common in eastern U.S., especially the upland soils of southern Appalachian Mountains ..... *Pinus echinata* (**Shortleaf pine**)
15. Needles 7–12 long, three needles in a cluster; mostly native to Atlantic coastal states and New England ..... *Pinus rigida* (**Pitch pine**)
15. Needles 12–45 cm long 16
16. Three needles in a cluster, each 12–30 cm long, yellow-green to gray-green; cones 10 cm long, open and deciduous when mature; common in western U. S. ..... *Pinus ponderosa* (**Ponderosa pine**)
16. Three needles in a cluster, each 20–45 cm long, bright green, densely tufted, at ends of stout branch tips, common in southern U. S. ..... *Pinus palustris* (**Longleaf pine**)
17. Cones with long stalk; cone scales thin; seeds with wing longer than seed itself 18
17. Cones with short stalk; cone scales thick; seeds with wing shorter than seed or lacking 20
18. Needles straight, slender, and flexible, 5–13 cm long, dark bluish-green 19
18. Needles often twisted, relatively stiff; cones 40 cm long on long stalk; common in west slopes of Sierra Nevada ..... *Pinus lambertiana* (**Sugar pine**)
19. Cones 12 cm long; common in eastern U. S.; needles 8–13 cm long ..... *Pinus strobus* (**Eastern white pine**)
19. Cones 20 cm long; common in western U. S.; needles 5–10 cm ..... *Pinus monticola* (**Western white pine**)
20. Cone cylindrical, about 12 cm long, opening at maturity; seeds with a very short terminal wing (sometimes wingless) ..... *Pinus flexilis* (**Limber pine**)

TABLE 15.1 WINTER KEY TO SOME COMMON TREES *(continued)*

20. Cone ovoid, about 6 cm long, remaining closed at maturity; seeds wingless.....*Pinus albicaulis* (**Whitebark pine**)
21. Center of scale tips depressed.....22
21. Center of scale tips not depressed.....23
22. Leaves narrow and flattened; cones 1–2.5 cm long.....*Sequoia sempervirens* (**Coastal redwood**)
22. Leaves small, short, and pointed; cones 5–10 cm long.....*Sequoiadendron giganteum* (**Sequoia**)
23. Center of scale tip ending in a sharp point; cones about 1–2 cm in diameter; foliage gray-green.....*Cupressus macnabiana* (**Cypress**)
23. Center of scale tip not sharply pointed; cones about 0.75 cm in diameter.....*Chamaecyparis lawsoniana* (**Port Orford cedar**)
24. Deciduous tree; leaf scars alternate or in a spiral.....25
24. Deciduous tree; leaf scars opposite or whorled.....47
25. Vascular bundle scars, 3 or more in a V-shaped or crescent-shaped line.....26
25. Vascular bundle scar, 1 (or seemingly 1), arranged in a circle or irregularly scattered.....41
26. Stipules or stipule scars present.....27
26. Stipules or stipule scars absent.....34
27. Terminal bud present.....28
27. Terminal bud absent.....29
28. Pith circular in outline; bud scales sticky; buds not stalked.....*Populus spp.* (**Cottonwood**)
28. Pith triangular in outline; bud scales with feltlike surface, buds on short stalks; conelike inflorescence of female flowers, often in clusters.....*Alnus spp.* (**Alder**)
29. Leaf scar does not completely surround axillary bud.....30
29. Leaf scar completely surrounds axillary bud.....*Platanus spp.* (**Sycamore**)
30. Single hoodlike scale covering each bud.....*Salix spp.* (**Willow**)
30. Several overlapping scales covering each bud.....31
31. Older bark of tree peeling in thin, papery sheets; lenticels forming horizontal lines.....*Betula spp.* (**Birch**)
31. Bark not in paper-thin sheets or lenticels not in horizontal lines.....32
32. Buds asymmetrical in appearance; bud scales usually red, occasionally greenish.....*Tilia spp.* (**Basswood, Linden**)
32. Buds symmetrical in appearance; bud scales brown.....33
33. Tips of buds flattened; pith of internode appears chambered in longitudinal cut; bark of tree trunk and older branches with raised, vertical, corky ridges.....*Celtis occidentalis* (**Hackberry**)
33. Tips of buds not flattened; pith solid; bark usually without raised corky ridges.....*Ulmus spp.* (**Elm**)
34. Terminal bud present.....35
34. Terminal bud absent.....37
35. When twigs cut lengthwise, pith is chambered; buds stain fingers when crushed.....*Juglans nigra* (**Black walnut**)
35. Pith not chambered.....36
36. Branches often with corky ridges of bark; bud scales with tiny hairs along margin; fruit a persistent globose head of beaked capsules.....*Liquidambar styraciflua* (**Sweet gum**)
36. Branches without corky ridges; twigs often with obvious lenticels, usually bitter to taste; bud scales without tiny hairs along margin.....*Prunus spp.* (**Cherry and Plum**)
37. Vascular bundle scars in sets of 3.....38
37. Vascular bundle scars, 5 or more.....39
38. Twigs angular in cross section; stipular spines often present.....*Robinia pseudoacacia* (**Black locust**)
38. Twigs more or less round in cross section; stipular spines absent, in older trees or trees in the wild large branched spines are often present on the trunk (the branched spines are seldomfound on cultivated trees 40 years old or less).....*Gleditisa triacanthos* (**Honey locust**)
39. Leaf scar V-shaped, partly surrounding the axillary bud.....*Rhus spp.* (**Sumac**)
39. Leaf scar semicircular or triangular.....40
40. Pith yellowish-tan; twigs foul-smelling when bruised.....*Ailanthus altissima* (**Tree of heaven**)
40. Pith pink to salmon; twigs not foul-smelling when bruised.....*Gymnocladus dioica* (**Kentucky coffeetree**)
41. Vascular bundle scar, 1 or seemingly 1.....42
41. Vascular bundle scars, 4 to many, arranged in a circle or irregularly scattered.....43
42. Terminal bud absent; twigs gray, not aromatic when bruised.....*Diospyros spp.* (**Persimmon**)
42. Terminal bud present; twigs green, aromatic when bruised.....*Sassafras spp.* (**Sassafras**)
43. Terminal bud present.....44
43. Terminal bud absent.....46
44. Stipule scars barely extending back from upper corners of leaf scars.....*Quercus spp.* (**Oak**)
44. Stipule scars partially or completely encircling twig.....45
45. Bud scales, several, overlapping; buds long-pointed.....*Fagus spp.* (**Beech**)
45. Bud scales, 2, forming a hood over bud; leaf scars circular.....*Liriodendron tulipifera* (**Yellow poplar, Tulip tree**)

TABLE 15.1 WINTER KEY TO SOME COMMON TREES *(continued)*

46. Visible bud scales, 4 or more.....*Morus spp.* (**Mulberry**)
46. Visible bud scales, 2 or 3; buds and twigs reddish, occasionally greenish; twigs zigzag in appearance.....*Tilia spp.* (**Basswood, Linden**)
47. Leaf scars whorled, mostly in 3s.....*Catalpa spp.* (**Catalpa**)
47. Leaf scars opposite.....48
48. Vascular bundle scars small and numerous, forming U-shaped lines.....*Fraxinus spp.* (**Ash**)
48. Vascular bundle scars distinct and separate.....49
49. Vascular bundle scars, 3.....50
49. Vascular bundle scars, 5 or more.....51
50. Terminal buds large and conspicuous; axillary buds tiny and barely visible to unaided eye.....*Cornus spp.* (**Dogwood**)
50. Terminal buds slightly larger than the easily seen axillary buds.....*Acer spp.* (**Maple**)
51. Terminal bud absent; spongy pith occupying at least ½ of the diameter of the twig.....*Sambucus spp.* (**Elderberry**)
51. Terminal bud present; pith small; stout twigs, large leaf scar with vascular bundle scars in V-shaped pattern.....*Aesculus spp.* (**Buckeye, Horse chestnut**)

LABORATORY TOPIC 16

Bioprospecting for Medicinal Plants

BACKGROUND

Every culture has evolved a time-tested tradition of herbal remedies. Some have been written down and preserved, as in the *Ebers Papyrus* from the classical Egyptian period or the *Badianus Manuscript,* a repository of Aztec botanical cures. Hippocrates, the Greek scholar credited with being the father of Western medicine, wrote extensively on plants that heal. In fact, most of the earliest physicians were also botanists because they were expected to prepare their own medications directly from plants they grew or collected from the wild. Western medicine split from herbalism during the advance of chemistry in the nineteenth century, when it became possible to identify the active principle within a plant extract and synthesize the chemical in the laboratory. *Simples*, herbal remedies that used plants directly to make healing teas or salves, became old-fashioned and were discounted by medical practitioners.

Today, however, there is renewed interest in investigating the botanical knowledge of the centuries. Relatively few medicinal plants have been scientifically evaluated, but some that have been analyzed have proven to be invaluable to modern medicine. For example, digitoxin, the standard treatment for many heart patients, is still extracted from the purple foxglove. Aspirin, the number one over-the-counter medication for fever and pain, was created by extracting salicylic acid from willow bark and then modifying it in the laboratory to make it easier to stomach. Presently in the United States, 25–50% of all prescription drugs are either extracted from plants directly or the plant principle is the template from which synthetic compounds are derived. Also, the use of nonprescription botanical drugs is rising rapidly, from 2.5% in 1990 to 12.1% in 1997, as more Americans turn to alternative treatments.

The science of healing is irrevocably linked to the study of plants and their unique chemistry. Plants are literally chemical factories, manufacturing an impressive number of distinctive compounds. It has been said that no chemist could ever imagine the complex structure of taxol, which is extracted from the bark of the Pacific yew and has proven effective in treating ovarian and other forms of cancer. The usefulness of plants in medicine is due specifically to a class of chemicals called **secondary products**. These compounds were initially labeled secondary because at the time they were discovered no obvious purpose for them could be discerned, unlike **primary products**, which were obviously necessary to a plant's metabolism.

We now know that plants produce a great diversity of secondary products; in fact, it has been estimated that the total approaches 400,000. Why do plants produce so many secondary products? Consider their life styles. Plants are by nature stationary and unable to run away from threatening herbivores; figuratively, they are "sitting ducks." How can a defense be mounted when the plant can neither move away nor put up a physical fight? The answer appears to be a chemical defense, and accounts for the tremendous number and variety of secondary products noted. Many of these compounds are **bioactive**, having a physiological effect upon herbivores, and these are the compounds that have been tapped for use in medicine.

In this lab, you will investigate the bioactivity of some herbal remedies by taking the first step and performing a bioassay.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Identify secondary compounds that play a major role in the defensive strategy of plants.
2. Perform a standard bioassay to test for the presence of bioactive compounds.
3. Recognize commonly used herbal remedies.
4. Use the scientific method and practice experimental design.

EXERCISE A: Investigating Herbal Remedies With the Shrimp Bioassay

A **bioassay** uses a living organism to test the toxicity of chemicals. One of the earliest bioassays was employed by miners who worked in underground coal mines. One

by-product of coal mining is the release of methane, a deadly, odorless gas. Caged canaries would accompany the miners on their journey deep into the earth to act as a bioassay for the presence of methane. If the birds died, methane concentrations in the mine were at dangerous levels, a warning that the miners should evacuate.

You will be using a bioassay to detect for bioactivity in plant extracts. The rationale behind the bioassay is that if the active ingredient in a plant can impair herbivores, as shown by killing off brine shrimp, perhaps it can be used to kill off cancer cells or to inhibit the spread of an infection. Also, since the classification of a plant principle as toxic versus therapeutic is often a matter of dosage, any evidence of lethality to the test organisms can indicate that the plant has the potential to act as a natural medicine at a lower concentration.

Many different organisms have been used in laboratory bioassays. The water flea (*Daphnia*) and wrigglers (mosquito larvae) are two common examples, but you will be working with an equally well-known organism of choice, the brine shrimp.

Brine shrimp, *Artemia salina*, are microscopic crustaceans (fig. 16.1). Commonly known as sea monkeys, they thrive in extremely salty environments, such as the Great Salt Lake and ponds at saltworks. The life cycle of a brine shrimp is about a year long. Newly hatched eggs produce larvae, or nauplii, that go through 15 molts before reaching the size (13 mm in length) of sexually mature males and females. Brine shrimp are filter feeders, feeding on bacteria and algae that can also tolerate the highly saline conditions of their environment. When environmental conditions are unfavorable—for example, when a pond dries out—fertilized eggs encyst (become covered with protective cases). These cysts are extremely durable and remain dormant until conditions are favorable again.

You will perform the brine shrimp bioassay on selected healing herbs. Some suggestions of herbs to test are listed in table 16.1, or you may bring in a plant you have collected from the wild or bought from an alternative health store.

FIGURE 16.1 BRINE SHRIMP (*ARTEMIA SALINA*).

Materials Needed for Exercise A

Aquarium pump	Micropipetors
Beakers, 50 ml	Mortar and pestle
Brine shrimp	Pasteur pipets
Dissecting microscope	Petri dishes
Gang valve, 4 way	Pipet pumps
Graduated pipet, 5 ml, or 10-ml graduated cylinder	Plastic tubing
	Seawater
Marker pen	Template vial
Medicine droppers	Test tube rack
Methanol, 100%	Vials, 2-dram

Procedure for Exercise A

1. Organize into research teams of four students. Select an herbal preparation to investigate from table 16.1, or an herb of your own choosing. Using a mortar and pestle that is clean and dry, grind the dried plant material into a powder, the finer the better. Weigh out 0.1g of the powder, and place it in a small (50 ml) beaker. Add 10 ml of 100% methanol to the plant powder. Let this set for 25 minutes. The methanol is a solvent that should extract any bioactive compounds from the plant material.
2. While the methanol is dissolving any bioactive compounds in the plant material, set up the air lines (fig. 16.2). Connect one end of the air line to the aquarium pump and the other end to a gang valve. From the gang valve, connect four air lines and a Pasteur pipet at the end of each line. These air lines will be used to evaporate the methanol from the plant extract. It is essential that the methanol be completely

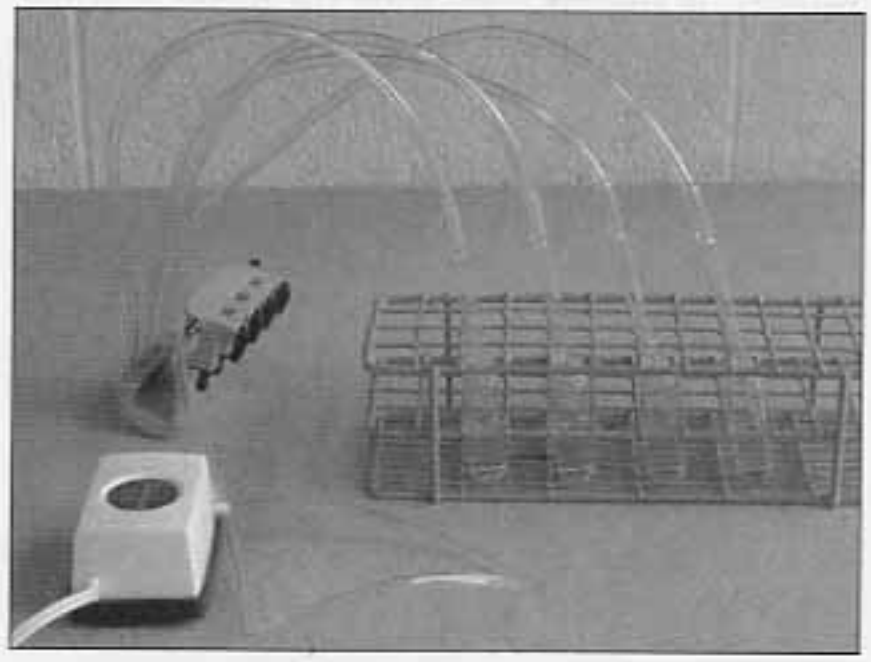


FIGURE 16.2 EXPERIMENTAL SETUP.

TABLE 16.1 FOLK BOTANICALS AND THEIR REPORTED MEDICINAL USE

SCIENTIFIC NAME	COMMON NAME	PART USED	REPORTED MEDICINAL USE.
Calendula officinalis	Calendula	Flowers	Combats fungal, bacterial, and viral infections
Echinacea purpurea	Purple coneflower	Root	Helps fight off flu and colds
Hydrastis canadensis	Goldenseal	Rhizome	Antiseptic/antibiotic, Fights infections
Panax ginseng	Ginseng	Root	Speeds recovery from illness
Camellia sinensis	Green tea	Unfermented leaves	Inhibits many cancers
Vaccinium oxycoccos	Cranberry	Fruits	Clears up infections
Valeriana officinalis	Valerian	Root	Tranquilizer

evaporated from the extract because methanol in itself would be toxic to the brine shrimp.

3. Set up and label the vials or test tubes for this experiment. Each test will require 10 vials. Label a set of five vials 1A–5A and label a second set 1B–5B. Vials 1A and 1B are the seawater controls. Only seawater will be added to these vials, as described in step 8. No plant extract will be added to vials 1A and 1B. Vials 2A and 2B are the methanol controls. You will add 50 μl (microliters) of 100% methanol to each of these vials. No plant extract will be added to vials 2A and 2B.
4. Tubes 3A–5A and 3B–5B are the experimental vials. These will contain three concentrations of the plant extract. To vials 3A and 3B, you will add 5 μl of the plant extract. To vials 4A and 4B, you will add 50 μl of the plant extract, and to vials 5A–5B you will add 500 μl of the plant extract.

