Evaluating Biologicals

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WHAT ARE CROP BIOSTIMULANTS?

- Currently no legal definition
  - More popular in Europe than in the U.S.
  - Definition is evolving

- 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."

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

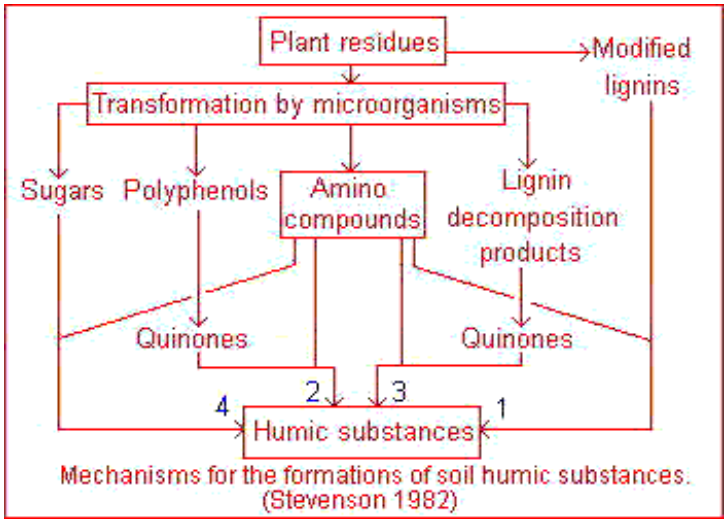
- Various products have been marketed for many years
  - Regional committee developed in 1980 to test claims of biostimulants and other nonconventional products
    - NCR-103; NCERA 103
    - http://extension.agron.iastate.edu/compendium/index.aspx

CATEGORIES OF BIOSTIMULANTS

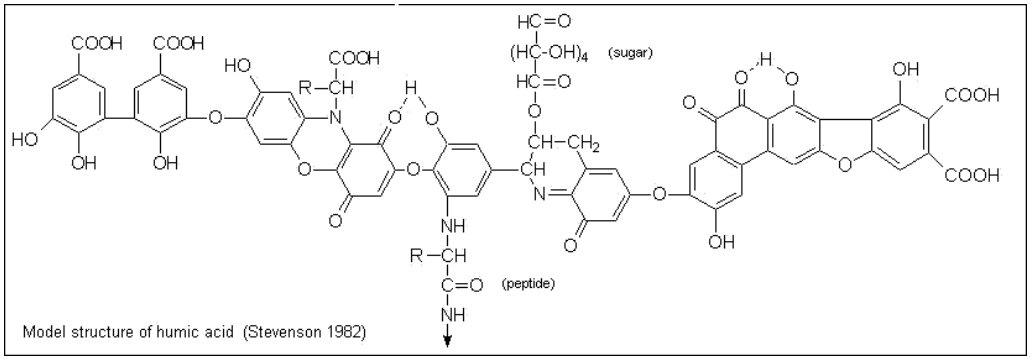
1. Humic substances
2. Amino acids and other N compounds
3. Chitosans
4. Seaweed extracts
5. Beneficial microorganisms
   - Bacteria – plant growth promoting rhizobacteria
   - Fungi – often mycorrhizae
6. Applied individually or in combination; promoted as “natural”

HUMIC SUBSTANCES (HS)

