DPG | Phytomedizin Online

Falko Feldmann

Efficacy and risks of „biorationals“ in organic and integrated pest management

8th International Symposium Plant Protection and Plant Health in Europe
jointly organised by DPG, JKI and HU-Berlin

Book of Presentations and Abstracts

urn:nbn:de:0294-PMO-PPPHE-2017-4

Conference Fee and Venue

Conference fee:
100 Euro (regular, early bird)

The conference fee includes complementary coffee, tea or beverages and a joint dinner.
For further payment modalities please refer to symposium website.

Registration at the symposium website is 15 July to 30 November 2017.

The conference language will be English.

Programme information:
Further details will be given at the symposium website.

Contact :

Falko.Feldmann@julius-kuehn.de

The symposium bureau will be open at

12 December 2017 from 16:30 — 18:30
and at
13 December 2017 from 8:00 — 9:00

The symposium »Plant Protection and Plant Health in Europe« is organised jointly by the *German Society for Plant Protection and Plant Health* (DPG, www.phytomedizin.org), the *Julius Kühn-Institut* (JKI, www.julius-kuehn.de) and the Albrecht Daniel Thaer-Institut für Agrar- und Gartenbauwissenschaft (www.hu-berlin.de).

It will be held at the Julius Kühn-Institut, Messeweg 11-12, 38104 Braunschweig.

Accommodation: Braunschweig offers a large number of hotels. Please visit the Symposium website.

www.ppphe.phytomedizin.org

Symposium Committee

Falko Feldmann & Johannes Hallmann
German Phytomedical Society (DPG) & Julius Kühn-Institut (JKI) Braunschweig, DE

Georg F. Backhaus & Stefan Kühne
Julius Kühn-Institut (JKI), Quedlinburg, DE

Carmen Büttner
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Jozef Kotleba
Slovenská Rastlinolekárska Spolocnost (SRS), Nitra, Sk

Vladimir Rehák & Petr Harašta
Ceska Spolecnost Rostlinolekarska (CSR), Prague, CZ

Ewa Matyjaszczyk
Institute of Plant Protection (NRI), Poznan, PL

Günter Neumann & Markus Weinmann
University of Hohenheim (UH), Hohenheim, DE

Manfred Raupp
Biofector Project, Madora, Lörrach, DE

Alexandra Makulla
Federal Office of Consumer Protection and Food Safety (BVL), Braunschweig, DE

Sabine Andert
University of Rostock (UR), DE

Oral and Poster Contributions

Please submit your proposal via our symposium website until
30.09.2017

www.ppphe.phytomedizin.org

Plant Protection and Plant Health in Europe

Efficacy and risks of „biorationals“ in organic and integrated pest management - acceptable?

8th International Symposium
jointly organised by DPG, JKI and HU-Berlin

13 – 14 December 2017
Braunschweig, Germany

Julius Kühn-Institute
Messeweg 11-12
38104 Braunschweig

Invitation

www.ppphe.phytomedizin.org

The Symposium Topic

Integrated Pest Management (IPM) and Plant Protection in Organic Agriculture (PPOA), should be science - based decision-making processes that identify and reduce risks from pests and pest management related strategies. They coordinate the consideration of pest biological factors, environmental conditions, and all available instruments to prevent unacceptable levels of pest damage, while concurrently combining economical means with the least possible risk to people, property, resources, and the environment.
We use the widely known term "biorationals", as an operative expression to speak about certain kinds of components of plant protection strategies, which are assumed to have advantages concerning risk characteristics on the one hand while at the same time provide acceptable efficacy in reducing pest impact. Nevertheless it is not our intention to propose a new legal category!
The products we want to speak about are often materials that are biologically-derived or, if synthetic, structurally similar and functionally identical to a biologically occurring material. Micro-organism, plant extracts, basic substances, semiochemicals, as well as non-pesticidal products like biostimulants, biological yield enhancers, plant health promoters, and soil conditioners are a matter of discussion.

Such "biorationals" do not reveal sufficient efficacy against pests alone but are useful to be integrated in plant protection strategies.
In addition, the risk-evaluation requirements under national and European regulatory frameworks of these diverse "biorationals" are very different from each other or there is even a lack of regulatory infrastructure to ensure that "biorationals" get a targeted risk assessment and approval procedure.

On this background, the symposium wants to work out

- a critical perspective on the risk and efficacy evaluation of „biorationals"
- an overview of agricultural and socio-economic experiences with „acceptable" instead of „sufficient" efficacy in pest managment strategies
- impediments to introduce „biorationals" under the existing Sustainable Use Directive 2009/128
- a conclusive statement to promote „biorationals" for use in agriculture

Preliminary Programme

13.12.2017

09:00	**Words of Welcome**
	Dr. G.F. Backhaus, President of JKI
	Section 1: Definitions
09:30	**What are we speaking about? Introduction and Discussion**
	Falko Feldmann (JKI, DE)
10:30	Break
11:00	**A rational for Bio-Products in organic plant health systems**
	Friedhelm von Mering, Peter Röhrig & Jutta Kienzle (BÖLW)

Preliminary Programme

13.12.2017

11:45	**„Biorationals" in IPM strategies**
	Ewa Matyjaszczyk (NRI, PL)
12:30	Break
Section 2: „Biorationals" in agricultural practice	
13:15	**Biorational use in Brasilian agriculture**
	Jose Pereira da Silva Jr. (EMBRAPA, BR)
14:00	4 oral contributions (15 min each)
15:00	Break
	Section 3: Risk and Efficacy Evaluation of „Biorationals"
15:30	**The underestimation of fungal toxicity**
	Holger Deising (University of Halle)
16:15	3 Oral contributions (15 min each)
17:30	**Poster presentation**
18:30	Joint dinner

14.12.2017

	Section 4: Regulatory framework of „Biorationals"
9:00	**Registration of „Biorationals" in practice**
	Geraldine Meunier (Bayer CropScience)
9:30	2 Oral contributions (15 min each)
10:00	**Acceptable vs. Sufficient Efficacy**
	Petr Harasta (CSR) & Jozef Kotleba (SRS)
10:30	2 Oral contributions (15 min each)
11:00	Break
11:30	**Promotion of „Biorationals"**
	Discussion
13:30	End of symposium

www.ppphe.phytomedizin.org

Presen-tation-ID		
		Wednesday, 13 December 2017
	Venue:	**Julius Kühn-Institut Braunschweig**
	09:30	**Words of welcome and introduction** **Prof. Dr. Georg Backhaus, President of the Julius Kühn Institute - Federal Research Institute for Cultivated Plants, Braunschweig, Dr. Alexandra Makulla, BMEL, Dr. Falko Feldmann, Deutsche Phytomedzinische Gesellschaft e.V.**
		Workshop
	09:45	**Definitions: What are we speaking about?** Chair: Falko Feldmann
		Biorational or biological - different concepts with impact on practical use Markus Weinmann
		Session 1: „Biorationals“ in agricultural practice I Chair: Falko Feldmann
1-1	10:30	**A rational for Bio-Products in organic plant health Systems** Friedhelm von Mering, Peter Röhrig & Jutta Kienzle (BÖLW)
1-2	11:00	**„Biorationals“ in IPM strategies - the state of the art** Ewa Matyjaszczyk (NRI, PL)
1-3	11:30	**Acceptable vs. Sufficient Efficacy in plant protection - what does it mean for the farmer?** Petr Harasta (CSR) & Jozef Kotleba (SRS)
1-4	12:00	**Assessment methods for biostimulants** Günter Neumann (Uni Hohenheim)
	12:30	**Break for Lunch**
		Session 2: „Biorationals“ in agricultural practice II **Chair: Ewa Matyjaszczyk**
2-1	14:00	**Micro-organsims between plant protection and biostimulation** Jörg Geistlinger (Hochschule Anhalt-Bernburg)

2-2	14:30	**Biorational use in Brasilian agriculture** Jose Pereira da Silva Jr. (EMBRAPA, BR)
2-3	15:00	**Biorational use in Hungarian agriculture** Borbala Biro
	15:30	**Break**

Session 3: Biosafety of „Biorationals“

Chair: BVL?

2-4	16:00	**Identity of microbial "biorationals" - efficacy of microbial consortia** Kornelia Smalla (JKI Braunschweig)
2-5	16:30	**Clean biostimulant inoculants - an illusion?** Markus Becker (Bactiva) ?
2-6	17:00	**The underestimation of fungal toxicity** Holger Deising (University of Halle)
2-7	17:30	**Discussion**
	18:00	**Working dinner in JKI**

Thursday, 14 December 2017

Session 3: Regulatory framework for "Biorationals"

Chair: Ewald Sieverding

3-1	08:30	**Registration of „Biorationals“ in practice** Geraldine Meunier (Bayer CropScience)
3-2	09:00	**Double standards to be applied for "Biorationals"** Christina Donat

3-3	09:30	**Regulatory differentiation between Plant Protection and Biostimulation** NN. BMEL/Brüssel
3-4	10:00	**Discussion of the regulatory framework**
	10:30	Break

Session 4: Promotion of "Biorational" use

Chair: Mering BÖLW?

4-1	11:00	**The WG of Sustainable Plant Protection ?** Silke Saydeeh?
4-2	11:30	**Improving the availability of plant protection solutions - the ECEG** Leinhos et al.
4-3	12:00	**The BIOFECTOR PROJECT database** Rolf Mäder?
4-4	12:30	**Conclusions**
4-5	13:00	**End of Symposium**
	14:00	**Workshop: Commentation of current public documents related to symposium topic** Chair: Falko Feldmann
	17:00	**End of workshop**

PLANT PROTECTION & PLANT HEALTH IN EUROPE

Julius Kühn-Institut

Bundesforschungsinstitut für Kulturpflanzen

Federal Research Centre for Cultivated Plants

Efficacy and risks of “biorationals” in organic and integrated pest management - acceptable?

8th International Symposium on Plant Protection and Plant Health in Europe

DPG

JKI Julius Kühn-Institut

HUMBOLDT-UNIVERSITÄT ZU BERLIN

IAPPS

We welcome
72 delegates
from 14 countries

What are we talking about?

Integrated Pest Management
following Directive 2009/128/EC

Source: JKI

Conventional Pesticides
Pesticides with higher risk to health and environment…

Biorationals
Biological Pesticides with low negative impact to health and environment, such as botanicles, microbials, basic substances…

Biological Control
Predators, parasitoides, nematodes, viruses, fungi, endophytes…

Physical & Mechanical Control
Pruning, weeding, mulching, traps, barriers, flaming…

Cultural Control
Site & plant selection, fertilization, sanitation, rotation…

Statement:

„Biorational or biological – different concepts with impact on practical use“

Markus Weinmann, University of Hohenheim

‚Biorationals'

- Natural Low Risk Substances (incl. Microorganisms)
- Basic substances

- Pheromone & Kairomone (Semiochemicals)

- Biostimulants, especially. microorganisms

- Soil improvers
- Organic fertilizers
- Mineral fertilizers

Efficacy of ‚Biorationals‘

‚Biorationals‘	**Efficacy in assessment**
• Natural Low Risk Substances (incl. Microorganisms)	• **Moderate – good (variable)**
• Basic substances	• **Not tested**
• Biostimulants	• **Tested in future?**

Sufficient information provided to customer/user?

Are there hidden risks?

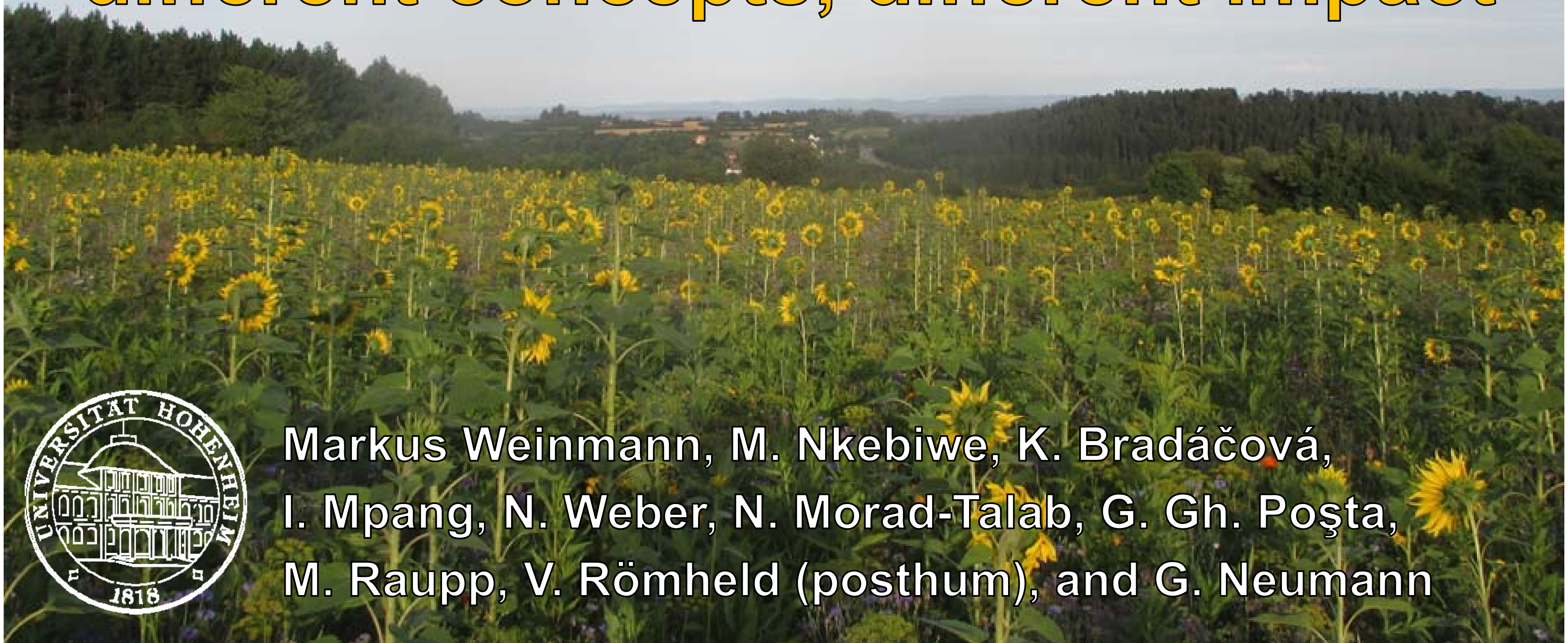
We hope that the symposium will answer the following questions:

- Which advantages do biorationals have for organic and integrated plant production systems?
- How effective are biorationals and how should we declare the efficacy for the user?
- Are there risks overseen during the assessment of microbial products?
- Is the regulatory framework sufficiently organised for biorationals?
- How could we promote biorational use and which impediments exist for intensifying the use?

Let´s go!

Plant Protection and Plant Health in Europe: Efficacy and risks of „biorationals“ in organic and integrated pest management - acceptable?

8th International Symposium, 13 - 14 December 2017, Braunschweig, Germany



Bio-rational or bio-logical: different concepts, different impact

Markus Weinmann, M. Nkebiwe, K. Bradáčová, I. Mpang, N. Weber, N. Morad-Talab, G. Gh. Poşta, M. Raupp, V. Römheld (posthum), and G. Neumann

BIOFECTOR was funded by the European Union within the 7th Framework Programme, Grant Agreement No. 312117

Bio-Rational or Bio-Logical: Is there a Difference ?

“In the beginning was the Word, …”
(John 1,1)

Bio: from Greek βίος, bios, ‘life’

Logical: from Greek λόγος, logos, ‘word, reason’

Rational: from Latin ratio, ‘reason’

“I cannot the mere Word so highly prize;
I must translate it otherwise …”
(Goethe: Faust I, 1808)

What is Reasoning ?

