Benefits of Inoculating Legume Crops with Rhizobia in the Northern Great Plains*

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Abstract
Inoculation of forage and grain legumes with rhizobia is an important process to maximize biological N_2 fixation capacity in these crops. Inoculation has the potential of increasing dry matter yield, N yield, and residual N levels. However, yield responses to inoculation are not universal in the region. In fields that have previously grown the same grain legume crop (i.e. contain an endemic rhizobial population in the soil), positive yield responses occur from one-third to one-half of the time. Yield responses to inoculation are dependent upon many factors, but legume species and soil N levels prior to seeding are two important factors. However, given the modest cost of inoculation compared to the potential agronomic benefits, producers are well advised to seriously consider inoculation of their legume crops in all circumstances.

Introduction
Biological N_2 fixation by legume-rhizobia symbioses is vitally important as an N input in agroecosystems in the northern Great Plains of North America. Inoculation of grain and forage seed at planting is generally recommended to maximize the potential for nodulation and N_2 fixation in these crops. However, producers in this region commonly raise questions on legume inoculation. What benefits can we expect from inoculating grain and forage legumes with rhizobia? Do we always need to inoculate legume crops, even if a field had previously grown the same inoculated crop? Can we depend upon inoculation to meet all the N requirements of the legume crop?

The objective of this article is to provide background information on the inoculation of legume crops and to review the benefits of inoculation of legume crops with rhizobia in the northern Great Plains (i.e. the northern United States from Montana to Minnesota and the southern regions of the three Canadian prairie provinces, Alberta, Saskatchewan and Manitoba). The primary focus of the review is to address the question of whether producers always need or should inoculate their legume crops. Comparisons of inoculant formulation (peat-based, liquid, or granular; e.g. 17) and its placement (seed treatment or seed row placement; e.g. 23) are not addressed. The review focuses on responses of grain legumes, although inoculation of forage legumes is also briefly addressed.

Benefits of Rhizobial Inoculation
Bacterial species within the genera *Allorhizobium*, *Azorhizobium*, *Bradyrhzobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (commonly referred to as rhizobia) can infect, nodulate and symbiotically fix N_2 in legumes. However, legumes express ‘host specificity’, meaning that only certain species or subspecies (i.e. biovars) of rhizobia will infect certain species of legume (Table 1). For example, pea is infected by *Rhizobum leguminosarum* biovar *viciae*, while common bean is infected by *Rhizobium leguminosarum* biovar *phaseoli*. Sometimes different legume crops (e.g. faba bean, lentil and pea) can be infected by the same species or biovar of *Rhizobium*, however, even within these crops often certain strains of rhizobia are more effective in one crop compared to another (8). Commercial rhizobial inoculants have been available for legume crops since the 1890's (12). Currently, a wide variety of commercial legume inoculants are available for use in the northern Great Plains (Table 2).

Producers inoculate their legume crops to increase yields and decrease the input costs of N fertilizer. Yield responses to inoculation, even in fields with soil rhizobial populations sufficient to infect the particular legume host, are common (but not universal) in field trials in the northern Great Plains (Table

3). However, even where yield responses are not evident, inoculation may still have benefits by increasing seed N levels and N levels in plant residues (e.g. 22). Economic premiums are paid for higher N content in legume grains in some markets (e.g. Ag Processing Inc., Omaha, NE) and higher seed/tissue N content is important when grain or forage is being used in livestock rations. Likewise, although higher residual N levels in soil and stubble may not be an immediate benefit to producers, residual N is an important component of the beneficial rotational effect of legumes in cropping systems (for review, see 28).

