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FUNGAL EXPLORATIONS: LESSONS AND ACTIVITIES TO HELP INTRODUCE FUNGI IN THE HIGH SCHOOL CLASSROOM

By

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ABSTRACT

FUNGAL EXPLORATIONS: LESSONS AND ACTIVITIES TO HELP INTRODUCE FUNGI IN THE HIGH SCHOOL CLASSROOM

By

Thomas Eric Abramson

Most high school biology curricula include very little information about the fungi. Since most high school teachers depend on either commercial texts or the state curriculum to guide their instruction, the omission of the fungi is not surprising as both sources then to focus on plants and animals, while mentioning the fungi in passing.

The lessons included in this thesis are designed to help highlight the impact that fungi have had on human history and the roles they play in a forest ecosystem. Most lessons are lab/activity oriented, focusing on the scientific process. They are also designed to enhance rather than replace an existing curriculum. Using these lessons to accentuate the funge can help improve the quality of instruction regarding fungi in the general biology classroom.

DEDICATION

This thesis is dedicated to my mother, Joanna Mae Abramson, who infused in all of her children a love of education and through great personal sacrifice strove to make educational opportunities available.

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I would first like to acknowledge the contributions of my wife, Debbie, and my children, Shelby and Sarah, who's cooperation made this endeavor enjoyable.

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Finally, I would like to thank Dr. Johann Bruhn and Dr. Dana Richter of Michigan Technological University for their assistance in preparing the Humongous Fungus lab.

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INTRODUCTION

Constantine Alexopoulos uses the following paragraph as an introduction to his text Introductory Mycology, Second Edition (1962).

> "The systemic study of fungi is only 250 years old, but the manifestations of this group of organisms have been known to man for thousands of years - ever since the first toast was proposed over a shell full of wine, and the first loaf of leavened bread was baked. Yet, even today, in a science-conscious world, a world in which the nucleus of the atom has become a household word, few people realize how intimately our lives are linked with those of the fungi. It can be said truthfully that scarcely a day passes during which all of us are not benefited or harmed directly or indirectly by these inhabitants of the microcosm."

Bryce Kendrick, in his book The Fifth Kingdom (1985), uses a "dream picnic" to illustrate how fungi are important. His hypothetical feast includes delicious pate flavored with black truffle, bread raised by the actions of yeast, and cheeses flavored with the by-products of molds. The wine and other alcoholic beverages were created by yeast fermentation. Even the grass which cushioned the picnickers, and the trees which shaded them, survive with the help beneficial fungi or could be killed by the actions of fungal pathogens.

Biotechnology is currently a hot topic in biology, but the first biotechnologists were probably ancient humans who utilized the products of fermentation to their advantage. The

exploration of the earth was probably hastened as nomadic tribes were able to increase their range because of the availability of preserved fermented foods. The intoxicating effects of alcoholic beverages have been known for thousands of years and continue to devastate society today. Who can measure the impact that this discovery has had on human civilization?

The history of the world has been affected directly and indirectly in many other ways by fungi. For example, Peter the Great was halted in his attempt to capture Constantinople and gain access to warm-water ports when his troops and their horses were poisoned by ergoty grain at the mouth of the Volga River in 1722, just one example of a major outbreak of ergotism, a common problem of the times. As grain crops were replaced by the new world potato, ergotism gradually became less of a problem. Ironically, though, another fungal pathogen caused the Irish Potato Famine of the mid-nineteenth century, resulting in millions of deaths due to starvation and the emigration of additional millions of citizens to the United States and other countries.

The development of intensive agriculture has been similarly affected by fungi, especially the pathogenic ones.

The European coffee plantations of Ceylon (now Sri Lanka), for example, were virtually wiped out in less than twenty years by an airborne pathogen (a rust) thought to have originated in Ethiopia. This destruction led H. Marshall Ward, an Englishman sent to try to save the coffee crop, to question the wisdom of the plantation method of monocultural agriculture. Today, tea has replaced coffee both as a major crop in Sri Lanka and as the hot beverage of choice in Europe, especially England. Coffee production in tropical South and Central America, begun in the 1700's, was initially spared the scourge of the disease, due in part to geographical isolation and in part to quarantine efforts. However, the rust fungus has also reached these crops, first being reported in Brazil in 1970. Efforts to contain the pathogen continue today with limited success.

Fungal pathogens have an equally dramatic and deleterious effect on forest ecosystems. The chestnut trees which once dominated the deciduous forests of the eastern United States, for example, were nearly wiped out by a blight introduced accidentally in the early twentieth century. North American elm trees were also decimated by a pathogen introduced during the same time period. Today, in spite of quarantine laws and other efforts to control their spread,

introduced disease agents threaten the beautiful flowering dogwoods of Appalachia, the valuable butternut of the Great Lakes region, and other important commercial trees.

Saprophytic fungi, while serving an essential role in the cycling of nutrients in most terrestrial ecosystems, can also have had and continue to have a negative commercial impact. Lumber can be discolored by such fungi, thus reducing its value. Fungal induced rots reduce the caloric value of firewood and can also destroy the integrity of buildings, furniture, and any other wooden structure. The same organisms that cause the rotting of bookshelves may cause the disintegration of the books as well.

Finally, millions of tons of food is lost annually to rot-inducing fungi after the crop leaves the field and is placed in storage, moved in transit, or placed on the shelves of stores. Millions of dollars are spent in drying or refrigerating the crops and in developing novel waty to rpevent this loss.

Fungi fill yet another important niche in the forest and other ecosystems. Some fungi in the forest soil may form a close symbiotic association with the roots of trees and produce a mycorrhiza, or "fungus root", which somehow aids in the plants survival. It is estimated that such associations

occur in 90% of all vascular plants. Researchers continue to try to find ways to inoculate soils with mycorrhizal fungi in order to enhance seedling and young tree survival. Some commercial inocula are now being marketed.

In spite of the general importance of fungi, most people know very little about them. Gail Schumann, in her book Plant Diseases: Their Biology and Social Impact (1991), stresses the need for greater understanding of plant pathology. She argues that although plant diseases have changed the course of human history, people know little about plants or their pathogens, most of which are fungi. She maintains that we haven't learned the lessons of the past and thus may be doomed to repeat them. Additionally, she argues that it is the duty of the citizens to be sufficiently informed to make rational decisions about such controversial agricultural issues as pesticide use, release of genetically engineered organisms, and genetic reserves.

Several personal observations confirm the lack of understanding of fungi and plant pathology that Schumann decries. Bins of fruit and other foodstuffs confiscated at an airport give testimony to the willingness of people to risk agricultural disaster in order to save a few dollars or enjoy a

delicacy unavailable in the United States. Citizens harvest oak firewood from an oak wilt die-off area in spite of warnings that by doing so they may transfer the fungal pathogen to uncontaminated areas. A local community replaces its stately, monocultural rows of elm trees destroyed by Dutch elm disease with monocultural rows of Norway maple. A major paper company harvests mixed hardwood forests and replaces them with red and jack pine megaplantations. Finally, the forests around Crystal Falls, Michigan are filled with curious tourists searching for the "humongous fungus", only to be disappointed when they learn that most of the mass of the fungus exists as subterranean hyphal filaments and not as one mushroom covering 37 acres.

This general ignorance concerning the fungi reflects the science curriculum that most people experienced, not only in high school, but also, in some cases, in college. I must confess that until my exposure to this important group of organisms at Kellogg Biological Station, my knowledge of fungi and their roles was limited. I taught very little about them, and often eliminated any discussion of them if I was pressed for time.

I believe that I am not alone in this tendency. I assert that most teachers did not write the curriculum that directs

their course content. Instead, most use their text as their principal guide in determining what they teach. If that is true, it is no wonder students remain generally ignorant about the fungi. A survey of three high school biology and life science texts, including Biology: Living Systems (Oram, 1979), Biology: An Everyday Experience (Kaskel, Hummer, Daniel, 1988), and Biology: Living Systems (Oram, 1994), shows the usual cursory treatment of the fungi. This treatment typically includes the classification and morphology of some fungi and a discussion of molds, yeasts, and mushrooms. Their importance as decomposers is mentioned in passing or in conjunction with bacteria. These texts averaged more than 750 total pages but the discussion of fungi took up fewer than 10 pages on average . Kasker, et.al. devoted fewer (7) pages to the fungi, but did mention the role of fungi as plant pathogens. Three full paragraphs were devoted to the topic. Also, Oram discusses the Irish Potato Famine and the development of penicillin in his more recent text.

The text-driven curriculum is becoming a thing of the past in Michigan. Because of Public Act 25 and other legislation recently passed, committees in schools across the state are struggling to write new core curricula in many areas,

including science.

Many schools are replacing their existing curricula with one either modeled after or adapted from the Michigan Essential Goals and Objectives for Science Education (K-12) published in 1991 by the Michigan State Board of Education. This shift in focus is generally good in that it should result in an outcomes based, activities based science program addressing not only scientific knowledge but also scientific processes and scientific values.

Unfortunately, this overall improvement in science education may be balanced by reducing even further discussions of the fungi. If the state objectives are used directly, almost no mycology or plant pathology will be taught. In fact, the word fungus appears only once in this document, in connection with the five kingdom classification system, and no mention is made of plant diseases.

I do not wish to propose that a basic course in mycology be included in either the Michigan Essential Goals or a local curriculum. I do believe, however, that the fungi are very important organisms and that some knowledge of them would be valuable to the average citizen. Because of this belief, I have sought to create a series of activities designed to teach

students basic mycology and illustrate some of ways fungi impact their lives daily.

In Michigan Essential Goals and Objectives for Science Education: New Directions For Science Education (1991), the Michigan State Board of Education states that the primary purpose of K-12 science education must be scientific literacy. I have used this concept of general scientific literacy as a guide in designing each lesson and activity included in this mycology unit. Additionally, the Board suggests that to fulfill this role, and to help students master the objectives outlined in their document, many Michigan schools will need a thorough reexamination of their science programs. During this reexamination, they recommend the following key goals: 1. Emphasizing understanding over content coverage 2. Emphasizing learning that is useful and relevant outside of school 3. Emphasizing scientific literacy for all students 4. Promoting interdisciplinary learning 5. Developing support systems for teachers. These 5 goals have also been considered as lessons were designed for this unit.

To emphasize understanding rather than content coverage, I have attempted to minimize facts and terms while stressing general scientific principles, skills, and procedures

as they apply to fungi. The lessons in this unit are in most cases activity oriented. Too often, students are forced to study science from a textbook without the benefit of experiencing what they have read, leading to vocabulary-based, fact oriented instruction. This "facts first" method has long been defended as a legitimate instructional method, but the idea is refuted by the State Board in Michigan Essential Goals. They state:

> "Vocabulary-based approaches to science teaching are sometimes rationalized with the claim that "basic facts" must be learned before students can engage in the "higher order thinking" required by in-depth activities. There is now a large body of research-based knowledge that supports the belief that the "facts-first" approach to science teaching is practically and developmentally inappropriate. Even young students ask many questions about the world and have developed many strategies for finding answers to their questions. Thus, most students engage in activities requiring "higher order thinking" **before** they learn "basic scientific facts."

The "facts-first" method is also critiqued in Science for All Americans: Project 2061 (American Association for the Advancement of Science, 1990), where it is argued that scientific investigations naturally begin not with memorization of terms but with questions about nature.

To stimulate this important learning process, it is stated that "Students need to get acquainted with the things around them - including devices, organisms, materials, shapes, and numbers - and to observe them, collect them, handle them, describe them, become puzzled by them, ask questions about them, argue about them, and then try to find answers to their questions."

Many activities provided in commonly available commercial biology texts involve organisms or ecosystems that aren't found locally, which makes this "getting acquainted" period difficult to initiate. The activities included in this unit, however, certainly are relevant to students. What student has never experienced the rotting of food at some level, or perhaps seen trees or other plants destroyed by disease? It is important that they learn about the organisms which cause these phenomena and perhaps learn to control them. The forest fungi lessons are especially applicable for similar reasons in the northern regions of Michigan, where forest ecosystems predominate. In fact, the forest and forest products industry ranks second only to tourism in economic importance in Michigan's Upper Peninsula.

The lessons have been designed to allow for maximum flexibility of instruction. It is unlikely, due to time

constraints, that the entire unit described in this thesis will be added to an existing curriculum. In fact, doing so would directly contradict goal 1 from above. Instead , it is hoped that these lessons can be used to improve an existing curriculum. In some cases the activities with fungi may be simply an alternative to other organisms commonly used, whereas in others the fungal approach to a basic biological concept or topic may be superior. For example, culturing a mold from strawberries is as easy as and perhaps safer than culturing bacteria. Molds are also more visible than bacteria. Similarly, the large hyphal fungus *Armillaria* can be used effectively to illustrate the concept of tissue rejection in any context.

Several of the activities in this unit were designed to review or introduce skills and concepts needed by a truly scientifically literate person. The United States Immigration activity includes a review of graph construction. It also serves as an introduction to the Irish Potato Famine activity, where germ theory and other vital scientific concepts are introduced. In the *Armillaria* labs, protocols for collecting and isolating specimens from the wild are introduced, as are basic sterile techniques for the lab. Finally, in the wood rotting labs the

scientific method and good experimental technique must be used to prove or disprove student generated hypotheses.

Several activities in this unit effectively promote interdisciplinary learning. The immigration of various groups to and their impact on the United States is studied in American and World History. The Irish Potato lesson accentuates how scientific knowledge and technology intertwines with historically important events. Similarly, the Fermentation activities include a discussion of the impact the development of this early form of biotechnology had on early and present societies. The "Dogwoods" article, in addition to showing how an introduced pathogen can impact an ecosystem and public policy, could be used in an English classroom as a reading exercise, allowing for "science across the curriculum." Such practice exercises also may be helpful to students in taking the text criticism portions of their State Proficiency or MEAP tests.

Finally, teacher support systems have been incorporated within this unit. Teacher notes, provided for most lessons, include helpful hints to make preparation for each lesson easier as well as sources for materials. Once the activities have been tested and revised for maximum effectiveness,

presentations are planned at regional and state science conventions so that a maximum number of people will be made aware of this unit.

UNIT IMPLEMENTATION

My ignorance of fungi and their importance in various ecosystems first became evident at Michigan State's Kellogg Biological Center during the summer of 1991 as Dr. Dennis Fulbright discussed and demonstrated his work with chestnut blight. He not only effectively illustrated the devastation caused on the forest ecosystems of the eastern United States by the introduced blight fungus (of which I was totally unaware), but also suggested that the isolated fungus that he worked with could be used to illustrate basic biological principles such as Koch's postulates.

Later, in early 1992, reports of the discovery of a single, 37+ acre clone of the fungus *Armillaria bulbosa* in the forests of Michigan's Upper Peninsula appeared in the scientific and popular media. It was touted as the largest living organism on earth. Although the reports trivialized serious research being conducted by Dr. Johann Bruhn, then of Michigan Technological University, and others, they did stimulate questions from my students regarding fungi. The nature of their questions indicated a general ignorance concerning this important group of organisms. I saw this as an opportunity to address the student's apparent lack of information. I contacted Dr. Bruhn

at Michigan Tech and he agreed enthusiastically that there was the potential to adapt some of his work so it could be used effectively in the high school setting.

Through my contact with Dr. Fulbright and Dr. Bruhn, I became convinced that not only did I need to include more information about fungi in my biology course, but also that it was possible to do it in fun, relevant, and educationally sound ways. From this conviction evolved a plan for a unit to accent the importance of fungi from two points of view - their role in the ecosystem (in this case, forests) and their role in history. Over a period of three to four years, several activities and labs were designed to complement the basic information provided in each subunit. These activities and labs are intended to be used in a first year biology or life science class, probably in ninth or tenth grade. Extentions provided with some of the activities would make them adaptable at a higher level, perhaps in a second year biology course, for example.

All of the activities were tested in what may be considered a typical first year high school biology class. Although it is listed as "college prep", the class actually contained students of all ability levels, including special needs students.

The activities are summarized in the outline which follows. A brief description of the labs and activities in each category follows the outline and a complete set of the activities can be found in the appendix.

UNIT OUTLINE

I. FUNGI OF THE FORESTS

A. INTRODUCTION: A brief text to highlight several of the important roles of fungi of the forest. (Appendix I)

B. STUDIES OF A HUMUNGOUS FUNGUS (Appendix IIA)

1. Lab 1 Finding and Collecting *Armillaria* . Instruction on finding, identifying and collecting this important pathogen and decomposer.

2. Lab 2 Isolating and Culturing *Armillaria* in the Lab. Procedures for obtaining pure tissue isolates of field collections of *Armillaria* are outlined.

3. Lab 3 Who's Who Among the Fungi. Somatic compatibility tests for identifying*Armillaria* individuals are described.

4. Teacher Notes. An aid to teachers outlining short cut methods, helpful hints, recipes for culture media, etc.

C. THE EFFECTS OF WOOD ROTTING FUNGI (Appendix IIB)

1. Lab I Changes in Mass During Rotting. Describes student procedures for examining the change in mass as wood rots.

2. Lab 2 Changes in Wood Strength Due to Rotting. Students are asked to design and perform a test to determine the effect of rotting on the strength of wood samples.

D. A DISEASE IN THE DOGWOODS Students are asked to critique an article taken from National Wildlife magazine. (Appendix IIC)

II. FUNGI IN HISTORY: IMPACTS OF FUNGI ON SOCIETY

A. IRISH POTATO FAMINE (Appendix IID)

1. Activity 1 United States Immigration. After analyzing four decades of immigration data, students are asked to present a hypothesis to explain the increase in Irish immigration to the United States.

2. Activity 2 Irish Potato Famine. A follow-up to Activity 1, students learn about the Irish potato famine and its impact primarily on the United States and Ireland.

B. FERMENTATION (Appendix IIE)

1. Historical Background. A description of the development of probably the first example of biotechnology.

a. Teacher notes

b. Student introduction

2. Lab 1 Yogurt A protocol for the production of yogurt by acid fermentation in the classroom.

3. Lab 2 Cheese A protocol for classroom production of soft cheese using rennet.

4. Lab 3 Alcohol Production. Hints for the production of common alcoholic beverages (beer and wine) and possible classroom applications.

5. Lab 4 Measuring yeast fermentation rates. A modification of a commonly performed high school biology lab that makes measuring the production of carbon dioxide as a function of fermentation rate easier and more direct.

DESCRIPTION OF UNIT ACTIVITIES

I. FOREST FUNGI

Almost all biology curricula include at least one ecology unit, and most of the time forest ecology is at least mentioned. The relationships of producers and consumers of this common biome are usually stressed. The roll of decomposers is often included, but the fact that most of the decomposers of trees are fungi is often omitted. The role of fungi as potential pathogens is rarely mentioned. Neglecting these important roles creates a simplified view of the forest ecosystem, as clearly the presence of fungal pathogens or the absence of fungal decomposers would severely limit the

growth potential of the forest. In fact, it could be said that in the forest, the fungi as a group are as important as the trees themselves.

