

# **What an Ag-Professional should know about the effectiveness and consistency of biologicals in aiding N nutrition**

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## **Asymbiotic N-fixing organisms**

**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*.**

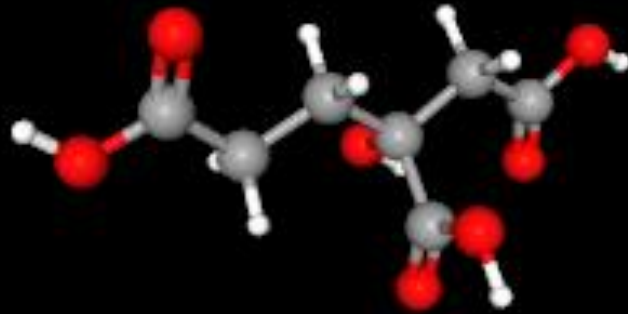
**To convert 1 N<sub>2</sub> to 1 NH<sub>3</sub> requires 16 ATP molecules (produced during photosynthesis) and 8 electrons.**

**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

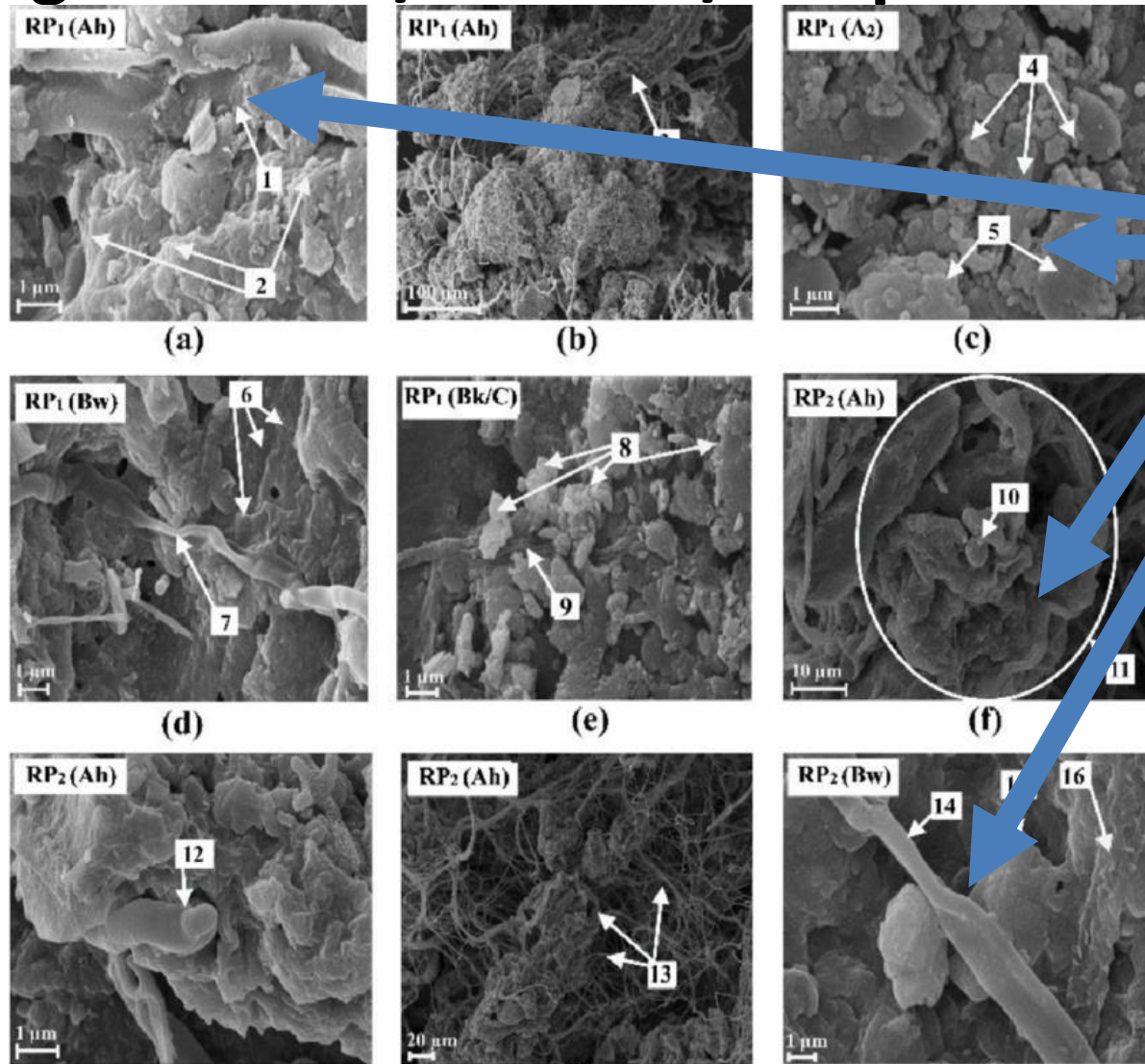


## **Asymbiotic N-fixing organisms**

**Energy sources for asymbiotic N-fixing organisms close to plant roots are the root exudates that surround many roots, and also includes components of soil organic matter and residue 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



**Root Exudates/  
biofilms**

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.

## Examples of asymbiotic bacteria

*Azotobacter* spp

*Clostridium* (certain species)

There are many species, some more efficient than others  
almost all have activity 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.**

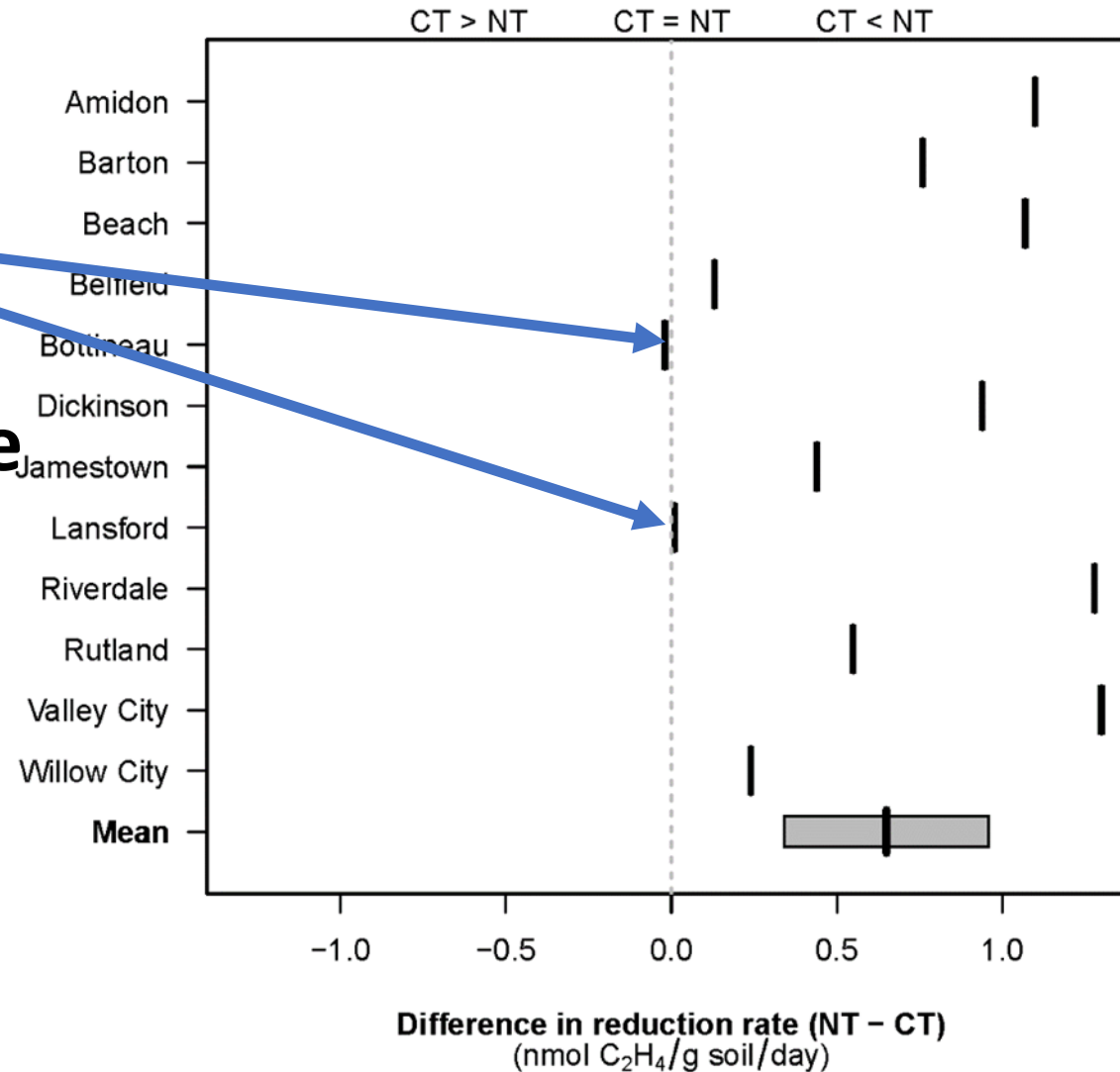
**Part of this credit probably comes from the increased microbial biomass under no-till that protects N from loss.**

