



JOURNAL OF THE NACAA

ISSN 2158-9429

VOLUME 17, ISSUE 2 – DECEMBER, 2024

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Efficacy of Foliar and Seed Applied Biofertilizers in Dryland Wheat

Abstract

Biological products, such as biofertilizers, are increasingly being marketed to the agricultural industry to improve crop nutrient efficiency. Most biofertilizer products were initially developed for use in corn and soybeans and have not been investigated as intensively in winter wheat or in the arid growing regions of the inland Pacific Northwest. Two biofertilizer products, Envita and Fresh Tracks Universal Microbes (FT), were examined in soft white winter wheat in North Central Oregon during the 2022-2023 crop year. Envita is a foliar applied nitrogen fixing bacteria, while FT is applied with seed and contains several strains of bacteria to increase nitrogen, phosphorus, and sulfur availability to the crop. Neither biofertilizer tested provided a significant response or a positive return on investment, possibly impacted by drought during grain development. More years of research are needed to see if biofertilizers may provide a significant response under different environmental conditions, especially in dryland cropping systems that experience high levels of variability in precipitation between years. Additional years of research at multiple locations need to be completed with biofertilizers before farmers should consider widely using them.

Introduction

Biofertilizers are primarily free-living bacteria and fungi that when applied to plants may enhance nutrient uptake, stimulate natural process (such as nitrogen fixation), reduce plant stress, and improve crop quality (Kumar, 2018; Nadeem et al., 2013). Most biofertilizers contain only microbes without any nutrients included, but microbes may increase nutrient availability in the soil for crop use. There are several different classes of biofertilizers including humic and fulvic acid, seaweed extracts, liquid manure composting, and beneficial bacteria and fungi. Beneficial bacteria and fungi are the primary biofertilizers that are increasing in availability. Commonly used bacteria are nitrogen fixers, potassium and phosphorus solubilizers, and plant growth promoting rhizobacteria (Mahdi et al., 2010). Many commercial biofertilizer products contain naturally occurring rhizobacteria that can supply nitrogen to crops (Cocking, 2003).

Agricultural crops depend on nitrogen fertilizers to maintain optimum yields (Peoples et al., 1995). However, these inputs can be costly and, despite the atmosphere being 78% nitrogen, most plants cannot access it directly (Galloway et al., 2003). Due to several global factors, the availability and price for nitrogen fertilizers has gone through extreme volatility in the last several years. Due to supply chain issues, the price for fertilizers doubled in 2022, but has decreased in recent years (USDA ERS, 2024). In addition, prior to the recent increase, prices quadrupled between 1999 and 2008 (Williamson, 2011). Soil acidification is also increasing due to the continued use of synthetic nitrogen fertilizers (Schroder et al., 2011) and is becoming an increasing challenge for wheat producers in the inland Pacific Northwest (McFarland and Huggins, 2015). If biofertilizers can effectively supplement nitrogen fertilizer use, it may help slow the rate of soil acidification while reducing fertilizer costs.

Over the last decade, hundreds of commercial biofertilizer products have been developed by agricultural companies marketed to farmers to apply to their crops (Owen et al., 2015). Though the development of biofertilizers continues to increase, widespread farmer adoption and peer reviewed studies are lacking, especially in the Pacific Northwest. In addition, much of the completed research has focused on corn and

soybeans grown in high rainfall environments (30+ inches of annual rainfall) compared to dryland wheat production in the inland Pacific Northwest (8 to 16 inches of annual precipitation).