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



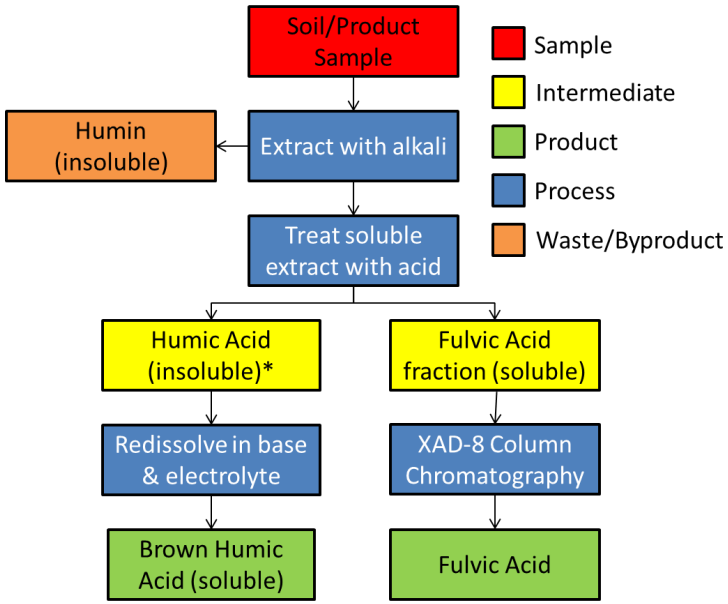
Mechanisms for the formations of soil humic substances. (Stevenson 1982)



Model structure of humic acid (Stevenson 1982)

COMMERCIAL HS PRODUCTS

- Sources of commercial humic substances
  - Peat soils
  - Composts
  - Leonardite (oxidized lignite or compressed peat)



http://oceanagrollc.com/standard-humic-acid-testing-protocols-a-review/

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

- Foliar application
  - Some indication of increased root and shoot growth

- In general, effects of HS on plant growth are not consistent and depend on a number of factors

ACCOMPLISH LM DATA

- Yield increase due to starter at 2 of 5 locations
- Yield increase due to Accomplish LM at 1 of 5 locations

		10-34-0 Rate (gal/ac)				Product Rate (qt/ac)		
Variable	Site	0	2.5	5		0	1	2
		----------bushels per acre----------						
Corn Grain Yield	1	157a	160a	162a		*156b*	*160a*	*163a*
	2	193a	194a	190a		193a	192a	193a
	3	179a	184a	183a		188a	180a	179a
	4	*174b*	*192a*	*181b*		183a	184a	180a
	5	*95b*	*92b*	*112a*		106a	91a	102a

HS META-ANALYSIS (ROSE ET AL., 2014)

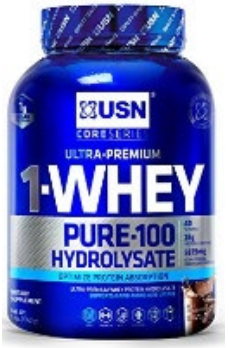
- Response to HS was affected by:
  - Source of HS (compost HS > lignite HS)
  - Rate of application
  - Lesser extent crop type & growing conditions

- 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 much higher than rates recommended for commercial HS products used in the field

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 nitrogen 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
  - 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

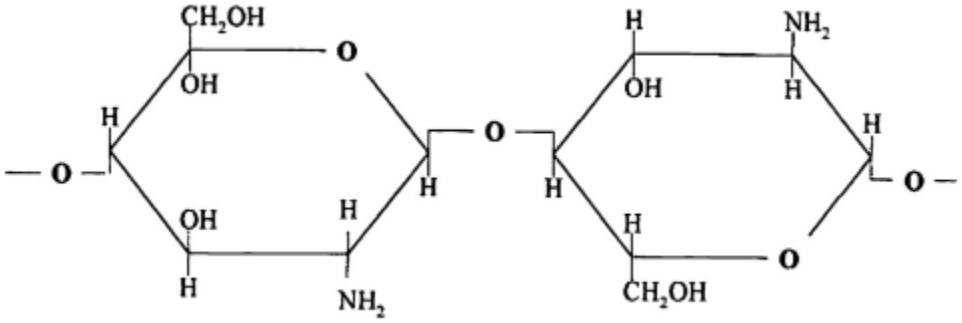
- Most effects are based on greenhouse studies
  - Soil and foliar application methods

EFFECTS ON PLANT GROWTH -AMINO ACIDS

- Commercial products are available, but consistent crop responses are not common
- Amino acids are a readily available source of N for microbes
  - Direct effects of amino acids or other small N compounds on plant growth in the field are questionable; more likely indirect by stimulating microbes
- Foliar application
  - Repeated application has been reported to increase yield in some horticultural crops

