



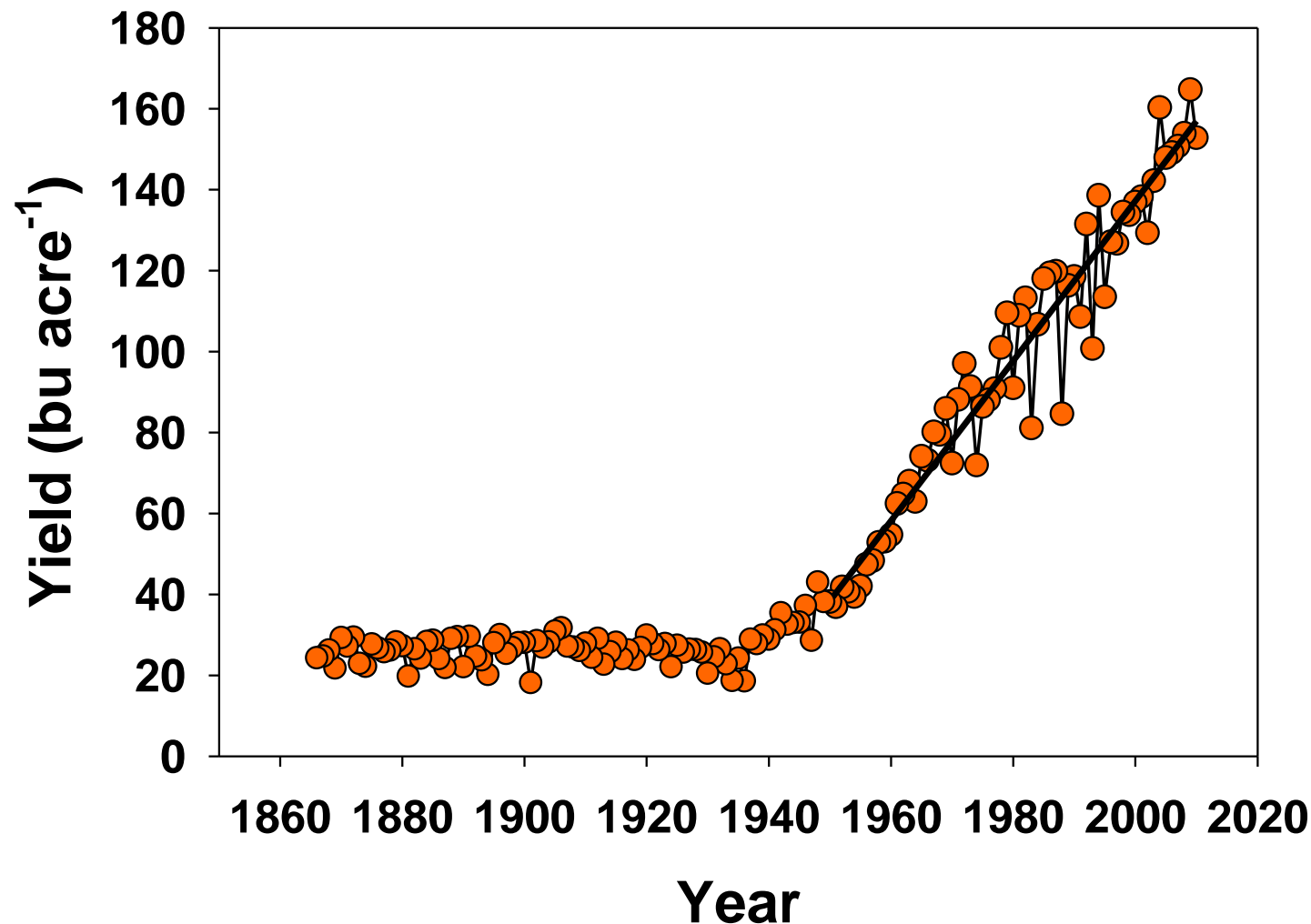
What do we know about PGRs, biostimulants, and biologicals?

Jason Haegele, PhD



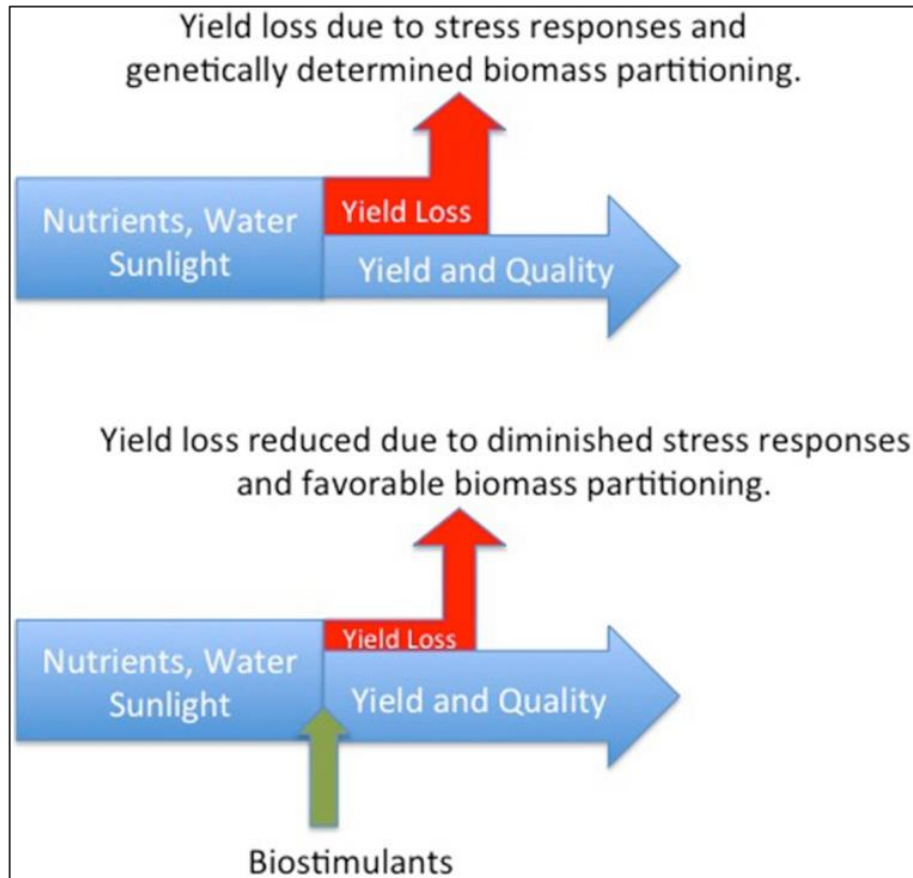
U.S. corn yields from 1866 to 2010

How did we get here and do we keep improving?



Source: USDA-NASS, 2011

Why PGRs, biostimulants, and biologicals?



Source: Brown and Saa. 2015. Biostimulants in agriculture. Frontiers in Plant Science 6:671.

- These products are not fundamental building blocks for yield, and are incapable of replacing essential nutrients, water, and photosynthesis.
- The benefits of these products likely result from stress mitigation or small improvements in nutrient use or water use efficiencies (yield enhancement).

Definition of plant growth regulators

FIFRA Definition [Sec 2(v)]

“...any substance or mixture of substances intended, through physiological action, for accelerating or retarding the rate of growth or rate of maturation, or for otherwise altering the behavior of plants or the produce thereof...”

