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Chapter 13

Biological Nitrogen Fixation

Nature's Partnership for Sustainable Agricultural Production

NifTAL Center for BNF Technologies

Many agriculturally important plants in the legume family can use nitrogen (N) from the atmosphere for growth through biological nitrogen fixation (BNF). Legume BNF involves a symbiosis between legume plants and the rhizobia that live in nodules on their roots. There are economic, environmental, and agronomic benefits from using BNF in cropping systems.

All living things require nitrogen. It is a key element of amino acids, the building blocks of proteins. There is a huge reservoir of N in the atmosphere. Approximately 80% of the air consists of nitrogen gas (N_2), but plants cannot use atmospheric N directly to make protein. The gaseous N must first be converted, or "fixed," into forms plants can use.

Unless fertilizer N is applied, most plants obtain their nitrogen from natural sources in the soil. Natural reserves of soil N are normally low, so N fertilizers must be added to increase plant growth. "Fixing" the N from the air to manufacture fertilizers like urea requires extremely high temperatures and pressures, and the use of fossil fuels. Because the energy requirements for production are high, and N is needed in large amounts, N fertilizers are an expensive input for crop production.

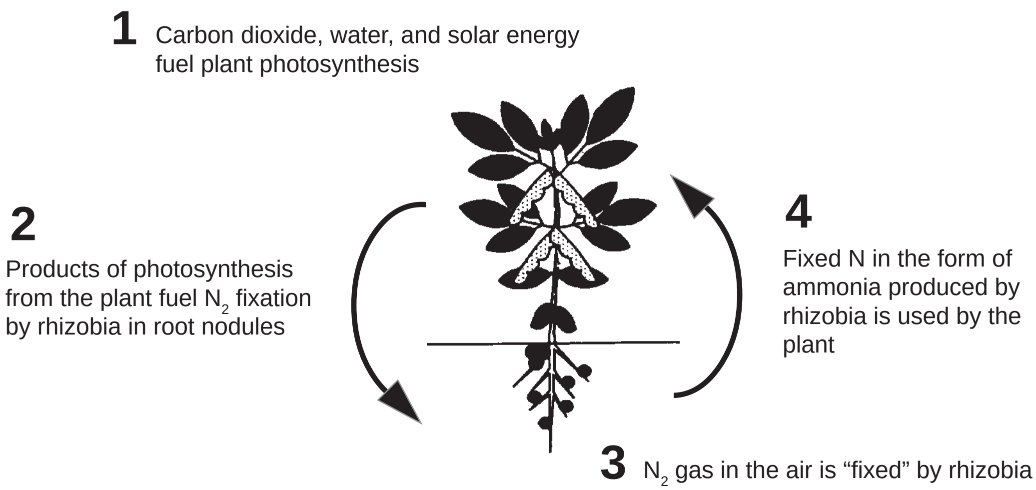
Nature has an alternative method of providing N to plants and enriching soil N resources—biological nitrogen fixation. Many members of the legume plant family, such as beans, peas, alfalfa, and leucaena, have the special ability to use BNF to meet their N needs. Legume BNF involves a remarkable symbiosis, or mutually beneficial relationship, between the plant and N-fixing soil bacteria called rhizobia. The rhizobia invade the host plant's roots and cause the formation of structures called nodules. Within these nodules, the rhizobia use enzymes to biologically convert ("fix") N_2 gas from the air into a form of N that can be used by its plant host to make proteins. In turn, the plant provides the rhizobia with products of photosynthesis: sugars and carbohydrates that fuel the bacteria and the BNF process (see Figure 13-1).

Benefits of using BNF

Economics: BNF reduces costs of production. Field trials have shown that the N captured by crops due to the use of rhizobia inoculants costing $3.00/ha is equal to fertilizer N costing $87.00.

Environment: The use of inoculants as alternatives to N fertilizer avoids problems of contamination of

Figure 13-1. The legume-rhizobia symbiosis.



water resources from leaching and runoff of excess fertilizer. Utilizing BNF is part of responsible natural resource management.

