Plant Pathology

Concepts and Laboratory Exercises

THIRD EDITION

Edited by

Bonnie H. Ownley

Robert N. Trigiano

CRC Press
Taylor & Francis Group

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Preface

We thank those instructors who have adopted the first two editions of *Plant Pathology Concepts and Laboratory Exercises* as a guide for their classes. We also are grateful to them and their students and colleagues for providing invaluable feedback and criticism of the previous editions. We have incorporated many of their ideas into this new, third edition, which includes combining concept and laboratory chapters into one presentation, almost all figures in color, more technical presentations of some topics, a chapter on safety in the laboratory, treatment of organic agriculture and disease, and more extensive chapters about disease diagnostics. We have also improved the binding of the book, which is now spiral bound, allowing the students to access any page easily.

This edition of *Plant Pathology Concepts and Laboratory Exercises* is intended to serve as a primary text for introductory courses and furnishes instructors and students alike with a broad consideration of this important and growing field. It presents many useful protocols and procedures and thus serves as a valuable reference to researchers as well as students in beginning and advanced plant pathology and allied biological sciences courses. The book is intentionally written informally to some extent as it provides the reader with a minimum number of references, but does not lose any essential information or accuracy. Broad topic chapters are authored by specialists with considerable experience in the field and are supported by one or more laboratory exercises illustrating the central concepts of the topic. Each chapter begins with a "Concept Box" highlighting some of the more important ideas contained within the chapter and signals students to read carefully for these primary topics. There is an extensive glossary, which appear as bolded words in each chapter. Collectively, the laboratory exercises are exceptionally diverse in nature, providing something for beginning to advanced students. Most importantly, the authors have successfully completed the exercises/experiments many times, often with either plant pathology or biology classes or in their own research laboratories. All the laboratory protocols are written in procedure boxes that provide step-by-step, easy-to-follow instructions. A unique feature of this text is that the authors have provided the expected results of each of the experiments in general terms. At the end of each exercise, there are a series of questions designed to provoke individual thought and critical examination of the experiment and results. Our intention is that instructors will not attempt to do all the experiments in each chapter, but rather select one or two for each concept that serves the needs and interests of their particular class. For an advanced class, other experiments may be assigned to resourceful students. We caution instructors and students to obtain the proper documents for transport and use of plant pathogenic organisms and to properly dispose of cultures and plant materials at the end of the laboratory exercises. We also support mandatory safety training that is typically available online at many institutions.

This book is divided into five primary sections: Introductory Concepts, Groups of Plant Pathogens and Abiotic Disorders, Plant–Pathogen Interactions, Epidemiology and Disease Control, and Special Topics. Chapter 1 in Part I introduces students to the basic concepts of plant pathology including some historical perspectives, fundamental ideas of what is disease, how disease relates to environment, the host, and time, and provides a very broad overview of organisms that cause disease. Chapter 2 is a new topic in the third edition and describes laboratory safety, media preparation, and solutions. Chapter 3 introduces students to the fundamentals of microscopy, which is a topic often omitted in biological textbooks. Part II includes chapters that detail various disease-causing organisms, plant parasitic plants, and the causes of abiotic diseases. This section begins with a consideration of viruses (Chapter 4), prokaryotic organisms (Chapter 5), and nematodes (Chapter 6). Chapter 7 provides a very broad overview of pathogenic species in the Oomycota (fungus-like organisms) and pathogenic true fungi. The next eight chapters are devoted to species in the Oomycota and various phyla of fungi followed by chapters that focus on soilborne plant pathogens, parasitic seed plants, and disorders caused by abiotic agents. Part III explores plant–pathogen interactions in Chapters 19–21 including treatments of virulence factors, pathogen attack strategies, extracellular enzymes, host defenses, and disruption of plant function. Part IV is anchored with an extensive chapter (Chapter 22) outlining the basic concepts of epidemiology, which is followed in turn by several chapters detailing various strategies for disease control, including host resistance (Chapter 23), plant–fungal interactions (Chapter 24), cultural management of plant disease (Chapter 25), chemical control of disease (Chapter 26), use of microbial control agents (Chapter 27), and integrated pest management (IPM) strategies (Chapter 28). The concluding chapter in this

section is an often suggested topic, organic agricultural and plant disease (Chapter 29). Part V is devoted to the treatment of plant disease diagnostics (Chapter 30) and identifying disease-causing organisms using molecular techniques (Chapter 31). Chapter 32 relates fungal and bacterial physiology/nutrition to disease via extracellular enzyme production. This chapter contains many valuable techniques that are applicable to other fields of science. Lastly, Chapter 33 provides explanations and exercises for molecular techniques used in plant pathology and other fields of study.

It is our hope that students and instructors find the format, level, and amount of information contained in the book to be appropriate for an introductory course and some advanced courses. The presentation style has been used very successfully in other books and with the addition of the extensive glossary, useful case studies, and concept boxes, students should find the format stimulating and conducive for learning. We invite and welcome your comments and suggestions for improvements.

B.H. Ownley
R.N. Trigiano
The University of Tennessee

Acknowledgments

We wish to recognize and applaud the extraordinary efforts and talents of all the contributing authors—their creativity, support, advice, understanding, and especially patience throughout the process of developing the third edition of *Plant Pathology Concepts and Laboratory Exercises* were phenomenal. We express our gratitude to the Tennessee Institute of Agriculture for permitting us the time and financial support necessary to complete this project. We thank our colleagues and students who suggested changes to the chapters and the arrangement in the text. We extend very special thanks to Alan S. Windham (The University of Tennessee) and David Shew (North Carolina State University) for the fantastic images donated to this project. We also express our sincere gratitude to our families for always supporting us during the completion of this book.


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Editors

Dr. Bonnie H. Ownley is a professor of plant pathology in the Department of Entomology and Plant Pathology at The University of Tennessee in Knoxville. She received her B.S. in biology from the University of North Carolina at Chapel Hill, M.S. in microbiology from Auburn University, Alabama, and PhD in plant pathology, with a minor in soil science, from North Carolina State University, Raleigh. She was a postdoctoral research fellow with the U.S. Department of Agriculture (USDA), Agricultural Research Service, in the Root Disease and Biological Control Research Unit at Pullman, Washington, and a visiting plant pathologist in the Plant Pathology Department at Washington State University before joining the faculty at The University of Tennessee.

Dr. Ownley's research and teaching programs are focused on the etiology, biology, ecology, and environmentally sustainable control of plant pathogens on a variety of food, fiber, and biofuels crops. Her work in biological control of plant diseases is recognized internationally. She has published more than 120 research papers, book chapters, conference proceedings, and popular press articles and has received numerous grants from the USDA, state agencies, private industry, and commodity groups to support her research, teaching, and outreach projects.

Dr. Ownley is the Director of Graduate Studies for the Department of Entomology and Plant Pathology. She is a dedicated teacher and strong proponent of experiential and service learning. Her teaching portfolio includes graduate courses on mycology, phytobacteriology, and soilborne plant pathogens. She has mentored and trained 19 graduate students and served on the research committees of more than 40 additional students. Her teaching has extended beyond the university to include multiple biotechnology workshops for middle and high school teachers across the State of Tennessee and experiential learning summer programs for middle and high school students.

Dr. Ownley has been recognized with numerous awards and honors for her research, teaching, and academic outreach programs, as well as service to the university and community. She has served in leadership roles for the American Phytopathological Society and as Senior Editor for *Phytopathology*. She is currently President of the Faculty Senate of The University of Tennessee, Knoxville. Her service to the university has been wide-ranging, including multiple administrative and faculty search committees, program initiatives, policy development, unit reviews, and strategic planning. Dr. Ownley has worked to improve the workplace and learning environment for faculty, students, and staff through her service to the university, from the department to the system level. Having often been the only woman at the table in the early part of her career, she is committed to eliminating bias and discrimination and educating others that inclusion of underrepresented minorities and women will multiply the possibilities and improve the innovation, creativity, civility, and sense of community of the organization.

Dr. Robert N. Trigiano received his B.S. degree with an emphasis in biology and chemistry from Juniata College, Huntingdon, Pennsylvania, in 1975 and an M.S. in biology (mycology) from the Pennsylvania State University, State College, Pennsylvania, in 1977. He was an associate research agronomist working with mushroom culture and plant pathology for Green Giant Co., Le Sueur, Minnesota, until 1979 and then a mushroom grower for Rol-Land Farms, Ltd., Blenheim, Ontario, Canada, during 1979 and 1980. He completed a PhD degree in botany and plant pathology (comajors) at North Carolina State University at Raleigh in 1983. After concluding postdoctoral work in the Plant and Soil Science Department at The University of Tennessee in Knoxville, he was appointed an assistant professor in the Department of Ornamental Horticulture and Landscape Design at the same university in 1987, promoted to associate professor in 1991 and to professor in 1997. He served as interim head of the department from 1999 to 2001. He then joined the Department of Entomology and Plant Pathology at the University of Tennessee in 2002 and was interim head from 2012 to 2013. In 2015, Dr. Trigiano was selected as an Institute Professor at The University of Tennessee Institute of Agriculture.

Dr. Trigiano is a member of the American Phytopathological Society (APS), the American Society for Horticultural Science (ASHS), and the honorary societies of Gamma Sigma Delta, Sigma Xi, and Phi Kappa Phi. He received the T.J. Whatley Distinguished Young Scientist Award (The University of Tennessee, Institute of Agriculture) and the Gamma Sigma Delta Research individual and team Award of Merit at the University of Tennessee. He is the recipient of the publication awards for the most outstanding educational and ornamental papers in

ASHS and in the Southern region ASHS L. M. Ware distinguished research award. In 2006, he was elected a fellow of the American Society for Horticultural Science. Dr. Trigano was awarded the B. Otto and Kathleen Wheeley Award of Excellence in Technology Transfer and founder and manager of Creative Agricultural Technologies, LLC by the University of Tennessee Research Foundation in 2007. He has been an editor for *Plant Disease*, ASHS journals, *Plant Cell, Tissue and Organ Culture*, *Plant Cell Reports* and is currently the editor-in-chief of *Critical Reviews in Plant Sciences*. Additionally, he has coedited ten books, including *Plant Tissue Culture Concepts and Laboratory Exercises*, *Plant Pathology Concepts and Laboratory Exercises*, and *Plant Development and Biotechnology*.

Dr. Trigiano has received research grants from the United States Department of Agriculture (USDA) and Forest Service, Horticultural Research Institute, and from private industries and foundations. He has published more than 200 research papers, book chapters, patents, and popular press articles. He teaches graduate courses in scientific writing and molecular techniques and has presented numerous workshops on English scientific writing in Germany, the People's Republic of China, and Brazil. His current research interests include molecular markers for breeding ornamental plants, population studies of pathogens and native plants, diseases of ornamental plants, somatic embryogenesis, and micropropagation of ornamental species.

Contributors

Richard E. Baird
Mississippi State University
Starkville, Mississippi

Peter Balint-Kurti
United States Department of Agriculture
Agriculture Research Service
Plant Science Research Laboratory
North Carolina State University
Raleigh, North Carolina

Anton Baudoin
Virginia Polytechnic Institute and State University
Blacksburg, Virginia

D. Michael Benson
North Carolina State University
Raleigh, North Carolina

Ernest C. Bernard
The University of Tennessee
Knoxville, Tennessee

Kira L. Bowen
Auburn University
Auburn, Alabama

Judith K. Brown
The University of Arizona
Tucson, Arizona

Carolee T. Bull
Pennsylvania State University
University Park, Pennsylvania

David M. Butler
The University of Tennessee
Knoxville, Tennessee

Lori M. Carris
Washington State University
Pullman, Washington

Christina Cowger
United States Department of Agriculture
Agriculture Research Service
Plant Science Resesarch Laboratory
North Carolina State University
Raleigh, North Carolina

Bert Cregg
Michigan State University
East Lansing, Michigan

Tom Creswell
Purdue University
West Lafayette, Indiana

Marc A. Cubeta
North Carolina State University
Raleigh, North Carolina

Margery L. Daughtrey
Cornell University
Ithaca, New York

Ricardo Manuel de Seixas Boavida Ferreira
Institute of Agronomy
Technical University of Lisbon
Lisbon, Portugal

Leonardo De La Fuente
Auburn University
Auburn, Alabama

Ann Brooks Gould
Rutgers University
New Brunswick, New Jersey

Kimberly D. Gwinn
The University of Tennessee
Knoxville, Tennessee

Denita Hadziabdic
The University of Tennessee
Knoxville, Tennessee

Jong Hyun Ham
Louisiana State University
Baton Rouge, Louisiana

Kathie T. Hodge
Cornell University
Ithaca, New York

Alejandra I. Huerta
Colorado State University
Fort Collins, Colorado

Teresa M. Jardini
Washington State University
Pullman, Washington

Steven N. Jeffers
Clemson University
Clemson, South Carolina

Steven T. Koike
University of California Cooperative Extension
Salinas, California

Kurt H. Lamour
The University of Tennessee
Knoxville, Tennessee

Blanca B. Landa
Institute for Sustainable Agriculture
Spanish National Research Council
Cordoba, Spain

Marie A.C. Langham
South Dakota State University
Brookings, South Dakota

Larry J. Littlefield
Oklahoma State University
Stillwater, Oklahoma

Stacy J. Mauzey
University of California Cooperative Extension Service
Salinas, California

Dmitri V. Mavrodi
The University of Southern Mississippi
Hattiesburg, Mississippi

Olga V. Mavrodi
Washington State University
Pullman, Washington

Rebecca Ann Melanson
Louisiana State University
Baton Rouge, Louisiana

Sara Alexandra Valadas da Silva Monteiro
Institute of Agronomy
Technical University of Lisbon
Lisbon, Portugal

Sharon E. Mozley-Standridge
Middle Georgia State University
Cochran, Georgia

Lytton J. Musselman
Old Dominion University
Norfolk, Virginia

Daniel L. Nickrent
Southern Illinois University
Carbondale, Illinois

James P. Noe
University of Georgia
Athens, Georgia

Kevin L. Ong
Texas AgriLife Extension Service
Texas A&M University
College Station, Texas

Bonnie H. Ownley
The University of Tennessee
Knoxville, Tennessee

Laura E. Poplawski
The University of Tennessee
Knoxville, Tennessee

David Porter
University of Georgia
Athens, Georgia

Melissa B. Riley
Clemson University
Clemson, South Carolina

Timothy A. Rinehart
United States Department of Agriculture
Agriculture Research Service
Southern Horticultural Laboratory
Poplarville, Mississippi

Erin N. Rosskopf
United States Department of Agriculture
Agriculture Research Service
Horticulture Research Laboratory
Fort Pierce, Florida

Craig S. Rothrock
University of Arkansas
Fayetteville, Arkansas

Isael Rubio
University of Wisconsin
Madison, Wisconsin

Gail Ruhl
Purdue University
West Lafayette, Indiana

Robert E. Schutzki
Michigan State University
East Lansing, Michigan

Barbara B. Shew
North Carolina State University
Raleigh, North Carolina

H. David Shew
North Carolina State University
Raleigh, North Carolina

Nina Shishkoff
United States Department of Agriculture
Agriculture Research Service
Foreign Disease-Weed Science
Fort Detrick, Maryland

Otmar Spring
University of Hohenheim
Stuttgart, Germany

Terry N. Spurlock
University of Arkansas
Monticello, Arkansas

C. Elizabeth Stokes
Mississippi State University
Starkville, Mississippi

Linda S. Thomashow
Root Disease and Biological Control Research Laboratory
Washington State University
Pullman, Washington

Robert N. Trigiano
The University of Tennessee
Knoxville, Tennessee

Phillip A. Wadl
Vegetable Research Laboratory
Charleston, South Carolina

David T. Webb
University of Hawaii
Honolulu, Hawaii

David M. Weller
Root Disease and Biological Control Research Laboratory
Washington State University
Pullman, Washington

Alan S. Windham
The University of Tennessee
Knoxville, Tennessee

Jason E. Woodward
Texas A&M AgriLife Research and Extension Center
Lubbock, Texas

David I. Yates
The University of Tennessee
Knoxville, Tennessee

Ana B. Zacaroni
Federal University of Lavras
Lavras, Brazil

Ning Zhang
Rutgers University
New Brunswick, New Jersey


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Part I

Introductory Concepts


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1 What Is Plant Pathology?

H. David Shew and Barbara B. Shew

Concept Box

- Most plants are healthy most of their lives; disease is the exception.
- The science of plant pathology had its beginnings in the late blight epidemics of the 1840s in Ireland and Europe.
- The germ theory of disease is the foundation of plant pathology.
- Plant disease is the result of a continuous interaction between a plant and a pathogen in a favorable environment.
- A disease cycle is the series of steps in the interaction of a host and pathogen from inoculation through pathogen reproduction and survival.
- Major pathogens of plants include fungi, fungus-like organisms, bacteria, viruses, nematodes, and parasitic seed plants.
- Diagnosis is the art of identifying disease based on symptoms and signs and associated factors.
- Koch's postulates are a set of rules to establish if a pathogen is the cause of a disease.
- Disease impacts include making plants and plant products scarce, dangerous to consume, and more costly to obtain.

CONCEPT OF PLANT HEALTH

Most plants, for most of their lives, are healthy! This is fortunate, because healthy plants are the foundation of the earth's terrestrial ecosystems. They are the source of the nutrients that sustain the interdependent organisms that together make up a stable ecosystem. Plants capture energy from the sun, and this energy provides food for large and small herbivores, the carnivores that eat the herbivores, and the scavengers that degrade the remains, including those of the plants themselves. Plants also provide energy for a variety of microorganisms that live in and on them, some of which are parasites that cause disease.

The plants that we observe in natural ecosystems are a product of natural selection. They have adapted to the biotic and abiotic environments of the ecosystem that they support by growing and reproducing more efficiently than their competitors. However, evolution is a dynamic process, and ecosystems are subject to changes due to climate change, introduction of new plant and animal species, introduction of exotic pathogens to the undisturbed ecosystem, and adaptation by existing microorganisms. In fact, coevolution with microorganisms, including those that are capable of causing diseases, is an important part of the long-term adaptation of a plant species to its environment. In this dynamic interplay between plants and their microbial companions, the pathogen sometimes gains the advantage and epidemics flare. In agroecosystems, many of the natural checks and balances of natural ecosystems are removed, so epidemics may occur more often and become very severe unless disease management practices are implemented.

In the following chapters, you will be introduced to the broad scientific discipline known as plant pathology. The primary goal of the text and the associated laboratory experiments is to raise your awareness of the importance of plant pathogens and plant diseases. The chapters will introduce you to the vast array of organisms that cause plant diseases and will allow you to experience the dynamic nature of the interactions between microbes and plants and to understand how we have successfully and unsuccessfully attempted to manage the organisms that cause plant diseases.

DEVELOPMENT OF PLANT PATHOLOGY

Plant pathology is a very broad and diverse scientific discipline. It integrates information from all of the core disciplines dealing with plant biology, plant production, microbial biology, and ecology to understand the dynamic interactions that result in disease. The concept that links all of these disciplines together within the science of plant pathology is the concept of disease: how it starts, develops, and spreads, and how it is prevented or managed. Literally, plant pathology is the science that studies plant suffering (pathology: pathos = suffer, and logy = study of). Plant pathologists attempt to improve plant health and crop productivity through the study of plant diseases, so that the severity and impact of diseases (suffering) can be alleviated.

