Biostimulant Research Overview

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Biostimulant Benefits

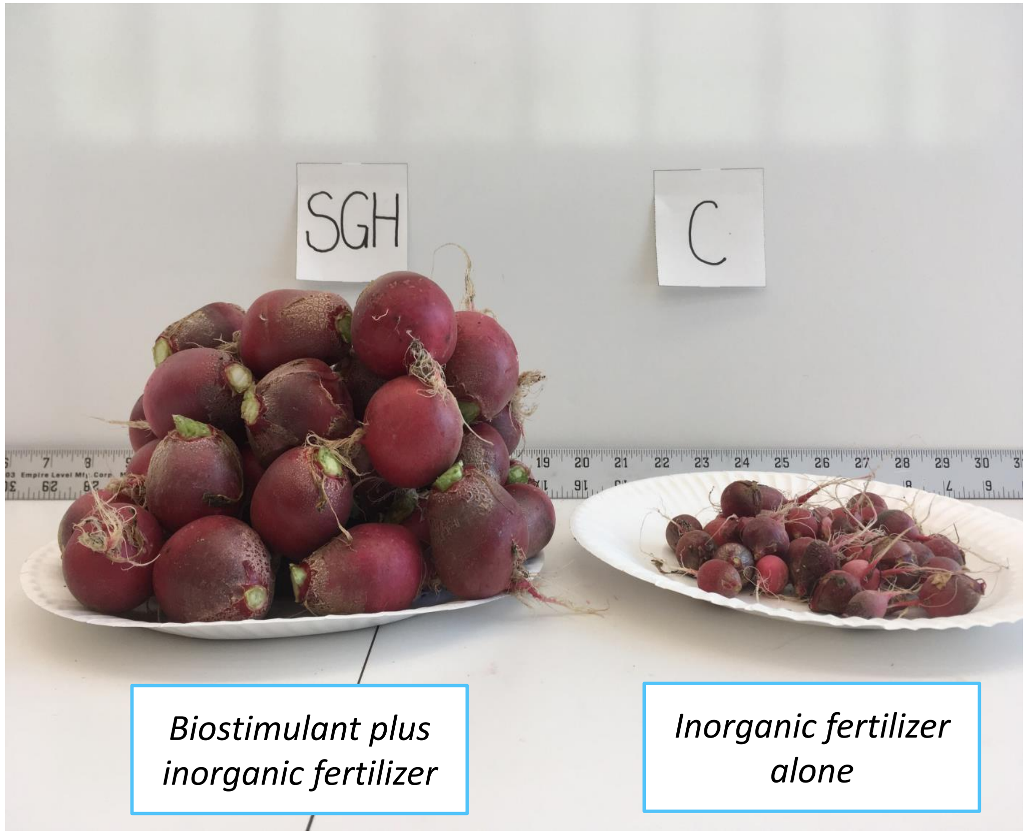
Increase yield ~400%+ over inorganic fertilizer with minimal, low-cost inputs

