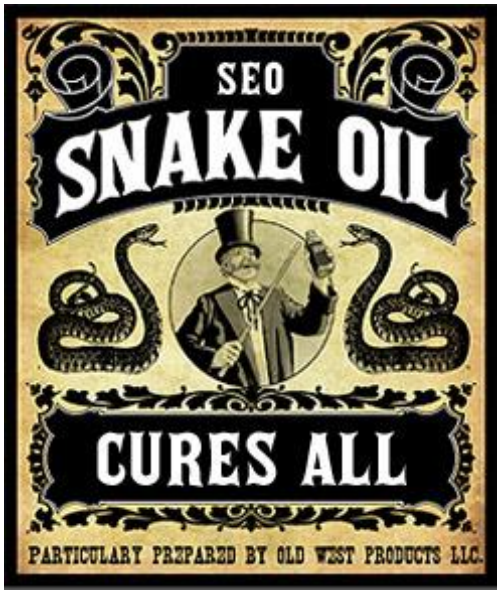
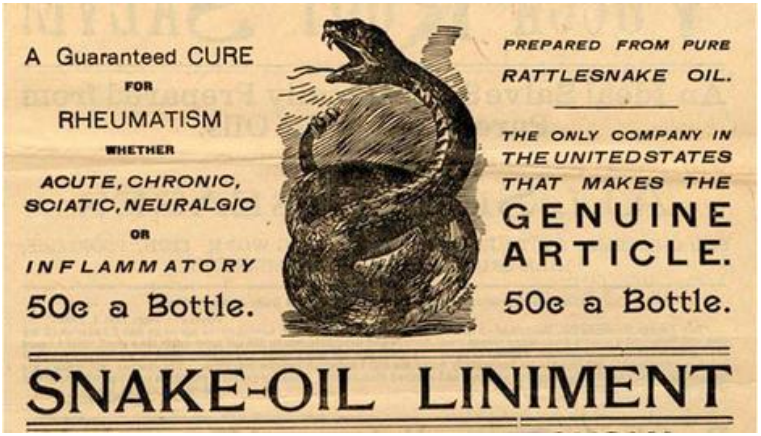
Biostimulants, Plant Stress and the Microbiome

Cal ASA, Feb 2017

Patrick Brown
University of California, Davis

Snake Oils and Jungle Juices





Biostimulants

($2bn industry)

EBIC

European Biostimulants Industry Council

EBIC Definition of Biostimulant–

"A material that, when applied to a plant, seed, soil or growing media - in conjunction with established fertilization plans, enhances the plant's nutrient use efficiency, *or* provides other direct or indirect benefits to plant development or stress response."

North American Definition of Biostimulant–

"They are derived from natural or biological sources.."

Joe Kloepper

frontiers in Plant Science | Crop Science and Horticulture

< Archive

January 26th, 2017

REVIEW ARTICLE

Front. Plant Sci., 26 January 2017 | https://doi.org/10.3389/fpls.2016.02049

Biostimulants in Plant Science: A Global Perspective

Oleg I. Yakhin[1,2*], Aleksandr A. Lubyanov[2], Ildus A. Yakhin[2] and Patrick H. Brown[3]

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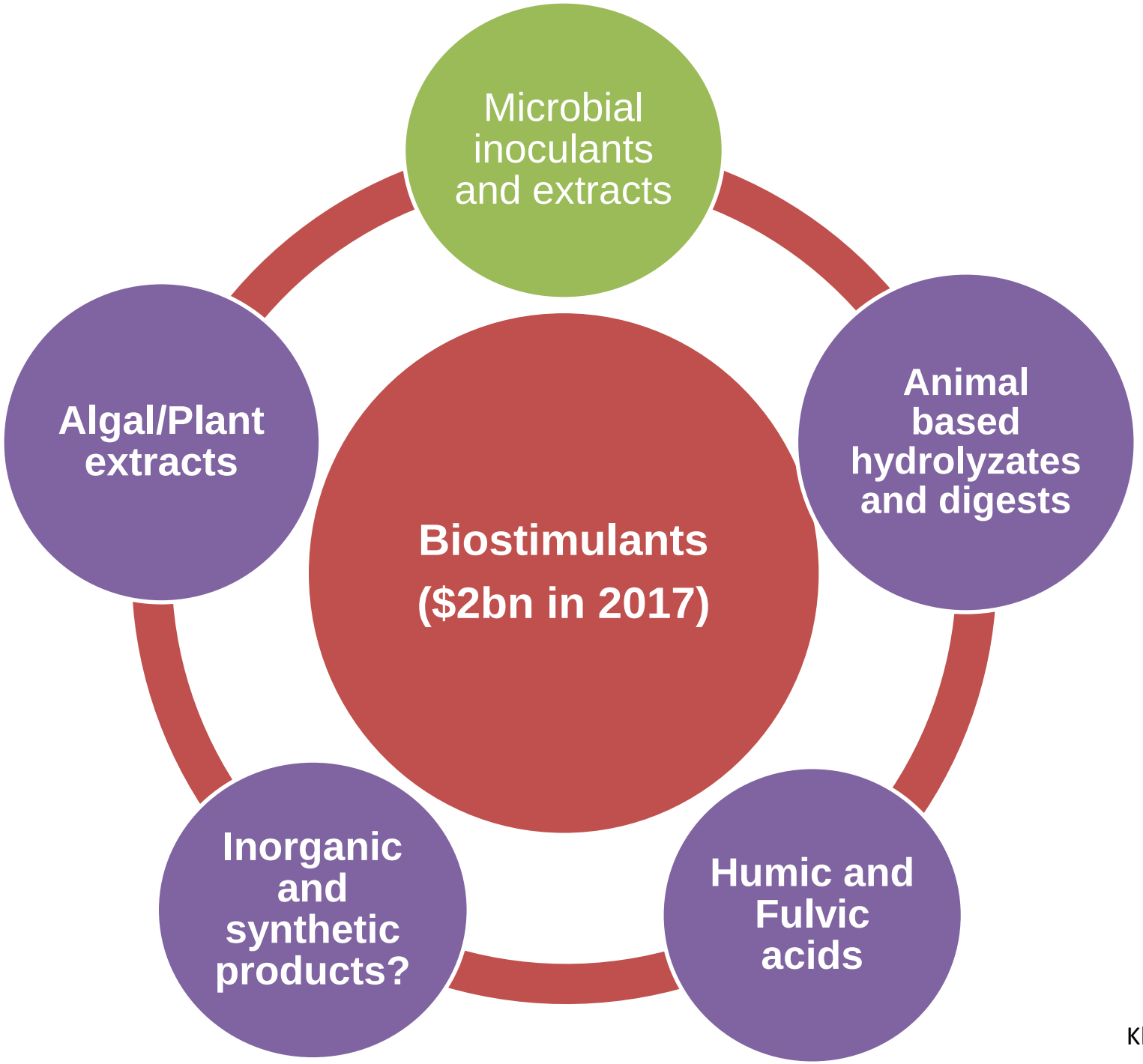
[2]R&D Company Eco Priroda, Ulkundy, Russia
[3]Department of Plant Sciences, University of California, Davis, Davis, CA, USA

‘a formulated product of biological origin that improves plant productivity as a consequence of the novel or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds’.


Microbial inoculants and extracts
Animal based hydrolyzates and digests
Humic and Fulvic acids
Inorganic and synthetic products?
Algal/Plant extracts
Biostimulants
($2bn in 2017)

Kloepper

Outline

- Physiological Rationale for Biostimulants
  - Yield Gaps and Challenges to Optimizing Productivity
- New Science New Ideas
  - Biostimulants
  - The Microbiome
  - Priming and Resilience

Physiological Rationale for Biostimulants

Stress Hypothesis

- Abiotic ‘stress’ occurs in all environments and as a consequence yield rarely reaches full potential.
  - Biostimulants enable plants to respond more effectively to stress
  - Biostimulants ‘short-circuit’ normal molecular constraints to productivity

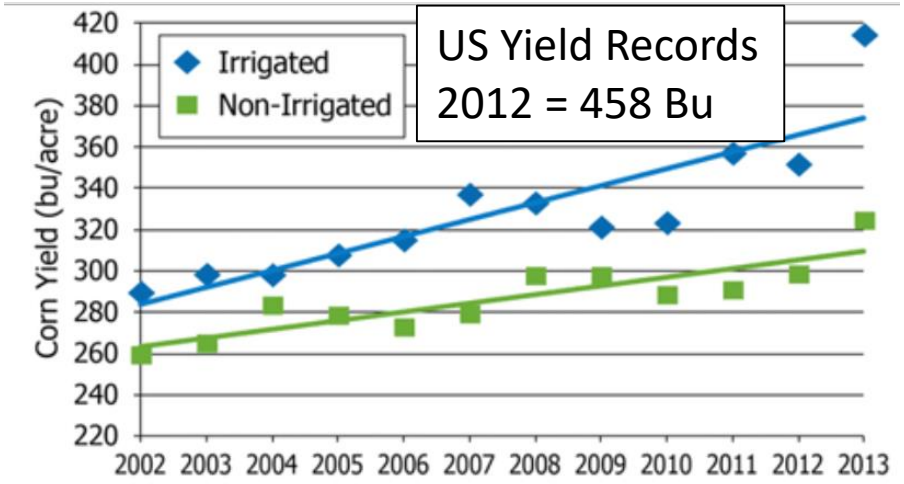
Microbiome Hypothesis

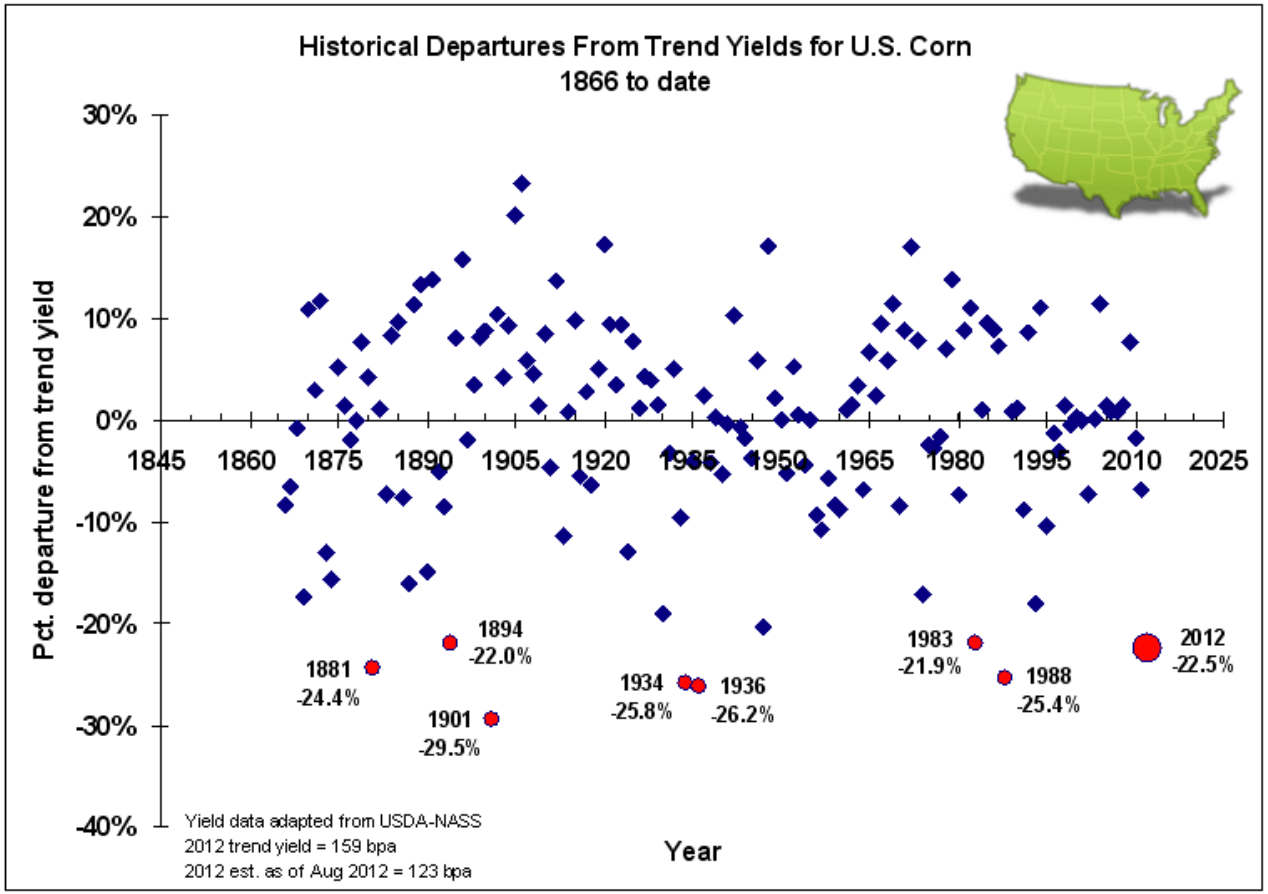
- Microbes in the environment have beneficial effects on crop growth
  - Biostimulants can favorably alter the plant microbiome
  - Biostimulants can ‘mimic’ the beneficial effects of microbial metabolites.

Brown and Saa, 2015 FIPS

Crops Rarely Achieve Maximal Yield Potential

US Corn Yields

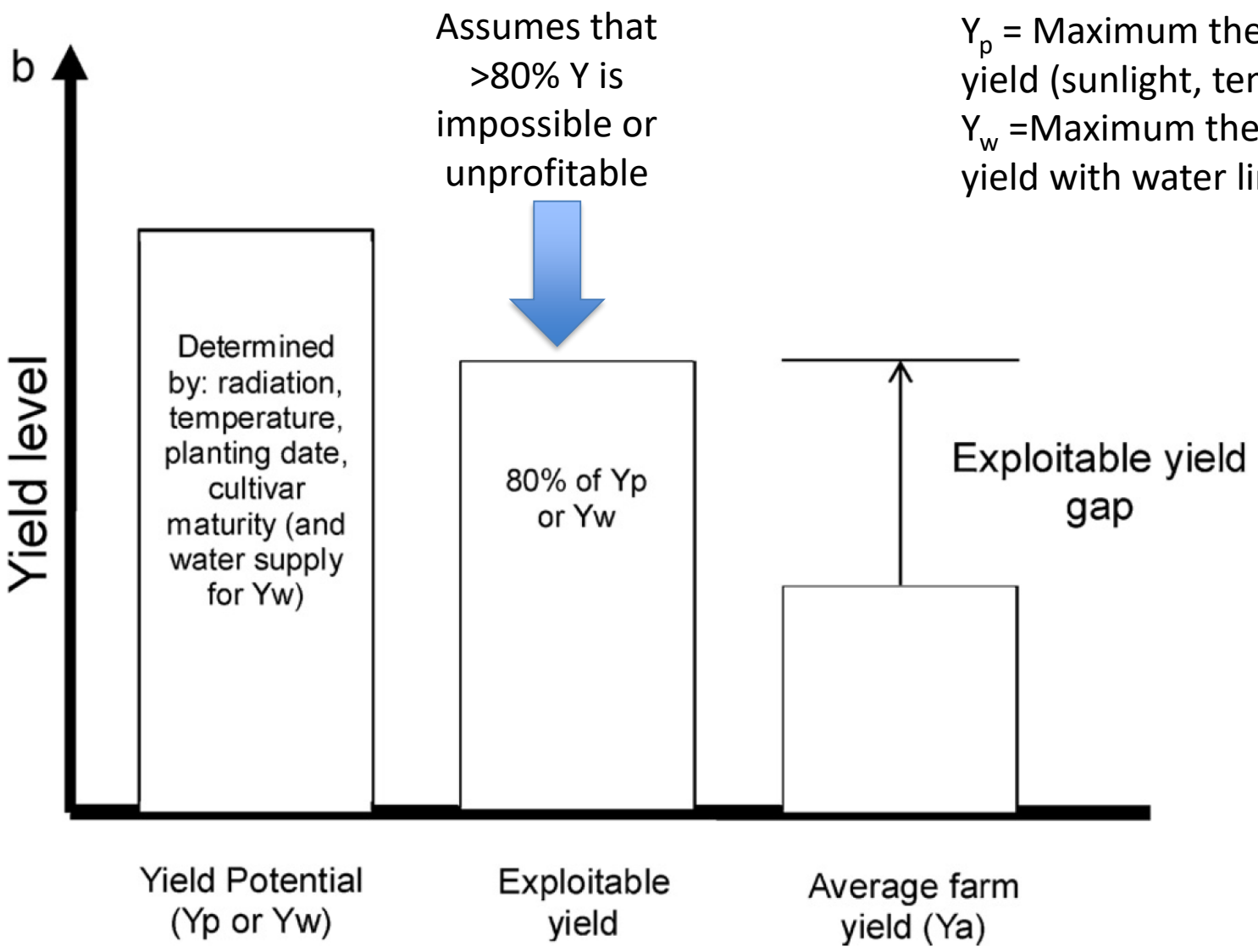




Difference between US expected average yield based on historical trends and actual yields.

2012 expected average US yield was 158 Bushels.

Yield Gap Analysis



M.K. van Ittersum et al. / Field Crops Research 143 (2013) 4–17

MAIZE GROWTH STAGES

Flowering and Grain Fill

Disease and stalk borer control important

Critical moisture requirement

Heat, Drought, Wind, N-P-K-Ca-Zn-B

Rapid Vegetative

Germination and Emergence

Weed control

Cold, Waterlogging, Drought, N-P-K Zn-Mn

Hidden stresses occur in the growth of all crops and this reduces yield.