   With the addition of a final volume of seawater (described in step 8), the concentrations of the extract tested will be 10 μg/ml (vials 3A, 3B), 100 μg/ml (vials 4A, 4B), and 1,000 μg/ml (vials 5A, 5B). The experimental protocol is outlined in table 16.2.
5. Two members of the research team have the task of counting out the brine shrimp. Ten brine shrimp will be added to each vial. Obtain a small sample of water from the brine shrimp aquarium. Each person doing the counting should have a dissecting microscope, a petri dish, and a medicine dropper. Create a small puddle of brine shrimp on one of your petri dish lids. Using the medicine dropper, count out 10 brine shrimp and move them to a new puddle in another petri dish lid. Make sure no cysts are included. The cysts are spherical and golden-brown in color. Also, make sure the puddle contains enough seawater to keep the brine shrimp from drying out before they are placed in one of the vials. Add to the puddle as need be from the pure seawater. Repeat this procedure until you have 10 puddles, each with a count of 10 brine shrimp.
6. Obtain a vial template that has a marker line at the 5 ml measure. Using this template and a marking pen, mark off the 5 ml mark for each of your 10 vials (1A–5A and 1B–5B series). The level line will be used later to make sure each vial contains a total volume of 5 ml of seawater.
7. After 20 minutes have elapsed, measure out with a micropipetor the volumes of plant extract as outlined in step 3 and table 16.2 to vial 3A–5A and 3B–5B. Measure out 50 μl (step 3 and table 16.2) of methanol each to vials 2A and 2B. Start the evaporative process by plugging in the aquarium pump and putting 4 air lines with pipets in test tubes 5A, 5B, 4A, and 4B. These test tubes contain the greatest volumes of methanol and will take the longest to be evaporated off, so they must be started first. Make sure the toxic methanol is completely evaporated from the extract. Expect a 30–60 minute drying time for vials 5A and 5B. As these extracts are evaporated completely, move to the next set of vials, but make sure you change the pipet with each new vial to avoid contamination. The other vials will take much less time. In fact, you may find that as you progress down the concentration line, the vials with smaller quantities of methanol have completely air-dried and will not require the air line at all.
8. Once the extracts and the methanol control vials have been completely dried, use the pipet pump to measure out and add 2 ml of plain seawater to each vial including tubes 1A and 1B. Then add a puddle of 10 brine shrimp to each vial. Now bring up the total volume in each vial by adding seawater to the level of the 5 ml line.
9. Label the test tube rack with the date, time, lab section, plant tested, and name of the research group members. Place the rack in a safe area away from hot lamps and direct sunlight.
10. After 24 hours, assess the bioassay. Empty the contents of a single vial into a petri dish lid. Rinse out the

TABLE 16.2 EXPERIMENTAL PROTOCOL

TEST TUBE TREATMENT	AMOUNT OF PLANT EXTRACT (μL)	FINAL CONCENTRATION OF EXTRACT (μG/ML)
1A and 1B (seawater controls)	None, only seawater	0
2A and 2B (methanol controls)	50 μl of methanol only	0
3A and 3B	5 μl	10
4A and 4B	50 μl	100
5A and 5B	500 μl	1,000

vial with a small amount of plain seawater and also dump this into the petri dish lid. Count the living brine shrimp using the dissecting microscope and record the numbers in worksheet 16.1. Also calculate and record the percent survivorship for each vial and the mean for each treatment.

TERMS TO KNOW

bioactive compounds 221
bioassay 221
brine shrimp (*Artemia salina*) 222
primary products 221
secondary products 221

QUESTIONS FOR REVIEW AND DISCUSSION

1. Another standard bioassay uses mosquito larvae or water fleas. Like brine shrimp, mosquito larvae and water fleas are aquatic organisms. Why are aquatic organisms the choice for so many bioassays?
2. If you do see significant lethality of brine shrimp in response to an herbal extract, what would be the next step(s) in developing a useful natural medicine?
3. One problem with some herbal remedies is that the same plant species collected from different populations or environments do not show the same effectiveness when tested in bioassays. How can you account for these discrepancies?

ADDITIONAL RESOURCES

Alkofahi, A., J. K. Rupprecht, J. E. Anderson, J. L. McLaughlin, K. L. Mikolajczak, and B. A. Scott. 1989. *Search for new pesticides from higher plants in insecticides of plant origin*. Washington, D.C.: American Chemical Society.

Blackwell, W. H. 1990. *Poisonous and medicinal plants*. Englewood Cliffs (NJ): Prentice Hall.

Cox, P. A., and M. J. Balick. 1994. The ethnobotanical approach to drug discovery. *Scientific American* 269(12):82–87.

Jackson, D. D. 1989. Searching for medicinal wealth in Amazonia. *Smithsonian* 19(11):94–103.

Krochmal, A., and C. Krochmal. 1973. *A guide to the medicinal plants of the United States*. New York: Quadrangle/The New York Times Book Co.

Levetin, E., and K. McMahon. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Nicholson, R. 2000. Az—Tech medicine. *Natural History* 108(10) 54—58.

Plotkin, M. J. 2000. *Medicine quest: In search of nature's healing secrets*. New York: Viking/Penguin Putnam.

Schar, D. ed. 2000. *Prevention's guide: Healing herbs*. Emmaus (PA): Rodale.

Swerdlow, J. L. 2000. Nature's Rx. *National Geographic* 197(4):98–117.

Swerdlow, J. L. 2000. *Nature's medicine: A chronicle of mankind's search for healing plants through the ages*. Washington, D. C: National Geographic.

ON THE WEB

Ask Dr. Weil, Drweil.com
http://www.pathfinder.com/drweil/
HerbMed
http://www.herbmed.org/
CIEER: Centre for Ethnomedicinal Education and Research
http://www.cieer.org/directory.html
Ethnobotanical Leaflets
http://www.siu.edu/~ebl/
Indiana University Molecular Structure Center
http://www.iumsc.indiana.edu/index.html
Wayne's Word: A newsletter of Natural History Trivia
http://daphne.palomar.edu/wayne/wayne.htm

OTHER ACTIVITIES

You may also test over-the-counter herbal supplements in pill form. In addition, it might be interesting to compare the efficacy of identical herbal supplements from different companies.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 16-1 EXERCISE A: INVESTIGATING HERBAL REMEDIES WITH THE SHRIMP BIOASSAY

Plant tested: _____

CONCENTRATION OF EXTRACT	NUMBER OF SHRIMP SURVIVING AFTER 24 HRS		% SURVIVORSHIP SURVIVING SHRIMP / TOTAL SHRIMP		MEAN
	SERIES A	SERIES B	SERIES A	SERIES B	
Seawater control					
Methanol control					
10					
100					
1,000					

1. What is the purpose of having both seawater and methanol controls in this experiment?

2. Why is it necessary to test the extract at various concentrations?

3. Did your extract show significant bioactivity as tested by the brine shrimp bioassay? At what concentration(s)?

4. Do the results of your bioassay substantiate the claims made for this herbal remedy? Why or why not?

TABLE 16.2 EXPERIMENTAL PROTOCOL

TEST TUBE TREATMENT	AMOUNT OF PLANT EXTRACT (μL)	FINAL CONCENTRATION OF EXTRACT (μG/ML)
1A and 1B (seawater controls)	None, only seawater	0
2A and 2B (methanol controls)	50 μl of methanol only	0
3A and 3B	5 μl	10
4A and 4B	50 μl	100
5A and 5B	500 μl	1,000

vial with a small amount of plain seawater and also dump this into the petri dish lid. Count the living brine shrimp using the dissecting microscope and record the numbers in worksheet 16.1. Also calculate and record the percent survivorship for each vial and the mean for each treatment.

TERMS TO KNOW

bioactive compounds 221
bioassay 221
brine shrimp (*Artemia salina*) 222
primary products 221
secondary products 221

QUESTIONS FOR REVIEW AND DISCUSSION

1. Another standard bioassay uses mosquito larvae or water fleas. Like brine shrimp, mosquito larvae and water fleas are aquatic organisms. Why are aquatic organisms the choice for so many bioassays?
2. If you do see significant lethality of brine shrimp in response to an herbal extract, what would be the next step(s) in developing a useful natural medicine?
3. One problem with some herbal remedies is that the same plant species collected from different populations or environments do not show the same effectiveness when tested in bioassays. How can you account for these discrepancies?

ADDITIONAL RESOURCES

Alkofahi, A., J. K. Rupprecht, J. E. Anderson, J. L. McLaughlin, K. L. Mikolajczak, and B. A. Scott. 1989. *Search for new pesticides from higher plants in insecticides of plant origin*. Washington, D.C.: American Chemical Society.

Blackwell, W. H. 1990. *Poisonous and medicinal plants*. Englewood Cliffs (NJ): Prentice Hall.

Cox, P. A., and M. J. Balick. 1994. The ethnobotanical approach to drug discovery. *Scientific American* 269(12):82–87.

Jackson, D. D. 1989. Searching for medicinal wealth in Amazonia. *Smithsonian* 19(11):94–103.

Krochmal, A., and C. Krochmal. 1973. *A guide to the medicinal plants of the United States*. New York: Quadrangle/The New York Times Book Co.

Levetin, E., and K. McMahon. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

Nicholson, R. 2000. Az—Tech medicine. *Natural History* 108(10) 54—58.

Plotkin, M. J. 2000. *Medicine quest: In search of nature's healing secrets*. New York: Viking/Penguin Putnam.

Schar, D. ed. 2000. *Prevention's guide: Healing herbs*. Emmaus (PA): Rodale.

Swerdlow, J. L. 2000. Nature's Rx. *National Geographic* 197(4):98–117.

Swerdlow, J. L. 2000. *Nature's medicine: A chronicle of mankind's search for healing plants through the ages*. Washington, D. C: National Geographic.

ON THE WEB

Ask Dr. Weil, Drweil.com
http://www.pathfinder.com/drweil/
HerbMed
http://www.herbmed.org/
CIEER: Centre for Ethnomedicinal Education and Research
http://www.cieer.org/directory.html
Ethnobotanical Leaflets
http://www.siu.edu/~ebl/
Indiana University Molecular Structure Center
http://www.iumsc.indiana.edu/index.html
Wayne's Word: A newsletter of Natural History Trivia
http://daphne.palomar.edu/wayne/wayne.htm

OTHER ACTIVITIES

You may also test over-the-counter herbal supplements in pill form. In addition, it might be interesting to compare the efficacy of identical herbal supplements from different companies.

LABORATORY TOPIC 17

Bioactive Drugs in Action

BACKGROUND

Many of the most useful bioactive chemicals—those that exert effects upon the physiology of organisms—have been isolated from plants, and many of these have been identified as **alkaloids**. Alkaloids are organic compounds containing rings of nitrogen, and as their name implies, they are alkaline, or basic, in solution. Alkaloids have a bitter taste, and according to standard terminology, most are named to end with the suffix —ine. Alkaloids often act upon the nervous system by mimicking the effects of **neurotransmitters**, natural chemicals of the nervous system. The action of alkaloids varies with the drug and the dosage. They may be stimulants, depressants, or even hallucinogens. Some common examples of plant alkaloids are cocaine, morphine, nicotine, caffeine, and quinine.

A second group of physiologically active substances from plants are the **terpenes**. Terpenes belong to the largest group of plant chemicals and serve a variety of functions in plants. They are the principal ingredients in plant-derived perfumes, soaps, flavorings, dyes, and many medicines. The **lactones** are a group of terpenes that have drawn the attention of researchers because they are often the active ingredient in many herbal preparations. **Glycosides** are another grouping. The defining characteristic of glycosides is the presence of a sugar group (usually glucose), but it is the compound attached to the sugar that defines the biological activity of a glycoside. Glycosides may be classified as cyanogenic, cardioactive, or saponins. **Cyanogenic glycosides** release cyanide. Plants containing this type of glycoside are usually considered poisonous rather than therapeutic. **Cardioactive glycosides** affect the circulatory system, specifically the heart. Depending upon the dosage, preparations from plants with this type of glycoside can poison by causing heart failure or act as a medicine by strengthening the contractions of a weakened heart. **Saponins** have a steroidal component. Steroids are physiologically important because many hormones are steroids. The saponins from some plants have been used as precursors for creating synthetic hormones in the laboratory. Examples of glycosides include salicin, the base for aspirin, and aloin, the collective name for many of the principal ingredients in aloe vera.

In this laboratory topic, you will observe the actions of several plant-derived **bioactive drugs** upon a test animal that demonstrates the effects of these chemicals on human physiology.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Recognize a variety of bioactive drugs derived from plants.
2. Know the value of an animal model system to test and to predict the effects of bioactive drugs on human physiology.
3. Use the scientific method and practice experimental design.

EXERCISE A: The Blackworm Model to Test Bioactive Drugs

To test the actions of bioactive drugs derived from plants, you will work with a segmented worm, or **annelid**. In these worms, the body is organized into repeated rings or segments. Annelids can be found in terrestrial, freshwater, and marine environments. There are three classes of annelids, based on the relative number of bristles, or **setae** on the body wall. You will be studying a species within the **oligochaetes**, annelids that have few bristles—in this case, only four per segment.

Our experimental animal is the California blackworm, or mudworm (*Lumbriculus variegatus*) (fig. 17.1). It is found throughout North America at the edge of freshwater ponds, lakes, and marshes. This free-living animal survives by ingesting organic debris and microscopic algae. It is a small worm, only 2.5–5.0 cm in total length. It has a **closed circulatory system**, much like our own in that the blood is contained within blood vessels. Two major blood vessels, the ventral and dorsal vessels, run lengthwise from head to tail, and several lateral vessels connect the two in the anterior segments. The dorsal (along the backside) blood vessel acts as the heart, pumping the blood through wavelike contractions of its

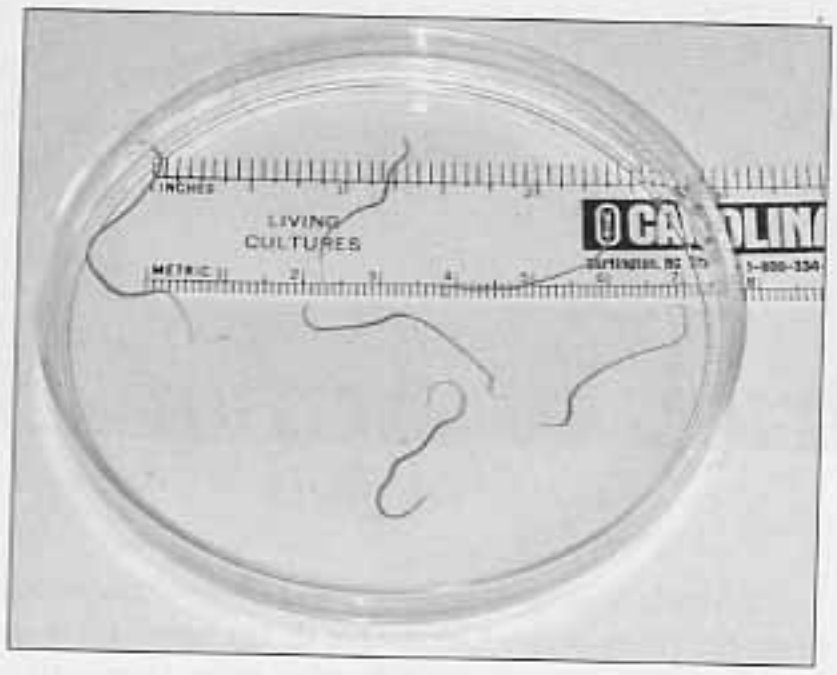


FIGURE 17.1 THE CALIFORNIA BLACKWORM (*LUMBRICULUS VARIEGATUS*).

muscular walls. The blood of the worm is also like ours in that it is bright red due to the presence of a hemoglobin-like pigment (erythrocuruorin) that transports oxygen. Unlike our circulatory system, this pigment is not contained in red blood cells but is dissolved in the plasma.

Since the body wall of the worm is transparent, you will be able to observe the blood directly as it pulses through the dorsal vessel. By immersing the worms in various plant extracts, you will observe the bioactive effects of these drugs on the worms' circulatory systems. It is expected that the pulse rate of the California blackworm will be affected by the plant drugs just as our circulatory system is affected by these same drugs. Please note that the worms will not be harmed by this testing.

The plant extracts or plant-derived drugs available for testing are listed in table 17.1.

Materials Needed for Exercise A

California blackworms (*Lumbriculus variegatus*)
Compound light microscope
Kimwipes
Marker pen
Observation chamber
Paper cups, 5-oz size
Pan of spring water for fresh worms
Pan of spring water for used worms
Pasteur pipet, large bore
Test solutions of nicotine, caffeine, kava kava, digitoxin, ephedrine, or pseudoephedrine
Spring water
Worm wrangling tools
Watch or clock with second hand

Procedure for Exercise A

1. You will need a lab partner for this exercise. One student observes and counts the pulsation rates of the test worms, while the other student acts as a timer and recorder of the data.
2. Decide upon a bioactive plant extract or drug to test. Label one paper cup as the control and three other paper cups with the appropriate concentrations of the drug or extract solutions. Obtain a compound microscope, observation chamber, Pasteur pipets (one for each solution and concentration), and a worm wrangling tool.
3. With the Pasteur pipet, select four worms from the culture pan and place them in a cup of spring water. Make sure the worms are approximately the same length. Do not pick worms that have recently regenerated tail segments. Regenerated segments will be unpigmented.
4. The next step is to determine the baseline pulse rate in spring water (before treatment) for each of the four worms. Using the pipet, place one worm in the observation chamber (fig. 17.2). Add enough spring water so that the worm will not dry out, but do not overfill or the worm will leave the confines of the trough. The water level should just fill the trough. Absorb any

TABLE 17.1 BIOACTIVE PLANTS AND THEIR PRINCIPLES

COMMON NAME	SCIENTIFIC NAME	PART USED	ACTIVE PRINCIPLE	DRUG CLASSIFICATION
Coffee tree, tea bush, and other species	*Coffea* spp., *Camellia sinensis,*	Leaves	Caffeine	Alkaloid
Ephedra, ma huang	*Ephedra sinica*	Stem	Ephedrine, pseudoephedrine	Alkaloid
Foxglove	*Digitalis purpurea*	Leaves	Digitoxin	Glycoside
Kava	*Piper methysticum*	Rhizome	Kawain	Lactone
Tobacco	*Nicotiana* spp.	Leaves	Nicotine	Alkaloid

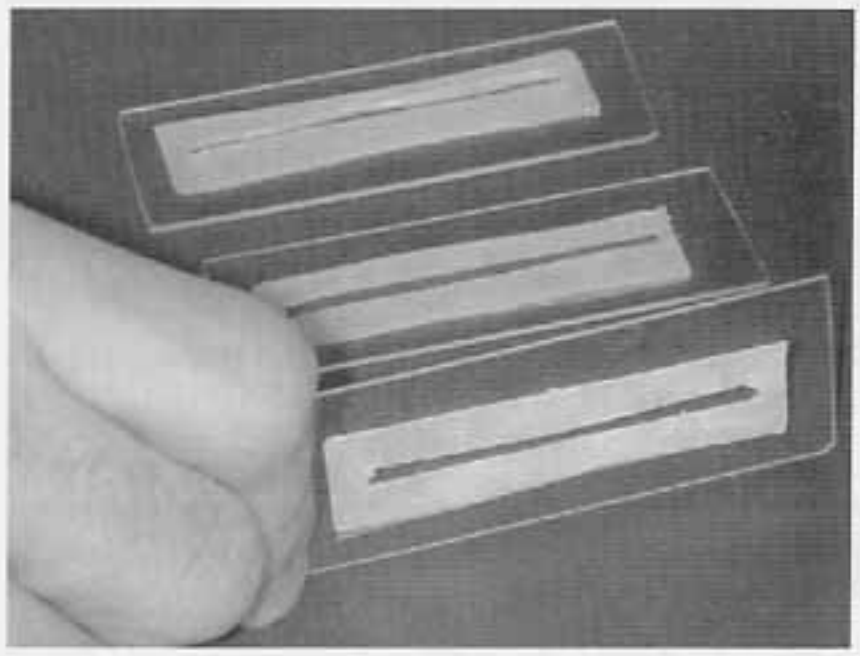


FIGURE 17.2 OBSERVATION CHAMBER.

excess water with a kimwipe. Do not add a coverslip. Let the worm crawl around and adjust to its new surroundings for a couple of minutes.

