Asymbiotic N-Fixing Organisms-Natural Populations and Commercial Products: What You Should Know and What Questions to Ask

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Point # 1- Asymbiotic N-fixing organisms are a natural part of soils

Organisms, usually a species of bacteria, that have the ability to fix atmospheric N (N_2), transforming it into NH_3, which is immediately attached to a 'carbon-skeleton', safening it.

The fixation requires energy, which when conducted in soil comes from organic matter.

Asymbiotic N-fixing organisms

Evidence for asymbiotic N-fixing organisms finds that these organisms were active 1.5 billion years ago- some of the oldest organisms found in the fossil record.

(Boyd & Peters, 2013, Frontiers in Microbiology)

Compared with about 59 million years ago for symbiotic N-fixers (Sprent and James 2007, Plant Physiology)

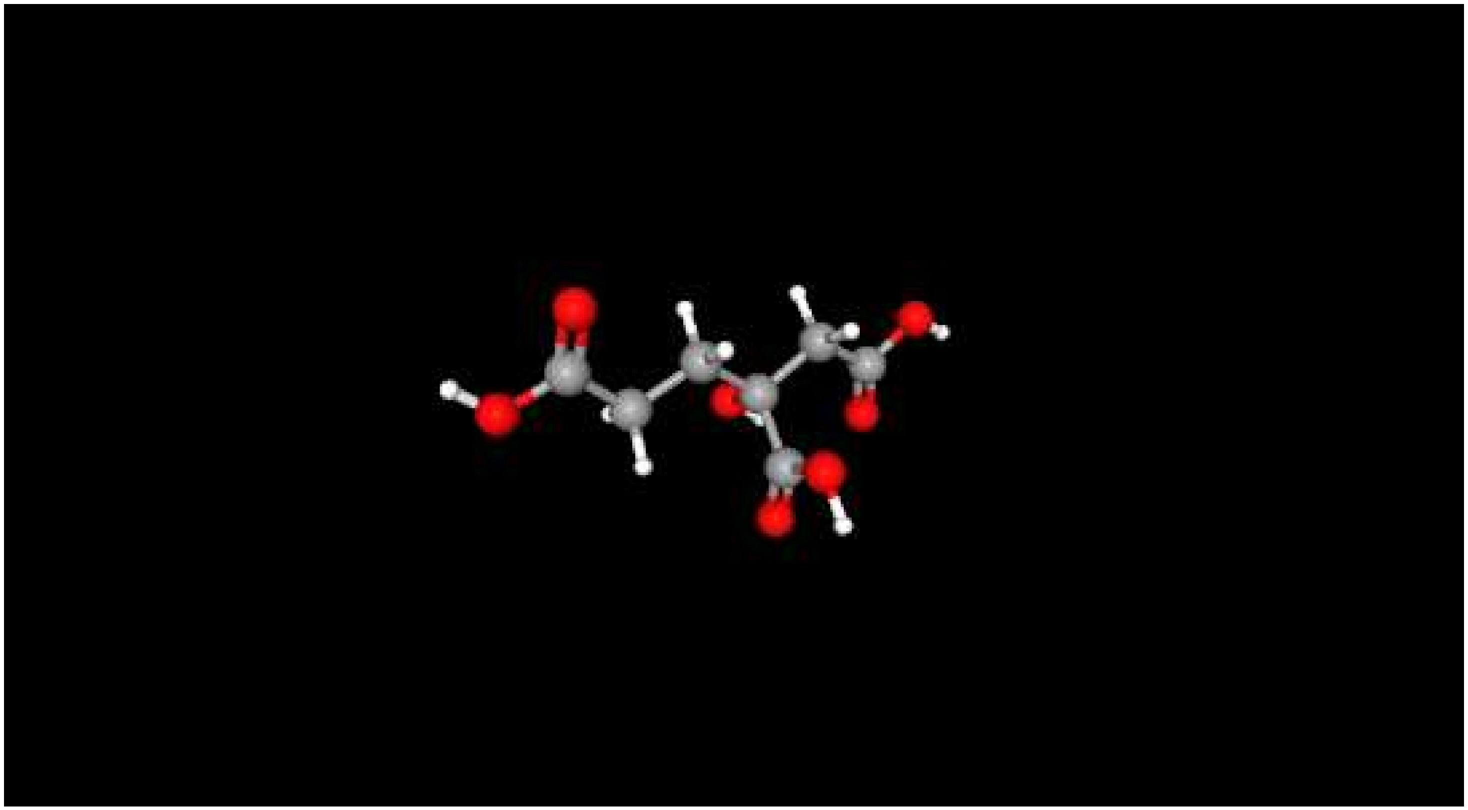
Asymbiotic N-fixing organisms

N-fixation is an energy-expensive process.
The enzyme that serves as ‘fixation facilitator’ in bacteria is *nitrogenase.*
To convert 1 N_2 to 1 NH_3 requires 16 ATP molecules (produced during photosynthesis) and 8 electrons.

Energy limits N fixation.
(Smercina et al., 2019, Applied Environmental Microbiology)

For comparison, production of 1 peptide bond in protein synthesis requires only 5 ATP (still considered ‘high energy requirement’)

Nitrogenase enzyme



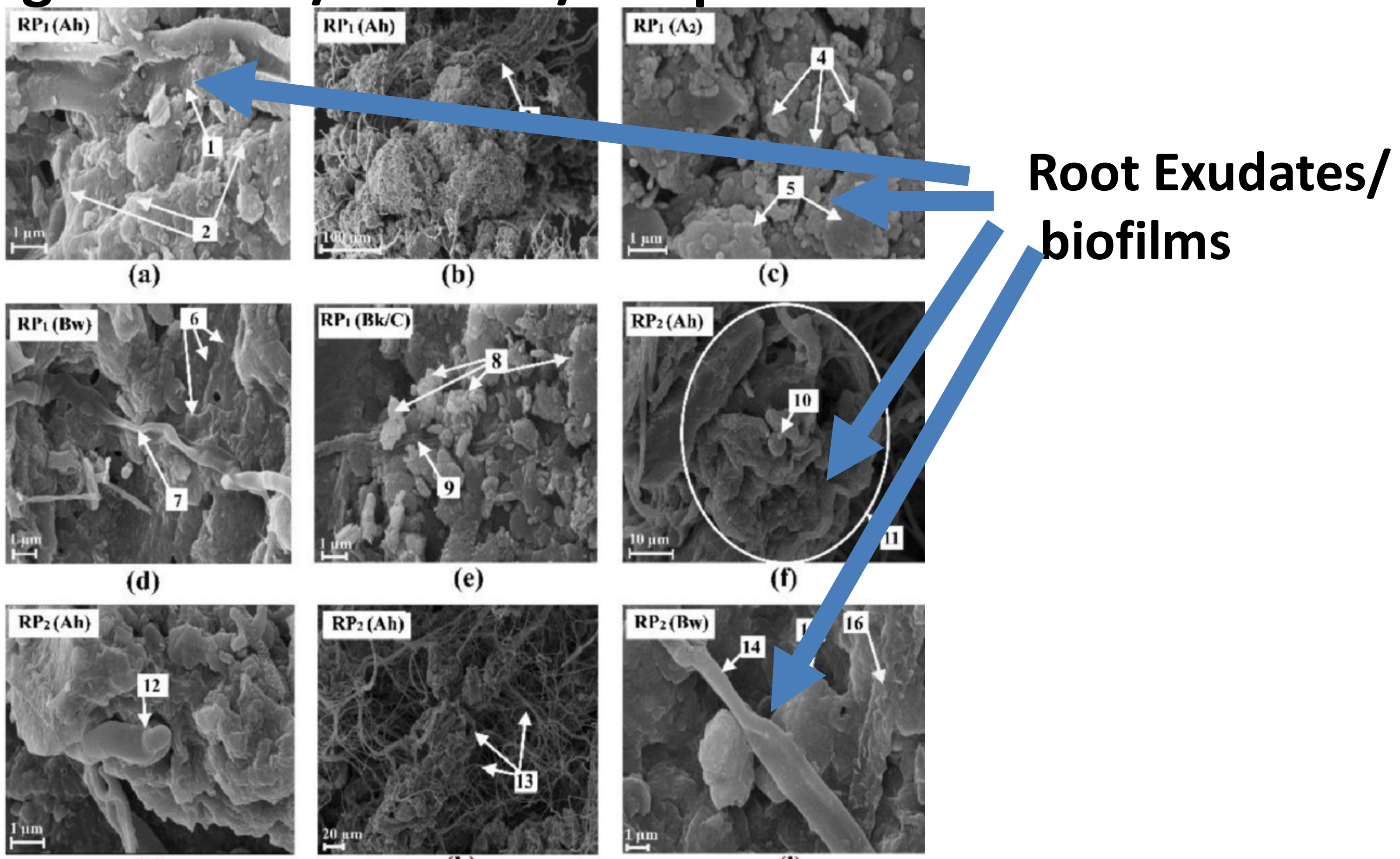
$C_7H_{19}Fe_7MoNO_7S_{9-12}$

Substrates to meet Asymbiotic bacteria energy requirement

Energy sources for asymbiotic N-fixing organisms close to plant roots are the <u>root exudates</u> that surround many roots, and also includes components of <u>soil organic matter</u> and <u>residue</u> decay intermediary compounds in the bulk soil.

N-fixing organisms that exist inside the plant tissue have to receive substantial energy from the plant within which they reside.

Images of root/exudate/soil particle interfaces



RP 1 images (SEM) of fine root (1), root exudates/biofilms (2), fine roots (3), clay mineral grains (4), clay mineral aggregates (5), root exudates/biofilms (6), organic filament (7), fine clay aggregates (8), fine root (9), and RP 2 images (SEM) of aggregates with abundant root remnants (10), pollen (11), root tap protruding through clay aggregate coated by exudates (12), dominant network of fine roots (13), and organic filament, primary mineral, clay minerals coating organic filament (14, 15, 16). From Razzaghi et al., 2017.

Diversity of Asymbiotic bacteria types-

A Chinese/Tibetan paper, using genetic screening, found Asym-bacteria from 6 Phyla, 13 Classes and 43 genera (Li et al, 2021, Front. Ecol. Evol. 13 August. Vol 9)

Genera include species from *Azotobacter, Ocrhobactrum, Sphingomonas, Opitutus, Clostridium, Pseudomonas* and ‘a host of others’.
There are many species, some more efficient than others,
Their activity is linked to substrate and soil condition.

Asymbiotic N-fixing bacteria are in most soils.

Their activity increases when tillage decreases. ***Food & Housing***

Lamb, Doran and Peterson, 1987
Nonsymbiotic dinitrogen fixation in no-till and conventional tillage SSSAJ 51:356-361

Recorded greater activity with no-till, but concluded that it was not great enough to contribute to any N credit. They considered the values from incubation to be values that might be experienced in the field. But the disturbance of soil probably killed billions of N-fixing critters, so values are index.