"reckoning of consequences"

(Hobbes, 1660: The Leviathan, V)

Rational → calculation

"inner dialogue of the soul with itself"

(Plato, ca. 360 BC: Sophist, 263e3-5)

Rational → soul inherent logic

What are Bio-Rationals ?

(Bio-) agents or processes with specific action against harmful organisms, but limited or no effect on non-target organisms

(e.g. Horowitz et al., 2009, in the text book "Biorational Control of Athropod Pests", Springer)

Rationality is the purposeful calculation of the most efficient means and procedures to realize goals.

(e.g. Max Weber, 1922)

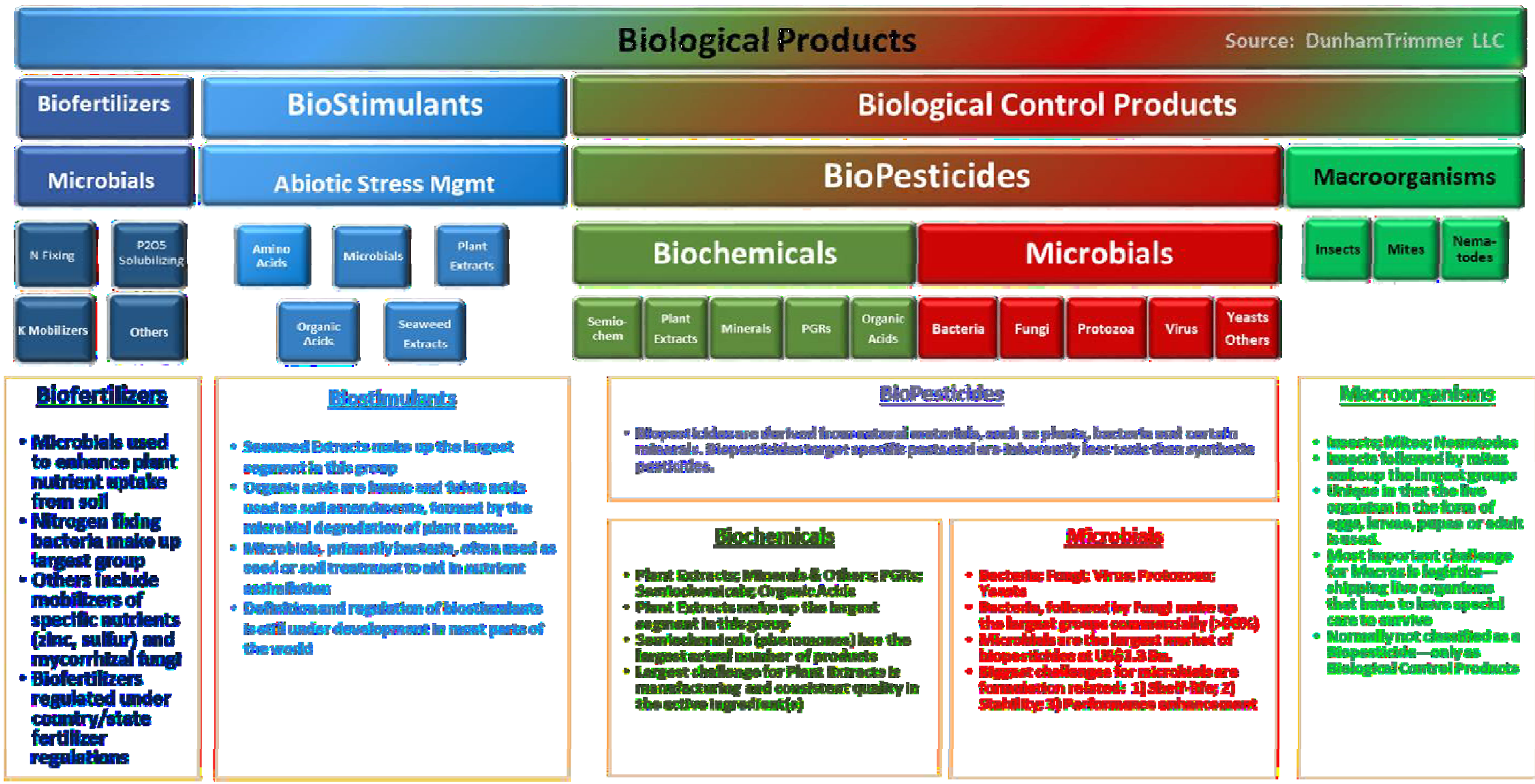
"*The use of the word 'biorational' should be avoided to prevent confusion because of the diversity of the definitions applied to the term*."

"*The term 'biorational' is inherently defective, in that it implies that chemical pesticides are irrational.*"

(Hall & Barry, 1995; cited in Crump et al., 1999)

Global Relevance of Bio-Logicals

Market Overview by DunhamTrimmer



Presented by Willem Ravensberg, IBMA at the MiCROPE conference in Vienna, 07. December 2017

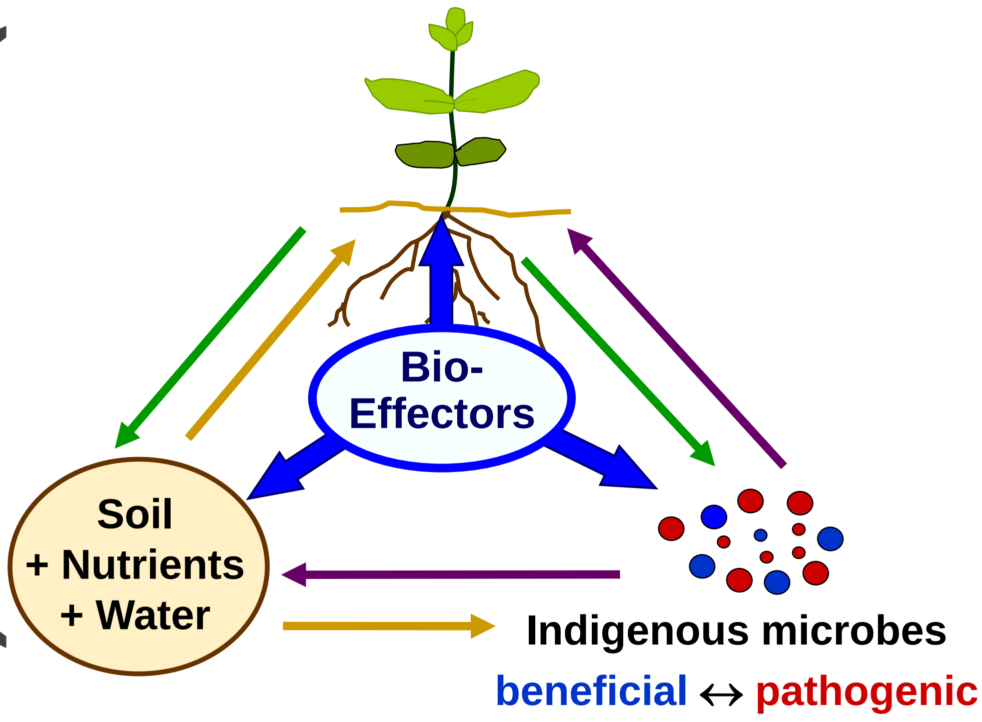
What are Bio-Logicals ?

Utilization Groups / Intension

- Bio-stimulants
- Bio-fertilizers
- Bio-pesticides
- Bio-control agents

Bio-logicals

Active Ingredients / Mode of Action



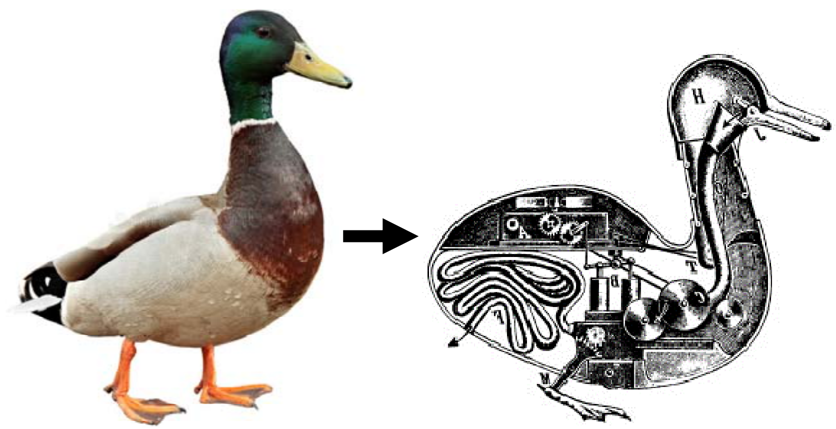
Functional implementation or activation of biological mechanisms, especially those interfering with soil-plant-microbial interactions.

No direct input of mineral nutrients or toxins in the sense of fertilizers or pesticides.

Is it Time for a Paradigm Shift in Natural Science?

Reductionism/positivism

- Systems / living things are fully determined by their components
  - → Genetics
  - → Physico/chemical factors
- Only empiric knowledge is valid



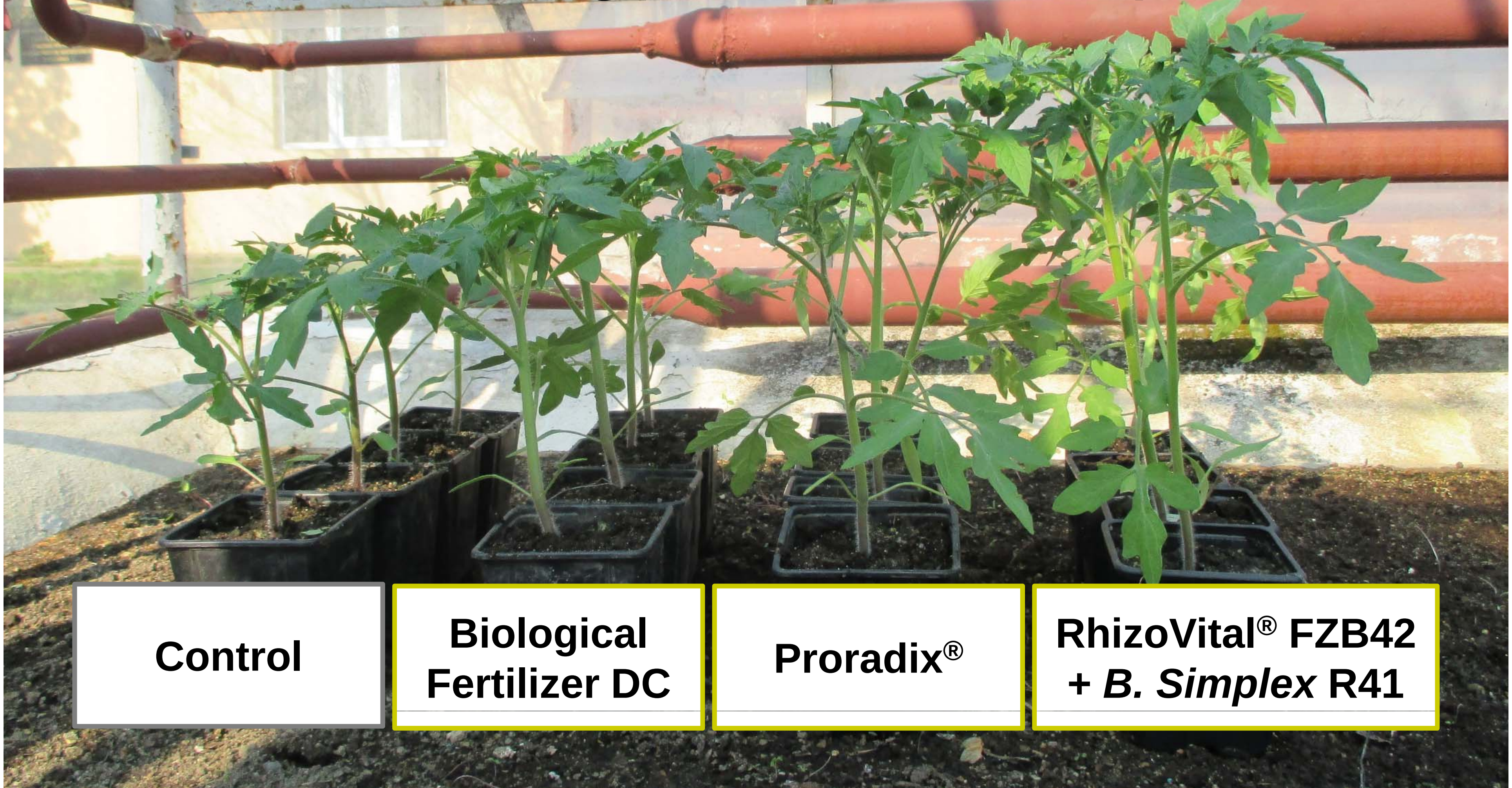
Holistic view

- Reality is complex and creative
  - → Feedback loops
  - → Synergistic interactions
  - → Spontaneity and active learning
- Organisms are organized life forms

The ordered whole is other than the sum of its parts !

Tomato greenhouse trial

Bio-effector treatments improved the shoot growth of tomato plants during pre-culture in small pots.



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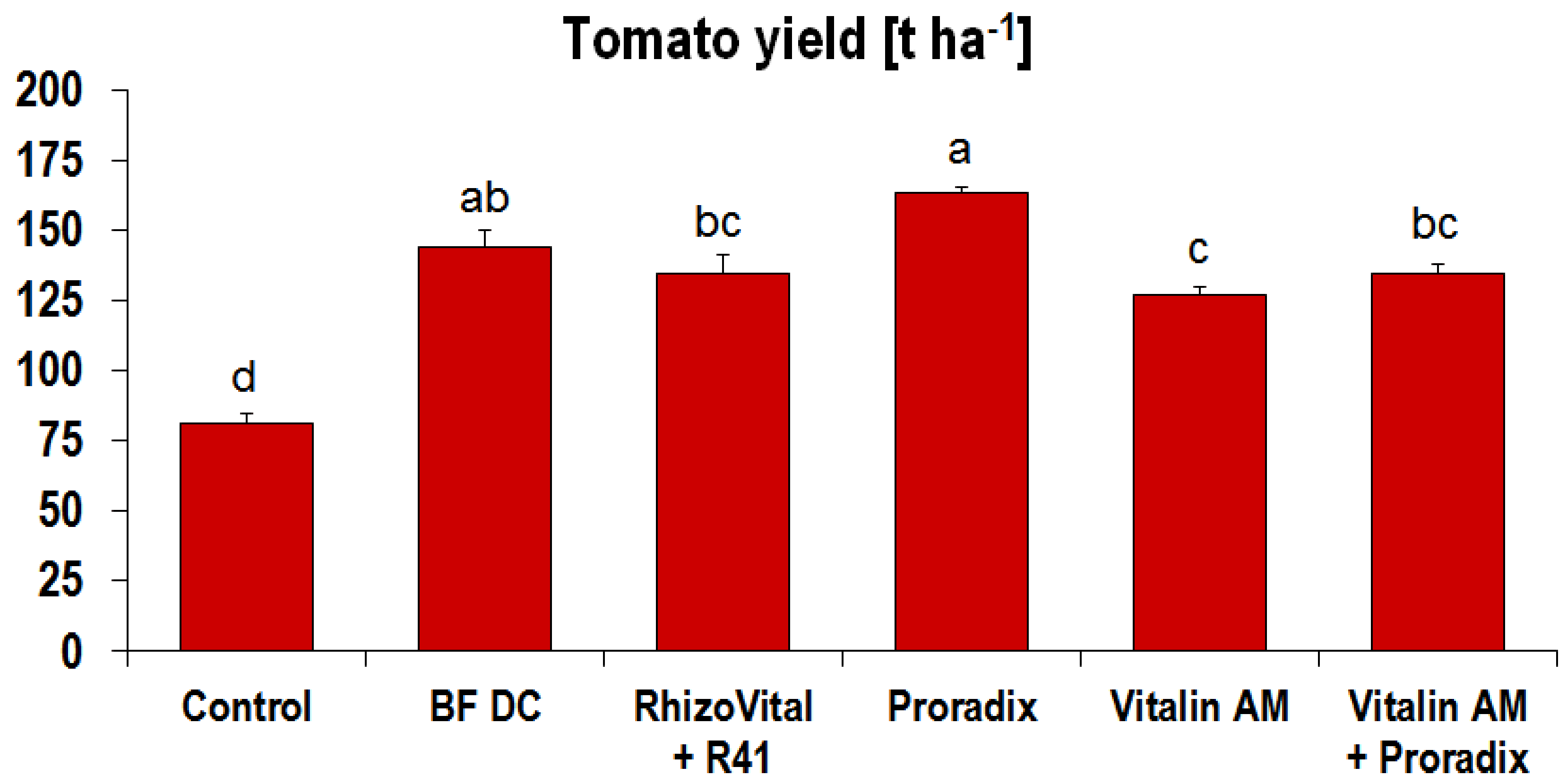
Tomato greenhouse trial