**But a part of the credit, perhaps 25-33% may come from greater asymbiotic activity in long-term no-till.**

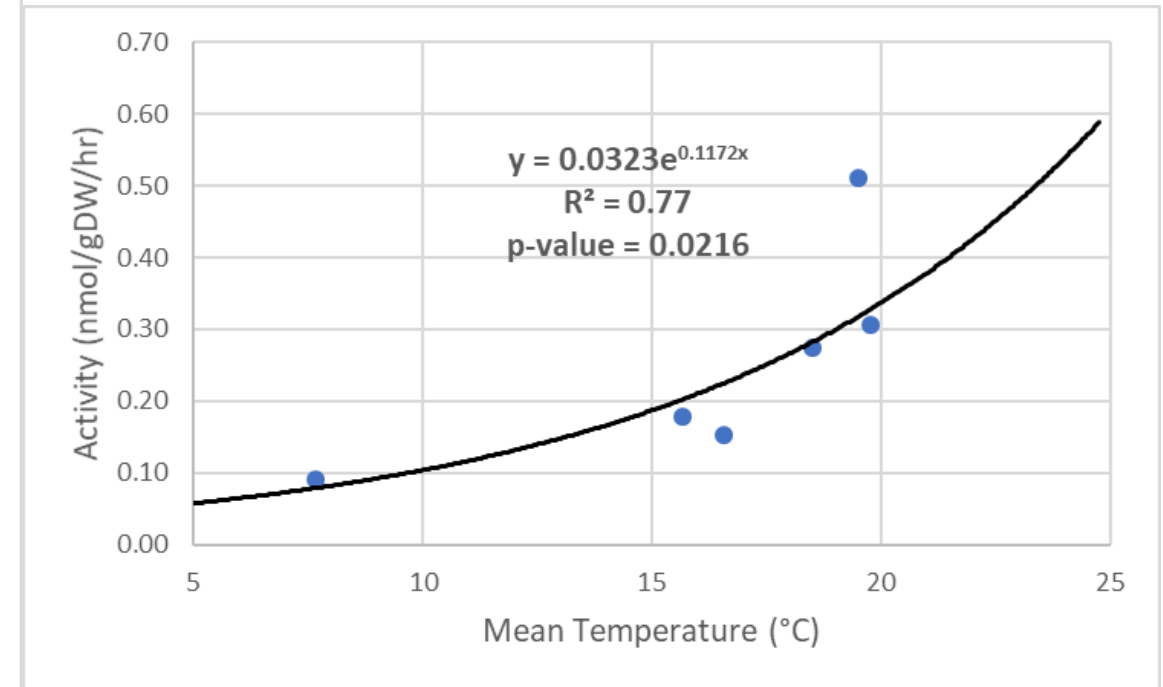
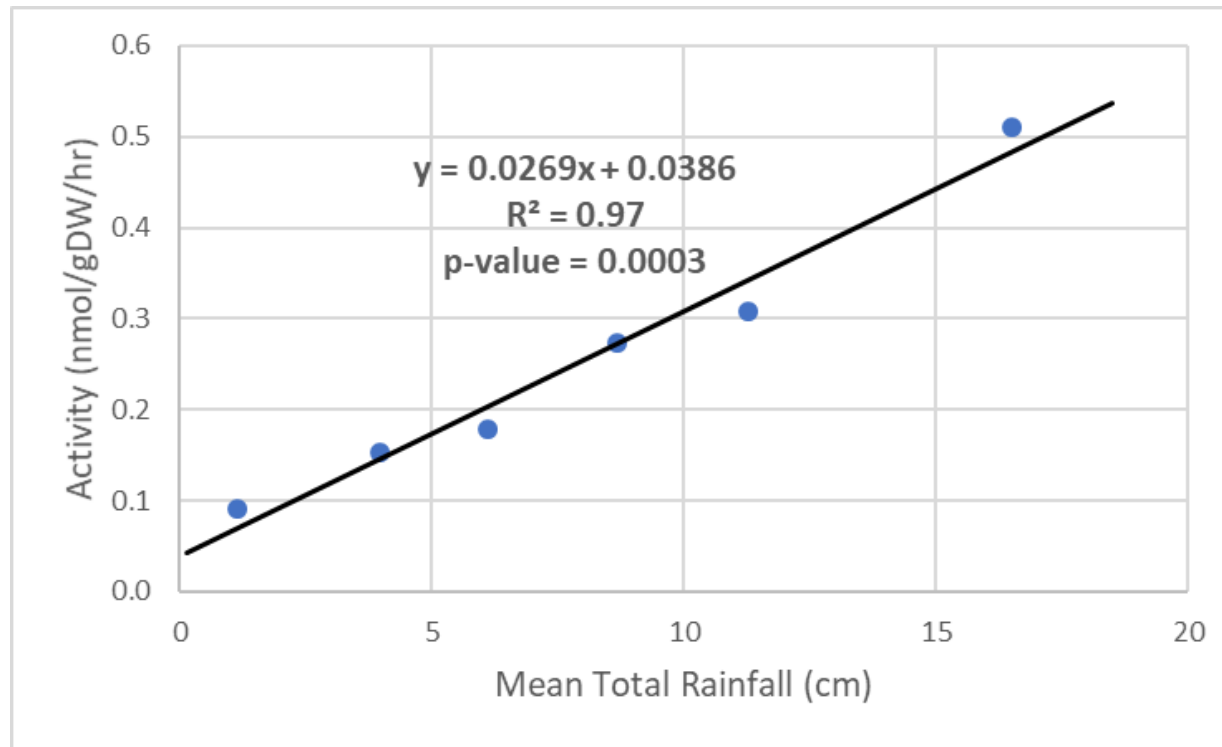
**Franzen et al. 2019, SSSAJ**

Took paired no-till/conventional till across state.

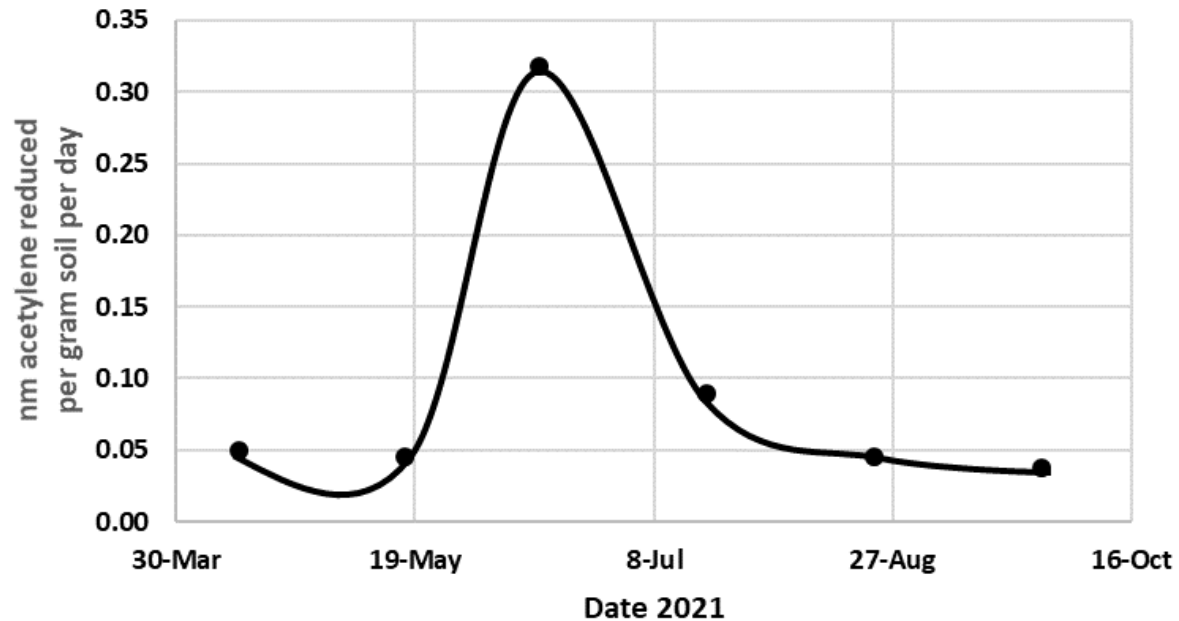
Turns out the 'conventional till' site across the fence was one-pass shallow tillage, so the same tillage category



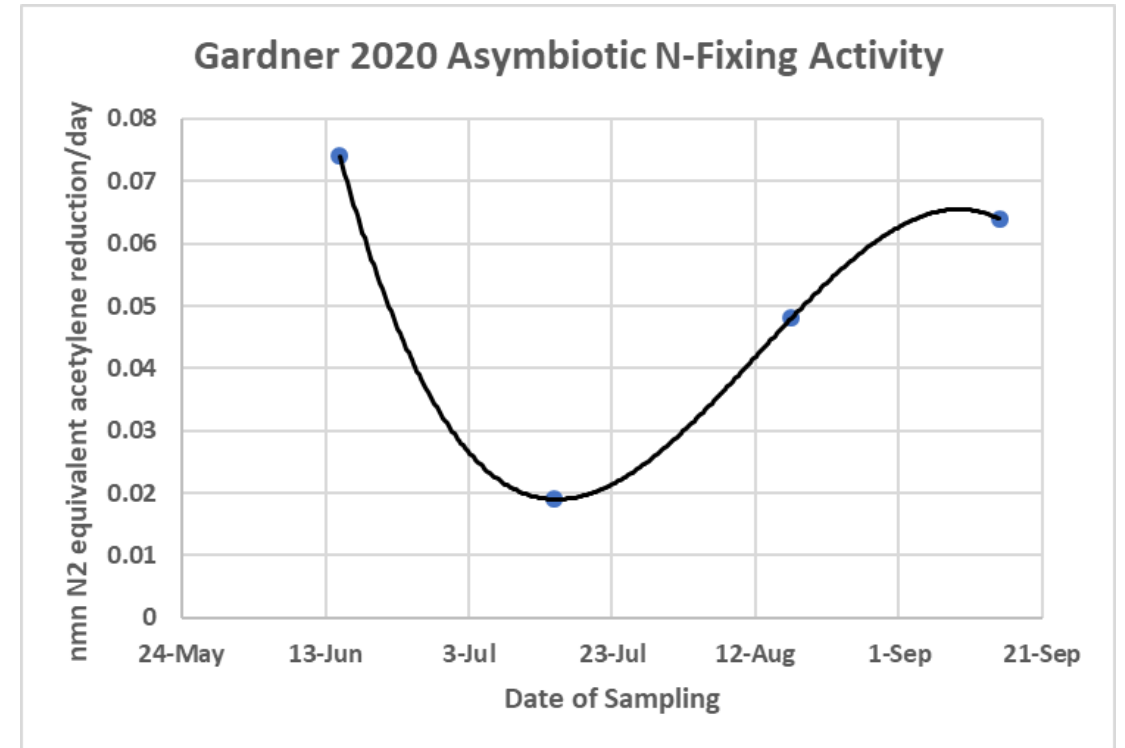
**In 2020 and 2021, 6 sites in eastern North Dakota were sampled each month for asymbiotic N fixing activity. Change in activity was related to rainfall within 30 days before sampling and mean air temperature.**