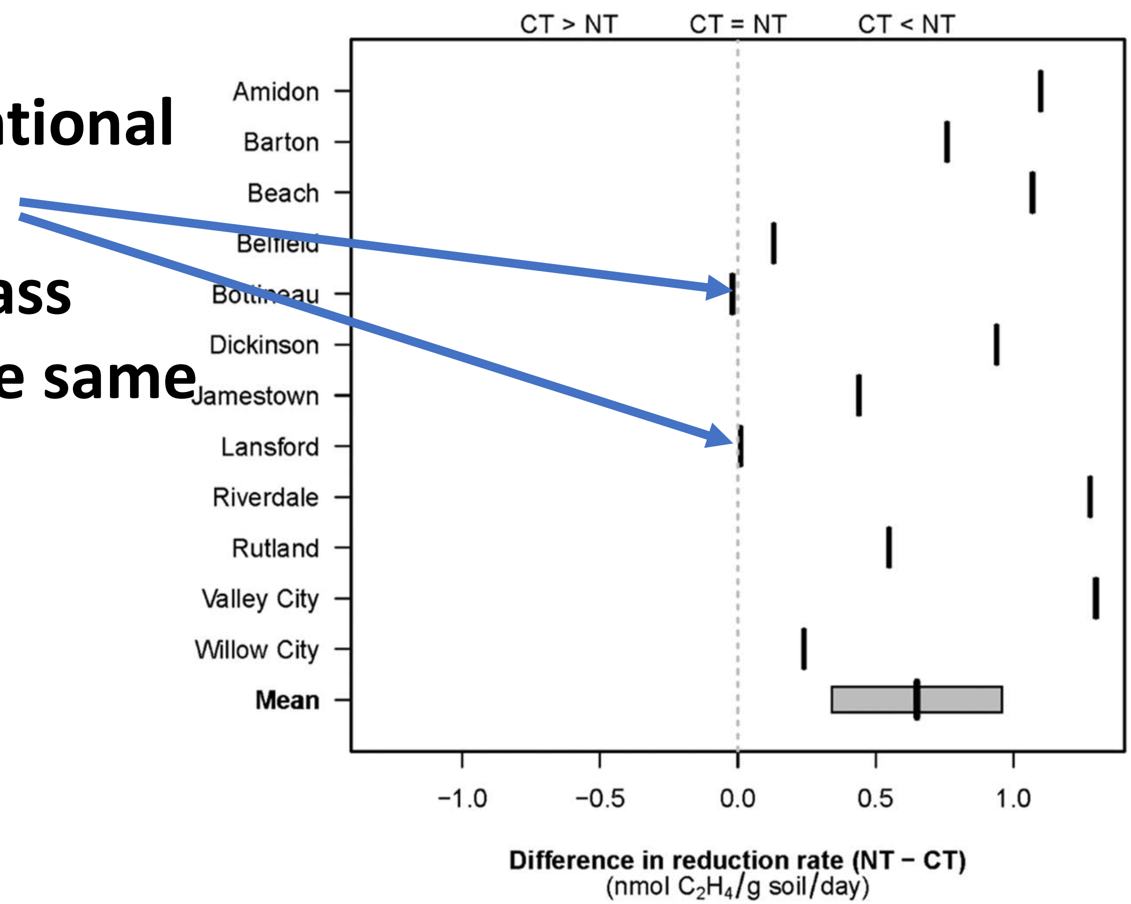
In North Dakota, there is an N credit for 6 or more years of continuous no-till, one-pass shallow tillage, shank strip-till, amounting to 40-50 pounds N per acre.

Part of this credit probably comes from the increased microbial biomass under no-till that protects N from loss. But a part of the credit, perhaps 25-33% may come from greater asymbiotic activity in long-term no-till.

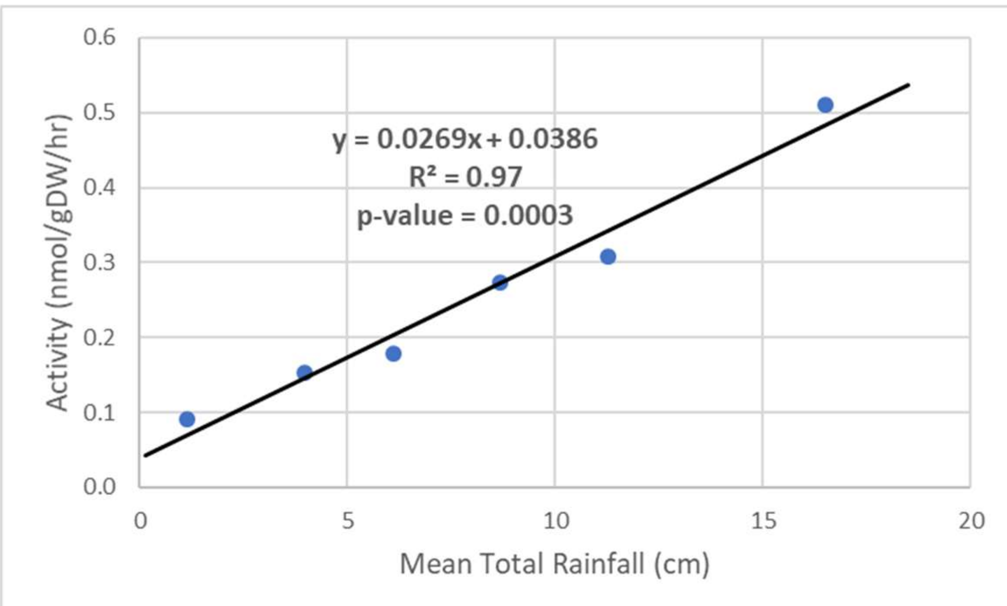
Franzen et al. 2019, SSSAJ

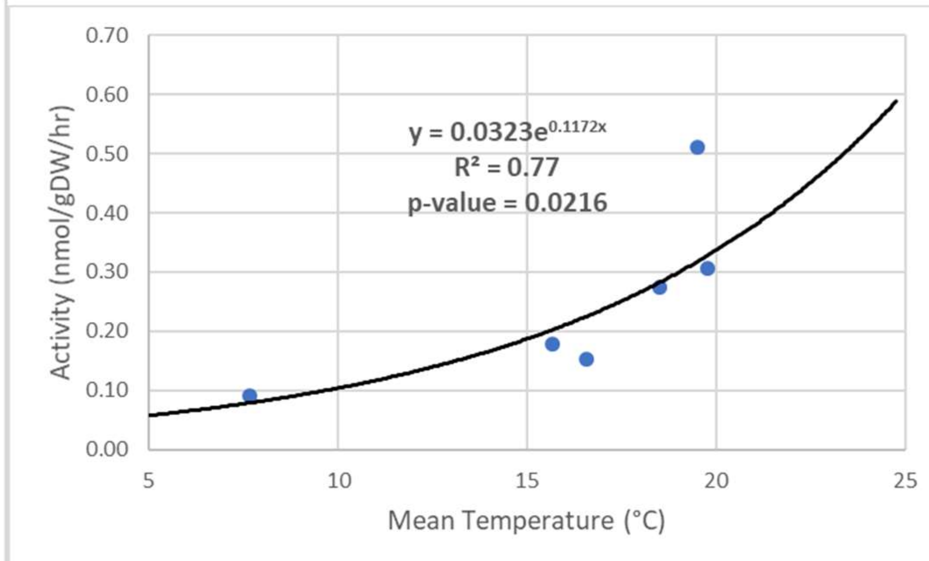
Took paired no-till/conventional till across state.

Turns out the 'conventional till' site across the fence was one-pass shallow tillage, so the same tillage category

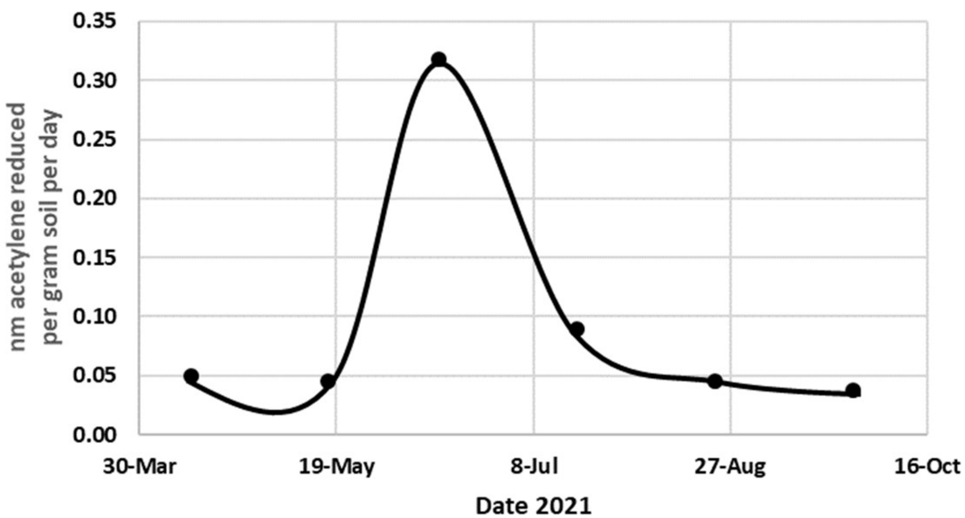


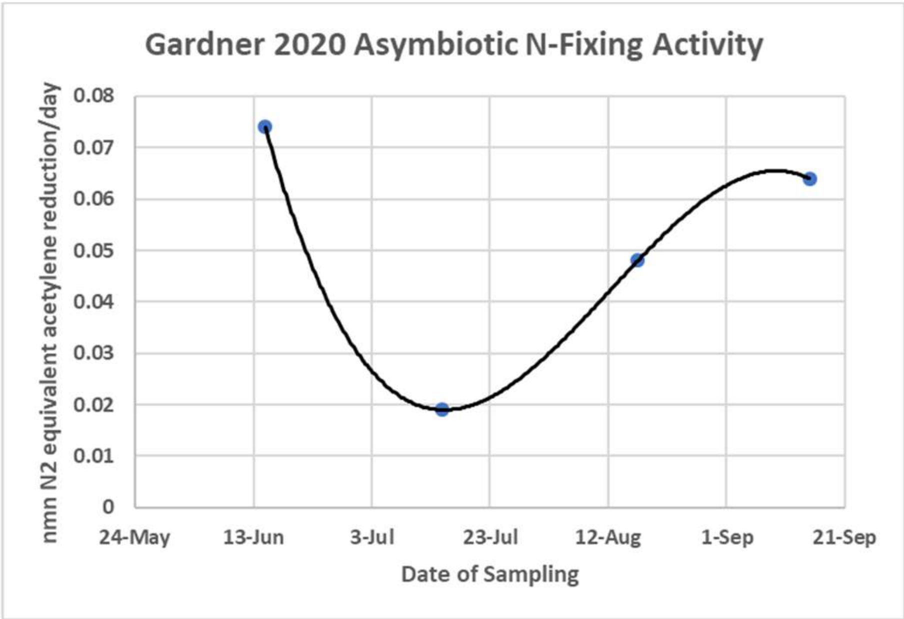
From 2019 to 2021, 6 sites in eastern North Dakota were sampled each month for asymbiotic N fixing activity. Change in activity was related to rainfall within 30 days before sampling and mean air temperature.





The relationship of N-fixing activity to soil condition made a large impact on the trend of activity over a season





Jamestown, 2021. Period from late May to early July moist, then very dry.

Gardner, 2020, Period for July sampling, soil was saturated in a high clay soil.

Summary of what we know about native activity-

<u>Greater in long-term no-till</u>. Their activity increases when their 'homes' are not destroyed and there is sufficient food to support their N-fixation.

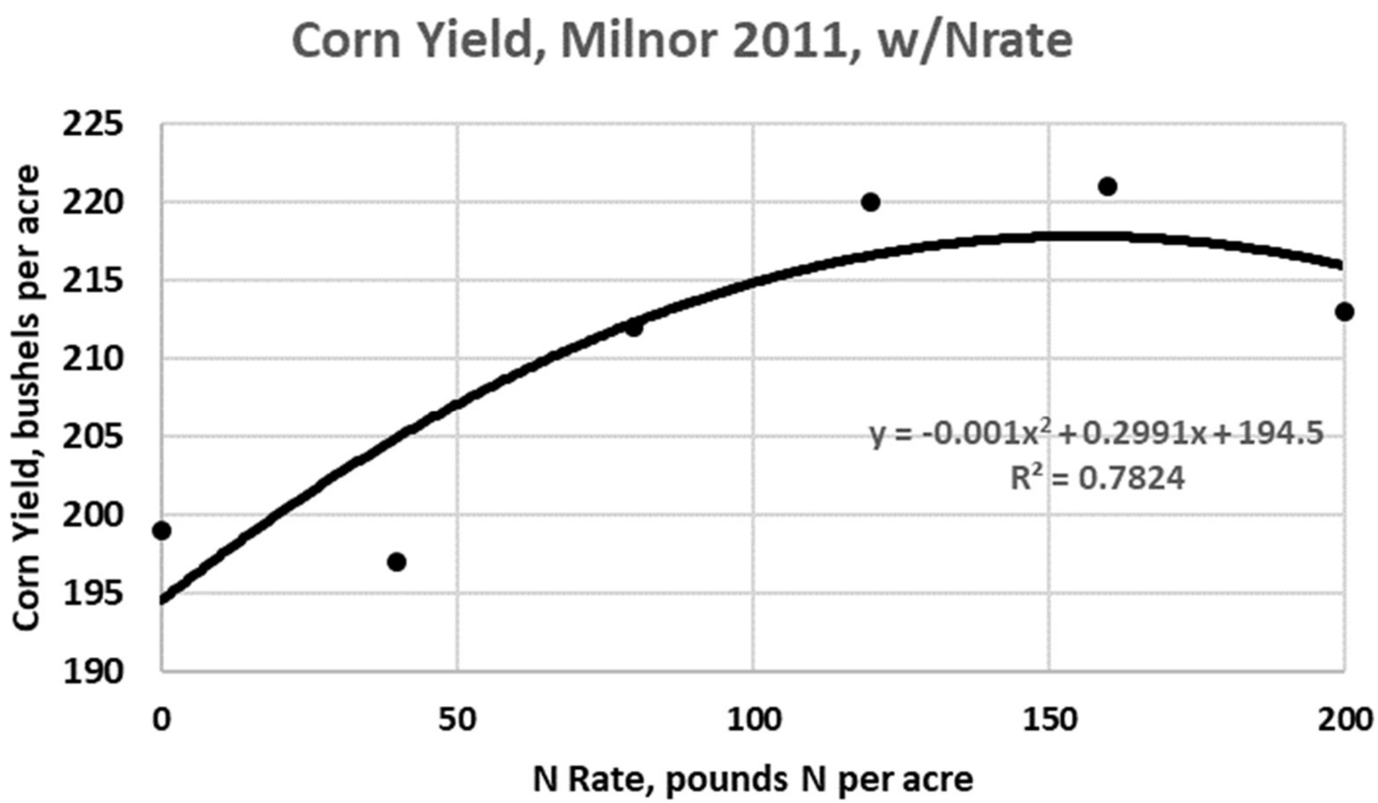
<u>Moist soil and warm conditions favor N-fixation</u>. Dry soil conditions, saturated soil conditions, and cold soil temperatures inhibit their activity.

