A New Source of Nitrogen for Maize from the Bacterial Product *PROVEN*

Crop Physiology

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Objectives: ***Determine the nitrogen fertilizer replacement value of PROVEN applications, through changes in plant growth and grain yield production.***

Introduction:

Currently, maize (*Zea mays* L.) plants acquire their nitrogen (N) from the soil (mineralized N from organic matter) and from applied fertilizers or manure.

Changes in topography and soil types within individual fields leads to field spatial variability in the N-supplying power of soils, which can result in areas of insufficient N supply even in fertilized fields.

N_2-fixing bacteria, such as *PROVEN* (Pivot Bio, Berkeley, CA, USA), can tap into a third pool of N, atmospheric N_2 gas, that is currently unavailable for plant N uptake.

It is unclear, however, how much of the plant's N can be provided by *PROVEN*, or when in life cycle any additional N is accumulated, which was the basis of this research.

Materials and Methods:

Nitrogen Application:
Urea was applied pre-plant broadcast and incorporated at rates of 0, 45, 90, 135, and 225 kg N ha^{-1}.

In-Furrow Application:
Across N rates, plots were either left untreated as a control or treated with *PROVEN* in-furrow at planting at a product rate of 1 L ha^{-1} (Figure 1). *PROVEN* was applied with a SureFire (Atwood, KS) planter-mounted system blended with water as a carrier at 112 L ha^{-1} total volume (Figure 2).

Environments:
Champaign (40° N; 2019, 2020, and 2021) and Nashville (38° N; 2021), IL.

Cultural:
A commercial maize hybrid was planted at 89,000 plants ha^{-1} following soybean under conventional tillage.

Design:
Experimental units at each site were plots, four rows wide, 0.76 m row spacing, and 11.4 m long, arranged in a randomized complete block design with six replications.

Figure 1. Applying *PROVEN* in-furrow at planting.

Figure 2. Planter-mounted Sure-Fire in-furrow system.

Special thanks to Pivot Bio for supporting this research.

Results:

PROVEN Affects Early Season N Accumulation

In-furrow applications of *PROVEN* increased V8 total N accumulation (Table 1), through increases in both leaf and stalk partitions (data not shown).

PROVEN-treated plants accumulated enough N to replace 22.5 kg ha^{-1} of synthetic fertilizer at intermediary N rates (Table 1).

Additionally, soil samples from the seed furrow at V8 suggested that *PROVEN* application tended to increase soil nitrate levels (Figure 3).

Table 1. The effect of *PROVEN* and N fertilizer rate on V8 total N accumulation for maize averaged over four site-years.

	V8 Total N Content	
	Treatment	
Nitrogen Rate	None	*PROVEN*
kg N ha^{-1}		
0	31.6	33.0
45	38.0	41.6
90	45.2	48.3
135	51.0	51.7
225	51.3	52.7
Average	43.4	45.5*

* Significant difference compared to control at $\alpha \leq 0.01$.

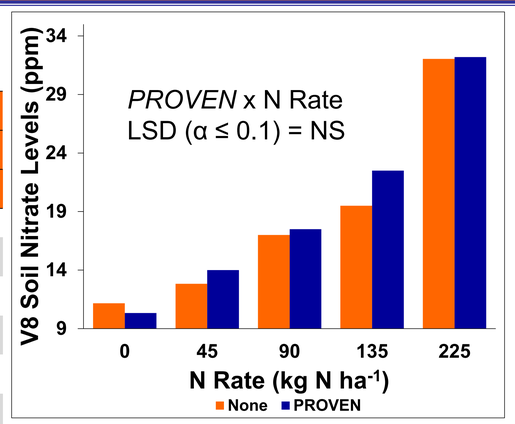

Figure 3. The effect of *PROVEN* and N fertilizer rate on V8 soil nitrate levels for maize averaged over two locations in 2021.

What Caused the Increase in V8 Plant N Accumulation?

Increased N accumulation at V8 was due to greater plant biomass production, while maintaining similar N concentration (Figure 4).

Leaf N concentration levels were not affected by in-furrow treatment, an indication that tissue tests will not show any effect of *PROVEN* application (Figure 4).

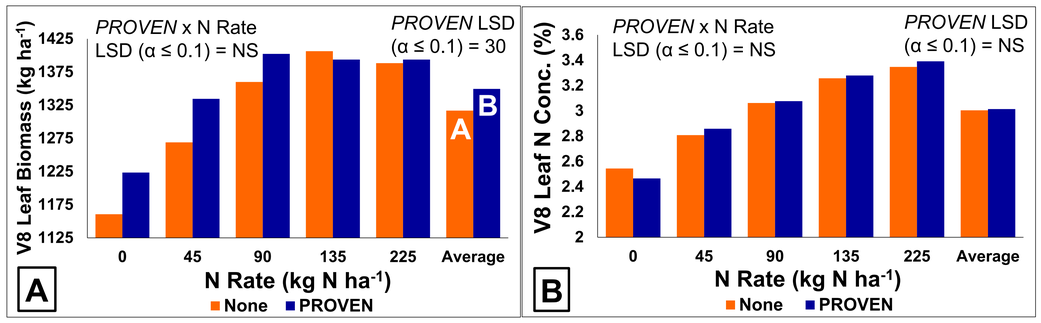

Figure 4. The effect of *PROVEN* and N fertilizer rate on (A) V8 leaf biomass and (B) N concentration for maize averaged over four site-years. Different letters indicate significant average difference of *PROVEN*.

How Does *PROVEN* Affect Late Season Parameters?

By flowering (VT/R1) *PROVEN* applications led to greater total biomass production (Table 2) as well as greater N accumulation (data not shown).

PROVEN-treated plants produced more reproductive tissue biomass (earshoot) (Table 2), suggesting greater grain yield potential.

While there was no effect of *PROVEN* application on grain yield, there were some trends of increased yields (Table 2). When moderate levels of N were applied, *PROVEN* treatment led to yield benefits ranging from 0.1 to 0.2 Mg ha^{-1}.

Table 2. The effect of *PROVEN* and N fertilizer rate on VT/R1 total and reproductive tissue plant biomass accumulation and yield for maize averaged over four site-years.

	VT/R1 Total Biomass		VT/R1 Repro. Biomass		Grain Yield	
	Treatment					
Nitrogen Rate	None	*PROVEN*	None	*PROVEN*	None	*PROVEN*
	kg N ha^{-1}				Mg ha^{-1}	
0	7565	7535	830	784	6.7	6.7
45	8912	9202	1085	1208	8.1	8.3
90	9302	9953	1245	1391	9.5	9.7
135	9953	9739	1336	1342	10.8	10.9
225	9966	9918	1387	1404	11.7	11.7
Average	9097	9276*	1177	1226*	9.3	9.4

* Significant difference compared to control at $\alpha \leq 0.05$.

Conclusions:

- ***PROVEN* microbes enhanced vegetative N accumulation, indicating a N replacement value of nearly 30 kg N ha^{-1}.**
- **Increases in plant N accumulation were due to a greater production of plant biomass. A leaf tissue test will NOT show a *PROVEN*-related treatment response.**
- **The greatest biomass, N accumulation, and grain yield responses occurred when moderate levels of N were applied with *PROVEN*.**