



Inoculation of Forage and Grain Legumes

Legumes have the ability to form a mutually beneficial (symbiotic) relationship with certain soil bacteria of the type or “genus” *Rhizobia*. The benefit to the plant, and thus to the grower, is that these bacteria can take (fix) nitrogen from the air (in soil spaces) and make it available to the plant (see Symbiotic Nitrogen Fixation, page 3). The amount of nitrogen fixed can meet the needs of the plant and leave nitrogen in the soil for following crops.

Species-specific inoculation

The genus *Rhizobia* is divided into various species and subdivided into multiple strains. *Rhizobia* bacteria are fairly specific as to which legumes they will infect, form nodules on the roots of, and for which they will fix nitrogen. Legumes effectively nodulated by the same *Rhizobia* species are termed cross-inoculation groups (Table 1). The specific bacteria to nodulate the legume you are planting may be present in the soil, especially if that legume has been previously grown in the same field. However, to ensure the availability of the correct species and an effective strain of that species, inoculation—adding the bacteria—is practiced. Inoculation is recommended when the legume being planted has not been grown in that field in the past three years or with every planting of a high-value crop. Because inoculant is inexpensive and easy to apply, it is good insurance of proper nodulation and nitrogen availability. But be sure to buy an inoculant specific for the legume you are planting.

Inoculation techniques

Inoculum is not magic dust—it contains bacteria that must be kept alive. All packages of inoculum have an expiration date. After this date, the bacteria may not be alive and the inoculum should not be bought or used. Heat and direct sunlight kill bacteria in stored inoculum, even while packaged. Since a short period of heat can reduce the number of live *Rhizobia*, the package should be kept in a cool place and out of direct sunlight—even when taking it home from the store (keep it off the dashboard). The preferred storage place for inoculum is the refrigerator (do not freeze).

Live bacteria may be added to the soil (direct-soil application) or to the seed (seed-applied inoculant).

Direct-soil application

Granular forms of inoculum may be placed in the seed row via the insecticide box of a planter or through the

fertilizer or grass seed box of a drill. (Clean the box before inoculum is placed in it.) The granules flow freely through field planting equipment, and their flow should be calibrated and metered.

Frozen or concentrated liquid cultures of inoculant may be diluted to a slurry, then added to a water-filled tank for spray application into the seed row.

Inoculant should not be mixed with either pesticide or fertilizer if applied to the seed row. When seeding forage legumes, it is recommended that fertilizer be applied separately.

Application of inoculant directly to the soil has been quite effective. However, the greater surface area being covered by the inoculant requires more of the material. This is especially the case when narrow-row soybean planting is practiced. Therefore, the method is more expensive than seed inoculation.

Table 1. Cross-inoculation groups of legumes and *Rhizobia*.

LEGUME GROUP	INOCULANT GROUP*	RHIZOBIA SPECIES
Alfalfa and sweet clover	A	<i>R. meliloti</i>
True clovers	B	<i>R. trifolii</i>
Peas and vetch (true)	C	<i>R. leguminosarum</i>
Soybean	S	<i>R. japonicum</i>
Birdsfoot trefoil	K	<i>R. loti</i>
Crownvetch	M	<i>R. spp.</i>

*Letters indicate manufacturer's reference to cross-inoculation groups.

Seed-applied inoculant

Inoculum to be mixed with seed before planting is available on a variety of carriers; the most common carrier is peat. Peat has proved to be better than most other carriers in preserving live bacteria under unfavorable conditions (high temperature, late planting).

Inoculating seed. When inoculating seed, two conditions must be satisfied to get good nodulation: (1) the roots must be in contact with the *Rhizobia* bacteria, and (2) the *Rhizobia* must be alive and able to infect the plant root.

For the bacteria to be in contact with the roots of every plant, inoculum should cover each seed. To achieve the best distribution, the inoculum should be mixed with seed in a large space rather than in a planter seedbox—on a tarp-covered floor, in a tub, in a cement mixer (paddles removed), or in the bed of a pickup.

Using an adhesive (a “sticker”) helps the inoculant adhere to each seed. This is especially important with small-seeded forage legumes, which need more inoculant per unit of seed-surface area. Table 2 shows the advantage, in number of nodules formed, of using a sticker during inoculation. Both commercial and homemade stickers are effective. A homemade sticker can be prepared as a 1-in-10 dilution of syrup or molasses; diluted cola or milk also can be used.

Table 2. Effect of inoculant and use of sticker on soybean root nodulation.

