

Biological Nitrogen Fixation

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Forage legumes are popular choices in pasture systems for several reasons including lengthening the grazing season and improving pasture nutritive value. An additional benefit of using forage legumes relates to the plant's ability to fix atmospheric N (N), thus reducing or eliminating the need for N fertilizer. Besides providing the legume plant with the required N for its own growth, this fixed N is also shared via several mechanisms with other non-N-fixing species such as associated forage grasses. This document describes the process by which forage legumes fix atmospheric N and provide N to the pasture system.

Plant-Bacteria Relationship

The legume plant's ability to fix atmospheric N is due to a *symbiotic* relationship with bacteria of the genus *Rhizobium*. In a symbiotic relationship, both the plant and bacteria contribute and both also receive as a result of their association. The bacteria that perform the role of atmospheric N fixation are host specific. For example, winter peas, alfalfa, and cowpeas are all legumes, but each requires a different *Rhizobium* species to effectively fix atmospheric N. Even within the clover group, many clovers require different *Rhizobium* species to bring about the desired N fixation.

For new establishments, seed of legumes are required to be *inoculated* with the proper *Rhizobium* spp. That is, the seed is coated with a sticking agent and the bacteria applied directly to the seed. This places the bacteria in close proximity to the seed at the time of germination. Infection of the plant by the bacteria takes place at the root hair, which indicates infection with a curling response.



Plant response Rhizobium infection is referred to as *nodulation*. Nodules appear as small lumps on the roots of legume plants. It is inside these nodules that N fixation occurs. As noted before, both the plant and bacteria are involved in the fixation process.

Inoculation Guidelines

- Match the legume species to the appropriate commercial inoculants. It is critical to purchase the correct inoculant. The exact legume species (examples: white clover, arrowleaf clover, alfalfa, cowpeas) should be listed on the inoculant package.
- ✓ Protect the package of inoculant from sunlight and high temperatures. Remember that the *Rhizobium* bacteria are live organisms that can die if they get too hot or too dry.
- ✓ Inoculate just prior to planting. If planting is delayed, keep the seed in a cool place until needed.
- ✓ Use inoculants with some type of sticker (adhesive) that will help keep the seed and inoculant together during the planting and germination process.

Plant Role in Nitrogen Fixation

Besides furnishing the bacteria a place to reside (inside the nodules), the legume plant provides the following key components necessary for N fixation.

- ✓ *Leghemoglobin*: This important enzyme helps maintain low levels of oxygen inside the nodule.
- *Energy*: The process of N fixation requires energy, which is provided by plant photosynthesis.

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Bacteria Role in Nitrogen Fixation

Just as the plant provides key components necessary for N fixation, so too do the bacteria living in the root nodules. These components are listed below.

- Nitrogenase: This enzyme actually breaks the strong atmospheric di-N molecule and causes it to be available to the plant.
- ✓ **Uptake hydrogenase**: This important enzyme increases the energy efficiency of the N fixation process. The uptake hydrogenase enzyme also ties up oxygen to protect the nitrogenase enzyme in much the same way leghemoglobin does.

Rhizobia and the Environment

Environmental aspects that promote plant growth are also required for effective colonization of the host plant by *Rhizobium* spp. Those environmental aspects of importance are noted below.

- ✓ **Soil pH**: Ideally soil pH should be 5.5-7.5. Below a pH of 5.0, N fixation is reduced and will cease below a pH of approximately 4.0.
- ✓ **Soil structure**: Good structure leads to good aeration and drainage. Although oxygen destroys the ability of the bacteria to fix atmospheric N, the bacteria themselves are aerobic microbes and require adequate oxygen.
- Temperature: Must be within a range suitable for microbial activity. As temperatures are reduced, bacteria activity is reduced and below freezing, most N fixation ceases. Likewise, elevated temperatures during summer can cause N fixation to slow or stop as soil temperatures become elevated.
- ✓ **Soil moisture**: An adequate supply of moisture is necessary for good bacterial activity. Generally, if there is adequate moisture for plant growth, there is adequate moisture for the bacteria.
- ✓ Adverse effects on N fixation: Certain N compounds such as ammonia, nitrates, nitrites, and amino acids may suppress both the activity and synthesis of the nitrogenase enzyme. Ammonia has a direct effect, while nitrates and other N compounds may give rise to ammonia. This is an explanation as to why, in the presence of N fertilizer, less N is fixed by the legume plant.

Sharing the Nitrogen

Nitrogen fixed by the legume plant is available for use by associated non-N-fixing pasture plants by several methods. Most common methods of N transfer are:

- Recycling N-containing compounds through the grazing animal as fecal material or urine.
- Recycling N-containing compounds as decaying legume plant above ground and below ground organic matter.
- Possible transport of N-containing compounds from legume plant roots and nodules directly to other plant species roots by certain types of soil microbes. This is variable due to soil type and nutrient status.
- ✓ Leaching of N-containing compounds from legume plant leaves during precipitation events. This is probably a minor component to the overall N sharing aspect.
- Possible leaking of N-containing compounds from legume plant roots and nodules for uptake by other plant species roots. This is another minor component of N sharing.

Regardless of the method, forage legumes can contribute significant quantities of N to the pasture system and reduce or eliminate the need for inorganic N fertilizer. (See Table 1).

Summary

In order for forage legumes to fix atmospheric N, there must be an adequate population of the appropriate *Rhizobium* bacteria in the soil where the legumes grow. Soil moisture, pH, temperature, and structure must be adequate to promote N fixation. The role of the plant in is to furnish carbohydrates used by the bacteria as energy sources for metabolism and for reaction with liberated N to form nitrogen-containing compounds for plant use. The plant also provides protection from oxygen contamination by furnishing the enzyme leghemoglobin.

The role of the bacteria is to furnish the nitrogenase enzyme required for fixation of atmospheric N. The bacteria also furnish the uptake hydrogenase enzyme necessary for energy efficiency and possible protection of the nitrogenase enzyme from oxygen contamination.

Nitrogen fixed by forage legumes can be shared with other associated non-N-fixing plants helping to reduce or eliminate N fertilizer requirement, thus helping to reduce input costs associated with livestock production systems.

Table 1. Dry matter	(DM) & nitrogen (N)	vield of crimson clover &	a nairy vetch over a 3-y	ear period at Overton, TX

Species	Avg DM Yield (lbs/ac)	DM Yield Range (lbs/ac)	Avg N Yield (lbs/ac)	N Yield Range (lbs/ac)	Avy Recovery ² (%)	Range Recovery (%)		
Crimson clover	4092	3943-4356	90.6		44.0	15.8-63.6		
Hairy Vetch	3196	2549-3548	111.5	92.2-122.5	55.3	29.7-70.6		
¹ Smith, 1986 ²	² Recovery is the amount of N contained in subsequent non-N fertilized warm-season annual grass, pearlmillet							

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