Regional studies on commercial asymbiotic N-fixation products

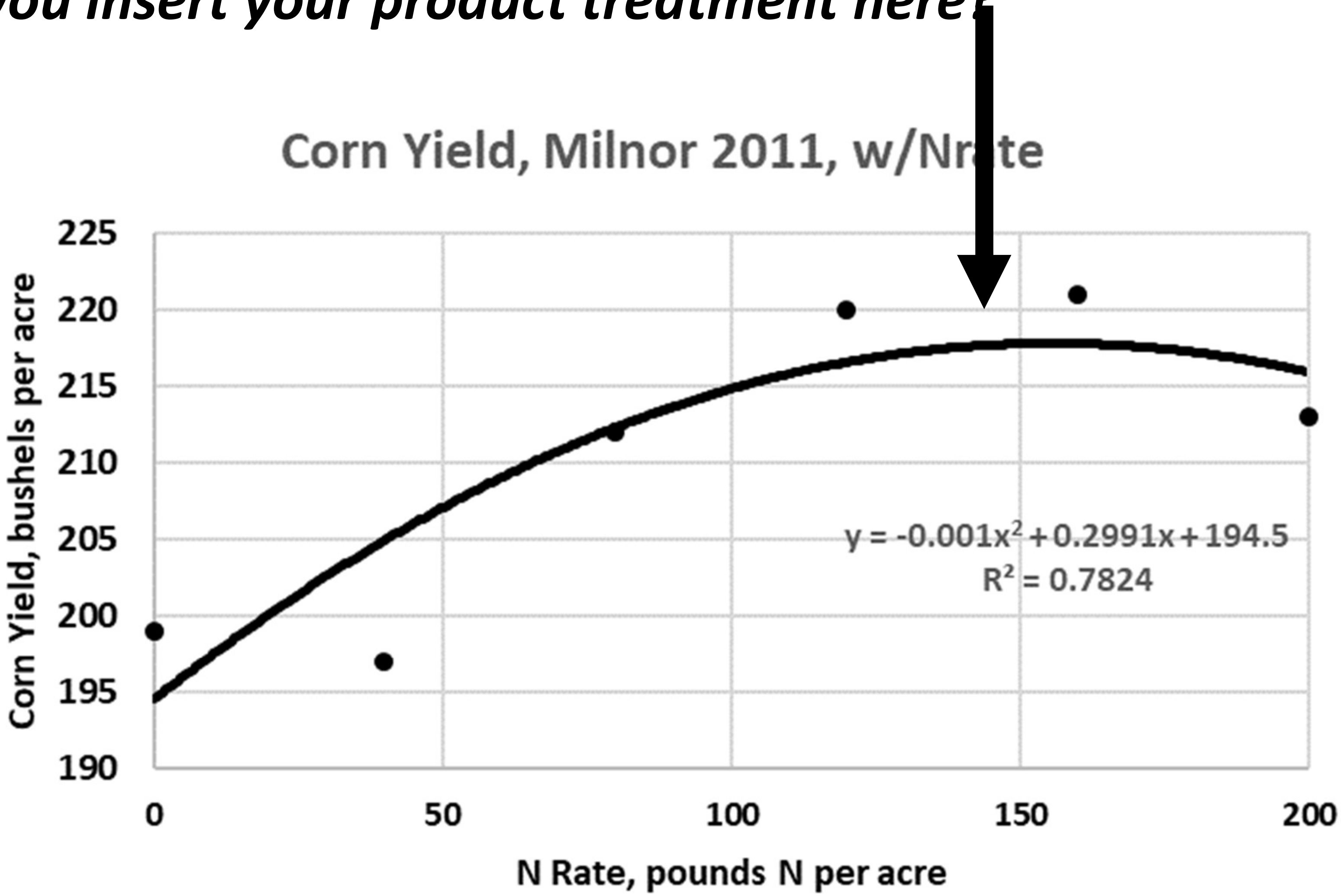
Products tested-

- **Envita, Azotic North America** *Gluconacetobacter diazotrophicus*
- **Utrisha, Corteva Agriscience** *Methylobacterium symbioticum*
- **ProveN, PivotBio** *Klebsiella variicola*
- **ProveN 40, PivotBio** *Kosakonia sacchari* & *Klebsiella variicola*

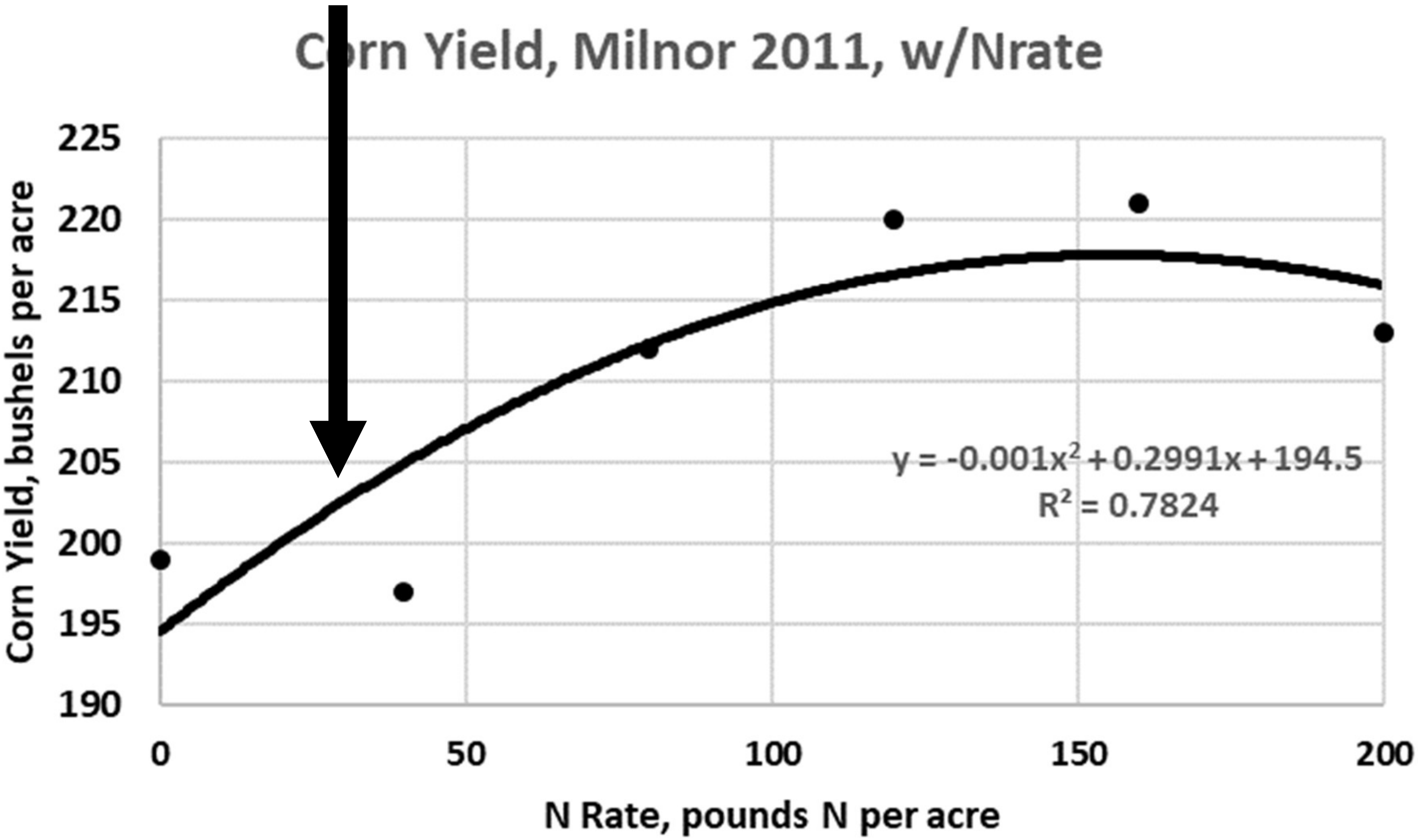
How to set up an N-rate experiment, or an experiment with a different N product or biological N provider-



Would you insert your product treatment here?



Or here?



Corn Yield, Milnor 2011, w/Nrate
225
220
215
210
205
200
195
190
Corn Yield, bushels per acre
0
50
100
150
200
N Rate, pounds N per acre
y = -0.001x² + 0.2991x + 194.5
R² = 0.7824

North Dakota studies on Envita and Utrisha

4 locations- Prosper, Absaraka, Oakes (irrigated), Carrington area

Protocol-

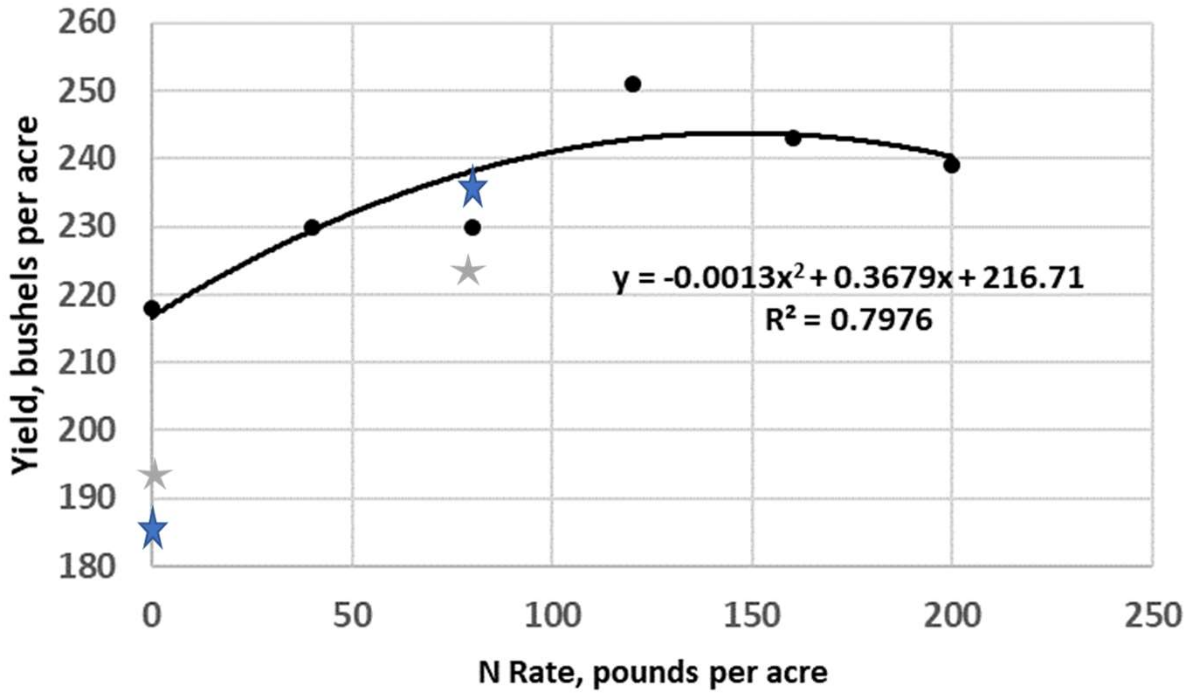
N rate studies, 0, 40, 80, 120, 160 and 200 pounds of N

