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Summary
Nitrogen fertilizer is one of the largest input expenses for grain crops. Biological products are currently available that contain nitrogen-fixing bacteria to supply nitrogen to a crop throughout the growing season. Pivot Bio PROVEN (developed for corn), RETURN (developed for sorghum) and experimental product versions were evaluated in grain sorghum at Manhattan, KS, in 2020, 2021, and 2022. Products were compared with an untreated check at five rates of nitrogen fertilizer. Products were applied at planting in-furrow in a volume of five gallons of water solution per acre. Crop response was characterized by several in-season parameters plus grain parameters and yield. In all years, the interaction of the product with nitrogen fertilizer was not significant for any parameter, indicating that neither version of the product affected how sorghum responded to nitrogen rates. However, most parameters significantly responded to nitrogen fertilizer, indicating that grain sorghum responded to nitrogen supply in all years. In 2020 and 2021, the product did not affect any of the response parameters. In 2022, the experimental product increased canopy normalized vegetative difference index (NDVI) by 8%, biomass by 21%, and nitrogen uptake by 20% early in the growing season compared to the untreated check. Biomass, nitrogen concentration, nitrogen uptake, grain yield, and other parameters quantified at late vegetative stages or later did not differ from the untreated check for either version of the product. Although one product had a positive effect on early-season growth in one year, mid- to late-season biomass and grain yield were not affected.

Introduction
Reducing input costs and minimizing potential for fertilizer nitrogen (N) losses while maintaining or enhancing yields are of interest to grain crop producers. Naturally occurring, N-fixing bacteria that associate externally with roots of grass species have been documented for decades. Versions of these organisms that continue to fix N in the presence of soil N are the basis of biological products appearing on the market that claim to supply N to a growing crop throughout the growing season. Biological N-fixing products have been available for corn for a few years, but products developed for grain sorghum have been released more recently and have fewer independent evaluations. Evaluations of the performance of these products in regions where grain sorghum is typically grown are necessary to better understand their efficacy for the target crop. The goal of these experiments was to evaluate biological products based on N-fixing bacteria applied in-furrow at planting in typical grain sorghum production scenarios.

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The specific objective was to determine if these products modify the response of grain sorghum to increasing rates of N fertilizer.

Procedures

Experiment Sites and Agronomic Management

Field experiments were conducted near Manhattan, KS, in 2020, 2021, and 2022. The experiments were located on a Wymore silty clay loam with 0 to 1% slopes. Soil samples from the upper six inches of the soil profile had pH of 6.1 to 6.4, and organic matter of 2.6–2.7%. Soybean was the previous crop before the 2020 and 2022 experiments and wheat was the previous crop before the 2021 experiment. A small grain cover crop was planted after wheat in fall 2020 and after soybeans in fall 2021 to generate uniform residue and reduce residual soil N. The cover crop was terminated with herbicides at least three weeks before planting the sorghum experiments. Soil samples from the top 24 inches of the profile indicated that approximately 8, 6, and 22 pounds of nitrate N per acre was present at sorghum planting in 2020, 2021, and 2022, respectively. Sorghum was planted with a no-till planter equipped with residue managers and double-disc furrow openers at 75,000 seeds per acre on June 2, 2020, June 10, 2021, and June 6, 2022. Medium-late maturity sorghum hybrids were planted each year: Pioneer 84P68 in 2020, 84P72-N281 in 2021, and 84G62 in 2022. Herbicides were applied at planting and as needed during the growing season to effectively control weeds. Grain was harvested when it reached harvest moisture (12 to 16%), which was October 29 in 2020, October 22 in 2021, and October 21 in 2022.