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



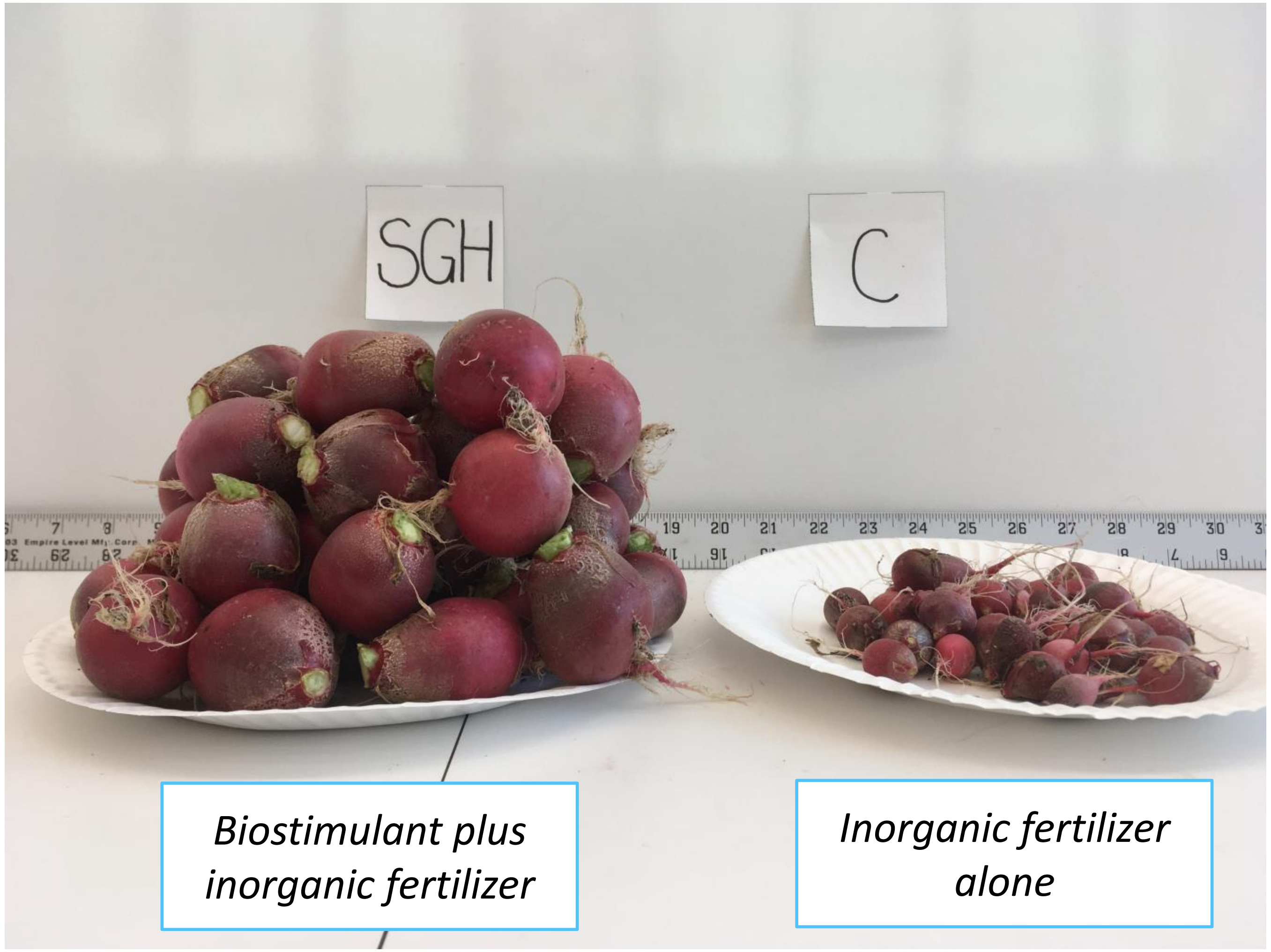
Simultaneously increase yield and sustainability

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 organic
  - ✓ 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.

Greenhouse Results



Biostimulant plus inorganic fertilizer

Inorganic fertilizer alone

Treatment (Left to Right)

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

Trials done in 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

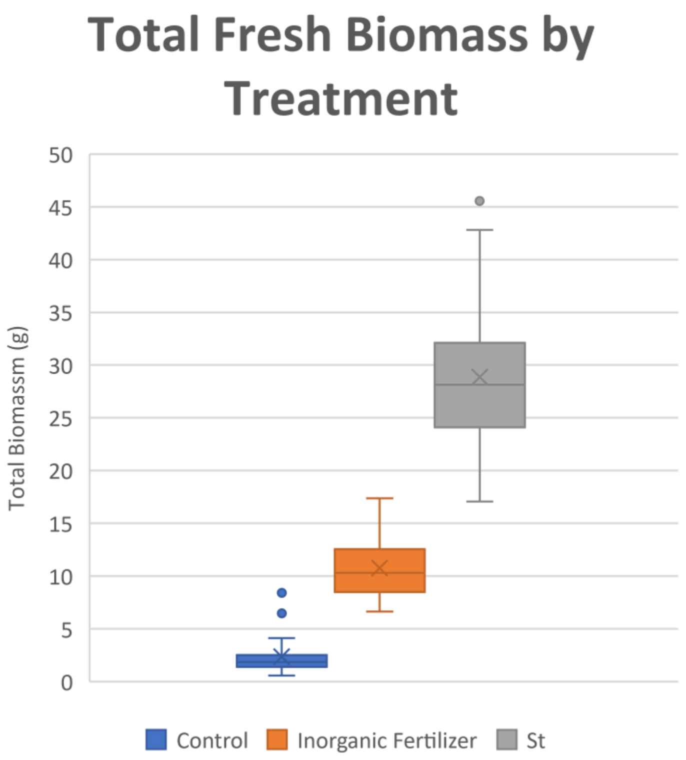
Biostimulant plus inorganic fertilizer

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



In January 2020, we tested the improved formula on radishes.