plus 0 and 80 with Envita in-furrow with 7-23-5

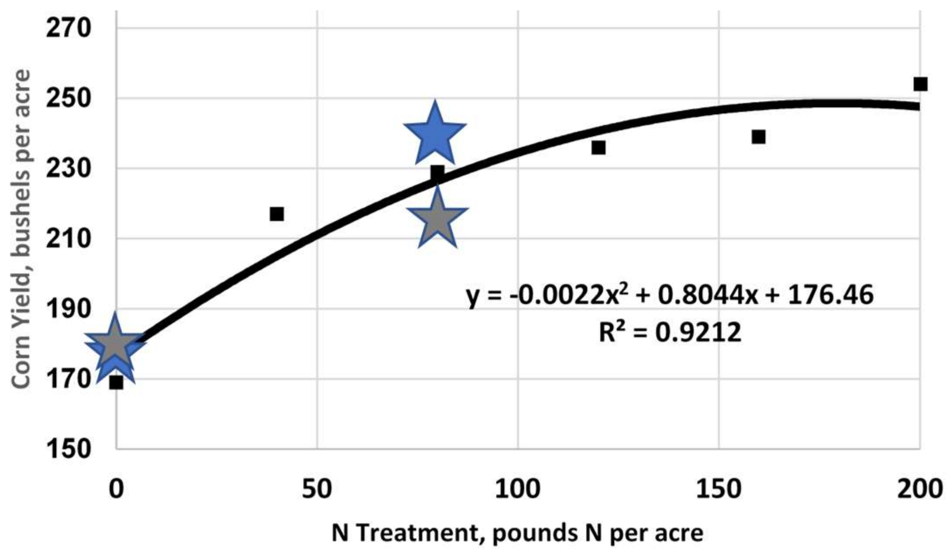
(all treatments received 2.5 gallon per acre 7-23-5)

plus 0 and 80 with Utrisha post at V6.

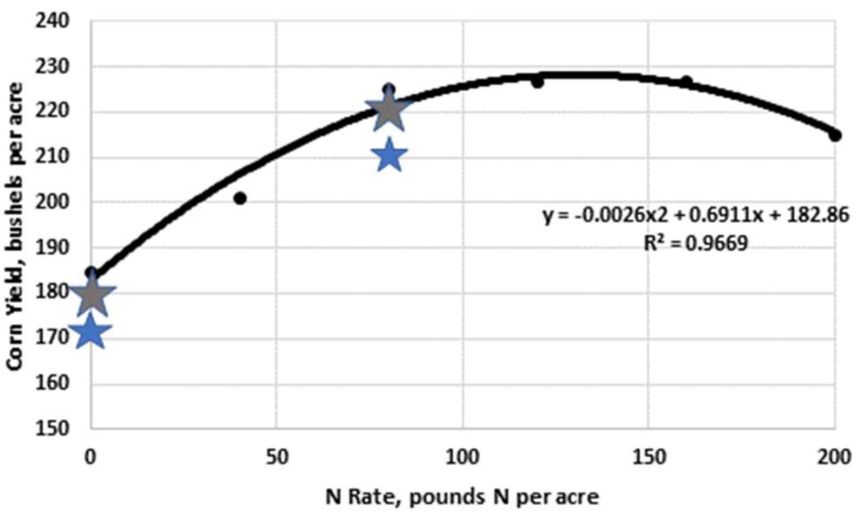
4 replications



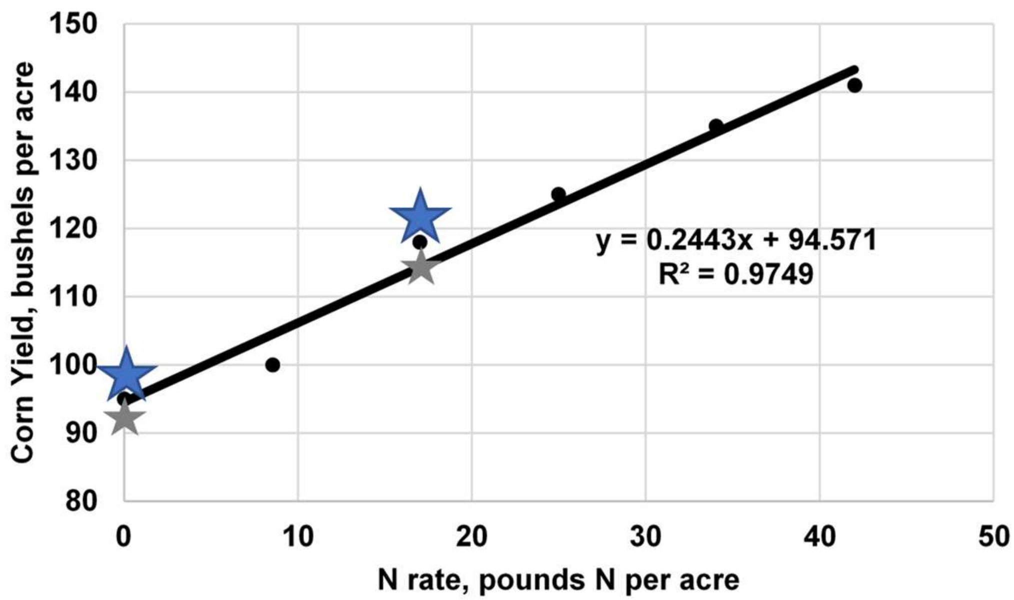
Absaraka response of corn to N treatment and N rate with additives.
Blue stars indicate treatments (0 and 80 lb N/acre) with Utrisha post-applied V6.
Gray stars indicate treatments (0 and 80 lb N/acre) with Envita in furrow at planting.



Prosper response of corn to N treatment and N rate with additives.
Blue stars indicate treatments (0 and 80 lb N/acre) with Utrisha post-applied V6.
Gray stars indicate treatments (0 and 80 lb N/acre) with Envita in furrow at planting.



Carrington response of corn to N treatment and N rate with additives.
Blue stars indicate treatments (0 and 80 lb N/acre) with Utrisha post-applied V6.
Gray stars indicate treatments (0 and 80 lb N/acre) with Envita in furrow at planting.



<u>Oakes</u> **response of corn to N treatment and N rate with additives.**
Blue stars indicate treatments (0 and 17 lb N/acre) with Utrisha post-applied V6.
Gray stars indicate treatments (0 and 17 lb N/acre) with Envita in furrow at planting.

Neither Envita nor Utrisha increased yield compared to the 0 and 80 (and 0 and 17) lb N/acre rates without at the North Dakota sites.

Minnesota trials 2019 and 2020

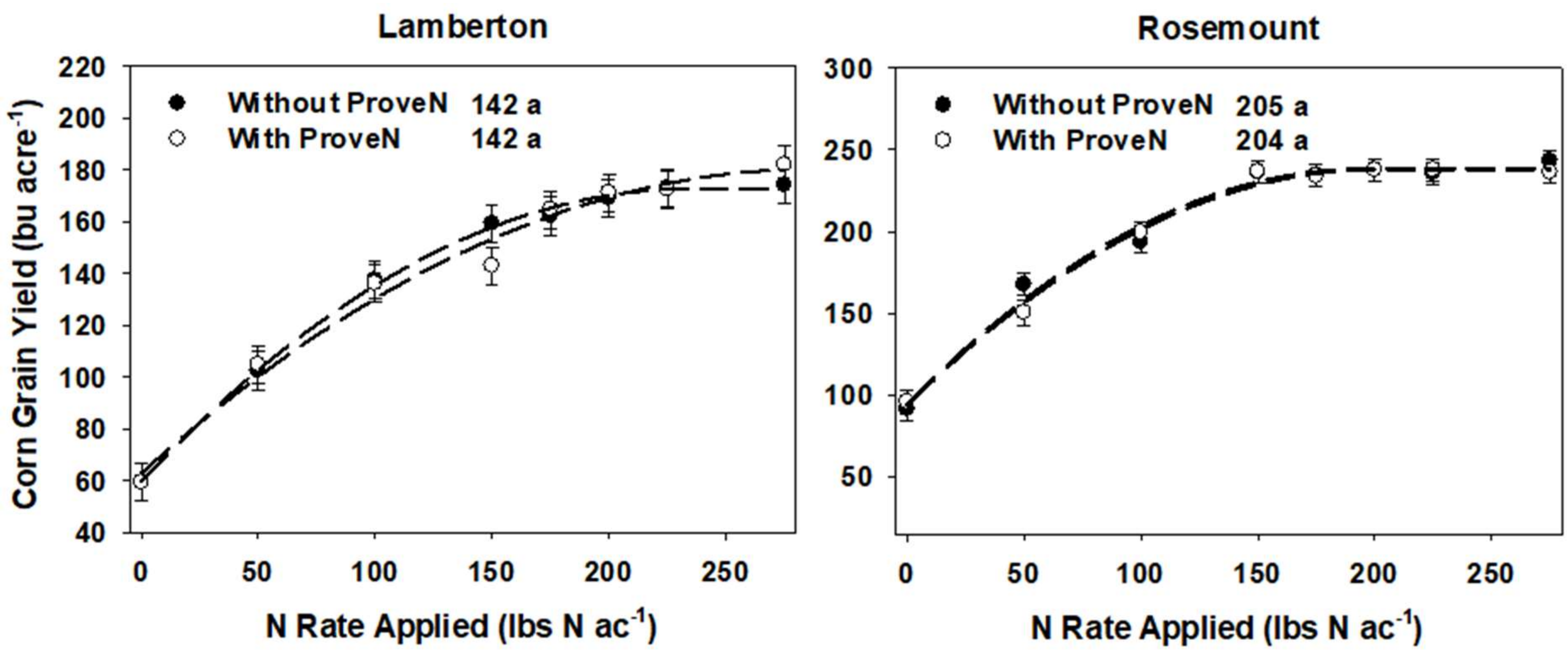
Currie, Kaiser and Vetsch,

2021 North Central Extension-Industry Soil Fertility Conference Proceedings

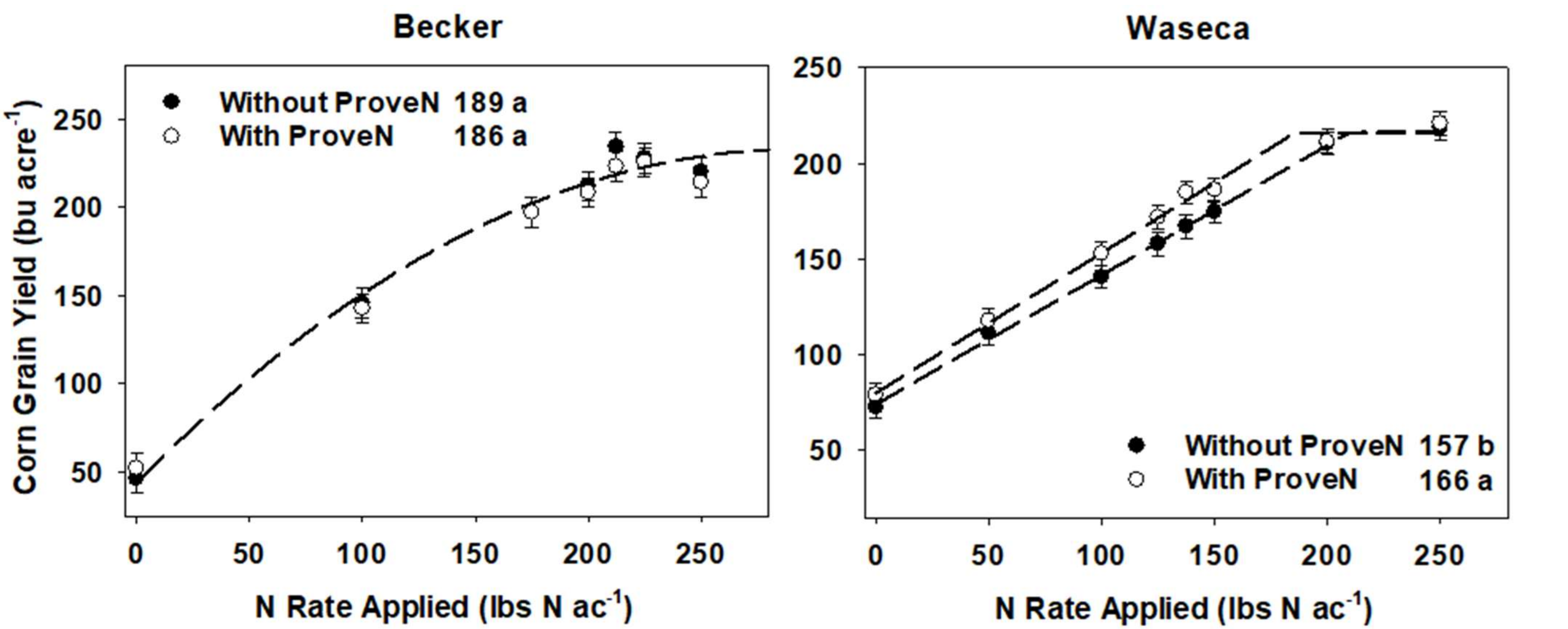
Compared N rates with and without ProveN