Does not include:

- **Plant nutrients/nutritional chemicals,**
- **Trace elements,**
- **Plant inoculants,**
- **Soil amendments,**
- **Vitamin-hormone horticultural products**

Hormones regulate all states of the plant life cycle

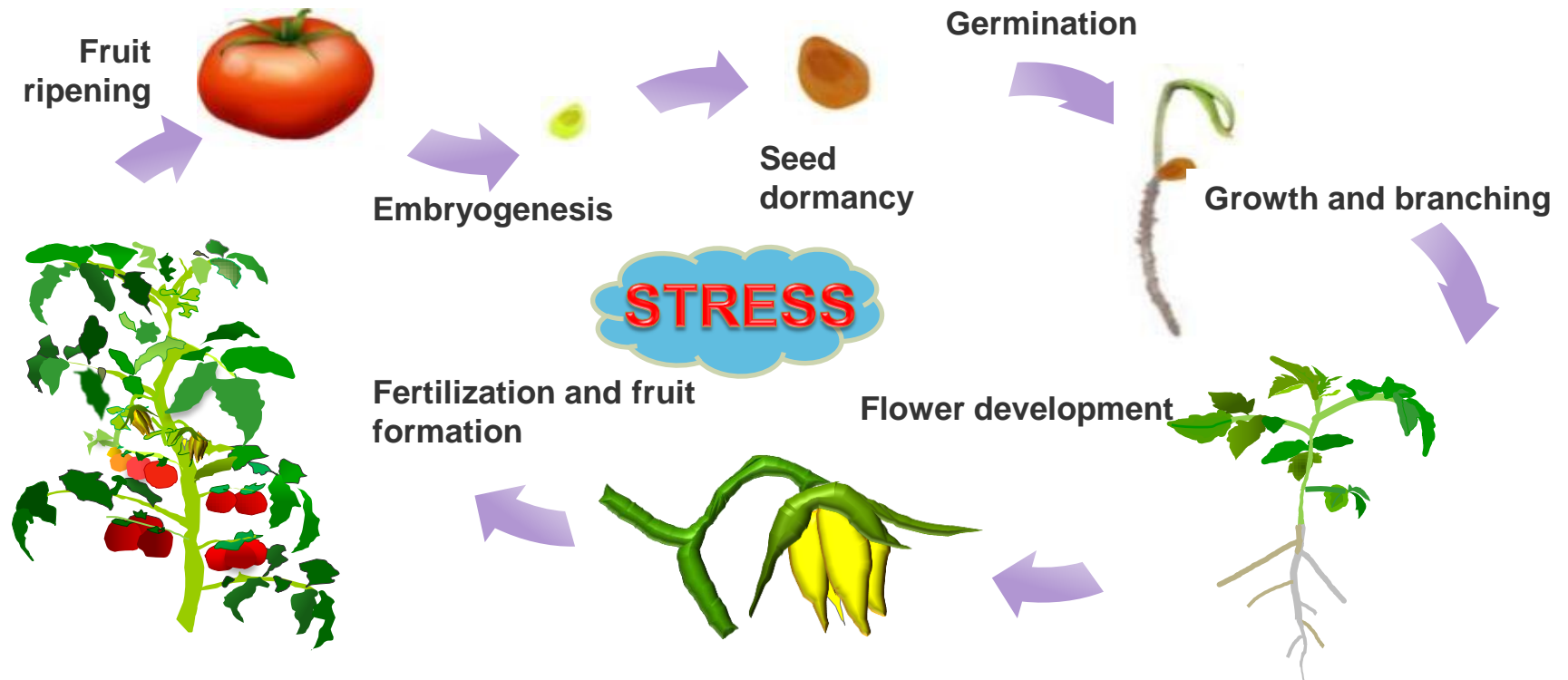
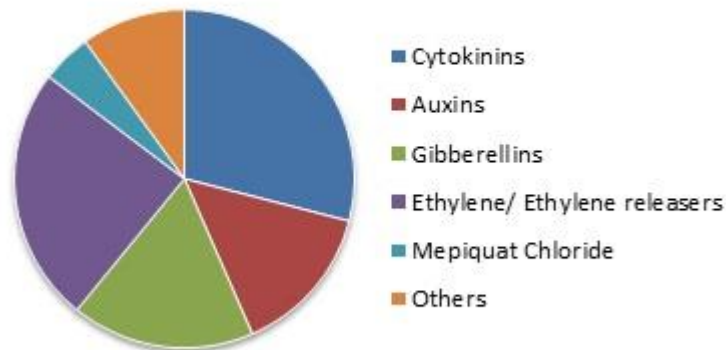


Image Source: Teaching tools in plant biology; American Society of Plant Biologists, © 2011.

Plant growth regulators in agriculture

Plant Growth Regulators Market Share,
By Chemicals Type, 2015 (%)



Source: IndustryARC Analysis, Expert Insights

- **Systematic use of PGRs in crop production began in the 1930s.**
- **PGR use is widespread in high value crops, and is an indispensable component of some production systems (e.g., pineapple, seedless grapes).**
- **GA inhibitors for height and lodging management.**
- **Mixtures of cytokinin, auxin, and gibberellic acid in row crop production.**