"Studies of a Humungous Fungus" is a series of activities designed to accentuate the impact fungal pathogens may have on a forest ecosystem and stems from the work of Dr. Johann Bruhn and others from Michigan Technological University. Dr. Bruhn's study focused on evaluating the impact of extra low frequency (ELF) radio waves, emitted by a submarine communication antenna system, on seedling survival rates in surrounding red pine plantations. The study lead to discoveries about the dynamics of the spread of*Armillaria bulbosa* , one of many *Armillaria* species that cause "shoe string root rot". It was in studying the spread of this fungus that Dr. Bruhn discovered that one clone of the fungus had infected trees in an extremely large area. This clone was to later become famous as the Humongous Fungus.

Armillaria showed promise for use in a high school curriculum for several reasons. First, it is commonly found in almost all forest ecosystems of Michigan. In the Upper Peninsula, plantations of red pine abound, and most have some degree of *Armillaria* infection. I have personally collected

trees infected with the fungus from several sites in the central U.P. It is in these artificial monocultural forests that the presence of the pathogen is most obvious, often causing large open areas in an otherwise thriving plantation.

Armillaria is also easy to identify and collect. The fungus is most visible as a white "fan" of mycelial material, usually at the base of the stem of a living tree or at the base of a stump. The black "shoestring" rhizomorphs can be easily recognized with practice. One can collect mycelial fan samples simply by taking the lower part of a stem of a living tree - making sure to include some roots- or by chopping a segment out of an infected stump. Small segments of rhizomorphs can simply be clipped off, best from the new growth at the tip. Basidiocarps (called honey mushrooms) can also be collected at the appropriate times of the year.

Finally, *Armillaria* can be cultured in the lab with commonly available materials. In fact, it is somewhat easier and safer to grow in the lab than bacteria because *Armillaria* cultures do well at room temperature and pose no threat to human health.

Lab 1 of the "Humongous Fungus" series begins with a site visit, the best site would be a relatively new (3-6 year)

pine plantation. In this setting, the patterns of infection sometimes can be generally recognized. However, to really understand infection patterns and to predict effective methods of controlling the spread, one must know which fungal clone is infecting which trees. This can be determined by taking samples of fungus from selected trees for culturing in the laboratory. Once cultured in pure form in the lab, somatic compatibility tests can be done to determine whether or not the samples came from the same clone. This information could then be used in a variety of ways, such as to predict future infection patterns or to propose a containment strategy.

Lab 2 describes the culturing and transferring process.

Lab 3 gives the procedure for the somatic compatibility test.

In addition to the applications described above, pure cultures of *Armillaria* could be used in a number of other interesting ways. The confrontations between unlike clones in the somatic compatibility tests are very distinct and could be considered analogous to tissue rejection in organ transplants and are also similar to the antigen-antibody reaction in Ouchterlony gel tests. *Armillaria* cultures sometime respond dramatically to changes in environmental conditions, allowing

innumerable opportunities for student experimentation. Some changes can favor the formation of rhizomorphs, while others can stimulate basidiocarp formation. *Armillaria* will also fluoresce if grown on the proper medium. In fact, several biological supply companies market prepared plates of *Armillaria mellea* specifically because of this unusual fluorscent trait.

The first goal of the "Wood Rotting" labs is to highlight the roll of fungi as the major decomposers of wood or wood products. In Lab 1, mass changes caused by fungal induced rotting are measured, while in Lab 2 the strength of rotted wood is compared to that of sound wood.

A second, equally important goal of these labs is to review the scientific approach to a problem. In Lab 1, students are provided with the purpose, materials, methods, and even a data table and then are asked to make measurements and answer questions based on the data and their understanding of the decomposition process. In Lab 2, however, they are asked to devise their own experiment to measure the effects of rotting on the strength of wood. Minimal guidelines, either oral or written, are provided for the experimental procedure and write up of the exercise. Students must "do" science in

order to complete this Lab.

The "Disease in the Dogwoods" activity also serves many purposes. The article concerns the impact of an introduced fungal pathogen, anthracnose, on the flowering dogwood of the Appalachian Mountains. It addresses the properties of the fungus itself, the far-reaching ecological effects of the death of the dogwoods, and the possible political and legal ramifications of trying to control the spread of anthracnose or other introduced pathogens. The format of the activity is consistent with a "text criticism" segment found on the State of Michigan's High School Science Proficiency Test . It thus provides the students with valuable experience in extracting information from written text as they will be asked to do when they take this required test later in their high school careers.

II. FUNGI IN HISTORY: IMPACTS OF FUNGI ON SOCIETY

A. Irish Potato Famine

It is important that students understand the impact that scientific discoveries have on society as a whole. Although they benefit from new technological developments daily, they often still view science as something studied by individuals outside the mainstream and having very little effect on their

lives. In Essential Goals (1991), the Michigan Board of Education addresses this misconception and prescribes an entire set of related "Reflecting on Scientific Knowledge " objectives to be included in the curriculum of each high school in the state. Students, according to the Board, must show that they can, for example, "Discuss the historical development of key scientific concepts and principles", or "Describe the historical, political, and social factors affecting the developments in science."

The activities found in the section on the Irish Potato famine were designed to meet the scientific knowledge objectives set by the Michigan Board of Education. Using a historically significant case study involving a fungal pathogen, the students will be able to determine how the level of scientific knowledge influences politics, society, and history. The students are also asked to apply the scientific principles addressed in the activities to the present and to make predictions for the future.

These activities are intended to enhance units already in place in the classroom.. For example, the lessons on the Irish Potato Famine could be interjected into a unit on farm ecology, or perhaps they could be used to help teach life cycles, or

maybe used to help show the important differences between asexual and sexual reproduction. Several activities are also intended to allow the student apply important skills, such as graphing and text critique.

The first activity found in the unit on fungi in history- United States Immigration- requires the students to graph several sets of data, serving as a review of graphing skills. The activity concludes by asking students to hypothesize about the causes of the death and emigration of so many Irish people in the time periods specified. These questions are meant to encourage students to look for connections with what they have graphed and the information they may have obtained in other contexts. They also will serve as the lead - in for the second activity.

Of course, the potato famine in Ireland caused the death and emigration. The text of the second lesson - Irish Potato Famine-describes the complex set of circumstances that resulted in the failure of the potato crop and lead to the famine. Students are asked to describe how the biology of the potato and the fungus, the political organization of Ireland, and the lack of knowledge of causes of diseases all contributed to this great tragedy. They are directed to look for parallels

between past and present agricultural practices and to identify ways to help prevent similar tragedies in the future.

B. Fermentation

The application of fermentation and the development of fermentation technology changed the course of human history. Yet, this development is often understated in most traditional biology courses. Authors of texts seem fond of discussing the reactants and products of this form of cellular respiration, often comparing it to aerobic respiration in humans. Often they include a lab involving the measurement of yeast respiration rates, but usually fail to go beyond that. The "Fermentation" activities in this part of the unit were included to provide a relevant and hopefully exciting alternative to current presentations which should enhance the traditional approach to the study of cellular respiration.

The fermentation section begins with a student introduction which is written in a "text critique" format and is intended to be used to supplement the traditional text. The introduction not only describes alcoholic and lactic acid fermentation, but also addresses the historical importance and technological applications of these processes.

Upon completing the introductory lesson, students

procede to Activities 1 and 2, making yogurt and cheese, which provide them with an opportunity to utilize lactic acid fermentation to produce an edible product, not unlike our ancient ancestors may have done. Students are asked to observe, measure, describe, and enjoy the results of fermentation. The focus is on the scientific process rather than on thestudy of terms and descriptions from a science text.

Activity 3, beer brewing, has a focus similar to activities 1 and 2, but with even greater potential to generate student interest.

Activity 4, measuring yeast respiration rates, is an improvement on a unit often included in many biology courses. By using this method, more accurate measurements of carbon dioxide production during alcoholic fermentation can be made, making conclusions and comparisons more reliable.

EVALUATION

FUNGI OF THE FORESTS

The development and testing of the "Humongous Fungus" lab series, detailed in Appendix IIA, depended on the collection of *Armillaria* samples from the forest and subsequently culturing them in pure form in the lab. The collection of the samples posed no problem to the students. However, in spite of their sincere and continued effort, contamination of cultures could not be eliminated. In four separate trials, all cultures were found to be contaminated by other fungi or bacteria and had to be destroyed.

I attributed the failure of the first attempt, in September of 1992, to lack of student experience. Students tended to fumble the tools, forget to cover Petri dishes, and commit other errors in technique. Within days, it became clear that all samples were contaminated and would not be usable for the subsequent labs.

Although the cultures were not usable, students became more familiar with the procedure and became more aware of the importance of sterile technique. We wrote the experience off as a dry run and decided to try again. Several students volunteered to return with me to the sample site to collect

samples for a second trial. Although the cultures taken from these samples, collected in October of 1992, seemed cleaner at first, contamination slowly began to appear in some samples. Eventually, all cultures in this second trial were ruined by contamination.

The patterns of contamination on the second trial plates suggested that the source of the unwanted fungi was airborne spores. In a well equipped college lab, an air flow hood could be used to help avoid such contamination. Since most high schools are not so equipped, an alternative "mini-hood" was constructed as described in Appendix IIA. Unfortunately, winter came before the effectiveness of the mini-hoods could be tested by the students.

The third trial was attempted the following fall. Again, contamination destroyed all cultures in spite of the use of the hoods. A fourth set of samples and cultures, collected the same season, met a similar fate. It was becoming clear that student sampling and culturing from the field was not going to be effective due to contamination.

If poor student technique in sampling and culturing at the first stage was the source of the contamination, a solution would be for the teacher to do the initial culturing. By doing

this, the subsequent labs could be salvaged and the study could go on. This alternative, however, would be very time consuming for the teacher and thus would probably not be doable.

A second option that I chose to explore was to obtain pure cultures of *Armillaria* from some other source such as one of several biological supply companies that sell "glow in the dark" fungi kits, which are cultures of *Armillaria mellea* on a special bread crumb agar. Several subcultures of these could be prepared and used instead of student collected samples. The advantage of this option is that it is less time consuming. The major disadvantage is that it would reduce the study to a simulation and modification of the lab procedures would be necessary.

I purchased a commercial *Armillaria mellea* plate in the spring of 1995 to see if subculturing would be feasible. However, even before I opened the Petri dish, I could see that it was contaminated by a mold and that it would be pointless to continue. Time constraints prevented me from trying again that year. It was late in the fall of 1995 that I obtained a second sample for another try at subculturing. This time the purchased culture was clear of any obvious contamination and

the subculturing was a success. It is possible to create a classroom set of one *Armillaria mellea* clone from a single plate. If other clones were obtained, a simulation could be set up mimic what might happen in an actual plantation. Maps could be created to show sample sources and scenarios of disease spread could be created. Students could then be asked to present control strategies, much the same as they would have in the real plantation. Although not quite the real thing, such a simulation could still be used effectively as a laboratory model of a real problem.

The "wood rotting" labs, detailed in Appendix IIB, were introduced in the spring of 1992 with questions regarding the cause of wood rotting. In spite of having recently studied nutrient cycles and the role of decomposers, most students responded that it was caused by moisture. Upon reading the introduction of Lab 1, students were reminded that decomposers, particularly fungi, cause the rotting of wood.

The results of Lab 1 varied. Some students test samples gained mass while others lost mass. Students were usually able to identify the cause of the additional mass as water gained from the soil and inadequate drying procedure but were less able to explain the loss of mass. Students noticed the

changes in color and texture typical of rotted wood, and in some cases reported molds growing on the test sticks.

I got the impression from student responses to the evaluation questions that even after doing Lab 1 they still believed that rotting was a physical change brought about by the absorption of water by the samples. Those students whose samples lost mass generally did not recognize the role of fungi in the decomposition of the woody material, even though they often reported the appearance of fungi on the sticks. It was particularly revealing that only a small portion of the students predicted that fungal activity would be limited if the jars would have been sealed and thus less rotting would have occurred.

The primary objective of Lab 2 was to allow students a chance to design and perform a test to compare the strength of rotted to fresh wood samples. Using the guidelines from the introduction, pairs of students wrote procedures to be approved before they could proceed. Volunteers presented their experimental procedures to the class, which was instructed to identify problem areas. The peer critiques were very severe in many cases, and from the questions being asked it was clear that students were becoming quite aware of the

difference between an effective lab procedure and a poor one. I also saw the need to limit materials. Some proposed procedures, although they probably would work, simply were too extravagant for the situation. In the end, the students completed the labs and produced write-ups which met the criteria outlined in the instructions of the lesson. I noticed, however, that although students were able to write and perform a test to compare the wood strengths, they often had difficulty in organizing their data. This is probably because data tables and graphs are usually provided in the lab activities, thus limiting students chances of developing this important skill.

The "Disease in the Dogwoods" activity, shown in Appendix IIC, was intended to supplement existing information on forest ecology and to highlight the role of fungi as forest pathogens. It was written in a "text criticism" format to give students practice in extracting answers to questions as they read segments of the article.

Students were reasonably successful answering the questions. 15 objective questions regarding the causes, impacts, and physical factors affecting the spread of the disease were almost never missed as over 90% of 40 students

answered these questions correctly. Student success was somewhat lower on inference questions, however. More than 20% of the students missed a question relating to disease resistance in Chinese dogwoods, and a similar number were unable to propose actions that would prevent the introduction of diseases in the future.

FUNGI IN HISTORY: IMPACTS OF FUNGI ON SOCIETY

Like the "Dogwood" article, the U.S. Immigration and Irish Potato Famine activities, found in Appendix IID, were designed to highlight the roles of fungi while supplementing an existing curriculum.

The "Immigration" activity was written to serve as an introduction to the "Famine" activity. Students were asked to utilize four decades of immigration data provided to graph the changes in immigration patterns. They were asked to describe possible impacts that such changes in population might have had on both the United States and Ireland and were able to do all of these with a high rate of success. In one section of twelve students, for example, only 1 student got more than two questions wrong.

The last question of the "Immigration" activity was, however, probably the most important in relating to the second

activity. Very few students were able to correctly identify the potato famine as the cause of the massive changes in Ireland and the United States. For example, in one class of 22 students, only 1 student answered correctly, mentioning the potato famine. 2 more students came close to the proper answer, relating the cause to starvation or lack of food. Common incorrect causes listed were war, invasion, or emigration from Ireland due to the attractiveness of jobs and economic opportunities in the United States. This lack of knowledge is almost unbelievable considering the magnitude of the tragedy and certainly confirms the premise that students need to be taught more about fungi and their impact on society.

The Irish Potato Famine activity not only identified the cause of the changes researched in the "Immigration" activity, but also provided more information regarding the complex circumstances that led to the disaster. The activity was again written in the "text critique" format and questions related to 5 general objectives were included to measure student understanding. The five objectives are listed in the first column Table 1, which follows. Column II shows the number of students out of the sample class size of 16 that successfully answered at least 80% of the questions related to each

objective.

TABLE 1

COLUMN I	COLUMN II
I. Identify the role of fungi in the potato famine (6 questions)	13 (81%)
II. Identify the physical factors contributing to the potato famine (3 questions)	12 (75%)
III. Identify biological factors contributing to the potato famine (7 questions)	9 (56%)
IV. Identify political factors contributing to the potato famine. (I question)	5 (31%)
V. Relate lessons of potato famine to the present and the future. (7 questions)	7 (44%)

The data above shows that although the students were able to glean facts from written material quite well (objectives I, II, and III), they were less able to identify relationships and draw conclusions from the facts they read (objectives IV and V). This may indicate a lack of student experience with this type of question, but also should be expected due to the higher level thinking skills required to answer such questions.

The "Fermentation" activities, in Appendix IIE, were used to supplement the information on fermentation provided in the text, which tended to focus on abstract chemical equations and ignored the practical applications of fermentation as well as

the historical and societal importance of the discovery of fermentation.

The student introduction, again written as a "text critique", told the story of this important discovery in what I considered a less "dry" and abstract way without ignoring the important chemical changes taking place. Students were asked a variety of questions to test their understanding of the fermentation process and its importance in history. Of 37 students answering the questions in the spring of 1996 only 9 (24%) were able to answer 80% or more of the twenty questions correctly. 32% of the students 60% or fewer of the questions correctly. Because of this lack of success, extra time was spent to review and discuss fermentation more fully. During this discussion, student comments indicated that although some of material was difficult to understand, many items were of interest. The methods of production of the first cheeses and chicha brought forth the most comments.

In the past, a classroom discussion of fermentation was followed by a lab activity designed to measure the rates of yeast respiration. The lab protocol used was typical of many published labs in that it provided a pre-lab discussion including the objectives, materials, procedure, and data tables

needed by the student. I felt that students were not getting what they should out of the lab, mainly because they had too much information provided and the measurements that they took had no meaning. In the spring of 1996 I decided to redesign the activity to put more responsibility for writing and completing the lab on the students.

After the general discussion of fermentation mentioned above, students were told to design a lab to measure yeast respiration rates and compare the rates of respiration in hot and cold conditions. Students were given few instructions, other than that they would be given yeast and would have access to laboratory glassware. They also had their texts to use as a reference, and were told to have a write up - including a title, objectives, a materials list, a procedure, and necessary data tables-ready to go the next day.

The next day, the students were allowed to proceed with their lab, provided that their procedures passed a safety check. Most students tried to perform a lab similar to one described in the text, but from the onset, students began to experience problems. Many neglected to provide the yeast with a substrate. Most could see no evidence of respiration due to other unexpected technical problems, and often students were

not careful to limit variables. Students began to recognize that they needed to make changes. Some began to make revisions but soon ran out of time.

The next day, students were asked to discuss their results. Students were quickly able to list problems with their procedures. When they identified the need for a standardized way of measuring yeast respiration, they were provided with the lab procedure included in this fermentation lesson.

Although students at times had difficulty manipulating the measuring apparatus, they were not only able to effectively compare the respiration rates at different temperatures, but were also able to compare the rates using different substrates. Their write ups generally met the requirements specified in the lab protocol and indicated a high level of understanding.

Not only were students asked to answer evaluation questions as they did the activities, they were also asked questions related to fermentation on a chapter test to check for long term retention of knowledge gained. The questions related to the general objectives shown in Column I of Table II. Column II indicates the number of students (out of a class of

36) that answered at least 80% of the questions correctly.

TABLE 2

COLUMN I	COLUMN II
I. Matched organisms with fermentation type	9 (25%)
II. Identified products and reactants of fermentation	14 (39%)
III. Recognized important fermentation products	16 (44%)
IV. Interpreted experimental data from graph	12 (33%)
V. Wrote a brief fermentation experiment including	
hypothesis	19 (53%)
procedure	18 (50%)
limited variables	21 (58%)

The data suggest that students retained the laboratory related objectives (Objective V) at a higher rate than those objectives related to facts and abstract chemical reactions (Objectives I - IV). The data would also suggest that more activities to compare and contrast fermentation types and show important fermentation products would help the students retain more of that information.

