Inoculating Legumes

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Nitrogen is an essential element in living things. Proteins, nucleic acids, and amino acids contain nitrogen. Nitrogen is therefore is an essential element for plant growth and is often limited in soils. The atmosphere contains almost 80 percent nitrogen, which occurs as a stable, chemically un-reactive gas unusable by plants. Nitrogen can be provided to enhance the productivity of plant communities by applying fertilizer. Nitrogen fertilizers are expensive and can have potentially detrimental environmental impacts if over used or applied at the wrong time.

Alternatively, nitrogen can be supplied through a process called *nitrogen fixation* by growing legumes. Legumes form a symbiotic relationship with certain species and strains of soil bacterium (*Rhizobium*) that convert atmospheric nitrogen to the chemical form ($NH4^+$) usable by plants. When the young legume roots become *inoculated* or infected, the bacteria penetrate into the cortical cells of the root hairs causing the formation of nodules around the site of infection where the bacteria multiply. The term “infection” often refers to a harmful effect of bacteria. In the case of *Rhizobia* inoculation or infection the effect is beneficial.

Photo Credit: Minnesota Agricultural Experiment Station

The host plant supplies energy to the bacteria via photosynthate and the bacteria supply usable nitrogen to the host plant. The fixed nitrogen supplies most if not all the needed nitrogen for the growing legumes. With perennial legumes some of the nitrogen becomes available for other nearby plants and vegetation. Nitrogen fixation is an important biological function that can be utilized in natural resource conservation. Important applications include:

1. Providing nitrogen in nitrogen-poor environments, such as degraded soil material typically encountered in critical area sites.
2. Providing nitrogen to sustain diverse grassland communities.
3. To enhance protein content and productivity of pasture and hay.
4. To provide nitrogen in annual cropping system.

The value of the nitrogen fixed depends on factors such as species, quality of the legume stand, growing conditions, degree of nodulation, and price of nitrogen (Table 1).

Table 1. Value of fixed nitrogen.

Crop	N fixed, lb/A/year	N value, dollars/acre		
		0.25/lb-N	0.35/lb-N	.045/lb-N
Alfalfa	150-250	38-63	53-88	68-113
Red Clover	70-200	19-50	26-70	34-90
White Clover	75-150	19-38	26-53	34-68
Vetch, lespedeza, other forage legumes	50-150	13-38	18-53	23-68
Source: Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2002. Southern Forages. Potash and Phosphate Institute and Foundation for Agronomic Research, Norcross, Georgia.				

Maximum nitrogen fixation will occur only if certain conditions are present. The specific strains and species of nitrogen fixing bacteria must be present in the soil for the legumes grown. Poorly nodulated legumes grow less optimally and fix less nitrogen (Table 2). Fields that have a history of growing certain legumes generally have enough bacteria present for inoculation and nitrogen fixation to occur. Disturbed soils, fields with no previous history of growing a particular legume, and sandy, low organic matter soils likely do not have enough of the specific strain of bacteria present to ensure adequate nodulation.

Because the *Rhizobia* bacteria do not thrive in acid environments, the soil pH must be above 6.0. The legume roots must be actively growing; therefore, optimum soil moisture and soil phosphorus is critical. An abundance of available nitrogen in the soil will interfere with nitrogen fixation as plants expend less energy absorbing available soil nitrogen compared to acquiring nitrogen symbiotically.

Photo Credit: Jennifer Dean

Nitrogen fixation can be determined in the field by splitting and inspecting the root nodules. Active nodules are pink to red in color. The most definitive time to inspect the roots is two to three weeks after emergence or two weeks after harvesting or closely grazing of perennial legume forages.

Table 2. Dry matter production and nitrogen content of two hairy vetch varieties.

Legume	Dry Matter (lbs/ac)	Actual Total Nitrogen (lbs/ac)	Percent Nitrogen
Pennington Vp-07 Hairy Vetch (Not Inoculated)	2658.5	86.9	3.27
Pennington Vp-07 Hairy Vetch (Inoculated)	4631.0	144.9	3.13
Pennington Vp-08 Hairy Vetch (Not Inoculated)	3001.6	117.1	3.90
Pennington Vp-08 Hairy Vetch (Inoculated)	4888.2	180.4	3.69
Source: Terry Taylor and Mike Plumer, University of Illinois, "Utilizing Legume Winter Cover Crops". http://web.extension.uiuc.edu/carbondalecenter/downloads/18791.pdf			

There are no simple tests to reliably determine if the desired strains of bacteria are present in the soil prior to planting. Inoculating legume seeds prior to planting is the only way to ensure the proper bacteria will be present and come in contact with the legume roots. Fortunately, identified species and strains of bacteria have been isolated for specific native and introduced legumes and are commercially available (Table 3 and 4). Commercial inoculants are available in granule, liquid, or peat based formulations, and pre-inoculated seeds. The bacteria on pre-inoculated seeds that are coated will remain viable for as long as 12 months if stored under cool, dry conditions. Peat based formulations are most common for introduced and native legumes. The products contain live bacteria and are applied directly to the seed or to the soil with the seed.

