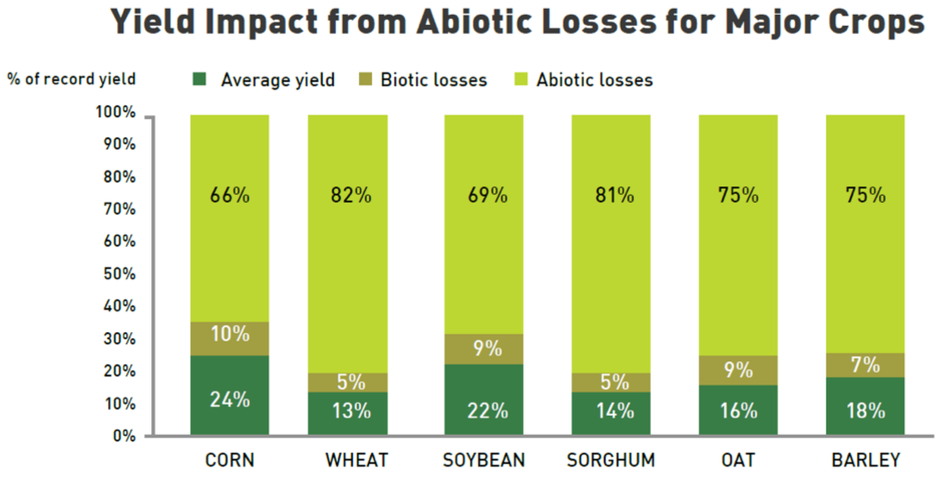
Biostimulant Overview

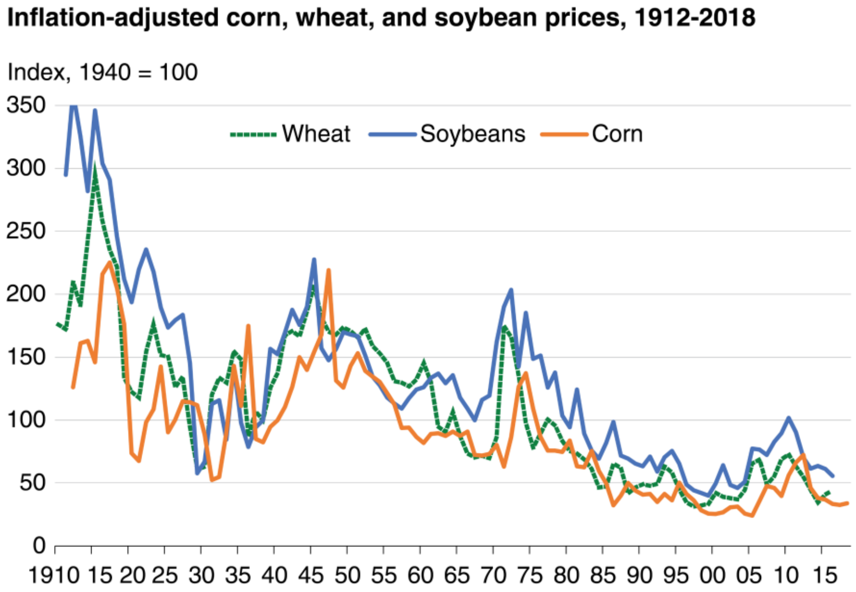
Dr. Graeme Berlyn, Kevin Gallagher

Graeme.Berlyn@Yale.edu

Kevin.Gallagher@Yale.edu

March 2021

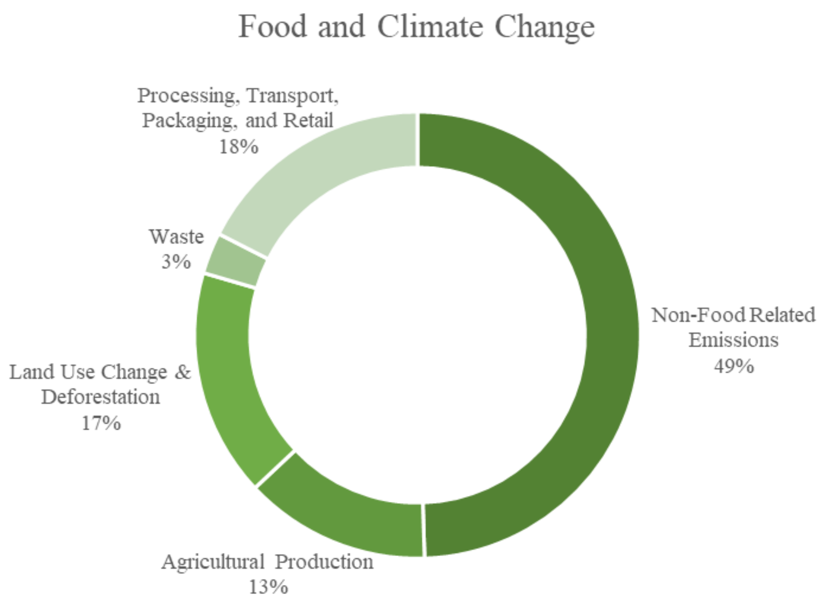


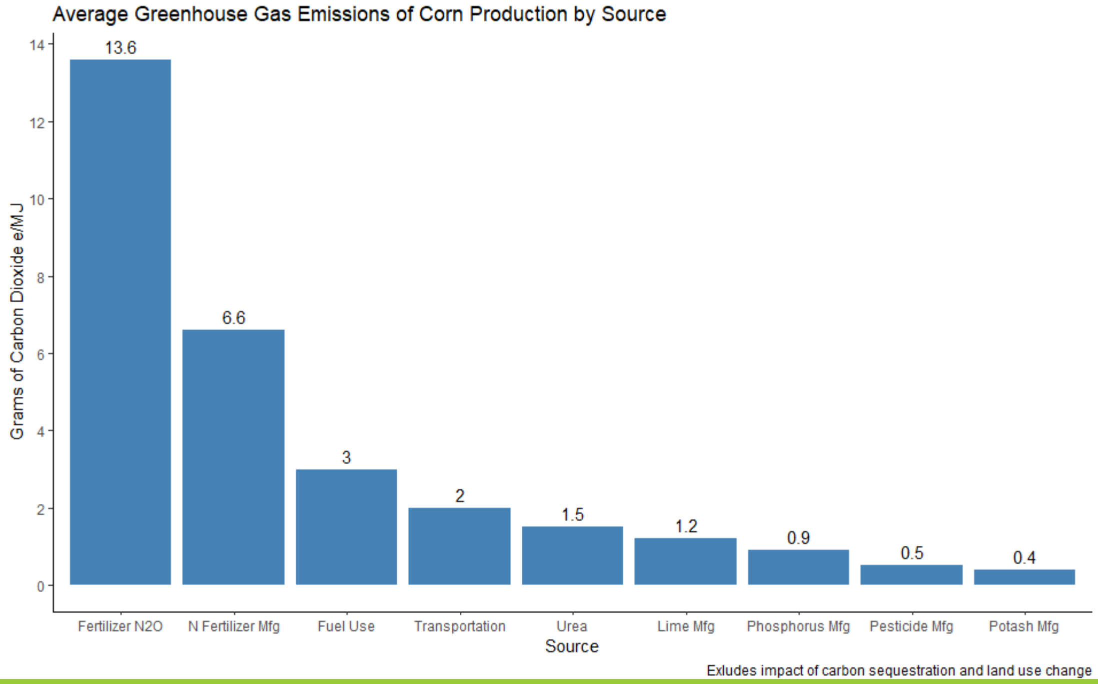


The Challenges

<u>Financial</u>

- Environmental stress reduces agricultural productivity, reducing crop yield and profitability
  - Plants are unable to achieve their full genetic potential and farmers are leaving yield on the table
- Farmers are not economically situated to adopt more sustainable practices because of low prices and rising costs
- Half of all inorganic fertilizers applied is not absorbed by plants
- Soil degradation costs U.S. corn farmers a half-billion dollars per annum





The Challenges

Environmental

- Agriculture contributes between 11% and 33% of global greenhouse gas emissions