The making of yogurt (Activity 1), allows such comparisons . Students are often surprised at the sour flavor due to the lactic acid formed. Most were more accustomed to the sweetened commercial varieties. The optional bacterial count also accentuates the organism responsible for the

fermentation. The other two labs, the making of fresh milk cheese and the brewing of beer, are also options which could be used to show the importance of fermentation.

CONCLUSION

Species that make up the Kingdom Fungi play an important role in bioprocessing the earth's natural resources and in doing so have had a great impact on human history. Societies today of course are still affected daily by fungi and the quality of society's future may depend on our ability to manipulate the activities of fungi. In spite of their important role, these organisms are virtually ignored in many high school science curricula. Therefore, the primary objective of the lessons and activities included in this thesis was to provide a variety of ways which could be used within an existing curriculum to highlight fungi and the many ways they may be important to us.

I think this primary objective was met. In doing the activities included in the Forest Fungi section , students witnessed the impact of a pathogen in a forest ecosystem and were able to apply concepts learned in the classroom to the plantation system of growing trees even though they were unsuccessful in culturing *Armillaria* in the lab. They also determined the effect of fungal decomposers on wood products and read about the impact of an introduced pathogen on a complex forest ecosystem. In the Fungi in History lessons,

students read how the interaction of several factors lead to the Irish potato famine, learned how the discovery and application of fermentation technology changed history, and did activities to investigate the process and products of fermentation.

This unit was also intended to be activity oriented, with students being given the responsibility of not only doing activities as directed, but also, in some cases, designing the activities themselves. I think two activities, the wood strength lab and the yeast respiration lab, were especially effective because of this approach. It was evident that students not only were more able to interpret the results of each activity, but they also had a greater understanding of the processes of science after completing these labs. Because of the success of this hands on approach, I have begun to apply it in other courses I teach. Although it is more time consuming, students seem to be retaining more.

Some of the activities, while not lab oriented, address other important science skills. The Immigration activity, for example, reviewed graphing skills. Several activities were written as text critiques, with questions requiring students to extract and interpret information from written material.

Students in Michigan schools must show proficiency in these skills in order to obtain an endorsed diploma.

The Humongous Fungus labs proved to be a major disappointment. Although I had helped culture hundreds of isolates of *Armillaria* while at Michigan Tech, students were unable to obtain pure cultures in the high school lab using a procedure similar to the one used at Michigan Tech. In spite of considerable time and effort spent, this first hurdle could not be cleared. Hence, subsequent labs and investigations could not be investigated more fully. Commercial sources of *Armillaria* proved to yield pure plates, but the lessons would be less meaningful and thus less effective than those using student samples from local forests.

I do not intend to give up on developing these labs. Due to a recent remodeling of our school, I feel that there is a greater chance for success in obtaining pure fungal cultures. Also, the students in the upcoming biology class are very dedicated and able students and will give their best effort to be successful. We will again attempt to obtain pure cultures in the fall of 1996.

Additionally, I have already discussed with officials from a local paper company the possibility of conducting a long

term study in one of their new plantations. In the study, students would compare the survival rates of seedlings planted by different methods and under varying conditions. *Armillaria* will probably have an impact on the survival of the seedlings, and its role as a pathogen could be studied in this context.

I also intend to improve and extend some of the fermentation labs. I would like to improve the yogurt lab by addressing the chemical changes more fully. For example, I would like to use a pH meter interfaced with a computer to measure changes over time as the fermentation takes place. Plotting such information could be used to estimate bacterial population growth rates. The cheese lab needs to be refined to make it more practical. Finally, I intend to make the beer activity more appropriate by adapting it to focus on the importance of alcohol as a fuel rather than as a beverage. Biology students will study the biological aspects of the fermentation process while chemistry students will focus on the distillation process which would result in the purer alcohol needed for burning. Two similar procedures, both potentially applicable in the high school setting, will be tested in the fall of 1996.

APPENDICES

Appendix I

The Forest Fungi: Background Information

"The systematic study of fungi is only 250 years old, but the manifestation of this group or organisms have been known to man for thousands of years - ever since the first toast was proposed over a shell full of wine, and the first loaf of leavened bread was baked. Yet, even today, in a science-conscious world, a world in which the nucleus of the atom has become a household word, few people realize how intimately our lives are linked with those of the fungi. It can be said truthfully that scarcely a day passes during which all of us are not benefited or harmed directly or indirectly by these inhabitants of the microcosm" (1).

While the above statement is certainly true about fungi in general, it is especially true about the fungi of the forest. Many people have witnessed the devestation of American forests caused by *Cryphonectria* (*Endothia*) *parasitica*, the chestnut blight fungus. The introduction of *Ceratocystis ulmi*, the fungus that causes Dutch elm disease, threatened the American elm with extinction. Apple growers must contend with *Venturia inaequalis*, the primary causal agent of apple

scab, which can decrease the value of a harvest or even cause permanent damage to an orchard. Commercial foresters must deal with a variety of fungal pathogens which can threaten trees from the seedling stage in the greenhouse until the time they are harvested. The potential harm caused by fungi doesn't end with the harvest of trees, however, as forest products are also susceptible to colonization by saprophytic fungi. Lumber can be discolored by such fungi, thus reducing its value. The caloric value of firewood is reduced as fungi rot it. Rotting fungi can also destroy the integrity of buildings, furniture, or any other wooden structure. Finally, the same organisms that cause the rotting of bookshelves may cause the disintegration of the books as well.

Many fungi of the forests are beneficial. For instance, the importance of the decomposers in the carbon cycle is well studied. Fungi serve that function in the forest, breaking down leaf litter and other organic matter which otherwise would build up to incredible depths and releasing carbon that will be used as a raw material for photosynthesis.

Some forest fungi are edible. Morel mushrooms (*Morchella spp.*) are considered delicacies and are sought by an increasing number of people. Somewhat ironically, the

delicious honey mushroom (*Armillaria spp.*) is the fruiting body of the same species that causes "shoestring" root rot in many tree species. *Amanita muscaria* is often consumed even though it contains a toxic substance, muscarin, which can cause death if taken in large doses. It seems that muscarin will induce hallucinations in small doses and is used in this capacity during the religious rites of certain Native American tribes. *Psilocybe mexicana* and other related species are also noted for their ability to produce hallucinations and other ecstatic effects. The hallucinogenic substance in these mushrooms, psilocybin, has been experimentally produced and was once studied as a possible treatment for schizophrenia (2).

Some fungi in the forest soil may form a close symbiotic association with the roots of trees and produce a mycorrhiza, or "fungus root", which aids in the plant's survival. It is estimated that such associations occur in 90% of all vascular plants (3). Researchers continue to try to find ways to inoculate soils with mycorrhizal fungi in order to enhance seedling and young tree survival. Some commercial inocula are now being marketed.

Fungi are important in a forest ecosystem but their

importance is often overlooked. The primary goal of this background and the activities which appear as other appendices is to illustrate the importance of fungi as pathogens, decomposers, and as beneficial mycorrhizae formers. It would be difficult to understand how the fungi fill any of these roles, however, without first knowing about the organisms in general. Providing this important background information is a secondary goal of this unit.

WHAT ARE FUNGI?

At present, biologists use the term fungus (pl. fungi; L. fungus = mushroom) to include nucleated, spore-bearing, achlorophyllous organisms which generally reproduce sexually and asexually, and whose usually filamentous, branched somatic structures are typically surrounded by cell walls containing cellulose or chitin, or both (4).

To put it more simply, the basic unit of a fungus is the cell including cell wall, plasma membrane, nucleus with a nuclear envelope, and various organelles within the cytoplasm. Chlorophyll is not present making the fungi heterotrophic, that is, they must use organic compounds as the primary source of energy. The primary constituent found in the cell walls is chitin, but the total composition of cell walls in the various

species of fungi is not consistent nor clearly understood.

The life cycles of the fungi are highly variable. Some fungi go through their entire life cycle as individual cells performing all functions, without differentiation into tissues. More often, however, the body of a fungus, the thallus, is composed of tubular, branching filaments called hyphae that may or may not have crosswalls (septa). Several hyphae enmeshed are called mycelia.

Reproduction in fungi is varied but generally is said to be sexual (perfect) or asexual (imperfect). Sexual reproduction involves the union of two compatible nuclei. The total sexual process consists of three distinct phases. The first phase is plasmogamy in which the union of the two protoplasts brings two or more compatible nuclei into close association. Plasmogamy is followed by karyogamy, the actual fusion of two nuclei. Prior to fusion, the nuclei are in the monoploid (1n) state; after fusion the combined nucleus is in the diploid (2n) state. The sexual process is completed when the diploid nucleus undergoes meiosis, forming four monoploid nuclei. Depending on the species, the nuclei may then undergo one to several mitotic divisions.

Asexual reproduction involves the formation of

propagules that have not resulted from the union of compatible nuclei. Generally, the asexual state is repeated many times during a season, whereas the sexual state occurs only once. Both stages result in the formation of spores, but it is thought that the asexual state functions primarily in the propagation of the species by producing large numbers of individuals, whereas the sexual state functions primarily in the adaptive survival of the species by producing recombinations of individuals.

CLASSIFICATION OF FUNGI

In the currently predominant 5 kingdom scheme the fungi are classified in a separate kingdom, though in the past they were considered a part of the protist kingdom. More specific classification of the fungi is sometimes confusing because of the high degree of variation found in the fungi. In addressing the difficulty in the classification of fungi in his text Introductory Mycology 3rd Edition, (1962) Alexopoulos states:

> "There is a tendency for the beginning student to regard these various taxonomic categories as concrete and stable, and more or less sacred. Such an attitude will lead to disappointment with the first attempt to identify an unknown organism. You should understand, above all, that living organisms are constantly evolving, and that any attempt to

> pigeonhole them into a system of classifications bound to meet with difficulties. All systems of classification are nothing more than the attempts of man to organize his knowledge, and are strictly man-made. Even when our knowledge of fungi becomes much greater than it is at present, any attempt to draw hard and fast lines between taxonomic categories will be futile, because the categories themselves are only human concepts and intermediate forms are bound to exist and to arise by hybridization and mutation."

Perhaps a practical approach is best. As Blanchard and Tattar state in their Field and Laboratory Guide to Tree Pathology (1981),"The question becomes: Can the organism be identified so that published research information can be utilized? Last year's organism and this year's name do not change control strategies. The fungus is still the same fungus."

With this practical approach in mind, let us consider only two of the most important classes of fungi associated with wood. Both are distinguished by their unique spore-bearing structures. The Class Ascomycetes are sometimes called the sac fungi because of the saclike spore case called an ascus. Ascomycetes are known to cause a variety of diseases such as foliage diseases, wilts, and cankers. The Class Basidiomycetes are called the club fungi because of the

clublike basidia that bear the spores. Further, most Basidiomycetes produce fruiting structures that bear the basidia. These "basidiocarps" are commonly recognized as mushrooms, puffballs, and conks. Diseases most commonly attributed to Basidiomycetes include rust diseases and wood decay.

REQUIREMENTS FOR FUNGAL GROWTH

Whether the fungi is a pathogen or a wood rotter, the basic requirements for growth are similar. Blanchard and Tatter state that the three factors that affect the development and destructiveness of disease are susceptibility of the host population, virulence of the pathogen, and an environment favorable to the pathogen. Further, they explain that reductions in host susceptibility, or in pathogen virulence, or changes to less favorable environmental factors for the pathogen, will reduce the severity of a disease.

W.H. K. Findley, in his book Timber Pests and Diseases (1967), makes a similar statement but lists five conditions that must exist for a fungus to develop. They are, considered in detail:

I. **Source of infection**. Spores, most often airborne but also transmitted by a variety of animals, are a common

source of infection. Fungi produce incredible numbers of spores that remain viable for extended periods of time. Most soils contain fungi that are active and it is therefore possible for infection to spread directly by hyphae growing from the soil. Fungal pathogens have also been known to spread from contaminated trees to healthy trees via root grafts.

2. Suitable substratum. A suitable substratum must be present on which the spores can germinate, and from which the fungus can derive its nourishment. Spores that cause leaf spot or other leaf diseases require nothing more than a moist leaf surface in order to germinate. Bark, however, is not a suitable substrate so fungi that infect the woody portion of trees must first gain entry through a wound or other opening. Similarly, direct infection by soil pathogens often requires a suitable opening through which to enter, although some species of *Armillaria*, the honey mushroom, have specialized mycelia called rhizomorphs that can aggressively penetrate root tissue.

Fungi can derive nourishment from trees in a great variety of ways. Powdery mildews produce germ tubes which penetrate the walls of the leaf epidermal cells. The plasma membranes are not penetrated, but the fungus forms absorbing

structures known as haustoria. The fungus proliferates over the leaf surface, obtaining nourishment from hausteria in the epidermal cells.

Fungi may also obtain nourishment by digesting the wood tissue itself. Wood is composed of three main organic components, namely cellulose, hemicellulose, and lignin. Cellulose is composed of molecules of glucose, a monosaccharide formed through photosynthesis from atmospheric carbon dioxide. Glucose molecules are linked together to form long cellulose chain molecules. Hemicelluloses are chemically related to cellulose in that both are carbohydrates. Separation of cellulose and hemicelluloses is based on their respective solubility in alkali; cellulose is not soluble in a 17.5 percent solution of caustic soda (NaOH), whereas hemicelluloses are soluble (5). Lignin is the cell-wall component that differentiates wood from other cellulosic materials produced in nature. Lignin is not a carbohydrate; it is predominantly aromatic in nature. However, the chemical structure and reactivity of lignin are not completely known. The composition of lignin differs between softwoods ("guaiacyl" lignin) and hardwoods ("syringyl" lignin), and also varies especially among different hardwood species.

Considering this variable composition of lignin, it is easy to understand why many fungi often are very host specific. For instance, the birch polypore is by far the most common fungus found on birch in this country but it is rarely found on any other tree.

Some fungi have the enzymatic capacity to digest only the cellulose (and hemicellulose) portion of the wood. This causes the wood to darken in color and to crack up into brick shaped pieces which crumble easily into a brown powder. These are referred to as "brown rots". There are other fungi (mostly those that grow in hardwoods) that can, in addition to their attack on cellulose, break down the lignin through the action of oxidizing enzymes. These fungi cause the wood to become paler in color, and may reduce it to a fibrous white mass. Rots of this kind are commonly known as "white rots".

A fourth and minor component of wood is worth mentioning here. Wood may contain various materials collectively called extractives. They are not part of the wood substance, but are deposited in cell lumina and cell walls. The proportion of extractives varies from less than I percent (e.g. poplar) to more than 10 percent (e.g. redwood) of the oven dry weight of the wood. It is thought that a higher extractive

content imparts some degree of decay resistance to wood as these extractives are toxic to fungi. This is the main reason why some species, such as redwood, oak, chestnut, and black locust, for example, are more durable than others, and why heartwood is generally more durable than sapwood of the same species (6).

3. **Moisture requirements**. In practice, moisture content is by far the most important factor controlling the development of fungi on trees, timber, and also on many other organic products (7). This moisture is necessary both for the germination and growth of a fungus. In fact, for the germination of the spores it is generally necessary not only for the substratum to be moist but also for the relative humidity of the surrounding atmosphere to he high.

Moisture in wood is found either in the cell cavities - called "free water" - or absorbed in the cell walls themselves. The amount of free water varies by season and by species. While very little water is lost from a living tree or a freshly cut log, free water will begin to be lost from sawn timber as it begins to season. When all the free water has evaporated from the cell cavities, and the walls begin to dry out, the "fiber saturation point" is said to have been reached. This

saturation point varies but generally lies between 20-35 percent depending on wood species.

Species of fungi differ in their moisture requirements but in general wood must contain at least 20 percent moisture based on oven dry weight of the wood. Estimates vary as to optimum moisture levels for fungal growth, but a reasonable range is from 25-50 percent. At lower levels the wood is not attacked but the fungi do not die; they have the ability to remain dormant until conditions again become favorable. It is interesting that the hydrolysis of cellulose by a fungus releases water which effectively raises the moisture content of the wood. Also interesting is that although moisture is required for fungal growth, too much moisture will check fungal growth. The critical point of growth or no growth is reached when all spaces between cells are saturated with water, thus eliminating the required supply of oxygen. Thus, wood can be protected from decay by submerging it in water. It is thought that the wood for the instruments made by the famous Antonio Stradivari was stored in this way.

In a living tree the moisture content of the sapwood is generally too high to permit fungal growth. It is only when a fungus is introduced into the dead and dryer heartwood that

decay of a trunk can occur. To prevent the growth of fungi on lumber, the moisture content must not only be lowered but lowered rapidly. The most effective way to do this is with a kiln. Kiln drying also allows the moisture content to be reduced to about 12 percent as opposed to 18 percent for air drying. The lower moisture content also helps prevent excessive shrinkage and cracking of the wood when a product is used in a heated room.

4. Oxygen Requirements. Most fungi are aerobic organisms and thus need at least some air for their growth. Many species die if deprived of it, although survival in a dormant state is also possible.

The amount of air and the amount of moisture in wood are closely related because the spaces not taken up in the mass of wood by water are occupied by air. Again, it is for this reason that wood-rotting fungi cannot grow on wood that is waterlogged.

5. Temperature. Temperature requirements of fungi vary within a wide range, from 2 to 20 degrees centigrade; the optimum range is 20-36 degrees centigrade, depending on the species (8). Temperatures slightly below the minimum and above the maximum limits may not kill the fungi, but will tend

to retard or halt their activity. Fungi are killed only by very low or very high temperatures; more effective are high temperatures in combination with high relative humidities. Such conditions are employed in a kiln-drying process. Thus, this process not only removes the moisture necessary for fungal growth, but also sterilizes the wood if done for a sufficient amount of time.

FUNGAL PATHOGENS

Let us consider three common tree diseases in order to better understand how basic knowledge of pathogenic organisms can be put to use to either prevent the disease or lessen its impact.

DUTCH ELM DISEASE

Dutch elm disease was discovered in Cleveland, Ohio, in 1930 and around the port of New York in 1933. The causal fungus, an ascomycete, was introduced on elm-veneer logs imported from Europe. Soon the disease appeared along railroad rights-of-way and at ports of entry. Since then, it has spread and is reported in 42 states (as of 1979). The disease has caused extremely heavy losses of elms, both wild and those planted as shade trees. The losses are probably most evident in urban areas where boulevards once lined with

majestic elms are now less attractive as the trees have been replaced with less asthetic species.

Dutch elm disease is first evident as yellowing and wilting occur on leaves of one to several branches in the upper crown during late spring or early summer. The affected leaves quickly turn brown and die. These symptoms are repeated on progressively larger branches and often involve the entire tree by the end of the summer. Severely infected trees often die in the current season and those surviving the winter usually are dead by the end of the next season.