5. Place the observation chamber under the scanning (4 × objective) or low power (10 × objective) of the compound light microscope. Use the lowest possible setting of the lamp so as not to dry out or "cook" the worm.
6. Using the worm wrangling tool, lightly touch the worm to coax it into a position that allows you to view the dorsal blood vessel. Now you are ready to determine the basal pulse rate of your worm.
7. Count the pulse rate from a mid-body location by watching the wave of blood flow in a single body segment. As the timer keeps track of time, count the number of pulses for at least 15 seconds and, if possible, 30 seconds. Repeat for two additional readings. Convert each pulse rate to beats per minute, and record it in worksheet 17-1 at the end of this laboratory topic. Return the control worm to a cup of spring water for 15 minutes, and then redo the pulse determination.
8. For each drug treatment, you will take pulse readings at three concentrations. Before the worms are immersed in the treatment concentration, a baseline pulse rate must be taken in springwater as in step 7. After the baseline pulse rate is determined, immerse the treatment worm in a cup with the particular drug concentration for 15 minutes. At the end of the exposure time, place the drug-exposed worm in the observation chamber. Fill the observation chamber with spring water (not the drug solution!). Redo the pulse determination as before and record in worksheet 17-1.
9. After each drug treatment, rinse the worm briefly in spring water and then place the worm in the pan marked "used worms." It is very important to prevent contamination by rinsing the observation chamber thoroughly with spring water after a pulse reading is taken on a drug-treated worm.
10. Calculate the means for both before and after treatment, and analyze the data in worksheet 17-1.

TERMS TO KNOW

alkaloids 227
annelid 227
bioactive drugs 227
cardioactive
glycoside 227
closed circulatory
system 227
cyanogenic glycosides 227
lactones 227
neurotransmitters 227
oligochaetes 227
saponins 227
setae 227
terpenes 227

QUESTIONS FOR REVIEW AND DISCUSSION

1. In what way does the California blackworm (*Lumbriculus variegatus*) make an ideal experimental animal for testing bioactive drugs?
2. Why have so many plants that have been identified as toxic been used medicinally?
3. What is the expected effect of a stimulant upon pulse rate? Of a depressant?
4. Why is it important to test drugs or extracts at various concentrations?
5. Why is it important to measure the pulse rate of a worm in spring water?

ADDITIONAL RESOURCES

Alkofahi A., J. K. Rupprecht, J. E. Anderson, J. L. McLaughlin, K. L. Mikolajczak, and B. A. Scott. 1989. *Search for new pesticides from higher plants in insecticides of plant origin.* Washington, D.C.: American Chemical Society.

Blackwell, W. H. 1990. *Poisonous and medicinal plants.* Englewood Cliffs (NJ): Prentice Hall.

Cox, P. A., and M. J. Balick. 1994. The ethnobotanical approach to drug discovery. *Scientific American* 269(12):82–87.

Drewes, C. D. 1996. Those wonderful worms. *Carolina Tips* 59(3):17–20.

Jackson, D. D. 1989. Searching for medicinal wealth in Amazonia. *Smithsonian* 19(11):94–103.

Krochmal, A., and C. Krochmal. 1973. *A guide to the medicinal plants of the United States.* New York: Quadrangle/The New York Times Book Co.

Lesiuk, N. M. 1999. Blackworms, blood vessel pulsations, and drug effects. *American Biology Teacher* 61:48–53.

Levetin, E., and K. McMahon. *Plants and society,* 2d ed. New York: McGraw-Hill Companies, Inc.

Nicholson, R. 2000. Az —Tech medicine. *Natural History* 108(10):54—58.

Plotkin, M. J. 2000. *Medicine quest: In search of nature's healing secrets.* New York: Viking/ Penguin Putnam.

Schar, D. ed. 2000. *Prevention's guide: Healing herbs.* Emmaus (PA): Rodale.

Swerdlow, J. L. 2000. Nature's Rx. *National Geographic* 197(4):98–117.

Swerdlow, J. L. 2000. *Nature's medicine: A chronicle of mankind's search for healing plants through the ages.* Washington, D. C.: National Geographic.

ON THE WEB

Ask Dr. Weil, Drweil.com
http://www.pathfinder.com/drweil/
HerbMed
http://www.herbmed.org/
CIEER: Centre for Ethnomedicinal Education and Research
http://www.cieer.org/directory.html
Ethnobotanical Leaflets
http://www.siu.edu/~ebl/
Indiana University Molecular Structure Center
http://www.iumsc.indiana.edu/index.html
Wayne's word: A newsletter of Natural History Trivia
http://daphne.palomar.edu/wayne/wayne.htm

ADDITIONAL ACTIVITIES

With the help of your instructor, obtain and investigate other plant derived drugs and extracts to test on the blackworm.

NAME _____

DATE _____

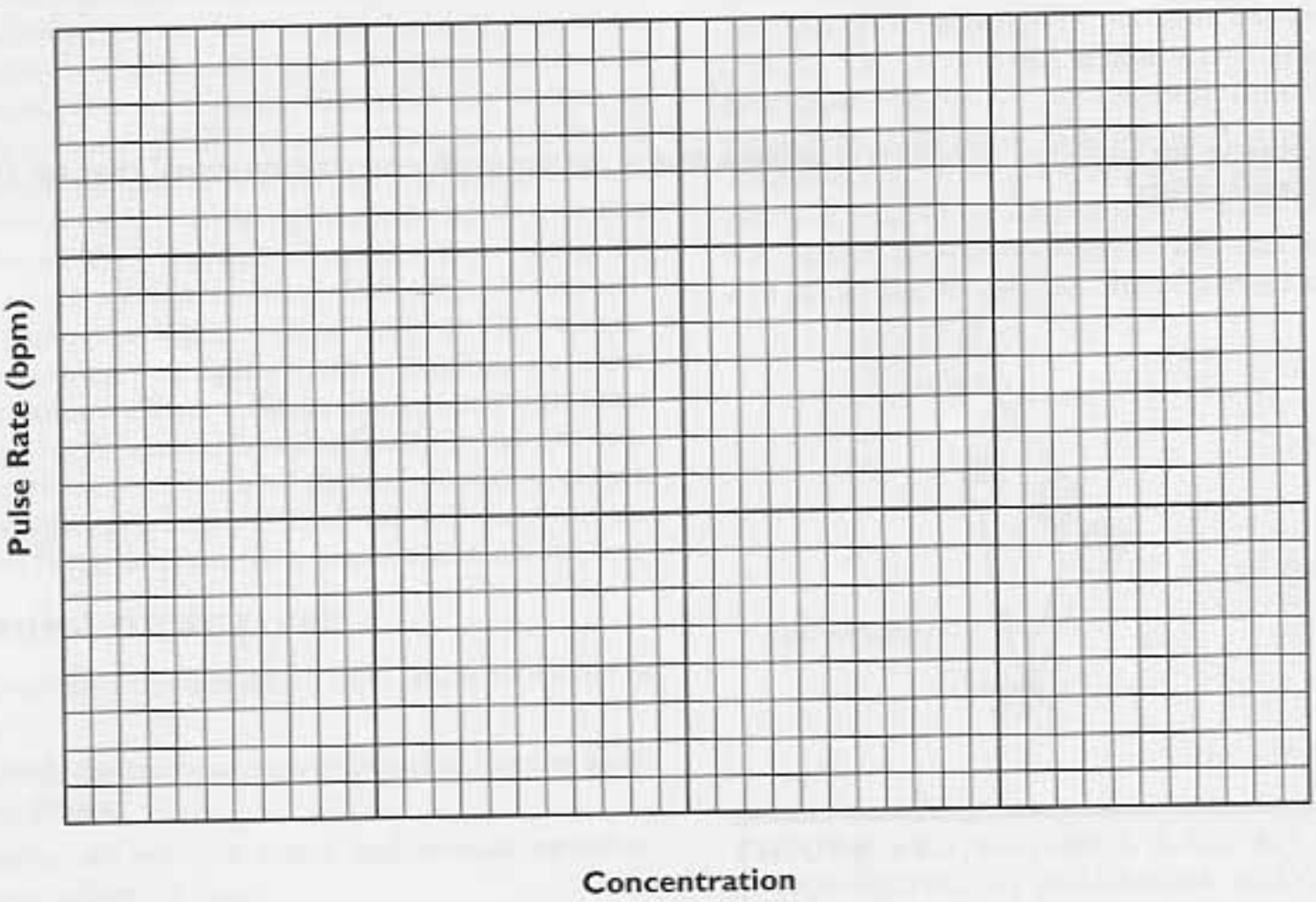
LAB SECTION NUMBER _____

WORKSHEET 17-1 EXERCISE A: COMPARISON OF PULSE RATES (BPM) OF THE CALIFORNIA BLACKWORM

Plant tested: _____

DRUG CONCENTRATION	INITIAL PULSE RATE (BPM)				FINAL PULSE RATE (BPM)			
	Trial 1	Trial 2	Trial 3	Mean	Trial 1	Trial 2	Trial 3	Mean
Spring water control								

1. To see general trends, make a bar graph to show the changes (before and after) in pulse rate as a function of concentration.



NAME

DATE

LAB SECTION NUMBER

WORKSHEET 17-1 EXERCISE A: COMPARISON OF PULSE RATES (BPM) OF THE CALIFORNIA BLACKWORM *(continued)*

2. What conclusions can be drawn about the effects of the bioactive drugs tested upon pulse rate in the California blackworm? Do your findings substantiate the effects reported in the medical literature? Why or why not?

3. Did you observe any conflicting recordings in testing a drug or extract at different concentrations? How can you explain these conflicts?

LABORATORY TOPIC 18

The Fungus Among Us

BACKGROUND

The Kingdom Fungi contains a large diversity of organisms, from inconspicuous molds and yeasts to large mushrooms, bracket fungi, and puffballs. Fungi, which can be found everywhere in the environment, are **absorptive heterotrophs**. They secrete enzymes that break down large, complex organic molecules such as starch or cellulose in the surrounding substrate. The fungal hyphae then absorb the smaller breakdown products, such as the glucose molecules that result from the breakdown of starch or cellulose.

As absorptive heterotrophs, fungi exist as saprobes, pathogens, or symbionts. The vast majority of fungi are **saprobes**, which are responsible for the natural decay and decomposition that occurs in the environment. But saprobes also cause unwanted spoilage of food and materials (including paper, fabrics, and building materials) indoors, where they are able to grow on substrates such as carpeting, shower curtains, insulation and wallpaper. Any time excess moisture is present indoors, molds will grow. Fungal **pathogens** attack a variety of plants and animals. Some fungi are obligate pathogens and are only able to survive within a specific host species. Other fungi are able to attack a range of hosts. Still others are opportunistic pathogens, which exist as saprobes but are capable of becoming pathogens when conditions are right. Fungal symbionts form intimate associations with other organisms to the mutual benefit of both. Fungi also contribute positively to our lives through food, beverages, and medicinal products. Today's lab will focus on the Kingdom Fungi and how these organisms affect our lives.

LEARNING OBJECTIVES

After completing this laboratory topic, students should be able to:

1. Identify the major groups of organisms in the Kingdom Fungi.
2. Distinguish between sexual and asexual reproductive structures in fungi.
3. Understand that the atmosphere naturally contains a diversity of fungal spores.
4. Recognize lesions on plants caused by pathogenic fungi.
5. Understand the contribution fungi make to our diet.
6. Describe the fermentation process that leads to wine production.

EXERCISE A: Fungal Diversity

Fungus-like organisms occur in both the Kingdom Protista and the Kingdom Fungi. In this lab, we will look at several groups of organisms in the Kingdom Fungi. The Division **Zygomycota** includes a large group of fungi that have very simple structures. There are two characteristic reproductive structures in this group. The asexual reproductive structure is the sporangium, within which asexual spores are produced. The **sporangium** (pl. sporangia) develops on a specialized hypha called a **sporangiophore** (fig. 18.1*a*). The sexual reproductive structure is the **zygospore** (fig. 18.1*b*), which results from the fusion of compatible gametangia. The common black bread mold, *Rhizopus stolonifer*, is a member of this division. One group of fungi in the Zygomycota produce **mycorrhizae**, a symbiotic association between a fungus and the roots a of plant. The mycobiont (fungal partner in the associ-

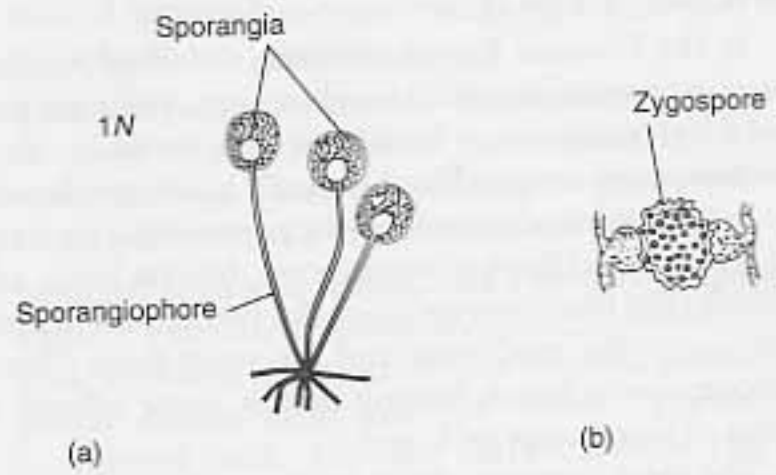


FIGURE 18.1 RHIZOPUS, A MEMBER OF THE ZYGOMYCOTA. (A) SPORANGIA, ASEXUAL REPRODUCTIVE STRUCTURES. (B) ZYGOSPORE, A SEXUAL REPRODUCTIVE STRUCTURE.

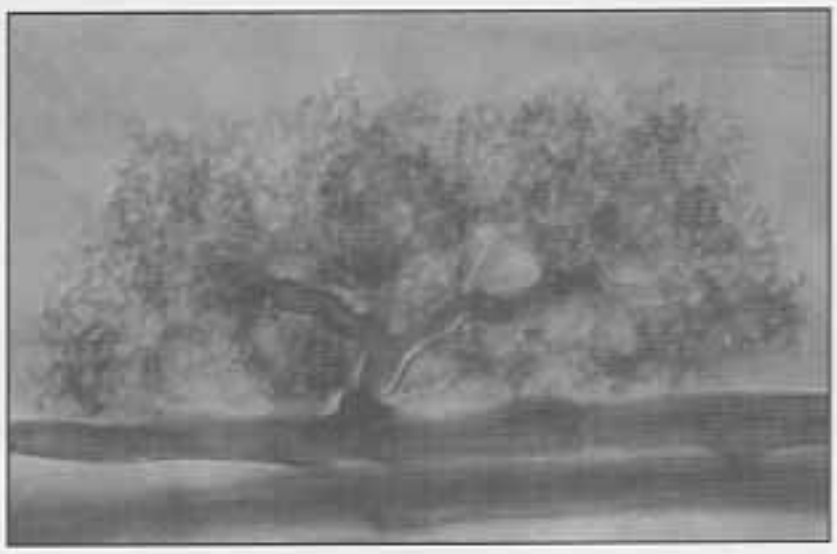


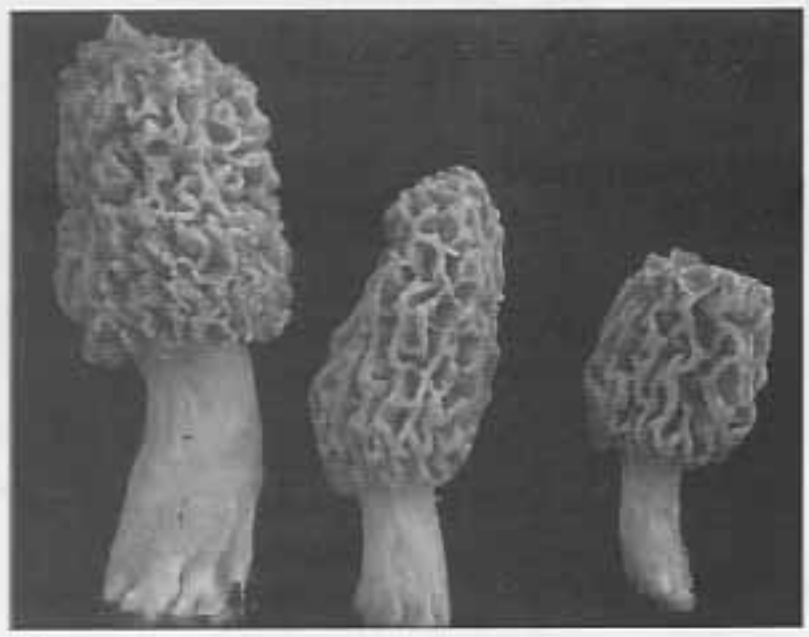
FIGURE 18.2 VESICULAR-ARBUSCULAR (VA) MYCORRHIZAE. AN ARBUSCULE IS VISIBLE WITHIN THE PLANT CELL.

ation) aids in mineral uptake (especially phosphorus), and the plant furnishes carbohydrates for use by the fungus. It has been estimated that 95% of all vascular plants have mycorrhizal. Although several different types of mycorrhizae exist, the mycorrhizal fungi in the Zygomycota are known as vesicular-arbuscular (VA) mycorrhizae. They produce highly branched structures called arbuscules within the plant cell (fig. 18.2). The exchange of nutrients occurs through these arbuscules.