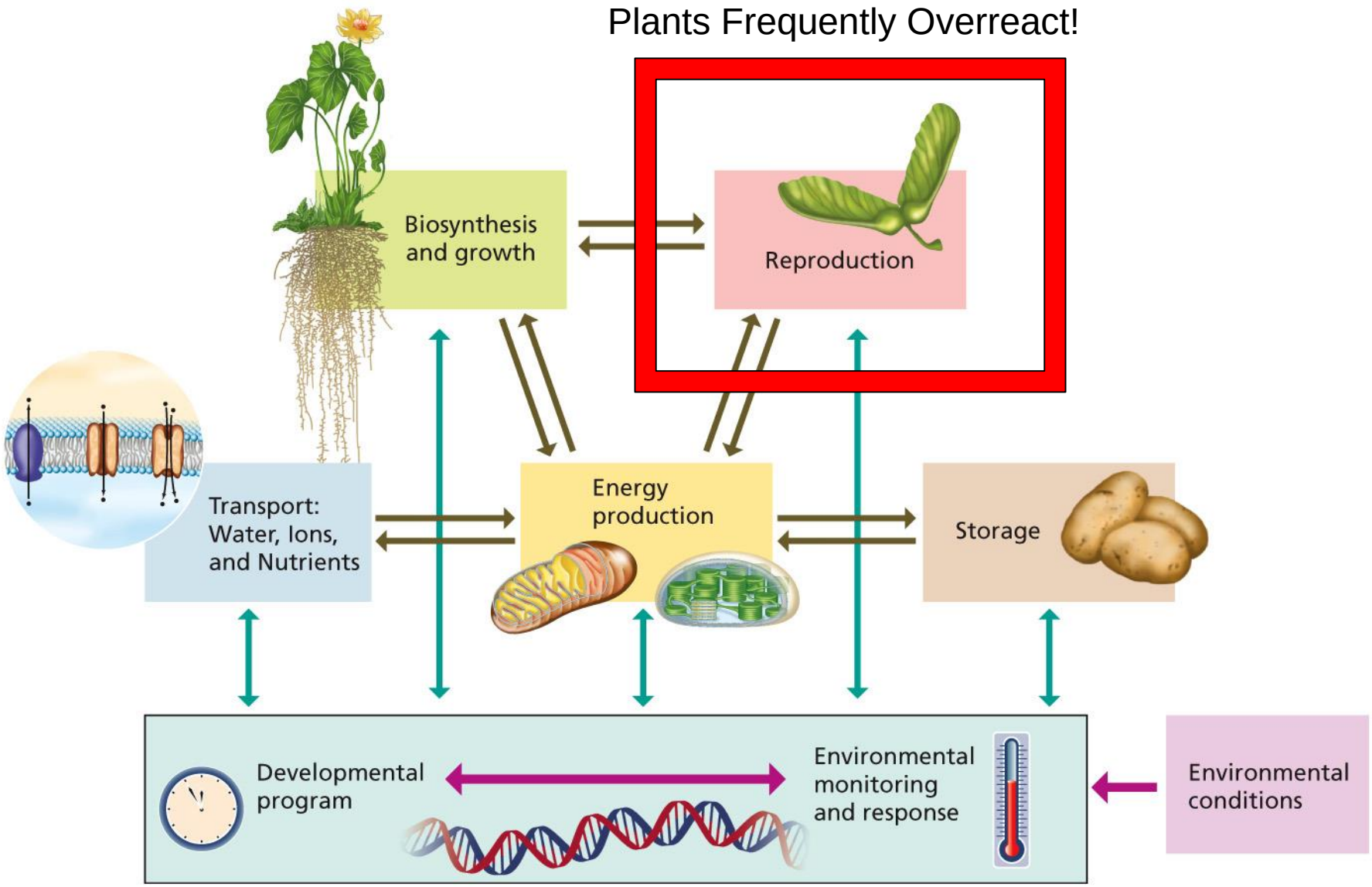
Questions:

- What are these stresses and how do plants react?
- Can we identify, prevent or mitigate these stresses?

V0	V2	V5	V8	V10	V12	V14	VT	R1	R2 - R3	R3 - R4	R5 - R6
Days after Emergence*											
0	7	21	32	38	44	49	56	63	70 77 84	91 98 105	112 119	160
Germination and emergence. Planting depth 5-8 cm.	Plant population established. Growth point 20-25 mm below surface. 5 Leaf – cob and tassel initiation.	Cob development.	Active growth – leaves and cob development. Brace root development. 12 Leaf – cob size determined.	Pollination. 5-10 Days.	Kernel development.	Grain filling. Nutrients are transported to cob.	Physiological maturity. End of mass gain.	Ready for harvest. 14% Moisture.			
Vegetative Growth Stages							Reproductive Development Stages				

* The number of days varies between different growth classes and environments.

Plants constantly attempt to balance growth with the need for survival. The perception of stress results in an immediate reallocation of energy to ‘defensive’ strategies that can compromise yield.



***PLANT PHYSIOLOGY AND DEVELOPMENT 6e*, Figure 24.1**

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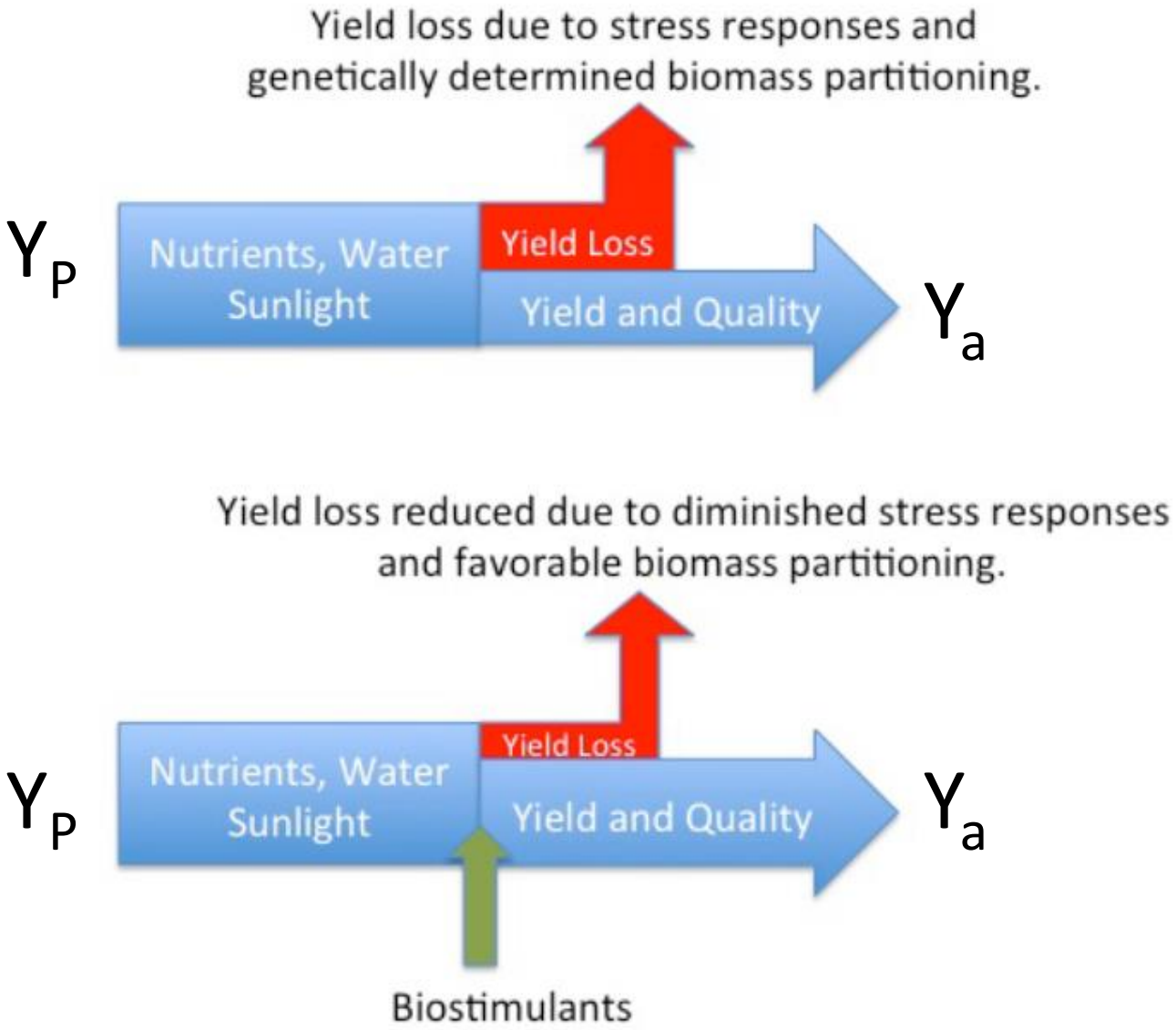

Brown and Saa, 2015 FIPS

Table 24.1 Physiological and biochemical perturbations in plants caused by fluctuations in the abiotic environment

Stress can vary dramatically either temporally, spatially or by intensity

TABLE 24.1 Physiological and biochemical perturbations in plants caused by fluctuations in the abiotic environment

Environmental factor	Primary effects	Secondary effects
Water deficit	Water potential (Ψ) reduction Cell dehydration Hydraulic resistance	Reduced cell/leaf expansion Reduced cellular and metabolic activities Stomatal closure Photosynthetic inhibition Leaf abscission Altered carbon partitioning Cytorrhysis Cavitation Membrane and protein destabilization ROS production Ion cytotoxicity Cell death
Salinity	Water potential (Ψ) reduction Cell dehydration Ion cytotoxicity	Same as for water deficit (see above)
Flooding and soil compaction	Hypoxia Anoxia	Reduced respiration Fermentative metabolism Inadequate ATP production Production of toxins by anaerobic microbes ROS production Stomatal closure
High temperature	Membrane and protein destabilization	Photosynthetic and respiratory inhibition ROS production Cell death
Chilling	Membrane destabilization	Membrane dysfunction
Freezing	Water potential (Ψ) reduction Cell dehydration Symplastic ice crystal formation	Same as for water deficit (see above) Physical destruction
Trace element toxicity	Disturbed cofactor binding to proteins and DNA ROS production	Disruption of metabolism
High light intensity	Photoinhibition ROS production	Inhibition of PSII repair Reduced CO_2 fixation

***PLANT PHYSIOLOGY AND DEVELOPMENT 6e*, Table 24.1**

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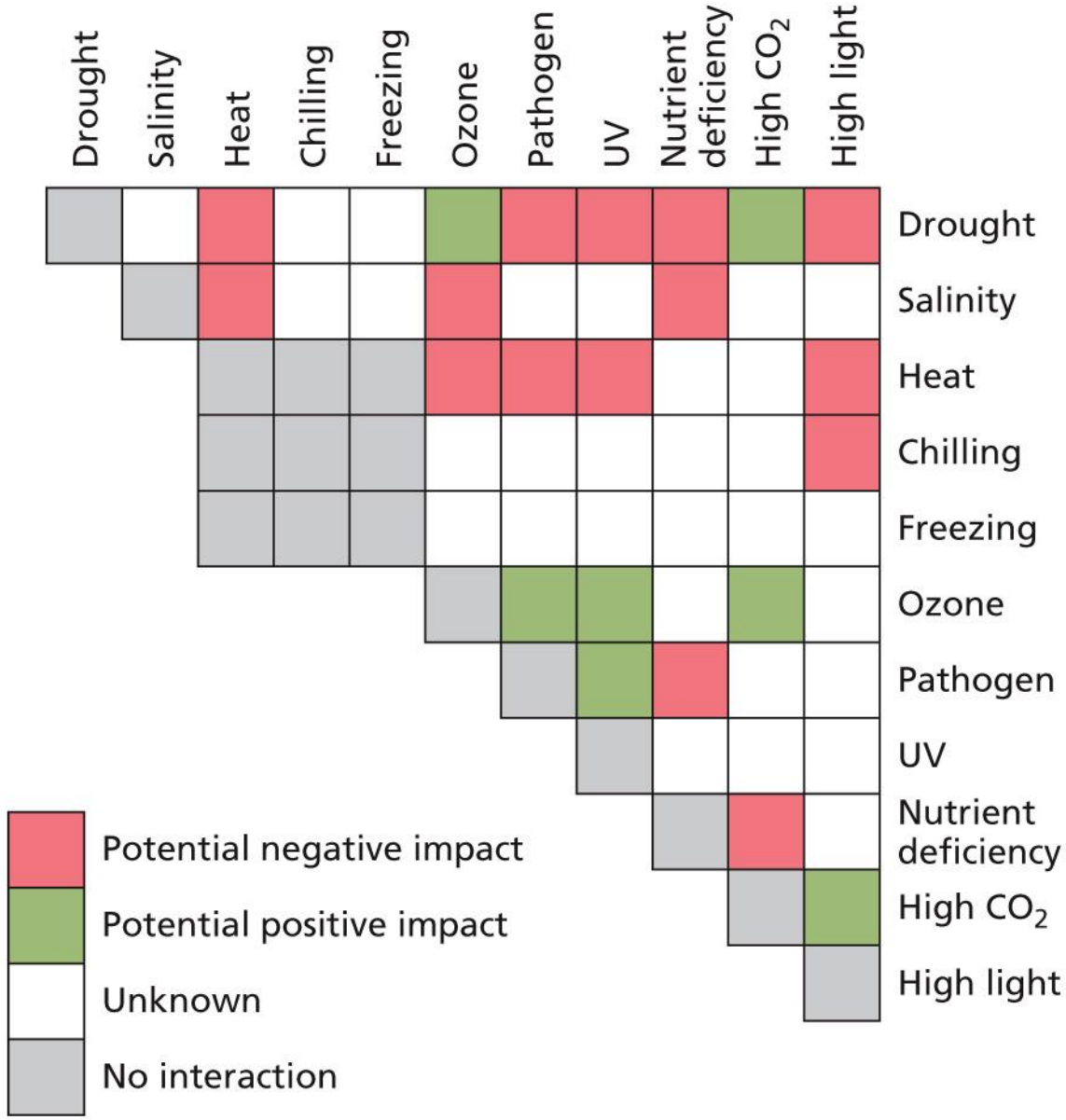
Figure 24.7 Stress matrix



***PLANT PHYSIOLOGY AND DEVELOPMENT 6e*, Figure 24.7**

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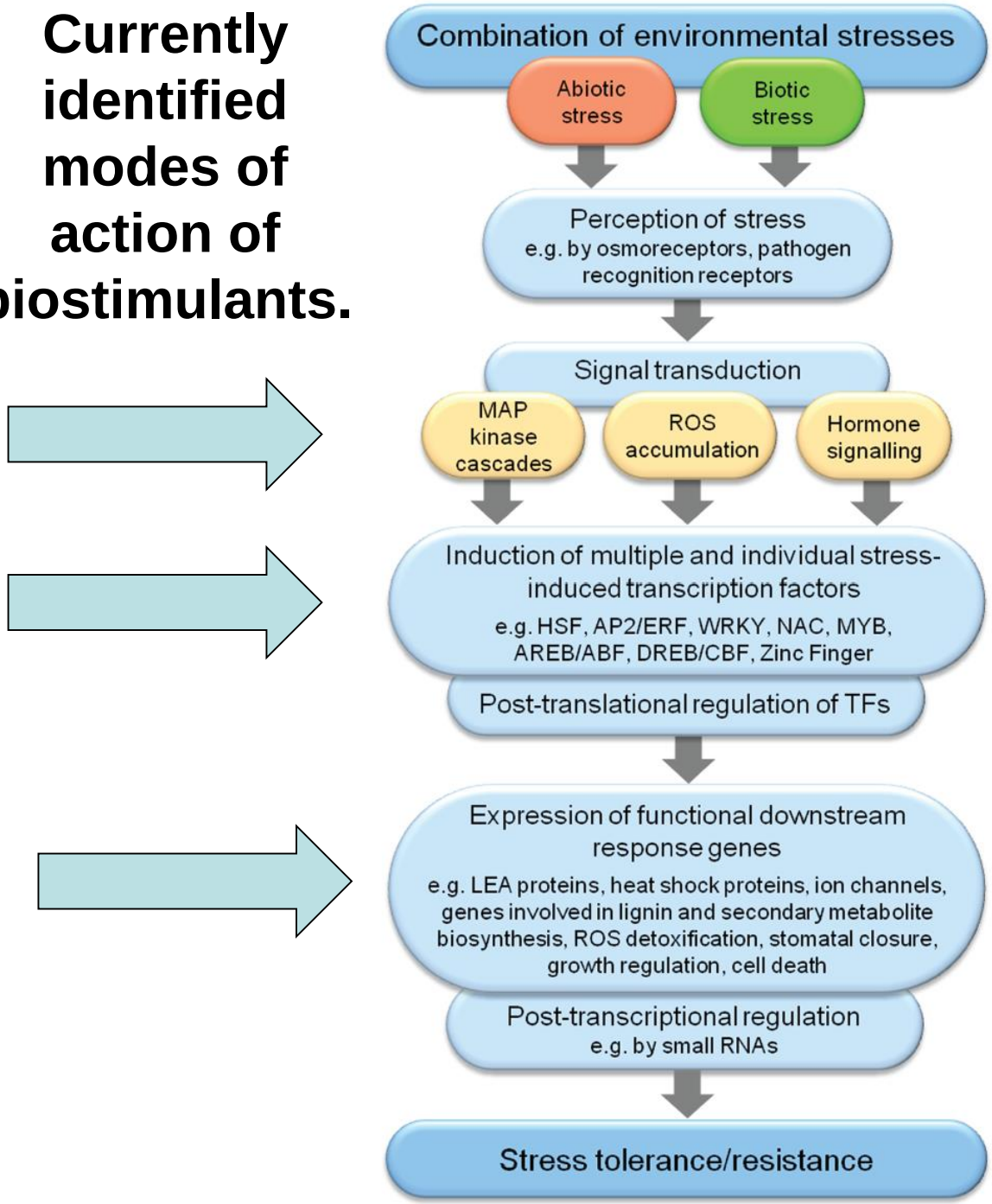
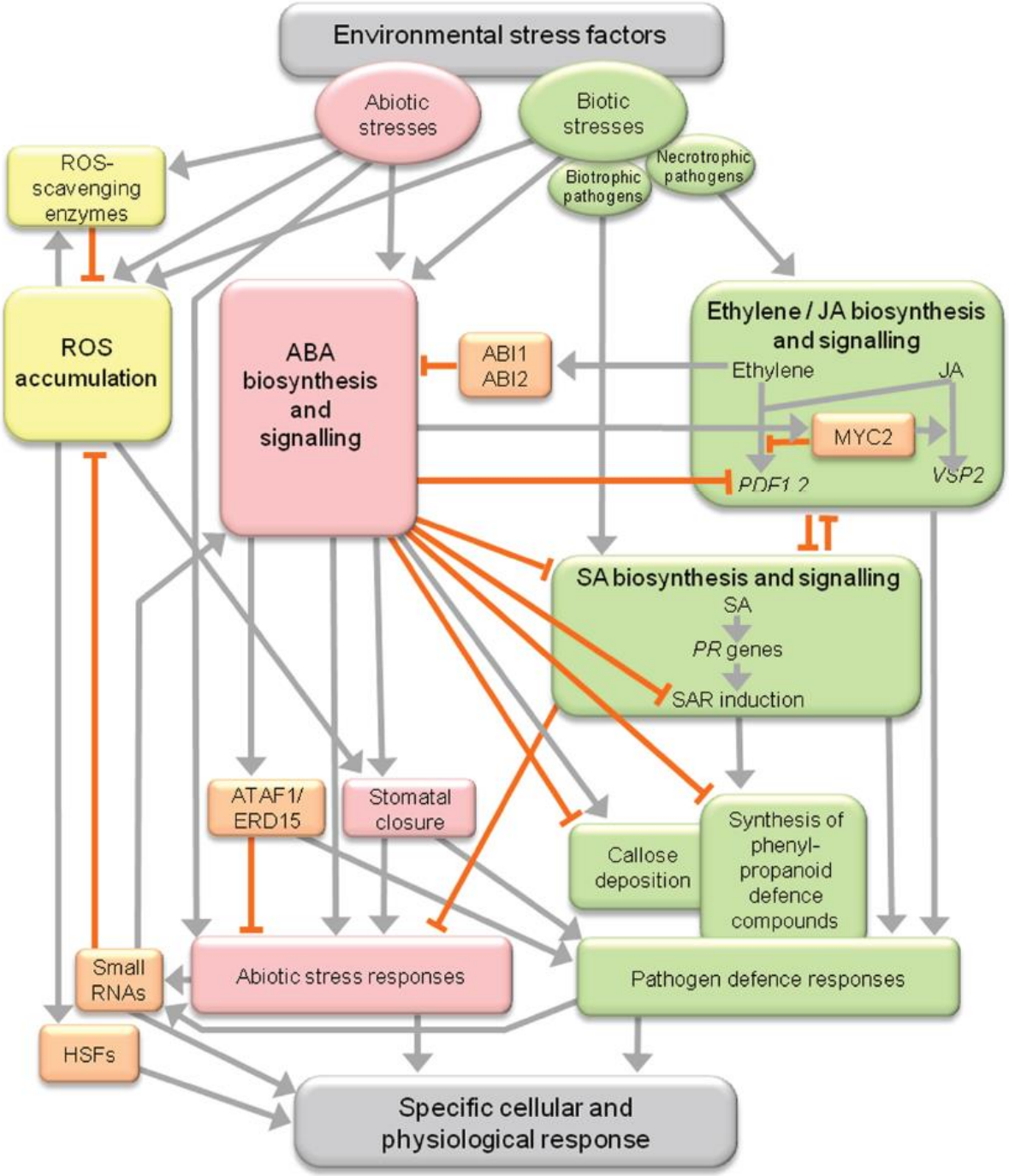
Currently identified modes of action of biostimulants.



