ECOS Inquiry Template

1. CONTRIBUTOR'S NAME: Carl Rosier

2. NAME OF INQUIRY: DO BACTERIA (MICROORGANISM) ENHANCE PLANT GROWTH

3. GOALS AND OBJECTIVES:

   a. Inquiry Questions:
      1. Are there microorganisms that limit plant growth?
      2. Are there microorganisms that enhance plant growth?
      3. If a microorganism does enhance plant growth, can all plant species take advantage of this?
      4. How would a microorganism enhance plant growth (i.e. what would the bacteria do for the plant)?
      5. If a microorganism does help plants grow is there a cost to the plant?

   b. Ecological Theme(s):
      1. That some microorganisms are beneficial to plant growth and form symbiotic relationships with certain species of plants.
      2. The relationship between plant and microorganism can impact where certain plants are found.

   c. General Goal:
      1. Introduce the concept that microorganisms can enhance plant growth.
      2. Discuss how microorganisms help plants gain nutrients.
      3. Discuss how the symbiotic relationship between bacteria and plant root is formed.
      4. Discuss the meaning of symbiotic relationships. Discuss mutualism relationships, and compare them to other relationships (parasitic, and commensalisms).
      5. Discuss the cost associated with bacteria/host plant relationship.
      6. Compare the energy required by humans to create Nitrogen fertilizers with that of plants investment into nitrogen fixation.

   d. Specific Objectives:

   Academic: Students learn that certain microorganisms are capable of enhancing plant growth and that this relationship is unique to only a certain group of plants.

   Experimental: Students are able set up the experiment and make observations while their plants are growing, as well as decide how to measure the plants at the end of the experiment.

   Social: Students can work in groups of 3-5 this promotes teamwork and a sharing of responsibilities.

   Communication: at the end of the experiment students present their results and offer conclusions to explain their observations.

   e. Grade Level: 5th

f. Duration/Time Required:

Prep time: 3-4 hours. This includes pre-germinating seeds, sterilizing soil, preparing plant containers, and purchasing bacteria inoculant.

Implementing Exercise During Class. 1 hour to setup the experiment and 1 hour to collect data and interpret results.

Assessment: 30 minutes.

4. ECOLOGICAL AND SCIENCE CONTEXT:

a. Background (for Teachers): Most students will readily accept the concept that microorganisms cause diseases in plants ("germ theory"). Yet some bacteria in the soil form mutualistic relationships with plant roots. One particular type of symbiotic bacteria are called *Rhizobium,* which forms nodules on the roots of legume plants such as peas, beans, and clover (to name only a few). These bacteria are important with respect to plant nutrition because they take atmospheric nitrogen (N^2 "gas") and convert it into NH^{4+} (ammonium) a process called nitrogen fixation. This is important because plants cannot use nitrogen gas (N^2) as a nutrient source however, plants can readily use ammonium. The fact that plants cannot use nitrogen gas is somewhat ironic as 78% of the earth's atmosphere is composed of N^2. Furthermore nitrogen in the form of ammonium is a very important macronutrient and greatly influences plant growth. When plants are deprived of ammonium their leaves appear yellow and their overall growth is stunted.

The formation of root nodules by *Rhizobium* is a complex process that involves signaling from the host root and bacteria as well as morphological changes within the host root. The roots of legume plants secrete an intricate substance (organic compound called "flavonoids") that informs surrounding *Rhizobium* bacteria that a host root is present. The nitrogen-fixing bacteria emit an organic substance of their own called nod factors. This nod factor informs the host root that the bacteria are present and the root begins to prepare for their arrival. Root hairs of the host plant begin to flatten "taking a spatula shape". The root hair forms a pocket that allows colonization of the bacteria. Once the bacteria enter this pocket the root begins to curl and encloses the bacteria forming the nodule. The area within the nodule is devoid of oxygen, this is very important, as the enzymes necessary to convert N^2 to NH^{4+} will only occur under anaerobic conditions.