- Modern agricultural practices deplete soil, making agriculture a net source of atmospheric carbon
- 50% of all fertilizers applied are not absorbed by plants and pollute the environment
- Approximately 2/3rds of agricultural greenhouse gas emissions come from N fertilizer use
- No cost effective and scalable carbon sequestration technologies commercially available

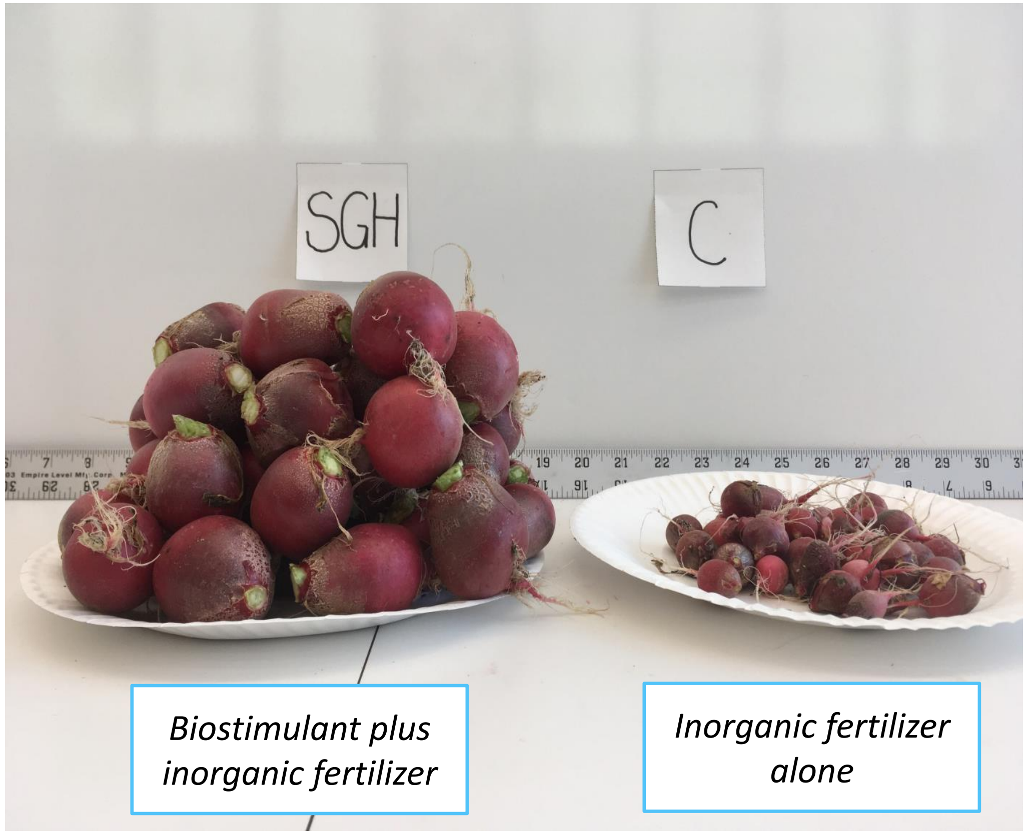
The Biostimulant Solution:

Enable farmers to grow more food on less land while becoming more sustainable

The Technology

Biostimulant Benefits

- *Increase yield 200%+ over inorganic fertilizer with minimal, low-cost inputs*
- *Increase sustainability and decrease dependence on inorganic fertilizers and pesticides*
- *Mitigate the effects of climate change on agriculture and elevate crop stress tolerance*
- *Increase the quality of crops, elevate carbon sequestration, and promote a healthy rhizosphere*
- *Formula ingredients are safe, non-toxic, all-natural, and organic*

