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Using the Appropriate Legume Inoculant for Conservation Plantings

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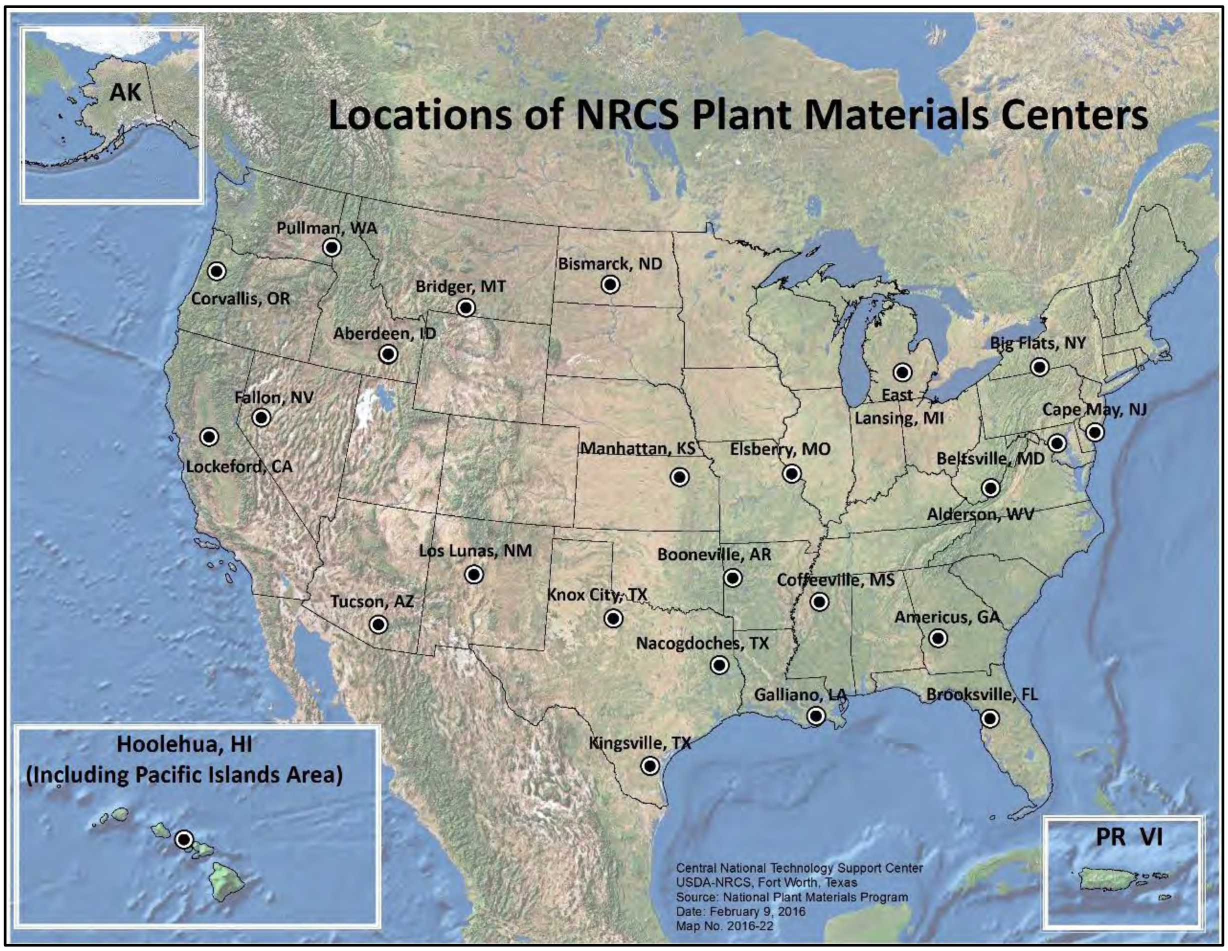
Preface

The U.S. Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) Plant Materials Program has been involved in the evaluation of conservation plants and planting technology for more than 80 years. This Technical Note explains the symbiotic relationship between legumes and rhizobial bacteria as well as the benefits of inoculating legume crops with rhizobia. It contains practical information on choosing the right inoculant for a legume crop or cover crop, considering field conditions prior to inoculating and planting, handling and applying the inoculant, and checking for successful nodulation of the crop after planting.