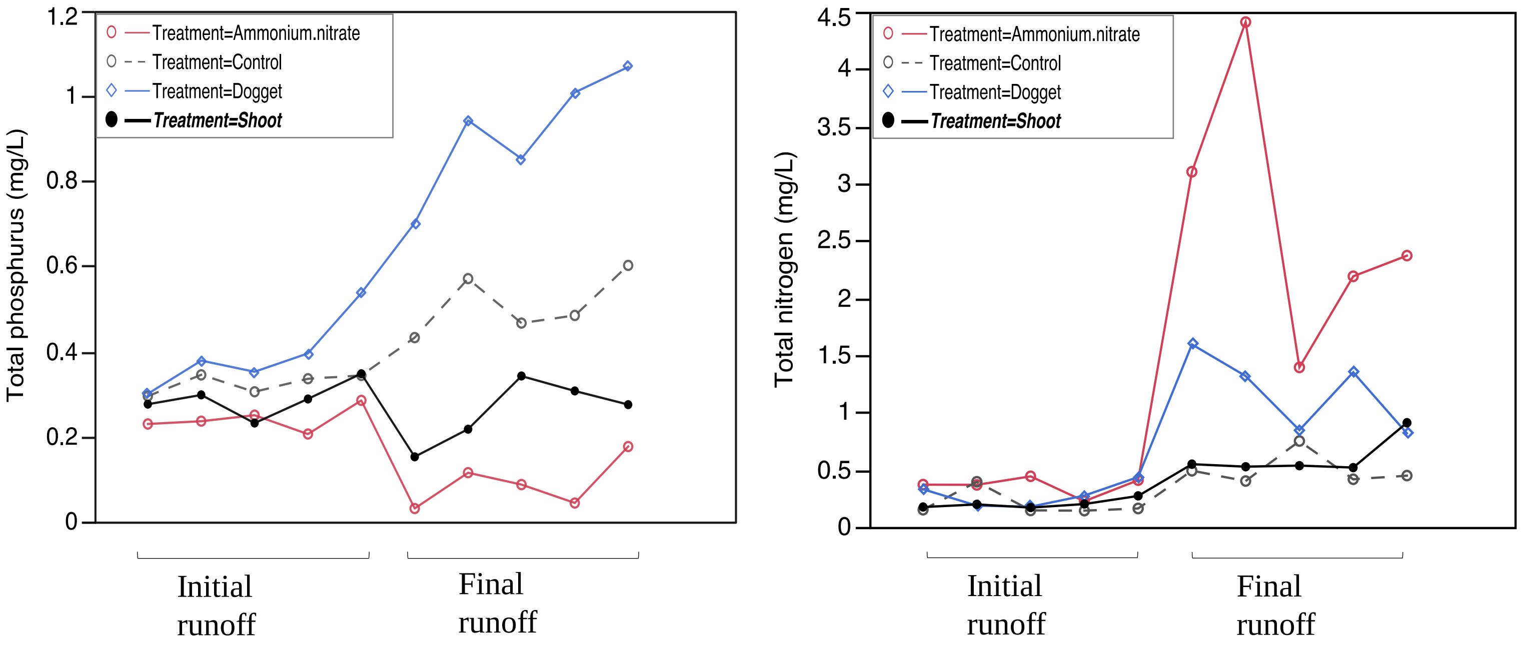
The biostimulant, applied once a week, increased yield 2,205% over the water control and 484% over plants with fertilizer alone.

