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Nitrogen Fixation and Inoculation of Forage Legumes [1]

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Nitrogen Availability

All plants must have nitrogen (N) for growth. Approximately 110 million tons of N are required for the world's annual food production but only 7 million tons are supplied by the fertilizer industry; the rest come from legumes. Legumes are plants, like peas, beans, soybean, alfalfa, clover, and aeschynomene, which have special bacteria in their rooting system and make use of N from the air. The air we breathe is 78% nitrogen gas, and 21% oxygen. There is ~35,000 tons of free N above every acre of land, but this gaseous form is unavailable to plant or animal life. Fortunately, nature has provided us with a simple and cheap method of obtaining some of this N from the atmosphere by growing legumes.

Symbiotic Nitrogen Fixation

Soon after a legume begins to grow, special N-fixing bacteria that reside in the soil invade the tiny root hairs and multiply in large numbers. The legume roots, in reaction to this infection, form tumor-like swellings called **nodules** on the root surface (see Figure 1). Bacteria inside the nodules absorb air from the soil and convert (**fix**) gaseous N into ammonia (NH_3). The association between the legume host plant and the nodule bacteria is mutually beneficial (**symbiotic**). The plant furnishes the necessary energy that enables the bacteria to fix gaseous N from the atmosphere and pass it on to the plant for use in producing protein. This partnership is known as **symbiotic N fixation.**

Most, but not all, legumes have the capacity to fix N. The quantity of N fixed depends on several factors, such as (1) the kind of legume, (2) the effectiveness of the N-fixing bacteria, (3) the soil conditions including pH and N fertilizer, and (4) availability of necessary plant food such as carbohydrates, phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), molybdenum (Mo), copper (Cu), and boron (B). For example, estimates of N fixed in a growing season for alfalfa are 100-200 lb/A; berseem clover, 50-210 lb/A; red clover, 50-200 lb/A; white clover, 50-150 lb/A; hairy vetch, 100 lb/A; and aeschynomene, 50-150 lb/A. In soils that are well supplied with N

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fertilizer, there may be little or no fixation because the plants use available N in the soil and do not encourage the bacteria to fix more. As a result, the greatest N fixation is obtained in soils low in available N.

Bacterium Specificity and Inoculum Selection

Legume plants only grow vigorously if they have functioning nodules, and this depends upon their roots encountering the appropriate **bacteria strains** in the soil. Bacteria involved in nodule formation and symbiotic N fixation belong to the genera *Rhizobium* and *Bradyrhizobium***.** *Rhizobium* species are fast-growing, acid-producing, N-fixing bacteria associated with many temperate pasture legumes such as alfalfa, clovers, medics, trefoils, vetches, and lespedezas. *Bradyrhizobium* species are slower-growing, alkaline-producing bacteria associated with soybean and many tropical legumes such as cowpeas, carpon desmodium, stylosanthes, aeschynomene, and vigna.

Each legume selected for planting must be matched with sufficient quantity of compatible N-fixing bacteria in order to achieve an effective symbiotic N-fixing relationship. Closely related legume species can be grouped according to their N-fixing-bacteria compatibility into cross-inoculation groups. In some instances, these groups have been used to designate the scientific names for the N-fixing bacteria; e.g., bacteria that nodulate peas and vetches are classified as *R. leguminosarium,* clovers (white, red, crimson, and subterranean) are nodulated by *R. trifoli,* beans by *R. phaseoli,* alfalfa, black medic, and sweet clover by *R. meliloti,* lotus and lupine by *R. loti,* and soybeans by *Bradyrhizobium japonicum.* However, within some cross-inoculation groups only certain bacteria strains are compatible with the particular legume being grown. Therefore, a producer must select the specific strain (inoculum type) that is compatible with the legume being grown.

A major brand of N-fixing bacteria inoculum sold in Florida is the Nitragin® Brand produced by the Lipha Tech Company, Milwaukee, WI. A grouping of the major cross-inoculation groups and inoculum types for legumes commonly grown in Florida, using the Nitragin® Brand designation for inoculum type, is provided in Table 1. To ensure effective symbiotic N fixation, producers must be certain they match the proper inoculum type with the legume to be grown.

Inoculation of Legume Seeds

The soil is the natural habitat of N-fixing bacteria but too often our soils do not have either the proper kind of nodule-forming bacteria or enough of them to really bring about good legume growth. In nature, the vigorously nodulating N-fixing bacteria strains are usually found in soils where the particular legume species originated. When a legume is grown for the first time in a new area, it is crucial to ensure that the appropriate N-fixing bacteria strains are present in the soil. The physical process of applying specific N-fixing bacteria to seed or soil at or before planting is called **inoculation.** The purpose of inoculation of legume seed is to coat seeds with a sufficiently high number of viable N-fixing bacteria of the correct strain to provide early and effective nodulation of that legume in the field.

Effective Nodulation

Effective nodulation takes place within four weeks of planting. Nodule size and shape vary among the different legumes, with soybean and peanuts having larger, round nodules and clovers, alfalfa, and summer forage legumes having smaller, round or cylindrical nodules. Visual sampling of the number of nodules and interior nodule color can indicate the status of N fixation in a legume plant. Nitrogen fixation in legumes is highly correlated with the content of a reddish pink pigment inside the nodules called **leghemoglobin**. A cross section of a functional nodule made with a pocket knife should reveal a pink to dark-red coloration, whereas a greenish color may indicate ineffective nodulation. Effective nodulation is generally indicated by vigorous growth of the legume itself and by improved growth and green color of grasses associated with forage legumes.