Efficiency: Legume inoculants do not require high levels of energy for their production or distribution. Application on the seed is simple compared to spreading fertilizer on the field. Long-term leguminous tree crops are self-sustaining through BNF.

Better yields: Inoculants increase legume crop yields in many areas. BNF often improves the quality of dietary protein of legume seed even when yield increases are not detected.

Increased soil fertility: Through practices such as green manuring, crop rotations, and alley cropping, N-fixing legumes can increase soil fertility, permeability, and organic matter to benefit nonlegume crops.

Sustainability: Using BNF is part of the wise management of agricultural systems. The economic, environmental, and agronomic advantages of BNF make it a cornerstone of sustainable agricultural systems.

Legumes comprise one of the most important plant families in agriculture. Nitrogen-fixing members of this family include important food grains like soybeans, peas, beans, and peanuts; forage crops like alfalfa and clover; and useful trees like leucaena and acacias. By inoculating legume seeds with appropriate rhizobia, farmers can ensure that they take advantage of the benefits of BNF listed above. Estimates of the amounts of N fixed by various legumes are listed in Table 13-1.

Inoculating legumes with rhizobia to maximize BNF

- Many soils do not have sufficient numbers of appropriate rhizobia for maximum BNF.
- Rhizobial inoculants and legume crops must be properly matched.
- There are several methods of inoculating legumes.
- Inoculants require some special care to maintain their viability.

Although BNF is a natural process, many soils do not have sufficient numbers of appropriate rhizobia for effective symbioses. Inoculating legume crops with compatible rhizobia ensures maximal BNF. Inoculation is especially important when introducing new legumes to an area.

Table 13-1. Estimates of the amount of nitrogen fixed by various legumes (FAO 1984).

Plant	Scientific name	Nitrogen fixed (kg N/ha/yr)
Horse bean	*Vicia faba*	45–552
Pigeon pea	*Cajanus cajan*	168–280
Cowpea	*Vigna unguiculata*	73–354
Mung bean	*Vigna mungo*	63–342
Soybean	*Glycine max*	60–168
Chickpea	*Cicer arietinum*	103
Lentil	*Lens esculenta*	88–114
Peanut	*Arachis hypogaea*	72–124
Pea	*Pisum sativum*	55–77
Bean	*Phaseolus vulgaris*	40–70
Leucaena	*Leucaena leucocephala*	74–584
Alfalfa	*Medicago sativa*	229–290
Clover	*Trifolium* spp.	128–207

Inoculation is simply the process of applying suitable live rhizobia to the soil where they can infect the roots and form effective nodules. Inoculant producers grow pure cultures of selected, highly effective strains of rhizobia. The cultures are usually mixed with a carrier material like powdered peat. Each gram of high-quality peat inoculant can contain a billion rhizobia.

There are several inoculation methods. Granular inoculants can be applied directly to the field. Powdered inoculants can be mixed with water for irrigating the soil. This liquid application is suitable for inoculating trees in a seedling nursery. By far, the most common method of inoculating crops is to coat the seed with the powdered inoculant. The rhizobia are then "planted" in the soil with the seed, and can infect the emerging root as the seed germinates. Some dealers sell preinoculated seed, but these are not recommended because the viability of the rhizobia is often low by the time they are planted.

There are several general variations of the seed inoculation process. While the simplest is to dust the seeds with the powdered inoculant, the use of a liquid "sticker" is recommended so the inoculant adheres to the seed. Commercial stickers are recommended. If they are not available, a 10% sugar-water solution or vegetable oil can be used. Farmers should follow the inoculation rates and procedures described by the inoculant producers.

Sources of commercial inoculants are listed at the end of this section on BNF. Like fertilizers, inoculants are inputs that can increase plant growth, but they are very different than chemical fertilizers in several respects. Some important points to consider when using inoculants:

- Rhizobia are living organisms. Use inoculants before their expiration dates, and protect them from sun and heat.
- Never expose inoculants to temperatures above 35°C or below freezing. Inoculants should be stored in sealed bags at 4–25°C.
- Inoculate seeds just before planting and protect the coated seeds also from heat. Cover furrows and, if possible, irrigate soon after planting.