TREATMENT	NODULES PER PLANT
No inoculant	0.0
Inoculant, no sticker	0.7
Inoculant, plus commercial sticker	2.7
Inoculant, plus sugar sticker	2.7

Source: University of Kentucky

Mix seed with enough sticker to just moisten all seeds. Too much liquid may cause premature germination of the seed. To the moistened seed add inoculant and mix to coat the seeds. Air-dry by spreading the coated seed in the shade. Drying may be speeded by adding additional peat-based inoculant or finely ground limestone. The seed must be dry to flow properly through the planter. Calibrate the seeder with inoculated seed when setting desired seeding rate. Seed should be planted as soon as possible after inoculation because bacteria begin to die in the drying process. If not planted within 24 hours, reinoculate.

The rate of inoculant to use depends on the amount of time elapsed since the legume was last grown in that field and the conditions for bacteria survival at the time of planting. Start with the manufacturer’s recommendations. If the soil is dry and germination of the seed is expected to be delayed, then a higher rate of inoculant is required to make up for loss of some *Rhizobia*. For soybeans being planted into a new field, three times the normal rate of inoculant is recommended. A good way to achieve this is to moisten the seed with liquid inoculant applied at the normal rate, then mix seed with twice the normal rate of peat-based inoculant.

Preinoculated seed. Forage seed may be purchased already inoculated. One of two methods of preinoculation is generally used: (1) impregnation with *Rhizobia* by a vacuum process or (2) pelleting with fine limestone. The pelleted type of preinoculated seed is generally preferred on the basis of research that shows that bacteria live longer on pelleted seed and that this type of preinoculated seed results in formation of a greater number of root nodules.

Preinoculated seed should be handled in the same way as packaged inoculum. Several precautions can ensure better results. Check for an expiration date on the seedbag tag, store and transport the seed out of direct sunlight and heat, and plant the seed as soon as possible. If you believe

that bacteria may have died, then reinoculate the seed. Since water or the sticking solution causes the lime content of pelleted seed to gum up, use mineral oil (0.5 to 1.0 ounce of oil per pound of seed) to adhere new inoculum to seed. Plant immediately.

Other factors affecting nitrogen fixation

Rhizobia bacteria require the availability of molybdenum (Mo), a soil element. In Pennsylvania, Mo is generally present in soils in sufficient quantity, but its availability is affected greatly by the soil pH (Figure 1). Soil into which a legume is being planted should be limed to raise the pH to between 6.5 and 7.0.

Some inoculants or preinoculated seed may be sold with combinations of Mo and a fungicide. These additional treatments tend to reduce the number of live *Rhizobia* and generally are not recommended.

When establishing forage legumes, 20 pounds of nitrogen per acre is often recommended as a starter application. However, if conditions at planting are favorable for quick seed germination and seed has been inoculated, this starter nitrogen fertilizer is often unnecessary.

Do not apply nitrogen fertilizer to established legumes. Legumes can fix all the nitrogen they need for growth. Research has shown that nitrogen fertilizer does not increase yield of established legumes if the plants are effectively nodulated. In fact, fertilizer nitrogen discourages nodulation and inhibits nitrogen fixation.

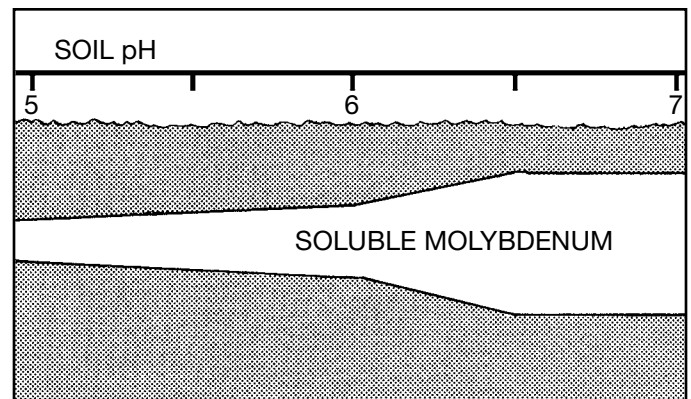


Figure 1. The relationship of soil pH and molybdenum availability. (The width of the band indicates the relative amounts of soluble molybdenum in the soil solution as influenced by pH.)

SYMBIOTIC NITROGEN FIXATION

Air is almost 80 percent nitrogen (N). Although air-supplied nitrogen is the primary source of N for the fertilizer industry, as well as the source used by legumes, it does not come free. Nitrogen in air exists as two N atoms that are triple-bonded together; in effect, the atoms are glued, stapled, and taped together, not to be undone without a great expenditure of energy.

In the industrial (Haber) process, petroleum energy is used to break the triple bond, and three hydrogen ions from natural gas or another petroleum product are added to each N atom. Therefore, N fertilizer prices increase as energy prices rise.

