CHECKING FORAGE LEGUME Nodulation

This publication discusses legume nodulation, which is essential for nitrogen fixation—the biological process that adds nitrogen to the forage system. Inoculation of legume seeds assures that the correct bacteria are available to form the nodules on the legume roots. See the publication Forage Legume Inoculation *(AG-719) for information on inoculating legumes.*

Legumes provide high quality, highly digestible, high protein forage. Forage producers also grow legumes to increase total nitrogen (N) in the soil-plant system. This can reduce nitrogen fertilization costs. Legumes can only "fix" nitrogen if they have abundant and active nitrogen-fixing nodules. The nodules "house" the microscopic rhizobia bacteria that convert nitrogen gas in soil pores to forms of nitrogen (nitrate and ammonium) that can be used by plants. ***Top growth and flowering are not proof of effective nodulation.*** Managers should check nodulation of annual and perennial legumes each growing season. Soil conditions can change and become unfavorable for legume nodules and nitrogen fixation.

- Check perennials 3 – 4 weeks after regrowth begins or at "green-up."
- Check new stands 4 – 6 weeks after planting or seedling emergence.

Any time you notice field areas where legume plants appear pale or stunted, check the roots for active nodules. This may occur mid-season. Early vigorous top growth can be attributed to available soil nitrogen; legume plants grow well using soil nitrogen, but decline when soil nitrogen availability declines because the legume is not nodulated and fixing nitrogen.

Figure 1. Nodulated lespedeza and white clover roots

Compared with soil and forage sampling, checking nodulation is easy!

- Check five to six random sites around each field.
- Select sites that are on different planting passes.
- Using a shovel, dig up one or two plants with their roots at each site (it is not necessary to dig deeper than 10-12 inches). Do not simply pull up plants; nodules may remain in the ground because they may not be firmly attached to roots.
- Carefully break away or rinse away the soil from the root mass.
- Look at the roots. Depending on the legume species, nodules may be on the plant's secondary or fine roots; sometimes they are on the taproots.

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Figure 2. One active nodule. The interior of each nodule is pink because of leghemoglobin—the same chemistry that causes blood to be red.

Nodules are small oval, round, or lobed bumps protruding from the roots with a whitish color. Nodule size varies; many forage legumes have small nodules: 15 – 20 nodules will fill a spoon. Some clover nodules are very small; 100 nodules could fit on the same spoon! The nodules should be obvious and abundant, although the actual number depends on many factors. In general there should be a minimum of 20 well-developed nodules per plant. Note that nodules may be in clusters.

• Check if the nodules are active and fixing nitrogen by pinching several nodules in half. They should be a fleshy pink inside. If nodules are green or white inside, they are NOT fixing nitrogen.

White nodules are inactive—they may be immature or perhaps the wrong type of rhizobia formed the nodule. (Other rhizobia are often present in the soi andc can create an inactive nodule)

Green nodules usually indicate that the nodule is dying or senescent. If a plant is never stressed, and soil conditions unchanged, a nodule lifespan can be the same as the plant's lifespan. But, when the legume plant begins to die (or senesce), so do the active nodules. If a plant loses leaves or fine roots, nodules usually die. Stresses that effect plant leaves, fine roots and nodules include overgrazing, insect damage, high soil temperatures, drought, and flooding.

***Every* legume plant in a growing stand should have active nodules.** Active nodules should be present on all legume plants that are growing and photosynthesizing. It is unusual for one single plant in a stand to fail to nodulate. After checking 6 – 10 sites in a field, use these guidelines:

Figure 3. Poorly nodulated red clover with a poorly developed root system. No nitrogen fertilizer was applied. Before inoculation of seed, the packet of inoculant spent several hours on a sunny dashboard. A lack of pink nodules means that NO nitrogen is being added to the soil -plant system.

• If there are active pink nodules on legume roots at all 6 – 10 sites in the field, the entire field is probably well-nodulated.

• If some plants appear to be well-nodulated and some not well-nodulated, check the field more intensively. Check the roots of both vigorous and less vigorous plants. Use the same technique you would use for soil sampling: Identify natural sub-areas of the field and sample within each sub-area (see www.soil.ncsu.edu/publications/Soilfacts/AG-439-30/).

• If you do not find nodules on any plant ***or*** if more than 10 percent of the plants are poorly nodulated, review the field situation. See the next section.

NO ACTIVE NODULES?

Nodules are not active and will not be pink if the legume is not growing.

As noted above, legumes can lose nodules following stress. Loss of nodules may follow grazing, mowing, waterlogging, high temperatures, and dry periods.

• Was inoculant applied? Was it the proper type of inoculant (cross-inoculation group) for the legume?

• Was it applied properly using a "sticker"? Was the proper sticker (not toxic motor oil or soda pop) used?

• Was the inoculant or pre-inoculated seed handled and stored properly at all times between factory and planting? Check with your supplier. Mishandled inoculum will not form nodules. On a hot day, a planter breakdown with the hopper full of freshly inoculated seed could result in poor nodulation.

• Was the legume planted in good soil moisture conditions? Have soil temperature and moisture conditions been reasonable since planting? Waterlogged or compacted soils restrict air flow into the soil, resulting in poor nodulation.

• Has the weather been suitable for nodule survival? Very hot, dry soil conditions at or after planting will destroy rhizobia before they can form nodules. Nodules are more resistant to soil conditions, but hot, dry conditions or flooding that stresses the plants can cause nodules to die off and disappear. Timely re-inoculation or replanting may be required. This is why legume stands should be checked each season.

