FORAGE LEGUME Inoculation

Rhizobia are bacteria that add nitrogen to a forage system by forming a special association with legume roots. Inoculation of legume seeds ensures that the correct bacteria are available to form active nitrogen fixing nodules on the legume roots. This publication explains the proper way to inoculate legume seeds with rhizobia so seeded stands will be well-nodulated and add nitrogen to the forage system. Information on nodulation is provided in Checking Forage Legume Nodulation *(AG-720).*

Adding a legume to a forage system provides two benefits:

1. Legumes improve the digestibility and protein content of forage. Livestock are able to digest and use more of the carbohydrates and proteins in legume-grass mixtures than in grass forage alone; consequently, the forage provides more nutrients. Protein concentrations tend to be between 12 to 28 percent in legumes compared with concentrations of 8 to 18 percent in well-fertilized grasses.

2. Legumes can add nitrogen to the plant-soil-animal system through *nitrogen fixation*. A 30 to 50 percent legume stand in a grass field in North Carolina can add 80 to 150 lbs of nitrogen (N) per year to the system—***if*** the legumes are well-nodulated and fixing N. Inoculation helps to ensure successful nodulation and N fixation.

LEGUMES AND SYMBIOTIC NITROGEN FIXATION

Legumes can add N to a soil-plant system because they have an association with microscopic bacteria called *rhizobia*. Rhizobia are microscopic organisms that form small round or oblong bumps called *nodules* on the roots of legume *host* plants. The legume host plant supplies carbohydrates (sugars) to the rhizobia in a root nodule, and the rhizobia that live in the root nodule supply N to the plant. This mutually beneficial relationship is called *symbiosis*. The N fixing process is called *symbiotic nitrogen fixation*. The N fixed by the rhizobia and transferred to the legume comes from the air. Soil pores allow air, which includes N in the form of a gas, to reach the root zone. This is why compacted or waterlogged soils are poor environments for N fixation. Symbiotic N fixation by legume roots depends upon these key factors: the rhizobia species and the extent of its population, soil condition, and soil fertility. Pasture managers who inoculate legume seed with rhizobia to ensure N fixation in their forage systems must understand these key factors and handle the inoculant correctly.

Nitrogen is a component of plant and animal tissues and an especially important part of proteins and hormones. N is essential for plant and animal growth. Although 80 percent of the air we breathe is made of N, it is not in a form that can be used by nonlegume plants. Symbiotic N fixation is one way that nitrogen enters the soil-plant-animal system. Symbiotic N fixation is an essential part of the earth's nitrogen cycle: 60 percent of all N enters the soil system via symbiotic nitrogen fixation. Lightning, rainfall, and organic matter recycling also supply N to the soil system. Fertilizer manufactured using natural gas is the other source. Without soil N, either from manures, organic matter, fertilizer, or legume N fixation, plants and root systems will be stunted or die.

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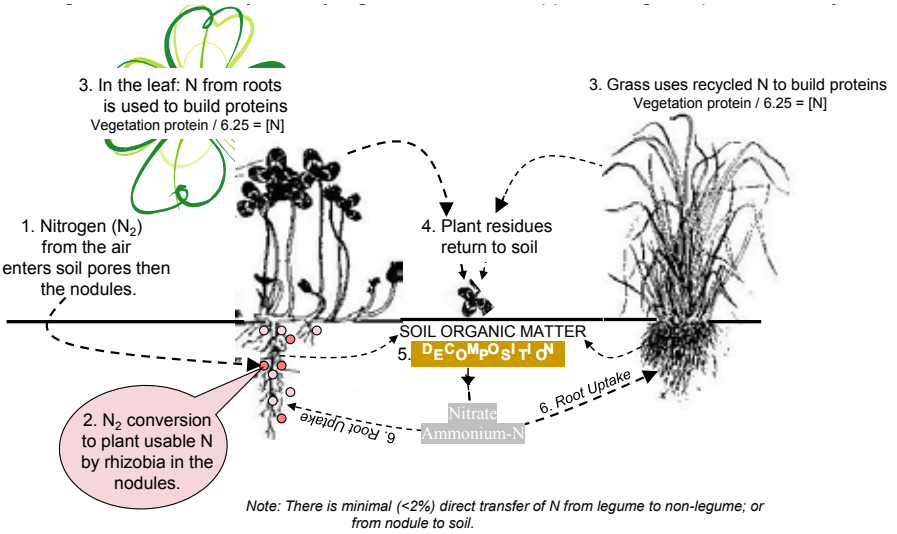

Figure 1. Nitrogen added to the system by legume N fixation supplies the grass plant *indirectly*.