In symbiotic N fixation, as is the case with legumes, these same steps are required of the *Rhizobia* bacteria. Sugars or carbohydrates of a legume infected with *Rhizobia* are the energy and hydrogen source used by the bacteria to fix N from the air at high energy costs to the plant. If the plant can avoid these costs by taking up N from the soil, it will. Therefore, N fertilization inhibits nodule formation and N fixation.

Infection by the *Rhizobia* bacteria is somewhat similar to an infection in the body. The bacteria enter through a susceptible location, travel inward, establish themselves, and multiply (Figure 2). In the case of legumes, the bacteria are welcome as long as they are of a certain species that the plant somehow recognizes. Infection enters through a

root hair, then grows back to the base of the root hair. Multiplication of the bacteria and enlargement of the root cells form a nodule.

Inside the nodule, an enzyme called nitrogenase drives the N-fixation reaction. Similar to the catalyst used in the industrial process, the enzyme also contains molybdenum (Mo). Therefore, availability of this element in the soil is important to the legume. Nitrogenase is peculiar in that contact with oxygen ruins the enzyme. The bacteria and the plant are faced with an engineering problem: how to get the nitrogen out of air that also contains oxygen. A sophisticated system to accomplish this involves a protein called leghemoglobin that is capable of binding to oxygen and removing it from the presence of nitrogenase. Leghemoglobin, like the protein hemoglobin in our blood, binds to and transports oxygen. The combination of the protein with oxygen makes both blood and the inside of an oxygen-free nodule red or pink, which is why these colors indicate an active N-fixing nodule.

Fixed N is used in the plant to make amino acids, the building blocks of proteins. The amount of N that is fixed depends on many factors that include the specific legume and the health of the plant. Because the *Rhizobia* rely on the plant to supply carbohydrate, maintaining a healthy stand fosters N fixation. The majority of the N fixed is removed with the crop; however, residual N is often available to the next crop. That residual N reduces the requirement for applied N. Table 3 lists the amounts of N that may be available to the crop that follows various legumes.

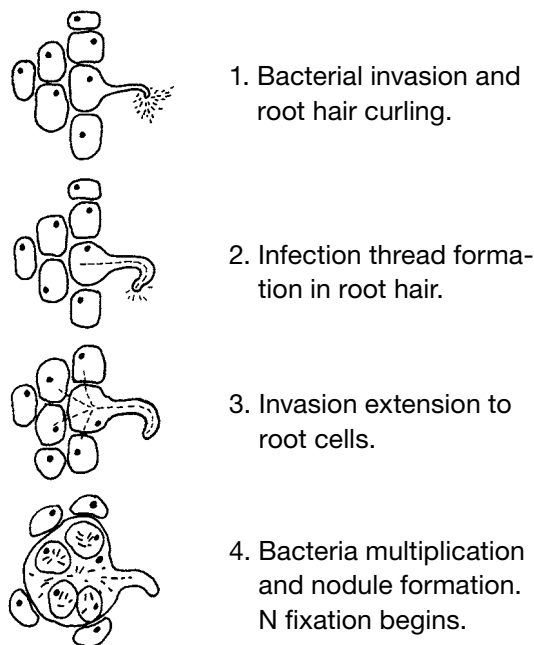


Figure 2. The infection process of legume roots by *Rhizobia* bacteria.

Table 3. Residual nitrogen contributions from legumes.

Alfalfa

First year after alfalfa

50%–75% stand 110 lbs/A

25%–49% stand 80 lbs/A

<25% stand 40 lbs/A

Second year after alfalfa

50%–75% stand 50 lbs/A

Red clover and trefoil

First year after clover or trefoil

25%–75% stand 40 lbs/A

Soybeans

First year after soybeans harvested for grain 40 lbs/A

Source: 1985–86 Penn State Agronomy Guide

Checking roots for effective nodulation

To check for effective nodulation two to four weeks after germination, carefully dig around and remove several plants. Then wash the roots in a bucket of water. Look for nodules and examine their distribution. Effective nodules generally are clustered around the taproot (Figure 3). Slice and observe the interior of several nodules. Nodules that have been actively fixing nitrogen have a red or pink interior. Nodules with white or pale-green interiors are ineffective.