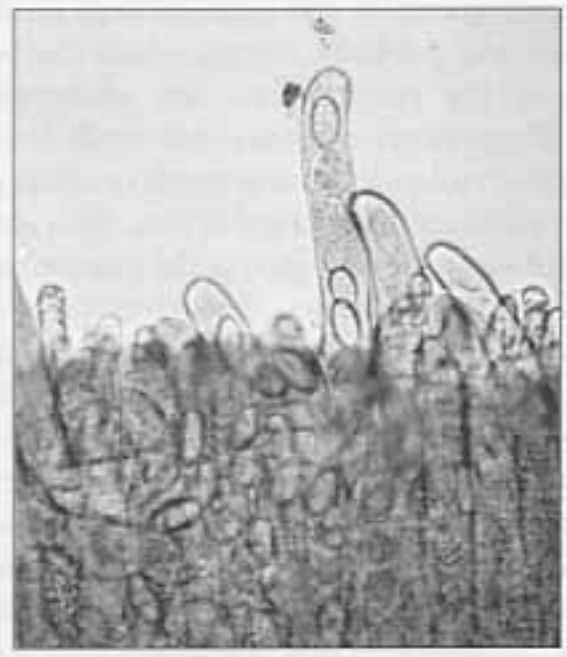
Members of the Division **Ascomycota** produce sexual spores called **ascospores** within a saclike structure called an **ascus**. There are 8 ascospores within each ascus. Some members of this division are microfungi (microscopic fungi), while others produce a large **fruiting body** (reproductive structure). Among those with fruiting bodies are the morels and cup fungi (fig.18.3*a*); the depressions on these fruiting bodies are lined with asci (fig. 18.3*b*). Most yeasts belong to the Ascomycota, but they do not produce a fruiting body.

In the Division **Basidiomycota**, the fungi produce sexual spores that are called basidiospores. These are produced externally on a **basidium** (pl., basidia). Four **basidiospores** are produced on each basidium. Basidia line the gills of mushrooms and the pores of bracket fungi (fig. 18.4). In addition to mushrooms, bracket fungi, and puffballs, the Basidiomycota includes two groups of plant pathogens—the rust fungi and the smut fungi. These pathogens, which lack fruiting bodies, cause billions of dollars of crop losses each year.

The most common fungi in the environment are a large group of asexual fungi whose sexual stage is unknown. They grow easily on most substrates and are commonly called molds. Most are believed to be members of the Ascomycota even though the sexual stage has not been identified. They form asexual spores called



(a)



(b)

FIGURE 18.3 MORELS ARE MEMBERS OF THE ASCOMYCOTA. (A) FRUITING BODIES. (B) ASCOSPORES WITHIN THE ASCUS.

conidia (sing., conidium) on structures called **conidiophores** (specialized hyphae that give rise to conidia). Asexual fungi occur in the soil, on leaf surfaces, and on contaminated foods and building materials. The spores are dispersed by wind, and they can easily be isolated from the air.

Materials Needed for Exercise A

Compound and dissecting microscopes

Cultures of *Rhizopus*, *Penicillium*, and *Aspergillus* (do not open the lid)

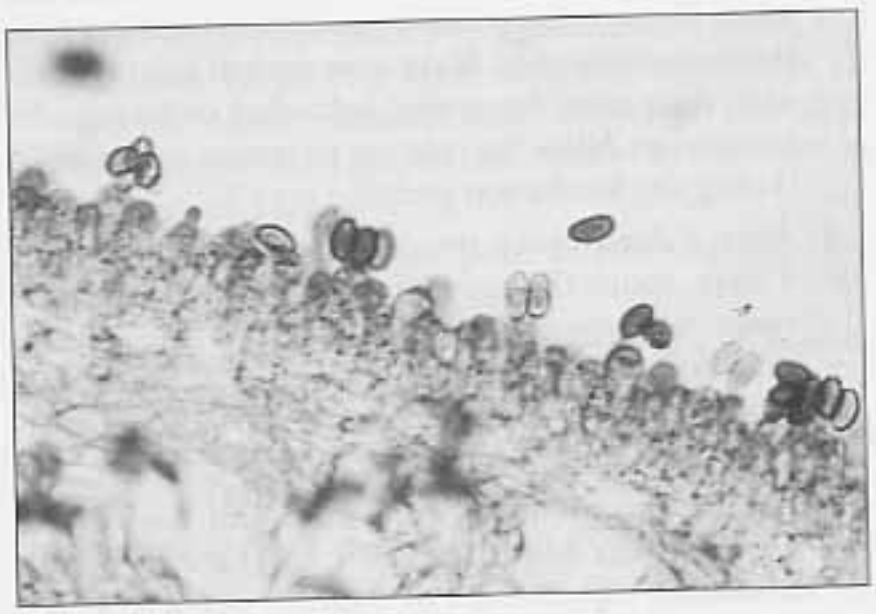
Cup fungi and morels

Ear of corn infected with *Ustilago maydis*

Prepared slide of a mushroom gill

Prepared slide of *Peziza* asci

(a)



(b)

FIGURE 18.4 MUSHROOMS ARE MEMBERS OF THE BASIDIOMYCOTA. (A) FRUITING BODIES. (B) BASIDIA WITH BASIDIOSPORES LINING THE GILLS OF MUSHROOMS.

Prepared slide of *Puccinia graminis*, uredial stage on wheat

Prepared slides of *Penicillium* and *Aspergillus* conidiophores and conidia

Prepared slides of *Rhizopus* reproductive structures

Samples of fresh or dried mushrooms, bracket fungi, puffballs, and stinkhorns

Stained roots containing mycorrhizae

Stalks of wheat infected with *Puccinia graminis*

Yeast culture in liquid medium

Procedure for Exercise A

1. ***Rhizopus* culture and prepared slides.** Using the dissecting microscope, examine the culture of *Rhizopus*. Do not open the lid of the petri dish. You should be able to see the sporangiophores, with the spherical sporangia at the tip (see fig. 18.1). With the compound microscope, examine a prepared slide showing the sporangia and zygospores. You should be able to distinguish these structures as well as the hyphae that make up the colony.
2. **Vesicular-arbuscular mycorrhizae.** Obtain a prestained root and place it on a microscope slide. Cover with a drop of the storage fluid and add a coverslip. Examine first under low power and the high power. Look for arbuscules within the root cells. Draw one of these in the following space.
3. **Yeast culture.** Place a drop of the yeast culture on a slide. Add a coverslip and examine the slide under the microscope. Find yeast cells that are budding.
4. **Asomycete specimens and *Peziza* prepared slide.** Examine the fruiting bodies that belong to the Division Ascomycota. Your instructor may have several different cup fungi and morels available for you to examine. Each cup, or depression, in the morel is lined with asci. Now examine the prepared slide of *Peziza*. This is a widely distributed genus that produces a cup-shaped fruiting body. You should be able to find the asci lining the cup, and you should also be able to see 8 ascospores within each ascus. Why are there 8 spores? You may need to refer to your textbook to find the answer.
5. **Basidiomycete specimens.** Examine the representatives of the Basidiomycota that your instructor has available in lab. There should be mushrooms, bracket fungi, puffballs, and maybe other types as well.
6. **Infected corn and wheat, and *Puccinia* prepared slide.** Examine the infected plant material. The stalks of wheat are infected with *Puccinia graminis*, which causes stem rust of wheat. The corn is infected with *Ustilago maydis*, which causes corn smut. Both fungi are also members of the Division Basidiomycota. Now examine the prepared slide of *Puccinia graminis* showing the uredial stage on wheat. Can you distinguish the fungal spore cluster (called a uredium or uredial stage) from the host tissue?

7. ***Aspergillus* and *Penicillium* cultures and prepared slides.** *Aspergillus* and *Penicillium* are very common asexual fungi. Although mycologists know that these fungi are members of the Ascomycota, the asexual stage is so common (and the sexual stage is so rare) that we can still consider them asexual fungi. Using the dissecting microscope, examine the cultures of these fungi. Can you see the asexual reproductive structures? Examine the prepared slides of these genera. Can you find the conidiophores of these fungi? Draw a conidiophore and several conidia of each genus in the following space.

Penicillium	*Aspergillus*

EXERCISE B: Airborne Fungi

Fungi reproduce by spores, and the majority of fungal spores are dispersed by wind. Fungal spores are therefore a natural component of the atmosphere. In some climates, fungal spores are present year-round, although they tend to be most abundant in late summer and fall. Many people are allergic to fungal spores both in the atmosphere and indoors. Spore concentrations in the atmosphere can reach levels of up to 200,000 spores per cubic meter of air although this is extreme, and more typical levels from the spring through the fall range between 5,000 and 25,000 spores per cubic meter of air. There are thousands of different types of spores in the atmosphere. In this exercise, you will perform an experiment to see how many colonies develop on culture plates exposed at various indoor and outdoor locations.

Materials Needed for Exercise B

Five or six culture plates containing malt extract agar or other suitable culture medium

Marker pen for labeling the lids

Tape to seal the plates

Procedure for Exercise B

1. Obtain from your instructor several petri dishes containing culture medium. Select locations for exposing the petri dishes. If the weather is mild, you might choose several outdoor settings, such as a wooded area, park, city street, and suburban lawn. Select at least one indoor location as well. If it is winter or raining, you might want to select all indoor locations. Your instructor may specify certain locations or have other suggestions. Also, you might want to expose the plates at different times of the day to see if the same types of fungi are present at different times.
2. When you are at each location, remove the lid from the dish. The open dish should be exposed for 10 minutes. Do not put the dish on the ground. After the 10 minutes are up, close the dish and tape the lid on. Be sure to write the location and your initials on the lid.
3. Bring the exposed petri dishes back to the lab, and place them in the room-temperature location your instructor indicates. Make sure natural light is available, since many fungi need light–dark cycles in order to develop. Allow the cultures to remain undisturbed during the incubation period.
4. After 3 days, check the cultures for growth. After 5 days, count the number of colonies. It is usually easier to count the colonies from the reverse side. Be sure to keep the lids closed. Remember that spores are easily airborne and many people are allergic to the spores.
5. Record the number of colonies and answer the questions on worksheet 18-1 at the end of this laboratory topic.

EXERCISE C: Fungal Foods and Fermented Products

Life would be dull without some of the products of fungi. Many mushrooms and other fleshy fungi are eaten as vegetables or gourmet delicacies. Fungi are responsible for the formation of several cheeses; camembert and blue cheese (including Gorgonzola, Roquefort, Stilton, etc.) are the product of fungal metabolism. Your instructor may have some of these available in lab.

The activities of yeast produce both bread and alcoholic beverages through the process of **fermentation**, the anaerobic respiration of glucose to produce ethanol and carbon dioxide. The glucose is only partially broken down, and only 2 molecules of ATP are produced for each molecule of glucose. In baking bread, the carbon dioxide causes the dough to rise (the alcohol vaporizes during baking). In brewing, the alcohol is the product of interest, and the carbon dioxide usually bubbles off. Both beer and wine are produced through fermentation by yeast. In this exercise, we will inoculate grape juice with two different types of yeast (champagne yeast and bakers' yeast). We will check these later in the week for both carbon dioxide and alcohol production.

Materials Needed for Exercise C

Four balloons

Four sterile 250 ml-flasks

Grape juice

Hygrometer to measure sugar/alcohol content

Stock cultures of champagne yeast and bakers' yeast

Tape measure

Procedure for Exercise C

1. Review the instructions about sterile technique in Laboratory Topic 2.
2. Wipe your lab bench with a cotton ball dipped in 15% bleach solution.
3. Pour some grape juice into a graduated cylinder, and measure the percent sugar using the hygrometer. (If a refractometer is available, it can be used instead of the hygrometer.) Record this number in the chart on worksheet 18-2.
4. Using sterile technique, pour 150 ml of grape juice into each of four sterile flasks.
5. Use a sterile 5-ml pipet and pipetor to add 5-ml of the champagne yeast stock to two flasks.
6. Use a second pipet to add 5 ml of the bakers' yeast stock to two flasks.
7. Carefully cover each flask with a balloon.
8. Use a tape measure to measure the diameter of the balloons on day 4 and day 7. Enter the measurement on worksheet 18-2.
9. Measure the sugar/alcohol content again in 7 days.

TERMS TO KNOW

absorptive heterotrophs 233
Ascomycota 234
ascospore 234
ascus (pl., asci) 234
Basidiomycota 234
basidiospores 234
basidium (pl., basidia) 234
conidiophores 234
conidium (pl., conidia) 234
fermentation 236
mycorrhizae 233
saprobes 233
sporangiophore 233
sporangium 233
Zygomycota 233
zygospores 233

QUESTIONS FOR REVIEW AND DISCUSSION

1. Describe the asexual spores produced by fungi in the Division Zygomycota.
2. Why are there 8 ascospores within the ascus in ascomycetes?
3. Where do the basidiospores form in bracket fungi?
4. If the outdoor concentration of fungal spores is 10,000 spores per cubic meter of air, how many spores do we inhale when we are outside for 15 minutes? (*Hint*: With moderate activity, we inhale 10 liters of air per minute. How many liters are there in a cubic meter?)
5. The uredial stage is called the "repeating stage" of the life cycle in rust fungi. What does this mean?
6. Most fungi are saprobes. What does this mean? Where do saprobes get their nutrients? What is the value of these organisms to the environment?

ADDITIONAL RESOURCES

Kendrick, B. 2000. *The fifth kingdom*, 3rd ed. Newburyport (MA): Mycologue Publications, Focus Text.

Levetin, E., and K. McMahon. 1999. *Plants and society*, 2d ed. New York: McGraw-Hill Companies, Inc.

ON THE WEB

American Phytopathological Society
http://www.apsnet.org/

The University of Edinburgh, The Microbial World, The Fungal Web
http://helios.bto.ed.ac.uk/bto/microbes/fungalwe.htm#crest

University of California—Berkeley, Introduction to the Fungi
http://www.ucmp.berkeley.edu/fungi/fungi.html

OTHER ACTIVITIES

1. Typically, there are about 1,000 to 10,000 spores in every gram of soil. To isolate fungi from soil, bring a spoonful of soil from near your home or dorm room. Weigh out 1 g, and add to 9 ml of sterile distilled water. Do a dilution series by transferring 1 ml of this first tube to a second test tube containing 9 ml of distilled water. Repeat until you have reached 10^{-3} and 10^{-4} dilutions. Using a sterile pipet, transfer 1 ml of the 10^{-3} suspension to a petri dish containing malt extract agar plus streptomycin. Carefully spread the soil suspension over the entire surface of the agar. Repeat with the 10^{-4} suspension and a second petri dish of agar. Allow the dishes to incubation for approximately 5 days at room temperature. Count the number of

colonies. If you assume that each colony developed from a single spore, calculate how many spores were in 1 g of your soil sample.

2. Lichens are symbiotic associations between a fungus and an alga. The algal partner provides carbohydrates from photosynthesis, while the fungus supplies water and minerals. Lichens are grouped into three categories on the basis of overall form: crustose lichens, which form a crustlike growth on rocks and other substrates; foliose lichens, which form a leaflike body; and fruticose lichens, which have an erect, branched body. Lichens are often deeply pigmented and have been used for centuries as a source of natural dyes. Examine the lichens available in lab and determine if they are crustose, foliose, or fruticose.

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 18-1 EXERCISE B: AIRBORNE FUNGI

LOCATION	NUMBER OF COLONIES

1. Can you tell any differences between the appearance of the fungi from the outdoor samples and the indoor samples?

2. Were there differences at different times of the day?

3. Would you say that the indoor locations you selected were contaminated?

4. The spores that landed on the culture plate produced colonies using nutrients available in the medium. In the natural environment, what might these fungi be growing on?

NAME

DATE

LAB SECTION NUMBER

WORKSHEET 18-2 EXERCISE C: FUNGAL FOODS AND FERMENTED PRODUCTS

	DIAMETER OF BALLOON	PERCENT ALCOHOL
Bakers' yeast—Day 1	0	
Bakers' yeast—Day 4		Do not measure
Bakers' yeast—Day 7		
Champagne Yeast—Day 1	0	
Champagne Yeast—Day 4		Do not measure
Champagne Yeast—Day 7		

Which type of yeast was quicker to ferment the sugar in the juice? Why?

APPENDIX A

Science as a Process

Science is more than a mere collection of facts. Science is a process of acquiring new knowledge. Science is a systematic way of discovering new explanations based on empirical evidence about the natural world. Science is a **way of knowing**. Science is also a social endeavor.

To the general public, science is often misunderstood. A typical image of a scientist is some geek in a lab coat surround by beakers spewing chemicals. Science is much more.

Science is a process for understanding the inner workings of our universe. Scientific explanations are based on **empirical** observations or experiments. These observations can be made by the scientist directly or with the aid of technology to extend his or her perceptions. Scientific explanations strive to distinguish between cause–effect relationships and mere correlation or coincidence. Scientific explanations are said to be tentative, and the explanations are modified as more evidence is uncovered. The current explanations of the world are those that best fit the available evidence to date. Finally, scientific explanations are made public to other scientists. In this way, every explanation is scrutinized by other scientists.

So what is the process of science? First, scientists observe. They look at natural objects, such as the planets or living plants, and look for patterns or discrepancies. Their observations lead to wonder and speculation about the natural world. Usually scientists make a conscious effort to distinguish what is known from what still needs to be known. The connections between the known and the unknown are questions. A scientific question is based on observations and seeks answers about the natural world. Some questions are too broad to answer; other are beyond the realm of science to answer. From this pool of questions, scientists select a realistic question and formulate a **hypothesis**, or possible explanation, for the phenomenon. If the hypothesis is correct, a scientist can make a prediction about what they will observe if they design an experiment or test of the hypothesis. Scientists test their predictions by performing a controlled experiment or by making more observations in the field. Often several alternative hypotheses are tested and compared to each other based on the strength of the supporting data. Hypotheses are accepted or rejected based on the empirical evidence. Usually the explanations are based on a statistical view of nature. Scientists observe samples of the natural world, rather than all possible events. In this way, scientific explanations are probabilistic, not absolute.

A **scientific theory** is a synthesis of facts and explanatory concepts about a phenomenon of nature. This definition is different from the common colloquial use of the word theory, which often means a "hunch" or "guess." A scientific theory is a generalized explanation derived from many experiments and many observations. A scientific theory is useful not only to answer some of the original questions, but also to guide further investigations by suggesting new questions. As more evidence is collected, all scientific theories are retested and again scrutinized. Some theories may eventually be rejected, some are modified, and some are supported even more. A scientist always continues to observe, to question, and weigh the strength of evidence.

