Protocols for Field-Scale Assessments of Biofertilizers and Biostimulants Applied to Enhance Nutrient Use Efficiency of Grain Crops

Background on protocol development

Beginning in early 2018, the NutrientStar Science Review Panel began to consider the emerging biofertilizer and biostimulant market and how best to inform farmers and their advisors about such products - especially those that make fertilizer efficiency claims. Discussion was around the fact that many biofertilizers and biostimulants are already being sold and many more are under development, but there are no standardized protocols for field-scale evaluations of the nutrient use efficiency achieved by these products, and little - if any - published studies in the scientific literature about the performance of such products.

Over several months, the panel deliberated and carefully developed a set of protocols for field scale evaluations of the nutrient use efficiency of these products. The NutrientStar team shared a draft of the protocols with industry representatives and presented the protocols at a special session during the Tri-Societies meetings in November 2018 to generate additional discussion and feedback. The version of the protocols presented here reflects the feedback received at the session and feedback from industry about a revised draft posted to the NutrientStar website (www.nutrientstar.org) shortly after the session.

Purpose of Protocols

The protocols described here are for *field-scale* evaluations of biofertilizers and biostimulants that claim a nutrient use efficiency benefit for major agronomic crops such as corn, wheat, and soybean. Protocols for laboratory, greenhouse, and small-plot field studies to develop new biofertilizers and biostimulants are *not* included.

With this protocol, the NutrientStar program aims to enable rigorous evaluation of the nutrient use efficiency achieved with biofertilizer and biostimulant products.[1] The protocol is designed to generate results that will be as accurate and reproducible as possible, providing scientists, farm advisors and farmers with reliable information about the effectiveness of the products.

There are no legal definitions of biofertilizers or biostimulants. We used the following definitions as we developed these protocols:

- A biofertilizer is a formulated product containing one or more microorganisms that may enhance the nutrient status (and the growth and yield) of plants by either replacing soil nutrients and/or by making nutrients more available to plants and/or by increasing plant access to nutrients (Malusá and Vassilev, 2014). Phosphorus solubilizing bacteria and

[1] The protocols are for products that do not require registration with the US Environmental Protection Agency (USEPA).

fungi are examples of biofertilizers that are advertised to enhance phosphorus uptake and increase yields.

- A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content (du Jardin, 2015). Humic acid amendments are a type of biostimulant commonly advertised to optimize plant health and yields.

The protocols proposed here for both types of products are *relative to their performance in terms of nutrient uptake, use and efficiency under field-scale conditions*.

I. Protocols for Field-Scale Evaluations of Biofertilizers and Biostimulants Applied to Enhance Nutrient Use Efficiency of Grain Crops

A. Number of trials and geographical distribution

1. A minimum of 20 trials in 2 years is recommended
2. The 20 trials should be located across the geographical area where the product will be sold and should include evaluations on the major soils and in the environmental conditions within the sales area.

B. In-field protocols – Minimum requirements

1. There should be a minimum of two treatments: one treatment with the biostimulant or biofertilizer and one without.
2. Treatments should be replicated at least four times.
3. Treatments should evaluate the product based on the advertised claims about the benefits of the product. For example, if a product is advertised to lower the rate of fertilizer, the product should be evaluated with a rate of fertilizer that is lower than the farmer typically applies with 50-70% of normal a good target; if a product is advertised to enhance yield at a full rate of fertilizer, the product should be evaluated at a full rate of fertilizer application.
4. Viability testing of the product should be completed immediately before implementation of evaluation trials if the product is a living organism.
5. The minimum length of a plot should be 62 meters (200 feet). Plots 400 meters long (1200 feet) or longer are preferred.
6. The minimum width of a plot should be the width of the combine used to harvest the trial plus at least 4 rows on either side of a plot to eliminate border effects.
7. Harvest should be completed by making at least one pass through a treatment strip that has at least a 4-row non-treatment border without mixing grain from two treatment strips.
8. The yield monitor should be set for loads or regions to identify the different strips.
9. Fertilizer applicators or sprayers should be calibrated before applying treatments.

