Fungi Workshop Curriculum

Fungi Biology Questions:

- What are fungi?
- How do fungi cells differ from plant and animal cells?
- How do fungi reproduce?

Fungi Environmental Questions:

- How do fungi function in natural ecosystems?
- How do fungi interact with plants and animals?
- How do fungi function in agricultural systems?

Sterile Environment Laboratory Safety and Standards:

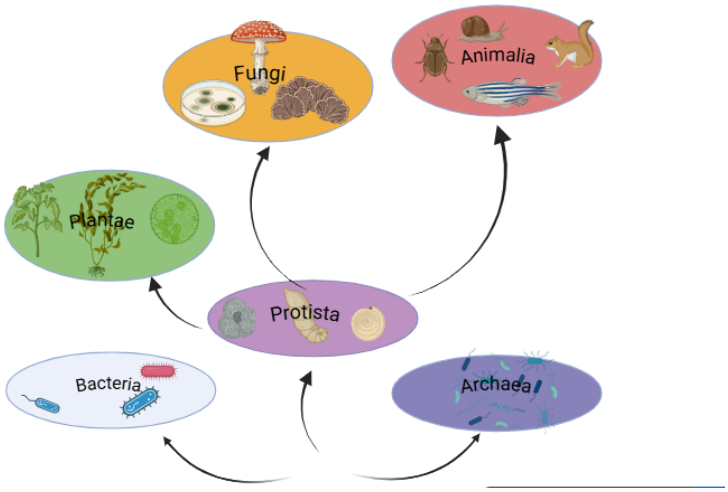
- What are the basics of mushroom cultivation?
- What are the laboratory safety standards in mushroom cultivation?

Objectives:

- Educate hobbyists on the basics of fungi biology.
- Educate hobbyists on the basics of how fungi function in our world.
- Educate hobbyists on the basics of sterile environment lab work and mushroom cultivation.

Lesson Notes:

What are fungi?



- **"Fungus"** is an umbrella term. When most people think of fungi, they think of mushrooms.The label of "fungus" encompasses millions of species of **mushrooms**, **molds**, and **yeasts**. The mushrooms that we eat on pizza are biologically the same kingdom as the mold that grows on the old crust, or the yeast that raises the dough. We

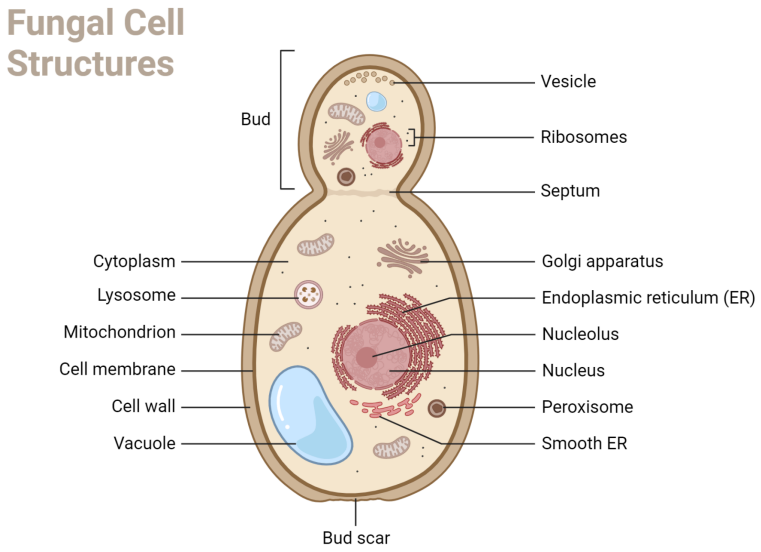
eat fungi every day, even if we aren't aware of it. Fungi range massively in size, from the giant puffball mushroom to the tiny single-celled yeast.

- **Fungi** are classified as a kingdom of life -- like plants and animals. Fungi are more closely related to animals than plants, despite the fact that their stationary lifestyles are so similar to that of plants. Fungi and animals both ingest nutrients rather than create their own nutrients like plants do.