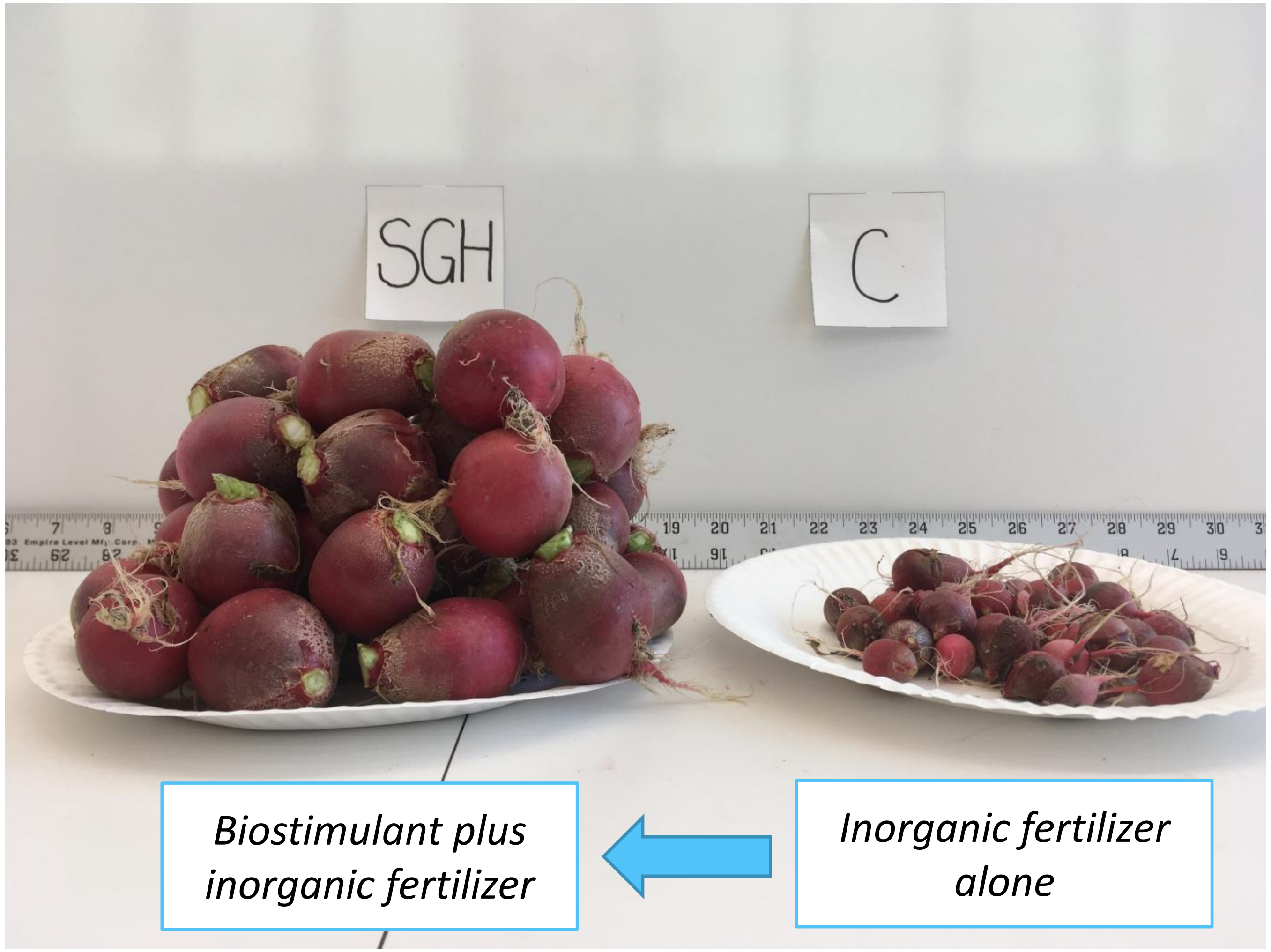


Simultaneously increase yield and sustainability

What are Biostimulants?

- Plants experience environmental stress, which decreases agricultural productivity by an estimated 78%[1]
  - Mitigated via genetic modification and/or biostimulants
- Biostimulants are:
  - Non-fertilizer products with beneficial effects on plant growth in relatively small amounts
  - A growth blend of vitamins, marine algae, amino acids, co-enzymes, and other bioactive materials
  - Stimulate metabolic growth processes
- Increase crop growth, yield, health, and nutritional value
- Analogous to giving a plant a nutrient shot
- They are:
  - ✓ All natural and non-hormonal
  - ✓ Water soluble
  - ✓ Biologically safe and non-toxic
  - ✓ Does not build up in soils

1) Boyer JS. *Plant productivity and environment*. Science. 1982 Oct 29;218(4571):443-8. doi: 10.1126/science.218.4571.443. PMID: 17808529.



<u>Treatment (Left to Right)</u>

Water; Inorganic Fertilizer; Biostimulant Formula A; Biostimulant Formula B

Trials done in Yale's Greeley Greenhouse

Root yield of four Yale formula variants (S, SGA, SAA, SA) vs. inorganic fertilizer control (C)

Representative leaf samples from the above experiment

Biostimulant plus inorganic fertilizer

Inorganic fertilizer alone

Experiment on paper birch (Betula papyrifera) of Yale Formula, a prior formula, Miracle Grow, and water

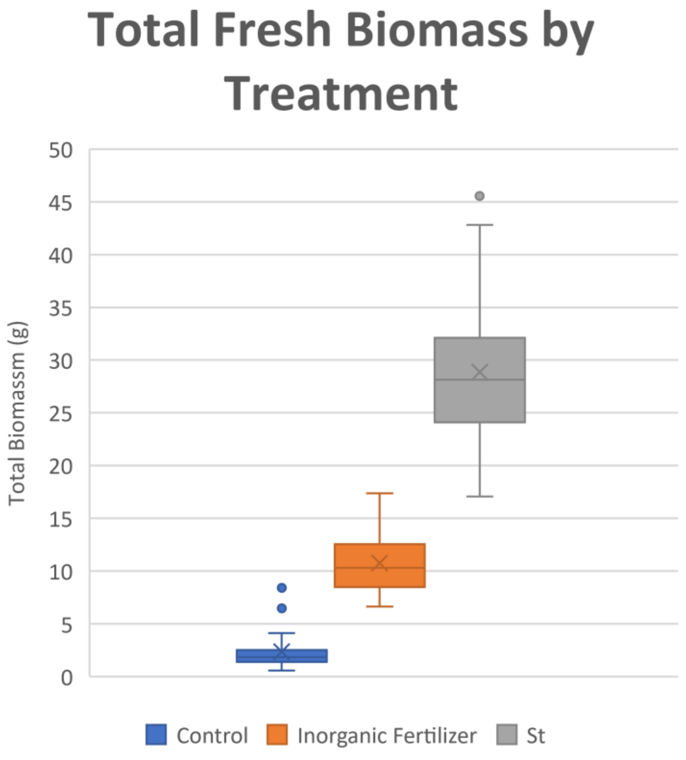
Root biomass of fertilizer control (C) vs biostimulants

Shoot biomass of fertilizer control (C) vs biostimulants

Treated

Fertilizer only

Water only



In January 2020, we tested the improved formula on radishes. The biostimulant, applied once a week, increased yield 1,122% over the water control and 216% over plants with fertilizer alone.

