

Inoculating Alfalfa and Clover Seed

Alfalfa and other legume plants that have no nodules or nodules containing ineffective bacteria are common causes of low production or complete failure of plantings. These failures can result in loss of the stand and the investment in soil preparation, fertilizer, and seed used to make the planting. In many cases these problems have been caused by failure to follow the basic rules of legume seed inoculation, as neither alfalfa nor clovers can be grown economically without effective nodulation.

Nitrogen fixation is important

Nitrogen is essential to plant and animal nutrition, as it is a vital ingredient of amino acids, protein, and other essential materials. The atmosphere contains about 80 percent nitrogen by volume, but this vast amount of free atmospheric nitrogen is unavailable to most plants or animals. However, nitrogen can be converted to a usable form by alfalfa or other legumes when growing in association with *Rhizobia* bacteria. These bacteria grow within modified parts of the plant roots called nodules. The bacteria obtain food energy from the legume root and convert gaseous atmospheric nitrogen (N_2) into ammonia (NH_3). This process is known as nitrogen fixation. The NH_3 is then used to make amino acids, which are the building blocks of protein. The bacteria and the host legume both benefit from this symbiotic relationship. The legume receives its nitrogen from the bacterial by-products, and the bacteria receive portions of their food supply from the legume plus a protected growth environment within the nodule. As the nodules mature and the roots decay, nitrogen by-products are returned to the soil with a net increase in nitrogen fertility. When legumes are grown for hay, much of the fixed nitrogen is removed from the field in the forage.

Bacteria invade the legume root

The *Rhizobia* bacteria enter the legume through root hairs on the young roots. This may occur as early as 4 to 12 days after seed germination.

The original infection rapidly develops into visible nodules 3 to 5 weeks after plants emerge, depending on the plant species and its growth rate.

Not all *Rhizobia* are the same

Legume bacteria are of different kinds. Bacteria that fix nitrogen in alfalfa and sweetclover will not fix nitrogen in clover, peas, and other legumes. Commercially prepared inoculant cultures are available for *specific crops*. The correct culture must be used when inoculating each type of legume seed.



Certain strains of legume bacteria are more efficient in fixing atmospheric nitrogen than others. Therefore, it is important to establish conditions that will permit root infection by the most efficient strains of bacteria. Nodules formed on legume roots by effective bacteria are large. Interiors have a red or pink color when compared with the smaller, more pale, ineffective nodules.

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To examine roots for effective nodules, carefully lift the plant with a shovel or fork and gently wash the roots to expose nodules. Then cut the nodules in half with a pocket knife and examine the interior for color.

The development of highly effective nodules is necessary to inhibit root infections by other strains of bacteria. For most successful nodulation, it is important that the original infection result from an efficient nitrogen-fixing strain. To improve the chances for infection by desirable strains, surround each legume seed with large numbers of viable *Rhizobia* of desirable strains and create soil conditions most favorable for their survival.

Conditions affecting successful inoculation

Bacteria placed on the seed before planting must survive in the soil for several weeks until seeds germinate and root hairs are developed so that infection and nodulation can take place. During this time, the number of bacteria on the seed may be greatly reduced or all bacteria eliminated, resulting in failure of nodules to develop. Several conditions reduce the number of bacteria:

- **Exposure to sunlight and drying** are two conditions that often occur together. Drilled seed may be exposed to sunlight (ultraviolet rays) after inoculation and before planting, and broadcast seed may be exposed both before and after planting. Drying conditions are often common in poorly compacted seedbeds, which lose soil moisture readily. Avoid this problem by drilling the seed into moist soil or into dry soil just ahead of a rain.

- **Exposure to high temperatures** may occur any time during storage by the wholesaler, retailer, or grower. Store inoculum in the refrigerator at a temperature above freezing. Inoculated seed is often exposed to high temperatures when planted in dry soils during summer months.

- **Unfavorable soil conditions** such as acidity, low fertility, and the presence of antibiotic substances are common. Liming of acid soils and proper fertilization will help overcome these problems. High levels of soil nitrate also reduce infection and nodulation of legumes by *Rhizobia*. Pelleting legume seed with inoculum, an adhesive, and lime is helpful when seed is exposed to light and drying or other adverse conditions. Seed should be re-inoculated, however, if it has been in storage for several weeks.

- **Banding seed in contact with acid fertilizers**, or with heavy metals such as boron, zinc, or molybdenum that are applied as micronutrients, may

destroy *Rhizobia*. Proper band placement at seeding time will prevent seed from contacting the fertilizer materials.