2 locations in 2019, Lamberton and Rosemount

2 in 2020, Becker and Waseca



No yield response to ProveN at 2 sites in 2019



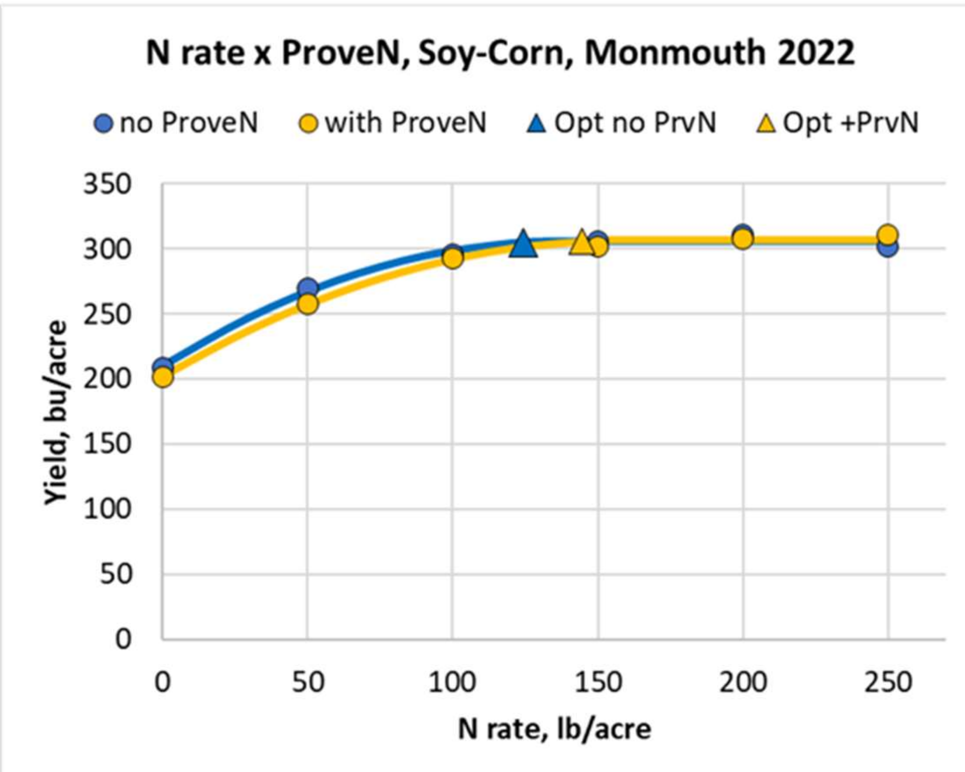
No response to ProveN at Becker in 2020

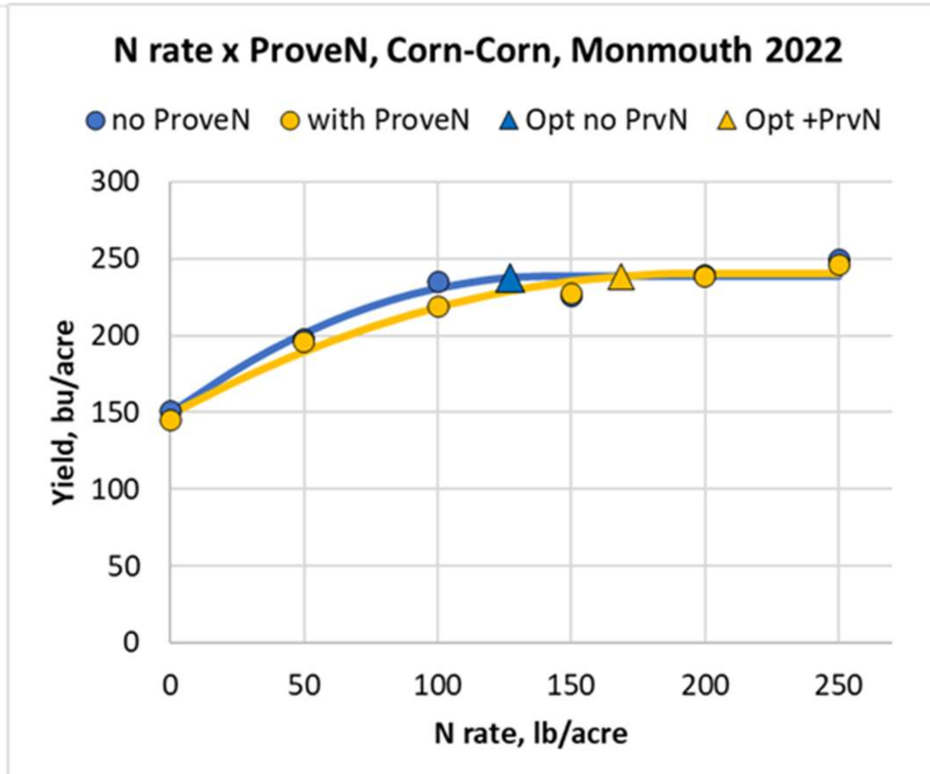
A mean 9 bu/acre increase with Prove N in 2021 at Waseca.

~125 lb N/acre rate + ProveN about equaled 145 lb N without.

University of Illinois, 2022 trials with ProveN 40

E.D. Nafziger, funded by Illinois Fertilizer & Chemical Association



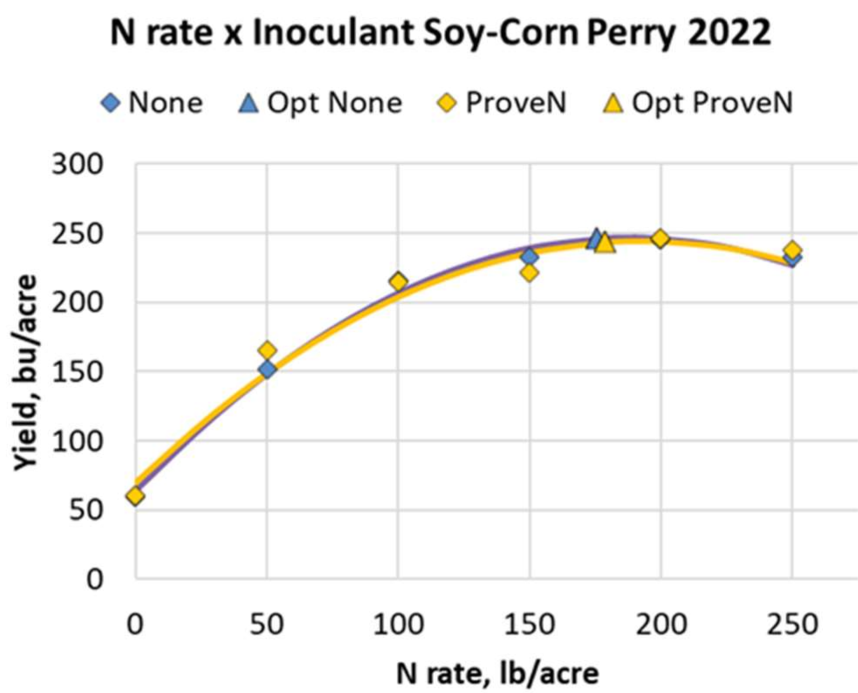


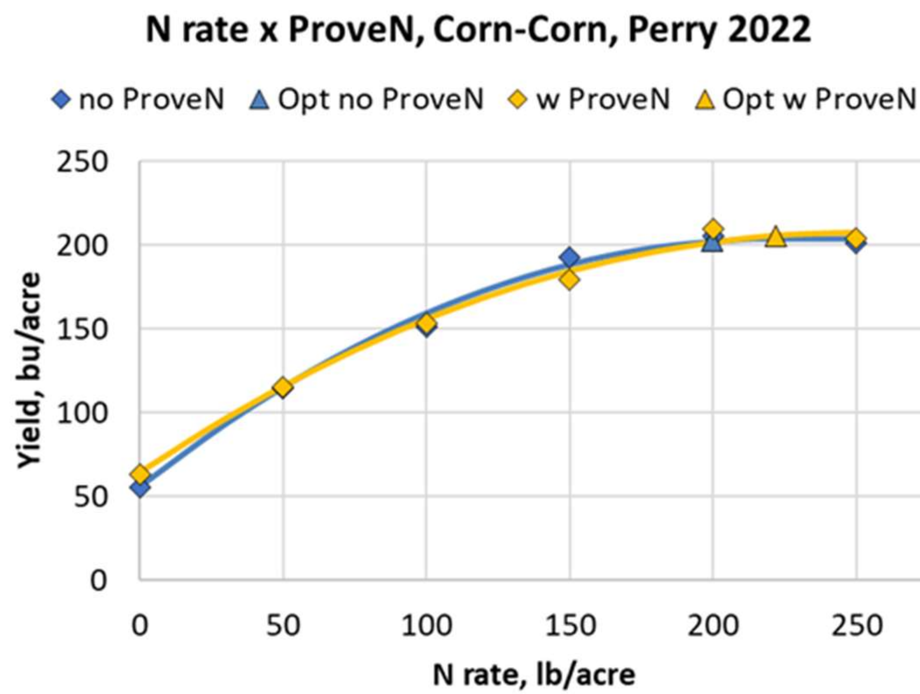
Split plot design- N rate main plot, ProveN in split.