Dutch elm disease is caused by *Ohhiostoma lumi* (Buism.) C. Mor. which is transmitted to susceptible elms either by root grafts or more importantly by insect vectors. The European bark beetle (introduced into the United States around 1909) is the most common vector and transmits the disease as it feeds on twigs. The larger native elm bark beetle transmits the disease as it feeds on larger branches. The fungus enters the vascular system through the feeding wounds and proliferates in the xylem vessels. The invaded vessels become nonfunctional due to the activities of the pathogen, and xylem sap is no longer transported through them. The pathogen progressively invades the xylem vessels and eventually the

entire tree dies as the pathogen completely invades and kills the vascular tissue. Female elm bark beetles then may enter the bark of dead and dying elms and deposit eggs in galleries between the inner bark and xylem. Eggs hatch and the emerging larvae tunnel under the bark and eventually pupate. When the adults emerge they are contaminated with spores of the fungi produced on special spore structures that line the galleries. The adult beetles can then transmit the pathogen to healthy trees where the cycle will continue.

Federal and State research workers, in cooperation with the United States Department of Agriculture, suggest four ways to combat dutch elm disease:

I. Reduce the population of beetles by eliminating elm material required for breeding. Elms should be surveyed for disease symptoms early in the spring and as often as possible throughout the summer. All symptomatic elms found between April and September should be destroyed within 30 days to prevent their colonization by beetles that later carry the fungus to healthy trees. All dead elm wood should be destroyed, either by burning or by disposal in a landfill. Diligence is required to search out and destroy all possible beetle breeding sites.

2. Protect healthy elms from feeding beetles. This is done by spraying with an insecticide. Methoxychlor has been commonly used in the United States for this purpose. Spraying must be thorough. All bark surfaces must be completely covered to prevent bark beetle feeding. Spraying may be done in the dormant period (fall, winter, early spring) to be effective during the period of high bark beetle activity beginning in mid to late spring.

3. Prevent underground transmission of the fungus from a diseased tree to adjacent healthy trees by root grafts. This may be done using a chemical barrier made by injecting a soil fumigant as soon as the disease symptoms appear. A mechanical barrier can be made by digging a narrow trench 75 centimeters deep between the diseased and healthy trees. The trench should extend midway between the diseased and healthy trees, beyond their drip lines.

4. Plant trees that are resistant to Dutch elm disease. The Siberian elm (*Ulmus pumila* L.) and the Chinese elm (*Ulmus parvifolia* Jacq.) are both very resistant to the disease but lack the growth patterns that made the American elm such a popular shade tree. Hybrids that are both disease resistant and attractive are being developed and some are being made

available to the nursery industry.

CHESTNUT BLIGHT

Chestnut blight was believed to be a disease of minor importance when it was first reported by the late Hermann Merkel, who found a few infected trees in the New York Zoological Park in 1904. Fifty years later, however, the disease had wiped out the chestnut stands along most of the tree's principal range. This is the most devastating disease ever to attack forest trees.

Chestnut blight is caused by the fungus *Cryphonectria parasitica* (Murrill) Barr, which enters the host through wounds in the bark. Germ tubes that enter these wounds are produced by both conidia and ascospores. The fungus spreads through the bark cortex, forming mycelial fans that penetrate the inner bark and kill the cambium. The outer bark over the diseased tissue swells and eventually begins to crack. The formation of a "canker" results as the host produces callus tissue to try to prevent further spread of the fungus. Orange fruiting structures called pycinidia extend above the canker and produce spores called conidia. Conidia may be passively dispersed by rainsplash, or actively dispersed by insects, birds, and other animals. After the pycnidia are produced,

other spore producing structures called perithecia are formed from the same fungal tissue. These perithcia are embedded in stroma and have long necks that extend to the bark surface. Bicellular ascospores are forcibly discharged and carried by the wind to new hosts. The fungus continues to grow in a susceptible host until the tree is girdled.

There is no effective way of treating chestnut blight. There is no danger of extinction of the chestnut tree, however, because the tree sprouts from the roots and stumps of dead trees. One idea being pursued as a control of the blight is the development of trees resistant to the pathogen. The development of naturally resistant trees is hindered by the fact that sprout trees are often killed before sexual maturity is reached. Systemic chemicals are also being investigated as a method of control. Experiments by Dr. Dennis Fulbright of Michigan State University and others on hypovirulent strains of the fungus show promise as control measures.

SHOESTRING ROOT ROT

One species of the fungus which causes shoestring root rot gained fame in early 1992 as the worlds largest living organism. Using advanced genetic techniques, researchers reported that they had identified and individual of *Armillaria*

bulbosa that occupies a minimum of 15 hectares, weighs in excess of 10,000 kg, and has remained genetically stable for more than 1,500 years (9).

Of more practical importance is the impact of shoestring root rot on populations of living trees, particularly commercial plantations of red pine and other species important for our growing paper industry.

Shoestring root rot is caused by several species of the genus *Armillaria*, including *Armillaria mellea* (Vahl. ex Fr.) Kummer and *Armillaria bulbosa*. The pathogen is a persistent soil fungus that is common to most forest soils worldwide. It can persist as a saprophyte in a vegetative state indefinitely in dead roots and can migrate to roots of healthy trees via rhizomorphs, which are the thick black strands of mycelia that look like shoestrings and give the organism its common name. These structures are thought to function in transport of materials to the growing regions of the fungus. After penetration of the healthy tree, the pathogen progressively invades the roots and buttress area where it kills the cambium and later decays the xylem beneath. It also may cause cankers around the root collar which often girdle the tree and cause its death. Infected trees are also subject to windthrow due to

wakened support of the root system.

Armillaria generally does little damage in healthy tree stands, causing limited root rot and butt rot while decaying woody debris and moribund rootwood (10). However, trees with root injury, recently transplanted trees, or trees that have suffered severe stress, such as prolonged drought or insect defoliation, are often attacked. Thus, controlling these stress factors when possible will permit healthy trees to stay healthy.

While armillaria root rot is not a major problem in healthy forest, it can be a problem in plantations, especially where indigenous forests are converted to conifer plantations. Mortality rates as high as 36% have been reported in such plantations (11). Mortality rates can be reduced by:

I. Selecting disease resistant nursery stock. Unfortunately, pines are not very resistant to this disease. Stock grown in local nurseries are more resistant than those imported from distant nurseries.

2. Proper site preparation. Some chemicals used to kill indigenous trees may favor the growth of effective competitors to *Armillaria*, while others (such as 2,4,5-T) favored the growth of decay species easily replaced by

Armillaria (12). Uprooting of stumps and the use of root rakes to reduce the available food base for the pathogen are also effective ways to reduce infection and mortality in the young trees.

3. Proper site selection. Sites should be selected not only based on the food base characteristics but also on soil characteristics and available moisture. High risk sites should be avoided.

In practice, armillaria root rot is not a significant problem if a site is well prepared. Ken Weyers, a forester with Mead Paper Company in Escanaba, Michigan, reports that in a well prepared site mortality of seedlings is very low. If a contamination problem is thought to exist at the time of planting, an additional 50-75 seedlings are planted per acre (at a cost of about 13 cents per seedling) to allow for those lost to the disease.

MYCORRHIZAL FUNGI

Mycorrhizae are fungus-feeder root associations in which the fungus is actually invasive in host cells and/or tissues. From this standpoint, the fungus could be considered pathogenic. However, mycorrhizal roots are considered to play a major role in root absorption of soil nutrients and

water. They are considered to be essential for rapid development and continued growth of transplanted trees. They are also thought to be valuable in protecting root tissue from pathogenic soil fungi.

There are two major groups of mycorrhizae: (1) ectomycorrhizae and (2) vesicular-arbuscular mycorrhizae (endomycorrhizae). Ectomycorrhizal roots are the most easily seen due to their modification of the anatomy of the feeder roots. Ectomycorrhizal roots have a fungus covering (mantle) over the outside of the feeder root and intercellular hyphae throughout the cortex (Hartig net). These structures are thought to present barriers to invasion of the roots by soil fungi and, therefore, to protect the tree from root diseases. Most ectomycorrhizal associations are caused by basidiomycetes in the mushroom and puffball groups. Vesicular-arbuscular (VA) mycorrhizae do not modify the feeder root anatomy and are not as easily detected as ectomycorrhizae. VA mycorrhizal roots have intracellular hyphae in the root cortex, and the invading fungus often produces vesicles and specialized branched haustoria (arbuscules) in this tissue. Most VA mycorrhizal associations are caused by phycomycete fungi in the genus *Endogone.*

Research is ongoing to develop effective ways to improve tree seedling survival by encouraging ectomycorrhizal development on container grown plants. Inoculation of spores into greenhouse soil is the most common method being developed. Several companies (including Abbott Laboratories and Sylvan Spawn Laboratories, Inc.) have developed and are marketing inocula. Tests have shown the effectiveness of these inocula to be highly variable. It has been proposed that some commercial inocula are not effective because they are not able to compete successfully with the indigenous fungi (13). Inoculation with indigenous species known to form mycorrhizae with target trees has shown much promise. Survival rates for red pine seedlings inoculated with the ectomycorrhizal fungus *Laccaria bicolor* and planted on the Baraga Plains, in Baraga County, Michigan were as much as 20% higher than uninoculated seedlings (14).

SUMMARY

Fungi are very important organisms in a forest. Diseases such as Dutch elm disease and chestnut blight are caused by fungi. The appearance and ecology of forests in the United States has been forever changed by these introduced diseases.

These fungi and others have caused and continue to cause incalculable financial loses to those associated with the forest industry.

Forest fungi also are beneficial in many ways. Many edible fungi can be harvested from the forest. They also play an important role as decomposers. Mycorrhizal fungi seem to benefit trees by aiding in the absorption of water and minerals through the roots.

As our knowledge of the fungi grows, so should our ability to control or at least lessen the impact of the many fungal disease. Also, we may learn to take better advantage of mycorrhizal fungi to benefit our forest products industry. Who knows what other suprises we will find as we increase our knowledge of these complex and important organisms?

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Appendix II: Laboratories, Activities, and Teacher Notes

A. Studies of a Humongous Fungus

1. Lab 1 Finding and Collecting Armillaria
2. Lab 2 Isolating and Culturing Armillaria
3. Lab 3 Who's Who Among the Fungi
4. Teacher notes

B. Effects of Wood Rotting Fungi

1. Lab 1 Changes in Mass During Rotting
2. Lab 2 Change in Wood Strength Due to Rotting

C. A Disease in the Dogwoods

D. The Irish Potato Famine

1. Activity 1 United States Immigration
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E. Fermentation Technology

1. Teacher Notes
2. Introduction to Fermentation Technology
3. Activity 1 Making Yogurt
4. Activity 2 Making Fresh Milk Cheese
5. Activity 3 Brewing Beer
6. Activity 4 Measuring Yeast Fermentation Rates

Appendix IIA

STUDIES OF A HUMUNGOUS FUNGUS

LAB 1: FINDING AND COLLECTING *Armillaria*

INTRODUCTION

Armillaria species are remarkably successful components of many natural forests. Members of the genus are important decomposers of both cellulose and lignin, and are thus important in mineral cycles. Many tree and shrub species of different strata, particularity in boreal and temperate forest ecosystems, are susceptible to root infection by *Armillaria* species, especially when the host plant is under stress. They may also play minor roles as mycoparasites (parasites on other fungi) and mycotrophic associates with some nonphotosynthesizing plants (e.g. orchid mycorrhizae).

In the midwestern United States, thinned hardwood forests and clear-cut areas that are being converted to conifer plantations are excellent collecting sites. Collecting becomes easiest several years after cutting.

Three kinds of *Armillaria* structures may be recovered in the field: 1) rhizomorphs, 2) mycelial fans, and 3) mushrooms. Rhizomorphs are dark-colored cord-like infection structures which grow through the soil as a highly branched network. Their function is to explore the soil for wood, and to colonize it if possible. Rhizomorphs may be found between the bark and wood of recently killed hardwood trees or stumps, and even growing through the wood of damp, well rotted aspen logs on the forest floor. Colonization of conifer wood usually results in the production of mycelial fans. Mycelial fans are white, felt-like sheets of fungal filaments which grow between the wood and bark, spreading from the point of infection on the roots upward to the stem. Mycelial fans kill

the vascular cambium and phloem wherever they develop. Mycelial fans of Armillaria may be collected from stumps of most conifer species, or from the roots and lower stems of dead conifer seedlings and saplings. Finally, *Armillaria* mushrooms, sometimes called "honey" or "stump" mushrooms, can be collected on or around hardwood stumps or even from the bases of infected conifers in the late summer or autumn. Mushroom collections are especially valuable, because both vegetative (diploid) tissue and single basidiospore (monoploid) isolates may be derived from them.

MATERIALS NEEDED

Axe or hatchet	Knife	Hand saw
Basket	Waterproof Marker	Tags
Notebook	Pruning shears	Paper Bags

PROCEDURE

Important General Information

I. Each sample should be tagged or marked in the field for reference. Collection date, exact location, and species of infected tree should be recorded in a notebook.

2. Proper care and handling of samples will greatly improve success in later attempts to isolate the fungus into pure culture. Paper or waxed paper bags should be used for rhizomorphs, mushrooms, and other small samples to reduce the amount of water condensation. Free water on the sample favors growth of contaminating bacteria and fungi. Samples should be transported to the lab as quickly as possible and kept at room temperature and out of the sun. Isolations should be attempted within 48 hours of collection to minimize the chances of contamination.

Collecting Armillaria

3. From rhizomorphs Rhizomorphs attached to infected tree

roots are most easily visible and may often be collected along with a mycelial fan. Rhizomorphs between the bark and wood on hardwood logs or stumps may be collected in a similar way. Rhizomorphs in the soil are often difficult to distinguish from fern roots (Rhizomorphs have a brown to black brittle outer rind which protects the slightly elastic white core tissue. Actively growing rhizomorphs may have a pale tip.) and are thus difficult to collect. Rhizomorph collecting may be made easier by placing potatoes or aspen stakes in the soil for several months to allow rhizomorph invasion. This technique is called "baiting".

4. From mycelial fans. First, use a knife to check for the presence of mycelial fans beneath the bark of suspected infected trees at the ground line. If none are found, another potential sample should be located. Once the presence of fans is confirmed, a sample of wood with attached bark (and the mycelial fan sandwiched between) should be collected. To collect from a dead conifer seedling or sapling, use an axe or hatchet to chop in a circle around the base of the stem to sever lateral roots. Pull the tree from the ground and use the pruning shears to trim the roots, leaving some of the larger ones for possible isolations. Use a saw to trim the stem, leaving the 10-20 centimeters above the soil line. This is the section from which isolations are most easily made.

If the mycelial fan is to be taken from a stump, the portion of the stump needed must be chopped out. If possible, the bark should be left intact to help prevent contamination of the fan.

5. From mushrooms. Mushrooms are the fruiting structures of *Armillaria* , analogous in many ways to the fruit of flowering plants. *Armillaria* mushrooms occur singly or in clumps on or around decaying wood. Use a knife to collect them. Try to collect the entire mushroom (including the base of the stem) for careful identification of each specimen. With study and practice, *Armillaria* can easily be distinguished from other mushrooms with similar habitats and appearances. Try to collect mushrooms that are opened (with their gills

exposed) since mushrooms with their gills still covered have not begun to produce spores. Immature mushrooms are nevertheless excellent sources of vegetative isolates.

STUDIES OF A HUMONGOUS FUNGUS

LAB 2: ISOLATING AND CULTURING *Armillaria* IN THE LAB

INTRODUCTION

Samples of *Armillaria*, whether from mycelial fans, rhizomorphs, or basidiocarps (mushrooms), can be grown in pure cultures on various types of agar. These pure cultures will later be used for a variety of experiments.

Isolates will be first cultured on water agar. This medium is almost devoid of nutrients and *Armillaria* will grow very slowly on it. Other contaminating fungi will often grow faster and can be thus identified. Such contaminants can then be cut out.

Once it is reasonably certain that contaminants have been removed, the cultures will be transferred and maintained on a medium containing 1.5% malt extract which will serve as a nutrient for the fungus. It is these vigorous colonies that will be used in later experiments.

It will be extremely important that sterile methods are used throughout the culturing activities if contamination is to be kept at a minimum. Some contamination is almost impossible to avoid, but poor technique can lead to excessive contamination which will often spoil an experiment. Techniques to insure sterility will be outlined at each important stage of the process.

MATERIALS NEEDED

For tissue isolations.

10% bleach solution	Bunsen burner (or boiling water)
sampling needles	knife

paper towel	paper for covering surfaces (newspaper)
cheese cloth	agar plates
spray bottle	mini hood (if available)

Additional materials for single spore isolations.

shiny black paper	vials of sterile water
dissecting microscope	pipette

General preparations.

I. Spread paper on the floor and place your sample on it. Using a knife, clean as much dirt and debris as possible from the sample. For stem and stump mycelial fan samples, mist with 10% bleach solution to keep dust down and to kill surface contaminants.

2. Sterilize the surface of your lab table by spraying with 10% bleach solution and wiping with paper toweling.

3. Cover a portion of the table surface with paper. Dampen the paper with a spray of 10% bleach solution.

4. Cover another portion of the table with cheese cloth and mist it with 10% bleach solution. Place your agar plates on this cloth. OPTIONAL. If a mini hood is available, place this over the cheesecloth and agar plates. Working with the agar plates under the mini hood will help reduce contamination by keeping fungal spores in the air from landing on the agar.

5. Use a Bunsen burner to sterilize several sampling needles. (This will allow you to always have a cool needle to work with.) Place the needles on something that will keep the tips off the table surface. Needles may also be sterilized by placing them in boiling water for 30 seconds.

Isolating from a mycelial fan.

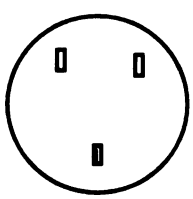
1. Place the sample from the floor onto the paper on the table surface.

2. Use a knife to cut a rectangular "window" through the outer bark. Gently lift the section of bark to expose the mycelium beneath it, in the cambial zone between the wood and bark. Try to find a section that contains the young, almost pure white mycelium. Older mycelial tissue is more likely to be contaminated.

3. Use the sharp sampling needle to cut a small square (about 2 mm X 2 mm) of mycelial tissue. A small section is likely to contain fewer contaminants. Gently lift the section from the sample.

4. Transfer the small section of tissue to the Petri dish containing the water agar medium. To minimize contamination, lift the Petri dish cover as little as possible and always keep the cover directly over the surface of the agar.

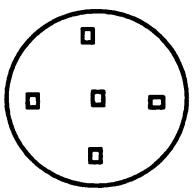
5. Put a total of three samples on each of two Petri dishes. Space them about 1 cm. from the edge of the dish and as far away from each other as possible. Below is a suggestion for arranging your samples on the dish.



