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Natural resource Conservation Service
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Legume Seed Inoculation

Plant Materials Technical Note

Background

Legumes are broadleaved plants capable of fixing atmospheric nitrogen for their use via a symbiotic relationship with bacteria. These bacteria belong to the genus *Rhizobia*, and are commonly found in soil. *Rhizobia* infect the legume through root hairs and form nodules on the legume's root system. Fixation of atmospheric nitrogen occurs within these nodules through a complex biochemical process.

The number of nodules present on the root system is directly related the amount of nitrogen fixation that can occur; the greater the number of nodules, the greater the potential for nitrogen fixation.

Legumes are moderately specific as to which *Rhizobia* species they utilize. Furthermore, one should never assume the presence of a compatible *Rhizobia* species is in the soil prior to planting any legume. To insure the proper *Rhizobia* species is present, seed should be inoculated with the bacteria prior planting. These inoculants are relatively inexpensive and are available through most commercial sources selling legume seed. Properly inoculating legume seed with the proper *Rhizobia* is important in developing healthy, productive stands of legumes.

Purpose

This is a continuation of a series of technical notes aimed at assisting conservation planners in developing successful conservation plantings. Previous technical notes focused on proper seed bed preparation (TX-PM-10-07), planter calibration (TX-PM-10-04), sprayer calibration (TX-PM-11-05), management of conservation plantings (TX-PM-10-03), and calculating seeding rates (TX-PM-12-02. The purpose of this technical note is to provide guidance on methods of applying inoculants to seed, the proper storage of inoculants and pre-inoculated seed, and how to determine effective nodulation on legumes root systems. A table of commonly used legume species and their corresponding inoculants is also provided as reference for conservation planners.

Proper Handling of Inoculants and Seed

The *Rhizobi*a bacteria in commercial inoculants are living organisms. They should not be exposed to heat, direct sunlight, excessive moisture, or other adverse conditions. Each package should have an expiration date and be used by this date to insure maximum viability of the bacteria. It is easy to forget the small packages of inoculants contain living organisms and throw them on the dash board of a vehicle or lay them on a hot table top or metal planter lid while getting seed ready to plant. Such conditions can create excessive heat quickly and are detrimental to the *Rhizobia* in the inoculant. In order to insure the maximum effectiveness of the inoculant always follow the checklist below:

Inoculants come in pre-measured packets for specific seed quantities, and are ready to mix. Note its powdered graphite consistency of the inoculant seen between the packages.

1. Select the proper inoculant for the legume species.
2. Check the expiration date on the package before using inoculants to insure they are viable or will still be viable by the expected planting date.
3. Always store seed and inoculants in a cool dry place out of direct sun, some brands recommend refrigeration.

4. Do not mix inoculants with fertilizers or pesticides during planting, fertilizer salts and chemicals can kill the bacteria.
5. Graphite, lime, or other commercial, dry seed lubricants can be used to facilitate seed flow through the planter without harming the *Rhizobia*.
6. Some seed from commercial sources, particularly clovers, comes with a seed treatment which includes the inoculant. Always ask the supplier if the seed is pre-inoculated if the seed appears to be treated or have seed coating applied, and store as you would any inoculant.

Inoculating Seed

Most inoculants come as a very fine powder that looks similar to dry graphite lubricant, and are available through commercial seed dealers. This powder must adhere to the seed to be certain the *Rhizobia* is carried into the soil with the seed during planting. This is accomplished by using a sugary liquid solution as a sticking agent to moisten the seed and adhere the inoculant. The solution may be made by adding sugar to water, diluting syrups, or even using sugar containing soft drinks and sodas. Place the seed in a large, open topped tub or other similar container; a small concrete or soil mixer works well if available. While stirring the seed, mix in a cup or two of the sugar solution. The seed should be damp, but not saturated; no standing liquid or wet, clumped seed should be visible. Once the seed is mixed thoroughly with the solution, gradually add the inoculant powder while continuously mixing, approximately 6.5 ounces of inoculant per 100 pounds of seed. It is easiest to do small batches rather than large amounts of seed unless a mechanical mixer is available. Seed should be planted within 24 hours of being inoculated to insure the viability of the *Rhizobia*.