# 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-**

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

**Moist soil and warm conditions favor N-fixation.  
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*

**MicroAZ-ST, TerraMax**    *Azospirillum lipoferum* and *Azospirillum brasilense*

# **North Dakota studies on Envita and Utrisha**

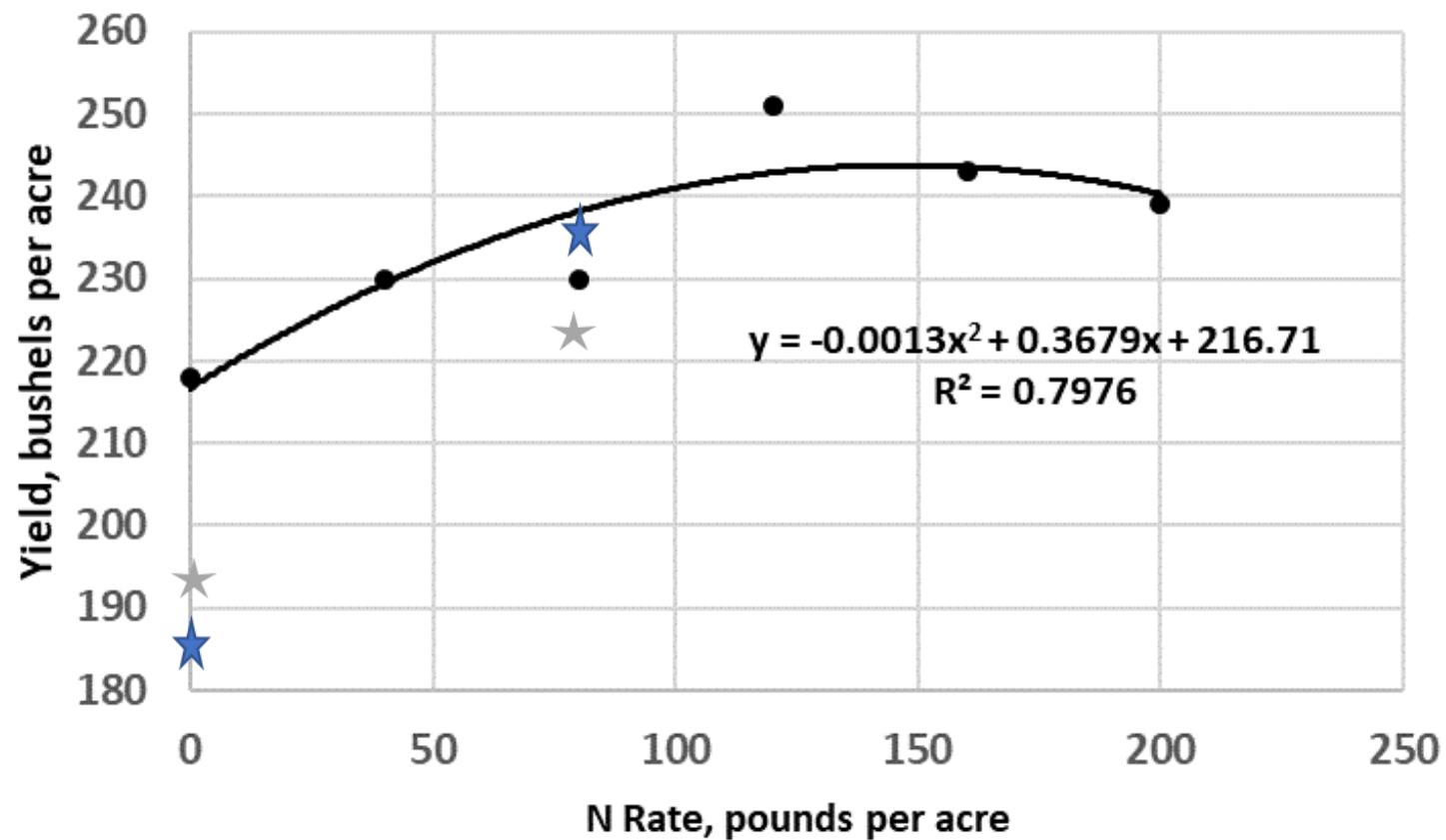
**4 locations- Prosper, Absaraka, Oakes (irrigated), Carrington area**

## **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**

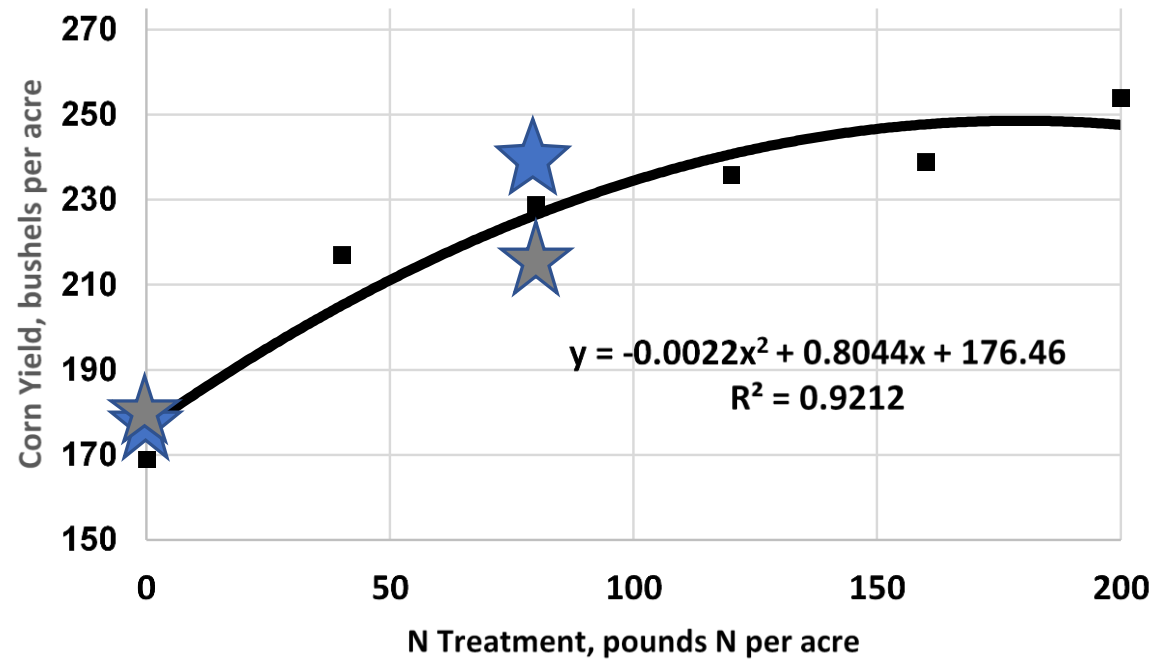




**Absaraka response of corn to N treatment and N rate with additives.**

**Blue stars indicate treatments (0 and 80 lb N/acre) with Utrisha post-applied V6.**

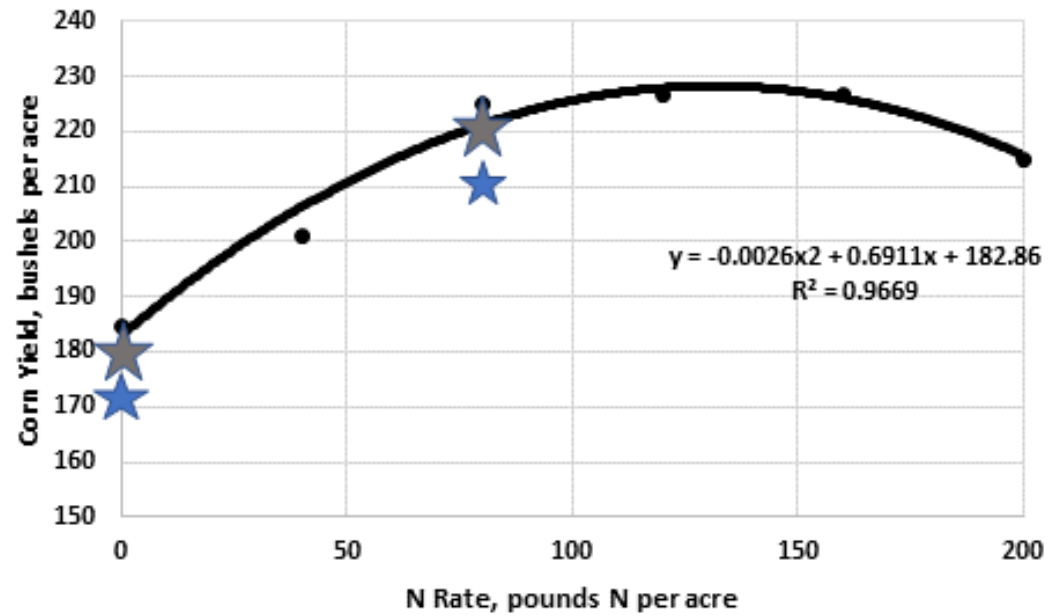
**Gray stars indicate treatments (0 and 80 lb N/acre) with Envita in furrow at planting.**



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.

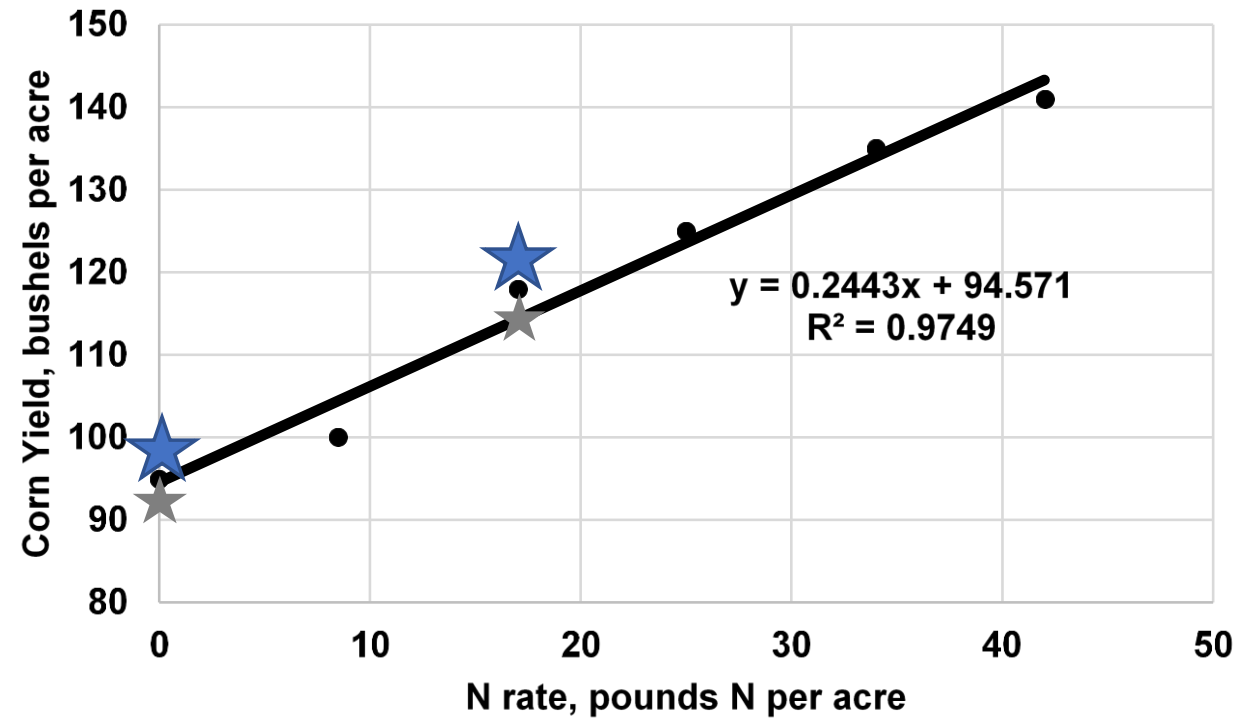
Gray stars indicate treatments (0 and 80 lb N/acre) with Envita in furrow at planting.