Rhizobia and legumes have specific requirements, and they must be properly matched. Legumes that are effectively nodulated by the same rhizobia belong to the same "cross-inoculation" group. Commercial inoculants contain tested, selected rhizobia that are effective on legumes in specific groups listed on the label. Always check the label to be sure you have the proper inoculant: different legumes require different inoculants.

Common methods of seed inoculation

(The inoculant producer usually provides specific directions for the use of the product.)

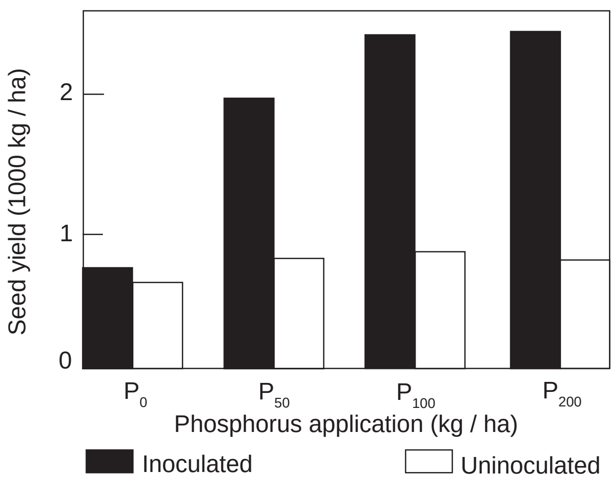
The two-step method: Seeds are first coated with a sticker solution at a rate of about 15 milliliters/kg seed, depending on the size of the seed. Inoculant is added to the sticky seeds at a rate of 5–20 g/kg seed.

The slurry method: Powdered inoculants are mixed with water or sticker solution to form a slurry. Seeds are mixed with the slurry until they are coated.

Seed pelleting: Seeds are first inoculated by the slurry or two-step method. Before the seeds dry, they are mixed with finely ground, sifted lime or phosphate to form a protective pellet.

Seed dusting: Powdered inoculants are simply shaken over the seeds. This method is the least satisfactory because relatively few rhizobia stick to the seed.

Figure 13-2. Response of a soybean crop in Hawaii to inoculation with rhizobia, with various levels of phosphorus fertilizer added.



Factors that affect a crop's response to inoculation

- Inoculation failure may be caused by loss of viability of the inoculant rhizobia.
- Environmental and management factors that affect the growth potential determine a plant's BNF requirement.
- When soil N sources are high enough to meet the crop's N requirements, legumes will not use BNF.
- Soils may have many native rhizobia that can infect the plant and fix N for the crop.

Viability of inoculants

Farmers in many parts of the world have received dramatic increases in yields due to inoculating their legumes, but positive results do not always occur. Inoculation failure is sometimes due to the loss of viability of rhizobia in the inoculant due to exposure to heat or prolonged storage. Other causes may be improper handling, application, or planting methods that cause the rhizobia to die on the seed.

Plant growth potential

If growth is limited by other factors, good inoculant quality and proper application will not increase yields. For example, Figure 13-2 shows that there was little difference between yields of inoculated and uninocu-lated soybeans in a P-deficient soil in Hawaii when no phosphorus was added, yet when 50 or more kg/ha of P was applied, inoculation resulted in dramatic yield increases.

This example demonstrates an important point: BNF is dependent on the plant's total growth potential. This type of study can help to identify situations in which farmers can get the maximum benefit from their inputs and from BNF. Phosphorus is the most limiting nutrient in the soil in which the experiment shown in Figure 13-2 was conducted. When the lack of P limits plant growth, as shown in Figure 13-2 when zero P was applied, inoculation has little effect on yields. When P is added to the soil, N becomes the most limiting nutrient, and inoculation results in large yield increases; however, the added P does not significantly improve the yields of the uninoculated plants. As the plant's growth potential improves with added P from P_{50} to P_{100}, the benefits of BNF also increase. Notice that when P applications are increased from 100 to 200 kg/ha, the benefits due to inoculation do not increase. At this point some other factor, possibly weather or the genetic yield potential of the variety, limits further yield increases.