Is it possible for a biostimulant to positively influence plant response to multiple stresses?



THE 2ND WORLD CONGRESS ON THE USE OF BIOSTIMULANTS IN AGRICULTURE
Monday 16th - Thursday19th November, 2015
Florence Convention Centre, Italy
ORGANISED BY NEWAG INTERNATIONAL

Patrick Brown
Co-Chairman of the Congress Scientific Committee

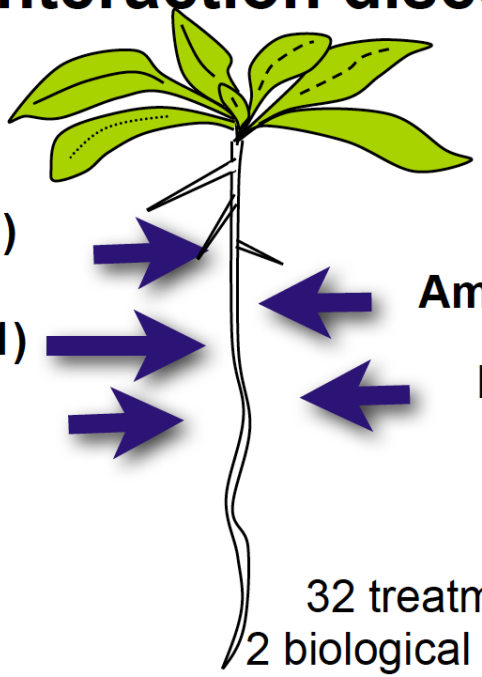
University of California, Davis
USA

How plant respond to a combination of signals:

Are biostimulant effects triggered by a cocktail of molecules?

Gabriel Krouk
2nd world congress on Biostimulants
Florence 2015

Experimental Design. Combinatorial Treatments lead to Signal interaction discovery.



Auxin (0/1)

Cytokinin (0/1)

ABA (0/1)

Ammonium (0/1)

Nitrate (0/1)

32 treatments x
2 biological replicates

Treat #	NO3 (1mM)	NH4+ (1mM)	Auxin 500 nm	Cytok 500nm	ABA 1µM	KCl	DMSO	Genotype
1	0	0	0	0	0	2	3	Col
2	0	0	0	0	1	2	2	Col
3	0	0	0	1	0	2	2	Col
4	0	0	0	1	1	2	1	Col
5	0	0	1	0	0	2	2	Col
6	0	0	1	0	1	2	1	Col
7	0	0	1	1	0	2	1	Col
8	0	0	1	1	1	2	0	Col
9	0	1	0	0	0	1	3	Col
10	0	1	0	0	1	1	2	Col
11	0	1	0	1	0	1	2	Col
12	0	1	0	1	1	1	1	Col
13	0	1	1	0	0	1	2	Col
14	0	1	1	0	1	1	1	Col
15	0	1	1	1	0	1	1	Col
16	0	1	1	1	1	1	0	Col
17	1	0	0	0	0	1	3	Col
18	1	0	0	0	1	1	2	Col
19	1	0	0	1	0	1	2	Col
20	1	0	0	1	1	1	1	Col
21	1	0	1	0	0	1	2	Col
22	1	0	1	0	1	1	1	Col
23	1	0	1	1	0	1	1	Col
24	1	0	1	1	1	1	0	Col
25	0.5	0.5	0	0	0	1	3	Col
26	0.5	0.5	0	0	1	1	2	Col
27	0.5	0.5	0	1	0	1	2	Col
28	0.5	0.5	0	1	1	1	1	Col
29	0.5	0.5	1	0	0	1	2	Col
30	0.5	0.5	1	0	1	1	1	Col
31	0.5	0.5	1	1	0	1	1	Col
32	0.5	0.5	1	1	1	1	0	Col

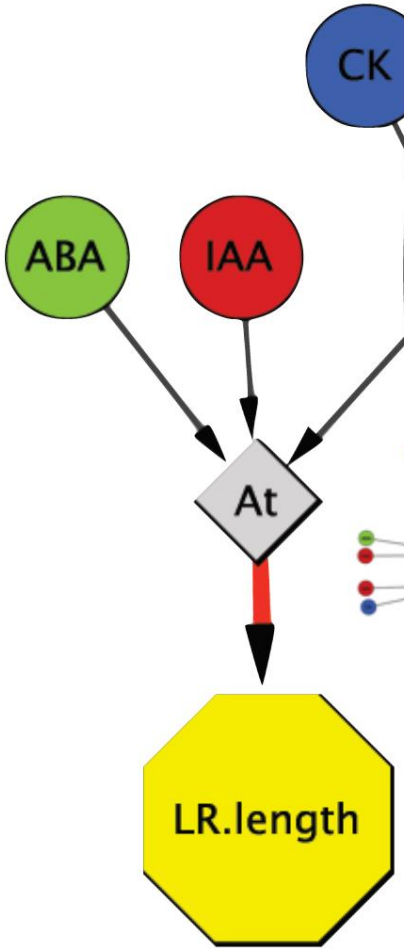
Root trait measurements w/ Daniela RISTOVA

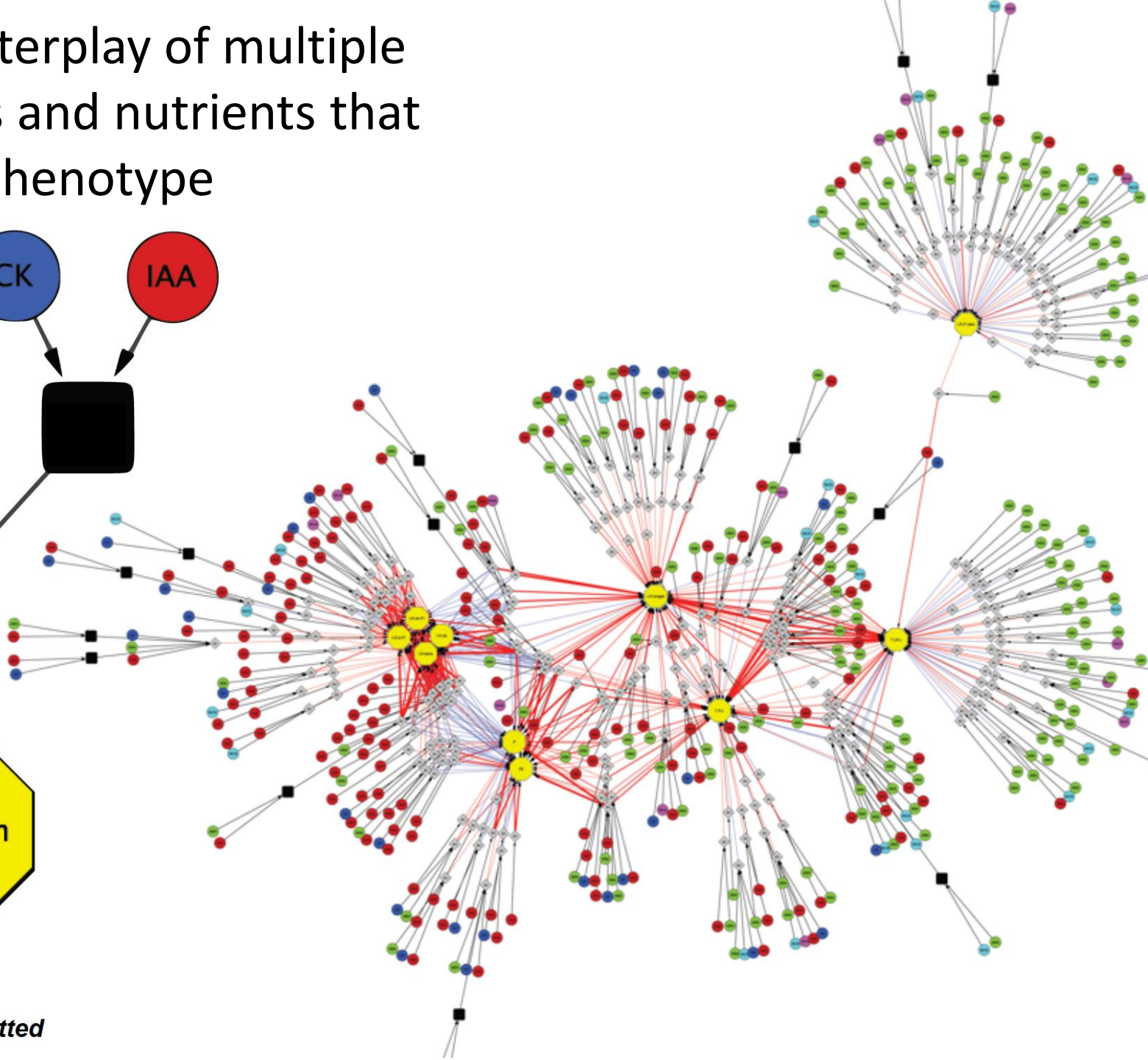
64 ATH1 Affy chips

ANALYSIS of VARIANCE

Ristova *et al submitted*

It is the interplay of multiple hormones and nutrients that regulate phenotype





Ristova *et al submitted*

Gabriel Krouk

Perspectives for Biostimulants:

Combinations of molecules in biostimulants can have dramatically distinct effects on gene expression and/ or physiological plant response.

Don't seek for only one molecule in your soup.

Think different: think combinatorial.

EMERGENCE

But perhaps a single molecule of discrete function is all you need

Stress Hypothesis:

- Abiotic stress limits crop productivity and select biostimulants have been shown to mitigate this stress

- Rarely is a single stress present, more commonly multiple stress occurs together (Drought and heat, salinity and water stress…)

- The pathways involved in stress perception and response are complex and poorly understood- results WILL be unpredictable

Question: There is inevitably a metabolic cost to up-regulation of stress protective genes – how do we achieve an appropriate balance?

Can a single biostimulant by active against multiple stresses?

Physiological Rationale for Biostimulants

Stress Hypothesis

- Abiotic ‘stress’ occurs in all environments and as a consequence yield rarely reaches full potential.
  - Biostimulants enable plants to respond more effectively to stress
  - Biostimulants ‘short-circuit’ normal molecular constraints to productivity

Microbiome Hypothesis

- Microbes in the environment have beneficial effects on crop growth
  - Biostimulants can favorably alter the plant microbiome
    - Live cultures
    - Substrate enrichment
  - Biostimulants can ‘mimic’ the beneficial effects of microbial metabolites.

The human microbiome, an initiative of the National Institute of Health

10X more microbial cells and 100X more microbial genes than human
(because microbial cells are 10-110 times smaller than human cells the human microbiome weighs only 7 ounces)

HMP

Human Microbiome Project

"We're not individuals, we're colonies of creatures."
Bruce Birren, Broad Institute

Diabetes, Parkinsons, Alzheimers, Obesity

Skin bacteria affect how attractive we smell to malarial mosquitoes

http://commonfund.nih.gov/hmp/

http://blogs.discovermagazine.com/notrocketscience/category/bacteria/microbiome-bacteria/

PeerJ

PEER-REVIEWED

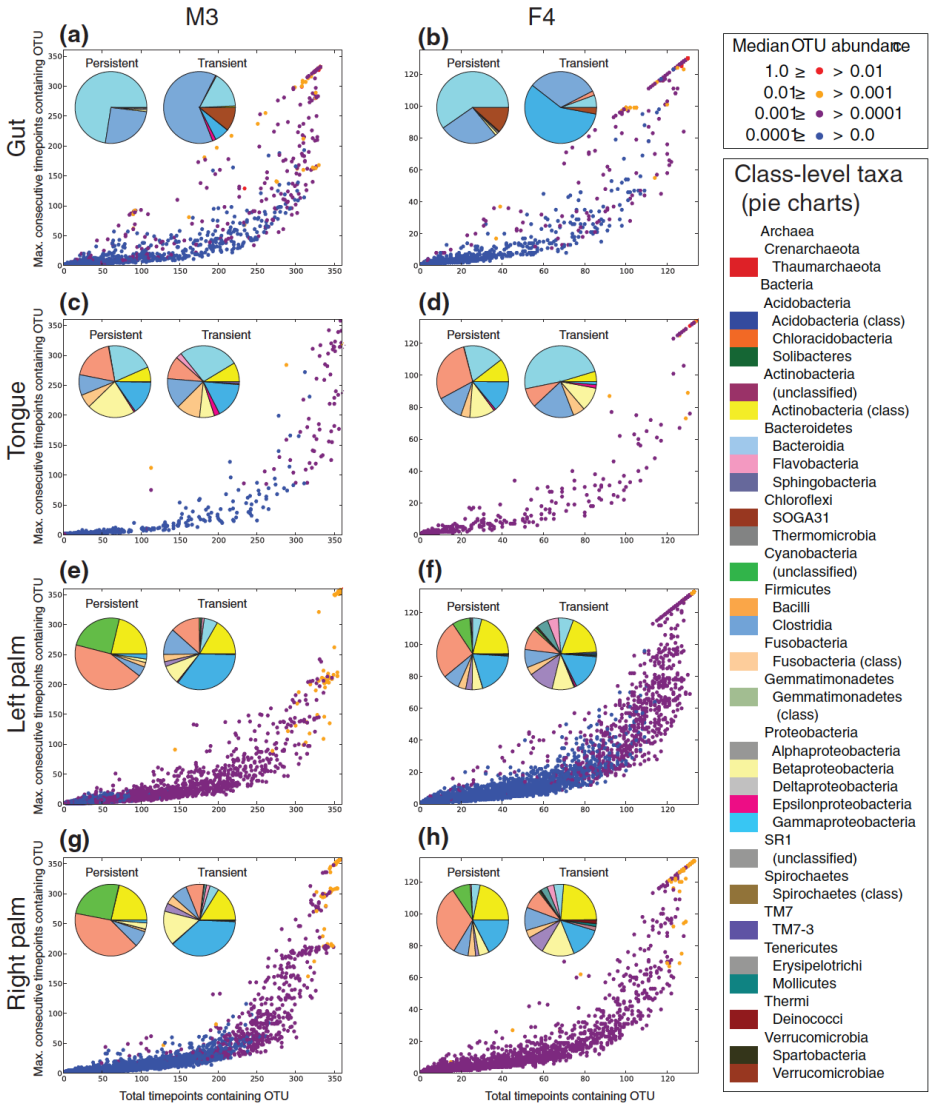
Humans differ in their personal microbial cloud

Ecology | Microbiology

James F. Meadow[1,2], Adam E. Altrichter[1,2], Ashley C. Bateman[1,2], Jasc
GZ Brown[1,3], Jessica L. Green[1,2,4], Brendan J.M. Bohannan[1,2]

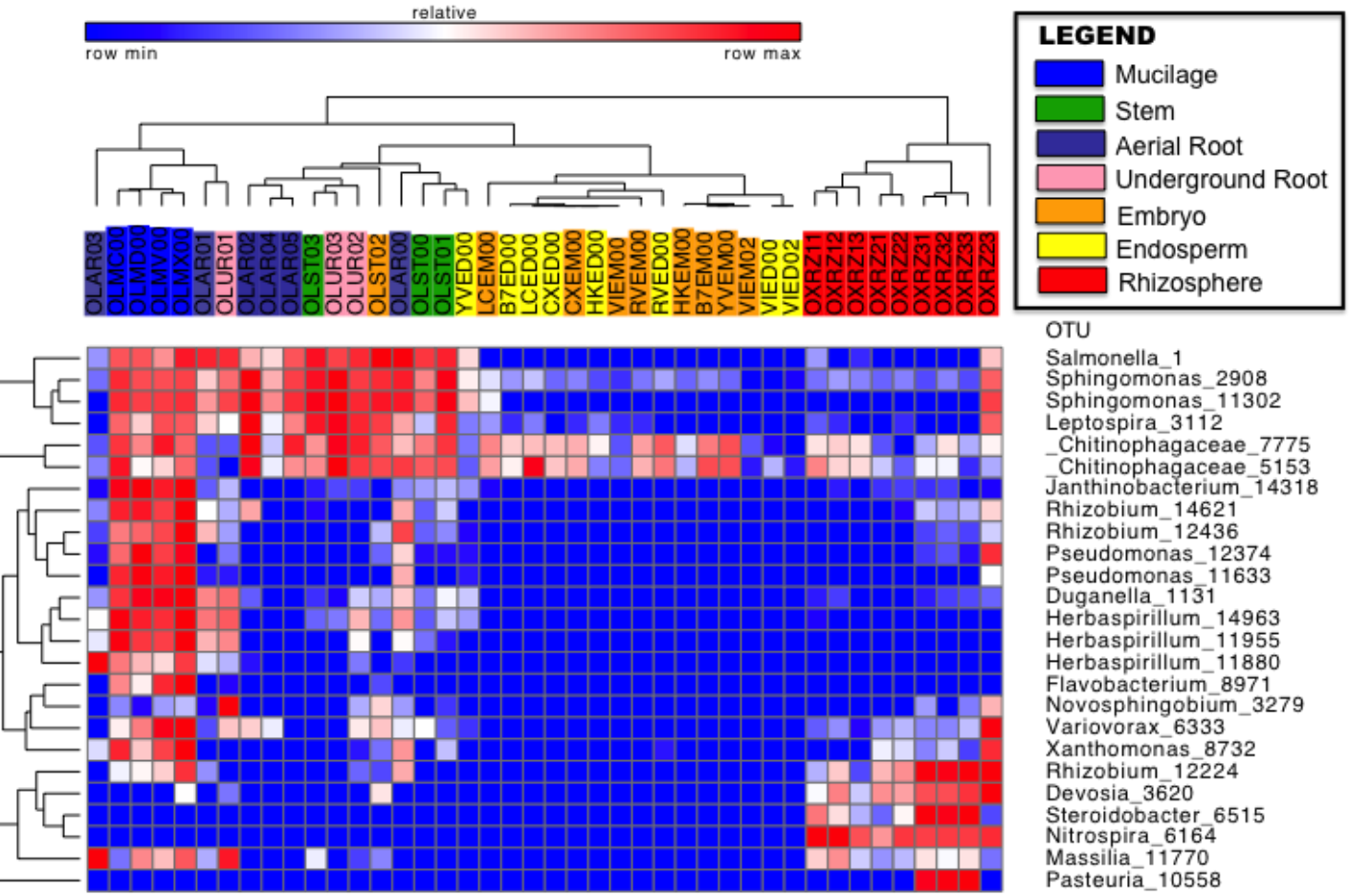
Published September 22, 2015

Our skin is host to multitudes of microbes



Biostimulants, the Microbiome and Plant Priming

The Mucilage of Tototenpec Maize Hosts N fixing Bacteria the associated microbiota required for N fixation (oxygen consumption, substrate conversion, nitrogen transport etc.)