Treatments and Experimental Design

Treatments included biological products evaluated at five rates of N fertilizer. The two (2020) or three (2021 and 2022) levels of product were: Check = no product, PROVEN = commercial product for corn in 2020, or RETURN = commercial product for sorghum in 2021 and 2022, and Experimental = experimental product from the same company containing different bacterial strains in 2021 and 2022. The five rates of N fertilizer were 0, 30, 60, 90, and 120 pounds N per acre. All levels of product were evaluated at all levels of N fertilizer for a total of 10 (in 2020) or 15 (in 2021 and 2022) treatments. Each plot consisted of four rows by 98 feet in 2020, 75 feet in 2021, and 60 feet in 2022. Biological products were diluted in non-chlorinated water per the manufacturer's protocol and applied in furrow at planting in a volume of five gallons per acre. Nitrogen fertilizer was applied at the five rates between sorghum rows before planting as 28% urea ammonium nitrate (UAN) below surface residue with a 4-row coulter applicator. The experimental design was a randomized complete block with six replications.

Data Collection and Analysis

Sorghum response to biological products and N fertilizer was evaluated throughout the growing season. Plant stands and phytotoxic responses were evaluated soon after emergence. Sorghum biomass and N status were evaluated nondestructively during vegetative stages with a Trimble GreenSeeker that provided normalized vegetative difference index (NDVI) values. NDVI values were between 0 and 1 with greater values associated with greater plant biomass. Sorghum biomass and N uptake were quantified destructively at S3 (growing point differentiation) in 2021 or S2 (five leaf collars) in 2022. The S6 (half bloom) and S9 (physiological maturity) development stages were sampled in both 2021 and 2022. All samples were obtained by harvesting all plants in three feet of row to determine dry biomass and N concentration. The product of biomass and N concentration provided an estimate of N uptake. Days to half bloom was determined as the number of days between planting and the day when at least half of plants in a plot displayed anthers at least halfway down the panicle. Grain yield was estimated by dividing the mass of grain harvested from the center two rows of each plot by the harvested area of each plot in acres and adjusting to a constant moisture of 12.5%. Grain moisture and test weight were determined using a DICKEY-john GAC II Grain Moisture Analyzer. Grain N was determined by combustion analysis in 2020. Data were subjected to analysis of variance to determine significance of treatment factors and mean separation. Treatment effects and interactions were declared significant when the probability of a greater F > 0.05 (95% confidence).

Results

The interaction between biological product and N fertilizer was not significant for any response variable in any year (Tables 1, 2, and 3), indicating that sorghum response to N fertilizer was similar for all product treatments. Therefore, only product and N rate means are presented for each year.

2020

The NDVI, grain N, grain moisture, and grain yield all responded significantly to N fertilizer, indicating an N-responsive site (Figure 1a, Table 1). NDVI plateaued after the first increment of N fertilizer, grain yield leveled off at 60 pounds of N/acre and greater, and grain N had significant increases up to 90 pounds of N per acre. Biological product did not significantly affect any response parameter in 2020.

2021

Dry conditions in June after planting delayed emergence of sections of row, but plant and head populations within the harvested area of each plot did not differ with treatment. No phytotoxicity was evident after emergence for any treatment.

The NDVI, days to half bloom, N uptake, test weight, and yield all responded significantly to N fertilizer, indicating an N-responsive site (Figure 1b, Table 2). NDVI had significantly greater values with the first increment of N fertilizer at early growth stages and had greater values with all increments of N fertilizer at the last sampling date on July 27. Increasing rates of N fertilizer reduced days to half bloom, which was more than 6 days less at the highest rates compared to the unfertilized 0 pounds N per acre. Test weight was greater with applications of 90 and 120 pounds of N per acre compared to 0, 30, and 60 pounds per acre. Increments of 30 lb N/acre produced significant grain yield increases up to 60 lb N/acre. An additional 60 lb N/acre produced another significant yield increase. Biological product did not significantly affect any response parameter in 2021, including N uptake and yield.

2022

Sorghum was planted into wet soils after several weeks of above-average precipitation, but adequate residue and subsequent rains resulted in uniform, although somewhat reduced, emergence. Relatively dry conditions during portions of July, August, and

September were interspersed with only one major precipitation event in late July and several smaller events in August and September.

