Maximizing nitrogen fixation between rhizobia and legume crops AGVISE Soil Fertility Seminars January 2024





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Synthetic fertilizers are feeding the world





• Often the main economic input for farmers

Synthetic fertilizer

- Use may be restricted in future?
- 30% reduction in fertilizer emissions goal by 2030 in Canada

Farmers and industry say government's emissions plans at odds with export goals

Groups ask for goals to be based on emissions per bushel but this may not reduce emissions long-term



Calgary

Misinformation, lack of consultations on fertilizer emissions hurting farmers, Alberta producers say

f У 🛛 🥌 in

'We're just getting kicked ... and this is just yet another thing lining up to give us a boot,' farmer says



Elise von Scheel · CBC News · Posted: Aug 29, 2022 3:00 AM MT | Last Updated: August 29



Symbiotic nitrogen fixation

- Legumes are pillars of sustainable agriculture
- Sustainable source of nitrogen fertilizer



Symbiotic nitrogen fixation

 Bacteria called rhizobia provide "fix" atmospheric nitrogen into usable forms for plants in organs called root nodules



Symbiotic nitrogen fixation



 Farmers can inoculate legume crops with rhizobia to try to harness symbiotic nitrogen fixation in their farm systems

Inoculation with rhizobia

- Different legume crops are nodulated by different rhizobia
 - Soybean Bradyrhizobium
 - Chickpea Mesorhizobium
 - Peas, lentils, faba beans *Rhizobium*
 - Alfalfa *Sinorhizobium*



- Different product types available
 - **Granular**
 - Peat
 - Liquid







Should farmers inoculate?

Inoculation history

 Carrington REC research suggests not necessary for 5 years after first planting soybeans Frequency of soybean yield responses, grain yield and protein differences between experiments with or without a soybean history when seed is inoculated with *Bradyrhizobium japonicum* formulations at planting. (Carrington Research Extension Center, 2003-2007a and 2012a; NDSU Oakes Irrigation Research Site, 2007b).

Number								
Site year ¹	Number of treatments	Soybean history	treatments higher than check	Yield without inoculation	Mean yield with inoculation	Grain protein of the check	Mean grain protein inoculated	
2003a	38	No	38	32.8	38.8	31.5	35.0	
2004a	23	Yes	0	29.1	28.9	33.5	34.5 (NS)	
2005a	25	Yes	0	39.6	39.6	33.5	33.8 (NS)	
2007b	7	Yes	0	55.9	55.9	35.1	35.1	
2007a	11	No	11	46.1	50.7	32.0	34.1	
2012a	6	Yes	0	56.1	56.1	34.6	34.6	

¹All site years Carrington except 2007b treatment study at Oakes.

Soybean Field Production Guide for North Dakota



• Soil/weather considerations

- Drought
- Flooding
- Acidic pH
- Salinity
- Iron deficiency
- High residual N



Photo credit Bob Nichols USDA

Photo credit University of Missouri

Impact of iron deficiency chlorosis on nodulation







Yes / No / Cheap Insurance

Agronomic considerations
No-till rows (Hans Kendal)



• Can we be more precise about inoculant decisions?



- Checking for nodulation
 - Dig up plants at V3/V4 stage and check for nodules
 - If none, and plants show signs of N limitation (yellowing), consider applying N





Should farmers inoculate?



Can we be more precise about inoculant decisions?

- Next generation soil testing will include SOIL BIOLOGY
- Quantification of rhizobia in field soils
 - Soybeans
 - Peas/lentils
 - Chickpeas



Schlaeppi and Bulgarelli, MPMI, 2015

 Quantitative PCR assays to estimate rhizobia numbers in field soils







 Potentially lower inoculant persistence in Western ND with dryer soil moisture

			Years Since		Average	Overall	Tentative Inoculate
Sample	Location	Region	Soybeans	Irrigation	Rhizobia per	Rhizobia	Recommendation
S100	Carrington	Eastern ND	1	No	1,208,939	High	No
S101	Medina	Eastern ND	3	No	695,195	High	No
S103	Hettinger	Western ND	2	No	Not Detected	Low	Yes
S104	Hettinger	Western ND	3	No	16,777	Medium	Next Year
S105	Hettinger	Western ND	6	No	3,901	Low	Yes
S106	Hettinger	Western ND	4	No	4,937	Low	Yes
S107	Madison	Western ND	2	No	19,406	Medium	Next Year
S108	Dickinson	Western ND	4	No	Not Detected	Low	Yes
S109	Alexander	Western ND	1	Yes	1,176,471	High	No
S110	Alexander	Western ND	1	No	581,132	High	No
S111	Alexander	Western ND	5	Yes	411,419	High	No
S112	Alexander	Western ND	5	No	12,593	Medium	Next Year
S113	Alexander	Western ND	2	No	4,769	Low	Yes
S114	Williston	Western ND	2	No	20,738	High	No





 Irrigation may enhance rhizobia populations in dry areas

Sample	Location	Region	Years Since Soybeans	Irrigation	Average Rhizobia per	Overall Rhizobia	Tentative Inoculate Recommendation
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	la	har - ND			1 300		
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 General alignment with ~5 year rule for re-inoculation







How to residual rhizobia impact inoculant success?