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.



Oakes 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.**

## **Experiments from Minot, ND (Bortolon, 2022)**

**Spring wheat with and without Envita foliar  
and Micro AZ seed treatment and foliar- No benefits**

**Canola with and without Envita foliar –No benefit**

**Corn with and without Envita foliar  
and Micro AZ seed treatment and foliar- No benefits**

# **Minnesota trials 2019 and 2020**

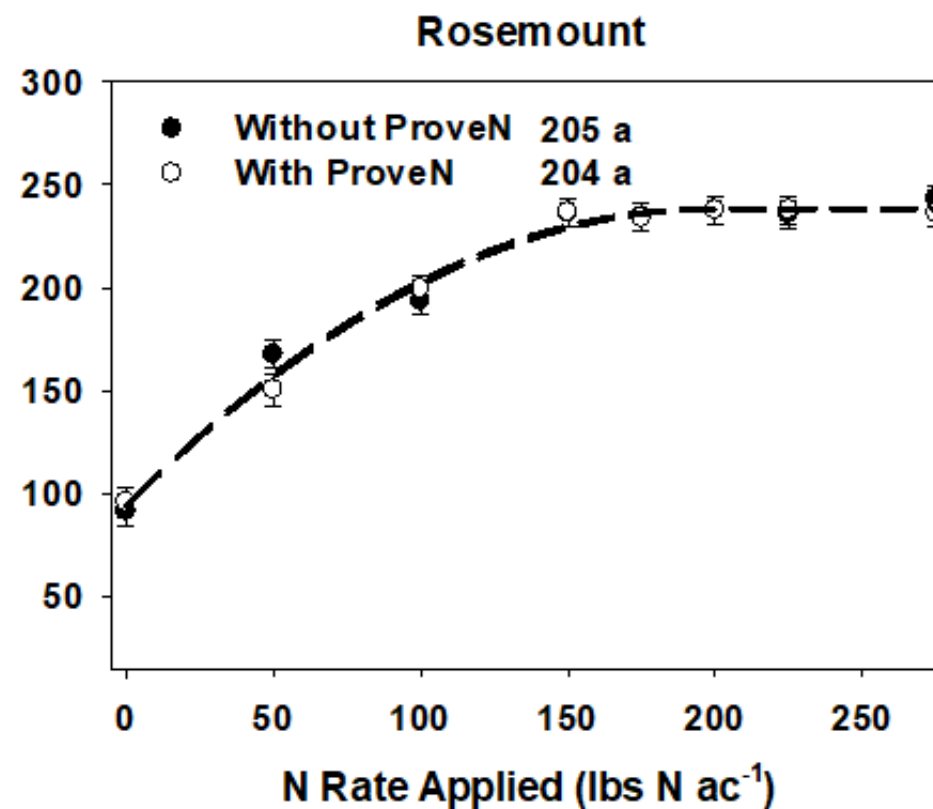
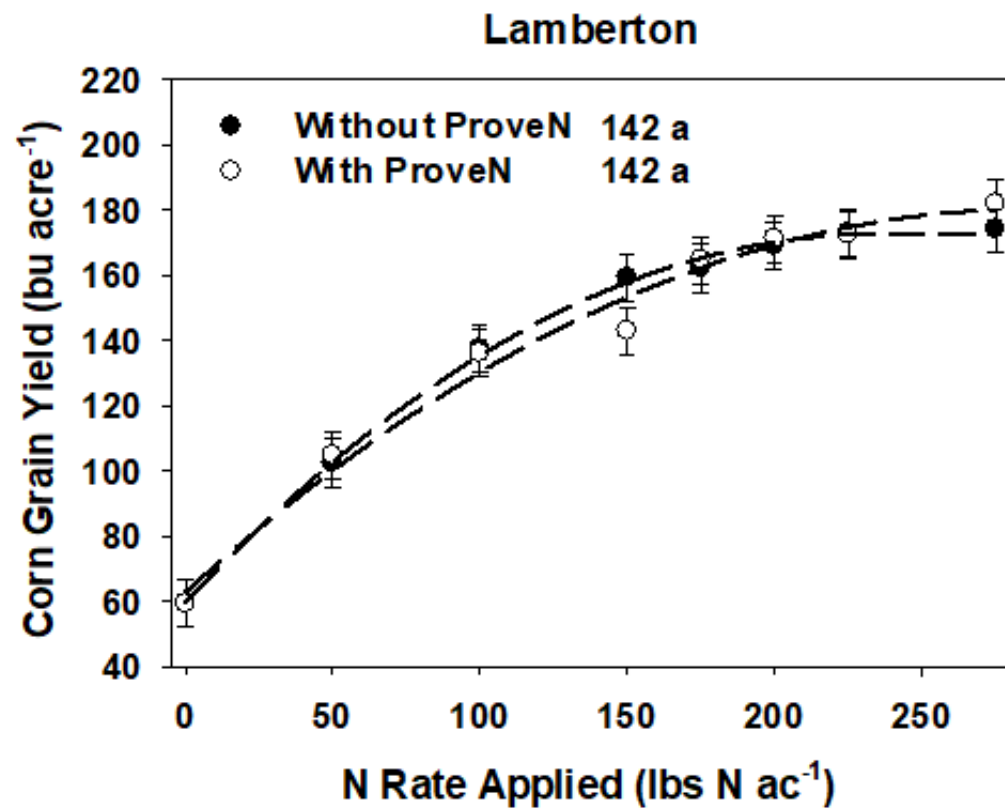
**Currie, Kaiser and Vetsch,**

**2021 North Central Extension-Industry Soil Fertility Conference Proceedings**

**Compared N rates with and without ProveN**

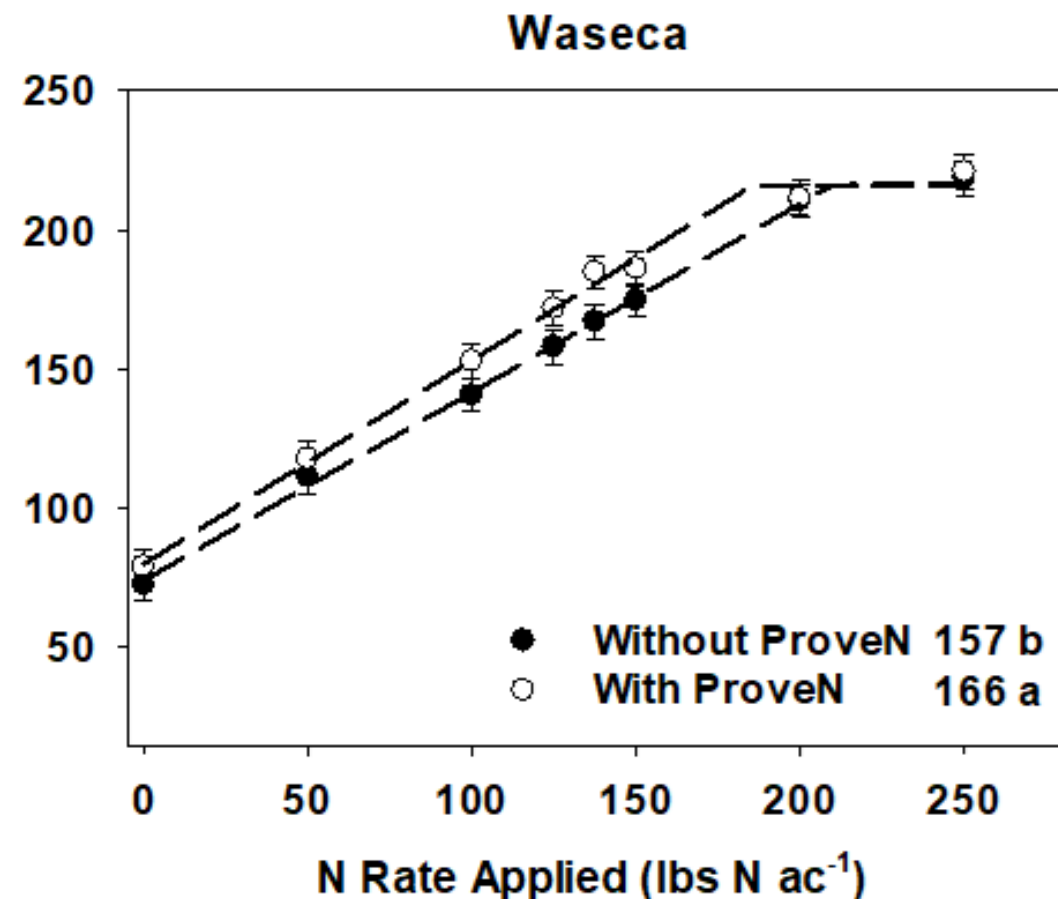
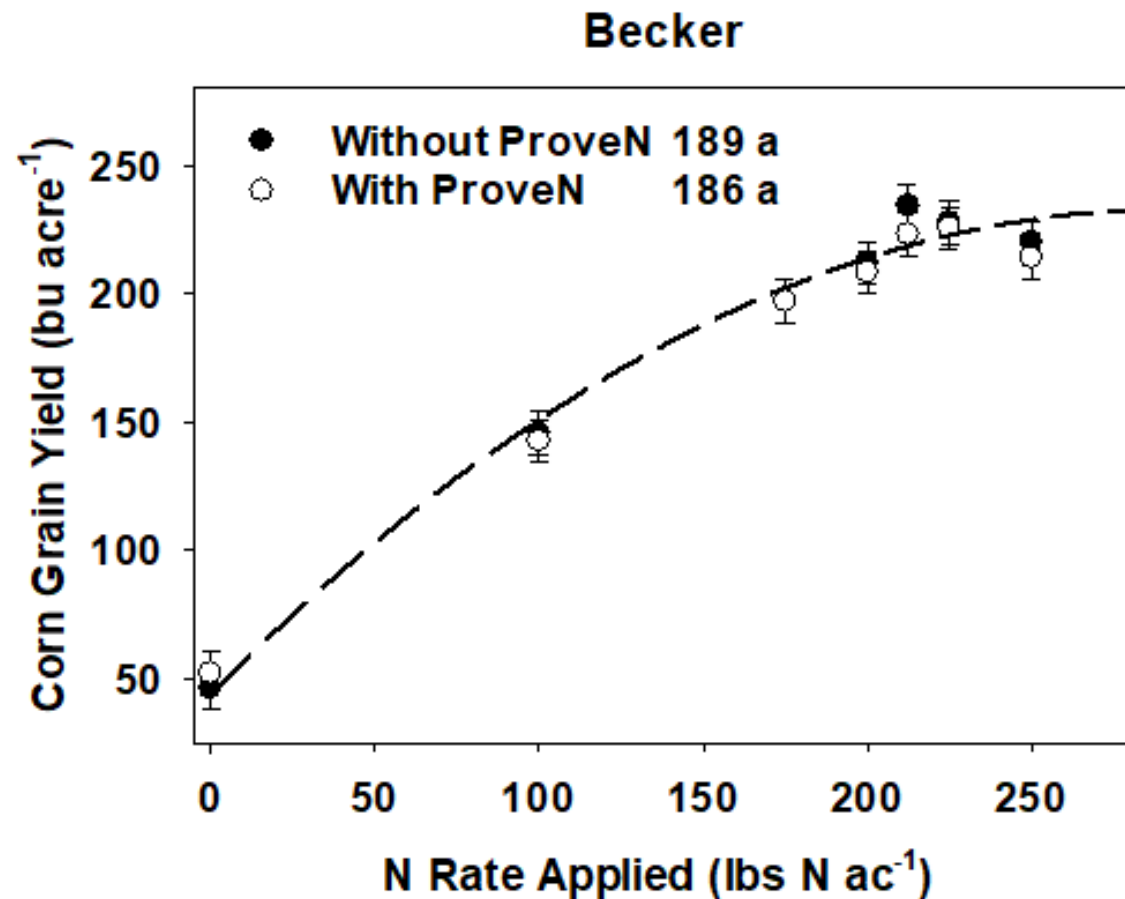
**2 locations in 2019, Lamberton and Rosemount**