As people moved from foragers to cultivators and began to rely on harvests of food and fiber from cultivated crops, their awareness of plant diseases must have increased. Much like today, the diseases that early agrarians observed no doubt ranged from minor to devastating. There are numerous references to blights and mildews in religious texts, including the Hebrew Bible, and in early Greek and Chinese writings. However, over several thousand years, there was little advancement in the understanding of the causes of disease and in the development of disease management strategies, largely because the biological basis of a disease was unknown. A disease was due to bad weather, toxic air, celestial events, imbalances of the sap, or divine intervention. The fact that microorganisms were only first observed in the seventeenth century, following the invention of the microscope, is hardly surprising. Even then, it would be nearly 200 years before the relationship between diseases and microorganisms was conclusively established. With few exceptions, people, including most scientists, instead believed in **spontaneous generation**. In the case of plant diseases, this belief or theory held that microbes were the result and not the cause of disease or decay.

It took multiple epidemics of the late blight disease of potato in Ireland and other areas of Europe in the 1840s, and the tragic events that followed, to provide the impetus for the founding of the science of plant pathology. The germ theory of disease provided the biological basis of the science. Within 15 years of the epidemics of late blight, Julius Kühn published the first textbook of plant pathology, concluding that both parasitic and nonparasitic factors resulted in plant abnormalities (disease). In the 1860s and 1870s, at least five additional textbooks were written about diseases of different groups of plants. The science of plant pathology continued to develop in response to the need of societies to understand the causes of plant diseases and to find the means to control them. It was the successful demonstration that disease problems could be alleviated by applying this new knowledge that led to the rapid development of plant pathology as a science in the late nineteenth and early twentieth centuries.

Germ Theory of Disease

Germ theory of disease was the single most important discovery in the early development of plant pathology. It states that germs (living organisms) cause diseases. A review of the history of plant pathology reveals that multiple scientists developed early evidence for the role of microbes in disease causality. It is beyond the scope of this chapter to list all those findings, but perhaps Prevost presented the most convincing results in his comprehensive studies of the bunt (smut) disease of wheat in the late eighteenth and early nineteenth centuries. His experimental approach led to a thorough description of the pathogen, its development in the plant, and even the approaches for controlling the disease. His treatise on this disease in 1807 should have provided the evidence needed to establish the germ theory of disease in plants, but his peers rejected the work as unsound (probably meaning too controversial).

It was not until the 1840s that a plant disease drew enough attention from scientists of the day to begin the science of plant pathology. The disease, late blight of potato (Figure 1.1a), ravaged potato crops throughout much of Europe and was especially devastating to the people of Ireland. The suffering that resulted from the consecutive years of epidemics was made worse by the dependence of the Irish population on a single food crop. Ireland was dominated by a landholding arrangement in which poor tenant farmers raised wheat, oats, barley, and other cash crops for export while depending almost exclusively on potatoes for their own sustenance. Even as cash crops continued to be exported, severe epidemics of late blight led to widespread starvation, sickness, and death. The Great Famine resulted in an estimated 1 million deaths and the mass emigration of at least a million more people from Ireland.

The multiple late blight epidemics in the 1840s found a better-prepared scientific community and a more profound need to understand the devastating effects of plant diseases than ever before. Many scientists uncovered important clues in the aftermath of the Great Famine, but the scientific approach used by Anton deBary finally led to the understanding of the true cause of late blight and other plant diseases. In 1861, deBary published his first work on the relationship of the pathogen, *Phytophthora infestans*, to late blight of potato. Much like Prevost, deBary described the development of the disease, from inoculation to symptom development and production of a new generation of spores on inoculated potato tissues. He also demonstrated the survival of the pathogen in potato tubers. He was able to duplicate all the stages of the disease cycle and repeat these stages in controlled

(a) (b) (c)

FIGURE 1.1 Symptoms of diseases studied to confirm the germ theory for different pathogen groups. (a) Destruction of a potato crop by late blight of potato caused by the fungus-like organism *Phytophthora infestans*. (b) Blighted terminal of ornamental pear caused by the fire blight bacterium, *Erwinia amylovora*. (c) Alternating light and dark green areas on tobacco leaves caused by tobacco mosaic virus. ([a] Courtesy of Marc Cubeta. With permission. [b, c] Courtesy of H.D. Shew.)

inoculations with the pathogen. In key experiments, deBary inoculated potato plants and compared them under identical conditions with plants that had not been inoculated (controls). When only the inoculated plants became diseased, it was clear that infection by *P. infestans* was the cause of late blight, and the germ theory was validated. By the time deBary published his work in 1861, many other scientists had begun to support the germ theory of disease on other plants, but deBary is credited with providing the conclusive proof for this theory and is often referred to as the founder of plant pathology.

The germ theory for other groups of plant pathogens followed the pivotal work of deBary. Burrill and his student, Arthur, developed the evidence for bacteria as pathogens of plants in the late 1870s and early 1880s in their pioneering work on the fire blight disease of pear (Figure 1.1b). It would be almost 20 additional years before the writings and studies of E.F. Smith helped to garner wide acceptance of the germ theory for bacteria. Acceptance of the germ theory for viruses would soon follow from the findings by three different scientists, Mayer, Ivanowski, and Beijerink, who all worked on tobacco mosaic caused by *tobacco mosaic virus* (Figure 1.1c). The extremely small size of viruses and the fact that they could not be cultured like fungi and bacteria introduced many problems in completing this work. Viruses were thought to be toxins or fluids, and Beijerink referred to them as a *contagium vivum fluidum*, a contagious living fluid. He also used the term virus (Latin for poison) to describe this type of pathogen. The physical nature of viruses was not known until after the invention of the electron microscope in 1931.

WHAT IS PLANT DISEASE?

Communication is based on the assumption that the people who are communicating understand each other. It is important to have a basic understanding of the terms used in any branch of science so that you can communicate effectively to others in the field. This is especially true when there are multiple definitions for a given term, such as disease. If asked to define disease, it is doubtful that any two people in a group or a class will have the exact same definition. However, there would be little disagreement about several key components that ought to be included in any definition of disease. Broadly speaking, disease refers to some type of abnormal condition or something that causes an organism to deviate from a healthy condition. It is important to understand that there is no clear line of demarcation between health and disease. Recognizing that a plant is diseased can be difficult and often requires the artful knowledge of a trained diagnostician. Regardless, most definitions of disease imply that the characteristics of a healthy, normally functioning plant are known. For example, the glossary of the American Phytopathological Society defines disease simply as "the abnormal functioning of an organism," and in 1968, the National Academy of Science defined plant disease as "a harmful alteration of the normal physiological and biochemical development of a plant." These are perfectly acceptable definitions of disease, but for the purposes of this volume, additional components are needed for our working definition. For this volume, we define plant disease as *a condition detrimental to the normal development of a plant resulting from the continuous*

interaction between the plant and a causal agent leading to the production of symptoms. There are four key components of this definition, with several separating disease from other detrimental conditions affecting plants.

First, disease is *detrimental* to the development of a plant. Disease may impact any stage of plant development, vegetative or reproductive. Some diseases, known as **damping off**, occur only on seedlings, others occur only on mature or senescing plants, and still others occur throughout the life of a plant. Many things can be detrimental to plant health, so this component of our definition does not separate disease from the negative effects of the physical or chemical environment, nor from damage by organisms such as insects, voles, and other plant-consuming pests.

Second, disease is the result of a *continuous interaction* between the plant and a pathogen. Disease is a dynamic process and takes time to develop; it is not the result of an instantaneous event. When you hear expressions like "the disease happened over night," beware. Although visible symptoms may show up seemingly overnight, the disease process is well underway by the time symptoms become evident. The continuous and progressive nature of disease is one of the components of our definition that separates disease from injury.

Third, disease results from the activities of a *causal agent*. Most people would agree that diseases are caused by something. Specifically, causal agent refers to a **pathogen**, an organism that can cause disease. Usually, a single agent causes a disease, but some diseases are caused by two or more pathogens acting together. As we have seen, the germ theory of disease is a fundamental concept of plant pathology. This is another component of our definition that separates disease from injury and implies that disease is contagious; pathogens can be spread and infect neighboring plants. Injury is not contagious; it is not capable of being moved or spread.

Fourth, disease leads to the expression of *symptoms*. Symptoms are the evidence that something has altered the normal development or appearance of a plant (Figure 1.2). Plants respond to the presence of disease in multiple ways, but symptoms typically fall into groups or categories based on the part or processes of the plant affected, as we will see later. Symptoms may be minor, barely detectable, or severe, up to plant death. Some symptom types are unique to specific diseases, but some are common to many different causes, just as fever in humans can indicate anything from a slight cold to bubonic plague. Furthermore, the presence of symptoms is not unique to disease and thus does not separate disease from injury. By keeping these four components in mind when you think of disease, you can begin to develop a conceptual framework to build upon as we introduce and discuss the many complexities of diseases in plants.

In our narrow sense of disease, biotic organisms cause all plant diseases. In a broader view of disease, abiotic factors may also cause disease. Most authors consider these abiotic diseases to be *disorders*, because typically either they are not the product of a continuous interaction, or they are not contagious (do not have a pathogen associated with the damage), or both. Disorders can result from nutritional deficiencies or toxicities, exposure to harmful levels of air pollutants, flooding, drought, and many other causes. While this broad sense of disease is helpful to understand and diagnose plant problems, plant pathology is developed with a focus on the nature of diseases caused by living pathogens.

FIGURE 1.2 Abnormal development in rose caused by a viral infection. Note the proliferation of shoots typical of Rose rosette disease in this shrub rose. (Courtesy of H.D. Shew.)

Symptoms and Signs of Plant Diseases

A **symptom** is the visible expression of a disease. Names of symptoms are generally descriptive of the primary abnormality that we see, such as leaf spot, wilting, or stunting. However, some diseases produce a whole **syndrome**, a series of symptoms that are characteristic for that disease. For example, yellowing, wilting, and death of all the plant parts that are above ground typically indicate that the real problem is in the roots, perhaps caused by a root rot disease. Symptoms are often used in the common name of a disease. For example, black root rot is a common name that is used to describe a disease that results in the development of black and rotted roots on a plant. Common names can also be misleading, as many diseases have more than one common name. For example, the disease of peanut caused by the fungus *Sclerotium rolfsii* may be called southern stem rot or white mold.

Plants have a limited ability to express the abnormal or harmful effects of a disease or disorder. Symptoms thus fall into discrete categories based on the type of damage caused, the part of the plant affected, and when it occurs in relation to the development of the plant. A disease may be characterized by one or more symptoms that are diagnostic

for that disease, but in other cases additional information is needed to determine which disease (or diseases) is present.

The most common symptom of plant disease is **necrosis**. Necrosis is browning or blackening of host tissues brought about by cell death. This is a very broad category of symptom, and there are numerous necrotic symptom types. A localized area of necrosis is a **lesion**. Perhaps the most common and most easily observed necrotic symptom or lesion is leaf spot. A **leaf spot** is a localized area of necrosis on a leaf. Leaf spots may be very characteristic in some cases, or nondescript in others (Figure 1.3a), and typically have a defined size and shape. Another common necrotic symptom is a **canker**, a sunken area on the main stem or trunk of a plant, sometimes with raised margins (Figure 1.3b). A different type of sunken lesion that occurs on leaves, stems, and fruits is **anthracnose** (Figure 1.3c).

Necrosis may also extend across tissue types. For example, **blight** is a rapid blackening of host tissue and may include leaves, stems, and flowers (Figure 1.1a and b). A general (not localized) type of necrosis is called **rot**. Another descriptive term is often used along with rot to describe a disease, such as root rot, ear rot, stem rot, soft rot, fruit rot, and so on. Finally, necrosis that begins at the top of a plant and progresses downward is a **dieback** (Figure 1.3d). This symptom is typical for root rot and canker diseases, but also is common with abiotic factors that impact root growth.

Symptoms of disease may include various types of color changes. These symptoms may affect leaves, flowers, and fruits. The most common color change is **chlorosis**, which is yellowing due to lack of chlorophyll in leaves. A specific type of chlorosis around a necrotic spot is a **halo** (Figure 1.3a). Leaf spots may have borders of distinct colors as well, such as red or purple. These colors are distinctive symptoms for certain diseases. Loss of color, or **bleaching** of tissue, is also characteristic for some diseases. For example, diseases caused by viruses (Chapter 4) result in many patterns of color variation including **mosaic** (Figure 1.1c), **mottle**, and **ringspot**. A color-breaking virus is famous for driving "Tulipmania" in the seventeenth century. Tulips with variegated flowers were highly prized for their unique color patterns, and collecting them became an obsession with the Dutch. The mania for tulips led speculators to pay much higher prices for even a single blub. Finally, the bubble burst and Tulipmania ended. Today, color breaking in tulips and the variegated flowers and foliage seen in many ornamental plants are the result of selection for genetically inherited traits and not caused by a virus.

A common response of plants to pathogens is the production of overgrowths or galls. A **gall** is a localized swelling or overgrowth of host tissue, which results from cell enlargement (hypertrophy) and cell proliferation

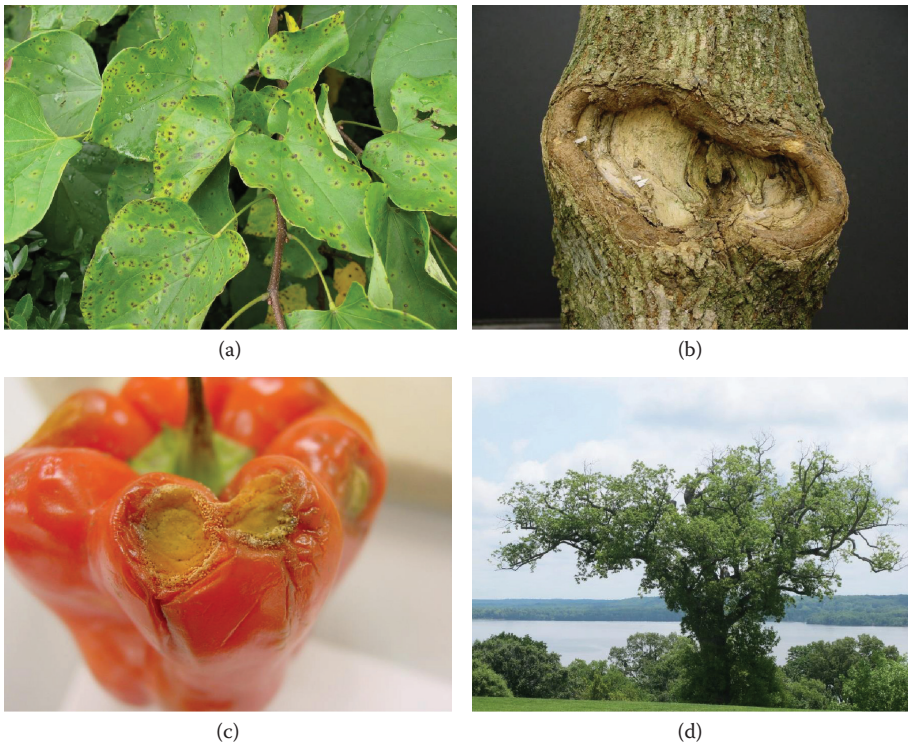


FIGURE 1.3 Common symptoms of plant diseases. (a) Leaf spot with chlorosis surrounding the spots. (b) Canker with rings of callous tissue around the infected area. (c) Sunken lesion of anthracnose on bell pepper. Note the abundant production of tan-colored spores in the sunken area. (d) Dieback of elm caused by the Dutch elm disease. (Courtesy of H.D. Shew.)

(hyperplasia) (Figure 1.4a and b). Multiple pathogen groups cause galls. Another common growth abnormality is **distortion**, or an abnormal formation or twisting of tissues and organs, especially leaves and fruit.

Symptoms may affect either the entire plants or the localized organs or tissues. **Wilt** is a general response to loss of water brought about by diseases that impede or degrade the vascular system. Wilts may occur as a result of stem cankers and root rots but are most commonly associated with the infection of the vascular system. **Vascular wilt diseases** are caused by pathogens that infect the xylem (Figure 1.5). Wilting may affect all of the plant or occur on one side of the plant or even one side of a leaf as specific areas of the xylem are plugged. One-sided wilting is called unilateral wilting. Another common whole plant symptom is stunting. **Stunting** is a reduction in plant size compared with an uninfected plant growing under the same conditions (Figure 1.6). Stunting is a common symptom in many virus-infected plants and plants that have root rot diseases.

(a) (b)

FIGURE 1.4 Examples of galls. (a) Small gall of corn smut, with an infected and swollen single kernel. (b) Small elongated gall (bottom) and a cross section through a large gall on pine caused by Fusiform rust disease. Note the very large growth rings in the galled tissue in the cross section. (Courtesy of H.D. Shew.)

FIGURE 1.5 Wilting of snapdragon caused by a plugging of the xylem tissue. The disease is a vascular wilt caused by the fungus *Verticillium dahliae*. (Courtesy of B.B. Shew.)

A **sign** is a part of the pathogen on or in the plant that is visible to the unaided eye. Signs may be very large or barely visible without magnification (Figure 1.7). In the broadest sense, a sign could include any visualization of a pathogen on or in the diseased plant, even those pathogens that, like viruses, can only be seen under extremely high magnification. However, we will confine our discussion to signs that can be seen with the unaided eye.

Signs are most easily observed with diseases caused by fungi. During periods of high humidity, fungi often produce visible vegetative growth or spores on infected plants. Some of our most important plant diseases are named for the signs they produce. For example, **rust diseases** (Chapter 14) are named for the rust-colored spores produced in abundance on their hosts (Figure 1.8a), **powdery mildews** (Chapter 12) are named for the powdery appearance of the hyphae and spores that the powdery mildew fungi produce on the surfaces of their hosts (Figure 1.8b), **smut** diseases (Chapter 14) are characterized by the black dusty spores produced in

FIGURE 1.6 Stunting of tomato in the production field due to viral infection. (Courtesy of H.D. Shew.)

FIGURE 1.7 Cluster of basidiocarps (sign) of the root rot pathogen *Armillaria*. (Courtesy of B.B. Shew.)

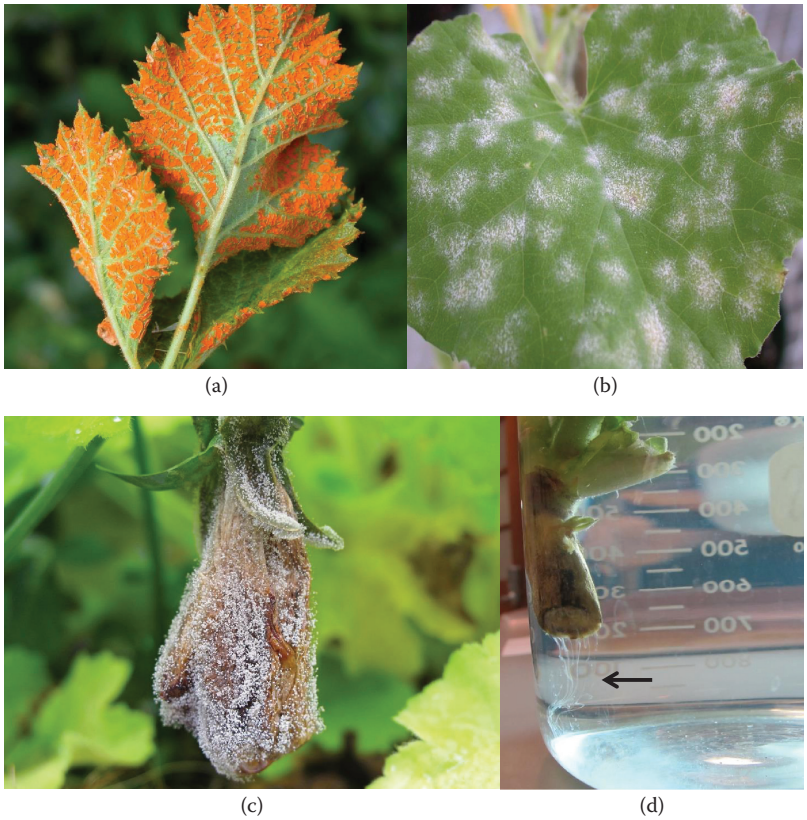


FIGURE 1.8 Examples of signs of plant pathogens. (a) Spores of the orange rust pathogen on blackberry. (b) Hyphae and conidia of powdery mildew on cucumber. (c) Abundant conidial production by *Botrytis cinerea*, the cause of gray mold on pansy. (d) Bacterial streaming (arrow) from the cut end of a tobacco stem infected by the vascular wilt bacterium *Ralstonia solanacearum*. (Courtesy of H.D. Shew.)

their hosts (Figure 1.4a), and **downy mildews** (Chapter 8) are named for the downy growth on the undersides of leaves. Pathogens that produce fuzzy masses of spores, or visible clumps or colonies of hyphae, are often referred to as molds (Figure 1.8c). All of these diseases also produce symptoms, but they are named and diagnosed by the signs they produced.