Most of the N fixed in association with legumes is not directly transferred from legumes to grass plants growing with them in the field. The fixed N accumulates in the legume leaves, stems, and roots. It becomes available to other plants only *after* the legume tissues die and are decomposed by soil organisms, releasing N into the soil. At this point, the "fixed" N is finally available for forage uptake. In well-structured, aerated soils with lots of organic matter, the N may be stored and recycled among soil organisms before plant uptake. Soils with more organic matter have greater biological activity and N turnover and storage capacity. A small percentage of the fixed N directly enters the soil system from the nodule. There is no evidence that significant N is directly transferred from legume to nonlegume plant roots.

Rhizobia Bacteria

Many types of soil rhizobia bacteria can form nodules on legume roots. The right type (species) of rhizobia must be in the soil to form the symbiotic relationship with the roots of a particular legume. Different legume types have different root nodule shapes and sizes because each legume associates with a specific type of rhizobia. Without it, no root nodule will form and no N will be fixed. In rare cases, if the wrong type of rhizobia comes in contact with the roots, the legume will form white or brown nodules that do not fix N.

Field History and Rhizobia in the Soil

Many types of rhizobia live naturally in the soil. Pasture managers must be certain that legume seeds are planted with the correct type of rhizobia so that they can form active nitrogen fixing nodules that will increase N in the soil and forage system.

Is inoculation necessary if the same type legume has been grown recently in a field? Some writers suggest that rhizobia surviving in the soil will be available to inoculate and form nodules on the roots of the newly planted legume crop. Technically, this is correct. Practically, however, it is risky. Rhizobia do live independently and carry over year-to-year in some soils, but the bacteria are not distributed evenly throughout the soil and the population numbers can be very low. For successful inoculation, the rhizobia must be in direct contact with the newly developing roots. Rhizobia survive best in soils with lots of organic matter and live roots. Heavy clay soils with low or no organic matter are among the worst for rhizobia. Especially in dry, hot soil conditions (such as those during summer in North Carolina's piedmont and coastal plain) rhizobia survival season to season can be low. Rhizobia that associate with small-seeded, shallow-planted, cool-season forage legumes are vulnerable to heat and drying. Large-seeded forage legumes are planted at deeper planting depths where the soil environment is often more favorable for rhizobia survival.

RHIZOBIA POPULATIONS

In good soil conditions, rhizobia reproduce very quickly: 2 become 4, 4 become 8, 1,000 become 2,000, and so on. This pattern and rate of multiplication is called *exponential growth*. In hot dry conditions, the populations can crash and die out quickly. Exponential growth explains why legume inoculation is a "numbers" game. The more live rhizobia added, the greater the chances that some will survive less-than-ideal conditions and that enough will come into contact with the new legume roots. Researchers estimate that at least 1,000 rhizobia must come into direct contact with each new root at the right time (within 4 days of the root forming) for successful nodulation.

Soil Fertility and Rhizobia

Legumes will grow in less-than-ideal pH and fertility, but N fixation will be reduced and the N added to the forage system will be less than it could be. For successful N fixation, fields with legumes should be well-fertilized with everything *except* N. Be sure your soil test recommendations are for legumes. Adequate phosphorus and potassium (potash) must be available for nodules to form. More nodules are formed and more N is fixed by each nodule when soil phosphorous and potassium are at optimal concentrations. Sulfur is another nutrient important for legumes. Nitrogen fertilization will cause the rhizobia to fix less atmospheric N. Plants and rhizobia will use "free" soil N if it is available instead of fixing N. In addition, when fertilizer or manure N is available, many grasses will use it to outgrow (and outcompete with) the legume. If establishing a *pure* legume stand, *without grasses*, a light application (30 lb/acre) of N fertilizer is sometimes recommended.