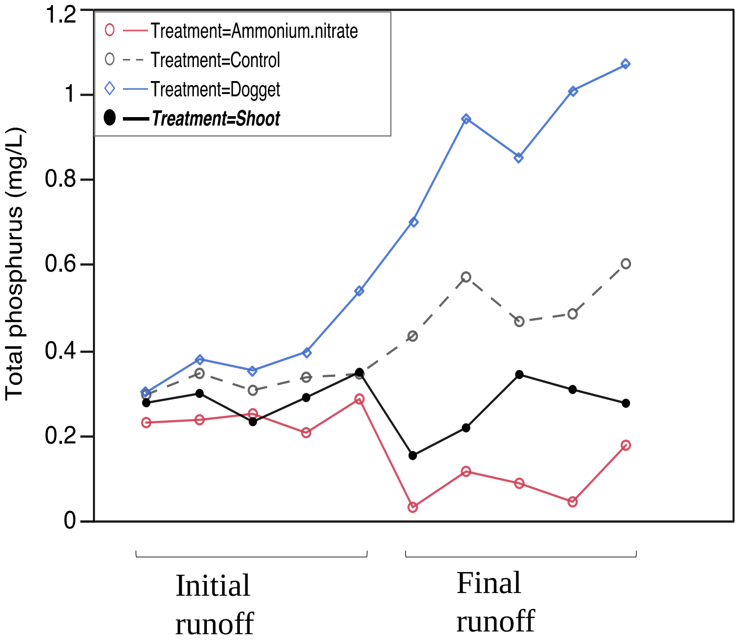
Results unpublished. n = 40 x 5. All differences significant at $p < 0.001$

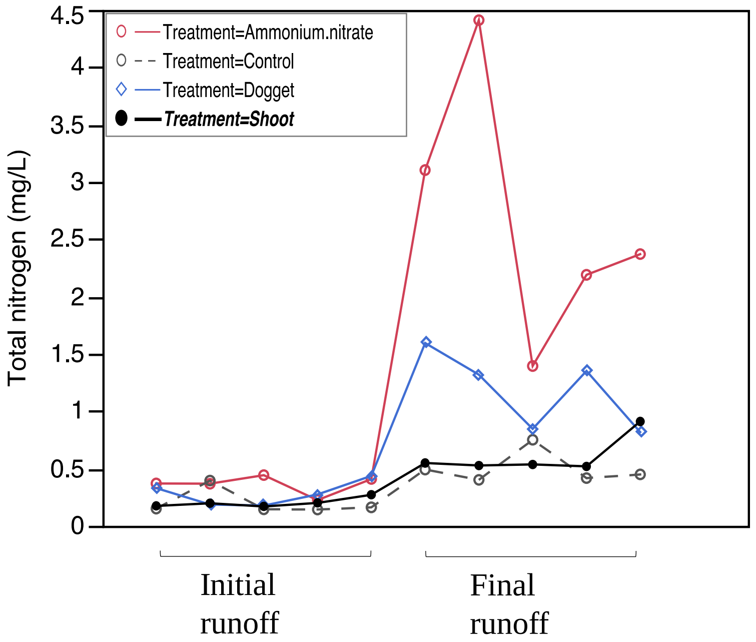
How they Work

- They protect against biotic and abiotic stressors at the molecular level
- They create a self-reinforcing feedback loop:
  - Promote increased root biomass, which allows greater water and nutrient uptake and stimulates vascular development
  - Increase hydraulic conductivity that lowers water and nutrient stress in the leaves
  - Enhance photosynthesis and carbon sequestration, leading to more protective and other secondary carbon compounds

Plant Derived Reagents	→	Greenest Organic Growth
Amino Acids		Superior Growth
Vitamin C		Green Aesthetics
Vitamin B		Sustainability
Carbohydrates		Efficiency

Improved Sustainability





Nitrogen and Phosphorus runoff of the Yale biostimulant formula and inorganic fertilizer in paper birch. The biostimulant runoff was comparable to the control

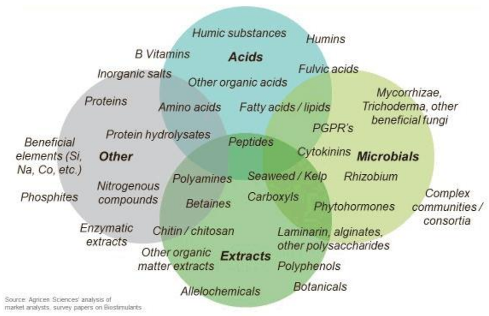
Unlike inorganic fertilizers, biostimulants:

- Do not immediately start leaching into the water table
- Produce no detectable increase in nitrogen runoff

Approximately half of all the inorganic fertilizer applied is not taken up by the plants and pollutes the water table

Lowering fertilizer use would reduce pollution, prevent ecological damage (e.g. eutrophication), and lower dependence on unsustainable mined minerals (e.g. potassium)

Biostimulants reduce the required amount of fertilizer by 50%-100% to maintain yield, increase nitrogen use efficiency, and increase yield ~200%+ when added in addition to fertilizer



Fertilizer control, Neptune's Harvest, SN-14, Yale Formula

Neptune's Harvest, Yale Formula, SN-14

Competing Organic Fertilizers

Current commercial biostimulants and 'organic fertilizers' lack the main bioactive components of the Yale proprietary formula and have lower levels of available nitrogen