Results from the production phase



Bio-effector treatments induced strong yield increased in tomato production (Tukey test, $p < 0.05$, data not normally distributed)

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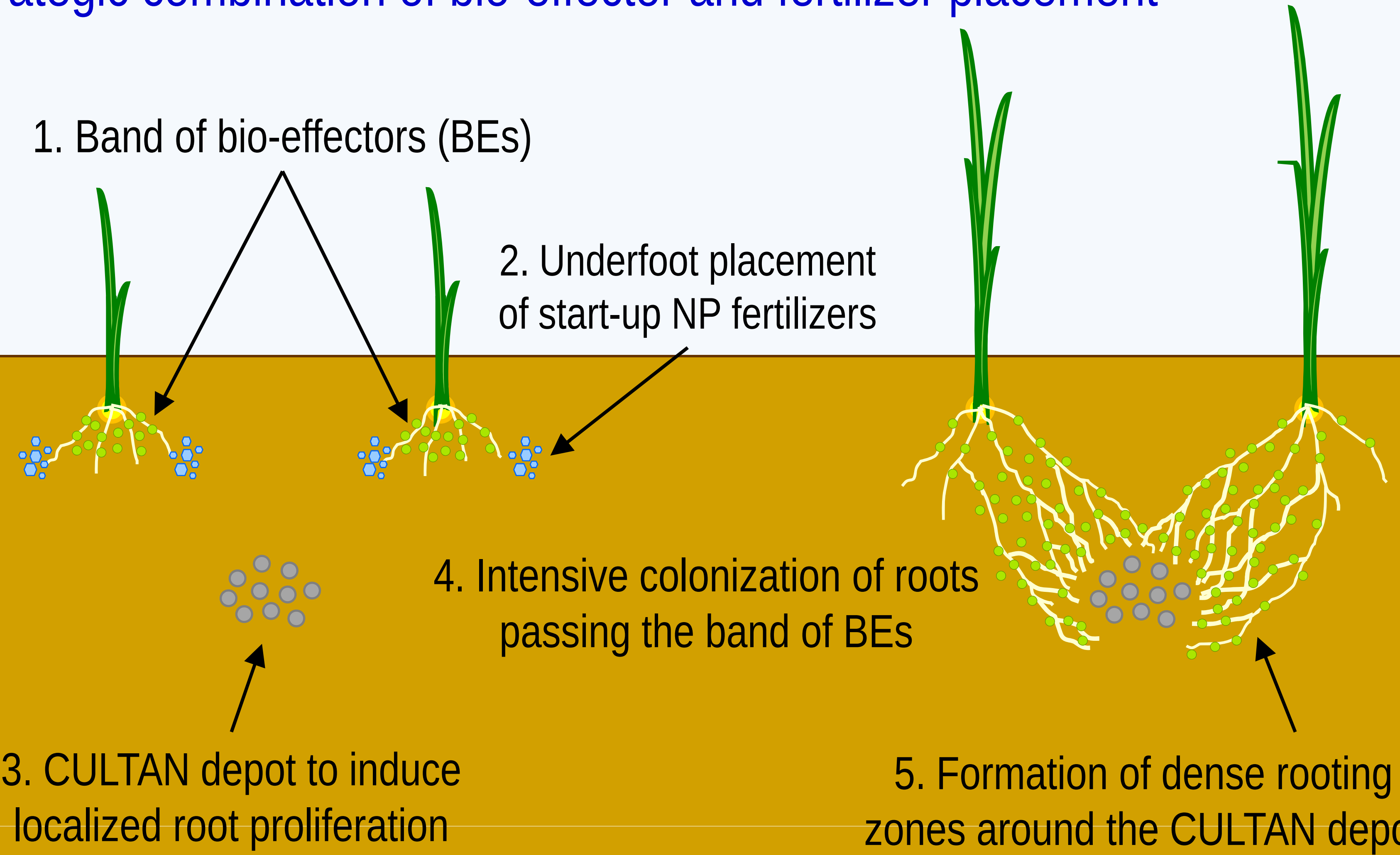
Placement strategies in Maize

Strategic combination of bio-effector and fertilizer placement



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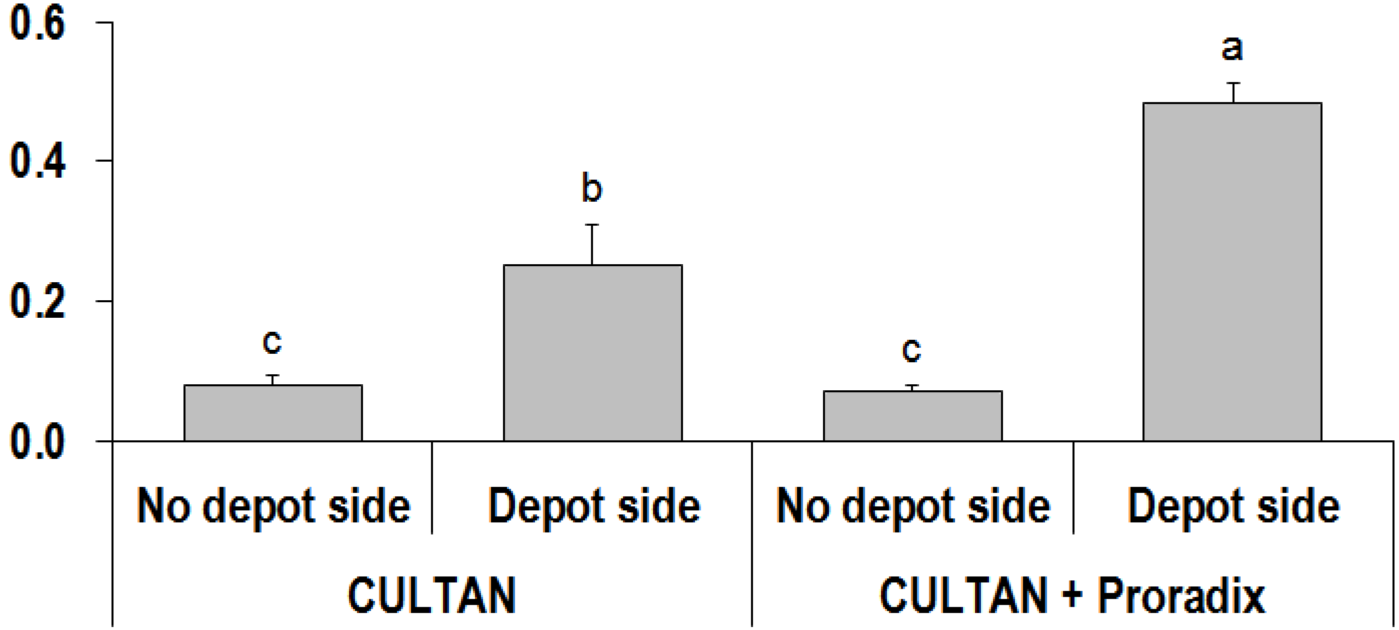
Placement strategies in Maize

Rooting density

CULTAN depots induced the formation of dense rooting zones

Placement strategies in Maize

Root length density per soil volume at 12 weeks after sowing, 7 weeks after CULTAN application [cm cm^{-3}]



Higher root density at the depot side compared to the side without depot.
Proradix® further increased the root density at the depot side.
(Tukey test; $p < 0.05$; data not normally distributed)

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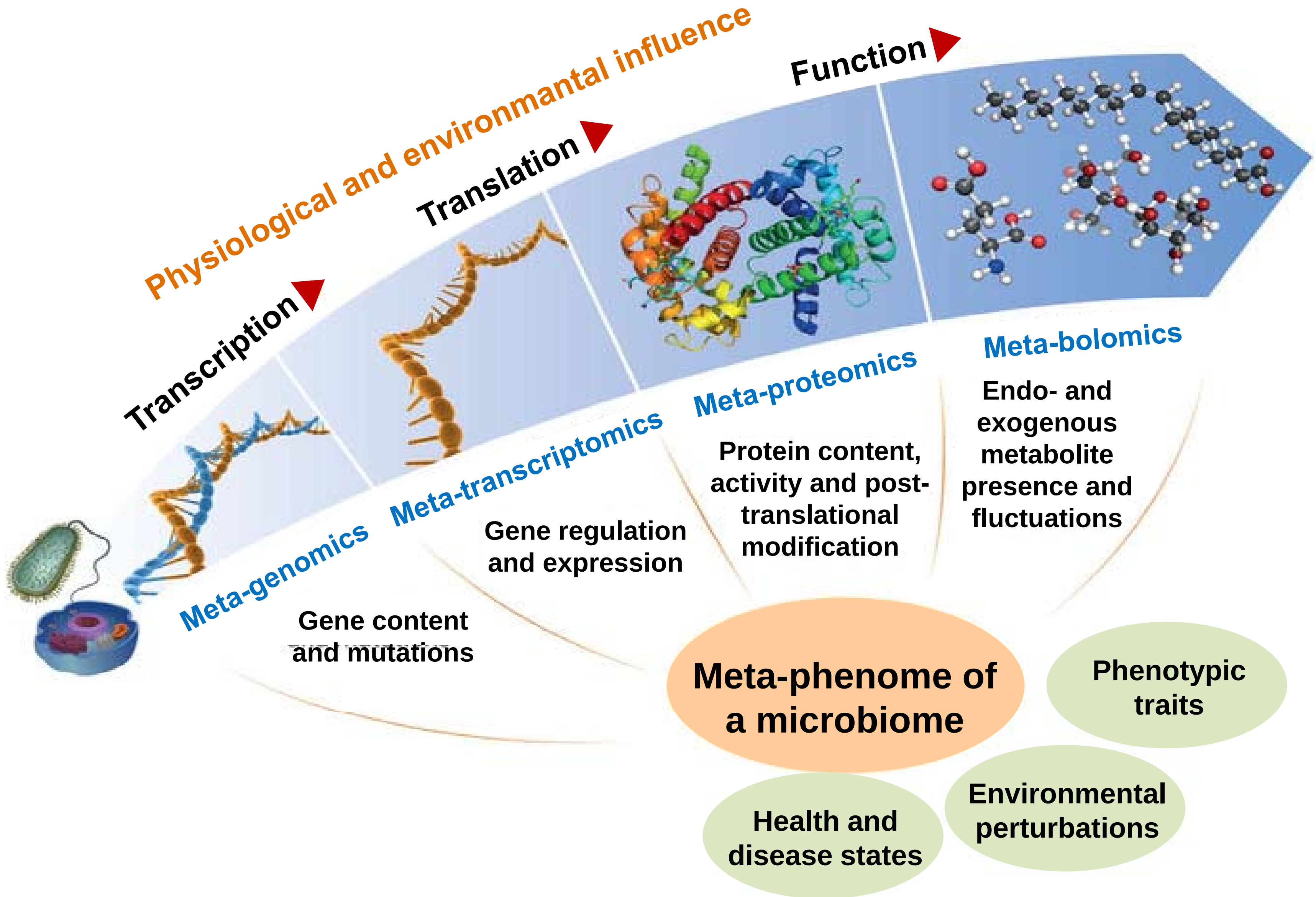
Microbiomes are Meta-Phenomena:

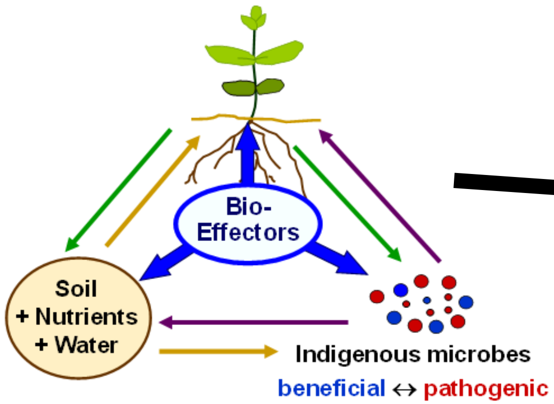


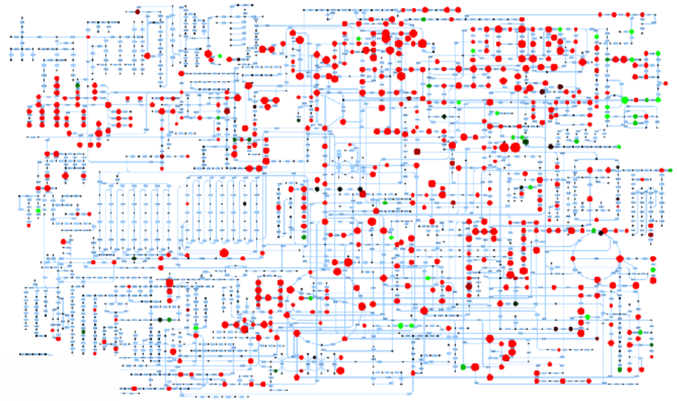
Jansson and Baker (2016). Nature Microbiology 1, 16049

Better understanding of functional traits by multi-omics ?

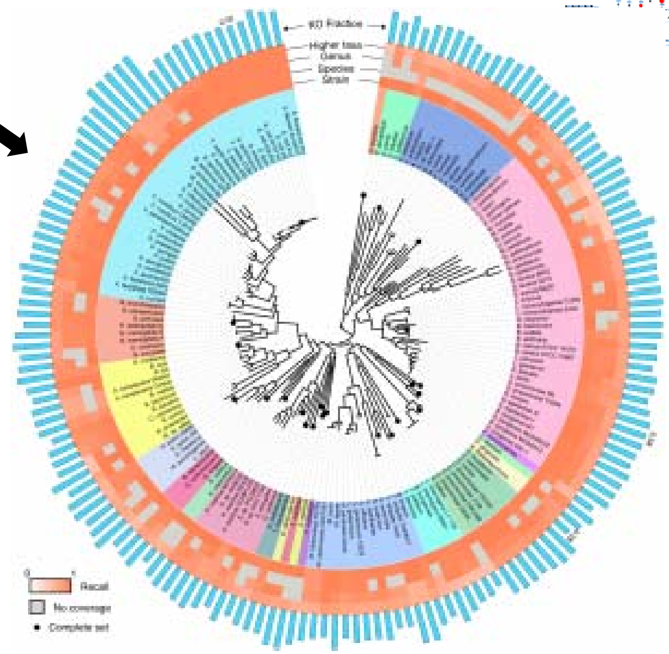
Future Grand Challenge ?