Rhizobia do not tolerate low pH. The target pH for most soils should be 6.5 to 7.2. On most soils, a pH below 6.0 results in reduced or no N fixation. Low pH also risks low to no molybdenum availability. Molybdenum is an important micronutrient for N fixation. On very high organic matter soils, such as the N.C. coastal "blacklands," a pH above 6.2 can reduce soil manganese availability, which will negatively affect legume growth; target a pH between 5.5 and 6.

INOCULANTS AND INOCULATION

Nodulation and N fixation are not automatic processes. The soil environment must be suitable for both legume growth and for the rhizobia. The correct rhizobia must be present in the soil as the root develops. The proper rhizobia should be added to the seed-soil system in a process called *inoculation*. An *inoculant* is a commercial product, often (but not always) mixed with a powdered peat base, that is used to introduce millions of live rhizobia into soils. Because they contain live organisms, inoculants must be treated with care.

Inoculants are usually added to the soil directly with the seed at planting. The goal of inoculation is to be sure that the new roots of each legume seedling come into direct contact with the correct type of live rhizobia. If soil conditions and the inoculant handling are adequate, the rhizobia will form active, pink nodules and begin N fixation within 6 weeks of planting.

Without the correct live rhizobia inoculant, other rhizobia types present in the soil may form nodules that fix little or no N. The center of inactive nodules will be white or brown, not pink.

INOCULATING RAW SEED

Some forage legume seed is sold "raw" or uncoated. Raw seed is sometimes all that is available of a particular legume. The correct rhizobia inoculant must be purchased and applied before planting raw seed to ensure that the legume plants fix nitrogen. The inoculant must be applied by the grower before the legume seed is planted. Follow these guidelines:

1 ***Use the right inoculant, and make sure it has been handled properly.*** Each legume species needs a specific type of rhizobia to form active nodules. Note that some types of rhizobia form nodules on more than one type of legume. Commercially, eight mixtures of rhizobia types are available. (These mixtures are called "cross-inoculation groups.") If inoculating legume seed, be sure to purchase an inoculant with the type of rhizobia that will form *active* nodules on the legume being planted.

Forage legume inoculant is usually sold as a powder mixed with ground peat. The rhizobia themselves are microscopic and not visible. The mixture is usually sold in small plastic packets about 5 inches × 7 inches. Each packet treats 50 to 100 pounds of seed, depending on type. All the species that can be inoculated by the rhizobia in that packet are listed on the packet. Use these guidelines when buying inoculant:

- Know the exact species of legume you are planting. *"Clover" is not exact enough.*
- Try to purchase the correct inoculant where you purchase the legume seed. Be sure you are buying inoculant for the type of legume or clover you are planting. CHECK the PACKAGE LABEL. Be sure it lists the legume you want to inoculate.
- Check the package expiration date. Be sure the inoculant has not passed its expiration date. Fresher is better. Unlike some old seeds, old inoculant will *not* be effective, even if it is properly stored.
- Purchase from a reputable supplier who stores and handles the inoculant properly. Inoculant can easily get overheated or frozen in warehouses and vehicles or during shipping. If the inoculant gets overheated or frozen—even for a few hours—purchase a new supply. All the rhizobia will not die instantly, but the mishandled inoculant will not be very effective. You can use old inoculant without harm to plants, but it will not result in good nodulation.
- Store the inoculant in a cool, dry place and out of the sunlight.

- Never store the inoculant below 40°F or above 90°F. Rhizobia are quickly affected by temperature in dry conditions.

CAUTION

Some legume inoculants may have the correct type of rhizobia for a specific forage legume, but it may also include a fungicide or other products with the inoculum; be sure the additives are labeled for use in forages. Check with the manufacturer.

For example, see these Web sites:
http://www.histick.com/about.htm
http://www.nitragin.com/canada/company/news.cfm

Figure 2. Many inoculant products are available. Use the proper inoculant for the legume being planted, and handle it properly.