One commercial biofertilizer product of interest to dryland wheat producers in the inland Pacific Northwest is Envita, produced by Azotic North America LTD. The active ingredient is a naturally occurring, food-grade gram negative bacteria, *Gluconacetobacter diazotrophicus*. This strain is an endophytic bacterium that provides nitrogen to the host plant from atmospheric nitrogen. It was originally isolated from sugarcane plants in Brazil (Cavalcante and Dobereiner, 1988). *Gluconacetobacter diazotrophicus* can enter plants through roots, stems, and leaves (James et al., 2001). Envita is applied as a foliar spray in the springtime when cereal crops are in the two to six leaf growth stage. It can be tank mixed with most herbicides, but does not tolerate 2,4-D or MCPA that have antibacterial properties. Azotic North America LTD claims that Envita can maintain wheat yields when synthetic nitrogen is reduced by a rate of 27% or can boost yields when applied with a full rate of nitrogen. Azotic North America LTD has completed several trials with corn, but only one study with wheat. They tested Envita on spring wheat in South Dakota and reported an increase in yield by 7% compared to an untreated control at the same fertilizer rate. Research has also examined Envita with spring wheat in parts of Canada, but these studies have not yet been published under peer review and show a variable response. However, research has been limited on winter wheat in the inland Pacific Northwest.

Fresh Tracks Universal Microbes (FT) produced by Fresh Tracks LLC is another commercial biofertilizer of increasing interest to wheat farmers in the inland Pacific Northwest. FT can be applied as a foliar spray or mixed with seed during planting. Research testing FT in winter wheat is also limited, but it has been used more widely with other high value crops. FT contains four different strains of bacteria: *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, and *Bacillus megaterium*. FT also contains an additional 6 strains of bacteria that are not listed on the label for proprietary reasons. The genus *Bacillus* is one of the predominant bacterial genera found in soil. *Bacillus* spp. serve multiple ecological functions in soil ecosystem from nutrient cycling to conferring stress tolerance to plants. These strains are reported to improve nutrient

efficiency, increase microbial respiration, and increase the amount of nitrogen and phosphorus available for crop use (Etesami et al., 2023). *Bacillus megaterium* is known to oxidize sulfur and increases phosphorus availability by breaking down organic phosphorus in the soil (Subhashini, 2015).

The objective of this study was to determine the impact of using two different commercially available biofertilizer products, Envita and Fresh Tracks Universal Microbes (FT), in an arid dryland cropping region on winter wheat comparing grain yield, grain test weight and protein, nitrogen levels in wheat tissue, total nitrogen uptake in wheat, post-harvest soil nutrients, and financial return on investment.

Methods

Experimental design and location

The trial was located at 2,000 ft above sea level near the town of Moro, Oregon, in North Central Oregon with Walla Walla silt loam soils in a 10-12 inch precipitation zone. Precipitation in Moro during the 2022-2023 crop year (September 2022 – August 2023) was slightly below average at 10.18 inches (Figure 1). Rainfall was well below average during May and June. The soft white winter wheat variety LCS VooDoo was seeded on October 17, 2022, at a seeding rate of 80 lbs of seed per acre with rows planted north to south at a bearing of 23°. Nitrogen rates were selected based on soil nitrogen tests and reduced by 25% from standard university recommendations across the entire field (Lutcher et al. 2007). 70 lbs of nitrogen per acre were applied using liquid ammonium thiosulfate (12% nitrogen and 26% sulfur), along with starter fertilizer NACHURS 6-24-6 (6% nitrogen, 24% phosphate, and 6% potassium). The trial was seeded with a 40 ft no-till disc drill into a field that has been in no till wheat for the last decade. Plots in the field were arranged in a randomized block design and replicated four times. Plots measured 40 feet wide by 600 ft long (during harvest the middle 35 ft of each plot was harvested). Treatments included Fresh Tracks Universal Microbes (FT) mixed with seed at planting, spring foliar application of Envita, and untreated control. Products were acquired directly from manufacturers and all applications followed labeled rates and instructions. FT was

applied at the labeled rate of 30 grams per acre by mixing with seed in the grain auger when filling the grain cart. Envita was applied down the middle 36 ft of treated plots with a UTV mounted boom sprayer on May 11, 2023, at the labeled rate of 3.2 oz per acre when winter wheat was at the four leaf stage. A non-ionic surfactant was used at a rate of 0.1% per volume of spray solution (applied at a rate of 17 gallons of spray solution per acre).

