Fungal Fighters Supplemental Materials

Fungal Fighters - A Fungal Competition Lab Module for Budding Microbiologists

Lai, E.Y., Mejia, E., Parker, I.M., & Gilbert, G.S. (2024)

Fungal Fight Club

Module Type: multi-week lab activity **Timeline/duration:** 4 weeks (2 block period labs per week)	**Concepts:** microbiology, fungi, plant diseases **Skills:** fungal culturing, sterile technique, microscopy, testing predictions, data collection and analysis

Background

Why this matters:

Plants play an essential role in our lives, providing much of our food and maintaining ecosystem health. Here in California, we produce over half of the nation's produce all while being one of the world's biodiversity hotspots! However, plants can be threatened by a wide range of diseases, most of which are caused by fungi. As scientists, we learn about these diseases by identifying pathogenic species and running experiments to understand their structure, behavior, and impact on plants.

Starting Point for Inquiry:

Students will build on previous knowledge of ecosystem interactions and energy flow. Students may conventionally recognize fungi as mushrooms or organisms responsible for decomposition. However, this module will look into fungi as agents of disease, specifically in their role in infecting plant populations. With each student collecting environmental samples from their home or around school, we will explore the diversity of fungal species interacting with the plants we regularly encounter. Students will isolate fungi using sterile technique and make observations of their cultures to make informed hypotheses for the later parts of the module.

Special context:

This module synthesizes cross-cutting concepts in biology and ecology with particular emphasis on applications to agriculture. The lab procedures additionally introduce students to lab techniques for microbiology research.

Learning goals:

1. Understand and describe the impact of fungal diseases on plants, from an individual plant to plant populations and ecosystems
2. Learn skills for microbiology research: sterile technique, microscopy, culture isolation
3. Practice making observations on organism phenotype
4. Practice collecting data and analyzing results using mathematical tools
5. Practice developing informed and testable hypotheses

Module

Timeline:

	Block period 1	Block period 2
Week 1	Lab overview/introduction Pour agar plates	Plant diseases discussion Plate diseased leaf tissue
Week 2	Isolate pure culture of fungi	Check fungal growth Start fungal fight matchups
Week 3	Measure final growth + complete growth rate section of worksheet	Assess group fight winners Set up final matchup(s)
Week 4	Microscopy	Final matchup assessment

Links to Detailed Procedures and Worksheets:

- Procedures document
- Student worksheet

Assessment Methods:

- **Technique:** When practicing sterile technique, we can see visually when a sample or culture is contaminated. Agar plates that are left exposed to the air used with unclean tools will grow colonies of foreign spores.
- **Calculations:** Students will conduct measurements and calculations to compare the physiology of their isolated fungi. Students will show in their lab worksheets how they calculated rates and areas. In week 2, students will measure the lengths of their fungal culture to calculate the growth rate. In week 3, students will trace their fungal culture off of matchup plates to calculate the area.
- **Biological concepts:** Instructors should also discuss concepts of plant structure/function, fungal structure/function, and ecological interactions between species. Potential questions to assess understanding of material:
    - **Plant structure:** Our fungi are all collected from leaf samples. How does a foliar disease impact a plant? What are all the ways that diseases could affect plants?
    - **Symbiotic relationships:** Fungi and plants can interact in many ways. List and describe the types of relationships between these two types of organisms.
    - **Ecological impacts:** Outbreaks of novel diseases (such as Sudden Oak death) can devastate forests and other ecosystems. How do researchers study pathogens? What characteristics do researchers investigate?
    - **Agricultural impacts:** Disease outbreaks are a huge concern for farmers. How does our study of fungal growth connect to this larger issue?

Possible pitfalls:

- Some fungal species can not be cultured in a laboratory setting. This lab begins with collecting diseased leaf samples from home or around the school based on a photo guide of known culturable fungal pathogens. If a student uses a non-culturable sample during week 1, they can subsample from a lab mate in week 2.

Glossary:

Pathogen	Noun	A fungus, bacterium, virus, or other microorganism that can cause disease. Also referred to as an infectious agent or a germ
Biotroph	Noun	(Plant) pathogens that can only grow on plant tissue that is still alive
Necrotroph	Noun	(Plant) pathogens that actively kill host tissue as they colonize and then consume the dead remains
Endophyte	Noun	A bacterium or fungus that lives within a plant without causing apparent disease
Saprotroph	Noun	An organism that consumes plant material that is already dead
Spore	Noun	A microscopic reproductive unit capable of giving rise to a new individual
Hypha	Noun	Long branching filament of a fungus (plural: hyphae)
Septum	Noun	The hyphae of fungi are divided into cells by internal walls called septa (singular: septum, plural: septa)
Mycelium	Noun	The vegetative part of a fungus, consisting of a network of hyphae
Sterile	Adj.	Free from bacteria and other living microorganisms; totally clean
Contaminate	Verb	To make impure or unsuitable by contact or mixture with something unclean, e.g. contaminating microorganisms
Culture (in microbiology)	Verb	The growth of microorganisms, such as bacteria, fungi, or tissue in laboratory conditions
Isolate (in microbiology)	Verb	To separate a strain from a natural, mixed community of living microbes in order to identify the microbe(s) of interest
Inoculum	Noun	The population of microbes introduced to the culture medium for reproduction

NGSS Standards

HS-LS2 Ecosystems: Interactions, Energy, and Dynamics

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HS-LS2-1. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.
HS-LS2-2. Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales.
HS-LS2-6. Evaluate claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem.

