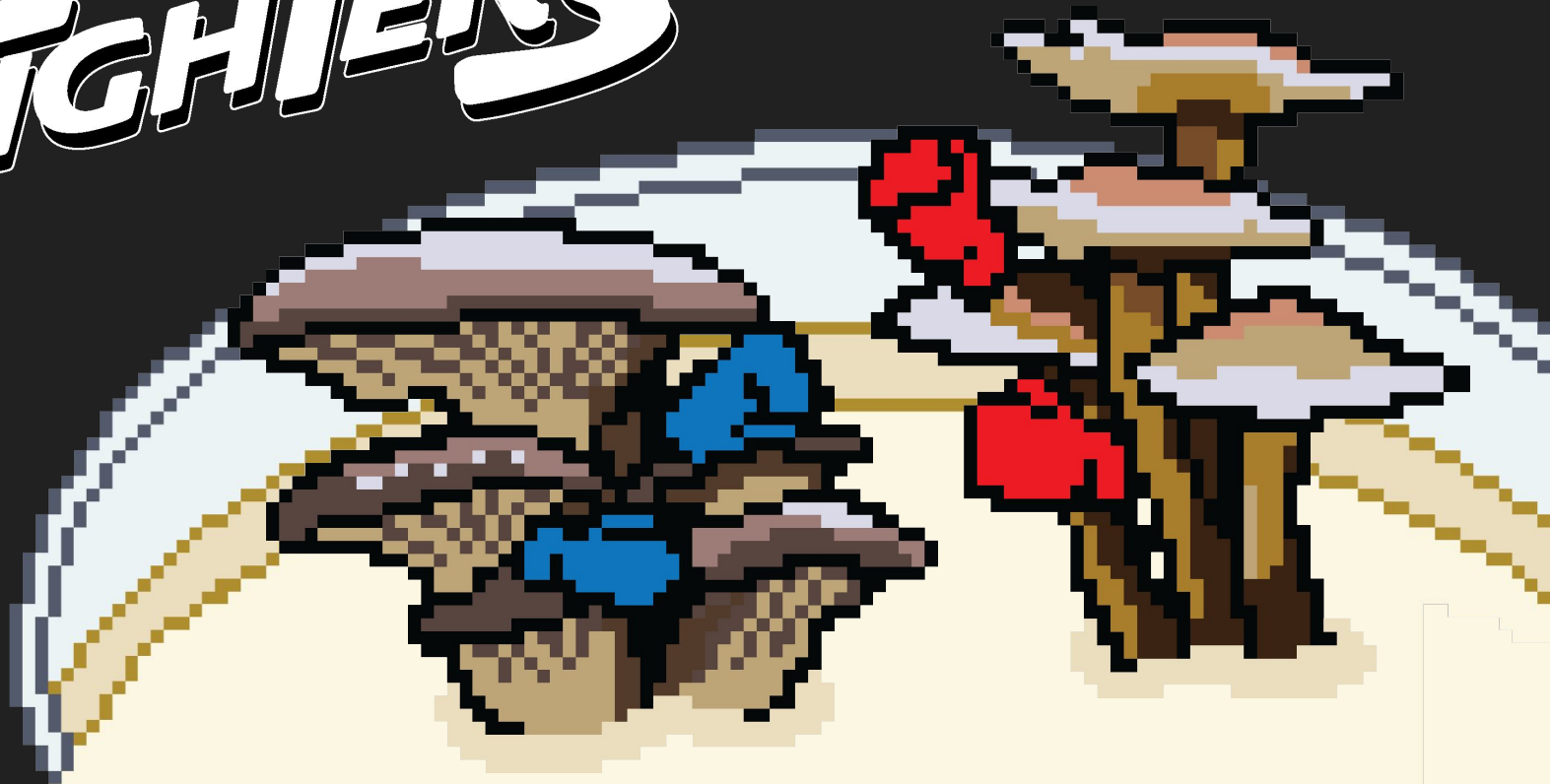
Fungal Fighters



Plants in the ecosystem

Plants play a huge role in our lives, providing us with food, air, shelter, and more!

In California, we are one of the world’s biodiversity hotspots AND we produce over half the nation's produce! But plants are threatened by lots of issues.

So how do we as scientists study and resolve these threats?

Plants get sick too!

How do we identify the pathogens affecting plants?

How can this information help us manage disease?

Gray mold on strawberries

Black spot on roses

Fruit rot on squashes

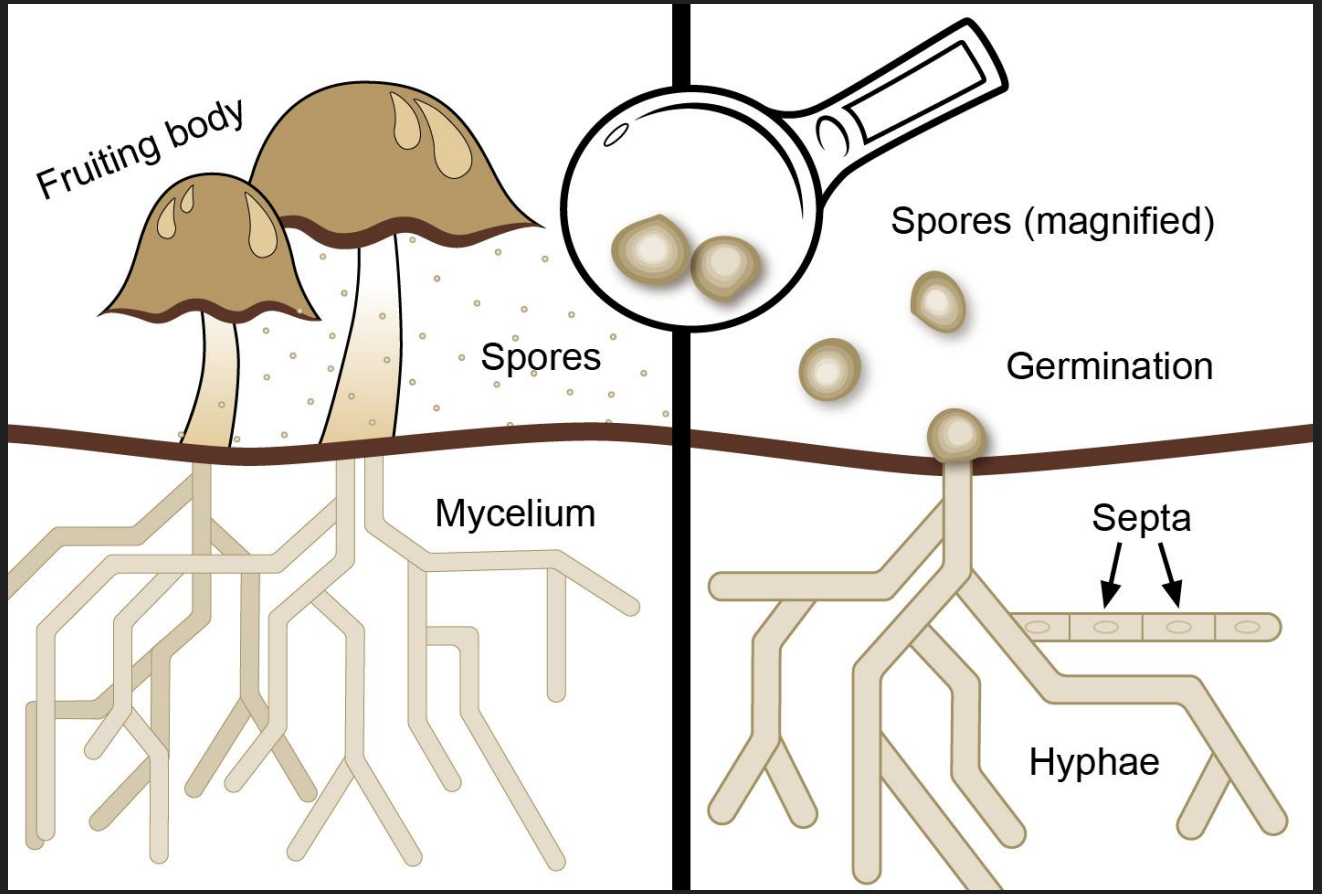
But aren’t *these* fungi?

Do you typically seen mushrooms growing out of a leaf?

The fungal kingdom is *super* diverse and many hide from plain sight!

Hill et al. Frontiers in Microbiology 12 (2021)

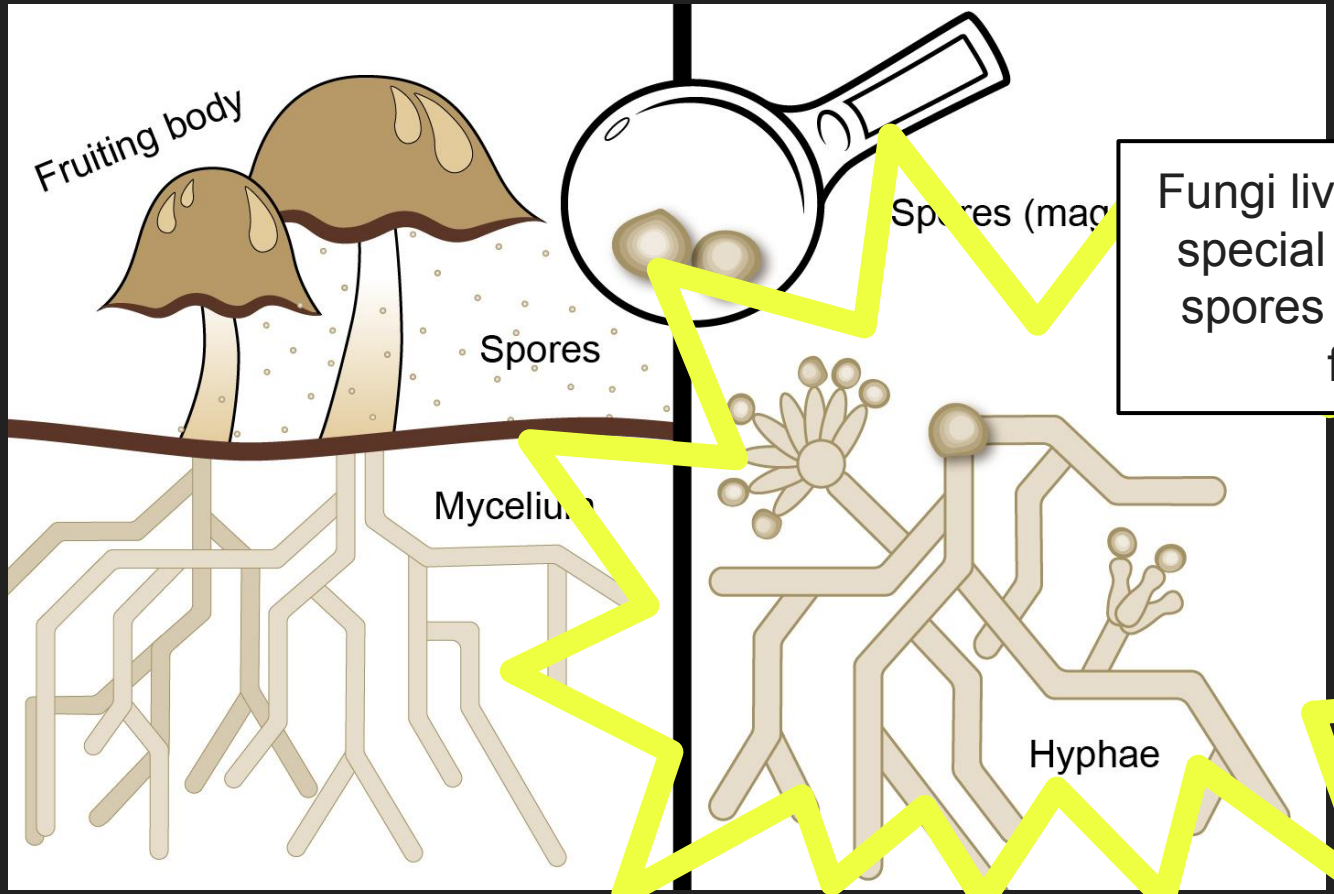
Typical fungus life cycle and structures