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**



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

Improved Sustainability

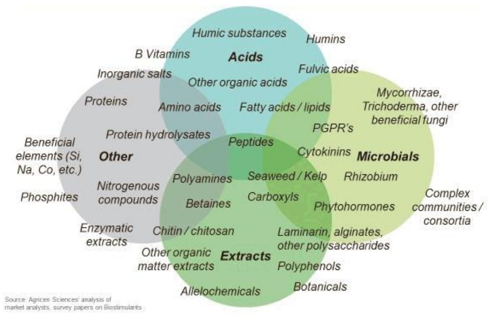
Unlike inorganic fertilizers, biostimulants:

- 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 and increase yield ~400%+ when added in addition to fertilizer



Other 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

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

Neptune's Harvest, Yale Formula, SN-14

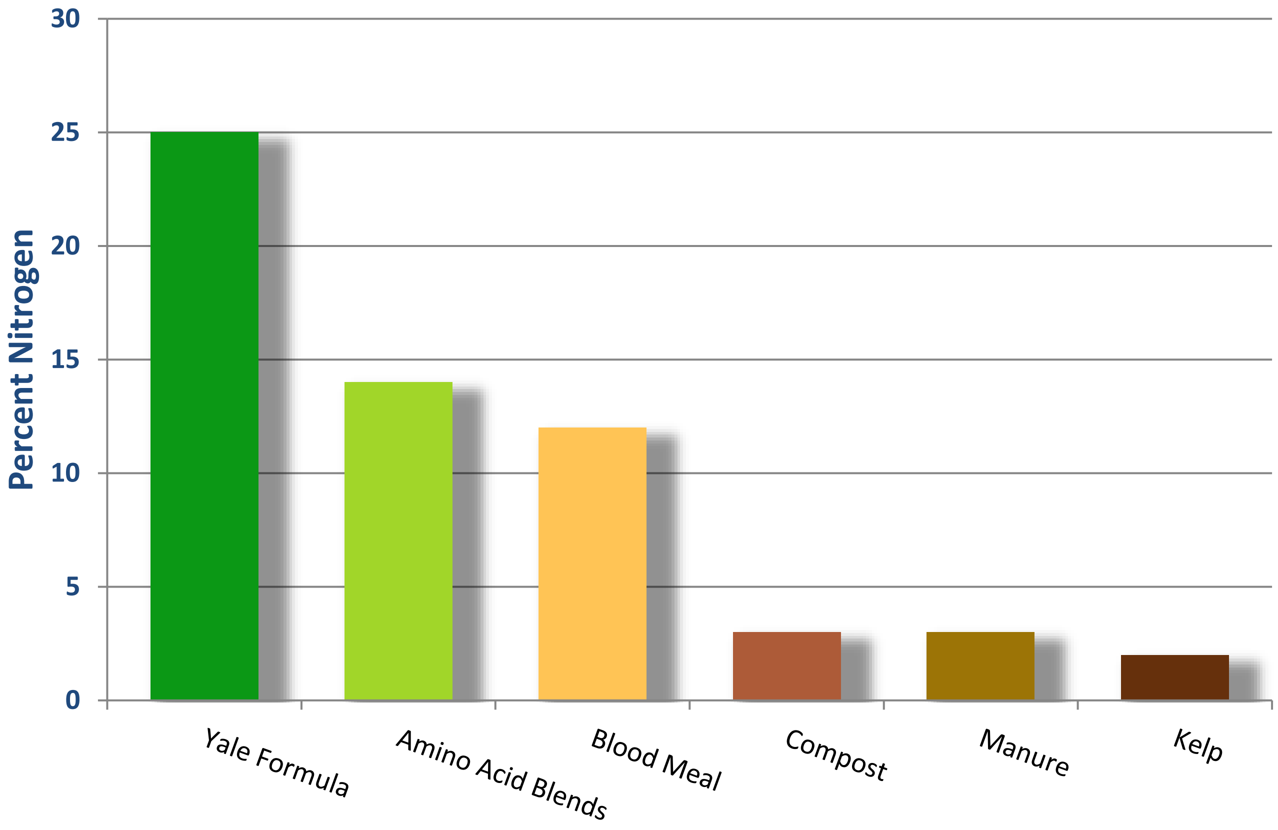
Testing against selected current market solutions:

- 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 ~400+% 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