Bacteria (Chapter 5) are extremely small and single-celled, so they are not visible except in mass. Signs of bacteria include streaming and ooze, which is a combination of bacterial cells, extracellular slime produced by bacteria as they cause disease, and host cells (Figure 1.8d). Streaming is most frequently used to diagnose vascular wilts caused by bacteria but can also be used to diagnose leaf spots and other necrotic diseases. The most obvious sign of a nematode disease is the presence of adult females of a certain group of nematodes called cyst nematodes (Chapter 6). The enlarged, globose body of the female can be seen on the surface of infected roots. It changes from white to dark brown as she dies, forming a **cyst** that contains the nematode's eggs. Viral diseases do not have signs, as they are too small to be seen.

THE DISEASE TRIANGLE

As you have seen, disease is a product of complex interactions between host and pathogen. Clearly, if either host or pathogen is absent, there will be no interaction and disease cannot occur. However, host and pathogen will coexist without interacting unless the environment is favorable for infection and disease development. Thus, disease occurs only when host, pathogen, and environment come together at the right time and in the right place. The classic disease triangle (Figure 1.9) illustrates this fundamental concept.

Pathogens

With few exceptions, plant diseases are caused by microorganisms that are also parasites on their host. That is, they live in close association with the plant and derive all or most of their nutrients from it. Not all parasites are pathogens; in some cases, a parasite causes little harm to its host. The parasite becomes a pathogen when it impairs normal plant function through its activities, causing disease. Each species of plant pathogen causes

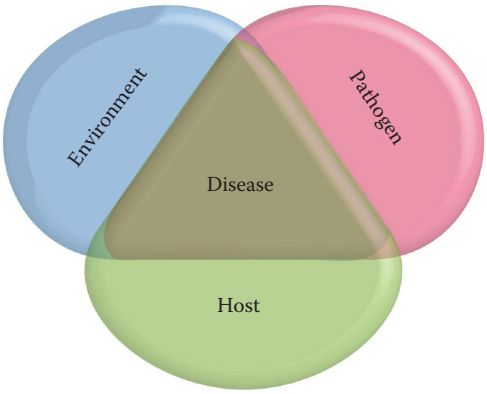


FIGURE 1.9 Disease triangle, illustrating the interaction of host, pathogen, and a favorable environment leading to disease. (Courtesy of Arlene Mendoza-Moran. With permission.)

FIGURE 1.10 Range of resistance responses observed in peanut to *Cylindrocladium* black rot disease. (Courtesy of Jerald Pataky. With permission).

disease on a limited number of plant species. The **host range** of a pathogen consists of all the host species on which it causes disease. Some plant pathogens have a host range of only a single host species, whereas others can attack hundreds of species across many plant families. **Pathogenicity** is the ability of a particular plant pathogen species to cause disease on a particular plant species. Pathogen species and individuals within a species vary in **aggressiveness**, which is the relative ability of the pathogen to inflict damage on its host: pathogens that cause severe symptoms are highly aggressive, whereas those that cause mild symptoms are nonaggressive. Pathogenicity and aggressiveness in the pathogen are inherited traits, but aggressiveness can also vary with the age, life stage, nutrient reserves, or previous exposure to adverse or favorable conditions of the pathogens.

Plants

Most plants are healthy during most of their lives. Just as a given species of pathogen has only a limited number of potential plant hosts, any plant species is host to only a limited number of plant pathogens. The plant species must be **susceptible** to the pathogen for disease to occur. A susceptible host is one that is capable of being attacked by a pathogen. **Susceptibility** (Latin: take up, sustain) is a measure of how well a host can sustain a pathogen's development. Therefore, within a host plant species, susceptibility exists along a continuum and can range from extremely high to low. If the plant actively reduces, delays, or prevents the development of a pathogen or disease, it has some form of **disease resistance** (Chapters 20 and 23). This resistance varies along the same continuum as susceptibility from very low (susceptible) to very high (Figure 1.10). In some cases, resistance is so high among some members of a normally susceptible host species that no disease is apparent at all. Often, however, resistance or susceptibility within a host species is a matter of degree and is described relative to a standard or average type. Like pathogenicity and aggressiveness, susceptibility and resistance are inherited traits. Susceptibility may also vary with age, growth stage, or the condition of the plant before infection. Injuries, nutrient excess or deficiencies, and stresses like drought or cold can **predispose** the plant to infection or make it more vulnerable to disease. Conversely, vigorous plants are usually less likely to become infected or diseased than less healthy ones.

Environment

Often, we think of the environment in terms of physical (e.g., temperature and moisture) and chemical factors (e.g., pH and nutrients). However, the biotic environment also profoundly affects host, pathogen, and disease. The biotic environment includes insects, nitrogen-fixing bacteria, beneficial and antagonistic microbes, competing pathogens, weeds, earthworms, and many other organisms. The physical characteristics and population density of plants may also affect the environment. For example, leaves shade the soil, transpiration increases humidity, and dense plantings reduce wind circulation, creating microclimates that can favor pathogen growth and disease development. Conversely, disease can change the environment near the infected plant, sometimes making that environment more or less favorable for further disease development. For example, defoliation may reduce shading and result in increased air circulation, whereas rotten fruits or vegetables may release moisture and nutrients that promote further infection and decay. Since disease develops over time, these processes may speed up, slow down, or even stop as the environment changes.

Each species of plant and pathogen has a range of environmental conditions that are optimal for growth. Plants are adapted to nearly every environment on earth, but all green plants require sunlight, oxygen, water, nitrogen, and an array of other essential elements. Likewise, plant pathogens can be found wherever there are plants.

In very general terms, plant pathogenic fungi and bacteria tend to thrive in moist (but not necessarily wet) soil and high humidity. The environment that favors a disease will be found within the overlapping range of environments that favor both the host and the pathogen. Sometimes, an environment that is best for the plant is likewise very favorable for the pathogen and thus disease. For example, high soil moisture and fertility may promote rapid plant growth, but at the same time encourage pathogen infection, growth, and reproduction. Conversely, the same conditions may inhibit both plant and pathogen, so that no disease develops. For example, plants usually suffer little disease during dry periods because, like plants, most pathogens need ample moisture to survive and infect. Naturally, some pathogens have evolved to thrive in dry conditions and can take advantage of a drought-stressed host. Disease often develops when the environment is not ideal for the pathogen, but is even more unfavorable for host. For example, seed germination may slow down or even stop in cold, wet soil. This makes seedlings vulnerable to infection by a variety of soil-inhabiting pathogens, even those that prefer or grow optimally at warmer soil temperatures.

Understanding the dynamic balance between host, pathogen, and environment is a key to developing sound disease management strategies. Actions that promote the general health and resistance of the host, remove or impair the pathogen, and shift the physical, chemical, or biotic environment help to reduce or prevent diseases and the losses that they cause.

DISEASE CYCLES

Disease develops when a host and a pathogen interact in a sequential series of events. Because these events recur over time, they can be envisioned as a cycle, paralleling to varying degrees of life cycles of the two organisms. Plant pathologists refer to the continual repeating steps in the interactions between a host and a pathogen as a **disease cycle**. Often, the major stages or steps in disease development are depicted in a diagram that helps us to visualize how a disease progresses from beginning to end (Figure 1.11a and b). Disease cycle diagrams and the concepts they represent provide an excellent framework for understanding how disease develops and even how it can be managed.

In most climatic regions, plants complete a single cycle of growth, development, and reproduction per year. Even in tropical regions, plants grow in cyclic patterns that usually correspond to seasonal changes in the environment. Crop plants are mostly cultivated as annuals, with some notable exceptions, including fruit and tree crops, so they have a single cycle of growth per year. The majority of pathogens have the capacity to complete many life cycles per year, but some are limited to a single cycle. The number of cycles of disease that occur per year (or host growth cycle) is one of the most important characteristics of a particular disease. **Monocyclic** diseases complete one disease cycle per year (Figure 1.11a). **Polycyclic** diseases complete multiple cycles of disease per year (Figure 1.11b). Disease cycles that repeat or extend over more than one year are referred to as **polyetic** diseases.

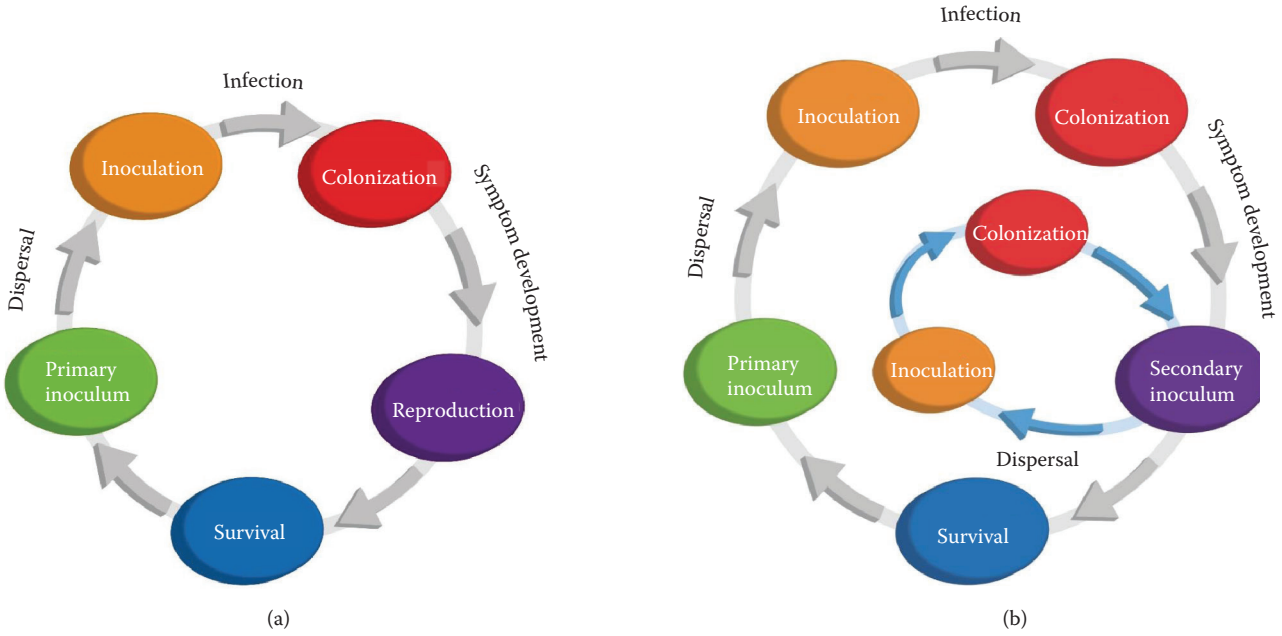
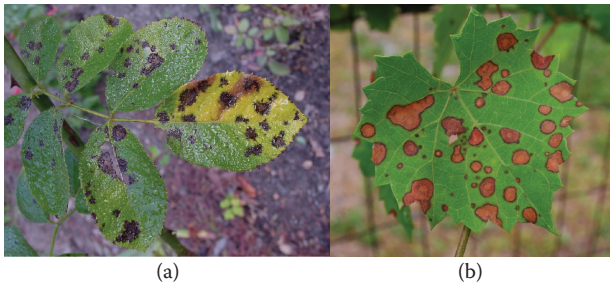


FIGURE 1.11 Graphical representation of the most common types of disease cycles. (a) Monocyclic disease, with only one cycle per plant-growing season. (b) Polycyclic disease, with multiple cycles of disease per plant-growing season. (Courtesy of Arlene Mendoza-Moran. With permission.)

Although the specific features of each disease cycle are unique, some general features are present in all cycles. Plant growth begins from a dormant stage, for example, germination of a seed, growth from tubers, bulbs, or vegetative cuttings, or bud break in perennial species. Similarly, pathogens typically start new growth from a dormant stage. The dormant stage or period of inactivity for a pathogen is referred to as **survival**. Pathogens use a variety of strategies and structures to survive periods when the host is not present or when the environment is not favorable for growth. Specific strategies for survival by specific types of pathogens are discussed in Chapters 4 through 17.

Survival structures are a type of **inoculum**, the part of the pathogen that can infect a plant. The inoculum that begins a disease cycle is referred to as **primary inoculum** (also known as initial inoculum, overseasoning or overwintering inoculum). Germination or regrowth of the pathogen may be dependent on the presence of host exudates or totally independent of the host. For example, soybean cyst nematodes require a specific organic molecule exuded from soybean roots to induce the emergence of juveniles from the survival cysts. In contrast, production of ascospores, the primary inoculum of the Camellia petal blight pathogen *Ciborinia camelliae*, is based solely on environmental conditions. Likewise, the pathogen may actively find the host by swimming or growing toward it or may be carried passively in air, soil, water, or other agents to the host. In all the cases, the success of the primary inoculum in finding, contacting, and infecting a host will determine whether disease will occur. If the primary inoculum is not successful in finding a host, then disease will not develop and no new inoculum will be produced in the next season. Because monocyclic diseases complete only one cycle of infection per year, they depend entirely on infections from primary inoculum. While infection by primary inoculum is also required for polycyclic diseases, it is less important in determining the amount of disease that ultimately develops, because even a few infections from primary inoculum can give rise to many cycles of infections during the year. The chances of finding an **infection court**, a site on a host where the pathogen can infect, are very small for the typical microscopic plant pathogen. **Inoculation** of a host occurs when inoculum finds an infection court. Pathogens compensate for the low chance of success by any one unit of inoculum by producing very high number of individuals. The likelihood that the pathogen will find an infection court may be enhanced by its dispersal mechanisms. Pathogens disseminated by wind and rain may have a very low chance of finding a host, whereas pathogens that are disseminated by a vector have a much higher chance of success. A **vector**, an organism that transmits a pathogen, finds the host for the pathogen. Unfortunately, human activities also inadvertently move pathogens along with host plants. As we will see, this is the case with many of our most devastating epidemics, such as the chestnut blight that killed billions of chestnut trees in the eastern United States during the early twentieth century.

If the environment is favorable following inoculation, infection of the host occurs. **Infection** is the establishment of a food relationship between the host and pathogen and is the second stage of the disease cycle. Pathogens may infect directly through host tissues, but most use natural openings such as stomata or wounds in the plant that are caused by various types of injury. Once infected, all subsequent growth of a pathogen in a host is called **colonization**. Pathogens have specific patterns of colonization within host tissue(s), primarily based on how the pathogen obtains nutrients from host cells during pathogenesis. **Pathogenesis** (meaning: origin of suffering) is the sequence of events that occur during disease development. As the pathogen colonizes the host, it accumulates nutrients that it uses for growth and reproduction. The capture of these nutrients from the host takes place over time. The time between inoculation and reproduction is the **latent period**. This period may be shorter or longer than the **incubation period**, which is defined as the time between inoculation and symptom development. For example, powdery mildew fungi often produce new inoculum before any symptoms are present. Organisms that reproduce on dead tissue or after symptoms appear have latent periods longer than the incubation period. Inoculum that is produced on infected tissue and that is capable of infecting a new host immediately is termed **secondary inoculum**. If the inoculum is dispersed to and infects other plants within the same growing season, it is a polycyclic disease (Figure 1.12a and b). These secondary cycles of infection can result in devastating epidemics such as the potato late blight epidemic discussed earlier. Ultimately, the host will die or the environment will become unfavorable, slowing and then halting disease development on the infected plant. Late in disease development, a pathogen may produce survival structures that will allow it to remain dormant until the time when new host plants are again available. The disease cycle is



(a) (b)

FIGURE 1.12 Examples of splash dispersed pathogens. (a) Black spot of rose, with lesions of various sizes; splashing rain or water disperses spores to other leaves or to neighboring plants. (b) Leaf symptoms of black rot of grape, showing multiple generations of infections on the leaf caused by spores splashing from lesions with active sporulation. (Courtesy of H.D. Shew.)

now completed and will start again when all the factors necessary for the disease occur in the same time and space.

TYPES OF PLANT PATHOGENS

Trophic Levels

Pathogens can be separated into groups based on their trophic lifestyle; that is, how they obtain nutrients. With the exception of some parasitic seed plants, all plant pathogens are **heterotrophic**, which means they must capture their nutrients from another organism. Organisms that use live plant cells as their only source of nutrients are called **biotrophs**. Since they can exist only as parasites, they are also referred to as **obligate parasites**. Parasites that obtain their nutrients from cells that they kill through the production of toxins and enzymes are called **necrotrophs**. Although they feed and reproduce on dead cells, these organisms are not saprotrophs since they killed the host cells prior to using them as nutrients. **Saprotrophs**, on the other hand, have no parasitic phase and derive nutrients from nonliving sources. While some necrotrophs can live as saprotrophs when a susceptible host is not available, others compete poorly with true saprotrophs or even lack the ability to live independently from their hosts. **Hemibiotrophs** are pathogens that begin their relationship with their host as biotrophs, but become necrotrophic in later stages of pathogenesis. Biotrophic pathogens tend to have narrow host ranges, but some hemibiotrophs and necrotrophs also have a limited host range. Some necrotrophs, such as *S. rolfsii*, have a very extensive host range, attacking hundreds of hosts across many plant families. This fungus kills host cells and tissues by producing a very potent toxin, oxalic acid, and copious amounts of cell wall-degrading enzymes. This nonselective strategy of interacting with plants leads to the very wide host range. Not all pathogen groups fit well into these trophic levels. For example, viruses and viroids do not directly absorb and metabolize nutrients, so they do not have a true trophic lifestyle. However, they are obligate parasites in the sense that they replicate only inside living cells.

Fungi

Fungi (singular: fungus) are the most abundant group of plant pathogens. They cause many thousands of different plant diseases, producing a wide range of symptom types, including spots, blights, rots, galls, and wilts. Fungi belong to the Kingdom Fungi and the branch of science that studies fungi is **mycology**. Fungi find and explore new substrates by vegetative growth via microscopic filamentous threads called **hyphae** (singular: **hypha**). Hyphae are composed of individual fungal cells laid end to end. Growth occurs at the tips of the hyphae, or with branching followed by tip growth. When a potential food source is found, they secrete enzymes that break down complex molecules into simple compounds that are readily absorbed and used for growth, reproduction, and survival. In substrates rich in nutrients, the fungus can produce masses of hyphae called a **mycelium**. Mycelium may be visible to the unaided eye and sometimes is diagnostic. When grown in a sterile culture medium, the mycelium that forms is called a **colony**.

Fungi reproduce by the production of **spores**. Spores come in many sizes and shapes and play many roles in the sometimes-complex life cycles of fungi. They allow the fungus to find new sources of food as wind, rain, or vectors disperse them. Spores are the primary survival structure for most fungi and give rise to new growth when conditions become favorable for growth.