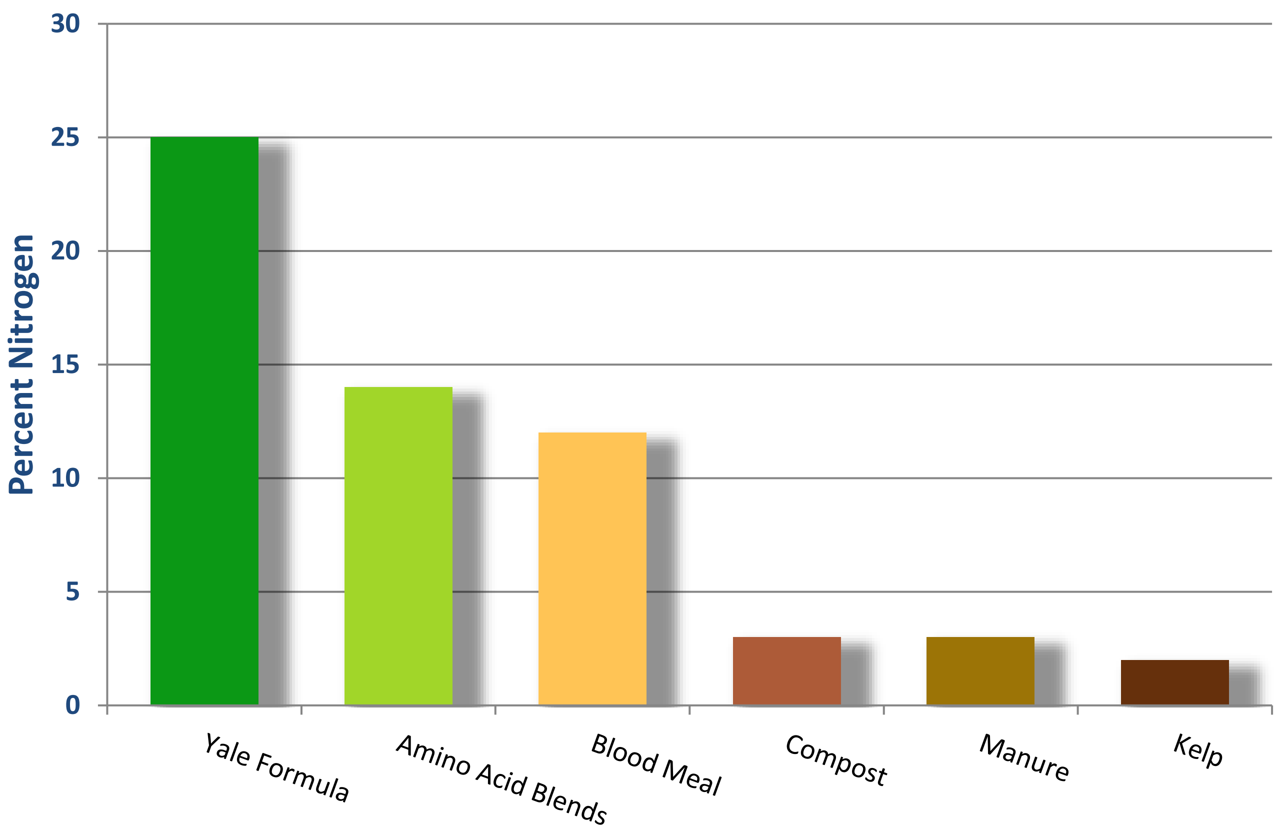
Testing against selected current market solutions:

- No significant outperformance of current available products over the inorganic fertilizer control
- The Yale formula performed statistically significantly better than current solutions

Organic Nitrogen Comparison

The Yale formula has the highest nitrogen concentration among organic competitors

There are other products on the market that only contain humic acids and marine algal extracts that are not as effective, as they lack the most bioactive compounds and do not create an as strong feedback loop.



Summary

Increase growth rate, yield, and plant vigor

Increase yield 200%+ over fertilizer alone

Reach blooming and commercial stages earlier

Elevate resistance to disease, insects, and extreme weather events

Improve nutritional value

Natural, organic, and safe ingredients

Improve sustainability and ecological impact

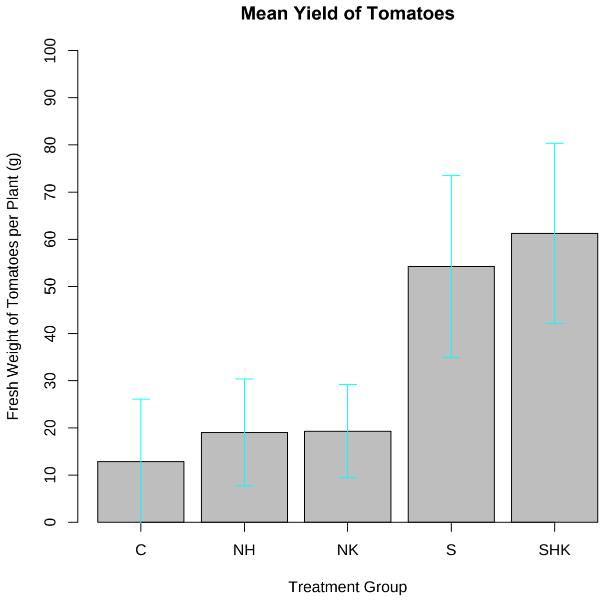


Figure 1: Mean Yield of Tomatoes
The Yale formula increased the yield of tomatoes by 321% in greenhouse trials

Vision

Mission:

To promote sustainable agriculture, mitigate climate change, and increase agricultural productivity

Value Proposition:

Enable farmers to grow more food on less land while becoming more sustainable

Environmental impact from:

- *Increase in yield results in accelerated carbon sequestration*
- *Decreased fertilizer use (50%-100%), leading to lower emissions of nitrous oxide*
- *Water quality improvement due to decreased nutrient runoff*
- *Promote accumulation of carbon in soil, turning agriculture from a net source to a net sink of carbon and improving soil quality*
- *Generate sellable carbon credits*

Kevin Gallagher
- Yale University, Statistics and Data Science; Economics
- Agriculture venture experience

Dr. Graeme Berlyn
- Professor in the Yale School of the Environment
- Invented the first organic biostimulant in the 1990s
- Has since perfected his formula

Founding Team

- Team currently of Dr. Graeme Berlyn and Kevin Gallagher
- Together, we are co-founding Shoots to bring our research out of the lab and into farms across the country
- Dr. Berlyn has been a professor of plant physiology at Yale since 1960 and has published eight peer-reviewed studies on organic biostimulants

Thank you

Dr. Graeme P. Berlyn

Graeme.Berlyn@Yale.edu

Kevin Gallagher

Kevin.Gallagher@Yale.edu

(203) 505 – 2774

"A nation that destroys its soil destroys itself."

- Franklin D. Roosevelt

Appendix

Academic Literature

Berlyn, G.P. and R. O. Russo. 1990. The use of organic biostimulants in nitrogen fixing trees. NIFTRR. 8: 1-2.

Berlyn, G. P. and R. O. Russo. 1990. The use of organic biostimulants to promote root growth. Below Ground Ecology 1: 12-13.

Russo, R. O. and G. P. Berlyn. 1990. The use of organic biostimulants to help low input sustainable agriculture. Journal of Sustainable Agriculture 1: 19-42.

Russo, R. O. and G. P. Berlyn. 1992. Vitamin-humic acid-algal biostimulant increases yield of green bean. HortScience 27: 847.