**2 in 2020, Becker and Waseca**



**No yield response to ProveN at 2 sites in 2019**





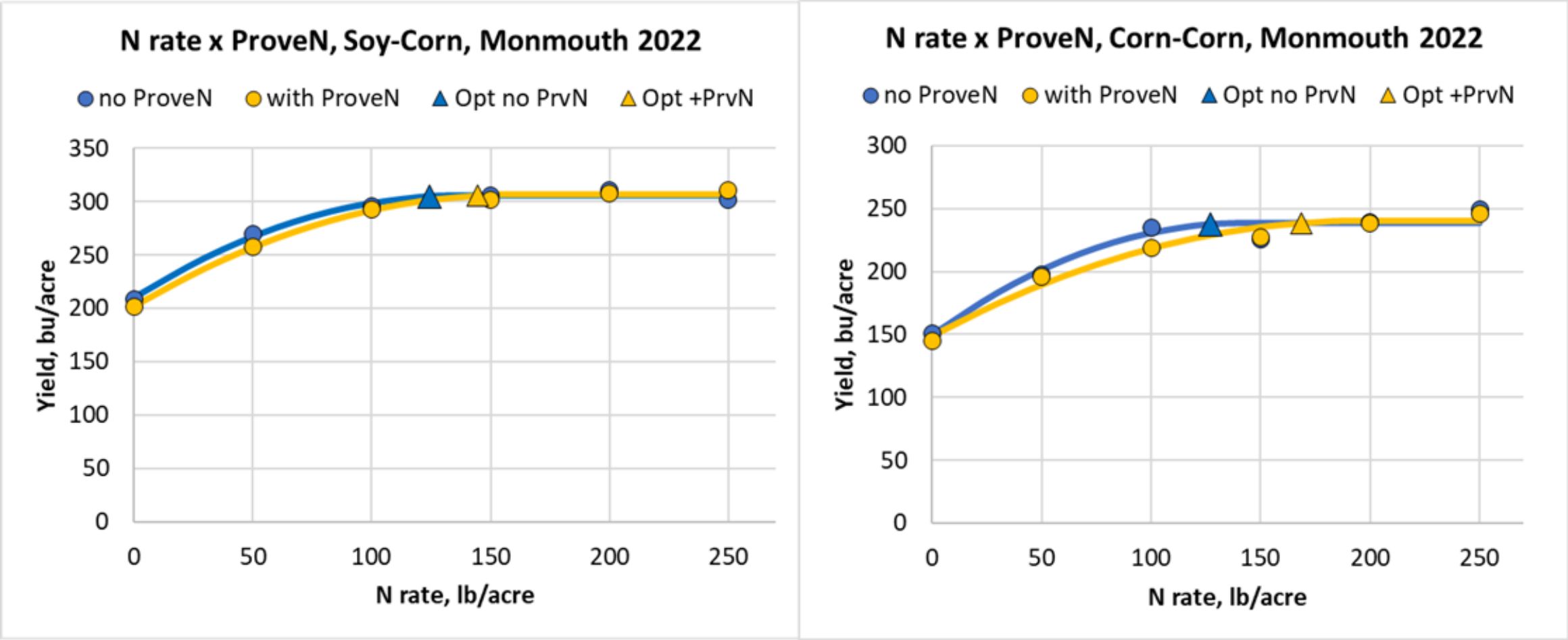
**No response to ProveN at Becker in 2020**

**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, ProvenN in split.

## **Monmouth**

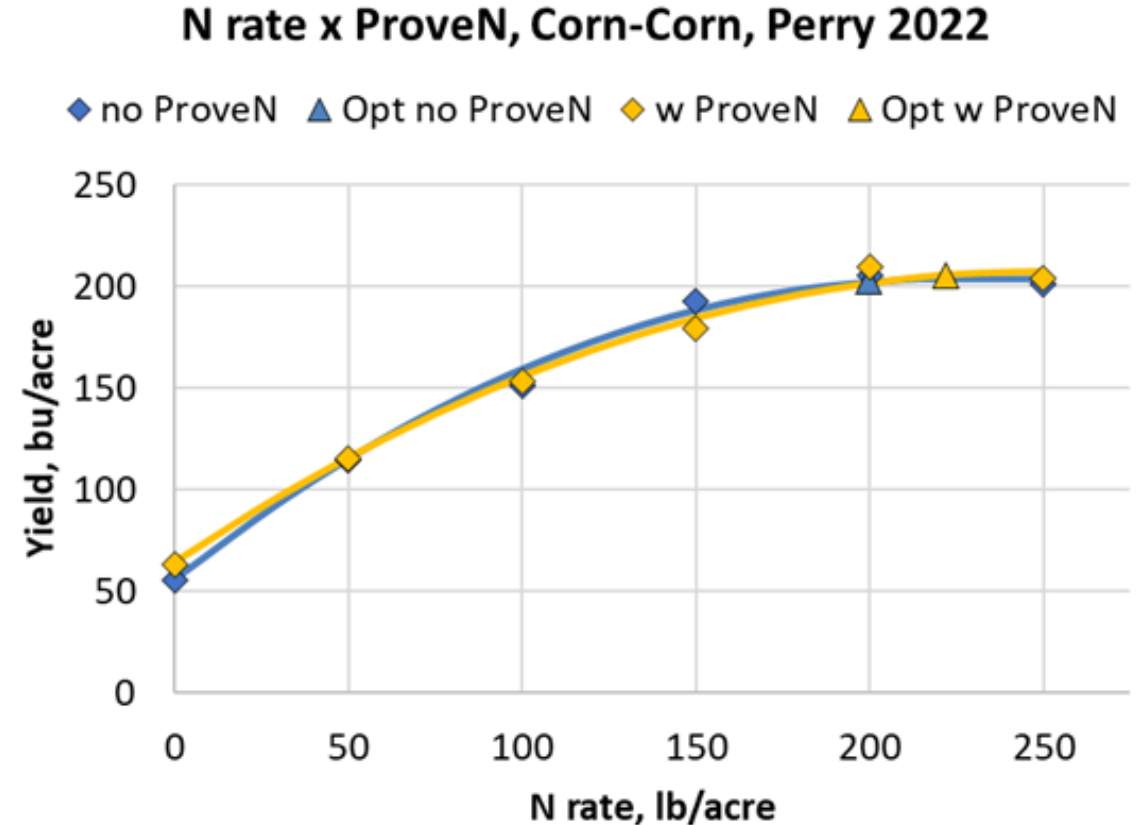
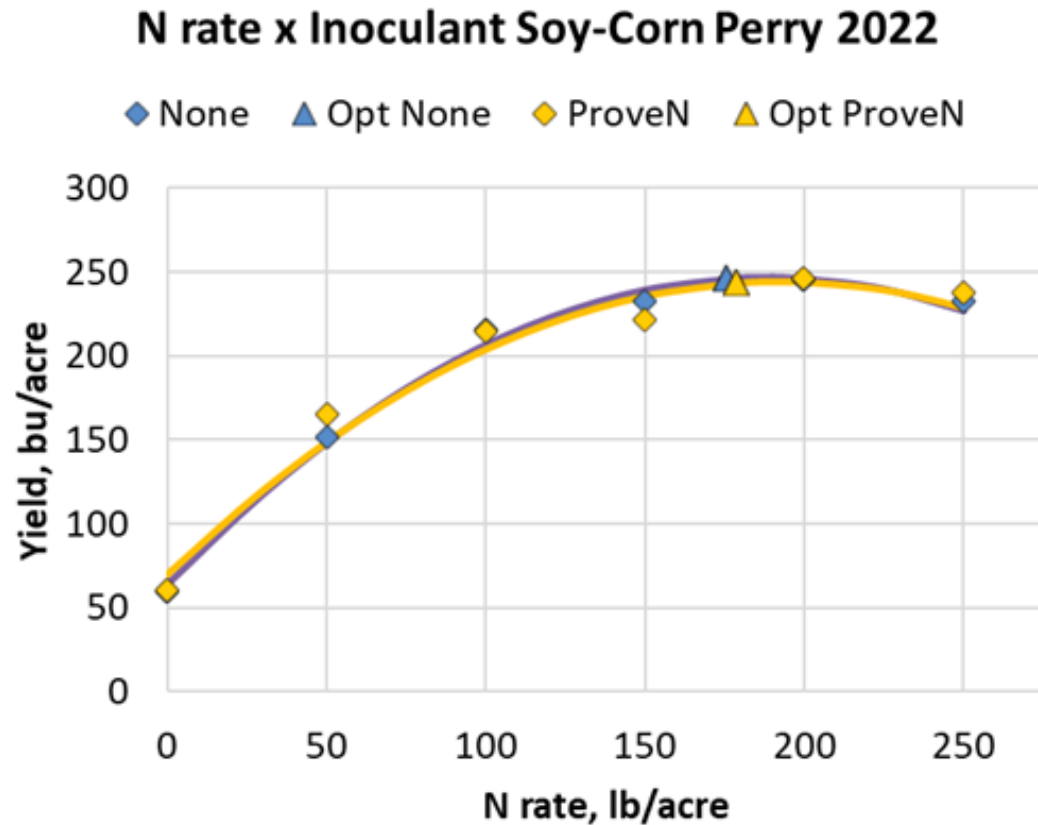
**Soybean-corn, No main effect of ProveN or  
Nrate X ProveN interaction.**