Meta-Analyses to evaluate Multi-Omics BIG DATA !

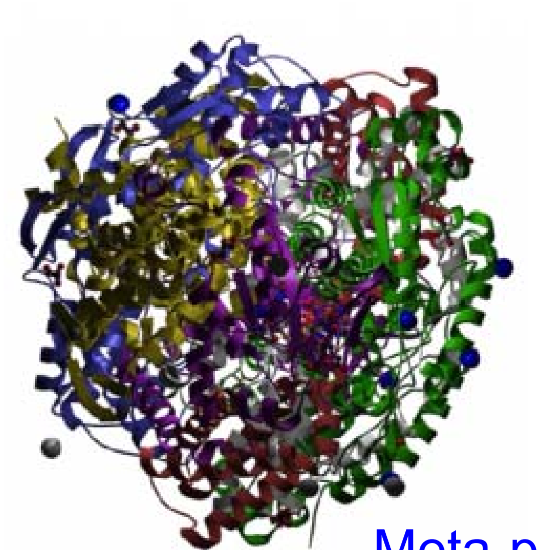




Meta-bolomics:
tens of thousands of
metabolites



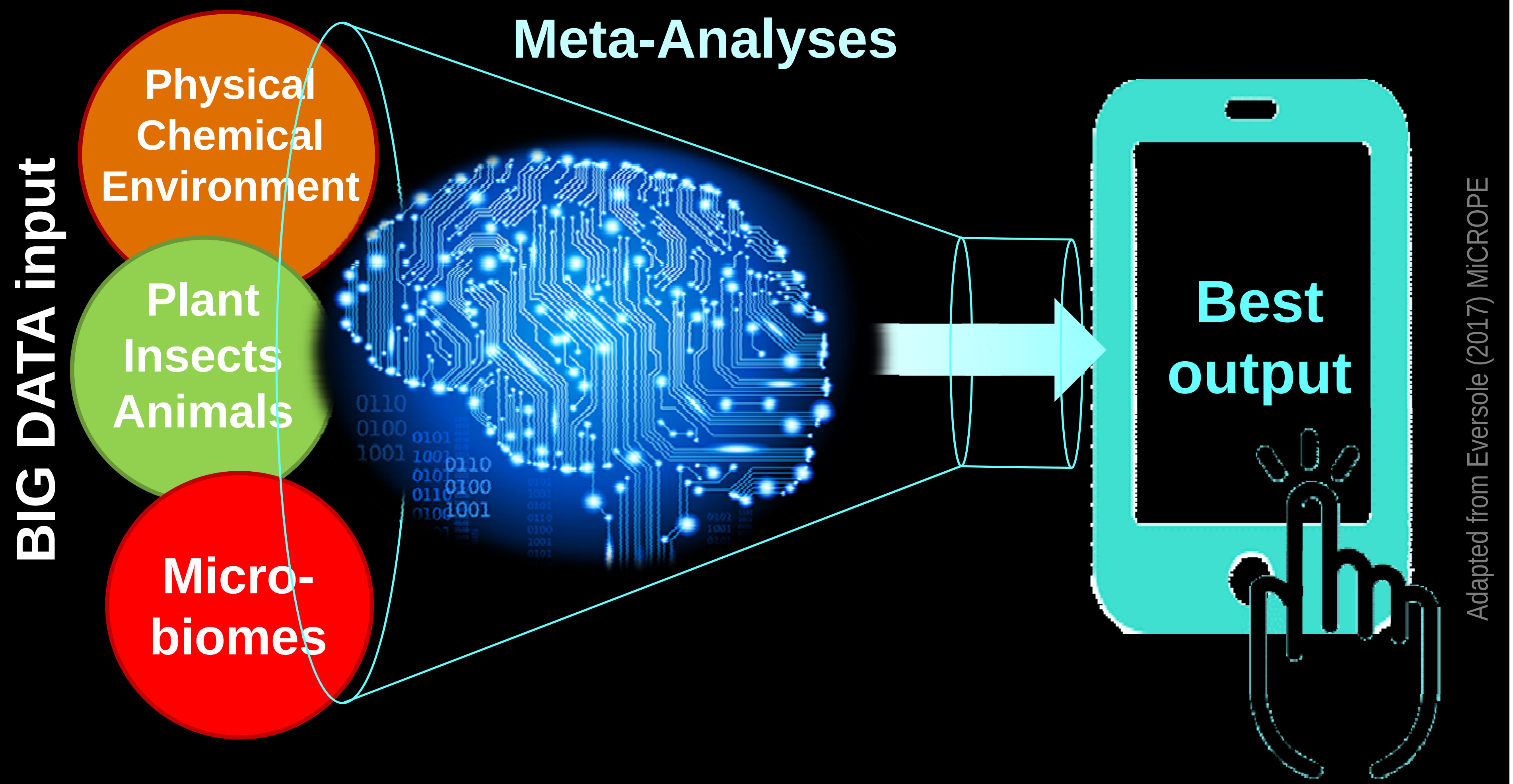
Meta-genomics:
millions of genes



Meta-proteomics:
tens of thousands of proteins

Adapted from Jansson and Baker (2017)

Vision of an Evidence Based Agricultural Science



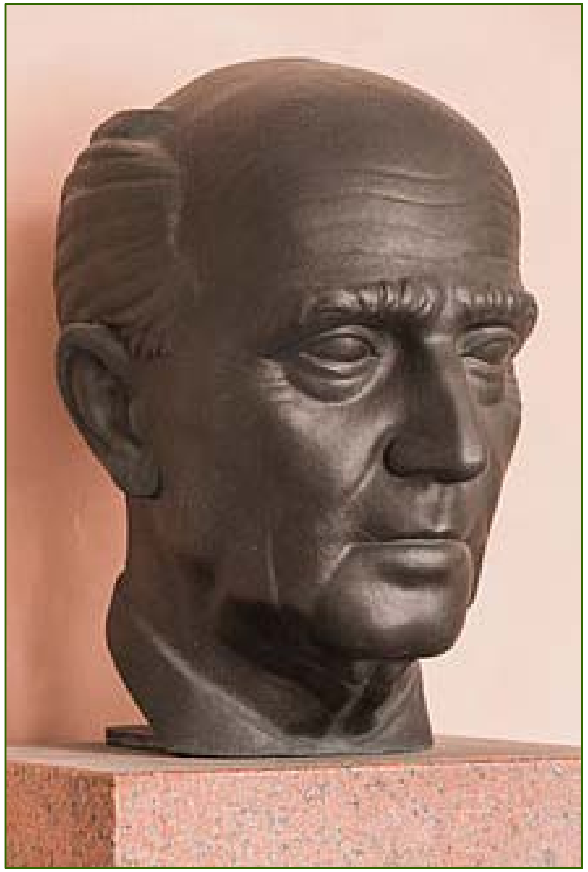
Producing recommendations based on computational management of empirical data or reason-able learning?

Are Meta-Physical Questions Dispensable in a holistic Meta-Worldview ?

- **What is the best (n or n + 1) ?**
- **What is the optimal ?**
  - Individual ↔ social interests
  - Economic ↔ ecological imperatives
- **What is justice ?**
  - Objective criteria
  - Distinction of good ↔ bad
- **What enables true knowledge?**
  - Empirism (posteriori)
  - Reason-ability (apriori)

Legal Positivism [from Latin ponere, ‘to put’]

Laws are commands of human beings recognized as legal authorities



Bust of Hans Kelsen 1881-1973,
University of Vienna

“*Nature is an aggregate of objective data linked together in terms of cause and effect*”

Norms can only come from the will.

Nature can only contain norms if a will had put them there.

This would presuppose a Creator God, whose will had entered into nature.

Legislation of Bio-pesticides in the European Union

REGULATION (EC) No 1107/2009: concerning the placing of **plant protection products** on the market

Article 2.2:

"*… shall apply to substances, including* ***micro-organisms having general or specific action*** *against harmful organisms …*"

Article 77, ***Guidance documents****:*

"The Commission may, …, adopt or **amend** technical and other guidance documents … concerning micro-organisms, pheromones and biological products, for the implementation of this Regulation."

- REG. (EU) No 283/2013: **data requirements** for active substances
  PART B MICRO-ORGANISMS
- REG. (EU) 2017/1432 **approval of low-risk active substances**

Scientific understanding of “Bio-logical control”

Bio = living → activity of living organisms

Bio = biotic → mechanisms, processes and products related to living organisms

Bio = biological → reason-able use of knowledge on living organisms and their vital processes

“Biological control is ***based on human’s understanding*** *of living organisms which is implemented for the purposeful management of natural controls …”*

(Barbosa and Braxton, 1993)

Legislation of Bio-stimulants and Bio-fertilizers

Proposal for a REGULATION (EC) laying down rules on the making available on the market of CE marked fertilizing products

Bio-stimulants: are not as such nutrients (independent of the nutrient content), but nevertheless stimulate plant nutrition processes tolerance to abiotic stresses, quality traits

Bio-fertilizers: not extra defined (included under bio-stimulants)

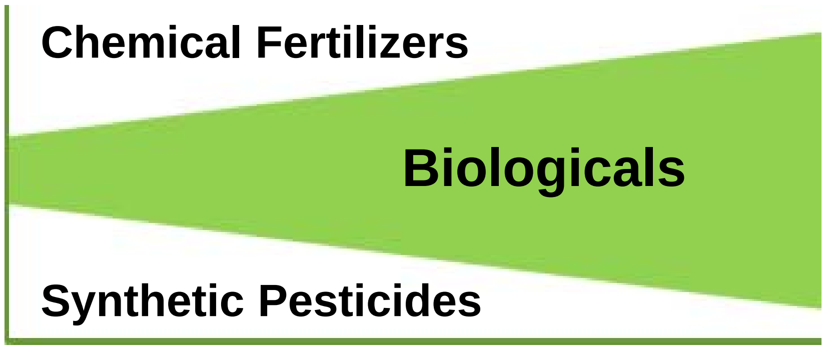
Positive list for Microorganisms:

- *Azotobacter* spp.
- Mycorrhizal fungi
- *Rhizobium* spp.
- *Azospirillum* spp.

Outlook on the role of bio-logicals in agriculture

Increasing interest from big companies and regulatory authorities

- Success rates screening of new microbial products 100 times higher than for chemical compounds
- Costs for product development 10 times lower
- Are regulatory policies compromising future developments?



The global market for agricultural “biologicals” has been estimated to be worth 15 billion US $ in 2016 and is projected to reach 20 billion US $ by 2021 (CAGR of 6 %).

Bio-pesticides having the largest share.

(age - LW 1/2017; Market Data Forecast, 2017; Ravensberg, MiCROPE, 2017)

Conclusions

- A strict legal categorization as bio-pesticides or bio-stimulants (bio-fertilizers) may hamper a holistic view of plant ecology and agricultural problem-solving

- A one-sided functional classification of microbial agents as bio-pesticides would disregard many of their other beneficial traits that could be reasonably used in integrated strategies for sustainable plant nutrition.

- The term “bio-effector” is more appropriate when the active agent and not the purpose of a specific application is meant

Cordial Thanks for Your Attention !

Markus Weinmann, M. Nkebiwe, K. Bradáčová, I. Mpang, N. Weber, N. Morad-Talab, G. Gh. Poşta, M. Raupp, V. Römheld (posthum), and G. Neumann

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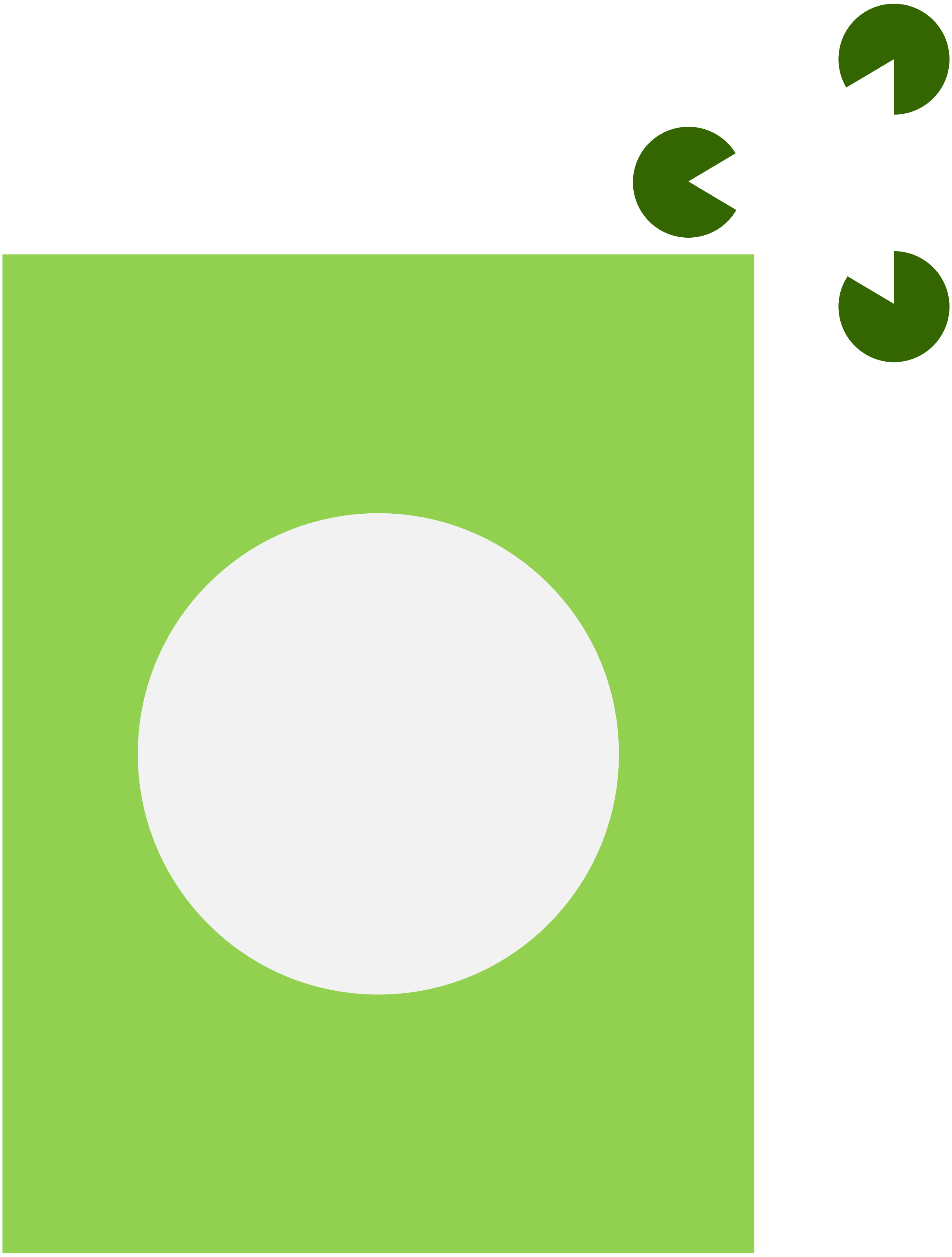
BIOFECTOR was funded by the European Union within the 7th Framework Programme, Grant Agreement No. 312117

categorization into bio-stimulants, bio-fertilizers or bio-pesticides may hamper a holistic view of plant ecology and agricultural problem-solving

term “bio-effector” as defined in Chapter 1.1, when the active agent and not the purpose of a specific application is meant more appropriate and scientifically correct

PLANT PROTECTION & PLANT HEALTH IN EUROPE



WP08: Major Topics since September 2016

- **Combination Strategies**

➡ Bio-Effectors + Ammonium and/or Phosphate Fertilizers

➡ Bio-Effectors + Organic Fertilizers

➡ Bio-Effectors + Bio-Effectors in “Combi Products”

- **Stress Mitigation**

➡ Micronutrients and Silicates against cold / drought stress

- **Cost efficient and effective Application Strategies**

➡ Placement of Bio-Effectors and/or Fertilizers

➡ Spray Application of Bio-Effectors (e.g. against Cold Stress)

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What are Bio-Rationals ?