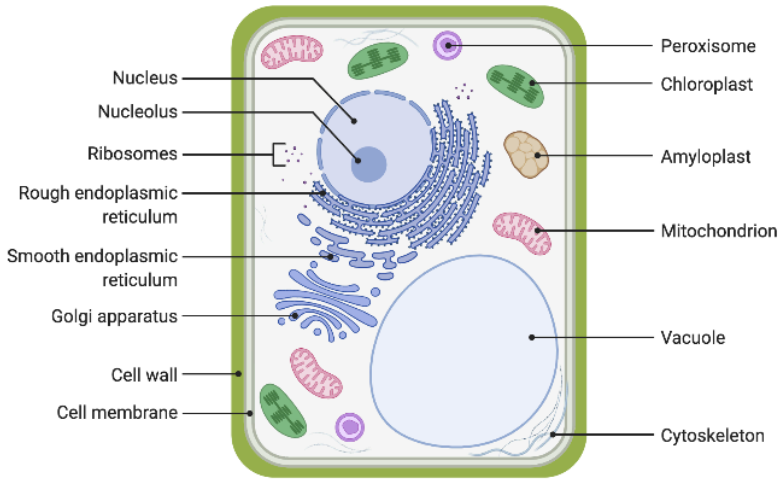
- Fungi are incredibly common in our world, but we just don't always see them. They're everywhere--under the soil, in the bark of the tree in your backyard. Yeast spores are also incredibly common in the air, we breathe them in daily. They aren't harmful. In fact, wild yeast in food and drinks is what creates fermentation, like in kimchi or kombucha. Yeast cells ingest sugars and produce alcohol and carbon dioxide. Cultivated yeast, like what you buy in the store, are better at surviving in higher alcohol contents. That's why there's beer yeast vs wine yeast vs bread yeast.

- There are two main classes of fungi-- **higher fungi** and **lower fungi**. This is a very informal form of classification, but essentially, **higher fungi** usually form large **fruiting bodies**, known as mushrooms. Higher fungi include mushrooms and some **slime molds** (which are a more rudimentary form of mushroom, essentially). The term **lower fungi** encompasses molds and yeast, which are mainly microscopic. Lower fungi also have different cellular structures, which I will briefly touch on. I will be focusing mostly on higher fungi for the rest of this workshop, but I will answer questions about lower fungi if anyone has any.

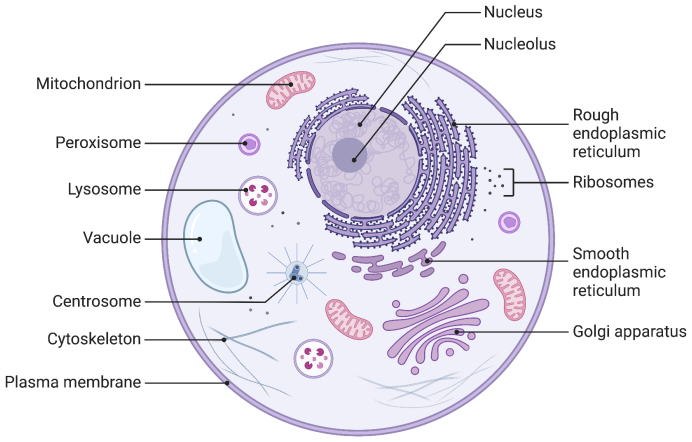
How do fungi cells differ from plant and animal cells?