The relationship between *Rhizobium* and the plant can be classified as symbiotic or mutualism depending on the definition used. Symbiotic relationship is often defined as a long- term physical contact between individuals of different species. Where individual specie lives permanently on or within its host. In the case of *Rhizobium* and its plant host the more accurate definition of mutualism applies. The plant provides carbon in the form of sugars and carbohydrates to the bacteria in exchange for ammonium. Mutualism occurs when both species benefit from the relationship, but can survive independently outside the union as well. The instructor can also introduce other relationship types such as parasitic and commensalisms and compare them to mutualisms.

Nitrogen is such an important nutrient that tons on chemical fertilizers are produced every year to meet the needs of industrial agricultural practices. The production of commercial fertilizers containing NH^3 (ammonia: can be used by plants as well) relies heavily on the Haber-Bosch process where atmospheric N_2 and H_2 are converted to NH^3. This process is a high pressure/high temperature reaction (130-680 atm, 340 -420^oC) that utilizes massive amounts of fossil fuels. By the year 2050 commercial production of ammonia based fertilizers will reach 120 Tg/yr. One teragram (Tg) is equivalent to one million metric tones. Plant roots colonized by *Rhizobium* are able to complete this process via nitrogenase enzyme using sugars fixed during photosynthesis.

Plants that have the ability to form a beneficial relationship with *Rhizobium* would be perceived as maintaining a competitive advantage over plants that cannot form this relationship. If this were the case “why is the earth not covered with legume plants??” Tradeoffs can best answer this question. In all biological systems tradeoffs occur, the ability to perform one function comes at the cost of another. Plants that are colonized by *Rhizobium* most provide food to their bacterial companion in the form of sugars and carbohydrates. In this case the plant has to divert resources that could be utilized for growth. Leguminous plants need full sunlight to facilitate this relationship. In open environments nitrogen fixing plants flourish however, as the canopy closes they are quickly replaced. This occurs because leguminous plants sacrifice growth for nitrogen.

b. Background (to present to Students):

Pre-experiment discussion

The instructor can begin discussing with their students symbiosis. Most students will be aware of the classical symbiotic relationship clown fish and anemone. The teacher can then ask the students to provide other symbiotic relationships. Next the instructor can ask “do microorganisms and plants form symbiotic relationships?”. At this point the instructor can being to describe the symbiotic relationship of plants and rhizobia.

Post-experiment discussion

After completion of the experiment the students observe their plants and decide if their predications are valid. Here the teacher can ask the students to share with the class the outcome of their experiment. The teacher can then begin a discussion on the effectiveness of commercial inoculants. Is it worth while to purchase these inoculants, or are microorganisms truly everywhere.

5. MOTIVATION AND INCENTIVE FOR LEARNING: This is a fun hands on experiment where the student is able to maintain their plants. During this time the student is allowed to make observations comparing the experimental treatments. At the end of the experiment the student can directly access the impact of bacterial colonization via root nodule formation.

6. VOCABULARY:

<u>*Rhizobium*</u>*:* A genus of heterotrophic soil bacteria capable of forming symbiotic nodules on the roots of legume plants to fix atmospheric nitrogen

<u>nitrogenase:</u> The bacterial enzyme responsible for converting atmospheric nitrogen (N2), which is unavailable for plant utilization, into ammonium (NH4+), which is a nitrogenous compound readily utilized by plants.

<u>Symbiosis</u>: situation in which two dissimilar organisms live in close association.

Parasitism: relationship between two species in which one benefits while the other is harmed.

Commensalisms: relationship between species that is beneficial to one but is netural or of no benefit to the other.

Mutualism: relationship between two species in which both benefit.