"*The use of the word 'biorational' should be avoided to prevent confusion because of the diversity of the definitions applied to the term*."

"*The term 'biorational' is inherently defective, in that it implies that chemical pesticides are irrational.*"

(Hall & Barry, 1995; cited in Crump et al., 1999)

"*substances or processes that when applied in a specific system or ecological context have little or no adverse consequence for the environment and non-target organisms, but cause lethal or other suppressive or behavior modifying action on a target organism and augment the control system*". (Horowitz et al., 2009)

BUAS: Combination Strategies in Tomato

Experimental set-up at Timisoara, Romania:

Plant: Tomato (*Lycopersicum esculentum* Mill.) var. Primadona, Hazera

Nursery substrate: 45 % manure, 30 % garden soil, 15 % peat, 10 % sand

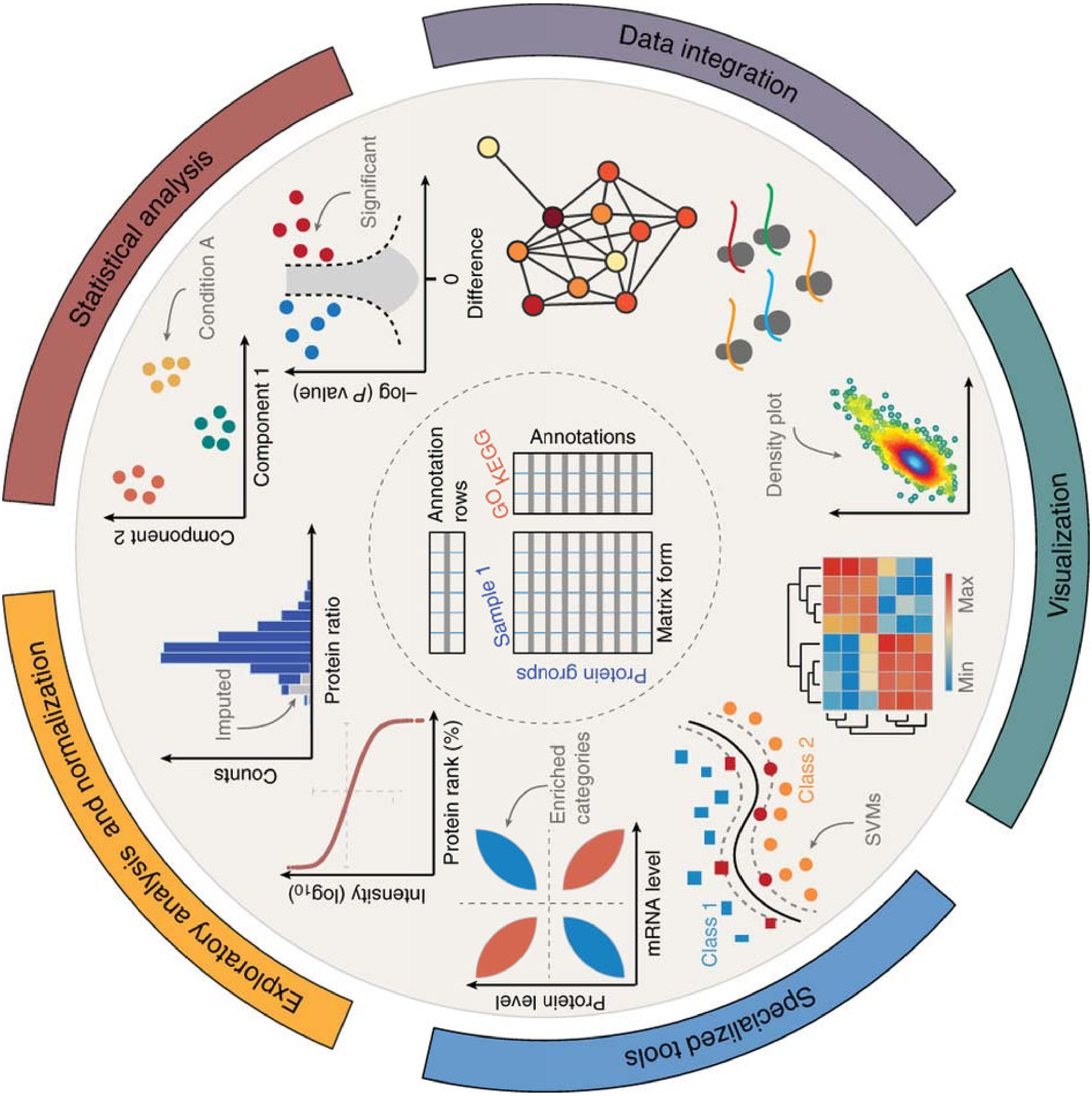
Cultivation substrate: Clay peat substrate (Patzer, Sinntal), ca. 10 liter pot^{-1}

Basal fertilization: 140 mg N, 70 mg P, 149 mg K $liter^{-1}$ + micronutrients

Bio-effector treatments, applied as suspension in water:

Microbial bio-effector product	1st Application: during nursery in small pots	2nd Application: at transplanting into big pots
Proradix® WP	$2.6*10^8$ cfu $plant^{-1}$	$3.3*10^9$ cfu $plant^{-1}$ or not
RhizoVital® FZB42+R41	each $2.0*10^8$ cfu $plant^{-1}$	each $2.5*10^9$ cfu $plant^{-1}$
Biological Fertilizer OD	$1.0*10^7$ spores $plant^{-1}$	$1.3*10^8$ spores $plant^{-1}$
ECAG 2895 (Mix)	$3.8*10^3$ cfu in total $plant^{-1}$	$4.7*10^4$ cfu in total $plant^{-1}$, repeated every 2 weeks

BIOFECTOR is funded by the European Union within the 7th Framework Programme, Grant Agreement No. 312117


Data integration
Statistical analysis
Visualization
Specialized tools
Exploratory analysis and normalization
Significant
Difference
0
−log (P value)
Condition A
Component 1
Component 2
Annotation rows
GO KEGG
Annotations
Sample 1
Matrix form
Protein groups
Density plot
Max
Min
Imputed
Protein ratio
Counts
Protein rank (%)
Intensity (log10)
Enriched categories
mRNA level
Protein level
Class 1
Class 2
SVMs

WP08: Overall Highlights

Country	Crops	Bio-Effectors	Fertilizers	Soil P level	Plant growth results
Germany	Wheat	NH_4^+ + Microbial	Min. / org.	low	Tendential increases
Germany	Maize	NH_4^+ + Microbial	Min. / org.	low	Regional differences
Italy	Maize	NH_4^+ + Microbial	Organic	very low	Improved early growth
Czech	Maize	NH_4^+ + Microbial	Mineral	low	No improvement
N. Ireland	Wheat	Algae etc.	Mineral	unknown	Manek improved yield
Germany	Wheat	Algae etc.	Mineral	low	Manek improved yield
Germany	Maize	Si, Mn/Zn	Mineral	high	Improved early growth
Romania	Wheat	Microbial	Organic	low	Annual variations
	Maize				Annual variations
	Tomato			very high	Improved yield
Hungary	Tomato	Microbial	Organic	very high	Improved yield + quality
Israel	Tomato	NH_4^+ + Microbial	Mineral	low	Tendential increases

BIOFECTOR is funded by the European Union within the 7th Framework Programme, Grant Agreement No. 312117

Bund Ökologische Lebensmittelwirtschaft

A Rationale for „Bio“ in Organic Farming

Friedhelm v. Mering, BÖLW e.V.

BOLW
Bund Ökologische Lebensmittelwirtschaft

Organic farming: principles and legislation I

Global definition (IFOAM 2005):

"Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved."

IFOAM ORGANICS INTERNATIONAL

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Organic farming: principles and legislation II

Legal definitions of organic farming:

§ EU Organic Regulations 834/2007 & 889/2008

§ NOP, JAS,… (87 Countries in total)

Key elements in **all** legal standards:

✓no synthetic pesticides,

✓no synthetic fertilizers,

✓no GMO

Plant protection strategies in organic farming

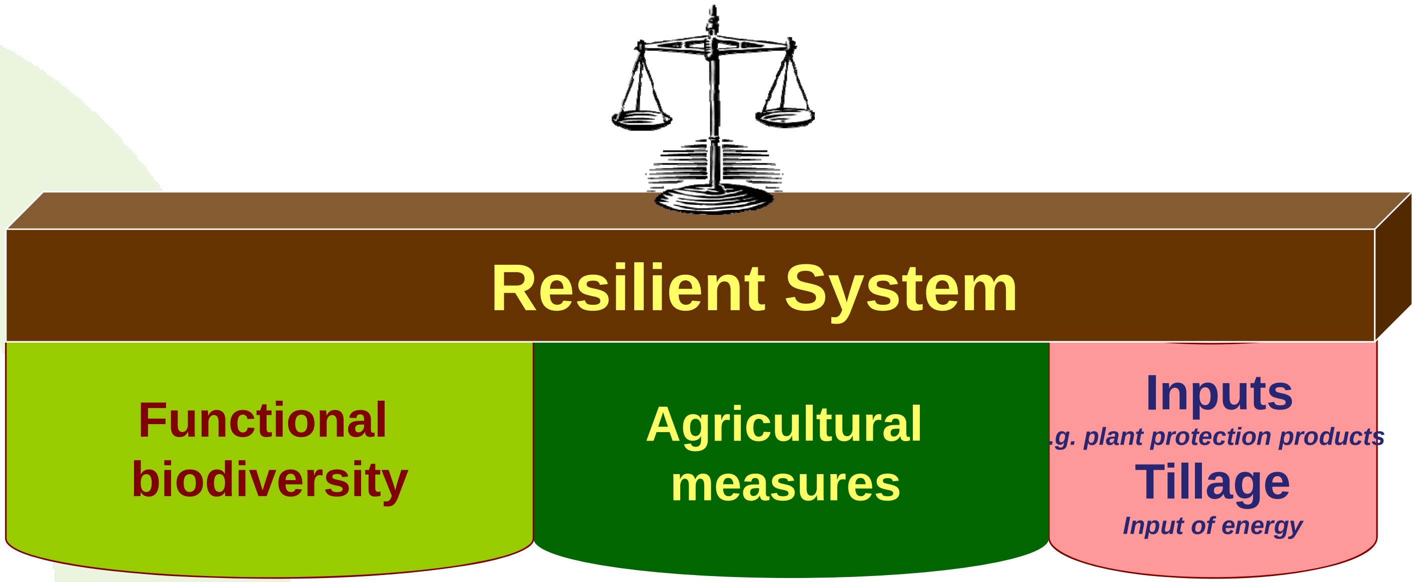
Principles and legislation provide **narrow framework**:

☞ emphasis on **prevention** of calamities (crop rotation, crop management, resistant varieties, functional biodiversity)

☞ minimal dependency on inputs

☞ no application of PPP on ≈ 95 % of organic area

☞ all inputs must be **naturally occurring substances** and must be „in line with the principles of organic farming“ (EGTOP)



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ANNEX II

Pesticides — plant protection products referred to in Article 5(1)

Note:

A: authorised under Regulation (EEC) No 2092/91 and carried over by Article 16(3)(c) of Regulation (EC) No 834/2007
B: authorised under Regulation (EC) No 834/2007

1. Substances of crop or animal origin

Authorisation	Name	Description, compositional requirement, conditions for use
A	Azadirachtin extracted from *Azadirachta indica* (Neem tree)	Insecticide
A	Beeswax	Pruning agent
A	Gelatine	Insecticide
A	Hydrolysed proteins.	Attractant, only in authorized applications in combination with other appropriate products of this list
A	Lecithin	Fungicide
A	Plant oils (e.g. mint oil, pine oil, caraway oil).	Insecticide, acaricide, fungicide and sprout inhibitor.
A	Pyrethrins extracted from *Chrysanthemum cinerariaefolium*	Insecticide
A	Quassia extracted from *Quassia amara*	Insecticide, repellent
A	Rotenone extracted from *Derris* spp. and *Lonchocarpus* spp. and *Terphrosia* spp.	Insecticide

Note: all substances = **naturally ocurring** substances!

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PPP in organic farming

☞ Low efficacy (most, not all products)

☞ (Very) limited number of inputs authorized

Inputs very important as tools in holistic/combination strategies!

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What term to define inputs for organic farming?

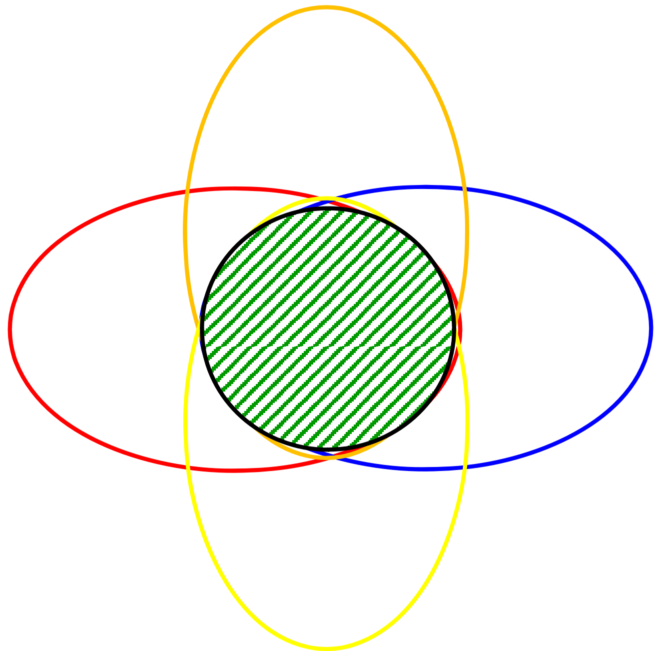
„Botanicals“?

„Semiochemicals“?

„Minerals“?

„Biorationals“?

Naturally occurring substances!



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Why naturally occurring substances?

Environmental fate of „new“ (= synthetic) substances is never predictable ☞ **precautionary approach** in organic farming systems

Concentration of naturally occurring substances:

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Challenges for risk assessment

Naturally occurring substances are „special“ and should be regulated as a separate category – with risk assessments based on specific Guidance Documents and evaluated by specially trained staff

Guidance must take natural exposition into account
☞ Applying similar „safety factors“ as for synthetic substances can result in test conditions that do not mirror natural environments

Many substances are „multiple actors“ (e.g. PPP & fertilizer)

Most substances are not „highly efficient“, but their efficacy is high enough if used in combination strategies

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Challenges for registration

PPP Registration process is adapted to „syntheticals“ – not really suitable for most naturally occurring substances

Regulation 1107/2009/EC ☞ Guidance documents for assessment of botanicals, pheromones and microorganisms (but not for minerals)

High cost of generating data for a registration dossier
limited possibility for the protection of Intellectual Property
Limited market, especially for selective products
☞ Low return on investment
☞ No investment ☞ no registration ☞ limited number of substances!

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Thank you!

Any Questions?