Plant (phyto)hormones

Naturally-occurring growth substance

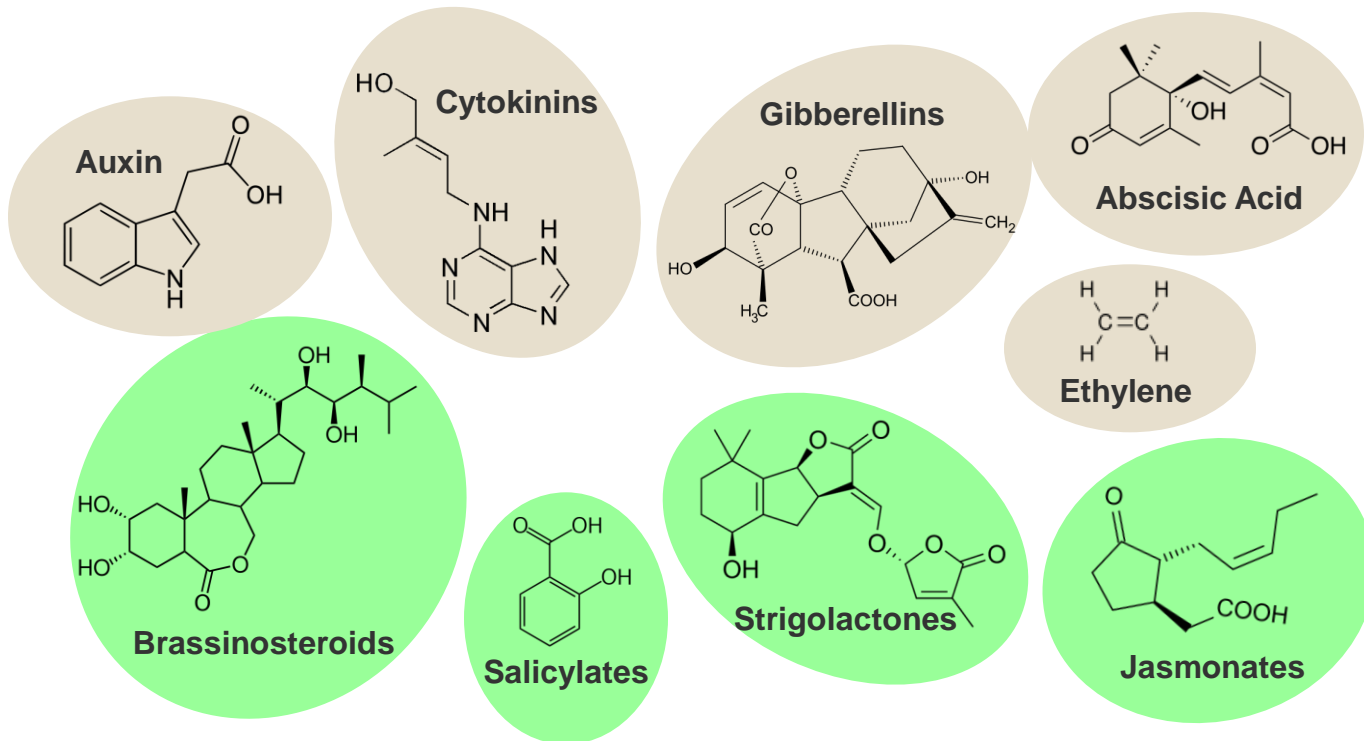


Image Source: Teaching tools in plant biology; American Society of Plant Biologists, © 2011.

Non-hormonal substances

Not naturally occurring

- **NAA, 2,4-D (auxin herbicides)**
- **Mefluidide (cytokinin inhibitor)**
- **Mepiquat chloride (GA synthesis inhibitor)**
- **S-ABA (abscisic acid agonist)**
- **Ethephon (ethylene agonist)**
- **1-MCP (ethylene inhibitor)**



**Ambient
UTC**

**Heated
UTC**

**Heated
1-MCP**

Image Source: University of Illinois Crop
Physiology Laboratory

How might yield be increased by the use of plant growth regulators?

- Plant growth regulators influence the accumulation of biomass and its partitioning between root and shoot, as well as, reproductive development.
- Mechanistically, grain yield results from interception of solar radiation and its conversion into biomass.
 - **Rapid emergence and vegetative growth.**
- A portion of this biomass, typically 50 to 55% in a well managed crop, will be partitioned into grain.
 - **Manage seed abortion under stress, and maintain leaf photosynthetic activity through grain filling.**

Promotion of seed germination by GA

- GA is a signal for germination.
- After planting, imbibition triggers GA production in the embryo, which in turn triggers production of hydrolytic enzymes (e.g., amylase) which begin to remobilize nutrients stored in the endosperm.

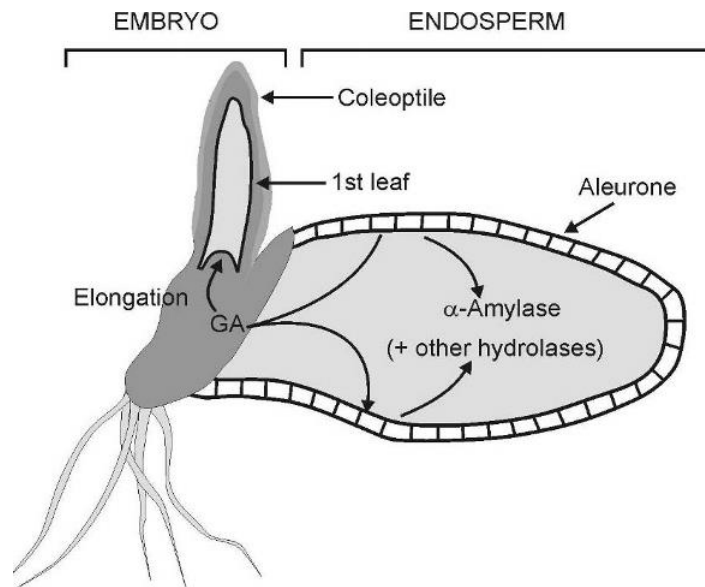


Image source: Woodger, Jacobsen and Gubler, Gibberellin action in germinated cereal grains, in Hormones, Davies (editor), 2010.

Hormones during vegetative development

- The balance between auxins and cytokinins establishes partitioning between root and shoot as well as development of apical and lateral meristems.
 - Partitioning between the root and shoot is influenced by environmental and cultural factors.
 - In general, changes in the soil environment (e.g., decreased water availability or nutrient supply) will favor the root, while changes in light intensity will favor the shoot.
- Gibberellic acids promote growth *via* elongation of internodes.

Role of auxin in root growth and architecture

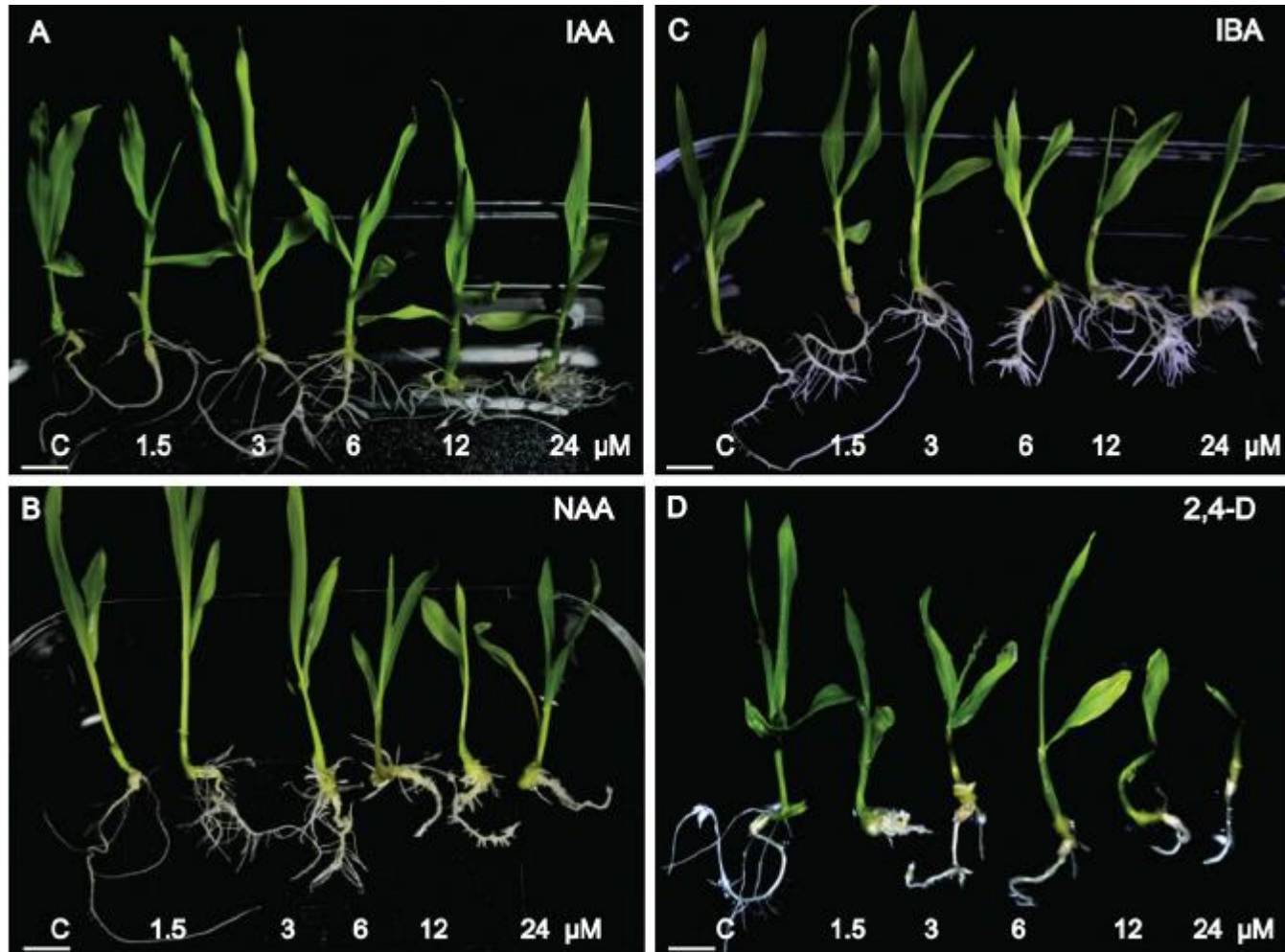
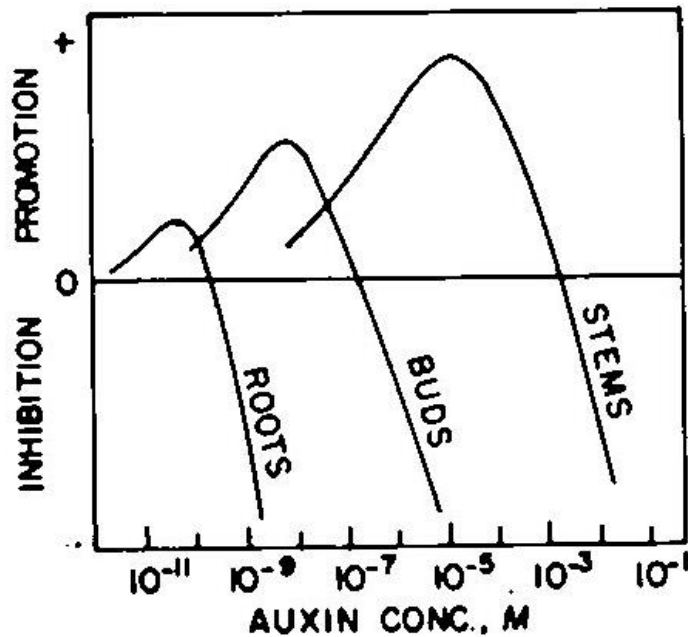