HS.Matter and Energy in Organisms and Ecosystems

HS-LS2-4. Use mathematical representations to support claims for the cycling of matter and flow of energy among organisms in an ecosystem.

Performance Expectation

Science and Engineering Practices:	
Using Mathematics and Computational Thinking Engaging in Argument from Evidence	Calculate growth rates of individual cultures and measure areas of fungal growth on competition plates. Using information collected in the growth rate section of the lab, students predict outcomes of fungal competition. Students may be validated to find that fast growing fungi are good competitors or find that other fungal traits play key roles in competition.
Disciplinary Core Ideas:	
LS2 Ecosystems: Interactions, Energy, and Dynamics ● LS2.A: Interdependent Relationships in Ecosystems ● LS2.C: Ecosystem Dynamics, Functioning, and Resilience	Students learn about how pathogenic fungi interact with and cause disease in plants in both agricultural and natural settings. Students then discuss how disease outbreaks can have catastrophic impacts on the plant community and broader ecosystem.
Cross Cutting Concepts:	
Cause and effect Structure and function	Understand how fungal pathogens infect plants, and cause both localized disease effects as well as broader ecosystem impacts. Examine fungal structures at a microscopic scale and discuss the functions of these structures.

Sample Collection Guide

Look for leaves that are still living and mostly green, but with dark brown or black spots. Necrosis: dead tissue, Chlorosis: yellowing due to loss of green chlorophyll

Madrone

Live Oak

English Ivy

Eucalyptus

Sunflower

Bellbean

Plum

Garrya

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Fungal Fighters Lab Worksheet

Microbiology Vocabulary

Fill in the term: recall from in-class slides and/or refer to the lab procedures

_____ free from bacteria, fungi, viruses, or other living microorganisms

_____ to make impure by contact or mixture with something unclean (such as from surfaces or airborne spores)

_____ the growth of microorganisms, such as bacteria, fungi, or tissue in laboratory conditions

_____ the solidified growth medium used to grow microorganisms on

_____ to separate a strain from a natural, mixed population of living microbes in order to identify the microbe(s) of interest

_____ the population of microbes introduced to the culture medium for reproduction

Fungal plant diseases

Types of fungi living inside plants - fill in the term

	Pathogens that consume living plant tissue, therefore keeping its host alive
	Pathogens that actively kill host tissue as they colonize
	A fungus that lives within a plant without causing disease

Fungal Fighters Lab Worksheet

How did we visually identify diseased plants for this lab? *1 sentence answer*

Plant disease researchers

What are some other microbes that cause disease in plants? *List one.*

Give an example of a research method for studying plant diseases.

Isolate a pure culture

Observations on fungi cultured from your leaf sample

Each leaf sample could have one or multiple fungi living inside

How many distinct fungal colonies do you see growing on your plate? _____

Describe the colors, textures, and growth patterns of the different fungi you observed (you might not have as many as 3)

1.

2.

3.

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Fungal Fighters Lab Worksheet

Isolate a pure culture

After growing a single fungal culture for 5 days, observe and take notes about your species

Color(s)

Texture

Shape/size

Growth rate

Predict

How long do you think your fungus will take to grow 1 cm?

Brainstorm: What traits could make a fungus especially good at growing fast?

Fungal Fighters Lab Worksheet

Measure growth

Record the distance grown each day, measure centimeters to one decimal point (eg 2.5 cm)

Days since start	Distance grown

Calculate growth rate

Growth rate measures the change in size [distance] over a period of time [days]. Using this equation, Growth rate = $\frac{(total\ distance\ grown)}{days}$ *fill in numbers and calculate:*

Early growth rate = $\frac{distance\ grown\ in\ 3\ days}{3\ days}$ = _____

My early growth rate: _____

Later growth rate = $\frac{distance\ grown\ in\ 7\ days - distance\ grown\ in\ 3\ days}{7\ days - 3\ days}$ = _____

