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Forage Legume Inoculation

Dirk Philipp
Assistant Professor -
Animal Science

John Jennings
Professor -
Animal Science

Inoculation – What Is It?

Inoculation is the introduction of specific microorganisms into the soil. Typically, these microorganisms are specific strains of bacteria (*Rhizobium,* pl. *Rhizobia*) that "fix" nitrogen from the soil atmosphere into a form which can be used by the legume or other plants growing in association with it. Farmers normally accomplish legume inoculation by applying the bacteria in a peat-based material to the seed prior to planting.

Clover, alfalfa, vetch, trefoil and lespedeza are common forage legumes. They are important sources of protein, and well-inoculated plants do not require nitrogen fertilization for high yields. These plants have developed a symbiotic relationship (partnership) with *Rhizobia*. In this symbiotic relationship, the *Rhizobia* take nitrogen gas from the soil air and convert it into ammonia, which is quickly converted into amino compounds and protein. The legume provides the bacteria with a home in root nodules, nutrients and energy. In turn, the bacteria provide nitrogen to the legume through this "fixation" process.

The specific *Rhizobium* species is often not present in the soil in sufficient numbers to be effective in nodulating the roots of a specific forage legume. Inoculating the legume seed with the proper *Rhizobia* at planting introduces the necessary numbers of bacteria into the soil. As the seedling develops, the bacteria infect microscopic root hairs. The bacteria and the root cells multiply until eventually a nodule (gall-like structure) forms on the root. The nodule is the actual site of nitrogen fixation.

The amount of nitrogen "fixed" under optimum weather and soil conditions illustrates the significance of the symbiotic legume-*Rhizobium* relationship (Table 1).

Table 1. Amount of Nitrogen Fixed by Well-Nodulated Legumes Under Favorable Field Conditions

Legume	Pounds N Fixed/Acre/Year
Alfalfa	150 to 200
Soybean	60 to 100
Vetch	50 to 150
Cowpeas	44 to 132
Clovers	50 to 200
Lespedeza	50 to 100

Why Inoculate?

As mentioned previously, a sufficient number of effective *Rhizobia* for a given legume may not exist in the soil unless the specific legume has been grown in the previous two or three years and it was well-nodulated. Adding the appropriate bacteria through seed inoculation results in early formation of effective nodules for nitrogen fixation. This ensures an adequate supply of nitrogen to the seedling after the seed and soil nitrogen reserves are exhausted.

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Methods of Inoculation

To successfully establish a legume stand, sufficient numbers of the correct *Rhizobium* must be kept alive on the seed for nodule formation. There are many methods of inoculating seeds. All methods require **thorough mixing** of the legume seed with the appropriate inoculum in a tub or other large container. While in the past acacia gum was used as a sticking agent, there appear to be no commercial sticking agents on the market today.

Most forage legume seed is both small and slick-surfaced, which makes it difficult to ensure that large numbers of *Rhizobia* stick to the seed. For these reasons, always use sticking agents. Use the inoculation procedure that follows:

Inoculation Procedure for 25 Pounds of Seed

1. Use a commercial sticking agent and follow the manufacturer's label directions, or prepare a sticking agent as follows:

   a. Mix 2 ounces of syrup or molasses with 8 ounces of water. Shake or stir well, OR
   b. Mix 1 cup of sugar with 2 cups of water, OR
   c. Just use milk.

   Do not use cola drinks or other carbonated beverages. The pH of such beverages is near 2.0 and is harmful to the *Rhizobia*.

2. Add about 1/3 bag of fresh inoculant (about 2 ounces) to about 1 cup of the sticking agent. Mix to form a black slurry.

3. Place 25 pounds of seed in a tub or similar container.

4. Add the black slurry (6 to 7 ounces) to the seed in the tub and thoroughly mix. Be sure to coat every seed.

5. Add the remainder of the bag of dry inoculant to coat and dry the seeds.

6. Allow seeds to dry in the shade, stirring occasionally.

7. Plant inoculated seed as soon as possible or keep in cool, shaded condition for no longer than one to three days. **Do not leave the bag of inoculants or inoculated seed in direct sunlight because sunlight kills *Rhizobia*. As a general rule, do not mix inoculated seed with fertilizer. The salts and acids in the fertilizer will kill the *Rhizobia*. However, inoculated seed may be mixed with dry fertilizer if the mix is planted immediately.**

8. If molybdenum or fungicides are needed, apply them to seed after inoculation and plant immediately. (Generally, inoculants premixed with a fungicide and/or molybdenum have fewer viable *Rhizobia* than other inoculants and should not be used.)

Preinoculated and Lime-Coated Seed

Preinoculated and/or lime-coated (lime-pelleted) seed are available in Arkansas. Preinoculation is the process of sticking inoculant to the seed at the processing plant before the seed is bagged. Sometimes preinoculated seed are also coated with finely ground limestone. This seed is referred to as lime-coated, preinoculated seed. Such seed is ready to plant without further inoculation. Do not use water or sticking agents because this will cause the lime coating to "gum up."

When planting, always cover seed with soil to speed germination and protect the *Rhizobia* from sunlight. Be careful to avoid planting too deeply. Do not plant clover or alfalfa seed deeper than 1/4 inch deep.

Inoculants Are Specific

Rhizobia can be grouped according to the legume host which they nodulate. For example, those which will effectively nodulate soybeans will not cause nodules to form on clover. The cross-inoculation groups and *Rhizobium*-legume associations are illustrated in Table 2.

