Soil Bioactivators: What Do We Know? What Don't We Know?

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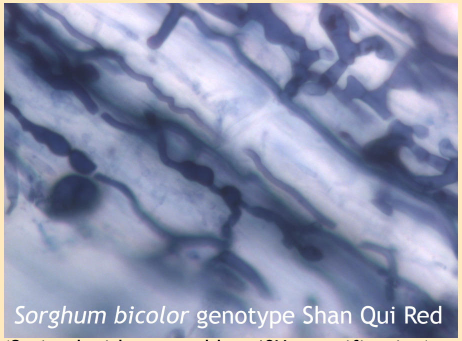
Main Points to Be Addressed

- Background on bioactivators/biostimulants
- Microbial Ecology 101
- Applications
- Currently available bioactivators

Long history of bioactivator use

- **Rhizobia** with leguminous crops
  - Fixes nitrogen for plant use ($N_2 \rightarrow NH_3$)
  - Association recognized in late 1800
  - Use in Midwest farming since early 1900

- **Mycorrhizal fungi**
  - Uptake of some nutrients
  - Improve drought resistance
  - Improve soil quality


Sorghum bicolor genotype Shan Qui Red
(Stained with trypan blue 40X magnification)

(Sylvia et al. 2005)

Common Terms

- Bio-activators
- Bio-stimulants (phyto-stimulator)
- Biologics
- Bio-inoculants
- Bio-formulations
- Bio-additives
- Bio-fertilizers

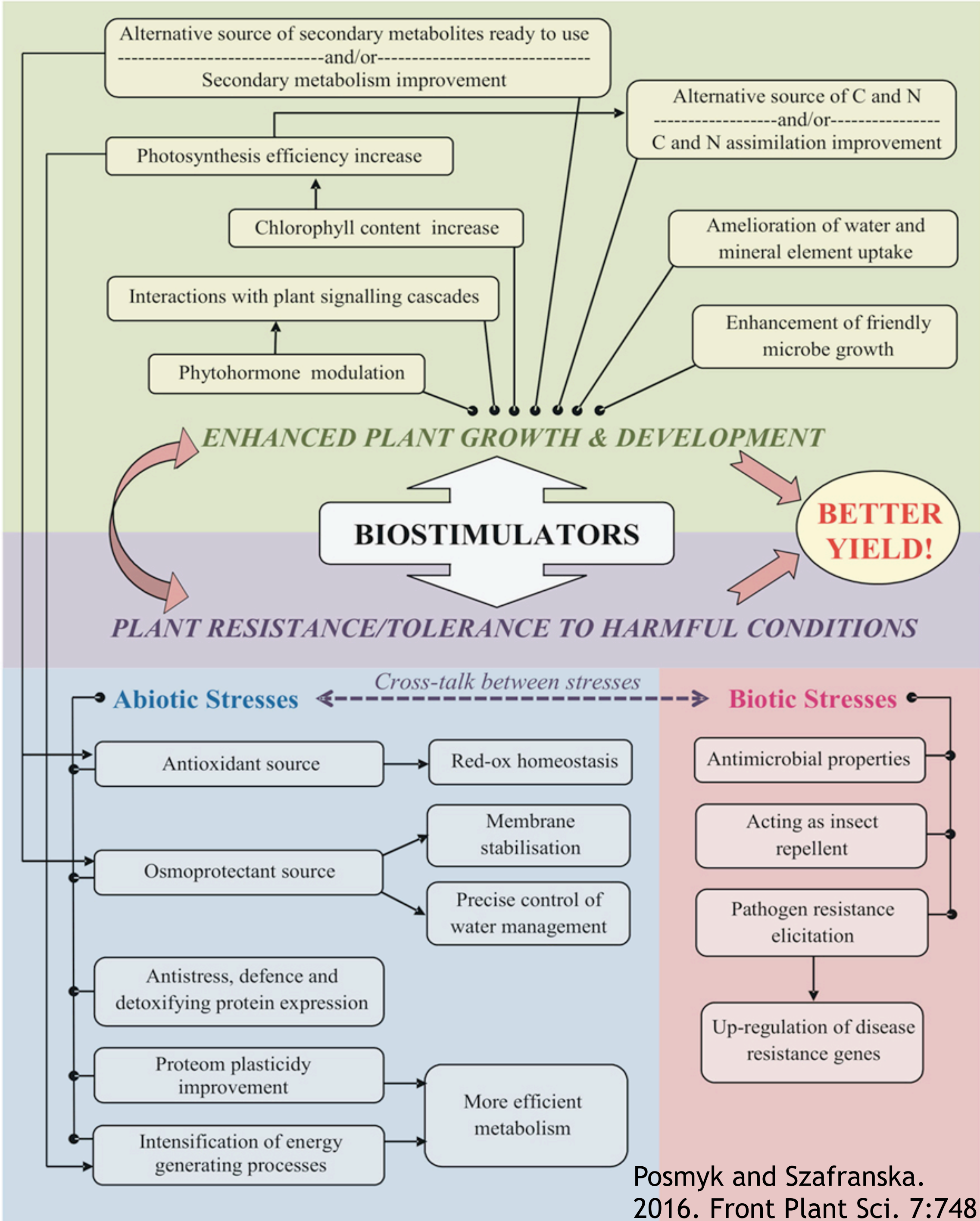
Biostimulants

"Plant biostimulants contain substance(s) and/or micro-organisms 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."

European Biostimulants Industry Council
http://www.biostimulants.eu/

Reasons for biostimulant use

- Increase available nutrients
- Improve crop growth and yield
- Improve crop quality
- Pathogen/disease suppression
- Provide non-traditional plant nutrients
- Improve soil quality/health
- Increase beneficial microbes



Posmyk and Szafranska. 2016. Front Plant Sci. 7:748

Desired biostimulator traits

1. Non-toxic, safe for animals and environment
2. Easily and actively taken up by plants from environment
3. Of natural origin or easily synthesized in laboratories
4. Not expensive
5. Dissolves in different solvents: water, alcohols but also lipids – that facilitates the use of various application methods
6. Easily penetrates cell compartments
7. Improve plant resistance to adverse conditions and help generate tolerance to stresses

Posmyk and Szafranska. 2016. Front Plant Sci. 7:748

Includes substances or microbes

Most commonly used

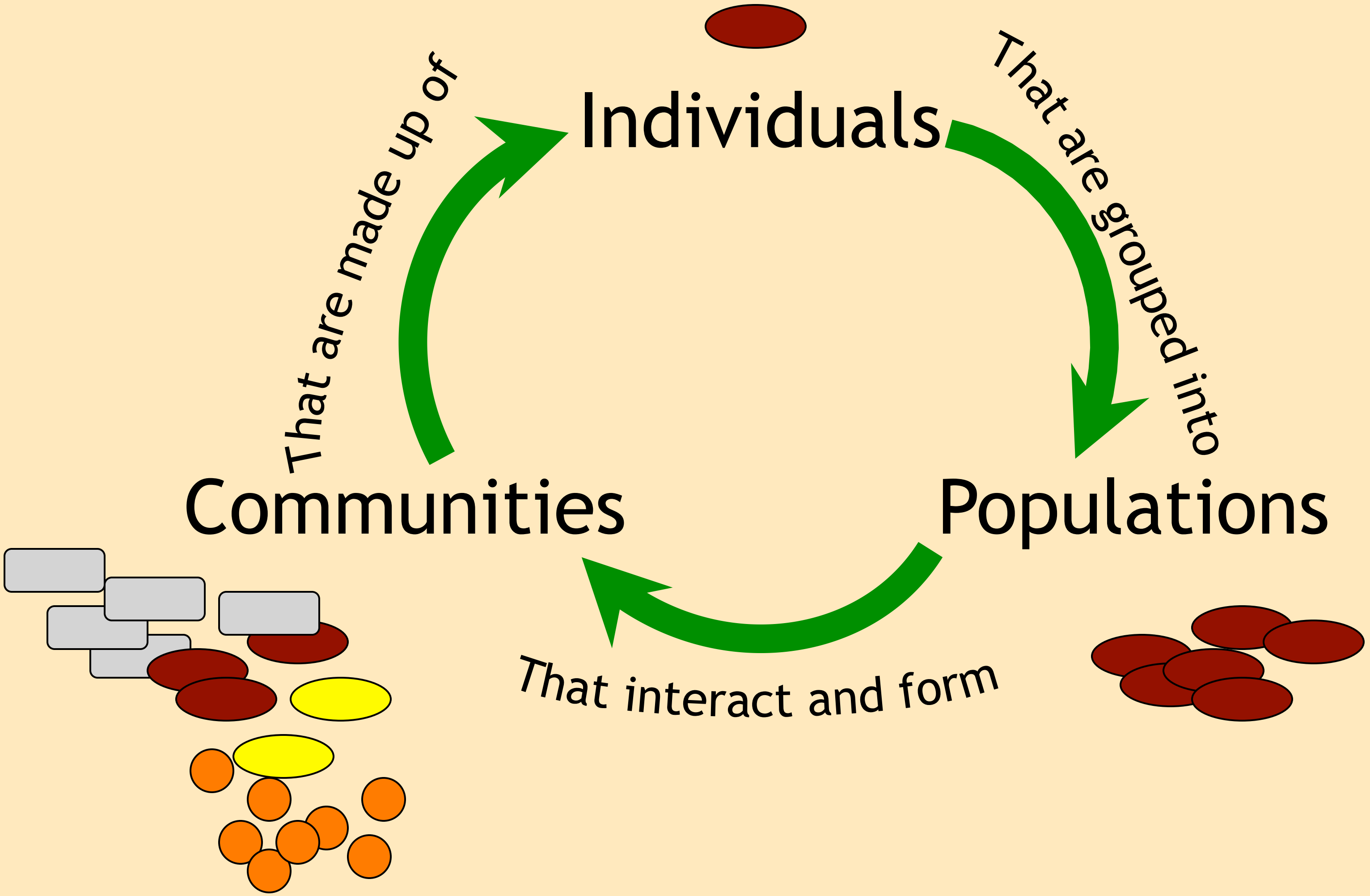
- Beneficial bacteria
- Beneficial fungi
- Humic acids and fulvic acid,
- Seaweed extract
- Protein hydrolates (amino acids, peptides)
- Chitosan and other biopolymers
- Inorganic compounds (beneficial to only some plants, e.g., silica)