Monmouth

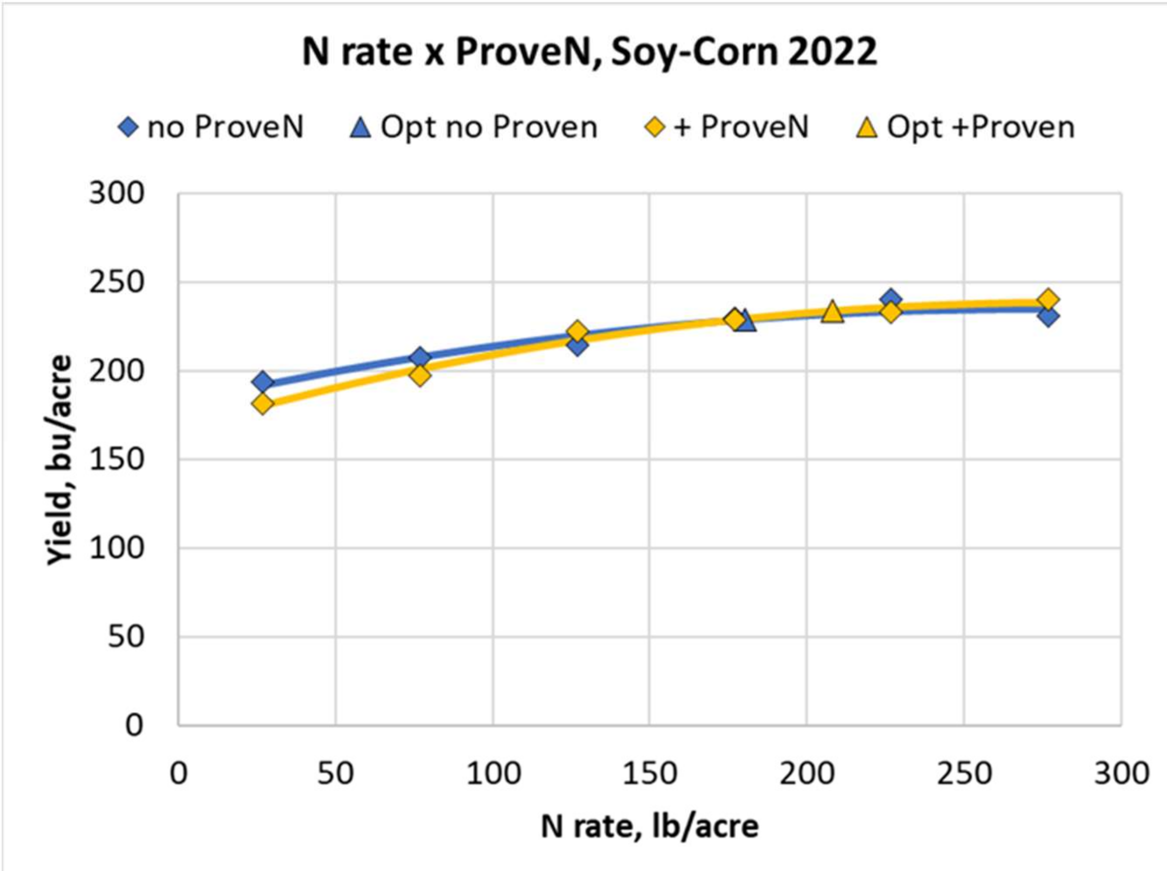
Soybean-corn, No main effect of ProveN or Nrate X ProveN interaction.
Optimum N rate without 124 lb/acre
Optimum N rate with Prove N 144 lb/acre

Optimum yield without 304.8 bu/acre
Optimum yield with 305.9 bu/acre





No differences in yield or optimum N rate with or without Prove N. Perry is in WSW Illinois



Similar split plot at Marion (southern Illinois)

No differences in yield, optimum N rate

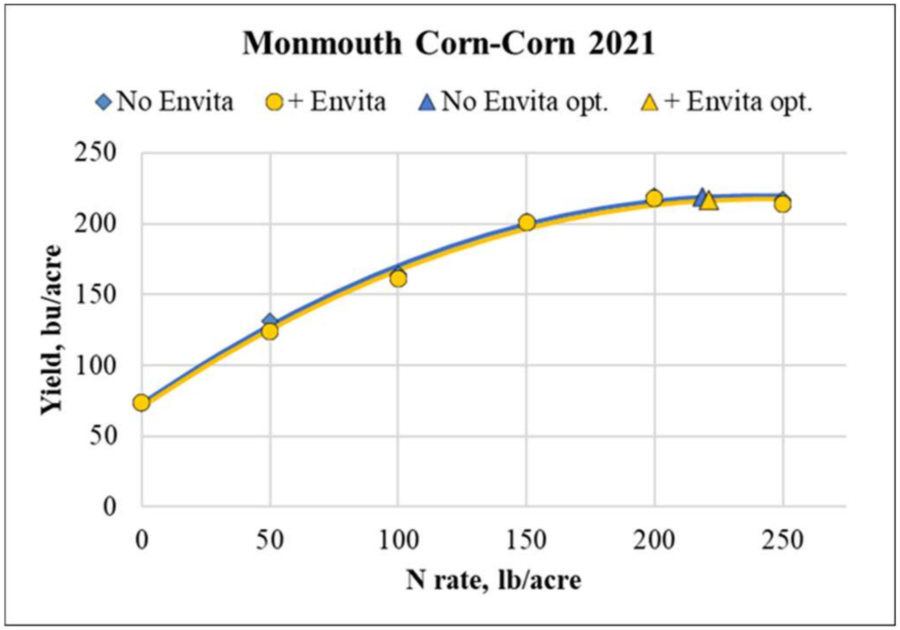
Strip trial-

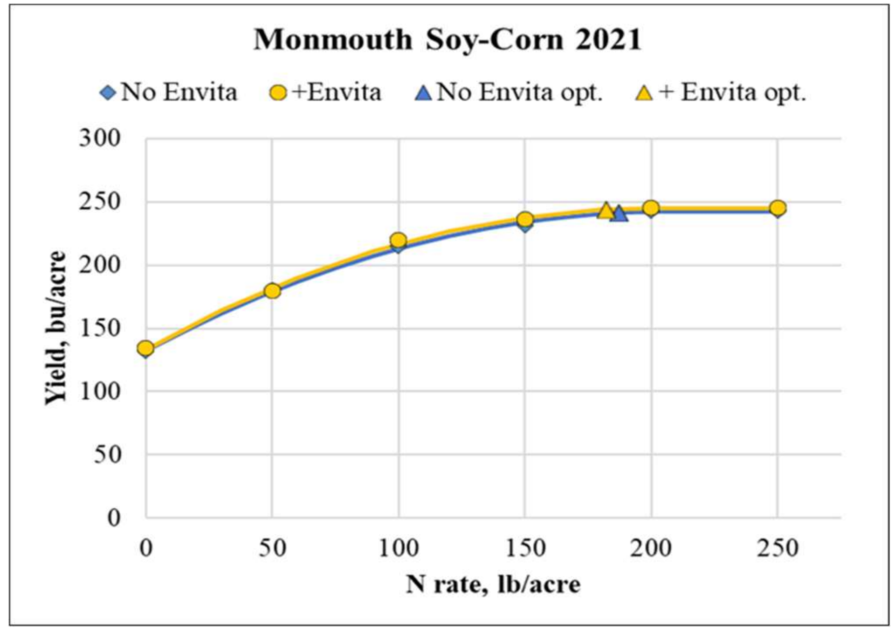
Marion, 160 pounds of N strips replicated 3 times, with or without ProveN.

No Yield difference treated or untreated.

Urbana- Prove-N applied as seed treatment.

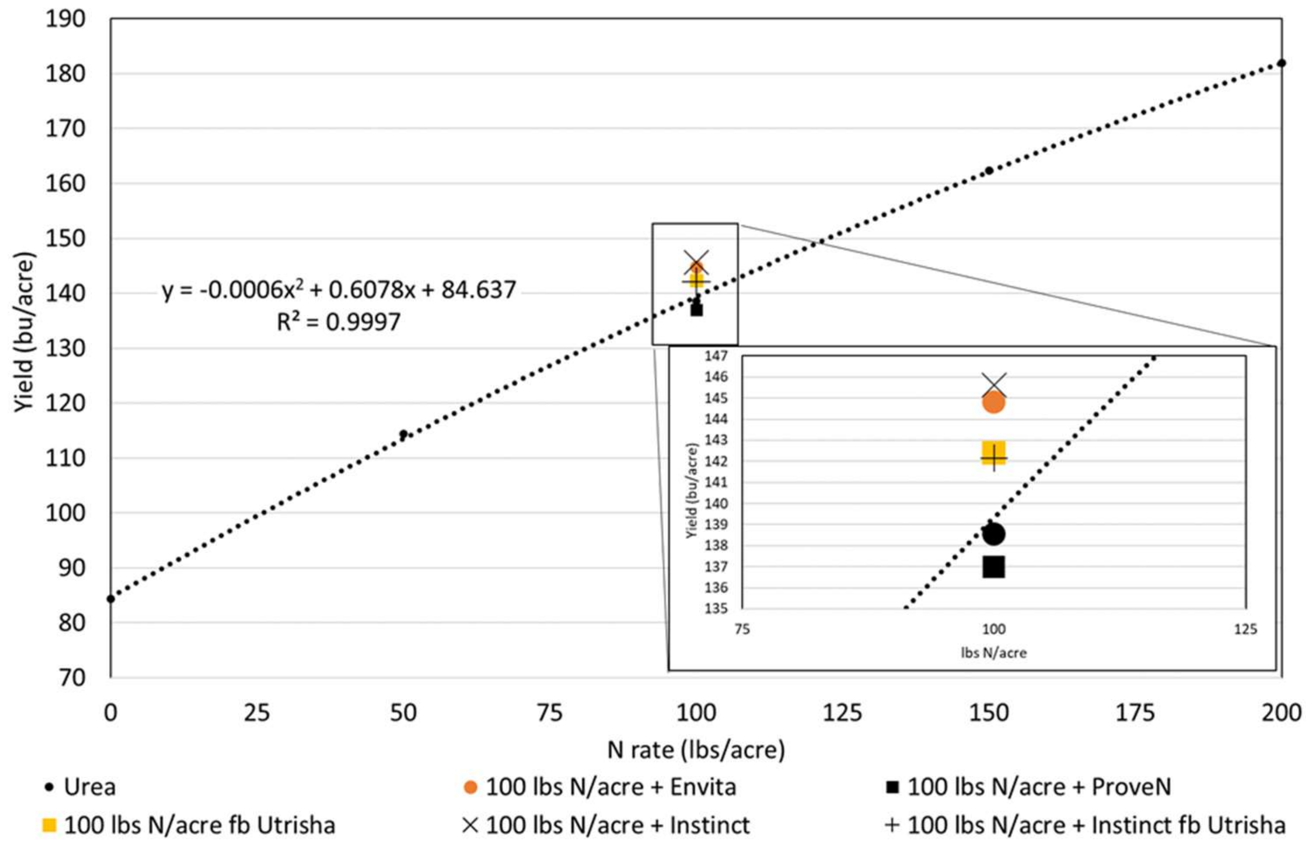
Seed Treatment	N rate lb/acre	Yield, bu/acre
Untreated	150	199 a
Untreated	200	213 a
Treated	150	201 a
Treated	200	214 a





Illinois studies with Envita
No benefit to its use

University of Missouri,Envita, Utrisha, ProveN and Instinct 2020-2022, **Novelty, Missouri (Kelly, et al.) 3 site years. Combined includes 2 site years with no Envita benfit and 1 with Envita benefit**



Yield increase with Envita (orange circle) and Instinct (a nitrification inhibitor- Nitrapyrin) The Envita yield increase was equivalent to about 12 lbs N/a.

