GROW
NEXT GEN

Nitrogen Fixation and Legumes
Teacher Instructions

This Lesson can be presented as a teacher prepared demonstration or as student planting event. It can be adapted to as short as a 2-3 day lesson or as long as 6-8 weeks depending on your space and time limitations. The instructions for planting are the same, it could be the teacher planting 3-4 weeks ahead of the lesson to show the nodule formation in various soils, or students preparing and tracking growth over a longer period of time. See **Experiment** handout.

Biological Nitrogen Fixation and Legumes
Information to use with **Nitrogen Fixation and Legumes powerpoint**
Discussion Points

-**Nitrogen (N)** is the element that plants require in the greatest amount.
- Nitrogen deficiency is the most common factor limiting plant growth and development in most agricultural and forest ecosystems
- Farmers apply ammonium and nitrate based fertilizers to make sure that their plants have enough nitrogen to produce a healthy crop

-Limited availability of nitrogen is closely tied to inhibited growth because nitrogen is a necessary component in many biologically important organic compounds
- Amino acids and nucleic acids are both products of biosynthetic pathways that use **ammonium (NH_4^+)** and **nitrate (NO_3^-)** as building blocks
- Proteins are made from nitrogen rich amino acids
- Symptoms of nitrogen deficiency include:
    - Interveinal **chlorosis,** yellowing of leaf tissues, of older leaves
      -Nitrogen can be moved from older tissues to new, allowing for a plant's newest leaves to remain green while its oldest are chlorotic
    - Slenderness and slow developing
    - Purple coloration
      -Excess buildup of carbohydrates may be used in synthesis of **anthocyanins**, pigments responsible for the purple colors in plants

-Nitrogen can be found in many forms throughout all global ecosystems
- The air is composed of almost 80% molecular nitrogen (N_2), meaning that all terrestrial life is surrounded by nitrogen
    - This large reservoir of nitrogen is unavailable to plants in its atmospheric state

-N_2 gas is extremely stable
- Made of two nitrogen atoms bound together by a **triple covalent bond**, this type of chemical bond is enormously unwavering and requires a high amount of energy to activate a reaction
    - temperatures exceeding 200˚C
    - pressures greater than 200 atmospheres
    - presence of metal catalysts

-N_2 gas must somehow be converted into useable forms, such as ammonium (NH_4^+) and nitrate (NO_3^-); this process is called **nitrogen fixation**.
- There are multiple natural processes to fix nitrogen

- Lightning creates highly reactive free atoms from water vapor that create nitric acid (HNO_3)
- Ozone (O_3) can react with nitric oxide (NO) gas to form nitric acid (HNO_3)
- The vast majority is performed by bacteria or blue-green algae.

-Microorganisms fix N_2 gas into ammonia (NH_3) which in the presence of water converts to ammonium (NH_4^+) in a process called **biological nitrogen fixation**

- Energy-wise, this process is extremely costly
- Requires the protein catalyst, **nitrogenase,** to exchange electrons
- Because of its electron exchange properties, nitrogenase is very susceptible to damage from strong electron acceptors like oxygen
    - This leads to the microorganisms regulating nitrogen fixation only under **anaerobic** conditions, when an environment lacks the presence of oxygen
    - Some bacteria create heavily walled cells that allow them to create a "local" anaerobic environment, while others maintain high levels of respiration to decrease oxygen concentrations
    - Others wait for anaerobic conditions such as flooding
- The majority of nitrogen fixing prokaryotes are found living freely throughout terrestrial soils

-Some nitrogen fixing prokaryotes have formed symbiotic relationships with higher plants

- The bacteria provide a host plant with fixed nitrogen in exchange for carbohydrates, other nutrients and a safe place to reproduce
- The soil microbes infect and live inside specific root tissues in organs called **nodules**.
- Within nodules, the bacteria are provided all the nutrients they need to survive and flourish causing them to produce hundreds of times more fixed nitrogen then they could if found in the soil alone
- Host plants also provide the microorganisms with an oxygen free anaerobic environment, allowing for continuous nitrogen fixation

-One family of plants, *Fabaceae* (**Legumes**), has mastered symbiotic relationships with N_2 fixing bacteria

- Legumes include: the pea, clover, lentils, beans and soy
- The majority of its species have evolved associations with specific soil microbes, **rhizobia**

-Both host plants and microbes have varying levels of ability to live in a symbiotic relationship

-For example: soybean (*Glycine max*) only plays host to *Bradyrhizobium japonicum*, while the common sweet pea can host multiple species from the genus *Rhizobium*

-Host plants and soil microbes communicate with one another via chemicals, proteins and transcription factors

- The host plant secretes chemical attractants into the soil surrounding from its root hairs; these chemicals cause the rhizobia to bind to the root hairs (**Fig. 1A**)

- Upon binding to the root hairs, rhizobia produce *nod* proteins. These proteins act as transcription factors, inducing the expression of genes that encode for enzymes that create the microbes specific Nod factor
  - **Nod factors** communicate with legume hosts to induce nodulation and curling of the root hair cell around the rhizobia (**Fig. 1B**)
  - A particular legume host responds to a specific Nod factor, this acts as a "lock-and-key" mechanism that allows for the specific host-microbe interaction

    -Some plants respond to multiple Nod factors, allowing for relationships with multiple rhizobia species