Du Jardin 2015 Scientia Horticulturae 196:3-14

Before going further: Ecology

- What is already present in soil?
- What role do they play?
- Factors that need to be considered.

Ecosystems are Comprised of:



Number of Microbes in soil

Table 1. Prokaryotic abundance as determined by fluorescence microscopy and total genomic diversity in prokaryotic communities calculated from the reassociation rate of DNA isolated from the community (*9*). Community genome complexity is described as numbers of base pairs (bp). Genome equivalents are given relative to the *Escherichia coli* genome (4.1×10^6 bp).

DNA source	# cells in g (0.2 tsp) of soil	Community genome complexity (bp)	# species in g (0.2 tsp) of soil	Ref.
Forest soil	4.8×10^9	2.5×10^{10}	6000	(*8*)
Forest soil, cultivated prokaryotes	1.4×10^7	1.4×10^8	35	(*8*)
Pasture soil	1.8×10^{10}	(1.5×10^{10})–(3.5×10^{10})	3500–8800	(*22*)
Arable soil	2.1×10^{10}	(5.7×10^8)–(1.4×10^9)	140–350	(*22*)
Pristine marine sediment	3.1×10^9	4.8×10^{10}	11,400	(*8*)
Marine fish-farm sediment	7.7×10^9	2.0×10^8	50	(*8*)
Salt-crystallizing pond, 22% salinity	6.0×10^7	2.9×10^7	7	(*9*)

10 Billion

Torsvik et al. 2002. Science 296: 1064-1066

Role of Microbes in Ecosystems

- Functional powerhouse
- Base of biogeochemical cycles
  (e.g. Degradation of plant materials)
- Survival of all other organisms dependent on microbes
  - Pathogenesis
  - Beneficial association
    (e.g., rhizobia)

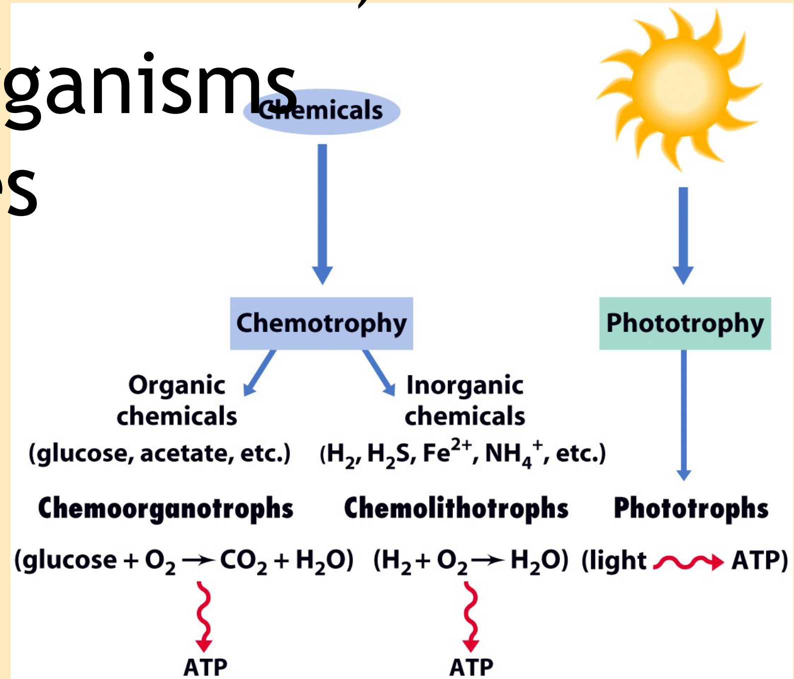
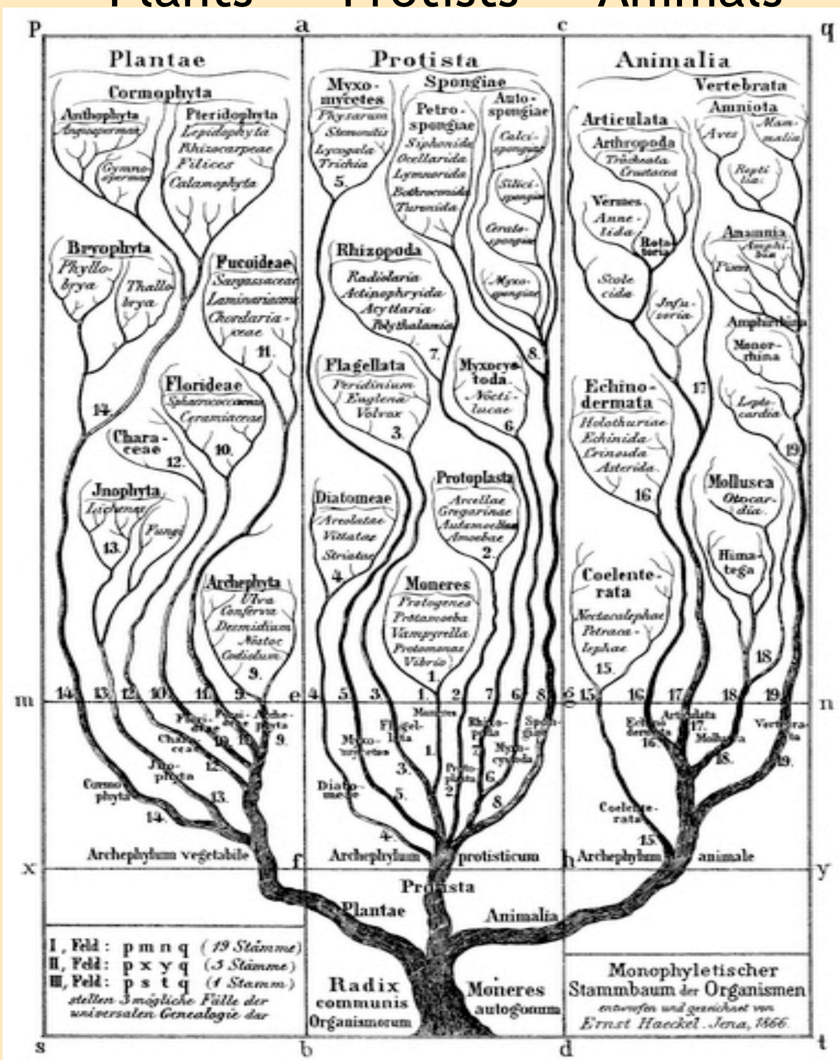


