#### An Overview of Crop Biostimulants: Do They Work and What Can You Expect?



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# **Topics Addressed**

- Types of crop biostimulants
  - -Biologicals
  - -Microbials
  - Modes of action
- Do they work in the field?
   Case studies
  - Corn
  - Potatoes





#### What are Crop Biostimulants?

- Currently no legal definition in the U.S.
  - -Initially more popular in Europe than in the U.S.
  - Working definition part of the 2018 Farm Bill
  - Some states/industries adopting Farm Bill definition

#### European Biostimulants Industry Council Definition

"Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/ benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality (independent of its nutrient content)."

## What are Crop Biostimulants?

Another definition:

"Substances/microbes provided in minute quantities that promote plant growth" (du Jardin, 2015)

- Not fertilizers
- Not pesticides
- Not soil conditioners

• Various products have been marketed for many years

## NCERA 103

- Specialized Soil Amendments and Products, Growth Stimulants, and Soil Fertility Management Programs
  - Regional committee developed in 1980 to test claims of biostimulants and other nonconventional products

-<u>https://NCERA (ncera103.org)</u>

 Compendium of Research Reports on Use of Non-Traditional Materials for Crop Production



# 2018 Farm Bill

(c) PLANT BIOSTIMULANT.—For the purposes of the report under subsection (a), the Secretary—

(1) shall consider "plant biostimulant" to be a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield; and

(2) may modify the description of plant biostimulant, as appropriate.



## 2018 Farm Bill

- USDA agrees to coordinate a multi-stakeholder workgroup to develop framework
  - Additional players: EPA, FDA, TFI, AAPFCO, AAPCO, NASDA, INDUSTRY
  - Goal common definitions/rules/labeling for states/industry to follow
  - A push to adoption the Farm Bill definition for certification
    - Does not promise efficacy
    - Provides research guidelines for testing
    - Certifies that companies have conducted proper research to verify claims

## **Categories of Biostimulants**

- Biologicals organic compounds (not living)
  - Humic substances
  - Amino acids and other N compounds/Sugars
  - Chitosans
  - Extracts Seaweed/other plants
    - Chelates, affect enzymes, membrane permeability, antioxidants, stimulate microbes, biocontrol; hormonal activity

#### Microbials - Beneficial microorganisms

- Bacteria plant growth promoting rhizobacteria
- Fungi often mycorrhizae

#### Applied individually or in combination



#### Humic Substances (HS)

- Constituents of soil organic matter
  - -Decomposition products of plants, animals, microbes
    - Peats, Composts and Leonardite
  - -Occur naturally in soil (OM breakdown)
  - -Categorized by molecular weight



Sample Intermediate

Product

Process

Extract with alkal

Humin

insoluble)

#### **Reported Modes of Action for HS**

#### Under conditions of adequate nutrition in solution

- Stimulation of root growth length and secondary growth
- Complex metal cations (iron, zinc etc.)
- Increase in membrane permeability
- Stimulation of nutrient uptake (esp. P)
- Foliar application
  - Some indication of increased root and shoot growth
- In general, effects of HS on plant growth are not consistent and depend on several factors



#### HS Meta-Analysis (Rose et al., 2014)

- Response to HS was affected by:
  - Source of HS (compost HS > lignite HS)
  - Rate of application
  - Lesser extent crop type & growing conditions

#### HS increased shoot and root growth by 15-25%

- Half the studies failed to increase growth by 5%
- High variation increases risks to farmers
- Rates in meta-analysis that promoted growth were <u>much higher</u> than rates recommended for commercial HS products used in the field



## Amino Acids and Other N Compounds

- Amino acids and peptides are protein breakdown products
  - Often termed protein hydrolysates
  - Protein is split with acid, alkakli or enzymes
  - Derived from animal or plant waste material



## Amino Acids and Other N Compounds

- Protein hydolysates have multiple uses:
  - Media for animal and plant cell culture
  - Animal feeds
  - Dietary supplement for humans
  - Crop production
- Source of N that is easier to digest than protein
  - For animals
- Plants can use inorganic N
  - Effect in plants is not due to the N supply





## Amino Acids and Other N Compounds

- Reported Modes of Action in Plants
  - Some amino acids have a chelating effect
    - Increase micronutrient availability and acquisition
    - Decrease heavy metal toxicity
  - Regulate enzymes involved in N assimilation
  - Antioxidant activity scavenge free radicals
    - Increase tolerance to stress salt, heat, chilling
  - Indirect effect increase microbial biomass and nutrient cycling
- Applied in furrow and foliar quarts to gallon/A

## Chitosans

- Linear polysaccharide composed of glucosamine
- Made by treating the shells of shrimp (chitin) with sodium hydroxide
- Uses:
  - Cancer treatment
  - Dietary supplement (we
  - Wine making
  - Agriculture