Defining characteristics

- Heterotrophic: acquire energy by breaking down the substrate they live in to become food
- Chitin fibers in their cell walls
- Make spores for reproduction

However…



Fungi living inside plants have special hyphae that produce spores without macroscopic fruiting bodies

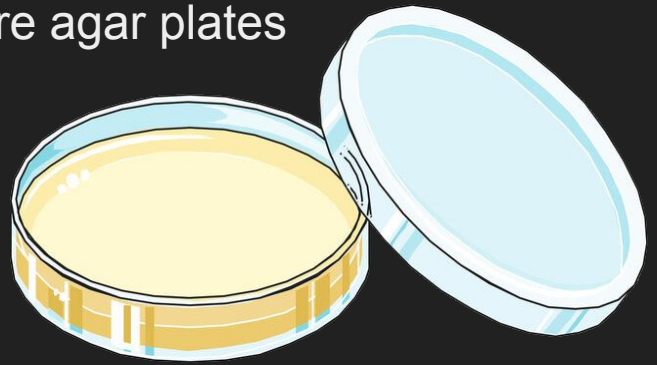
How do we study plant diseases?

1. Bring affected leaves into the lab to collect the fungal pathogen
2. Characterize the fungus species by running tests
3. Apply this information to crops or plants of concern

How do we study plant diseases?

Part 1 starts this week

Today: prepare agar plates



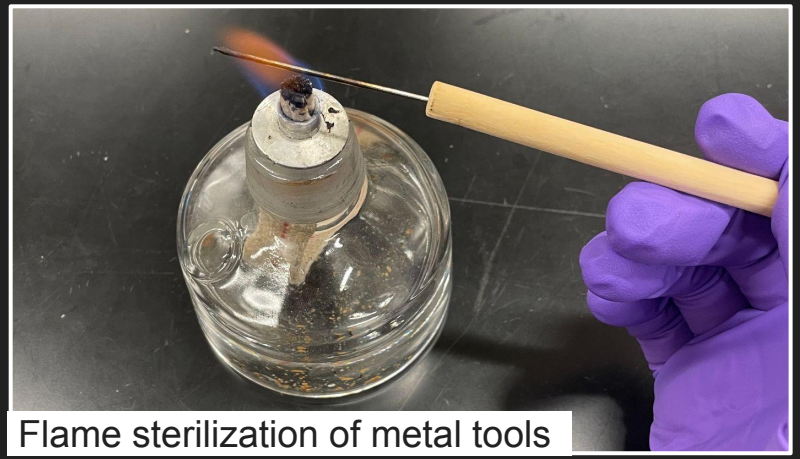
Wednesday: collect diseased leaves

Thursday: begin culturing fungi

Microbiology terms

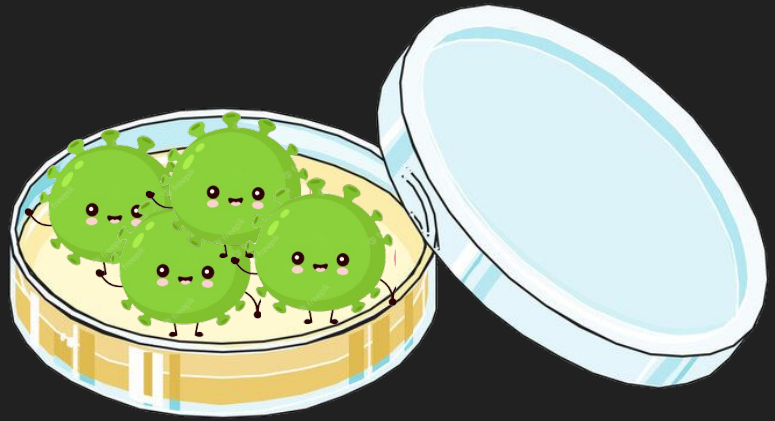
Sterile: free from bacteria, fungi, viruses, or other living microorganisms

Surface sterilization with chemical solution


Flame sterilization of metal tools

Contaminate: To make impure by contact or mixture with something unclean (such as from surfaces or airborne spores)

Microbiology terms

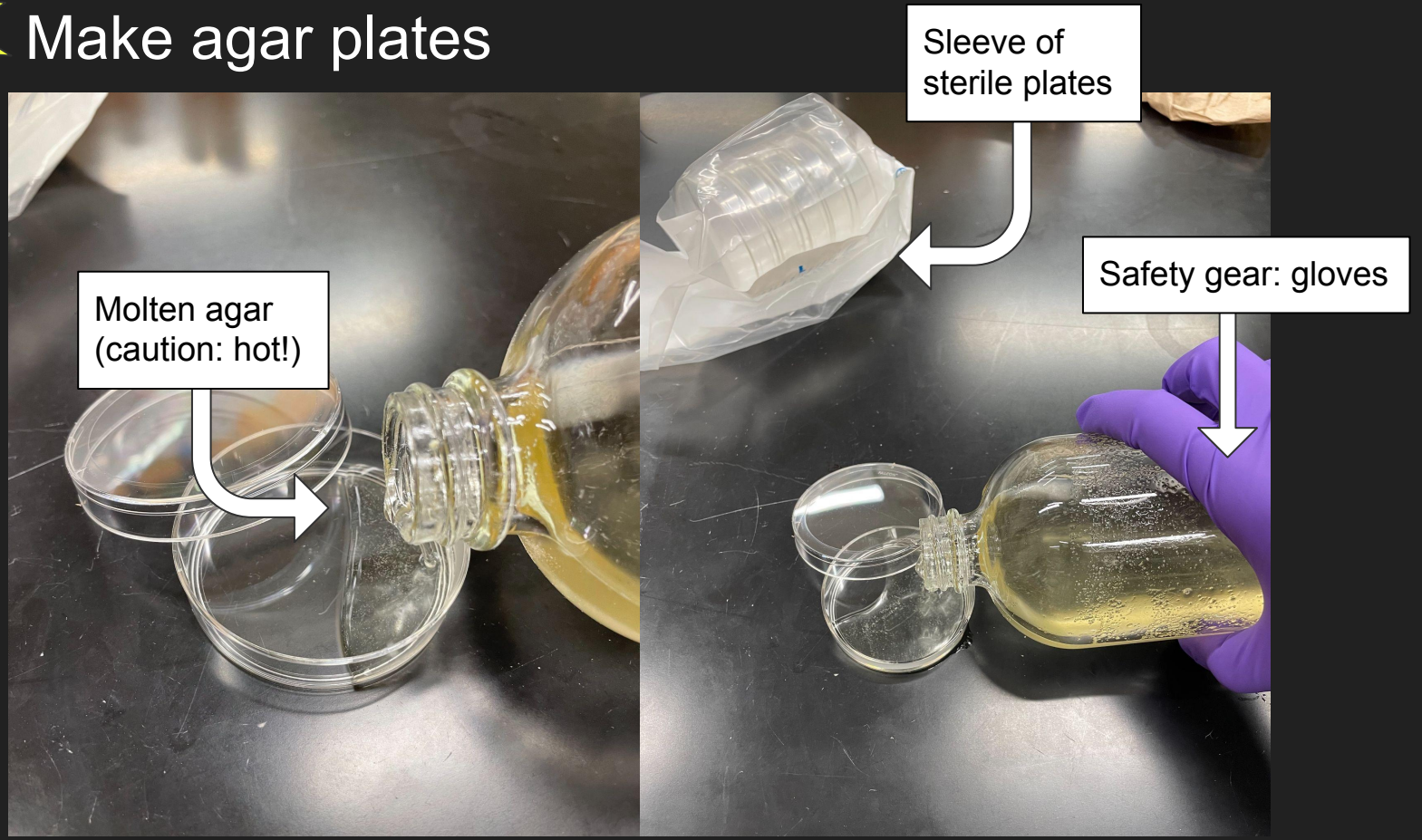


Culture: The growth of microorganisms, such as bacteria, or fungi, or tissue in laboratory conditions

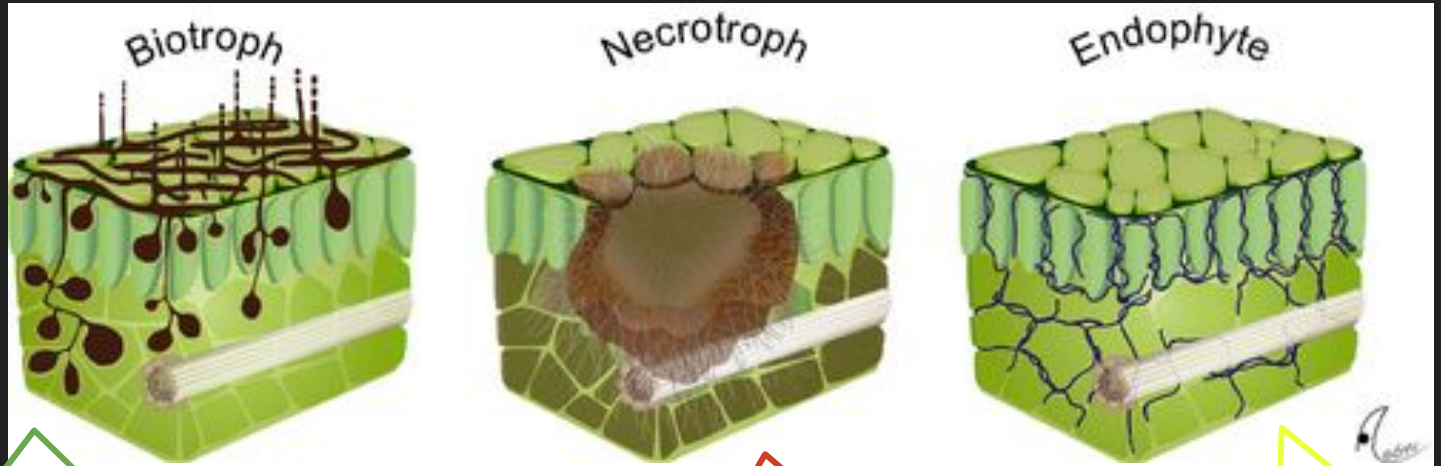
Agar plate: An agar plate is a Petri dish that contains a growth medium solidified with agar, used to culture microorganisms.

