service in ACTION

Colorado State University Cooperative Extension

no. .305

Application and storage of legume seed inoculants

Mark A. Brick and Ted L. Davis[1]

Quick Facts

- Legumes have the ability to convert atmospheric nitrogen (N_2) to useable ammonia nitrogen for the plant.
- Inoculation is the process of introducing commercially prepared rhizobium bacteria into the soil; commercial inoculants are available that are applied directly to soil or seed.
- Each legume species requires a specific species of rhizobia to form nodules and fix nitrogen.
- Inoculum and preinoculated seed should be stored in a cool environment (35-60°F) without being exposed to sunlight.
- Inoculum packages usually are labeled with an expiration date; do not purchase outdated inoculum, since rhizobial viability may have declined significantly after that date.

The air we breathe contains over 78 percent nitrogen in the form of nitrogen gas (N_2). Legumes have the unique ability to form a symbiotic relationship with rhizobia bacteria to convert atmospheric nitrogen gas to ammonia nitrogen that is useable by the plant. This relationship occurs in specialized root tissue, referred to as nodules. Some legumes, such as alfalfa, can produce enough ammonia to supply all nitrogen needs of the plant (Table 1), therefore, supplemented nitrogen fertilization usually is not needed.

The relationship between legume and rhizobia species is mutually beneficial, hence it is termed symbiotic. This relationship occurs when the bacteria invade plant root hairs and multiply in the outer root tissue. This invasion triggers the plant to form tissue that serves as a protective enclosure around the bacteria. The plant supplies energy (food) to the bacteria in the form of photosynthate, while the bacteria convert nitrogen gas to ammonia in the nodules. This is done during a series of biochemical events with enzymes produced by the bacteria in association with the host plant.

Several rhizobia species have been identified and defined by their ability to form nodules on specific legume species. Each legume requires a specific species and strain of rhizobia to form nodules. Commercial inocula are labeled relative to the plant species for which the rhizobia are appropriate and highly effective. It is necessary to apply the correct inoculant to assure successful nodulation and effective nitrogen fixation.

Inoculation

Legume inoculation is the process of introducing commercially prepared sources of rhizobia for the purpose of promoting nitrogen fixation. This usually is done by applying inoculum directly to the seed prior to planting. It also can be done by metering the inoculum into the seed furrow during planting.

If the same legume crop was grown in the field previously, there is a good chance that the soil already contains the correct rhizobial species for nodulation. However, the native rhizobial populations found in soil often are less effective in nitrogen fixation potential. A nodule that is actively fixing nitrogen, will be pink to reddish when cut open, rather than tan (ineffective) or green (dying).

Commercial inoculants are composed of rhizobial strains selected for maximum fixation

[1]*Mark A. Brick, Colorado State University Cooperative Extension specialist and associate professor; and Ted L. Davis, manager Colorado Seed Growers; both department of agronomy (7/87)*

potential. It should be noted, however, that even though more efficient strains are introduced into the soil, there is no guarantee these strains will compete well with native strains for entry into plant roots. Many studies have been conducted on the application of commercial inoculants into soils that already contain the correct rhizobial bacteria.

In some cases, a significant yield increase has been observed, whereas in other situations no response occurred. Soils with a low pH (acid soils) often have poor survival of the rhizobia. One way to evaluate the response of inoculants is to test several inoculants and an untreated control in fields using replicated strip tests. When in doubt about the rhizobial population in a field, it is a good practice to apply inoculum, especially if the legume has never or has not recently been grown in that field.

Proper soil fertility must be maintained to assure nodulation and nitrogen fixation. Some legumes can normally obtain most of their nitrogen from the atmosphere through symbiotic nitrogen fixation. Attempts to supplement the legume nitrogen supply by fertilization is usually counter-productive since plants tend to cease nitrogen fixation in the presence of high soil nitrogen. Phosphorous and potassium can affect nodulation and hence nitrogen fixation. Research has shown that additions of phosphorous and/or potassium increase the number of nodules formed, fresh weight of nodules, and amount of nitrogen fixed per nodule.