## Effects and Modes of Action for Chitosans

- Effects on plants seed treatment or foliar application
  - Aids in defense of pathogens biocontrol of fungi and nematodes biopesticide registered by EPA
  - Stimulates growth; increases photosynthesis; stimulates nutrient uptake
- Modes of action
  - Affects cell membranes; alters DNA; activates defense genes



# **Seaweed Extracts**

- Used in agriculture for thousands of years
  - Nutrient source; compost





https://www.indiamart.com/proddetail/seaweedextract-liquid-zyme-solution-3815782562.html



#### Seaweed Extract Uses

 Like amino acids – extracts are also consumed by humans as a dietary supplement



- Applied to soil or directly to the plant as a foliar application
- Generally applied with the seed or as a foliar application; often combined with fertilizer
   1.5 to 2 qts/A



#### Seaweed Extracts - Mode of Action

- Rates applied are too low to be of direct nutrient benefit
- Rates applied are also too low to have direct plant hormone effects
- Foliar application may stimulates production of hormones within the plant, which in turn may affect growth and stress tolerance

## **Biologicals - Beneficial Microorganisms**

- Practice of microbial inoculation has been used for centuries
  - Rhizobium for legumes; Nitrogen fixation
- Microbial inoculants; applied to soil or leaves
  - Classified as biopesticides or biofertilizers
  - Grey area as some are considered biocontrol agents
- Fastest growing segment of the biostimulant industry
  - New DNA techniques for analyzing the soil microbiome soil health
  - Interest in more sustainable practices climate change mitigation



## **Types of Microbial Inoculants**

- Free living bacteria, fungi, mycorrhizal fungi
- Derived from soil, plants, plant residues, composted manure, and water

- Isolated from the rhizosphere or within the plant (endophytes)





### **Examples of Beneficial Bacteria**

#### • Free-living nitrogen fixers

- Azospirillium, Azobacter
- Bacillus polymyxa, Clostridium pasteurianum
- Endophytes that fix N Envita, Utrisha
- Genetically modified N fixers ProveN



#### -Azospirillium is found in close contact with roots

Can supply 7-12% of N for wheat

#### Phosphorus solubilizers

- Pseudomonas spp., Azospirillium, Bacillus spp., etc.
- Produce organic acids and phosphatase enzyme

http://agriculturers.com/azospirillum-la-bacteria-del-suelo-como-bio-fertilizante-en-la-agricultura



### **Examples of Beneficial Bacteria**

- Iron transport chelators
  - Bacillus, Pseudomonas, Streptomyces
  - Siderophore production

#### Volatile organic compounds (VOCs)

- Paenibacillus, Bacillus strains
- Alcohols, ketones, hydrocarbons
- Biocontrol properties
- Growth stimulation increased auxin production



<sup>(</sup>Delaplace, et al., 2015)

- Production of plant growth regulators
  - Azospirillium spp. produces auxin & gibberellins
  - Bacillus subtlis increases cytokinin

## Is Inoculation with Microbes Effective?

- Field studies with microbes and other biostimulants so far have been inconsistent
- Each teaspoon of field soil contains over 1 billion bacteria
- Beneficial microbes must compete with the existing microbial community
  - They often lose out
- Nitrogen fertilizer application shuts down N fixation



## Research Should be Scientifically Sound

- Do not rely on testimonials
- Be careful of research that does not include proper controls
- Recommended N rate vs. 20% less N + biostimulant will not be enough to show that a biostimulant is effective

   A proper study should also include 20% less N without a biostimulant
- Ideally all N rates tested should compare with and without a biostimulant
- Try on a small scale first before using it on the whole farm



#### **Recent Publication on Biostimulants**

- Performance of Selected Commercially Available Asymbiotic N-fixing Products in the North Central Region
- Summary of field trials with:
  - Envita Gluconacetobacter diazotrophicus
  - Utrisha Methylobacterium symbioticum
  - ProveN Klebsiella variicola, genetically modified
  - <u>https://www.ndsu.edu/fileadmin/snrs/Files/SF2080\_Performance</u> of Selected N-fixing Products.pdf



**Selected Commercially Available** 

NDSIL EXT

NDSU

EXTENSION

Performance of

Abacteria, have the ability to 'th' nitrogen (h) fitters the atmosphere with its abundint as hogs, and produce Nitry whoth prival associated with plants of trugi. Fossil avidance indicates that asymbolic. Horse wore present and active 15 billino years ago (Bydy and Plever, 2013). This compares to the appearance only about 50 million years ago of the more commonly income which the Hubing bacteria associated with legumes (Bjerert and James, 2007).