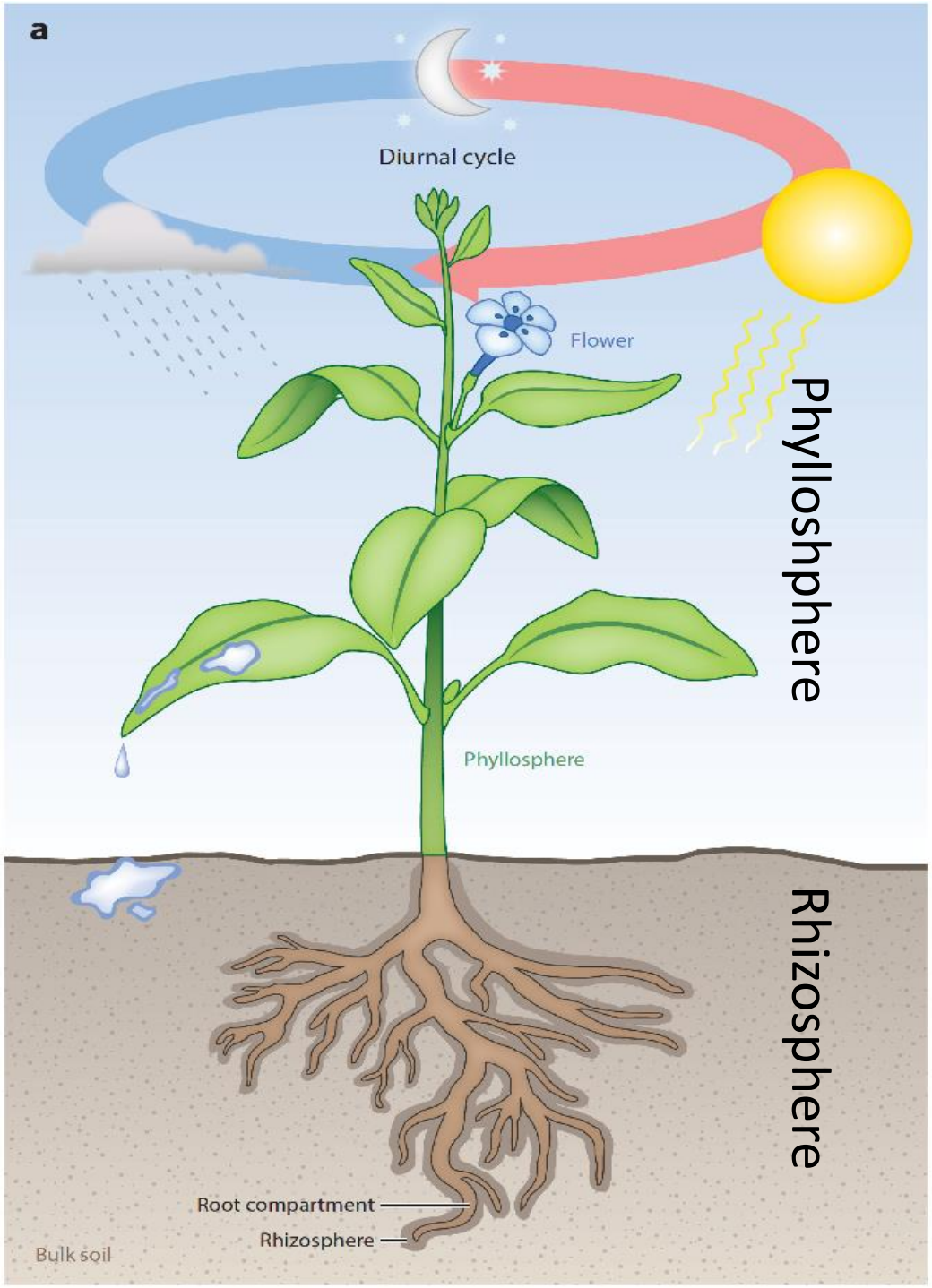


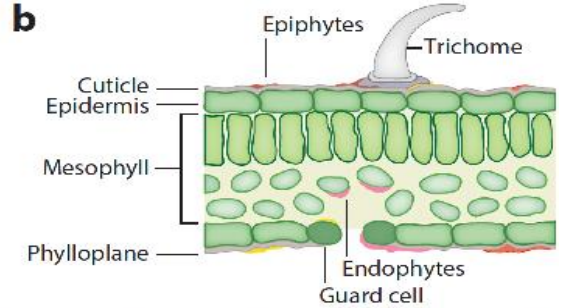
Oxygen Scavenging Bacteria

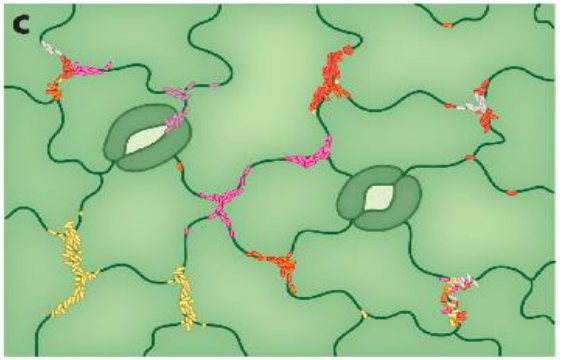
Nitrogen Fixing Bacteria

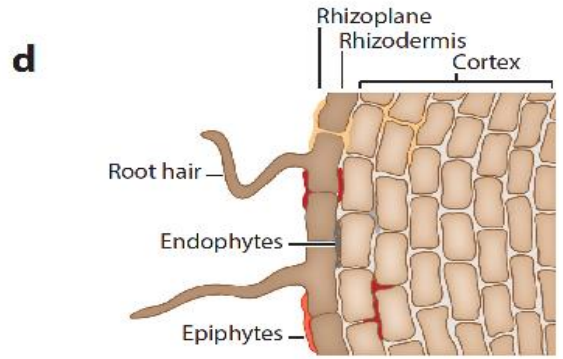
Ammonium Transport Bacteria

Heat Map: Red = High Expression, Blue = Low Expression


a
Diurnal cycle
Flower
Phyllosphere
Phylloshphere
Rhizosphere
Root compartment
Rhizosphere
Bulk soil


b
Epiphytes
Trichome
Cuticle
Epidermis
Mesophyll
Phylloplane
Endophytes
Guard cell