Legume:

1. plant, such as the soybean, that bears nitrogen-fixing bacteria on its roots, and thereby increases soil nitrogen content.
2. Any of the plants of the order Fabales (including peas, soybeans, and clover) important in nitrogen fixation. Legumes develop bacteria-harboring root nodules; from atmospheric nitrogen, the bacteria form compounds that can be taken up by plants and animals.

Nitrogen (N^2 “gas”)

1. colorless, tasteless, odorless gas that is the most abundant constituent of dry air.
2. One of the nine macronutrients. Nitrogen is a major component of plants; it is a building block of amino acids, proteins, and nucleic acids (genetic material), chlorophyll, and enzymes. Though nitrogen is in air, it is only available to plants once it has been fixed by soil microorganisms.

NH^3 (ammonia): A compound of nitrogen and hydrogen (NH3), found in fertilizers

NH^{4+} ammonium: A form of nitrogen that is available to plants. It is found in fertilizer, and is one of the first forms of nitrogen released as crop residues and organic fertilizers decay.

Nitrogen fixation: The process in which bacteria convert biologically unusable nitrogen gas (N2) into biologically usable ammonia (NH3) and nitrates (NO3-).

Flavonoids: are the largest class of plant phenolics (large organic compounds), they are Derived form the shikimic and malonic pathway (metabolic pathways).

Nod factor:

1. Nod factors are monoacylated chains of 3-5 B-1,4linked N-acetyl glucosamine units with various chemical modifications.
2. Nod factors stimulate specific genes in the plant that are required for the nodulation process to continue.

7. SAFETY INFORMATION: Students are working with soils so eye protection is necessary. Also if the instructor decides to have the student perform the sterilization step caution should be taken when working with bleach.

8. MATERIALS LIST (including any handouts or transparency masters):

1. Soil should be collected from at least two areas: one with high disturbance and one in pristine condition.
2. Legume seed (bean or pea)
3. 10% Bleach solution
4. Growing containers (paper cups are a cheap alternative).
5. Area with high light at least 14-16 hours per day.
6. Commercial legume inoculant

PROCEDURE

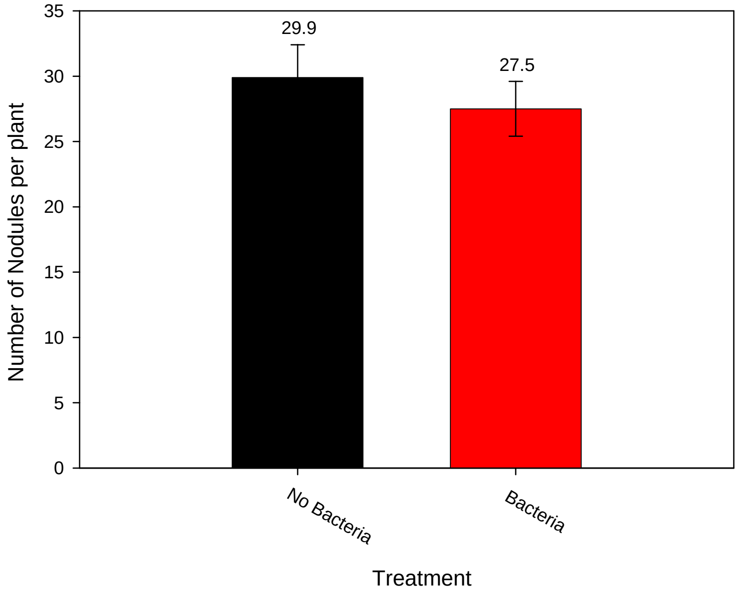
A) Surface sterilize the legume seed by soaking them in a 10% bleach solution for 2 minutes, next rinse the seed in clean water to remove bleach solution. This step should remove most of the bacteria attached to the seed coat without destroying the seed embryo.
B) Pre-germinate legume seed on a wet cloth. This step is optional however; we found that the students had an easier time working with germinated seeds.
C) Collect soil from at least two areas, these should areas such as a garden (location where Rhizobia should be present) and an area of disturbance such as a road side or an area of heavy traffic (an area where Rhizobia should be absent).
D) Each soil sample will be split into two treatments, one will receive the Rhizobia inculumn and the other will not. Rhizobia can be purchased at most plant nurseries.
E) Plants should receive at least 12-16 hours of light per day. Do not fertilize plants with fertilizers high in nitrogen this will prevent the plant from forming nodules with the Rhizobia.
F) After 4-5 weeks of growth the students harvest the plants. This entails clipping the shoots at the root collar, and weighing this material in order to determine shoot biomass. Next the students examine the roots and count nodules for all treatments.
G) At the end of the experiment the students compare shoot biomass and the number of nodules formed on each root for each treatment.