Figure 2-8 Brock Biology of Microorganisms 11/e

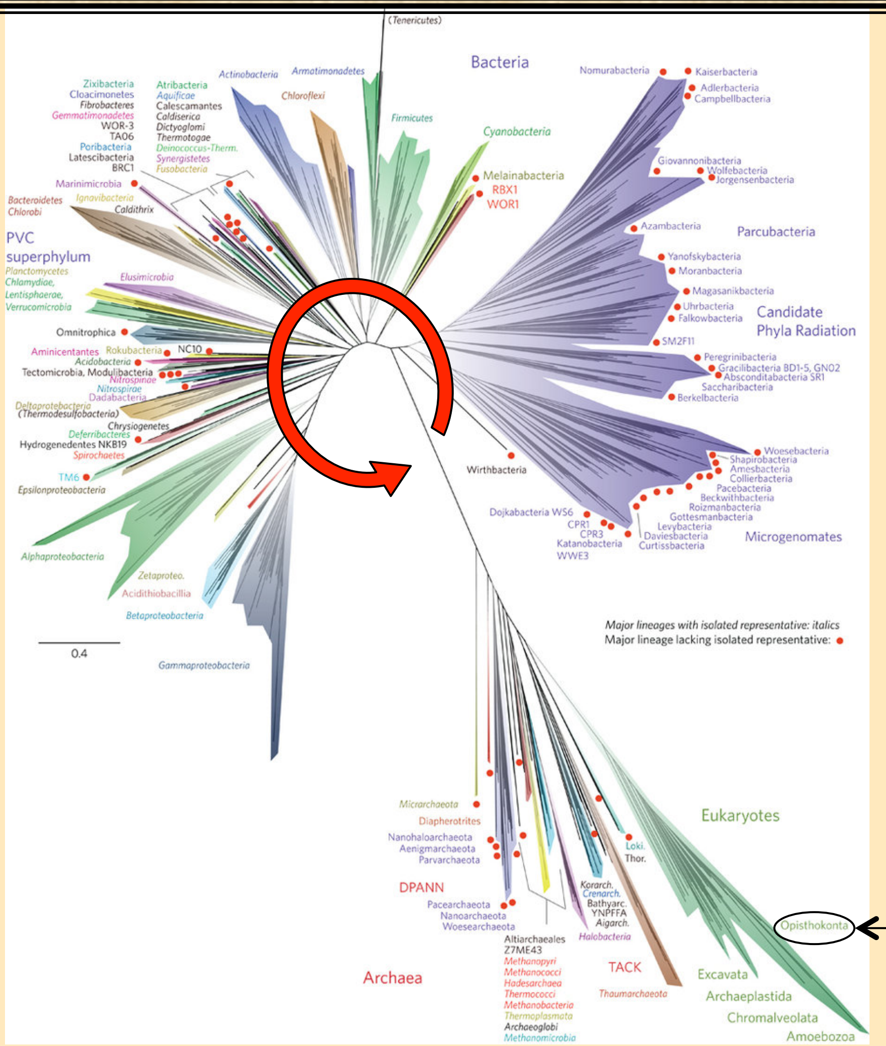
Tree of Life: Initial

Plants Protists Animals



- Tree of Life first proposed by Charles Darwin in 1859 "On the Origin of Species"
- Figure of tree produced by Ernst Haeckel in 1866 "*Generelle Morphologie der Organismen*"

Tree of Life: Current



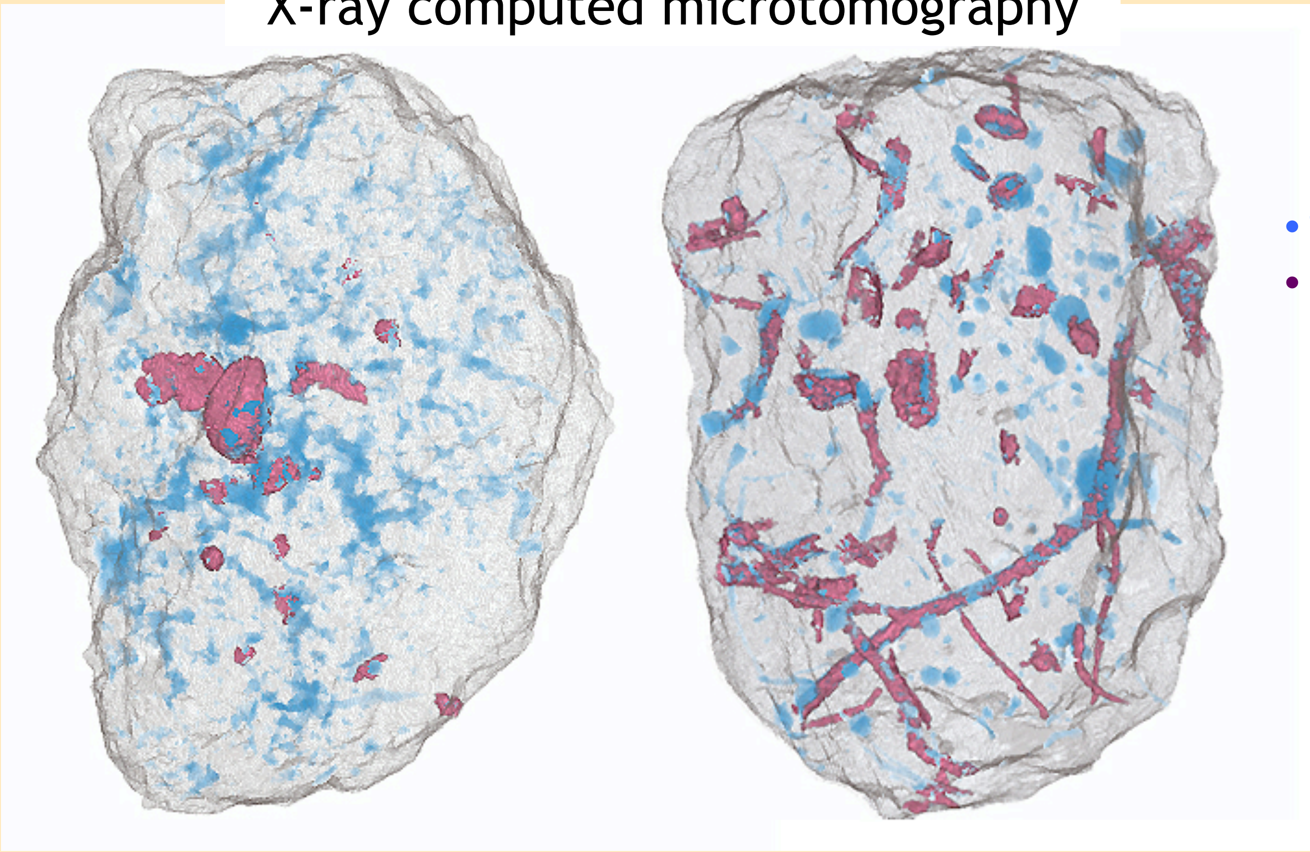
Tree based on ribosomal protein sequences

Microorganisms are the most diverse group of organisms on earth

Hug et al. 2016. Nature Microbiology 1:16048
doi:10.1038/nmicrobiol.2016.48

Distribution of microbes in soil

X-ray computed microtomography



- Soil pores
- Particulate organic matter

Certain microbes have preference for particular pores sizes

Soil aggregate under conventional agricultural management

Soil under organic management with red clover cover crops

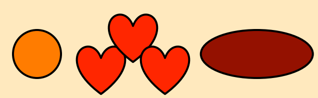
https://www1.aps.anl.gov/aps-science-highlight/2015/soil-study-maps-out-microbe-real-estate

Kravchenko et al. 2014. SSAJ 78:1924

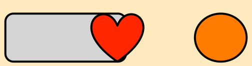
Interaction among cells

- Attributes of each individual contributes to functions of an ecosystem
- Interactions occur between individuals

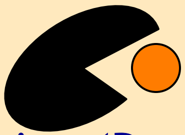
Mutualism
Both partners benefit



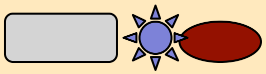
Synergism
Greater benefit from combining two



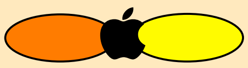
Commensalism
Only one partner benefits



Predation (Parasitism)
One organism consumes another



Ammensalism
Growth inhibited by toxin



Competition (Antagonism)
Competition for one nutrient

Bioactivators being sold

- Bacteria
- Fungi
- Mixtures of bacteria and/or fungi
- Humic/fulvic acids
- Seaweed extract

What could bacteria/fungi be providing?

