Lab V-2

Observing the Effect of Rhizobia on Plant Growth

Equipment and Materials

You'll need the following items to complete this lab session. (The standard kit for this book, available from *www.thehomescientist.com*, includes the items listed in the first group.)

Materials from Kit	
□ Ammonium nitrate[1]	□ Inoculating loop
□ Fertilizer, nitrogen-free (concentrate A)	□ Rhizobia inoculum[1]
□ Fertilizer, nitrogen-free (concentrate B)	□ Ruler, mm scale
□ Fertilizer, nitrogen-free (concentrate C)	□ Seeds, bush lima bean
□ Graduated cylinder, 10 mL	
Materials You Provide	
□ Gloves	□ Pencil
□ Balance	□ Plastic wrap (Saran or similar)
□ Chlorine laundry bleach	□ Soft drink bottle, 2-liter
□ Foam cups, 16 oz./500 mL	□ Vermiculite (or other sterile growth medium)
□ Lamp, fluorescent plant (optional)	□ Water, distilled
□ Paper towel	

[1]Ammonium nitrate is included only in kits shipped to Hawaii. Rhizobia innoculum is included in kits shipped to the other 49 states. Kit users in the continental US and Alaska can use the instructions provided here to complete this lab session. Kit users in Hawaii ONLY should modify these instructions. Rather than using Rhizobia, make up the nitrogen-free fertilizer solution as described later in this session. Use half of that solution to grow lima beans without nitrogen. To the other half of the solution, add about a quarter teaspoon of ammonium nitrate per liter of the diluted nitrogen-free fertilizer working solution to make a fertilizer solution that includes nitrogen, and use that solution to fertilize the other half of your lima bean seedlings.

If you purchase materials separately rather than using the kit, make sure to obtain a rhizobia culture that is suitable for lima beans. Rhizobia is available in many variants, each of which is specific to a particular plant or range of plants. For example, rhizobia that is suitable for alfalfa or clover does not work for lima beans, and vice versa. Rhizobia cultures sold in lawn and garden stores may contain a mixture of many rhizobia variants that works with several plants. Read the label.

If you obtain lima bean seeds separately, make sure they are not pretreated with rhizobia or an insecticide. To ensure the seeds you use are not contaminated (including those provided with the kit), surface-sterilize them as described later in the session.

??? Placeholder to separate Notes

The best growth medium is sterile vermiculite, available in small bags in any lawn and garden store. You can also use sand (sold in most big-box home centers). If you use sand, wash it first to remove soluble materials by stirring it up in a bucket full of tap water and pouring off the water. Repeat this washing two or three times. After washing, sterilize the sand by spreading it in a thin layer on a pizza pan or similar oven-safe container and heating it for at least 30 minutes at 450 °F. Allow the sand to cool and then transfer it to the planting containers.

??? Placeholder to separate Notes

Commercial fertilizers cannot be used in this lab session because they contain nitrogen. If you do not have the kit, you can prepare a suitable nitrogen-free fertilizer by adding the following to 10 liters of water:

1.0 g of calcium sulfate

0.1 g of iron(III) sulfate (ferric sulfate)

2.0 g of magnesium sulfate

8.0 g of potassium hydrogen phosphate (K_2HPO_4)

2.0 g of potassium dihydrogen phosphate (KH_2PO_4)

For best results, you should also include trace amounts (a few milligrams per liter) of salts of boron, chlorine, copper, manganese, molybdenum, sulfur, and zinc. The three-part fertilizer concentrate included with the kit is made to a different formula that includes all of these trace elements, and is sufficient to produce more than 10 liters of working-strength fertilizer solution.

Background

Biologists study the interactions of species in ecosystems to learn more about each species than they could learn by examining that species in isolation. In many cases, there is little or no direct interaction between species. For example, wolves and grasses neither harm nor benefit each other other than incidentally. (A wolf may damage a grass population by urinating on it; conversely, wolf feces may fertilize another grass population.)

When species do interact directly, one species may benefit to the detriment of the other, a state called *parasitism*. For example, tapeworms may infect a wolf. The tapeworms benefit; the wolf is harmed. In another type of direct interaction, called *commensalism*, one species benefits without benefit or harm to another species. For example, squirrels build nests in oak trees. The squirrels benefit by having a secure haven from predators while the oak trees neither benefit nor are harmed by the interaction.

The final type of direct interaction between species is called *mutualism*, in which each species benefits from the presence of the other. For example, woodpeckers eat insects that infest the bark of trees. The woodpecker benefits from the tree providing a ready source of food, while the tree benefits by the woodpecker keeping down the population of insects that might otherwise damage the tree.

