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# K-12 Partnership Lesson Plan

# Emily Grman

# *Invisible Friends Underground*

## Overview

In an inquiry‐based activity, students will learn about mycorrhizal fungi and how to find them by collecting roots, staining them with ink and vinegar, and looking for fungi under the microscope.

**Objectives**

At the conclusion of the lesson, students will be able to:

* Use a microscope
* Prepare wet-mount samples
* Understand one example of a mutualistic interaction and how it can change depending on the environment
* Formulate research questions
* Make predictions about what will affect the abundance of a specific type of organism and collect data to test their predictions
* Make and interpret graphs of data

**Length of Lesson**

One 1-hour class period for learning about fungi and sampling roots

One 1-hour class period for staining roots

One or two 1-hour class periods (or longer) for looking at roots under the microscope, collecting data, making graphs, and discussing the results

**Grade Levels**

6th-college, with varying levels of student independence

**Standards covered (NGSS)**

Disciplinary Core Ideas:

* **MS-LS1-1**: conduct an investigation to provide evidence that living things are made of cells; either one cell or many different numbers and types of cells
* **MS**-**LS2-1**: analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem
* **MS-LS2-2**: construct an explanation that predict patterns of interactions among organisms across multiple ecosystems
* **MS-LS1-5**: construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms

Cross Cutting Concepts:

* Structure and function

Science and Engineering Practices

* Asking questions and defining problems
* Planning and carrying out investigations
* Analyzing and interpreting data

**Materials**

* Microscopes capable of 100x magnification
* A hot plate or two
* Balance to weight out KOH
* Graduated cylinder capable of measuring 25 mL
* Safety goggles
* KOH pellets
* Forceps/tweezers
* Tissue cassettes
* Schaeffer black ink
* White household vinegar
* Eye droppers
* Microscope slides
* Plastic cover slips
* pH strips
* Slotted kitchen spoon
* Plastic cups
* Two 1L pyrex beakers
* Trowels for collecting root samples
* Ziplock bags for collecting root samples
* Student worksheet
* Photo guide and powerpoint

**Background**

Mycorrhizal (pronounced “MY‐CO‐RISE‐AL”) fungi are a specific kind of fungus that do not decompose (at least not very much) and do not make mushrooms. They also do not spread disease or rot food. But they do something really fascinating—they form a mutualism with the majority of land‐plant species on the planet. There are at least four types of mycorrhizal fungi. We are dealing with the most common kind in this part of the world, arbuscular mycorrhizal fungi. These fungi associate with the roots of grasses, flowers, and some trees and shrubs. The fungi have long, very thin root‐like structures called hyphae (“HY‐FEE”) that grow out into the soil and take up nutrients. They are better at doing this than plants, because fungal hyphae are thinner than plant roots. The fungi also grow into plant roots, but they don’t damage root cells or cause disease. Instead, the fungi give away these nutrients to plants that need them—but not for free. Fungi need sugars from plants. Scientists think that this type of fungi can *only* get sugar from plants, but scientists are actively researching whether there may be some other kind of food the fungus can use. So in exchange for the nutrients the plant desperately needs, the plant gives away sugar. Most plants have an abundance of sugar, fixed through photosynthesis. So most of the time, this trade of nutrient‐for‐sugar is beneficial for both the plant and the fungus. Therefore, it’s a considered a mutualism. This mutualism is critically important for plant growth and survival. In fact, it may have enabled the first plants to colonize land.

However, things are not always so straightforward. Sometimes plants have plenty of nutrients (for example, in a fertilized yard or garden), so they don’t need the extra help from the fungus. What happens then? Or, sometimes plants don’t have so much extra sugar, for example if grown in the dark. Will the plant stop giving away sugar and stop letting the fungus grow into its roots? Or will the fungus steal sugar and parasitize the plant? My dissertation research investigated this question. Many other differences among habitats could cause differences in the amount of fungi inside plant roots. For example, fungal hyphae are very fragile and get broken up when the soil is tilled. Tilled or disturbed soil might have fewer fungi. Different kinds of plants might be more or less dependent on fungi. For example, plants that typically live in disturbed soils (e.g., weeds or annual plants) might not interact with fungi as much as plants that typically live in undisturbed habitats. Additionally, scientists think that some plants might be better at taking up nutrients by themselves than other plants—for example if they have very fine roots with lots of absorptive surface. These plants might have less need for mycorrhizal fungi, so they might not have as many fungi in their roots.