- Plant growth hormones
- Phytochemicals (signals to plant)
- Antimicrobials
- Enzymes
  - Increase nutrient availability
  - Suppress pathogens

NON PLANT FOOD INGREDIENTS:

Microorganisms ....Bacteria................................ <1%

Genus species

Bacillus licheniformis ....$1x10^3$ cfu/ml*

Bacillus megaterium ....$1x10^3$ cfu/ml

Bacillus pumilus..........................$1x10^3$ cfu/ml

$1x10^3$ or 1,000 cfu /ml

*colony forming units/milliliter

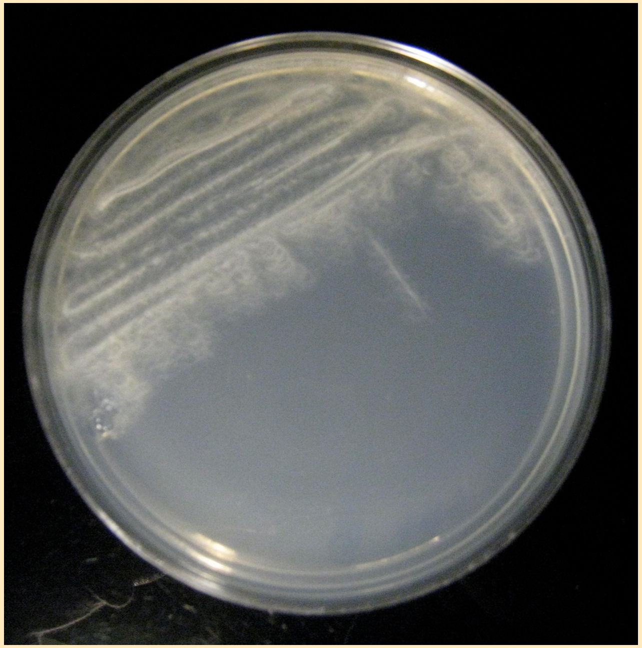
Inert Ingredients:

Water-based culture medium.......................... 99%

For use as a microbial soil amendment for improving the conversion of organic and inorganic fertilizers into plant-available forms.

Bacillus species

- Root colonizing free-living bacteria
- Produce GA and IAA that stimulate plant rooting and growth
- Solubilize phosphorus compounds

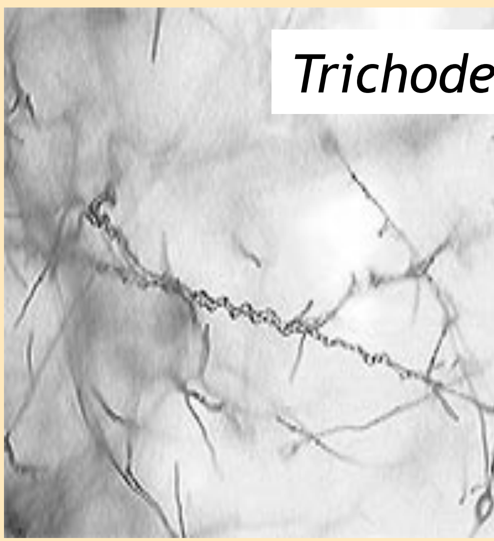


Bacillus grown in the lab
Possible P solubilizer

Microbial seed inoculant for improving nutrient availability for increased yield potential

MINIMUM GUARANTEED ANALYSIS

ACTIVE:	*Bacillus amyloliquefaciens TJ1000*	2.1×10^8 cfu/g
	Trichoderma virens GI-3	5.0×10^7 cfu/g
INERT:	talc-based carrier, 81.8%	



http://jgi.doe.gov/wp-content/uploads/2013/11/Trichodermacoiling.jpg

http://www.lagrotecnico.it/immagini/Bacillus_amyloliquefaciens.jpg

Trichoderma

- Free-living fungus that colonize the rhizosphere
- Common cellulose degrader
- Used to suppress plant diseases caused by fungi, bacteria, and viruses

SOIL DRENCH INOCULUM

AUXILARY SOIL AND PLANT SUBSTANCE
NON-PLANT FOOD INGREDIENT
CONTAINS NON-PLANT FOOD INGREDIENT.

ACTIVE INGREDIENTS:

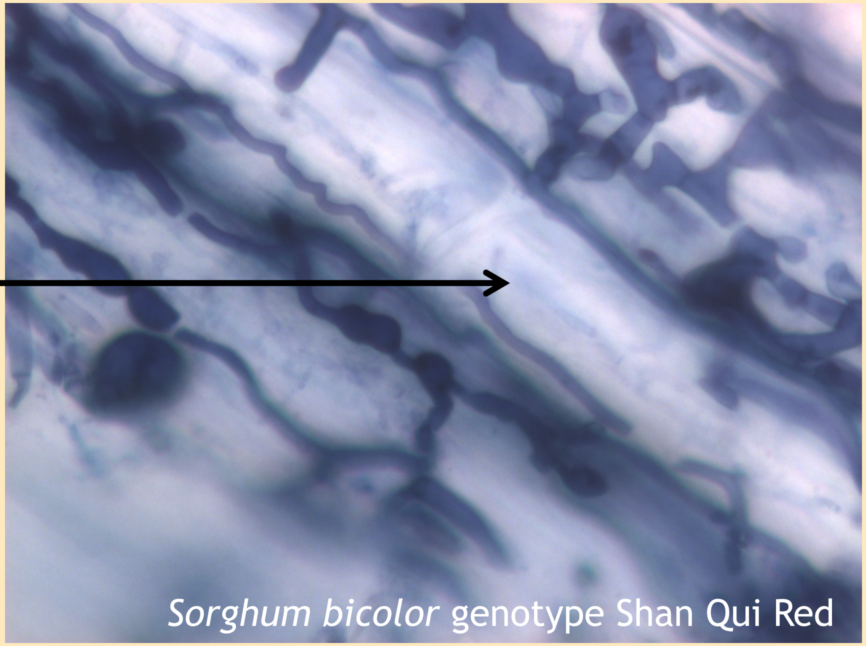
Contains eight (8) species of mycorrhizal fungi:

Glomus intraradices .... minimum 17 propagules/cm^3
Glomus mosseae .... minimum 17 propagules/cm^3
Glomus aggregatum .... minimum 17 propagules/cm^3
Pisolithus tinctorius .... minimum 3.2 million propagules/cm^3
Rhizopogon villosulus .... minimum 80,000 propagules/cm^3
Rhizopogon luteolus .... minimum 80,000 propagules/cm^3
Rhizopogon amylopogon .... minimum 80,000 propagules/cm^3
Rhizopogon fulvigleba .... minimum 80,000 propagules/cm^3

35.0% .... Kelp Extract (ascophulum nodosum, microbe food)
35.0% .... Humic Acids (Derived from leonardite.)

Mycorrhizae

- Fungi that forms a symbiotic relationship with plants
- Arbuscular mycorrhizal fungi enters root cells
- Ectomycorrhizal fungi external to root (mainly associated with trees)



(Stained with trypan blue 40X magnification)

Benefits of mycorrhizae

- Enhanced nutrient uptake (phosphorus & micronutrients)
- Disease, drought and salinity resistance
- Resistance to high metal concentrations

A Plant Growth Regulator For Crops

KEEP OUT OF REACH OF CHILDREN

CAUTION

PRECAUTIONARY STATEMENTS

ENVIRONMENTAL HAZARDS

Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwater or rinsate.

PHYSICAL OR CHEMICAL HAZARDS

Not For Use, Sale or Resale in California

ACTIVE INGREDIENT:

Cytokinin, as Kinetin ......0.5% w/w

OTHER INGREDIENTS: ..99.5% w/w

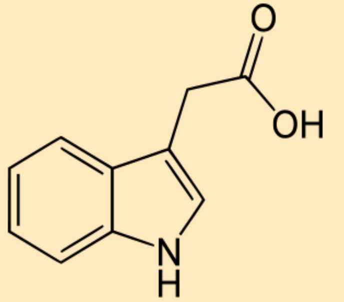
TOTAL 100.0% w/w

Growth regulators or hormones

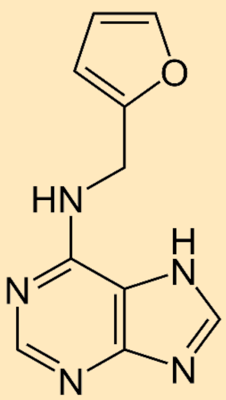
- Signal molecules occurring in plants in low concentrations
- Responsible for directing all aspects of plant development
- Also found in fungi and bacteria (but role unknown) secondary metabolites

Classes of phyto-hormones

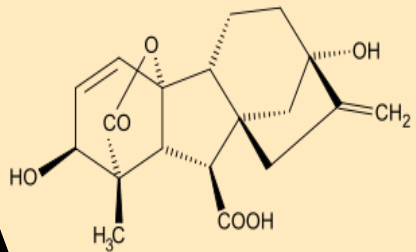
- Abscisic acid
- Auxins - IAA
- Cytokinins - kinetin
- Ethylene
- Gibberellins - GA