Anyone can approach the pursuit of knowledge from the scientific perspective. The mechanic who observes smoke coming from an engine often formulates a hypothesis about what is wrong based on prior observations. If he is a good mechanic, he systematically collects more observations—for example, by checking the condition of the spark plugs or the timing of the distributor. Often this leads directly to diagnostic tests or experiments. Each test has its own prediction of what the mechanic expects to happen if his hypothesis is correct. The mechanic may not realize that he is systematically testing each hypothesis until he has collected enough evidence to make a convincing argument about what is causing the smoke.

The mechanic is more interested in solving a problem, while a botanist may be more interested in understanding the underlying relationship revealed by nature. But both the mechanic and the botanist base their explanations on the observations they make. In either case, if their explanations are not supported when additional observations are made, they must reject the old explanations and seek new ones. In this way, the process is self-correcting and based on the empirical evidence. The

mechanic who accepts an incomplete or incorrect explanation for the smoke will most likely not solve the problem, and his customer will return in a rotten mood. The explanation accepted by the botanist is also only as good as the strength of the data.

SCIENTIFIC INQUIRY

The process of inquiry in science is often described as the **scientific method**. However, in all reality, scientists use many methods to develop a clearer understanding of the world, such as mathematical modeling or direct observation. The so-called "scientific method" usually refers to the use of controlled experimentation to test hypotheses. Because this is a powerful method, it is explained in depth here. The scientific method usually includes the following steps:

1. Observing
2. Questioning
3. Formulating a hypothesis
4. Designing an experiment to test hypothesis
5. Making a prediction
6. Conducting an experiment, collecting data
7. Analyzing the data
8. Interpreting the results
9. Drawing conclusions
10. Communicating the results to others

Step 1: Observing

Observing the natural world is the start of any investigation. Often it is done without much thought, but soon patterns appear—for example, trees lose their leaves in the fall, or the sun always rises in the eastern sky. Occasionally, events that do not fit these patterns occur. These exceptions may be as important as the events that follow the patterns.

To approach observations as a scientist, you should strive to record all you observe, both the details and the overall impressions. As you look more carefully, you will see features you overlooked before. Every scientist must first be skilled in observing. You may take advantage of technology to extend your senses, such as by using a microscope or a precise temperature gauge. Either way, these are still observations of real phenomena.

Step 2: Questioning

Your observations should spark your curiosity and help you pose meaningful **questions**. These questions are the links between what you already know and what you still need (and want) to discover. Questions represent problems in need of solutions. Often, the discrepant events stimulate more questions than the patterns do.

To approach a problem scientifically, you should record your questions. You may find that some of the questions are so broad they cannot be answered using the current technology. You may also find that some questions are trivial and not of great significance or relevance. Eventually, you will choose questions that are worth answering and within your capacity to answer. You can then narrow your investigation to answer these questions one at a time.

Step 3: Formulating a Hypothesis

Hypotheses come directly from the questions. A **hypothesis** is a possible explanation for the problem. In many ways, it is an educated guess, often based on prior knowledge and insight into the problem. Once stated, you can test your hypothesis.

Step 4: Designing an Experiment to Test the Hypothesis

Before proceeding, you must decide what type of evidence or data is needed to test your hypothesis. In some cases, you can answer your question by making more observations of the phenomenon. For example, you may observe the behavior of butterflies as they pollinate flowers. In other cases, you may find that conducting an experiment in which you manipulate one part of the environment is the best way to answer your question. The nature of the investigation should be matched to the question and the nature of the data needed to address the question. Since you will be testing your hypothesis, you should ask yourself how you can best get the evidence needed to make a good decision.

An **experiment** allows you to control your system so that your conclusions are less ambiguous. To accomplish this control, you must consider the factors that may affect your phenomenon. We call these factors variables; they can be either dependent or independent. The value of a **dependent variable** is determined by the value of an **independent variable**. For example, the distance traveled by milkweed seed may be dependent on the wind velocity, whereas the reverse is not true. In this case, the distance is the dependent variable, and the wind velocity is the independent variable. In a **controlled experiment**, only one independent variable is allowed to change, while the other independent variables are held constant. The investigator should recognize that additional variables may influence the dependent variable but may not be able to be controlled. Any **uncontrolled variables** should be recognized, and if possible their potential impact should be minimized. Remember, though, that living systems are by nature quite complex and variable. That is why you need to repeat your experiment several times and include as many **replications** of the experimental units as you can reasonably handle.

Variables also can be discrete or continuous. A **discrete variable** is divided into distinct groups, such as dif-

ferent species of plants. A **continuous variable** has an unlimited number of intermediate values, such as the velocity of the wind or the dry weight of the seeds.

The type of **statistical tests** to be conducted should be decided in advance, based on the type of data to be collected. The nature of the data analysis can dictate the manner in which experimental units are arranged or randomly selected for measurement. Consulting with a statistician prior to conducting the experiment can save time later. This advance planning will ensure that the experiment is reproducible should you or someone else need to repeat it.

Step 5: Making a Prediction

Before conducting the experiment, make a **prediction** for each hypothesis. The easiest way to do this is to rewrite your hypothesis as an "If—then" statement. The prediction is one more way to see if the data to be collected will address the problem. If not, adjustments can be made while the experiment is still in the planning phase.

Step 6: Conducting an Experiment, Collecting Data

To some people, conducting the experiment is the part of the process they love. To others, it is the monotonous yet necessary phase of the scientific method. The researcher needs to take special note of any unusual or unexpected results. Care should be taken to avoid any bias in the **data collection**. Measurements should be as precise and accurate as necessary to provide meaningful data.

Step 7: Analyzing the Data

Data should be summarized in tables or graphs in a manner that best explains the major results. The raw data should not be presented in totality, but rather processed to reveal trends or comparisons.

The appropriate statistical analyses should be conducted to **summarize data** and reveal any significant differences among treatments or statistical correlation. The data should meet the sampling criteria.

Presentation of the summarized data depends on what the researcher wants and needs to illustrate. A table can be a compact way of presenting exact values or data that do not fit into a simple pattern. Tables are also useful for classifying information. Bar graphs or histograms are best for illustrating differences between treatments, especially if the treatment types are discrete. Line graphs are appropriate for plotting continuous data to show trends or interactions between two or more variables. Pie charts are best for showing and comparing relative parts of a whole. Photographs or detailed line drawings are the best way to describe an entire object. Data connected to geographical sites can be displayed on graphical maps. Qualitative data is best explained in written descriptions.

The data analysis should go back to the original hypothesis and experimental design. First, you should keep in mind why the data were collected in the first place, making sure to process the data so that you can address your question and test your hypothesis. Second, you should consider how to convince other scientists that your interpretation of the data is sound.

Step 8: Interpreting the Results

After summarizing the data, you are ready to **interpret the results**. You should decide whether you accept or reject your hypotheses. Sometimes the experiment suggests new insights into the relationships behind the problem. You may generate new questions that can lead you in new directions. You should also compare your results with the studies of other scientists. Do your data agree or disagree with other studies?

Step 9: Drawing Conclusions

In this step, you prepare a convincing argument about what you learned from your experiment. Based on the interpretation of your data and the data of others, you can make a **conclusion** about the phenomenon under study. Be sure to stay within the boundaries of the data and study. Unsupported speculation extends your conclusions beyond your evidence and is thus unscientific. Your conclusions should be supported by your data, your empirical evidence. If you do not have enough data, you should repeat or redesign the experiment. Often, conclusions are small steps toward a generalized theory that is made after several experiments put all the pieces of the puzzle together.

Step 10: Communicating the Results to Others

The final step of any scientific study is to present your results to others. Science is a social endeavor. You are not done with your investigation until you attempt to **convince others** that your experimental design was sound, your data meaningful, and your conclusions appropriate. You should communicate your findings to your classmates. Present your original question, your experimental design, your summarized data, and your conclusions. What did you learn? What do you still want to know?

APPENDIX B

Field Trip to a Health Food Store

Health food stores typically sell many items besides "healthy" foods. A visit to such a shop can underscore many concepts from this course. Although it would be easy to spend several hours roaming around the store just looking at the produce and exotic food items available, this field trip has definite objectives. Consider this exercise a scavenger hunt. You will be looking for various items in certain categories. For each of the categories, you will list the items found, other information requested, and the unit cost. For the unit cost, indicate the price per package, per bottle, or per pound, or however the product is sold.

Look for foods or products in the following categories:

1. Find 7 different types of grains available in bulk as whole grains. List the grain, its principal use, and the unit cost.

   a. _____

   b. _____

   c. _____

   d. _____

   e. _____

   f. _____

   g. _____

2. Find 7 types of edible vegetable oils. List the oil, the source (i.e., whether it is from a seed, a fruit, etc.), and the cost.

   a. _____

   b. _____

   c. _____

   d. _____

   e. _____

   f. _____

   g. _____

3. Find 7 different types of legume seeds. List the legume, grams of protein per serving, and the unit cost.

   a. _____

   b. _____

   c. _____

   d. _____

   e. _____

   f. _____

   g. _____

4. Find 7 types of essential oils that have medicinal applications. List the oil, its principal use, and unit cost.

   a. _____

   b. _____

   c. _____

   d. _____

   e. _____

   f. _____

   g. _____

5. Find 7 types of produce that are new to you. List the produce, its type (fruit or vegetable), its country of origin, and the unit cost.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

6. Find 7 meat substitutes that are available for vegetarian diets. List the product, the main plant ingredient, and the unit cost.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

7. Find 7 types of unsweetened snack foods, each prepared from a different crop, (i.e., chip-type or nut-type foods, not cookies or candy). List the food item, the crop used, and the unit cost.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

8. Find 7 types of plant-based cosmetics (soaps, shampoos, hand creams, and shaving creams, as well as typical cosmetics). List the item, the plant material used, and the unit cost.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

9. Find 7 types of herbs or spices that are available fresh or in bulk (not packaged). List the item, the plant part used, and the unit cost.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

10. Find 7 types of juices that are new to you. (This disqualifies the usual orange juice, apple juice, tomato juice, grapefruit juice, etc.). List the juice, and indicate whether it is made from a fruit or a vegetable, its country of origin, and the unit cost.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

APPENDIX C

A Taster's Sampler of Caffeine Beverages and Foods

Caffeine is a small, bitter-tasting alkaloid found in tea leaves, coffee and cocoa beans, cola nuts, and other plant sources. Tea, coffee, cocoa, and cola are popular global drinks, and all are plant-based caffeinated beverages. They have been valued for centuries for their stimulating properties as well as for their flavors and aromas.

In this exercise, you will prepare and sample an assortment of caffeine beverages and foods.

COFFEE

Coffee is made by seeping in boiling water the roasted and ground beans (seeds) of the coffee tree. There are many species of coffee trees, but the commercially important ones are *Coffea arabica, C. canephora (robusta),* and *C. liberica.*

1. Examine the living coffee trees with flowers and fruits. What is the composition, arrangement, and venation pattern of the leaves?

2. If present, examine the flowers of the coffee tree. Are the flowers terminal (at the end of branches) or axillary (in the axile of leaves)? Do the flowers have a scent? Is the scent fragrant, musty, foul, or other? Do you think coffee flowers are wind or animal pollinated? Why?

3. What are the number of sepals, petals, stamens, and carpels? Is the flower regular or irregular? Monocot or dicot?

4. If present, examine the coffee fruit, called a cherry. Is it fleshy or dry? Determine the fruit type.

5. The flavor and aroma of a cup of coffee depend much upon the roasting of the beans. Light roasts are produced at lower temperatures (212–218°C /414–424°F) than are dark roasts (240–250° C/464–482°F.) As the temperature of roasting increases, sugars caramelize, turning the seed from the color of ivory to dark brown. At the highest temperatures, carbonization begins as the sugars burn, and carbon darkens the bean further. Compare the beans from several different types of roasts. Arrange the beans from light to dark roasts, and record the color of the beans in the following space.

6. The best coffee is grown in the cooler climate of subtropical and tropical highlands where there is plentiful rainfall and no danger of killing frost. Examine several brands of coffee, and list the premier areas of coffee cultivation.

7. Coffee can be prepared in a variety of ways. Enjoy and sample several coffee preparations (e.g., espresso, latte, cappuccino, caffe mocha), and write how they are prepared, in the following space:

TEA

Strictly speaking, tea is made from an infusion of the leaves of *Camellia sinensis*, the tea plant. Harvested tea leaves are treated differently to produce black, green, or oolong teas.

Black teas are fully fermented, during which the tannins in the leaves become oxidized and turn black in color. Oolong teas are fermented for a shorter period, and that's why their leaves are lighter in color than black teas. Green teas are not fermented at all and the tannins remain colorless. Firing a blast of hot air stops the fermentation process. Black tea is the most widespread tea worldwide, although green teas have always been favored in the Orient and are gaining popularity in the West.

Tea leaves are also graded or sized. The three main grades of black and oolong teas (from small to large) are orange pekoe, pekoe, and souchong. There are also broken grades, which are not an entire leaf but cuts of a leaf; these include broken orange pekoe, broken pekoe, broken pekoe souchong, broken orange pekoe, fannings, and dust. Broken orange pekoe is much sought after as it is a very small size and contains the leaf tip. In green tea, buds, unopened young leaves, are highly prized.

1. Prepare a proper pot of tea. Bring water to boil. Add a small amount of boiling water to the empty tea pot. Swirl to warm the pot, and then discard the water. Add 1 level teaspoon of loose tea for every 8 ounce cup of water. For black teas, let the infusion seep for 3-5 minutes. Oolong teas require more time, from 5–12 minutes. Green teas are ready after a minute or two. Using a tea strainer, pour the tea into individual cups.
2. Examine a variety of teas, and determine whether they are black, oolong, or green.
3. Examine a variety of loose teas, and see if you can distinguish some of these grades.
4. Tea grows best at higher elevations in subtropical or tropical climates where there is abundant rainfall and no danger of frost. Examine the tea packaging to find out the major tea-growing regions in the world. List your findings here:

Tea tasters are employed by tea companies to select and purchase the tea harvests from around the world. A tea taster has a palate so well developed that

by "slurping" a tea sample (a spoonful sprayed against the back of the mouth, producing the characteristic sound), the taster can identify where a tea was grown, when it was picked, how it was processed, and in this way, its value.

Look for the desirable and undesirable qualities of taste, grade, and color as listed in tables C1 and C2 as you sample a variety of black teas.

5. Blends of tea often incorporate herbs, spices, or other flavorings. List the ingredients for some perennial tea blends, such as Constant Comment and Earl Grey, and any others available.

6. Herbal teas, or tisanes, are made from plants other than *Camellia sinensis*. Most do not contain caffeine, but there are some exceptions. Maté tea is made from the leaves of a holly (*Ilex paraguariensis*) and does contain low levels of caffeine. Sample the variety of herbal teas available, and list at least five different herbs as well as the part of the plant used in the preparation.

HOT COCOA AND CHOCOLATE

1. The beans of the cacao tree (*Theobroma cacao*) are the source of both cocoa and chocolate. In processing, the harvested beans (seeds) are roasted at temperatures of 120–140°C/248–284°F for almost an hour. Chocolate liquor is then expressed from the large cotyledons of the seeds. The chocolate liquor is solidified to make bakers' chocolate. Examine a sample of bakers' chocolate, and if you wish, taste it. What does it taste like?

2. If the fat is removed from the chocolate liquor, the end product is a brown cake that is pulverized into cocoa powder. Sample the taste of cocoa powder, and compare it to bakers' chocolate. Check out the ingredients on the cocoa powder package. What has been added to reduce the acidity of the cocoa powder? This processing, called dutching, was named for the originators of the process.

3. The fat removed from the original chocolate liquor is cocoa butter. White chocolate is primarily cocoa butter with added spices; it contains no chocolate liquor at all. What spices and ingredients are found in white chocolate?

4. Examine various cosmetic products containing cocoa butter as the primary ingredient, and list them in the following space. What are the uses of cocoa butter?

5. In the making of confectionary chocolate, more cocoa butter is added to the chocolate liquor. Cocoa butter melts at body temperature, and that's why "melts in your mouth" is characteristic of quality chocolate. Cheaper chocolate candies are often processed with cheaper vegetable oils that replace the cocoa butter and the melting point. Many other ingredients (vanilla, whole milk, sugar, fruits, nuts, etc.) may be added to the chocolate base to achieve the desired taste sensation. Sample a variety of chocolate candies. Enjoy!

TABLE C1 DESIRABLE QUALITIES IN A TEA

TERM	DEFINITION
Attractive	Uniform color and leaf grade
Autumnal	Teas grown in this season
Body	Full-strength, rich taste
Bold	Leaves are too big for assigned grade
Bright	Lively taste
Character	Quality that pinpoints area of cultivation
Chunky	Tea with large-sized tips; desirable quality
Color	Varies with growing conditions and grade
Coppery	Desirable color of tea; denotes quality
Even	Bright coppery tea; no unevenness in color
Golden tip	Desirable; proper withering and rolling
Large	Leaf is larger than usual for its assigned grade
Neat	Well-made; quality tea
Orthodox	Tea picked and rolled by hand
Pungent	Brisk taste; desirable
Shotty	Well-made and rolled
Small	Leaves smaller than usual for assigned grade
Strength	Tea makes a presence in the mouth
Stylish	Neat; premium-quality leaf
Well-made	Beautiful leaf color; even texture and size

TABLE C2 UNDESIRABLE QUALITIES IN A TEA

TERM	DEFINITION
Bakey	Unpleasant taste due to high temperatures during firing
Burnt	Undesirable taste due to excessive heat during processing
Dry	Slightly bakey
Dull	Lifeless color
Flaky	Flat open leaf, improperly withered and rolled
Flat	Not brisk; flavor lost due to aging or improper storage
Moldy	Moldy taste
Musty	Moldy taste
Old	Flavor lost to aging
Thin	Tea lacks body; overwithered and or inadequate fermentation
Wild	Harsh/thin, undesirable; end-of-season teas

APPENDIX D

Notes to Instructors

NOTES TO INSTRUCTORS

LABORATORY TOPIC 1: CELLS OF CRYSTAL AND COLOR

EXERCISE A: PLANT CELLS WITHOUT WALLS

1. Buffer solution is approximately 13% mannitol or 0.625 M mannitol (FW 182.2). Prepare by dissolving 56.94 g of mannitol and add distilled water to bring to final volume of 500 ml. Adjust the pH to 6–7 by adding drops of 0.1N NaOH. Distribute 10 ml of buffer solution to each student team.
2. For each student group, measure out 0.1 g of pectinase and 0.2 g of cellulysin. These should be added to 10 ml of buffer solution immediately before use to make an enzyme concentration of 1% pectinase and 2% cellulysin (cellulase). Purchase pectinase and cellulysin from Sigma.
3. Protoplasts can be viewed directly through the petri dish or purchase microslides from Ward's to view at higher magnifications.
4. Ward's sells a Protoplast Isolation Kit (86W 8009) and Protoplast Fusion Kit (86W 8008) to use with geranium petals. The kits work well, and all you need to supply are the geranium plants with contrasting flower colors. Ward's will also sell the plants for additional cost.