Plant Cell



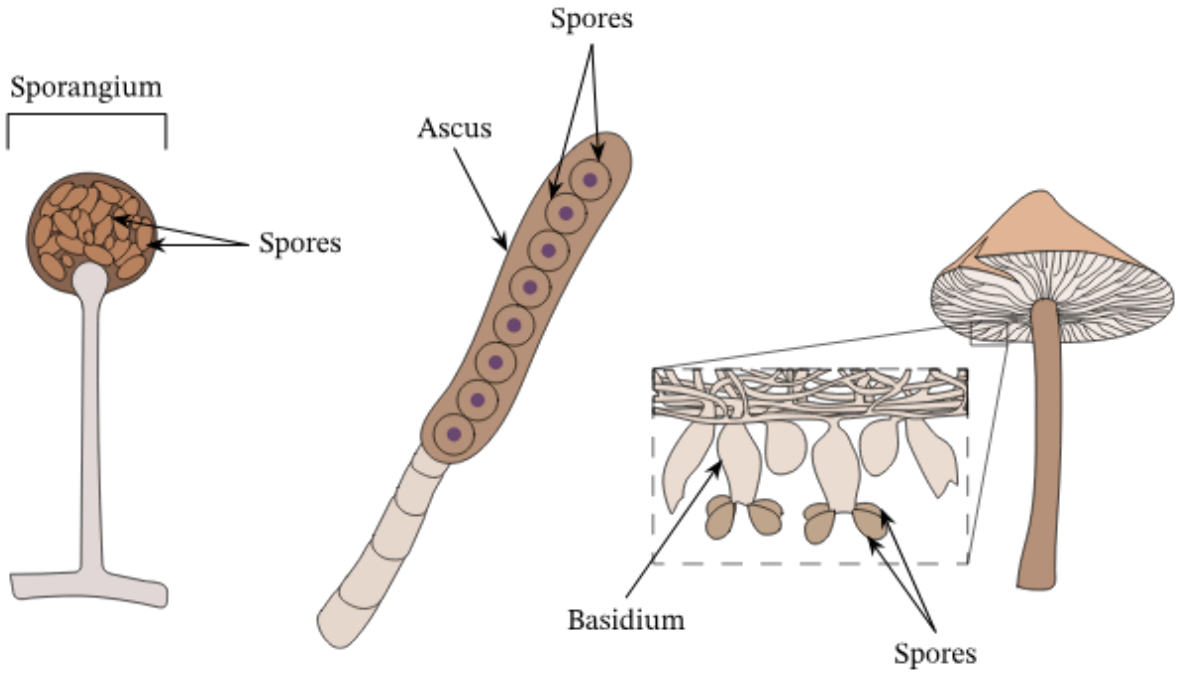
Structural Overview of an Animal Cell



- Fungal cells are structurally very different from plant and animal cells. They produce "**buds**", genetically identical cells that grow from the mother cell. This allows fungi to reproduce asexually. This is the biggest difference. Internally, fungal cells look very similar to animal cells. But fungi have cell walls, similar to plants.

- Fungal cell walls contain **chitin**, a strong compound that gives fungal cells their structure. This is the same compound that is found in insect exoskeletons.

How do fungi reproduce?



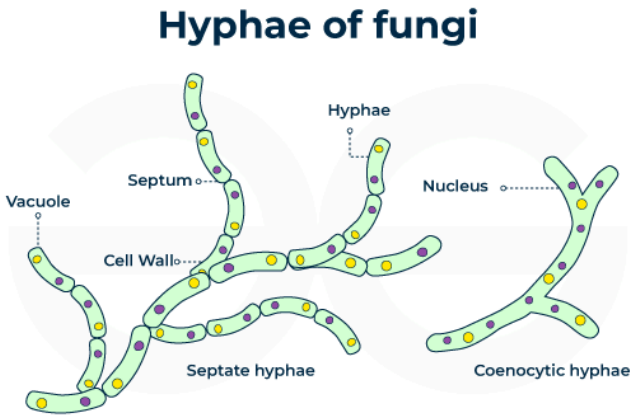
- Fungi reproduce both **sexually** and **asexually.** Some fungi species reproduce exclusively via sexual reproduction, some produce exclusively via asexual reproduction, some reproduce with both. **Asexual** reproduction occurs when the fungi mates with itself, producing spores. This is not an active process, like in animals. Two haploid cells (sex cells, think sperm and egg cells, which each contain exactly half the amount of DNA as a normal cell) merge to create a **spore** cell. The asexually produced **spore** cell is genetically identical to the parent fungus. **Sexual** reproduction occurs when a fungus mates with another fungus of the same species, and the same process occurs. The **spore** cell is genetically half of each parent fungus.

- Higher fungi produce spores through **fruiting bodies**, i.e. mushrooms. They only produce fruiting bodies when they have the ideal conditions-- usually a warm, moist environment. Some fungi species prefer the cold. But high humidity is essential in mushroom production. Mushrooms are essentially fruit. Underneath the cap, in the gills, are microscopic structures called **sporangia**. These are what form the spores. Usually hundreds to thousands of spores form per mating event.

- Fungal spores are single cells that can travel and create a whole new fungal system. Most are **immotile** (they can't move), but some lower fungi spores have **flagella** (little tails that allow them to swim). Spore cells are usually airborne.

- Fungal spores are usually not dangerous in the air, but some molds produce spores that can damage human lungs. Some people are more sensitive to this form of irritation.

How do fungi function in natural ecosystems?



- Fungi exist primarily in their **asexual** state, as **mycelium**, a network of root-like structures that usually exist underground or within plants.

- Mycelium “roots” are called **hyphae.** There are two forms of hyphae, the form on the left shows the structure of higher fungi hyphae. The form on the right shows the structure of lower fungi hyphae.

- Hyphae structures are actually also present all throughout the fruiting bodies of mushrooms themselves-- almost like veins.