- Is there a benefit to inoculation when rhizobia are already present?
 - Soybeans
 - No inoculation, liquid inoculation and granular inoculation treatments





Audrey Kalil (Williston REC)

Mike Ostlie

(Carrington REC)







Kristin Simons (Carrington REC)

How to residual rhizobia impact inoculant success?

 Field trials in collaboration with NDSU Research Extension Centers (Carrington, Hettinger, Williston)

N.D. Agricultural Experiment Stations







• Response to liquid inoculant when soybean rhizobia tested as low



How does it change with different crops?

Big difference between soybean and dry beans/peas/alfalfa = abundance of native rhizobia in the soil (even without inoculant history)

How to residual rhizobia impact inoculant success?

- Is there a benefit to igodolinoculation when rhizobia are already present?
 - Peas, lentils and chickpeas ulletin sites with high and low residual rhizobia present
 - No inoculation, liquid ulletinoculation and granular inoculation treatments





Audrey Kalil (Williston REC)







(Hettinger REC)



Lucas Andes (Geddes lab NDSU)





Is there a benefit to inoculation when rhizobia are already present?



How to residual rhizobia impact inoculant success?

Increased yield from inoculation in chickpea by ~20% (when rhizobia low!)



Yield Tesponse to Inoculant (Williston)



How to residual rhizobia impact inoculant success?



High nodulation and rhizobia number in plots that have never had peas/lentils



How to residual rhizobia impact inoculant success?



High proportion of noninoculant rhizobia in root nodules

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So, peas/lentils are different from soybeansmany natural rhizobia that can nodulate them in our soils



Is that a good thing?

Rhizobium competition problem

"Less effective native rhizobia that are locally adapted to soils outcompete the beneficial inoculant, limiting the nitrogen that can be derived from symbiosis"



- Sampling of pea rhizobia across ND
 - Create a culture collection of the pea nodulating rhizobia from across ND and assess their effectiveness and competitiveness
 - 1000 rhizobia!!











Mia Haugan (NDSU UGRA) Grace Loegering (NDSU UGRA) Natalie Visich (NDSU UGRA)

Sampling of alfalfa rhizobia across ND

- Create a culture collection of the alfalfa nodulating rhizobia from across ND and assess their effectiveness and competitiveness





Tania Gupta (NDSU UGRA)



Jake Schumacher (NDSU UGRA)

NAFA" REALIZANCE

500 rhizobia!!

igodol





Mendosa-Suarez et al. PNAS (2020)

• Enormous variation in rhizobium quality in ND pea fields



- Garrett Levin
- Enormous variation in rhizobium quality in ND alfalfa fields



Modified from Mendosa-Suarez et al. PNAS (2020)

 Plasmid-IDs for high throughput measurements of effectiveness and competitiveness

 Molecular barcodes allow detection of rhizobia in each nodule



Modified from Mendosa-Suarez et al. PNAS (2020)

 Nitrogen fixation "biosensor" allows prediction of nitrogen fixation ability in each nodule



Modified from Mendosa-Suarez et al. PNAS (2020)





 Highly competitive rhizobia from ND soils. Differences in different soils





 Some differences in the most competitive rhizobia by plant host





Highly competitive rhizobia from ND soils vary in quality



 Upcoming field trials to explore increased nodule occupancy by strains screened for elite competition with our cultivars in our soils



How can we enhance symbiotic nitrogen fixation between rhizobia and legumes NOW?

- Precision Ag
 - Consider residual nitrogen
 - Check rhizobia populations?
- Soil health
 - Keep pH from becoming too acidic
 - Manage IDC



How can we enhance symbiotic nitrogen fixation between rhizobia and legumes IN THE FUTURE?

- Rhizobium genetics
 - Find elite strains that match to the varieties farmers are planting and the soils theyre growing in
- Plant genetics
 - Breed for enhanced symbiosis (eg. under drought in soybean)



Acknowledgements



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North Dakota Soybean Council Our World Is Growing.







- Associative diazotrophs
 - Microbes that can fix nitrogen in a free-living state (no root nodule)
 - Can we derive SOME benefit from associative N fixation? (will never completely replace Nfertilizers)





- Not a replacement for fertilizer
 - Even at best can only supply ~10-20% of N needs
- Potential to "fill the Nitrogen gap" during the growing season and boost yields



Emerging biologicals

Envita (Azotic Technologies)

• Gluconacetobacter diazotrophicus

Proven (Pivot Bio)

• Kosakonia sacchari + Klebsiella variicola

Utrisha (Corteva)

• *Methylobacterium symbioticum*



Commercializing nitrogen-fixing microbes for cereal crops



- An opportunity to explore to maximize yield?
- A lot of work to be done to vet these new technologies
 - Agronomists!!
 - Farmer driven testing
 - use an untreated check!!
 - What works for your farm? How consistently?



2021 Field Trial – Lenore, MB