SUMMARY OF REGIONAL STUDIES IN CORN ON SELECTED COMMERCIAL ASYMBIOTIC N-FIXING ORGANISMS AND SUGGESTIONS FOR COMPANIES DEVELOPING SIMILAR PRODUCTS

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ABSTRACT

This is a summary of N rate studies from the North Central region which include treatments with selected commercial asymbiotic N-fixation organism products. Farmers are encouraged to remain curious of new products, but also skeptical; testing the products of interest through replicated strip trials their farms to determine whether the products have value to their operation. In conversations over the past several months with companies interested in developing asymbiotic N-fixation products, the following are frequent topics of concern:

- The organisms need to be kept alive through transportation and storage intervals between manufacturer, shipper, warehouses, distributor, dealer, and finally on farm storage before use.
- There should be a method of analysis developed to determine whether the organism is alive and functioning in the soil/plant after application.
- The organism should be able to ‘win a war’ with native microorganisms in order to survive and perform its function.
- The organism should be adapted to variable moisture, soil pH and soil salts in order to perform its function.

INTRODUCTION

The source of N for crops in this region is N from mineralized organic matter and residue decomposition, release of N from ‘fixed’ non-exchangeable ammonium in the interlattices of smectitic clay minerals, fertilizer N, N from atmospheric deposition and N released from the activities of N-fixing organisms. Most agronomists are aware of the contribution of symbiotic N-fixing bacteria to soybean and other legumes. However, it is common for agronomists to be unaware, or at least dismissive of the N contribution of asymbiotic, or non-symbiotic, free-living N fixing organisms. A more detailed description of the activities of these organisms is provided in Franzen et al., 2022 https://www.ndsu.edu/agriculture/extension/publications/performance-selected-commercially-available-asymbiotic-n-fixing-products ; however, the following is an abridged version.

Asymbiotic N-fixing organisms, primarily accepted to be bacteria at present, are active in most soils. It is one reason among others why when a fertilizer applicator ‘skips’ an area in an intended corn field, the yield is not zero. The limitation to their activity is food and housing. In a conventionally-tilled field (fall chiseled, once or more, and one to two field prep trips in the spring with a field cultivator or other finishing tool) the housing for the bacteria is serious disrupted and even destroyed several times a year. Some bacteria survive, but many die. Also, in a conventionally-tilled soil, residue is exposed to oxygen resulting in a fast decomposition by a limited number of organisms. The resulting decomposed material is a less desirable food source compared to leaving residue alone in a more anaerobic environment.

In a long-term, continuous no-till field, the tiny nooks where bacteria produce a microenvironment hosts many organisms, including evidence shows asymbiotic N-fixing organisms. The food in a long-term no-till soil is much more diverse and therefore supports a greater array of organisms at a higher biomass compared to a frequently tilled system. A paired-sample study in North Dakota, with one sample at each paired location from a long-term no-till soil, compared to a sample directly across the fence or road in a similar soil series showed that asymbiotic N-fixing organism activity was much higher in long-term no-till soil compared to the conventionally-tilled relative (Franzen et al., 2019). The higher activity of the asymbiotic N-fixing organisms may contribute up to about one-third of the long-term no-till N credit provided in the N calculators for corn, spring wheat/durum, sunflower and 2-row malting barley in North Dakota (https://www.ndsu.edu/pubweb/soils/N_calculators/).

Asymbiotic N-fixing organisms are very sensitive to the soil environment. They function best when the soil is warm and moist. Dry soils support very low active and activity in saturated soils was near zero in a recent study (Franzen et al., 2023). Activity is low during spring thaw in North Dakota, and increases as the soil warms. Activity thereafter is a function of soil moisture.

THE COMPILATION DOCUMENT OF NCERA-103 RESEARCHER EXPERIMENTS THROUGH 2022

In November, 2021, the Agricultural Experiment Station North Central Committee on Specialized Soil Amendments and Products, Growth Stimulants, and Soil Fertility Management Programs met after the North Central Extension-Industry Soil Fertility Conference in Des Moines, as it has met for decades. The discussion was largely centered on the new asymbiotic N-fixing products on the market and the intention of most around the room to conduct some work with them in 2022. A few researchers already had a little experience with one of more of the products. It was resolved for each researcher to contribute their methods/results at the 2022 committee meeting and compile the results in a single document. There was general hope around the room that the products would have value, enabling reduction of farmer-applied N rates, which despite the efforts of many researchers and extension soil fertility people in the region tend to be greater than published recommendation in print or in N calculators.