Classification of fungi is based on the type of sexual spore produced. For example, the **ascospore** is the sexual spore of the **Ascomycota** (Chapters 11 through 13) and the **basidiospore** is the sexual spore of the **Basidiomycota** (Chapters 14 and 15). A large group of fungal plant pathogens, the **mitosporic fungi** (formerly Deuteromycota) or imperfect fungi, produce only asexual spores called **conidia**. These imperfect or mitosporic (Chapter 13) fungi may lack a sexual cycle entirely or they may reproduce sexually only under very specific and rare conditions so that the sexual stage is unknown. Finally, the **sterile fungi** do not produce spores at all. These fungi propagate and survive either as hyphae or in structures made of masses of hyphae such as **sclerotia** (singular: **sclerotium**).

Many plant pathogenic fungi produce both sexual and asexual spores. Historically, these different spore stages were each given a distinct genus and species name known as a Latin binomial. This dual naming system led to the use of the terms **teleomorph** for the sexual stage, **anamorph** for the asexual stage, and **holomorph** for both the stages. For example, one of the most common plant pathogens is known mostly by its anamorph name, *Rhizoctonia solani*. The teleomorph and holomorph name for the organism is *Thanatephorus cucumeris*, but this name is rarely used because the organism is observed in its anamorph stage most of the time. The dual naming of organisms contradicts the purpose of naming organisms with a unique Latin binomial, which was to eliminate confusing nonstandard common names, thus allowing people to communicate more accurately. The confusion caused by having two names for one organism has led to an effort to establish one scientific name for all organisms (one organism: one name), including plant pathogenic fungi.

Fungi are phylogenetically more closely related to animals than plants. Like plants, fungi have cell walls, but these walls are made primarily of **chitin**, which is also the main component of the exoskeletons of insects and crustaceans. Unlike animals and vascular plants, fungi spend most of their life cycle as **haploid** organisms, meaning they have only one set of chromosomes in their nuclei. Cells may contain one or more nuclei,

and cells are separated by the formation of **septations**. These hyphae are thus called septate. The septate hyphae may have characteristic features such as color, branching pattern, or the presence of distinctive structures, but few fungi can be identified by hyphal characteristics alone.

Fungi use multiple strategies to survive. As we have seen, some fungi can survive as saprotrophs in the absence of a host. Others survive as the same spores found during pathogenesis and epidemic development, or they may produce highly specialized survival spores. In some cases, fungi survive in reproductive structures produced on or inside living or dead host tissues. Other fungi survive by producing various kinds of thickened vegetative cells or clumps of cells, including **stromata** (singular: **stroma**), **chlamydospores**, and **microsclerotia**. Sclerotia are very common vegetative survival structures that may survive for years in the absence of a host. Under favorable conditions, they may germinate directly, producing mycelium that acts as primary inoculum, or in some species they may germinate indirectly to give rise to sexual or asexual spores that are primary inoculum. Pathogenic fungi may survive as hyphae in tissues of perennial hosts. The pathogen begins growth as the host begins a new life cycle. For example, in Fusiform rust of pine, the fungus survives vegetatively in perennial galls and produces a new set of spores on the galls each spring (Figure 1.4b).

Oomycetes

A group of organisms very similar to fungi in appearance and function are the Oomycota (Chapter 8). These fungus-like organisms include many important pathogens, including the late blight pathogen, *P. infestans*. Important pathogens in this group include the downy mildews and members of the genera *Phytophthora* and *Pythium*. Appearance can be deceiving, because this group of pathogens may look like fungi, but they are more closely related to plants than animals. Like plants, these organisms have cellulose cell walls, but unlike plants, they are heterotrophic and obtain their nutrients by absorption. These organisms belong to an entirely different kingdom of organisms, the Stramenopila. Most organisms in this group produce characteristic biflagellate swimming spores called **zoospores**, so they are sometimes called water molds. The sexual spore is the **oospore**. Also, unlike fungi, hyphae of the Oomycota do not generally have septations; however, septations are found at the base of reproductive structures. Because the hyphae are mostly **nonseptate**, cells are multinucleate (**coenocytic**), and streaming of the cytoplasm and other cellular contents may be evident upon microscopic examination. Individual nuclei are diploid (2N), with haploid cells produced only inside sexual structures called **oogonia** and **antheridia**. Oospores germinate to produce mycelium or they may produce **sporangia**. These sporangia may in turn bear zoospores or produce mycelium, depending on environmental conditions. Some species of oomycetes produce sexual spores very rarely and some species produce sporangia but never zoospores. These pathogens survive using similar structures and strategies as fungi. Survival structures include oospores, chlamydospores, and hyphae in infected or infested plant debris.

Bacteria

Plant pathogenic bacteria cause a wide range of symptoms and diseases in plants (Chapter 5). Common symptoms of bacterial infection include leaf and fruit spots, soft rots, cankers, galls, and vascular wilt. Plant pathogenic bacteria are unicellular **prokaryotes**; that is, they are organisms that lack a nucleus and other membrane-bound organelles such as mitochondria and chloroplasts. Plant pathogenic bacteria are heterotrophic organisms that attain nutrients by absorption. They secrete enzymes and toxins to kill cells and break down potential sources of nutrients. Bacteria are much smaller than fungi, typically less than 3 μm in length (a human hair is about 75 μm in diameter), and they have relatively simple morphologies, especially compared with fungi and nematodes. With few exceptions, plant pathogenic bacteria have cell walls and are rod-shaped; many also have one or more flagella arranged in various patterns on the cell. A mass of bacteria is called a colony, with color, shape, and morphology of the colony being important characteristics. Cells are often coated with a slime layer, and some bacteria produce colonies that appear distinctly slimy in culture. However, bacteria are very difficult to identify from their appearance under the microscope or in culture. Typically, a range of laboratory tests are needed to identify bacterial species based on their ability to break down different types of carbohydrates and other nutrients under different cultural conditions. Sequences of specific genes are also useful for identification. In addition to the bacteria that have cell walls, the phytoplasmas and spiroplasmas are wall-less bacteria that cause important plant diseases. These wall-less forms are the only obligate parasites among the plant pathogenic bacteria. Most bacteria are present on host surfaces and are intercellular when inside the host. They do not penetrate host cells.

Bacteria multiply by **binary fission** (splitting into two), with cells dividing rapidly when nutrients are available. Bacteria do not reproduce sexually, but bacterial cells sometimes exchange genetic materials through **conjugation** and the transfer of **plasmids**, which are small pieces of DNA found inside the bacterial cell. Bacteria also obtain new DNA and new variability via absorption of free DNA, **transformation**, or as a result of movement from cell to cell via infection by viruses called bacteriophages, **transduction**.

Bacteria require wounds or natural openings to penetrate their hosts. Once inside a host, they reside in the intercellular spaces and produce many of the same types

of weapons in pathogenesis as fungi. Unlike many other types of bacteria, most plant pathogenic bacteria do not produce survival spores that are highly resistant to heat and other adverse environments. It is important to note, however, that spore-forming bacteria living on and in plants are a major cause of contaminated produce and that their control is critical for maintaining food safety. Because of their small size and lack of specialized survival structures, plant pathogenic bacteria have evolved somewhat different survival strategies than eukaryotic plant pathogens. Plant pathogenic bacteria typically survive in groups of cells in a **biofilm**. The biofilm is secreted by the bacteria and protects them from harmful external factors. It also allows the bacterial cells to communicate effectively and sense when it is appropriate to initiate life cycle events such as attempting to infect a host. Bacteria survive in vectors, or in biofilms as epiphytes, in host plant debris, in and on seeds, or in soils.

Nematodes

Symptoms caused by plant parasitic nematodes include wilting, yellowing, stunting of entire plants or organs, root or leaf lesions, and galling (Chapter 6). Nematodes are unsegmented roundworms of the phylum Nematoda. Most nematodes are free-living saprotrophs or predators, but many are also important animal or plant parasites. All plant parasitic nematodes are biotrophs; they must feed on living plant cells in order to obtain nutrients. Nematodes have specific feeding habits, feeding either as **ectoparasites**, from outside the host, or as **endoparasites**, from inside the host. Ectoparasites and endoparasites may be either **migratory**, moving from cell to cell, or **sedentary**, staying in one place once a feeding site is established. Most plant parasitic nematodes feed on the roots of plants, but there are some genera that feed on leaves and at least one genus feeds in the xylem of trees.

Nematodes are identified based on their morphological features, as they are readily observed with a low-power microscope. They range in size from 300 µm to almost 4000 µm (4 mm) in length. Many nematodes are long and slender, but some are sausage-shaped and others take on a globose appearance as they mature. Typically, male and female nematodes mate to produce eggs, referred to as **amphimitic** reproduction, but in some species females can produce viable eggs without mating, a process known as **parthenogenesis**. All nematodes produce eggs, and the juveniles differentiate and undergo a molt before hatching. The **second-stage juvenile**, which emerges from the egg, is the first infective stage. Three additional molts occur as the nematode grows and matures. All plant parasitic nematodes have a hollow protrusible stylet that they use to penetrate host cells and obtain nutrients. Various enzymes, toxins, and growth regulators are injected into the host cell during feeding. Although they are biotrophic, migratory nematodes may kill host cells during feeding. In contrast, sedentary feeders must repeatedly feed from the same cells over extended periods without killing them.

Nematodes may have specialized survival structures or stages. Nematodes may survive as eggs, as quiescent or resting larvae, or as adults. Cyst nematodes survive as eggs inside the hardened body of adult female. The wheat seed gall nematode can survive for many years living in a desiccated state inside a wheat seed.

Viruses

Viruses are noncellular entities that do not directly absorb and metabolize nutrients (Chapter 4). However, they are obligate parasites in the sense that they depend entirely on living host cells for the basic materials and cellular mechanisms necessary for their replication. Viruses can replicate only inside living cells. Viruses cause some of the most devastating diseases of plants. Many symptoms caused by viruses, including wilting, yellowing, necrosis, stunting, and lesions, are similar to those caused by other pathogens. Viruses also cause symptoms that are distinctive, including color breaking, mottles, ringspots, vein clearing, and unusual patterns of plant growth.

Viruses are very small (measured in nanometers) in comparison with other pathogens discussed earlier. Virus particles are only visible by electron microscopy and may be rod-shaped, filamentous, or bacteria-like, or have a geometric three-dimensional form (**isometric**). Individual virus particles are called **virions** and are composed of either RNA or DNA surrounded by a protein coat (**capsid**). Viruses are classified based on the organization of their genome, the shape of the particle, and their natural means of transmission and host range. Depending on the virus species, the DNA or RNA can be single- or double-stranded and the synthesis of new nucleic acid may proceed in either a positive or negative direction. Single-stranded RNA species are the most common viruses in plants. In addition, the virus genome may be organized as a single strand or it may be **multipartite**, that is, broken up into two to four separate pieces. Virus species are given descriptive names rather than Latin binomials. Species are named for the host on which they are originally described, then by the most common symptoms associated with the virus, and then the word virus. For example, tomato spotted wilt virus was first observed on tomato, the primary symptoms are spots and wilt, and it is a virus. Virus names are commonly made into acronyms, for example TSWV for tomato spotted wilt virus. The host range of viruses varies greatly from a few species to many hundreds, and the host for which the species is named may not be the host most severely affected. Dispersal of viruses, with the notable exception of tobacco mosaic virus, is highly dependent on a specific relationship with a vector. The types of vectors vary with the virus, but insects are the most common vectors. Among insects, the

most common vectors are aphids and whiteflies. Noninsect vectors include mites and plant parasitic nematodes, fungi, and plants. Viruses have very limited survival capacity once outside of its host or vector, so survival is typically in their host, in alternative perennial or annual hosts (including weeds), or in their vectors. These alternative hosts or vectors serve as primary sources of inoculum for epidemics caused by viruses, as do propagative materials like tubers and cuttings.

Parasitic Seed Plants

The vast majority of plant species are **autotrophs**, that is, they produce their own food by photosynthesis, but some species have evolved as parasites of other plants (Chapter 17). In addition to the presence of the plant itself (signs), symptoms of infection with parasitic seed plants include yellowing, poor growth, stunting, wilting, swellings or galls, and excessive branching known as **witches' brooms**. Parasitic plants either lack roots entirely or produce highly modified roots that penetrate the host in order to obtain its nutrients. The common true mistletoes infect the stems of trees but produce their own chlorophyll. They rely on the host plant only for mineral nutrition and water. On the other hand, dwarf mistletoes absorb minerals, plant nutrients, and water from the stems of their conifer hosts, greatly reducing the tree's productivity. They also stimulate the formation of witches' brooms and other growth abnormalities. Species of the vine dodder are very common parasites on stems of both herbaceous and woody plants. Most cause little harm, but others can result in extensive overgrowths and damage to plants. Dodders can also vector certain plant viruses. Some parasitic plants, including witchweed and broomrapes, attach to host roots and use the host's nutrients for their own development. These plants have wide host ranges, which include important crop species such as corn, sorghum, beans, cowpeas, and other legumes. These parasites can be devastating to yield and productivity and can be particularly difficult to control.

Parasitic seed plants reproduce by producing flowers and seeds. As with other higher plant species, they are separated into groups based on their morphology and phylogeny. Parasitic seed plants generally survive as seeds. The seeds are adapted to be dispersed by a variety of mechanisms, including forcible discharge in dwarf mistletoes, and dispersal by birds and other animals in the case of true mistletoes. Other species of parasitic plants are very prolific producers of seeds that survive in soil until a susceptible host is grown.

DISEASE DIAGNOSIS

Earlier in this chapter, we defined disease as *a condition detrimental to the normal development of a plant resulting from the continuous interaction between the plant and a causal agent leading to the production of symptoms*. We can use this definition to look at how diseases are diagnosed (Chapter 30).

Diagnosis begins with observation of the entire plant, including the roots. The diagnostician must first identify the plant and know the characteristics of a normal plant of the same species and variety. This can be a daunting task, considering the thousands of plant species and varieties under cultivation around the world. As we saw with color breaking in tulips, an unusual plant type may be inherited genetically or may be the symptom of a disease. On the other hand, symptoms such as stunting can be subtle and hard to distinguish without the knowledge of normal plant appearance and development.

As the diagnostician examines the plant, they look for symptoms and signs. Signs are usually highly diagnostic of a particular disease because they immediately point to the pathogen. Some symptoms are so diagnostic that no further investigation is needed. More commonly, however, individual symptoms are only clues. For example, wilting is a symptom of many diseases, which can only be distinguished by checking for additional symptoms like root rotting or vascular discoloration or by attempting to culture the pathogen.

Observations or information about the location and history of the problem are equally important. This information will help the diagnostician to separate diseases (resulting from a continuous interaction) from injuries and disorders. The diagnostician must consider the setting: where was the plant growing and how was it cultivated? How quickly did the symptoms appear and where are they distributed in the field, greenhouse, or landscape? As diseases are contagious, we expect them to increase over time and appear in clusters or clumps. In contrast, disorders often develop very quickly and tend to affect all of the plants in an area. Recent weather, for example, rainfall, temperature, cloudy periods, humidity, and frosts, must be considered. Warm, humid periods are ideal for many blight diseases, but rapid death of tender leaves could also indicate a recent frost. Taken together, the recognizable associations of signs, symptoms, timing, location, and history constitute a syndrome or signature that is typical of a particular disease.

Because any plant species is host to only a limited number of pathogens, it is possible to narrow the range of possible causal agents to a fairly manageable few once the signs, symptoms, and history have been investigated. A **host index**, such as the U.S. Department of Agriculture Fungal Database (http://nt.ars-grin.gov/fungaldatabases/), lists all the hosts on which a pathogen has been identified, and most are searchable by host or pathogen. The host index can help to guide the diagnostician as they consider whether additional culturing or diagnostic tests are necessary to identify the causal agent.

When signs are not visible, the affected parts of the plants may be checked microscopically for evidence of the

pathogen. Often, the diagnostician can observe the mycelium of fungal or bacterial cells but cannot find spores or other identifying characteristics. In those cases, the diagnostician can attempt to obtain pure cultures of the organism in order to identify it or perform additional diagnostic tests.

Although signs and other direct evidence of a pathogen strongly indicate it as the causal agent, it is important to consider whether the pathogen identified agrees with all the other observations that have been made. The diagnostician should consult reliable references that describe the disease and verify that the observations fit published descriptions before making the final diagnosis.

Often, the exact cause of a disease cannot be determined with certainty. Sometimes, critical information is lacking. It may not be possible to culture the pathogen, either because it is an obligate parasite or because it has died. In other cases, a potential pathogen can be identified, but the symptoms or other observations do not match those described as being caused by the organism in question. Finally, an organism may be positively identified, but not previously described as a pathogen on the host. In that case, the diagnostician may decide to perform Koch's postulates in order to confirm the organism as the cause of the disease.

Koch's Postulates—How We Determine the Cause of a Disease

In the 1870s, the scientist Robert Koch began a series of experiments that would change the science of pathology. From the 1850s to the 1870s, many scientists conducted scientifically sound experiments to prove the germ theory of disease in plants. In the 1860s, Louis Pasteur completed his famous experiments showing that microbes developed on boiled substrates only if they were exposed to air after boiling. This work conclusively disproved the theory of spontaneous generation. However, there was still no widely accepted standard of proof that a specific microbe caused a specific disease.

Koch worked with anthrax, a lethal disease of sheep. He was able to see the bacteria in the blood of infected animals and not in healthy animals. He was also able to initiate disease in healthy animals by injecting them with blood from infected animals. But the question remained: how could he prove that there was not something else in the blood of the infected animals that caused anthrax? He needed reliable methods to **isolate** the bacterium from the blood of the infected animals by growing it in pure culture, free of all other organisms. He could then introduce the purified pathogen into healthy animals and see if disease developed. As momentum increased for studying the importance of the microbial world, Koch and others developed improved techniques for isolating and growing organisms in pure culture. In 1876, Koch published his results and developed the germ theory of disease for anthrax. In the early 1880s, he developed a set of rules known as **Koch's postulates** (Chapter 31). These postulates are now used as a guideline for establishing proof of pathogenicity; that is, proof that a specific pathogen is the cause of a specific disease (Figure 1.13).

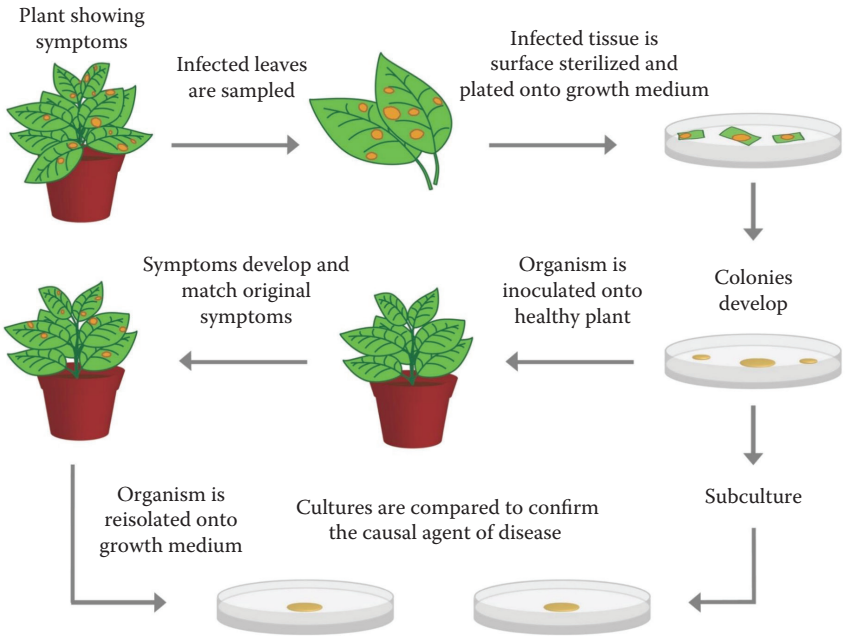


FIGURE 1.13 Diagram of the steps involved in Koch's postulates. These four steps are used to demonstrate that a specific organism causes a disease. Modifications of these steps are needed for pathogens that cannot be cultured. (Courtesy of Arlene Mendoza-Moran. With permission.)