EXERCISE B: COMPONENTS OF THE PLANT CELL

To prepare Sudan III solution for staining elaioplasts in avocado fruit, mix 70 ml of 95% ethanol in 30 ml of distilled water in a beaker. Heat gently. Add Sudan III crystals slowly with stirring until no more will dissolve. Decant. Place in dropper bottles.

EXERCISE C: PLANT ULTRASTRUCTURE

If you have an Electron Microscope facility, collect SEM and TEM micrographs for student folders. Add a question or worksheet for each micrograph to ensure that students carefully examine the collection. If not, bring in SEM and TEM atlases for students to examine.

EXERCISE D: CRYSTAL PERSUASION

If you wish, Triarch and Ward's sells prepared slides of *Ficus elastica* leaf cystoliths.

EXERCISE E: PLANTS TO DYE FOR

1. Note that betacyanins, found in beets (Chenopodiaceae) and species within the Aizoaceae, Amaranthaceae, Cactaceae, Nyctaginaceae, Phytolaccaceae, Portulacaceae and a few other families within the Caryophllales, are alkaloids and not natural pH indicators. They will not show the color change as the anthocyanins in red cabbage juice will.
2. It is best to pre-mordant the wool before dyeing. Alum and cream of tartar can be purchased from the spice section in the supermarket. Use natural (undyed) wool or wool that has been dyed natural or white. Using a 1,000-ml beaker, dissolve 105 g of alum and 1 g of cream to tartar in 500 ml of distilled water that has been heated to approximately 60°C. Add a length of wool (about 15 g), and simmer for an hour. Let cool. Dye directly or store the wool in a sealed plastic bag to keep moist until ready to dye.

LABORATORY TOPIC 2: CELL DIVISION AND CLONING

EXERCISE A: CELL DIVISION

1. For the root tip squash, broad bean roots work very well since the chromosomes are larger than those in onion. However, this same exercise can be done with onion root tips if you prefer. For broad bean, allow the seeds to germinate for 7 to 10 days. Soak the seeds in water for about 30 minutes and then plant in perlite. Keep them moist. After one week, dig up the seeds and check for secondary roots. You should use the secondary roots for the exercise. Depending on conditions in your lab, they may need another few days. Usually many secondary roots form from the primary root of each germinating seed. In fact, 5 or 6 seeds may be enough for a whole class. If you need to save some time in lab, you may want to harvest the roots and place in Carnoy's fixative (3:1 ethanol: acetic acid);

however, students get the connection with a growing root tip better when they harvest the roots themselves.

2. Toluidine blue stain works very nicely for staining chromosomes. To prepare 200 ml of stain, dissolve 0.2 g of toluidine blue O (Sigma T-3260) in 200 ml of 0.1 M benzoate buffer, pH 4.4 (0.25 g benzoic acid, 0.29 g sodium benzoate, 200 ml distilled water).
3. The results with broad bean root tips are often outstanding, and students may want to keep the slides. One quick method is to seal the slides with clear nail polish. Although not permanent, the slides will last for a few weeks or longer.

EXERCISE B: CLONES FROM TISSUE CULTURE

1. It is important to stress sterile technique, since the cultures can easily become contaminated. You should demonstrate how to sterilize instruments by dipping in alcohol and passing through a flame. Explain the safety precautions necessary when using alcohol near an open flame. If a laminar flow hood is available, have the students use it. This will dramatically cut down on the contamination. If not, make sure the students clear their lab benches and wipe the counters down with 70% ethanol. If it is possible to work in an area with limited air flow, this will also cut down on contamination. Nevertheless, many students will have contaminated cultures. One reason for using 3 or 4 petri dishes is to increase the chances of having one uncontaminated plate. Note: If you are using a laminar flow hood, make certain you use a shielded burner not a regular bunsen burner.
2. Tobacco works very well for tissue culturing, and this is a good opportunity to explain the value of tobacco as an experimental tool. Although students hear a lot about the negative aspects of tobacco, they should realize its positive value in research. The hormone concentrations given in step 4 are those specifically for tobacco callus. If you use other plants, you may need to find the correct concentrations for that plant. When cutting sections of tobacco stem, make sure the students avoid the nodes. Culturing works best with parenchyma cells in the pith. When the students trim off the epidermis, they can also remove the cortex and vascular tissue as well, leaving just the thin cylinder of pith.
3. For culture media, the same basal medium is used for the callus growth medium and the differentiation medium; only the levels of hormones differ. The callus growth medium is poured into petri dishes. You can use test tubes, but it is more difficult for students to retrieve the callus from the tubes. For differentiation of shoots and roots, the medium should be dispensed into magenta jars. If these are not available, you can use baby food jars. Both need to be sterilized before you pour the media.
4. Media can be made from scratch, but it is more convenient to use prepackaged media. Murashige and Skoog basal medium with sucrose and agar is available from Sigma (M-9274). Each package makes one liter of medium, which is enough for 50 petri dishes or about 20 magenta jars. Follow the directions on the package to prepare the medium. Adjust the pH to 5.8 with 1N NaOH prior to autoclaving. Place a stir bar in the flask before autoclaving. After autoclaving, add the hormones as follows: Both hormones should be separately dissolved in 1 or 2 ml of 1N NaOH (they are not water soluble) and filter-sterilized prior to adding to the culture medium. Allow the media to cool somewhat (but don't let it solidify). Place the culture medium flask on a magnetic stir plate and stir at medium speed for about one minute to distribute the hormones. Use culture media within a week of preparation. Prolonged storage can cause the hormones to degrade.
   a. For the callus growth medium, use 2.0 mg IAA per liter of medium and 0.2 mg kinetin per liter of medium.
   b. For the differentiation medium, use 0.02 mg IAA per liter of medium and 2.0 mg kinetin per liter of medium.
5. Ideally, cultures should be incubated in a growth chamber, but excellent results can be achieved using a bank of cool white fluorescent bulbs at room temperature in the lab.

EXERCISE C: CLONING HERBS FROM CUTTINGS

1. Many herbs root very easily from cuttings, but there are some things to watch out for. Make sure that the herbs are actively growing. Often herbs planted outdoors become dormant in the fall and do not root well. You might want to purchase some from a local nursery or have them growing in a greenhouse if one is available.
2. Make sure that you use a loose potting soil so that you will be able to dig the plants up easily in 2 weeks. A perlite-peat mix should work well. Have the students be careful when they dig up the plants, check for roots, and then repot.

LABORATORY TOPIC 3: PLANT TISSUES—THE FABRICS OF OUR LIVES

EXERCISE A: PLANT TISSUES

1. In this exercise, the students look at a mixture of both fresh and prepared slides. Students will need some practice in making thin sections. Encourage them to try several sections. It is sometimes helpful to have the students make a number of sections, float them in a bowl of water, and then select the thinnest one to mount on a microscope slide.

2. Toluidine blue and phloroglucinol-HCl are the two stains used in this exercise. The directions for preparing toluidine blue are given in the previous section. (see Laboratory Topic 2, Exercise A, Notes to Instructors.) Phloroglucinol-HCl is prepared by dissolving 1.0 g of phloroglucinol in 100 ml of water. Mix 1:1 with concentrated HCl. This reagent colors with age and probably should be prepared fresh about every month or 6 weeks. Store in brown bottles.

EXERCISE B: ECONOMIC FIBERS

1. Burlap is a good source of jute. Both sisal rope and manila hemp rope should be widely available at hardware stores. Tapa cloth should be available at craft shops.

EXERCISE C: PAPERMAKING

1. Although it is possible to make your own mold and deckle, papermaking kits are available that provide everything you need to make paper. Some even include dried plant materials. These kits are generally inexpensive; you should be able to find kits for around $20 or less. Look for them in craft shops, hobby shops, and even toy stores. These are not available from biological supply houses.
2. This part of the lab is time consuming, and you will need more than one kit for a large class. You should consider having some students work on papermaking while others are doing Exercises A and B, and then switching.

LABORATORY TOPIC 4: PLANT ARCHITECTURE

EXERCISE A: ROOTS TO ANCHOR AND ABSORB

1. Germinate radish seedlings 3–4 days prior to lab to see good root hairs. Keep in moist chambers since root hairs collapse readily in the air. Quickly transfer several seedlings to petri dishes lined with moistened filter paper for student examination.
2. Although carrots could be used instead of parsnips, the parsnip is preferable because it is white and the stains show up much better.
3. I_2KI turns blue-black in the presence of starch. To prepare I_2KI stain, dissolve 20 g of KI (potassium iodide) in 1,000 ml of distilled water. Using a magnetic stirrer, add 4.0 g of iodine to the potassium iodide solution. It will take some time for the iodine to dissolve. Store in brown bottles, as I_2KI breaks down in light. Dispense in dropper bottles.
4. Phloroglucinol-HCl will stain red the lignin found in secondary cell walls of xylem and sclerenchyma tissues. To prepare phloroglucinol-HCl stain, dissolve 1.0 g of phloroglucinol in 100 ml of distilled water. Then mix 1:1 with concentrated HCl. Store in brown bottles. Prepare fresh every month.
5. Prepared slides of *Ranunculus* (buttercup) root x.s. can be purchased from a number of biological supply houses (e.g., Carolina Biological). Make sure to purchase the mature root to show the endodermis.
6. Prepared slides of *Elodea* root x.s. are available from Carolina Biological. The root of this hydrophyte completely lacks conducting tissue, especially notable in the lack of any xylem. Living *Elodea* can be purchased from local aquarium stores or ordered from the biological supply houses.
7. Prepared slides of epiphytic (aerial) orchid root x.s. are available from Carolina Biological and Ward's. It has been suggested that the velamen, the multiple epidermis of aerial orchid roots, is capable of absorbing and storing water and minerals. The cells of the velamen are large, thick-walled, and dead at maturity.

   The fiery reed orchid (*Epidendrum radicans*) is the example of an epiphytic orchid with velamen that is put on display. It is very easy to maintain and propagate.

EXERCISE B: THE NUTS AND BOLTS OF STEM ANATOMY

1. The stainless steel nut-and-bolt microtome is from Dickey (1995). The diameter size of the nut is ¾-in., which makes a ½-in. diameter well. The bolt is 1-¼ in. long. These can be purchased from the local hardware store.
2. For the embedding medium, we recommend Paraplast, a mix of paraffin and plastic polymers available from Carolina Biological, but you can use paraffin wax. Melt before class time.
3. Most of the paraffin can be removed from the nuts and bolts by scraping with a dissecting needle. Soaking in xylene will remove any remaining traces of paraffin.
4. Methylene blue moves up the xylem, staining the vascular bundles. To prepare methylene blue, dissolve 1.5 g of methylene blue in 100 ml of 95% ethanol for stock solution. Dilute 10 ml of stock solution to 90 ml of distilled water.

EXERCISE C: LEAVES FOR IDENTIFICATION

1. Collect and distribute to student pairs, leafy twigs to illustrate leaf characteristics and to use in the leaf key.
2. Prepared slides of *Ligustrum* leaf x.s. can be obtained from Ward's, Carolina Biological, and other biological supply houses.
3. Prepared slides of *Aloe* leaf x.s. can be obtained from Carolina Biological.

EXERCISE D: LEAF SKELETONS—A VICTORIAN CRAFT

One-hundred percent lye can be purchased inexpensively from the grocery store. It is sold as a drain opener. Red Devil is a common brand.

EXERCISE E: SUPERMARKET BEAUTY

Purchase a variety of vegetables that illustrate supermarket botany. Provide a knife for cross-sectioning to examine internal anatomy. Check out the plant list for Laboratory Topic 13: Foods from Underground and Far Away to avoid repetition. Many of the starchy vegetables and some exotic produce are covered in a similar exercise in this lab topic.

LABORATORY TOPIC 5: PLANTS DO IT ALL—PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION

EXERCISE A: THE INS AND OUTS OF CO_2

Using Vernier CO_2 gas sensors to monitor levels of CO_2 in closed systems allows students to ask meaningful questions, design experiments, and collect data within a normal 3-hour lab period. If Vernier CO_2 gas sensors are not available, increasing CO_2 levels can be detected with a NaOH trap that is titrated to determine the amount of CO_2 produced.

1. If CO_2 levels do not change in 45–60 minutes, try increasing the number of plants or living material.
2. High light intensity is critical for some plants to detect decreases in CO_2 via photosynthesis. The lamp needs to be placed behind a water-filled heat shield to prevent scorching of leaves.
3. Care should be used when handling sodium pyrogallate. This is best handled in an exhaust hood and not inhaled.
4. To make surface impressions of leaves to see the stomata, cellulose acetate works well. In the past, clear fingernail polish was a good source of cellulose acetate. If diluted by half with acetone, this solution can easily be applied to the surface of leaves, allowed to dry, and then peeled off. If cellulose acetate peels are difficult to remove, placing leaves in a warm oven can help pull leaf tissue from the cellulose acetate. The peels can be examined directly with the compound microscope. If contrast of the surface is too faint, a standard stain such as cotton blue can be added to the cellulose acetate solution.
5. Some fingernail polishes available today have other ingredients that affect the leaf impressions. You may need to try several brands before finding a suitable solution. A camel hair brush can be used to apply the cellulose acetate, but it should be cleaned thoroughly with acetone between uses.
6. Another way to see stomata is to examine a leaf of a purple *Zebrina*. The purple anthocyanins are deposited in the normal epidermal cells. The guard cells do not have the pigment, so they appear almost fluorescent against a purple background.

EXERCISE B: SAVING FOR ANOTHER DAY—STORING STARCH

For this exercise to work well, it is important that half of the plants be kept in the dark for at least 4 days. Often, if the period is shorter than this, there will still be residual starch. The activity listed in the "Other Activities" using a black-and-white negative works well if you start with a plant from the dark and then attach the negative and grow in the light for another 4 days.

EXERCISE C: TRANSPIRATION

Cobalt chloride strips can be purchased from several biological and chemical supply companies. Alternatively, the strips can be made by soaking filter paper in 5% cobalt chloride solution (5 g cobalt chloride in distilled water; total volume of solution = 100 ml). The filter paper can be air-dried on paper towels and then dehydrated in a drying oven. Strips should be stored in a sealed jar over calcium chloride when not being used. Cobalt chloride strips can be reused if dehydrated before use.

EXERCISE D: CORN CLOUDS

This exercise can be connected with a tour of modern farming practices. It is an excellent way of relating plant growth and physiology to food production.

LABORATORY TOPIC 6—SAY IT WITH FLOWERS

EXERCISE A: FLOWER STRUCTURE

Have a variety of different flowers available in the lab. Make sure you have single flowers and inflorescences. Also make sure to provide both animal- and wind-pollinated flowers.

EXERCISE B: POLLEN MORPHOLOGY

1. Only a limited number of pollen slides are available commercially from biological supply houses. For the pollen in this exercise, you will need to make the slides yourself. The pollen can be collected from the field if you plan ahead, but it may be easier to order the pollen from a lab that prepares extracts for allergy injections. Four of these pollen types will also be needed to make slides for Exercise C. Greer Laboratories, Inc. (P.O. Box 800, Lenoir, NC 28645, 828-754-5327) stocks a wide variety of pollen types and charges about $3 to $8 per gram for common pollen types. Other labs around the country also have pollen available. One gram of each pollen type will be plenty.
2. Once you obtain the pollen, make permanent pollen slides using a glycerin-jelly mounting medium containing basic fuchsin or phenosafranin to stain the pollen.
3. Glycerin-jelly mounting medium contains 20 g of gelatin, 70 ml of water, 60 ml of glycerol, and 2.4 g of phenol. Prepare as follows: Boil distilled water. Measure out 70 ml and pour into a beaker containing 20 g of gelatin. Boil again to dissolve the gelatin. Remove from the heat. Add 60 ml of glycerol and 2.4

g of phenol (use a mask and gloves when handling the phenol). Stir well to mix. Add about 4 mg of basic fuchsin or phenosafranin. (Both dyes stain the exine, or outer pollen wall, a reddish-purple color.) Stir well to distribute the stain evenly. Pour the mounting medium into small bottles and refrigerate for storage. The mounting medium solidifies as it cools.

4. To prepare slides, place the jar of glycerin-jelly in a water bath at about 50°C or above. This will melt the mounting medium. Put a drop on a slide, quickly stir in a sample of the pollen, and mix well. (Try using a toothpick to pick up a small sample of the pollen.) Add a coverslip and press down slightly. Some of the mounting medium may ooze out. After the mounting medium solidifies, use a razor blade to trim off excess glycerin-jelly that has oozed out and hardened. Many people do not consider glycerin-jelly a permanent mounting medium and suggest sealing the slide with clear nail polish; however, this recipe has produced slides that have lasted over 10 years without sealing. (*Note:* Use great care when cleaning the slides. If you rub hard you can disrupt the mounting medium.)
5. *Juniperus* pollen has a thin exine (outer wall). Often, when mounting the pollen in any type of mounting medium, the intine (inner wall) swells and ruptures through the exine. Tell students to ignore any ruptured grains and to look for intact ones.
6. *Pinus* pollen has two large air bladders. Students often think this pollen looks like a Mickey Mouse hat or a toreador's hat. Sometimes air gets trapped in the air bladders, and the bladders look black under the microscope.
7. The pollen stain used for making wet mounts by the students contains 16 ml of glycerol, 35 ml of 95% ethanol, 49 ml of distilled water, and 3 mg of basic fuchsin or phenosafranin. Place in dropper bottles. It will last for years.