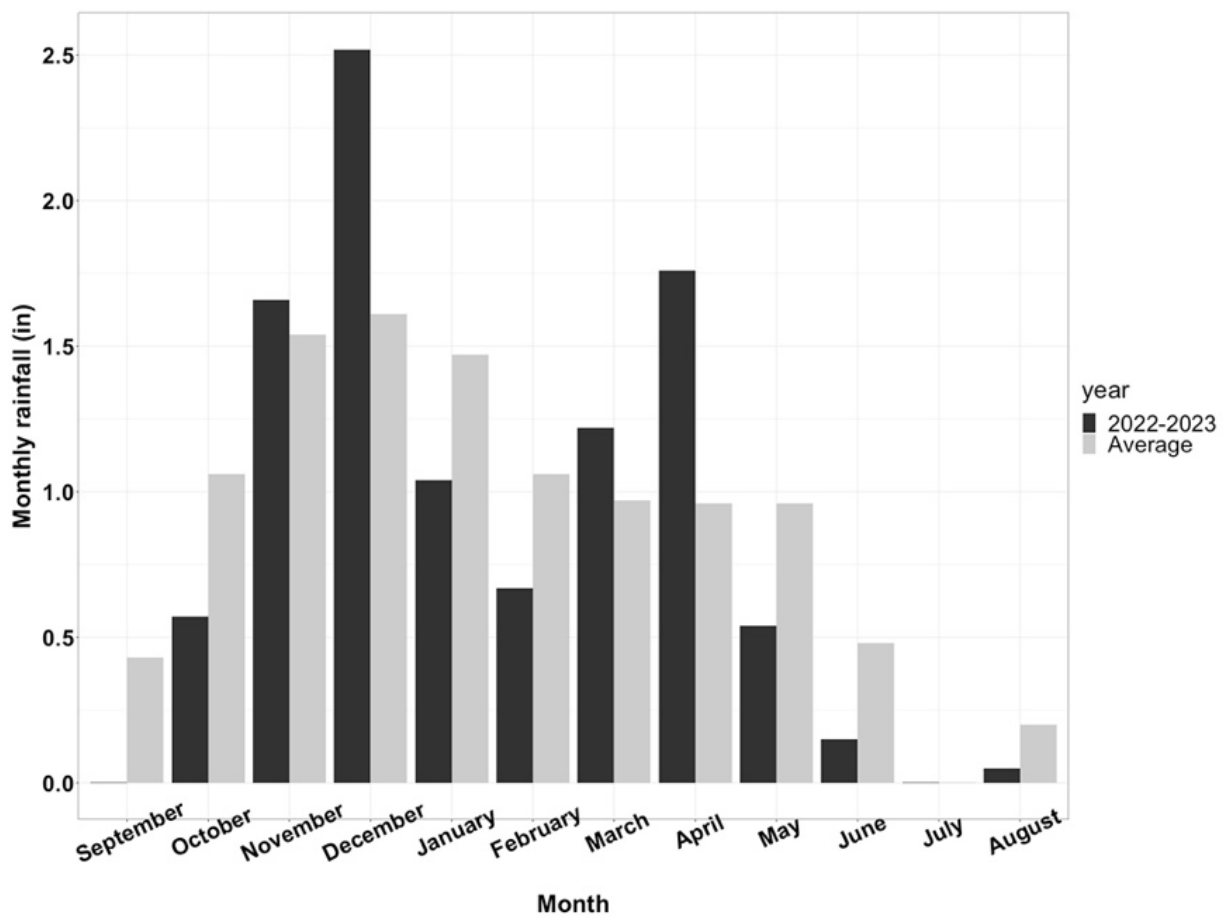


Figure 1. Monthly rainfall for the Sherman Experiment Station in Moro, OR for the 2022-2023 crop year (September 2022 – August 2023) and 30 year average.