Inoculation of legumes with a reliable inoculant that is specific for that legume is essential. Commercial inoculant manufacturers have selected rhizobial strains for their inoculants which are (1) more competitive with other soil microorganisms and (2) more efficient in fixing nitrogen than many native strains in soils. Commercial inoculants may contain more than one strain of *Rhizobia* for a given inoculation group and as such are termed wide-spectrum types. This enhances the potential of the inoculum to form nodules on the host legume and efficiently fix nitrogen.

When purchasing an inoculant, be certain that the package you buy is labeled specifically for the legume you will plant. Failing to do

this may result in poor nodulation and short life of the legume stand. However, legumes that are not naturalized to our region should **always** be inoculated with the specific inoculum for them or their cross-inoculation group. Notice that Table 2 lists four clover groups. Arrowleaf clover is listed alone in Group IV. Inoculants for the three other clover groups will not be effective for arrowleaf clover, for example.

Recently, the market and demand for inoculants has decreased. Inoculant companies have responded to this situation by combining inoculant strains, e.g., for several clover species in one single product.

Table 2. Cross-Inoculation Group and *Rhizobium*-Legume Association

Cross-Inoculation Group	Legumes Included	*Rhizobium* Species
Alfalfa	Alfalfa Black medic Burr clover (medic) Button clover (medic) Sweetclovers (yellow and white)	*Rhizobium meliloti*
Bean	Beans	*Rhizobium phaseoli*
Clover I	Berseem clover Crimson clover Lappa clover Persian clover Rose clover	*Rhizobium trifolii* strain
Clover II	Rose clover Subterranean clover	*Rhizobium trifolii* strain
Clover III	Alsike clover Ball clover Hop clover Ladino clover Red clover White clover	*Rhizobium trifolii* strain
Clover IV	Arrowleaf clover*	*Rhizobium trifolii* strain
Lupine	Lupines	*Rhizobium lupini*
Pea	Caleypea Garden peas Lentils Vetches Winter peas	*Rhizobium leguminosarum*
Soybean	Soybeans	*Bradyrhizobium japonicum* strain
Cowpea	Alyce clover Cowpeas Lespedeza Lima bean Peanut Kudzu	*Bradyrhizobium japonicum* strain
Trefoil	Birdsfoot trefoil	*Rhizobium loti*

*Note that arrowleaf clover requires specific inoculum. It will not cross-inoculate with other clovers.

Therefore, it may be difficult to obtain a specific inoculant for a particular legume on the market.

It is best for both retailers and farmers to store inoculants in a refrigerator to keep them cool. **Do not** freeze the inoculant. Inoculants contain living organisms and must be protected from harsh conditions (freezing, heat, salts, etc.). If a refrigerator is not available, store the inoculum in a cool, dark place until ready for use.

Commercial inoculants have expiration dates displayed on the packages. (Preinoculated seed should also have an expiration date marked on the bag.) If the expiration date has passed, do not purchase the inoculant. **Always** reinoculate if there is any doubt about the viability of *Rhizobia* on preinoculated seed. It is inexpensive insurance.

Recognize Effective Nodules

Nodule size, shape and number vary with the host plant and *Rhizobium* strain. Red and white clovers have club-shaped and lobed nodules. Alfalfa nodules are more branched and longer, while cowpea, peanut and soybean nodules are spherical. Mature, effective (nitrogen-fixing) nodules are often clustered on the primary root and have pink to beefsteak red centers. If nodules have green or white centers, they are not actively fixing nitrogen and are ineffective. Ineffective nodules are usually small and scattered over the entire root system. Nodules form only on new roots and first appear when the first trifoliate or compound leaves appear, or within two to three weeks after seedling emergence. The exact lifespan of a given nodule is not known. Nodules on older roots may naturally senesce (die) with time.

The pattern of nodule growth, decay and shedding is not the same in all legumes. Following forage harvest, nodules of most legumes are usually shed, and new nodules form when root growth is renewed. However, alfalfa nodules may remain attached to the roots after forage is harvested. Looking for effective nodules earlier than two weeks after cutting or close grazing could lead to wrong conclusions about the nitrogen-fixing status of the plant.

Factors Affecting Nodulation

Factors that adversely affect the host legume, the *Rhizobia* or both may reduce nodulation. Exposure to sunlight, fertilizer, salts, soil acidity, undesirable soil moisture levels and high soil temperatures reduce the survival of *Rhizobia*. Temperatures above 90°F may kill *Rhizobia*, whether in inoculants packages, in the soil or in nodules.

Most seed fungicides and molybdenum can be toxic to *Rhizobia* during prolonged exposure. Usually, treated seed can be inoculated with the peat- or humus-based inoculants and planted within a few hours with no ill effects. Inoculants which are premixed with fungicides and/or molybdenum have significantly lower numbers of viable *Rhizobia* than inoculants which do not contain fungicides and/or molybdenum.

Conclusion

Without proper inoculation, new stands of alfalfa or other legumes may turn yellow (become nitrogen deficient) and perform poorly throughout their lifespan. To ensure good nodule formation, (1) buy the correct inoculum, (2) check the expiration date, (3) correctly apply the inoculant to the seed and (4) plant under favorable conditions.

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DR. DIRK PHILIPP is an assistant professor - animal science for the University of Arkansas Division of Agriculture and is located in the Department of Animal Science at the University of Arkansas, Fayetteville. **DR. JOHN JENNINGS** is a professor - animal science for the University of Arkansas Division of Agriculture and is located in the Department of Animal Science in Little Rock.

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