Part 1

Make agar plates



Types of fungi living inside plants



García-Guzmán & Heil 2013

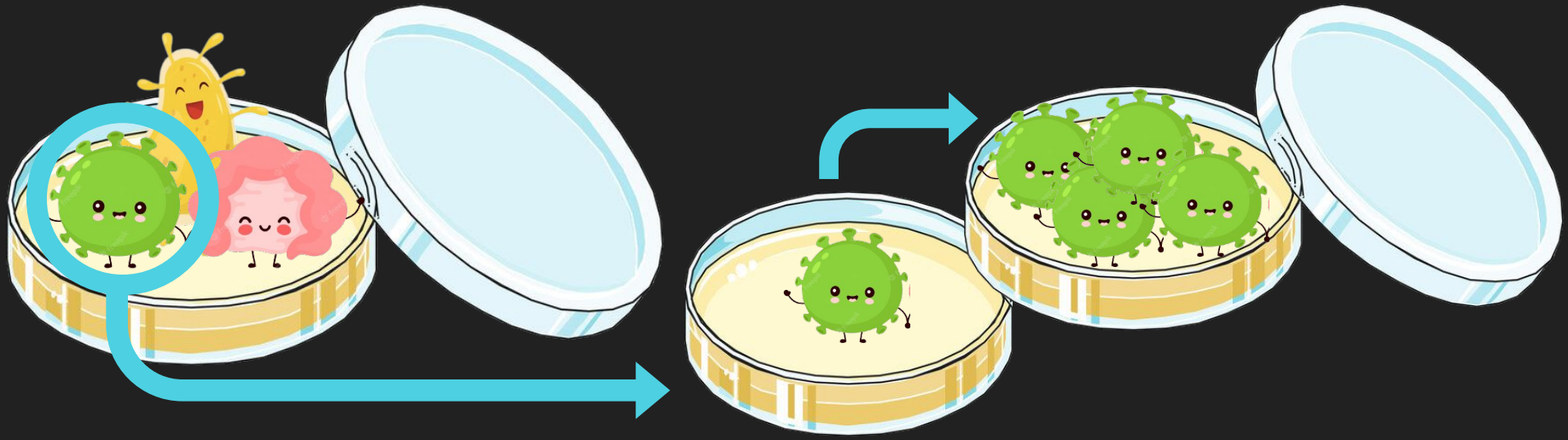
Pathogens that consume living plant tissue, therefore keeping its host alive

Pathogens that actively kill host tissue as they colonize

A fungus that lives within a plant without causing disease

Microbiology terms

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

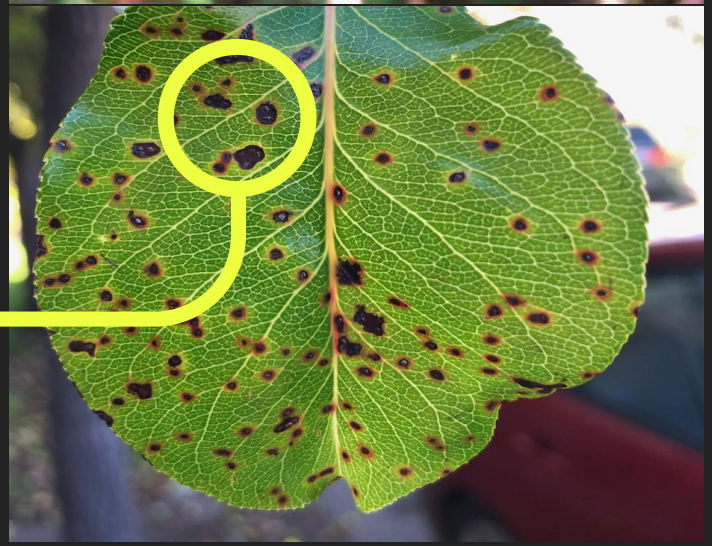


Inoculum: Microbes introduced to the culture medium for reproduction

Collecting diseased plants

Look for leaves that are still living and mostly green, but with dark brown or black spots, sometimes surrounded by a yellow halo

Try to get different plant species in your lab group to culture different fungal species



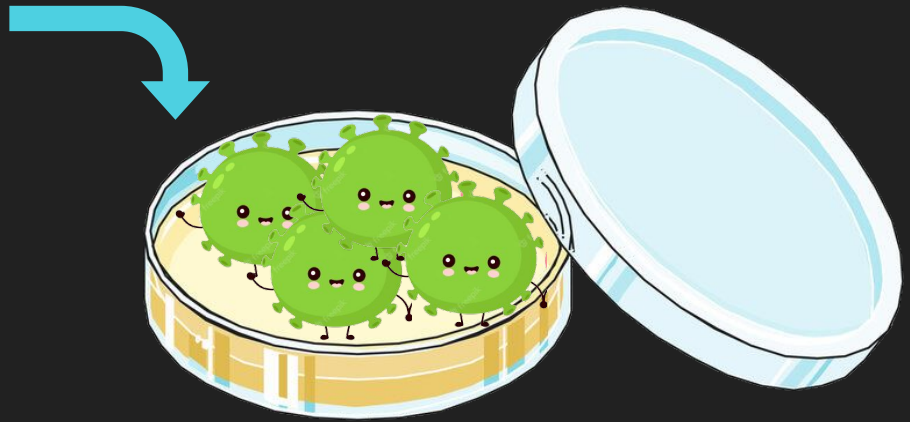
Necrosis: death of cells or tissue

How do we study plant diseases?

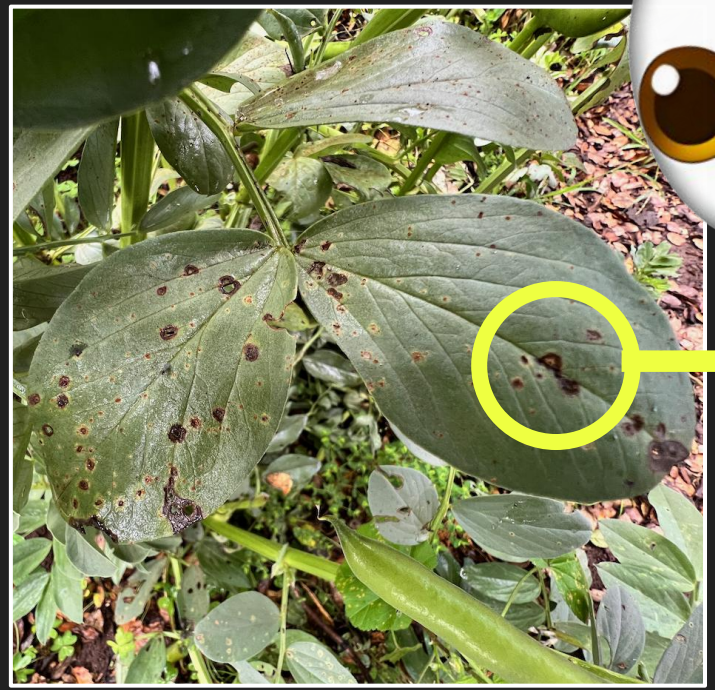
1. **Culturing the fungal pathogens**

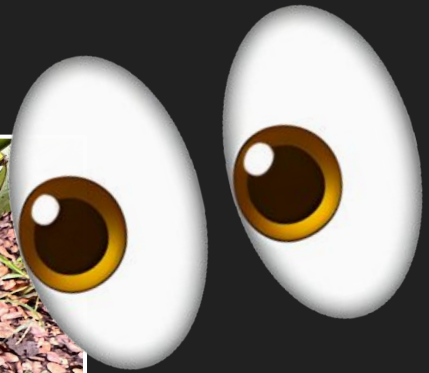
   Monday: prepare agar plates

   Thursday: begin culturing fungi



Culture: The growth of microorganisms, such as bacteria, or fungi, or tissue in laboratory conditions





Necrosis: death of cells or tissue

How do we study plant diseases?

1. Bring affected leaves into the lab to collect the fungal pathogen
2. Characterize the fungus species by running tests
3. Apply this information to crops or plants of concern

How do we study plant diseases?

2. Apply this information to crops or plants of concern

Let’s look into a couple of projects plant-disease researchers are working on at UCSC and CSU MB

Plants get sick too!

How do we identify the pathogens affecting plants?

How can this information help us manage disease?

Gray mold on strawberries

Black spot on roses

Fruit rot on squashes

Research question/topic of interest

Picture of researcher

1-2 sentence description of the project

Researcher name

Position/year in school

Research or career goals

Study system:

Field picture if there is a field component to the project, or pictures of the plant and microbe species

Methods picture:

Could be a conceptual diagram, a lab bench setup, or process pictures

Short description can be on the picture or put in speaker notes

Methods picture:

Could be a conceptual diagram, a lab bench setup, or process pictures

Short description can be on the picture or put in speaker notes

Methods picture:

Could be a conceptual diagram, a lab bench setup, or process pictures

Short description can be on the picture or put in speaker notes

Methods picture:

Could be a conceptual diagram, a lab bench setup, or process pictures

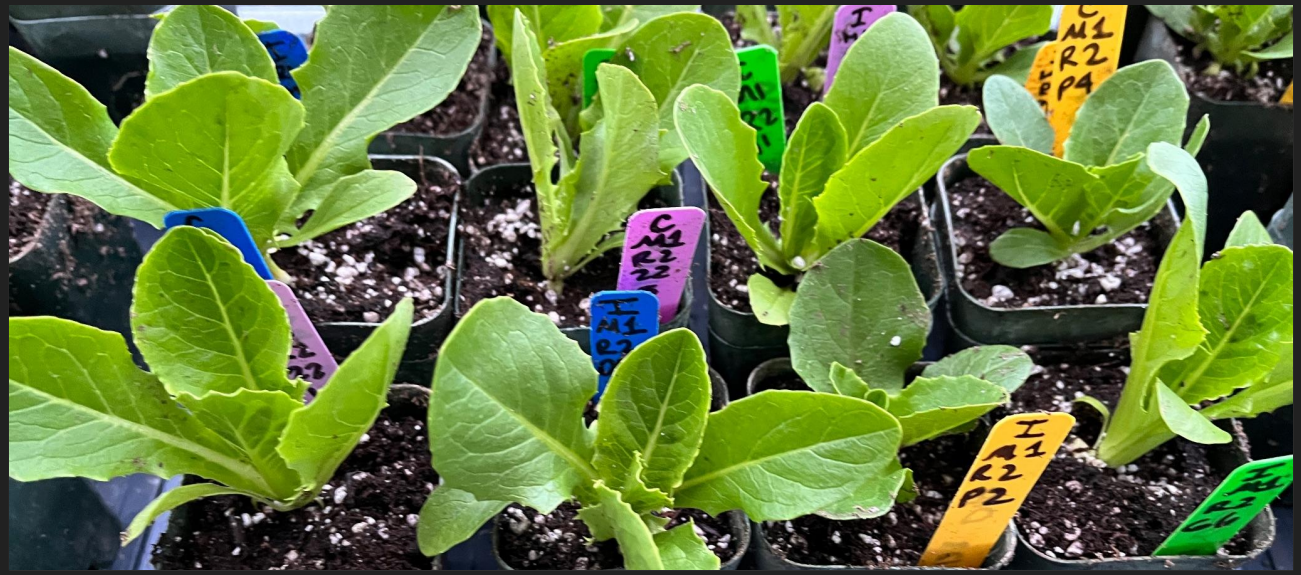
Short description can be on the picture or put in speaker notes