Data collection

Wheat tissue was sampled when wheat was in the hard dough stage in early July. A total of eight linear feet of crop row was sampled per plot by clipping at the ground level from four different two ft long sections. Samples were weighed after being completely dried. A sub sample was taken from each sample and sent to a laboratory to determine total nitrogen concentration (%) through combustion analysis. Using tissue test results and known dry weight of tissue samples, total nitrogen uptake was calculated for each plot. The trial was harvested on July 27, 2023, using a combine with a 35 ft header with 2.5 ft between the edges of each plot. Yield was determined using a weigh wagon with one lb accuracy after harvesting each plot. Grain samples were sent to a lab to determine test weight, protein, and moisture for each plot. Soil samples were taken from the top foot of soil in early August using hand augers. Eight sampling points per plot were mixed into one composite sample per plot. Samples were sent to a laboratory to determine nitrate nitrogen (NO₃), ammonium nitrogen (NH₄), pH, organic matter, sulfur (S), phosphorus (P), and potassium (K).

Data analysis and return on investment calculations

Analysis of variance (ANOVA) was used to test for treatment effects at a significance value of $p < 0.05$ for each of the sampled variables. A post hoc Tukey test was planned to be used for pairwise comparisons if ANOVA results indicated a significant effect. All analyses were conducted using R (R Development Core Team, 2023). An economic return on investment was calculated by determining total revenue for each treatment using average yield, grain protein, price premiums for grain protein offered by the grain elevator, and the average price of \$7.18 per bushel for soft white wheat in August 2023. The cost of purchasing biofertilizer products was subtracted from the total revenue calculated for each treatment. Envita cost \$14 per acre to purchase and FT cost \$12 per acre to purchase. This analysis did not include a cost for applying biofertilizers as both products are either mixed in with seed or tank mixed with herbicide and do not require an additional separate application from what a producer is already doing. All other expenses in producing the crop were not included, as only the cost of purchasing the biofertilizer differed between treatments (i.e., the cost of fertilizer and herbicide was not included as they were applied at the same rate across all treatments).

Results

Neither biofertilizer produced a significant response ($p < 0.05$) in any of the examined variables (Tables 1, 2). Envita increased yield by only 1.1 bushel per acre compared to control and FT treatments (80.6 bushels per acre vs. 79.5 bushels per acre) and was not statistically significant ($p = 0.83$, Table 1). Grain quality was not significantly different across treatments, though there was some variability in protein (Figure 2). Nitrogen in wheat tissue and nitrogen uptake was also not significantly increased with biofertilizer treatments (Figure 3). Soil variables showed limited variability between treatments and were not significantly impacted by biofertilizer treatment (Table 2). In terms of financial return on investment, neither biofertilizer provided a positive return on investment. Applying Envita came at a cost of \$1.07 per acre, while applying FT cost \$8.02 per acre.

Table 1. Mean and standard deviation (\pm) for grain yield, grain quality, total nitrogen, and nitrogen uptake, for wheat treated with Envita, Fresh Tracks Universal Microbes (FT), and control. Significance values from ANOVA analysis also included for each variable.

Treatment	Grain Yield and Quality			Tissue Tests	
	Yield (bu/acre)	Protein (%)	Test Weight (lb/bu)	Total Nitrogen (%)	Nitrogen uptake (lbs/acre)
Control	79.49 \pm 3.89	10.2 \pm 0.7	61.3 \pm 0.5	0.83 \pm 0.05	95.39 \pm 10.44
Envita	80.61 \pm 2.42	9.6 \pm 0.4	60.95 \pm 0.3	0.81 \pm 0.15	98.61 \pm 14.34
FT	79.49 \pm 2.31	9.7 \pm 0.5	60.9 \pm 0.4	0.82 \pm 0.03	101.37 \pm 7.56
Significance (p value)	0.83	0.24	0.37	0.97	0.76

Table 2. Mean and standard deviation (\pm) for post harvest soil nutrients sampled in wheat research plots treated with Envita, Fresh Tracks Universal Microbes (FT), and control. Significance values from ANOVA analysis also included for each variable.

