BIOLOGICAL NITROGEN SUPPLIERS FOR SOYBEANS

V. Winnikes de Barros, and S.N. Casteel
Purdue University, West Lafayette, IN
vwinnike@purdue.edu (859)436-4788

ABSTRACT

Soybeans are known to require more N than most crops, largely due to the high N levels found in their seeds. The most important source of N for soybean plants is the biological N fixation process. However, high yields (above 70 bu acre^{-1}) could limit the capability of this process to supply the plant's N demand. This study aims to investigate the use of non-rhizobial biological N suppliers, their ability to provide N to the soybean plants and potentially fill the N demand gap. The study was conducted at three sites in Indiana with different fertility characteristics: high fertility (West Lafayette), intermediate fertility (Wanatah), and sulfur-deficient (LaCrosse). At each site, two non-rhizobial biological N suppliers, Envita® (*Gluconacetobacter diazotrophicus*) and Utrisha-N® (*Methylobacterium symbioticum*), were applied under four fertility regimes: no fertilizer; 40 lb acre^{-1} of N; 20 lb acre^{-1} of S; and 40 lb acre^{-1} of N plus 20 lb acre^{-1} of S. The experimental design followed a 4 x 3 factorial arrangement with an additional untreated control resulting in 13 treatments. The treatments were replicated five times in each location, resulting in 65 experimental plots per study site. The evaluated parameters were plant nutrient content at R2 and R4 growth stages, yield, seed weight, and grain oil and protein concentrations.

INTRODUCTION

A large amount of nutrients is demanded for high-yield crops, and since its importance for the composition of enzymes and other proteins needed for photosysnthesis, a large amount of N is required (Sinclair and Horie, 1989 as cited in Salvagiotti et al., 2009). It is known that soybeans usually require more N than other crops, largely due to high N levels found in their seeds (Sinclair and de Wit, 1975 as cited in Ciampitti et al., 2021). The most important source of N for soybean plants is the biological N fixation process (Ciampitti et al., 2021), however, high yields (above 70 bu acre^{-1}), could limit the capability of this process to supply the plant's N demand (Ciampitti & Salvagiotti, 2018). This context makes it interesting to improve the N supply for the soybean plants utilizing different biological N sources. This study investigates two non-rhizobial biological N suppliers, which are Envita® and Utrisha-N®.

Envita® is a biological product produced by Azotic that consists of *Gluconacetobater diazotrophicus* bacteria. According to the manufacturer the bacteria are able to enter the plant both through the root zone, when applied in-furrow, or leaf stomata, when applied as a foliar spray. Once inside the plant, the bacteria colonizes the plant cells and create small vesicles or "air pockets" that have the ability of capturing nitrogen from the atmosphere. The bacteria then repopulates within the cell.

Utrisha-N® is a biological product produced by Corteva that consists of *Methylobacterium symbioticum* bacteria. Acording to Corteva, the bacteria enters the plant through the stomata and enters the leaf cells. Once in the plant cells, the bacteria converts N_2 from the air into ammonium, which results in a constant supply of amino acids to the plant.

This study aims to evaluate the efficiency of the two non-rhizobial biological nitrogen suppliers in providing nitrogen to soybean plants and their subsequent impacts on crop yield under contrasting environmental conditions. Specifically, the research investigates their performance in both low nitrogen supply environments, where additional N input may enhance plant growth, and high-yield environments, where greater nitrogen demand is expected. It is hypothesized that these products will improve soybean yield, with a stronger effect in high-fertility soils due to increased crop nutrient demand, while also demonstrating the potential to supply nitrogen effectively in low-N environments, contributing to overall nitrogen-use efficiency.

MATERIALS AND METHODS

The study followed a 4 x 3 factorial structure and was arranged in a randomized complete block design (RCBD) having 4 fertility regimes and 3 biological treatments, plus the addition of 1 extra untreated control, resulting in a final number of 13 total treatments. The 13 treatments were replicated 5 times in each experimental site, resulting in a final number of 65 small scale (10ft x 50ft) plots per location. Field trials were established in 3 locations within the state of Indiana with different fertility characteristics and were conducted throught the 2023 and 2024 seasons. Soybeans were planted in 15 in wide rows at a 140,000 seeds/acre seeding rate. Fertilizers were hand broadcasted on the small plots after planting. Biological treatments were sprayed at V6 growth stage with CO_2 backpack sprayer.