For additional information on specific species of plants mentioned in this publication, please see the USDA PLANTS database at https://plants.sc.egov.usda.gov/home or contact the nearest Plant Materials Center or plant materials specialist https://www.nrcs.usda.gov/wps/portal/nrcs/main/plantmaterials/contact/directory/ and/or the Land Grant Universities that serve the State. For specific information on soils and soil health, please see USDA NRCS soils website at http://www.nrcs.usda.gov/wps/portal/nrcs/site/soils/home/. Additional technical resources are on the National Plant Materials Program website at http://www.plant-materials.nrcs.usda.gov/.



Introduction

Plants of the legume family (Fabaceae) can form a symbiotic, or mutually beneficial, relationship with unique species of nitrogen-fixing bacteria called rhizobia (*Rhizobium, Bradyrhizobium,* and other species). When legume plants are colonized by rhizobia, small structures called nodules form on the plant roots. The rhizobia live inside these nodules where they convert or "fix" atmospheric nitrogen (N_2) to plant-available ammonia (NH_3). The host plant uses the fixed nitrogen and provides the rhizobia with nutrients in return. Fixed nitrogen can improve yields and increase protein content of the crop. It can also improve soil fertility for adjacent plants and subsequent crops.

Different legumes have evolved to have symbiotic partnerships with various species of rhizobia, so it is critical to make sure that each legume crop has the appropriate rhizobia present in the soil as root development begins (Snyder and Hankins, 1987). When a legume crop is planted on a field for the first time, there is a good chance that the right rhizobia species are not present in the soil. Unfortunately, no simple test exists to determine if rhizobia are already present in the soil. To guarantee the successful formation of nitrogen-fixing nodules, the legume seed must be inoculated with the appropriate rhizobial bacteria at planting.

Based on the relatively inexpensive cost of inoculants and the high cost of nitrogen fertilizers, the addition of rhizobial inoculants when seeding a legume species is a wise investment in nutrient management and crop production.

Benefits of Inoculation

There are many benefits of inoculating legume seed. These can include, but are not limited to:

Nitrogen-fixing nodules on the roots of field pea in a diverse cover crop mix. Photo by Derek Tilley.

- **Increased forage production.** Forage production may increase from 10 to 100% depending on specific soil conditions.

- **Increased crop yields.** Soils with average fertility may have yield increases from 15 to 25%.

- **Improved protein content.** Inoculated alfalfa has tested 3.5% higher for protein.

- **Increased soil nitrogen for subsequent crops.** Nitrogen added to the soil through fixation will vary depending on legume species, rhizobial population, amount of plant biomass removed (harvest with low residue vs. incorporation of biomass as green manure), soil nitrogen (N) and phosphorus (P) status, soil pH, moisture, and temperature.

Most of the nitrogen fixed by rhizobia goes to the above ground portion of the host plant and does not become available in the soil until the host plant dies and decomposes. However, plant roots can release some ammonium into the rhizosphere for uptake by adjacent non-legume plants (Walley et al., 1996). Adjacent plants also benefit from the nitrogen released into the environment when root nodules die and break down during the growing season (Schwartz, 2014). If legume plants are harvested with low residue, very little fixed nitrogen may be left for subsequent crops (Schwartz, 2014). Table 1 lists nitrogen additions to the soil by some commonly used legumes. Sometimes this added soil nitrogen is expressed as N credits for the subsequent crop.

Table 1. Pounds of nitrogen added to the soil per acre per year by legumes under optimum conditions.

Legume	lb N/ac/yr	Reference
Alfalfa	40-100	Brown et al., 2010; Killpack and Buchholz, 1993
Clover, Crimson	70-130	Clark, 2007
Clover, Red	70-150	Clark, 2007
Pea, field	90-150	Clark, 2007
Soybean	15-60	Killpack and Buchholz, 1993
Sweetclover	90-170	Clark, 2007
Vetch, Hairy	60-120	Undersander et al., 2015
Vetch, Woollypod	100-250	Clark, 2007

Choosing the Right Inoculant

Inoculate legumes with the appropriate rhizobial inoculant for each legume species planted. Using the correct inoculant will maximize nitrogen fixation. In the past, various tables containing "cross-inoculation groups" of legumes and rhizobia were published, sometimes with conflicting information. More recent research has shown that the relationship between rhizobial species and legume species is not 1:1 and there is overlap between the groups (Schwartz, 2014).