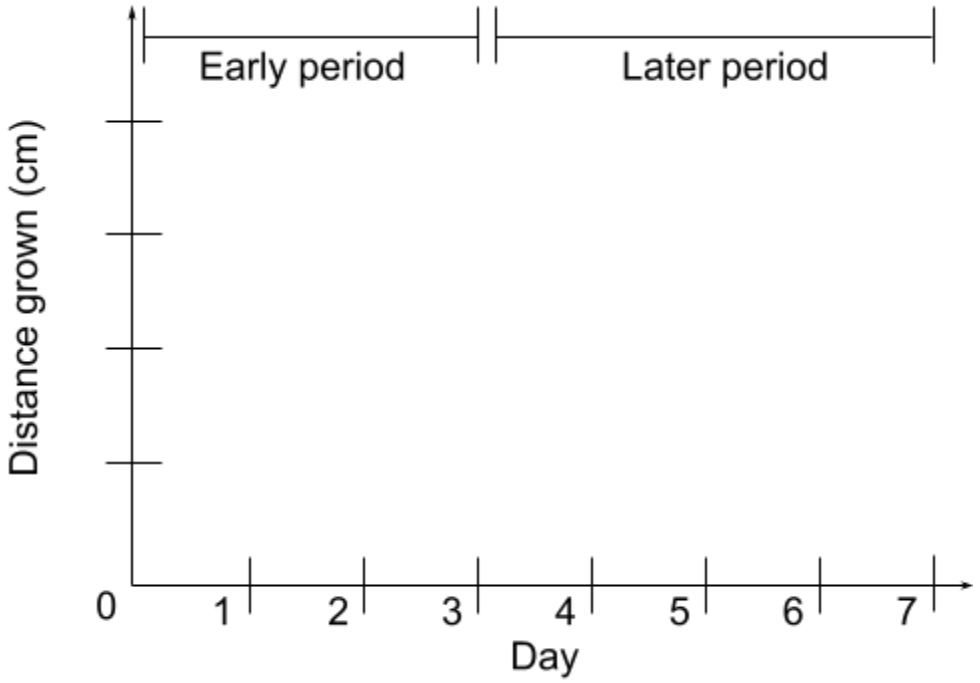
My later growth rate: _____

Growth rate is (circle) **faster**, **slower**, or **the same** at the early period relative to the later period.

Fungal Fighters Lab Worksheet

Plot your data

Label the y-axis with centimeter values based on your measurements and plot the distances grown by day 3 and by day 7. Draw one straight line from (0,0) at the bottom left corner to the day 3 distance, then draw a second straight line from the day 3 distance to the day 7 distance.



Compare with labmates

Whose fungus grew the fastest? _____

How close was your prediction to your actual growth rate?_____

_____

Whose guess was closest to their actual growth rate in your group? _____

Fungal Fighters Lab Worksheet

Fungal fights

Predict

Based on observations of the growth of your group's fungi so far, guess whose fungus in your group will win the fungal fights:

Brainstorm: What other traits could make a fungus good at competing with other fungi?

Lab group identification

Have each member of your lab group select a sharpie color to mark their fungal competitor. Make sure to label each plate to keep track of your matchups.

Name	Color

Fungal match-ups

Calculate the total number of paired match-ups based on the number of people in your group

Fungal Fighters Lab Worksheet

Outcomes

Trace the shape of each fungal colony onto a clear sheet and calculate the areas

Matchup 1	Name: Name:	Grid squares: Grid squares:	Final area: Final area:
Matchup 2	Name: Name:	Grid squares: Grid squares:	Final area: Final area:
Matchup 3	Name: Name:	Grid squares: Grid squares:	Final area: Final area:
Matchup 4	Name: Name:	Grid squares: Grid squares:	Final area: Final area:
Matchup 5	Name: Name:	Grid squares: Grid squares:	Final area: Final area:
Matchup 6	Name: Name:	Grid squares: Grid squares:	Final area: Final area:

Is there a clear fight champion?

Whose fungus is the winner of your group? _____

Was the fastest growing fungi also the best competitor in your group? _____

Fungal Fighters Lab Worksheet

Viewing Fungi with a Microscope

Station 1:

Observe your fungus under the microscope. Describe what you see here:

Color, dark or bright _____

Shape(s) _____

See if you can find these structures (use reference images to help) and draw them here

Spores	Hyphae
Magnification: _____	Magnification: _____

Station 2:

Take descriptive observation notes of your fungus

Color, dark or bright _____

Shape(s) _____

See if you can find these structures (use reference images to help) and draw them here

Spores	Hyphae
Magnification: _____	Magnification: _____

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Fungal Fighters Lab Worksheet

Station 3:

Take descriptive observation notes of your fungus

Color, dark or bright _____

Shape(s) _____

See if you can find these structures (use reference images to help) and draw them here

Spores	Hyphae
Magnification: _____	Magnification: _____

Station 4:

Take descriptive observation notes of your fungus

Color, dark or bright _____

Shape(s) _____

See if you can find these structures (use reference images to help) and draw them here

Spores	Hyphae
Magnification: _____	Magnification: _____

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Fungal Fighters Lab Worksheet

Station 5:

Take descriptive observation notes of your fungus

Color, dark or bright _____

Shape(s) _____

See if you can find these structures (use reference images to help) and draw them here

Spores	Hyphae
Magnification: _____	Magnification: _____

Station 6:

Take descriptive observation notes of your fungus

Color, dark or bright _____

Shape(s) _____

See if you can find these structures (use reference images to help) and draw them here

Spores	Hyphae
Magnification: _____	Magnification: _____

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