Locations

• **West Lafayette – IN:** high fertility environment
• **Wanatah – IN:** intermediated fertility environment
• **LaCrosse – IN:** sulfur deficient environment

Table 1. Locations of experimental sites.

Soybean varieties and planting dates

Location	2023		2024	
	Variety	Planting date	Variety	Planting date
West Lafayette	P31A73E-Illevo	May 6th	P31A73E-Illevo	May 4th
Wanatah	P28A65E-Illevo	May 18th	P28A65E-Illevo	May 22nd
LaCrosse	P18A73E	May 2nd	Becks 3300E	May 7th

Table 2. Soybean varieties and planting dates.

Fertility regimes

• **No fertilizer**
• **Nitrogen** = 40.0 $lb.acre^{-1}$ via Urea
• **Sulfur =** 20.0 $lb.acre^{-1}$ via Pelletized gypsum

• **N + S =** 40.0 lb.acre^{-1} + 20.0 lb.acre^{-1} via Urea + Pelletized gypsum

Table 3. Fertility regimes with application rates and fertilizer sources.

Figure 1. Fertilizer spreading.

Biological treatments

• **No biological**
• **Utrisha-N® (Corteva):** *Methylobacterium symbioticum* – 5.0 fl.oz.acre^{-1}
• **Envita® (Azotic):** *Gluconacetobacter diazotrophicus* – 0.18 fl.oz.acre^{-1} + 5.0 fl.oz.acre^{-1} of NIS (Activator 90)

Table 4. Biological treatments with application rates.

Figure 2. Biological spray application.

Data colection

For both the 2023 and 2024 seasons soil fertility was determined by soil sampling the study sites before the fertilizer application at 0-8 in depth. Yield was determined by harvesting the center of the plots using a combine harvester and then adjusting yields to 13% grain moisture. Grain subsamples were collected to dermine protein and oil contents through NIR analysis and also grain weight. For the 2023 season, plant nutrient content was determined for both the R2 (full bloom) and R4 (full pod) growth stages through leaf sampling of the most recent mature leaves.

Statistical analysis

SAS 9.4 was used to run proc GLM with main level factors, and interactions were tested with appropriate error terms. Interactions are reported and means separated according to Fisher's Protected $LSD_{0.1}$.

RESULTS AND DISCUSSION

The only analyzed parameter in which the biological products had a significant positive effect was the 2023 R4 nitrogen leaf content at a high fertility environment, West Lafayette – IN, where treatments that received Utrisha-N had a higher leaf N content on the pooled results.

%	None	Envita	Utrisha-N	Pooled	
None	4.7	4.6	5.1	4.8	**b**
Nitrogen	4.9	4.9	4.9	4.9	**b**
Sulfur	5.7	5.5	5.5	5.6	**a**
N + S	5.4	5.5	5.7	5.5	**a**
Pooled	5.2	5.1	5.3		
	B	**b**	**a**		

Table 5. R4 nitrogen leaf content at West Lafayette in 2023.

Sulfur was the biggest contributor factor for yield gains in all locations and years. With an emphasis on the low fertility environment, LaCrosse – IN, where a gain of 10.9 bushels per acre was observed in 2024.

$bu.acre^{-1}$	None	Envita	Utrisha-N	Pooled	
None	57.5	56.6	57.7	57.3	**c**
Nitrogen	58.9	54.9	54.2	56.0	**c**
Sulfur	67.4	66.9	70.2	68.2	**b**
N + S	72.6	70.0	73.9	72.2	**a**

Table 6. Grain yield at LaCrosse in 2024.

Preliminary conclusions

The results show that S was the responsible for the fertility effects observed. The biologica N suppliers were not able to overcome the limited supply of N at LaCrosse, which is the S deficient and low biological N fixation soil. There was no biological effect or interaction effect at Wanatah, which is the moderate fertility soil. The biological N suppliers were able to increase the N supply in a high yield environment.

Considerations

It is important to further study what drives the efficiency of the biological products, their working mechanisms and how they are impacted by other sprays during the season.

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