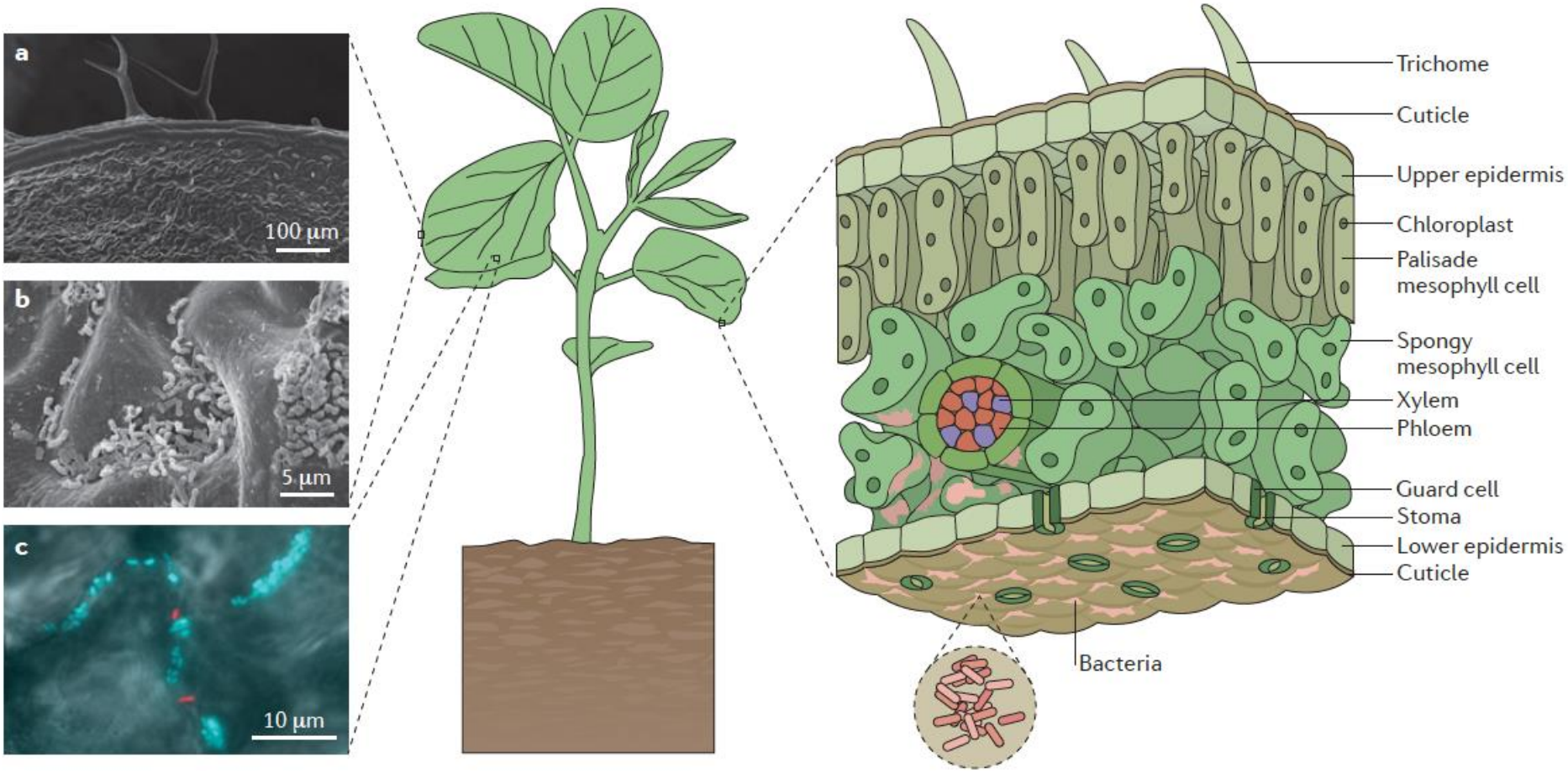

c


d
Rhizoplane
Rhizodermis
Cortex
Root hair
Endophytes
Epiphytes

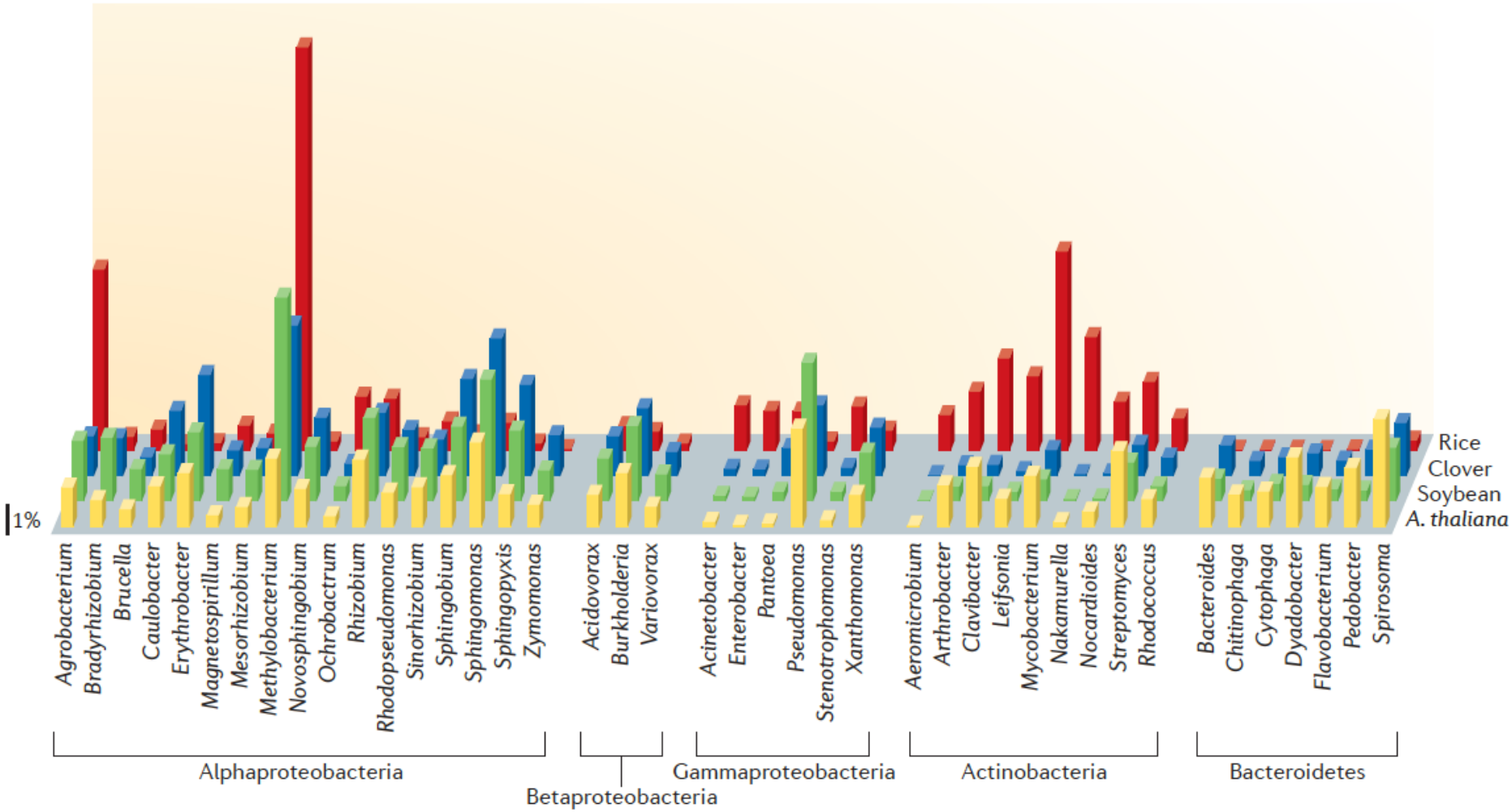
e

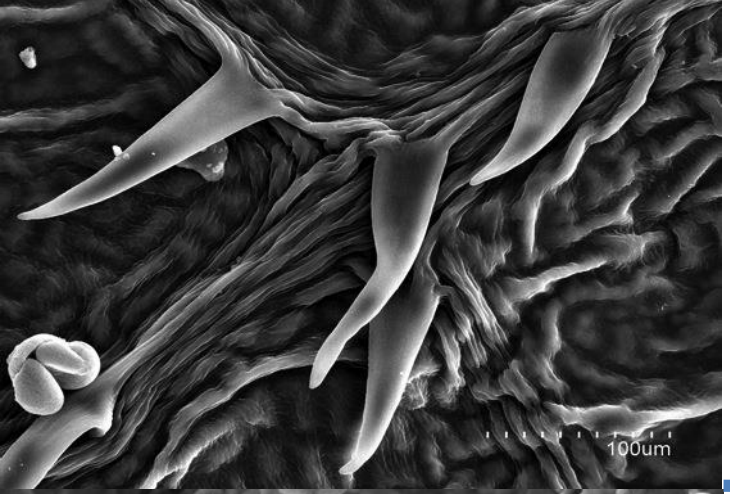
Endosphere

The Microbiome of the Plant is the Most Diverse Biological Environment on Earth

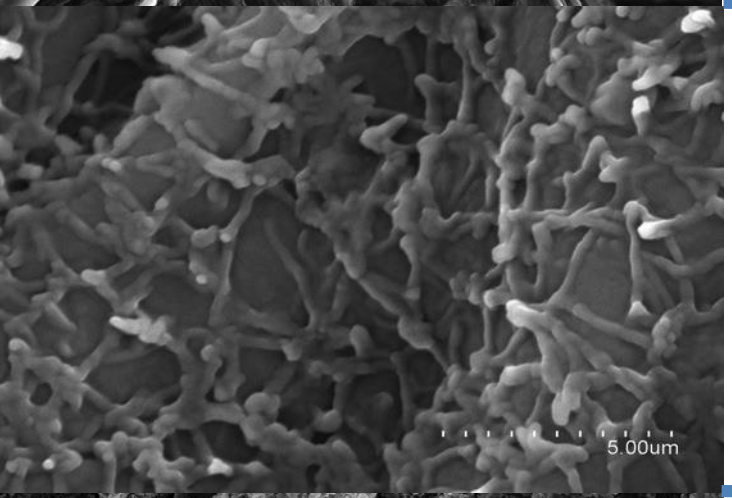


Microbial Populations Vary by Species, Environment and Agronomic Management

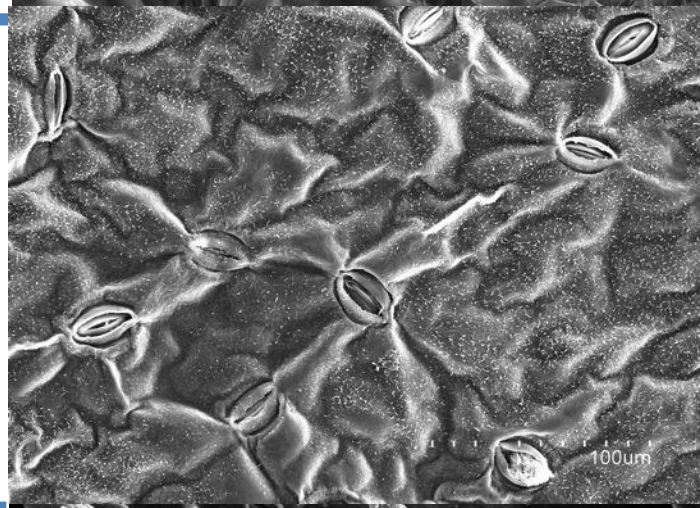


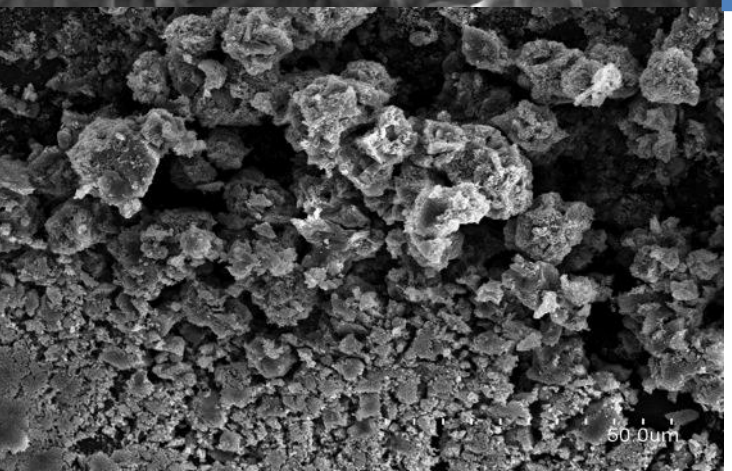


Cherry

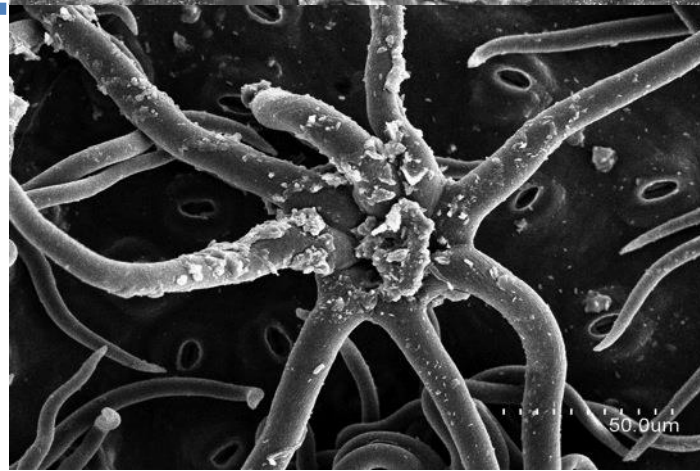


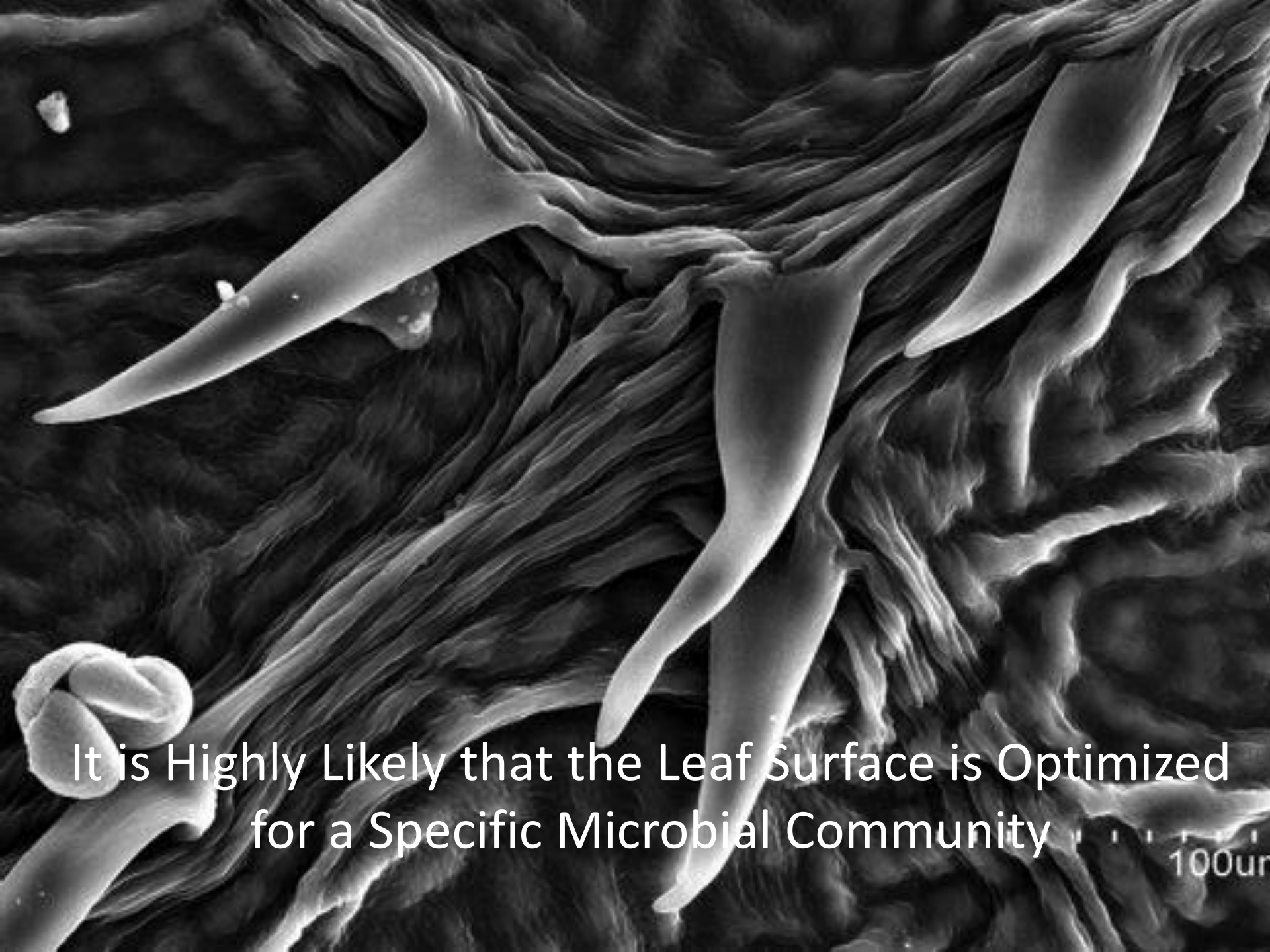
Rose



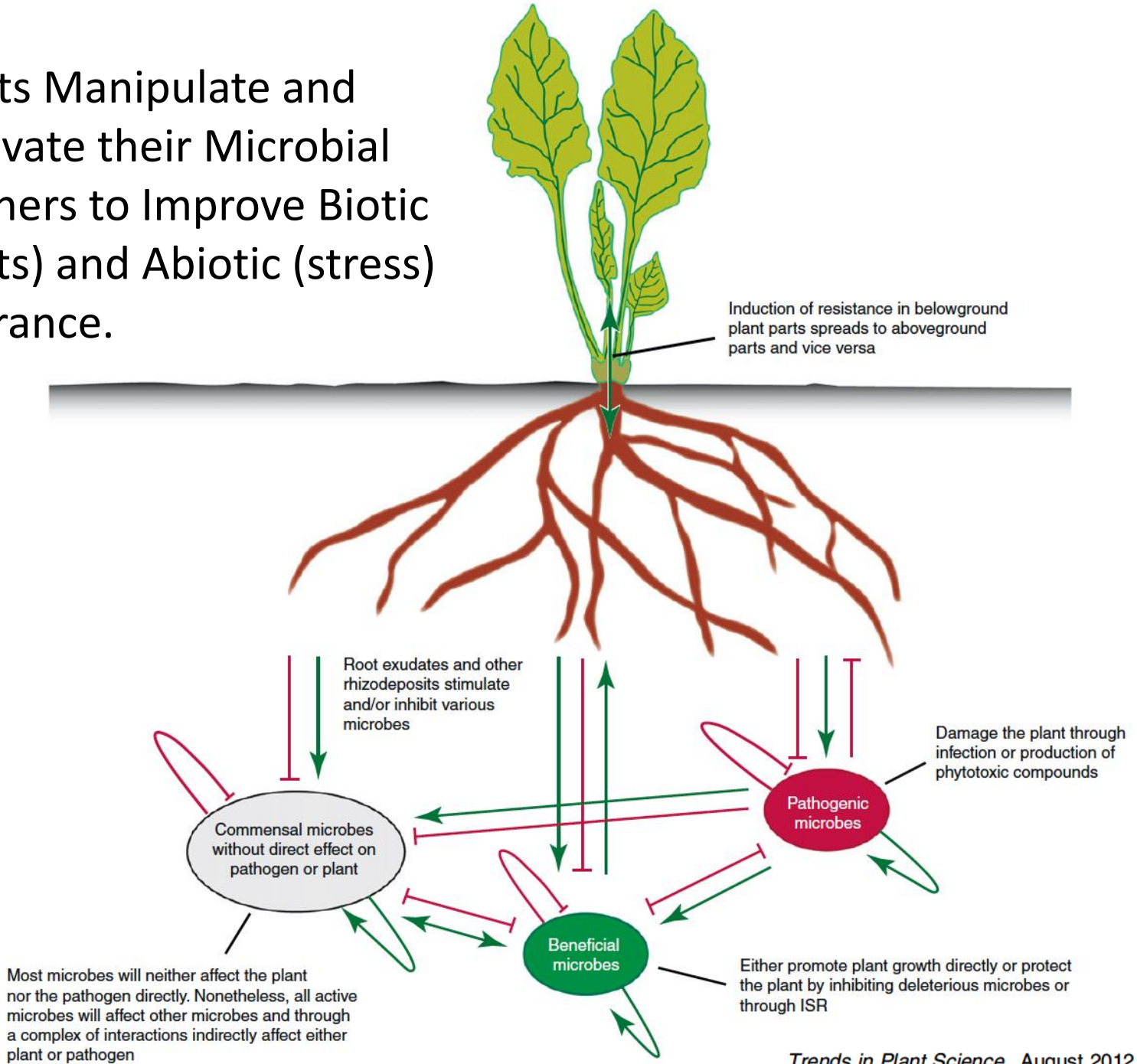


Oak




It is Highly Likely that the Leaf Surface is Optimized for a Specific Microbial Community
100um

Plants Manipulate and Cultivate their Microbial Partners to Improve Biotic (pests) and Abiotic (stress) Tolerance.

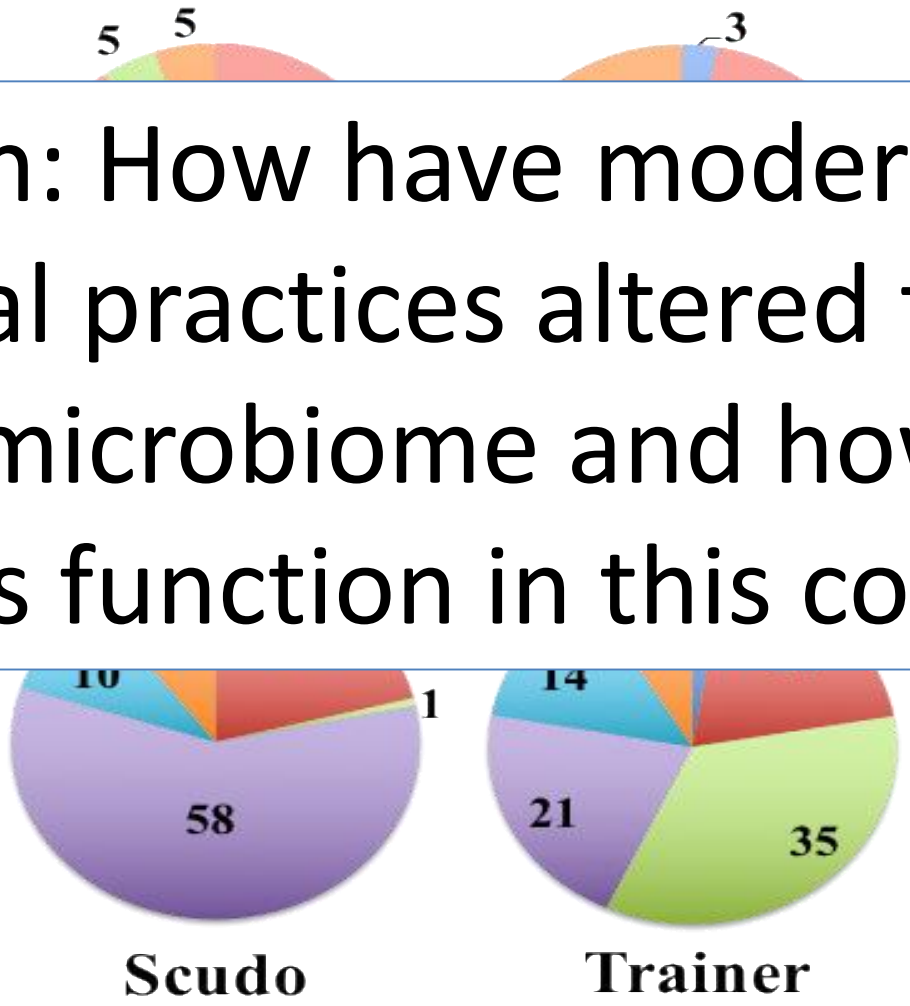


Trends in Plant Science August 2012, Vol. 17, No. 8

Foliar Biostimulants alter Phyllosphere Bacterial Communities

Prof. Maurizio Ruzzi

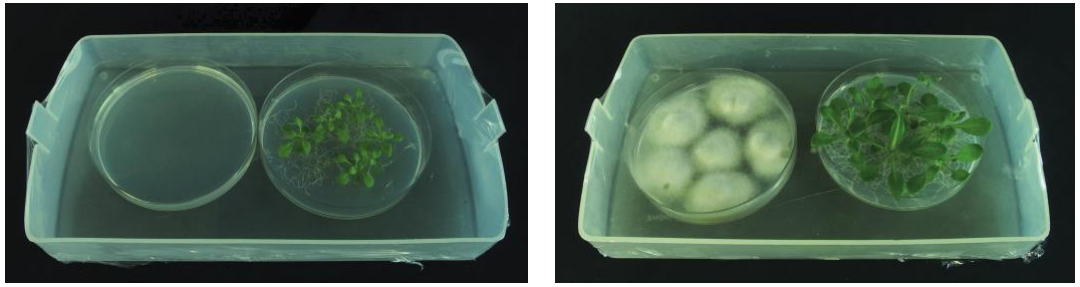
Question: How have modern agricultural practices altered the agricultural microbiome and how can biostimulants function in this context.



Exposure of Plants to Microbial Volatile Compounds Promotes Growth

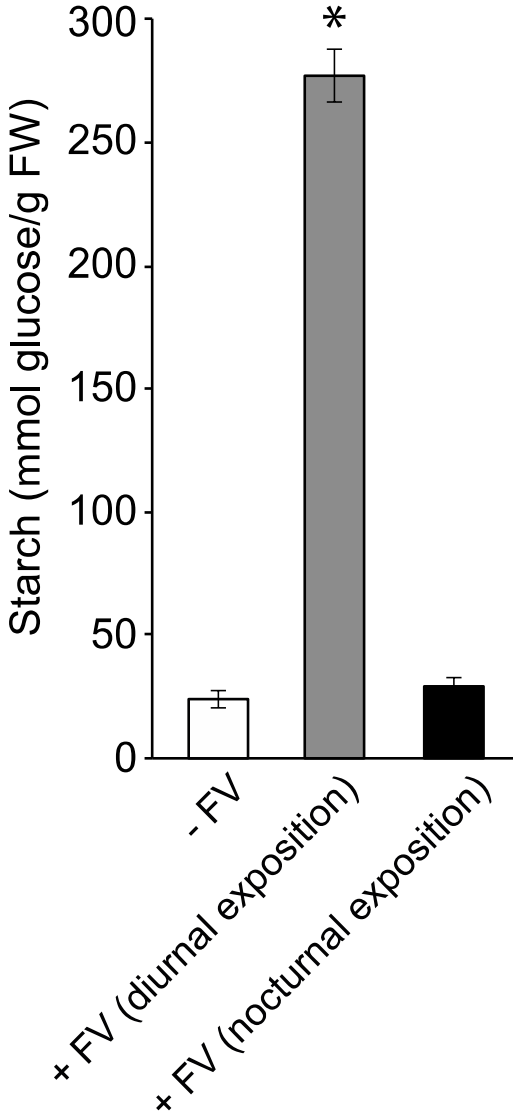
(Javier Pozueta Romero)

A



B

C



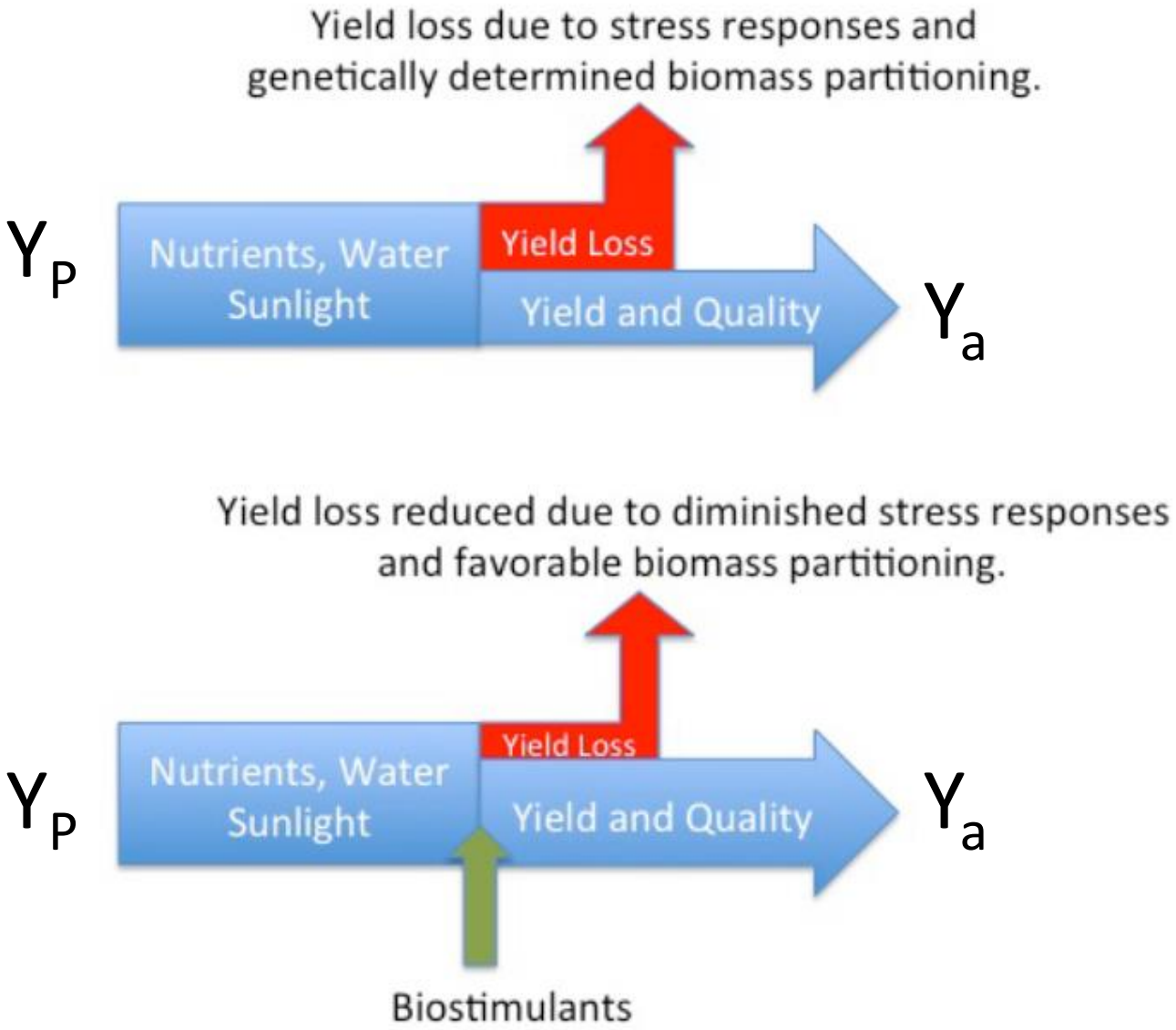
Physiological Rationale for Biostimulants and Microbiome Manipulation

Stress Hypothesis

- Abiotic ‘stress’ occurs in all environments and as a consequence yield rarely reaches full potential.
  - Biostimulants enable plants to respond more effectively to stress
  - Biostimulants ‘short-circuit’ normal molecular constraints to productivity

Microbiome Hypothesis

- Microbes in the environment have beneficial effects on crop growth
  - Biostimulants can favorably alter the plant microbiome
    - Live cultures
    - Substrate enrichment
  - Biostimulants can ‘mimic’ the beneficial effects of microbial metabolites.
  - The microbiome produced biostimulants or ‘detect’ stress