Other forms of commercial inoculation products are available for some types of legumes, such as soybeans and peanuts. Some are liquids, some are dry, and some can be applied directly to seeds. Some products combine inoculant with other substances, such as minerals or fungicides, to help plant establishment and growth. Except for alfalfa and forage soybeans, granular and liquid forms of inoculum are not readily available for most forage type legumes in 2009.

Some seed treatments, including fungicides, seed disinfectants, or bactericides, are ***TOXIC*** to rhizobia. Check with the seed company about inoculating treated seed.

2 ***Use the correct amount of inoculant.*** In North Carolina's environmental conditions, use 2 to 4 times the manufacturer's recommended amount of inoculant.

3 ***Use a sticking agent to apply the inoculant to the seed.*** Inoculum is usually sold in small packets mixed with peat as a carrier. The powdered peat with rhizobia must stick to the raw seed to ensure good contact with young emerging roots. When the inoculant in dry peat is mixed directly with seeds in a planter, fewer nodules are produced on the resulting roots than when a sticking agent is used to "stick" the inoculant and peat to the seeds. Commercial inoculant sometimes includes a "sticking agent." These combinations are effective, but the product and all additives should be labeled for ***forages***. Sticking agents can be purchased, or use the following recipe to "stick" powdered peat and inoculum to ***raw seed***:

- Make a corn syrup or sugar solution (a 1:4 solution of corn syrup and cool water) by mixing 1 cup (8 oz) corn syrup with 1 quart (32 oz) water for 50 pounds of seed.
- Use 5 oz (100 grams or about ½ cup) of dry inoculum to treat 50 pounds of seed. Add ¼ cup of the dry inoculum to the syrup solution, and mix to make a slurry. (The remaining ¼ cup of inoculum will be used later.)
- Put the seed in a tub and add the slurry. Mix until all seeds are coated. (A small cement mixer is great for mixing inoculant sticker and seed).
- Add the remaining ¼ cup of inoculant, and allow the seed to dry in cool shade. Wet sticky seeds can clog the planter. Adding more inoculant speeds the drying process. It is not possible to have too much inoculant.
- Plant within 48 hours.

The above recipe makes enough sticking agent to treat 50 pounds of seed. To make enough sticking agent for 100 pounds of seed, double the quantities. Use 8 oz of dry inoculum (227 grams or about 1 cup), 2 cups of corn syrup, and 2 quarts of water.

CAUTION

Do ***NOT*** use carbonated drinks (Coke, Pepsi, or any soda pop) to stick the inoculant to the seed. The acidity in soft drinks kills rhizobia. Water is not a good a sticker, because the inoculant detaches as the water dries. Do ***NOT*** mix inoculated seed or inoculum with any fertilizer. Direct contact with fertilizers will kill rhizobia.

CAUTION

Warehouses and vehicles are all situations where inoculum can easily get overheated or frozen. If the inoculum gets overheated or frozen—even for a few hours—get new inoculant. All the rhizobia will not die instantly, but the mishandled inoculum will not be very effective. You can use old inoculant without harm, but it will not result in good nodulation.

Seeds planted with inoculant that has been heated or frozen may be poorly nodulated, and the resulting plants will not add nitrogen to the system.

PRE-INOCULATED SEED

Many forage legume seeds are sold as pre-inoculated. The seeds usually have a clay or lime-based coating that includes large numbers of the proper type of rhizobia. Once planted, the thin coating dissolves with moisture, activating the rhizobia. If you purchase pre-inoculated seed, the proper rhizobia are in the seed coating. Be sure to calibrate the planter for the coated seed. Planting rates for pre-inoculated seed usually need to be 15 to 30 percent higher than for raw seed. *For example, if the general recommendation for white clover seed is 4 to 6 lb/acre, plant 6 to 8 lb/acre to plant the same number of seeds for a successful stand.* For coated seed, follow the seed company's recommended seeding rates.

Ask how the pre-inoculated seed has been handled and stored. Age, heat, sunlight, freezing, and moisture all destroy rhizobia in the coating and the ability of the inoculant to nodulate the legume (see photos). The coating does not protect the rhizobia from the environment. The seed may still be viable and germinate, but the rhizobia will not effectively nodulate the roots. Even when properly stored in cool, dry conditions, the rhizobia in the coating remain active for only a few weeks or months.