CHITOSANS

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



EFFECTS AND REPORTED 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

FIELD RESEARCH WITH WHEAT – HADWIGER, 2013

- "The conditions that enabled chitosan to increase wheat yield were so influenced by environmental or cultural practice that in a given year the chitosan benefits could deviate both up and down from the average 10% yield increase and reflected on the product credibility…"

- "On the plus side, chitosan can also increase yields and is edible, without being allergenic, mutagenic, or carcinogenic. Thus, the application of chitosan directly to the plant part to be consumed is not of major concern."

SEAWEED EXTRACTS

- Seaweed used in agriculture for thousands of years
  - Nutrient source; compost
- Process developed in the 1950s to produce liquid extracts
  - Mostly made from brown seaweeds
    - *Ascophyllum nodosum, Fucus, Laminaria, Sargassum, & Turbinaria spp.*
  - Proprietary process includes:
    - Includes extracting with water, acids, alkalis with or without heating
    - Physical disruption – low temperatures; high pressure

SEAWEED EXTRACT COMPOSITION

- Polysaccharides (long chain sugars)
- Carrageenans (polysaccharides with sulfur)
  - Flucoidan, alginates, laminarin
- Macro and micronutrients
- Various plant hormones – auxins, gibberellins, cytokinins, etc.

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
- Some evidence that foliar application stimulates production of hormones within the plant, which in turn may affect growth and stress tolerance

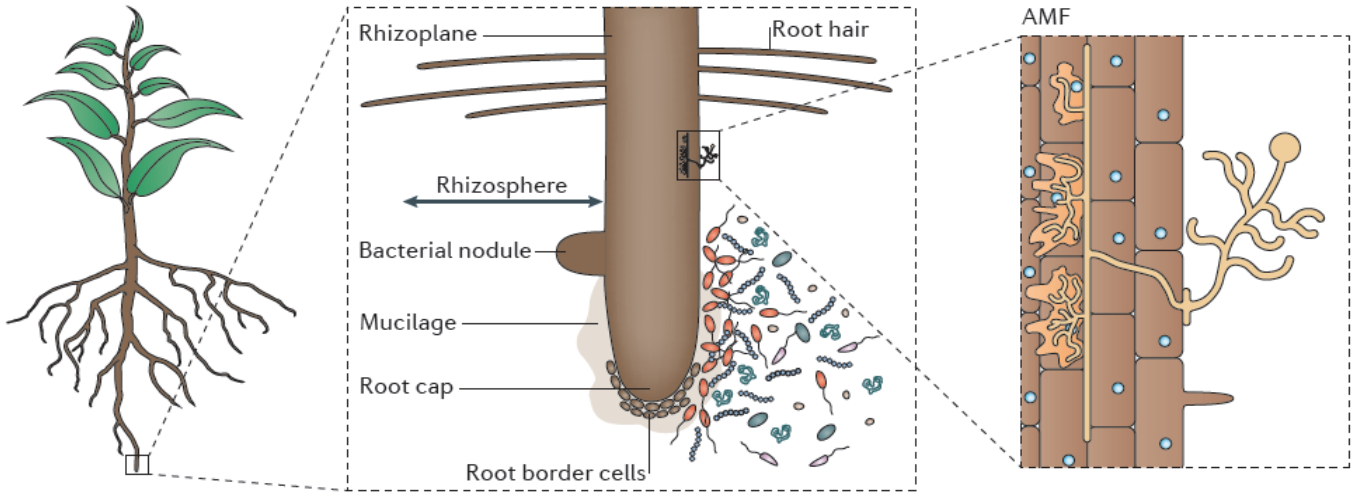
BENEFICIAL MICROORGANISMS

- Practice of microbial inoculation has been used for centuries
  - Rhizobium for legumes; Nitrogen fixation
- Often termed microbial inoculants
  - 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
  - Interest in more sustainable practices