Note that while one type of interaction between species may be commensalist, another type of interaction between those same species may be mutualist. Returning to the oak trees and squirrels provides such an example. The oak trees provide acorns as a food source for the squirrels, benefiting the squirrels. In turn, the squirrels bury acorns over a wide range. Some of those acorns are forgotten and subsequently germinate, benefiting the oak tree. Overall, then, the relationship between oak trees and squirrels is mutualist.

Also note that when more than two species are involved, the type of interaction depends on the species-pairing point of view. From an insect-tree POV, the relationship is parasitic; the insect benefits to the detriment of the tree. From a bird-insect POV, the relationship is also parasitic; the bird benefits to the detriment of the insect. From a bird-tree POV, the relationship is mutualist; both benefit at no detriment to either species.

In this lab session, we'll examine a mutualist relationship between a plant, the bush lima bean, and a bacterium called rhizobia. Legumes, including the bush lima plant, are commonly infected by rhizobia, which form colonies as nodules in the root structure of the plant. The bacteria benefit by having a nice cosy nodular home amongst the plant's roots. These nodules do no harm to the plant, and in fact benefit it greatly.

To understand why, it's important to understand that plant growth requires six *macronutrients* (arbitrarily defined as those that typically make up 1% or more of the dry weight of the plant) and ten *micronutrients*, some of which are present only at sub ppm (parts per million) levels. All ten of the micronutrients—boron (B), calcium (Ca), chlorine (Cl), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), sulfur (S), and zinc (Zn)—must be obtained from the soil. Three of the macronutrients—carbon (C), oxygen (O), and hydrogen (H)—are obtained from carbon dioxide gas and water. Two of the remaining three macronutrients—phosphorus (P) and potassium (K)—must be obtained from the soil.

The final macronutrient, nitrogen (N), may be obtained from the soil or from atmospheric nitrogen with the aid of *nitrogen-fixing bacteria*, such as rhizobia. From a plant's point of view, nitrogen is both abundant and rare. Nitrogen is abundant in the sense that our atmosphere is about 79% nitrogen gas. Unfortunately, plants cannot use nitrogen gas directly but must obtain it in the form of soluble nitrates in the soil, which may be present in inadequate amounts to sustain plant growth. That's where nitrogen-fixing bacteria come in. They convert atmospheric nitrogen gas into soluble nitrates, making the nitrogen available to plants.

In this lab session we'll investigate the growth of the common lima bean, *Phaseolus lunatus*, under controlled conditions. We'll grow two plants in vermiculite, which is a sterile, nutrient-free type of soil that provides only support for the plant. We will provide each plant with nutrients in the form of a fertilizer that contains all of the nutrients named above with the exception of nitrogen. We'll inoculate one of the seeds with Ryzobia before planting it, and plant a second seed as a control without rhizobia, and compare the growth of the plants with and without nitrogen-fixing bacteria present.

> This experiment requires successful germination and growth of two lima bean plants, one with and one without rhizobia present. If you start with only two seeds, there is a reasonably high probability that one of the plants will fail to germinate,

ruining the experiment. Planting two (or more) seeds each without rhizobia and with rhizobia greatly increases your chance of success.

Procedure V-2-1: Grow lima beans with and without rhizobia

1. If you have not already done so, put on your goggles and gloves.
2. Rhizobia is very common in the environment, so we need to make sure that our lima bean seeds are not contaminated. To surface-sterilize the seeds, prepare enough of a mixture of one part chlorine laundry bleach to ten parts water to fill a foam cup to depth of 5 cm or so. Immerse the lima bean seeds in the diluted bleach solution and allow them to soak for a couple of minutes, swirling the contents periodically. Empty the bleach from the cup, and rinse the seeds thoroughly in running tap water to remove all bleach residue. Allow the seeds to drain on a paper towel until you need them.
3. Prepare and label two 16-ounce (500 mL) foam cups. Use a pencil to poke several small drainage holes in the bottom of each cup. Cover the holes with a single layer of paper towel.
4. Fill each foam cup with vermiculite to within 2 cm from the top.
5. Press a lima bean seed into the center of the vermiculite in the first cup to a depth of about 2 cm. Cover the seed with vermiculite. Gently pour distilled water onto the surface of the vermiculite until water comes out of the drain holes on the bottom of the cup. Set that cup well aside from your work area.
6. Press a lima been seed into the center of the second cup and use the inoculating loop to transfer a small speck of rhizobia to the well containing the seed. Cover the seed and rhizobia with vermiculite. Again, gently pour distilled water onto the surface of the vermiculite until it drains from the holes in the bottom of the cup.
7. Wash your hands thoroughly before proceeding. If you have an alcohol-based hand sanitizer, also use it to ensure that no rhizobia is transferred to the first cup.
8. Place both cups under a plant grow light or on a windowsill or other area where they'll get sunlight for most or all of the day.
9. Record the details in your lab notebook.
10. Water both cups with distilled water daily. Allow the water to drain completely each time, and then cover the cup with plastic wrap between waterings until the seeds germinate. Observe the two cups at least daily. Note any indications of germination, such as the first appearance of stem or leaves, in your lab notebook. If you have a digital camera, make a photographic record of your observations.
11. After the seeds germinate, transfer 20 mL of fertilizer concentrate A from the kit to an empty, clean 2-liter soft drink bottle and half-fill the bottle with water. Transfer 4.5 mL of fertilizer concentrate B to 500 mL of water, swirl to mix the solution, and add that solution to the 2-liter bottle with swirling to mix the solutions. Transfer 2 mL of fertilizer concentrate C to 500 mL of water, swirl to mix the solution, and add that solution to the 2-liter bottle with swirling to mix the solutions. (Do not be concerned if there is some cloudiness or a slight precipitate in the mixed solutions.) Water the young plants every day or two, alternating between distilled water and the fertilizer solution. (If there is cloudiness or a slight precipitate in the fertilizer solution, agitate it to re-suspend the precipitate before each use.) Use enough liquid to dampen the vermiculite thoroughly. Do not cover the cups with plastic wrap once the seeds have germinated.
12. After six weeks, measure the heights of both plants and record it in your lab notebook. Carefully remove the plants from the cups, and gently shake off and rinse off all of the