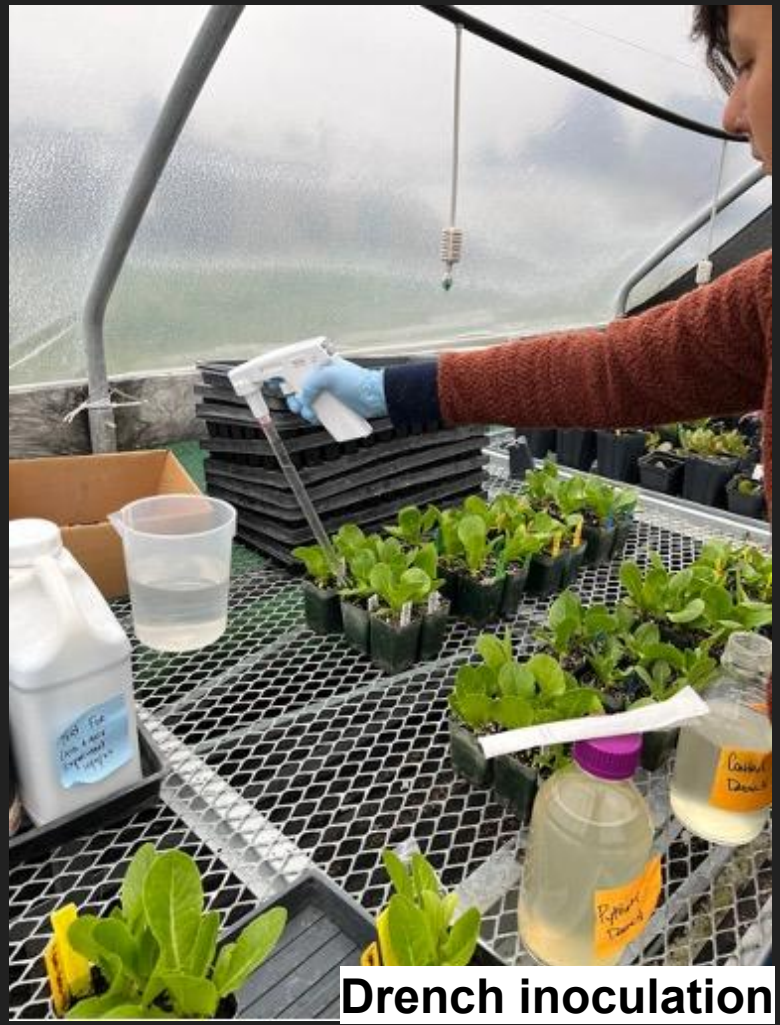
Developing tools to evaluate tolerance to Pythium wilt

Jasper (left) and Alex (right)

Agricultural Plant and Soil Sciences at CSUMB

We aimed to optimize protocols to successfully inoculate lettuce plants to test germplasm resistance and tolerance to Pythium wilt - a root rot disease caused by oomycetes (fungus-like microbes).





Drench inoculation

Dip inoculation

Infected roots

Assessing Pythium wilt tolerance in lettuce varieties

We aimed to determine any possible resistance or tolerance of lettuce varieties against Pythium wilt. We found that severe above-ground Pythium wilt symptoms were accurate predictors of infection, but already too late for any management intervention.

Tyler

CSUMB Biology B.S. Graduate

Mock inoculated

5 days post inoculation

Inoculated with *Pythium uncinulatum*

5 days post inoculation

How do we study plant diseases?

2. Characterize the fungus species by running tests

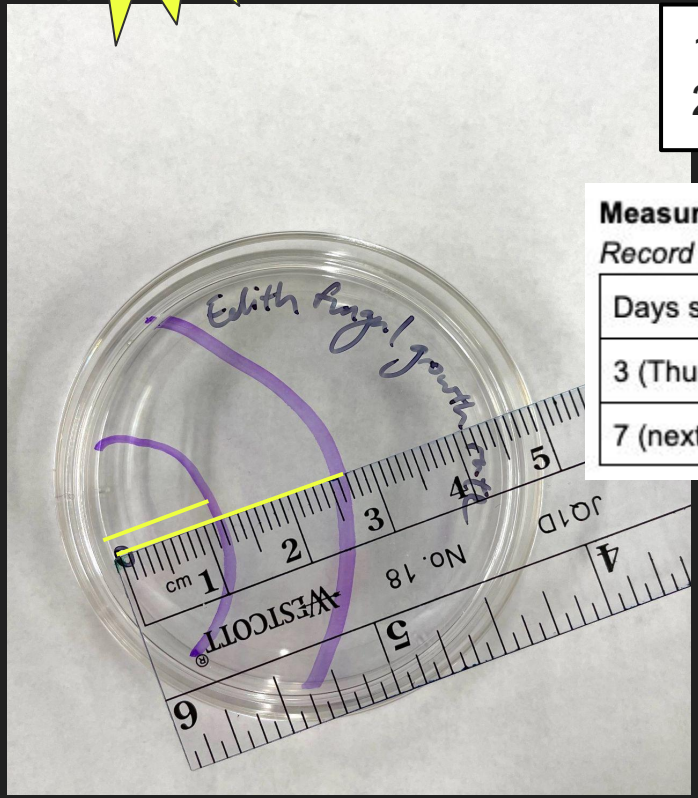
Monday: isolate pure culture, start plates to measure growth rates

Thursday: check in on growth rates, start plates with fungal fight matchups

Part 4

Growth rate

1. Outline fungal growth area again (7 days since start)
2. Fill out your worksheet and begin calculating growth rate



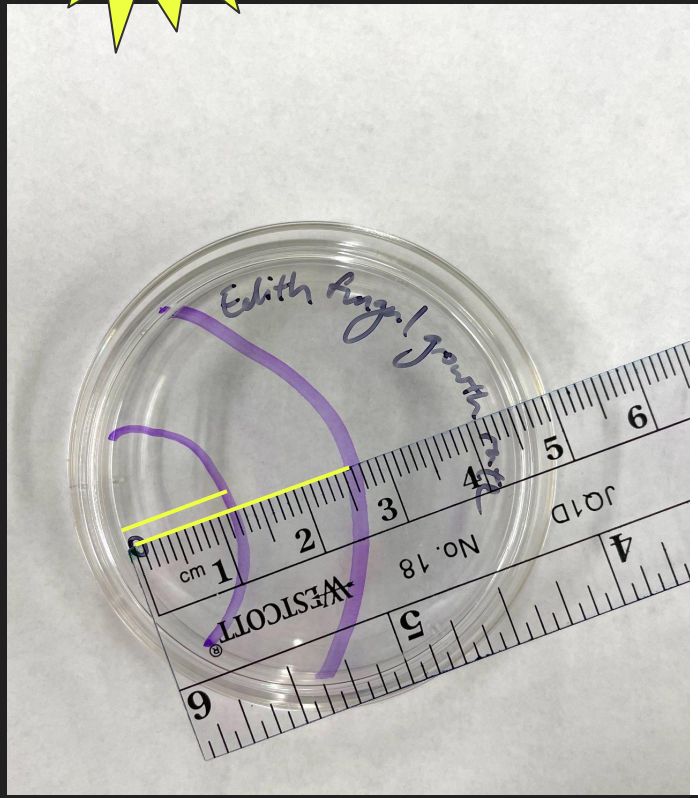
Measure growth

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

Days since start	Distance grown
3 (Thursday)	1.2
7 (next Monday)	2.7

Part 4

Growth rate



Calculate growth rate

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

Early growth rate = $\frac{\text{distance grown in 3 days}}{\text{3 days}} = \frac{\text{1.2 cm}}{\text{3 days}}$

My early growth rate: 0.4 cm/day

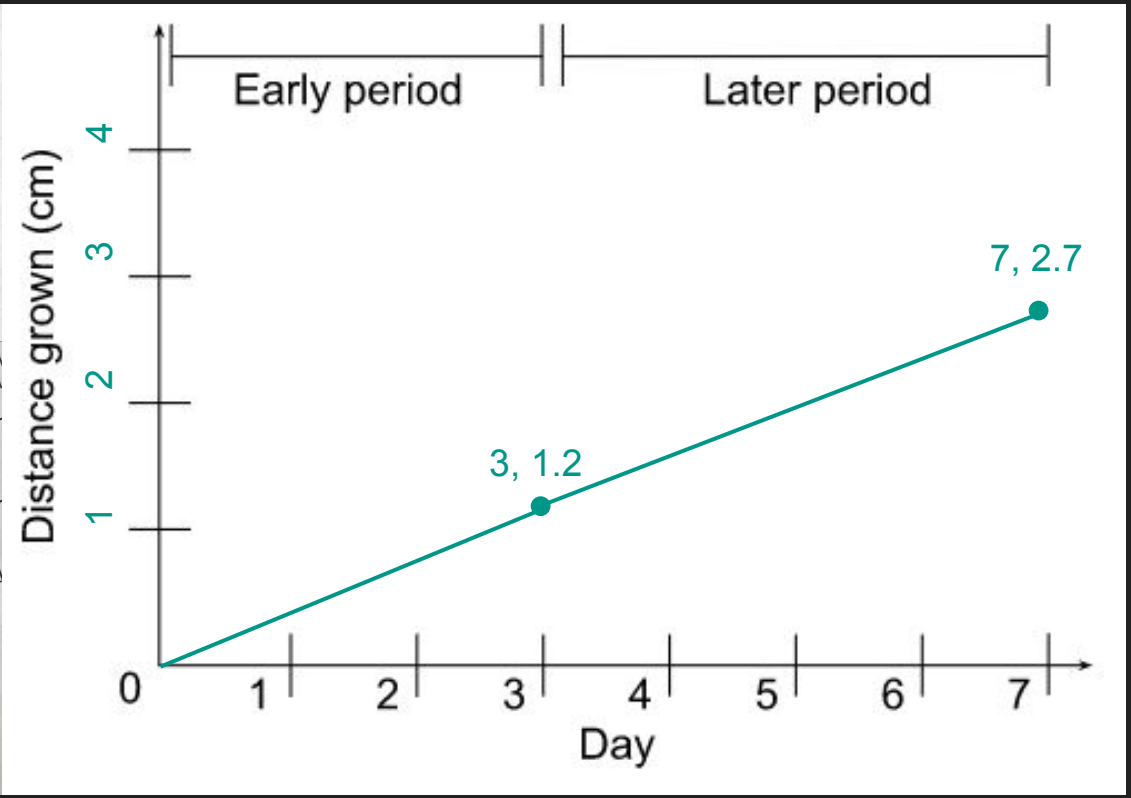
Later growth rate = $\frac{\text{distance grown in 7 days} - \text{distance grown in 3 days}}{\text{7 days} - \text{3 days}} = \frac{\text{2.7 - 1.2 cm}}{\text{4 days}}$