What should happen?

At the conclusion of this experiment we expected the treatments without the incolumn added would not form nodules. However, this was not the case we found the same number of nodules on all of the plants regardless of the treatment (Figure: 1) . Furthermore we discovered that shoot biomass did not differ between treatments as well (Figure:2) .

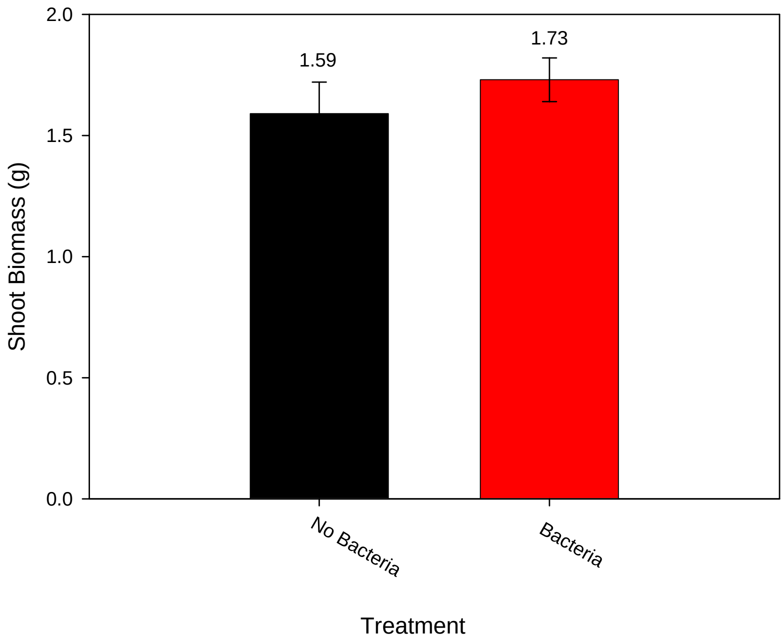
Results

Figure: 1 Number Nodules



This figure describes the average number of nodules formed in each treatment. The data indicates that there is no significant difference in amount of nodules formed in either treatment.

Figure: 2 Shoot Biomass



This figure describes the average shoot biomass or each treatment (bacteria inoculant vs. no-inoculant). The data suggests that there is no significant difference between treatments.

9. METHODS/PROCEDURE FOR STUDENTS:

Pre-investigation work: the purpose of this inquiry is to introduce students to plant microbe interactions (symbiosis). Students should already have a basic knowledge of microorganisms. The instructor can facilitate this instruction through lectures or interactive web sites. The instructor can lead a variety of discussions “what is symbiosis” “can microorganisms aid plant growth”would the production of microorganism inoculants be worthwhile.

b. Investigation work: Students can work alone, pairs or groups of no more then 5 individuals. For our investigation each student was given soil from two different sites, rhizobia inoculant, planting containers, and pre-germinated legume seed. They were instructed to begin the no bacteria treatment first in order to prevent cross contamination.
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Once the planting is complete students are asked to write out their predictions and/or draw pictures of what they think will happen. The primary message of this investigation is microorganisms are everywhere, and that purchasing bacteria inoculants is a futile endeavor.