You and your students can also study this question—by collecting roots from plants growing around the schoolyard, in the schoolyard biofuels plots, or near the students’ homes. Or, you can conduct experiments growing plants in the classroom and see if you can predict what kinds of conditions or what kinds of soil will get you the most fungi, or the fewest fungi. It is easy to grow mycorrhizal fungi in experiments—just make sure you include at least 2‐3 tablespoons of topsoil in the pot when you plant the seeds. Commercial inoculum sources of fungi are also available—in fact some garden supply stores are convinced they will improve plant growth. Scientific studies have not always shown this to be the case—sometimes fungi from natural soil are just as good or better for plant growth than purchased inoculum. However, most kinds of potting soil do not have mycorrhizal fungi in them. In experiments in the classroom, you can compare the amount of fungi in the roots of different kinds of plants or in the roots of plants raised in different kinds of environments. You can compare the abundance of fungi inside roots grown in soil from different habitats.

This activity is designed to provide you with the background information, skills, and tools to explore a variety of questions about mycorrhizal ecology. There are an infinite number of active avenues of research on the interactions between plants and mycorrhizal fungi. I have suggested lots of interesting, testable hypotheses in the previous two paragraphs and I encourage you and your students to brainstorm some more. The activity is appropriate for a wide range of student ages. I have suggested ways to modify the activity according to student age, and teachers should feel free to simplify or expand according to the needs and interests of their students.

### Activities of the session

1. Review the background information on the ecology of arbuscular mycorrhizal fungi using the powerpoint.
2. Decide on a research question or comparison you would like to make. Examples of questions students could ask: Do invasive plant species have fewer fungi in their roots than native plants? Do annual plants have fewer fungi than perennials? Do plants grown with different sources of fungi (e.g. commercial inoculum vs. topsoil, garden soil vs. soil from a natural area) have different amounts of fungi in their roots? Do plants grown in the shade have different amounts of fungi than plants grown in full sunlight?
   1. For older students (e.g. college age), students can break into groups of 3‐4 and each group can work on their own question.
   2. For younger students, the entire class could focus on a single question that they brainstorm in class discussion. The class can break into groups of 3‐4 for sample collection and labwork.
3. Design an experiment or sampling scheme to answer the question. Keep the design simple, and make only one or two comparisons (except for very advanced students). Keep in mind general rules of experimental design and be sure to replicate (collect at least 5 and preferably more identical samples for each comparison).
4. Go in the field to collect samples! Use the trowels provided to dig up plant roots and place each sample in a labeled ziplock bag. You will need only a few roots from each plant to analyze for fungi.
   1. Older students should make sure they collect independent replicates for each comparison.
   2. For younger students who are working as a class to answer a single question, each group should collect 1‐2 samples of each sample type so that the full range of samples are present in each student group.
5. Bring the samples back into the lab for analysis. This procedure is detailed below in the Lab protocol section, and should be modified based on the maturity of the students. Boiling KOH is very caustic and can cause irreversible injury to soft tissues such as eyes, nasal passages, and skin.
   1. College age students should be capable of safely and responsibly using the chemical, following the safety rules outlined below. It might be best to set up stations in the classroom with hotplates and have student groups rotate through the stations as they are ready.
   2. For younger students, it may be best for teachers to perform steps involving boiling KOH and the ink/vinegar solution.
6. Using the microscopes, look at the stained roots and quantify fungi. To identify fungi inside plant roots, refer to the handouts with labeled pictures of fungi. There are also many web resources with additional pictures. The INVAM website (http://invam.caf.wvu.edu) is particularly good; look for pictures of different fungal species by clicking on The Fungi, then Species ID, then the individual species names. To quantify fungi, have the students haphazardly choose a single root and determine whether fungi are present or absent at a single place in the root that intersects the micrometer in the eyepiece. Record, using a tally sheet, whether fungi were present or absent at that single location in the root. Repeat this for many randomly selected root segments, preferably at least 30 per root sample. Then calculate the percent of root segments with fungi present for each sample (this is the percent of root system colonized by fungi).
   1. For more advanced students, this can be an opportunity to discuss the importance of random sampling. (How can we randomly select locations in the root sample? What would happen to our estimates of fungal abundance if we did not select locations randomly?)
   2. For younger students, this level of quantification may be difficult, and students can simply visually estimate the percent of root system with fungi in them, or rank samples in order of fungal abundance. The sample with the fewest fungi should be given a rank of 1 and the sample with the most fungi should be given the highest rank. If students are working in groups, each group can rank their samples independently of other groups.
7. Plot the data in a graph. Depending on the type of data students collected, it may make the most sense to plot the average percent root colonization with a bar graph.
   1. More advanced students should also plot error bars indicating some measure of variation within each category, such as standard deviation.
   2. For younger students, compile the sample ranks from all the student groupson the blackboard. You can average the ranks of all the samples within acategory and make a bar graph with those averages. The class should work together to analyze and plot the same dataset.
8. Discuss the results. Did they support your prediction? Why or why not? Are there follow‐up experiments you would like to do?
   1. If time permits, older students can make brief (5 min) presentations to the rest of the class summarizing their question, prediction, results, and explanation.
   2. Younger students should discuss reasons why replication is necessary. What would have happened if we only collected one plant of each type?