6. Label the lids of the dishes to identify the sample. Include the sample number, your initials, and the date.

7. Incubate the sample for 4-6 days at room temperature and in the dark, checking for contaminants every two days. Remove any contaminants by carefully cutting the agar around the contaminated site and lifting the agar from the plate.

8. Once it appears that the samples are growing well and are free of contamination, they should be transferred to 1.5% malt extract (1.5 M) agar. Working under a mini hood if possible, or at least on sterilized cheesecloth, use a sampling needle to cut small sections of the fungus and place them on the 1.5 M plates. This time, put 5 sections on each plate and arrange them in a pattern similar to this:



9. Incubate these 1.5 M plates for another 4-6 days, when they should be ready for the next activity.

10. A reference culture of your sample should be kept. To do this, transfer a small section of the fungus onto the surface of a 1.5 M agar slant. The cap should be placed loosely on the slant and then parafilmed. These slants should be refrigerated and may be kept for up to a year.

Isolating from a rhizomorph.

l. Obtain long segments of rhizomorph samples. Trim the samples to about 5 cm.

2. Soak the rhizomorph sections in 10% bleach solution for about 30 seconds to kill surface contaminants.

3. Trim small segments (about l5 cm) of rhizomorph and treat them as described in steps 4-10 in the previous section.

Isolating from a basidiocarp.

1. Obtain the mushroom and clean any dirt off of the cap. Also, trim the dirt from the stipe. Remember, if single spore isolates are to be made, a mature cap must be saved.

2. Place the sample on the paper covered table top.

3. Cut the cap of the mushroom with a sterilized knife to expose the fresh, white internal cap tissue. Samples from gill tissue or from wormy, discolored caps are very likely to be contaminated.

4. Use a sharp sampling needle to cut a small section (about 2mm X 2mm) of the white tissue.

5. Transfer 3 such sections of tissue to the 2 water agar plates. Label the lid of each Petri dish and incubate and transfer a previously described.

Isolating single spores.

I. Making a spore print

a. Trim the stipe of the mushroom so the cap will lie flat when it is placed gill side down on the table top.

b. Place the cap on a piece of shiny black paper.

c. Cover the paper and cap with a piece of paper towel that is moistened with water.

d. Let the cap stand on the paper for about 30 minutes. Carefully lift the cap off of the paper. The spore print should appear white or off-white against the black background.

2. Transferring and diluting the spores.

a. Cut the paper beneath the spore print into thin strips. Place the strips into a vial containing 10 ml of sterilized water. Cap and shake the vial to disperse the spores.

b. Use a pipette to transfer 1 ml of the water from the

first vial into a second vial and dilute with 9 ml of sterilized water. (You should now have a solution that has a spore concentration that is 1/10th the original concentration.)

c. Repeat the procedure from b two more times to get spore concentrations that are 1/100th and 1/1000the the original concentration, respectively.

3. Plating and incubating the spore samples.

a. Three plates will be cultured from each of the diluted spore samples. Culturing is accomplished by pipetting varying amounts of spore containing water from each vial onto water agar plates according to the following suggestion:

Plate I - 1.0 ml Plate 2 - .5 ml Plate 3 - .2 ml

Put the water onto the surface of the agar. Allow it to stand for 5-10 minutes and then pour off the excess water by carefully uncovering and inverting the Petri dish over a piece of paper towel.

b. Allow the plates to sit at room temperature for about 24 hours to allow the spores to germinate.

c. Examine the samples under a microscope after 24 hours for evidence of spore germination.

d. Using a sterile needle, cut out a single germinating spore and place it on a plate containing 1.5 M agar. Plate 10-15 spores in this manner. Remember sterile technique! Incubate these single spore isolates for 7-10 days, at which time it will be ready to use.

STUDIES OF A HUMUNGOUS FUNGUS

LAB 3: WHO'S WHO AMONG THE FUNGI

INTRODUCTION

In April of 1992, a group of researchers, including Dr. Johann Bruhn, then of Michigan Technological University, reported the discovery of an individual fungus in the Upper Peninsula of Michigan that covered 37 acres and weighed an estimated 110 tons. The fungus was heralded as the largest living thing on earth. Shortly afterward, researchers reported finding an even larger one in the Pacific Northwest. One might ask how they knew that they are dealing with only 1 individual and not several individuals (sometimes called clones or genets). In other words, how do you tell one fungal individual from another.

In the Michigan study, individuals (genets) were identified two ways - by using somatic compatibility tests and by mitochondrial DNA comparisons. In this activity, somatic compatibility tests will be done to determine if the fungal samples collected and cultured earlier are from the same or from different genets. DNA isolations are difficult and very expensive to do and thus will not be done in this activity.

To do somatic compatibility tests, two sample isolates (from your earlier labs) will be placed in very close proximity to each other on the surface of a 3% maltose extract (3M) agar plate. After allowing time for the fungal isolates to grow, the interactions between the two samples will be observed. If the samples are from the same individual (genet), they will grow together with no differences observed between cultures. This is called a positive result. If the samples are from different genets, they will not grow together. A definite zone of inhibition called a barrage zone will be observed, either as a clear zone or as a dark, melanized line (called a

pseudosclerotial plate). Such a response would be recorded as a negative result.

Sometimes many samples must be compared to one another and keeping track of the results may be difficult. A Punnett square may be helpful. An illustration is shown below using four samples labeled A-D.

	Samples				
	A	B	C	D	
A	+	X	X	X	
B	-	+	X	X	X's are placed to avoid duplication.
C	-	-	+	X	
D	+	-	-	+	

The positive results when any sample was confronted with itself would be expected. The positive results between A and D indicate that these samples are from the same genet.

MATERIALS NEEDED

Spatulas	Parafilm	Indelible marker
10% bleach solution	Cheesecloth	Mini hood if available

Samples on 1.5 M agar (from Lab 2)
Plates of 3M agar

PROCEDURE

I. Clean the surface of your lab table by misting it with 10% bleach solution and wiping with a paper towel.

2. Place cheesecloth over an area of the table top and mist it with bleach solution. Put your mini hood (if available) over the

cheesecloth.

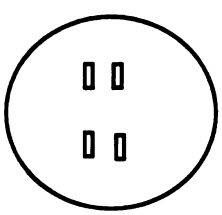
3. Sterilize several spatulas by heating them to red hot in a Bunsen burner flame. Allow them to cool by placing them on something that will keep the sterile part off the table top.

4. Obtain your samples and your 3M plates and place them on the cheesecloth (and under the mini hood, if available).

5. Use a sterilized spatula to cut a small rectangle of one of the samples and place it near the midline of the 3M plate.

Remember sterile technique!!

6. Cut a small rectangle of another sample in a similar manner and place it very near the other sample on the opposite side of the midline. Actually, two confrontations can be done on each 3M plate. A suggested arrangement of the samples is shown below.



7. Seal the cover on the Petri dish with a band of parafilm.

8. Label the bottom of the dish to identify each sample. Incubate the plates at room temperature and in the dark for at least 10 days.

9. After inoculation, read the results and record them as described in the introduction.

STUDIES OF A HUMONGOUS FUNGUS
TEACHER NOTES

FINDING *Armillaria*

Armillaria is a widespread genus with ten species identified in North America. They are usually all collectively called "honey mushrooms". Basidiocarps can be found on stumps of most common hardwood species at certain times of the year, usually from late September to mid to late October. Fruiting season varies, however. I have collected mushrooms as early as mid July. Conifers that have been killed by the fungus may also have mushrooms sprouting from their bases. They may also grow on dead logs and often appear to grow in open areas but are probably growing on covered wood. Mycelial fans can be found on the bases of hardwood stumps and dead conifer stems. Simply peel the bark away from the base to reveal the characteristic white fan. Rhizomorphs can often be collected with the mycelial fan. As mentioned in the introduction of student Lab 1, rhizomorphs are difficult to collect from the soil or leaf debris as they are easily confused with other structures such as fern roots. However, the fungus can be "baited" with aspen stakes or potatoes buried in the soil. Rhizomorphs will invade the aspen or potato tissue and can then be collected.

Further information on finding and identifying *Armillaria* basidiocarps can be found in any of several good books dealing with fungi. Several are listed as references. The following description of *Armillaria mellea* , from Roger Phillips' Mushrooms of North America (1991), is provided for convenience.

> "**Cap** 3-15 cm across, very variable, convex then flattened and centrally depressed or wavy; ochre, tawny to dark brown, often with an olivaceous tinge; covered in dark hairy scales, especially at the center. **Gills** attached or slightly descending stalk, nearly distant, narrow; whitish then

yellowish becoming pinky-brown and often spotted darker with age. **Stem** 60-150 x 5-15mm, stuffed to hollow; whitish becoming reddish brown. **Veil** partial leaving a thick whitish to yellow cottony ring on upper stem. **Flesh** white. **Odor** strong. **Taste** astringent. **Spores** ellipsoid, smooth, nonamyloid, 8-9 x 6-9 micrometers. **Deposit** whitish. **Habitat** in small or large clusters at the bases of trees or near stumps. Common. Found widely distributed throughout North America. **Season** August - November. **Edible** but must be cooked. Some cases of severe stomach upset have been reported after eating this mushroom. Eat in small quantities the first time you try it".

MATERIALS NEEDED

The following is a list of materials needed for a class of 20 working in 10 groups to do all of the activities. Additional notes on these materials are provided after the list.

Axe	Knives (10)
Saw	Labels
Paper or waxed bags	Spray bottles (10)
Pruning snips	Baskets or large paper bags (10)
Bleach	Sampling needles (50)
Bunsen burners (10)	Cheesecloth (1 large roll)
Shiny black paper	Permanent markers (10)
Small lab spatulas (10)	Vials (at least 10)
Parafilm (I roll)	Agar Plates (see notes)

Agar slants (see notes)
1 ml Mohr pipettes (10)
Paper for covering surfaces
Dissecting microscopes (10)

GATHERING AND STORING SAMPLES OF *Armillaria*

The procedure for collecting mycelial fans and

rhizomorphs is well described in the student lab sheets. Although it is best to use these samples immediately, the stems or stump pieces can be stored for a short time if they are kept cool. Sample contamination becomes more likely as the length of the storage period increases.

Basidiocarps will become unusable if they are stored for more than a day or two. Therefore, tissue cultures and spore prints should be taken immediately after collecting if possible. Shiny black paper good for making spore prints is often used as a spacer in packages of photographic paper. Talk to your local commercial photographer. Once spore prints are cut and placed in vials of water, they may be frozen for later use. They will remain viable indefinitely in this condition.

NOTES ON PREPARING CULTURE MEDIA

An autoclave is necessary for the preparation of the agar media. A pressure cooker can be used if an autoclave is not available. Agar should be sterilized at 17 psi for at least 30 minutes.

Agar preparations are reasonably simple. 800 milliliters of agar will fill about 100 Petri dishes. The recipes for the media used in the activities are:

Water Agar (WA) - 2% agar. For example, to prepare 800 milliliters of agar, use 16 grams of solid agar and dilute to 800 milliliters in a 1 liter Erlenmeyer flask. Cover the top with foil and autoclave as described in the paragraph above.

1.5% malt extract agar (1.5 M) - 2% agar with 1.5 % malt extract. For example, to make 800 milliliters of this medium would require 16 grams of solid agar and 12 grams of malt extract.

3% malt extract agar (3M) - 2% agar with 3% malt extract. For example, to make 800 milliliters of this

medium would require 16 grams of solid agar and 24 grams of malt extract.

15 x 60 mm disposable Petri dishes work well in these activities. A case of 500 costs about $70. Small screw cap vials work well for spore collections and for making permanent reference collections of isolates.

If a group wanted to do only one tissue isolation and confront it with only one other isolate, they would require 2 WA plates, 2 - 1.5M plates, and I - 3M plate. If the isolate is to be made part of a reference collection, an agar slant would also be needed. If they wanted to isolate single spores from only one sample, they would require 9 WA plates and 10-15 - 1.5M plates.

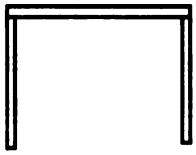
Agar plates should be poured in sterilized areas using sterile technique. Also, they should be poured in areas with minimal air movement. To avoid running out of culture plates they can be poured in bulk in advance (1 to 2 months) and stored (inverted and in a cool, dark place). Make sure that the plates are stored in sealed sleeves to minimize contamination and dehydration.

TIPS ON HANDLING AND TRANSFERRING *Armillaria* CULTURES

Size 18 hypodermic needles are flatter and much sharper than ordinary teasing needles. When mounted on a wooden handle they make excellent sampling and transferring tools. A safety pin tip can also be modified to make a good tool. Heat the tip to red hot in a burner flame and flatten using a hammer. Cut the tip section of the pin off and mount it on a wooden handle.

A mini hood, with three wooden sides and a plexiglass top, helps reduce contamination from airborne fungal spores. A plan for making one is shown below.

2 sides - 8" x 161/2" x 3/4 " thick
l back - 8" x 24 1/4" x 3/4" thick
l piece 1/8" plexiglass - 16" x 24"



Use a table saw to cut a groove (the width of a saw blade, about 3/8" deep) about 1/4" from one edge of the wood pieces. The plexiglass top will slide into this groove when the mini hood is assembled.

Nail the side pieces to the back using small finishing nails. Make sure grooves are lined up.

Slide the plexiglass top into the grooves until it locks into the back piece.

Petri dishes containing isolates can be stored and incubated in any dark place that remains at about room temperature. Plates should be put back in plastic sleeves to reduce contamination and dehydration and to make storage neater. They should be stored inverted to prevent water from condensing on the surface of the agar.

Agar slants that are to be kept as part of a permanent reference collection should be refrigerated. They will remain viable for up to a year. They should be freshened after that time by transferring the culture to a 3M plate, incubating the plate, and then transferring the fresh culture to a fresh agar slant.

OTHER NOTES

Single spore isolates are more difficult to obtain than tissue isolates. A compound microscope may be helpful in finding the germinating spores. It may also be helpful to mark the surface of the agar by scratching it with a sterile needle.

Otherwise, the spores that are on the surface are extremely difficult to find. A steady hand is necessary to pluck only 1 germinating spore for culturing. For this reason, single spore isolation and related labs may be better done as a demonstration or eliminated altogether.

The appearance of the confrontations between two isolates may vary. Negative interactions are most noticeable when dark pseudosclerotial plates form between the isolates. When the interaction is less obvious, it is often valuable to move the plate to get various views of it. Also, a clear zone may show up better is lighting is varied.

Axes, knives and pruning snips can be dangerous if not used correctly. It may be wise to do any chopping of trees and roots yourself. Stress safety to students working with knives.

Sometimes it is difficult or even impossible to obtain pure cultures of *Armillaria* from the wild. However, commercial sources of this fungus, sold as "glow in the dark fungi", are available from many biological supply companies and can be used to simulate these labs. A classroom set of plates can be cultured easily from one plate and these plates can then be used as outlined in activity 3. Several sources must be used to give both positive and negative interacions.

Appendix IIB

THE EFFECTS OF WOOD ROTTING FUNGI

LAB 1: CHANGES IN MASS DURING ROTTING

INTRODUCTION

In all likelihood, you come into contact with wood every day. Wooden houses, decks, fence posts, utility poles, railroad ties, signs, steps, and, of course, number two pencils are part of your daily life.

Wood is an important renewable resource that is lightweight, strong, resistant to chemicals, and easy to work with. Unfortunately, the design of some wooden structures make them susceptible to fungal decay. Wood that is subject to high moisture conditions, such as exposed beams and decking, and wood in direct contact with the soil, such as fence posts, are prime targets for fungi. As fungi break down the wood many changes occur, some of them making the wood unfit for many uses.

In this activity, you will be placing wood samples into soil. The fungi present there will likely cause rotting. You will compare the mass of fresh wood to the mass of the same samples after they are rotted. You will also compare the amount of mass change in two or more types of wood, or perhaps compare the rotting of a variety of wood in two or more different types of soil.

MATERIALS NEEDED

Wood pieces
Jars or other containers
A pencil or marking pen
A balance
Soil

PROCEDURE

PART I: Setting up for the incubation of the wood samples.

1. Obtain the wood samples. Label each piece clearly to make identification easier later. Label at least three pieces (of each species if more than one species of wood is to be tested) TEST, and another 3 pieces CONTROL.

2. Determine the total mass of the TEST and CONTROL pieces to the nearest 0.1 gram. Record these beginning masses in the data table.

3. Obtain a jar for each type of wood being tested and fill each about 1/2 full with soil. The soil should be moist (but not too wet). Add a small amount of water if necessary.

4. Place the TEST wood samples in the jars so that most of the length is buried in the soil. Only one type of wood should be placed in each jar. Label the jar with your group name and the type of wood.

5. Cap the jars loosely and place them in a shaded area for incubation. The jars should be allowed to sit for six to eight weeks. Check them every two weeks and record any changes you see in the table provided.

6. Place the CONTROL pieces on a shelf or some other area designated by your teacher. These pieces should also be checked for observable changes every 2 weeks.

PART II: Calculating the change in mass.

7. After the incubation period is complete, remove the wood samples from the jars. Scrape off any excess dirt and allow the samples to air dry for one more week.

8. Measure the mass of the samples of each type of wood. Record these masses in the data table.

9. Calculate the mass change using the following formula:

$$\text{Mass Change} = \frac{(\text{Beginning Mass} - \text{Final Mass}) \times 100\%}{\text{Beginning Mass}}$$

10. Record the mass changes for each of the wood samples in the data table.

11. REMEMBER TO SAVE YOUR WOOD PIECES FOR LAB 2.

TABLE I: MASS MEASUREMENTS

TYPE OF WOOD	BEGINNING MASS	END MASS	% CHANGE

TABLE II: OBSERVATIONS OF ROTTING WOOD

WEEK	APPEARANCE
_____	_____
_____	_____
_____	_____
_____	_____

QUESTIONS

1. Explain the change in mass you observed. If the wood sample lost mass, where did it go? If the wood gained mass, where did it come from?

_____

_____

2. What differences, other than any change in mass, did you observe between the TEST and CONTROL pieces of wood?

_____

_____

3. Why was a CONTROL necessary for this activity?

_____

_____

4. Which type of wood showed the greatest change in mass as it rotted?

_____

_____

5. Which type of wood would you use in the areas of your house most susceptible to fungal decay? Explain.

_____

_____

6. How can fungal attack of wood be prevented?

_____

_____

7. How do you think the results of this activity may have been different if the jars had been sealed?

_____

_____

8. How do you think the results may have been different if dry sand was used instead of soil?

_____

_____

THE EFFECTS OF WOOD ROTTING FUNGI

LAB 2: CHANGE IN WOOD STRENGTH DUE TO ROTTING

INTRODUCTION

Wood changes in many ways as it undergoes fungal decay. In Lab 1, mass changes were observed. Changes in appearance of the wood, such as in color or texture, might also have been observed. The purpose of this activity is to devise a way to determine if the strength of wood changes due to rotting. You will use the strength test you develop on the samples from Lab 1 to compare rotted wood to fresh wood. If you did not do Lab 1, instructions for the preparation of wood samples are provided in PART I of the procedure outlined below.