OLD PRE-INOCULATED SEED

According to the manufacturers of seed coatings, the inoculum in the coating on old (last season's) seed has only a 50 percent chance of having enough live rhizobia to effectively nodulate the legume. Do not plant last season's pre-inoculated seed without re-inoculating with fresh live rhizobia.

RE-INOCULATING PRE-INOCULATED SEED

If you are uncertain about the storage conditions or past handling of pre-inoculated seed, adding fresh powdered inoculant will help ensure good nodulation. To re-inoculate, you need to "stick" the fresh powdered inoculant to pre-inoculated seed without dissolving the coating:

- Use mineral or vegetable oil as a "sticker." ***Motor oil and petroleum products are toxic to rhizobia.*** Do not use water, sugar solutions or soda pop because they will dissolve the coating before planting and clog up the planter.
- Mix the seed with mineral or vegetable oil (½ ounce of oil per pound of seed. For example, use 12 ounces of oil for 25 pounds of seed.)
- Once the seed have a thin coating of oil, dust with the correct peat-based inoculum. Depending on seed size, one packet of inoculum will coat 50 to 100 pounds of seed.
- "Dry" the seed in cool shade, out of direct sunlight.
- Plant as soon as possible ***within 48 hours*** after applying the fresh inoculant.

RHIZOBIA ARE PERISHABLE

Whether in pre-inoculated seed or powdered inoculum, rhizobia are perishable. Check the expiration date. Fresher is better! Expired inoculant will contain fewer live rhizobia and form fewer effective nodules than fresh inoculants.

Rhizobia bacteria are much smaller than seeds and more sensitive to soil conditions. *A legume seed can germinate and survive even when rhizobia in the inoculant or soil cannot.* Rhizobia are sensitive to heat and cold. An afternoon on the dashboard or bed of a truck (or in an uncooled warehouse) on a sunny day will kill rhizobia bacteria—whether they are on coated pre-inoculated seed or in a powdered form mixed with peat. Sunlight, specifically UV light, will destroy rhizobia.

When buying pre-inoculated seed or powdered inoculant, ask how the product has been handled and stored before purchase. Store inoculant in a refrigerator (not a freezer).

Figure 3. A comparison of results from inoculation treatments

A. Red clover plant fertilized with N fertilizer. The plant looks great but is not fixing N in the soil. Seed were inoculated when planted, but no nodules formed because the N fertilizer was readily available to the plant roots.

B. Red clover produced by seed inoculated after the inoculant's expiration date.

C. Red clover produced by properly inoculated seed planted without N fertilizer.

D. Red clover produced by seed inoculated after the inoculant was stored on a truck dashboard.

E. Red clover produced by seeds that were not inoculated. The seeds were planted without N fertilizer.

F. Alfalfa produced by inoculated (right) and not inoculated (left) seed in a 1980 Wake County field trial.

CHECKING LEGUME ROOT NODULATION

Remember to check nodulation 4 to 6 weeks after planting seeds. Nodules should be evident and active 3 to 4 weeks after inoculated legumes emerge. If plants are not well-nodulated (20 or more nodules per plant) or if the nodules are not pink inside, they will fix only minimal nitrogen. For all the details, see another Extension publication, *Checking Forage Legume Nodulation* (AG-720).

Figure 4. Examples of poor and proper nodulation
A. White clover roots at 6 weeks after seeding. Seed were inoculated with mishandled inoculant. The inoculant spent half a day on a truck dashboard in the sun the day of planting.
B. White clover plants at 6 weeks after planting. Seed on the left were properly inoculated. Seed on the right were not.

RESCUE INOCULATION: INOCULATING LEGUME STANDS AFTER PLANTING OR ESTABLISHMENT

Sometimes legume seeds are planted without inoculant. *If you are about to plant and realize you don't have inoculant, it is better to delay planting until seed can be properly inoculated than to depend on a rescue treatment for inoculation after planting.* Sometimes the handling of the inoculant results in poor nodulation. Sometimes the reason for poor nodulation is not clear. A rescue inoculation treatment will NOT help a failed legume seeding where the seeds did not germinate or emerge or when soil conditions are not adequate.