Indole-acetic acid *



Kinetin **



Gibberellic acid ***

*Public Domain, https://commons.wikimedia.org/w/index.php?curid=701798
**By Azulene at English Wikipedia - Public Domain, https://commons.wikimedia.org/w/index.php?curid=3257320
*** By Minutemen using BKchem 0.12 - Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=1906246

Field testing of products

- Collect as much information as you can before deciding to use a biologic
- eg., field test by Purdue researchers
  - Jim Camberato**
  - Bob Nielsen
  - Jason Lee (PhD student)

IOWA STATE UNIVERSITY
Agronomy Extension

http://extension.agron.iastate.edu/compendium/index.aspx

NCRA: NCERA-103 | Product List

Compendium of Research Reports on Use of Non-Traditional Materials for Crop Production

by NCR-103 Committee[1]
"Non-Traditional Soil Amendments and Growth Stimulants"

Change View A A A

Default
Print Version

Search: humic acid | Any Words | Search | Clear
Example: surfactant

Your search for **humic OR acid** Returned 77 Results.
Page: 1 of 8 Previous Page Next Page

1.) Stimulation of Plant Growth by Humic Substances
Soil Biology, Microbiology and Biochemistry. Stimulation of Plant Growth by Humic Substances1 YONG SEOK LEE AND RICHMOND J. BARTLEir2 ABSTRACT Humic substances prepared by different techniques of extraction and from different sources of organic materials were tested for their effects on growth of corn seedlings and algae. Stimulating effects were confirmed with optimum concentrations about 5 ppm C as Na-humate for corn and 60 ppm for algae. With corn, the increase was 30 to 50% in nutrient

2.) A Volatilization Study Of "Extend"
A Volatilization Study of "Extend" Results and Discussion: Study I was initiated on 27 February with a broadcast application of 500 lbs/A of 28% UAN solution (140 lbs N) with and without "Extend" onto bare soil in a greenhouse flat. The treated areas were covered with a collection system devised to entrap volatilized ammonia in a boric acid system, which was later titrated with a known strength acid according to Bremner (1965) A boric acid color change, of red to blue-green, was observed after 4

3.) Report of Laboratory Studies with a Product Called PROMESOL 30 that is Sometimes Promoted as "Liquid Lime"
Report of Laboratory Studies with a Product Called PROMESOL 30 that is Sometimes Promoted as "Liquid Lime" T.R. Peck Department of Agronomy University of Illinois In the fall of 1972 an Illinois Agribusinessman asked my assistance in a cursory evaluation of Promesol 30. This product had been represented to him as an alternative to agricultural lime for altering soil pH of an acid soil. Approximately an 8 oz. bottle was supplied to me along with a product specification sheet in Spanish (attached,

4.) Beneficial Effects of Humic Acid on Micronutrient Availability to Wheat
1744 SOIL SCI. SOC. AM. J. VOL. 65, NOVEMBER–DECEMBER 2001 D.C. Coleman, D.F. Bezdicek, and B.A. Stewart (ed. Defining Rhoades, J.D. 1982. Soluble salts. p. 167–179. In A.L. Page (ed. soil quality for a sustainable environment. SSSA Spec. Publ. no. Methods of soil analysis. Part 2. Chemical and microbiological 35. SSSA, Madison, WI. properties. 2nd ed. Chemical and microbiological properties. Marrs, R.H. R.D. Roberts, R.A. Skeffington, and A.D. Bradshaw. Agron. Monogr. no. 9. ASA and SSSA, Madis

5.) Oat Agronomic and Grain Quality Responses to Growth Regulators
Agronomy Journal. NOTES 443 be done to promote uniform germination will make color within cultivars more uniform. Another possibility would be to germinate seeds on blotters, transplant to sand, and place early germinating seeds in different rows from slow germinating seeds. In this way, variation in color due to emergence rate could be determined. OAT

This electronic compendium provides information on non-traditional materials marketed for use in crop production in the north central region of the USA. It is a collection of research abstracts and reports released by scientists in State Agricultural Experiment Stations. This electronic compendium contains material previously published in the compendium (1985) and supplements 1 and 2.

The NCR-103 Committee takes no responsibility for comments or conclusions expressed in any of the reports contained within this collection. The purpose of the Committee activity is to provide a single, more conveniently used file of references for researchers, extension personnel, crop advisers, and agency personnel who have interest or assigned responsibilities in the use of non-traditional materials. Inclusion of information on materials and/or expression of trade names does not represent approval or disapproval, implied or otherwise, for a product for use in crop production.

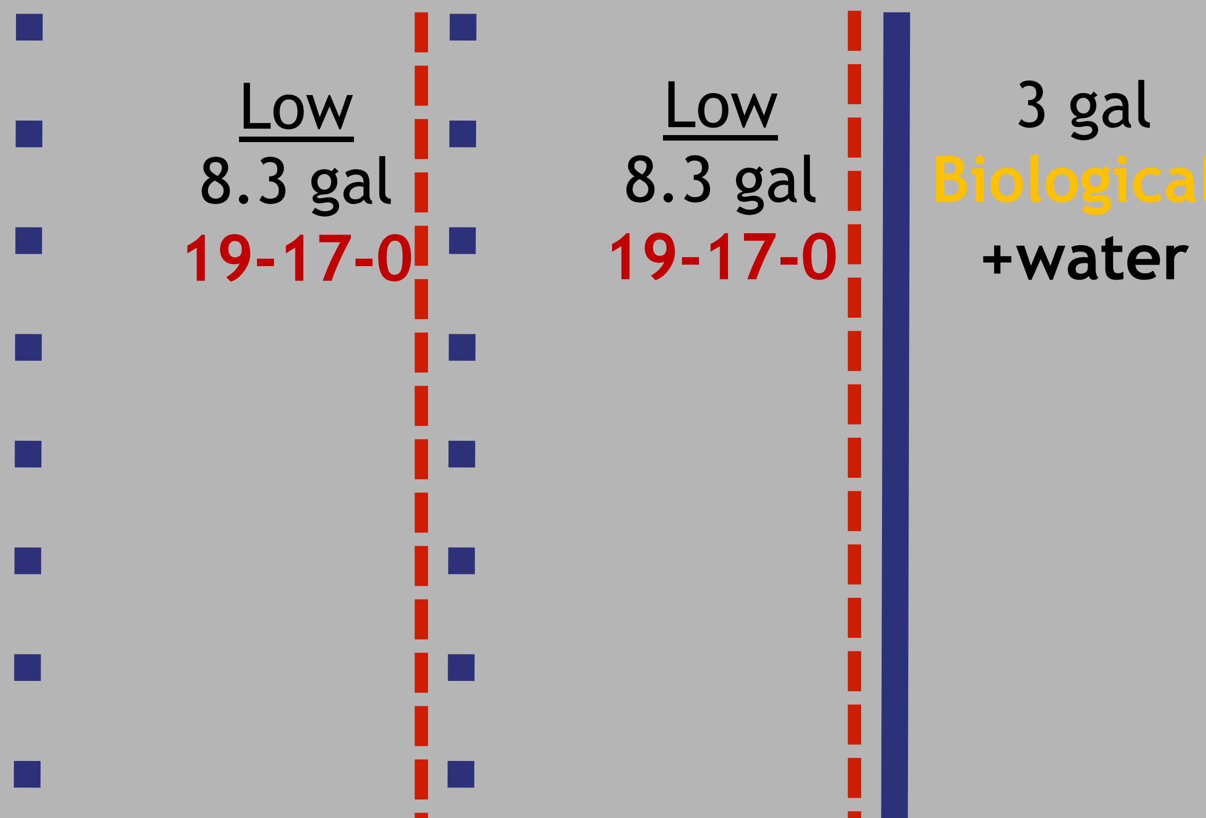
The individual reports have been placed into an electronic database that is searchable for any text.