The postulates are as follows:

1. There must be a constant association between the presence of the pathogen and the disease.
2. The pathogen must be isolated into pure culture from the symptomatic host.
3. The pathogen must be inoculated into a healthy host of the same species or variety and the original symptoms reproduced.
4. The pathogen must be reisolated from the inoculated host and be identical to the original pathogen.

It is important to note that Koch's postulates are guidelines for the proof of pathogenicity and must be modified somewhat when applied to those pathogens that cannot be grown in pure culture. In those cases, the pathogen is grown on host plants and not in sterile medium. Interestingly, this is the approach taken by deBary in confirming *P. infestans*, which is very difficult to culture, as the cause of late blight of potato.

IMPACTS OF PLANT DISEASE

Plant diseases have wide-ranging impacts on plants and the organisms that depend on them for their existence. There are many examples of disease in natural and agroecosystems that have had devastating consequences for humans. In general, plant diseases matter to humans because they (i) make food scarce, sometimes to the point of malnutrition or even famine; (ii) increase the cost of food and other products due to yield losses or increased production costs; (iii) make products dangerous for consumption by producing toxins; (iv) damage natural habitats; (v) reduce the esthetic value of produce, flowers, trees, and other plants and plant products; (vi) degrade environmental quality due to loss of key species or some methods of disease control; and (vii) inhibit trade in plant products due to the threat of pathogen movement across geographic or political boundaries. Although not all diseases are dramatic in their effects on plants, most cultivated plants have important diseases that must be managed each year to prevent some of the problems described earlier.

Occasionally, plant diseases are beneficial to humans. For example, corn smut produces edible galls that are highly prized in Mexican cuisine, while certain fungi produce beautiful grains and staining patterns in wood products. In some cases, plants are deliberately inoculated with plant pathogens or managed so that the desirable or beneficial product will be produced.

As we have already seen, plant diseases develop only when all elements of the disease triangle, host, pathogen, and environment, come together at the same time and place. New diseases may occur when host or pathogen is introduced in a new place for the first time. On the other hand, existing diseases can become important or reemerge when one element of the existing disease triangle changes or shifts. What factors lead to these changes and subsequent impacts? Why do some diseases cause extensive losses and others remain minor or unimportant? If we focus on the disease triangle, it is clear that some of our most important epidemics have come about by a significant and often abrupt change in the balance between pathogen, host, and environment.

Exotic Pathogens

Worldwide, many of the most devastating epidemics come about after the introduction of an exotic pathogen. An **exotic pathogen** is one that is introduced into a new geographic area. Of course, the presence of this pathogen becomes noticeable only when it finds a susceptible host, growing in an environment also favorable for the pathogen. This new host is often a relative of one of the pathogen's established hosts; likewise, the plant may already be host to a closely related species of the pathogen. In these cases, host and pathogen have coevolved through a kind of trench warfare, in which each has developed defensive and offensive strategies that keep each partner partially at bay. However, the exotic pathogen may be extremely aggressive on its new host and the host highly susceptible to the exotic pathogen because these partners have not evolved together. The list of epidemics caused by exotic pathogens is a long one, with examples present on all continents and across all pathogen types. In most cases, people inadvertently introduce these pathogens. Well-known examples of epidemics caused by exotic pathogens include late blight of potato, chestnut blight, white pine blister rust, Dutch elm disease, Jarrah dieback in eucalyptus and other species, downy mildew of grape, fire blight of apple and pear, citrus canker, plum pox virus, dogwood anthracnose, Camellia petal blight, and many others. These exotic pathogens, along with numerous insect pests, are the principal justification for establishing regulations and plant quarantine laws by all countries.

An excellent example of an exotic pathogen that resulted in total devastation of a host is the chestnut blight pathogen, *Cryphonectria parasitica*, which was brought into the United States. The American chestnut was the dominant tree in the eastern half of the country and was very important in many aspects of the economy. It has been said that the chestnut tree was used in rural mountain areas from the cradle to the grave. The wood was used not only for building houses and furniture but had many other uses because of its resistance to decay. The same tannins that made the wood resistant to decay were extracted and used in leather tanning. Chestnut trees were an abundant source of nuts for humans and wildlife. The chestnut blight epidemic destroyed billions of chestnut

trees from Georgia to Maine and changed Eastern forests forever. What happened?

Cankers from chestnut blight were first observed on trees in the New York Zoological Gardens in 1904. Within just 4 years of the original observation, the disease was present in multiple states in the Northeast, and within 10 years, it was widespread throughout the northeastern states and as far south as North Carolina. All efforts to stop the spread were ineffective. Over 4 billion trees were lost in less than 30 years, and the American chestnut population was destroyed by the early 1950s. The pathogen does not kill roots, so young trees that develop from root sprouts can still be seen occasionally, only to be killed by the pathogen that is now endemic in eastern U.S. forests.

The pathogen was most likely introduced in many locations in small cankers on Japanese and Chinese chestnuts, which have coevolved with the pathogen and are not severely damaged by it. The disease was not reported on Chinese chestnuts until 1913, well after the disease had become epidemic in the United States. In contrast, *C. parasitica* found a highly susceptible host in the fast-growing American chestnut, which had natural growth cracks that served as ideal infection courts for both wind- and rain-dispersed spores. Casual vectors, such as birds that picked up spores on their feet, also moved the pathogen. The pathogen thrived in a highly favorable environment, uninhibited by host resistance or natural antagonists.

Chestnut blight is one of three major diseases that led to the passing of the Plant Quarantine Act of 1912, which was intended to prevent further catastrophes like chestnut blight. The disease changed the composition of eastern hardwood forests and changed the way of life for many people who depended on the tree. There are extensive efforts to breed resistance to the blight pathogen into American chestnut, with seedlings now planted in natural forests to determine whether they will be able to survive.

Exotic Hosts

Sometimes new diseases result when a new host is introduced into an area where it finds a pathogen in wait. This **exotic host** completes the disease triangle, but its impact is usually not as devastating as observed with exotic pathogens. For example, when the Dutch introduced a New World (Americas) crop, tobacco, into Indonesia in the 1860s, the crop thrived until it was planted in an area on the island of Sumatra where a new disease developed. This root and crown rot disease, caused by the oomycete *Phytophthora nicotianae*, was described in 1896 and named black shank. The black shank pathogen was eventually spread on tobacco back to the Western Hemisphere and then to most tobacco-producing areas of the world. If tobacco had never been introduced to Sumatra, it is possible that millions of dollars of annual losses to black shank would have been avoided. A change in the host itself may result in a drastic change in its response to a pathogen. An excellent example of this occurred in the hybrid corn crop in the United States during the late 1960s and early 1970s. Hybrid corn is grown because yields are higher and hybrid plants are more vigorous than their inbred parents, a phenomenon known as hybrid vigor. Production of hybrid seed requires a male and female parent, with the female inbred line being pollinated with pollen from the desired male line. In corn, this meant that the pollen-bearing tassels of female (seed-bearing) plants had to be removed to prevent self-pollination. This was accomplished by hand detasseling, a very labor-intensive job. Plant breeders took advantage of the discovery of a cytoplasmically inherited trait that caused male sterility. A seed parent with male sterile cytoplasm did not have to be detasseled. The Texas cytoplasmic male sterile (Tcms) trait was soon used in almost all seed corn produced in the United States. While the nuclear genes varied in the corn varieties planted from Florida to Wisconsin, their cytoplasm was identical. This genetic uniformity in the cytoplasm was about to spell disaster for the corn crop in 1970. What happened?

Southern corn leaf blight, caused by *Bipolaris maydis*, was a minor disease of corn in the 1960s. Year-to-year losses varied slightly, but the disease was not considered to be a severe threat to corn. In the late 1960s, localized areas of unusually severe disease caused by this organism were observed. When an early season epidemic began in Florida in 1970, one of the worst epidemics in the history of U.S. agriculture was afoot. All the elements of the disease triangle came together to cause a severe epidemic that year. The environment was highly favorable for southern corn leaf blight throughout the entire summer, and several hurricanes helped to spread inoculum up from the South through the Corn Belt. The epidemic spread throughout most of the U.S. corn crop, with losses exceeding a billion dollars (about $6 billion in 2013 dollars). The epidemic sent the seed corn industry into a crash program to breed inbred corn lines without the Tcms trait before the next season. Within 2 years, Tcms was gone from corn germplasm sold in the United States.

The genetically uniform host had been selected for a variant in the pathogen population that was highly aggressive on corn that carried the Tcms trait. This pathogen variant, race T, produced a toxin that specifically recognized and acted on a cytoplasmic trait associated with Tcms. This type of recognition is common with host-specific toxins produced by some fungi. The southern corn leaf blight epidemic uncovered the genetic vulnerability of a major food crop and forever changed our perceptions of the hazards of genetic uniformity. The

epidemic demonstrated the importance of cytoplasmic inheritance of traits and clearly showed how a minor pathogen could cause a devastating epidemic when a susceptible host selects for variants in the pathogen population. As discussed in Chapters 23 and 26, strong selection pressures such as host resistance genes and site-specific fungicides result in significant shifts in pathogen populations and increased disease, but this epidemic illustrated that host factors other than disease resistance genes could also have significant impacts on the pathogen and thus disease.

Environment

Even when a host and pathogen are well established in the same place and time, disease will not develop without a favorable environment. People have long recognized that environment plays a role in disease outbreaks, even attributing disease itself to bad air (miasmas) or to excess rain in the days before the germ theory was established. As already discussed, late blight of potato became especially severe in the cloudy, wet summers of the mid-1840s in Ireland and Northern Europe. Likewise, the wet summer of 1970 contributed to the severity of the southern corn leaf blight epidemic of that year. Because of the importance of environment on disease, epidemics of some diseases are very sporadic, with no or minimal disease observed in a season following a severe epidemic. Sclerotinia blight of rapeseed in the Northern Great Plains provides an excellent example of how the environment impacts host and pathogen in disease dynamics. The fungal pathogen *Sclerotinia sclerotiorum* survives in soil as sclerotia, and when soil is cold and saturated for extended periods of time, the sclerotia germinate to produce tiny mushroom-like structures (apothecia) that produce millions of ascospores. Ascospores infect flowers, so disease is severe when the environment favors simultaneous production of ascospores of the pathogen and blooming of the host. Disease problems are minimal if the environment is unfavorable, and the infection court is not present when inoculum is produced. The reliance of this and many other epidemics on specific environmental conditions often allows plant pathologists to forecast or advise growers of potential disease outbreaks based on weather. Growers can then take steps to prevent disease losses through spray programs and other management strategies.

Although we often think of environment in terms of weather, the cultural environment also plays a very important role in disease development. Rotation, tillage, fertility, irrigation, planting date, pruning, and harvesting are examples of cultural practices that may influence disease development. A change in cultural practices can lead to unexpected changes in the balance between host, pathogen, and environment, leading to serious outbreaks of diseases that were of lesser importance previously.

An excellent example of how favorable weather in combination with a change in cultural practices led to major epidemics is seen with Fusarium head blight (FHB), or scab, of wheat and barley in the Upper Midwest of the United States in the 1990s. Although epidemics of this disease had been somewhat common earlier in the twentieth century, scab was considered a minor disease and attracted little interest in the years leading up to the 1990s. All of this changed with a devastating epidemic in 1993 and outbreaks that continued through 1997. Direct losses of over a billion dollars (more than $1.6 billion in 2013 dollars) and additional indirect losses of $4.8 billion were attributed to FHB in 1993, one of the greatest losses caused by a plant disease in North America in a single year. An estimated 70 million tons of barley were lost in the 1993 epidemic alone, and an estimated 100 million acres of wheat and barley were affected. In addition to yield losses, FHB results in contamination of the grain with a toxin called deoxynivalenol (DON). Contaminated grain is hazardous to humans and animals and leads to feed refusal, particularly in swine. Therefore, animal feed, grain, and flour are monitored for DON contamination, and rejection of contaminated products results in losses in addition to gross yield losses. Infected grain can continue to produce DON during malting, posing a health hazard and causing excess foaming (gushing) in beer. Epidemics were especially severe in the Red River Valley of the Dakotas and Minnesota, and many farmers faced foreclosure and bankruptcy.

FHB is commonly caused by the fungal pathogen *Fusarium graminearum*, although other species of *Fusarium* can also cause head blight. The host range of *F. graminearum* includes wheat, barley, corn, and other grain and grass species. The fungus overwinters on the debris of previous crops and produces asexual spores (macroconidia) on the overwintered debris. These macroconidia are one source of primary inoculum. Under relatively warm, wet, humid conditions, the overwintered fungus also produces sexual structures (perithecia) that bear ascospores. The conditions that favor ascospore production also favor infection and colonization of the host. Both the splash-borne macroconidia and the wind-blown ascospores can infect the grain head during flowering. Under favorable conditions, colonization continues, and depending on the time of infection, infected florets may produce no seed at all, produce a shriveled grain called a tombstone, or produce normal-looking seeds contaminated with DON.

The spring and summer of 1993 were extremely wet in the Red River Valley. Rainfall was 250%–600% of normal in that year. In addition, in typical years, rainfall accumulation is greatest in June, before plants produce heads and florets, and July is fairly dry. In 1993, however,

rain continued in July, during the entire period of flowering and grain fill. Although less extreme, above normal rainfall patterns continued during the mid-1900s along with continued outbreaks of FHB. Cultural changes also played a major role in the scab epidemics of the 1990s. Traditionally, grains were produced with clean tillage, meaning that weeds and crop debris were buried by use of plowing, disking, and other tillage operations. However, with the advent of better herbicides and soil conservation programs, growers began to adapt conservation or minimum tillage practices. These practices are beneficial to soil, but they also result in increased amounts of debris on the soil surface, which may be colonized by the pathogen. In addition, production of corn, which is also a host of *F. graminearum*, began to expand into wheat production areas. Corn debris is more resistant to breakdown than debris from wheat and other small grains, also resulting in an increase in overwintering inoculum due to a change in the cultural environment.

FHB continues to cause losses to grain crops in the United States, Canada, China, and other countries. As a result of the epidemics of the 1990s, a major effort to develop management strategies and tactics was undertaken in the United States. This scab initiative emphasizes breeding for resistance to both FHB and DON accumulation. In addition, risk assessment tools and weather-based FHB alerts are available to growers throughout the affected areas, so that they can identify periods when the environment favors outbreaks of FHB.

SUGGESTED READINGS

There are many books and articles on plant pathology-related topics. We list a few of those that will allow you to read further on the topics that we have briefly described in this introduction. By having an understanding of the basic concepts in plant pathology, you will be better prepared to critically evaluate both scientific and popular articles on plant disease and how they affect our lives every day.

Agrios, G. N. 2005. *Plant Pathology* (5th edition). San Diego, CA: Academic Press.

Ainsworth, G. C. 1981. *An Introduction to the History of Plant Pathology*. Cambridge, UK: Cambridge University Press.

Horst, R. K. 2013. *Westcott's Plant Disease Handbook* (8th edition). Dordrecht, the Netherlands: Springer.

Large, E. C. 2003. *The Advance of the Fungi*. St. Paul, MN: American Phytopathological Society.

Lucas, J. A. 1998. *Plant Pathology and Plant Pathogens*. Oxford, UK: Blackwell Science.

Maloy, O. C., and T. D. Murray. 2001. *Encyclopedia of Plant Pathology* (Vols. 1 and 2). New York, NY: John Wiley and Sons.

Matossian, M. K. 1989. *Poisons of the Past. Molds, Epidemics, and History*. New Haven, CT: Yale University Press.

Schumann, G. L., and C. J. D'Arcy. 2010. *Essential Plant Pathology* (2nd edition). St. Paul, MN: American Phytopathological Society.

Schumann, G. L., and C. J. D'Arcy. 2012. *Hungry Planet. Stories of Plant Disease*. St. Paul, MN: American Phytopathological Society.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2 Laboratory Skills
Safety and Preparation of Culture Media and Solutions

Robert N. Trigiano and Bonnie H. Ownley

Concept Box

- Laboratory safety is very important. Specific rules are in place to prevent most accidents and should be practiced at all times.
- Preparation of culture media requires the following three steps: combining the ingredients, sterilizing the medium (typically by autoclaving), and dispensing the media into suitable vessels such as Petri dishes or flasks.
- Heat-liable materials in solutions, such as antibiotics and amino acids, may be sterilized by filtration.
- Knowledge about how to make molar, normal, and buffer solutions are necessary for proper experimentation.

Laboratories are an integral part of any science-based learning experience as they are wonderful opportunities to reinforce the ideas presented in lectures and to develop research and critical thinking skills. If done properly, laboratory exercises can be used as occasions to improve organizational, collaborative, and written skills as well. The lessons learned by participating and completing laboratory experiments provide great benefits to students as the exercises help to prepare them to undertake advanced undergraduate and graduate studies. Obviously, we strongly believe that experiential learning is an important facet of instruction in plant pathology as most of the chapters in this book include laboratory exercises. Fortunately, for many students, most introductory plant pathology courses have incorporated some form of laboratory-based learning.

In this chapter, we will focus on three primary areas in laboratory work, namely safety, preparation of culture media, and preparation of solutions. We will not explore all possibilities and specific research techniques as these are adequately represented in the individual chapters of this book and do not warrant additional explanations. However, there are certain common elements to all experiments that bear discussion. We have assumed that most students have had limited exposure to laboratory work, and hence, we will start from the beginning.

The first consideration to any laboratory experience is *safety*. Each experiment presents its own safety concerns, but there are a few general precautions that apply to all situations. By following these basic rules, students may have a safe and productive experience. We suggest that the safety officer of the unit be asked to conduct a safety presentation for the class and/or that, if available, students should be strongly encouraged to take training in chemical and biological safety offered by their institution.

Before the first laboratory exercise, students should be instructed to

- Wear appropriate clothing and shoes. Open-toed shoes are not permitted in the laboratory. Long-sleeved shirts and long pants provide some protection for your skin and should be worn if working with chemical hazards. Lab coats also provide protection and are strongly recommended.
- Remove all jewelry before lab starts and store them in a secure place.
- Eating and drinking are not permitted in the laboratory. Food for human consumption should never be brought into the laboratory.
- Do not apply cosmetics or handle contact lenses while in the laboratory. Chewing gum or use of any tobacco products is strictly prohibited. Leave all books (except your laboratory notebook), coats or jackets, and hats outside of the laboratory.

- Consider constraining long hair, so that it does not get in your way.
- Never pipette anything, even distilled water, by mouth. Mechanical pipetting devices must be used.
- When leaving the lab for the day, wash hands with soap and water.

