Lab 6A: Microscopic Assessment of Mycorrhiza - Part 1

What can I expect to learn in lab today?

You will gain experience in assessing the degree of mycorrhizal infection of Western Wheatgrass (Agropyron smithii)

You will gain additional experience in basic techniques of microscopy

You will gain additional experience in basic statistical techniques used to correlate the data obtained in this lab with data obtained by the range management laboratory students

Assessment Pre-Test

The development of this lab was partially funded by the National Science Foundation (NSF). This lab is part of a larger project designed to help strengthen the observational skills of undergraduate science students. To help your instructor monitor the effectiveness of the lab please complete the pre-test on the following page. This test is used only for anonymous assessment purposes and has nothing to do with your grade for this lab or for the course. Simply answer the questions to the best of your ability. If you have no idea of the correct answer (cannot even make a likely guess) please select answer D, I do not know.

When you have completed the pre-test, please carefully tear it out of the lab manual and give it to your instructor. You must complete this pre-test and the regular pre-test at the end of the lab before beginning the lab.

Microscopic Assessment of Mycorrhiza

Pre-Test

1 What is mycorrhizae?	A beneficial association between soil fungi and plant roots B An organism that promotes Nitrogen fixation in crops C The fungus that causes root knot disease D I do not know
2 What is the purpose of utilizing an analysis of variance (ANOVA) to analyze experimental data?	A There is no purpose, ANOVA is a laboratory test, not a statistical test B To determine if there are statistically significant differences among experimental treatments C To exclude invalid data D I do not know
3 How does mycorrhizae affect nutrient uptake in plants?	A It promotes greater N fixation in crops and rangeland grasses B It generally enhances nutrient uptake C It generally decreases uptake of P and Zn in rangeland grasses D I do not know

4 What is meant by the term resolution in reference to microscopy?	A The sharpness of an image as it appears in the viewfinder B The maximum possible degree of magnification C The smallest distance between two features that can be seen as separate objects D I do not know
5 In what way can mycorrhizae contribute to more sustainable agricultural systems?	A By facilitating more efficient use of nutrients and water B By reducing dependence of synthetically manufactured fertilizers C Both A and B are correct statements D I do not know
6 Which of the following statements related to fungi are correct ?	A Most fungi are unicellular B Some fungi are involved in mutually beneficial relationships with other organisms C Neither A or B is correct D I do not know
7 How does mycorrhizae affect water uptake in plants?	A Mycorrhizae in soil reduces water uptake by plants B Mycorrhizae in soil can enhance drought resistance of plants C Mycorrhizae in soil is not related to water uptake by plants D I do not know

8 What is the purpose of regression in the analysis of data analysis?	A To examine historical trends in experimental data B To assess the potential for bias by researchers C To determine if the dependent variable in an experiment is linearly related to the independent variable or variables D I do not know
9 Which of the following statements correctly describes the process of bright field microscopy?	A The specimen to be viewed appears dark against a bright background B The specimen appears bright against a dark background C Neither A or B is correct D I do not know
10 Which of the following statements correctly describe procedures and/or equipment required for the assessment of mycorrhizae?	A Potassium chloride B Atomic Absorption spectrophotometer C Dissecting microscope D I do not know
11 Which of the following statements correctly describe the relationship between mycorrhizae and western wheatgrass?	A The portion of roots infected is estimated by a gridline method B The mycorrhiza are stained with either Tryptan blue or Tryptan green stain C Both A and B are correct statements D I do not know

12 Which of the following statistical tests can be used to assess the statistical relationship between mycorrhizal infection and wheatgrass productivity?	A ANOVA B Linear regression C Both A and B are correct D I do not know

Introduction: Foundational Principles of Mycorrhiza

We live in a time of unprecedented agricultural productivity. With the adoption of high yielding hybrids and high levels of fertilization and irrigation, crop yields have reached record highs. To view information about current and historical crop yields, a useful web site is the National Agricultural Statistics Service site.

Despite these impressive technological accomplishments, thoughtful observers have raised the issue of sustainability. In other words, how sustainable are these high yields, given that they are based on external nutrient inputs in the form of synthetically produced fertilizers? Are there more sustainable ways that plants can obtain nutrients from soil? These questions are only some of the questions that are fueling the growing interest in sustainable agriculture.

One potential key to addressing this important question is the further understanding and utilization of a plant-fungus association known as **mycorrhiza**. The word mycorrhiza is derived from words meaning fungus root. Several types of soil dwelling fungi (Basidiomycetes, Ascomycetes and Zygomycetes) form beneficial associations with the roots of many plants. As a result of this symbiotic relationship the plants receive greater access to essential mineral nutrients in the soil (e.g. phosphorus), and the fungi receive energy in the form of photosynthetically derived carbon compounds. A number of different types of mycorrhizal associations have been identified, including:

- Vesicular-arbuscular mycorrhiza (VAM) - also known more recently as simply arbuscular mycorrhiza or AM, and
- Ectomycorrhiza (ECM)

The hyphae of VAM penetrate inside the root cells. For this reason VAM have also been known as endomycorrhiza. The hyphae of ECM grow around the root cells without actually penetrating them and are known as ectomycorrhiza.