- **Age of inoculum** affects the number of bacteria. All commercial inoculants are dated. Do not accept inoculants past their expiration dates.

Adverse soil and planting conditions can be partially overcome by applying a greater number of *Rhizobia* bacteria to increase the probability that enough bacteria will survive until roots are developed and infection can occur. Under unfavorable conditions a large number of bacteria may result in successful nodulation, while a small number may result in failure.

Commercial inoculants

Several types of commercial inoculants are available. The dry peat cultures have proved most successful. They have a good shelf life when stored in cool, dark areas and are easy to handle at planting time. Do not use inoculum beyond its expiration date.

Pre-inoculated seed

Pre-inoculated seed has been marketed for several years. Results from its use have been variable. The use of additional inoculant at seeding will provide cheap insurance and reduce the risk of failure in legume establishment, particularly if the pre-inoculated seed has been in storage for a long time.

Steps for seed inoculation

Legume inoculation will be more successful if you follow a few simple steps and provide suitable soil pH and physical conditions.

- Use inoculants specifically labeled for the legume you are treating. Mixtures of bacteria strains usually are not as effective as preparations labeled for one specific legume.

- Use only fresh, age-dated inoculant purchased from dealers who store their supplies in cool, dark places to minimize deterioration. Best storage conditions are provided by refrigerators with temperatures just above freezing.

- Store the inoculant in a refrigerator until used.

- Dampen the legume seed, using as little liquid as possible. Approximately 1 pint of liquid per 100 pounds of seed is required. Use milk (whole, condensed, or skim), diluted pancake syrup, or gum arabic solution as an adhesive. Consult OSU Extension Circular 1086, *Making Lime-Pelleted Seeds*, for details on using gum arabic

as an adhesive for lime-pelleted seeds. Mix the seed and liquid thoroughly until every seed is moist but not wet enough to cause the seeds to stick together. If too much liquid is added, add a handful of finely ground agricultural limestone. Do not use containers or mixers contaminated with seed disinfectants or fertilizer materials that might be toxic to legume inoculants.

- Add the inoculant to the seed in small quantities until *at least* the amount recommended by the manufacturer has been applied. Two to three times the suggested amount can be used without difficulty. Mix thoroughly until every seed has come into contact with the inoculant. When planting under conditions that are not ideal, increase the inoculant rate.

- Be sure inoculated seed does not come into direct contact with the fertilizer.

- Plant seed into a well-prepared, firm seedbed *immediately* after inoculation. Avoid exposing the seed to sunlight, severe drying conditions, or high temperatures. If seed is not planted within 24 hours, repeat the inoculation step because the bacteria from the previous treatment may have been destroyed by drying.

- When planting, leave seedbed surface packed to minimize exposure of seed to sunlight and drying conditions. Broadcast seedings should be covered and firmed by a cultipacker or roller.

- Plant seed just before a rain or into moist soil. Cultipacking the soil after seeding will help to maintain moisture near the seed.

When nodules fail to develop

Improperly nodulated legume stands can be identified by light yellow foliage. Entire leaf surfaces turn yellow, with symptoms first appearing on the lower leaves. These symptoms are caused by nitrogen deficiency. Verify the diagnosis by examining the roots. Compare the size and color of any nodules that are present with nodules

of a healthy plant. Apply nitrogen fertilizer to a small strip to determine if plants are deficient in N. An application rate of 100 to 150 pounds per acre will result in pronounced color differences to plants inadequately nodulated.

Sulfur deficiency also causes alfalfa leaves to turn yellow, and this may be confused with a nitrogen deficiency caused by poor nodulation. However, the younger, upper leaves turn yellow first on sulfur-deficient plants.

If a perennial legume seeding contains a few well-nodulated plants, the stand might be salvaged by fertilizing lightly with nitrogen (40 to 50 pounds of N per acre) to maintain the non-nodulated plants temporarily, with a possibility that they may develop effective nodules. Continued application of nitrogen to maintain a stand is not recommended. Applying fresh inoculant through irrigation water or spray application during a rainy period has not been effective in correcting a poorly nodulated stand.

Summary

For successful legume nodulation:

- Correct soil acidity with lime application (or lime-pelleted clover seed)
- Fertilize to correct soil nutrient deficiencies (see OSU Extension Fertilizer Guides)
- Prepare a fine, firm, moist seedbed
- Select the correct inoculant culture for the legume
- Store the inoculant in a cool refrigerator until used
- Mix dampened seed with fresh inoculant (See OSU Extension Circular 1086)
- Plant seed immediately after inoculation
- Do not drill inoculated seed directly with fertilizer
- Cultipack the soil after seeding

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