Seed suppliers should provide the correct inoculant for each type of legume seed they sell. Consult with the seed supplier and read manufacturer labels in order to select the most appropriate inoculant for each legume crop. If purchasing inoculant separately, check the product package label and company literature for the name of the legume(s) the product is intended to treat.

Table 2 provides a list of rhizobia commonly found in commercially available inoculant and the legumes they can inoculate. This table is not intended to list every rhizobial species that can have symbiosis with the legumes listed. However, Table 2 may be useful for situations where the desired legume is not listed on any inoculant product labels or company literature, or to check if an "all-purpose" inoculant contains suitable rhizobia for the intended legume crop or cover crop mix.

Field Conditions

Re-inoculation is recommended when the legume species has not been grown in a crop field in the past three years. In high-value crops, it is probably prudent to inoculate at every planting to avoid unnecessary yield losses.

Soil temperature and soil pH can affect the survival and ultimate effectiveness of inoculants. Most rhizobia cannot live in soils with a pH less than 5.0 and do best at a pH of 5.5 or higher. Lime acidic soils to achieve appropriate pH prior to planting inoculated legume seed. High soil pH and/or salinity can also prevent effective legume nodulation. However, as soil pH and salinity are improved, legume nodulation is expected to increase unless there are other limiting factors.

Vetch in a diverse cover crop mix. Note the absence of nitrogen-fixing nodules on roots. Photo by Marlon Winger.

Soil testing prior to planting an inoculated legume crop is important. Nitrogen fixation requires adequate levels of P, potassium (K), sulfur (S), and micronutrients (Flynn and Idowu, 2015). If soil tests indicate high N or if nitrogen fertilizer has recently been applied, be aware that high levels of nitrate can reduce or inhibit rhizobial colonization and nodulation. If soil test N is very low, legume crops may benefit from a small amount of starter N before they begin fixation. While some legume crops (e.g. common beans) require supplemental N, other legume crops (e.g. alfalfa) can supply most of their N needs through fixation if nodulation is successful. High soil N is acceptable when planting a cover crop mix containing legumes because the plants will use up excess soil N even though the legumes may not have many nodules or fix much nitrogen.

Soil moisture is very important for successful inoculation and nodulation. Do not plant inoculated seed into dry soil unless irrigation is planned.

Inoculant Handling and Storage

Because inoculants are cultures of living organisms, they are perishable and have a short shelf life. They should be transported and stored according to the manufacturer's temperature recommendations and never used past their expiration date. Contact suppliers well in advance of planned seeding dates to ensure the availability of viable inoculant.

Never mix the inoculant with fertilizer or pesticide unless specifically labeled for that use. Avoid contact with substances that are acidic or basic. Agricultural lime or sulfur should be applied to the field well in advance of planting inoculated seed.

Heat and sunlight can kill rhizobial bacteria. Do not allow the inoculant to be exposed to direct sunlight or heat when transporting. Mix the seed and inoculant in a shady location out of direct sunlight. Plant seed within 24 hours after mixing with inoculant; after this period, it is best to re-apply inoculant to the seed.

Inoculation Procedures

Always follow the manufacturer's recommendations regarding the type of inoculant to use and the amount to be added to the legume seed. The inoculant package label will specify which legume species the product is intended to treat. Due to the different formulations and carriers used in developing inoculants, each manufacturer will have a different requirement for the amount needed.

Inoculants are available in dry, granular, or liquid formulations. Dry inoculants require a sticking agent such as commercial "sticker" products (follow label directions) or 10% solutions of powdered milk, corn syrup, molasses, or sugar in water. Soft drinks like soda are sometimes used, but these are very acidic (pH 2-4) (Reddy et al., 2016) and can potentially harm the rhizobia.

Inoculants can be mixed and applied to the seed by one of the following methods:

Cover crop seed inoculated in a cement mixer using the slurry method. Photo by Mary Wolf.

1. **Pre-inoculated Seed –** Inoculant is applied to the seed prior to sale to the producer. Seed inoculated in this manner must be stored under cool conditions until planting to ensure viability of the rhizobial bacteria.