**Safe use of boiling KOH and ink/vinegar solutions:**

* Be aware of the location of eyewash stations.
* If a vent hood is available, set up hot plates there. If a hood is not available, avoid breathing fumes and minimize the time solutions are boiling.
* Wear eye protection (goggles) and gloves when working with solutions. Dish gloves or latex gloves work.
* Be careful not to splash: these solutions can damage clothing and injure skin.

**Lab protocol (preparing roots for microscope):**

1. Remove roots from soil samples with forceps and put in tissue cassettes.
2. Use pencil to label the smooth slanted edge of cassettes with sample name or number.
3. Put cassettes in plastic cups of water and rinse 3‐4 times.
4. Mix 2.5% KOH solution (12.5 g KOH powder to 500mL water) and bring to a boil on a hot plate in the 1L beaker. If you have 30 or fewer cassettes of roots, you can do them all in one batch. If you have more than 30 cassettes, you will probably need to do them in batches of less than 30.
5. Carefully place tissue cassettes in boiling KOH and boil for 3‐5 minutes.
6. Remove the cassettes from KOH using the slotted kitchen spoon. Save the KOH— you will need to neutralize it with vinegar (an acid) before disposing of it (instructions below). Put the cassettes in a plastic cup full of tap water.
7. Rinse roots several times in tap water.
8. Mix 5% ink/vinegar staining solution in the second 1L beaker (25 mL Schaeffer black ink to 500mL vinegar) and bring to boil on hot plate.
9. Put cassettes in boiling staining solution for 3 minutes. Again, if you have more than 30 cassettes, you will need to do them in batches.
10. Using the slotted kitchen spoon, remove the cassettes from the stain and soak in a plastic cup of water with a few drops of vinegar for 15 minutes.
11. If necessary, you can store cassettes containing roots in tap water overnight. If you will be storing them for longer than a day or two, put them in tap water in the fridge. They will keep for a few months.
12. To view roots and mycorrhizal fungi under the microscope, remove the roots from the cassettes with tweezers. Use the tweezers to spread the roots on a microscope slide with a few drops of water. Cover with a plastic cover slip and view at 40x or 100x.

**Disposal information:**

* KOH solution: needs to be neutralized by adding vinegar (an acid). You can wait until after class to do this. SLOWLY add approximately 250mL vinegar to the 500mL KOH in your 1L beaker. Gently stir with the kitchen spoon, being careful not to splatter. After a minute or two, test the pH with the pH strips. If the pH is still above 10, add additional acid (~15‐20mL at a time) to the beaker. After each addition, stir, wait a minute or two, and then test the pH. The closer to neutral (7) you can get, the better. It will take approximately 300mL of vinegar to neutralize 500mL of KOH, but it is best to add the last 50mL slowly to be sure you don’t overshoot neutral. After neutralizing, the solution can be flushed down the drain with lots of water.
* Ink/vinegar staining solution: can be flushed down the drain with lots of water as is.
* Stained roots—can be thrown in the trash.
* Cassettes are re‐usable, simply rinse with soapy water and let air‐dry.

**Resources**

* Photo key and student worksheet found on the “Invisible Friend Underground” lesson page on the KBS GK-12 website
* INVAM website: http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm. Click on the name of a genus of fungi to see lots of photos of what the fungi look like inside roots.
* Feel free to contact me for electronic files or with any questions about how to do this activity or adapt it for your students: grmanemi@msu.edu