Dr hab. Ewa MATYJASZCZYK, prof. PPI-NRI
Plant Protection Institute – National Research Institute, Poland

Braunschweig, 13-14.12.2017

BIORATIONALS in Integrated Pest Management strategies

Agenda

- **What the law says?**
- How the biorationals fit into IPM rules?
- Misunderstandings
- Availability
- Conclusions

IPM is obligatory in the EU

General principles of integrated pest management (…) are implemented by all professional users by 1 January 2014.*

*Directive 2009/128/EC

DIRECTIVE 2009/128/EC of 21 October 2009 **establishing a framework for Community action to achieve the sustainable use of pesticides**

"Directive establishes a framework to achieve a sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment and promoting the use of **integrated pest management** and of alternative approaches or techniques such as **non-chemical alternatives to pesticides**."

- '**integrated pest management**' means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and <u>other forms of intervention</u> to levels that are <u>economically</u> and ecologically justified and reduce or minimise risks to human health and the environment.

- '**non-chemical methods**' means alternative methods to chemical pesticides for plant protection and pest management, based on agronomic techniques (…) or physical, mechanical or biological pest control methods;

DIRECTIVE 2009/128/EC of 21 October 2009 **establishing a framework for Community action to achieve the sustainable use of pesticides**

- In very sensitive areas (such as Natura 2000 sites, parks and gardens, sports and recreation grounds, school grounds and children’s playgrounds): appropriate risk management measures should be established and low risk pesticides as well as biological control measures should be considered in the first place.

Agenda

- What the law says?
- **How the biorationals fit into IPM rules?**
- Misunderstandings
- Availability
- Conclusions

General principles of integrated pest management

(Annex III of the Directive 128/2009)

1. Prevention and/or suppression of harmful organisms.* (!)
2. Monitoring of harmful organisms.
3. Application of protection measures on the basis of the monitoring of harmful organisms and threshold levels.*
4. Preference of non-chemical methods over chemical methods if they provide satisfactory pest control.*
5. Preference of specific pesticides and of pesticides with the least side effects over the others.*
6. Keeping the use of pesticides to levels that are necessary.
7. Prevention of resistance development.*
8. Checking protection results.

Application of protection measures on the basis of the monitoring of harmful organisms and threshold levels.

Moderate effectiveness may be acceptable, e.g. **when the pest pressure is low**, when a product will be used as a component of an IPM programme (…)*

*EPPO PP 1/296 (1) approved in September 2017
Principles of efficacy evaluation for low-risk plant protection products

Preference of non-chemical methods over chemical methods if they provide satisfactory pest control

If both: biological and chemical plant protection products are registered for the same scope of use **and show similar efficacy**, biological products should be chosen in IPM rather than chemical.

To be considered:

- Cost
- Some biorationals are less effective in a short-therm but act longer

Preference of specific pesticides and of pesticides with the least side effects over the others

Some biorationals, especially microbial plant protection products and pheromones are very specific to a particular pest, also biorationals usually decompose easily and are less persistent than chemical pesticides.

Prevention of resistance development

Biorationals are **not** resistance proof!!!

But: their mode of action is often different than chemical pesticides.

If used interchangeably with chemical pesticides or other means of protection, can constitute a worthy tool in the resistance prevention strategy.

Agenda

- What the law says?
- How the biorationals fit into IPM rules?
- **Misunderstandings**
- Availability
- Conclusions

General principles of integrated pest management

(Annex III of the Directive 128/2009)

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2. Monitoring of harmful organisms.
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4. Preference of non-chemical methods over chemical methods if they provide satisfactory pest control.*
5. Preference of specific pesticides and of pesticides with the least side effects over the others.*
6. Keeping the use of pesticides to levels that are necessary.
7. Prevention of resistance development.*
8. Checking protection results.

Some biorantionals may have preventive action

- Supression of pest development
- Stimulation of crop defence system
- Improvement the conditions of plant growth or availability of nutrients allows the crop to grow better and be more robust to pest pressure

However unnecessary use of any preparations is hardly in line with the IPM principles

- Use of any product in IPM should be well though out.
- Claims like “Product X **should be** used in IPM strategies” are a **misuse**
- Promotion of a fertilizer or an unregistered product as a tool to control pest is **always against the law**
- Disturbing fact: Unregistered products of unknown composition in EU MS are promoted as “safe”, “environmentally friendly” and “suitable for IPM”

‘**Integrated pest management**’ means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and <u>other forms of intervention</u> to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment.

However unnecessary use of any preparations is hardly in line with the IPM principles

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Agenda

- What the law says?
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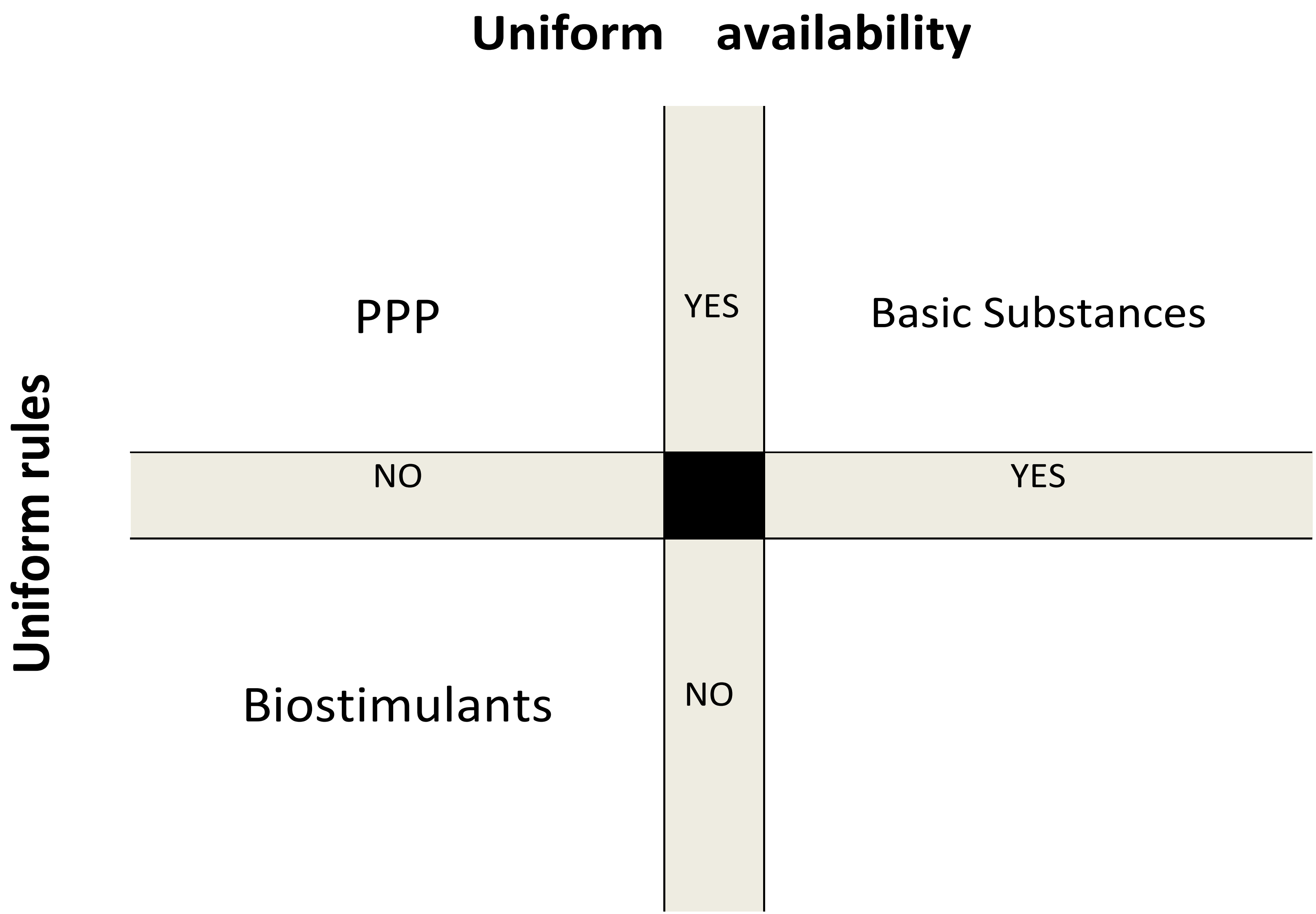
- Misunderstandings
- **Availability**
- Conclusions



Source: Matyjaszczyk E. 2017 Plant protection means used in organic farming throughout the European Union. Pest Management Science DOI: 10.1002/ps.4789

Agenda

- What the law says?
- How the biorationals fit into IPM rules?
- Misunderstandings
- Availability
- **Conclusions**

Conclusions

- Use of biorationals is clearly reccomended in IPM strategies.
- If biological and chemical plant protection products are registered for the same scope of use and show similar efficacy, biological products should be chosen in IPM rather than chemical.
- Biological PPP showing moderate effectiveness can be of use in IPM when the pest pressure is low

Conclusions

- Some biological PPP, especially microbial plant protection products and pheromones are very specific to a particular pest.
- Can constitute a worthy tool in the resistance prevention strategy.
- Biorationals usually decompose easily and are less persistent than chemicals.

Conclusions

- Some biological PPP may have preventive action.
- Unnecessary use of any preparations is not line with the IPM principles.

Thank you for your attention!

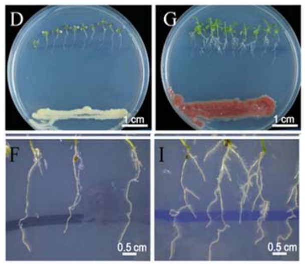
Assessment Methods for Biostimulants/Biofertilizers

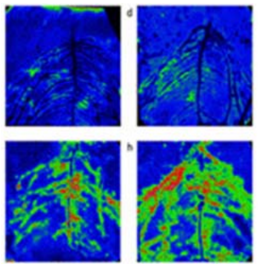
Achievements and challenges

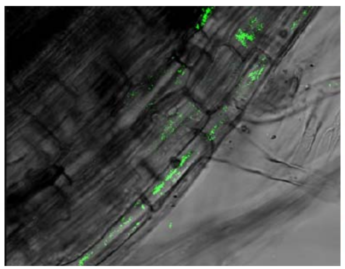
Günter Neumann - Institute of Crop Science (340h)

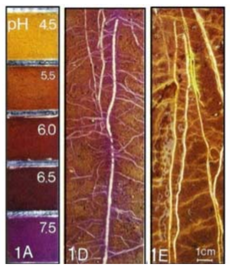
University of Hohenheim – Stuttgart – Germany

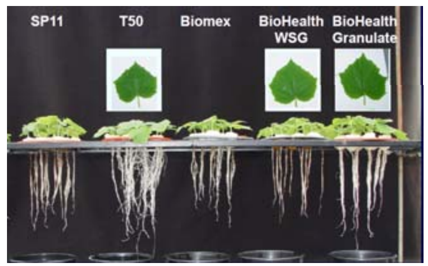
(guenter.neumannquni-hohenheim.de)

