# Nitrogen Fixation by Legumes

Guide A-129

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Cooperative Extension Service • College of Agricultural, Consumer and Environmental Sciences

## BIOLOGICAL NITROGEN FIXATION

Approximately 80% of Earth's atmosphere is nitrogen gas ($N_2$). Unfortunately, $N_2$ is unusable by most living organisms. Plants, animals, and microorganisms can die of nitrogen deficiency, surrounded by $N_2$ they cannot use. All organisms use the ammonia ($NH_3$) form of nitrogen to manufacture amino acids, proteins, nucleic acids, and other nitrogen-containing components necessary for life.

Biological nitrogen fixation is the process that changes inert $N_2$ into biologically useful $NH_3$. This process is mediated in nature only by N-fixing rhizobia bacteria (*Rhizobiaceae*, *α -Proteobacteria*) (Sørensen and Sessitsch, 2007). Other plants benefit from N-fixing bacteria when the bacteria die and release nitrogen to the environment, or when the bacteria live in close association with the plant. In legumes and a few other plants, the bacteria live in small growths on the roots called nodules. Within these nodules, nitrogen fixation is done by the bacteria, and the $NH_3$ they produce is absorbed by the plant. Nitrogen fixation by legumes is a partnership between a bacterium and a plant.

Biological nitrogen fixation can take many forms in nature, including blue-green algae (a bacterium), lichens, and free-living soil bacteria. These types of nitrogen fixation contribute significant quantities of $NH_3$ to natural ecosystems but not to most cropping systems, with the exception of paddy rice. Their contributions are less than 5 lb of nitrogen per acre per year. However, nitrogen fixation by legumes can be in the range of 25–75 lb of nitrogen per acre per year in a natural ecosystem, and several hundred pounds in a cropping system (Frankow-Lindberg and Dahlin, 2013; Guldan et al., 1996; Burton, 1972).

***Figure 1.*** **A legume plant root showing nodules attached to the roots.**

## LEGUME NODULES

Legume nitrogen fixation starts with the formation of a nodule (Figure 1). The rhizobia bacteria in the soil invade the root and multiply within its cortex cells. The plant supplies all the necessary nutrients and energy for the bacteria. Within a week after infection, small nodules are visible with the naked eye (Figure 1). In the field, small nodules can be seen 2–3 weeks after planting, depending on legume species and germination conditions. When nodules are young and not yet fixing nitrogen, they are usually white or gray inside. As nodules grow in size, they gradually turn pink or reddish in color, indicating nitrogen fixation has started (Figure 2). The pink or red color is caused by leghemoglobin (similar to hemoglobin in blood) that controls oxygen flow to the bacteria (Figure 2).

Nodules on many perennial legumes, such as alfalfa and clover, are fingerlike in shape. Mature nodules may actually resemble a hand with a center mass (palm) and protruding portions (fingers), although the entire nodule is generally less than 1/2 inch in diameter (Figure 3).

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*Figure 2.* **Nodules from a legume cut open to show the reddish-pink color that indicates an active, healthy nodule.**


*Figure 3.* **Nodules detached from the roots of a mature legume plant, with a centimeter ruler for scale.**

Nodules on perennials are long-lived and will fix nitrogen through the entire growing season as long as conditions are favorable. Most of the nodules (10–50 per large alfalfa plant) will be centered around the tap root.

Nodules on annual legumes, such as beans, peanuts, and soybeans, are round and can reach the size of a large pea. Nodules on annuals are short-lived and will be replaced constantly during the growing season. At the time of pod fill, nodules on annual legumes generally lose their ability to fix nitrogen because the plant feeds the developing seed rather than the nodule. Beans will generally have fewer than 100 nodules per plant, soybeans will have several hundred per plant, and peanuts may have 1,000 or more nodules on a well-developed plant.

Legume nodules that are no longer fixing nitrogen usually turn green and may actually be discarded by the plant. Pink or red nodules should predominate on a legume in the middle of the growing season. If white, grey, or green nodules predominate, little nitrogen fixation is occurring as a result of an inefficient rhizobia strain, poor plant nutrition, pod filling, or other plant stress.

The fixed nitrogen is not free; the plant must contribute a significant amount of energy in the form of photosynthate (photosynthesis-derived sugars) and other nutritional factors for the bacteria. However, some legumes are more efficient than others. Cowpea, for example, requires 3.1 mg of carbon (C) to fix 1 mg of N. White lupin, however, requires 6.6 mg of C to fix 1 mg of N (Layzell et al., 1979). A soybean plant may divert up to 50% of its photosynthate to the nodule instead of to other plant functions when the nodule is actively fixing nitrogen (Warembourg et al., 1982).

Any stress that reduces plant activity will reduce nitrogen fixation. Factors like temperature and water availability may not be under the farmer's control, but nutrition stress (especially phosphorus, potassium, zinc, iron, molybdenum, and cobalt) can be corrected with fertilizers. When a nutritional stress is corrected, the legume responds directly to the nutrient and indirectly to the increased nitrogen nutrition resulting from enhanced nitrogen fixation. Poor nitrogen fixation in the field can be easily corrected by inoculation, fertilization, irrigation, or other management practices.