An important micronutrient for nitrogen fixation is molybdenum. Soils with a pH below 6.0 usually have low molybdenum availability. Other soils that could have molybdenum deficiences include those that are strongly weathered or leached, sandy soils, or soils high in manganese and iron. If molybdenum is a limiting factor, it can be applied as a seed treatment with the inoculum. Some inoculants have molybdenum already incorporated. Read the package label for this information.

Commercial Inoculants

Three basic forms of commercial inocula are available including: 1) solid, 2) liquid and 3) freeze-dried. The most commonly used are solid, peat-based inoculants that can be purchased for seed or direct soil application. Liquid inoculants are available in broth culture or as frozen concentrate. Broth or frozen concentrates usually are mixed with water and sprayed into the seed furrow at planting. Since liquid inoculants must be kept frozen or refrigerated during shipment and storage, their availability is limited through normal distribution channels.

Seed-applied inoculants exist as planter box additives, preinoculated seed and custom inoculants. The planter box additive, where inoculant is mixed with seed in the planter box, is most common. This can be accomplished by applying dry inoculum or a slurry directly to seed. The dry method is least desirable because of uneven distribution and poor adhesion of inoculum to seed. The slurry is prepared by mixing the inoculum with water for better adherence of inoculum to the seed coat. Seed also may be pre-wetted before mixing dry inoculants with the seed. Do not leave dry inoculum in the planter box overnight, or let it get wet from rain or dew.

Many small-seeded legumes, such as alfalfa, are preinoculated by seed conditioners, distributors and dealers. After conditioning, a sticking agent is applied to the seed followed by the dry inoculum, or incorporating the inoculum into a seed coating. Preinoculated seed must be kept in a cool environment during shipping and storage. The seed should be used within one year of inoculation, or reinoculated prior to planting. Remember that rhizobia cells are living bacteria that must be kept viable until planting.

Custom inoculation usually is done on the farm or by the seed distributor. This process involves application of a nutrient-rich adhesive formulation, followed by a peat-based inoculum. This method assures viable inoculum if seed is stored properly following application and used within one year.

Seed inoculation should not be confused with chemical seed treatment. Most seed disinfectants, including fungicides, are toxic to rhizobia bacteria. Inoculum should not be applied to seeds that are treated with a bactericide, such as streptomycin. A furrow application of the inoculum should be used in these cases as the bactericide will kill the rhizobium. Although some rhizobia species are slightly tolerant to certain chemical compounds, inoculating chemically treated legume seed requires special precautions. Check with the inoculum manufacturer regarding combining products and avoid inoculum products pre-mixed with pesticides or other toxic chemicals.

Selecting the Correct Inoculant

Each legume species requires a specific species and strain of rhizobia. For example, the rhizobial species that nodulates alfalfa will not nodulate dry beans or soybeans. Plants mutually compatible with the same species of rhizobia were listed in earlier years in so-called "cross-inoculation groups." With ongoing research, the demarcations between these plant groups has become less distinct. When selecting inoculum, consult your supplier and read the package label to be sure of selecting the correct inoculum for the crop.

Storage of Inoculum

Inoculum contains living rhizobial cells that survive on an organic carrier such as peat. The rhizobia population declines, over time, even

under proper storage conditions. Most inoculum manufacturers put an expiration date on the package, therefore, do not purchase outdated inoculum, since the rhizobial population may have declined significantly after that date.

Optimum storage conditions for peat-base inoculum is under refrigeration; however, short-term storage at temperatures below 60° F is acceptable. It is best not to freeze peat-based inoculant, but if it does freeze, keep in a frozen condition for a minimum time. Inoculants should not be left in direct sunlight because ultraviolet rays and heat will kill the bacteria.

Purchase only the amount of inoculum or preinoculated seed needed for one planting season. When treating seed on-farm, avoid treating more seed than can be planted in one day. Inoculant packages provide information regarding the amount of seed or furrow that can be treated with a measured amount of inoculum.

Table 1: Amount of nitrogen fixed per acre by several legume crops.

	Estimates of nitrogen fixed per acre (lbs.)	
Crop	**Low**	**High**
Alfalfa	44	308
Cowpeas	44	132
Dry beans	50	150
Garbanzo beans	25	81
Peas	53	305
Soybeans	53	265