



## Nitrogen Fixation and Legumes Student Planting Instructions

**Experimental question:** Which growing media will result in the most root nodules forming on soybean roots?

You will be testing the following types of conditions to determine which soil encourages the most formation of root nodules: soybean field soil, sterile soil, sterile soil with inoculated seeds, sterile soil with fertilizer at various levels

### Materials:

Soybean seeds (or other legume)

Inoculant (N-DURE or other *rhizobia* containing inoculant for the legume you are planting)

Mass scale

10 ml graduated cylinder

plastic tubs (1 for each soil treatment)

Sterile soil

Soybean field soil

5-gal plastic bucket (large planting) or Styrofoam cup (small planting) (1 for each soil treatment)

Inoculate seeds:

1. Weigh 2 g of inoculant and place it into a container
2. Add 6 ml of water and mix until a uniform mixture is achieved
3. Weigh 100 g of seeds and place them into a separate container
4. Add 2 ml of the slurry to the seeds and stir until they are uniformly coated with the inoculant slurry
5. Remove seeds by straining them from the container and spread them on a piece of clean paper to dry
6. To reduce variables in the experiment, seeds in (-) soil inoculum regimes should still go through the slurry and drying process the same as inoculated seeds only without the addition of the inoculum

Prepare pots/cups for planting:

7. In large plastic bins, soak sterile potting mix using water until slightly moist. Fill pots with slightly moistened growing media to about 3/4s up the soil line
8. Soak other soils as needed to moisten to same level as sterile soil mix.
9. Spread slow release fertilizer evenly on top
  - a. 1X treatments in 5-gal containers should add a cup of fertilizer, in styrofoam cup, add 1 pinch
  - b. 0.25X treatments in 5-gal containers should add a quarter cup of fertilizer in styrofoam cup, add 1/4 pinch
  - c. 0X treatments should skip the fertilizer step
10. Continue to fill the pots to the soil line with slightly moistened soil

Plant seeds:

11. a. Using buckets of soil: Poke 8 one-inch deep holes in the top of the growing media using your finger



- b. Using Styrofoam cups: Poke 3 one-inch deep holes in soil
12. Place a soybean in each hole and lightly cover with dry growing media
13. Using a pipette or dropper, gently apply water to each seed (10-20 drops)
14. Label your groups pot/cups properly and place it on the proper lighted shelf

#### *Growing Tips*

- Adjust lights as the plants grow so that the lights are 10-15 cm from the growing meristem
- Keep room temperature above 60°F
- Check on plant hydration everyday and water as needed
  - Plants will need watered more often as they grow in size
  - If you lift the pot/cup and it feels light, it may need to be watered
- If growing for longer than 3 weeks, thin to 3-4 plants per pot; transplant from other pots if necessary
  - Thin and transplant as soon as you are confident in the establishment of the seedlings