EXAMPLE

During the drought in fall 2007, several producers in North Carolina 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, while the seed remained viable in the soil until rainfall, the hot dry soil killed the rhizobia before it could inoculate the roots and form nodules.

• Is soil fertility adequate? Proper pH is critical—rhizobia cannot tolerate acid soils. A pH of 6.5 is ideal for nitrogen fixation for most legumes. A minimum pH of 6 is recommended for legume production and nitrogen fixation on North Carolina soils, except organic soils. Liming is the most effective way to increase soil pH. Acid soils tend to be high in aluminum which is toxic to rhizobia. The legume plant and the rhizobia must have adequate supplies of phosphorus (P), sulfur (S), and molybdenum (Mo). Both molybdenum and sulfur can be limiting in some areas of North Carolina. Follow N.C. Department of Agriculture (NCDA) soil test recommendations for these nutrients. ***Check that your soil test recommendations are for legumes!*** Note that molybdenum availability is limited below a pH of 6.5. Note that the application of sulfur can reduce soil pH.

• Was nitrogen fertilizer applied? Nitrogen fertilizers interfere with the nodulation of legumes in several ways. Nitrogen fertilizers acidify the soil, reducing rhizobia numbers at planting. Legumes will take up fertilizer or manure nitrogen rather than provide sugars to rhizobia nodules; but if soil nitrogen is available, many grasses naturally grow faster and taller than legumes. At a pH of less than 6.5, grasses take up nitrogen more quickly and efficiently than do legumes. Grasses growing with legumes will use the fertilizer nitrogen to compete more aggressively with the legumes for light and moisture. Long-term shading stresses some legumes and could reduce nodulation. For best nodulation, avoid applying any nitrogen fertilizer to forage legumes, especially in mixed stands.

REDUCING CLOVER PERCENTAGE IN MIXED FORAGE STANDS

Occasionally grass stands are overtaken by legumes —especially clovers. A forage stand with more than 50 – 60 percent clover is often not desirable. Total forage yield per acre is reduced. A common tactic for reducing the clover percentage in a mixed forage stand is to apply nitrogen fertilizer. In many situations, 40 – 50 pounds/acre will reduce clover percentage without eliminating the legume completely.)

The timing of grazing or mowing and the height of the forage stubble also will influence the relative percentages of grass and legume in a stand. Appropriate timing and stubble heights depend on legume species and growing conditions.

• Were there any additional seed treatments? Growers should not add or treat raw seed, inoculated seed, or the field with any other substance, fungicide, or chemical. Do not mix inoculated seed (or pre-inoculated seed) with fertilizers. If possible, apply fertilizers to the field a few days prior to seeding. Any pre- or post-emergence herbicides or chemicals should be labeled for use with newly seeded legumes.

Figure 4. Properly inoculated white clover *with* **(A) and** *without* **(B) nitrogen fertilizer: same seedlot, planting date, and growing conditions.** Nitrogen fertilizers reduce nodulation and nitrogen fixation. The plant in photo A produced good forage and has a good root system, but it is not adding any nitrogen to the plant-soil system.

DEALING WITH POORLY NODULATED STANDS

If legume roots are not nodulated but soil and growing conditions are acceptable, it is usually because the legume seeds were improperly inoculated or not inoculated, or the seeds or plants have experienced *severe* stress. A rescue inoculation treatment to add the rhizobia

bacteria that form nodules is an option. Rescue treatments are not always successful, and the costs should be balanced against other options, such as the cost of replanting.

If legumes have white (or green) nodules the situation is more complex. White nodules are often associated with poor legume growth. White nodules are not fixing nitrogen. They may be immature nodules that will eventually fix nitrogen or impostors that are drawing sugars and starches from the legume root but not adding any nitrogen to the system. Recheck the stand after one or two weeks if white, brown, or green nodules are detected. Consult with an Extension agent or another expert. The stand may need to be killed outright and replanted.

Details on the rescue inoculation procedure are in another Extension publication: *Forage Legume Inoculation* (AG-719). Four to five weeks following a rescue treatment, recheck the field for nodulation. Soil and growing conditions must be satisfactory and 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 following various stresses, but data are limited. Rescue treatments have not been evaluated on fully established perennials that were once nodulated but no longer have active nodules. Some perennial legumes shed fine roots and nodules (and resident rhizobia) during plant dormancy or stress. Nodule loss followed by nodule redevelopment has been documented in white clover following cutting. Possibly the rhizobia in the immediate vicinity of the new roots reinoculate the roots when the plant begins new growth.

DEFINITIONS AND TERMINOLOGY

Inoculant – A product that is a combination of the rhizobia and a carrier, such as ground peat, that is used to inoculate legume seed.

Inoculation – The process of introducing or adding inoculum or inoculant to seed or soil.

Inoculum – Same as *inoculant.*

Nodulation – The action of a rhizobia inoculating and forming a nodule on a legume root.

Nodules – Also called *root nodules* or *legume nodules*. Nodules are the visible "structures" that rhizobia form. Nodules house a colony or group of rhizobia.

Rhizobia – Plural of rhizobium.

Rhizobium – The microorganism that actually infects the legume root or root hair and forms nodules.

Rhizo-bacteria – The term used for the entire group or collection of all different types of root-colonizing microorganisms, of which rhizobia are one.

Prepared by

Sue Ellen Johnson, *Assistant Professor and Extension Forage Specialist*
Department of Crop Science
North Carolina State University
se_johnson@ncsu.edu, (919) 513-1335

The author acknowledges the contributions made by these reviewers: Jim Dunphy, Julie Grossman, and Chris Reberg-Horton

Published by

North Carolina Cooperative Extension

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E10-51854

AG-720-W
01/2010 BS