My later growth rate: 0.375 cm/day

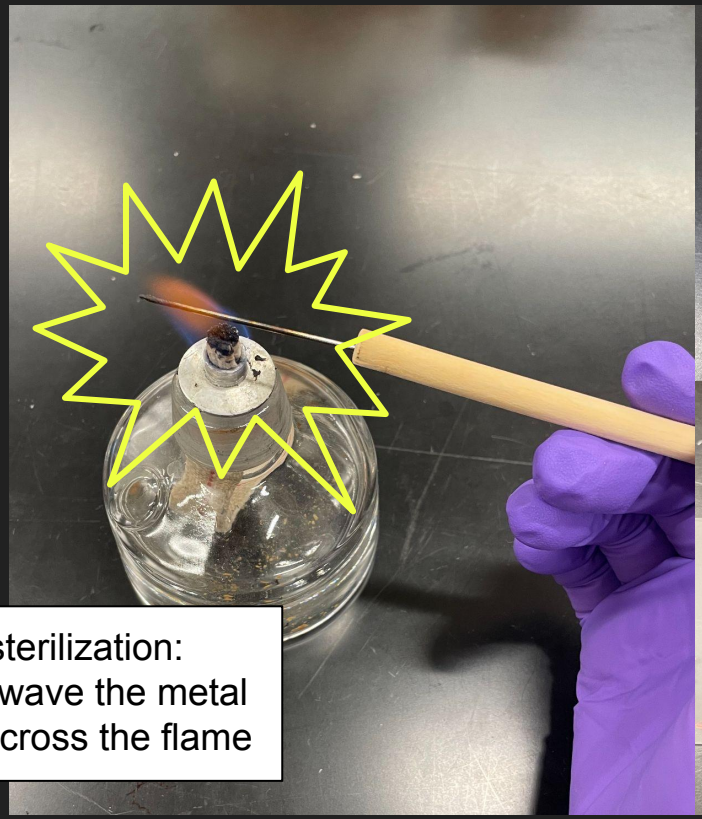
Growth rate is (circle) **faster**, **slower**, or **the same** in the early period relative to the later period.

Part 4

Growth rate

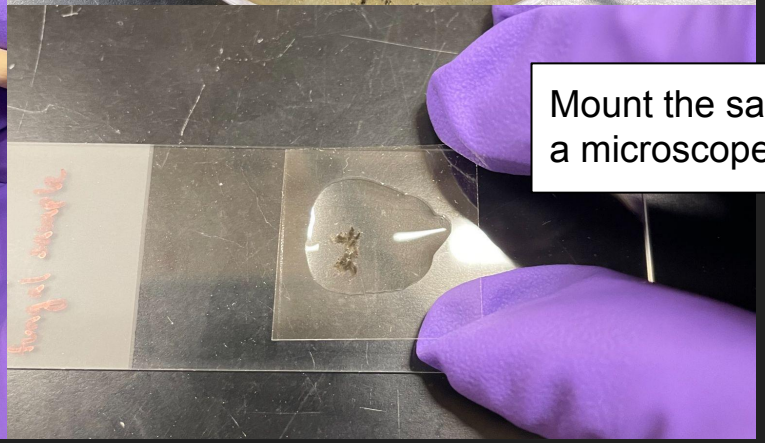


Viewing your fungi under the microscope



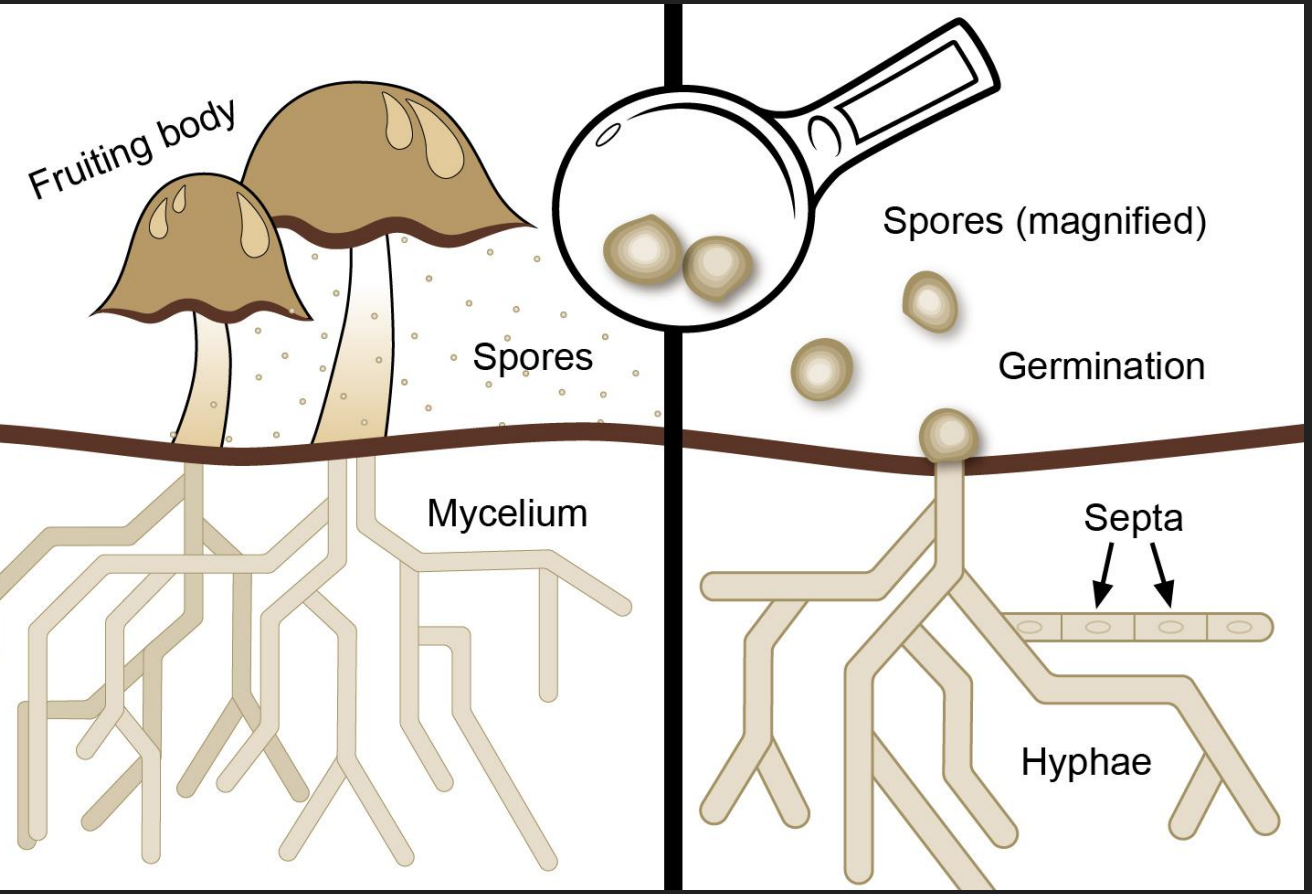
Flame sterilization: quickly wave the metal probe across the flame

