CORN RESPONSE TO NITROGEN FIXATION TECHNOLOGY IN UPSTATE MISSOURI

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ABSTRACT

Nitrogen is one of the most expensive corn input costs and is critical for grain production. Nitrogen (N) fixing bacteria convert atmospheric N into organic forms that can be utilized by the plant are common with legumes. The symbiosis between Rhizobia and legumes is a critical plant-microbe mutualism that is essential for high yielding soybean. Recently, an emphasis on developing technology to supply corn with additional N through biological processes has been the focus of several agribusinesses throughout the Midwestern U.S. A reduction in N rates using biological N efficiency enhancers could reduce environmental N loss (i.e. leaching and gaseous) commonly experienced in soils throughout Missouri. Biological N efficiency enhancers ("bugs-in-a-jug") may increase plant-available nitrogen and could be incorporated into our current nitrogen recommendation systems if there is a valid and repeatable increase in corn grain yields. The objective of this research was to quantify the N impact of biological management products (*Gluconacetobacter diazotrophicus*, *Methylobacterium symbioticum*, and *Klebsiella variicola* + *Kosakonia sacchari*) on corn response. Field research was conducted from 2020 to 2023 at the University of Missouri Lee Greenley Jr. Memorial Research Farm near Novelty. Leaf greenness was similar among treatments when combined over years. At nitrogen responsive sites, significant interactions in grain yield between years and biological management products indicate inconsistency of this technology due to environmental conditions, hybrids, and/or nitrogen management systems. A better understanding of responsive sites is critical for refining nitrogen recommendations using this technology.

INTRODUCTION

Many studies have shown that increasing nitrogen availability increases corn productivity (i.e. increased yields, biomass, plant height). In addition, it is very common for corn to use less than 50% of the synthetic fertilizer applied during the growing season. A University of Illinois study conducted in 2018 showed that fall applied NH_3 with nitrapyrin in a corn soybean rotation resulted in the corn nitrogen use efficiency to be 42.4% which was the highest among treatments (Griesheim et al., 2019). This has led many farmers to overapply nitrogen in order to help protect themselves against the risk of having nitrogen as a limiting factor affecting crop growth and development. Thus, applying biological N fixing products could provide an environmentally friendly way of supplementing nitrogen to corn which could reduce synthetic nitrogen sources like anhydrous ammonia and help keep the nitrogen in crop fields.

In a study by Tufail et al. (2021), *Gluconacetobacter diazotrophicus* (Envita, Table 1) applied to corn increased shoot and root dry weight 67% and 80%, respectively. When compared to the untreated control, *Gluconacetobacter diazotrophicus* increased N concentration in corn shoots when grown under moderate drought stress, severe N deficiency, and a combination of moderate and severe drought stress and N deficiency. These results have shown that the bacteria were able to colonize with the corn roots, increase plant N concentration, and increase plant growth. *Klebsiella variicola* and *Kosakonia sacchari* are both asymbiotic N fixing bacteria that are found in ProveN (Table 1). A study by Wen et al. (2021) showed that these bacteria increased corn yield by 5.2 bu ac^{-1} and reduced field variability by 8-25%.

METHODS

Experiment 1

Field research was conducted from 2020 to 2022 at the University of Missouri Lee Greenley Jr. Memorial Research Farm near Novelty. A summary of the biological stimulant active ingredient organism or common chemical name, application rate, and timings of N efficiency enhancers is reported in Table 1. Treatments included Urea at increasing nitrogen rates (0, 50, 100, 150, and 200 lbs of N per ac^{-1}) as well as biological products plus 100 lbs of N per ac^{-1}. Experiments were arranged in a randomized complete block design with six replications. Plots were 10 by 40 ft. Corn was planted in 30 inch wide-rows at 34,000 seeds ac^{-1}. Planting date and in-furrow applications of products occurred on 30 April 2020, 27 April 2021, and 10 May 2022. The in-furrow application was made at 18.8 gallons ac^{-1} at 5 psi with water as the carrier. The postemergence broadcast application was applied at the V5 stage of development with a CO2 propelled sprayer on the 12 June 2020, 10 June 2021, and 13 June 2022.

Leaf greenness was determined using a SPAD chlorophyll meter (Konica Minolta, Tokyo, Japan). Plant populations prior to harvest were determined from the entire length of one row. Grain weight, moisture, and test weights were determined for each plot using a plot combine (Wintersteiger Delta) equipped with a HarvestMaster GrainGage. The harvest dates for this study were 23 September 2020, 21 September 2021, and 28 September 2022. Grain yields were adjusted to 15% prior to analysis. Grain samples were collected and analyzed for starch, protein, and oil concentrations (Foss 1241, data not presented).