10. Yield monitors on combines should be calibrated before harvesting the strips.
11. If the biostimulant or biofertilizer is applied to seed, the seed for both the treated and untreated seeds should be the identical variety, and applied at the identical rate, time and placement as the control treatment
12. Control plots should be planted before treatment plots.
13. Equipment used to apply the biostimulant or biofertilizer should be cleaned between application of treatments containing the biostimulant or biofertilizer by using ethanol or another method known to remove or kill the biostimulant or biofertilizer to ensure no contamination of the other treatments.
14. All management practices such as tillage, pest control, seed variety, fertility, etc., should be identical across the entire experiment and based on recommended practices
15. Soil type should be similar across the experimental area
16. Artificial drainage, if present, should be the same across the experimental area.

II. Minimum Data Reporting Requirements for Field-Scale Evaluations of Biofertilizers and Biostimulants Applied to Enhance Nutrient Use Efficiency of Grain Crops

A. Soil and Landscape

Required information

1. Geolocation of field to 4 decimals or a tenth of a degree
If do not provide geolocation then provide:
    a. Slope
    b. Dominant soil series
    c. Drainage class
    d. Technology Extrapolation Domain (TED) number

2. If tile drainage in trial area:
    a. Yes or No

B. Cultural Practices & Management

Required information

1. N Fertilizer, previous and current years: rate, form, timing, placement for all applications
2. Manure, previous and current year: type, rate of application
3. Fertilizer other than N applied in year of experiment: rate, form, timing, placement
4. If a product requires foliar application, the rate, form, timing, placement, type of sprayer and nozzle size for all applications.
5. Previous two crops and current crop
6. Seed variety or hybrid

7. Herbicide applications: rate, type, application method, and timing
8. Insecticide applications: rate, type, application method, and timing
9. Fungicide applications: rate, type, application method and timing
10. Tillage practices from harvest of previous crop until harvest of experiment
11. Cover crop: species, planting date, termination method and date

Optional information to enhance evaluations

1. Manure applications: type, and manure applied past 10 years: yes/no
2. Tillage: depth and dates

C. Trial information

Required information

1. Routine soil test results for macronutrients, soil organic matter and pH
2. Duration of study – months, years
3. Dimensions of individual plot
4. Dimensions of entire trial
5. Planting date
6. Seeding rate
7. Row spacing
8. Dates for N applications
9. Active ingredient of product being tested
10. Concentration of active ingredient of product being tested
    If water mixed with product, provide:
    a. Cl in water
    b. Salt content
    c. pH
11. Harvest date
12. Harvest area
13. Harvest method
14. Individual strip yields
15. If individual strip yields are not provided, report mean yield and standard deviations by strip

Optional information to enhance evaluations

1. Measured nutrient plant uptake with and without the product

D. Environmental information

Required information

1. Daily rainfall two weeks before application of product to be tested until harvest of experiment

2. Daily ambient temperatures for the two weeks after application of treatments
3. Annual and seasonal rainfall
4. Deviations from normal rainfall – annual and seasonal
5. If a product's effectiveness is dependent on sufficient soil moisture for activation of the product at or shortly after the time of application, soil moisture should be reported at the time of application and for two weeks after application.
6. If a product's effectiveness is dependent on rainfall or irrigation for activation of the product at or shortly after the time of application, irrigation amounts and/or daily rainfall should be reported at the time of application and for two weeks after application.
7. If a product's effectiveness is dependent on soil temperature for activation of the product at or shortly after the time of application, soil temperature should be reported at the time of application and for two weeks after application.

Optional information to enhance evaluations

1. Growing degree days
2. Soil temperature for the two weeks after application of treatments
3. Soil moisture for the two weeks after application of treatments
4. Solar radiation

E. Economic information

Required information

1. Cost of product being tested per acre
2. Additional cost of application for product, if any

References

Du Jardin P. (2015). Plant biostimulants: definition, concept, main categories and regulation. Sci. Hortic. 196, 3–14. 10.1016/j.scienta.2015.09.021.

Malusá, E. and N. Vassilev. 2014. A contribution to set a legal framework for biofertilisers. Appl Microbiol Biotechnol (2014) 98:6599–6607. DOI 10.1007/s00253-014-5828-y.