Purdue, 2020

Envita trials, NE Exp. Station between Fort Wayne and Columbia, IN

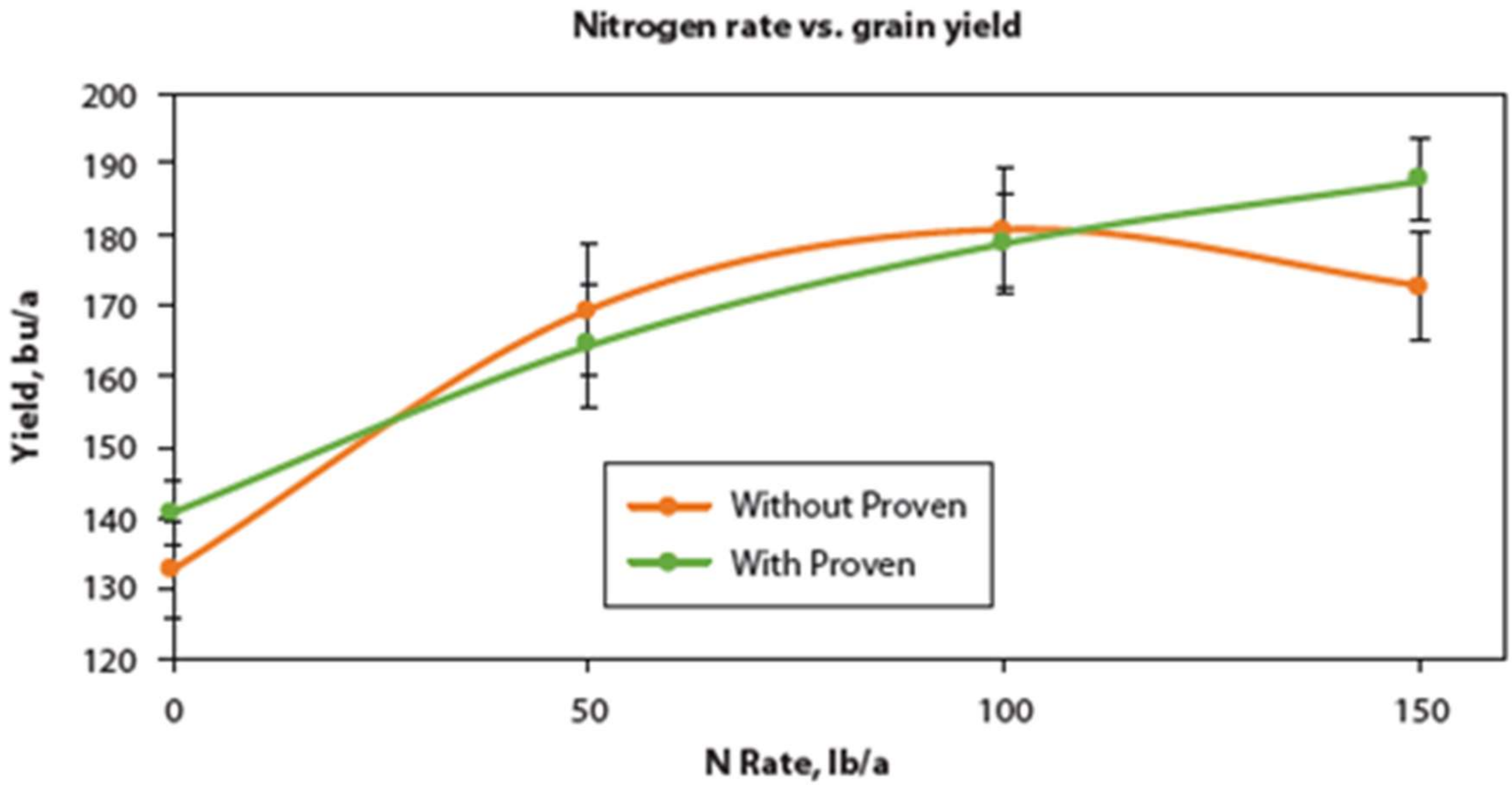
N rate, lb/acre	Grain yield, bu/acre
0	110 b
65	138 a
135	148 a
205	143 a
no	134
yes	136 NS

Camberato, 2020

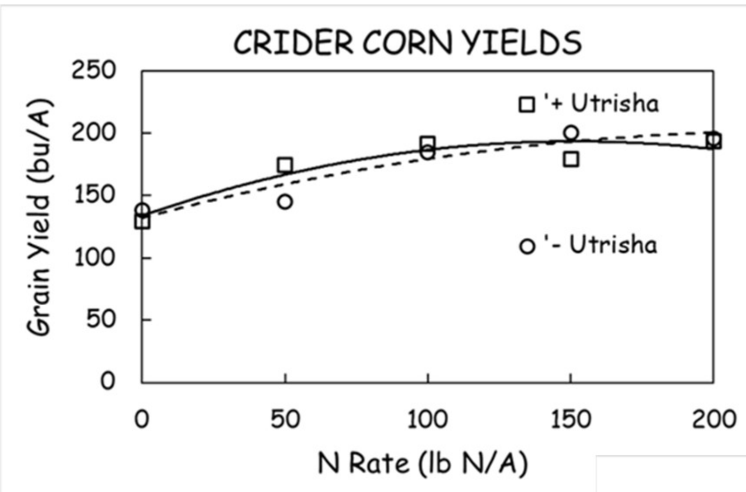
Michigan State University trials 2022

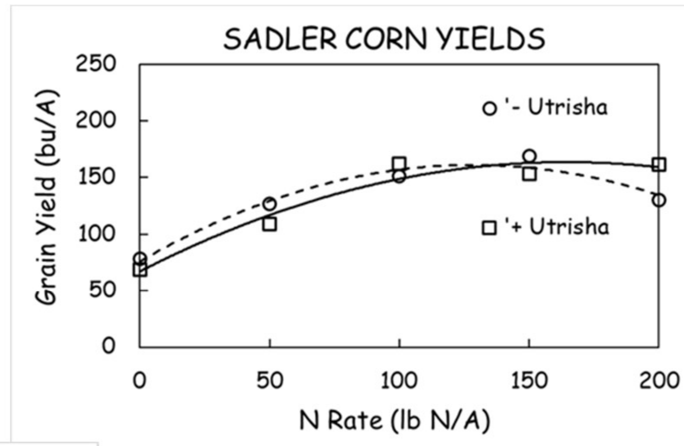
N rate, lbs/a	Corn Yield, bu/a			
	No additive	Envita	Utrisha	ProveN 40
60	130 bcd	148 ab	120 cd	119 d
110	154 a	148 ab	152 a	137 abc
180	160 a	145abc	139 abcd	154 a
Mean	148NS	147	137	137
Check	128 d			

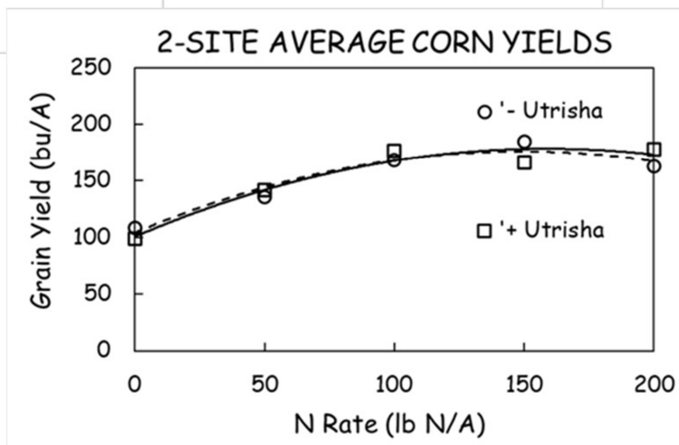
Kansas State trials, Manhattan, KS, 2020
no differences with or without.



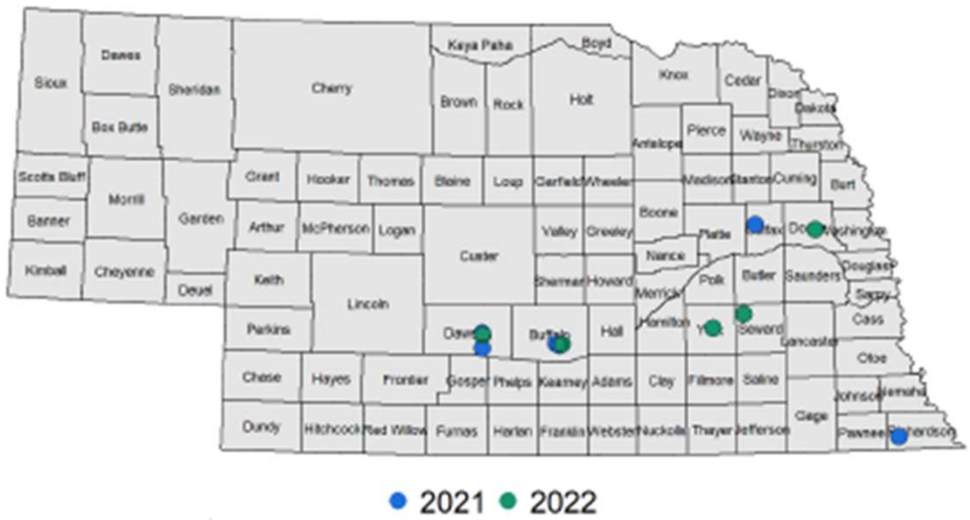
University of KY trials, 2 sites with very different soil series no difference with or without Utrisha foliar.







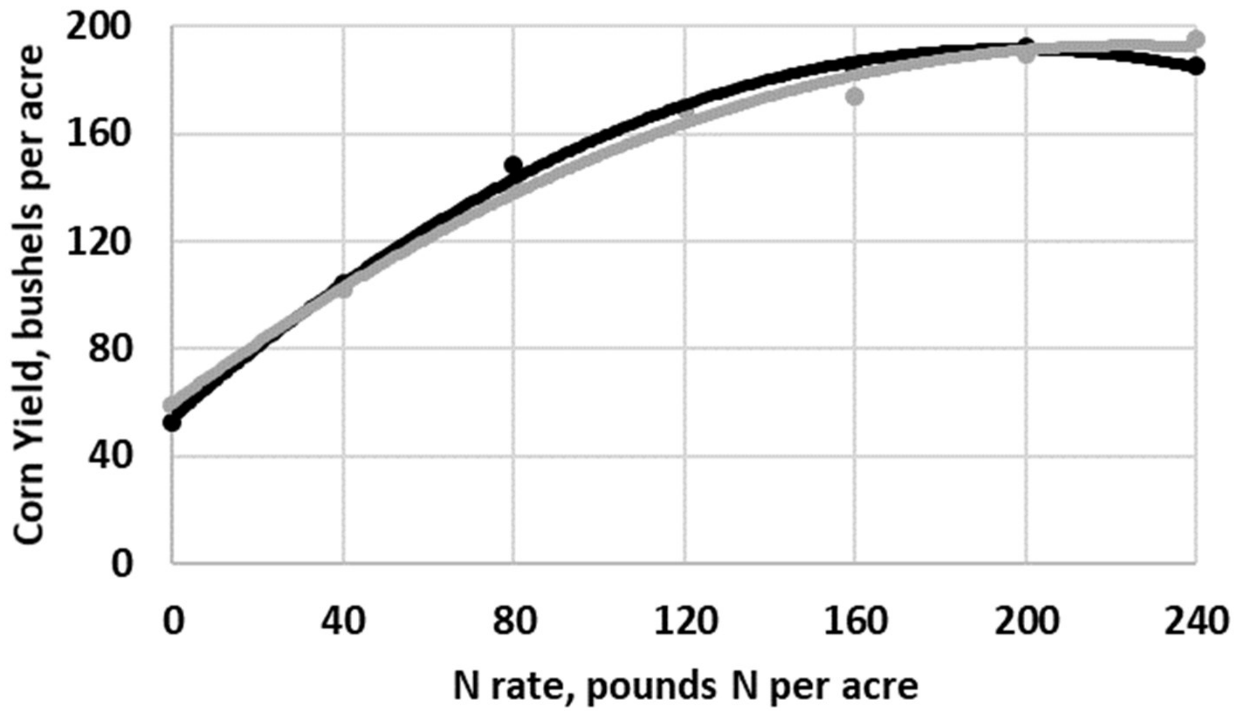
Nebraska on-farm network, data from L. Thompson, Univ of NE Ext.



5 sites with Prove N (2021) no differences in corn yield

6 sites with ProveN 40 (2022) no differences in corn yields

Ohio State Utrisha N rate experiment, 2022, Hoytville, OH
No difference between Utrisha (black) and without (gray).



Summary of results from 10 states.

No means no difference between same N rate with or without additive

Yes means a yield increase present at least 1 N rate

State	Envita IF†	Utrisha	ProveN	ProveN 40 IF	ProveN 40 ST
	Number of site years included in evaluations				
ND	4 No	4 No	--------	--------------	--------------
MN	1 No	--------	3 No/1 Yes	---------------	---------------
IL	2 No	--------	4 No	5 No	2 No
IN	1 No	--------	----------	-------------	--------------
MO	2 No / 1 Yes	3 No	2 No	1 No	-------------
KS	---------	--------	1 No	-------------	-------------
MI	1 No	1 No	-----------	1 No	------------
KY	-----------	2 No	----------	------------	------------
NE	-----------	--------	5 No	6 No	-------------
OH		1 No			
Total	11 No/1 Yes	11 No	15 No/1 Yes	13 No	2 No

Total corn experiments 54.

52 no benefit to yield over N rate alone.

2 benefits with N rate benefits 12-20 lbs N/a

Growers should be skeptical about new products

Point # 2-

Try new products and ideas on replicated strips on the farm.

Refer to L. Thompson, 2022 from
Proceedings of the North Central Extension-Industry Soil Fertility Conference
for ideas regarding on-farm testing and data analysis.