Russo, R. O. and G. P. Berlyn. 1992. The effect of an organic biostimulant (Roots ™) on the growth of loblolly pine *(Pinus taeda)* seedlings in greenhouse conditions. Agrociencia serie Recursos Naturales Renovables 2: 7-13.

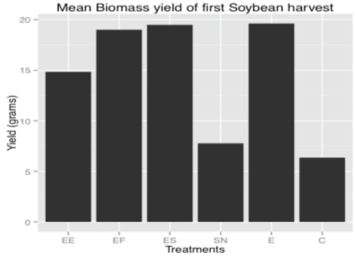
Russo, R. O., R.P. Poincelot, and G. P. Berlyn. 1994. The use of a commercial organic biostimulant for improved production of marigold cultivars. J. Home & Consumer Hort. 1: 83-93.

Berlyn, G. P. and S. Sivaramakrishnan. 1996. The use of organic biostimulants to reduce fertilizer use, increase stress resistance, and promote growth, p 106-112 in T. D. Landis and D. B. South (eds). National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW -GTR-389, Portland, OR: Department of Agriculture, Forest Service, Pacific Northwest Research Station

Sivaramakriishnan, S. and G. P. Berlyn. 1999. The role of site conditions in survival of hemlocks infested with hemlock woolly adelgid: Amelioration through the use of organic biostimulants. P. 201-204. Proceedings: Symposium on Sustainable management of Hemlock Ecosystems in Eastern North America, Durham, NH. U.S. Forest Service. Gen. Tech Report N-267, Newtown Square, PA.

SOYBEAN EXPERIMENT: HARVEST 1

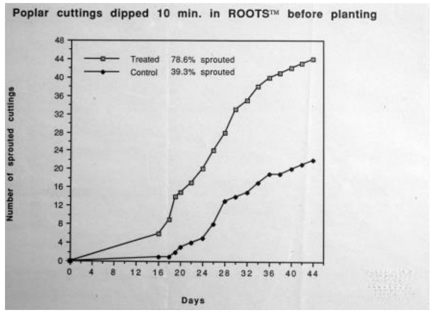
Treatments:
EE-Shoots + Vitamin E
EF-Shoots + Folic Acid
ES-Shoots + SOD (0.5g/l)
E-Shoots
SN-SN-14
C-Control
All were given 0.5g/l Miracle Grow at each treatment.



E had the highest mean pod yield

Figure 1. Mean yield of treatment from first harvest

Additional trial showing increase of formula vs fertilizer control



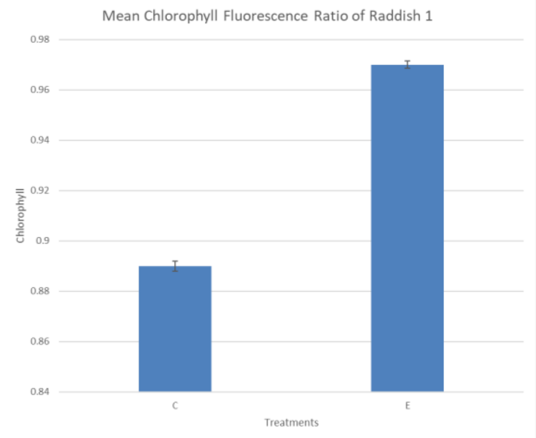
Biostimulants dramatically increase cutting survival rates

Table 1. Growth of loblolly pine seedlings three years after transplanting. Treated three times with ROOTS™ in the first year.

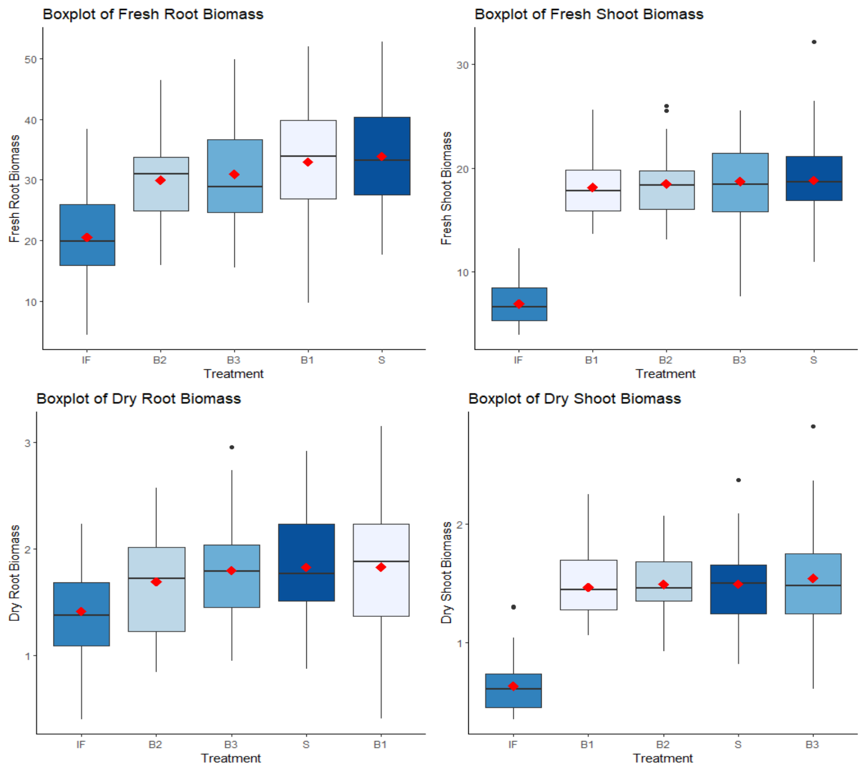
	ROOTS™	CONTROL A	CONTROL C
Survival (%)	85%	43%	77%
Total Height (cm)	181.6 a* (39.1)	145.5 c (30.2)	165.1 b (36.1)
Collar Diameter (mm)	40.1 a (11.4)	30.7 b (8.6)	34.0 b (9.6)

ROOTS™ treated N=82; Control A, N=41; Control B, N=74; * means with different letter in the same row are statistically different at 95% level. Values within parentheses are standard deviations

Biostimulants significantly increase transplanting survival rates



Biostimulants increase the chlorophyll florescence ratio, an indicator of the stress tolerance