**Optimum N rate without 124 lb/acre**

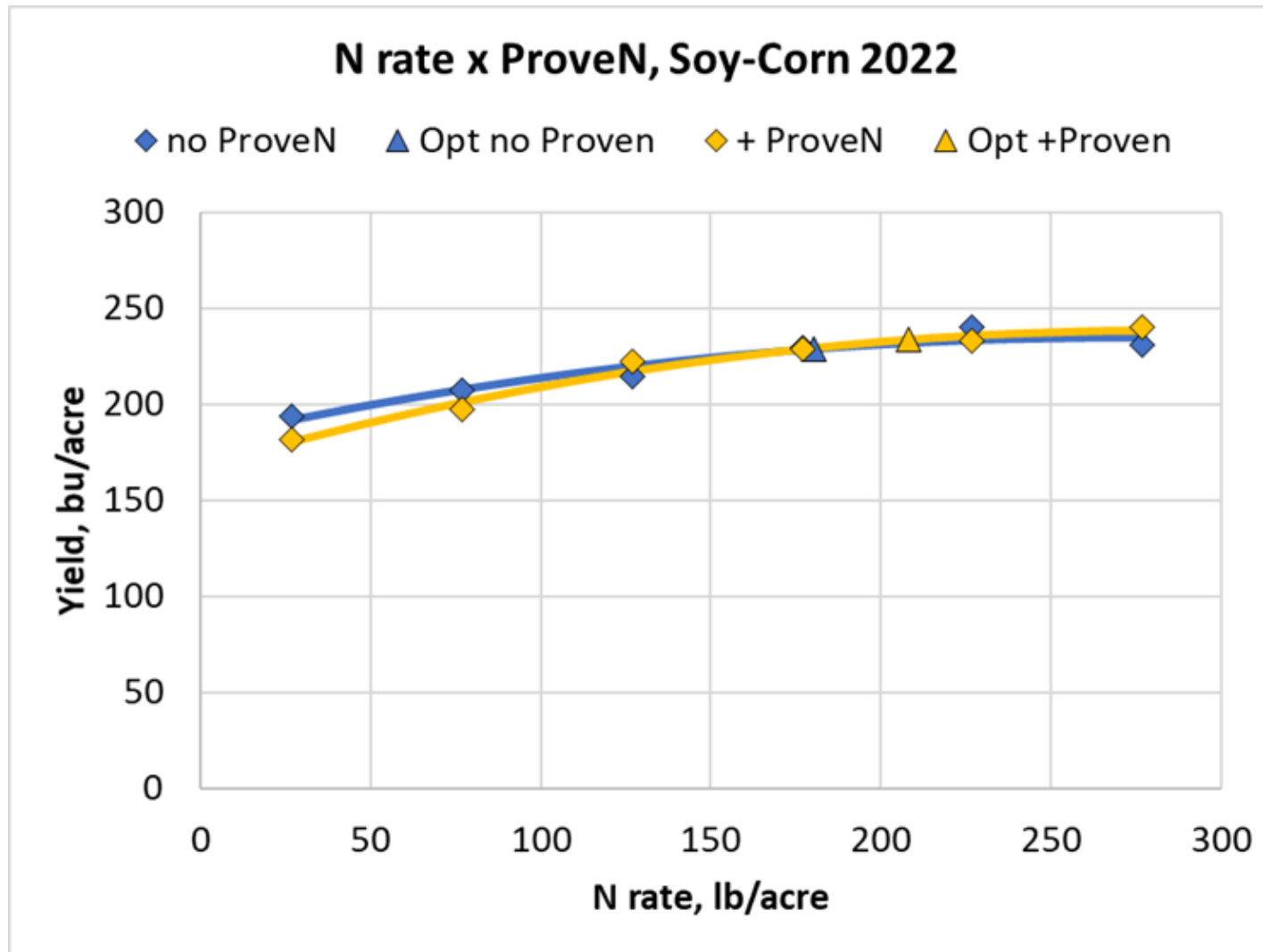
**Optimum N rate with Prove N 144 lb/acre**

**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 Proven N. Perry is in WSW Illinois**



**Similar split plot at Marion (southern Illinois)**  
**No differences in yield, optimum N rate**

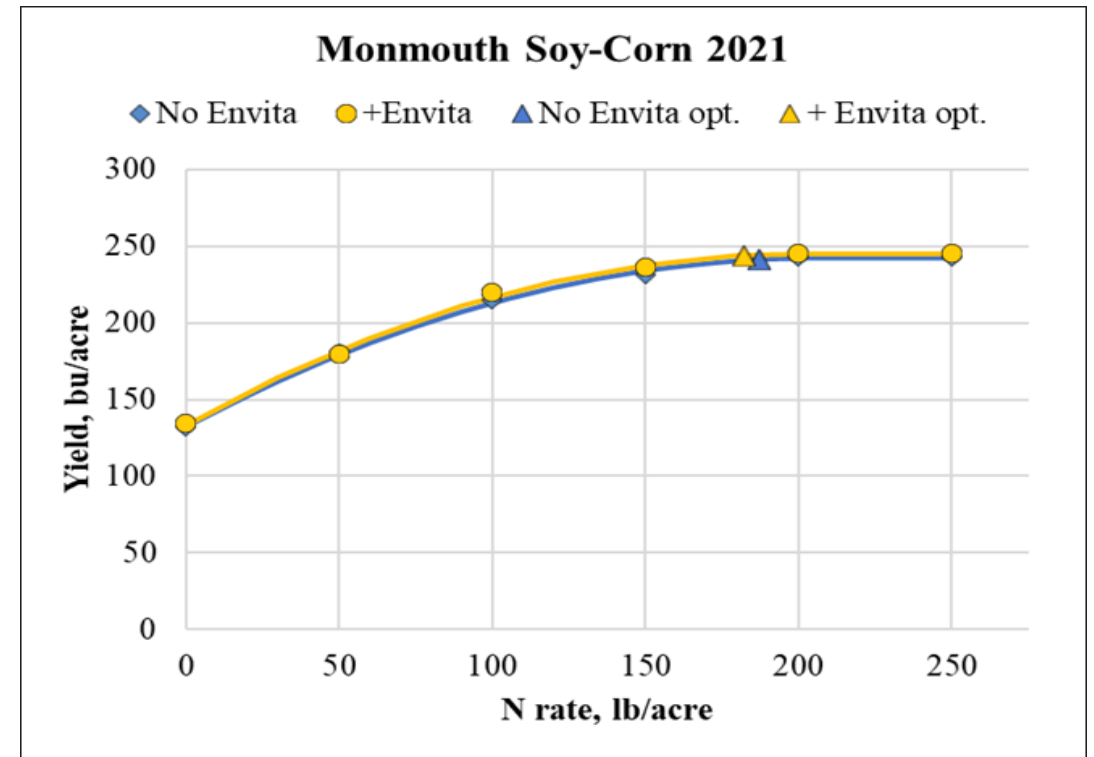
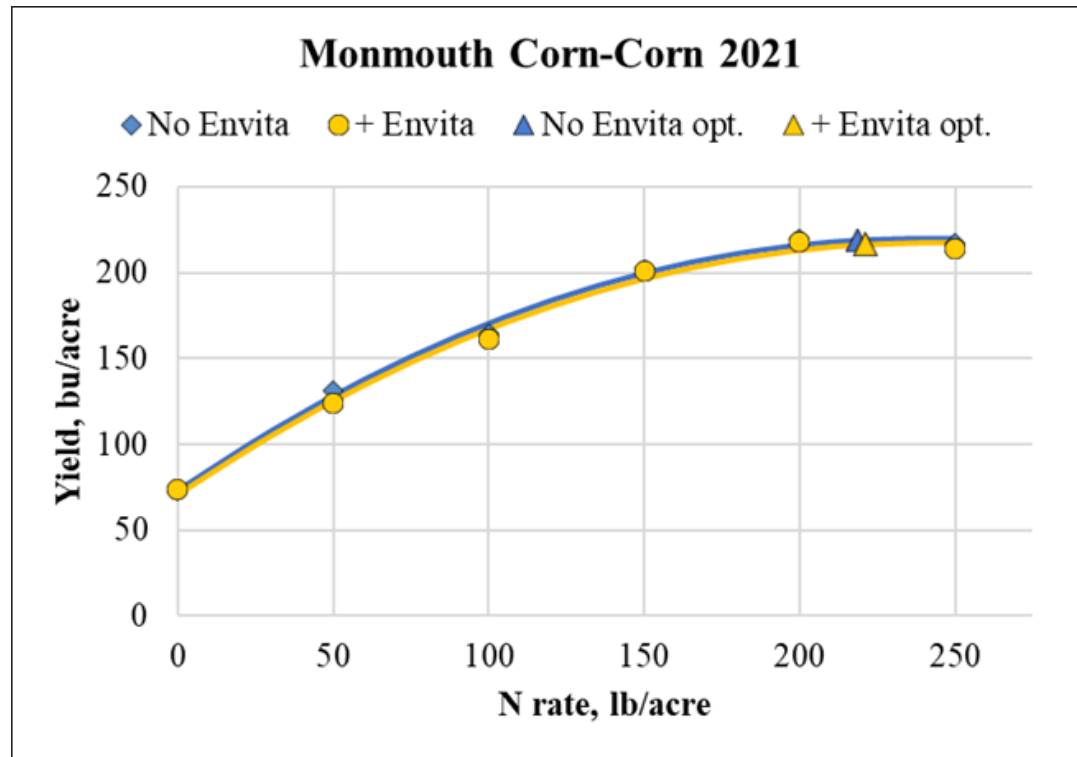
## **Strip trial-**

