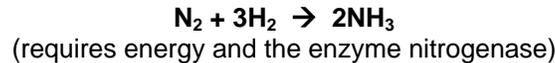


# Nitrogen Fixation by Forage Legumes

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## Why is nitrogen fixation important?

The air we breathe is 78% nitrogen (N<sub>2</sub>) by volume, yet many plants suffer N deficiency because this N is in a form unusable to plants. Some plants, however, have co-evolved with specific bacteria that can make this N available. When properly inoculated, legumes generally do not require N fertilizer because of this symbiotic relationship with beneficial bacteria.



## What are rhizobia?

Rhizobia are bacteria capable of fixing N in association with a plant. Bacteria that nodulate legumes are currently classified into 6 genera (*Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium*, and *Allorhizobium*). From a practical perspective, these are called “nodule bacteria” or **rhizobia**. Rhizobial species are specific to their host plant, so producers should be sure the strain of rhizobia is appropriate for the legume they are planting. Commercial inoculants often list on the packaging the legume species they can effectively ‘infect’ and nodulate. Even now, less than 15% of the roughly 20,000 classified species of legumes have been evaluated for nodulation. Many of the tropical forest legumes have not been evaluated but may have potential for development at some future time. The photo at right shows birdsfoot trefoil nodules.



## What happens during nodulation?

Nodules are the tissue (small swellings) on the root system of legumes that house the rhizobia. Signals in the form of organic molecules, called flavonoids, from the plant communicate with the rhizobia. In most legumes, root hairs near the growing root tip curl as rhizobia attach to the tip and an infection thread forms within the hair to allow bacterial entry. The plant produces a new meristematic region where rapid growth occurs to house the rhizobia and to provide them water and nutrients. The rhizobia, in return, use part of the nutrients provided to produce ammonia (NH<sub>3</sub>) from N<sub>2</sub>. The NH<sub>3</sub> is then converted into an organic compound for transport and use by the plant.

## Do I need to inoculate?

Inoculation is the process of adding effective rhizobia to the seed or soil before or during planting. The purpose of inoculation is to ensure that there are enough rhizobia of the correct type present in the soil to form an effective legume-bacterial symbiosis.

**The relatively small investment of time and money in inoculation is cheap insurance to ensure that the correct bacteria are present.**

When a new legume is first introduced in a field, the soil may not contain appropriate rhizobia. Suitable organisms must be added for adequate nodulation and N fixation. The difference between nodulated and non-nodulated plants is often striking. Once the

legume becomes established and is well nodulated, inoculation in subsequent years is usually not required. Rhizobia can function as free-living bacteria and are able to live in the soil even when forage legumes are not present.

**There is no simple test to tell whether suitable rhizobia are already present in the soil before planting.**

Where a specific legume has been grown in a region for some time and the roots are extensively nodulated, most soils contain abundant rhizobia and even non-inoculated plants are well nodulated. Because of abundance of rhizobia in these soils, added rhizobia often produce only a small fraction of the nodules formed and yields are unresponsive.

### Which inoculant should I use?

The rhizobia are specific in which legumes they will nodulate, and there are hundreds of different types of rhizobia. For example, alfalfa rhizobia will not nodulate clover, soybean, peas, trefoil, or crownvetch. Some companies may mix different rhizobia in one inoculant to be effective on more than one legume. **Do not use an inoculant for a legume if the legume species isn't listed on the package or company literature**