From Table 3, one can see that inoculation of grain legumes in soils already containing the appropriate rhizobia to infect the seeded legume crop will result in a significant yield response commonly one-third to one-half of the time, but sometimes much higher with some species (e.g. chickpea). There are multiple reasons for a lack of response to inoculation. Probably the most common reason is lack of non-N limiting conditions due to high mineral N levels in the soil (see Comments in Table 3). Alternatively, other factors (e.g. drought stress, hypoxia, phosphorus limitations, disease) maybe more limiting to growth than N (e.g. 1, 2, 4, 25). Finally, it is possible that the endemic soil population of rhizobia is sufficient in concentration and effectiveness to maximize nodulation and N_2 fixation in the grain legume (see below).

Endemic populations of soil rhizobia may arise from two sources, native strains and naturalized strains. Native populations of rhizobia are indigenous strains which nodulated wild legumes and which can also infect introduced legume crops (e.g. *R. leguminosarum* bv. *viciae* strains which nodulate wild *Vicia* and *Lathyrus* species can also infect fababean, lentil and pea). Naturalized populations of rhizobia are strains that originate from previously grown legume crops treated with commercial inoculant.

One may well ask if rhizobial inoculation of legumes in the northern Great Plains will make the crops self-sufficient for N, or if supplemental N is required to maximize yield. This is very much dependent on the legume in question. Table 4 cites studies which assessed yield responses to N fertilizer additions in inoculated pea crops. In each of these studies, non-N_2-fixing control treatments were shown to be responsive to fertilizer-N additions. The data in Table 4 indicate that in most cases inoculation of pea will result in a non-limiting amount of N for the crop. However, this observation cannot be extrapolated to all other legume species. Clearly this is not the case for common bean. Common bean is recognized around the world as being one of the poorer grain legumes for N_2 fixation (13). In the northern Great Plains, yield responses by inoculated common bean to N fertilizer are common (e.g. 21). As a result, inoculation of common bean is recommended in the northern Great Plains, but normally with the addition of supplemental N fertilizer (e.g. 11). Likewise, given its less than stellar levels of N_2 fixation, it is not surprising that of all the species listed in Table 3, common bean is the least responsive to inoculation. Unfortunately, there are not enough N fertilization response studies at sites shown to be responsive to N-fertilizer to conclude whether inoculation of the other major grain legumes (i.e. soybean, lentil, chickpea) on the northern Great Plains is sufficient to meet all of their N requirements.

Do We Always Need to Inoculate Legumes?

A very common question from producers is whether they always need to inoculate legumes at seeding, especially if they grew the same inoculated legume in a field in the recent past. There are two situations where producers are well advised to always inoculate: in "virgin" soils, and for biennial/perennial forage legumes.

Fields which have never been inoculated with the particular type of rhizobia required for a particular legume crop (i.e. taking host specificity into account) are sometimes referred to as "virgin" soils. There is clear evidence that if a particular legume crop is being planted for the first time in a field which has never grown an inoculated legume crop of the same inoculation group, a crop can significantly respond to inoculation (e.g. 3, 22). This is despite the fact that for some crops native strains of rhizobia may exist in the soil. For example, Chemining'wa (5) found that pea had dramatic growth responses to inoculation (up to 6 fold) at a native prairie site which were known to contain strains which inoculated wild *Lathyrus* and *Vicia* species. He also found that the DNA-plasmid profiles of rhizobia infecting *Lathyrus* species close to pea fields were genetically very different than the strains of rhizobia within the field.

Another situation where inoculation is clearly indicated is in the seeding of biennial/perennial forage legume crops (e.g. alfalfa, sweet clover). One could say that these crops are grown to produce nitrogen. These crops have very long growth periods (compared to annual crops at these northern climes) and great dry matter production potential, and consequently huge N demand. Peoples et al. (24) showed a good correlation between dry matter production and N_2 fixation capacity of a variety of legumes across a variety of environments. Forage legumes have a huge capacity to fix N_2 in the northern Great Plains (e.g. up to 416 lb N $acre^{-1}$ yr^{-1} for alfalfa; 15). Even if high levels of mineral N are available in the soil, alfalfa has the ability to fix significant amounts of N_2 (20).