10. ASSESSMENT:

Younger students can draw pictures of what they think will happen in each treatment or write out hypothesis of which treatment will grow larger. Older students could begin to design other experiments involving rhizobia.

11. EXTENSION IDEAS: This experiment could easily be extended. Students could investigate the quality of light on rhizobia/ plant interaction. Treatments could consist of growing plants in low and high light and determining the amount of nodules formed. Students cold also conduct fertilizer experiments, thus determining the influence of fertilizer on nodule formation.

12. SCALABILITY: This study could be scaled down for younger students where the instructor sets up the majority of the experiment and the student either write out or draw the hypothesis and observations. Advanced students can begin to design studies that would effect the symbiosis.

13. REFERENCES:

- http://edis.ifas.ufl.edu/SS180
- http://www.soils.wisc.edu/~barak/soilscience326/nitrogen.htm
- http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenFixation.html

14. LIST OF EXPERTS AND CONSULTANTS

1. Master Gardner
2. Microbiologist
3. Agronomist
4. Soil Biologist

15. EVALUATION/REFLECTION BY FELLOWS AND TEACHERS OF HOW IT WENT: This investigation went very well. The students were very excited and surprised to see that all of the treatments were similar. This experiment also enables the student to actually see the effect of bacteria on plants (i.e. nodule formation). Furthermore the student is able to investigate an actual product and determine its effectiveness.

16. Background Pictures

26.13 Roots of a legume (pea) with nodules

Image: 1 Root nodules of pea plants www.cartage.org.lb/.../ Nutrition/rhiz.gif

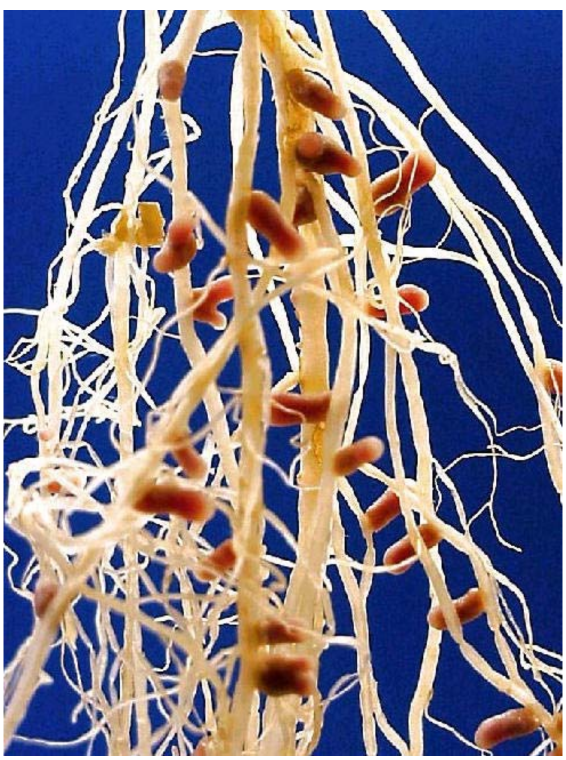


Image: 2 Root nodules of bean plants iris.cnice.mecd.es/.../ imagenes/rhizobium.jpg