The process used to fix N by asymbiotic organisms is highly energy intensive. The arrying that serves as the fixation facilitation in bacteria is nitrogenase. To convert one molecule of N<sub>2</sub> to one molecule of NH<sub>3</sub>, this requires 16.87 (denosine triphosphata) molecules (the chemical fault for many biological reactions) and 8 electrons. This compares to only SATP to produce one peptide bond in the production animo acidit, which is classified itself as a 'high energy requirement' (Smercina et al., 2019).

te energy required by asymbiotic organisms to fix N may come from a triety of sources depending on their spatial presence in the surface soil

consist of soil organic mater, and intermediary compounds during the process of plant/animal decay, or the decay or other microorganisms. A rich source of energy



Figure 1. Nitrogenose enzyme, C7H19Fe7MoN07S9-12 (PubChem http://pubchem.ncbi.nlm.nih.gov/compound/25199882).

#### Field Studies a Biostimulants Corn Case Study with Envita

- On-farm trial near Le Sueur, MN
- Product tested Envita
  - A new biological product reputed to benefit grain species by fixing nitrogen for the plant



LABEL VER. 190115-01

#### **Microbial Inoculant**

#### CONTAINS NON-PLANT FOOD INGREDIENTS:

Active Ingredient: Gluconacetobacter diazotrophicus 1	x 10 <sup>7</sup> CFU / ml
Total Other Inert Ingredients:	98% (w/v)
(polysaccharides: 1-10% (w/v) and water: 88-97% (w/v))	



#### Envita Instructions (from website)

- Envita microbes fix N in the plant (endophyte) – originally isolated from sugarcane
- Apply in furrow or as a seed treatment or foliar
- Reported to fix nitrogen quickly



Edge of corn root tip showing blue stained Envita bacteria within cells (scale bar = 10  $\mu m$ ).

Section of corn leaf showing blue stained Envita bacteria within cells.

Envita colonized (bright red) within the chloroplast of cells within a corn leaves.



#### On-Farm Trial Design (Corn P0339R)

- Three N Rates
  - Base N Rate 40 pounds/A at plant
  - Mid N Rate 40 pounds at plant + 50 pounds side dress = 90 pounds/A
  - High N Rate 40 pounds at plant + 120 pounds side dress = 160 pounds/A
- Two Microbial treatments (With or Without Envita)
   Envita (4 oz) applied with 2.5 gallons 10-34-0/A in furrow
- Four Replicated Blocks randomized complete block design (3 x 2 x 4 factorial = 24 plots/strips)
  - Each plot = 12, 30" rows wide length of field





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128c 187b 245a < 0.0001 191a 182b 0.0890 133 123 193 180 247 242

#### Illinois Envita Trials – 2021

Dr. Emerson Nafziger – University of Illinois

- 2 locations
- Corn-Corn and Soybean-Corn
- On-farm strip trials 4 reps
- 4 N rates and a control; +/- Envita
- Envita applied at labeled rates, both in-furrow (4 oz/A)
  and as foliar spray (3.2 oz/A) applied at stage V3



#### Illinois Envita Trials – 2021

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## Envita Summary

- Data does not support claims of increasing yields with Envita
- Envita was not effective in replacing corn N requirement
- Reasons for the slight decline in yield with Envita in the first study are not known. Not an expected response

# Field Studies a Biostimulants

Potato Case Study - 2023

- Sand Plain Research Farm, Becker, MN
- Product tested Utrisha
  - "Utrisha N is a biological solution that provides a variety of crops with an alternative, sustainable nitrogen source."



Active ingredient:

3%	•	Microbe
	Methylobacterium symbioticum	SB23, 3 x 107 CFU/g
97%	Total Other (Inert)	Ingredients (Carriers)
100%		Total

#### Small Plot Study with Chipping Potatoes

- Two varieties
- Three N Rates
  - Base N Rate 40 lbs/A at plant
  - Mid N Rate 120 or 160 lbs N/A
  - High N Rate -240 or 320 lbs N/A
- Two microbial treatments (With or Without Utrisha)
  - Foliar application Utrisha (5 oz/A) Late June
- Four Replicated Blocks randomized complete block design (2 x 3 x 2 x 4 factorial = 48 plots/strips)
   – Each plot = 4, 36" rows wide x 20 ft in length

# Minnesota Potato Utrisha Trial FL 1 FL 2



Utrisha effect – NS; N rate effect \*; Variety effect - NS

## Summary and Key Points

- Biostimulants are substances or microbes applied to soil, plants, or plant roots in small amounts that promote growth
- Modes of action for biostimulants vary
  - Direct on plant or indirect effects by stimulating microbes
  - Improved nutrient availability and uptake, N fixation
  - Act as a growth regulator or hormone
  - Protect against diseases
- Effects found in controlled lab experiments often do not translate to the field



## Summary and Key Points

- Responses in the field are inconsistent at best
  - Yield increases if they do occur are often small
  - Field variability ("background noise")
  - Biostimulants come at a cost
  - There are no silver bullets
- Evaluation of biostimulants should be based on replicated field studies over multiple years
- Test on a small area before using the product on the whole farm