EXERCISE C: PALEOECOLOGY—THE USE OF POLLEN TO STUDY PAST VEGETATION

1. If your campus has a palynologist (mostly likely in the geology department) who is doing work in paleoecology, you may be able to obtain slides that represent local sites.
2. The site mentioned in the lab exercise is Ferndale Bog in southeastern Oklahoma, and you can prepare pollen slides that simulate core samples from this site. These simulated slides are based on the percents from the actual samples. They differ in that there are fewer pollen types in the simulation.
3. Place each of the following pollen mixtures into a separate small bottle or vial. You may need to wash the pollen off the weighing paper with a small amount of distilled water (1.0 to 2.0 ml). Add additional distilled water to the vial to bring the total to 10.0 ml and mix well. The following pollen mixtures represent the major vegetation during the time periods from Ferndale Bog (*Note:* This is a simplified pollen profile, since other pollen types have been omitted.)
   a. 700 years ago (20 mg pine, 25 mg oak, 3 mg grass, 3 mg ragweed)
   b. 2,000 years ago (25 mg pine, 15 mg oak, 3 mg grass, 2 mg ragweed)
   c. 5,000 years ago (10 mg pine, 25 mg oak, 6 mg grass, 2 mg ragweed)
   d. 8,000 years ago (5 mg pine, 25 mg oak, 21 mg grass, 3 mg ragweed)
   e. 12,000 years ago (5 mg oak, 5 mg grass, 50 mg ragweed)
4. Label microscope slides with the time period. Place one drop of the pollen mixture on each slide. Place the slides on a slide warmer until all the water evaporates. Add a drop of melted glycerin-jelly mounting medium. Using a toothpick, stir well to distribute the pollen through the mounting medium. Add a coverslip and gently press down. Remove the slides from the slide warmer and allow the mounting medium to solidify. Remove any excess mounting medium. Repeat for the other time periods.
5. Interpretation: Ragweed is a pioneer plant that quickly invades disturbed areas. Dominance by grasses may indicate a prairie phase in succession and/or limited rainfall. Tree species indicate more rainfall and/or later stages in succession. Have the students describe what they think may have been the changes in vegetation and climate during the past 12,000 years.

EXERCISE D: DOES AIRBORNE POLLEN AFFECT HOW WE FEEL?

1. *Please note:* This exercise is seasonal. It works especially well during the ragweed season in the fall—late August through September in many parts of the United States and also during the spring tree pollination period, generally in April (February to April in the South). During other times of the year, this exercise probably will not work.
2. You will need to provide symptom diaries to your students. Students should use the one in their lab manual for summarizing their data the next week. Depending on the size of the class, you might want to distribute from 5 to 10 diaries each. The aim is to get a cross section of the students on campus.
3. About 20 to 25% of the population has allergies, and not all allergy sufferers are allergic to ragweed pollen (or spring tree pollen). So remind the students that *only a small percent of respondents will have symptoms* at any

one time of the year. However, if enough diaries are distributed around campus, the students should be able to see some trends that reflect the pollen counts.

4. This exercise has the potential to generate a great deal of data. Compiling all the data in class the next week might take a lot of time. You may want to decide ahead of time which symptoms to focus on in class if time is a problem. (The first three symptoms are good indications of hay fever, and the next three asthma.) If you have a computer in the lab, you might want to set up a spreadsheet and have the students enter their summary data into it prior to the start of lab.
5. Meteorological data is easy to find, but you may have greater difficulty finding the pollen count. Daily pollen counts are available in most medium to large cities. If there are none available in your locale, look for the nearest large city. Two Internet sites that might help you locate local pollen counts are the National Allergy Bureau (http://www.aaaai.org/nab/default.stm) and http://pollen.com.
6. Pollen counts tend to be higher on warm, windy days and lower on cool, rainy, and humid days. Ideally, students should see peaks in symptoms on the days that pollen increases and fewer symptoms when pollen counts are lower. However, this doesn't always occur, and one of the reasons is time lags. Often the published pollen counts are 24 to 48 hours old. Many counting stations report pollen counts to the media in the late morning; these represent the air sample collected on the previous day. These may not get broadcast (or printed) until that evening. Although it will be reported as the current pollen count, it represents the previous day. You may wish to see if the correlation improves when you take this lag into account.

LABORATORY TOPIC 7: FLESHY FRUITS AND FLYING SEEDS

EXERCISE A: DIVERSITY OF FLESHY FRUITS

It may be difficult to do all three exercises for Laboratory Topic 7 in one lab period unless the students are very efficient. Any fruits can be used for this exercise, depending on what is available. Most examples of fleshy fruits can be found at a local supermarket. The dry fruits can be collected from trees, shrubs, and herbs in the area, and stored until needed.

EXERCISE B: EDIBLE FLESHY FRUITS OF COMMERCE

Take advantage of any exotic or unusual fruits available. Use the "Additional Resources" to add additional commentary on these fruits, or encourage the students to find their own information.

EXERCISE C: DISPERSAL OF FLYING SEEDS

This exercise is a model system that challenges students to ask their own questions, design an experiment, and collect meaningful data. The entire process can be accomplished within a normal 3-hour lab if the whole focus is seed dispersal.

Note that the measuring devices and other materials needed will depend on the student-generated designs. As students design their experiments, make sure they consider adequate controls and ample replication. For some reason, many students are led to believe that three replicates are adequate for all experiments, even when they could drop 100 seeds from a height just as easily. Students should be encouraged to ask questions that they personally feel are meaningful. Finally, when students must present the results of their experiments and defend their data to their peers, they are more likely to take the process seriously.

LABORATORY TOPIC 8: GENETIC DIVERSITY OF OUR FOOD

Some of the exercises in this laboratory topic require advance planning. They should be started early in the term and extended over the entire semester. Most of the time, the plants do not require much tending except water and fertilizer. Seldom do students have the opportunity to see several generations of an organism within a single semester. This activity does require a slight modification of the allocation of class time.

The tasks that could be accomplished prior to the beginning of the semester include the vernalization of the turnip and Chinese cabbage for Exercise B and the planting of the parent population of Fast Plants for Exercise C. If students are given some time in the first lab period of the semester, they can get both experiments started. Pollination requires only a few minutes and can be accomplished at the beginning of other lab exercises.

Wisconsin Fast Plants (Rbr) seeds and kit supplies are available through Carolina Biological as well as on the Web site (www.fastplants.org). More detailed instructions on the care and maintenance of the Fast Plants are available through the same web site. This site also suggests several low-cost materials to use as growing containers and growth chambers. Remember that the keys to successful growth of the Fast Plants are 24-hour lighting, noncompacted soil mixture, constant moisture, and adequate fertilizer.

EXERCISE B: DO THEY REALLY BELONG TO THE SAME SPECIES?

Containers for growing the plants are easily made from 2-liter soda bottles. Fill a bottle with hot tap water and replace the cap. Hold the bottle firmly, and twist off the opaque bottom. Seal the holes on the bottom with black electrical tape.

EXERCISE C: *BRASSICA* EVOLUTION BY ARTIFICIAL SELECTION

Save the seeds from this exercise in an envelope for the use of next semester's students.

LABORATORY TOPIC 9: ALGAE—FROM DIVERSITY TO DESSERT

EXERCISE A: ALGAL DIVERSITY

Cultures are available through most biological supply companies including Carolina Biological and Ward's. They also have a limited selection of freshly collected brown and red algae. Preserved materials, herbarium specimens, and prepared slides are also available.

EXERCISE B: HUMAN USES OF ALGAE

1. Irish moss (*Chondrus crispus*) is available at stores that sell supplies for making beer, since it is used for precipitating proteins in the wort. It is generally sold chopped up (or flaked) in small bottles and is inexpensive. It is also available on the Internet in this form, and a few web sites even have freshly collected Irish moss available. To make the seaweed pudding, use the chopped-up form sold for brewing. Because it is chopped, you need to make absolutely certain all the algae is removed from the pudding to avoid a seaweed taste. If you are using fresh Irish moss, you may need to adjust the recipe; you might try 20 to 25 g instead of the 5 g dried. The recipe in the exercise will make enough for two students to try the pudding.
2. Dried edible seaweed, sushi, and even seaweed salad are becoming widely available in health food stores and also in regular grocery stores. Even in landlocked Tulsa, Oklahoma, several grocery stores regularly sell these. Many students are well acquainted with sushi today, while others need to be encouraged to try it. We recommend getting vegetarian types of sushi, since students are more willing to try sushi with carrots or avocado than with raw fish.

EXERCISE C: ALGAE AS INDICATORS OF WATER POLLUTION

1. This exercise utilizes the Palmer index, which was first published in the late 1960s. In 1975, *Carolina Tips* described the use of this index for educators. Ward's Biology sells a kit with samples that represent highly polluted, intermediate, and clean water sources (86 W 3056). This exercise is based on Ward's kit, although it can be successful with water collected locally. Photographs of the most common algae are included in the exercise; however, other algae may also be present. A useful reference for the other algae is *How to Know the Freshwater Algae*, 3rd edition, by G.W. Prescott, 1978, WCB/McGraw-Hill.
2. The directions call for students to do two horizontal traverses. Another option might be to have students work in pairs on two different water samples. After one traverse, have the students change slides with their lab partner. This way students will get to see two different water samples, possibly with different taxa present.

EXERCISE D: EFFECTS OF NITRATES AND PHOSPHATES ON ALGAL GROWTH

Bold's basal medium is a standard culture medium for the growth of many freshwater algae. The medium described here includes all the ingredients and should be prepared as described for algal culture maintenance. However, when preparing the medium for Exercise D, modifications will need to be made, as explained following these preparation instructions.

Bold's basal medium is prepared using stock solutions. Make up the following stocks:

1. $NaNO_3$ ..... 10 g/400 ml H_2O
2. $MgSO_4\,7H_2O$ ..... 2 g/400 ml H_2O
3. K_2HPO_4 ..... 4 g/400 ml H_2O
4. KH_2PO_4 ..... 6 g/400 ml H_2O
5. $CaCl_2$ ..... 1 g/400 ml H_2O
6. NaCl ..... 1 g/400 ml H_2O

To 936 ml of distilled water, add 10 ml of each stock solution and 1.0 ml of each of the following trace element solutions:

1. EDTA stock. Add 50 g of EDTA and 31 g of KOH (85%) to one liter of distilled water.
2. H-Fe stock. Add 4.98 g of $FeSO_4\,7H_2O$ to one liter of acidified water (999 ml of distilled water plus 1 ml of H_2SO_4).
3. Boron stock. Add 11.42 g of H_3BO_3 to one liter of distilled water.
4. Micronutrient stock. Add the following to one liter acidified water:
   a. $ZnSO_4$ ..... 8.82 g
   b. MoO_3 ..... 0.71 g
   c. $Co(NO_3)_2{\cdot}6\,H_2O$ ..... 0.49 g
   d. $MnCl_2$ ..... 1.44 g
   e. $CuSO_4{\cdot}5H_2O$ ..... 1.57 g

   Place in flasks or bottles and autoclave for 15 minutes. This medium can be solidified by adding 15 g of agar.

To modify for Exercise D: Omit the $NaNO_3$, K_2HPO_4, and KH_2PO_4 from the basal medium and bring the total up to one liter. Autoclave as described. Prepare the nitrate-phosphate solution as follows: Add 10 g of $NaNO_3$, 4 g of K_2HPO_4, and 6 g of KH_2PO_4 to 400 ml of distilled water. Stir until all the salts dissolve. Label the flask **N-P solution** and autoclave for 15 minutes. Students will be adding 0.1 ml, 0.4 ml, or 1.0 ml of this solution to tubes A, B, and C, respectively.

There are several possible methods to evaluate the results of the experiment. The simplest and quickest is to just compare the intensity of the green color. If a spectrophotometer is available, the density can be evaluated by measuring the absorbance at 750 nm. (Remember to always

use a water blank when using a spectrophotometer.) Microscopic evaluation can be done by using a haemocytometer or by using the counting method described in Exercise C.

LABORATORY TOPIC 10: YOUR PIECE OF THE SUN

Exercises A and B require students to keep track of their dietary consumption for an entire week. They need to be reminded of this a week in advance so they come prepared. All of the calculations can be accomplished during a lab session or outside of class time if needed.

The interactive Web site provided by the USDA is a powerful tool for completion of this lab. Alternatively, nutritional reference books can be used to extract the caloric and nutritional data.

LABORATORY TOPIC 11: LEAVES OF GRASS

EXERCISE A: A TYPICAL GRASS PLANT

The corn plants and trays of lawn grass need to be started from seed 2–4 weeks prior to the class meeting. These can be grown in the greenhouse or under grow lights. Both require adequate water and fertilizer. Grass seed should be sowed in loose soil to produce a carpet of grass. Allow to grow until inflorescences appear. If seed is available, plant a tray of Kentucky bluegrass (*Poa pratensis*) and Bermuda grass (*Cynodon dactylon*).

Samples of grains can be obtained from farmers or purchased from biological supply companies.

EXERCISE B: THE BOTANY OF BAKING

1. Specialty flours are available at most supermarkets. Often these flours are located apart from the normal baking goods in a section devoted to specialty foods, especially foods for special diets. Some of the available flours, such as buckwheat or soy, are not from cereal grains. Most of the specialty flours typically are not used to make bread. The flours may be added to wheat flour to change flavor or nutrition, but are seldom used alone. The dough and breads from these flours yield some interesting responses from the students.
2. The recipe for making bread used in this manual is very simple. You can adjust it to your needs and taste. For example, many students like to add a little salt for flavor.
3. Since this lab activity finishes with students eating the baked bread, care should be taken to remove any potential hazards from the area. Hazardous chemicals or living microorganisms must be kept far away from the bread. Ideally, this lab should be conducted in an area suitable for food preparation.

EXERCISE C: CRUDE GLUTEN CONTENT

Many individuals are allergic to gluten. A rapid gluten test kit is available from ELISA Technologies, One Progress Blvd., Alachua, FL 32615, http://www.elisa-tek.com. It can detect gluten at 200 ppm in 5 minutes. Another simple method for quantification of gluten is presented by H. Wieser in *Cereal Chemistry* 77:48–52, "Simple Determination of Gluten Protein Types in Wheat Flour by Turbidimetry," 2000.

EXERCISE D: BLUE CORN

If time allows, the amount of niacin can be determined with HPLC or microbiological assays before and after treating the blue corn flour with the alkaline solution. For specific instructions, see the official methods of analysis of the Association of Official Analytical Chemists (AOAC) (1970 onwards) Washington, D.C. A comparison of the thiamin levels of brown rice versus polished white rice is a comparable study.

LABORATORY TOPIC 12: THE LOWDOWN ON LEGUMES

EXERCISE A: SHIPSHAPE FLOWERS, SPLITTING FRUITS, AND NUTRITIOUS SEEDS

1. Collect a variety of legume flowers from whatever is locally available: black locust (*Robinia pseudoacacia*), Texas bluebonnet (*Lupinus*), vetch (*Vicia*), false indigo (*Baptisia*), *Wisteria*, etc. Or grow from seed in the greenhouse: sweet pea (*Lathyrus*), pea (*Pisum sativum*), or any of several types of beans (*Phaseolus vulgaris, Vicia faba*).
2. Mung bean (*Vigna radiata*) seedlings are often sold as bean sprouts in the produce section of the supermarket. Alternatively, you could purchase the seeds from Carolina Biological and sprout your own.
3. A bean soup mix available from the supermarket has a ready assortment of different beans and peas that can be easily distributed in the laboratory. Alternatively, your own assortment could be made from whatever dried bean/pea seeds are available at your supermarket or health food store.
4. Purchase tofu, soy milk, tofutti (tofu ice cream), or any other soy products for taste sampling.

EXERCISE B: THE NITROGEN GOES ROUND AND ROUND

1. To prepare crystal violet stock solution, weigh out 13.87 g of crystal violet dye. Add 100 ml of 95% ethanol. Stir. Filter through filter paper. Dilute 10 ml of the stock solution with 90 ml of water.
2. Dig up field clover or grow beans (*Phaseolus vulgaris*) and inoculate the soil with *Rhizobium* powder. *Rhizobium* powders (AA-15-7832) are available from Carolina Biological or from local nursery centers.
3. Alternatively, prepared microscope slides of *Rhizobium* in legume nodules can be purchased from Carolina Biological or Ward's.

EXERCISE C: SATURATED OR UNSATURATED? YOU BE THE JUDGE

1. The Sudan IV dye (powder) is soluble in lipids only. The more red color, the greater percentage of lipids are present in the substance being tested. The dye will not dissolve in substances that are not composed of lipids.
2. It will take about a week to see the results of the experiment on drying oils. Some good drying (polyunsaturated) oils are linseed, walnut, safflower, tung, and poppy seed. These can be purchased from an art supply store or a hobby store. Oils that will not dry (monounsaturated) include peanut, canola, and olive. Corn oil is intermediate in drying time.

   Linseed oil (high degree of polyunsaturation) dries in about 3 days. Cottonseed, walnut, safflower, poppyseed and sunflower oil were tacky after a week's exposure to the air; although polyunsaturated, they have fewer double bonds than linseed oil. Olive and peanut oils (monounsaturated oils) did not dry in a week's time.
3. Oils that are highly polyunsaturated absorb the iodine more quickly (thus becoming colorless). Sunflower oil takes about 2-3 hours to become completely colorless but is noticeably lighter in about an hour. Poppyseed and sunflower oils also become colorless in about the same time.

Oils that are have a lower degree of unsaturation and do not absorb the iodine and hence do not change color as quickly are olive, peanut, and canola.

Corn was intermediate in clearing time.

Have student teams pick two different oils and then compare class results at the end of the laboratory period.

Use I_2KI as the iodine solution for this test.

To prepare I_2KI, dissolve 20 g KI (potassium iodide) in 1,000 ml distilled water. Using a magnetic stirrer, add 4.0 g of iodine to the potassium iodide solution. It will take some time for the iodine to dissolve. Store in brown bottles as I_2KI breaks down in light. Dispense in dropper bottles.

EXERCISE D: LATHER UP

1. One-hundred-percent lye can be purchase inexpensively from the grocery store. It is sold as a drain opener. Red Devil is a common brand.
2. Palm oil can be purchased from East Indian or Middle Eastern food stores. It is also known as vegetable *ghee*. Coconut oil can be purchased from the same source, although it is often sold as a soap-making supply in hobby stores. If soybean oil is substituted for palm oil, stirring time will be about 30-40 minutes.
3. Food dyes *cannot* be used to color soap. Purchase dyes specifically designated for soaps from a hobby store or use spices for color. Purchase essential oils for soap fragrances from health food stores (aromatherapy section) or from hobby stores (soap-making section). Inexpensive plastic molds in a variety of shapes can be purchased from hobby stores.