In November 2022, the committee met again, and results and experimental details were provided before mid-December 2022 by all members of the committee. The document was prepared sent out to the committee for edits and the final version was published April 2023. NDSU Extension Circular SF2080. Performance of Selected Commercially Available Asymbiotic N-fixing Products. D. Franzen, J. Camberato, E. Nafziger, D. Kaiser, K. Nelson, G. Singh, D. Ruiz-Diaz, E. Lentz, K. Steinke, J. Grove,

E. Ritchey, L. Bortolon, C. Rosen, B. Maharjan, and L. Thompson https://www.ndsu.edu/agriculture/extension/publications/performance-selected-commercially-available-asymbiotic-n-fixing-products . The authors represented all of the North Central States with the exception of Iowa and South Dakota. Out of 51 trials of products on corn, using label rates and company instructions, 2 produced a statistical yield advantage over the N rate used alone, while 49 did not provide a yield improvement nor did it provide evidence that N rate might be reduced due to its use.

Summary table of results from corn trials on corn yield with N rate alone vs addition of amendment to N rate.

State	Envita IF	Utrisha	ProveN	ProveN 40 IF	ProveN 40 ST
	Number of site-years of product testing				
ND	4 No	4 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	---------------
MI	1 No	1 No	-------------	1 No	---------------
KS	---------------	--------	1 No	--------------	---------------
KY	-------------	2 No	--------------	--------------	-------------
NE	-------------	--------	5 No	6 No	--------------
OH	-------------	1 No	---------------	----------------	--------------
Total	8 No / 1 Yes	11 No	15 No / 1 Yes	13 No	2 No

TAKE-HOME MESSAGES FROM THE NCERA-103 EXPERIMENTS

There are two important messages that should come from these results. One is that it is OK that farmers are curious about new products; the NCERA-103 committee members were certainly curious about these N-fixing products or they would have had no interest in examining them. However, farmers should also be skeptical about new products. A better way to learn about new products is to test them on their farms. Note that the Nebraska data in the table as explained in the circular are all farmer replicated test strips. This is the way that farmers should test products or management techniques. Applying a product on thousands of acres based on claims, then thinking the product performed well or not well due to overall yields compared to those in the past is not helpful or informative. Neither is using it on one field compared to another. Each field has a different 'personality' and one field compared to another is likewise not helpful in indicating future performance of a product. Also, splitting a field is not helpful. If I three a rock towards the west half of a field and didn't throw a rock into the other, the half I threw the rock on would yield more than the one without the additional rock about half the time. Not helpful. The only way to truly have confidence on product or management difference performance is through replicated strip trials.

At the 2022 NC Extension-Industry Conference, the paper Thompson et al. (2022), was particularly helpful in guiding farmers onto a path towards better product testing. Using replicated N-rate strips within a field, the field had grower N-rate alone compared to strips of grower N-rate with addition of a product. Figure 1 represents a possible farmer strip-trial with use of biological product against N rate alone.

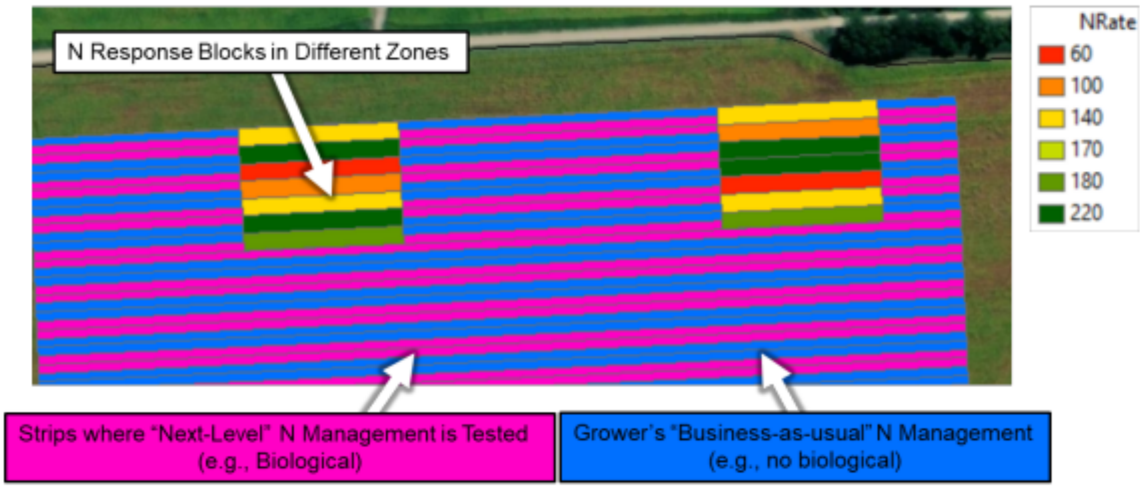

Figure 1. A possible field arrangement of replicated strips to test biological product against N rate alone.
(From Thompson et al., 2022, reproduced with permission.)