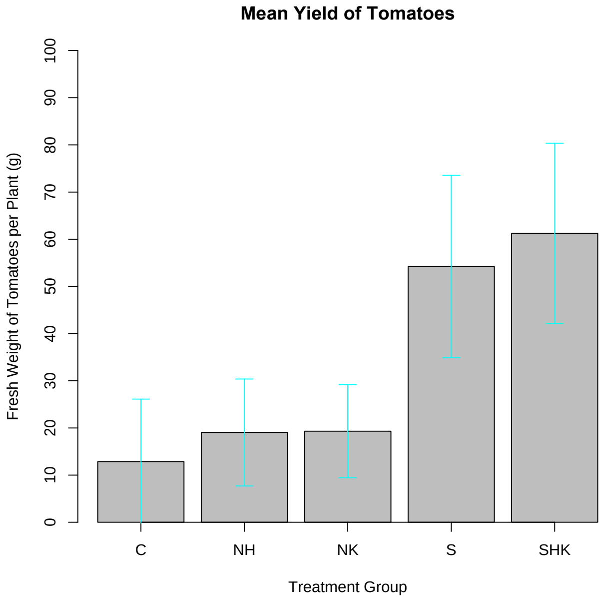


Figure 1: Mean Yield of Tomatoes
The Yale formula increased the yield of tomatoes by 321% in greenhouse trials

Basic Economic Units and Material Raw Costs at Scale

To achieve an estimated 200%-400+% increase in yield

Applied in small quantities

The seven ingredients are fungible chemical commodities

Vitamins, minerals, amino acids, co-enzyme proprietary mix

Per plant and acre economics displayed to the right

Treatment Cost Per Plant Sans Markup

Treatment	Quantity Applied	Cost per Month
Small Potted Plant	25 ml / week	$ 0.0024
Shrubs and Larger Plants	100 ml / month	0.0022
Large Trees	2 liter / month	0.0440

Material	Concentration	Price Estimate per kg at Scale	Cost per Liter
A	0.5 g / L	$ 5.00	$ 0.0025
B	0.1	19.00	0.0019
C	0.1	5.00	0.0005
D	1.0	6.00	0.0060
E	6.0	1.40	0.0084
F	1.0	2.50	0.0025
G	1.0	0.20	0.0002
Total	9.7 g / L		$ 0.022

Crop	Plants per Acre	Monthly Application Cost per Acre	Average Revenue per Acre
CBD Hemp	1,000 - 1,600	$ 2.37 - $ 3.78	$ 2,500 - $ 75,000
Tobacco	6,000 - 7,200	$ 14.19 - $ 17.03	$ 4,715
Corn	20,000 - 44,000	$ 47.30 - $ 104.06	$ 673
Tomato	2,600 - 5,800	$ 6.15 - $ 13.72	$ 29,392
Soybean	100,000	$ 236.50	$ 769
Lavender	3,000 - 5,000	$ 7.10 - $ 11.83	$ 120 - $ 2,000
Ginseng	280,000 - 560,000	$ 662.20 - $ 1324.40	$ 60,000
Viniculture	1,000 - 3,000	$ 2.37 - $ 7.10	$ 11,000

Hydroponics added directly to water until 9.7 g / L

Assuming no markup

Plants such as wheat and sorghum fertilized as a grass

Our Team

- Team currently of Dr. Graeme Berlyn and Kevin Gallagher
- Dr. Berlyn has been a professor of plant physiology at Yale since 1960 and has published eight peer-reviewed studies on organic biostimulants
- Most of his work on biostimulants is unpublished

