Energy efficiency and profitability can be improved by including legume plants, such as soybeans, in the rotations. These plants in collaboration with soil bacteria, have the capacity to convert atmospheric \( \text{N}_2 \) to plant \( \text{N} \) (N fixation). In soybeans, the soil bacteria responsible for this process are called *Bradyrhizobia japonicum*. Each plant type that has the capacity to fix N requires a different bacteria, and if the soil does not contain that bacteria, it cannot fix N. Tips for increasing N fixation are provided in Table 23.1.

Inoculums can be applied as an in-furrow granular or as a solid or liquid treatment to the seed. Inoculants are live organisms and should be treated carefully. The purpose of this chapter is to discuss soybean seed treatment with *Bradyrhizobia japonicum*.

**Table 23.1 Tips for increasing N fixation.**

1. Soybean seed should be inoculated:
   a. if the field has not been seeded to soybean within the previous 5 years;
   b. if the field is being converted from CRP to soybean;
   c. if soil has been flooded for over a week; and
   d. if soil has a pH less than 6.0 or greater than 8.5.
2. To minimize plant stress, follow P and K recommendations, seed at an appropriate time, and control pests.
3. Use care when handling or combining seed treatments; take note of all safety precautions from seed treatments.
4. Under moderate drought stress, monitor the soil N supply. N fixation is one of the first activities to be reduced under water stress. [http://arkansasagnews.uark.edu/4859.htm](http://arkansasagnews.uark.edu/4859.htm)
5. For corn-following-soybeans, subtract the appropriate N credit from the N fertilizer recommendation.
6. If cover crops cocktails include legumes, they should be inoculated with an appropriate bacteria.
7. N credits from soybeans and cover crops should be subtracted from next year’s N recommendation.
The importance of rotations in N fixation

Prior to the development of commercial N fertilizer industry, the primary source of N for row crop production was N₂ fixation by legumes. Different cultures have integrated legumes and other soil amendments using a variety of approaches. North American native peoples seeded corn, beans, and squash in the same area, while the Incas collected guano from the bird-islands.

In Europe, the linkages of the livestock with rotations that included legumes has been partially credited with providing the food needed for the English population to increase from 5.7 in 1750 to 16.6 million people in 1850. Associated with the population growth was a change in the rotational sequence from a crop rotation that included a cereal (oats, rye, wheat, and barley), legume (peas and beans), and fallow to a four-year rotation that included wheat, barley, turnips and clover. Turnips replaced fallow in the rotation, provided forage for livestock, and reduced weed pressure. The net result was wheat and pulse yields that increased 68% and 44% from 1750-1759 to 1850-1859. In addition, stocking densities for milk cows, sheep, and swine increased 46, 25, and 43%, respectively (Broadberry et al., 2010).

These cultures learned that soil fertility is critical for productive agriculture and that N fixation by legume crops increases the yields in following crops. N fixation is conducted by plants that have the capacity to form symbiotic relationships with N-fixing soil bacteria. In rice production, the free-living bacteria, blue green algae, provide N to rice, while in South Dakota soybean fields *Bradyrhizobia japonicum* have this capacity. Other legumes with this capacity are peas, clovers, and beans. In forest systems, N is fixed by both legume and non-legume plants. In row crop production, each legume requires a different N-fixing organism.

In N₂ fixation, energy is used to break the triple bond holding the two N atoms together. The equation that converts atmospheric N₂ to plant N is:

\[ \text{N}_2 + 8\text{H}^+ + 8e^- + 16 \text{ATP} = 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{Pi} \]

In this reaction, the ATP represents energy. This reaction is energy intensive and requires approximately 10 lbs carbohydrates for each pound of N produced. The ammonia (NH₃) is subsequently converted to an amino acid such as glutamine.

The protein complex that facilitates this reaction is nitrogenase, which is composed of an iron and molybdenum-iron proteins. N fixation by nitrogenase is inactivated when exposed to oxygen. Different organisms use different techniques to reduce O₂ inactivation. For example, in rhizobium, leghaemoglobin (closely related to our hemoglobin) is used to reduce O₂ concentrations, while in cyanobacteria N₂ fixation is conducted in specialized cells (heterocysts) where only photosystem I (oxygen is not produced) is used. N fixation can also be reduced by stresses that reduce plant activity.
In soybeans, N fixation is conducted by *Bradyrhizobia japonicum*. In this symbiotic relationship, the plant obtains N while the bacteria obtains energy required for growth and development. Nitrogen fixation is inhibited by a high nitrate concentration. After germination, *Bradyrhizobia japonicum* invade the seedling root hairs and nodules begin to form. The first nodules can be observed several weeks after seedling emergence at V2-V3. Active nodules turn red or pink when cut in half while inactive nodules are a grey color.

N fixation can be reduced:
1. when the temperatures are cold or hot (optimum temperature is between 60° to 80°F),
2. high soil nitrate levels,
3. drought,
4. excessive wetness, and
5. soil compaction.

**N fixing inoculants**
If *Bradyrhizobia japonicum* inoculants are needed, it is important to remember that they are live organisms. The basic choices are solid, liquid, and freeze-dried. Seed inoculations can be conducted as an in-furrow or directly to the seed. In-furrow materials generally are dry, granular materials. These products should be stored flat on a pallet at temperatures ranging from 8° to 12°C.

Direct seed treatments generally are dry or liquid materials that may be a broth culture or frozen concentrate. They can be metered and mixed with the seed by the auger when filling the planter or drill. The inoculants should be fresh, stored using appropriate techniques, and treated with care. Labeled directions should be followed. Care should be used when using seed treatments. Inoculants are very sensitive to Captan and PCNB.

When treating soybean seed:
1. check the expiration date,
2. do not expose the inoculants to heat or direct sunlight,
3. follow labeled directions for different mixtures, and
4. plant treated seed quickly—do not leave treated seed in the planter box overnight.

Liquid inoculants should be stored as cool as possible without freezing. Granular inoculants should be stored flat on a pallet (8-12°C).

**Impact on the following crop**
Nitrogen fixation by the soybean and other legumes can reduce the N requirement in the following crops. These credits are provided in Table 23.2. When no-tilling into legume green manure, use a half credit. For the second year following alfalfa and a legume green manure, use a half credit.

<table>
<thead>
<tr>
<th>Previous Crop</th>
<th>Population plants/ft²</th>
<th>Credit lbs N/acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean, edible beans, peas, lentils and other</td>
<td>--</td>
<td>40</td>
</tr>
<tr>
<td>annual legumes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa and legume green manure crop</td>
<td>&gt;5</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>3-5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>&lt;1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Cover crops**
If you are using cover crops in your rotation and the cover crop cocktail contains a legume, the legume seed needs to be inoculated with an appropriate organism to insure N fixation. N credits from cover crops currently are being evaluated.
References and additional information

Conley, S.P., and E.P. Christmas. 2005, Using inoculants in a corn-soybean rotation. SPS-100-W. Purdue University, Purdue Extension. Available at http://www.extension.purdue.edu/extmedia/sps/sp-100-w.pdf


Acknowledgements
Funding for developing this chapter was provided by USDA-NRCS, South Dakota Soybean Research and Promotion Council, USDA-AFRI, and South Dakota 2010 research program.