Biological & 2x2 starter trials

- 5 locations (2016)
- 5 treatments
  - Control -no 2x2 or pop-up
  - Low 2x2 - 8.3 gal 19-17-0
  - 2x2 + Bacteria 1
  - 2x2 + Growth regulator
  - 2x2 + Bacteria 2
  - 2x2 + Fungi

(Lee et al. unpublished)

Starter fertilizer treatments



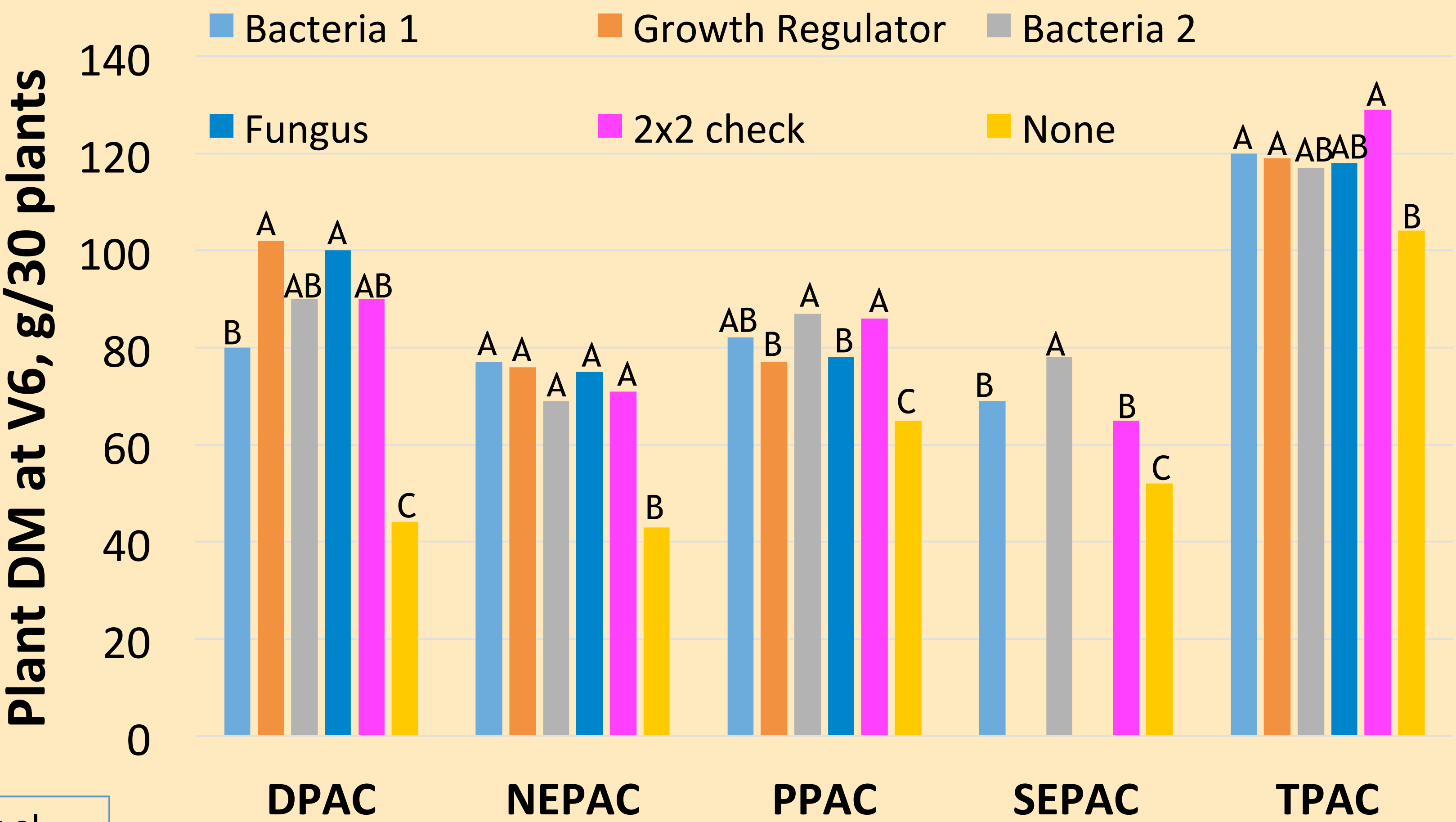
No 2x2 / no in-furrow

2x2 / no in-furrow

2x2 / biological in-furrow

(Lee et al. unpublished)

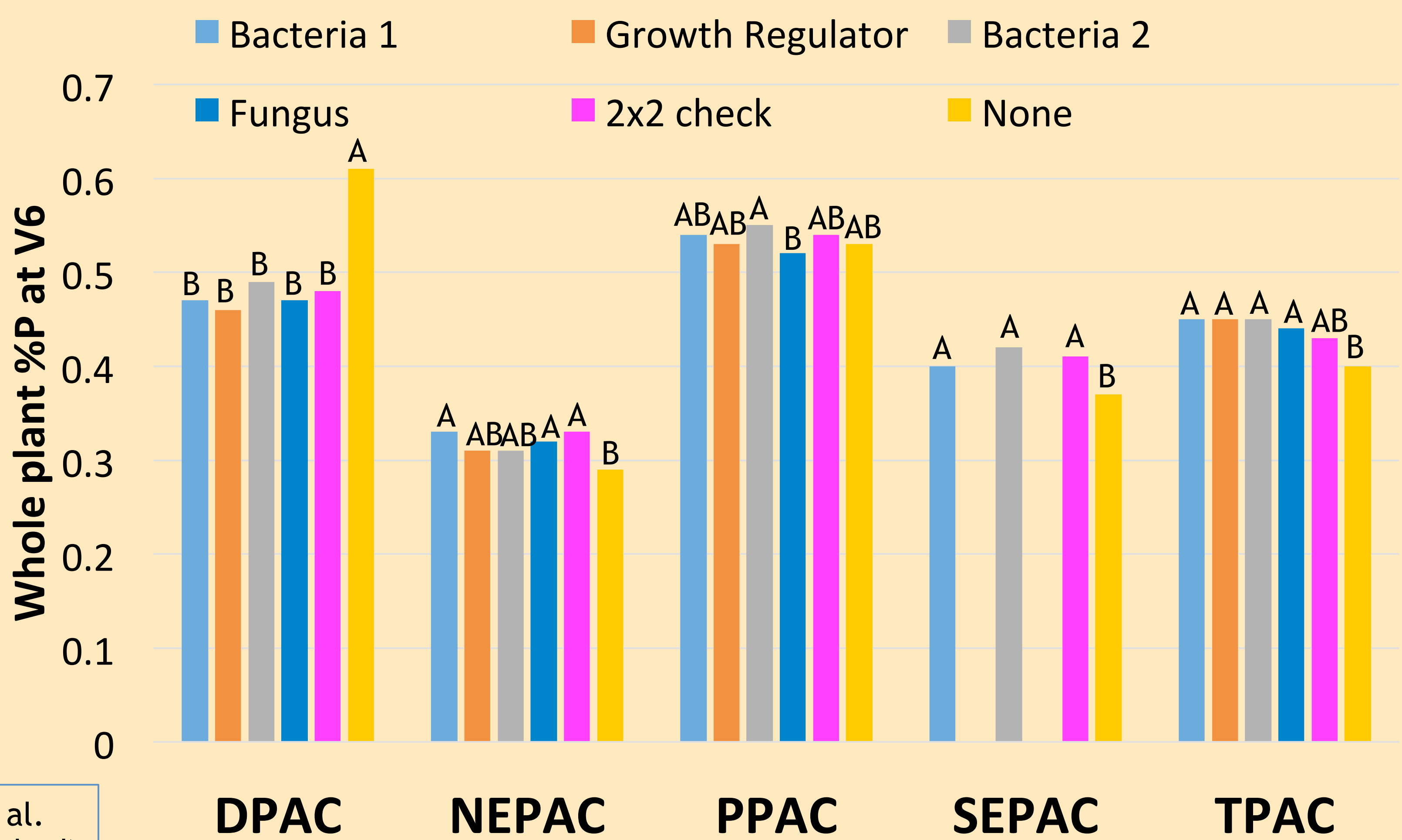
Plant dry weight was mostly unaffected by biologicals



(Lee et al. unpublished)