If not seeding into virgin soil or planting a multi-year forage legume, should producers always inoculate legume grain crops? Table 3 suggests that even in soils that have a rhizobial population which will nodulate a crop, yield responses to inoculation are common for most grain legumes. Why? These yield responses to inoculation will occur if the endemic rhizobial population in the soil is either not high enough or not effective enough to maximize nodulation and N_2 fixation. Kucey and Hynes (16) followed pea and bean rhizobia population levels in soil for up to 5 years after these crops. Although they found that the rhizobia populations continued to exists, their concentrations generally decline with time (by up to two orders of magnitude). The reason for the declines in rhizobia concentrations in the soil are not clear. The declines may have been due to the introduced strains not being competitive as other free-living bacteria in the soil, or that more frequent growth of the legume host crop is necessary to maintain concentrations of any rhizobia strains, regardless of their competitiveness. Chemining'wa (5) found pea rhizobia in fields that had not grown inoculated pea crops for up to 25 years. However, using DNA fingerprinting techniques, he found a high degree of genetic diversity in both plasmid and chromosomal DNA of pea rhizobia populations within and among 20 field sites in southern Manitoba. This high degree of genetic diversity, and lack of genetic similarity to commercial inoculant strains previously used in these field suggests, a rapid rate of evolution in soil rhizobial populations. These genetic changes in soil rhizobia are no doubt in response to selection for a saprophytic (i.e. free-living in the soil) rather than a symbiotic (i.e. within the legume) existence. Inoculant companies make substantial efforts to select and maintain efficient strains of rhizobia in their inoculants. The genetic changes that occur in endemic soil populations may explain why dependence upon native/naturalized rhizobia often results in lower yields compared to the use of commercial inoculants (Table 3).

Conclusions

To maximize the agronomic benefits of legume crops on the northern Great Plains, producers are well advised to always inoculate with rhizobia when planting a crop in a field for the first time (i.e. “virgin” sites) or for forage legumes. In cases where a field is known to have an endemic population of rhizobia in the soil, producers can still expect significant yield responses to inoculation of grain legumes approximately one-third to one-half of the time. The most common reason for lack of a yield response to proper inoculation of grain legumes is high residual N levels in the soil prior to seeding. However, given the modest cost of inoculation (currently approximately \$2 to \$3 $acre^{-1}$) compared to the potential benefits in yield and N inputs to a cropping system, producers are well advised to seriously consider inoculation of their legume crops in all circumstances.

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Table 1. Forage and grain legumes common to the northern Great Plains and rhizobia taxa which infect them.

Common name	**Latin name**	**Rhizobia species or biovars (bv.)**
Alfalfa	*Medicago sativa* L.	*Sinorhizobium meliloti*
Chickpea	*Cicer arietinum* L.	*Mesorhizobium ciceri*
Common bean	*Phaseolus vulgaris* L.	*Rhizobium etli* *Rhizobium leguminosarum* bv. *phaseoli* *Rhizobium tropici*
Faba bean	*Vicia faba* L.	*Rhizobium leguminosarum* bv. *viciae*
Lentil	*Lens culinaris* Medik.	*Rhizobium leguminosarum* bv. *viciae*
Pea	*Pisum sativum* L.	*Rhizobium leguminosarum* bv. *viciae*
Red clover	*Trifolium pratense* L.	*Rhizobium leguminosarum* bv. *trifolii*
Soybean	*Glycine max* (L.) Merr.	*Bradyrhizobium elkanni* *Bradyrhizobium japonicum* *Sinorhizobium fredii*
Yellow sweet clover	*Melilotus officinalis* (L.) Lam.	*Sinorhizobium meliloti*

Table 2. List of rhizobial inoculant companies active in the northern Great Plains*.