This "limiting factor" principle applies to other factors that affect plant growth. Anything that limits the yield of the legume will reduce the amount the farmer will benefit from BNF. If the legume crop yield is limited by the availability of an essential nutrient like P, as in the previous example, or any other factor like soil pH, water, or pests, the crop will not require much N. Legumes will not form many nodules or fix much N when N requirements are low, and the addition of rhizobia through inoculants will not increase yield.

In summary, farmers will get the most benefit from inoculation when they combine it with good crop management. It is important to use inoculants with other agricultural inputs that increase plant health and yield.

Available soil N

Legumes can get their N from BNF and from available N sources in the soil. Often they use both N sources — fixation and the soil—to meet their N requirements. Nitrogen in the soil comes from mineralized organic matter, manure, or residual fertilizer N from the previous crop. Legumes use these mineral sources of N because it requires less energy for the plant to take up N directly from the soil than to fix N_2 through BNF. If there is enough N available in the soil to meet plant requirements, there will be no added benefit from inoculation. If through good management the plant's growth potential increases, the resulting increased N requirement for N may be met by BNF.

Although legumes use available soil N first, a farmer who can establish effective BNF with his legumes will profit more than the farmer who adds fertilizer N to the crop. Legumes can have low fertilizer use efficiency, and the farmer would have to apply at least two to three times as much N in fertilizer as can be fixed by the legume. For example, the farmer would have to add 200 to 300 kg/ha N in fertilizer to get the same yield as a crop that gets 100 kg/ha of N from BNF.

Native rhizobia in the soil

Some soils have high populations of native rhizobia that are compatible with the legume crop. If the crop's requirement for BNF can be met by these native rhizobia, inoculation may not increase yield. Large native rhizobial populations often occur when legume crops are grown in the same field for many crop cycles or when crops have been previously inoculated and the rhizobia persist. Despite this occurrence, many farmers continue to inoculate their legumes each season because this practice is considered "cheap insurance." Native rhizobial strains may not be as effective as the tested strains in inoculants, and inoculation assures the farmer that there will be sufficient numbers of superior rhizobia for the crop.

Assessing BNF

Legume BNF can be assessed by nodule color, observations of plant growth, and field and pot trials.

Nodules should be visible on inoculated legumes within 21 to 28 days after planting. They should not be confused with nematode galls. The nodules can be detached from the roots, unlike the galls, which are actually root swellings. Root nodules have characteristic shapes, depending on the legume species. For example, soybeans, cowpeas, and peanuts have spherical nodules; clovers and leucaenas have elongated, finger-like nodules; and chickpea and alfalfa nodules are fan or coral shaped. The presence of many effective nodules on or near the tap root generally indicates that a quality inoculant has been properly applied to the crop.

Effective nodules usually are large and clustered on the primary and upper lateral roots. When they are sliced open, they have a deep pink or red color inside. In contrast, ineffective nodules often are small, numerous, and distributed throughout the root system. They have white or green interiors. A single plant may be nodulated by both effective and ineffective rhizobia.

Figure 13-3 shows commonly observed conditions and how to diagnose them. Pot and field experiments can provide further assessment of the need for inoculation and other management inputs that can improve BNF. These types of experiments should be conducted with care to ensure that there are proper controls. For further information on conducting tests to assess BNF, contact the NifTAL Center for BNF Technologies, 1000 Holomua Rd., Paia, Hawaii, 96779, USA.

Sources of inoculants

High-quality legume inoculants can be obtained from garden shops and other retailers of agricultural supplies, or directly from the following producers:

LiphaTech, 3101 W. Custer Ave., Milwaukee, Wisconsin 53209, USA. Phone (414) 462-7600, fax (414) 462-7186

MicroBio RhizoGen, 3835 Thatcher Ave., Saskatoon, Saskatchewan S7R 1A3, Canada. Phone (306) 373-3060, fax 306 374 8510, e-mail <mbrsask@sk.sympatico.ca>.