As soon as a problem with inoculation is recognized, organize a rescue inoculation treatment—the sooner the better. Experiments with rescue inoculation of legumes that weren't inoculated at planting have been successful in North Carolina. Rescue inoculation is costly. But if plants are not nodulated, the costs of wasted legume seed, lost N fixation, and a lost stand or season must be considered. Re-inoculation may be justified.

EXAMPLE

During the fall 2007 drought, several North Carolina producers planted forage legumes, including alfalfa, in very dry conditions. Eventually, once the rains started, the seed germinated – with good emergence and stands. However, the plants were pale and growth was poor. Apparently, the seed had remained viable in the soil until rainfall; but the hot dry soil killed the rhizobia inoculant before the seed germinated. Late-winter rescue inoculations resulted in successful nodulation, which saved the stands.

Roots must be actively growing to become nodulated. Rhizobia are most effective at nodulating roots in the first 4 weeks of root development. Because new roots and root hairs are developed during recovery from stress, established legumes might form new nodules during regrowth after stresses, but data is limited. Rescue treatments have not been evaluated on fully established perennial legumes that were once nodulated but no longer have active nodules. Nodule loss followed by nodule redevelopment has been documented in white clover after cutting.

Rescue inoculation treatments will be successful only if soil and growing conditions are satisfactory. There are two approaches to rescue applications of inoculant. Recheck the field for nodulation 4 to 5 weeks after the rescue treatment.

Method 1. For 1 acre, thoroughly mix 1 pound of the correct peat-based inoculum with 75 pounds of sand or 50 pounds of cottonseed meal, wheat

middlings, or sawdust. Drill this mixture into the planting if possible or uniformly broadcast this mixture on each acre needing inoculation. Time the treatment to coincide with a good rain, or use an evening application when heavy dew is expected.

Method 2. Mix a fine slurry of 1 pound (6 to 10 packages) of peat-based inoculant in 20 gallons of water for each acre. Apply as a broadcast spray at 20 gallons/acre. Clogging is likely with peat carriers. Large spray tips without screens and vigorous agitation will be necessary. Time the treatment to coincide with a good rain, or use an evening application when heavy dew is expected.

BUYING INOCULANT

Unfortunately, there are surprisingly few suppliers of forage legume inoculant in North Carolina. If you need to purchase inoculum, legume seed dealers should stock inoculant for the legumes they sell. Be sure to ask about inoculant handling and expiration date, and be sure to check that the inoculant is the correct type for the legume you plant! Local farm supply stores sometimes carry inoculant. Consider shipping temperatures when ordering inoculant from distant suppliers. Some companies require you to purchase a case or more of inoculant. Remember that inoculant expires and cannot be used successfully the next season.

SOME SUPPLIERS OF FORAGE LEGUME INOCULANTS IN NORTH CAROLINA AND ELSEWHERE IN THE SOUTHEAST

Adams-Briscoe Seed Co.
325 E. 2nd Street, Jackson, GA 30233
Telephone: (770) 775-7826 www.abseed.com

Cooper Seeds
1550 University Drive, Suite A, Auburn, GA 30011
Telephone: 1-877-463-6697 www.cooperseeds.com

Hancock Seed
18724 Hancock Farm Road, Dade City, FL 33523
Telephone: (352) 567-6971 www.hancockseed.com

Mixon Seed Co.
1438 Joe Jeffords Hwy., Orangeburg, SC 29116
Telephone: 1-800-922-1377

Southern States Cooperative, Inc.
Home Office: P.O. Box 26234, Richmond, VA 23260
Stores are located in North Carolina and Virginia.
Telephone: 1-804-281-1000
www.southernstates.com

Wyatt-Quarles Seed Co.
P.O. Box 739, Garner, NC 27529
Telephone: 1-800-662-7591
http://www.wqseeds.com/

The use of brand or service names in this publication does not imply endorsement of the products or services or criticism of similar ones not mentioned.

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