Company	Location	Product type(s)	Trade name(s)
Agribiotics Inc.	Cambridge, ON	Peat-based with sticker Liquid Frozen concentrate	PulseR Apex Power-Pack
Becker Underwood (Canada)(formerly MicroBio RhizoGen Corp)	Saskatoon, SK	Peat-based with sticker Liquid Granular	SelfStik** BioRhiz** Nodulator**
Becker Underwood (USA)(formerly Urbana Laboratories)	Saint Joseph, MO	Peat-based with sticker Clay-based Liquid Liquid Polymer Granular Frozen concentrate	Rhizo-Stick/HiStick Dormal Rhizo-Fix/ NOD + DryCoat/HiCoat Rhizo-Flo Frozen - Prep XP
Nitragin Inc. (formerly Liphatech Inc.)	Brookfield, WI	Peat-based Peat-based with sticker Liquid Granular	Nitragin Powder NitraStik Cell-Tech Soil Implant +
Philom Bios	Saskatoon, SK	Peat-based with sticker Peat-based with sticker or granular and containing *P. bilaiae*	N-Prove Tag Team

*List may not be comprehensive. Appearance of companies and products listed here are for information only and are not recommendations by the author.
**Also available in USA through Becker Underwood USA.

Table 3. Details of studies investigating yield responses of grain legumes to commercial rhizobial inoculation in soils containing native or naturalized populations of rhizobia available to infect the host crop.

Crop	**Location***	**Site years****	**Comments**	**Reference**
Chickpea	SK	6 of 6	Three inoculant formulations	18
	AB	2 of 2	Plots were irrigated	26
Common bean	AB	0 of 9	Yield responses to N fertilizer at 7 of 9 sites	21
	SK	4 of 4	Positive in four cultivars at each site	23
Pea	SK	6 or 18	Dryland conditions; no responses on two high NO_3-N sites (> 80 lb $acre^{-1}$)	2
	MB	2 of 10	Positive responses on the two sites with lowest preplanting NO_3-N	5
	SK & AB	1 of 4	Low NO_3-N sites	14
	SK	5 of 14	Granular generally superior to liquid	17
	AB	3 of 8	Sites previously grew pea	22
	AB	4 of 4	Plots were irrigated	26
Faba bean	SK	2 or 12	Dryland conditions; no responses on two high NO_3-N sites (> 80 lb $acre^{-1}$)	2
	MB	3 of 5	Elevated inoculant rates did not increase responses	7
	MB	2 of 3	Pea rhizobia not as effective as fababean rhizobia	8
	MB	1 of 3	No response at two high NO_3-N sites	9
Lentil	SK	7 of 18	Dryland conditions; no responses on two high NO_3-N sites (> 80 lb $acre^{-1}$)	2
	SK & AB SK & AB	7 of 8 7 of 8	Low NO_3-N sites Low NO_3-N sites	14 Hynes et
	AB	2 of 4	Plots were irrigated	26
Soybean	MB	1 of 2	Higher NO_3-N at non-responsive site	3
	MN	2 of 12	Mean of 71 lb NO_3-N $acre^{-1}$	19
	MN MN	11 of 12	Despite > 45 lb NO_3-N $acre^{-1}$	30 Wiersma

*AB = Alberta; MB = Manitoba; MN = Minnesota; SK = Saskatchewan
**Number of site years with positive yield responses of total number of site years

Table 4. Yield responses of pea to N fertilizer additions at sites where a non-fixing control was responsive to N fertilizer.

Location*	**Cropping System**	**N rate (lb acre^{-1})**	**Yield response to N addition**	**Reference**
SK	Monocrop	0, 95	positive	27
SK	Monocrop	9, 27, 45	nil	6
SK	Intercrop	9, 27, 45	nil	6
MB	Monocrop	9, 27, 45, 80	nil	29
MB	Intercrop	9, 27, 45, 80	nil	29
ND	Monocrop	20, 50	nil/positive**	10

* MB = Manitoba; ND = North Dakota; SK = Saskatchewan
**Positive response at only the higher N rate with 1 of 2 inoculant sources.