Emergency inoculation

If the lack of effective nodulation on a newly seeded crop is known or feared, then inoculant can be applied to the crop in the field. Nodulation deficiencies can be corrected, although not entirely overcome, by salvage (emergency) inoculation up to four weeks after seedling emergence. The grower may use one of several techniques and expect equal results. Rate of application of actual inoculant should be in the range of 1¼ to 1¾ pounds per acre regardless of the application method. The methods are:

1. Drilled application of a granular peat-based inoculant 1 inch deep.
2. Drilled application of inoculated sand. In this case, you need to inoculate sand just as you would seed and use a sticker. Inoculate an amount of sand for an application rate between 60 and 90 pounds per acre and drill 1 inch deep.
3. Sprayed application of a water-inoculum suspension. Prepare the treatment by combining powdered peat-based inoculant with about a quart of water and shaking to ensure adequate saturation. After sieving to remove large peat particles, add this suspension to a water-filled sprayer tank. Remove nozzle screens and spray uniformly over soil surface. Application by this method should be made only on a cloudy day just before rain is expected or irrigation is scheduled.

If the crop was fall seeded and if cold weather has set in, then salvage inoculation should be delayed until early spring when the soil is warmer.

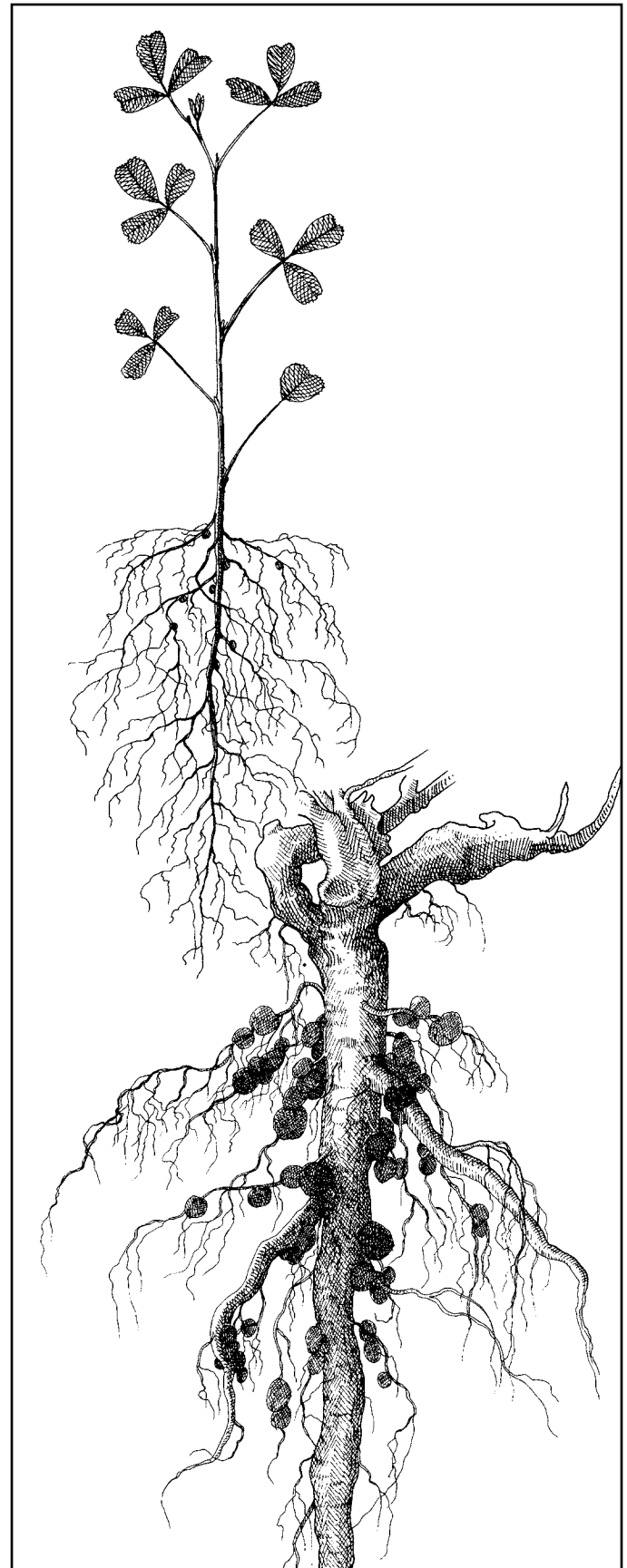


Figure 3. Distribution of nodules on seedling and established legume roots.

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Penn State College of Agricultural Sciences research, extension, and resident education programs are funded in part by Pennsylvania counties, the Commonwealth of Pennsylvania, and the U.S. Department of Agriculture.

This publication is available from the Publications Distribution Center, The Pennsylvania State University, 112 Agricultural Administration Building, University Park, PA 16802. For information telephone 814-865-6713.

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Produced by Ag Communications and Marketing.

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CODE # UC048 R1.5M07/08mpc3141