The instructor should complete the following on the first day of laboratory work:

- Give a tour and demonstrate the safety features of the laboratory, including eye protection (against hazardous solutions and UV light), emergency showers and eyewashes, fire extinguishers and alarms, and emergency phone numbers. The instructor should also outline the evacuation plan for the laboratory in case of an emergency, such as a chemical spill.
- Provide the location of the emergency first aid kit.
- Reinforce the idea that students should not work alone in the laboratory.
- Explain and demonstrate the use of acetonitrile gloves and under which situations they are needed. Never reuse gloves and never wear your gloves outside of the laboratory.
- Explain and demonstrate how to safely dispose of hazardous waste materials and sharp objects (needles, scalpels, etc.).
- Show students how to prepare a label for secondary storage (not the original container) of chemicals and solutions. The label should contain the following information: name, date, contents, and any specific hazard associated with the contents of the bottle.
- Provide an explanation of the "Right to Know" law in regard to any hazards associated with materials and procedures in the laboratory.
- Provide an explanation of Material Safety Data Sheets (MSDS) and how to use this information, including how to read the hazard codes on chemical bottles. A chemical hygiene plan should also be available for the laboratory.
- Explain the purpose and demonstrate the proper use of chemical fume hoods, biosafety cabinets, and laminar flow hoods.
- Demonstrate how to clean up liquid and solid chemical spills or contamination.
- Explain how to work with open flames (alcohol lamps and Bunsen burners) in the laboratory.
- Explain the hazards of handling items stored at low temperatures (–20°C and –80°C).
- Explain why it is necessary to report all accidents immediately to the instructor and seek medical attention if necessary.

We suggest that after completion of the safety presentation, each student sign and date a document that states that they have completed safety training for the laboratory. This should be filed as part of the safety training of students for the laboratory class.

Now that you are familiar with some basic safety materials and procedures, we will discuss preparation of culture media for growing microorganisms. Most of the organisms that will be used in the laboratory can be cultured on various artificial solidified (agar) or liquid nutrient media. There are generally four steps in the preparation of media: (1) measuring and combining the constituents, (2) sterilization, (3) dispensing culture medium into suitable containers, and (4) short- or long-term storage of culture media.

PREPARATION OF CULTURE MEDIUM—STEP 1: COMBINING INGREDIENTS

Many media are used in plant pathology, and several of them are commercially available as dehydrated agar- (a solidifying agent) or broth-based preparations. A commonly used base medium (notice that the singular form uses "ium" instead of "ia") for fungi is potato dextrose (PD) (= glucose, a simple sugar). Potato dextrose agar (PDA) is very simple to prepare from a purchased powdered product and typically contains 4 g potato infusion (primarily starch), 20 g dextrose, and 15 g agar.

Materials

Each student or team of students will need the following items:

- Commercially prepared PDA and PD broth
- Agar
- Top loading balance, spatula, and plastic weigh boats
- 1-L graduated cylinder
- Several 1-L Pyrex bottles with caps
- Distilled or deionized water
- Stir plate with magnetic stir bars
- Autoclave tape
- Permanent marking pen
- 125 cc (mL) rice or oat grains
- 250-mL graduated cylinder
- Aluminum foil

Follow the instructions in Procedure 2.1 to prepare media.

Procedure 2.1
Preparation of Culture Media

Step	Instructions and Comments
	A. Solid Culture Medium
1	Weigh 39 g of powdered PDA preparation into a large plastic weigh boat using a top loading balance. Clean up any spilled materials and never return unused or spilled materials to the stock bottle.
2	Combine the powder with 1 L of distilled water in a glass Pyrex bottle and stir for few minutes to dissolve the sugar and potato infusion—the agar will not dissolve.
3	Label the bottle with "PDA," your name, and date, and place a small strip of autoclave tape on the bottle (Figure 2.1). Lastly, place the cap on the bottle loosely—do NOT tighten the cap completely.
	B. Liquid or PD Broth Medium
1	Weigh 24 g of PD broth (without agar) in a large weigh boat and dispense into 1 L of distilled water. Stir the preparation until all of the powder is dissolved.
2	Label the bottle as before.
	C. Dilute Formulation of PDA, Such as 0.1 PDA
1	Weigh 2.4 g of PD broth (without agar) in a small weigh boat, dissolve completely in 1 L of distilled water, and then add 15 g of agar.
2	Clean up the balance and label the bottle with the same information as with the PDA.
	D. Water Agar
1	Dispense 15 g of agar into 1 L of distilled water and label the bottle as before. Mix, but note that the agar will not dissolve at room temperature.
	E. Rice or Oat Grain Culture Medium
1	Dispense about 150 cc or mL of rice or oat grains into a wide-mouthed 1-L flask. Add 125 mL of distilled water. Cap the flask with aluminum foil. Other grains can be used and the amount of water may need to be adjusted.

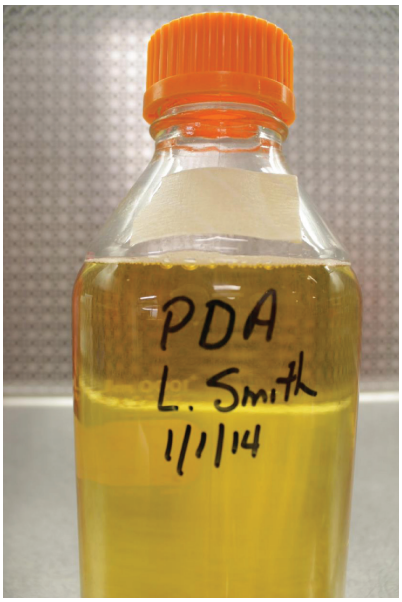


FIGURE 2.1 Label on medium bottle including type of medium (PDA), name, and date. (Courtesy of R.N. Trigiano.)

PREPARATION OF CULURE MEDIA—STEP 2: STERILIZATION

The media are now ready to be sterilized. Water and other ingredients that are used to prepare media always contain living microbes in the form of either bacterial cells or fungal hyphae and spores (bacteria or fungi), and these will grow unabated in media if not destroyed. In plant pathology, we need to have axenic (without a stranger) or pure cultures of organisms used in our studies. In other words, we only want our organism of interest to be present. However, there are instances where there is a need to have two or more organisms in the same culture, but that will not be discussed here.

The most frequently used option for sterilizing agar media, liquids, glassware, and instruments is by autoclaving at high temperature and pressure. There are times when certain heat-liable compounds (antibiotics, amino acids, plant growth regulators, fungicides, etc.) will need to be sterilized using a filter with small pores (0.22- or 0.45-μm diameter) that exclude contaminating

organisms. Heat sterilization of instruments and glassware in an oven at 204.4°C (400°F) for 2 h can be used, but typically is not. This discussion will focus on autoclaving and filtering for sterilization.

Materials

Each student or team should have the following items:

- Agar, liquid, and grain media prepared from Procedure 2.1
- Access to autoclave gloves
- 50-mL beakers covered with aluminum foil
- Twenty-five 125-mL screw-top Erlenmeyer flasks
- Large metal or "autoclave-safe" pan for holding flasks and bottles
- Empty 1-L storage bottles with screw tops
- Access to an autoclave

Follow the instructions in Procedure 2.2 to sterilize the media that was prepared using Procedure 2.1.

Procedure 2.2
Sterilization of Culture Medium

Step	Instructions and Comments
1	Place the bottles of media into a large pan. You may choose to dispense 25 mL of liquid PB medium into the 125-mL Erlenmeyer flasks at this time. If you choose to dispense after autoclaving, then the Erlenmeyer flasks must be autoclaved. First, add two drops of water to each flask and lightly tighten the cap.
2	Add a drop of water to each 50-mL beaker and cover with a square of aluminum foil. Add a few drops of water to each empty 1000-mL bottle and loosely tighten the cap. Caution: Do not tighten the cap on any bottle containing liquids to be autoclaved.
3	Securely tighten the door of the autoclave. Most recently purchased autoclaves have a selectable program. If this is the type of autoclave in use, select "liquid cycle" that provides at least 121°C and 15 lb. pressure per square inch (psi) for a minimum of 20 min and engage. If the autoclave is older, close and tighten the door, and open the valve that admits steam to the chamber. Begin timing (20 min) when the temperature reaches 121°C and pressure 15 psi.
4	Most modern autoclaves will automatically time the heat/pressure duration. When "liquid cycle" is selected, the autoclave is programmed to release the pressure in the chamber slowly to avoid super heating of the liquid media. If an older autoclave is in use, select slow exhaust. This may take some time to exhaust the pressure completely.
5	After the pressure in the chamber becomes "zero" and the temperature is below 90°C, it is safe to open the autoclave door. Be careful, use autoclave gloves, and keep your face away from the door. Open the door slowly and watch out for hot steam escaping from the chamber.
6	Remove the pans and place the bottles containing PDA on a magnetic stir plate and stir gently. Alternatively, media may be placed in a 60°C water bath until the temperature of the media equilibrates with the temperature of the water. Hot agar medium cannot be dispensed into plastic Petri dishes immediately as the dishes will warp and excess water will condense on the lids.
	Sterilization of Rice and Oat Grains
1	Place the flask containing the water and grain in the autoclave and set for liquid cycle for 90 min. Remove from the autoclave and place at room temperature overnight.
2	The first autoclave cycle will kill actively growing microorganisms, but not heat-resistant bacterial spores. The heat will activate spores, and they will "germinate" and reproduce vegetatively. Reautoclave the grain preparation with a liquid setting for 90 min the next day. "Double autoclaving" should produce sterile grain suitable for growing pure cultures of many fungi. The sterile grains may be aseptically transferred to test tubes or flasks and used to culture fungi.

PREPARATION OF CULTURE MEDIA—STEP 3: DISPENSING MEDIUM

Solidified and liquid media prepared in Procedures 2.1 and 2.2 may be stored for an extended time if desired (see Step 4), but if needed soon, they may be dispensed after the media has been "cooled." A "rule of thumb" is if the bottle containing the autoclaved medium can be held with a "bare" hand without discomfort, it is sufficiently cooled (50°C–55°C) to be poured and for any amendments, such as antibiotics that would have been destroyed or have diminished activity if autoclaved, to be added, and then dispensed. Agar medium may be dispensed into a number of containers, but Petri dishes of various sizes are typically used in the laboratory. In this exercise, agar medium (with and without augmentation) will be poured into Petri dishes and liquid medium dispensed into flasks.

Materials

Each team of students should have the following materials:

- Two sleeves of 10-cm diameter Petri dishes
- Four sleeves of 6-cm diameter Petri dishes
- 1000-µL pipette and sterile tips
- 10-mL syringe and 0.22-µm syringe-driven filter unit (Millipore Corp., Billerica, MA)
- Lab supply vacuum source or vacuum pump with rubber tubing and a bottle top filter (Millipore Corp., Billerica, MA)
- 10 mL water-based food dye solution (substitute for antibiotic solution that needs to be filter-sterilized)
- Disposable acetonitrile gloves
- 25-mL disposable pipettes and manual pipette pump or battery-operated pipette pump
- Two 50-mL sterile beakers covered with aluminum foil

Follow the instructions in Procedure 2.3 to complete this section.

After the medium in the Petri dishes has been solidified, the dishes may be placed in the original plastic sleeve for storage at 4°C. Be sure to write the name of the medium, date, and your name on the sleeve. If you have excess medium that was not poured, the bottle may also be stored at 4°C with identification of the date, type of medium, and who prepared it written on the bottle.

Procedure 2.3

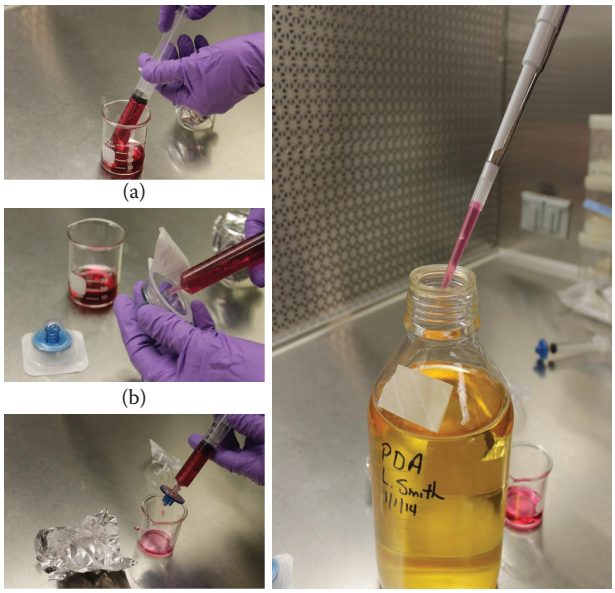
Augmenting Autoclaved Media and Dispensing Medium into Vessels

Step	Instructions and Comments
1	Wear acetonitrile gloves during this operation. Dissolve 100 mg of powdered dye in 10 mL of distilled water. Depending on the size of the class, these solutions may be shared with different groups.
2	Load the 10-mL syringe with the colored dye solution (this is a substitute for water-soluble antibiotics for demonstration purposes only) by drawing the solution into the barrel by pulling the plunger in the syringe upward (Figure 2.2a). Unwrap the sterile 0.22-µm filter and aseptically attach to the syringe—the tapered end of the filter unit should face outward (Figure 2.2b). Remove the aluminum foil from the sterilized beaker and express the dye solution through the filter into the beaker (Figure 2.2c). Adjust the 1000-µL pipette to "1000," place a tip on the end of the pipette and measure 1000 µL or 1 mL of the dye solution into the tip (Figure 2.2d). Dispense into the liter of PDA and stir. The effective concentration of dye solution is 10 mg/L.
3	Steps 3–5 can be completed using a laminar flow hood or on the lab bench using proper aseptic technique. A liter of medium is sufficient to pour 25 mL into each of forty 10-cm diameter Petri dishes or to pour about 15 mL into each of sixty-five 6-cm diameter dishes. Most investigators do not actually measure 25 mL, but approximate the volume poured into each dish. Lift the lid of the first dish, leaving an opening that allows you to pour the medium into the bottom of the Petri dish while holding the bottle of the medium in your other hand. Do not place the lid of the Petri dish on the lab bench. Immediately replace the Petri dish lid after pouring the medium (Figure 2.3a), then place an empty dish on top and repeat the process to pour medium into this dish. Do this in stacks of 10 dishes (Figure 2.3b). The bottle of medium should be swirled a number of times when pouring dishes because agar will settle at the bottom of the bottle. By pouring the dishes in this fashion, water condensate on the lids of the dishes is minimized. Save the original plastic sleeves that housed the sterile plastic dishes and slip the sleeves over a stack of about 20 Petri dishes after the agar has been solidified.

(*Continued*)

4 Typically, if the liquid medium does not require amendments, the medium is dispensed into the flasks and then autoclaved. However, if heat-sensitive materials must be added, follow Procedure 2.3 Step 2 and mix thoroughly. Aseptically remove a sterile 25-mL pipette (has a cotton plug in the barrel near the top) from the wrapping and mount with the milliliter gradations, so that you can easily see them. Draw 25 mL into the pipette and dispense into each 125-mL flask. The same pipette may be used to fill all of the flasks since all flasks are filled with the same medium. Completely tighten the screw cap of the flask.

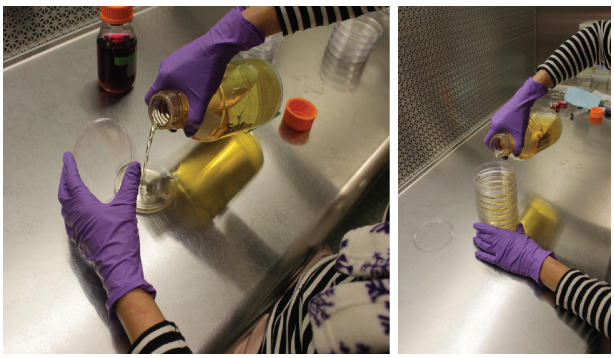
5 There may be occasions when large volumes of liquid medium may need to be filter-sterilized. For this, a bottle-top filter and a sterile bottle are used. Unwrap the bottle top 0.22-µm filter unit and place the membrane side of the filter on the opening of a sterile bottle (Figure 2.4a). Attach rubber tubing to the nipple on the filter and the other end to a vacuum source. Pour the medium in the reservoir and gently apply vacuum. The medium will be drawn through the membrane and into the sterile bottle (Figure 2.4b). The sterile medium may be dispensed into vessels.



(a) (b) (c) (d)

FIGURE 2.2 Syringe and filter sterilization of heat-liable compound. (a) Load solution into a 10-mL disposable plastic syringe. (b) Attach a 0.22-µm filter unit to the nipple of the syringe without touching the filter. (c) Remove aluminum foil cover from a small sterile glass beaker and express the fluid through the filter into the beaker. (d) Using a sterile pipette tip, transfer 1 mL of the sterile liquid into the medium, which has been autoclaved and cooled to about 50°C–55°C. Dispose of the filter and syringe according to the hazard level of the filtered solution. (Courtesy of R.N. Trigiano.)

To use the medium, melt the agar in a hot water bath or steamer. Agar remelts at 85°C. Do NOT autoclave the medium again as this may "caramelize" (change the structure of) the sugar component, which can alter the growth of microorganisms. Liquid medium in either bottles or flasks may also be stored at 4°C. Remember to mark the identification of the medium with type of medium, date, and name.



(a) (b)

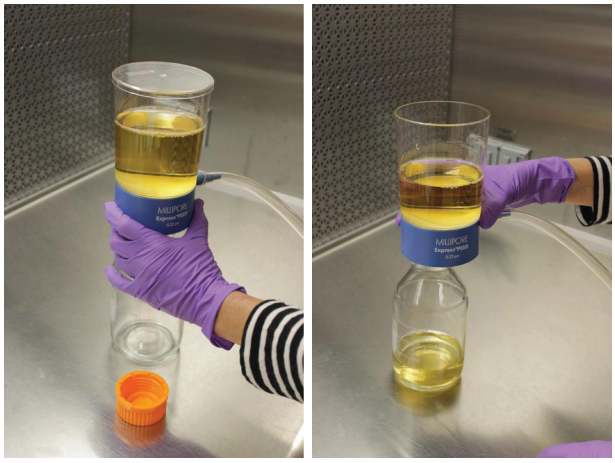
FIGURE 2.3 Pouring agar medium into Petri dishes. (a) Be sure that the medium has been cooled (50°C–55°C) before pouring about 25 mL into each 10-cm diameter Petri dish. This operation can be performed in a laminar flow hood or on the lab bench using aseptic technique. (b) Stack dishes when pouring the medium. This will prevent excess water condensation on the inner surface of the Petri dishes. Fill the empty medium bottle with hot water immediately after all of the medium is dispensed. After the agar has been solidified, the Petri dishes may be stored in the original plastic sleeve. Label the sleeve with the type of medium, date, and name. (Courtesy of R.N. Trigiano.)

PREPARATION OF SOLUTIONS

Occasionally, protocols will require that chemical solutions be made. The composition of most of the solutions generally falls into the following two categories: weight or volume/volume or percentage and molar and normal.

Materials

- Magnesium chloride (easy to dissolve and safe) (any suitable compound may be substituted)
- Disposable acetonitrile gloves
- Glacial acetic acid
- 100- and 1000-mL graduated cylinders



(a) (b)

FIGURE 2.4 Sterilizing a large volume of liquid medium using a bottle top filter. (a) Mount the bottle top filter onto the sterile storage bottle and fill the reservoir with the medium. Attach a vacuum source to the unit. (b) Gently pull the medium through the 0.22-µm filter membrane into the sterile medium bottle with the vacuum. Sterile medium may be either stored or dispensed to other vessels. (Courtesy of R.N. Trigiano.)

- Several glass 1-L storage bottles
- Chemical fume hood
- Milligram balance and small plastic weigh boats
- Top loading balance and large plastic weigh boats
- Magnetic stir bars and stir plate
- Succinic acid anhydrous (without associated water molecules); sodium succinate dibasic·6H_2O
- pH meter and electrode (recently calibrated with pH 4.0 and pH 7.0 standards)
- Several 1-L beakers
- Concentrated sulfuric acid

A. Making weight/volume (w/v) solutions. For this example, magnesium chloride will be used to make a 1% (w/v) solution. One milliliter [or 1 cubic centimeter (cc)] of water at room temperature weighs 1 g or 1000 mg. Follow the instructions in Procedure 2.4A to complete this exercise.