Treatment	Soil Tests						
	NO3 (lbs/acre)	NH4 (lbs/acre)	S (ppm)	pH	Organic Matter (%)	P (ppm)	K (ppm)
Control	14.22 \pm 0	24.02 \pm 3.41	4.0 \pm 0	5.83 \pm 0.1	1.5 \pm 0.08	22.75 \pm 1.5	386.25 \pm 19.86
Envita	13.34 \pm 7.16	23.13 \pm 3.56	3.25 \pm 0.5	5.83 \pm 0.1	1.48 \pm 0.08	22.75 \pm 0.5	388.5 \pm 28.44
FT	14.2 \pm 0	24.02 \pm 3.41	3.50 \pm 0.58	5.78 \pm 0.05	1.46 \pm 0.14	22.0 \pm 2.16	380.25 \pm 27.94
Significance (p value)	0.41	0.92	0.1	0.74	0.9	0.82	0.63

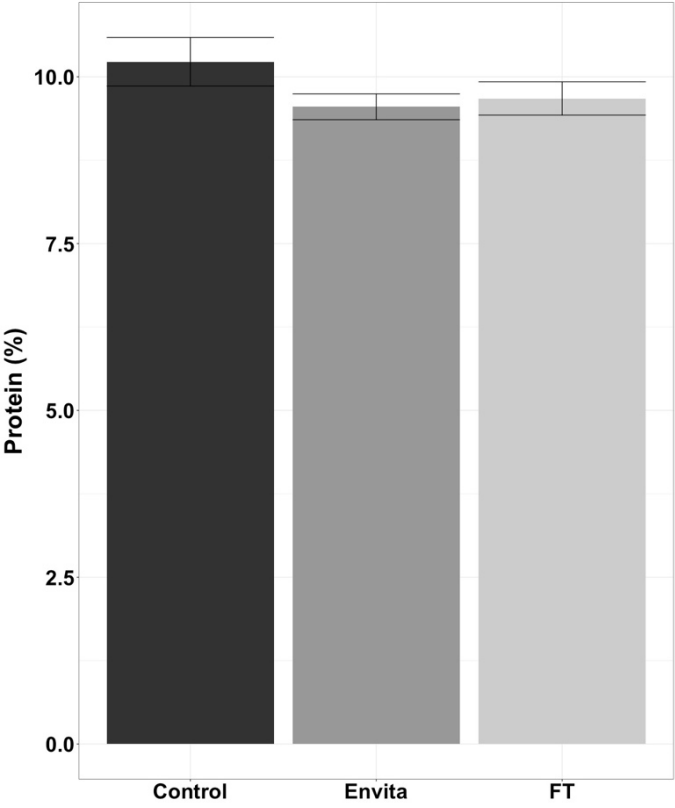


Figure 2. Average grain protein (%) for wheat treated with Envita, Fresh Tracks Universal Microbes (FT), and control. Error bars represent one standard error.

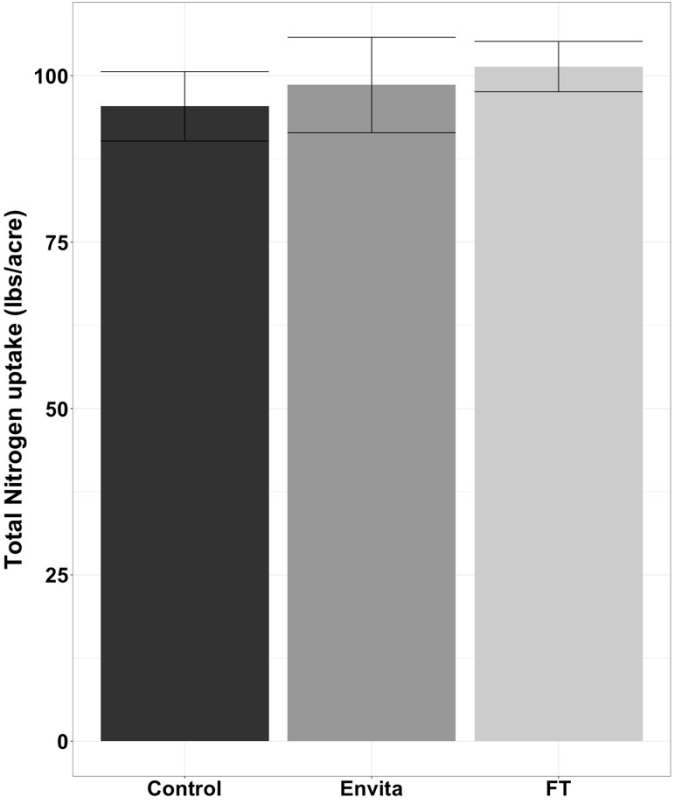


Figure 3. Average nitrogen uptake (lbs/acre) for wheat treated with Envita, Fresh Tracks Universal Microbes (FT), and control. Error bars represent one standard error.