    -While other legumes, respond only to only one Nod factor and can therefore only be colonized by one species or subspecies
- Nod factors cause the plant root cell to expand abnormally and curl around the growing rhizobia colony (**Fig. 1C**)
  - The rhizobia proliferate within the safety of the coiled root hair
- As rhizobia numbers build, the cell wall within the coil degrades, allowing for infection of the plant cell (**Fig. 1D**)
  - After infection, rhizobia cells travel down the infected root hair
- Upon reaching the end of the root cell, rhizobia are encased by plant membranes and spread to target subepidermal cells where N fixation takes place (**Fig. 1E**)

-As the nodule forms, the plant encases the subepidermal cells in protective tissues and creates a vascular system

- Protective tissues exclude oxygen, providing the bacteria with a perfectly anaerobic environment
- The newly developed vascular tissues provide the rhizobia with carbohydrates and other nutrients

-Under the nodules conditions, the rhizobia export fixed nitrogen in the form of ammonia, which is converted by nodular tissues into organic compounds like **amides** and **ureides**

- Quick conversion of ammonia is necessary since high levels of ammonia can be toxic for both the host plant and the rhizobia
- Amides and ureides are easily transported via xylem
- Ureides, in particular urea, can be long lasting in soils under certain conditions

-Because of this symbiotic relationship between legumes and rhizobia, farmers often have no need for nitrogen fertilizer applications for *Fabaceae* when their specific rhizobia are present

- In fields where the rhizobia are not present, farmers will inoculate the soil or seed with the host plant's specific microbe to increase yields

-Legumes are also commonly used in crop rotations to supply the soil with fixed nitrogen that may have been depleted during previous non-legume plantings

- This practice goes back as far as the Romans, when it was common practice to turnover legume crops to "manure" wheat and other nitrogen intense crops

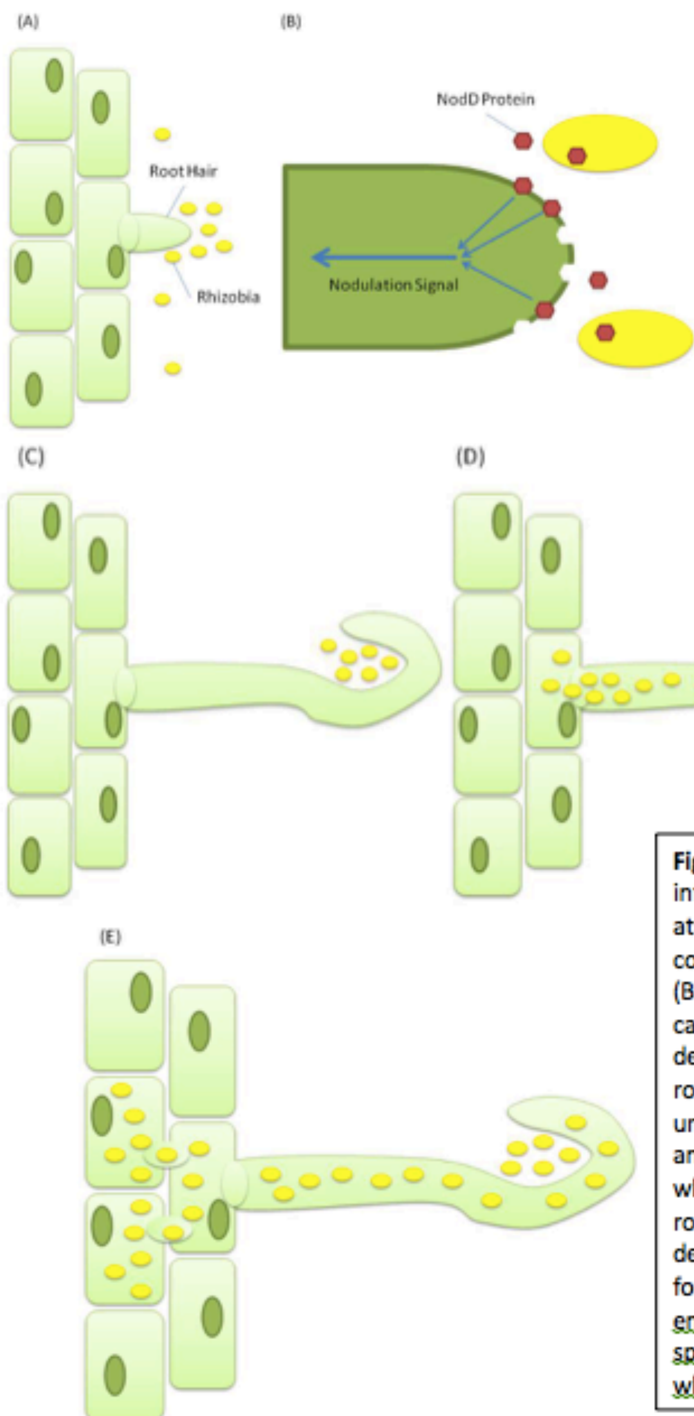

Figure 1: Nodulation and Rhizobia infection. (A) Rhizobia are attracted to root hair surface by compounds released by the root. (B) Rhizobia produce Nod factors causing a cascade of developmental signals within the root hair cell. (C) Root cell undergoes excessive elongation and curls around the Rhizobia, which proliferate within the coiled root hair. (D) Root cell wall degrades within the coil allowing for infection. (E) Rhizobia are encased by plant membranes and spread to target cells and cytosol where N fixation takes place.