A small cement mixer is an efficient and effective way to mix inoculants and seed

Inoculant Selection

Selecting the right *Rhizobia* will help increase nodulation and nitrogen fixation. The following table provides a list of legumes commonly used in conservation and cropping systems and their corresponding inoculants. If the legume being used is not listed on this chart, check for one in the same genus and use the corresponding inoculant(s). Additional resources for legume inoculants may be found at the following web site:

http://stephenville.tamu.edu/topics/forages/forage-species/nitrogen-fixation/

Rhizobium Inoculants for Legume Species

Common Name	Scientific Name	Inoculant Code
Alfalfa	*Medicago sativa*	A
Acacia, prairie	*Acacia angustissima*	EL
Bundleflower, Illinois	*Desmanthus illinoensis*	EL
Bundleflower, prostrate	*Desmanthus virgatus*	EL
Bundleflower, velvet	*Desmanthus velutinus*	EL
Clover, alyce	*Alyiscarpus vaginalis*	EL
Clover, arrowleaf	*Trifolium vesiculosum*	O
Clover, ball	*Trifolium nigrescens*	B
Clover, berseem	*Trifolium alexandrinum*	R
Clover, bur	*Medicago arabica*	N
Clover, button	*Medicago orbicularis*	N
Clover, crimson	*Trifolium incarnatum*	R
Clover, Persian	*Trifolium resupinatum*	R
Clover, purple prairie	*Dalea purpurea*	F
Clover, red	*Trifolium pratense*	B
Clover, rose	*Trifolium hirtum*	WR
Clover, subterranean	*Trifolium subterraneum*	WR
Clover, sweet (yellow or white)	*Melilotus officinalis*	A
Clover, white	*Trifolium repens*	B
Cowpeas	*Vigna unguiculata*	EL
Guar	*Cyamopsis tetragonoloba*	GU
Jointvetch	*Aeschynomene sp.*	EL
Lablab	*Lablab purpureus*	EL
Lespedeza, bicolor (shrub)	*Lespedeza bicolor*	EL
Lespedeza, common	*Kummerowia striata*	EL
Lespedeza, kobe	*Kummerowia stipulacea*	EL
Lespedeza, sericea	*Lespedeza cuneata*	EL
Lespedeza, roundhead	*Lespedeza capitata*	EL
Lespedeza, thunberg (shrub)	*Lespedeza thunbergii*	EL
Lupines	*Lupinus* spp.	H
Medic, black, bur, button	*Medicago* spp.	N
Mimosa, herbaceous	*Mimosa strigillosa*	EL
Pea, Austrian winter	*Pisum sativum*	EL
Pea, partridge	*Chamaecrista fasciculata*	EL
Pea, singletary, caley	*Lathyrus hirsutus*	C
Peanut, Spanish	*Arachis* spp.	P
Sainfoin	*Onobrychis viciifolia*	F
Soybean	*Glycine max*	S
Soybean, wildlife	*Glycine soja*	S
Sunn Hemp	*Crotalaria juncea*	EL
Ticktrefoil	*Desmodium* spp.	EL
Trefoil, birdsfoot	*Lotus corniculatus*	K
Vetch, big flower	*Vicia grandiflora*	C
Vetch, deer pea	*Vicia ludoviciana*	C
Vetch, crown	*Securigera varia*	C
Vetch, hairy	*Vicia villosa*	C

Checking For Effective Nodulation

Approximately 30-40 days after planting is an ideal time to check for the formation of nitrogen fixing nodules on the roots. Dig the plant up being careful to keep the root system intact and not destroy the nodules. Gently shake loose soil from the roots, and then wash the roots in a bucket of water to clear away any remaining soil. Nodules will appear as swollen, knot like structures attached to the roots or along the tap root. They usually form in clusters. The locations and number of nodules is species dependent and varies between legumes. Nitrogen fixation occurs via biochemical process using the enzyme nitrogenase which acts as a catalyst, along with molybdenum, to start the nitrogen fixation process. A soil test to determine molybdenum levels should be performed prior to planting to ensure it is present in sufficient concentration to facilitate nitrogen fixation. Nitrogenase will not function in the presence of oxygen. A protein similar to hemoglobin in human blood, leghemoglobin, binds with atmospheric oxygen allowing nitrogenase to continue the nitrogen fixation process. The leghemoglobin, when bound with oxygen, turns red, similar to hemoglobin in human blood. This red color can be seen by cutting the nodule open and indicates the nodule is active with the potential to fix atmospheric nitrogen.

Nitrogen fixing nodules seen on a soybean root with nodules cut open to reveal red color indicating an active nodule
Photo Credit: Jennifer Dean,
http://phys.org/news158926862.html

References:

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