Scoop up some fungus with the sterile probe



Mount the sample onto a microscope slide

Recall the typical fungus structures

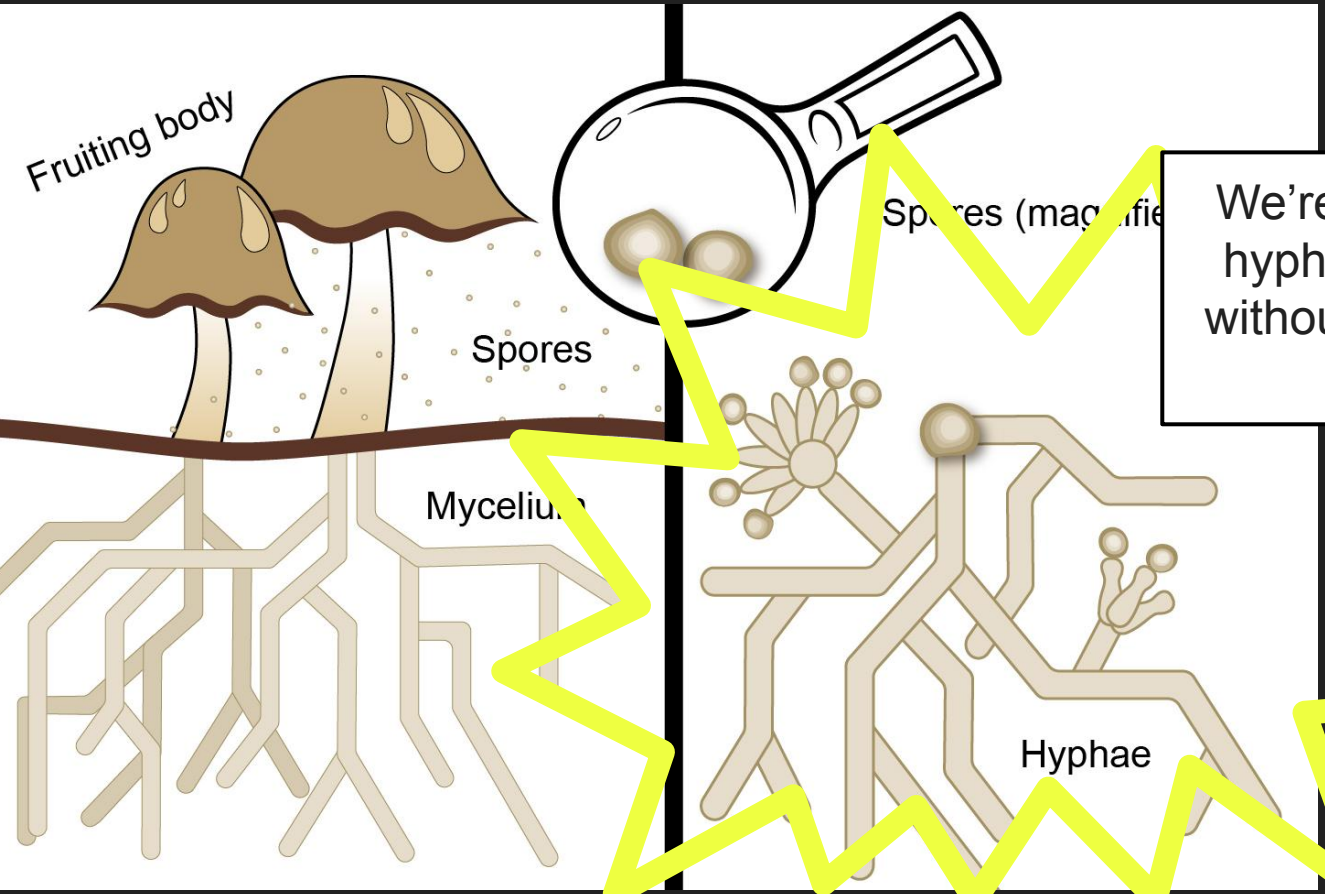


Spores: A small, reproductive unit capable of giving rise to a new individual

Septa: The hyphae of fungi are divided into cells by internal walls called septa

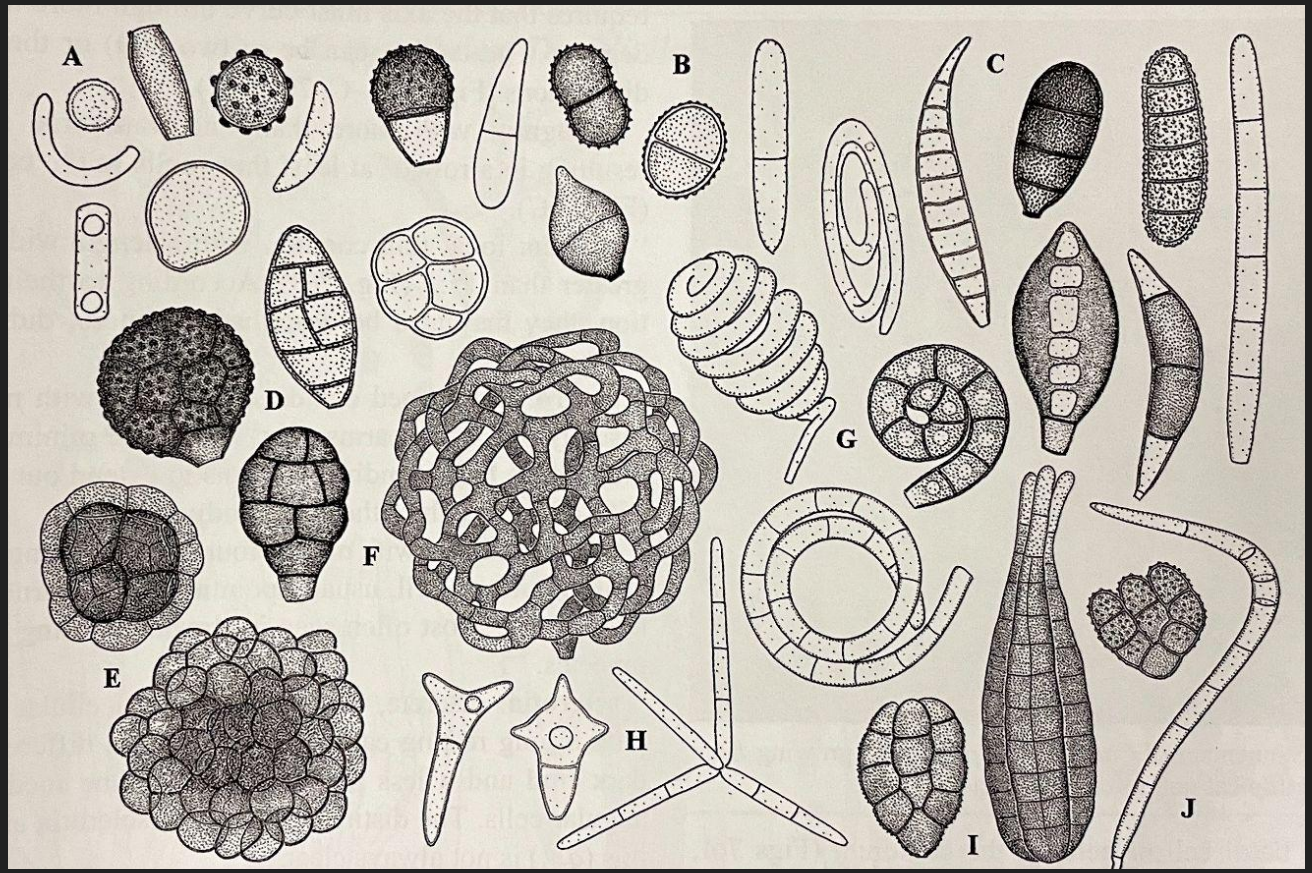
Hyphae: Long branching filaments of a fungus

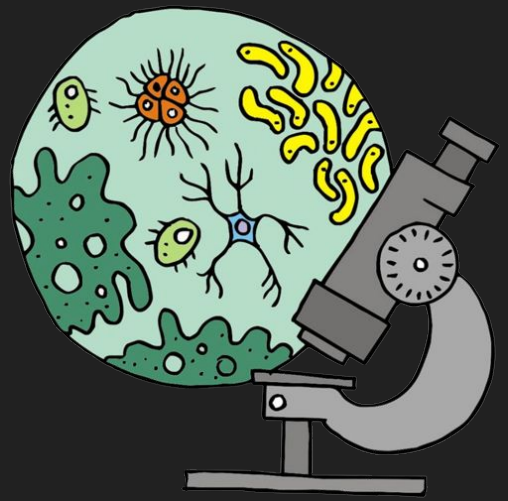
Our microscopy focus:



We're looking for specialized hyphae that produce spores without a macroscopic fruiting body

Spore shapes, sizes, and structures:





Part 6

Using microscope

1. Lower the stage all the way down
2. Turn the objective lens to the lowest magnification
3. Place fungal slide on the stage
4. View the slide through the eyepiece
5. Use the coarse and fine adjustment knobs to focus the image
6. Fill in your worksheet
7. Lower the stage and remove the slide before moving to the next station

Summary: How did we study plant diseases?

Cultured fungi from diseased leaf samples

Practiced sterile techniques to avoid contamination

Isolated a single species from the agar plate

Measured and compared growth rates

Identified spores and hyphal structures

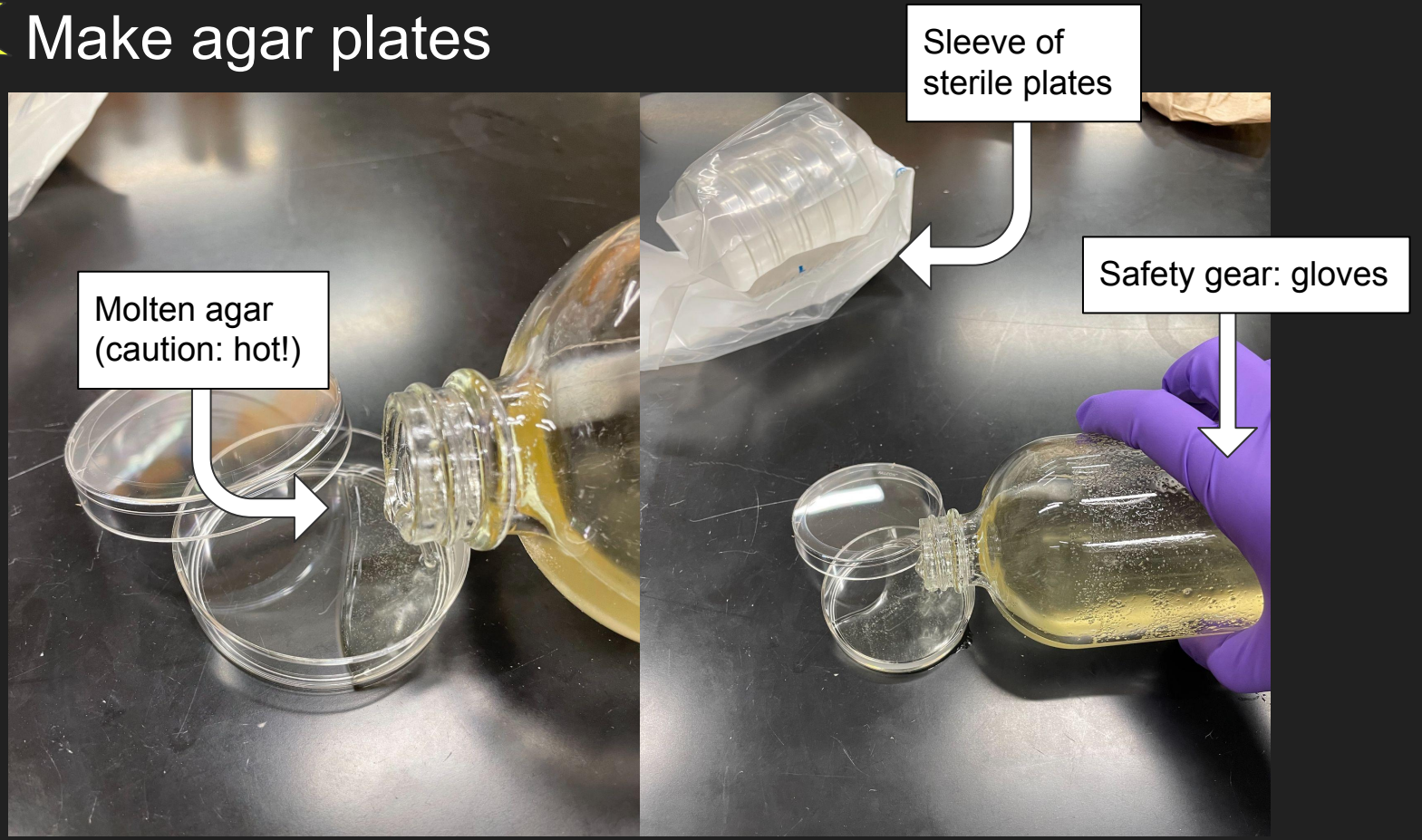
Measured competitive ability with fungal fights

Discussed other kinds of fungal diseases and how researchers look for solutions to protect agricultural crops