Different letters above bars indicate statistically significant differences

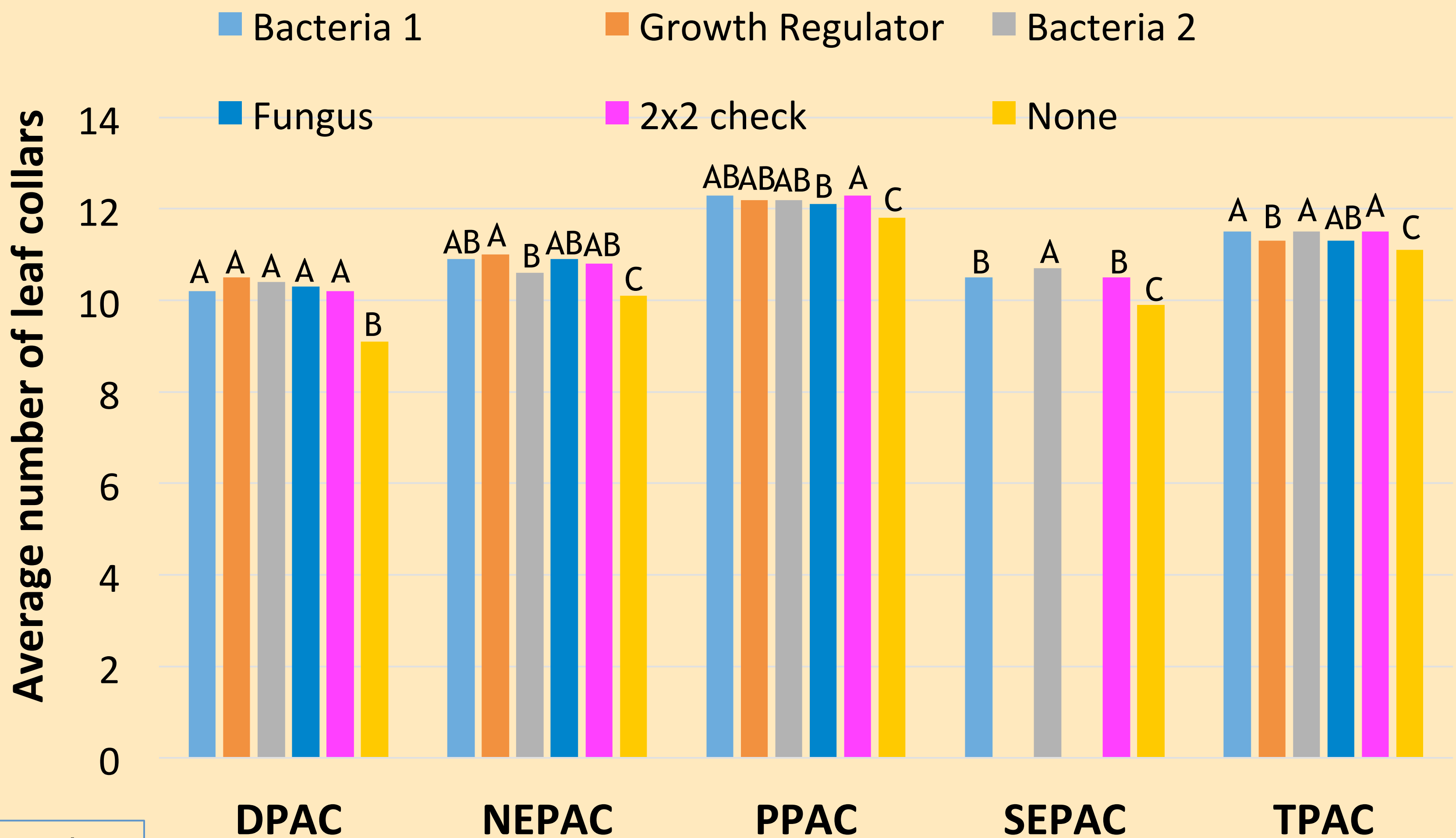
Whole plant %P at V6 was mostly unaffected by biologicals



(Lee et al. unpublished)

Different letters above bars indicate statistically significant differences

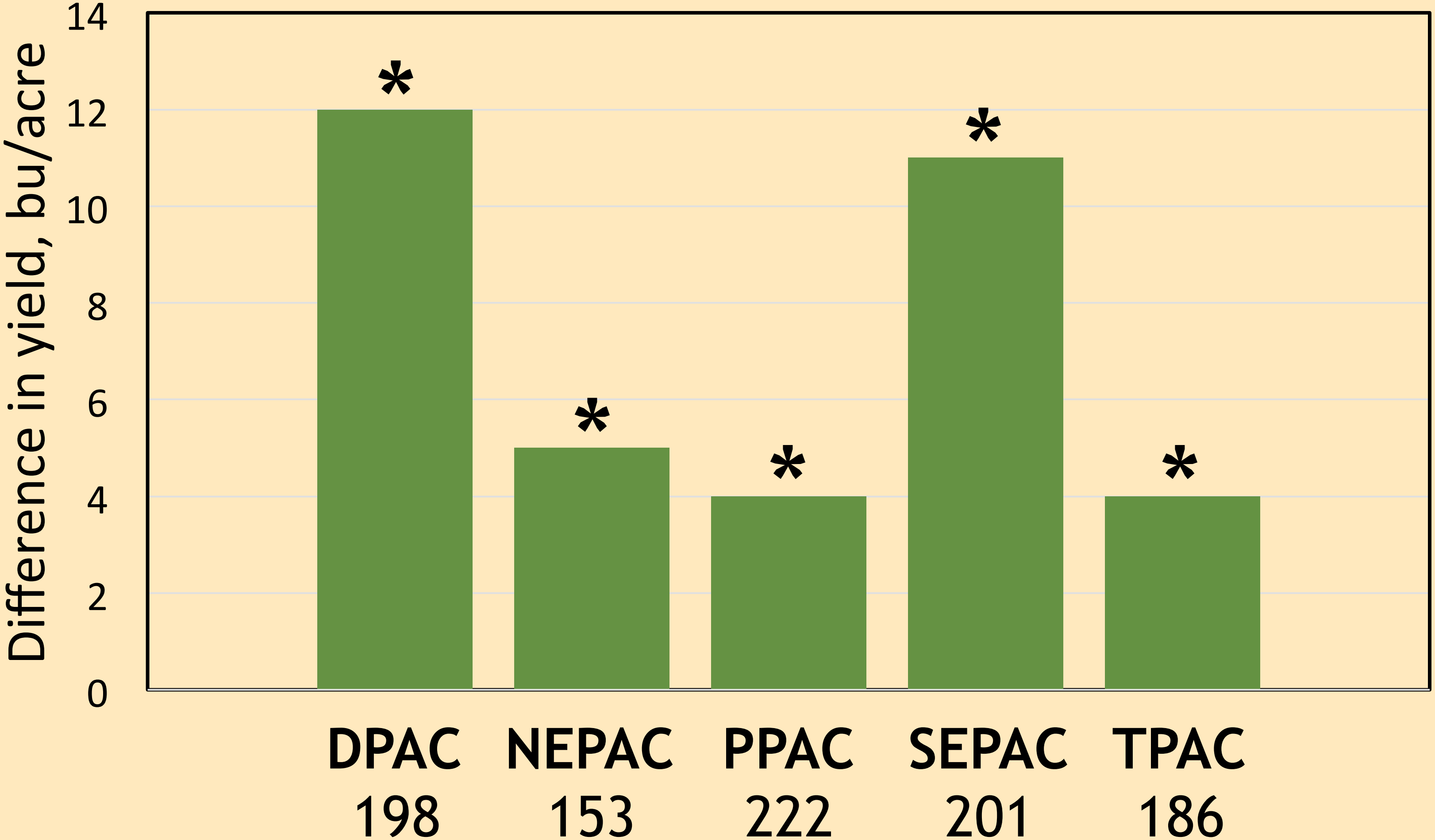
Rate of leaf appearance was mostly unaffected by biologicals



(Lee et al. unpublished)