- Anytime you lift up a log and see white tendrils on the soil -- that is a fungus growing in its asexual form. I recommend doing this next time you’re in the woods! The hyphae are probably also growing all throughout the log, since that is what they eat!

How do fungi interact with plants and animals?

- Fungi are all **decomposers** **--** meaning they break down nutrients and absorb them on a molecular level. Fungi do not *eat*, instead they release enzymes and acids through their hyphae, which breaks down organic material into base nutrients that they are able to absorb into their cells.

- Fungi play a key role in our ecosystem. The vast majority of higher fungi have a symbiotic relationship with plants. They “colonize” the roots of trees and vegetative plants, their hyphae growing interwoven with the roots. Hyphae are much thinner than plant roots, so fungi are able to be in contact with even more nutrients in the soil than plants. They break down the nutrients to a form that plants are able to absorb-- and in turn, the plants provide the fungi with carbohydrates, which fungi cannot produce on their own. Fungi that provide this service to plants are called **mycorrhizae.** Phosphorus absorption in plants would be close to impossible without fungi.

- Without fungi, plants would not be able to access key nutrients in the soil. The vast majority of dead plant material would also just sit, without returning to the soil.

- New research also shows that fungi allow plants to communicate with each other. Underneath the forest floor, there is a vast web of **mycelium** that connects every single plant with one another. Research shows that plants send simple signals to each other in the form of electric currents that run along the mycelium network.

- Fungi also provide services to animals, mostly in the form of food. Fungi are a complete protein and are a good source of selenium and phosphorus. They are a key component of many little creatures’ diets, like insects and nematodes. As mentioned before, they also allow us to ferment our food!

How do fungi function in agricultural ecosystems?

- They are essential in nutrient uptake, but they also provide fields with good soil structure. Good soil has structure in the form of aggregates (which we learned about in our last workshop on soil health!) and fungi provide just that, with their own mycelial network and with the organic material that fungi help to break down.

- Fungi are also key to many **bioremediation** methods. This is the act of removing harmful substances from soil. Studies have found that some fungi species are able to break down lead particles to a chemical form that is not harmful to plants or animals.

What are the basics of mushroom cultivation?

- Mushrooms are a great food source. Mushroom cultivation can be broken down into three main steps:

    1. **Inoculum Prep**
        - Mushroom inoculum is a **culture** (a sterile liquid or gel form of nutrient) of fungal **spores** or **hyphae**. You have to have a completely sterile environment to create mushroom inoculum, and it is actually quite hard to do. You need either a **laminar flow hood** (a sterile box with sterile air that

constantly blows outward) or a **still hood** (a sterile box with two holes for your arms).

- The environment needs to be sterile at ***all times***. Anything that goes in needs to be sanitized with 90% isopropyl alcohol. Any metal equipment that is being used should be dipped in isopropyl and then touched quickly to a flame- until the alcohol burns away. The flame technique can **only be used in a laminar flow hood and should not be used in a still hood.** It is the best method for decontamination, though, so that is why a laminar flow hood is recommended.
- Culture plates or tubes should be prepared with a recipe, essentially. It has all the nutrients that fungi need to live, in an agar gel. All plates or tubes need to be sealed and then sanitized in a pressure cooker (or a dishwasher on "sanitize" works too, if your cultures are completely watertight).
- In a sterile environment, a small square of mushroom **gills** (the underside where spores are produced) should be cut and then added to the plate or tube. This will allow the spores to colonize the plate or tube. You'll know it's ready when the mycelium starts to grow on the agar gel. Liquid inoculum, like we have today, consists of spores suspended in a sanitary liquid.

2. **Grain Spawn Prep and Inoculation**
    - **Grain spawn** is basically a substrate for mushroom mycelium to feed on and flourish. It consists of literally just grains. Rye and barley are common grain spawns, as are wheat or sorghum.
    - Grain spawn needs to be sanitized in the same way that culture plates or tubes are sanitized: in a pressure cooker. In a laboratory setting, we use **autoclaves** to sanitize, which are basically massive pressure cookers. Grain spawn can be sanitized in plastic bags, like you see today, or in mason jars.
    - Inoculation of the grain spawn needs to happen in a sterile environment, much like the inoculum prep. That is what we will do today. The inoculum needle needs to be inserted into the inoculation port. **The syringes contain 10 cc's of liquid. Each grain spawn bag only needs 1 cc of liquid.** The ratio is 1 cc to 2 pounds of grain spawn.