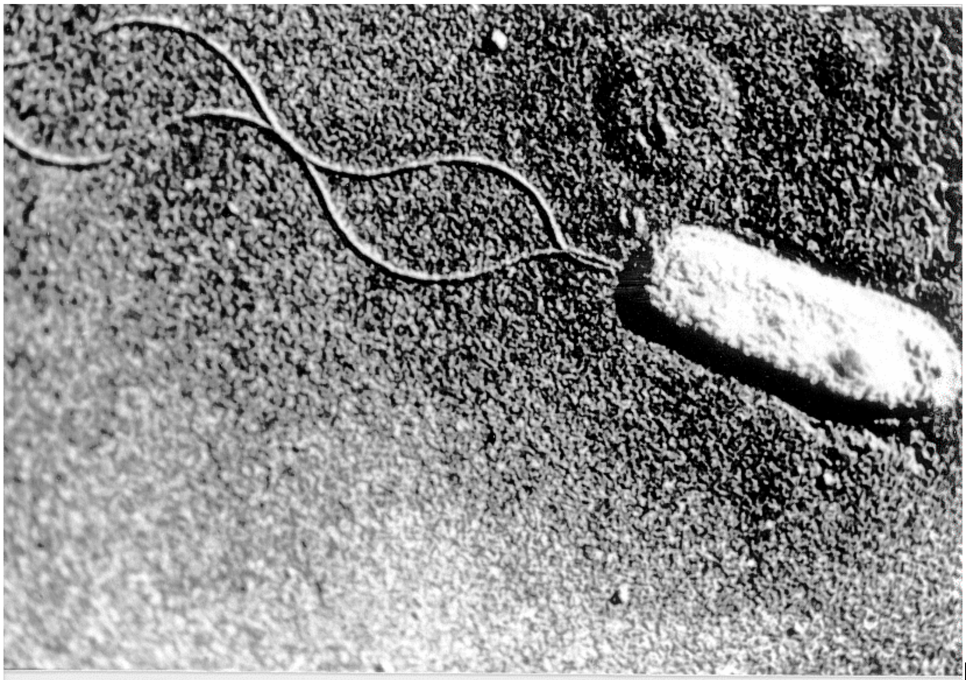


Image: 3
Scanning electron micrograph of *Rhizobium* the long strands coming off of the cell are flagella that enable the cell to move www.cnpab.embrapa.br/ servicos/baby/rizobio.htm

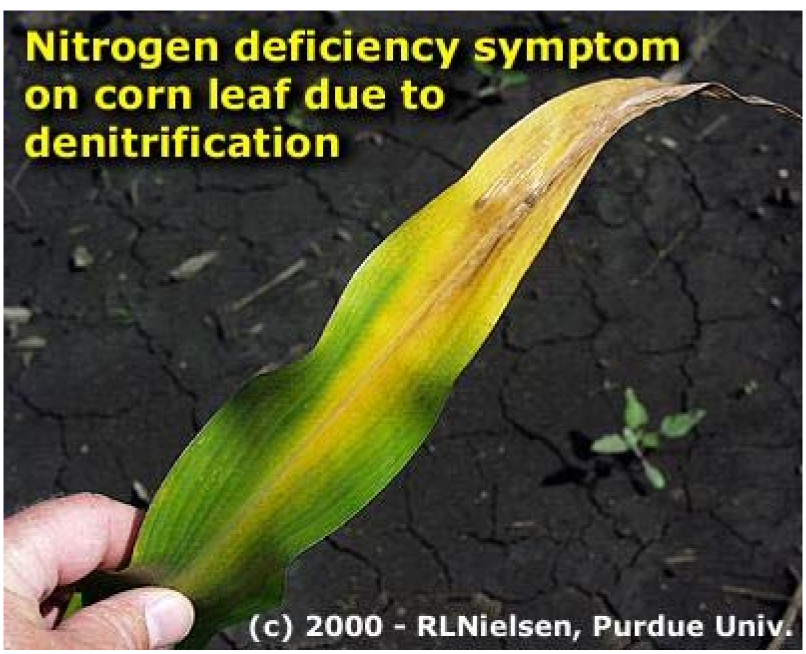


Image 2: Nitrogen deficiency in corn www.agry.purdue.edu/.../ 2000/P6290011.jpg

Image: 3 Growth responses due to nitrogen liminatitions www.hydromall.com/grower/images/nutrient_1.jpg