Urbana Laboratories, P.O. Box 1393, St. Joseph, Missouri, 64502, USA. Phone (816) 233-3446, (800) 892-2013, fax (816) 233-8295, Web <www.seedsolutions.com>.

Acknowledgments

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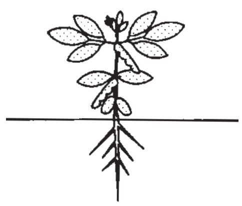
Burton, J. 1984. Legume inoculants and their use. Food and Agriculture Organization, Rome.

Singleton, P., P. Somasegaran, P. Nakao, H. Keyser, H. Hoben, and P. Ferguson. 1990. Applied BNF technology: a practical guide for extension specialists. NifTAL, Paia, Hawaii.

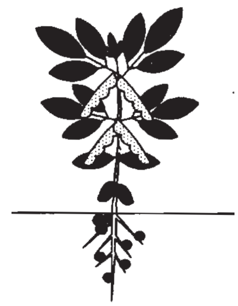
Figure 13-3. Legume growth conditions commonly observed in farmers' fields, and the explanations for them.



NOT INOCULATED

YELLOW PLANT
NO NODULES

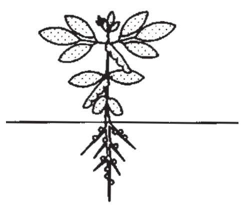
No native rhizobia capable of nodulating that legume.



INOCULATED

DEEP GREEN PLANT
LARGE NODULES, RED INSIDE

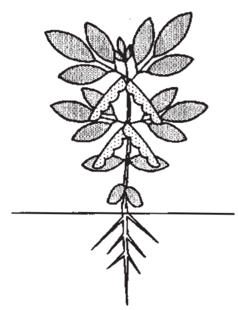
If uninoculated plants are yellow with no or only small nodules, then inoculant is highly effective and native rhizobia are ineffective.



NOT INOCULATED

YELLOW PLANT
MANY SMALL NODULES, WHITE INSIDE

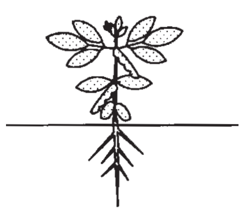
Native rhizobia ineffective at fixing nitrogen



NOT INOCULATED

GREEN PLANT
NO NODULES

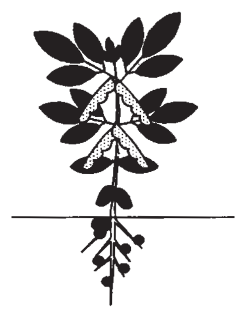
Soil high in mineral nitrogen. No compatable native rhizobia.



INOCULATED

YELLOW PLANT
NO NODULES

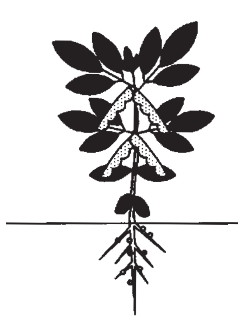
Inoculation failure. Improper rhizobia or rhizobia are dead.



NOT INOCULATED

DEEP GREEN PLANT
LARGE NODULES, RED INSIDE

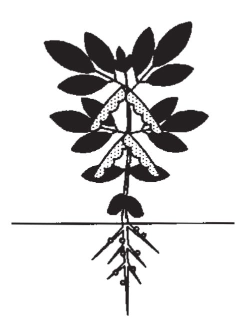
Sufficient effective native rhizobia. Inoculation not necessary.



INOCULATED

DEEP GREEN PLANT
SMALL NODULES

Soil high in mineral nitrogen. Nodules not fixing nitrogen.



NOT INOCULATED

DEEP GREEN PLANT
SMALL NODULES

Soil high in mineral nitrogen. Native rhizobia may be effective or ineffective.