TYPES OF MICROBIAL INOCULANTS

- Free living bacteria, fungi, mycorrhizal fungi
- Isolated from soil, plants, plant residues, composted manure, and water
  - Most isolated from the rhizosphere

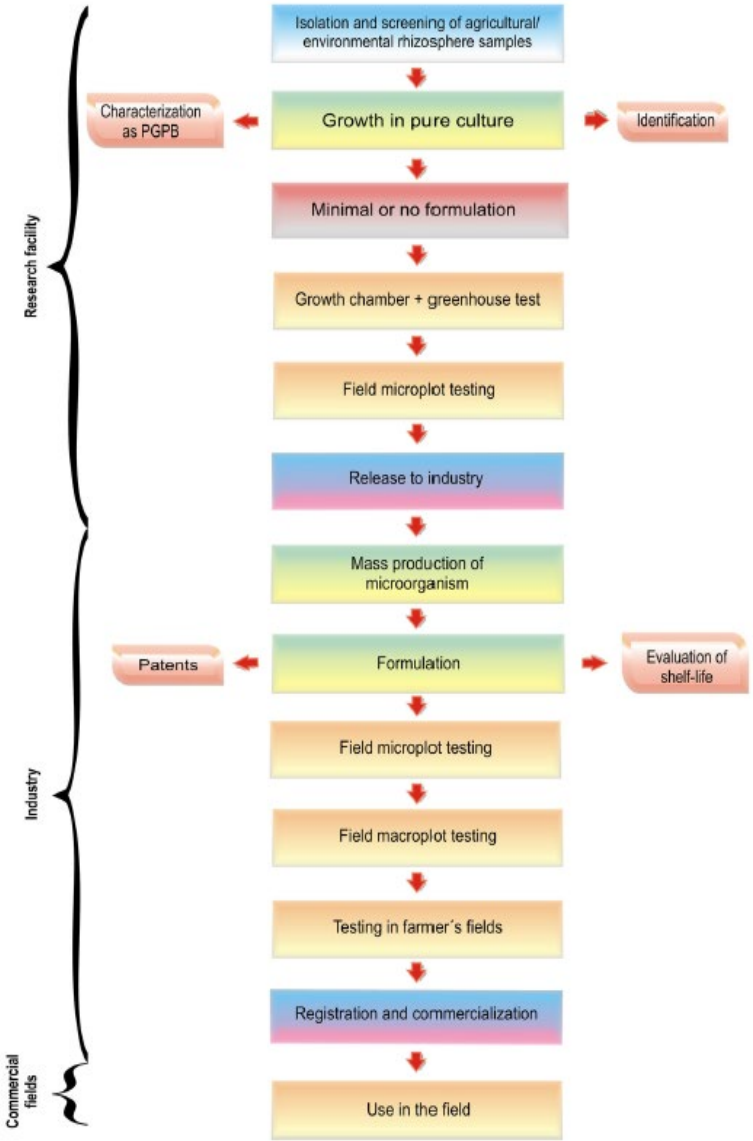
http://plantsinaction.science.uq.edu.au/content/421-rhizosphere



DEVELOPMENT OF INOCULANTS

- Factors to consider:
  - Plant species (root exudates)
  - Reproducibility over environments
  - Commercial formulation – must survive and be effective
  - Compatibility with fertilizers and pesticides

Bashan, et al., 2014



BENEFICIAL FUNGI

- Fungi have co-evolved with plants for millions years
  - Symbiosis to parasitism
- Fungal endophytes
  - Mycorrhizae – beneficial symbiosis with over 90% of all terrestrial plants
    - Associated with increased P uptake, Zn uptake, protects against some diseases, increases drought tolerance
    - Extends the root system to explore more soil (similar to root hairs)
  - Other fungal endophytes
    - e.g. Trichoderma
    - Transfer nutrients and also have biopesticidal activity

