

# Mycorrhizal Training class

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## Daily Class Schedule:

### Day one - Laboratory and Lectures

1. Welcome introduction and sampling
2. Lectures on mycorrhizae include:
  - An Overview
  - Development
  - Impacts on plants and soils
  - Functions in the environment
  - Variations
  - Identifications and quantifications
  - Inoculum production

### Day Two - Mycorrhizal Laboratory

1. Spore extractions
2. Spore quantifications
3. Root clearing and staining
4. Root colonization test
5. Inoculum Potential (MIP) test

### Day Three - Mycorrhizal Laboratory

1. Microscopic observation
2. Spore morphological identification
3. Inoculum Production
4. General Discussion

# How to select the right class for you?

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Mycorrhizal training is offered in three formats; on-demand (year around), ZOOM Meeting (three classes a year), and On-Campus class (once a year) as in this link <https://ifas-mycorrhizae.catalog.instructure.com/> . In all three formats, the online materials will be open for you six months after finishing the class. During the on-campus class, field sampling is in the first day or you can bring your own samples to work on. The other two formats (on-demand and zoom classes), you can finish the class, then do your own sampling to practice on. Help with your mycorrhizal project can be provided through email or one-hour zoom meeting that can be arranged in advance.

It is recommended to go through the needed materials to select the right class. If you have the materials and find instructions and videos are enough for, on-demand class could be the right one for you. If you have the materials and need instructions for lectures and laboratory protocols, the ZOOM class could be right for you. If you find in person and hand-on-experience is needed and you do not have the needed materials, then on-campus class could be the right one for you.

# MATERIALS

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## Sampling:

1. [Gloves](#)
2. [Tools to collect soil](#)
3. [Sample Bags](#)
4. [Sieves](#)
5. [Balance](#)
6. [pH Meter](#)
7. [Oven to dry subsamples for soil moisture calculation](#)
8. [Aluminum Weigh Boat – suitable for oven](#)
9. [Spoon or spatula](#)

## Spores Extraction:

1. [Grinder](#)
2. [Balance](#)
3. [Spoon or spatula](#)
4. [Funnel](#)
5. [50 ml Centrifuge Tubes](#)
6. [Blender](#)
7. [Large beaker or pitcher](#)
8. [Two sieves, 1000  \$\mu\text{m}\$  upper and 45  \$\mu\text{m}\$  micron down](#)
9. [Centrifuge](#)
10. [Table Sugar](#) to make solution of 60% concentration
11. [Microscope slides](#)
12. [Microscope](#)
13. [Pipettes, Pasteur Pipettes, or graduated dropper to take 0.5 ml volume](#)

### Roots Clearing and Staining:

1. [Microscope slides](#)
2. [Microscope](#)
3. Mounting Media - Polyvinyl-Lacto Glycerol PVLG ([Polyvinyl Alcohol](#) 16.6 g, Glycerol 10 ml, Lactic Acid 100 ml, and Water 100 ml)
4. [Tissue Cassettes](#)
5. 10% [Potassium Hydroxide, KOH](#)
6. [Hot Plate](#), [Water Bath](#), or [Autoclave](#)
7. 30% [Household Bleach](#) or 30% [Hydrogen Peroxide](#)
8. 1% [Hydrochloric Acid](#) or [Household White Vinegar](#)
9. Stain can be one of the following:
  - a. 0.05% [Trypan Blue](#) (33 ml Water, 33 ml [Lactic Acid](#), 33 ml [Glycerol](#), and 0.5 g Trypan Blue),
  - b. 0.03% [chlorazol black E](#), CBE (33 ml Water, 33 ml [Lactic Acid](#), 33 ml [Glycerol](#), and 0.3 g CBE), or
  - c. 5% [Ink](#) in vinegar.

### Mycorrhizal Inoculum Potential Test:

1. Pots of uniform volume
2. 10 grams of Soil Inoculum or Mycorrhizal Inoculum
3. Pasteurized soil culture media of 1:1 ratio of soil to sand.
4. Surface sterilized seeds of model plant, sweet corn, the one used by most mycorrhizal research.
5. 30% household bleach
6. Greenhouse of average 25°C and 12 to 16 h of sunlight.
7. Fertilizer, 0.01 P and 0.1 of all other nutrient concentration

### Inoculum Production:

1. Medium - soil or soil-less mix (1:1 sand and vermiculite, pH 6 ± 0.5).
2. Balance nutrient supply (Hoagland's solution concentration of 0.1x all nutrients and 0.01x P)
3. Neutral host (*Zea mays* as the most plant used)
4. Best Environmental conditions (16-hour light and 25 °C temperature)
5. Sterilized seeds (Remove coated fungicides with water and soap)
6. Sterilized pot containers