Discussion

Grain yield was increased by only one bushel where Envita was applied compared to FT and the control, indicating a negligible difference. Though FT was added earlier in the crop life cycle at the time of seeding, it did not produce any significant response compared to the control or Envita. The lack of response is not entirely surprising, as recent research in Mexico over several years with multiple biofertilizer products similarly did not find a beneficial response to biofertilizers - except for in soils that were nutrient deficient (Santillano-Cázares et al., 2022). Wheat yield and the effectiveness of these biofertilizers are both influenced by several biotic and abiotic factors that may have limited responsiveness (Ahmad et al., 2011). Drought development, nutrient availability, soil environment, and competition from other soil microbes are all likely contributing factors in the limited response.

Drought conditions that developed in May and June may have caused the wheat to be more water limited than nutrient limited and thus less likely to reflect changes in nutrient availability from FT or Envita. Grain test weight was higher than expected under drought conditions that developed across all treatments. Under extreme drought test weight is easily lowered and reflected by small and shriveled grain. This higher test weight might also be an indication that although drought conditions occurred, biofertilizer response was likely more limited by other factors. Despite slightly higher nitrogen uptake rates where biofertilizers were applied, grain protein was lower with Envita and FT. Lower protein is desired for soft white winter wheat, so the fact that biofertilizers did not significantly increase grain protein in this trial is a positive result. Farmers often receive price premiums for soft white wheat with protein below 10.5% protein. For other classes of wheat, however, high protein is desirable and it would be beneficial if biofertilizers could significantly increase grain protein. A considerable amount of research is needed to better understand the impacts of biofertilizers on nutrient uptake and grain protein.

The soil environment and nutrient availability may have also limited biofertilizer effectiveness. Post harvest soil nutrients showed limited variability and indicated a relatively high amount of soil nutrients in the top foot of soil remaining after the wheat

was harvested across all treatments, with the exception of nitrogen and sulfur. Soil tests after harvest showed an average total of 38 lbs of nitrogen remaining in the top foot from both ammonium nitrogen and nitrate nitrogen. The moderate amount of nitrogen remaining coupled with applying 75% of the recommended nitrogen fertilizer suggests that nitrogen was not a limiting nutrient. Perhaps if nitrogen levels had been more deficient, a yield response would have been generated as found in other recent biofertilizer research (Santillano-Cázares et al., 2022). It was also anticipated that Envita would have left more nitrogen in the soil, as it would enable wheat to access nitrogen directly from atmospheric nitrogen rather than from the soil. However, nitrogen uptake was not increased and soil nitrogen levels showed little variability between treatments. Sulfur levels in this trial were relatively low for the region (Lutcher et al. 2007) and showed some variability where biofertilizers were applied. Much of this variability can be attributed to the limited accuracy of sulfur soil tests. The reduced fertilizer rate also reduced the amount of sulfur applied as nitrogen and sulfur were applied together in the ammonium thiosulfate fertilizer.

Phosphorus levels above 15 ppm are considered high for wheat production in the region and this trial averaged 22.5 ppm, indicating a high level of phosphorus (Lutcher et al. 2007). Research in Mexico with corn and wheat found a positive response to biofertilizers only where soil phosphorus levels were moderately low (Santillano-Cázares et al., 2022). The high phosphorus levels suggest that it could have limited biofertilizer response. In addition, potassium levels were high and usually is not a limiting nutrient for wheat in the region (Lutcher et al., 2007). Soil pH was slightly acidic with an average pH of 5.8 across treatments and may have impacted biofertilizer effectiveness, as has been documented in other biofertilizer studies (Schütz et al., 2018). Soil organic matter was just below 1.5% across all treatments and likely had a neutral to positive influence on biofertilizer response (Schütz et al., 2018).