MATERIALS NEEDED

Wood samples from Lab 1 (or incubate as in Part I)
Weights
String or rope
Any other materials described in your procedure

PROCEDURE

PART I: Incubating the wood samples. (If necessary.)

I. Obtain the wood samples. You will need at least six(6) pieces of each type of wood to be tested. Label three (3)of them TEST and three (3) of them CONTROL.

2. Place the 3 TEST pieces in a large jar of moist (but not too wet) soil. Push them into the soil until they are mostly covered. The 3 CONTROL pieces should be placed somewhere where they will be clean and dry.

3. Cover the soil jar loosely and allow the test samples to incubate for at least 6 weeks.

4. After the incubation period, wash the test pieces to remove excess dirt and soak both CONTROL and TEST pieces in water for at least 1 day prior to doing PART II. (It is easier to get uniformly wet pieces than uniformly dry ones.)

PART II: Testing the strength of the test and control groups.

I. Devise a way of testing the strength of the wood pieces using materials readily available to you. Write our the step-by-step procedure for your test. When designing your test, consider the following:

a. Is your procedure understandable and easily followed?
b. Do you have a plan for recording and organizing the results of your test(s).
c. Do you limit the variables?
d. Is your method reproducible?
e. Is your method safe?

2. Have your written procedure approved by your teacher and proceed with the test.

3. Write up the results of your tests. In your write up include:
   a. The purpose of your test.
   b. The materials used in your test.
   c. The method of performing the test. (Your procedure can be used here)
   d. Your results. Include data tables or graphs that you may have used when recording your results.
   e. A discussion of the results. What differences did you observe between the rotted and fresh wood?
   f. Any improvements that you might recommend or any reasons you believe may account for unusual results.

Appendix IIC

A DISEASE IN THE DOGWOODS

INTRODUCTION

A mysterious disease first struck dogwoods in 1976, causing cankers, spots and diebacks. Not until 1991 did scientists identify a killer fungus. As this dogwood blight sweeps through 19 states, experts worry about food supplies for woodland wildlife.

The following article appeared in National Wildlife (April-May, 1993) and describes the effect of dogwood blight on forest ecosystems of, primarily, the eastern United States. Answer the questions about the blight as you read the article. (Used with permission from the National Wildlife Foundation.)

WHAT DIFFERENCE DOES THE DOGWOOD MAKE?
By Frank Kuznik

THE PROBLEM BECOMES EVIDENT

The crisp, rolling landscape of Virginia Tech's campus is vibrant with the sounds of cicadas and students, but tree doctor Jay Stipes notices nothing but ailing patients as he strides the grounds. Yellowing pin oaks starved for iron, elm trees kept alive with periodic injections, various species with lightning scars or lawn-mower cankers - and his current obsession, the blight ravaging that jewel of the Appalachian woodlands, the flowering dogwood.

"This one is loaded with it," says Stipes, stopping at a sick dogwood perhaps a foot in diameter. Though still topped by a resplendent crown, the tree is losing its lower leaves and branches, which are mottled and withering. "Here's your

dogwood anthracnose," Stipes says as he pulls off a leaf covered with brown spots. "And you can see how it's moving back into the branches. But it will probably never kill this tree. It's just too hot and sunny here."

Would that Stipes could say the same for the millions of dogwoods in the nation's forests under siege from the killer fungus. Since it first appeared nearly simultaneously in the Northeast and Northwest about 16 years ago, dogwood anthracnose has savaged its host species with a speed unmatched since the outbreaks of chestnut blight and Dutch elm disease earlier this century. The new disease attacks several native species, including both wild and cultivated trees of the widespread species known simply as the flowering dogwood. In a broad swath of woodlands stretching from Maine to Alabama, the flowering dogwood is literally vanishing.

The good news is that , unlike chestnut blight or Dutch elm disease, the curse striking dogwoods is fatal only under certain conditions. It flourishes in moist, cool mountain environments. In the warmer, drier flatlands, it attacks trees but generally does not kill them. "It's been in Atlanta now for about four years," says Robert Anderson, a plant pathologist with the U.S. Forest Service. "So far, it's disfigured the trees, but not taken them out completely."

Though dogwoods in hot, sunny spots will survive, the species will soon be only a memory in many forests, where it is an important food source for wildlife. Rabbits, deer, squirrels and a remarkable variety of game birds and songbirds rely on the dogwood to build up energy reserves to survive the winter. No studies have been made yet of the disease's long-term impact on wildlife-but clearly, the change will not be good. "From a wildlife standpoint, there really isn't anything to replace the flowering dogwood," says Robert Whitmore, professor of wildlife ecology at West Virginia University. "We don't know how animals are going to adapt when they're ready for a heavy dose of dogwood fruit and there isn't any."

That is particularly the case in the South, where the flowering dogwood - one of some 60 species of dogwood worldwide - grows abundantly. There dogwood festivals and dogwood boulevards and dazzling spring displays along popular venues such as the Blue Ridge Parkway. Postcards in gift shops explain the local folklore of the flower, with the stained tips of its blossoms said to represent the wounds of the crucified Christ and the jumble of angular central flowers his crown of thorns. The tree's year-round beauty and food value for wildlife has made the flowering dogwood perhaps the most-planted ornamental tree in the eastern United States.

Questions For Understanding

1. When (what year)and where (what general regions) did dogwood anthracnose first appear in the United States?

   When_____

   Where_____

2. How are dogwoods important :

   a. to wildlife _____

   _____

   b. to humans _____

   _____

3. Why are all dogwoods not killed by the disease?

   _____

   _____

THE DISEASE IS DESCRIBED

Dogwood anthracnose first appeared on a western

species of dogwood in Washington State in 1976. shortly thereafter the fungus struck its eastern cousin in Connecticut and New York. But not until the early 1980s was the disease recognized as some kind of new fungus. And not until 1991 did pathologists finally isolate the anthracnose organism.

"Most anthracnose will cause lesions on branches and leaves, then the plant's natural resistance will stop it," says Stipes, who's been teaching and researching tree biology at Virginia Tech for 25 years. "This one is unique; it's lethal to the whole tree. It's like a cancer that gets in your lymph system and just keeps moving and moving." Significantly, the blight has not proven fatal to related species such as the Chinese dogwood. This is one of the factors that led Stipes and other plant pathologists to conclude that the disease is an import, brought here unwittingly on the Chinese dogwood or some other exotic that long ago developed a tolerance for the fungus.

Once the fungus arrived, it spread down the East Cost; maps showing the disease's progression trace almost perfectly the spine of the Appalachian mountains. There conditions are the best for the fungus - shady, wet, and cool. It's carried by the wind, wind-driven rain and birds. The sweep from north to south suggests that many migratory birds play a key role in spreading the fungus.

At lower elevations, the blight seems less deadly. "I don't think it will ever be a big problem in the tidewater areas," says Stipes. "It just can't take the heat."

Stipes is back in his lab, talking amid a jumble of microscopes, refrigerators, petri dishes and other equipment when Kyle Thornham, who runs Virginia Tech's electron microscopy facility, walks in and reminds him of another factor helping the disease along - acid rain.

Stipes digs out a slide showing the acidity in East Coast rain and snow. The image forms a near-perfect match with the

disease map, the highest acidity framing the worst infection.

"I don't think this is entirely an acid -rain problem," Thornham says, pulling out a greatly enlarged photo of a dogwood leaf. Acid has definitely damaged the leaf surface, and in addition, fungus spores are invading. "Somehow it's very easy for this to happen."

While research continues on a micro level, the macro picture grows gloomier every year. The number of states invaded by the disease is now up to 19. It's in every county in West Virginia, and officials in Pennsylvania estimate they've lost more than half of their flowering dogwoods. Individual study plots reveal equally devastating effects. In Catoctin Mountain Park in Maryland, for example, plots surveyed in 1984 contained an average of 276 dogwoods per acre; by 1986, they had only 32 per acre. By 1988, not a single dogwood in the plots could be classified as "apparently healthy."

Surveys of seven southern states (Virginia, North and South Carolina, Georgia, Alabama, Tennessee and Kentucky) compiled by Robert Anderson show a dramatic increase in the number of acres infected by the dogwood blight, which jumped from 500,000 in 1988 to 9.1 million by the end of 1991. Worse, sites stripped of dogwoods show no sign of regeneration. "Young trees come up, but they're killed very quickly," Anderson Says. "I went back recently to the first place I saw the disease, in the Chattahoochee National Forest in 1988, and there isn't a single dogwood left."

Questions for Understanding

4. A pathologist mostly studies:
   a. plants b. diseases c. fungi d. ecology

5. Dogwood anthracnose is caused by:
   a. a fungus b. a virus c. a bacterium d. cold, moist weather

6. Dogwood anthracnose probably arrived in the United States
   a. on imported Chinese dogwood b. on the wind
   c. on migratory birds d. on imported lumber

7. How is dogwood anthracnose different than other anthracnose found in the United States?
   a. it causes lesions b. it is caused by a fungus
   c. it occurs on trees d. it is lethal to the whole tree

8. Why has the disease spread down the "spine" of the Appalachian Mountains?

_____

_____

9. How does acid rain appear to help the spread of the disease?

_____

_____

10. Why don't dogwoods killed by the disease regenerate?

_____

_____

THE EFFECTS OF THE LOSS OF THE DOGWOODS

No flowering dogwoods means no dogwood fruit in the fall, a time when a number of animals - in particular migratory and overwintering birds - depend on it. Dogwood fruit has one of the highest fat contents of any food in the forest - nearly 18 percent. That's important to both songbirds bulking up for a trans-Gulf migration and game birds such as turkey and grouse putting on layers of fat for the winter. Dogwood fruit is also high in calcium, the major component of

eggshells.

"We've documented of 40 species of birds that use dogwood fruit as a food item," says Robert Whitmore. "It's even been linked to the late breeding and fall migration of cedar waxwings, which eat dogwood berries quite a bit."

What's likely to happen once dogwood fruit is gone from the mountains?

"Birds will have to forage in a wider pattern, which means they'll need more energy to get as much food as they did before," says Whitmore. "Because of what you might call increased foraging costs, the individual animal will likely have lower fat reserves, which could mean it won't be able to fly as far south, or can't make it at all, or makes it in such lousy condition that it can't compete. The other thing birds can do is eat something else. The trouble is, there's not a lot of something else available at that time."

For bird populations already beset on other fronts, the food loss could be severe. "There're so many other contributing factors already knocking migratory songbird populations down," notes Craig Tufts from the National Wildlife Federation's urban wildlife programs. "We have habitat fragmentation on this end, loss of tropical forests where they overwinter, and fewer and fewer rich food bearing open spaces where they can stop and recharge in between. I'm afraid of what the impact of losing a significant food resource will be."

Questions for Understanding

11. The loss of the dogwoods would most likely affect populations of _____ the most.
    a. rabbits b. deer c. squirrels d. migrating birds

12. Dogwood fruit is especially valuable as an energy source because it is especially high in:
    a. fats b. carbohydrates c. proteins d. calcium

13. Dogwood fruit contains high amounts of the mineral _____, especially important in making eggshells.
a. sodium b. potassium c. calcium d. iron

14. What do scientists predict birds will have to do to replace dogwood fruit in their diets?

---

15. What factors, other than the loss of the dogwood, may be contributing to the decline of songbirds?

HOPE FOR THE FUTURE?

Dogwood anthracnose is not unstoppable. Homeowners can control it by pruning dead twigs and branches, mulching, improving air circulation and , if necessary, spraying trees with fungicides. But such measures are obviously impractical in the forest. Flowering dogwoods are vanishing, at least until some naturally disease resistant form spreads or the disease itself dies down. Neither process will occur in our lifetime. "It'll be 500 to 1,000 years before you see dogwoods in the woods again," Stipes predicts.

Except for the hardy few that survive the blight. University of Tennessee's Mark Windham and Stipes are currently studying a pair of trees found near Camp David, the presidential retreat in Maryland. Survivors are being culled by other plant pathologists as well, who are hoping that one of the trees holds a natural resistance to dogwood anthracnose in its genetic makeup that can be identified and eventually reproduced. "We feel we have a good propagation system - if we ever find a resistant tree," says Anderson.

Trees are much more likely to have survived because of a chance placement in open sun or on an airy, well drained hillside than because of genetic resistance. Finding a lone survivor somewhere in the woodlands which can tip the forces of natural selection back in favor of the flowering dogwood is considered by scientists to be a long shot at best.

But at this point, it's the only one that we have.

Questions for Understanding

16. List at least three ways homeowners can control dogwood anthracnose.

_____

_____

17. Why do you think the homeowner's methods are considered impractical for larger forests?

_____

_____

18. Why can Chinese dogwoods trees be resistant to anthracnose while no trees in the United States have been shown to be resistant to the disease.

_____

_____

19. Dogwood anthracnose is just one example of a disease introduced by accident into the United States. What could be done to prevent the introduction of other diseases in the future?_____

_____

Appendix IID

THE IRISH POTATO FAMINE

UNITED STATES IMMIGRATION

THE QUESTIONS WITHIN THIS ACTIVITY RELATE TO THE UNITED STATES IMMIGRATION DATA SUMMARIZED BELOW.

IMMIGRATION, 1821-1860

DECADE	ENGLAND, SCOTLAND, AND WALES	IRELAND	GERMANY	ALL OTHERS
1821-1830	25,079	50,724	6,761	60,875
1831-1840	75,810	207,654	152,454	163,207
1841-1850	267,044	780,719	434,626	230,862
1851-1860	423,974	914,119	951,667	283,774

I. Calculate the total immigration from all sources during the four decades for which data is given.

Decade	Total Immigration
1821-1830	_____
1831-1840	_____
1841-1850	_____
1851-1860	_____

2. What general trend do you see in immigration over the four decades? Why do you think this trend is observed?

_____

_____

_____

3. What effects do you think all of the immigrants had on conditions in the United States?

_____

_____

_____

4. From 1841-1850, the population of Ireland was reduced from 8.2 million to 6.6. What effect do you think this decrease had on conditions in Ireland?

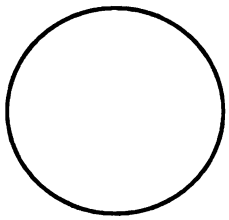
_____

_____

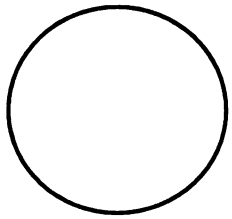
5. For each decade, calculate the percentage of the total immigration that each group accounted for.

	1821-1830	1831-1840	1841-1850	1851-1860
% English, etc.	_____	_____	_____	_____
% Irish	_____	_____	_____	_____
% German	_____	_____	_____	_____
% Others	_____	_____	_____	_____

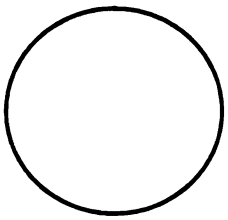
6. Use the data to construct a PIE GRAPH for each decade.



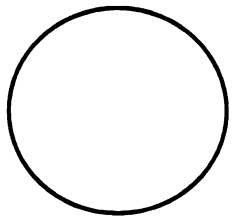
1821-1830



1831-1840



1841-1850



1851-1860

7. Notice the immigration of Irish into the United States. During which decade do Irish make up the largest proportion of the total immigration?

_____

8. What do you think caused this bulge of immigration during that decade?

_____

_____

9. Between 1841 and 1850, the population of Ireland decreased by I.6 million people. Only about 789,000 immigrated to the United States. What do you think happened to the other people?

_____

_____

THE IRISH POTATO FAMINE

The Island Nation of Ireland underwent many changes in the mid 1800's. Between 1841 and 1851, the country's population fell from 8.2 million to 6.6 million, a decrease of 1.6 million people (about 20% of the population). Approximately 800,000 of these people emigrated to the United States and Canada. The rest perished. In a 15 year period beginning in 1845, an estimated 1 million people perished and another 1.5 million people emigrated from Ireland.

What could have caused such death and destruction? Was it war that caused the deaths? Were the emigrants fleeing Ireland because of political or religious persecution?

The above reasons may have been a minor part of the problem,but most of the people were either fleeing from or dying from starvation. They were starving because of the failure of the potato crop in what has become known as the **Irish Potato Famine**.

In order to better understand what happened in Ireland, some history must be reviewed. Also, the biology of potatoes must be understood. Finally, the disease that caused the failure of the potato crop, called **late blight** and caused by a **fungus**, must be reviewed.

The Potato: History and Biology

The important plant that we know of today as the potato probably was first found in the Lake Titicaca region between Peru and Bolivia. It is estimated that they have been an important food crop since about 400 B.C.

The potato is in the same plant family (Solanaceae) as tomatoes, red and green peppers, tobacco, petunia, and some poisonous members such as the deadly nightshade. The family name comes from the Latin word solamen, which means "comforting", reflecting the sedative effects of some of the

chemicals (**alkaloids**)produced by some plants in the family. Potatoes produce alkaloids in green parts of the plant, but the only known effect of potato alkaloids is stomach and digestive system distress. If potato tubers are left in the light, they will also develop green color and alkaloids and should not be eaten.

All these plants have similar flowers, but it is not the flower of the potato that is important. Potatoes produce specialized structures called **tubers**, which are swellings on underground stems called stolons. These swellings store excess food in the form of starch. These starchy tubers are what are harvested and eaten and provide many people with food.

Potato plants will produce seeds. Potato growers, however, do not usually plant the tiny seeds from the green berries produced after flowering. Potatoes are grown instead from **vegetative propagation**: that is, growing a new plant from a piece of a "parent plant". Potato farmers plant small tubers or even pieces of tubers to maximize their planting stock. Each piece can grow into a new plant as long as a bud (called an eye) is present.

Vegetative propagation has several advantages. The tuber pieces, improperly known as "seed", contain food reserves so that a healthy green shoot pushes up through the soil more quickly than does the shoot from a tiny seed. In addition, each tuber piece grows into a plant that is genetically identical to the parent plant in color, taste, maturity, and other important characteristics. True seed, from the fruit of a plant, is the product of sexual reproduction, which results in genetic variation. Each seed produces a plant that is slightly different from all of its siblings. Genetic uniformity can be an advantage when planting, cultivating, harvesting, or marketing a product.

There are, however, some disadvantages to vegetative propagation. Since all plants in a field are genetically

identical, they are all equally succeptable to disease. If one plant in a field can contract and be destroyed by disease, then so can all of its neighbors. This genetic uniformity among the plants can therefore increase the chances that an entire crop will be lost. Also, since a piece of plant tissue is larger than most seeds, there is a greater chance that plant **pathogens** (the organisms that cause disease) will be carried from field to field on these pieces when crops are planted. Thus, many fields of a crop, although separated by great distances, may be infected with a disease

1. Define

   a. Alkaloids_____

   _____

   b. Stolon _____

   _____

   c. tuber _____

   _____

   d. vegetative propagation _____

   _____

   e. pathogen _____

   _____

2. Why must tubers be stored in the dark? _____

   _____

3. Give at least two advantages of vegatative propagation.

a. _____

b. _____

4. What are some disadvantages of vegatative propagation?

_____

_____

5. Why do you think farmers periodically allow plants to reproduce "naturally" (sexually, producing seeds) even though vegetative propagation is more convenient?