**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.**

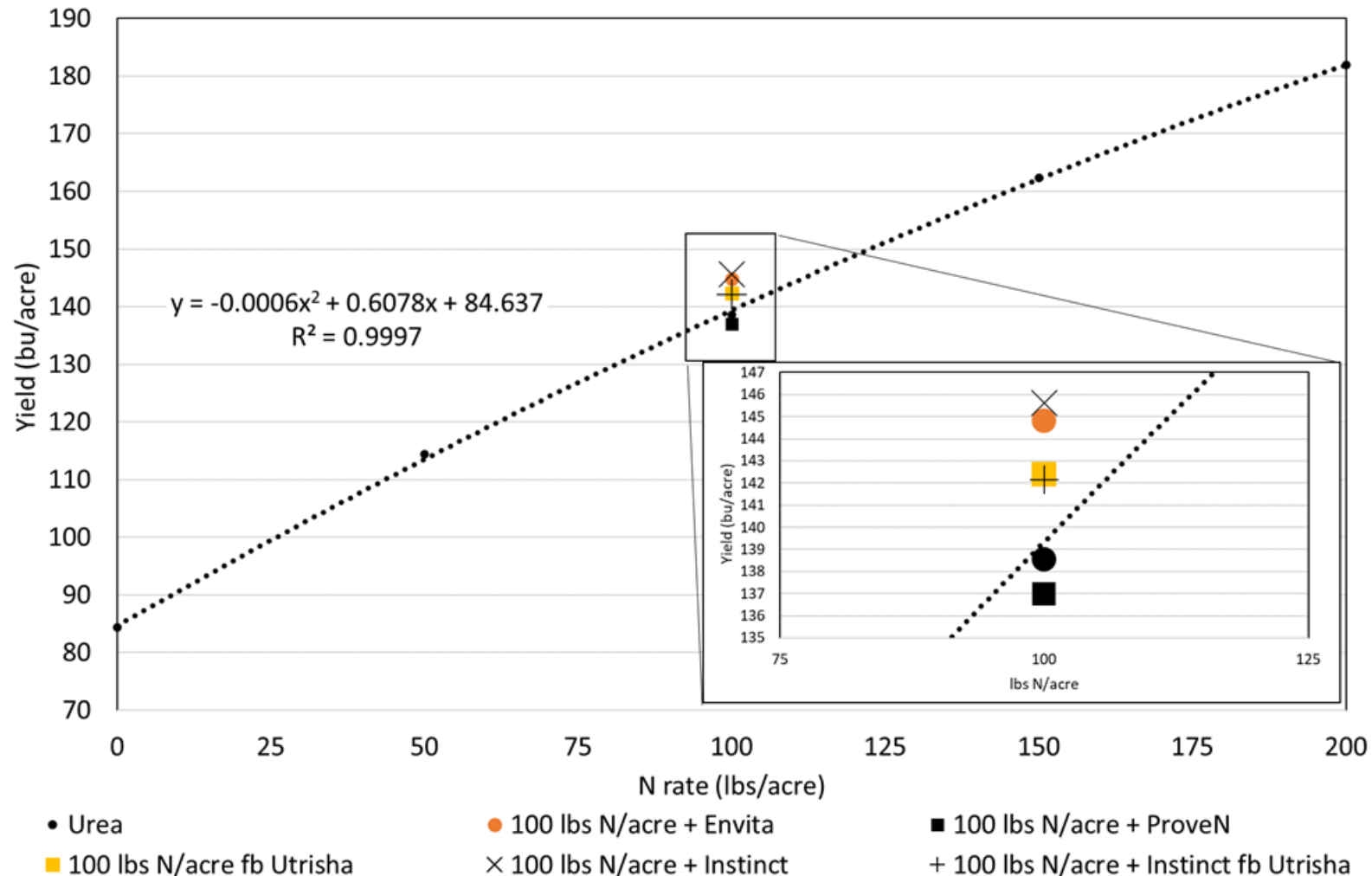
<b>Seed Treatment</b>	<b>N rate lb/acre</b>	<b>Yield, bu/acre</b>
<b>Untreated</b>	<b>150</b>	<b>199 a</b>
<b>Untreated</b>	<b>200</b>	<b>213 a</b>
<b>Treated</b>	<b>150</b>	<b>201 a</b>
<b>Treated</b>	<b>200</b>	<b>214 a</b>



**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 benefit 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**

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

**Camberato, 2020**

# Minnesota experiments on sugarbeet with BioRed & BioMate *Azotobacter vinelandii*, *Clostridium pasteurianum* (plus sugars and *Lactobacterium* sp)

	Sugarbeet Root Yield		Recoverable Sugar		Recoverable Sugar Yield	
Biostimulant	Crookston	Wood Lake	Crookston	Wood Lake	Crookston	Wood Lake
	--tons/a--		---lbs/ton---		----lbs/a----	
None	23.9	35.9	306	299 ab	7314	10670
Bio Red + Bio Mate	22.9	34.6	308	295 b	7093	10227
High Tide	25.3	34.7	304	301 a	7701	10474

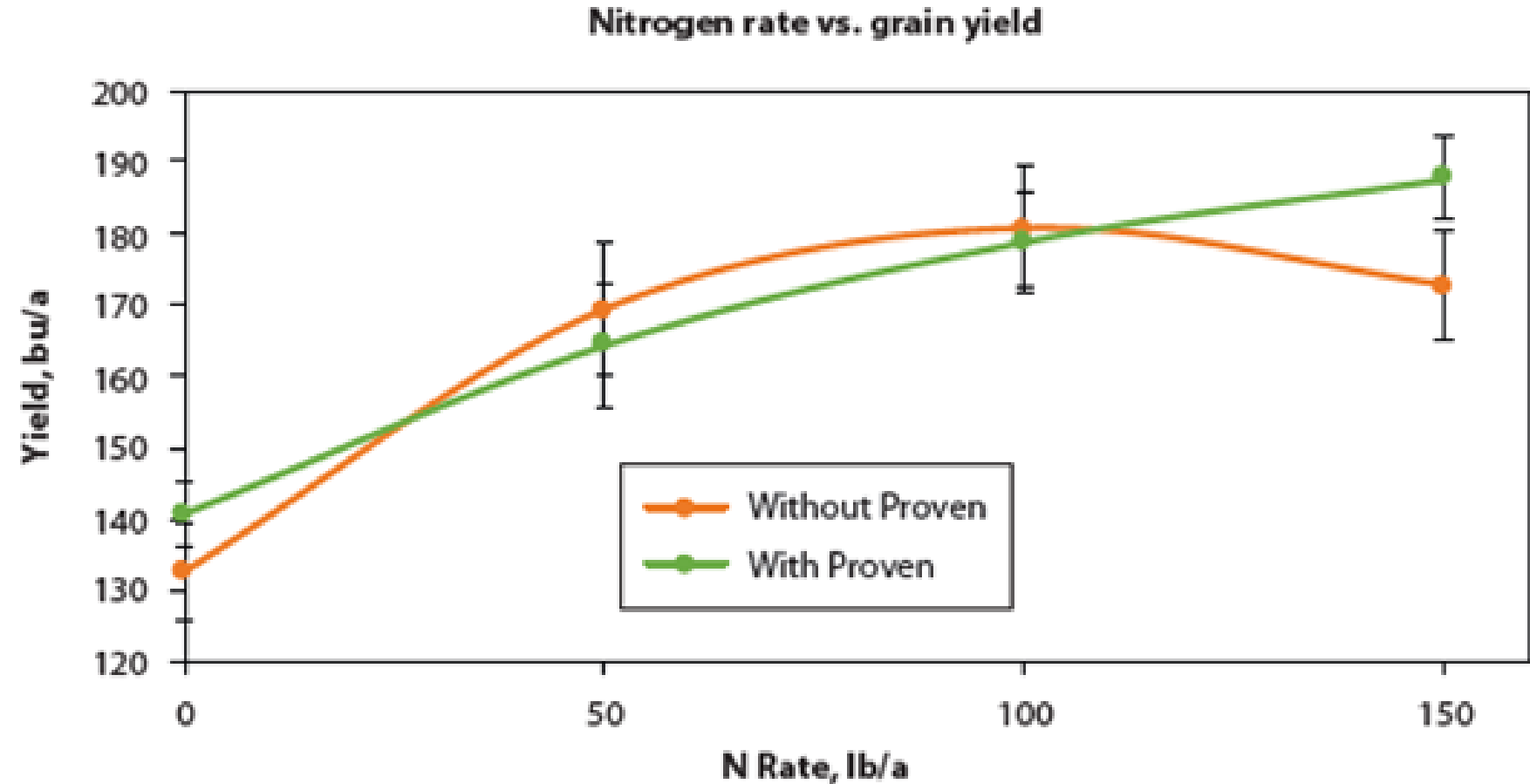
No improvement of Sugar yield or tonnage yield with additives over N rate.

# Michigan State University trials 2022

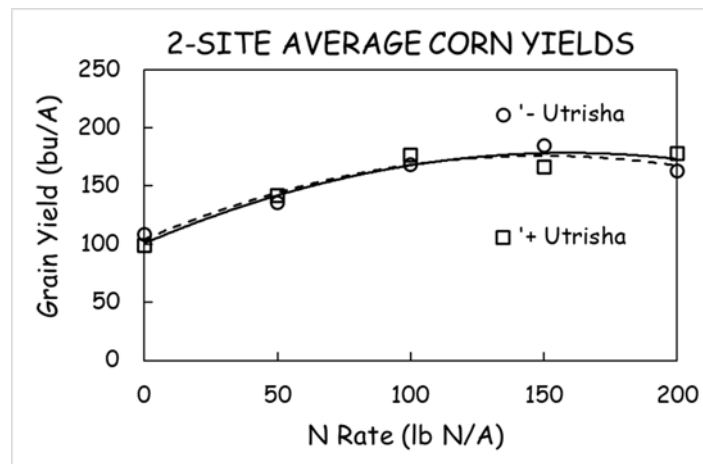
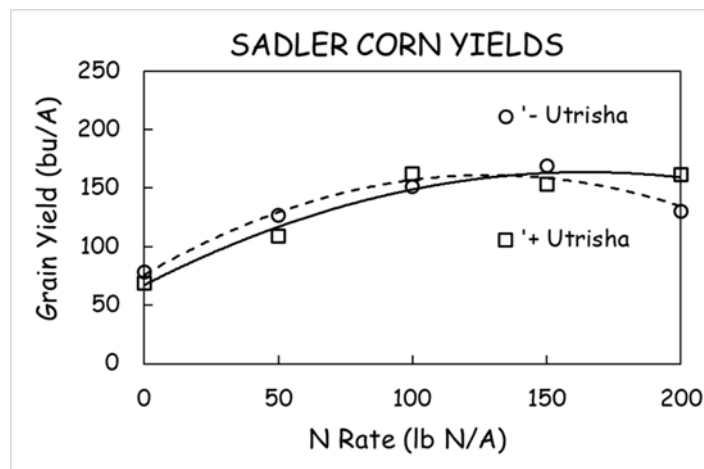
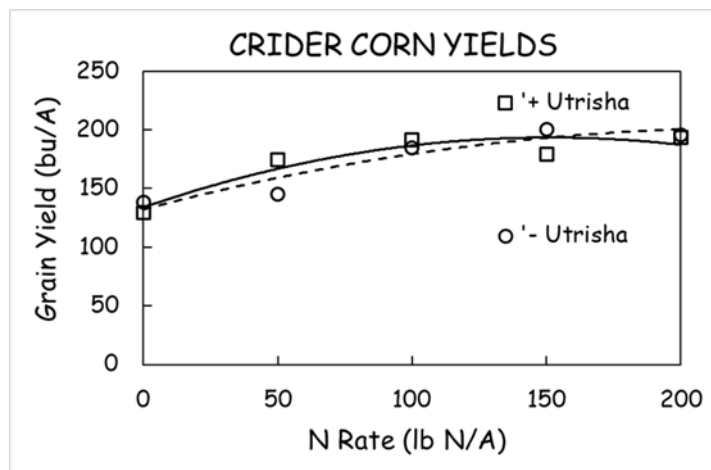
N rate, lbs/a	Corn Yield, bu/a			
	No additive	Envita	Utrisha	ProveN 40
60	130 bcd	148 ab	120 cd	119 d
110	154 a	148 ab	152 a	137 abc
180	160 a	145abc	139 abcd	154 a
Mean	148NS	147	137	137
Check	128 d			