Figure 1. White clover root nodules.

Commercial Inoculants

Commercial inoculants consist of finely ground peat mixed with the N-fixing bacteria which are intended for mixing with seed. Granular formulations of the peat-bacteria mixtures have also been introduced and are designed to be placed in the seed furrow at planting. By far, the ground, peat-based commercial inoculants are the most widely used. To purchase the proper inoculant, find the legume to be planted and then order the inoculant for that specific legume. Ensure that the package of inoculant is labeled with a serial number, the legumes for which the culture is to be used, the date of expiration, and storage method. Once purchased, it is important not to leave inoculant in trucks, in the sun, or exposed to heat. Instead, inoculant should be kept refrigerated to preserve the bacteria.

Other Requirements

There are several nutritional requirements for a nodulated legume. Small additions of N fertilizer to the soil at establishment may enhance nodulation of certain legumes, but in general, N-fixation decreases with increasing rates on N fertilizer. In the case of mixed grass-legume pastures, the addition of N fertilizer stimulates the grass component, which then suppresses the legume by increasing competition. The demand for P is high in the nodule in a number of tropical legumes. Hence the number and density of nodules may be stimulated by P fertilizer. Magnesium, more so than Ca, is important for N-fixing bacteria. Molbdenum, Fe, and B have been shown to be necessary for N fixation by legumes. Sandy soils with a pH <6.0 or soils high in Mn and Fe usually have low Mo availability. If Mo is a limiting factor, apply it as a seed treatment with inoculum. Some inoculants have Mo already incorporated.

The ability of N-fixing bacteria to persist in the absence of its host legume is crucial to successful establishment and persistence of legumes in pasture. Soil levels of Ca, P and K aid in N-fixing-bacteria survival. Inoculation can be beneficial to the establishment of effective N fixation on new seedlings of legumes in areas 1) where a legume of the cross-inoculation group has not been grown previously or 2) where N-fixing bacteria soil populations have been severely reduced by adverse soil conditions such as drought or soil acidity. If a particular legume has not been grown in a field for several years, inoculation of seed is generally recommended as “insurance” to ascertain maximum benefit from legume N fixation.

Recommendations

In using commercial inoculants, the following rules for successful inoculation should be observed:

1. Prepare a clean seedbed.
2. Inoculate in all cases when there is doubt as to whether bacteria of the proper strain are already present in the soil.
3. Purchase fresh inoculant for the legume variety you intend to plant and make sure to note the expiration date on the package and storage conditions in the store.
4. Store culture in a cool, dry place until ready for use.
5. For powdery inoculants, follow directions for each crop. Put a sticking agent such as pelgel, gum arabic, corn starch, or methyl cellulose on the seed, then add inoculant and

mix well with seed. Allow to dry under shade, never in direct sunlight. Only inoculate as much seed as you can plant in half a day. For the hot Florida conditions, apply at least twice the manufacturer's recommended inoculant rate to large-seeded legumes and four times the rate for small-seeded legumes.

6. Do not allow inoculated seeds to contact lime or fertilizer. Seed fungicides are also toxic to bacteria.

7. Plant inoculated seeds quickly, not later than 4 hours after inoculation, and cover with soil immediately. Pack soil with a roller or planter press wheel for row planting.

8. Apply granular inoculants in the seed furrow during planting at manufacturer's recommended rate.

Table 1. Cross-inoculation groups of field and forage legumes commonly grown in Florida and their specific inoculum-type requirements[1]

Cross-inoculation group	Inoculum type	Cross-inoculation group	Inoculum type
Clover group		**Lupine Group**	
Red clover	B	White lupine	H
White clover	B	Blue lupine	H
Ladino clover	B		
Ball clover	B	**Soybean Group**	
Alsike clover	B	Soybean	S
Crimson clover	R		
Berseem clover	R	**Bean Group**	
Persian clover	R	Garden bean	D
Strawberry clover	T	Kidney bean	D
Subterranean clover	WR	Pinto bean	D
Arrowleaf clover	O	Wax bean	D
		Scarlet runner bean	Phaseolus Spec.
Alfalfa Group			
Alfalfa	A	**Cowpea Group**	
Sweet clover	A	Aeschynomene	EL
Black medic	N	Alyceclover	EL
Bur clover	N	Carpon desmodium	EL
		Cowpea	EL
Vetch and Pea Group		Hairy indigo	EL
Hairy vetch	C	Kudzu	EL
Big flower vetch	C	Lespedeza	EL
Field pea	C	Partridge pea	EL
Austrian winter pea	C	Pigeon pea	EL
Rough pea	C	Peanut	EL
Common vetch	Vicia Spec.	Perennial peanut	EL
		Savanna stylo	EL
		Velvetbean	EL
		Vigna	EL

[1]Inoculum-type designation used in this table follow the designation of the Lipha Tech Company of Nitragin® Brand peat-based inoculum. This designation does not imply recommendation of this product over other similar product that may be available for sale, but rather is used as an aid to producers since this product is the one most commonly available in Florida.