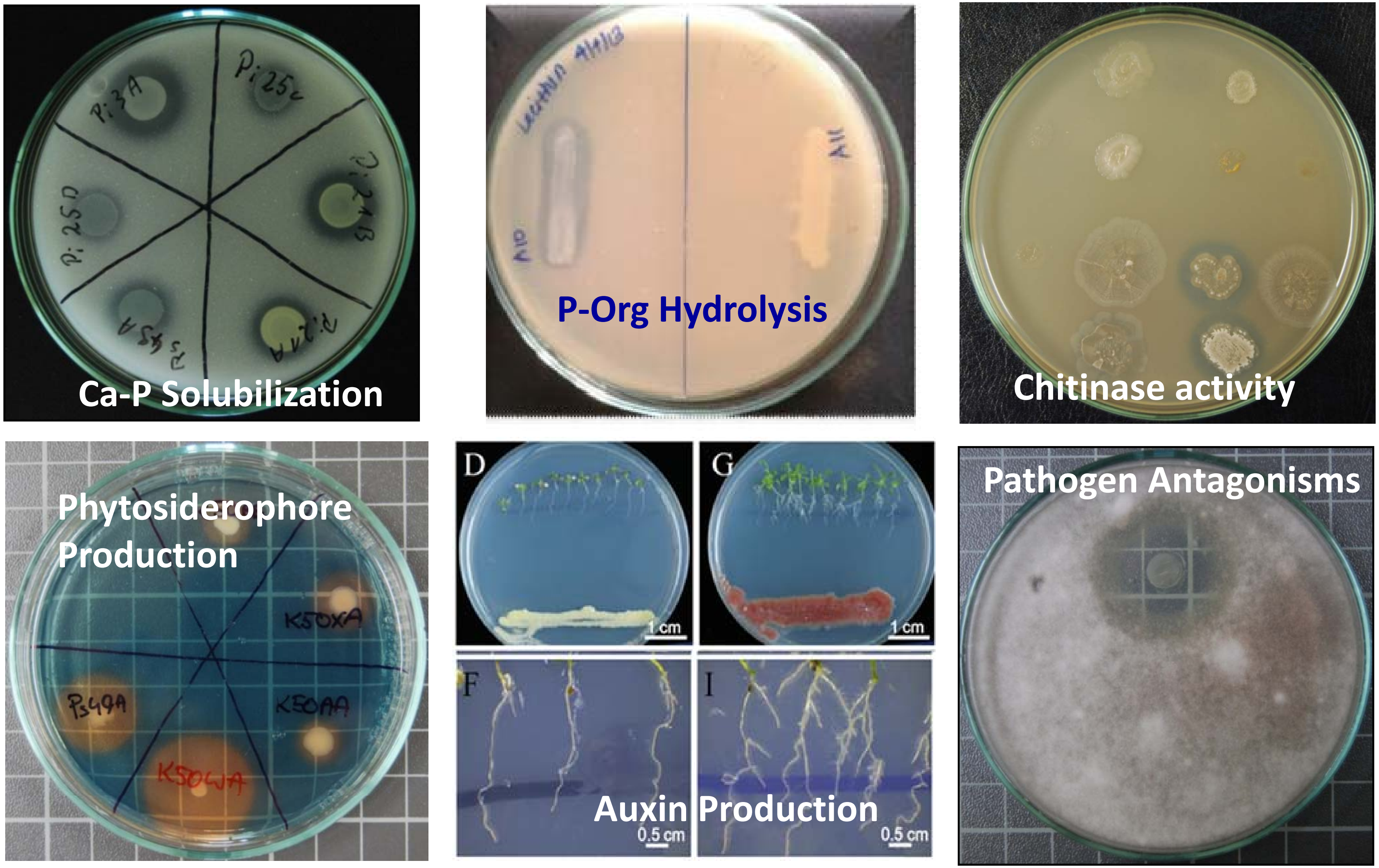






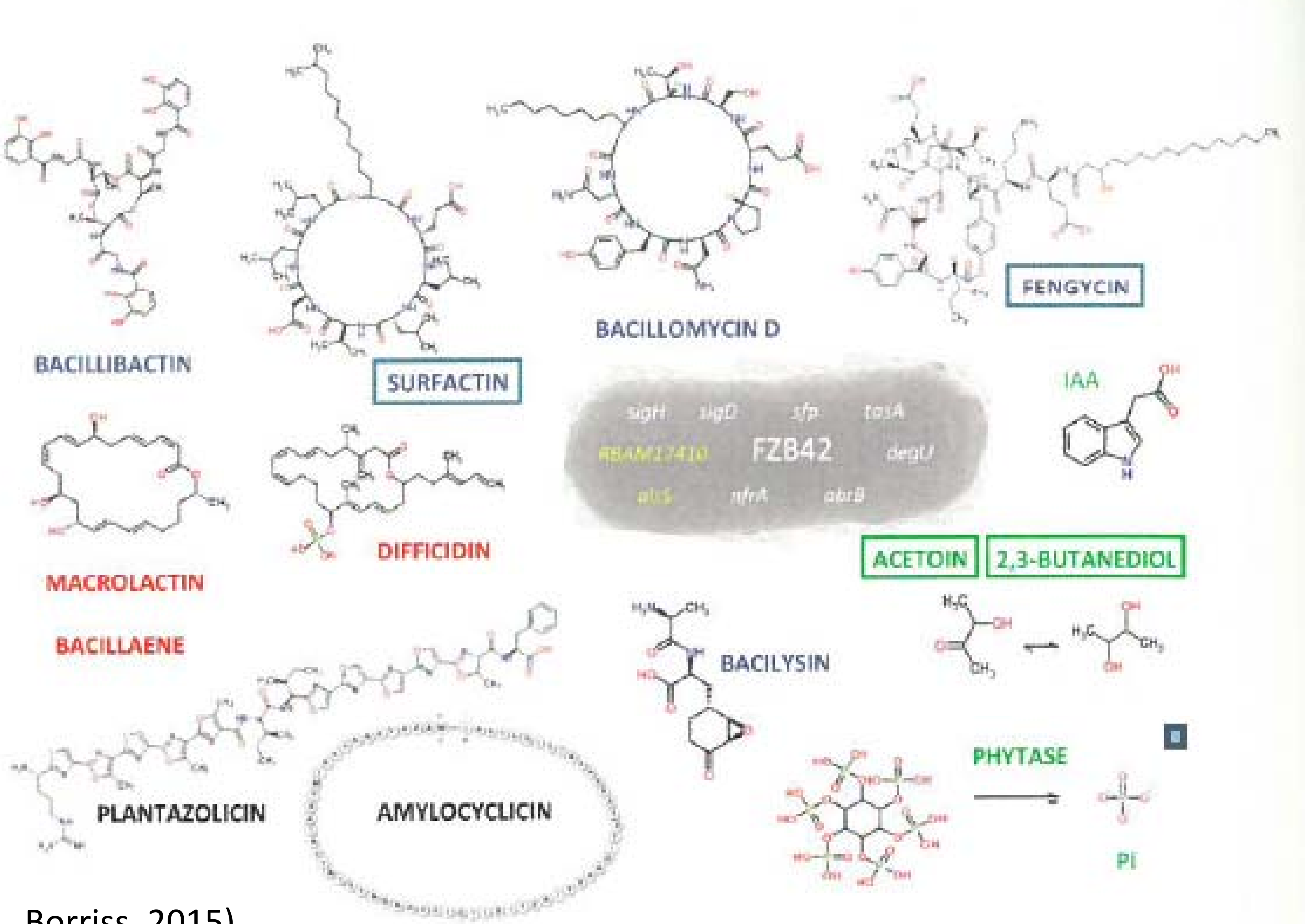






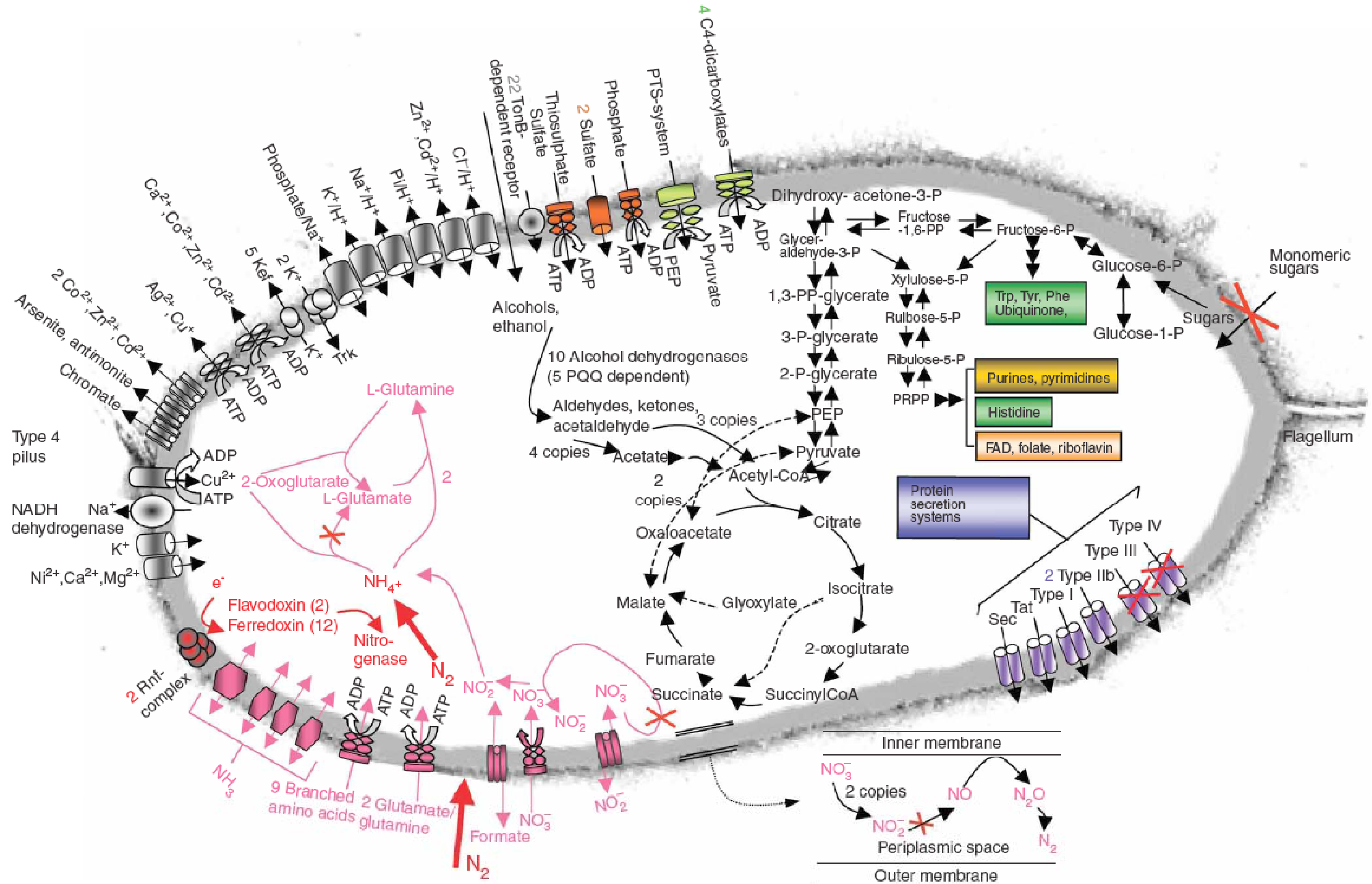
Testing metabolic properties of microbial inoculants

Secretory Metabolite Profiling in *Bacillus amyloliquefaciens* FZB42



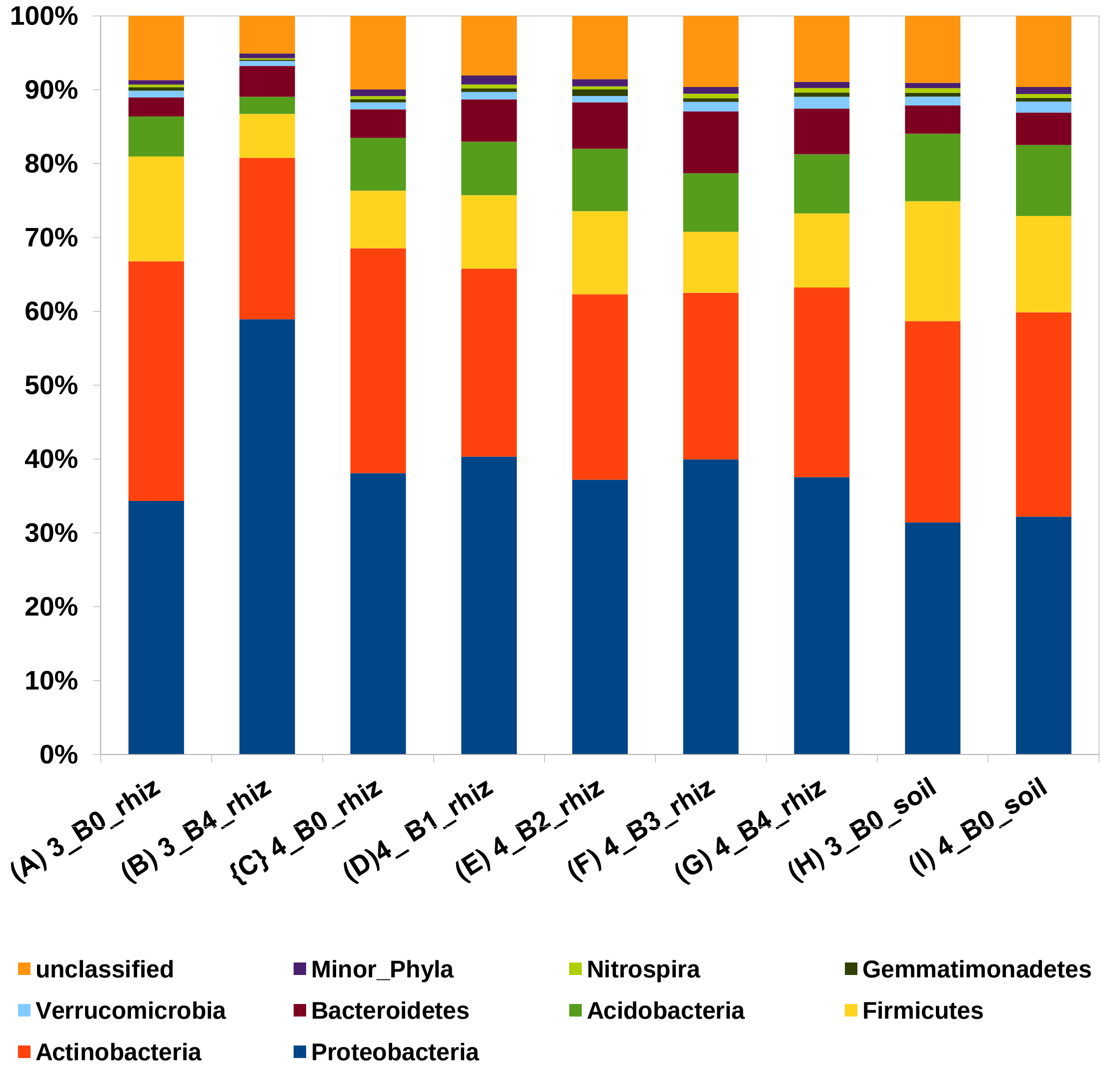
Borriss, 2015)

Genome sequencing to demonstrate
the genetic potential to interact with the environment



Krause et al. 2006, Nat Biotech 24: 1385

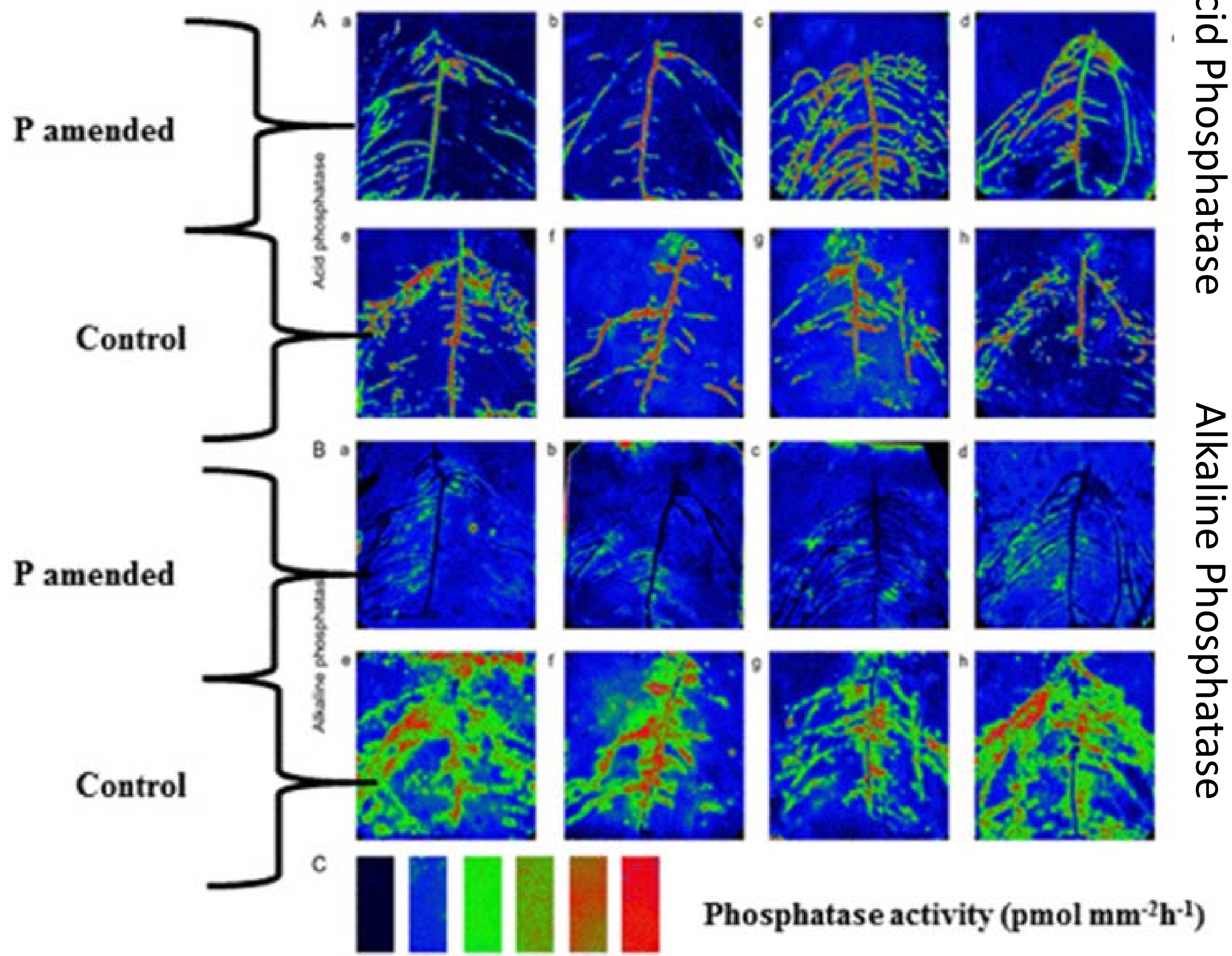
Metagenomics to study interactions with the soil microbiome



- Amplicon pyrosequencing of 16S rRNA genes obtained from rhizosphere and soil DNA from tomato revealed that ***Proteobacteria***, ***Actinobacteria***, ***Firmicutes*** and ***Acidobacteria*** were the dominant phyla in all treatments.

