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?

- 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

- Fruit ripening
- Embryogenesis
- Fertilization and fruit formation
- Flower development
- Germination
- Seed dormancy
- Growth and branching

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

- 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

- Auxin
- Cytokinins
- Gibberellins
- Abscisic Acid
- Ethylene
- Brassinosteroids
- Salicylates
- Strigolactones
- Jasmonates

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)

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.

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

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.

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


- 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

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

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?

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

Past examples of exogenous proline as an osmoprotectant

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

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: Clariva™ 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!

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)

Mechanisms associated with PGPR

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.

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.