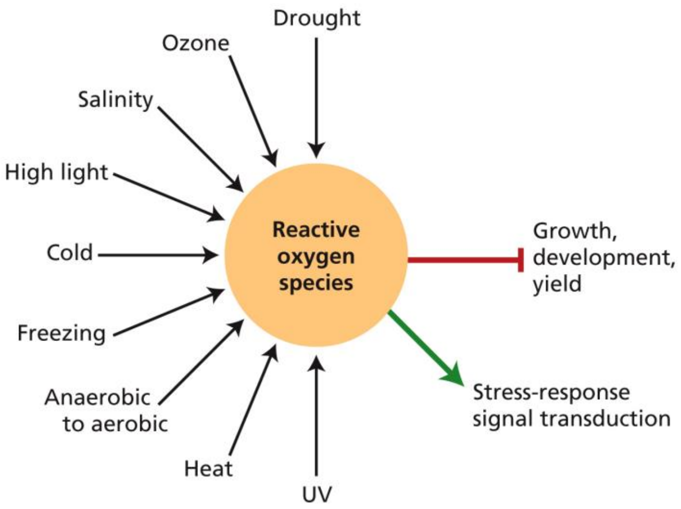
Additional results decomposing the increase in yield into root and shoot biomass

Additional Benefits and Results

Detailed Science

In small quantities, non-hormonal biostimulants have profound effects on plant growth and total biomass production. Plants are seldomly situated in an ideal environment in which they can achieve their full autotrophic capacity and maximum yield. Even in seemingly optimal environments, stressors are omnipresent, even if they are undetectable (Foyer et al., 1994). By stimulating metabolic growth processes, biostimulants mitigate these stressors' effect and regulate the response pathways at the molecular level. Also referred to as organic growth enhancers, biostimulants have been demonstrated to increase crop yield, overall plant biomass, plant vigor (Russo and Berlyn, 1990), crop nutritional value (Borsook and Berlyn, 2015), polyphenol content (Berlyn, Shields, and Young, 1995), plant vascular system development, and stress resistance. The effects are self-reinforcing: Increased biomass promotes high nutrient and water uptake, which, in tandem with the elevated hydraulic conductance from increased xylem development, reduces water and nutrient stress levels in leaves, which also benefited from the innate antioxidant capacity of the compounds. The reduced stress and elevated photosynthetic capacity increase the carbon to nitrogen ratio, carbon compounds available for growth, and overall biomass. This is achieved with natural, non-hormonal ingredients.

These compounds achieve the stress reduction predominately via their antioxidant capacity. Under high light fluxes, plants experience photooxidative stress, which is when the light-dependent photosynthesis reactions generate reactive oxygen species (ROS) when the chlorophyll absorbs more energy than it can physically use in photosynthesis. When the photoelectron chain is bottlenecked due to a lack of NADP or oxidized electron acceptors, the additional electron, usually from photosystem II, reduces an oxygen molecule. The resulting oxygen species, which are energetic molecules with unpaired electrons, can damage lipids, DNA, RNA, proteins, and is exceptionally harmful to chlorophyll. All stressors effect the electron transport chain or its supporting process to a certain degree and will result in ROS. Examples of ROS include singlet oxygen ($^1O_2$), hydroxyl radical ($HO^{\cdot}$), hydrogen peroxide (H_2O_2), and superoxide (O_2^-), one of the most detrimental. Extreme damage is irreparable and can result in mutagenesis and cell death. Reactive oxygen species are created by metabolic processes even under optimal conditions and have been shown to function in signaling (Shin et al., 2012). Plants have evolved antioxidant responses to detoxify these harmful compounds through both enzymatic and non-enzymatic processes that scavenge and neutralize ROS. An enzymatic process, superoxide dismutase (SOD) is an exceptionally powerful antioxidant that to reduces ROS into hydrogen peroxide. Hydrogen peroxide is then converted into water and oxygen in either the peroxisome, which is an organelle containing catalase (CAT), or via the ascorbic acid glutathione chain. Since chloroplasts do not contain catalase, it relies on the latter process. Non-enzymatic processes predominately include lower molecular mass antioxidants, which include ascorbic acid, flavonoids, and glutathione (Huang et al., 2019). The organic biostimulant hypothesis is founded on the observation that under stressful conditions, plants cannot produce the optimal amount of these antioxidant compounds and exogenously applying them can increase detoxification capacity and overall productivity (Berlyn and Beck, 1980).



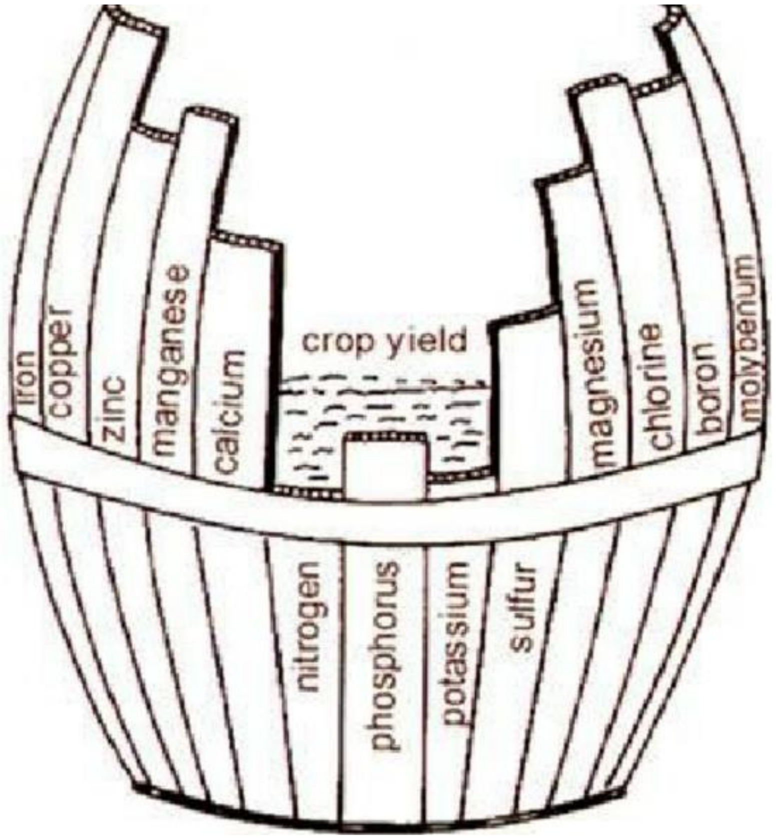
PLANT PHYSIOLOGY AND DEVELOPMENT 6e, Figure 24.4

Detailed Science Analogy

Plant growth follows Justus von Liebig's "Law of the Minimum" (1873), which states that if one necessary growth factor is deficient, even if all other vital factors are adequate, the limited element puts a ceiling on the maximum yield potential.