2. **Slurry Method –** Seed is mixed in a portable cement mixer or tub along with a slurry of dry inoculant and water or sticking agents. Liquid inoculant is also used in this manner. The amount of inoculant and liquid used per bag of seed will depend on the type of dry inoculant used. Amounts are usually listed on the inoculant package; however, a general guideline is 8 to 10 oz of fluid per 50 lb of seed. Allow the coated seed to dry in a cool, shady area before transferring to the planter box. Generally, the amount of liquid used is small enough that the drying process takes a short time, especially if the seed is spread out on a tarp. The slurry method is the most effective of the in-field inoculation methods. Make sure that all treated seed is removed from the planter boxes at the end of the day.

3. **Sprinkle Method –** Seed is sprinkled with dry or granular inoculant and mixed in a cement mixer, tub or directly in the planter box. Follow manufacturer instructions regarding how much inoculant to apply per bag of seed. This application method is not as effective as the slurry method because the inoculant does not adhere to the seed as well without liquid. Again, remove all seed from the planter boxes at the end of the day.

Checking for Nodulation

Not all root nodules fix nitrogen. Other soil bacteria that form root nodules may be present and can give the impression that nitrogen fixation is occurring when it is not. To be sure, cut open a few nodules and observe the color. Approximately 30-40 days after planting is a good time to check, although small nodules may be visible within a few weeks. A reddish or pink color indicates that nitrogen is being fixed. This color is due to the presence of leghemoglobin (a protein similar to hemoglobin in blood), which keeps oxygen away from the nitrogen fixation reaction. White, brown or green nodules are not fixing nitrogen.

Active root nodules on vetch roots. Note the reddish pink color. Photo by Derek Tilley.

Table 2. Rhizobial species commonly found in commercially available inoculants and the legume species with which they form symbiotic relationships.

Rhizobial Species	Legume Common Name	Legume Species
Bradyrhizobium japonicum	soybean	*Glycine max*
Bradyrhizobium lupinus	lupines	*Lupinus* spp.
Bradyrhizobium spp. and *Rhizobium* spp.	cluster bean (guar)	*Cyamopsis tetragonoloba*
Mesorhizobium cicer	chickpea	*Cicer arietinum*
Mesorhizobium loti	trefoil, birdsfoot	*Lotus corniculatus*
	trefoil, big	*Lotus pedunculatus*
Mesorhizobium loti, Rhizobium leguminosarum bv. *viceae*	vetch, crown	*Securigera varia*
Rhizobium leguminosarum bv. *phaseoli*	bean	*Phaseolus* spp.
Rhizobium leguminosarum bv. *trifolii*	clover, ball	*Trifolium nigrescens*
	clover, berseem	*T. alexandrinum*
	clover, crimson	*T. incarnatum*
	clover, kura	*T. ambiguum*
	clover, red	*T. pratense*
	clover, white	*T. repens*

Rhizobial Species	Legume Common Name	Legume Species
Rhizobium leguminosarum bv. *trifolii* (subterranean)	clover, subterranean	*T. subterraneum*
Rhizobium leguminosarum bv. *trifolii* (vesiculosum)	clover, arrowleaf	*T. vesiculosum*
Rhizobium leguminosarum bv. *viceae*	caley pea	*Lathyrus hirsutus*
	fava bean	*Vicia faba*
	lentil	*Lens culinaris*
	pea: winter, field	*Pisum sativum*
	vetch, common	*Vicia sativa*
	vetch, hairy	*Vicia villosa*
	vetch, chickling	*Lathyrus sativus*
Rhizobium spp. (cowpea)	acacia, prairie	*Acacia angustissima*
	bundleflower	*Desmanthus* spp.
	cowpea	*Vigna unguiculata*
	lablab	*Lablab purpureus*
	lespedeza	*Lespedeza* spp.
	mimosa, herbaceous	*Mimosa strigillosa*
	mung bean	*Vigna radiata*
	partridge pea	*Chamaecrista fasciculata*
	peanut	*Arachis hypogaea*
	pigeon pea	*Cajanus cajan*
	sunn hemp	*Crotalaria juncea*
	tick-trefoil	*Desmodium* spp.
Rhizobium spp. (cowpea); *Azorhizobium calinodans*	sesbania	*Sesbania* spp.
Rhizobium spp. (sainfoin)	sainfoin	*Onobrychis viciifolia*
	clover, purple prairie	*Dalea purpurea*
Sinorhizobium meliloti	alfalfa	*Medicago sativa*
	clover, sweet	*Melilotus officinalis*
	fenugreek	*Trigonella foenum-graecum*
Sinorhizobium meliloti, Sinorhizobium medicae	medic: black, burr, button	*Medicago* spp.

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