Different letters above bars indicate statistically significant differences

2x2 *Starter* yielded more than *no starter*

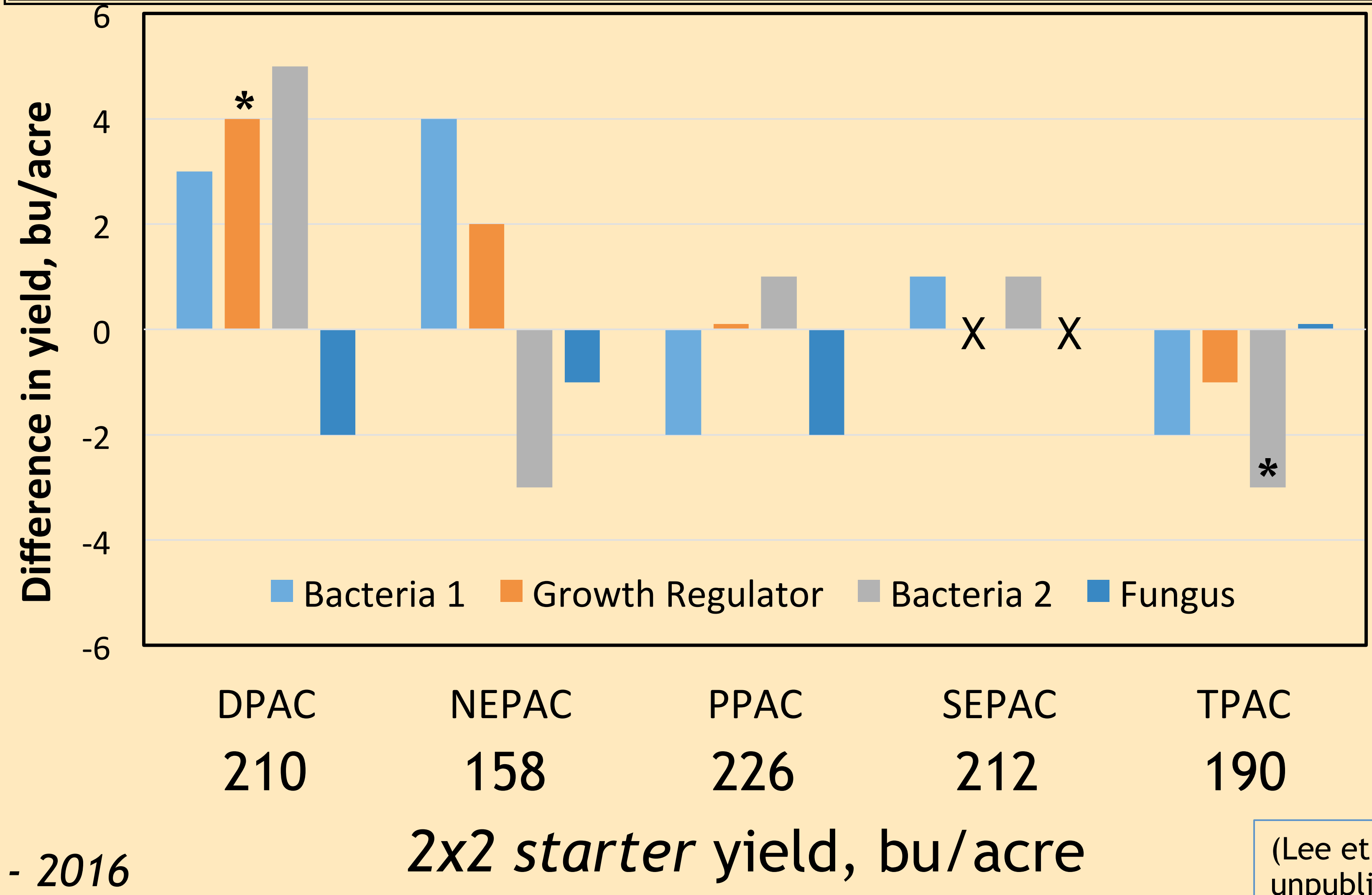


C/S - 2016

Relative to No Starter: yield, bu/acre

(Lee et al. unpublished)

In-furrow biologicals had small effect on yield



C/S - 2016

(Lee et al. unpublished)

On average in-furrow biologicals had no effect on yield

Bacteria 1	Growth regulator	Bacteria 2	Fungus
Yield difference due to biological, bu/acre			
+1	+1	0	-1

(Lee et al. unpublished)

Summary field test

- Commercial formulations of bacteria, growth regulator or fungus applied in-furrow with 2x2 starter fertilizer had **little to no effect on**
  - **Plant growth**
  - **Leaf appearance or**
  - **Yield**
- 2x2 starter fertilizer increased yield at 5 of 5 locations an average of 7 bu/acre

(Lee et al. unpublished)

Soil health test

Two commercial tests available

- Haney test
  - Soil extracts chemically analyzed for
    - N (total, organic, water soluble)
    - C (organic, water soluble)
    - P (organic, inorganic)
    - Minerals (Al, Fe, Ca & K)
  - Microbial activity using Solvita
    - CO_2 release from soils after drying and re-wetting

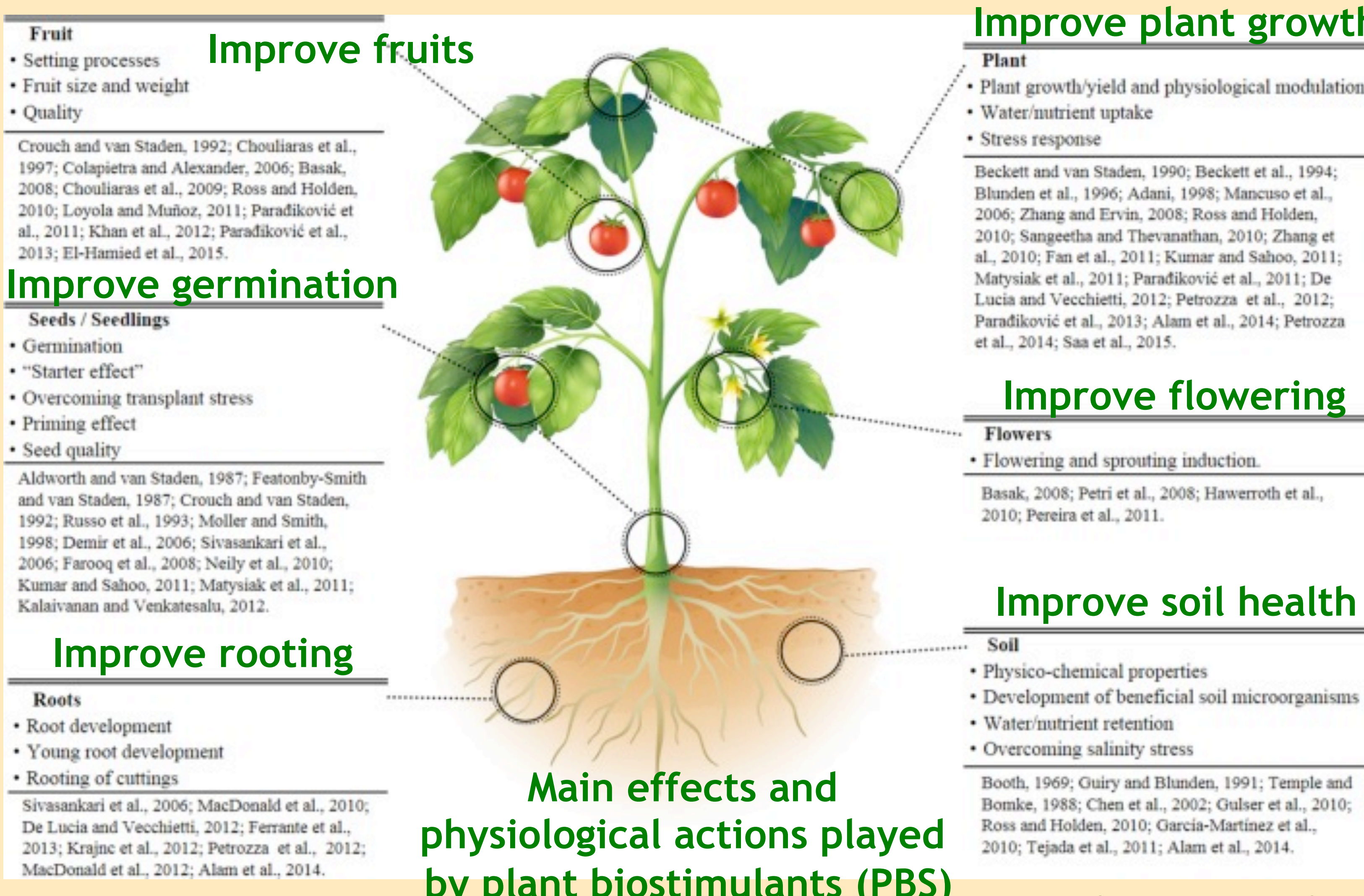
Soil health test

- Cornell test (comprehensive test)
  - Biological
    - Microbial activity, CO_2 respiration (e.g. Solvita)
    - Active C, available food source for microbes
    - Root health (optional)
  - Physical
    - Soil texture, aggregate stability, water capacity, surface/subsurface hardness
  - Chemical
    - Organic matter, mineralization N, soil protein
    - pH, nutrients, toxic elements (heavy metals)

Summary: What we know

- Microbial inoculants have been used successfully for over 100 years in agriculture
- Studies have demonstrated benefits to use of some biostimulants

Summary: Possible Outcomes



Povero et al. 2016. Frontiers Plant Sci 7:435

Summary: What we don’t know

- Why biostimulators seem to work in some locations and not others
- Contribution of different soil types with different indigenous communities
- Underlying mechanisms of some products
- Product quality: Formulations do not have oversight by any government agency

Questions