PROMOTING ADOPTION OF PRECISION NITROGEN MANAGEMENT TECHNOLOGIES THROUGH ON-FARM RESEARCH

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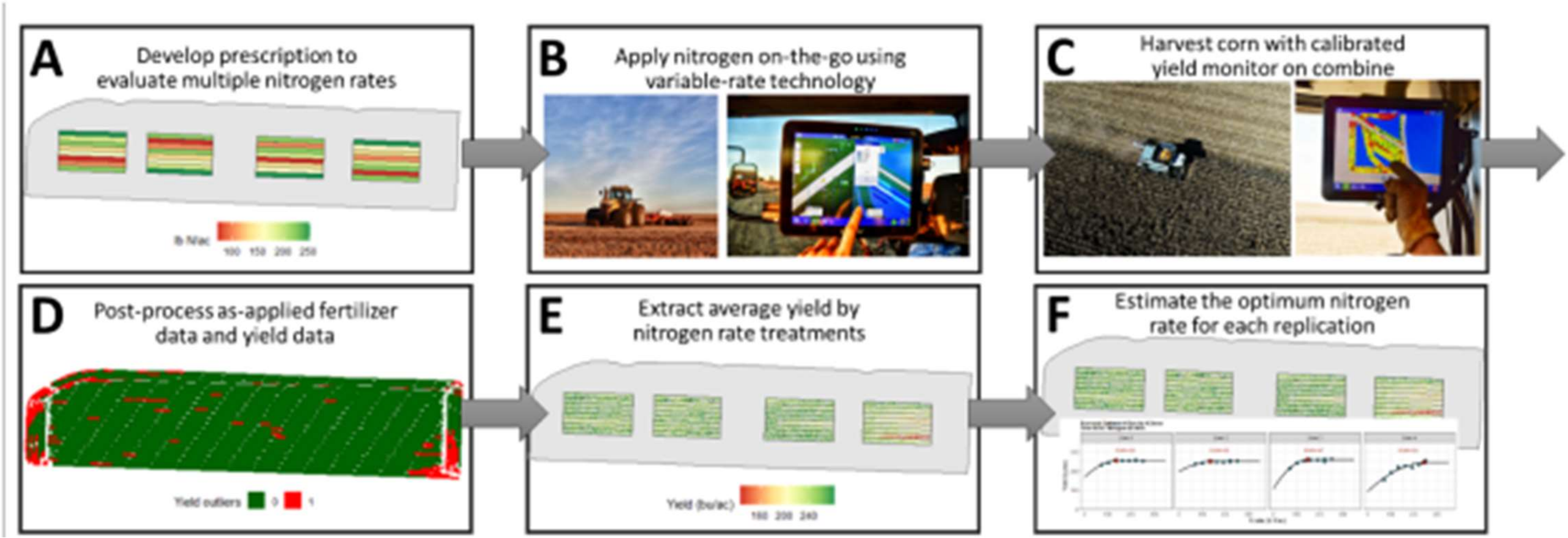
ABSTRACT

The Nebraska On-Farm Research Network helps farmers evaluate products and practices that impact the productivity, profitability, and sustainability of their operations. There are many technologies that have potential to increase nitrogen use efficiency (NUE) on corn and winter wheat but typically these technologies have low adoption. Concurrently, farmers have technologies such as GPS, yield monitors, and variable-rate application equipment on their farmers that enables them to easily conduct on-farm research to evaluate new technologies and products. Participating farmers evaluated commercially available nitrogen (N) management technologies across Nebraska and their impact on yield, profit, and NUE. We enabled farmer's hands-on experience with technologies that are relevant for their operation and promoted technology adoption. We also collected field data to validate and improve the technology tested. 40 trials are established each year in the three-year project. We utilized an innovative experimental design combining traditional strip trials with small N plots where all treatments are established with variable-rate fertilizer equipment on-the-go. An automated data processing tool was developed for data processing, analysis, and reporting. 98% of the experiments were successfully established in the first year of the study and 90% were analyzed using the automatic process. To measure impact, grower incremental changes in N management strategy and technology adoption were documented.

INTRODUCTION

Nitrogen (N) is critical for attaining higher crop yields; however, risks of environmental losses necessitate more precise fertilizer management. Predicting the economic optimum N rate (EONR) remains challenging due to spatial and temporal

Examples from Thompson Paper:



https://cropwatch.unl.edu/farmstat

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FarmStat

FarmStat is a statistical analysis tool that provides quick, accurate, and straightforward analysis for data from agricultural experiments. While FarmStat is provided at no cost, we do require you to register with your name and email address.

First Name Required

David

Last Name Required

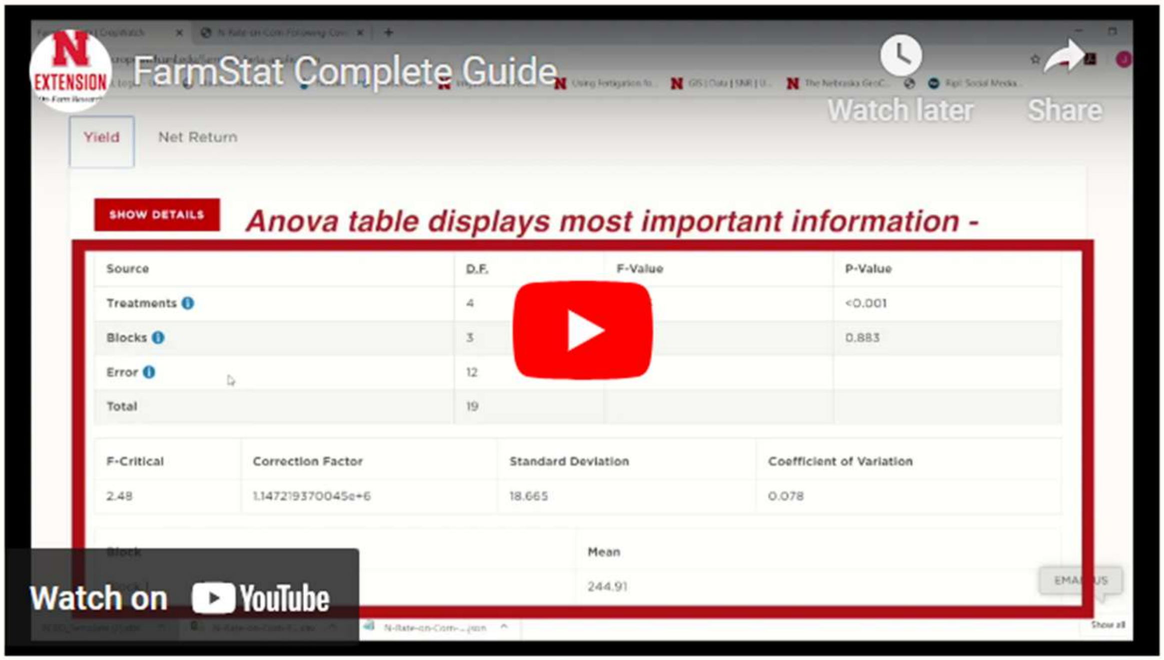
Franzen

E-Mail Required

david.franzen@ndsu.edu

FarmStat Beta Welcome

Thank You



Download FarmStat User Guide | Download CRD Excel Template | Download RCBD Excel Template https://cropwatch.unl.edu/farmstat-welcome

Farmers have GPS
Farmers have GPS yield monitors

Farmers have everything they need to replicate treatments and test product effectiveness.

Point 3-

There should be a quick method of analysis developed to determine whether the organism is alive and functioning in the container, the field or the plant.

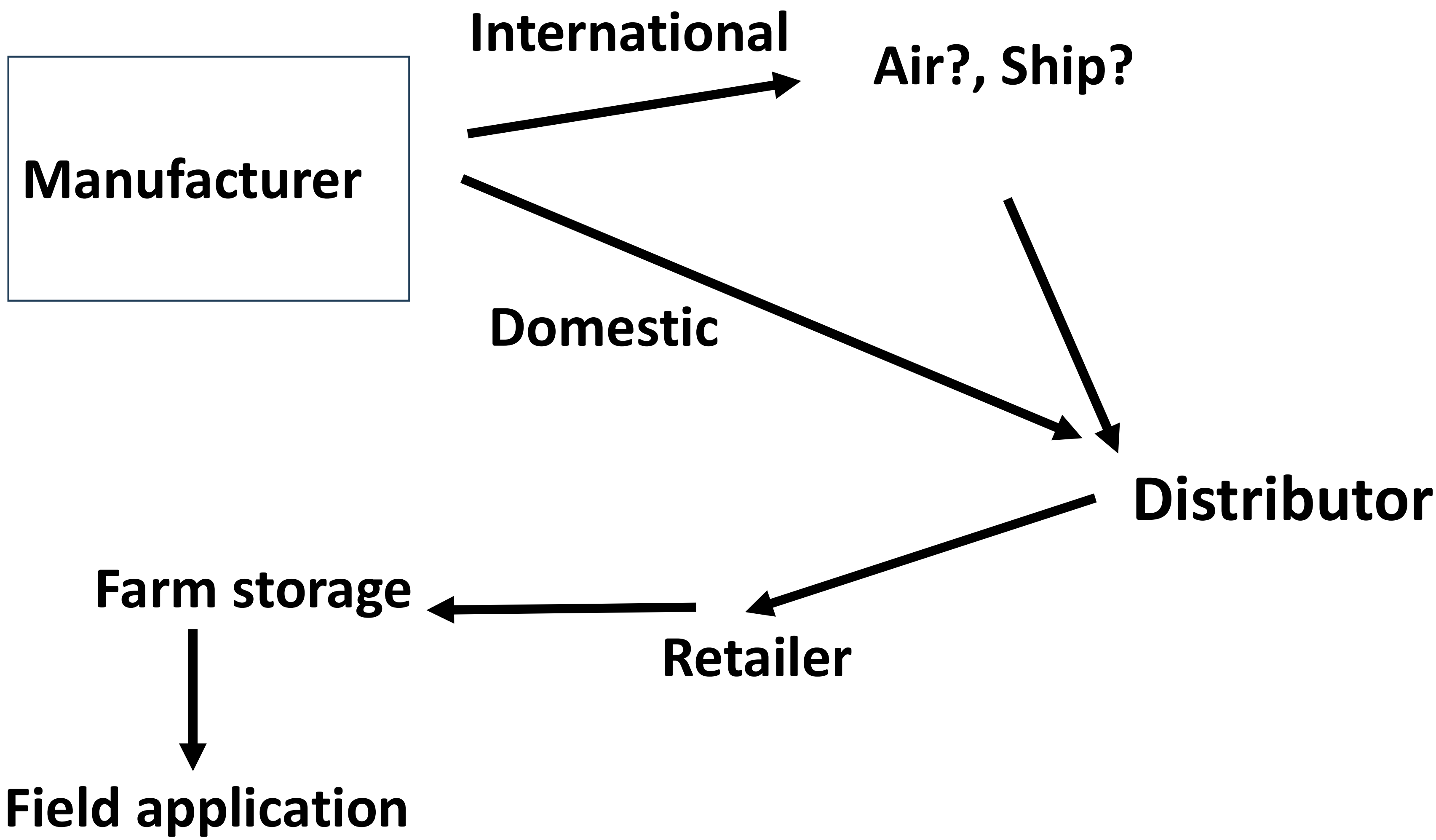
Point 4-

Organisms need to be kept alive through transportation and storage intervals between manufacturer, shipper, warehouses, distributor, dealer and on the farm awaiting application.

Storage and Disposal

Storage Conditions: Keep the product in a well-ventilated place. Store at room temperature, do not subject to temperatures below 39 °F (4 °C). Keep the product hermetically closed.

STORAGE AND HANDLING: DO NOT FREEZE. This product contains live non-pathogenic organisms. Store between 39°F to 46°F. Store in well ventilated buildings, away from foodstuffs and animal feed. Keep out of reach of children. Keep out of direct sunlight. DO NOT open product container until ready for use.


International
Air?, Ship?
Manufacturer
Domestic
Distributor
Farm storage
Retailer
Field application

Point 5-

Organisms should be able to compete and ‘win the war’ with native microorganisms in order to survive and perform its function.

Point 6-

Organisms should be adapted to variable moisture, variable soil pH and variable soil salts in order to perform its function.

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