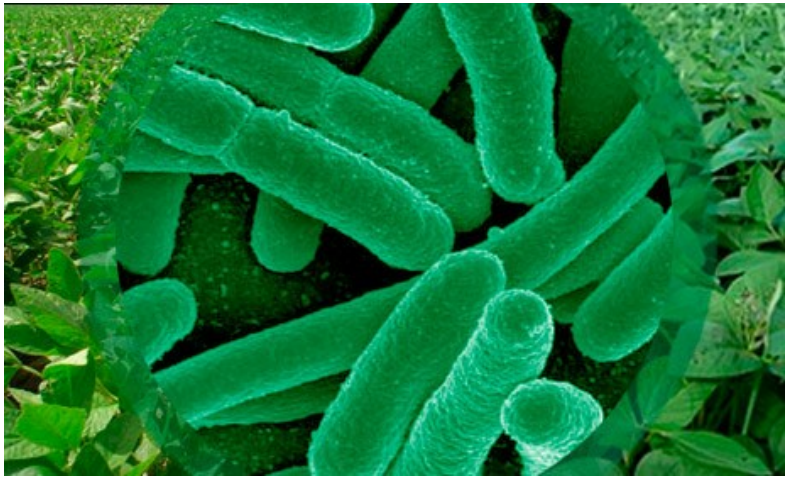
BENEFICIAL BACTERIA

- Two types of bacteria isolated
  - Plant growth promoting bacteria (PGPB)
  - Plant growth promoting rhizobacteria (PGPR)

- Mechanisms reported for stimulating plant growth and nutrient uptake
  - Asymbiotic nitrogen fixation (free living)
  - Nutrient solubilization (esp. phosphorus)
  - Production of chelating agents
  - Production of volatile organic compounds
  - Production of growth hormones

EXAMPLES OF BENEFICIAL BACTERIA

- Free living nitrogen fixers
  - *Azospirillium*
  - *Azobacter*
  - *Bacillus polymyxa*
  - *Clostridium pasteurianum*



  - *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
  - *Paenibacillus, Bacillus* strains
  - Alcohols, ketones, hydrocarbons
  - Biocontrol properties
  - Growth stimulation increased auxin production
- Production of plant growth regulators
  - *Azospirillium spp.* – produces auxin & gibberllins
  - *Bacillus subtlis* – increases cytokinin

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IS INOCULATION WITH BACTERIA BENEFICIAL?

- 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

2019 DATA – PROVEN (ROSEMOUNT MN C-C

- 2% increase in greeness measured as NDRE at V5
- 2% increase in SPAD value at R1 (ear leaf)
- 3% increase in plant mass at R1
- No increase in N uptake at V5 or R1
- No increase in Yield

SUMMARY AND KEY POINTS

- Responses in the field are inconsistent at best
  - There are no silver bullets
  - They come at a cost
- Do not use biostimulants based on testimonials
- Evaluation of biostimulants should be based on replicated field studies over multiple years
  - Compare apples to apples
- A more systematic approach is needed to define situations when a biostimulant may be beneficial

HOW DO WE TEST THESE PRODUCTS

- Yes/No comparisons do not necessarily prove a product works
- Need to better identify the mode of action for a product to be able to determine if/how it may work
- Small plot or lab/greenhouse studies are beneficial to determine if a mode of action work
- More intensive studies are needed followed by on-farm field trials
  - Yes/no trials can help in the end determine probability of response

HOW DO WE VIEW THESE PRODUCTS?

- Yield enhancers?
  - Unlikely they will increase yield if they are supposed to enhance nutrient supply and fertilizer is applied at optimal rates
- Promote fertilizer efficiency
  - More likely, but need to better test modes of action, doubtful fertilizer needs can be completely eliminated.
- Other
  - Do they have mystical or magical properties???

FINAL THOUGHTS

- On farm research in this case can provide some value if done across a large range in locations and the trials are set up properly
  - Helps give a probability of response that a product works
- Trial design is critical – cannot add too many treatments but need enough to test efficacy
  - Cannot over apply fertilizer
- Product costs still need to be offset


Thank You
Questions?
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