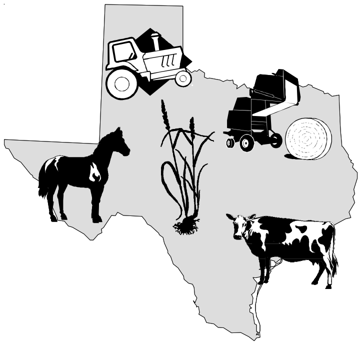

# FORAGES

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# Inoculation, Nodulation, Nitrogen Fixation and Transfer

by
Dr. Twain Butler
*Extension Forage Specialist*
*Stephenville*

Dr. Gerald Evers
*Professor*
*Texas Agricultural*
*Experiments Station*
*Overton*

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Soil & Crop Sciences Dept.
Texas Cooperative Extension
Texas A&M University
College Station, TX 77843-2474



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## Introduction

Nitrogen (N) is one of the most limiting nutrient for plant growth. A legume plant's ability to use nitrogen from the air is one of the best known benefits of growing legumes, but the least understood. Approximately 79% of the earth' atmosphere is nitrogen gas ($N_2$). However, this N is not in a form that plants can use. In reality, it is not the plant that removes N from the air, but *Rhizobium* bacteria, which live in small tumor like structures on legume roots called nodules. These bacteria can take $N_2$ gas from air in the soil and transform it into ammonium ($NH_4^+$), which is then used by the plant. This $NH_4^+$ is the same form as the $NH_4^+$-N in ammonium nitrate (34-0-0) and ammonium sulfate (2 1-0-0) fertilizer. The nitrogen fixation ($N_2$-fixation) process between the legume plant and bacteria is referred to as a symbiotic (mutually beneficial) relationship. Each organism receives something from the other and gives back something in return. *Rhizobia* bacteria provide the legume plant with N in the form of $NH_4^+$ and the legume plants provide the bacteria with carbohydrates as an energy source.

## Seed Inoculation

Inoculation is the process of adding the proper *Rhizobia* bacteria to the legume seed so that $N_2$-fixation can occur. Most legume species have a specific *Rhizobia* species requirement for inoculation. There are numerous strains of native *Rhizobia* bacteria that occur naturally in different soils. Some of these *Rhizobia* strains are capable of infecting a given legume species, but will vary in their efficiency to fix $N_2$. To ensure that an effective *Rhizobia* strain is present legume seeds are inoculated with the correct *Rhizobia* bacteria before planting.

When purchasing inoculant, be sure the legume species you want to plant is listed on the package and that the expiration date has not passed. The inoculant should be purchased when buying the legume seeds several weeks in advance of the estimated planting date. This allows time for the retailer to order the seeds and/or inoculant if not kept in stock. There are several brands of inoculant and the most effective ones are those, which have a large number of *Rhizobia* per gram of inoculant. Ground peat moss is used as a carrier for the bacteria contained in inoculants. Most inoculants contain a sticker that helps hold the inoculant to the seed such as HiStick and Pelinoc-Pelgel. Rhizobia bacteria are very susceptible to high temperatures. Be sure the inoculant is kept in a cool, dry location away from direct sunlight. Most inoculant companies recommend their products be kept in a refrigerator until used except for HiStick, which can be kept at room temperature. It is desirable to drill the inoculated seeds in the soil to help protect the bacteria from the sun and high temperatures.

*Rhizobia* bacteria begin dying as soon as the inoculated seeds are planted. The longer the seeds lies in the soil before germination, the fewer viable *Rhizobia* are present. If regular inoculant is applied to the seeds with water, buttermilk, or Coca-Cola as a sticker, the bacteria may only survive in the soil for about a week, however this depends on temperature and moisture. Inoculant containing a sticker or that is coated on the seed (clay-coated) provides more protection for the bacteria, which improves its survival to about 3-4 weeks. It is difficult to introduce a new legume species into a pasture that has had a native, naturalized, or different legume species growing on it for several years. The *Rhizobia* strain infecting the previously grown legume species will have built up a large soil population over the years. Due to overwhelming numbers (competition), the resident *Rhizobia* strain may occupy most of the infection sites on the new seeded legume and prevent infection by the introduced *Rhizobia* strain.

