Laboratory investigations of the Botanical kind: Nitrogen Fixation in Root Nodules

Objectives

- Examine root nodules in legumes
- Investigate the differences between legume (bean) and a non-legume (radish).
- View active cultures of Rhizobium.
- Recognizing relationships
- Hypothesizing
- Comparing and contrasting

Materials

- protective gloves
- 6 three-inch flowerpots
- sterile potting soil
- 2 mixing sticks
- 1.2 m L (1/4 tsp) of Rhizobium bacteria per pot
- 6 bean seeds
- 6 radish seeds
- 6 Alfalfa seeds
- 2 microscope slides
- 2 coverslips
- 1 prepared reference slide of a legume root-nodule cross-section infected with Rhizobium
- compound light microscope
- stereoscope or magnifying glass
- scalpel

Background

1. Define symbiosis, and give an example of three types of symbiotic associations.
2. Nitrogen-fixing bacteria and leguminous plants have a symbiotic relationship.
3. Root nodules are swellings in the roots of leguminous plants that are infected with nitrogen-fixing bacteria.
4. Rhizobium is a genus of nitrogen-fixing bacteria that lives in soil and infects the root nodules of leguminous plants.
5. Green root nodules indicate actively reproducing bacteria that are not fixing nitrogen. Pink nodules indicate bacteria that are actively fixing nitrogen but not reproducing.

PART A:

Growing the Test Plants

1. Fill three flowerpots with soil. Using a mixing stick, stir approximately 1/4 teaspoon of the Rhizobium mixture into each pot.
2. Plant 3 bean seeds in one pot and label it "Bean Rhizobium" and 3 radish seeds in the other and label it "Radish Rhizobium" and then plant and label a third pot for the Alfalfa seeds. Water each pot so that the soil is moist but not saturated.

Growing the Control Plants

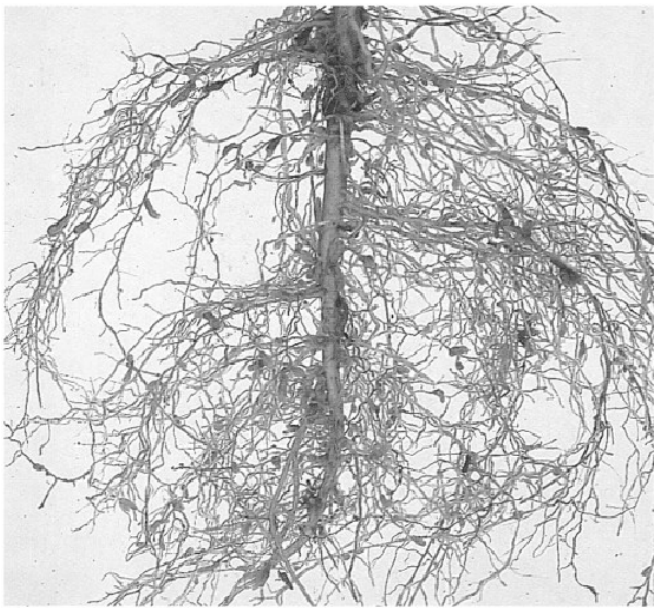
3. Have the lab partner who has not touched anything on the Rhizobium side of the room repeat the above procedure this time omitting the Rhizobium powder. Label the three pots with the seed name and the word control.
4. Place the plants where they will receive direct sunlight. Water the soil when necessary to keep the soil moist but not saturated. Do not fertilize these plants.
5. After approximately one week, check to see if both seeds germinated in each pot. If they did, remove the smaller seedling. The plants will be ready to be examined after six to eight weeks.
6. Clean up your materials and wash your hands before leaving the lab.

7. Monitor the plants each day. Give them enough water to keep the soil slightly moist, and keep them in direct sunlight. Note: The radish seeds will germinate faster than the bean seeds.
8. Once a week count and record the height of each plant, the number of leaves and color. (You will be graphing this data at the conclusion of this investigation so you may want to consider the use of a data table…. but that's up to you.)

PART B: Observing the Roots of Alfalfa and Beans

1. Prepare a data table similar to the one below. As you work, record your observations in your data table.
2. **CAUTION** Wear disposable gloves while handling plants. Do not rub any plant part or plant juice on your eyes or skin. Remove the alfalfa plant from the pot by grasping the bottom of the stems, inverting the pot and then gently pulling the plant out. Be careful not to injure the plant.
3. Carefully remove all dirt from the roots.

OBSERVATIONS OF RHIZOBIUM	
Appearance of nodule	
Color of nodule	
Number of nodules	
Number of pink nodules	
Number of green nodules	



4. View the roots of the bean plant under a stereoscope.
5. Compare the appearance of the bean root system with the photograph to the left. Note the formation of any nodules on your bean plant's roots. Draw a root with a nodule in your lab report, and label each structure.
6. **CAUTION** Use the scalpel with care. A scalpel is a very sharp instrument. Always make cuts with the blade facing away from your body. If you cut yourself, quickly apply direct pressure to the wound and call for your teacher. Remove a large nodule from the bean root and carefully cut it in half with a scalpel.
7. The pink nodules contain active nitrogen-fixing bacteria.
8. The green nodules contain bacteria but cannot fix nitrogen because they are actively reproducing. Rhizobium will begin fixing nitrogen only after it stops reproducing.
9. View the cross section under a stereoscope. Note the arrangement of bacteria within the cell. Draw a cell infected with bacteria in your lab report. Label the nodule, cell, and bacteria.

