

Exploring plants and soils: Mycorrhizal staining



· EXPLORE
SOILS ·

Summary:

Roughly 85% of vascular plants form partnerships with some form of arbuscular mycorrhiza. The fungus receives sugars from the plant as food and in return supplies the plant with phosphorus and water, using their extensive network of hyphae. These hyphae also build soil structure by binding the soil particles and producing sticky proteins. Knowledge of mycorrhizas is critical to understanding how plants interact with their environment and how they compete with each other. It is also a key concept in understanding the nutritional needs of plants and managing soil for the growth of plants.

Mycorrhizas are often taught using diagrams and microscope images shown in a presentation. Staining the mycorrhizal tissue within the plants roots allows you to easily see them with a compound microscope, and appreciate the structures (vesicles and arbuscules) the fungi form within the root tissues.

Learning Objectives:

- Seeing and understanding the different cell structures of plants and fungi
- Witnessing and gaining wider understanding of symbiotic relationships

Equipment:

- A selection of well established plant roots; grasses, tomato, cucumber etc.
- Old pan for boiling
- Glass bowl
- Cold water
- Potassium hydroxide at a concentration of 10% (wt/vol)
- White pickling vinegar
- Tweezers
- Measuring cylinder
- Compound microscope with x40 objective lens
- Pipette
- Glass slides
- Slide cover slips
- Shaeffer black permanent ink or Parkers/Quink Blue ink

Preparation:

- Estimated time 20 minutes.
- Gathering suitable mature plant roots
- Removal of soil from roots
- Potential need to mix Potassium hydroxide to correct concentration

Time Required:

- Introduction 10 mins, including safety
- Preparation if included, 15-20mins for gathering and cleaning materials
- Processing and staining root material 50 mins
- Examining and recording mycorrhiza ~20mins

Total timing 1 hour and 10 minutes.

Background Learning Needs:

- An understanding of what a symbiotic relationship is.
- Seen examples of what structures to find

Risk Assessment:

Hazard	Likelihood	Severity	Mitigation
Injury from working with boiling liquid/chemicals	Low	Medium/High	Use gloves when handling
Injury to eyes from chemicals	Low	Medium/High	Wear goggles if required
Site/local specific risks	Unknown	Unknown	Anyone running this activity is advised to conduct a risk assessment for the specific site and conditions

Description of Activities:

1. Place prepared roots in an old pan with the Potassium Hydroxide, the level of which is sufficient to cover the material.
2. Boil in the KOH for 5 minutes, however, thick roots such as bean roots may require 10 minutes.
3. Remove from pan and place in a mix of cold water and vinegar for 20 minutes.
4. Place root material in a bowl containing the staining mix (1:20 ink and vinegar) for 5 minutes.
5. Soak the root materials in a mixture of cold water and vinegar for a further 20 minutes to wash off the residual stain.
6. Rinse and place in cold water in a bowl.
7. Cut off small sections of the root and place on glass slide, use a pipet to add water and cover with a slide cover.
8. Place under microscope and move systematically along the length of the root, moving focus through the depth of the root searching for dark stained cells.
9. Record either via digital capture or drawing the stained cells that are located.
10. Discuss with others and compare to see if arbuscules have been found.

Extension:

Comparison: do different species of plants have different levels of mycorrhizal infection? Compare plants from differently managed soils - eg. organic and conventional farms.

Devise a fair way to record the extent of mycorrhizae in the roots - how could this be used to test hypotheses using statistical tests?
from the soil surface.