Data were subjected to ANOVA and means separated using Fisher's Protected LSD (P=0.1).

Experiment 2

The first year (2023) of a split-plot study was conducted at the University of Missouri Lee Greenley Jr. Memorial Research Center near Novelty, MO. The plot size was 10 by 30 ft and had four replications. DK62-44 was planted 24 May 2023 in 30 in wide-rows at 35,000 seeds ac^{-1}. In this study, we evaluated different biological nitrogen fixation products for nitrogen use efficiency in corn. The treatments included a control which had no biological product applied, UtrishaN at 5 oz per acre, and Envita at 3.2 oz per acre. In addition, each treatment was applied prior to 32% UAN at 0, 60, 120, 180, and 240 lbs N ac^{-1}. The biological nitrogen treatments were all applied preplant and the UAN treatments were all sidedress applied at V6.

A handheld SPAD Chlorphyll meter was used to collect chlorophyll content in leaves at V10 and again at VT (Konica Minolta, Tokyo, Japan). Satellite imagery was also recorded during these times. Stand counts were taken at VT. Ear weight and nutrient concentration were taken on 8 August 2023. Whole plant biomass weight and nutrient concentration were taken 19 September 2023. Grain weight, moisture, and test weights were determined for each plot using a plot combine (Wintersteiger Delta) equipped with a HarvestMaster GrainGage. The harvest date for this study was 25 September 2023. Grain yields were adjusted to 15% prior to analysis. Grain samples were collected and analyzed for starch, protein, and oil concentrations (Foss 1241, data not presented).

The data was analyzed using quadratic plateau model in R. The agronomic optimum nitrogen rate (AONR) was estimated for each biological treatment.

RESULTS

There was no significant interaction between years and treatments for leaf greenness in late June and plant population at harvest; therefore, data were combined over years (Table 2). Leaf greenness increased as N rate increased. All of the biological N management treatments had leaf greenness values similar to urea at 100 lbs of N ac^{-1}. Plant populations at harvest were 32,150 to 34,640 plants ac^{-1}. All treatments had plant populations that were similar or greater than the non-treated control. Plant populations of all treatments were similar to urea at 100 lbs N ac^{-1}.

Average yields over the three years were reported in Table 2. Grain yields increased as N rate increased. At 100 lbs N ac^{-1}, an in-furrow application of Envita increased average corn yields 6.3 bu ac^{-1} compared to urea applied alone but was only responsive one of the three years. Over the three years ProveN and Utrisha were no different from each other and the same as the 100 lbs of N treatment.

For experiment two we see increasing corn yields with increasing N rates (Figure 1). At 0 lbs N/acre yields are about 100 bu/acre. The greatest yield obtained was with non-treated urea at 240 lbs N/ac (178 bushels ac^{-1}, Table 3). The agronomically optimal N rate for urea in the absence of a biological N treatment was greater than Envita or Utrisha in 2023.

CONCLUSIONS

After four site years of data in Missouri, there have not been consistent yield advantages with using biological N products. More research is needed to better understand how the biology of these products interact under varying field conditions.

Table 1. Biological product active ingredient organism, trade name, application rate, and placement in 2020, 2021, and 2022 in Missouri.

Biological product	Trade name	Application rate	Application placement
Gluconacetobacter diazotrophicus	† Envita™	4.5 oz ac^{-1}	In-furrow
Methylobacterium symbioticum	‡ Utrisha™ N	5 oz ac^{-1}	Postemergence V4-V8
Klebsiella variicola + *Kosakonia sacchari*	⁋ ProveN™, ProveN®40	13-14 oz ac^{-1}	In-furrow

† Azotic North America. 2022. The Science Envita. https://azotic-na.com/science-behind-envita/. Accessed 13 Nov. 2022.
‡ Corteva. 2022. UtrishaTM N Nutrient Efficiency Biostimulant. https://www.corteva.ca/content/dam/dpagco/corteva/na/ca/en/files/brochure/DF-Utrisha-N-Technical-BrochureEnglish.pdf. Accessed 13 Nov. 2022.
⁋Pivot Bio. 2020. Pivot Bio ProveNTM Safety Data Sheet. https://www.pivotbio.com/hubfs/Safety%20Data%20Sheets/2022%20SDSPivot%20Bio%20PROVEN40%20LIF.pdf. Accessed 13 Nov. 2022.
⁋Pivot Bio. 2022. Pivot Bio ProveN®40 Safety Data Sheet. https://info.pivotbio.com/hubfs/Safety%20Data%20Sheets/Pivot-Bio-2020-08-07-PBP-Safety-DataSheet.pdf?hsLang=en-us. Accessed 13 Nov. 2022.