Image source: Martinez-de la Cruz et al. 2015. Auxins differentially regulate root system architecture and cell cycle protein levels in maize seedlings. *Journal of Plant Physiology* 176:147–156.

Role of auxin in root growth and architecture

- Transport of shoot derived auxin (IAA) to the root establishes an auxin gradient that influences primary and lateral root growth.
 - Length of root hairs
 - Lateral root initiation
 - Bi-modal effect of concentration on primary root length.



- Stems respond positively to auxin over a wide range of concentrations, while roots are inhibited at all but the lowest concentrations.

Image source: Gardner et al., Physiology of Crop Plants, 1985.

What are biostimulants?

- **“A group of ingredients that stimulate life.”**
- **Derived from natural or biological sources.**
- **Stimulate natural processes that enhance nutrient uptake or efficiency, tolerance to abiotic stresses, or crop quality when applied to plant or soil.**
 - **No direct action against pests.**
- **Many contain nutrients, but the effect of the biostimulant is independent of the nutritive benefit.**

Commonly cited benefits of biostimulants

Hamza and Suggars, 2001, TurfGrass Trends

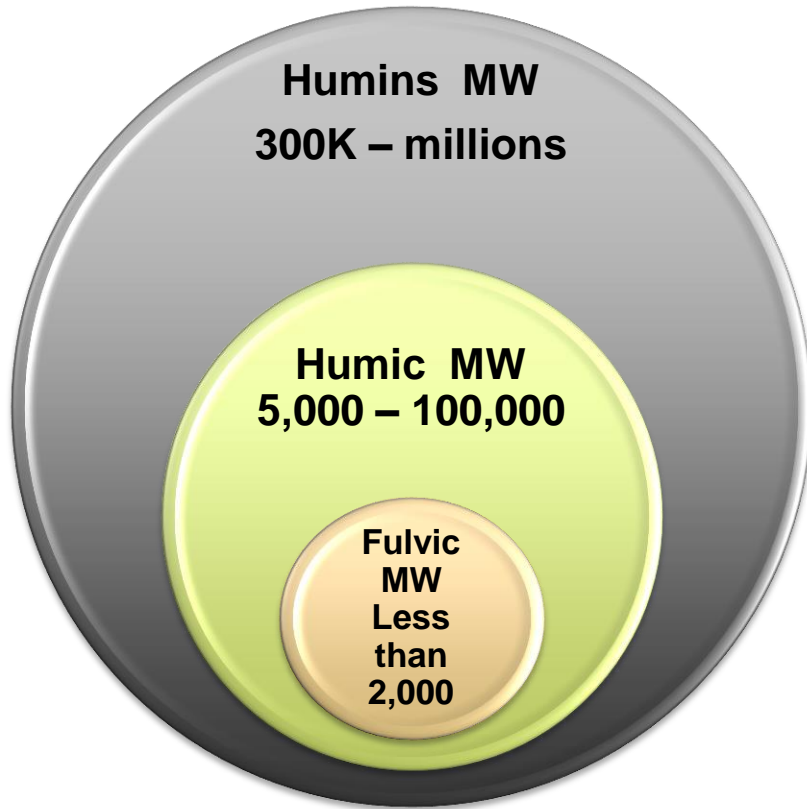
- Stimulate plant responses and work in all weather conditions
- Increase profits, cut operating costs, lead to 50% reduction in fertilizer
- Increase plant toxins, repelling pests
- Increase microbial root protection from soil pathogens
- Increase soil nutrient reserve up to 3000%
- Improve root development
- Build yields
- Improve taste and shelf-life
- Improve drought tolerance
- Increases nutrient uptake
- Stimulate plants' immune system
- Result in better performance
- Produce deeper roots
- Improve stress tolerance
- Accelerate establishment
- Increases cation exchange capacity
- Enhances fertilization and reduces leaching
- Detoxify chemical residues and heavy metals
- Make urea a long-life nitrogen
- Improve seed germination rates
- Increase stomata opening and plant transpiration

Ingredients contained in biostimulant products

Karnok, 2000, Promises: can biostimulants deliver?, Golf Course Mgmt. 68(8):67-71.




- Antioxidants
- Carbohydrates
- Chelates
- Chelated micronutrients
- Enzymes
- Humic/Fulvic acids
- Growth stimulators
- Lignin
- Manure extract
- Micronutrients
- Mycorrhizae
- Peptides
- Plant growth regulators
 - Cytokinin
 - Auxin or auxin precursors
 - Gibberellic acids
- Polysaccharides
- Proteins
- Seaweed or seaweed extracts
- Vitamins
- Amino acids
- And many others.....

Humic and fulvic acids – what are they?



- End-products of organic matter decomposition.
- Distinguished by size and solubility.
 - Humic acid generally larger, soluble in alkaline solution.
 - Fulvic acid smaller, soluble in both acid and alkaline solutions.
- Past studies have demonstrated that humic and fulvic acids have diverse effects on soil and plants:
 - Chelation of ions, greater nutrient availability and uptake.
 - PGR like activity, particularly smaller molecular weight compounds.