_____

_____

_____

The Potato in Ireland

Europeans did not know of potatoes until they were discovered by Spanish Conquistadors who were searching for gold in the Andes Mountains in the sixteenth century. Although the exact history is confusing, it is likely that the first potatoes arrived in Spain about 1570. Also, very importantly, it is likely that most of the potatoes later grown and harvested in Europe were taken from a relatively small supply of tubers that survived the lengthy trip across the Atlantic. **Thus, every plant in every field was nearly genetically identical.**

It is not known exactly when the potato was introduced into Ireland, but it is known that it was a well established food crop by 1800. Large amounts of nutritious food were produced on a regular basis, and because of this the population

of Ireland began to rise, from about 4.5 million in 1800 to more than 8 million in 1845.

It should be noted that the potato did not totally replace grain crops in Ireland. However, most of the grain grown and harvested was used by the poor farmers to pay rent to their wealthy landlords, most of whom lived in England. Additionally, a family could grow enough potatoes to feed themselves on half the land required to produce the same amount of food in grains.

The Famine Begins

The summer of 1845 began in promising fashion. In the spring the weather was warm and dry. The weather then changed to cool and rainy. In just a few weeks, the healthy green potato vines began to rot. When some tubers were dug from the ground they were already rotted, while some were sound. Even the sound ones, however, later rotted in the storage bins. Food was therefore in short supply that winter, and seed for the next season was even more scarce.

The summer of 1846 once again brought hope, with the potato plants growing green and healthy. Once again, however, the blight came and destroyed this hope. Winter again brought suffering and death due to starvation. Help from England was slow to come. The rich landlords there didn't really see any difference between this and other years because grain continued to be exported from Ireland throughout the blight years. The landlords continued to profit in spite of the starvation in Ireland. The people of Ireland were left with very little choice -either flee the country or die of starvation. In the 15 year period beginning in 1845, an estimated 1 million people died and another l.5 million emigrated from Ireland

6. What was there about the political organization in Ireland that led the Irish peasents to be almost entirely dependant on the potato for food?

_____

_____

_____

Late Blight: A Disease Caused By a Fungus

The people of Ireland struggled to understand what was causing the rotting of the potatoes. One idea was that the plants took up too much water in the rainy weather. But if that were the cause, why hadn't the rotting been seen in other years when the weather was equally rainy and cold?

In 1845, a white fungus was found on the blighted potato vines. A prominent German plant scientist (botanist), Anton deBary, performed experiments that proved the role of the fungus in causing the blight. He exposed potato plants to the cool, wet environmental conditions that favored blight. To some plants he applied spores from the fungus found on blighted plants, but others he kept as "control" plants and applied no spores. He found that though both sets of plants were exposed to the same favorable environment, only the plants inoculated with the fungus became blighted and died. It was clear that the plants did not rot away because they absorbed too much water. Instead, it must have been the fungus that was causing the disease. This idea (of living things causing disease) was new, and deBary's approach would soon be developed into what is now called the **germ theory**.

The fungus which causes potato blight is called *Phytophthora infestans*. Most of the fungus is found inside the plant tissue, where it exists as a network of tiny threadlike **hyphae** that penetrate between the cells. The hyphae absorb nutrients and water from the potato and in the process kill the

plant cells. A whole potato plant can be left a slimy mass in less than three weeks.

Under cool and humid conditions, the blight fungus will produce **spores**, which are the reproductive units of all fungi. Spore production in late blight begins with emergence of hyphae through the stomata (air exchange pores) of the potato plant. Lemon shaped **sporangia** (spore producing structures) are formed at the tip of these hyphae. The structures will appear as a whitish mildew which is barely visible on the surface of infected potato leaves and stems. Spores released from the sporangia will be dispersed by air currents to neighboring plants or even nearby fields. If the air is dry or at high temperatures the spores will die, but if they encounter cool moist conditions they will begin to change and can again infect a plant. Each spore is converted into about eight tiny zoospores, which can swim by wiggling a whiplike flagella. The zoospores may attach to a leaf surface, where a germination tube again invades the plant tissue . The hyphal filaments will begin to grow and the cycle starts all over again. The whole cycle can be repeated in as few as three to five days, allowing the fungus to colonize and destroy all available plant tissue.

Spores that don't encounter favorable cool and moist conditions do not die. They can remain in the soil or on harvested potatoes in an inactive state for long periods of time. Thus, they may be there to infect crops in the future if conditions again become favorable.

7. Define

a. hyphae _____

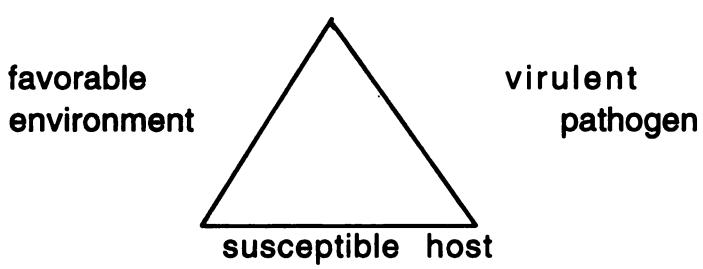
b. spores _____

c. sporangia _____

Summary

The fungal disease that is now called late blight devastated the potato crop in Ireland. The resulting famine decimated Ireland and impacted the rest of the world. But what lessons did we learn from this disaster? The lack of genetic diversity in the potato crops in Ireland contributed to the widespread destruction of the crop, yet we continue to plant large fields of single varieties of corn, wheat, and rice. The fungal pathogen responsible for the famine was most likely introduced to Ireland on imported "seed" tubers. In today's world, with people traveling more than ever and international trade of food crops on the rise, the possibility of pathogens being unintentionally transferred is great in spite of quarantines and import laws designed to prevent it. Is it just a matter of time before we have a modern Irish potato famine?

8. Below is an illustration of the disease triangle, a memory aid describing the three factors necessary for disease: a susceptible plant, a pathogen capable of causing disease in the plant, and environmental conditions favorable for disease development.



Describe how each condition for disease was completely met during the Irish potato famine.

_____

_____

_____

_____

9. Review the life cycle of the blight fungus, *Phytophthora infestans*. Tell how the following practices may prevent the spread of potato blight.

a. planting many potato varieties in small fields rather than large fields of a single variety.

b. hilling potatoes late in the season

c. collecting and burning vines after the harvest

d. not allowing freshly dug tubers to touch vines during harvest

e. not allowing "seed" tubers to be tranported from country to country.

10. Do you think that a disaster like the Irish Potato Famine could occur in the United States now? Explain your answer.

_____

_____

_____

_____

_____

Appendix IIE

FERMENTATION

TEACHER NOTES: FERMENTATION TECHNOLOGY

I. Introduction

A variety of definitions for biotechnology exist. One that incorporates components of all definitions is: Biotechnology is the application of organisms, and their cellular, subcellular, or molecular components, in order to provide goods, services, and environmental management. Considering this definition, it is safe to say that in all probability the first biotechnologists were Neolithic men and women who may well have preferred the taste of fermented cereals to raw grain. It is not difficult to imagine that they may have consumed this primitive malt added to water to make sweetened gruel. Wine, beer, and cheese followed as "fermentation technology" advanced.

Modern biotechnology has its roots in the works of nineteenth- century scientists, including Darwin and Mendel, and is growing out of the remarkable advances of bioscience in this century. The evolution of medicine has followed a similar path over the same periods of time. Knowledge of medicinal plants and their use, an ancient art, is still common in most contemporary societies, but modern health care includes the use of so-called miracle drugs and complex diagnostic machines.

When biotechnology is discussed in the classroom, it seems that the discussion automatically centers on the somewhat complex processes of cloning and recombinant DNA. The students, in many cases, have an inate mistrust of biotechnology because they don't understand the complex processes involved in what they perceive as biotechnology. The underlying goal of these activities is to demonstrate to

the students that biotechnology is not some nebulous new concept understood by only a few people with doctorate degrees but something that has been understood by humans for thousands of years.

The student introduction lesson is intended to be an overview of the biochemistry and history of fermentation - both acid and alcoholic. Appropriate textbook units should be used as resources if more in-depth information is desired.

Activity 1, the making of yogurt, and Activity 2, the making of cheese, serve as good examples of lactic acid fermentation, while activities 3 and 4 address alcoholic fermentation. The beer brewing activity, while sure to generate student interest, may not be appropriate in a secondary school setting.

II. Time Frame

A. Student Introduction : One 40 to 50 minute class period.

B. Activity 1 - Making Yogurt: One 50 minute class period to do culture. Culture must set for 15-18 hours (or overnight). Although lab protocol suggests testing every 3 hours, this time frame can be altered to fit your time constraints. Even a "before and after" test would be sufficient.

C. Activity 2 - Making Fresh Milk Cheese: About two 50 minute class periods. Set junket (end of step four) can be refrigerated overnight. Continue with step 5 on the second day.

D. Activity 3 - Brewing Beer: A total time of about 5 weeks broken down as:
Preparation of wort - I day
Primary fermentation - About 7 days
Bottling - I day

Conditioning - 7 days
Aging - 3 to 4 weeks

E. Activity 4 - Measuring yeast fermentation rates: One 50 minute lab period for lab procedure. Could be made into a 2 day lab with Part A the first day and Part B the second.

III. Other important notes

A. General

1. One basic rule in most labs is that no eating is allowed. The first two activities involve making an edible product. To ensure safety, you may want to exchange rooms and use the food science room for the production portions of the lab or at least set aside a specific lab or lab section to be used for this purpose. Also, set aside glassware and utensils to be used solely for use with edible products.

2. Cleanliness is a must. Contamination by wild bacteria or fungi will quickly ruin cheeses, yogurts, and beer. Devices used for production should at least be boiled prior to beginning the activities, or use an autoclave or pressure cooker for sterilization if one is available. A 10% bleach solution is recommended for cleaning table tops and other surfaces.

B. Activity 1

1. Improvise if you don't have an incubator. Gas ovens with permanent pilot lights and boxes with light bulbs have been used with success.

2. Perhaps a yogurt party on the second day would be a fun way to terminate the activity. Most

students find plain yogurt too tart, so suggest that they may want to bring something such as fruit preserves to sweeten it.

C. Activity 2

1. Look in your local community for the materials needed. Alternatively, several biological supply companies offer complete and inexpensive cheese making kits. A list of several supply companies is included in the resource section of this lesson.

2. A simple cheese mold can be made with 2 plastic cups. put small holes in the bottom of one of the cups. Put the cheese in the "holey" cup and press using the other cup.

D. Activity 3

1. Brewing supplies may be obtained locally at hobby shops or by mail. For a complete list of mail order sources write the American Homebrewers Association. The address is given in the resource section of this lesson.

2. Bottling is made much easier if a supply of "Grolsh" type bottles (those with stoppers attached) can be obtained.

3. It is important that the primary fermentation has stopped before the beer is bottled. If large amounts of carbon dioxide are still being produced in the bottled beer, the bottles may explode. That is the reason for the hydrometer test. If the specific gravity is not changing, it is likely that the fermentation is over.

E. Activity 4

1. Cake yeast is recommended. If dry yeast is to be used, a longer period must be allowed for the yeast/food mixtures to set. Also, commercial yeast vary greatly in their viability. It is best to try the procedure with your yeast before the students do the lab.

2. Bubbles of dye in the pipettes may break if made too small or not move effectively if made too big. 3-4 mm. bubbles have worked well. Tapwater with a few drops of food coloring added makes a nice dye.

3. Foods A,B,C, and D can be made from any type of sugar solution. Simply dissolve 20 grams of the sugar in 80 ml. of water. Some suggestions:

A - 20% sucrose (table sugar)
B - 20% lactose (doesn't dissolve well in water)
C - 20% maltose
D - 20% fructose

Try any other sweet juice or honey as a food. Experiment! Also, making one "food" pure water provides interesting results. Students seem to have a difficult time explaining why no gas is produced.

IV. Other Activities. Listed below are some other fermentation laboratory activities that may be applicable.

A. Winemaking. Quite easily done but takes longer to age than beer and again, it is debatable whether is should be done in the secondary school setting.

B. Making Tofu. Bean curd can be produced in much the same way as cheese. See references for books which describe the process.

C. Corn could be fermented to produce a distillable product. The use of the ethanol as a fuel could then be explored.

D. Field trips to a local cheese factory, brewery, or distillery might be possible.

V. Resources

A. Cheese and Yogurt

American Supply House
Box 1114
Columbia, Missouri 65205

Countryside General Store
Highway 19 East
Waterloo, Wisconsin 53594

New England Cheesemaking Supply Store
P.O. Box 85
Ashfield, Massachusetts 01330

The Plow and Hearth, Inc.
P.O. Box 530
Madison, Virginia 22727

B. Beer

American Homebrewers Association
Box 287
Boulder, Colorado 80306

C. General

Most biological supply companies have basic cheese making kits available at reasonable prices.

AN INTRODUCTION TO FERMENTATION TECHNOLOGY

A variety of definitions for biotechnology exist. One that incorporates components of all definitions is:
Biotechnology is the application of organisms, and their cellular, subcellular, or molecular components, to provide goods, services, and environmental management.

While modern biotechnology - including recombinant DNA technology , cloning, and many medical advances - may seem complicated and difficult to understand, other forms of biotechnology have been well understood for thousands of years. In fact, it is safe to say that in all probability the first biotechnologists were Neolithic men and women who may well have preferred the taste of fermented cereals to raw grain. It is not difficult to imagine that they may have consumed this primitive malt added to water to make sweetened gruel. Cheese, wine, and beer followed as "fermentation technology" advanced.

Two commonly used types of fermentation will be addressed in this introduction: lactic acid fermentation and alcoholic fermentation. In both, sugars are broken down anaerobically, but the organisms involved and the end products are different. In lactic acid fermentation, sugars are broken down by bacteria to produce lactic acid (and of course energy for the bacteria). This type of fermentation is important in the making of cheese and yogurt. In alcoholic fermentation, yeast break down the sugar into ethanol, carbon dioxide, and again energy for the yeast. This process is important in the baking of bread and in the production of alcoholic beverages.

Fermentation technology is considered to be the first example of biotechnology in human history. The following text details the development of three important examples of fermentation technology: cheesemaking, winemaking, and the brewing of beer. Questions for understanding follow each subunit and should be answered as each is completed.

QUESTIONS FOR UNDERSTANDING

1. Complete the table below to summarize the fermentation types discussed.

Name	Organism	End Product(s)

2. Write the complete chemical equation for the two types of fermentation. (Use your text as a reference if necessary.)

a._____

b. _____

CHEESE

There is really no good estimate of how long people have been making cheese. The earliest evidence known so far, a residue found in an Egyptian pot, dates from about 2300 B.C.. We do know that a variety of milks - from the familiar cow, sheep, and goat to the mare, water buffalo, and yak - have been made into cheese in many different parts of the world.

The advantages of converting milk into cheese are many and probably would quickly have become apparent to early agricultural societies. Cheese takes up about one tenth the volume of its original milk, making transportation easier. Cheese resists spoilage, and thus surplus milk could be stored for drier times. Finally, in making cheese, a generally bland food is converted into a food with a varied of complex flavors.

The first cheese was probably the result of prolonged bacterial action. Unlike yogurt, which remains rather homogeneous, cheese is fermented beyond the homogeneous state and becomes "curdled" due to the continuous buildup of

lactic acid. It is probable that a second and more efficient curdling agent, called rennet and extracted from the fourth (or true) stomach of a milk fed calf, was discovered when that organ was used as a bag for carrying milk. When this discovery was made is not known, but it certainly preceded Roman times.

Modern cheesemaking is not much changed from ancient times and can be divided into three basic steps. The first is precipitation of the protein casein into semi-solid curds (the liquid portion remaining is called whey). This is done first by lactic acid produced by bacteria infused into the warm milk and later by the addition of rennet. The second stage is the concentration of the curds. Any free whey is drained off, and the curds are cut, pressed, cooked, and salted to remove much of the rest. The final stage is the "ripening" or aging of the "green" curds. It transforms the initially rubbery and bland green curd into a smooth substance with a pronounced and complex flavor. The hundreds of varieties of cheeses are a result of small variations in the first, second, and especially the third steps of the cheesemaking process.

Some recent advancements in cheesemaking technology have resulted in an improved, more consistent product. Commercial preparations of pure microbes and rennet have been available since the turn of the century. Fungal enzymes are sometimes used in the place of rennet. Also, since 1935 most cheese is made from pasteurized milk.

QUESTIONS FOR UNDERSTANDING

3. The lactic acid which begins the curdling of milk in the making of cheese comes from

a. bacteria
b. the stomachs of milk fed calves
c. yeast
d. molds

4. The rennet important in the making of cheese originally came from

a. bacteria
b. the stomachs of milk fed calves
c. yeast
d. molds

5. The liquid produced in making cheese is called
   a. curds b. rennet c. whey d. casein

6. Many nomadic people, such as the Mongols of Ghengis Kahn's time, commonly ate cheese. Why do you think it was such an important food source for these "people on the go"?

_____

_____

ALCOHOLIC BEVERAGES

WINE

The subject of alcoholic beverages encompasses a long and colorful history. The enigma of fermentation - the transformation of fruits and grains into intoxicating fluids - attracted some of the best and some of the most headstrong scientists of the 19th century, and gave rise to the science of microbiology. (Louis Pasteur classic experiments (1857-1876) were the first to prove that fermentation is the result of microbial activity)

We will never know what the first alcoholic beverage was or how it was made, though it is possible to make a good guess. Yeasts, the micro-organisms responsible for alcoholic fermentation, generally cannot break down complex sugars or starches. It follows, then, that a liquid must be fairly sweet in order to be fermented. The juices of most wild fruits, however, are high in acid and low in sugar and thus probably could not be made into wine until some domestication had taken place. For this reason it is thought that the first alcoholic beverage was a wine produced perhaps 10,000 years ago when some honey was forgotten or stored for a week or two. The sweet date, as well as the sap of palm trees may also have been exploited very early. Once agriculture had gotten underway, and sweeter fruits had been propagated simply because they tasted better, many other kinds of wine

were possible. Grape wine may have become the predominant wine because the grape vine, a relative of Boston Ivy and the Virginia Creeper, is adaptable to various climates, is quick to mature, and is a heavy bearer compared to fruit trees.

Wine would probably have had two main attractions for its prehistoric discoverer. Like cheese, it is a partly spoiled yet edible food that resists further deterioration. This fact is probably overshadowed by the intoxicating effect that alcohol has on humans. Surely it was alcohol's intoxicating power rather than its antiseptic properties that made wines (and beers) so popular so early.