Table 2. Corn plant SPAD at VT and plant population at harvest averaged over years and corn yield response to biological N management treatments in 2020, 2021, and 2022. Corn grain yield was also averaged over years.

Nitrogen Treatments (lbs ac^{-1})	SPAD	Plant Population (plants ac^{-1})	Corn Grain Yield (bu ac^{-1})			
			2020	2021	2022	Average
0N	44.6	32,150	84.7	89.9	78.4	84.3
50N	47.3	34,440	101.9	124.5	117	114.4
100N	50.7	33,660	124.7	135.2	155.7	138.5
150N	51.5	33,940	166.9	156.3	163.7	162.3
200N	53.5	34,124	184.7	177.6	183.6	182
100N + Envita in-furrow	49.5	34,640	139.1	140.2	155.2	144.8
100N + ProveN in-furrow	50.5	34,130	123.9	130.4	156.6	137
100N followed by Utrisha postemergence	50.1	34,380	127.3	145.2	154.7	142.4
LSD (*P*=0.1)	1.7	1,180	9.9	9.9	9.9	5.7

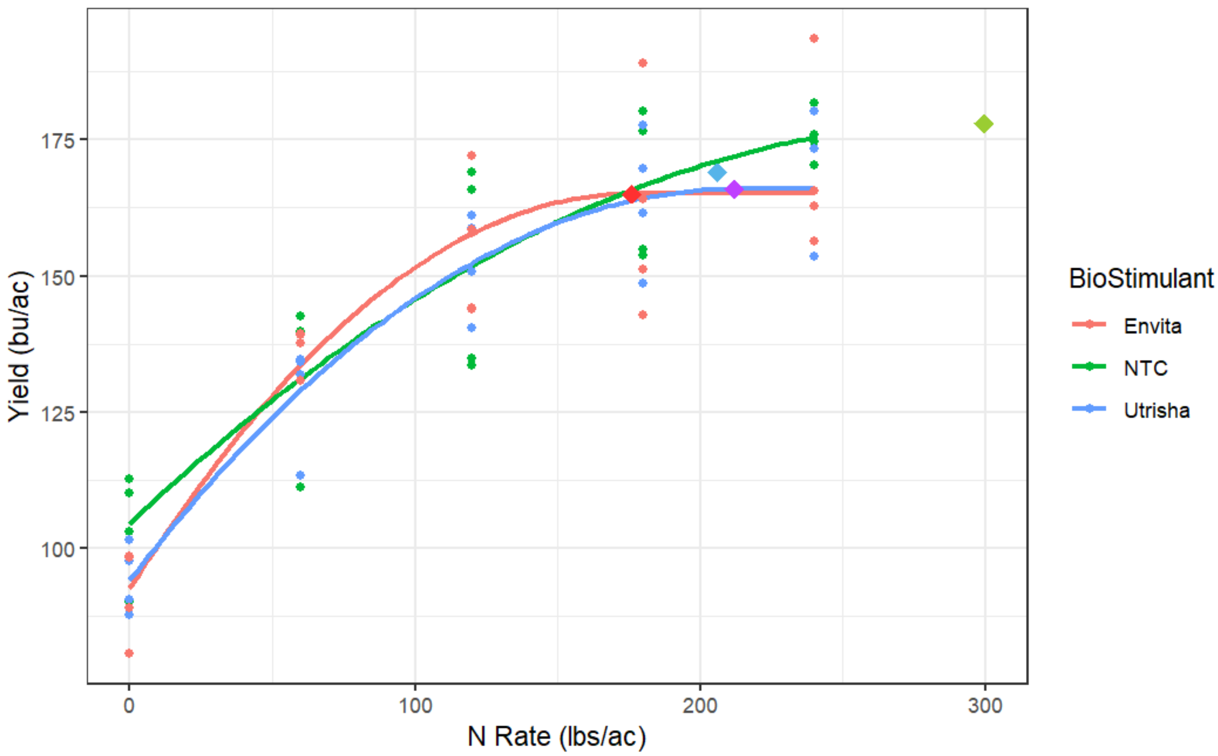

Figure 1. Corn grain yield response to UAN and biological N products in 2023. Diamonds represent the agronomically optimal nitrogen rates (Table 3).

Table 3. Agronomically optimal nitrogen rate (AONR) and grain yields at the agronomically optimal nitrogen rate (YAONR) for the non-treated control (NTC) and biological treatments.

Treatment	AONR (lbs ac^{-1})	YAONR (bu ac^{-1})
NTC	299.50	178.32
Envita	175.85	165.08
Utrisha	212.40	165.96

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