Kevin Gallagher

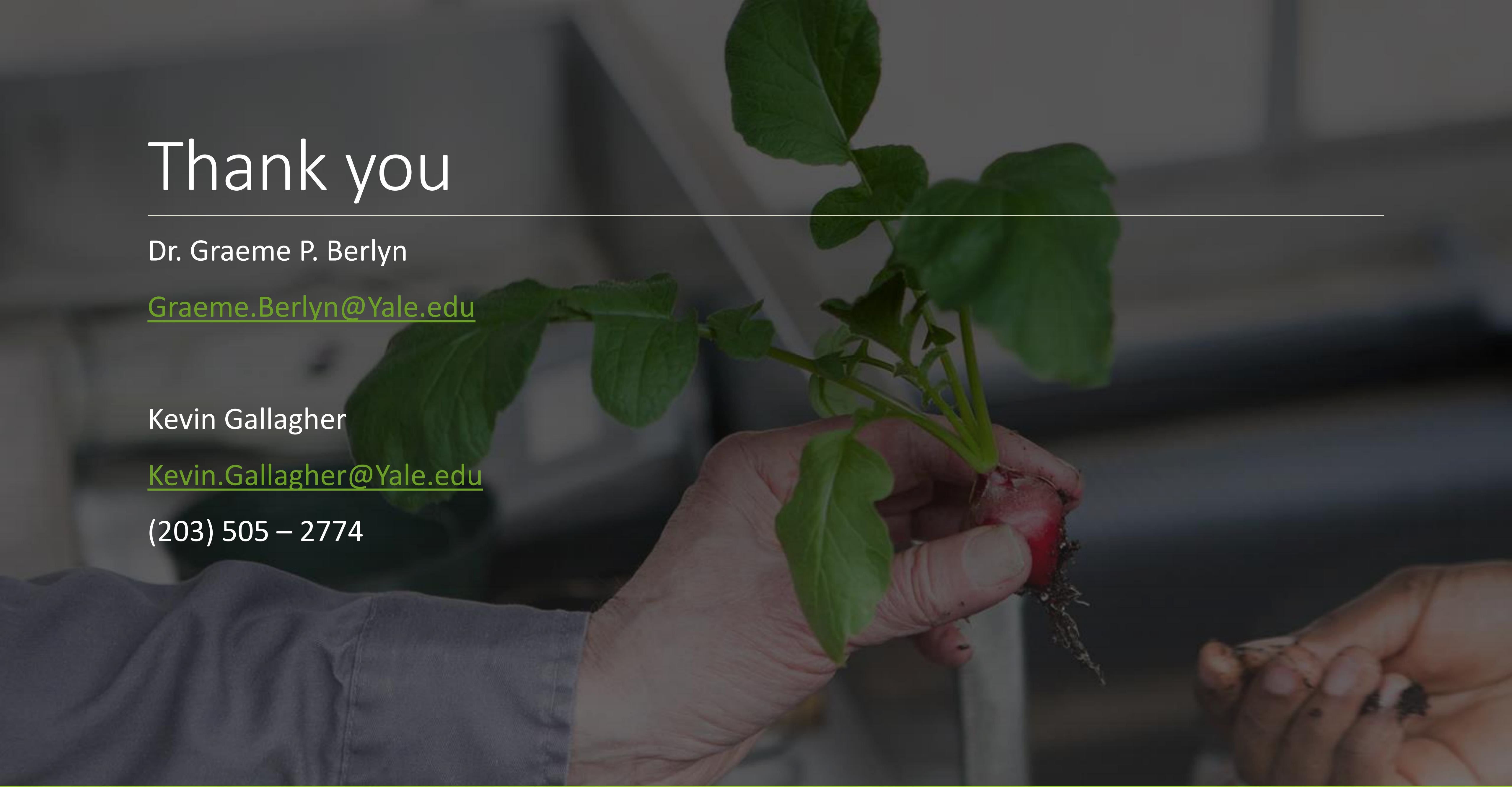
- Research assistant
- Yale University, class of 2021
- Statistics and Data Science; Economics

Dr. Graeme Berlyn

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

Objectives

- Near-term steps:
  - Network with private equity firms and industry professionals
  - Work with OCR on licensing, venture formation, and intellectual property matters
  - Replicate greenhouse experiment results in the commercial pilots in strategic partnership to determine commercial value
  - Develop optimal business plan and scaling strategy in consultation with OCR
- Venture objectives:
  - Commercialize the high-yield biostimulant formula via a startup
  - Increase yield of selected agriculture subsectors
  - Improve sustainability and reduce pollution of modern agriculture
  - Mitigate the impact of climate change on agriculture
  - Gain Dr. Berlyn the recognition I believe he deserves
- Preliminary strategy:
  - Target well capitalized and high-value agricultural sub-sectors
    - E.g. private equity backed companies, CBD hemp, grapes, ornamental bonsai, organic products, vanilla, designer hops, golf course turf, hydroponics, microgreens, tobacco, catnip, herbs, vegetables, fruits, berries
  - Upfront cost or marginal revenue profit share agreement



Thank you

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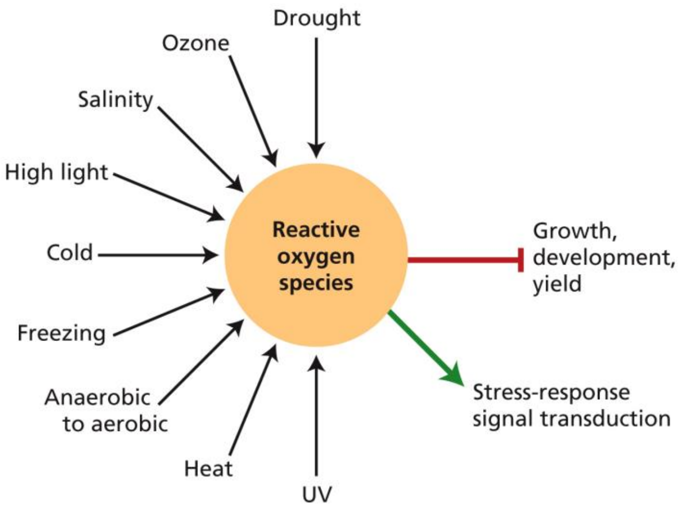
Appendix