Although winemaking has become very technical, the basic steps of winemaking are unchanged from ancient times. The steps are:

1. Cleaning of the fruit (in most cases grapes).

2. Preparation of a "must". Grapes are mashed (traditionally with the feet) creating a mass of juice, pulp, skin, and seeds.

3. Primary fermentation. In ancient times, wild yeasts were the fermentors. In modern times, the wild yeasts are killed and the must is inoculated with a culture of selected wine yeasts. Primary fermentation is sometimes called violent fermentation because large volumes of carbon dioxide are produced and considerable heat is generated.

4. Secondary fermentation. Liquid from the primary fermentation is drawn off and the pulp pressed to expel the liquid it contains. Fermentation is then continued at a gentler pace in a separate container.

5. Racking. Wine is racked (siphoned) to another container. Racking may be repeated several times.

6. Clarification. Finings - substances such as bentonite clay, gelatin, or isinglass - may be added to precipitate suspended matter. Alternatively, suspended matter may be removed by forcing the wine through a filter.

7. Aging. Wine is allowed to stand in closed containers to refine the flavor of the green wine. Aging time varies.

Thousands of wine varieties exist and, as with cheeses, result from alterations of one or more of the basic steps. For example, if the skin of red grapes are removed before the fast is fermented, a white or rose' wine will result. If skins are left in the mast, a red wine will result.

BEER

Wines were not the only alcoholic beverages known to the ancient world. Perhaps even before the grape was domesticated, people had discovered that starchy grains - barley, wheat, millet, and corn - could be treated so as to be fermentable.

Today, we know that it is necessary to break the starch down into its component glucose units in order to support the growth of yeasts. Historically, this necessary conversion has been done in one of three ways. The first technique, one that is very rare today, may have been the sole source of alcohol for pre-Conquest Peru. Chicha, a type of beer, was and is made by chewing on ground corn and letting a human salivary enzyme break down the starch into glucose and maltose. A second technique, invented in the Far East, uses a mold called koji (*Aspergillus oryzae*) to secrete the starch digesting enzyme to prepare rice for fermentation. The third technique, the one that is arguably the oldest and that predominates today, is malting. The grain is allowed to germinate for several days, during which time it generates enzymes that will break down the stored starch into glucose, the seedlings fundamental source of energy.

As with cheesemaking and winemaking, the basic process of brewing has changed little over time and includes the following steps:

1. Malting. Dry grain (mainly barley) kernel are soaked and allowed to germinate in order to accumulate the starch digesting enzymes, or amylases.

2. Drying and kilning. Drying the grain halts the enzyme activity and kilning produces the desired color and flavor.

3. Mashing. Malt is mashed in warm water, which revives the enzymes an results in a sweet, brown liquid called wort.

4. Addition of hops. Hops are thrown into the wort, and the two are boiled together. This treatment extracts the flavorful hop resins, inactivates the enzymes, kills any microbes present, and deepens the color of the wort.

5. Fermentation. The wort is fermented with yeast until the desired levels of sugar and alcohol are reached.

6. Filtering. The new beer is filtered to remove most of the yeast and ten aged for some time.

7. Clarification. The finished beer is clarified to remove precipitants, sometimes pasteurized, and packaged in bottles or kegs to be stored for later use.

QUESTIONS FOR UNDERSTANDING

7. In making both beer and wine, the organisms causing the fermenting are

a. yeast b. bacteria c. mold d. viruses

8. In the production of beer and wine, _____ is a major by-product.

a. alcohol b. carbon dioxide c. must d. water

9. Describe two reasons fermented beverages were useful to early humans. Which of these reasons probably accounts for their popularity throughout history?

_____

_____

_____

10. Winemaking parallels what other major development in agriculture? Why is this co-development important?

_____

_____

ACTIVITY 1: MAKING YOGURT (YOGHURT)

BACKGROUND

Yogurt is cultured milk. It has only comparatively recently become popular in the United States, but different cultured milks have been consumed for centuries in Eastern Europe, and in the countries of the Near, Middle, and Far East.

Cultured milk is produced by the action of bacterial micro-organisms which change the lactose (milk sugar) into lactic acid. The result is acid curd, with certain milk proteins forming a semi-solid mass. The curdled milk may then be consumed directly or flavored to mask the somewhat sour taste of the straight yogurt.

OBJECTIVES

1. To make yogurt from milk and live bacterial cultures prepared from commercial yogurt.

2. To observe changes that occur because of bacterial action on milk.

3. (Optional) Estimate the number of bacteria in 1 milliliter of yogurt.

MATERIALS (PER GROUP OF 2 STUDENTS)

1% milk (about 450 ml.)
Sample of commercial yogurt (about 5 ml.)
Metal tripod or ring stand
Wire gauze
600 ml. flask
3 containers (300 ml. capacity)
Aluminum foil
pH paper (neutral to acid range)
6 clean test tubes
6 plastic soda straws
Incubator

*Flavoring * = optional
*Microscope (with oil immersion lens)
*Stage micrometer
*Methylene blue stain
*Xylene
*0.01 ml. calibrated loop

PROCEDURE

1. Add 450 ml. of milk to the flask. Heat the milk to scalding. Stir to prevent a skin from forming.

2. Allow the milk to cool to room temperature. Measure the pH of the milk by touching a drop onto a strip of pH test paper. Also, make observations concerning taste, aroma, appearance, texture, etc. of the milk.
Record your observations in your data table.

3. Completely mix about 5 ml. of commercial yogurt into the milk. Pour 150 ml. of the now inoculated milk into each of the three containers. Label two of them with an A and the other TEST. Also include your name on the labels.

4. Transfer a column of yogurt from the test container to a test tube using a straw. Measure the pH and record other observations as in step 2. (Optional: Remove a loopful of milk with the calibrated loop and prepare a smear for the DMC procedure outlined on the next page).

5. Place aluminum foil covers on the cups and incubate them at 45 degrees centigrade.

6. Examine the test cup every 3-4 hours and repeat step four. Continue this periodic examination for 15-18 hours. (If optional bacteria counts become too high you may have to dilute the sample 1/10th to 1/100th)

7. After the yogurt has set (I day), refrigerate it, sweeten it to taste, and enjoy!

DATA

Complete a data table showing the changes you observed or measured as milk was changed to yogurt. This table is to be turned in with the conclusion questions answered completely.

CONCLUSION QUESTIONS

1. What change occurred in pH and yogurt was produced?

2. What accounts for the flavor changes of the milk as yogurt is produced?

3. Why must the milk be incubated at 45 degrees centigrade when making yogurt?

FOR FURTHER INVESTIGATION

1. What bacteria are used to culture yogurt? (Scientific name please)

2. What other dairy products involve bacteria in their production?

3. What special benefits to the consumer does yogurt offer?

4. How would the culturing of yogurt be different if milks with different milk fat content were used? (Try it on your own).

DMC(Direct Microscope Count) PROCEDURE FOR COUNTING BACTERIA

I. Use a 4 mm I.D. loop (delivers 0.01 ml) and prepare a smear of the milk over a I square centimeter area on a clean microscope slide. Air dry the smear and heat fix it by passing it rapidly through a lab burner flame.

2. Flood the smear with xylene for 1 minute to dissolve the fat.

3. Wash the xylene off with 95% ethyl alcohol and wash the alcohol from the slide with tap water.

4. Stain the slide for 15 seconds with methylene blue. Wash the stain off with tap water. Air dry the slide and examine under the microscope using the highest power and oil immersion.

5. Determine the number of bacteria by counting 25 fields. Use this count to estimate the number of bacteria in 1 ml. of the yogurt. The number of bacteria in the oil immersion fields multiplied by the MF (microscope factor, calculation given below) will give you the number of bacteria per ml.

MF (Microscope Factor) Calculation

1. Use a stage micrometer and measure the diameter of the oil immersion field. Convert the diameter measured in micrometers to millimeters. (For example, if the diameter measures 18 stage divisions, then it equals 180 micrometers or 0.18 millimeters.

2. The area of the oil immersion field is determined by squaring the field radius and multiplying it by Pi (3.14l6).

From our example: radius is half the diameter, or 0.09 mm

0.09mm x 0.09 mm = 0.0081square mm

(0.0081) (3.1416) = .025 square mm

3. The area in square millimeters is converted to square centimeters by dividing it by 100.

$$0.025/100 = .00025 \text{ square centimeters.}$$

4. The number of oil immersion fields in I square centimeter is calculated by dividing 1.0 by the figure obtained in step 3.

$$1 / .00025 = 4000$$

This number would be then multiplied by the number of bacteria counted in the 25 immersion fields to determine the number of bacteria in I ml. of yogurt.

ACTIVITY 2: MAKING FRESH MILK CHEESE

BACKGROUND

Yogurt is a cultured milk product but is not considered a true cheese because it is curdled by the formation of lactic acid. True cheeses are formed by rennet, an enzyme - containing product first isolated from the stomachs of milk-fed calves. Cheeses are usually classified as soft, semi-hard, and hard. Soft cheeses, such as fresh milk cheese, are the easiest to make because no aging is required.

OBJECTIVES

1. To produce fresh milk cheese using milk and commercially available rennet.

2. To observe the changes in milk as it is converted into cheese.

MATERIALS (Per group of 2 students)

milk (about 500 ml.)
rennet (tablet or liquid)
600 ml. beaker
large water container for water bath
mold (home made)
cheesecloth
slotted spoon
colander
string

PROCEDURE

1. Determine the mass of about 500 ml. of milk. Place milk into the 600 ml. flask and place it in the water bath. Heat the water bath until the water boils.

2. Remove the milk beaker from the water bath. Describe the appearance of the milk.

3. Dissolve 1/8 of a rennet tablet in cold water and add it to the warm milk. Stir well.

4. Cover the flask and leave it in a warm place for two hours until the junket (curdled milk) is set. Describe the appearance of the junket.

5. Line a colander with cheesecloth. Stand the colander in a bowl. Ladle small amounts of curd into the cheesecloth. You will notice that as you slice into the curd a watery liquid appears; this is the whey. Describe the appearance of the curds and whey. continue until all the curd is in the cloth.

6. Gather the corners of the cloth together and tie a piece of string tightly around it to make a bag. Allow the bag to drain for thirty minutes.

7. Mold the cheese by patting it into the homemade mold. Press to compact the cheese and squeeze any remaining whey out of the cheese. Wrap it in waxed paper and determine its mass.

8. The cheese is now ready to eat. consume within 2 to 3 days. Refrigerate the cheese if it is not to be consumed immediately. (See serving suggestions at the end of this lab.)

DATA

Construct and complete a data table in the space provided below. Include space for mass measurements and other observations made as cheese was made.

CONCLUSION QUESTIONS

1. Why is yogurt not considered a true cheese?

_____

_____

2. What is the liquid that comes out of cheese during draining called?

_____

3. What is cheese curd made of?

_____

_____

4. About what percentage of milk was converted into cheese in this activity?

_____

SERVING SUGGESTIONS

Eat the solid curd in traditional fashion - swimming in a bowl of whey - the storied curds and whey. Try adding sweat cream or sugar to taste.

Try the solid cheese mixed with canned or stewed fresh fruit.

FOR FURTHER INVESTIGATION

1. Describe additional procedures required for the production of semi-hard and hard cheeses.

2. Research the production of exotic cheeses such as Bleu or Swiss. Describe the organisms unique to such cheeses.

ACTIVITY 3 BREWING BEER (A DEMONSTRATION)

BACKGROUND

Brewing beer is one of the oldest examples of fermentation technology. for centuries, beer has been consumed as a beverage, as a medicine, and as a general tonic. In this activity a single stage brewing process will be used to produce a cheap, high quality beer.

OBJECTIVES

1. To brew a beer using yeast fermentation of a grain product.

2. To measure changes in beer as fermentation takes place.

MATERIALS (TO MAKE ABOUT 32 PINTS OF BEER)

Fermentation bucket (Plastic pail with a lid and airlock)
Hydrometer
Bottles
Bottle capper (Not needed with "Grolsch" type bottles)
Long handled spoon
1 can (about 3 pounds) plain light malt syrup
4 cups brewing sugar
I teaspoon water crystals (water hardener)
1 ounce hop pellets
I packet of top fermenting beer yeast

PROCEDURE

PART I: FERMENTATION

1. Clean and sterilize all equipment using a dilute (10%) bleach solution.

2. Take the label off the can of malt syrup and wash the

can. Immerse it in hot water for 5 minutes to soften the syrup.

3. Bring 3 liters of water to boil in a large pot. Add the malt syrup and sugar and stir to dissolve. Stir in the water crystals and add the hop pellets.

4. Reheat the mixture to a boil, taking care to avoid boiling over. (Watch for foam rising. If it does, remove from the heat until the foam falls.) Boil for ten minutes.

5. While the malt is boiling, put 12 liters of cold water into the fermentation bucket. Add the boiled malt to this water and stir briefly. You now have wort, or unfermented brewing solution.

6. Sprinkle the yeast on the surface of the wort and let stand open for ten minutes.

7. Stir the yeast into the wort, attach the lid, put water in the airlock, and affix the airlock to the lid. You're now ready to ferment the beer.

8. Allow the wort to ferment (at about 18-20 degrees centigrade). Check the specific gravity daily. Ferment for 7 days or until the specific gravity remains constant.

PART II: BOTTLING

1. Siphon the beer into another clean container, taking care not to disturb the sediment.

2. Pour 175 ml. of corn sugar or an equal amount of dried malt extract into a saucepan with 240 ml. of water. Heat gently to dissolve and stir this mixture into the beer.

3. Siphon the beer into bottles, leaving about 3 centimeters of air space above the beer. Cap the bottles.

4. Leave the bottles upright at room temperature for 1 week to condition.

5. Age beer at 10 degrees centigrade for 3 or 4 more weeks. During aging, the beer should clear completely. After this aging period, the beer is ready.

ACTIVITY 4 MEASURING YEAST FERMENTATION RATES

BACKGROUND

All living things respire - that is, they break down food to release energy for life activities. Different organisms show many forms of respiration. Yeast, for example, undergo alcoholic fermentation, a process by which carbohydrates are broken down, yielding as by-products ethanol and carbon dioxide. The amount of carbon dioxide released as fermentation proceed can be measured and can serve as an indicator of the respiration rate.

OBJECTIVES

1. To measure the rate of fermentation using a simple apparatus.

2. To compare the rate of fermentation at two different temperatures.

3. To compare the rate of yeast fermentation in different foods.

MATERIALS

Yeast cakes	6 Test tubes-Small (10mm. x 80 mm.)
2 Beakers (250 ml.)	One hole stoppers to fit test tubes
Rubber tubing	4 Mohr Pipettes (1 or 2 ml.)
Glass tubing	20% glucose solution
Warm water	Cold water (or ice cubes)
Thermometer	Wax marking pencil (or tape)
1 Beaker (50 ml.)	Graduated Cylinder
Colored Water	

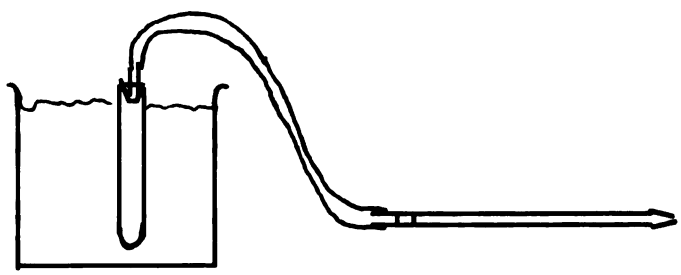
PROCEDURE

PART A: INFLUENCE OF TEMPERATURE ON YEAST FERMENTATION RATE

1. Pour 20 ml. of 20% glucose solution into a 50 ml. beaker.

2. Obtain a 1 cm. x 1 cm. piece of cake yeast. Put it into the beaker and mix well with the glucose solution. Allow the mixture to stand for 5 minutes.

3. While allowing the above mixture to stand, assemble two respiration measuring devices by (see diagram):
   a. Putting a small piece of glass tubing into a rubber stopper and connecting the rubber hose to it.
   b. Introduce a drop of dye into the large end of the pipette. The drop should be at least 3-4 mm. long.
   c. The pipette which should be placed flat on the table. **DO NOT** connect the rubber tubing to the pipette at this time. (A sheet of white paper may be used as a background to improve contrast.)
   d. Incline the tube to adjust the bubble so that it is near the zero point on the pipette.



4. Set up a warm water bath using warm tap water in a 250 ml. beaker and adjust the temperature to about 38 degrees centigrade. Similarly, set up a cold water bath and adjust the temperature to about freezing using ice cubes.

5. After the yeast-glucose mixture has stood for 5 minutes, use a graduated cylinder to measure 5 ml. of the solution and pour it into each of 2 test tubes.

6. Place a stopper/hose assembly into each of the test tubes and place them in the hot and cold water baths. Allow the tubes to sit in the baths for a few minutes before proceeding to step 7.

7. Connect the hose(s) to the large end of the pipette(s). Record the initial volume on your data table. Make ten measurements at 2 minute intervals as fermentation takes place and record these volumes in your data table.

8. Clean all equipment used and prepare for Part B.

PART B: INFLUENCE OF DIFFERENT FOODS ON FERMENTATION RATE

1. Prepare yeast/food mixtures of four different foods (labeled A,B,C,D) as in PART A, steps 1 and 2.

2. Prepare 4 test tube assemblies and in PART A, step 3.

3. Prepare a warm water bath as in step 4 of PART A.

4. Proceed as in steps 5-7 of PART A to measure the rate of fermentation in each of the 4 different foods. Don't forget to clean up all materials before leaving your lab area.

PART C: COMPARING CLASS AND INDIVIDUAL DATA

1. Calculate the total volume of gas produced in each test tube in PARTS A and B. Put your totals on the board and also record them in Table II.

2. Calculate the class averages for each part from the class data on the board. Record the class averages in Table II.

DATA: Record the volumes for all parts in the data tables provided.

Table I

	Part I		Part II			
Time	Warm	Cold	Food A	Food B	Food C	Food D

TABLE II TOTAL VOLUME IN 20 MINUTES

	YOUR DATA	CLASS AVERAGE
COOL WATER		
WARM WATER		
FOOD A		
FOOD B		
FOOD C		
FOOD D		

ANALYSIS:

Write a summary of PART A of this activity. Include in your summary:

a. the purpose of PART A.
b. a general equation for fermentation including reactants and products. (Use your text if necessary.)
c. how the fermentation rate was measured.
d. how different temperatures affected the fermentation rate. Include specific data from your lab in this discussion.
e. an explanation of why fermentation rates may be different at different temperatures.
f. an explanation of how class averages compare in general to your individual data.
g. several reasons why your data and class averages may not agree exactly.

Write a summary of PART B similar to the one for PART A. Remember, though, that PART B had a different objective than PART A.

Turn in your data tables and your summaries of PARTS A and B.

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