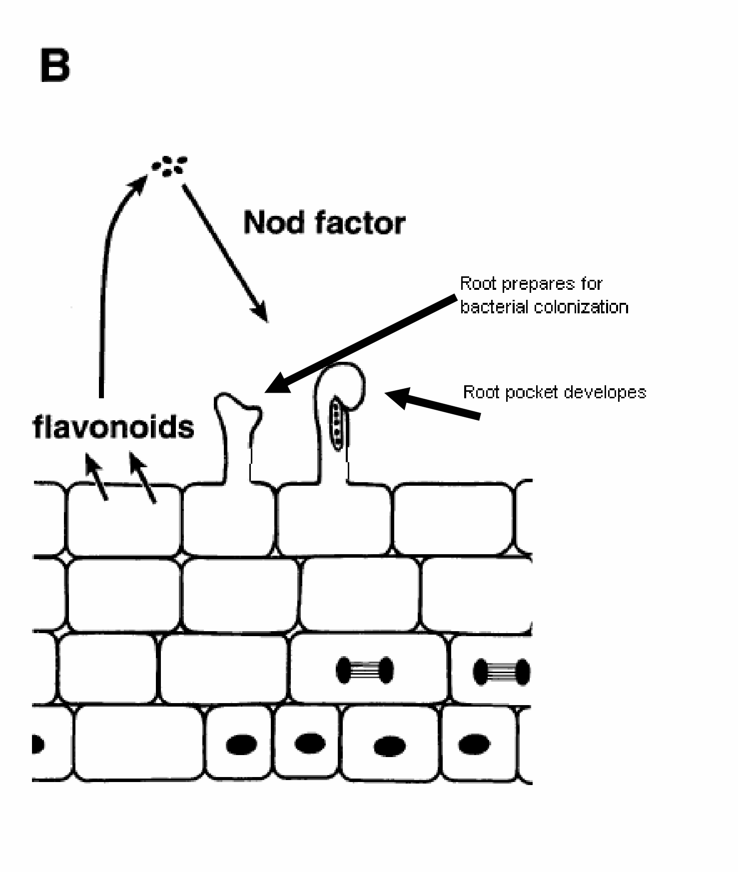


Image: *Rhizobium* colonization process and signaling mechanisms produced by both plant and bacteria. Hirsch A.M and Kapulnik Y (1998). Signal Transduction Pathway in Mycorrhizal Associations: Comparison with *Rhizobium* legume Symbiosis. Fungal Genetics and Biology 23 205-212

Do Bacteria Enhance Plant Growth?

Objective:

1. Determine if bacteria enhance plant growth.
2. Increase our understanding of soil microorganisms.

Treatments

1. Pea plants grown without bacteria
2. Pea plants grown with bacteria

Materials

1. Soil with bacteria
2. Soil without bacteria
3. Bacteria packet
4. Cups
5. Pen (sharpie)
6. Pea seeds

Methods:

Start with no bacteria treatment!!

1. Label two cups with your name(s), teachers name and no bacteria.
2. Place equal amounts of soil into each cup.
3. Make three ½ inch holes in the soil of each cup.
4. Place one pea seed into each hole
5. Cover holes with soil.

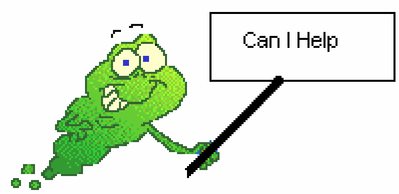
Bacteria treatment

1. Label two cups with your name(s), teachers name and bacteria.
2. Place equal amounts of soil into each cup.
3. Make three ½ inch holes in each cup.
4. Pour small amount of bacteria into each hole (not too much you have six holes to fill)
5. Place one pea seed into each hole
6. Cover holes with soil.

½ scale

Things to think about

1) Why did we do the no-bacteria treatment first?
2) Do you think the bacteria will help the plant grow?
3) What is your hypothesis?
4) How should we measure the plants at the end of the experiment??



Step 1

Step 2 (Bacteria Treatment)

Step 4 and 5