Brown and Saa, 2015 FIPS

THE 2ND WORLD CONGRESS ON THE USE OF BIOSTIMULANTS IN AGRICULTURE
Monday 16th - Thursday19th November, 2015
Florence Convention Centre, Italy
ORGANISED BY NEWAG INTERNATIONAL
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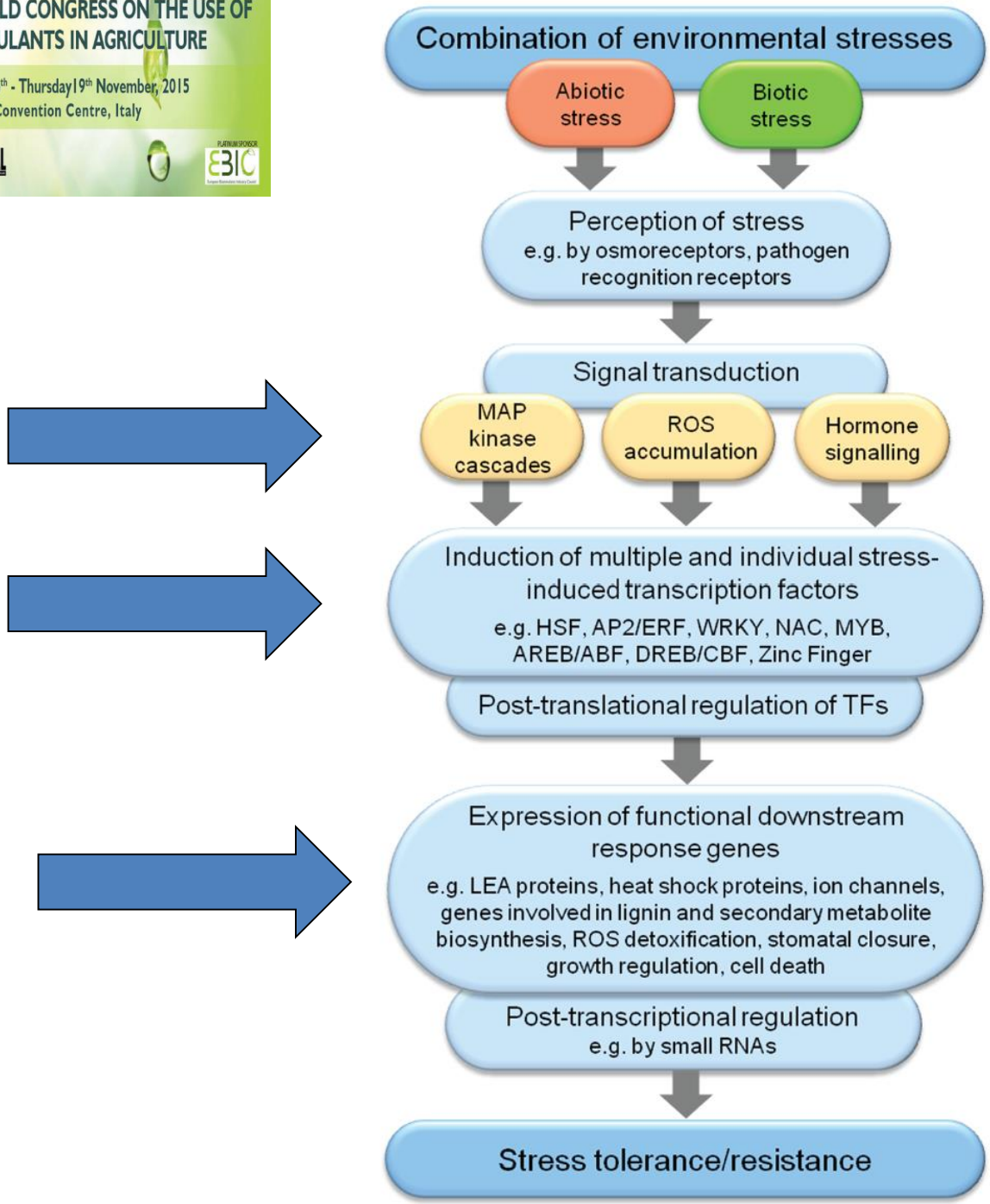

Cytokinin, ABA, Eth, Brassinoteroids

Currently identified modes of action of biostimulants.

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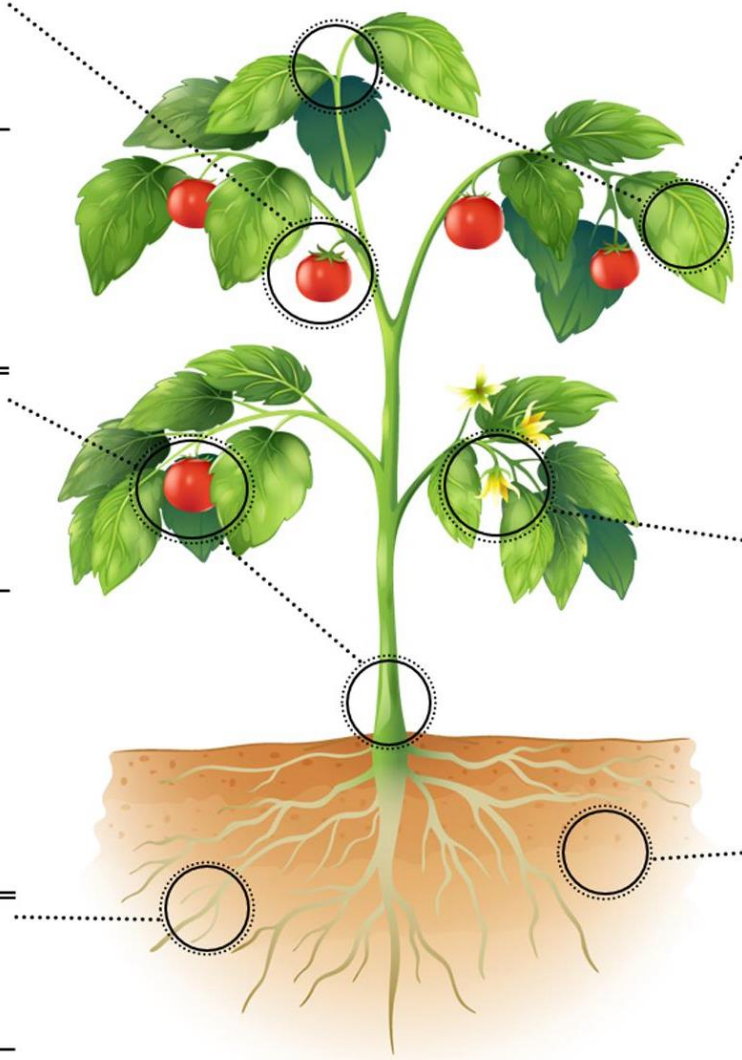
Published Responses to Biostimulants



Fruit
- Setting processes
- Fruit size and weight
- Quality

Crouch and van Staden, 1992; Chouliaras et al., 1997; Colapietra and Alexander, 2006; Basak, 2008; Chouliaras et al., 2009; Ross and Holden, 2010; Loyola and Muñoz, 2011; Parađiković et al., 2011; Khan et al., 2012; Parađiković et al., 2013; El-Hamied et al., 2015.

Seeds / Seedlings
- Germination
- "Starter effect"
- Overcoming transplant stress
- Priming effect
- Seed quality

Aldworth and van Staden, 1987; Featonby-Smith and van Staden, 1987; Crouch and van Staden, 1992; Russo et al., 1993; Moller and Smith, 1998; Demir et al., 2006; Sivasankari et al., 2006; Farooq et al., 2008; Neily et al., 2010; Kumar and Sahoo, 2011; Matysiak et al., 2011; Kalaivanan and Venkatesalu, 2012.

Roots
- Root development
- Young root development
- Rooting of cuttings

Sivasankari et al., 2006; MacDonald et al., 2010; De Lucia and Vecchietti, 2012; Ferrante et al., 2013; Krajnc et al., 2012; Petrozza et al., 2012; MacDonald et al., 2012; Alam et al., 2014.

Plant
- Plant growth/yield and physiological modulation
- Water/nutrient uptake
- Stress response

Beckett and van Staden, 1990; Beckett et al., 1994; Blunden et al., 1996; Adani, 1998; Mancuso et al., 2006; Zhang and Ervin, 2008; Ross and Holden, 2010; Sangeetha and Thevanathan, 2010; Zhang et al., 2010; Fan et al., 2011; Kumar and Sahoo, 2011; Matysiak et al., 2011; Parađiković et al., 2011; De Lucia and Vecchietti, 2012; Petrozza et al., 2012; Parađiković et al., 2013; Alam et al., 2014; Petrozza et al., 2014; Saa et al., 2015.

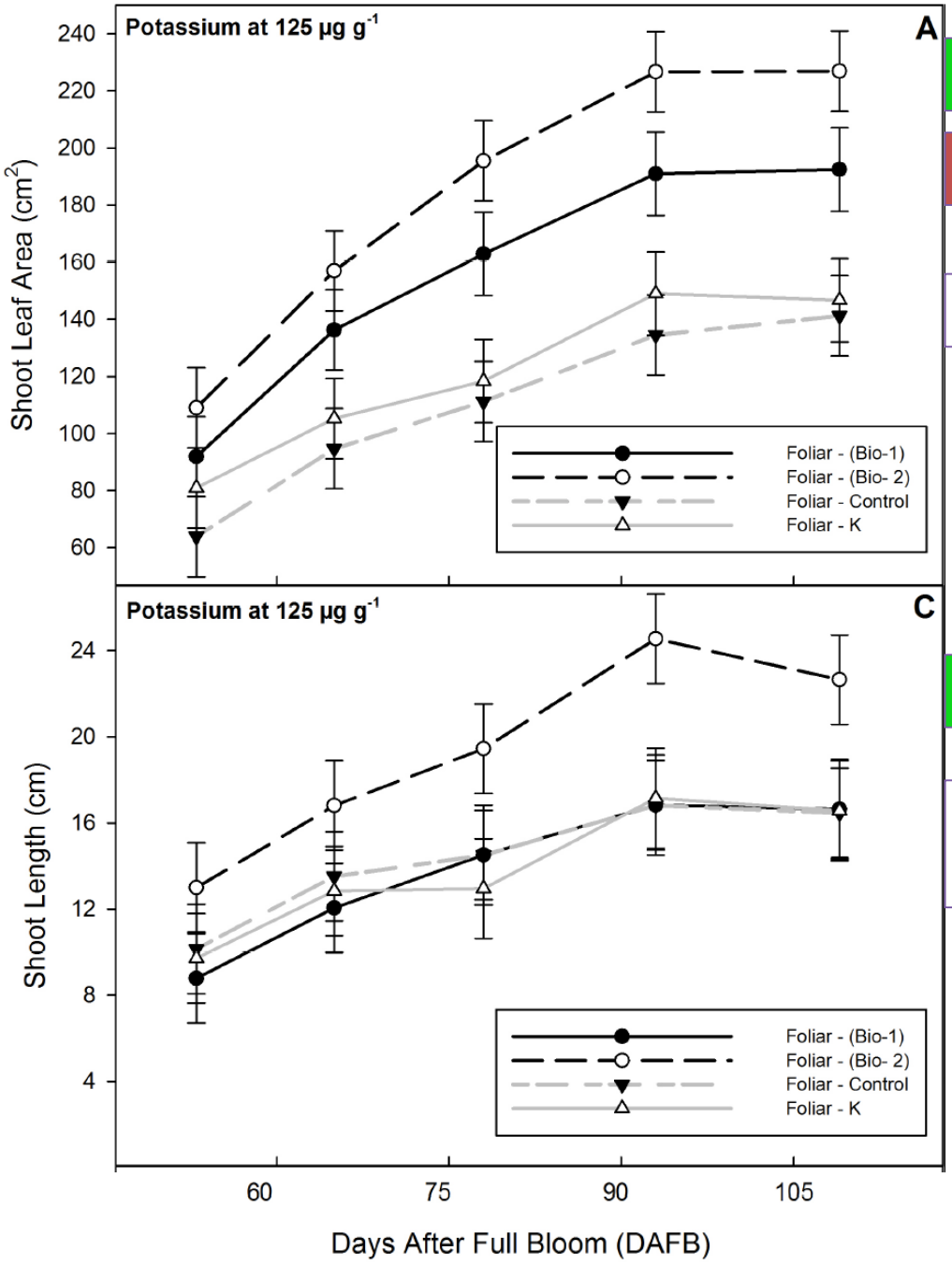
Flowers
- Flowering and sprouting induction.

Basak, 2008; Petri et al., 2008; Hawerroth et al., 2010; Pereira et al., 2011.

Soil
- Physico-chemical properties
- Development of beneficial soil microorganisms
- Water/nutrient retention
- Overcoming salinity stress

Booth, 1969; Guiry and Blunden, 1991; Temple and Bomke, 1988; Chen et al., 2002; Gulser et al., 2010; Ross and Holden, 2010; García-Martínez et al., 2010; Tejada et al., 2011; Alam et al., 2014.

Povero et al FIPS 2016



AgSpectrum

Valagro

Control/+K

180% greater Leaf Area

- Greater leaf size, leaf number, shoot length

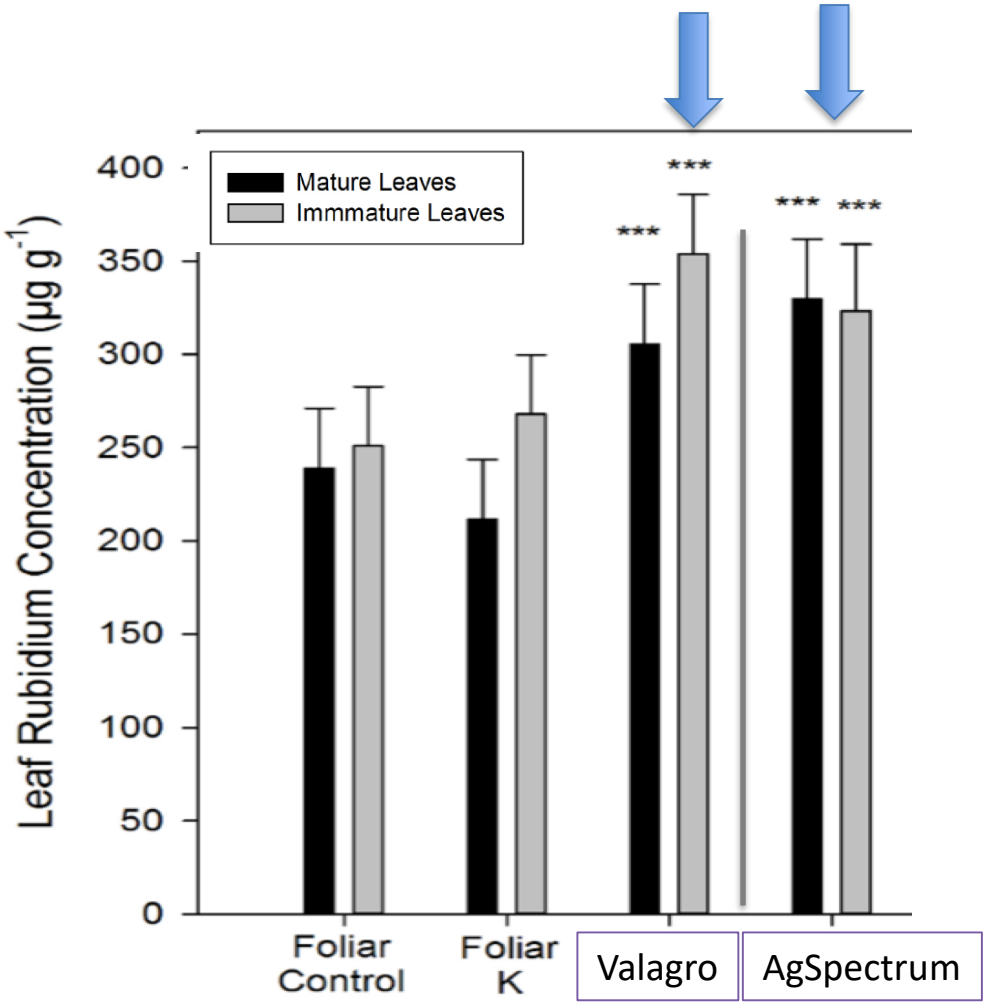
AgSpectrum

150% greater shoot length

Control/+K/ Valagro

Saa et al., 2014. FIPS

Potassium Uptake: Rb tracer, 14 day uptake period

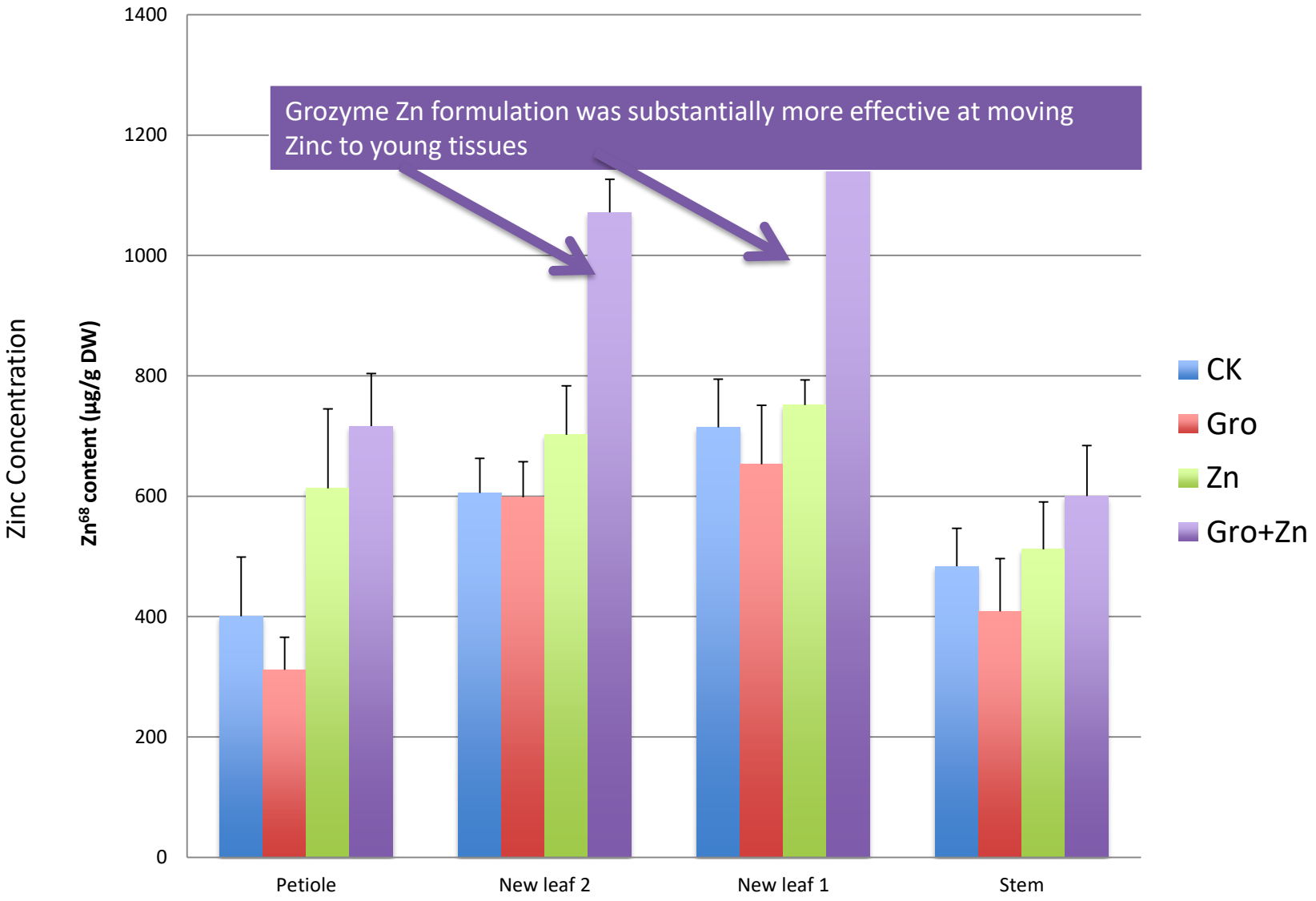


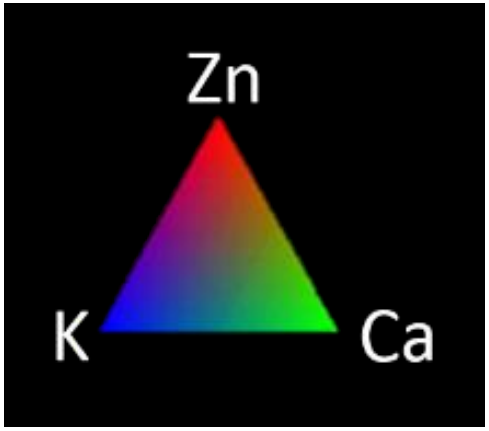
Both biostimulant products increased K (Rb) uptake and transport to young tissues.