Neither biological product nor N rate significantly influenced seedling phytotoxicity, plant populations, or heads per plant (not shown). These parameters typically would not respond to N rates except for possibly heads per plant. However, at nearly 60,000 plants per acre in this experiment, heads per plant were likely limited more by plant density than by N supply.

Most parameters responded significantly to N fertilizer rate, indicating an N-responsive site (Figure 1c, Table 3). NDVI responded to N fertilizer at all sample dates with significant increases up to 60 or 90 pounds of N per acre depending on sample date. Nitrogen application accelerated development, resulting in sorghum reaching half bloom nearly a week sooner with the first 30-pound application of N and nearly two weeks sooner with 90 pounds of N. At all sample times, N uptake increased as the amount of N applied increased. Both grain yield and test weight increased with N applications up to 90 pounds per acre with no additional increase at 120 pounds per acre.

Biological products had no significant effect on most response parameters. However, Experimental product increased NDVI and N uptake at sorghum stage 2, compared to Check and RETURN (Figure 1d, Table 3). This difference did not persist into later developmental stages or grain yield.

Conclusion

The fact that NDVI, bloom date, N uptake, grain test weight, and grain yield responded to 30-pound per acre increases in N application showed that grain sorghum was responsive to N supply in all experiments. The lack of significant differences between the 90 and 120 pound per acre N rates for most response parameters emphasizes the need to evaluate products at multiple N rates. Biological product did not affect how grain sorghum responded to changes in fertilizer N rate. Although the Experimental product resulted in greater early-season N uptake in one year, the biological products did not affect N uptake later in the growing season or grain yield.

Acknowledgments

This project was supported by funding and product from Pivot Bio, Berkeley, CA.

Table 1. Effect of in-furrow nitrogen-fixing biological product and nitrogen fertilizer on grain sorghum near Manhattan, KS, in 2020

	GreenSeeker July 17		Grain nitrogen		Grain nitrogen		Grain moisture		Grain test weight	Grain yield	
Factor	Probability of > F[1]										
Product	0.7313		0.4153		0.5191		0.3215		0.3395	0.1525	
N rate	0.0066		<0.0001		<0.0001		0.0063		0.2393	<0.0001	
Product × N rate	0.3444		0.1259		0.2807		0.9042		0.3936	0.8231	
Product	NDVI[2]		%		lb/acre		%		lb/bu	bu/acre	
Check	0.84		1.35		90		14.9		60.3	136	
PROVEN	0.84		1.33		91		14.9		60.1	138	
N rate (lb/acre)											
0	0.83	b[3]	1.18	c	73	d	15.1	a	59.9	125	c
30	0.85	a	1.23	c	81	c	15.0	ab	60.2	133	b
60	0.84	a	1.37	b	95	b	14.8	c	60.3	142	a
90	0.84	a	1.43	ab	102	ab	14.9	bc	60.0	142	a
120	0.84	a	1.47	a	103	a	14.9	bc	60.4	142	a

[1]Values > 0.05 indicate 95% confidence in no significant effect of that factor.
[2]Normalized difference vegetation index.
[3]Values within a factor within a column followed by the same letter are not significantly different.

Table 2. Effect of in-furrow nitrogen-fixing biological products and nitrogen fertilizer on grain sorghum near Manhattan, KS, in 2021

	GreenSeeker reading date				Days to bloom
	July 9	July 14	July 20	July 27	
Factor	Probability of > F[1]				
Product	0.1647	0.7795	0.7180	0.0766	0.9616
N rate	0.0377	<0.0001	<0.0001	<0.0001	<0.0001
Product × N rate	0.5352	0.6208	0.6276	0.6524	0.5354
Product	NDVI[2]				days
Check	0.72	0.78	0.79	0.81	61
RETURN	0.71	0.78	0.79	0.81	61
Experimental	0.73	0.78	0.79	0.82	61
N rate (lb/acre)					
0	0.70 b[3]	0.75 c	0.75 c	0.78 d	66 a
30	0.71 ab	0.77 b	0.78 b	0.81 c	62 b
60	0.73 a	0.79 ab	0.80 a	0.82 b	61 c
90	0.72 ab	0.79 b	0.80 a	0.82 ab	59 d
120	0.74 a	0.81 a	0.81 a	0.83 a	59 d