The presence of mycorrhiza in a crop ecosystem or a natural ecosystem provides a number of important benefits to the plants, including:

- Enhanced nutrient uptake
- Enhanced water uptake and drought resistance
- Enhanced growth rate and yields

Mycorrhiza occur naturally in relatively undisturbed terrestrial ecosystems such as natural grasslands, sometimes known as rangelands. It has been suggested that the increased use of mycorrhiza to increase nutrient and water uptake efficiency by forage plants may enhance the sustainability of natural grasslands and rangelands, which comprise approximately 50-60% of U.S. land resources.

Materials

1	Western Wheatgrass Roots
2	10% (w/v) Potassium Hydroxide (KOH)
3	0.03% (w/v) Chlorazol Black E Stain in lactoglycerol
4	Dissecting Microscope
5	Petri Dish with 1.2 cm grid
6	Autoclave or hot water bath
7	Distilled water
8	Lactic Acid
9	Glycerol
10	Large Beakers (600 ml, 1000 ml)
11	Plastic Screen
12	Scissors
13	Wash Tubs

Reagent Recipes

10% Potassium Hydroxide
Dissolve 100 g KOH in 1 liter distilled water to make 1 liter CAUTION!!! This is an exothermic reaction and KOH can be irritating to skin! Use safety goggles and gloves when handling this material
0.03% Chlorazol Black E Stain in Lactoglycerol
Chlorazol Black E Stain (CBE) can be purchased in powdered form from scientific suppliers. Lactoglycerol is a 1:1:1 mixture of lactic acid, glycerol, and water. To prepare about 1 liter of this reagent mix together 350 ml lactic acid, 350 glycerol, and 350 ml distilled water To prepare a 0.03% w/v solution of CBE dissolve 300 mg of CBE in 1 liter of lactoglycerol

Methods

1 Obtain samples of western wheatgrass roots from your instructor and/or from a class field trip to the range management research plots. Each group will obtain a sample from one of three plots. Be sure to record which plot your sample came from in the space provided on the results page. As much as possible, collect samples from areas with abundant wheatgrass populations. When digging up plants, go to a depth of at least 6-8 inches to ensure a good root sample.

2 Bring the samples back into the lab. Carefully cut off the shoots of the plants with scissors. Gently, gradually, and thoroughly wash off the soil from each root sample by repeatedly immersing in a plastic tub containing tap water. You may need to immerse roots a number of times and repeatedly change the water to obtain clean roots.

3 Select about 1-2 g of washed root tissue

4 Cut the roots into relatively small pieces, about 2-4 cm long

5 The root tissue will then be 'cleared' so that mycorrhiza inside the root will become visible under the microscope. To clear the roots place washed roots into a large beaker (500 - 1000 ml) containing 10% KOH. Gently stir the roots for a few moments. Place the beaker with the root/KOH mixture into either an autoclave at 121C, liquids setting (pressure cooker) for 1 hour or a 70 C water bath for about 2 hours as directed by your instructor.

6 After removing the samples from the autoclave or water bath, wash the roots several times with tap water to remove traces of KOH. Once the rinse water comes off relatively clean, proceed to the next step in the process.

7 Gently stir root segments into 0.03% Chorazol Black E (CBE) in a 500-600 ml beaker. (Note: Your instructor may suggest that you use smaller beakers depending on space availability in the autoclave) Place the beaker with the root/stain mixture into an autoclave (pressure cooker) at 121C, liquids setting for about 1 hour.

8 Your instructor will show you where to put your samples for storage until our next laboratory period.

Pre-Test

Microscopic Assessment of Mycorrhiza

1. What three production practices have resulted in dramatic increases in crop production in the past century?

- Briefly define the term mycorrhiza

- List 3 benefits mycorrhizal associations provide to ecosystems

- What is the name of the stain used in this procedure that allows us to view the mycorrhiza inside of western wheatgrass roots?

- What is the name of the procedure used to estimate the % root length that is colonized by mycorrhiza?

By my signature I affirm that I have read the laboratory, completed the pre-test above, and have a reasonable understanding of the procedures involved in this laboratory.

_____
Student Signature & Date

_____
Instructor Signature & Date

References

Alexopoulos, C.J., C.W. Mims, and M. Blackwell. 1996. Introductorv Mycology. John Wiley and Sons, Inc. New York. p. 625.

Anderson, R.C. and K.J. Roberts. 1993. Mycorrhizae in Prairie Restoration: Response of Three Little Bluestein (Schizachryium scoparium) Populations to VAM Inoculum From a Single Source. Restoration Ecology 1(June): 83-87.

Bethlenfalvay, G.J. and R.G. Linderman. 1992. Mycorrhizae in sustainable agriculture. ASA Special Publication No. 54. Agronomy Societv of America, Madison, WI.

Brundrett, M. C., Melville, L., and Peterson, R. L. 1994. Practical Methods in VAM Research. Mycologue Publications, Waterloo. p. 161.

Giovannetti, M., and B. Mosse. 1980. Laboratory studies on VA mycorrhiza. Estimating infection by VA endophytes. Journal Rothamsted Experimental Station, 1979 Report, p. 188.

Kormanik, P. P., and A. C. McGraw. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Methods and Principles of VAM Research (N. C. Schenck, Ed.), pp. 37-47. The American Phytopathological Society, St. Paul, MN.

McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular VAM fungi. Journal New Phytol. 115(3):495-50.