Effectiveness of these biological products could have also been impacted by the number of bacterial colonies present when they were applied. The lack of response in the examined variables suggests that the inoculation of wheat by bacteria was not effective due to either a lack of bacteria initially present or environmental conditions. The biofertilizers were acquired directly from manufacturers within one month of being

applied. These products were used within their expected shelf life and should have had high numbers of live bacteria present. However, a bacteria analysis was not completed prior to application, so it is possible that bacteria numbers were too low to see a response. Future applied research should include an analysis of the bacteria population in these products prior to applying. Our findings suggest that if farmers plan on using biofertilizer products, it would be wise to test products before applying. It is also possible that biofertilizers were not able to generate a response due to competition with the resident bacteria community already present (Bloemberg and Lugtenberg, 2001; Lugtenberg and Dekkers, 1999). A better understanding of the soil microbiome and how it interacts with introduced biofertilizers is needed before large scale adoption.

In terms of economics, these biofertilizer products would have had to increase yield by at least two bushels with the price of wheat at \$7.18 at the time of harvest in 2023 to cover the expense of purchase. Applying Envita resulted in a cost of \$1.07 per acre and FT at a cost of \$8.02 per acre. This indicates that these products are not favorable for a farmer to apply given the cost and without any significant response to other non-yield related factors, such as soil nutrients. When considering economics, it is important to also factor in premiums for grain quality factors, such as protein for soft white wheat. Lower protein in soft white wheat is preferred by Asian export markets using it for pastries and cakes. Grain protein was not significantly changed with biofertilizers, but was low enough to earn slightly different protein premiums. The protein with Envita averaged 9.6% (24 cents per bushel premium), protein for FT averaged 9.7% (23 cents per bushel), and the control averaged 10.2% (18 cents per bushel). This slight difference increased the yield value of the biofertilizer treatment by only five cents more compared to the control. Across enough acres this premium can increase profits, but given the input costs and lack of yield response this difference would need to be considerable to change the return on investment.

Conclusion

The implications from this study are limited by having only one year of data at one location. However, this research still provides timely and useful information for farmers

in Oregon considering if they should use these products or not. The lack of responsiveness in wheat and soil nutrients to both Envita and FT suggests that more research is needed by manufacturers, universities, and extension to better understand the effectiveness of these products before widespread farmer adoption. Clearly more years of research are needed to fully understand the environmental conditions that may improve the effectiveness of biofertilizer products in wheat and where they may or may not be beneficial for farmers to use. In years without drought development, biofertilizers may be more effective in this region when wheat is more limited by nutrients than water. Global reviews of biofertilizers suggest they may be more effective in drier climates. However, without more research, it is unclear how performance would change in a year with more precipitation (Schütz et al., 2018). It is important to recognize that even if microbes can improve soil health, this response is not always immediate nor is it always translated into yield (Dal Cortivo et al., 2020). The economical use of biofertilizers is complex as it depends on the current price for fertilizers, yield response, and price of wheat. It is critical that farmers and those working in the agricultural industry understand the positive or negative returns that using these biological products can create and their consistency across years. Additional research is needed under different precipitation patterns and across more than just one year to determine the true efficacy and consistency of using these biofertilizers in wheat in Oregon.

Acknowledgments

This work would not have been possible without the assistance and cooperation of Noah Williams allowing this research to take place in his field, plus his additional time, fuel, and equipment hours to seed trials and harvest using his equipment and weigh wagon. In addition, thank you to the Oregon Wheat Commission and Fresh Tracks Ag for providing funding to cover the expenses of laboratory testing for grain samples, tissue samples, and soil samples. Lastly, thank you to Wasco County OSU Extension interns, Keon Kiser and Theo Sandoz, for helping with field sampling and data analysis.

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