To this end, a common analogy for plant growth is a barrel with unequal staves, with each plank representing a different key nutrient. Just how filling up the barrel with water is limited by the shortest stave, so is plant growth limited by the nutrient in the shortest relative supply.

This theory has been expanded upon by Acheampong Atta-Boateng and Graeme Berlyn in the Limiting-Stress-Elimination Hypothesis (2019). Here, the biostimulant metaphorically patches the shortest stave by mitigating the impact of stress, thereby maximizing crop yield via antioxidant pathways.



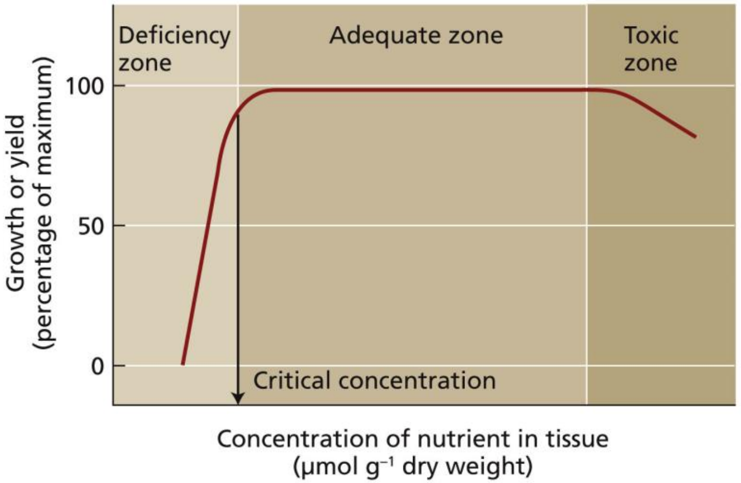
Distinction from Inorganic Fertilizer

Biostimulants function differently from inorganic fertilizer and trigger different metabolic pathways. Applying more inorganic fertilizer will not produce the same results as implementing biostimulants.

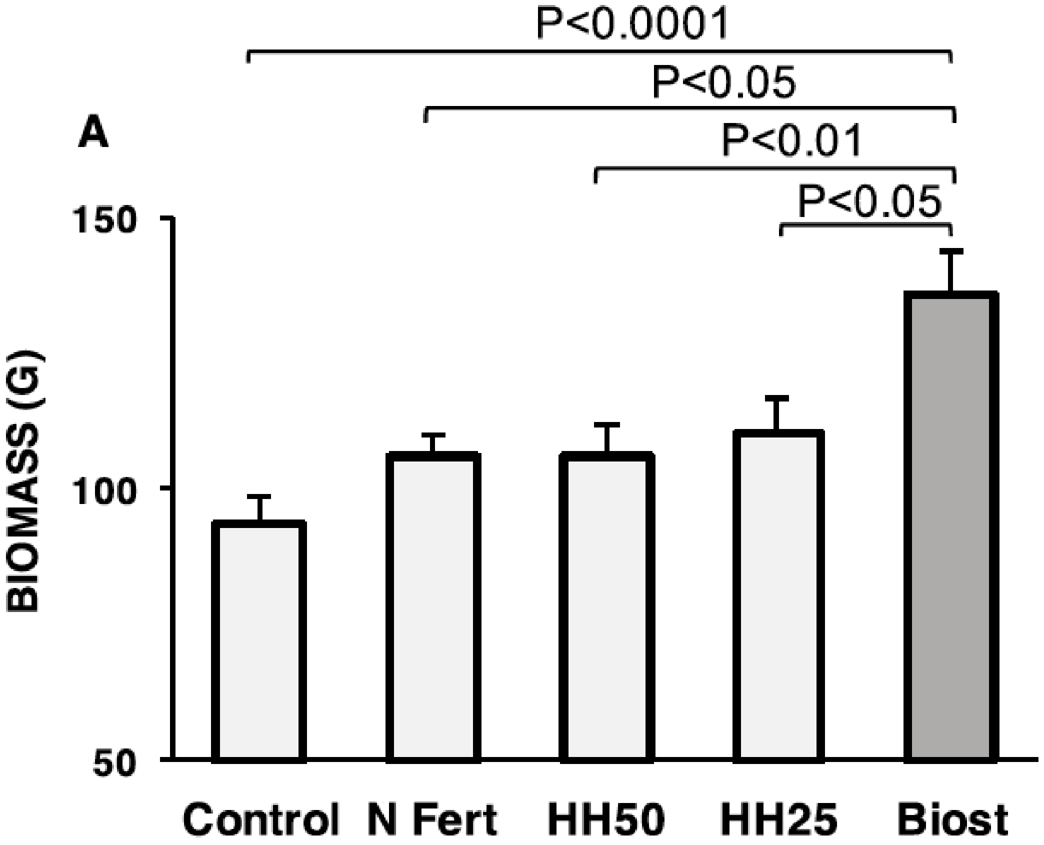
Mineral nutrition is vital for plant growth optimization and has been the center of agricultural practices since the NPK revolution of the 1950s and 60s following the discovery of the Haber-Bosch nitrate synthesis process earlier in the century. Essentially, there are three zones for nutrient applications. In deficient soils, crop yield increases linearly with fertilizer application. In soils with adequate nutrition, additional fertilizer has no effect. Eventually, too much fertilizer becomes toxic, resulting in chemical burns and senescence.

Biostimulants are not minerals like fertilizers. Rather, they are mixtures of bioactive substances that directly stimulate metabolic and antioxidant pathways.

Biostimulants can function in conjunction with fertilizer to maximize yield or completely replace fertilizer to maintain yield.



PLANT PHYSIOLOGY, 5e, Figure 5.4



HH25 and HH50 are two osmotic protectant treatments that are commercially available. N Fert is inorganic fertilizer, and the control was untreated

Field Trial: Cowpea Biomass in Ghana

Organic biostimulants are proven to increase biomass in the field under moderate stress conditions in cowpeas grown in Ghana's savanna.

Biostimulants outperformed osmo-protectants, inorganic fertilizer, and the control. The biostimulant-treated plants were not supplemented with fertilizer.

This suggests that biostimulants have significant applications in high stress environments, where they can replace fertilizer completely while also increasing yield. Biostimulants may have outsized benefits for subsistence farmer in developing economics, thereby promoting environmental justice.