vermiculite, making sure to retain all of the root structure and nodules. Allow the plants to dry completely and then weigh them. Record the weight of each plant in your lab notebook.

Review Questions

??? RBT: review questions/answers and add additional material after completing the labs.

??? Production: Please strip the answers (formatted as comments) from the final layout. RBT

1. Why did we use vermiculite rather than potting soil or ordinary soil from a lawn or garden?

    Potting soil or ordinary soil from the lawn or garden contains unknown amounts of various nutrients. Vermiculite is essentially expanded clay. It contains no nutrients of any sort, so using it allows us to control the nutrients provided to the plant specimens. Furthermore, potting or other soil may be contaminated with rhizobia bacteria.

2. In the absence of any information about the bacteria, what three hypotheses might you make about the effect of the bacteria on plant growth? Which of these hypotheses is more or less likely? Why?

    The first and most likely hypothesis (in the absence of other information) is that the bacteria would have little or no discernible effect on plant growth. All plants are surrounded by numerous species of bacteria, only a tiny fraction of which have any effect on plant growth. The second and third hypotheses, both equally likely and both less likely than the first, are that the bacteria would cause increased plant growth and that the bacteria would cause decreased plant growth.

3. What differences did you expect in the two cups? Why? Did your observations confirm or falsify your predictions? Could rhizobia infection of the magic beans account for the extreme size and rapidity of growth of Jack's beanstalk?

    Answers will vary, but a student might reasonably predict that the plant with rhizobia would flourish while the plant without rhizobia would exhibit stunted growth or even fail to germinate because it lacked nitrogen. Experimental results should confirm superior growth of the plant with rhizobia present, with the height 2.5% to 5% greater and the root mass 5% to 10% higher in the plant with rhizobia. These numbers rule out rhizobia as the cause of the extreme growth in Jack's beanstalk.

4. What are the controlled, independent, and dependent variables in this experiment?

    Controlled variables are those that are kept the same between specimens. In this case, those variables include the type of soil, planting depth, the amount of water or nutrient liquid provided, the temperature, amount of sunlight, and so on. Independent variables are those that differ between specimens. In this experiment (if it is carried out properly), the only independent variable is the presence or absence of rhizobia. Dependent variables encompass differences in observed results. In this experiment, the dependent variables we are measuring are the height of the plants and the mass of their root systems.

5. We used height and mass as a proxy in this experiment, on the reasonable assumption that they would fairly represent the actual question, which is more difficult to answer. What is that question, and why is it more difficult to answer?

    We don't grow lima bean plants to produce massive root structures, nor is a taller plant in any way more or less desirable than a shorter plant. What we're really interested in is producing lima beans for consumption, so the real question is whether the presence or absence of rhisobium affects the number or mass of lima beans produced. To determine this, we would have to allow the plants to grow to full maturity and count and weigh the beans actually produced by each plant. To ensure our results were statistically significant, we would have to grow many plants and harvest many beans.

6. Is the interaction between the plant and bacteria commensalist or mutualist? Explain.

Based on the effect on the plant, the relationship might be either. The plant clearly benefits, but does the bacteria? Yes, because the bacteria forms nodes on legumes rather than being randomly distributed in the soil, so the bacteria must gain some advantage from close proximity to the plant. If you have time, you can confirm this by culturing soil specimens from an area near the root system and from a more distant area. You'll find that the rhizobia count is much lower in areas distant from the root system.