Fungal Lab Procedures

Part 1

Make agar plates



Part 2

Plate a leaf sample: setup

Keep tweezers and scissors in 70% ethanol

Pre-made agar plates

Diseased leaf sample

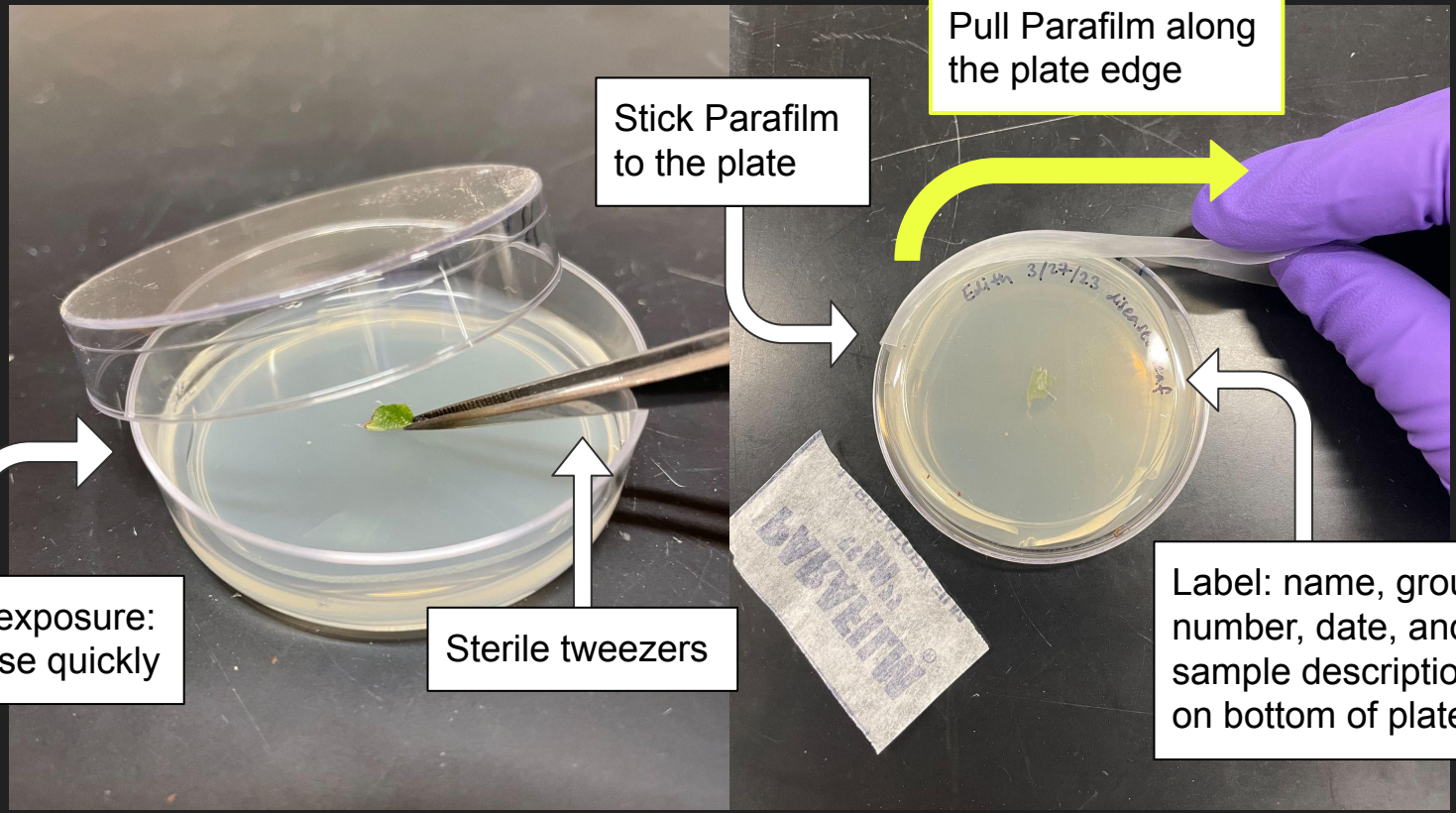
Clean workbench

Part 2

Plate a leaf sample: prep leaf sample

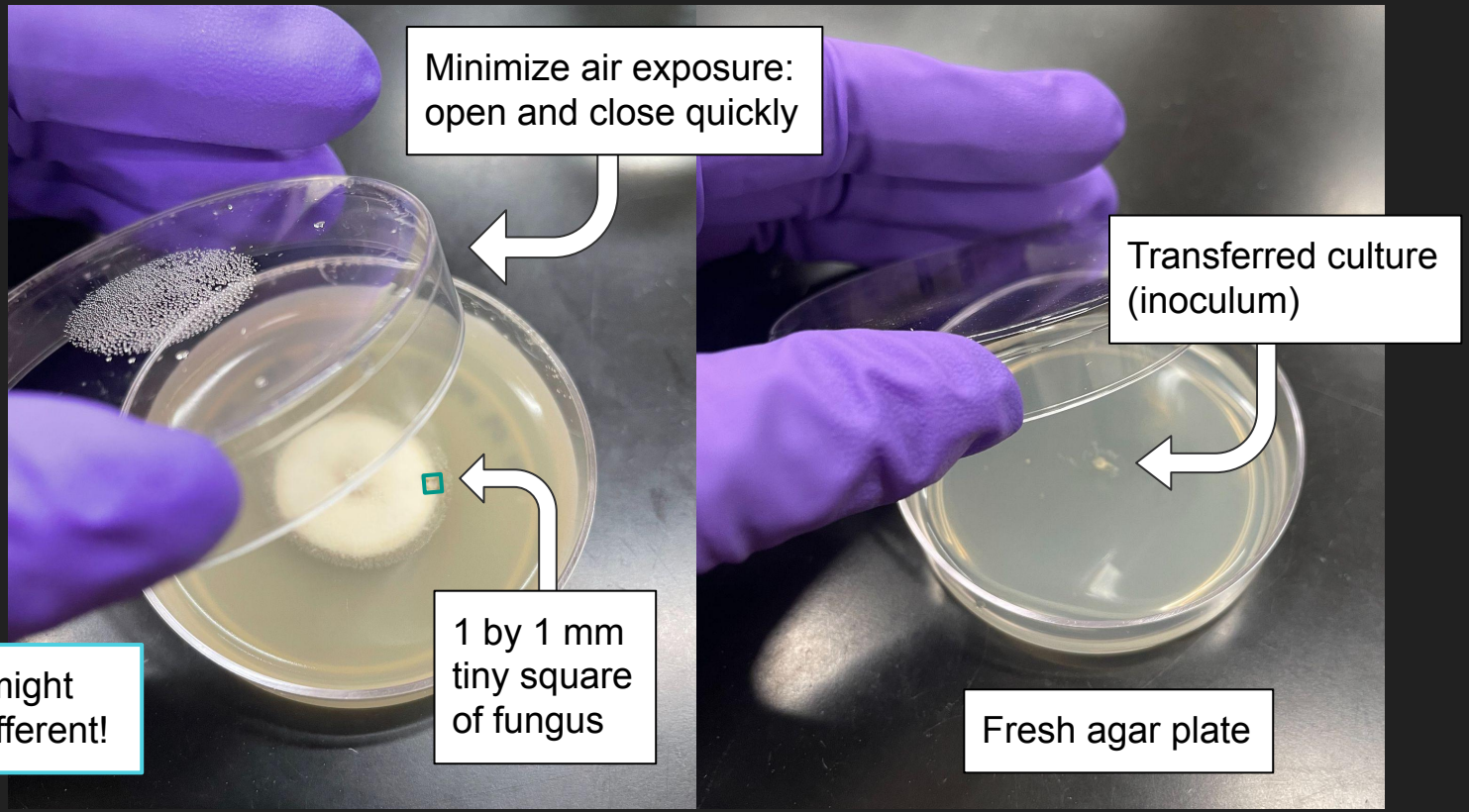
Part 2

Plate a leaf sample



Part 3

Isolate a pure culture



Your plate might look very different!

Part 4

Growth rate

Trace the outline of your fungal culture

Place the inoculum on agar near the edge of the plate

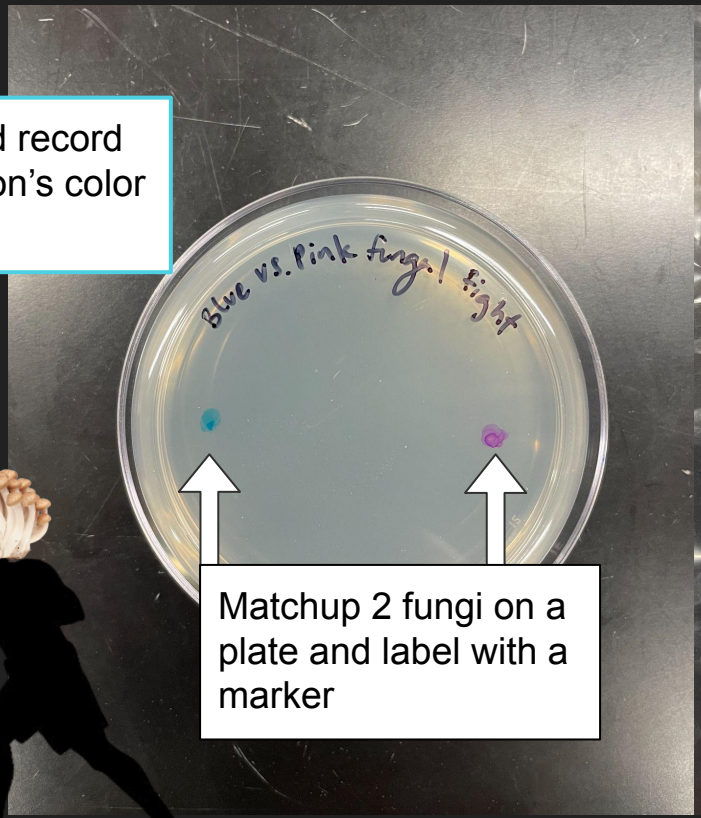
Measure and record: radius

Trace and measure again after 7 days

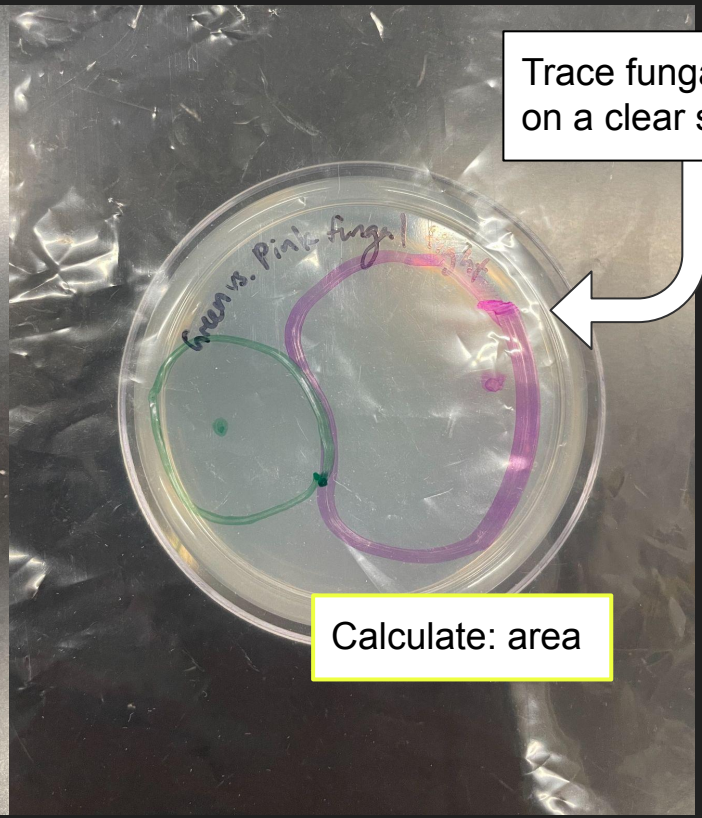
Part 5

Fungal fights

Assign and record each person's color (or shape)