Example of growth promotion of wheat seedlings by humic acid

Culture medium	Plant organ	Fresh weight, mg/plant	Stimulation, %
Water (control)	Root	93	0
	Shoot	185	0
Humic acid	Root	146	57.5 
	Shoot	252	36.2
Nutrition	Root	182	96.3 
	Shoot	342	84.9
Nutrition + humic acid	Root	203	119.0 
	Shoot	390	110.8

Source: Chen and Aviad. 1990. Effects of humic substances on plant growth. Humic substances in Soil and Crop Sciences; Selected Readings.

Seaweed extracts

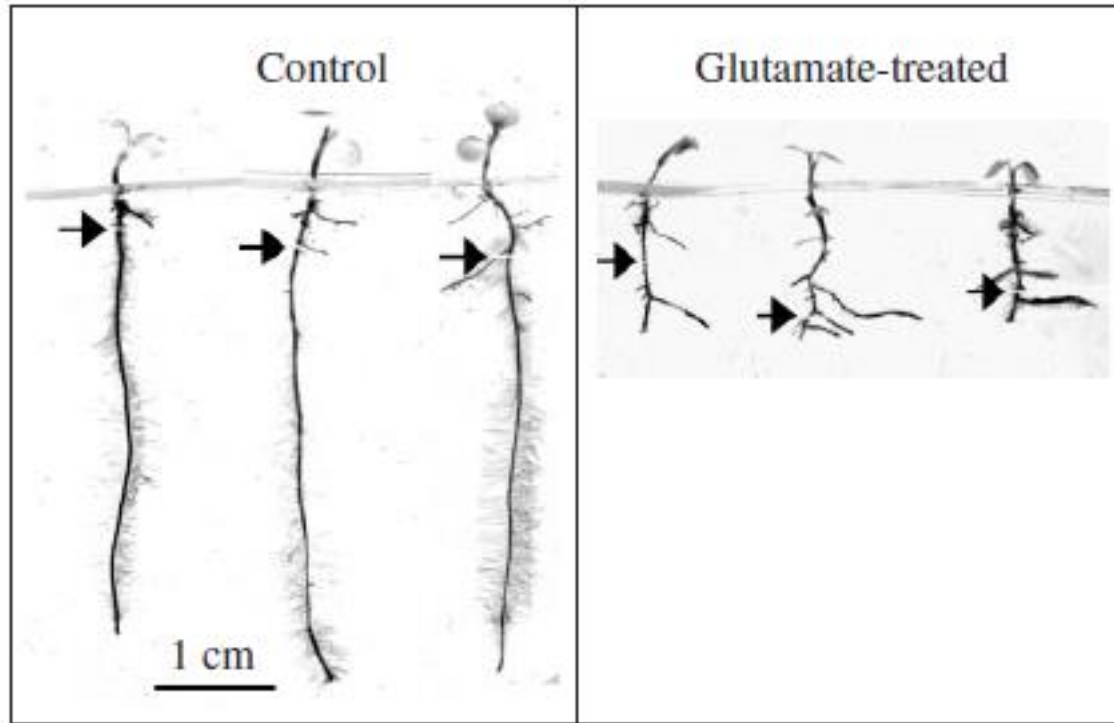


- Seaweed used as a fertilizer throughout history.
- *Ascophyllum nodosum* (brown seaweed)
- Seaweed extract is a diverse mixture of micronutrients, plant hormones, amino acids, polysaccharides, and other metabolites.

Non-nutritional aspects of amino acids

- Many reports of plant growth stimulation and stress tolerance associated with application of amino acids and 'protein based products'.
 - Individual amino acids
 - Structural amino acids (e.g., glutamine)
 - Non-protein amino acids and their derivatives (e.g., glycine betaine)
 - Protein hydrolysates
 - Animal or plant origin
 - Mixture of peptides and amino acids
- Certain plant growth regulators (auxin, ethylene, polyamines) have amino acids as their precursors.

A role for glutamate in establishing root architecture?



Source: Walch-Liu et al., 2006, *Annals of Botany*. 97:875-881.

Protein hydrolysates may impact C and N metabolism

- Past work has indicated that biostimulants comprised of protein hydrolysates enhance the activity of enzymes associated with primary C and N metabolism.
- Interaction between biostimulants and nutrition level is not clear in practice.

ALGAE EXTRACT AND MICRONIZED LEATHER AS BIOSTIMULANTS FOR EARLY-STAGE MAIZE FERTILIZATION

Authors: A. Trinchera, E. Rea, C.M. Rivera, S. Rinaldi, P. Sequi

The application of biostimulants to crops could represent a significant opportunity in order to prevent plant nutrient stress and increase crop yield: this is particularly relevant in Mediterranean areas, which are characterised by hot and dry climate and soils poor in nutrient content and organic matter. **On general terms, biostimulant properties are quite always associated to fertilizers based on algae extracts or hydrolysed proteins.** An alternative approach could be recognized in the application of solid fertilizers with specific properties, such to improve root development and following nutrient uptake, especially at the early stage of crop growth. In the present study, in a short-term pot trial, **we evaluated the effect of two different biostimulant products, a liquid and a solid, on maize root and shoot growth, under nutrient stress conditions.** A biostimulant based on algae extract, in liquid form (LB) at the rate of 100 \square L L⁻¹ and a solid (SB), based on micronized leather, at the rate of 100 mg L⁻¹, were added to an inert growing substrate. Seedlings of maize (Zea mais L. 'Suarta') were transplanted in pots and then grown in a greenhouse. Standard nutrient solution at 50% of the optimal concentration was supplied or not supplied (0%) to the pots, in order to evaluate if the presence of nutrient solution could emphasize the biostimulant properties of the considered formulates. **Results showed that both the maize primary root length and weight were significantly increased by both LB and SB application, particularly in absence of nutrient supply.** Also shoot length and weight of maize treated with LB without any nutrient supply was comparable to those obtained with 50% of nutrient solution. **The total fresh biomass produced with LB and SB addition without nutrient supply was the same obtained in presence of 50% of nutrient solution.**

Past examples of exogenous glycine betaine as an osmoprotectant

Table 1
Summary of the effects of exogenous application of glycine betaine on different plant species

Plant Species	Stress	Effect of exogenous GB	Reference
<i>Nicotiana tabacum</i>	Drought	GB induced improvement in growth and yield of water-stressed plants	Agboma et al. (1997b)
<i>Phaseolus vulgaris</i>	Drought	GB-treated plants showed a slower decrease in leaf water potential	WeiBing and Rajashekar (1999)
<i>Glycine max</i>	Drought	GB improved growth	Agboma et al. (1997c)
<i>Triticum aestivum</i>	Drought	(i) GB improved growth (ii) GB did not improve growth	Borojevic et al. (1980) Agboma et al. (1997a)
<i>Brassica napus</i>	Drought	GB did not improve growth	Mäkela et al. (1996)
<i>Zea mays</i>	Drought	GB improved growth of stressed plants	Agboma et al. (1997a)
<i>Lycopersicon esculentum</i>	Salt and high temperature	GB improved growth of stressed plants	Mäkela et al. (1998a,b)
<i>Oryza sativa</i>	Salt	(i) GB improved growth of salt-stressed plants (ii) GB improved shoot growth but not root growth (iii) GB-treated salt-stressed plants had lower Na ⁺ and higher K ⁺ in the shoot	Harinasut et al. (1996) and Lutts (2000) Lutts (2000) and Rahman et al. (2002) Lutts (2000) and Rahman et al. (2002)
<i>Arabidopsis thaliana</i>	Freezing temperatures	GB improved freezing tolerance (reduced freezing temperature from -3.1 to -4.5 °C)	WeiBing and Rajashekar (2001)
<i>Solanum tuberosum</i>	Low temperature	GB improved growth of stressed plants	Somersalo et al. (1996)
<i>Gossypium hirsutum</i>	Drought	(i) GB improved growth and yield of stressed plants (ii) GB did not improve growth and yield of stressed plants	Naidu et al. (1998) and Gorham et al. (2000) Meek et al. (2003)

Source: Ashraf and Foolad, 2007, Env. and Exp. Bot. 59:206-216.