B. Making volume/volume (v/v) solutions. Complete this exercise using a fume hood and protective eyewear. Follow the protocol in Procedure 2.4B to complete this exercise.

C. Making molar solutions. A mole of a substance, either an element or a compound, is its atomic weight (in grams) and contains 6.02×10^{23} or Avogadro's number of atoms or molecules. A one molar (1 M) solution is defined as this amount of the substance dissolved in approximately 1 L of water. Follow the instructions in Procedure 2.4C to complete this exercise.

D. Making normal solutions. Normality is based on molar equivalence of either H^+ or OH^- ions and is most often used for strong acids and bases. For example, hydrochloric acid (HCl) completely disassociates into H^+ and Cl^- ions. So, 1 mole of HCl (36.5 g) will yield 1 mole of H^+. In this case, 1 M = 1 Normal (1 N). However, sulfuric acid, another strong acid (H_2SO_4; 98 g/mole), completely disassociates into 2 moles of H^+ and 1 mole of SO_4^{-2}. Therefore, if 98 g sulfuric acid is dissolved in water, it yields 2 M or 2 N of H^+.

Known concentrations or normalities of acids (and bases) may be purchased from manufacturers, but these can be expensive. More typically, concentrated (almost pure) acid is usually obtained from the same sources. However, sometimes the manufacturer does not print the normality of the acid/base on the label. If this is the case, look for the specific gravity (s.g. or g/mL) of the liquid. The s.g. of most concentrated sulfuric acid preparations is about 1.84 g/mL or 1840 g/L. Because the contents of the bottle are 96% acid (see label), the amount of acid is 1840 g × 0.96 = 1766.4 g of acid per liter. This translates into about 18 moles (1766.4 g/98 g/mole) of sulfuric acid. Remember that each mole of sulfuric acid produces 2 moles of H^+; therefore, the normality of the solution is 2 × 18 or 36 N. This is a very caustic solution. Always wear protective clothing, eyewear, and rubber gloves while working in a chemical fume hood.

Follow the protocol outlined in Procedure 2.4D to make normal solutions.

E. Making buffer solutions—titration method. Buffers are used to control changes in pH and maintain the original pH of solutions when acids and bases are added to the solution. They have various applications in studies of the growth, enzyme activity, histology of tissues, and so on involving plant pathogens and their hosts. A simple definition of a buffer is the combination of a weak organic acid (e.g., succinic acid) with the salt of that acid (e.g., sodium succinate dibasic). In solution, the acid and salt of the acid are in equilibrium, so that when an acid or base is added, the equilibrium shifts to either the acid or base and pH remains relatively stable. The highest buffering capacity (can add the highest amount of acid or base without appreciably changing the original pH) of the solution is near the mid-range of the buffering system. Most physiological buffers are

Procedure 2.4
Making Solutions and Buffers

Step	Instructions and Comments

A. Making Weight/Volume (w/v) Solutions

1 To make a 1% (w/v) magnesium chloride solution, weigh 100 mg of the compound and dissolve in 9900 mg or 9.9 mL of water. Typically, this accuracy is not demanded, so 100 mg in 10 mL of water is sufficient. This yields a 1% solution.

B. Making Volume/Volume (v/v) Solutions

1 While wearing acetonitrile gloves, measure 70 mL of glacial acetic acid in a graduated cylinder and pour into a 1-L storage bottle.

2 Slowly add 930 mL of distilled water to the bottle; be sure that the mouth of the bottle is pointed to the interior of the chemical fume hood. Cap the bottle, mix, and label with name, date, and contents. This makes a 7% solution of glacial acetic acid.

C. Making Molar Solutions

1 In this example, 0.05 M solutions of succinic acid and sodium succinate dibasic will be made and used when buffers are discussed (Procedure 2.4E). A 0.05 M solution of succinic acid requires 0.05 M × 118.1 g/L = 5.91 g dissolved in 800 mL of distilled water contained in a beaker.

2 Pour the contents of the beaker into a 1000-mL graduated cylinder and bring the volume up to 1000 mL using distilled water. Label the bottle with contents, date, and name.

3 To make a 0.05 M solution of sodium succinate dibasic, weigh 0.05 M × 270.1 g/L = 13.51 g, dissolve as before and bring to 1 L with distilled water. Label this bottle also.

D. Making Normal Solutions

1 To make a 1 N solution of sulfuric acid, dilute the concentrated acid with water. Use this simple formula to determine how to dilute concentrated sulfuric acid to 1 N.

$V1 \times N1 = V2 \times N2$

where V1 = the volume in milliliter of concentrated acid to use
N1 = the normality of concentrated acid (36 N) (mL)
V2 = the desired volume of 1 N acid (1000 mL)
N2 = the desired normality (1 N)

2 Solve the equation for V1: $V1 = (V2 \times N2)/N1$ or $V1 = (1000\ mL \times 1\ N)/36\ N$
$V1 = 27.77$ mL of concentrated sulfuric acid

3 Add 27.77 mL of concentrated sulfuric acid to a 1000-mL graduated cylinder and bring to 1000 mL with distilled water to make a 1 N solution of sulfuric acid. It is best to hold the graduated cylinder at a 30° angle with the mouth pointed toward the interior of the chemical fume hold and slowly pour the water down the side of the cylinder. Care should be exercised when adding the water as heat is liberated (exothermic). Label the bottle with contents, date, and name.

E. Making Buffer Solutions—Titration Method

1 Make a pH 4.5, 0.05 M succinate buffer. Pour 300 mL of 0.05 M succinic acid solution (prepared in Procedure 2.4C) into a 1-L beaker containing a magnetic stir bar. Place on a stir plate and determine the pH of this solution, which should be below 4.5. Caution: stir slowly and be sure the pH electrode is not contacted by the stir bar.

2 Slowly add 0.05 M sodium succinate dibasic (made in Procedure 2.4C) to the succinic acid solution and monitor the change in pH. When the pH meter reads 4.5, the buffer is ready for use.

in the range of 0.05–0.10 M of both the acid and the base. The Henderson–Hasselbalch equation will predict how much of the acid and the salt should be added to achieve a buffer of a specific pH. However, this equation can be difficult to use. An alternative to making a mathematical calculation is to use a titration method to achieve the desired initial pH of the buffer.

Follow the protocol in Procedure 2.4E to make a succinate buffer.

Laboratory exercises are excellent learning experiences, but like anything else, there are real dangers. Follow and practice the safety guidelines presented in the beginning of this chapter. However, emergencies and accidents do occur in laboratories; try to prevent them, but if they happen, be prepared to respond appropriately to them.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3 Proper Use of Compound and Stereo Microscopes

David T. Webb

Concept Box

- Microscopes are used to magnify and examine small objects such as spores, hyphae, and fruiting bodies.
- A compound microscope is used to examine very small objects and typically has 10X ocular (eyepiece) lenses and 4X, 10X, 40X, and 100X (oil immersion) objective lenses. The total magnification can be approximated by multiplying the power of the ocular lens by the power of the objective lens, e.g., 10X × 40X = 400X.
- Stereo or dissecting microscopes are used to examine larger objects and typically have 10X ocular lenses and a variable (0.5X – 4.0X) objective lens.
- When using a compound microscope, begin with the lowest power objective lens (4X), focus, and then progress to higher magnification objective lenses. Never begin your examination with higher magnification (40X or 100X) objective lenses.
- Only lens paper should be used to clean lenses. Do NOT use paper toweling or laboratory wipes as these will scratch the lenses, which are very expensive.
- Measurements of objects with a compound microscope can be made using an ocular micrometer that has been calibrated with a stage micrometer. Dimensions are usually reported in micrometers.
- Commercially prepared slides of plant host and plant pathogen are usually stained with fast green and safranin O, whereas fresh sections of materials are typically stained with phloroglucinol, IKI, or toluidine blue O.

This chapter is written as if the reader is a microscope novice. Experience has taught us that it is best to assume that most students will know next to nothing about using a microscope correctly and that it is best to start from scratch. In some cases, you may have learned some bad practices that need to be corrected. This chapter also covers compound microscopes that have a field diaphragm and a condenser that can be centered and focused to achieve Koehler illumination. Many student scopes do not have these features as their condensers and field diaphragms are fixed or of limited flexibility. In the course of your career, you will encounter microscopes that have the ability to achieve Koehler illumination. At that point, this tutorial will be even more useful.

Although the compound microscope is the most commonly used biological instrument, it is often used improperly. This may not matter with very thin commercial slides at low to medium magnifications. However, proper alignment of the illumination system is essential for viewing thick sections, whole mounts, and highly magnified samples of fungi and bacteria. It is also crucial for studying unstained specimens and for photomicroscopy.

You will be using microscopes throughout your career. If you learn the simple lessons contained within this chapter, you will do much better work and see the exciting world of microscopy in a new light. The modified procedure that we present was developed by the German scientist August Köhler (1866–1948) and bears his name (Köhler, 1893). Recently, his ideas were used to make the EM 910 Electron Microscope by Zeiss. Thus, this procedure, which was introduced in 1893, has been of lasting value.

MONOCULAR, BINOCULAR, AND TRINOCULAR MICROSCOPES

Microscopes are partly categorized by the number of oculars they contain. The first microscopes had one ocular and therefore were monocular. Binocular scopes have two oculars, whereas trinocular scopes have three. The third ocular is modified typically for the use of a camera. This chapter explains the uses of binocular microscopes. The same principles apply to all of the preceding types. However, stereo or dissecting microscopes differ significantly from the typical compound microscope.

THE COMPOUND MICROSCOPE

Because the optical systems in a microscope are composed of many lenses, the term compound microscope is used. This is applied specifically to microscopes that are used to study thin sections with high-power objectives (also known as objective lens; Figure 3.1). Dissecting or stereo microscopes are used to examine larger, three-dimensional specimens at lower magnifications (Figure 3.2). They also have compound lenses, but they are not generally called compound microscopes. Both the types of microscope use **transillumination** (illumination through) in which light passes from the microscope base through the specimen and travels to your eyes through oculars. This requires a special transillumination base for stereo microscopes. **Epiillumination** (illumination from above) is typically used with stereo scopes but is not typically used with compound scopes. This chapter illustrates Zeiss and American Optical microscopes. Your microscopes may be somewhat different, but you should be able to transfer the terminology and procedures described herein to your instrument. First, we will examine compound microscopes and will discuss stereo scopes later.

Microscope Care and Handling

Please treat these instruments with great care—they are expensive and somewhat fragile.

- Value what they can do and handle them with respect.
- Always use two hands to carry microscopes. Place one hand on the arm, the curved area that connects the body to the stage and base, and the other hand under the base of the microscope (Figures 3.1 and 3.2).
- Do NOT carry scopes sideways or upside down, as the oculars and other parts will fall out.
- Use lens paper to clean all lenses on the compound scope before each lab and especially after using immersion oil. Do not use any other kind of paper except lens paper to clean microscope lenses.

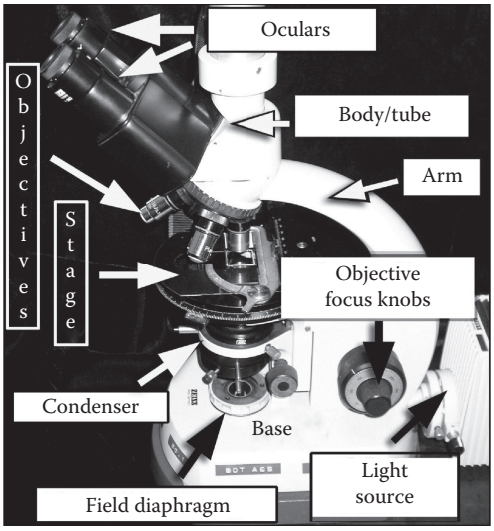

FIGURE 3.1 Zeiss Standard microscope showing major parts of a typical compound microscope. (Courtesy of D.T. Webb.)

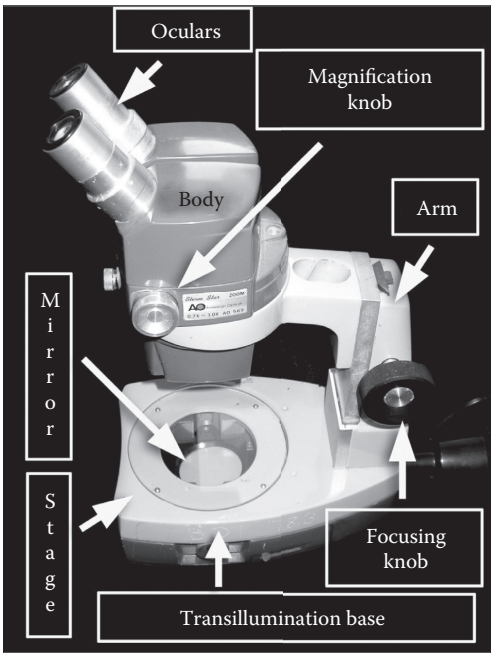

FIGURE 3.2 Typical stereo or dissecting microscope with transillumination base. This is an American Optical Stereo Zoom microscope. (Courtesy of D.T. Webb.)

- Do not use liquids (except where specified) when cleaning the lenses.
- Always use the correct focusing technique to avoid contact between any objective and your slide.
- Turn off the light when not using microscopes for long time periods.
- Carefully place the power cord or any other cords out of the way at your workspace.

- Always replace the cover on the microscope when you put it away.
- Deal with any problems immediately. Do not use the microscope if you cannot see your specimens clearly.

The Pathway of Light in a Typical Microscope

The light in a typical microscope traverses the following pathway:

Light Source → Mirror → Field Diaphragm → Condenser → Stage → Specimen → Objective → Body / Tube → Ocular → Eye or Camera

- Locate the major parts of your microscope by referring to Figure 3.1.

Light is provided by a bulb and is reflected through the field diaphragm, condenser, specimen, objective, tube, and ocular. There are various control knobs on the microscope that affect the light path. In addition, there are knobs for coarse and fine focus, as well as knobs to move the stage.

FOCUSING THE OBJECTIVES

The objectives are focused on the specimen by two sets of knobs that are located on both the sides of your microscope (Figure 3.1). The large outer knob is for coarse focusing. This should be used at the lowest magnification when you first place a specimen on the stage. It should be used with caution at higher magnifications. The smaller, central knob is for fine focusing and is used more at the higher magnifications, but is also used with low-magnification objectives.

- Locate the coarse and fine focusing knobs on each side of your scope.

Moving the Mechanical Stage

Light passes through the stage opening so that it can illuminate the specimen. The knobs that control the mechanical stage (stage transport knobs) are usually on the left side of the microscope as it faces you. One of these moves the stage from side to side (*x*-axis), whereas the other moves the stage in and out (*y*-axis). Most stages have x and y scales. These will allow you to record the precise location of the objects that you may want to relocate without searching the entire specimen. Slides are held in place by a mechanical slide holder.

- Locate the stage transport knobs on your microscope.
- Locate the x and y scales on your stage.
- Locate the mechanical slide holder and explore its mode of action.

USING THE CONDENSER

The condenser aligns and focuses light on the specimen. It may be equipped with a high-power condenser lens (HPCL) (Figure 3.3). This is used with 10× to 100× objectives, but is removed from the light path with low-magnification objectives that are typically 4× to 5×. The position of the HPCL may be controlled by a rotating knob. In some microscopes, it is moved in and out of the light path by a push–pull plunger or a lever. Failure to use this lens properly is the most common mistake that people make. The lens is typically left out of the light path at low magnification because it limits the **field** of illumination. A fully illuminated field is achieved with the HPCL out of the light path with low-power objectives. There may not be a large penalty for examining commercial slides at higher magnification with the HPCL out, but there is a severe visual penalty at higher magnifications and with fresh mounts.

- Locate the HPCL on your microscope and determine how to move it into and out of the light path.

Condenser-Centering Screws

A pair of screws, set apart by a 45° angle, are used to center the condenser. These are located along the back

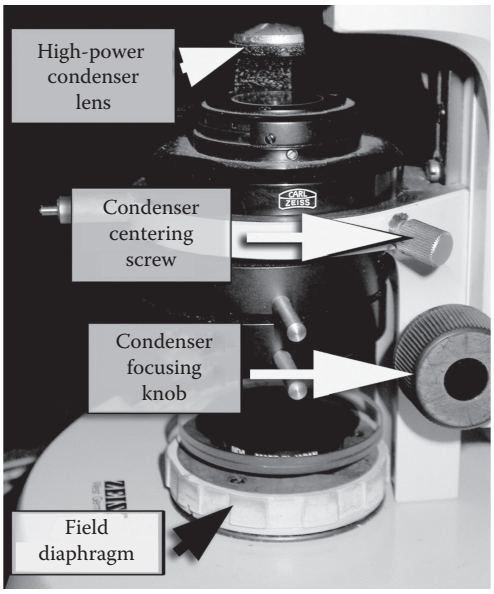

FIGURE 3.3 Side view of a Zeiss Standard microscope showing the high-power condenser lens, the condenser focusing screws, the condenser focusing knob as well as the field diaphragm. (Courtesy of D.T. Webb.)

right and left sides of the condenser on the Zeiss Standard (Figure 3.3). However, they may be found near the front of the condenser with other scopes.

- Locate the condenser-centering screws on your microscope.

The condenser is used to focus light onto the specimen from below. This is an extremely important, but poorly understood, function of the condenser. It is obvious that you must focus the objectives onto the specimen to see it clearly, but it is less obvious that you need to focus the condenser on the specimen so that it is properly illuminated. Imagine that the condenser is a magnifying glass and you want to start a fire. You need to move the magnifying glass to a position that produces the smallest focused beam of sunlight in order to start fire. That is exactly the same concept you need to keep in mind when you focus the condenser. This is done by rotating the condenser-focusing knob that is found on the right side of the condenser with the Zeiss Standard (Figure 3.3).

- Locate the condenser-focusing knob on your microscope.

Condenser (Aperture) Diaphragm

Finally, there is a lever that controls the aperture of the condenser or aperture diaphragm (Figure 3.4). I like to refer to this as the condenser diaphragm to prevent confusion with the field diaphragm. However, it has typically been called the aperture diaphragm. Partially closing this iris improves **contrast** (the difference between light and dark) especially at intermediate and high magnifications. It also increases the **depth of field**, which is very small at high magnifications.

Do NOT use this to increase or decrease brightness! This is the second most frequent mistake that people make. It is best to leave this completely open (rotated to the left on the Zeiss Standard) at the outset. Later, you will experiment with this to see its effects.

- Locate the condenser (aperture) diaphragm lever and manipulate it.
- Leave it in the open position for now.

USING THE FIELD DIAPHRAGM

The light source is usually housed in the base of the microscope. It passes through the field diaphragm that also contains an iris (Figure 3.5). The size of its iris diaphragm is controlled by rotating a knurled ring, which is concentric with it. The field diaphragm controls the area of illumination.

- Locate the field diaphragm on your microscope.
- Manipulate the knurled ring to open and close the iris inside.
- Leave it in the fully open position for now.

Using the Objective Lens

The magnification of an image is regulated by the objectives that are housed in a rotating nosepiece (Figure 3.6). To change objectives, rotate the nosepiece. Ensure that the low-power objective is in place before you start using your scope and when you are finished using it. This prevents damage to the objectives and your specimen.