Table 3. Rizobia species and cross inoculation groups for introduced legumes.

Cross-Inoculation Group	Legumes Included	Rhizobium Species
Alfalfa	Alfalfa Black medic Burr clover (medic) Button clover (medic) Sweetclovers (yellow and white)	*Rhizobium meliloti*
Bean	Beans	*Rhizobium phaseoli*
Clover I	Berseem clover Crimson clover Lappa clover Persian clover Rose clover	*Rhizobium trifolii* strain
Clover II	Rose clover Subterranean clover	*Rhizobium trifolii* strain
Clover III	Alsike clover Ball clover Hop clover Ladino clover Red clover White clover	*Rhizobium trifolii* strain
Clover IV	Arrowleaf clover*	*Rhizobium trifolii* strain
Lupine	Lupines	*Rhizobium lupini*
Pea	Caleypea Garden peas Lentils Vetches Winter peas	*Rhizobium leguminosarum*
Soybean	Soybeans	*Bradyrhizobium japonicum* strain
Cowpea	Alyce clover Cowpeas Lespedeza Lima bean Peanut Kudzu	*Bradyrhizobium japonicum* strain
Trefoil	Birdsfoot trefoil	*Rhizobium loti*
*Note that Arrowleaf clover requires specific inoculum. Arrowleaf will not cross-inoculate with other clovers		
Source: Jennings, John. *Forage Legume Inoculation.*, University of Arkansas, Cooperative Extension Service. http://www.uaex.edu/Other_Areas/publications/pdf/FSA-2035.pdf		

Table 4. Rhizobia species for selected native legumes.

Legume Species	Rhizobia species
Leadplant, *Amorpha canescens*	*Mezorhizobium amorphae*
Partridge pea, *Chameacrista fasciculata*	*Bradyrhizobium spp.*
Purple Prairie Clover, *Dalea purpurea*	*Rhizobium spp. (Dalea)*
White Prairie Clover, *Dalea candida*	*Rhizobium spp. (Dalea)*
Showy Tick Trefoil, *Desmodium canadense*	*Bradyrhizobium spp. (Desmodium)*
Roundhead Lespedeza, *Lepedeza capitatum*	*Bradyrhizobium spp. (Lespedeza)*
Milk Vetch, *Astragalus canadensis*	*Mesorhizobium huakuii*

Inoculation Procedure for 25 Pounds of Seed

Legume seed coats often are slick and smooth which prevent the inoculum from adhering to the seed to sufficiently coat the seeds with enough rhizobia to ensure adequate inoculation. Commercial sticking agents are available or a sticking agent can be prepared by mixing 1 cup of sugar with 2 cups of water. Do not use soft drinks as a sticking agent as their pH is near 2.0 and will harm the rhizobia.

1. Mix 2 ounces of a 6 ounce bag of fresh inoculant to about one cup of sticking agent to form a slurry. Follow manufacturer's instructions if a commercial sticking agent is used.

2. Place 25 pounds of seed in a tub or similar container.

3. Pour about 6-7 ounces onto the seed and mix thoroughly to coat all of the seed.

4. Mix the remaining inoculant into the tub to coat and dry the seeds. Allow the seeds to dry before planting.

5. Plant inoculated seed immediately if mixed with dry fertilizer. Do not store inoculated seed in direct sunlight.

Inoculum Suppliers

Philom Bios/Novozymes 3935 Thatcher Avenue Saskatoon, SK Canada S7R1A3 Phone: 888.744.5662 Fax: 306.975.1215 USA Representative Dorn Severtson Phone: 952.361.3495 Cell: 952.913.3941	Becker Underwood, Inc. 801 Dayton Avenue Ames, IA 50010 Phone: 800.232.5907 FAX: 515.232.5961
Nitragin/EMD Crop Bioscience 13100 West Lisbon Avenue Suite 600 Brookfield, WI 53005 Phone: 262.957.2000 Fax: 262.957.2121	INTX Microbials, LLC 200 W. Seymour P.O. Box 62 Kentland, IN 47951 Phone: 219.474.5510 FAX: 219.474.3700
CelPril – Bayer Crop Sciences 251 Oak Street Manteca, CA 95337 Phone: 209.823.1738 FAX: 209.823.8855	Plant Probotics 6835 Lindel Court Indianapolis, IN 46268 Phone: 317.329.7731 (native legumes)

Note: An attempt has been made to include the *Rhizobia* manufacturers and contact information that supply Illinois. Notify Brett Roberts at brett.roberts@il.usda.gov with necessary information if any manufacturers were omitted. Omissions were unintentional.

References

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