PART C: Observing the Roots of Radishes

1. **CAUTION** Wear disposable gloves while handling plants. Do not rub any plant part or plant juice on your eyes or skin. Remove the radish plant from the pot by grasping the bottom of the stem, inverting the pot and gently pulling the plant out. Be careful not to injure the plant.
2. Carefully remove all dirt from the roots.
3. Examine the roots of the radish plant under a stereoscope. Compare the radish roots with the roots of the alfalfa plant that you have already examined.

4. Are there any nodules on the roots of the radish plant?
5. Draw the root of the radish plant in your lab report. Label the drawing "Radish."

PART D: Preparing a Wet Mount of Rhizobium

CAUTION Handle the slide and coverslip carefully. Glass slides break easily and the sharp edges can cut you but more importantly Mr. O does not like to reorder slides.

1. Prepare a wet mount by placing part of a nodule from the root of a bean plant on a microscope slide, adding a drop of water, and covering with a coverslip.
2. Place the slide on a flat surface. Gently press down on the slide with your thumb; use enough pressure to squash the nodule. Make sure the coverslip does not slide.
3. Examine the slide under a microscope. Draw and label a cell and the arrangement of bacteria in the cell in your lab report. Note what power of magnification you used.
4. Compare your wet mount preparation with the prepared reference slide of Rhizobium and the photograph on the right. A cell infected with Rhizobium should have a similar appearance to the photograph at right.
5. Clean up your materials and wash your hands before leaving the lab area.

Analysis and Conclusions

1. Which plant had the most nodules?
2. How many nodules were found on the radish plants?
3. How do legumes become infected with bacteria in nature?
4. What kind of relationship exists between the legume plant and Rhizobium? How does this relationship benefit the legume plant? How does this relationship benefit the bacteria?
5. If you were to grow legumes without root nodules to use as experimental controls, why should you plant the seeds in sterile soil?

Presentation of Data

As part of your lab report you will need to submit the following:

1. your final copy of your weekly data chart
2. a graph of alfalfa control and alfalfa Rhizobium growth over time
3. a graph of radish control and radish Rhizobium growth over time
4. a graph of bean control and bean w/Rhizobium growth over time
5. A conclusion as to the benefit or detriment of Rhizobium to your plants
6. Two suggestions to improve the results of your lab
7. The usual analysis and conclusion

BOTANY ROOT NODULES LAB REPORT RUBRIC

Student Names:	Due Date:

This analytic rubric is used to verify specific tasks performed when producing a lab report. If the task has been completed, all points are awarded. If the task is incomplete half points may be awarded. No points are awarded if the task is not complete.

Category	Scoring Criteria	Points	Student Evaluation	Teacher Evaluation
Lab Introduction ***15 points***	The essential question to be answered during the lab is stated.	5		
	The hypothesis clearly shows it is based on facts. *(Background of Nitrogen-fixing bacteria and leguminous plants)*	5		
	A connection is made between the lab and the "real world" *(how might data from this lab improve life?)*	5		
Methods ***10 points***	Procedures are written in paragraph form and clearly state what was done to set up the lab. *(not written as numbered steps.)*	5		
	There are no "understood" procedures.	5		
Results ***25 points***	"Results" of a procedure are clearly recorded. *(include the* final copy of your weekly data chart*)*	5		
	Measurements, when required, show proper units. *(Write these as observations on the lab report.)*	5		
	a graph of alfalfa control and alfalfa w/Rhizobium growth over time	5		
	a graph of radish control and radish w/Rhizobium growth over time	5		
	a graph of bean control and bean w/Rhizobium growth over time	5		
Discussion ***35 points***	Summarize the essential lab data. (come to a conclusion)	5		
	Show how the essential data answers the lab question.	10		
	All analysis and conclusion questions answered	15		
	Identify the one area of the lab most likely for measurable experimental error. *(*2 suggestions to improve the results of the lab*)*	5		
Presentation ***15 points***	Report is printed in black ink on white paper using 12 point Times New Roman or Arial font double spaced with one inch margins on all sides with no visible corrections (**Warning**: this is not Word's default setting).	5		
	A diagram of the essential apparatus used in the experiment is drawn in the largest available white space on the front of the lab report.	5		
	Report is written in such a way that others could accurately duplicate the experiment.	5		
Participation ***10 points***	No group members were sited for safety or participation violations.	10		
Score	Total Points	110		
Self-evaluation	If the difference between the student evaluation and the teacher evaluation is more than 10 points, 5 points will be deducted from the teacher's score when the grade is recorded.			
Deadline	Reports will be accepted after the beginning of class for 3/4 credit. Papers turned after that time will be mulched for use in the greenhouse and receive ¼ credit.			