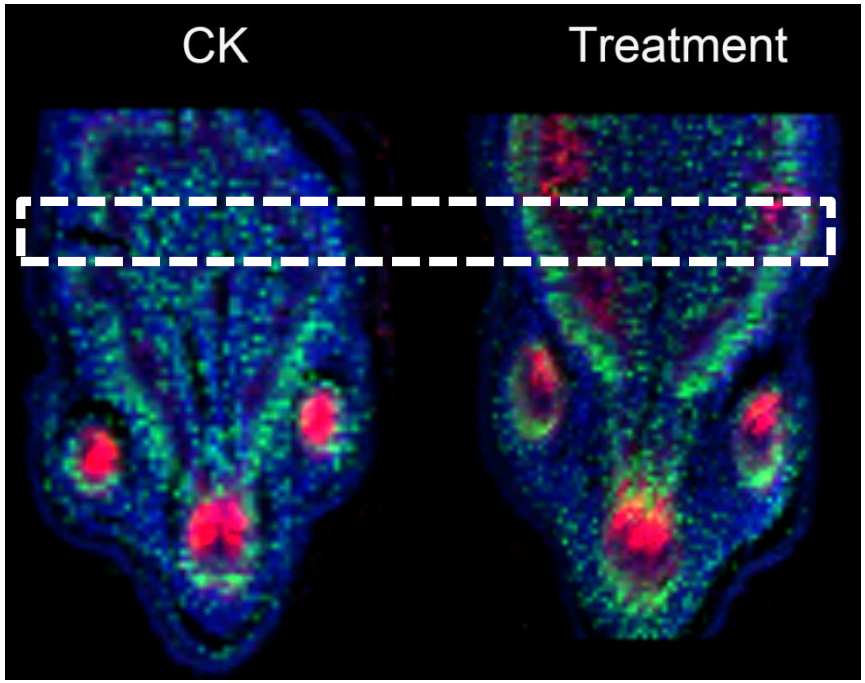
- 150% greater in mature leaves
- 170% greater in immature leaves

Saa et al., 2014. FIPS

Zn^{68} content in sunflower

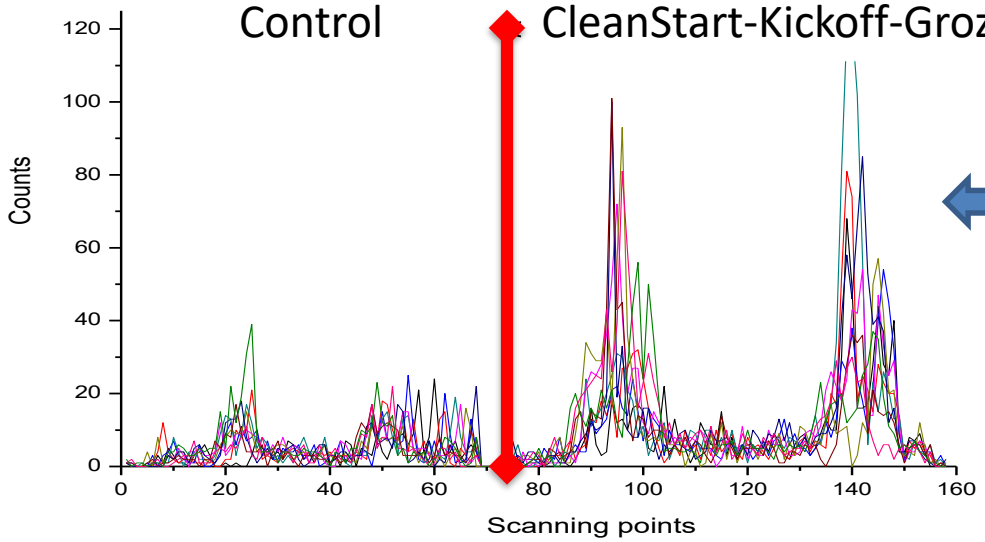






Main Stem

Floral and
Vegetative Buds



Zn

Plant Priming

BIOLOGICAL REVIEWS
Cambridge Philosophical Society
Biol. Rev. (2016), **91**, pp. 1118–1133.
doi: 10.1111/brv.12215

Priming and memory of stress responses in organisms lacking a nervous system

Monika Hilker[1,2*], Jens Schwachtje[3], Margarete Baier[4], Salma Balazadeh[5], Isabel Bäurle[5], Sven Geiselhardt[1], Dirk K. Hincha[6], Reinhard Kunze[7], Bernd Mueller-Roeber[5], Matthias C. Rillig[2,8], Jens Rolff[9], Tina Romeis[10], Thomas Schmülling[11], Anke Steppuhn[12], Joost van Dongen[13], Sarah J. Whitcomb[3], Susanne Wurst[14], Ellen Zuther[6] and Joachim Kopka[3]

Trends in Microbiology

Review

Engineering Microbiomes to Improve Plant and Animal Health

U.G. Mueller,[1,2,*] and J.L. Sachs[3,4]

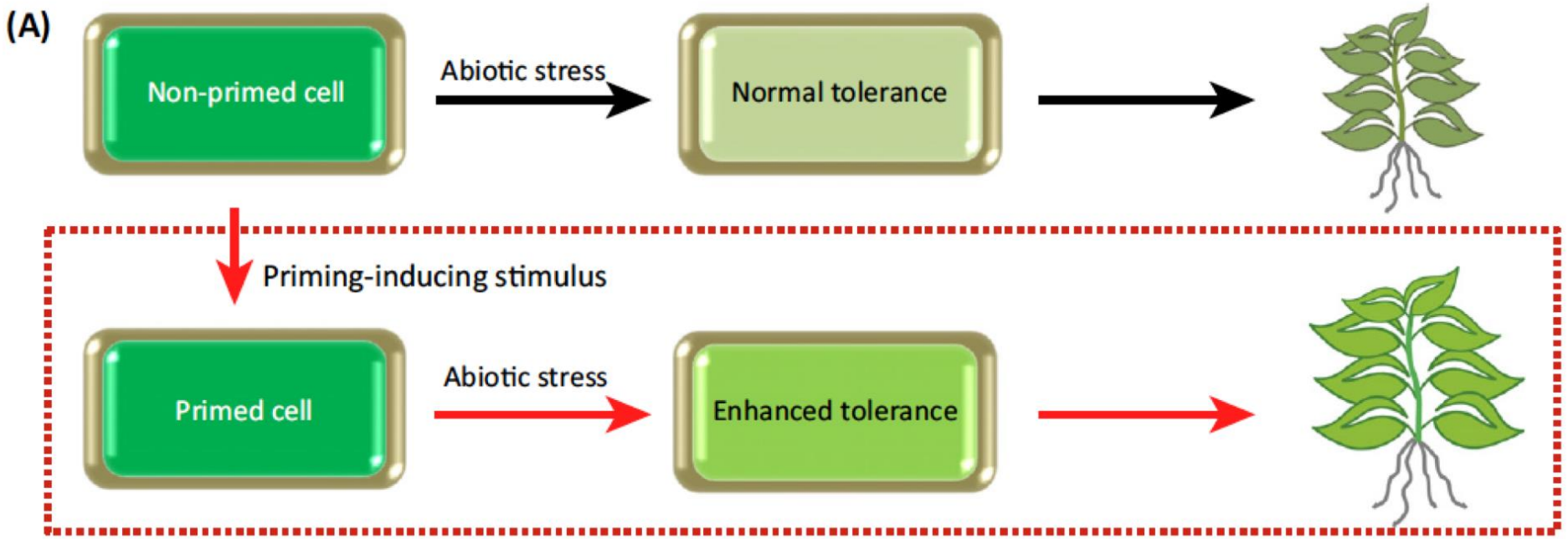
REVIEW

BOTANY

Reconsidering plant memory: Intersections between stress recovery, RNA turnover, and epigenetics

Peter A. Crisp, Diep Ganguly, Steven R. Eichten, Justin O. Borevitz, Barry J. Pogson*

PRIMING


(A)
Non-primed cell
Abiotic stress
Normal tolerance
Priming-inducing stimulus
Primed cell
Abiotic stress
Enhanced tolerance

Journal of Experimental Botany Advance Access published September 9, 2014

Journal of Experimental Botany
doi:10.1093/jxb/eru362
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

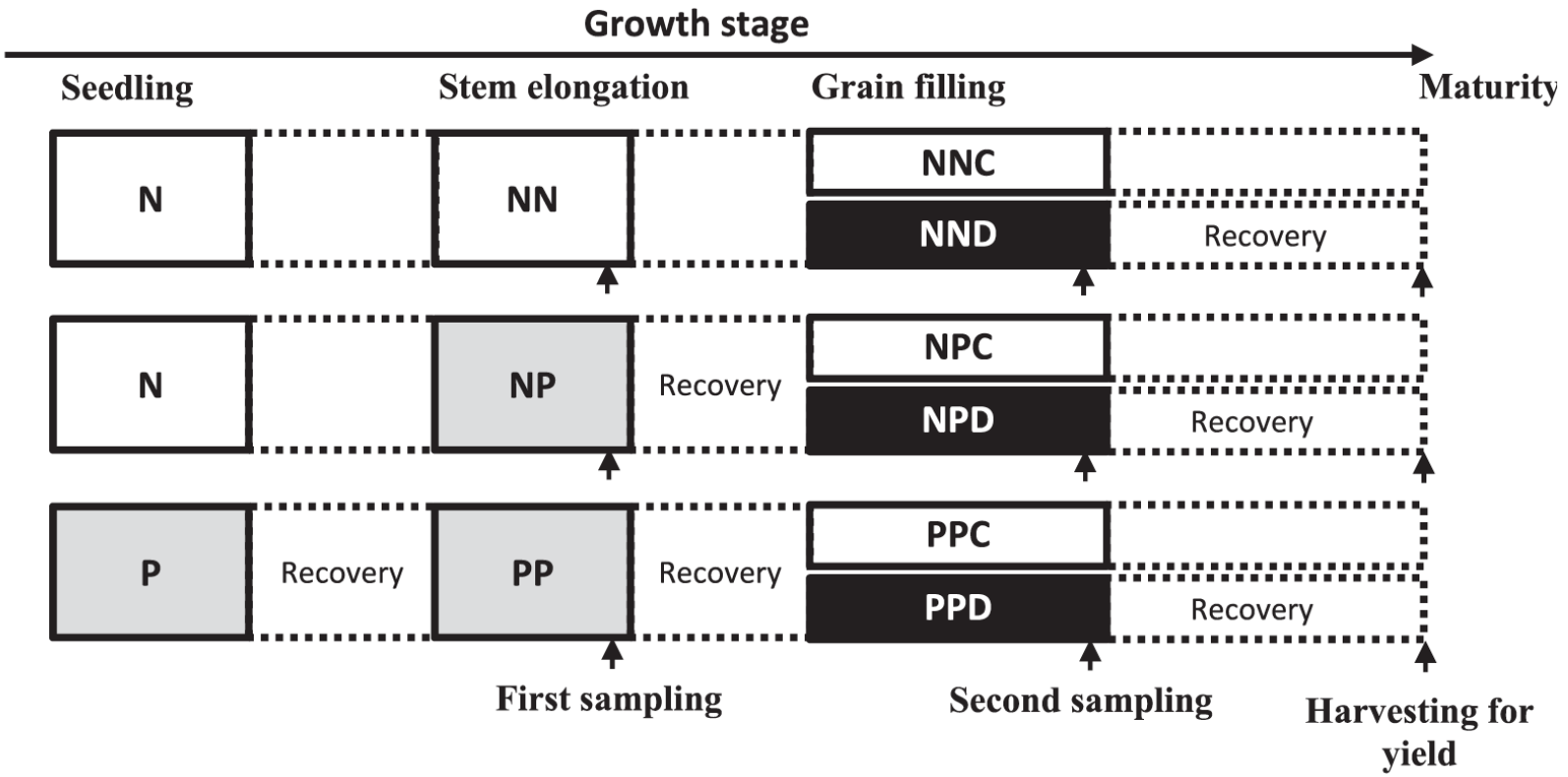
RESEARCH PAPER

Improved tolerance to drought stress after anthesis due to priming before anthesis in wheat (*Triticum aestivum* L.) var. Vinjett

Xiao Wang[1,2,3], Marija Vignjevic[2], Dong Jiang[1,*], Susanne Jacobsen[3,†] and Bernd Wollenweber[2*]

[1] Key Laboratory of Crop Physiology and Ecology in Southern China, Ministry of Agriculture/Hi-Tech Key Laboratory of Information Agriculture of Jiangsu Province, Nanjing Agricultural University, PR China
[2] Aarhus University, Faculty of Science and Technology, Institute of Agroecology, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
[3] Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Building 224, DK-2800 Kgs. Lyngby, Denmark

Wheat was treated with mild stress during early development and then subjected to more severe stress at grain filling.



N=No Stress C=Control
P=Priming Stress D=Drought

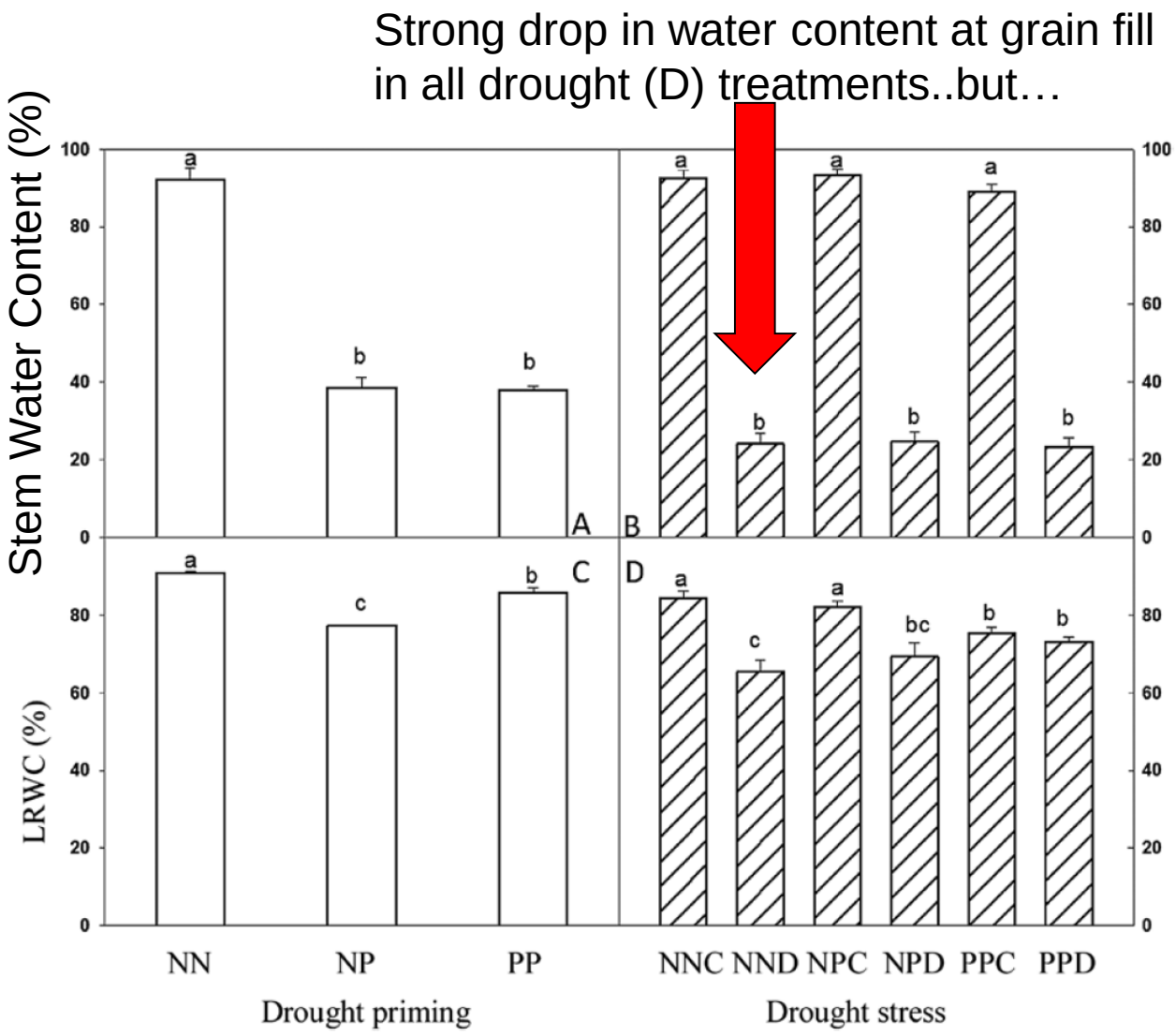
Influence of priming during development on stress tolerance at maturity: Stem Water Content

NNC: Non primed, non stressed

NND: Non primed and drought at grain fill

NPD: Primed at mid-vegetative and drought at grain fill

PPD: Primed early and mid, drought at grain fill



Influence of priming during development on stress tolerance at maturity: PHOTOSYNTHESIS