## NITROGEN FIXATION EFFICIENCY AND NITROGEN FERTILIZATION

Some legumes are better at fixing nitrogen than others. Common beans are poor fixers (less than 50 lb N per acre) and fix less than their nitrogen needs. Maximum economic yield for beans in New Mexico requires an additional 30–50 lb of fertilizer nitrogen per acre. However, if beans are not nodulated, yields often remain low, regardless of the amount of nitrogen applied. Nodules apparently help the plant use fertilizer nitrogen efficiently.

Other grain legumes, such as peanuts, cowpeas, soybeans, and fava beans, are good nitrogen fixers and will fix all of their nitrogen needs other than that absorbed from the soil. These legumes may fix up to 250 lb of nitrogen per acre and are not usually fertilized (Walley et al., 1996; Cash et al., 1981). In fact, they usually don't respond to nitrogen fertilizer as long as they are capable of fixing nitrogen. Nitrogen fertilizer is usually applied at planting to these legumes when grown on sandy or low organic matter soils to supply nitrogen to the plant before nitrogen fixation starts. If nitrogen is applied, the rate should not exceed 15 lb per acre. When an excessive amount of nitrogen is applied, the legume literally slows or shuts down the nitrogen fixation process (Delwiche and Wijler, 1956). It is easier and less energy consuming for the plant to absorb nitrogen from the soil than to fix it from the air.

Perennial and forage legumes, such as alfalfa, sweet clover, true clovers, and vetches, may fix 250–500 lb of nitrogen per acre. Like the grain legumes previously discussed, they are not normally fertilized with nitrogen. They occasionally respond to nitrogen fertilizer at planting or immediately after a cutting when the photosynthate supply is too low for adequate nitrogen fixation (Aranjuelo et al., 2009). However, $N_2$ fixation continues in the presence of high levels of soil N, but at reduced levels (Lamb et al., 1995). It is also important to note that $N_2$-fixing alfalfa is much more capable of removing excess nitrogen from soil compared to non-$N_2$-fixing alfalfa varieties (Russelle et al., 2007).

## NITROGEN RETURN TO THE SOIL AND OTHER CROPS

Almost all of the fixed nitrogen goes directly into the plant. However, some nitrogen can be "leaked" or "transferred" into the soil (30–50 lb N/acre) for neighboring non-legume plants (Walley et al., 1996). Most of the nitrogen eventually returns to the soil for neighboring plants when vegetation (roots, leaves, fruits) of the legume dies and decomposes.

When the grain from a grain legume crop is harvested, little nitrogen is returned for the following crop. Most of the nitrogen fixed during the season is removed from the field as grain. The stalks, leaves, and roots of grain legumes, such as soybeans and beans, contain about the same concentration of nitrogen as found in non-legume crop residue. In fact, the residue from a corn crop contains more nitrogen than the residue from a bean crop simply because the corn crop has more residue left after the harvest of corn.

A perennial or forage legume crop only adds significant nitrogen for the following crop if the entire biomass (stems, leaves, roots) is incorporated into the soil. If a forage is cut and removed from the field, most of the nitrogen fixed by the forage is removed. Roots and crowns add little soil nitrogen compared with the aboveground biomass.

## NITROGEN FIXATION PROBLEMS IN THE FIELD

Measuring nitrogen fixation in the field is difficult. However, a grower can make some field observations that can help indicate if nitrogen fixation is adequate in some of the common legumes.

If a newly planted field is light green and slow growing, suspect insufficient nitrogen fixation. This is often seen with beans and alfalfa. In a new field, the poor fixation is often attributed to the lack of native rhizobia to nodulate the legume, but the cause may also be poor plant nutrition or other plant stresses that inhibit nitrogen fixation. Small nodules should be present from 2–3 weeks after germination. If nodules are not present, consider the following options.

A. Replant using seed inoculated with the correct rhizobia.

B. Try to inoculate the plants in the field through the irrigation system or by other means. Caution: this technique often does not work and expert advice is needed.

C. Consider nitrogen fertilization to meet all of the plants' nitrogen needs. This may not be an option for a perennial legume such as alfalfa, especially if the field is kept in alfalfa for several years. Also, some legumes use soil or fertilizer nitrogen more efficiently if nodules are present.

If few or small nodules are present, sufficient soil nitrogen may not be available for the young plant before nitrogen fixation starts. The plant usually grows out of this condition, or a small amount of nitrogen can be applied. Also, inefficient native rhizobia may result in poor nitrogen fixation. Consider other soil stresses that may be inhibiting plant growth, especially plant nutrition and water stress.

If an established crop becomes nitrogen deficient in the middle of the growing season—when plant growth and nitrogen demands are greatest—poor or inefficient nitrogen fixation might be the cause. Nodules should be clearly evident, at about the size and number per plant as previously described, and should be pink or red in color. If only a few nodules are present, insufficient rhizobia numbers have limited nodulation, or plant stresses may be inhibiting nitrogen fixation. At this time, you may be able to remove a plant stress, but it is too late to inoculate if the nodules are mostly green, gray, or white since the native rhizobia are likely inefficient nitrogen fixers. The only choice may be to apply nitrogen fertilizer sidedressed on the present crop and to sufficiently inoculate the next legume crop. New Mexico State University Extension Guide A-130, *Inoculation of Legumes* (http://aces.nmsu.edu/pubs/_a/A130.pdf), describes when and how to inoculate legumes.

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