# Kansas State trials, Manhattan, KS, 2020

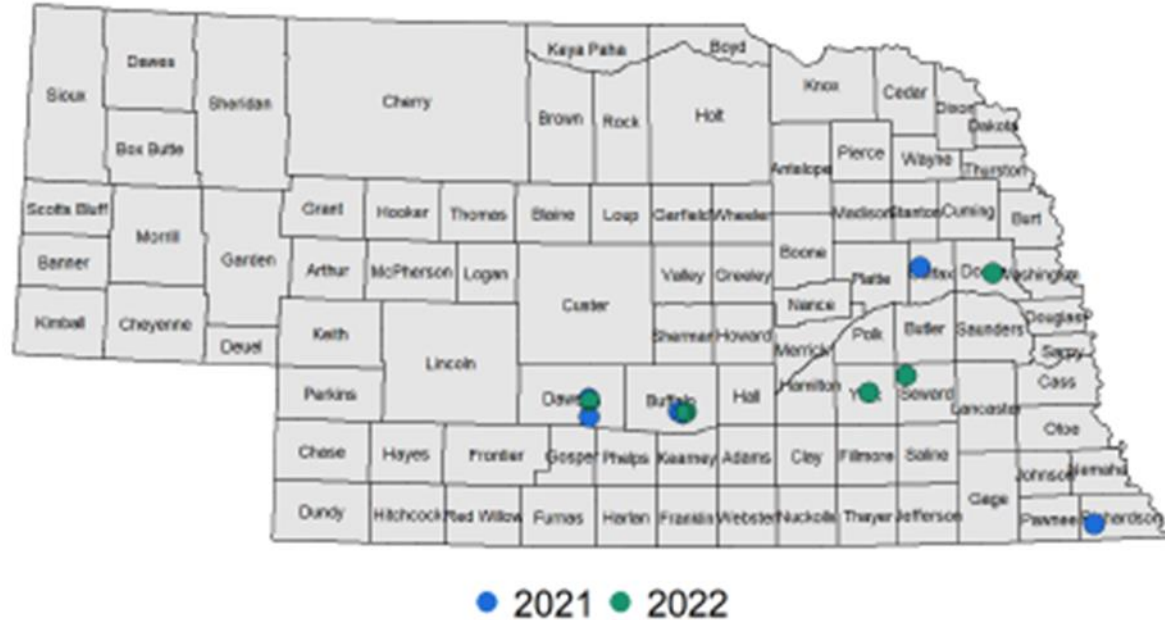
no differences with or without.



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



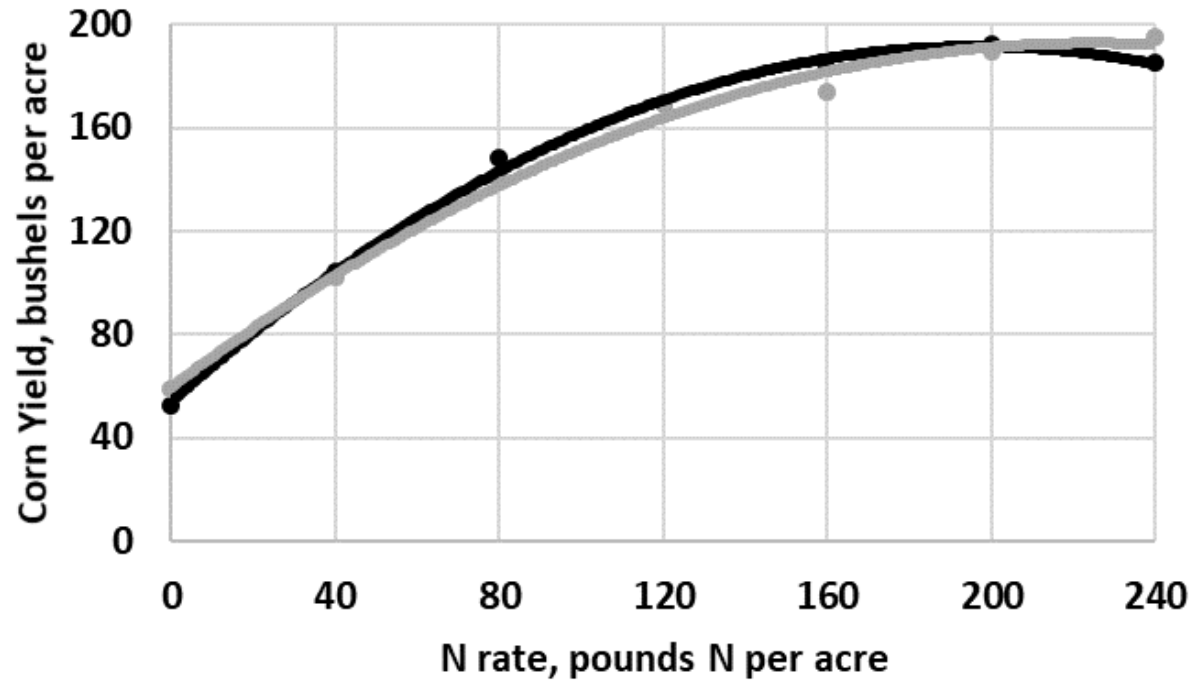
## Nebraska on-farm network, data from L. Thompson, Univ of NE Ext.



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†	Envita F	Utrisha	ProveN	ProveN 40 IF	ProveN 40 ST	MAZ‡ ST	MAZ F
	Number of site years included in evaluations							
ND	4 No	1 No	4 No	-----	-----	-----	1 No	1 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	1 No	11 No	15 No/1 Yes	13 No	2 No	1 No	1 No



**Total corn experiments 53.**

**51 no benefit to yield over N rate alone.**

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

**Sugarbeet- 2 experiments with Bio-Red/Bio-Mate  
no benefit to yield/sugar yield**

**Canola- 2 Envita foliar experiments (Minot) no benefits**

**Spring wheat with Envita foliar (2) and MicroAZ  
seed treatment (1) and foliar (1) no benefits.**

## **General comments about additives**

**Growers need to understand that since about 2008, the burden of research falls on the user.**

**Companies are good at 'development', meaning marketing, but research is sparse and results from University researchers may be controlled by the restrictions of signed confidentiality agreements.**

**Growers should be skeptical about new products**

**Try them 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**

L.J. Thompson, L.A. Puntel, T. Mieno, J. Iqbal, B. Maharjan, J. Luck, S. Norquest, J. G.  
C. P. Pinto, C. Uwineza

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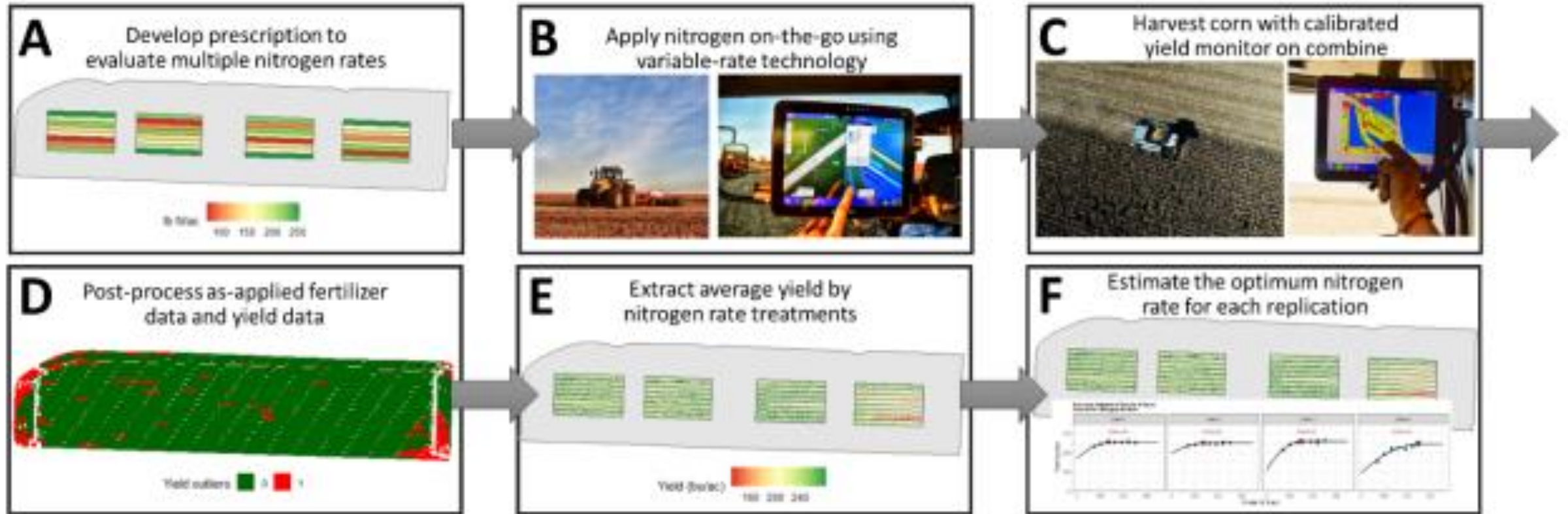
### **ABSTRACT**

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 farms 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

# Example from Thompson paper:



## Contact information:

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