<b>It is critical that the producer select the specific strain (inoculum type) that is compatible with the legume being grown.</b>			
Common Legume Name	Scientific Name	Nitragin Inoculant Group*	Becker Underwood Inoculant Group**
Adzuki bean	<i>Vigna angularis</i>	EL	cowpea/lespedeza
Alfalfa	<i>Medicago sativa</i> L.	A	alfalfa/sweet clover
Alsike clover	<i>Trifolium hybridum</i> L.	B	true clover
Annual medic	<i>Medicago spp.</i>	N	alfalfa/sweet clover
Berseem clover	<i>Trifolium alexandrinum</i>	R	true clover
Birdsfoot trefoil	<i>Lotus corniculatus</i>	K	birdsfoot trefoil
Chickpea (garbanzo)	<i>Cicer arietinum</i> L.	GC	garbanzo bean/chickpea
Cicer milkvetch	<i>Astragalus cicer</i> L.	<i>Astragalus</i> Sp. 1	special (milkvetch)
Common or field bean	<i>Phaseolus vulgaris</i>	D	dry bean
Cowpea	<i>Vigna unguiculata</i>	EL	cowpea/lespedeza
Crimson clover	<i>Trifolium incarnatum</i>	R	true clover
Crownvetch	<i>Coronilla varia</i>	M	crownvetch
Fava bean	<i>Vicia faba</i> L.	Q	pea/vetch
Field peas	<i>Pisum sativum</i> subsp. <i>arvense</i>	C	pea/vetch
Hairy vetch	<i>Vicia villosa</i>	C	pea/vetch
Korean lespedeza	<i>Lespedeza stipulacea</i> Maxim	EL	cowpea/lespedeza
Kura clover	<i>Trifolium ambiguum</i> Bieb	<i>Trifolium</i> Sp. 3	special (kura clover)
Lupine	<i>Lupinus</i> spp.	H	special (lupine)
Mung bean	<i>Vigna radiata</i> L.	EL	cowpea/lespedeza
Pigeon pea	<i>Cajanus cajan</i> <i>C. indicus</i>	EL	cowpea/lespedeza
Red clover	<i>Trifolium pratense</i>	B	true clover
Sainfoin	<i>Onobrychis viciifolia</i> Scop	F	special (sainfoin)
Sericea lespedeza	<i>Lespedeza cuneata</i>	EL	cowpea/lespedeza
Subterranean clover	<i>Trifolium subterraneum</i> <i>T. yanninicum</i> <i>T. brachycalcycinum</i>	WR	special (sub colver)
Sweetclover	<i>Melilotus officianalis</i> <i>M. alba</i>	A	alfalfa/sweet clover
White clover	<i>Trifolium repens</i>	B	true clover

This table lists the inoculant groups of two major inoculant suppliers for legumes commonly grown in Iowa (both companies also make inoculants for legumes not listed).

\*Nitragin, 13100 West Lisbon Ave., Suite 600, Brookfield, WI 53005 U.S.A (800 558-1003).  
 \*\*Becker Underwood, Inc., 801 Dayton Avenue, Ames, IA 50010 U.S.A. (800 232-5907). Special inoculants are available on request (check with company).

(call the company if in doubt). Pay particular attention to clover inoculates; different strains are used on a) red and white clovers, b) arrowleaf clover, and c) crimson and berseem clovers.

### How do I inoculant my crop?

The inoculant industry manufactures cultures ranging from small quantities for use with exotic seed to large-scale, fermenter-grown cultures mixed with peat, clay, or other suitable carrier for use with alfalfa and clovers. Inoculation must put the rhizobia in contact with the seed or root system. The grower has several options for seed or soil-applied inoculation. Organic producers must verify that any carriers contain products approved for their production system.

Seed applied—Inoculant may be surface applied to seed before planting. Peat (or clay), which protects the rhizobia and maintains viability, is a common carrier. The inoculant carrier must adhere to the seed and stay put until the seed enters the soil.

This is especially important with many small-seeded forage legumes because of their size and slick

A small cement mixer mixes the seed and inoculant uniformly, but also mixing in a tub, on a tarp, or in the bed of a pickup, will work. For small seed quantities, pouring seeds and inoculant between clean 5-gallon buckets also works well.

surface. To aid adhesion, the inoculant is often mixed with a sticker, such as milk or water (water may contain sugar, molasses, or gum arabic), and uniformly coated on the seed. (Commercial sticking agents are also available from many inoculant producers and generally work well.) You can make your own sticker by preparing a sugar solution of 1-cup sugar to 1-quart warm water. Slightly moisten the seeds with