- Always start viewing with the low-power objective (4× or 5×).
- Do not start by swinging the 10× to 100× objectives into position.
- Be sure that you rotate the nosepiece in the right direction. You do not have to switch from 4×

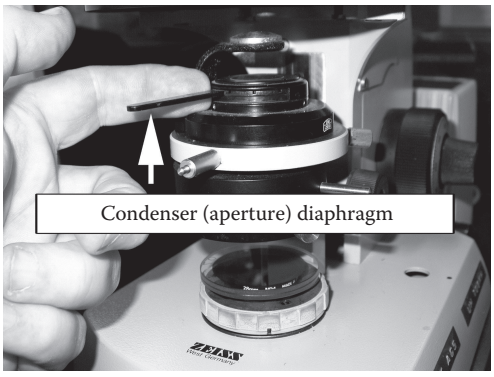

FIGURE 3.4 Lever that controls the condenser (aperture) diaphragm. It is completely open in this position. Rotating the lever to the right closes the iris diaphragm inside. (Courtesy of D.T. Webb.)

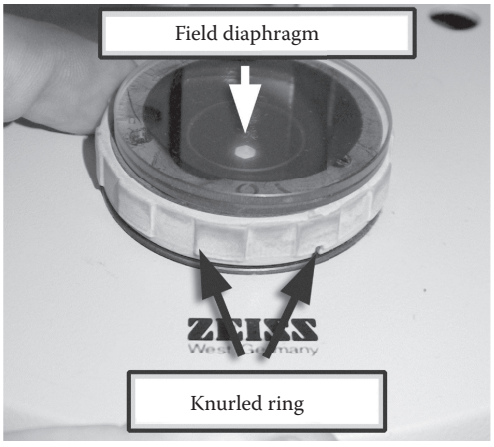

FIGURE 3.5 Field diaphragm that is almost completely closed. The knurled ring is used to open and close the iris inside. (Courtesy of D.T. Webb.)

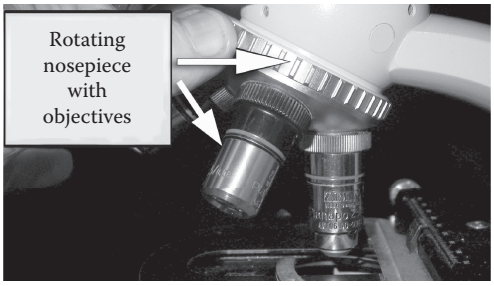

FIGURE 3.6 Rotating nosepiece with objectives. In this case, the nosepiece is rotated clockwise (left to right) to change objectives to higher magnifications. (Courtesy of D.T. Webb.)

to 100× because objectives may be damaged if they hit the specimen.

- You should focus on the sample with each objective before switching to the next. Focus on the sample using the low-power objective and rotate to the next lens (10×), and refocus and repeat this until you reach the magnification you plan to use. It is vital to focus on your specimen with the 40× objective before switching to 100×.

Higher quality microscopes have objectives that are **parfocal**, the ability to focus with one objective and switch to the next one and the one after that without refocusing. However, this rarely occurs, especially with student scopes. Special care must be used with fresh sections and whole mounts, which can be thick and irregular. Consequently, greater care must be taken when changing objectives. When in doubt, play it safe.

A typical microscope will have a series of objectives like the following 5×, 10×, 20×, 40×, and 100×. The length of the objectives is a rough indication of their relative magnification. However, their magnifications are engraved on them.

- Check the magnification of your objectives.

Most oil immersion objectives have a black line near their tip (Figure 3.7). However, this is not true for all manufacturers. The words "Oil" or "Oel" indicate that it is an oil immersion objective. Oil improves the image because it unites the coverslip and the objective and replaces air with oil. Immersion oil has the same refractive index as glass. Thus, less light scattering and refraction occur. The oil also protects the objective lens from getting scratched.

The markings, homogenous immersion (HI) or multiple immersion (Imm), indicate oil or multiple medium immersion objectives, respectively. Water immersion objectives are marked with the words "water," "waser," "water immersion," or WI. Furthermore, an objective with a **numerical aperture** greater than 1.0 (Figure 3.7) is probably an immersion objective. Oil is the most

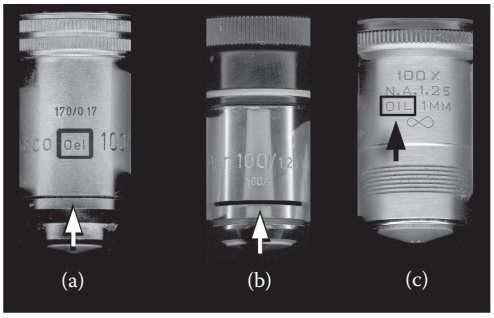

FIGURE 3.7 Oil immersion objectives from (a) Leitz, (b) Zeiss, and (c) American Optical (AO). Note the black lines (indicated by the white arrows) on (a) and (b), which indicate that they are oil immersion objectives. The AO objective (c) lacks this line. However, it has the word OIL inscribed on it as indicated by the black arrow and box. The word Oel (oil) is inscribed on the Leitz objective (a). (Courtesy of D.T. Webb.)

common immersion medium, but water and glycerin can also be used with Imm objectives.

- Check your 100× objective to see its markings.

The instructions for properly using oil immersion objectives can be found in Procedure 3.1.

For optimum results, oil should also be placed between the uppermost condenser lens and the bottom of the slide. However, the condenser needs to have a numerical aperture greater than 1.0 in order for the oil to have a beneficial effect. This may be impractical for routine studies, but should be used for critical examinations and for the most detailed microphotography. However, most oil immersion objectives require you to partially close the condenser aperture. This alleviates the need to add oil to the condenser lens because it effectively lowers the numerical aperture of the condenser, such that adding oil is no longer beneficial.

Using the Oculars

The oculars must be adjusted to suit both of your eyes. You should be able to adjust the interpupillary distance between the two objectives. This means that you can move them to match the distance between your eyes. Grasp the base of the ocular tubes or the plate at their base and gently spread them apart or draw them together. In some cases there is a dial that you can use to move the oculars. Either one or both oculars may be movable. There should be a scale and a reference line or dot that allows you to record the best spacing for your eyes. Thus, you can readily readjust the oculars to your personal setting if they have been moved. Follow the steps in Procedure 3.2 to adjust the interpupillary distance of your oculars.

Head position is very important. You need to find a comfortable distance for your eyes from the oculars

Procedure 3.1
Using an Oil Immersion Objective

Step	Instructions and Comments
1	Locate the area of interest in the center of the field and focus on this with the 40× objective.
2	Raise the objective to its upper limit. Swing the oil immersion objective into viewing position.
3	Place a drop of oil on the 100× oil immersion objective and place a small drop of oil on the coverslip. Do NOT look through the oculars.
4	While observing from the side of the stage with your eyes, focus on the objective and the specimen. Your eyes must be at the same height as the stage. Lower the objective lens carefully (use the coarse-focusing knob) until it just touches the oil on the coverslip. A light flash may be observed when the oil on the objective meets the oil on the slide.
5	Now look through the oculars and use the coarse-focusing knob to bring the specimen into rough focus. Use the fine-focusing knob to complete focusing on the sample.
6	Hereafter, avoid focusing down on the specimen with an oil immersion lens. Change the focus so that the objective is traveling away from the slide. If the image does not come into focus readily, carefully reverse the direction until it does. When in doubt, stop and ask for help. The lens might be dirty or there may be some other problem.
7	Important! Wipe the oil from the objective with lens paper when you are finished. Clean the objective until no more oil is visible on the lens paper. Wipe oil from the coverslip of the slide if it is to be saved.

Procedure 3.2
Adjusting the Interpupillary Distance of the Oculars

Step	Instructions and Comments
1	Position your head so that you can see through the oculars while focusing on a sample. Focus on a specimen using a 10× to 20× objective.
2	Grasp the base of the ocular tubes or use the dial located between the oculars.
3	Move the oculars so that they are as close together as possible. Carefully move the oculars apart until you see only one image.

in order to see things properly. This depends on the type and quality of your oculars and may require some experimentation. Most oculars can be used without eyeglasses that are corrected for nearsightedness or farsightedness. However, they are not compensated for astigmatism. If you have astigmatism, you need to use your eyeglasses or contact lenses. Oculars with eyeglasses engraved on them are suitable for use with glasses (Figure 3.8), but you are not required to wear glasses to use these.

It is important that each ocular is in focus for your eyes when you examine samples. Oculars that are capable of independent focusing will have a scale, a reference line, and a knurled ring on them (Figure 3.8). These markings may be on the ocular tube rather than on the oculars themselves. Follow the instructions in Procedure 3.3 to focus your oculars.

Koehler Illumination

The best resolution occurs when all the elements of the microscope are in perfect alignment and the iris diaphragms are properly adjusted to the best apertures for the objectives you are using. On simple microscopes, you may not be able to focus and align the condenser, but on the Zeiss Standard and many other microscopes, it is possible to do this and achieve "Koehler illumination." This makes a significant difference for viewing unstained and lightly stained samples, especially at high magnifications.

Centering the Lamp Filament

The first step in this process involves centering the lamp filament. This may not be possible with your microscope.

Procedure 3.3
Focusing Oculars for Your Eyes

Step	Instructions and Comments
1	Before you make any adjustments, place a slide on the stage and focus on the central part of the specimen with a 10× to 20× objective.
2	Block one of the oculars. Look through the other ocular with your matching eye (left eye → left ocular or right eye → right ocular) and focus on a fine detail in the center of the specimen with the objective focusing knobs at the rear of the scope.
3	Switch to the other ocular and look through it with the matching eye. Do NOT look through the other ocular while you are doing this!
4	Rotate the knurled ring of the ocular to bring the fine detail into sharp focus. You will need to stabilize this with one hand while you turn it with the other.
5	Check the first ocular to see that the image is still in focus with your other eye.
6	Both the oculars are now focused for your eyes.

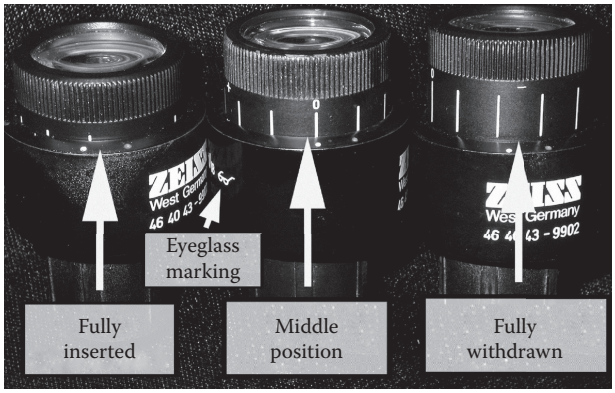

FIGURE 3.8 Zeiss oculars showing three focusing positions. These are focused by grasping the knurled ring at the top of the ocular and rotating it while holding onto the barrel below. The one on the left has been adjusted so that the rotating part of the ocular is fully inserted into the barrel. The one on the right shows the extreme opposite rotation. The one in the center is approximately in the middle. In this case the position of the ocular is indicated by the length of the white lines. In other cases there are numbers that can be used to designate the best focusing position for the ocular. Note the eyeglasses engraved on the middle objective. This indicates that this ocular was designed to be used with your glasses. (Courtesy of D.T. Webb.)

Furthermore, it is best done by someone who is very familiar with this process. Check the illuminator housing at the back of your microscope to see if there are any adjustable screws. If not, you cannot do this. A generic description of this is as follows:

- Turn on the microscope illuminator.
- Place a piece of paper over the field diaphragm.
- If the illumination is uneven, use the lamp-centering screws or rotate the lamp to get uniform illumination, or do both.

Focusing on the Specimen for Koehler Illumination

The recommended procedure for focusing on a specimen as part of achieving Koehler illumination is given in Procedure 3.4. However, when you decide to proceed, it is very important to focus on a specimen before doing anything else. After you have focused on it, you may have to move the specimen out of the light path the first few times you do this.

Focusing and Centering the Field Diaphragm

This is the heart of Koehler illumination. See Procedure 3.5 for the detailed steps in this process. Briefly, completely close the field diaphragm and use the condenser focusing knob to focus the field diaphragm until you see that it is a small polygon of light (Figure 3.9a). Use the condenser centering screws to center the image of the field diaphragm (Figure 3.9b). Partially open the field diaphragm and center it again (Figure 3.9c). Open the field diaphragm until the field is completely illuminated and stop.

Adjusting the Condenser (Aperture) Diaphragm

When working with 10× to 100× objectives, it is important to adjust the condenser diaphragm (Figure 3.4). This is especially true for translucent structures. Closing this iris increases contrast. Thus, something indistinct becomes sharp and something faint becomes dark. It also improves the depth of field, which is critically small at high magnifications. It is usually possible to close the iris and judge its effects subjectively (Delly, 1988). However, there is a "tried and true" procedure (Procedure 3.6) that you should know.

In practice, you can experiment with this while viewing a specimen and adjust it without removing the ocular. This is what I do when I want to take photos. I slowly close

Procedure 3.4
Focusing on a Specimen as Part of Koehler Illumination

Step	Instructions and Comments
1	Adjust the oculars so that they have the correct interpupillary position and are in focus for your eyes.
2	Use a commercial slide with obvious well-stained contents and move the specimen into the light path. Focus on the specimen with your low-power objective. Now, rotate the 10× objective into viewing position.
3	Watching from the side of the stage (not looking through the oculars), lower the 10× objective so that it comes closer to the coverslip.
4	Look through the oculars and rotate the objective focusing knobs so that the objective is retracted from the slide. Make a note on which direction the objectives retract.
5	Stop when the sample comes into focus. Moving the mechanical stage during this process may help.

Procedure 3.5
Focusing and Centering the Condenser

Step	Instructions and Comments
1	Use the 10× objective to focus on the center of a specimen. Reduce the illumination to a moderate level so that you do not hurt your eyes.
2	Check to see that the condenser (aperture) diaphragm is open, and ensure that the HPCL is in the light path.
3	Close the field diaphragm so that the circle of light becomes smaller. Observe the field diaphragm through the oculars when it is being closed (Figure 3.9). When the diaphragm is as small as possible, use the condenser focusing knob (Figure 3.3) to make the "circle" of light as small as possible (Figure 3.9).
4	You should see that the field diaphragm is NOT circular in outline, but has a polygonal shape (Figure 3.9). You may see a red or blue fringe as you bring the field diaphragm into focus. The best position is the one in between the red and blue fringes.
5	Use the condenser-centering screws to center the field diaphragm. Open the field diaphragm by rotating its knurled ring. It may not be perfectly centered. Perform final centering of the field diaphragm when it fills most of the field (Figure 3.10). Expand the field diaphragm just beyond the field of view and stop!
6	Repeat this with the 20× and 40× objectives. For critical work, this should be done for each objective. This is especially important for taking photographs and for examining minute, translucent specimens like fungi and bacteria. This is difficult to do with the 100× objective. However, if you achieve proper alignment with the 40× objective, the 100× will be similar.

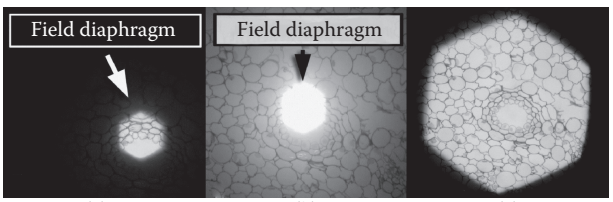

FIGURE 3.9 (a) Focused and uncentered field diaphragm, (b) closed and centered field diaphragm, and (c) partly open and centered field diaphragm. (Courtesy of D.T. Webb.)

this iris until I first see a perceptible change in the specimen and take a photograph. I close it some more and take another photo and repeat this for a third time. In reality, each specimen is different, and strict rules like those in Procedure 3.6 may not give the best results. Closing the condenser diaphragm also increases the depth of field. Thus, more regions of a three-dimensional specimen will be in focus. However, if it is closed too much, a flat indistinct image results.

The examples in Figure 3.10 show how the condenser iris increases contrast and depth of field. It shows

Procedure 3.6
Adjusting the Condenser (Aperture) Diaphragm

Step	Instructions and Comments
1	Complete all of the preceding operations (Procedures 3.1 through 3.5).
2	Place a lightly stained specimen in the light path. Focus on the specimen using a 20× to 40× objective lens.
3	Remove one of the oculars and look directly down the tube at the light field. Close the condenser diaphragm so that it occludes 1/4 to 1/3 of the area. This should give the best contrast.
4	Examine a specimen before and after adjusting this iris.
5	This should be done for each objective for critical viewing.

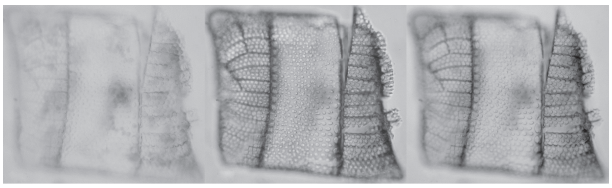

FIGURE 3.10 View of a diatom with condenser (aperture) diaphragm (a) completely open, (b) completely closed, and (c) partially closed. In (a), it is hard to see any detail and most of the subject is out of focus due to the shallow depth of field. In (b), more details are visible due to increased contrast, and there is a greater depth of field in (b) compared with (a). In (c), more details are visible, and there is a greater depth of field in (c) compared with (a). In this case, (c) should have been the best. However, (b) appears to be the best. (Courtesy of D.T. Webb.)

a diatom frustule that is very translucent. There is little detail when the condenser diaphragm is wide open (Figure 3.10a). When it is fully closed (Figure 3.10b), the contrast and depth of field are greatly increased. When the iris is closed 25%–30%, there is an improved contrast and depth of field with less theoretical potential for aberrations (Figure 3.10c). In this case Figure 3.10c should have been the best image, but Figure 3.10b appears to be the best.

- Experiment with the condenser diaphragm while viewing a lightly stained or unstained slide.
- Once you have achieved what you think gives the best image, remove one of the oculars and see how much of the field is occluded.

Throughout your career, you will be using different stains to study their effects on fresh specimens. Experiment with the aperture diaphragm as you study these. The condenser diaphragm can be used to great effect with this type of material. Although some of these procedures seem to be tedious, they will become routine as you progress in your work. Your results will be superior to others who do not know how to do this.

Simple Measurements with a Compound Microscope

In most cases, you cannot accurately determine the magnification of a compound microscope by multiplying the magnifications of the ocular and the objective lenses. This is a very common misconception. Microscope parts are not manufactured that precisely. Furthermore, the length of the microscope tube/body differs from one type of scope to another. This is especially true in photography because projection lenses of different magnifications are used in place of oculars and the total distance of the light path is different from that used with the oculars. We will work through Procedure 3.7 for calibrating an **ocular micrometer** that can be used to make direct measurements during observations.

Before we proceed, a quick review of the metric system will be helpful. A millimeter (mm) = 10^{-3} m, whereas a micron (μm) = 10^{-6} m. Consequently, 1 mm = 1000 μm, 0.1 mm = 100 μm, and 0.01 mm = 10 μm. A stage micrometer (Figure 3.11) is used to precisely determine the magnification.

Calibrating an Ocular Micrometer

The **stage micrometer** is the "known" micrometer in this process. It has finely etched distance calibrations on its surface. The largest dimensions from one end to the other are millimeters (Figure 3.11a and b). Each millimeter (1000 μm) is divided into 0.1-mm (100-μm) segments (Figure 3.11b). Each 0.1-mm segment is divided into 0.01-mm (10-μm) segments.

Ocular micrometers have precisely etched lines engraved on them. However, because of the differences in the optics of individual microscopes, they must be calibrated with a stage micrometer. Briefly, the stage micrometer and the ocular micrometer are brought together under the microscope at 10× so that