Past examples of exogenous proline as an osmoprotectant

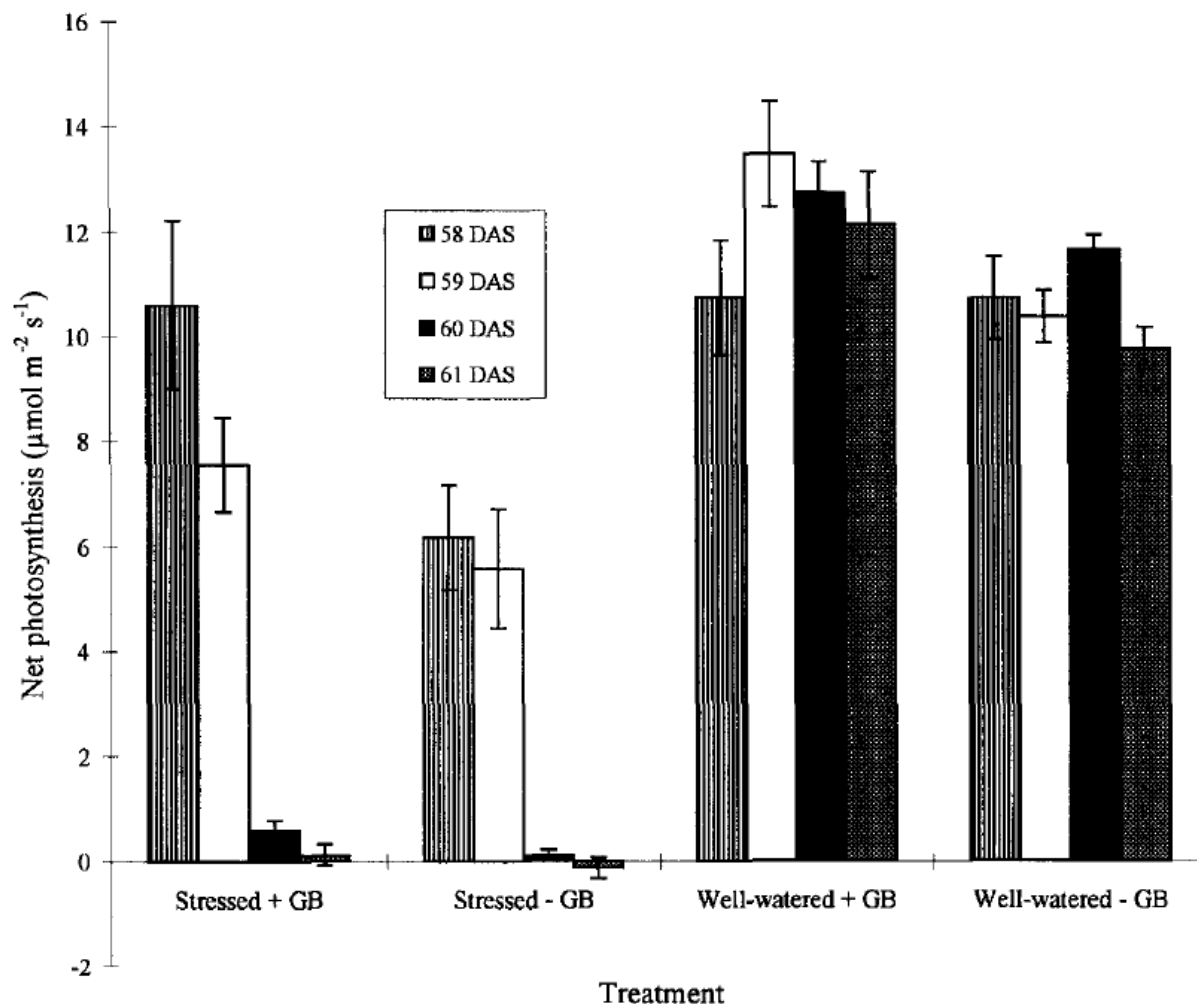
Table 2
Summary of the effects of exogenous application of proline on different plant species

Plant species	Stress	Effect of exogenous proline	Reference
<i>Distichlis spicata</i>	Salt	Proline accumulation was high in cells adapted to a concentration of salt	Heyser et al. (1989)
<i>Glycine max</i>	Salt	Proline application increased production of superoxide dismutase and peroxidase in stressed plants	Yan et al. (2000) and Hua and Guo (2002)
<i>Allenrolfea occidentalis</i>	Salt and drought	Proline neutralized the increased ethylene production in stressed plants	Chrominski et al. (1989)
<i>Hordeum vulgare</i>	Salt	Proline caused a decrease in shoot Na^+ and Cl^- accumulation in embryo culture cells	Lone et al. (1987)
<i>Allium cepa</i>	Salt	Proline resulted in mitigating the effect of NaCl on cell membrane disruption	Mansour (1998)
<i>Oryza sativa</i>	Salt	Proline did not alter leaf Na^+ and Cl^- contents in salt-stressed plants	Krishnamurthy and Bhagwat (1993)
<i>Nicotiana tabacum</i>	Salt	Proline promoted the growth of suspension cells under salt stress without maintaining a high ratio of K^+/Na^+	Krishnamurthy and Bhagwat (1993)
<i>Arabidopsis thaliana</i>	No stress	Proline caused damages to chloroplast and mitochondria ultra-structures	Hare et al. (2002)

Source: Ashraf and Foolad, 2007, Env. and Exp. Bot. 59:206-216.

An evaluation of the effect of exogenous glycine betaine on the growth and yield of soybean: timing of application, watering regimes and cultivars

Agboma et al., 1997, Field Crops Res. 54:51-64.