	Nitrogen uptake at sorghum developmental stage[4]			Grain test weight	Grain yield
	S3	S6	S9		
Factor	Probability of > F				
Product	0.7920	0.4597	0.7056	0.2550	0.5889
N rate	<0.0001	<0.0001	<0.0001	0.0027	<0.0001
Product × N rate	0.4786	0.2780	0.6777	0.8911	0.8984
Product	pounds/acre			lb/bu	bu/acre
Check	34	104	115	57.3	105
RETURN	33	101	111	57.2	105
Experimental	33	99	113	57.6	102
N rate (lb/acre)					
0	21 d	53 e	65 e	57.0 b	57 d
30	30 c	78 d	94 d	56.9 b	91 c
60	34 c	108 c	115 c	57.1 b	117 b
90	38 b	126 b	137 b	58.0 a	124 ab
120	44 a	142 a	154 a	57.9 a	132 a

[1]Values > 0.05 indicate 95% confidence in no significant effect of that factor.
[2]Normalized difference vegetation index.
[3]Values within a factor within a column followed by the same letter are not significantly different.
[4]S3 = growing point differentiation. S6 = half bloom. S9 = physiological maturity.

Table 3. Effect of in-furrow nitrogen-fixing biological products and nitrogen fertilizer on grain sorghum canopy and bloom date near Manhattan, KS, in 2022

	GreenSeeker reading date					Days to bloom
	June 30	July 8	July 14	July 22	July 28	
Factor	------------	------------	------------ Probability of > F[1]	------------	------------	------------
Product	0.0011	0.8684	0.7557	0.4844	0.2639	0.1693
N Rate	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Product × N rate	0.4894	0.2536	0.3751	0.0780	0.9201	0.3201
Product	------------	------------	------------ NDVI[2]	------------	------------	----- days ----
Check	0.39 b[3]	0.71	0.73	0.74	0.78	70.3
RETURN	0.38 b	0.70	0.73	0.74	0.78	70.5
Experimental	0.42 a	0.71	0.73	0.74	0.78	69.9
N rate (lb/acre)						
0	0.34 c	0.62 d	0.65 c	0.67 d	0.72 d	78.9 a
30	0.39 b	0.69 c	0.72 b	0.72 c	0.77 c	72.7 b
60	0.41 ab	0.72 b	0.76 a	0.76 b	0.79 b	68.4 c
90	0.43 a	0.75 a	0.75 a	0.78 a	0.81 a	65.7 d
120	0.42 a	0.75 a	0.77 a	0.77 ab	0.81 a	65.4 d

	Nitrogen uptake at sorghum developmental stage[4]			Grain test weight	Grain seed size	Grain yield
	S2	S6	S9			
Factor	------------	------------	------------ Probability of > F	------------	------------	------------
Product	0.0134	0.2962	0.2525	0.5724	0.6311	0.8631
N Rate	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
Product × N rate	0.8888	0.2222	0.2574	0.3639	0.6221	0.2246
Product	------------	pounds/acre	------------	------------	lb/bu ------------	-- bu/acre --
Check	20 b	85	93	59.3	26.1	88
RETURN	21 b	93	93	59.5	26.4	88
Experimental	24 a	87	100	59.5	26.3	89
N rate (lb/acre)						
0	11 d	44 d	60 c	58.0 d	25.3 b	49 d
30	16 c	56 d	66 c	59.1 c	26.2 a	63 c
60	21 b	81 c	102 b	59.6 b	26.8 a	95 b
90	30 a	117 b	114 b	60.3 a	26.3 a	116 a
120	30 a	143 a	135 a	60.3 a	26.8 a	118 a

[1]Values > 0.05 indicate 95% confidence of no significant effect of that factor.
[2]Normalized difference vegetation index.
[3]Values within a factor within a column followed by the same letter are not significantly different.
[4]S2 = five fully emerged leaves. S6 = half bloom. S9 = physiological maturity.