Most seed fungicides (or molybdenum) can be toxic to rhizobia during prolonged exposure. Usually, treated seed can be inoculated with an inoculant and planted within a few hours with little ill effect.

a small amount of water or sticker, and mix with inoculant. Only a small amount of liquid should be used to prevent the seeds from getting too wet. After

mixing, all the seeds should be evenly covered with small specks of inoculant. The seed is dried in a cool, shaded location (not exposed directly to sunlight) and planted the same day. Delayed planting can result in extensive loss of rhizobia. If not planted within 24 hours, reinoculate.

Preinoculation--Forage seed also may be purchased that is preinoculated. This is usually done by impregnation with rhizobia or by pelleting the seed with fine limestone (or even rock phosphate). In the later process, the sticker and inoculant are added and the seed is rolled in ground limestone.

Preinoculated seed may be quite variable in viable rhizobial numbers based on the time of inoculation and storage conditions since inoculating (the seed should be handled in the same manner as packaged inoculum, out of direct sunlight and heat). If you suspect that the rhizobia may have died because of poor handling or a long time since inoculation, then reinoculate the seed. If coated with limestone, consider using mineral or vegetable oil (0.5 to 1.0 ounce per pound of seed) as a sticker because the water or sticking solution may cause the pelleted seed to flow poorly.

**Many believe that applying the inoculant separately at time of planting is preferable.**

Inoculant poured on the top of seed in a planter box often will result in poor inoculation. This is a simple method, but inoculant and seed tend to become separated in the box and provides uneven coverage.

Soil applied—Inoculant may be band applied below the seed in peat, clay, or liquid form or applied in the seed row. Granular inoculants, although not common with forage legumes, may be placed in the seed row by using an insecticide box of a planter or through the fertilizer or grass seed box of a drill (clean the box thoroughly before use). Frozen or concentrated liquid cultures may be diluted to a slurry, then added to a water-filled tank for spraying into the seed row. Inoculant application directly to the soil often gives higher numbers of rhizobia but is more expensive than seed-applied inoculants. Never allow the inoculant to remain in a planting attachment in the hot sun for long. Remove unused inoculant from the attachment, seal it in the original bag, and store in an area protected from heat and sun.

*An inoculant contains rhizobia, up to a billion per gram (less than a teaspoon) that must be kept alive. Heat and direct sunlight can kill rhizobia in stored inoculants, even in unopened packages. The rhizobia also die naturally with time. Therefore, all packaged inoculants have an expiration date (**growers are advised not to use an inoculant past its expiration date**). Even a short period of exposure to heat (such as on the dashboard of a hot vehicle) may kill the rhizobia. The preferred storage location for inoculants (for both retailer and farmer) is in a refrigerator, but not a freezer. If a refrigerator is not available, store the inoculant in a cool, dark place until ready for use. The photo below shows alfalfa seeds inoculated with a peat-based inoculant.*



### Who makes forage legume inoculants? (This list is thought to be complete as of May 2005.)

Agribiotics Inc.  
<http://www.agribiotics.com/index.html>  
145 Turnbull Court  
Cambridge, ON N1T 1C6  
(800) 619-6773  
FAX (519) 624-8976

Philom Bios  
<http://www.philombios.com/index.html>  
318-111 Research Drive  
Innovation Place  
Saskatoon, SK  
Canada, S7N 3R2  
(306) 668-8220  
FAX (306) 975-1215

Becker Underwood, Inc.  
[www.beckerunderwood.com](http://www.beckerunderwood.com)

801 Dayton Avenue  
Ames, IA 50010  
(800)232-5907  
FAX (515) 232-5961

CelPril  
<http://www.celpril.com/>  
251 Oak Street  
Manteca, CA 95337 U.S.A  
(209) 823 1738  
Fax: (209) 823-8855

Nitragin  
<http://www.nitragin.com/>  
13100 West Lisbon, Ave., Suite 600  
Brookfield, WI 53005 U.S.A.  
(262) 957-2000  
FAX (262)-957-2121

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### Are my nodules fixing nitrogen?