Relationship between amino acids and plant hormones

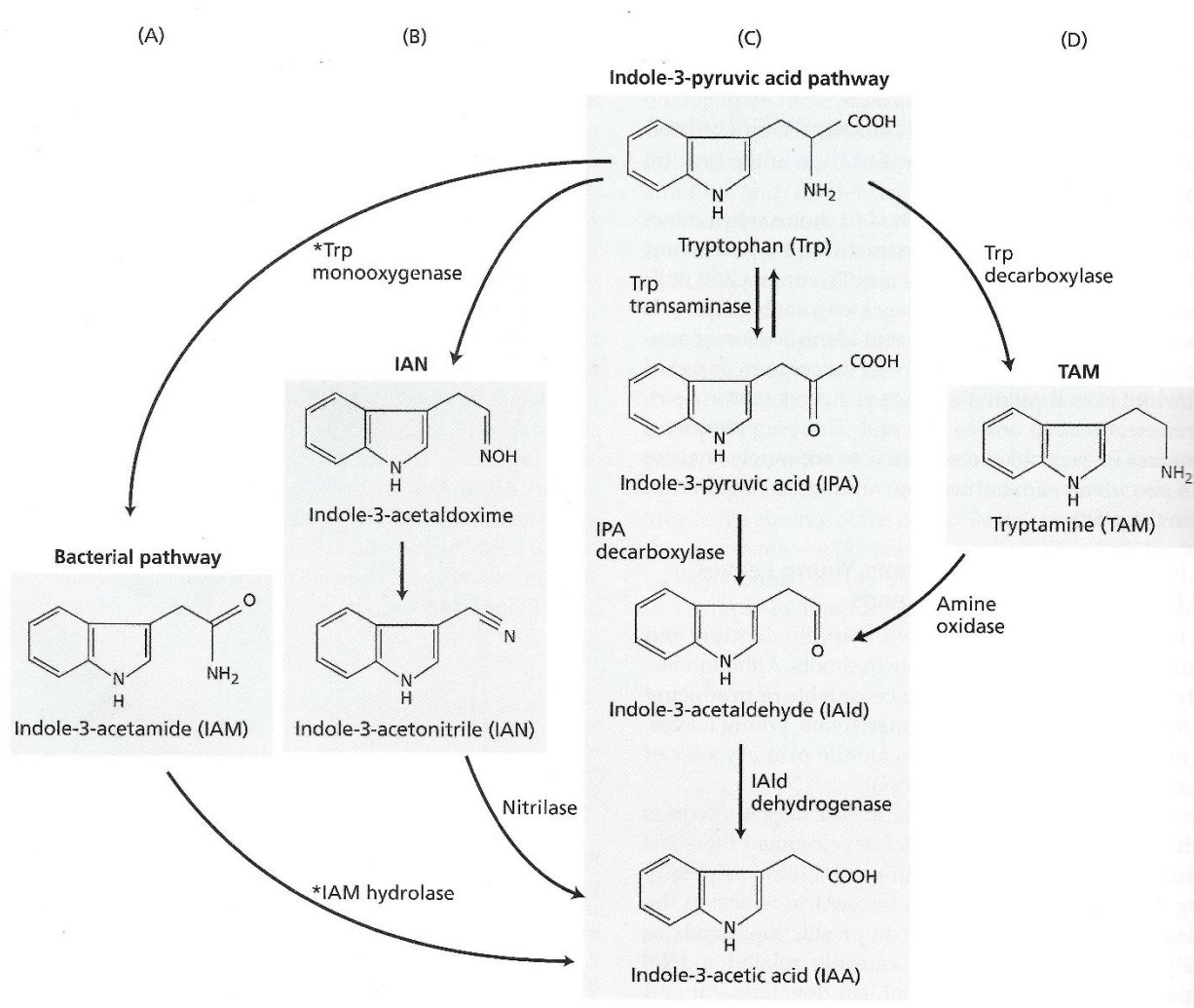


Image source: Fig. 19.6 from Taiz and Zeiger, Plant Physiology, 2002.

What are biologicals?

- Biologicals are live organisms that positively influence crop growth, development, health, or quality.
- These organisms exist naturally or are supplied to the crop as an inoculant, seed treatment, foliar spray, etc.
- Other types of biologicals may be referred to as 'biopesticides' and their use benefit the crop indirectly as a consequence of controlling insects or plant pathogens.
 - Example: ClarivaTM pn is bacterium applied as a seed treatment for the control of soybean cyst nematode.

We have been using biologicals in crop production for a long time!



FIG. 4.—INOCULATING SOYBEANS BY THE “MUDDY-WATER” METHOD

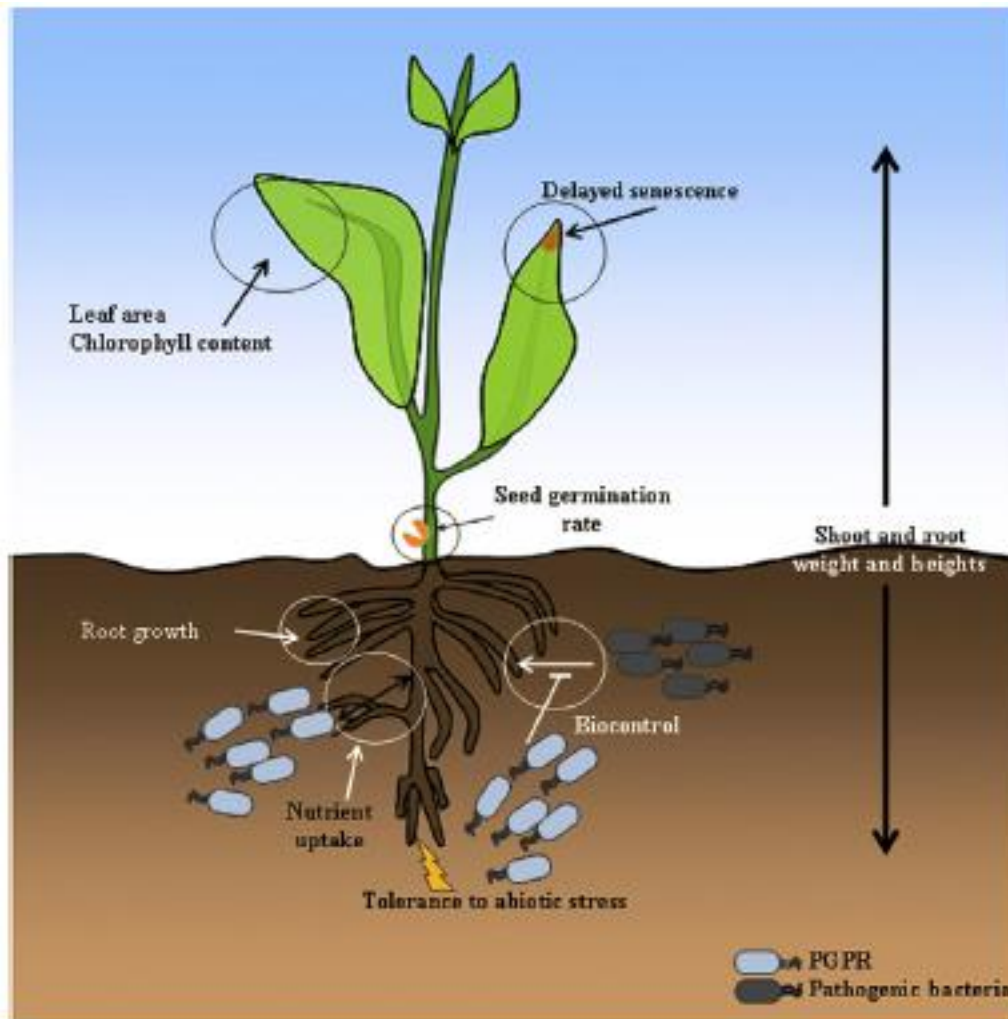
Well-inoculated soil must be used and every seed moistened with the muddy water. At the same time it is very important that an excess of water be avoided, for it injures the beans by causing the seed coats to “slip” and the result is a poor stand.