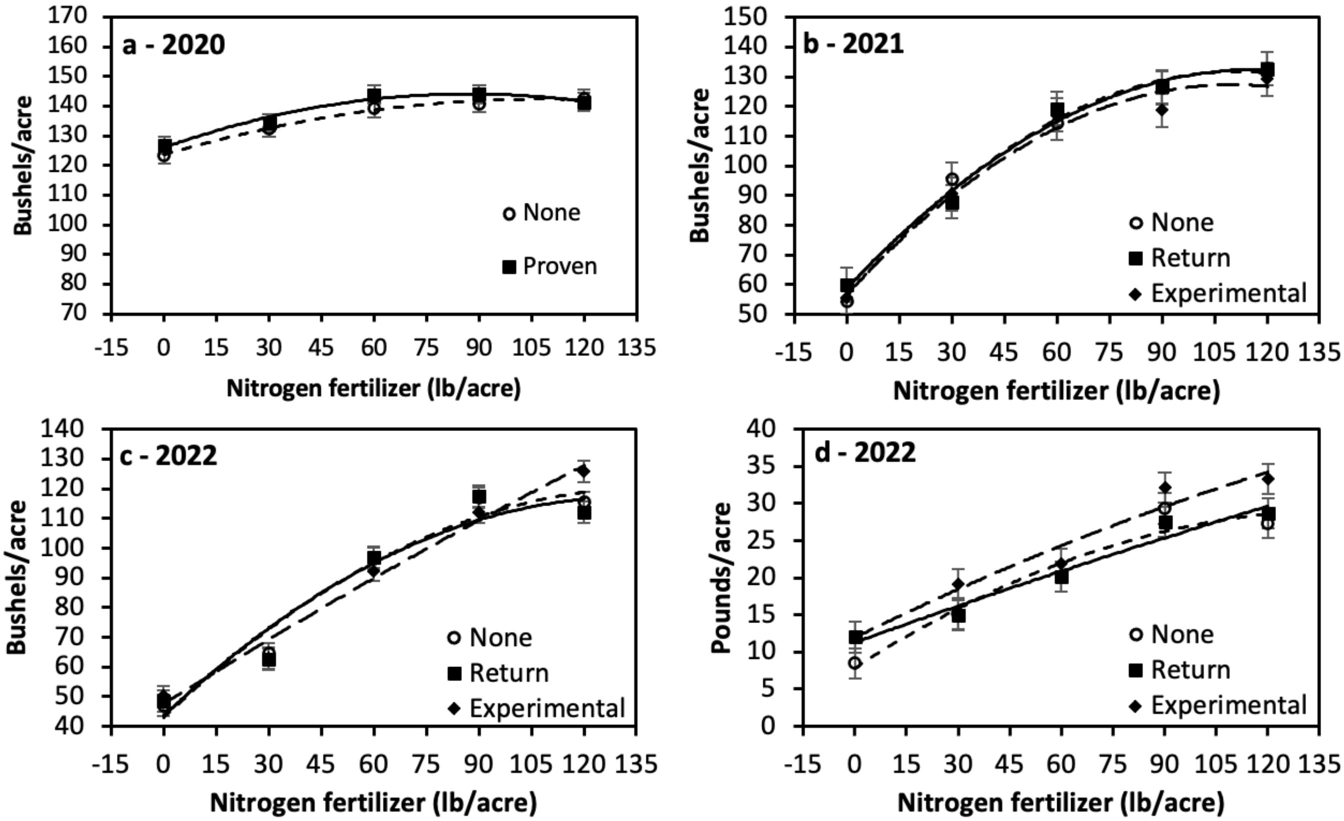

Figure 1. Nitrogen-fixing product and nitrogen fertilizer effect on grain yield in 2020 (a), 2021 (b), and 2022 (c), and on nitrogen uptake of 5-leaf sorghum in 2022 (d).