3. **Mycelium growth and mushroom harvest**
    - You'll get to watch the mycelium hyphae grow in real time. It could take a while-- be patient. Any mold growing in the bag means that the bag has been contaminated and needs to be tossed. The mycelium grows slowly in cold temps and quickly in higher temps. On a shelf in a dark place is the best place for quick mycelium growth. The fridge is a good place for slow growth.

- When the spawn bag is ***fully colonized,*** there will be white mycelium covering all of the grain. That is when it is ready to start fruiting. Mushrooms can be grown on grain spawn itself or inoculated grain spawn can be scattered in straw or over soil, to allow the mycelium to grow even more mushrooms.
- To grow in the bag, cut an **X** on the side of the bag without the inoculation port. Soak the grain spawn in water. Put the bag in a sunny, warm place with high humidity and mist with water every day. You'll see mushroom **pins** start to grow soon. Keep misting daily until harvest.

What are the laboratory safety standards in mushroom cultivation?

- **NO** food or drink anywhere near lab equipment at any time. This is a big one.

- When working with the flame technique, as mentioned above, protective eyewear is essential. We won't need them today, because we will not be using the flame technique to sanitize our equipment.

- Close-toed shoes and long pants (with no holes) are other common safety standards, but we aren't working with anything dangerous enough to warrant that today.

- To avoid contamination, it is vital that you **never** breathe on your cultures or grain spawn in the hood or still box. A mask is recommended to avoid this, but there is a common practice of keeping your head up while looking down so that your breath is never angled at your work. This is less necessary with a still box.

- **Always** wear gloves when working in the hood or still box. This is mostly to protect your hands. I have worked in a hood with no gloves, and spraying my hands constantly with isopropyl alcohol really dried them out!

- Be careful with the needles that we'll be using today. Ensure that you do not poke yourself.

- All equipment in the still box **stays in the still box.** Do not take anything out.

- Anything going into the still box **always** needs to be sanitized thoroughly with isopropyl alcohol. This includes your hands and arms! Anytime you take them out, they need to be sanitized again.

- Dip needles in the beaker of isopropyl provided **before and after** inoculation. Wipe with a provided chemwipe afterwards. **Do not take needles or tubes out of the still box.**

Vocab List:

Fungus- Or the plural **fungi**, is an umbrella term for any mushroom, mold, or yeast in the Fungi Kingdom of Life.

Higher Fungi- An informal grouping of fungi that consists of more complex species that usually produce **fruiting bodies** that are visible with the naked eye. Higher fungi also have **hyphae** with individual cells, otherwise known as **septate hyphae.**

Lower Fungi- An informal grouping of fungi that consists of simplistic species that are smaller than higher fungi. They also have **hyphae** with conjoined cells, otherwise known as **coenocytic fungi.**

Buds- A small, immature fungal cell that forms at the tip of a mature and fully formed mother fungal cell. It is genetically identical to the mother cell.

Chitin- An organic chemical that is present in the fungal cell wall. Its molecular structure makes it quite rigid in large quantities, thus it provides structure in the cell wall. It is also present in insect exoskeletons.

Sexual Reproduction- The production of a spore cell between two parent fungi. The spore cell is genetically half of each parent cell. This occurs when two parent hyphae find each other and merge.

Asexual Reproduction- When a fungus mates with itself and produces a spore cell. The spore cell is genetically identical to the parent fungus. This occurs when the correct conditions occur.

Spore- A single cell, essentially the fungal equivalent of a seed. A single spore can grow a whole new mycelial network.

Fruiting Bodies- What we know as mushrooms! This is where the spores of higher fungi are formed.

Sporangia- Structures on the underside of mushroom caps that produce spores.

Flagella- The little tails that allow spore cells to swim.

Mycelium- The term for a system of fungal hyphae. Usually white in color and can be insanely expansive- the largest living organism in the world is a mycelium in a forest in Oregon. It is estimated to weigh 35,000 tons and encompasses 3.5 square miles.

Hyphae- The “roots” of fungi. It is what fungi are, essentially. Hyphae flow all throughout mushrooms as well, like veins.

Decomposers- This is a term that encompasses fungi and bacteria, as well as many other microscopic organisms. Detritivores are also decomposers, but not all decomposers are detritivores.

Mycorrhizae- Fungi with a symbiotic relationship with plants.

Bioremediation- A method of cleansing toxins from soil using living organisms.

Culture- A technique of growing cells or organisms on artificial media.

Grain Spawn- The media that cultivated mushrooms are grown on. It includes any grain that mushrooms like to decompose. The grain is rich in nutrients that allows mycelium to flourish.