Matchup 2 fungi on a plate and label with a marker

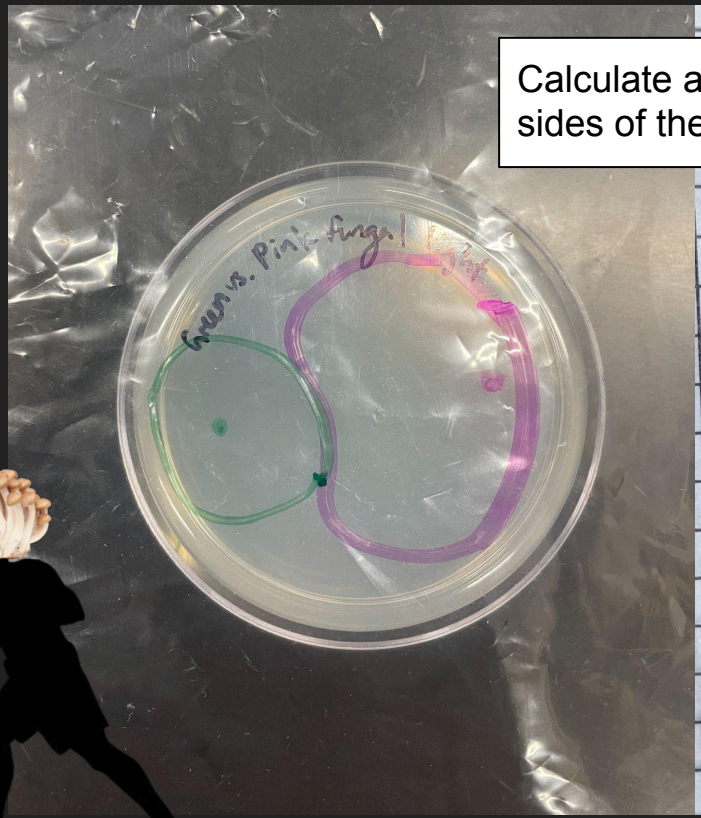


Trace fungal outlines on a clear sheet

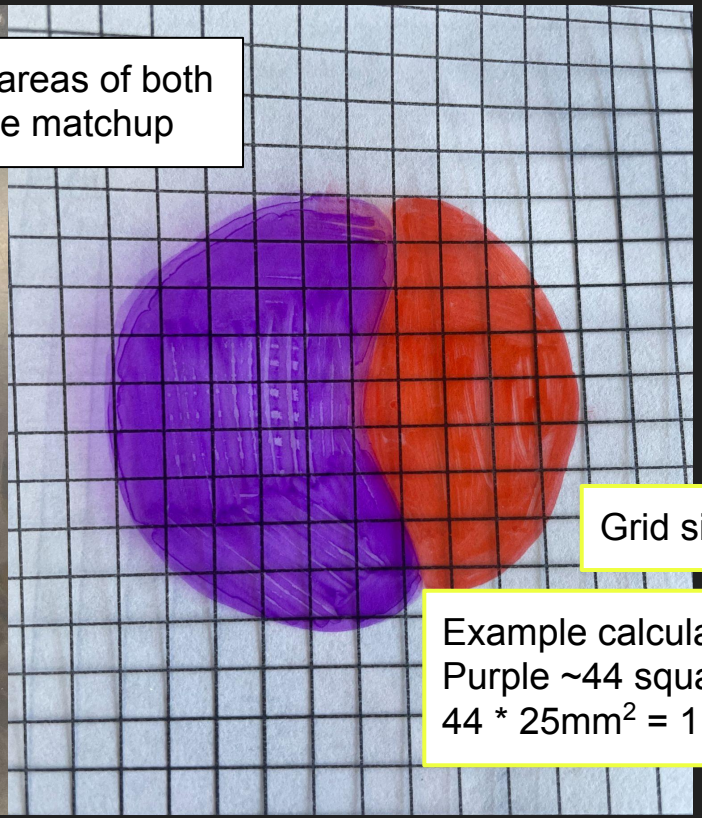
Calculate: area

Part 5

Fungal fights: calculating a winner



Calculate areas of both sides of the matchup

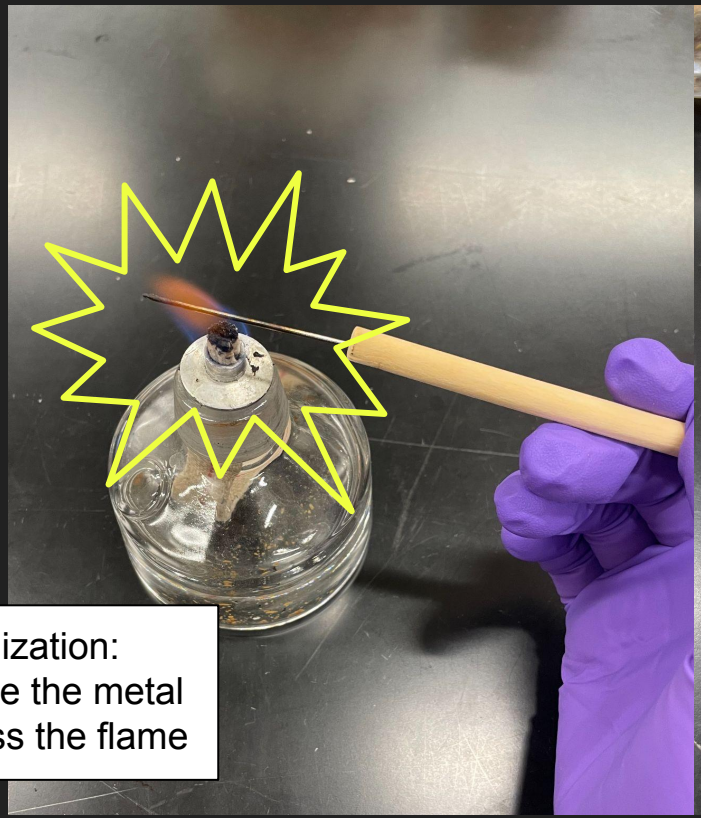


Grid size: 5mm

Example calculation
Purple ~44 squares filled
$44 * 25mm^2 = 1{,}100mm^2$

Part 6

Viewing your fungi under the microscope

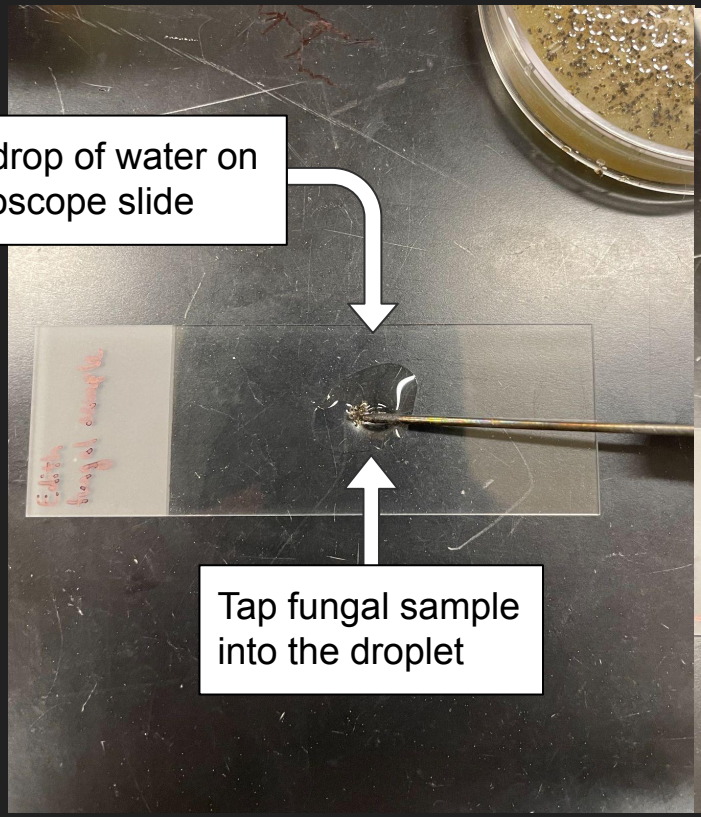


Flame sterilization: quickly wave the metal probe across the flame

Scoop up some fungus with the sterile probe (avoid the agar below)

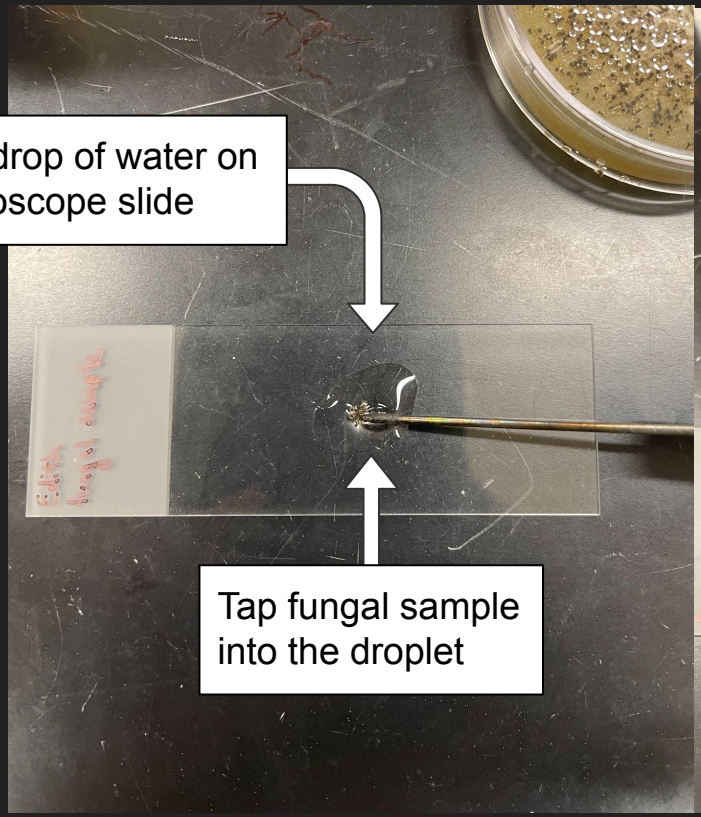
Part 6

Viewing your fungi under the microscope



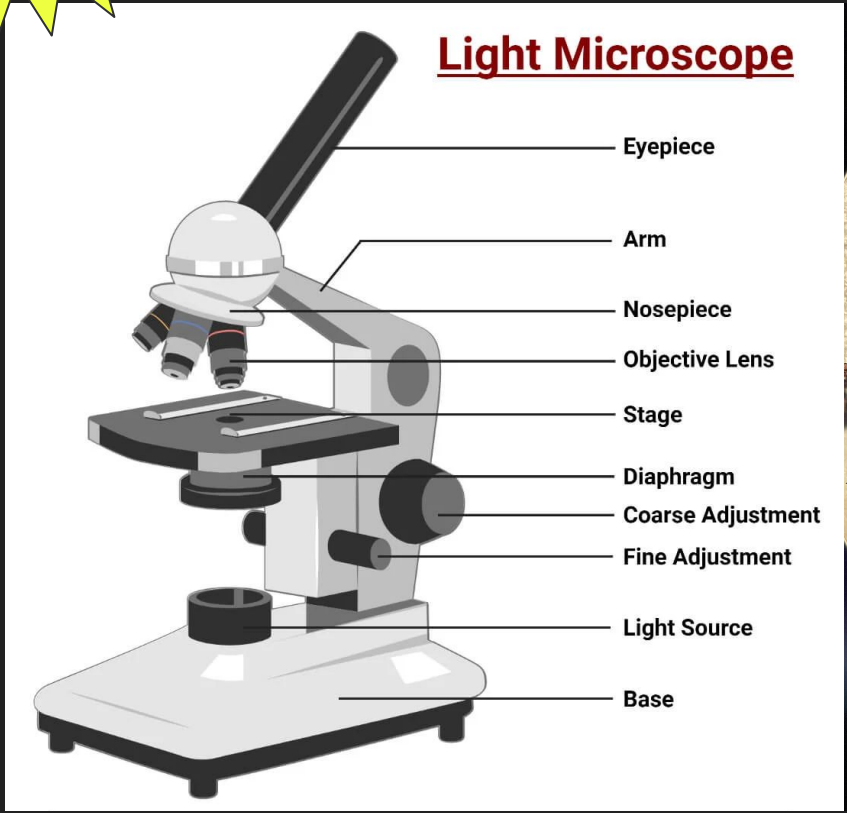
Part 6

Viewing your fungi under the microscope



Part 6

Viewing your fungi under the microscope



Distinguish between hyphae, spore producing structures, and spores