Nodules appear on the new roots in 2 to 3 weeks after the plant emerges. Nodule shapes vary with different legumes. Alfalfa nodules are small, branched, and elongated. Red and white clover nodules are club shaped and lobed. Cowpea, peanut, and soybean nodules are spherical.

**A simple way to tell if a nodule is fixing N is to cut it open. A red color indicates the presence of leghemoglobin and a capacity to fix N. White interiors indicate ineffective nodules. Green interiors indicate nodules that are past their prime.**

Three outcomes of inoculation:

- 1) **No nodulation:** suitable rhizobia are not present in the soil or high N levels prevent nodules from forming.
- 2) **Ineffective nodulation:** the nodules that form are not fixing good rates of N--they may even be taking N and carbon from the plant and reducing plant growth. Often these are smaller nodules scattered over the entire root system.
- 3) **Effective nodulation:** numerous nodules are present and fixing the N needs of vigorously growing plants. Often these nodules are larger and clustered on primary root systems, but nodules on lateral roots also may be effective.

### Is there a field test to indicate suitable rhizobia are present and fixing N?

If you wish to conduct a small field trial to determine if a) you have appropriate rhizobia in your soil and b) they are effective in fixing N, you may plant a SMALL area with the test legume. Mark plots and fertilize portions of the area with N fertilizer (perhaps 100-200 lb N/acre). Extensive nodulation of the plants in the non-fertilized area would mean the proper strains are present that can nodulate the legume. Compare the fertilized and non-fertilized areas. If growth is generally comparable in both areas, the rhizobia are effective in fixing N. If all plants grow poorly, factors other than N may be limiting growth, such as variety adaptation, acidity, fertility, moisture, compaction, or other.

**This field test is relatively simple but will take several weeks to complete.**

### Can plants already growing be inoculated?

Emergency inoculation of plants already growing in a field is possible but not desirable. After the young seedlings are up (wait at least 4 weeks after planting), check for adequate nodulation. If nodules are not present, one may mix the inoculant with a bulking agent (such as cottonseed meal, wheat middlings, sand, or limestone) and broadcast uniformly over the soil surface or drill into the soil. Alternatively, one may make a slurry of the inoculant and spray on the soil surface. Both surface applications depend on cool conditions and rains shortly after application to move the rhizobia into the soil. Under hot and dry conditions, it is necessary to drill the inoculum into the soil. Check with inoculant supplier on appropriate rates to use.

## Coordinator's supplemental information

<http://www.rhizobium.umn.edu/>

This page is a good discussion site.

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenFixation.html>

This site offers a good discussion of the legume infection process.

[http://www.rhizobia.co.nz/Rhizobia\\_Taxonomy.html](http://www.rhizobia.co.nz/Rhizobia_Taxonomy.html)

This page discusses the current taxonomy of rhizobia.

<http://commtechlab.msu.edu/sites/dlc-me/zoo/zdrmain.html>

This site is the "Microbe Zoo" and has good general discussion and electron micrographs of rhizobia and legume associations.

<http://www.nap.edu/readingroom/books/bnf/chapter1.html>

This page talks about the need for investment in nitrogen fixation research.

<http://www.ars.usda.gov/is/np/systematics/rhizobium.htm>

This is the homepage for the USDA-ARS National *Rhizobium* Germplasm Collection located in Beltsville, MD.

<http://mac122.icu.ac.jp/rls/rhrls.html>

This site contains a good discussion of the root hair involvement of establishing the rhizobia/legume symbiosis.

[http://edis.ifas.ufl.edu/TOPIC\\_Forage\\_Legumes](http://edis.ifas.ufl.edu/TOPIC_Forage_Legumes)

This site maintained by the University of Florida has a general discussion of forage legumes.

<http://www.extension.umn.edu/distribution/cropsystems/components/DC5963b.html>

This site is helpful in identifying perennial legumes.