Thought should go into what treatments should be compared. Is it best to compare a farmer-rate of 200 pounds N per acre to 200 pounds N per acre with amendment, when the state recommendations are 160 pounds N per acre? Probably not. A better comparison might be 120 pounds N per acre with amendment compared to the state recommendations. That way if the amendment produced N, the yields of the treatments when compared would justify reduction of N rate when the amendment was used, and still achieve similar yield to a higher rate from the state recommendations.

There are many accessible papers regarding on-farm replications. Kyveryga et al. (2018) recommendations making sure that the field chosen for the experiments will not include areas in which treatments might be confounded special areas of natural or manmade interference. The treatments should be randomized. The paper also includes strategies for blocking and even split-plot designs, all of which can easily be statistically analyzed. At the simplest, replicated strips analyzed with a simple t-test in Excel is a great improvement over something like splitting a field, which is a poor observation at best.

One of the results of publishing the NCERA-103 authored compilation of research trials has been an opening of communication with other startups of N-fixing organisms in the US and in Europe. I have visited in person, on the phone and on web-meetings with representatives and scientists from several industries and I have discussed with them what might be done to improve their chances in the marketplace when they decide the product is ready to sell. The discussions always settled on four points:

- The organisms need to be kept alive through transportation and storage intervals between manufacturer, shipper, warehouses, distributor, dealer, and finally on farm storage before use.

- There should be a method of analysis developed to determine whether the organism is alive and functioning in the soil/plant after application.
- The organism should be able to ‘win a war’ with other native microorganisms in order to survive and perform its function.
- The organism should be adapted to variable moisture, variable soil pH and variable soil salts in order to perform its function.

The asymbiotic N-fixing bacteria are living organisms. Although the firms were convinced that when the product left the point of manufacture, they all were concerned that they might not be viable by the time they reached the point of field application. If the product is produced overseas, what will be the conditions if air-freight instead of by ship. In a cargo-hold of a jet liner, the temperature can be adjusted between about 40 degrees F and 70 degrees F. Some bacteria have narrow temperature storage requirements. One product that was used in the North Dakota experiments recommended 42 degrees F. The flight from overseas can be 6 to 20 hours depending on where it originates, so the bacteria needs to be shipped at a temperature at which survival is assured; similarly, on a ship. Transport in a shipping container is subject to temperatures at the dock, on the ship, then again on the dock for an extended period of time. Will the bacteria survive?

If produced in the USA, shipment is still an issue. Will it be shipped to a distributor on a climate-controlled truck? Once it is dropped off at the distributor, are they equipped with a large fork-lift accessible climate-controlled storeroom? Then a distributor to a retailer, again is the transporting truck climate controlled, or subject to 100-degree temperatures in a hot spring? At the retailer, do they have a climate-controlled storeroom capable of handling the volumes anticipated. Finally, delivery to the farm. Does the farmer have climate-controlled storage capable of holding the bacteria live for 2-3 weeks in case planting is delayed due to rain or breakdowns? The logistics of delivery to the end-user is not a trivial exercise and some manufacturers are now aware of the issues that need to be overcome.

Presently, according to industry people I have visited with, there are no quick assays or analyses available to determine whether the organisms in the container, in the soil or plant are alive and functioning in the manner they need to function to benefit the end-user. These tests need to be developed to support the product and provide confidence to the farmer that the product is performing.

The organism needs to be able to compete with other organisms in the soil. The soil is alive with all kinds of macro- and micro-organisms all ‘thinking’ the same thought- ‘What’s for dinner today?’ If the ‘*Spéciale du jour*’ is the newly applied asymbiotic bacteria, the application was for naught. It is possible that one of the reasons that in controlled experiments newly released Rhizobium for soybean with claims of greatly improved N-fixation seldom result in more than a bushel if that of soybean yield improvement, is the lack of competitiveness of the new release. Regardless, competitiveness of any organism applied to the soil is important for its degree of benefit to the end-user.

Finally, conditions in soils across the North Central Region in terms of soil pH, temperature through the growing season, soil moisture conditions, and presence of soluble salts. Just in North Dakota, soil temperature when seeding spring wheat is often

35 degrees F at the beginning. Soil pH may vary in the same field from the high 4's to over 8. Soil moisture varies all over the region from very dry to very wet at times. Throwing bacterium into such an environment that has specific environmental demands for its performance without the screening successes through the possible extreme conditions would not be a successful venture unless luck was on its side.

CONCLUSIONS

To date, researchers associated with the NCERA-103 Committee of Specialized Soil Amendments and Products, Growth Stimulants, and Soil Fertility Management Programs have found a low frequency of N fertilizer rate replacement from the application of commercial asymbiotic bacterial products to corn. It is possible that future related products might be beneficial if manufacturers consider ways to maintain viability of the organisms from point of production to point of field application, are able to assay organism activity in the field, and screen organisms for their ability to compete with other soil organisms and remain active in a wide range of possible soil environments. Farmers should strive to be curious regarding new products, but also to be skeptical; testing products of interest in replicated trials on their farms.

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