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

Academic Literature

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

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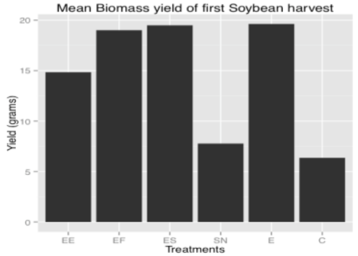
Russo, R. O., R.P. Poincelot, and G. P. Berlyn. 1994. The use of a commercial organic biostimulant for improved production of marigold cultivars. J. Home & Consumer Hort. 1: 83-93.

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

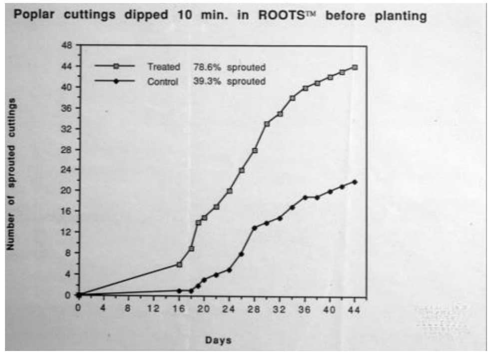
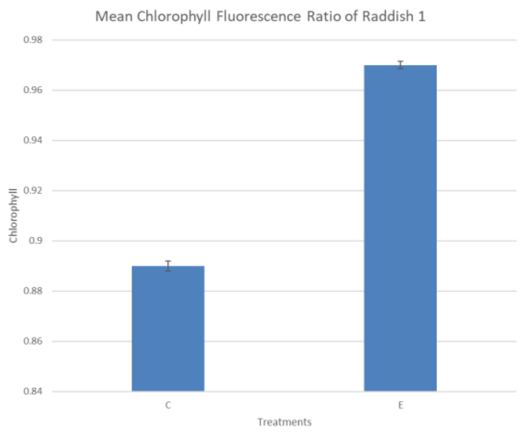
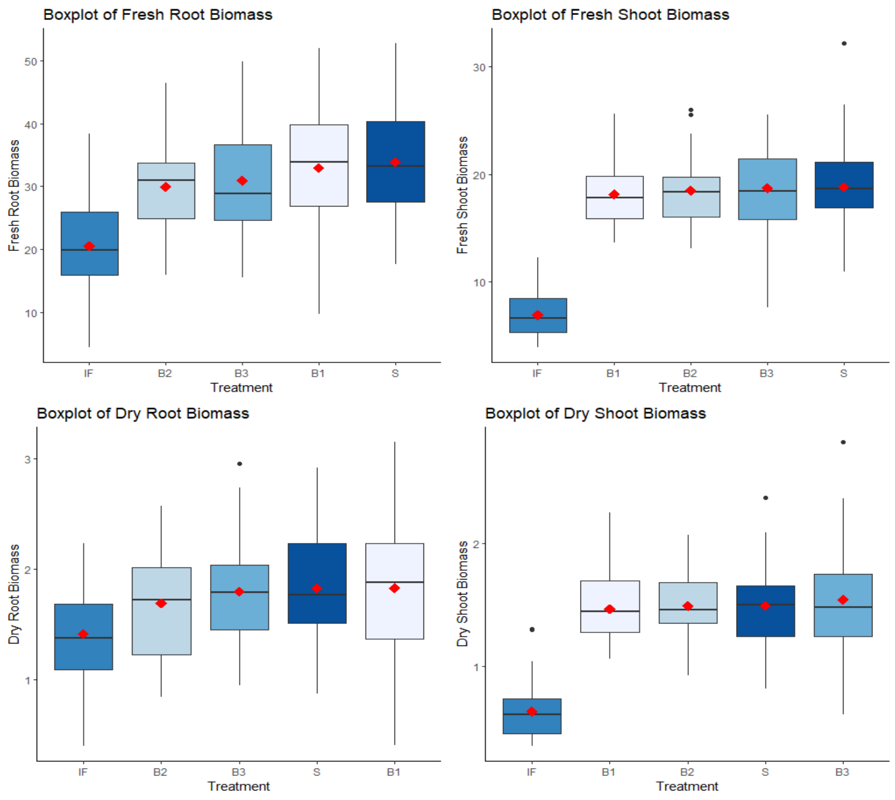


Table 1. Growth of loblolly pine seedlings three years after transplanting. Treated three times with ROOTS™ in the first year.