Source: “Soybean Production in Illinois”, University of Illinois Agricultural Experiment Station Bulletin no. 310, June 1928.

Examples of biological organisms used in crop production

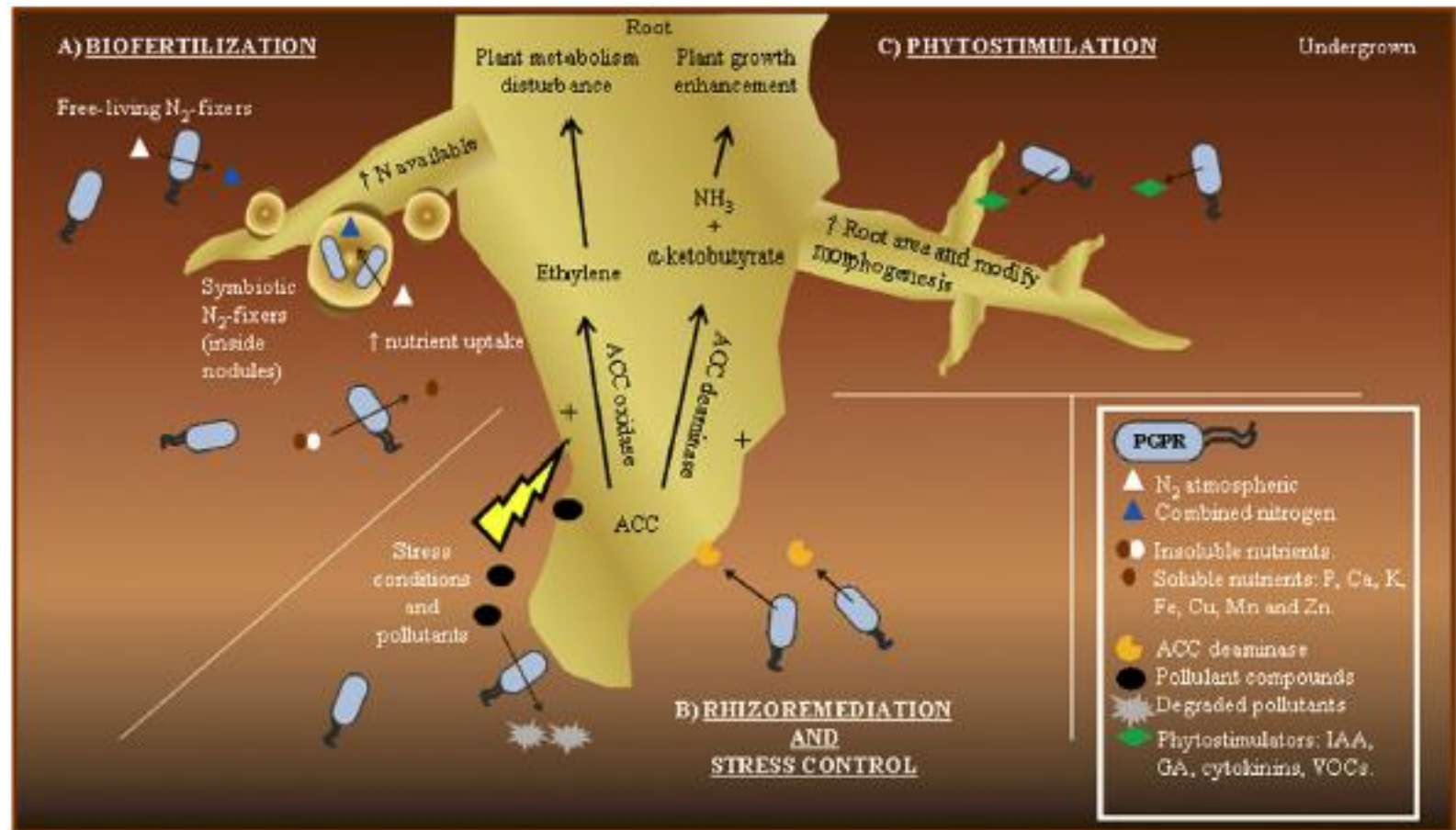
- *Bradyrhizobium japonicum* – soil bacterium that forms symbiotic relationship with soybean.
- *Azospirillum spp.* – soil bacteria that form symbiotic relationships with monocots like sugarcane.
- *Bacillus subtilis*, *B. pumilus*, *B. amyloliquefaciens* – spore forming soil bacteria that possess diverse functions.
 - Biopesticides
 - Soil nutrient availability
 - Production of plant growth regulators
- Fungi including *Trichoderma*, *Glomus*, *Pennicillium*

Plant growth promoting rhizobacteria (PGPR)



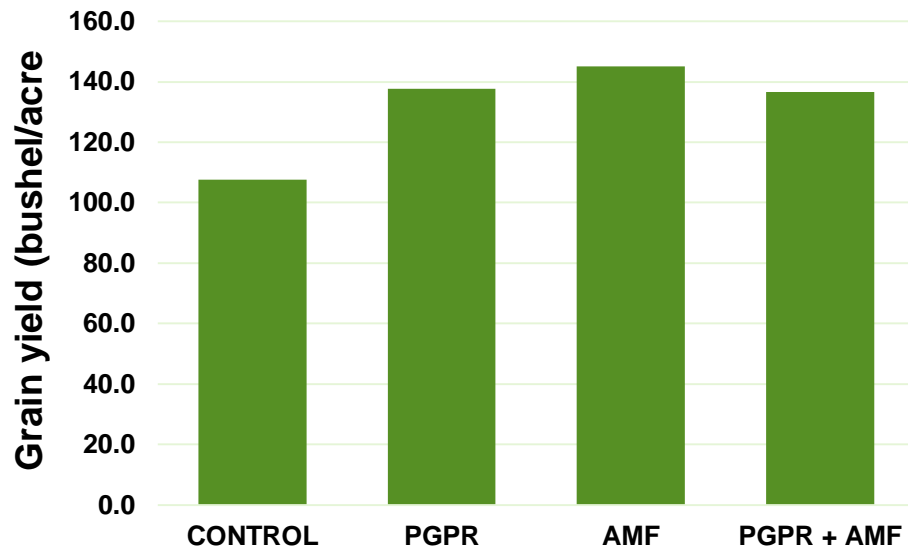
Source: Perez-Montana et al. 2014. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiological Research 169:325-336.

Mechanisms associated with PGPR



Source: Perez-Montana et al. 2014. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiological Research 169:325-336.

Example of corn grain yield response to microbial inoculation



- Researchers inoculated field grown corn plants with plant growth promoting rhizobacteria (PGPR), arbuscular mycorrhiza fungi (AMF), or a combination of both.
- In addition to increased yield, they also reported increases in uptake of N, P, and K.

Adesemoye, Torbert, and Kloepper. 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can. J. Microbiol. 54:876-886.

Evaluating product efficacy

What does success look like?



Conclusions

- **PGRs, biostimulants, and biologicals have a place in row crop production.**
 - **The underlying science is sound, but consistency has been difficult to achieve.**
- **Evidence suggests that these products often interact with plant nutrition, and are likely best used in combination with optimal fertility management.**
- **Predicting appropriate rates and application timings makes the use of these products in crop production challenging.**
 - **Environment, crop, growth stage, etc.**