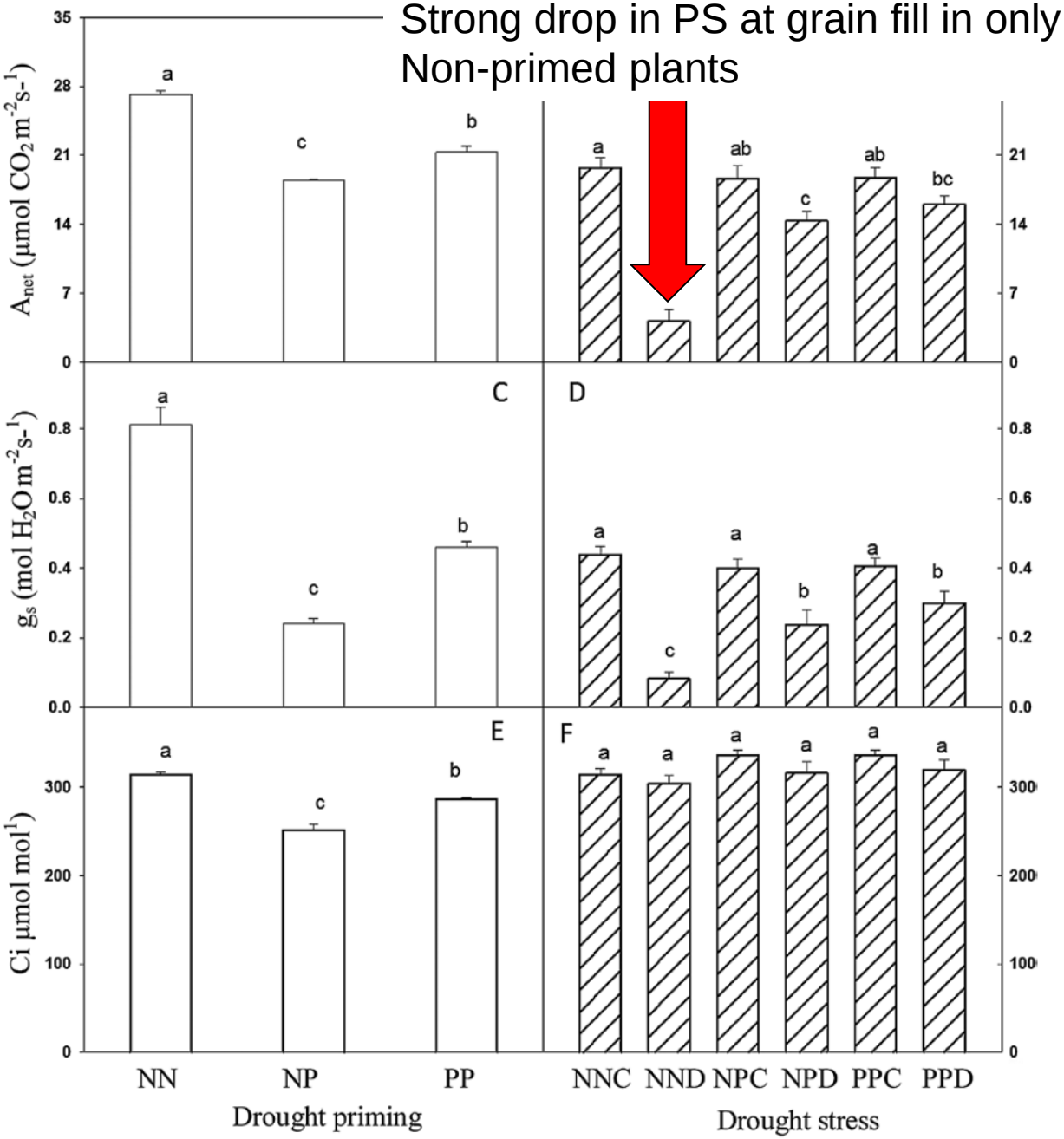
Strong drop in PS at grain fill in only Non-primed plants

NNC: Non primed, non stressed

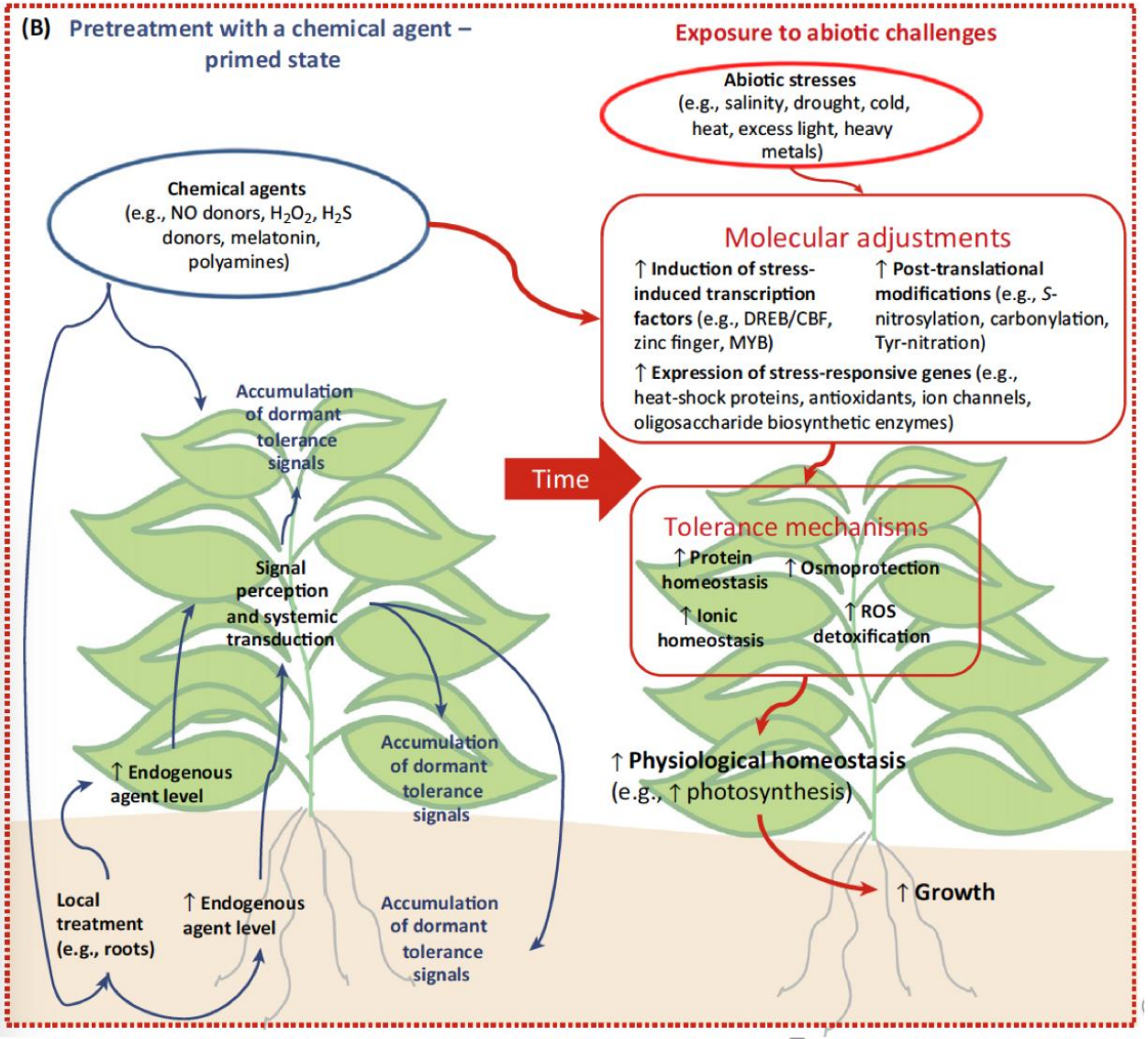
NND: Non primed and drought at grain fill

NPD: Primed at mid-vegetative and drought at grain fill

PPD: Primed early and mid, drought at grain fill



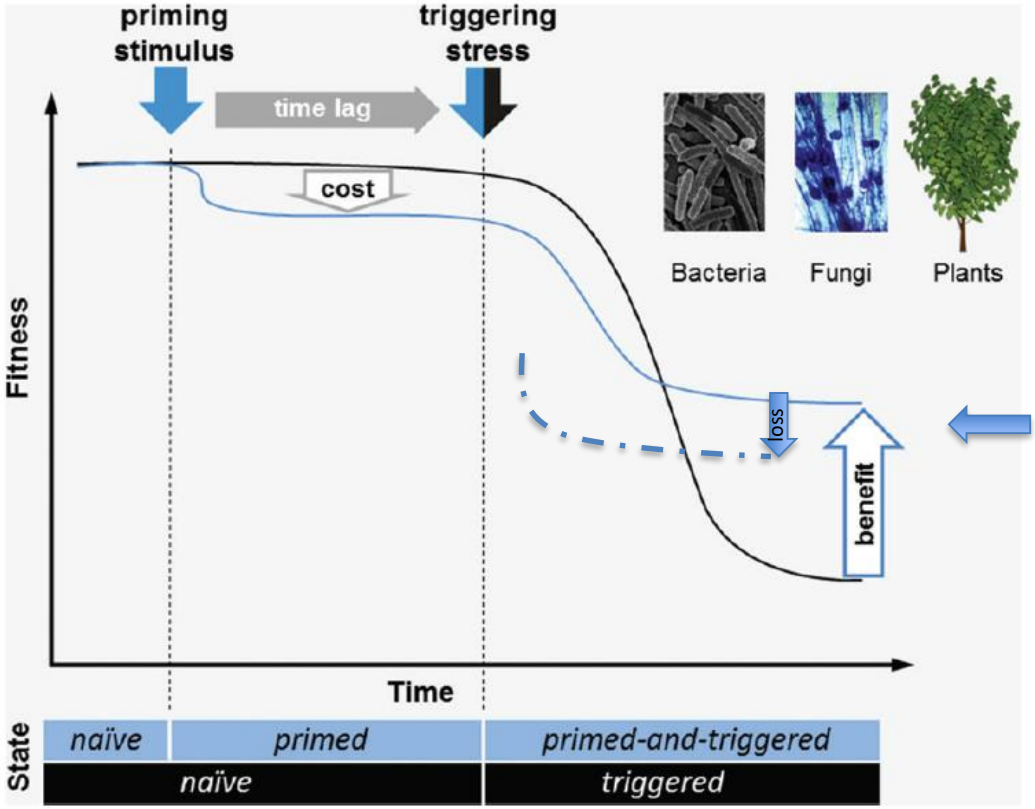
Can Plants be Primed for Stress Tolerance Using Synthetic Chemicals or Biostimulants or Microbiome?



How generic are priming events? Does a heat stress priming event protect against a water, glyphosate, cold…stress – or could an irrelevant priming event compromise a subsequent stress response?

Clearly there must be a 'cost' to priming, otherwise the plant would simply express these defense/response mechanisms at all times. What factors determine the extent of this cost and what factors mitigate it?

Can a priming event be triggered by application of discrete molecules rather than applying the stress itself?.

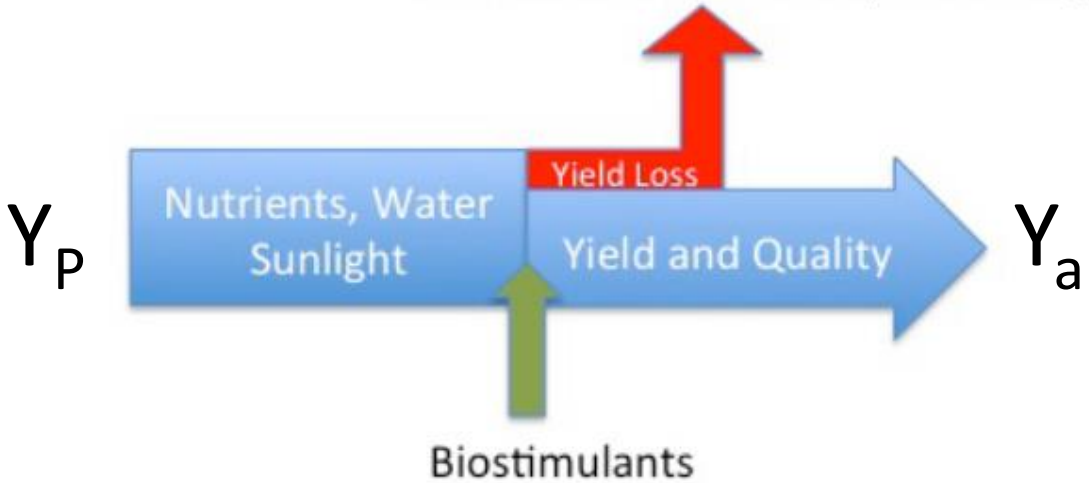


If the subsequent stress does not occur then there must be a yield drag. Clearly the agronomic use of priming requires a sound understanding and ability to predict the occurrence of stress.

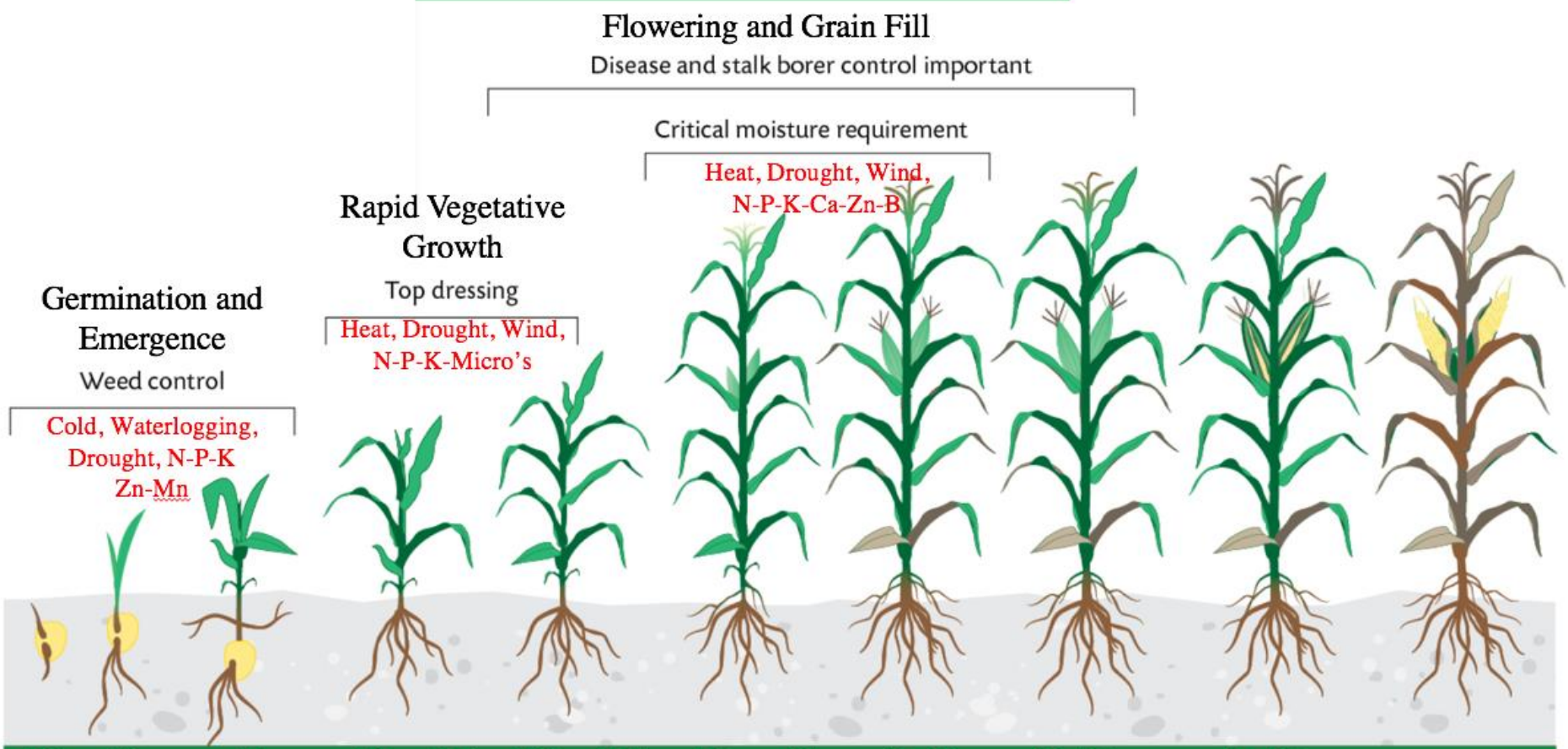
Biol. Rev. (2016), **91**, pp. 1118–1133.
doi: 10.1111/brv.12215

Yield loss due to stress responses and genetically determined biomass partitioning.

Yield loss reduced due to diminished stress responses and favorable biomass partitioning.



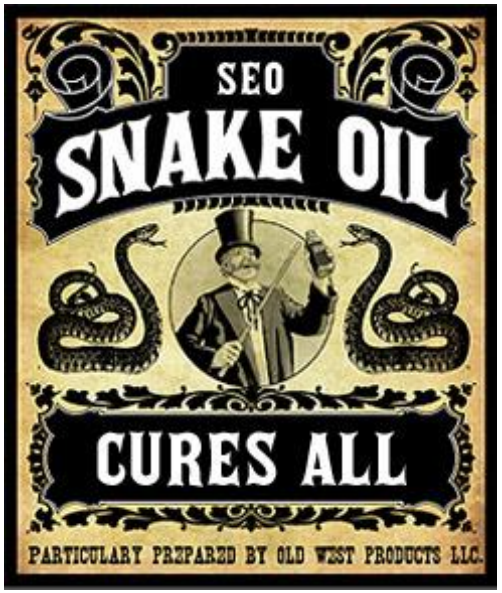
MAIZE GROWTH STAGES

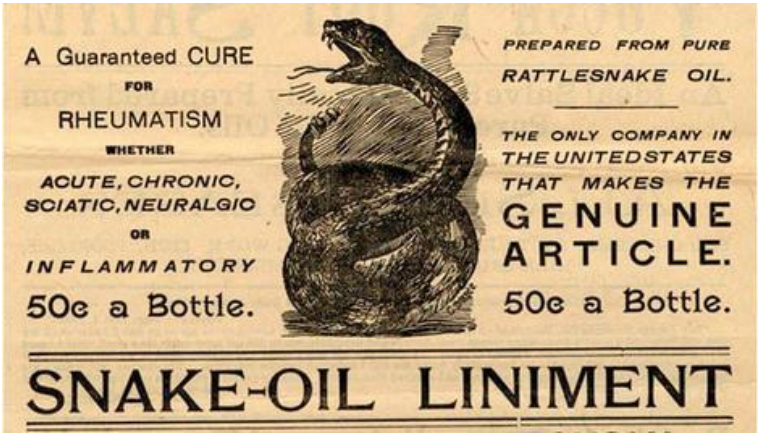


V0	V2	V5	V8	V10	V12	V14	VT	R1	R2 - R3	R3 - R4	R5 - R6
Days after Emergence*											
0	7	21	32	38	44	49	56	63	70 77 84	91 98 105	112 119	160
Germination and emergence. Planting depth 5-8 cm.	Plant population established. Growth point 20-25 mm below surface. 5 Leaf – cob and tassel initiation.	Cob development.	Active growth – leaves and cob development. Brace root development. 12 Leaf – cob size determined.	Pollination. 5-10 Days.	Kernel development.	Grain filling. Nutrients are transported to cob.	Physiological maturity. End of mass gain.	Ready for harvest. 14% Moisture.			
Vegetative Growth Stages							Reproductive Development Stages				

* The number of days varies between different growth classes and environments.

Snake Oils and Jungle Juices





Can of Worms

NEW AGRICULTURE INTERNATIONAL

3RD BIOSTIMULANTS WORLD CONGRESS

The World's Largest Event on Agricultural Biostimulants!

SAVE THE DATE!

Monday 27th - Thursday 30th November 2017
Hyatt Regency, Miami, Florida (USA)

1st Biostimulants World Congress, November 2012, Strasbourg, France - over 700 delegates from 55 countries in attendance.

Further information: www.Biostimulants2012.com

2nd Biostimulants World Congress, November 2015, Florence, Italy – over 1,200 delegates representing 750 companies and organisations from 69 countries in attendance.

Why Bother?
Basic Biological Discovery

Discovery of Novel Molecules

- Cytokinins – Coconut milk stimulated plant growth
- Brassinosteroids - Pollen from Brassica napus stimulated plant growth
- Strigolactones – Parasitic weeds can be stimulated by a molecule emitted by the host plant
- Jasmonic acid – Extract of wormwood (Artemisia absinthium) impacts on plant senescence.
- Giberrellins – Fungal infection with (Gibberella fujikuroi) altered plant seedling growth.

Microbiome – Stress Biology – Yield Gaps.

BIOSTIMULANTS
Maybe…Just
Maybe!