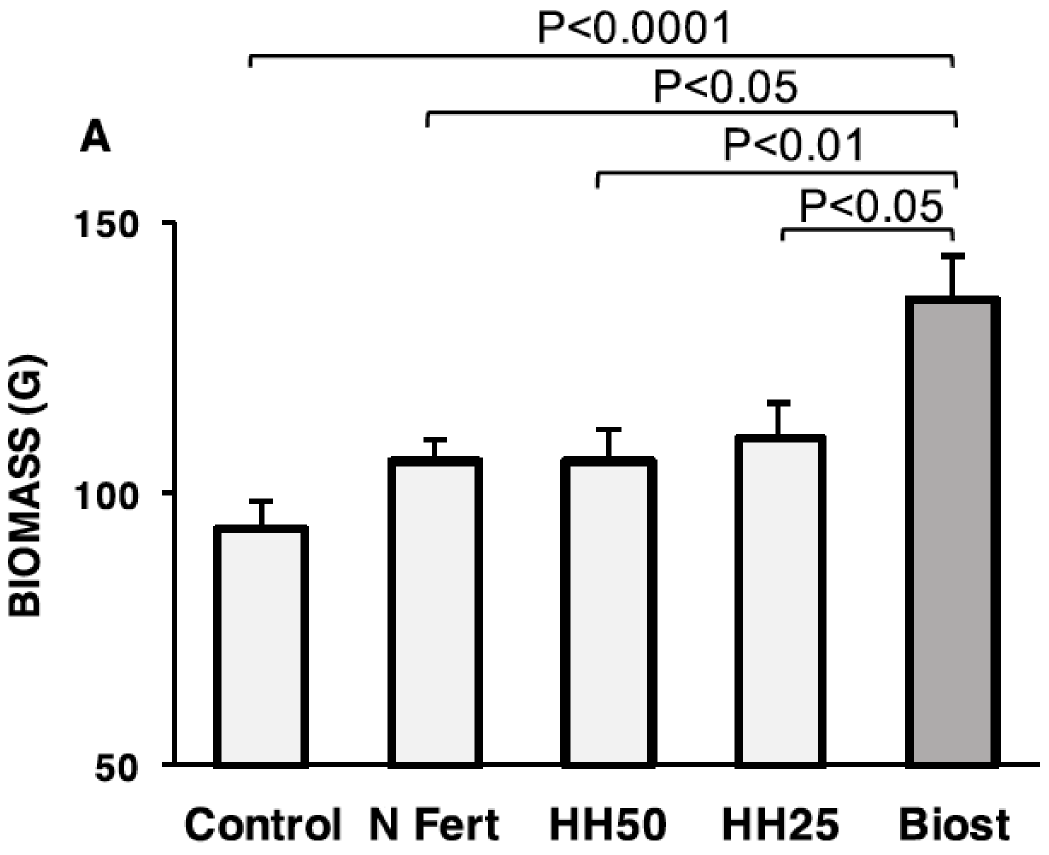
	ROOTS™	CONTROL A	CONTROL C
Survival (%)	85%	43%	77%
Total Height (cm)	181.6 a* (39.1)	145.5 c (30.2)	165.1 b (36.1)
Collar Diameter (mm)	40.1 a (11.4)	30.7 b (8.6)	34.0 b (9.6)

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





Other Benefits



COWPEA BIOMASS IN GUINEA TROPICAL SAVANNA, GHANA

In this study, the limiting stress is determined by testing which stress intervention favors higher sensitivity to physiological response in cowpea and its effect on total biomass yield. Further, the ecological hypothesis that supports the results is discussed. Also, the implications of choice of intervention for addressing yield gap in leguminous crop production in low resourced environments in low income regions such as sub-Saharan savanna landscapes are explored. To examine the preferred response strategy of cowpea, the effect of abiotic treatment and N fertilization on cowpea are evaluated.