## Nodulation

Nodulation is a highly specific process that is extremely complex. The first step in the establishment of symbiotic $N_2$-fixation is the attachment of host specific *Rhizobia* bacteria to the root hair tips. Legume roots exude flavenoids (specific phenolic compounds), which attract and stimulate bacteria growth around the root tips. Rhizobia bacteria secrete indole acetic acid (IAA), which acts like an auxin, causing the root hair to curl around the bacteria and forming a nodule. Once the nodule is attached, an infection thread penetrates the root, which allows for the bacteria and root to act as one unit. Ineffective *Rhizobia* strains will form many small nodules on the legume root, but fix little or no $N_2$. Effective *Rhizobia* strains that fix high rates of $N_2$ from fewer, but larger nodules that have dark pink or red centers, which indicate the presence of leghemoglobin. Since the enzyme responsible for $N_2$-fixation is poisoned by $O_2$, leghemoglobin is important because it supplies $O_2$ to the bacteria while preventing $O_2$ from the enzyme.

## Nitrogen Fixation and Transfer

Nitrogen fixation is the conversion of atmospheric $N_2$ to $NH_4^+$-N in the presence energy (ATP) and the nitrogenase enzyme complex. The bacteria encode an enzyme complex called nitrogenase (Mo-Fe, Fe-S protein), which is made up of molybdenum (Mo), iron (Fe), and sulfur (S). This enzyme complex is actually responsible for $N_2$-fixation.

$$N_2 \xrightarrow{\text{Nitrogenase}} 2NH_3$$

Rate of $N_2$-fixation is directly related to legume plant growth rate. Anything that reduces plant growth such as drought, low temperature, limited plant nutrients, or disease will also reduce $N_2$-fixation. Maintaining sufficient leaf area in a legume stand to intercept most of the sunlight is also critical to maintain a high growth rate to support $N_2$-fixation. When the legume plant matures and dies, nodules on the root system decompose and release the *Rhizobia* into the soil. If the same legume species is planted again the following year or volunteers from seeds, sufficient numbers of *Rhizobia* are usually present to provide good nodulation.

The $NH_4^+$ form of N is incorporated into organic acids in the root. These compounds are transported via the xylem in the plant. The primary pathways for N transfer from the legume to the soil are through grazing livestock and decomposition of dead legume plant material. The root system and unused leaves and stems of annual legumes die at plant maturity and are decomposed by soil microbes over time. Nitrogen contained in this plant material is released through this process and becomes available to other plants.

When legume forage is consumed by grazing livestock most of the N in that forage passes through the animal and is excreted in the urine and feces. Unfortunately about 25-50% of the N in the urine is lost through volatilization. Another problem is the distribution of feces and urine across the pasture. With continuous grazing at low stocking rates, much of the animal excreta is concentrated around local areas. Animal excreta distribution is improved with rotational grazing systems where stock density is higher.

## Summary

The quantity of $N_2$ fixed by legumes can range from none to over 200 lb $Ac^{-1}$. General estimates of nitrogen fixed in Texas range from 75 to 125 lb N/acre for annuals and about 150 lb N/acre for alfalfa. (Table 2). Proper inoculation is essential for nodulation and $N_2$-fixation. Factors that influence the quantity of N fixed are the level of soil N, the *Rhizobia* strain infecting the legume, amount of legume plant growth, how the legume is managed, and length of growing season. If given a choice, a legume plant will remove N from the soil before obtaining $N_2$ from the air through $N_2$-fixation. Once $N_2$ is fixed, the legume can utilize this for the legume's growth. This N can then be recycled for utilization by other plants.

**Table 1. Nitrogen Fixation Estimates for Winter Annual Legumes**

| Species | *Scientific Name* | Lb. $N/Ac.^{-1}$ $yr.^{-1}$ |
|---|---|---|
| Arrowleaf clover | *Trifolium vesiculosum* Savi | 113 |
| Ball clover | *Trifolium nigrescens* L. | 84 |
| Berseem clover | *Trifolium alexandrinum* L. | 243 |
| Burr medic | *Medicago polymorpha* L. | 131 |
| Button medic | *Medicago orbicularis* L. | 111 |
| Crimson clover | *Trifolium incarnatum* L. | 138 |
| Rose clover | *Trifolium hirtum* All. | 88 |
| Red clover | *Trifolium pratense* L. | 100 |
| Subterranean clover | *Trifolium subterraneum* L. | 143 |
| White clover | *Trifolium repens* L. | 38 - 348 |
| Alfalfa | *Medicao sativa* | 132 |
| Australian winter pea | *Pisum arvense* L. | 150 |
| Hairy vetch | *Vicia villose* Roth. | 89 |