LABORATORY TOPIC 13: FOOD FROM UNDERGROUND AND FAR AWAY

EXERCISE A: MAKING PLASTIC

1. Use cornstarch since it is mostly amylopectin, the branched form of starch.
2. The material acts like a liquid when stirred slowly and a solid when stirred quickly. It is difficult to move the spoon through when stirring quickly because there is not enough time for the branched amylopectin molecules to rearrange themselves.
3. The ratio of water to starch is critical. If there is too much water, the starch will always flow easily. Adjust the water:starch ratio if there are difficulties showing the fluid or solid nature of the starch.

EXERCISE B: STARCH GRAINS

1. I_2KI turns blue-black in the presence of starch. To prepare I_2KI stain, dissolve 20 g of KI (potassium iodide) in 1,000 ml of distilled water. Using a magnetic stirrer, add 4.0 g of iodine to the potassium iodide solution. It will take some time for the iodine to dissolve. Store in brown bottles, as I_2KI breaks down in light. Dispense in dropper bottles.
2. Polarizing filters, of course, can be bought for student microscopes but an inexpensive alternative is to purchase polarizing plastic sheets from Edmund Scientific (Tech Speck Linear Polarizing Laminated Film). Cut into 10 cm × 10 cm squares. Two per microscope. This is a one-time purchase since these filters can be used over and over.

You may use the flours of potato (the usual soluble starch sold by supply houses), corn, and arrowroot to show the Maltese cross under polarizing light. Also, a sample of sweet potato root works well. Illuminate the field on high. Completely open the diaphragm and use highest lamp setting to get the best results.

EXERCISE C: STORAGE ORGANS

1. Bulbs include daffodils, tulips, onions, and amaryllis. Lily bulbs lack the outer brown scales. Garlic cloves are bulbets.
2. Corms include elephant's ears, gladiolus, crocus, water chestnuts, and the tiny corms of the wildflower known as spring beauty (*Claytonia virginica*).
3. Rhizomes include canna, iris, lily-of-the-valley, shamrock (*Oxalis brasiliensis*) and ginger.
4. Tubers include caladium, white potato, and Jerusalem artichoke (*Helianthus tuberosum*).
5. Taproots include carrot, parsnip, and turnip.

6. Tuberous roots include spider plant (*Chlorophytum comosum*), sweet potato, and dahlia.

EXERCISE D: THE STARCHY STAPLES

1. Make sure the potato chips you bring in are not made from processed potatoes, as these will not show the ring of vascular bundles common to dicot stems.
2. Bring in examples of the four major varieties of white potato grown in the United States. Round white include Kennebec and Katahadin. Examples of russets are Idahos and Russet Burbank. Round red includes Red La Soda and Norland. A popular long white is white rose.
3. When the sweet potato is halved, find the visible lighter ring of tissue just inside the narrow cortex. This is the endodermis of the sweet potato.
4. Cassava (*Manihot esculenta*) may be available in the specialty produce section of a local supermarket or health food store. It also goes by the names manioc, yucca, or yucca root. If the intact root is not available locally, bring in tapioca pearls to examine the cassava starch grains. Some health food stores have cassava chips (again, these can be called yucca chips) available for sampling.
5. Again, true yam (*Dioscorea*) is becoming more widely available in specialty produce sections and health food stores in the mainland United States.
6. Elephant ears (*Colocasia ultissima*) corms are available at many gardening centers. Also, taro chips are popular at health food stores. Taro is also known as *dasheen*.

EXERCISE E: WHAT ARE YOU EATING?

Visit the specialty produce section of the local supermarket or health food store. Many hitherto exotic produce is now available in the mainland United States. Most can be served raw or with minimal preparation (i.e., boiling). If not available as raw produce, look for foods prepared from these plants.

LABORATORY TOPIC 14: THE SPICE OF LIFE

EXERCISE A: HERBS AND SPICES

Have a variety of herbs and spices available. Encourage the students to crush the whole spice or herb, so that they can smell the "aroma" of the spice.

EXERCISE B: ESSENTIAL OILS

1. Use the herbs grown from cuttings by the students during Laboratory Topic 2 or purchase potted herbs at a garden shop or fresh herbs (not dried) at a grocery store.
2. The glandular trichomes should be visible with a dissecting microscope; however, you may prefer a compound scope.

EXERCISE C: HOW HOT IS THAT JALAPENO?

1. If the students are not familiar with a dilution series, you might want to explain this part carefully.
2. You might treat this as a class exercise and have only five volunteers do all the taste testing for all the peppers. Different people have different sensitivities to the capsaicin, based on what they are used to. When you switch volunteers, it is hard to compare results from different peppers. However, the volunteers *must* drink water or milk and eat an unsalted cracker between the different peppers. Students may initially be hesitant to volunteer, but they soon get very excited about doing this exercise.

EXERCISE D: ANTIBIOTIC ACTIVITY OF SECONDARY PRODUCTS IN GARLIC

1. To prepare the roasted garlic, separate the cloves from a head of garlic, but do not peel. Wrap the cloves in foil and bake for 30 minutes at 150 °C (about 300°F).
2. Review sterile technique with the students.
3. Use actively growing cultures (about 18 hours old) of *E. coli* and *B. subtilis* as the source of inoculum for the plates. Grow the cultures in LB broth at 37°C.
4. Demonstrate to the class how to inoculate the plates so that cultures will develop a solid lawn of the test organisms. Stress that they should be rubbing lightly on the surface with the cotton swab to avoid gouging the agar.
5. Filter paper discs (blank sterile discs) are available from Wards (38 W 1600).

LABORATORY TOPIC 15: THE BEAUTY OF WOOD

EXERCISE A: THE COMPOSITION OF WOOD

Prepared slides can be purchased from most microscope slide companies or biological supply firms.

EXERCISE B: COMMON TYPES OF WOOD

Blocks of a wide collection of hardwoods can be purchased through many lumberyards and sanded. Alternatively, sets of wood samples are available through some of the biological supply houses. Some woodworking organizations provide samples to educators free of charge. Any planks of wood or pieces of furniture can be used to observe the figure of the wood and determine the angle of the saw cut.

EXERCISE C: TWIG MORPHOLOGY

Twigs can be collected from trees during the winter and saved until needed during other seasons. If leaves are removed, the twigs will resemble the description given for the winter twigs.

EXERCISE E: DENDROCHRONOLOGY—READING THE RECORD OF TIME

The preparation of tree cores takes some time, so this activity probably is best extended over at least two class

meetings. An alternative to the tree-ring analysis described in the manual is purchase of a dendrochronology kit. Fischer Educational has a Dendrochronology Tree-Ring Dating Kit Lab-Aids No. 52 CQS19369 (2000 catalog).

LABORATORY TOPIC 16: BIOPROSPECTING FOR MEDICINAL PLANTS

EXERCISE A: INVESTIGATING HERBAL REMEDIES WITH THE SHRIMP BIOASSAY

1. Purchase live brine shrimp (*Artemia*) or cysts from Carolina Biological, Ward's, or the local aquarium store. If you choose, hatching brine shrimp is extremely easy. Using Instant Ocean (also available from the same sources), prepare a 1.022 specific gravity salt-water solution (approximately 9 tablespoons) in a 1-gallon aquarium. Purchase an inexpensive Aquarium Systems Seatest Specific Gravity meter (marine hydrometer) from Ward's to achieve the correct specific gravity. Aerate to keep cysts in suspension. Maintain temperature between 28°C and 30°C. Hatching will take 24–48 hours. To harvest, use a lamp to attract the positively phototactic shrimp. Collect with a fine mesh net.
2. Purchase aquarium tubing, 4-gang valves, nets and pumps from Carolina Biological, Ward's, or the local aquarium store.
3. Two-dram vials may be purchased from Carolina Biological.
4. Obtain dried herbs from a health food store, or collect them fresh from the field and allow to air-dry for at least a week. This will concentrate the active principles and make it easier to grind the plant material into a fine powder.
5. The green tea extract was made from the contents of a green tea bag. Cranberry extract was made by grinding up pills of pure cranberry juice concentrate. Both showed biological activity.

Calendula flowers, valerian root, purple coneflower, goldenseal, and ginseng prepared from dried herbs did show some biological activity.

LABORATORY TOPIC 17: BIOACTIVE DRUGS IN ACTION

EXERCISE A: THE BLACKWORM MODEL TO TEST BIOACTIVE DRUGS

1. The observation chamber (Lesiuk and Drewes, 1999) is made with parafilm and a glass slide. Cut off six rectangular pieces of parafilm (6.5 cm × 1.5 cm). Remove the backing. Stack the parafilm sheets on top of one another on a glass slide. Obtain a hot plate and turn to a low heat setting. Heat the underside of the slide until the parafilm softens and becomes slightly clear. Remove from the heat and quickly place one of the parafilm backing papers on the Parafilm stack. With your fingers, apply gentle pressure to work out any air bubbles to the edge of the stack. The fingertip pressure will also ensure that the Parafilm sheets stick to each other. If the sheets do not appear to stick, reheat.

   With a sharp razor blade, trim the Parafilm around the edges of the slide. Next create a center trough to hold the worm. Cut out a 2 mm × 50 mm rectangle. Do a test run with a worm. If the worm has too much room (the trough is too long or too wide), reduce the dimensions. These observation chambers can be reused over and over. They are basically indestructible.
2. Worm wrangling tools (Lesiuk and Drewes, 1999) are made by attaching a short length of human scalp hair (2 cm in length) to the end of a wooden applicator stick. Use labeling tape to secure the end of the hair to the stick to that the hair extends approximately 1 cm beyond the stick.
3. The California blackworm (*Lumbriculus variegatus*) may be purchased from Carolina Biological. Care of the worms is described in *Carolina Biological Tips* 59(3), "Those Wonderful Worms" (August 1996). It is very easy to maintain a blackworm culture. All that is needed are a shallow dishpan, spring water, and fish pellets for food.
4. Be sure to make all drug test solutions in spring water. Pure caffeine (FW 194.2) can be obtained from Sigma. Test at 0.5 mM, 1.0 mM, 2.0 mM, up to 10 mM. Lesiuk and Drewes (1999) found that 3.0 mM concentration shows a maximum increase in pulse rate.
5. Prepare a crude extract of tobacco from a cigarette, as described by Lesiuk and Drewes (1999). Unwrap two cigarettes, weigh out 2g and add to 100 ml of 60° spring water. Stir continuously for 0.5 hour. Filter and cool. Dilute by 50% to obtain the stock solution. Test at full stock, half stock, and quarter stock strength. Nicotine generally lowers the pulse rate.
6. Kava kava at higher concentrations slowed the pulse to such a great extent that it was undetectable. Worms recovered after several minutes in spring water. At lower concentrations, it increased the pulse rate.

Dried kava kava root was obtained from the local health food store. Two grams of the dried root were added to 100 ml of spring water and heated to 60°C, with continuous stirring, for 20–30 minutes. It was then filtered, cooled, and diluted to half strength by adding an equal amount of spring water. This was the highest concentration that showed a complete depression of the pulse rate. All other concentrations (¼, ⅛, 1/16), stimulated the pulse rate.

Purchase ephedrine (FW 165.24) from Sigma. The pulse rate doubled at 1.2 mM. It may be difficult to purchase ephedrine because of federal laws regulating its use.

Purchase digitoxin (FW 764.9) from Sigma. At a concentration of 0.1 M, digitoxin lowered the pulse rate. At lower concentrations, there was no change.

LABORATORY TOPIC 18: THE FUNGUS AMONG US

EXERCISE A: FUNGAL DIVERSITY

1. We have suggested that students do not open the petri dishes containing actively growing cultures of *Rhizopus, Penicillium*, and *Aspergillus*, since many students have allergies and many fungal spores are common allergens.
2. The following technique will stain endomycorrhizal fungi in roots. The fungus will stain deep blue, while the root cells are pale blue or unstained. You can prestain the roots and have the students mount the roots on slides and look for VA mycorrhizae, or you can have the students do the entire staining procedure. However, the procedure may take 2 hours or more. Use great care when transferring the roots. They are often brittle following the clearing step.
   a. Endomycorrhizae (VA mycorrhizae) occur on the majority of plants in the environment, including grasses, legumes, and many agricultural crops. You can find VA mycorrhizae in the field or inoculate seedlings with endomycorrhizae inoculum, which is available commercially. A number of Internet sites supply inoculum at a reasonable price. (Golden Harvest Organics sells endomycorrhizae inoculum, which contains a mixture of *Glomus* species. Their Web site URL is http://www.ghorganics.com/index.html.) Try adding the inoculum to soil and planting tomato seeds, but be careful about the soil. If you use a potting soil that contains fertilizer, mycorrhizal growth will be inhibited. Harvest the plants when they are 3 to 4 weeks old.
   b. Dig up the plants and rinse the roots well. Harvest lateral roots that are 2 mm or smaller in diameter. Do not let the roots dry out. Cut the roots into segments that are from 1 to 3 cm in length.
   c. Place the root sections in a 100-ml beaker and add 10 to 15 ml of 10% KOH (w/v). Make sure all the roots are covered by the KOH. Place the beaker in an 80°C water bath for up to 2 hours. This step clears and decolorizes the roots. Very fine roots will only need 15 to 20 minutes, while larger roots will need a longer time. The correct amount of time is critical because roots that are not sufficiently cleared will still have cell contents that obscure the mycorrhizae. An alternative method is to autoclave the roots in 10% KOH for 15 minutes.
   d. Remove the beaker from the water bath and add one drop of 30% H_2O_2. Allow the roots to remain in this solution for 10 minutes.
   e. Place the roots in a petri dish and gently rinse with tap water for 20 to 30 seconds. Repeat the rinse three or four more times.
   f. Move the roots to a 100-ml beaker that contains 10 to 15 ml of 10% HCl. Make sure the roots are covered.
   g. After 5 minutes, transfer the roots to another beaker containing 10 to 15 ml of lactic acid with 0.05% aniline blue (also known as cotton blue). Place the beaker in an 80°C water bath for 30 minutes. Another widely used stain for fungi is acid fuchsin. Substitute the acid fuchsin for the aniline blue. It will stain the fungi bright pink with little or no stain in the root cells. Chlorazole black E can also be used.
   h. Transfer the roots into another beaker containing 85% lactic acid for 10 minutes to destain. Roots can also be stored in this solution for several weeks. Alternatively, the roots can be stored in 50% glycerol. Roots can be mounted for microscopic viewing in either storage fluid or water.
   i. **Note**: Some biological supply companies sell prepared slides showing mycorrhizal fungi in roots; however, these are generally **not** VA mycorrhizal fungi.

EXERCISE B: AIRBORNE FUNGI

Malt extract agar and potato dextrose agar are good culture media for isolating airborne fungi.

EXERCISE C: FUNGAL FOODS AND FERMENTED PRODUCTS

1. Champagne yeast and hygrometers are usually available at shops that sell wine-making and brewing supplies.
2. White grape juice works quite well.
3. Use round balloons designed to be filled with helium; these are usually quite durable.

Appendix A: Science as a Process

Appendix B: Field Trip to a Health Food Store

Appendix C: A Taster's Sampler of Caffeine Beverages and Foods

1. Appendix C could be a laboratory exercise or modified slightly to be a field trip to a supermarket or specialty store (e.g., health food store, coffee house, tea shop).

2. Potted *Coffea arabica* plants can be purchased from Ward's and are easy to grow. We have had ours for several years in the greenhouse and have watched them flower and produce fruit.
3. Visit a coffee house or purchase samples of coffee beans from a variety of roasts and ground coffees. Sample or prepare in lab espresso, latte, cappuccino and/or caffe mocha.
4. Visit the tea section in a supermarket or specialty store or purchase for the laboratory a variety of black, oolong or green teas, preferably loose, so the differences in the leaves can be observed. Likewise, observe the variety and/or prepare herbal teas. Sample the different varieties.
5. Purchase baker's chocolate, cocoa powder, white chocolate, and chocolate for tasting. Purchase or visit the cosmetic section of a specialty store or supermarket to find a variety of products that contain cocoa butter.

Suppliers

Carolina Biological Supply Company
(800) 334-5551
http://www.carolina.com

Edmund Scientific Co.
(609) 573-6250
http://www.edsci.com

Triarch
(800) 848-0810

Ward's
(800) 962-635-8439
http://www.WARDSCI.com

Sigma
(800) 325-3010
http://www.sigma-aldrich.com/order

Fisher Science Education
(800) 955-1177
http://www.fisheredu.com

LABORATORY MANUAL *for*
Applied Botany

The Aztecs and Mayans of Mesoamerica used the inner bark of trees to make paper. The cover drawing is an example of *amate*, a colorful folk art still seen in Mexico. Amate is derived from the word *amatl*, which means both paper and a type of fig tree in the language of the Aztecs. The inner bark of the tree is first stripped and then boiled in an ash solution to soften and purify the fibers. After the fibers are rinsed, they are positioned in a grid pattern on a wooden board and then beaten with a stone to mesh the fibers together to form the paper. After drying in the sun, artisans paint vibrant scenes of nature or village life.

While the Aztecs used the inner bark of trees to make paper, other societies have used other plant materials to produce a writing surface. The ancient Egyptians were the first to use botanical material. They prepared papyrus (origin of the word *paper*) sheets from the pith of the papyrus plant. Today papyrus sheets are used for decorative paintings, but they were used for written documents by people in the Mediterranean area for over 3,000 years. True paper is prepared from pulp, a watery suspension of plant cells. The first true paper was developed in China in the 2nd century. The inner bark of mulberry trees was the first plant used to prepare the pulp, and over the centuries, other types of plant material were used as a source of pulp including straw, leaves, stems, and even old cotton or linen rags. The cells in the pulp are matted into a thin layer and then compressed; however, the cells must be long enough to form a mat when the water is drained off. Typically these are tracheids, vessels, and fibers, but in papermaking terms, these are all called *fibers*. The use of wood pulp was introduced in 1840, and today most paper is prepared from wood pulp. Each year approximately one billion trees are cut down to satisfy our demand for paper. Today there is active research for alternate sources of pulp. One of the activities in this lab manual is preparation of paper from pulp produced using a variety of plant materials.