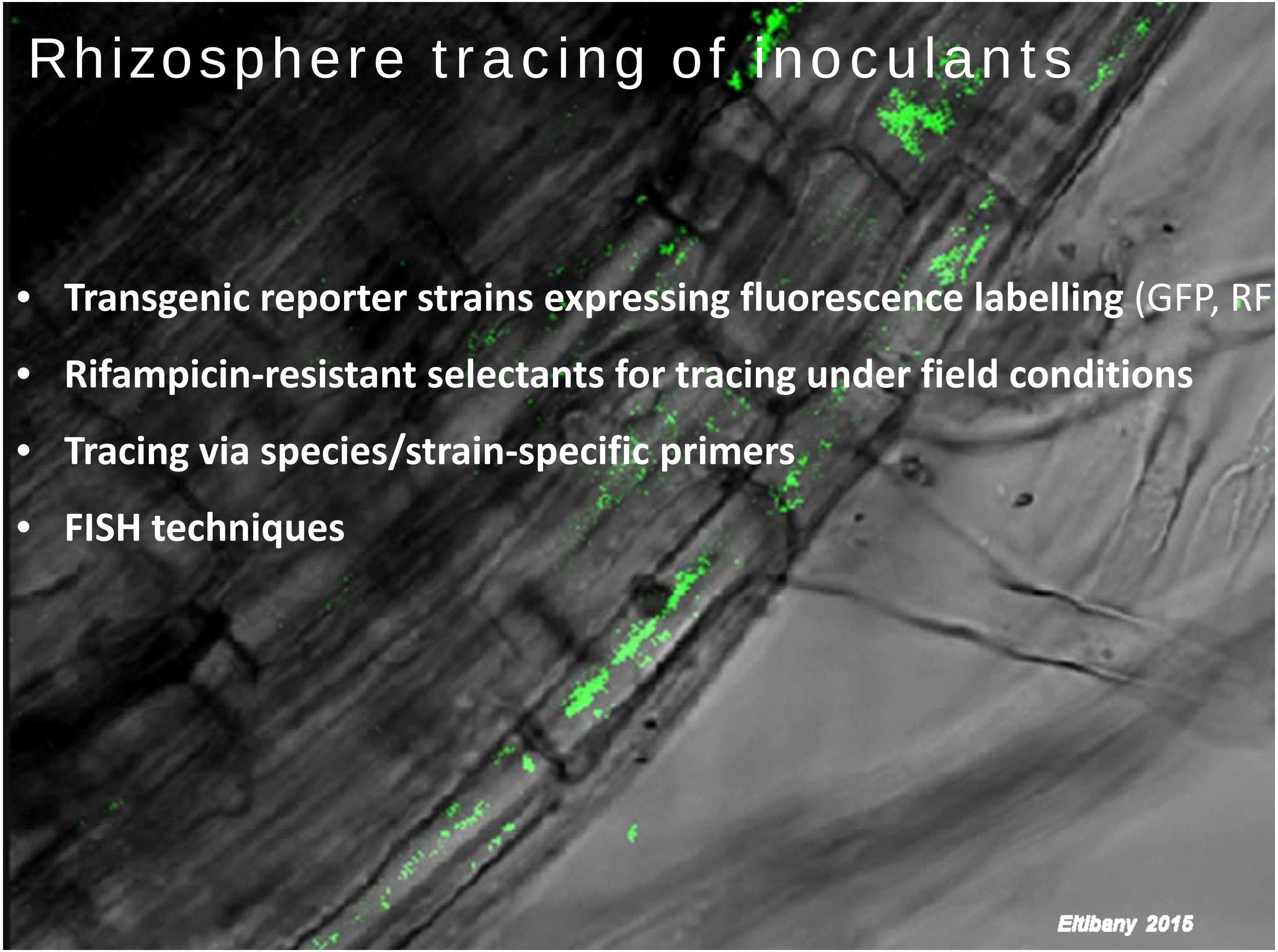
Functional characterization of plant PGPR interactions In the Rhizosphere

Zymographic detection of rhizosphere phosphatases by use of fluorogenic substrates and image analysis



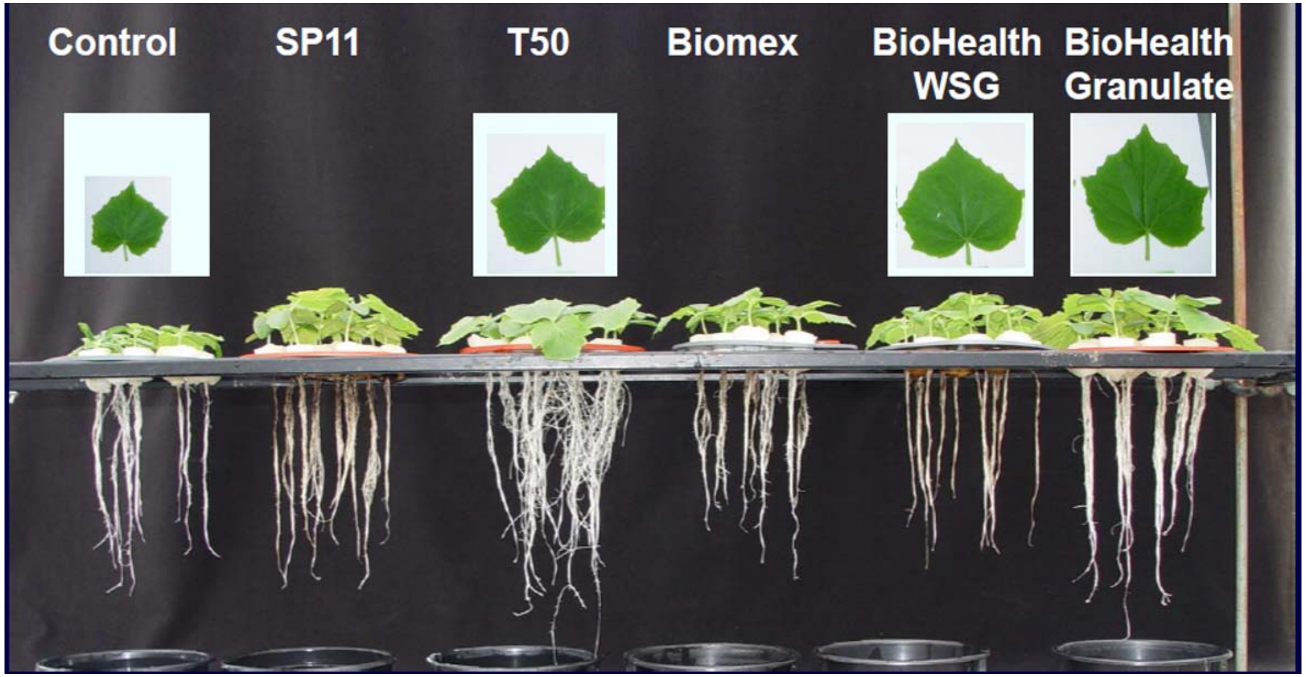
Marker enzymes for nutrient in the rhizosphere

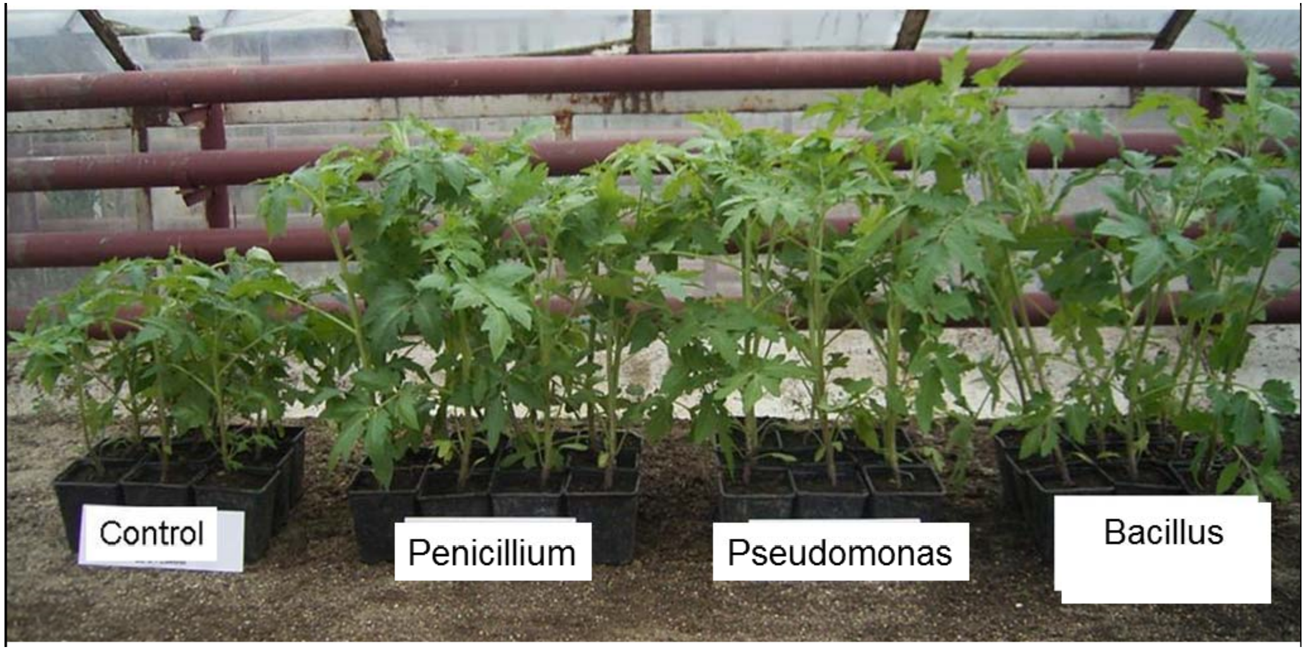
Expression of functional genes


Rhizosphere tracing of inoculants
• Transgenic reporter strains expressing fluorescence labelling (GFP, RF
• Rifampicin-resistant selectants for tracing under field conditions
• Tracing via species/strain-specific primers
• FISH techniques
Eltibany 2015

Screening for growth responses under controlled conditions

Akter et al.2007





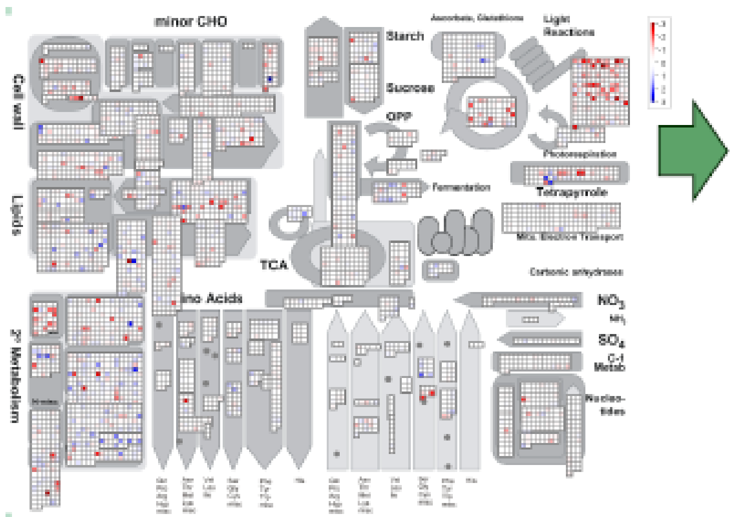
Greenhouse testing in soil culture

Posta et al. 2014

Root transcriptome changes in soil-grown maize plants In response to inoculation with microbial biostimulants

Whole transcriptome analysis

MapMan metabolism overview for whole transcriptome



Up-regulated BINs

- **Protein degradation** (Ubiquitin-mediated)
- **Hormones** (**ethylene** biosynthesis and signalling, cytokinin degradation)
- **Transcriptional regulation** (TFs): AP2/EREBP, WRKY, NAC, MYC
- **Phenylpropanoid/Flavonoid metabolism**
- Biotic stress: PR genes, SA (only FZB42)

Down-regulated BINs

- Protein synthesis
- DNA synthesis/ chromatin structure/ histones
- RNA processing
- Cell organisation, cell division

→ **No stimulation of auxin-related genes**

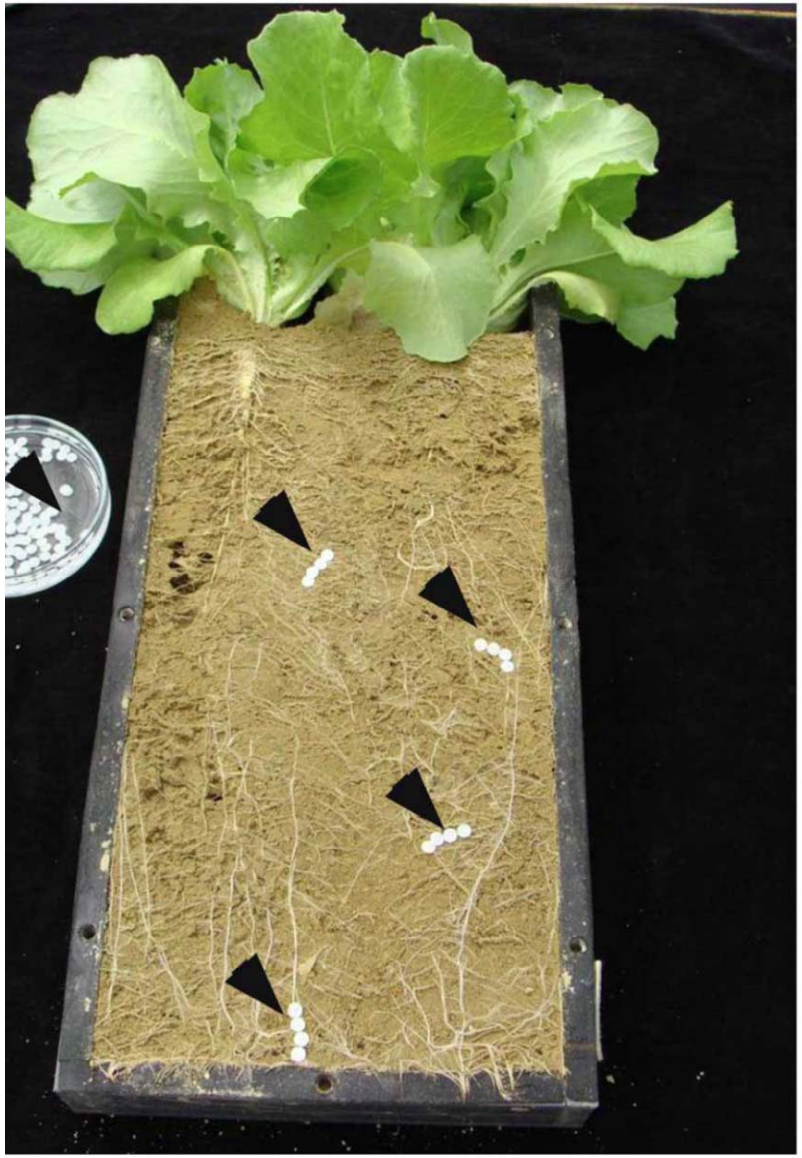
→ **Stress-related responses** (e.g. TFs, Ethylene, ubiquitin-mediated protein degradation) (Flick & Kaiser, 2012)

Weber 2016

GC-MS root exudate profiling of lettuce in different soils

Chemical group	Compound	Loess Loam	Alluvial Loam	Dilluvial Sand
Amino acids and amines	Alanine	+	+	++
	beta-Alanine	+	-	+
	Aspartate	+	+	+
	Glutamate	-	-	+
	Glutamine	+	+	+
	Glycine	+++	++	++
	Leucine	++	+	+
	Isoleucine	+	+	+
	Proline	+	+	+
	4-Hydroxyproline	+	+	+
	Pyroglutamate	++	+	+
	Serine	++	++	+
	Threonine	++	+	+
	Valine	++	+	++
	beta-Aminobutyric acid	+	+	+
	4-Aminobutyric acid	+	-	+
	Putrescine	+	+	+
Sugars and sugar alcohols	Glucose	+++	+	++
	Fructose	+++	+	++
	Mannose	+	-	-
	Maltose	+++	+	+++
	Trehalose	+++	+	+++
	Sucrose	+++	++	++
	Glycerol	+++	+++	+++
	Inositol	+++	+	+
Organic acids	Malate	+	+	+
	Fumarate	+	+	+
	Succinate	++	+++	++
	Lauric acid	++	+	+
	Benzoic acid	++	++	++
Others	Urea	+++	++	++
	Phosphate	+	+	+
	Ornithine	+	+	+

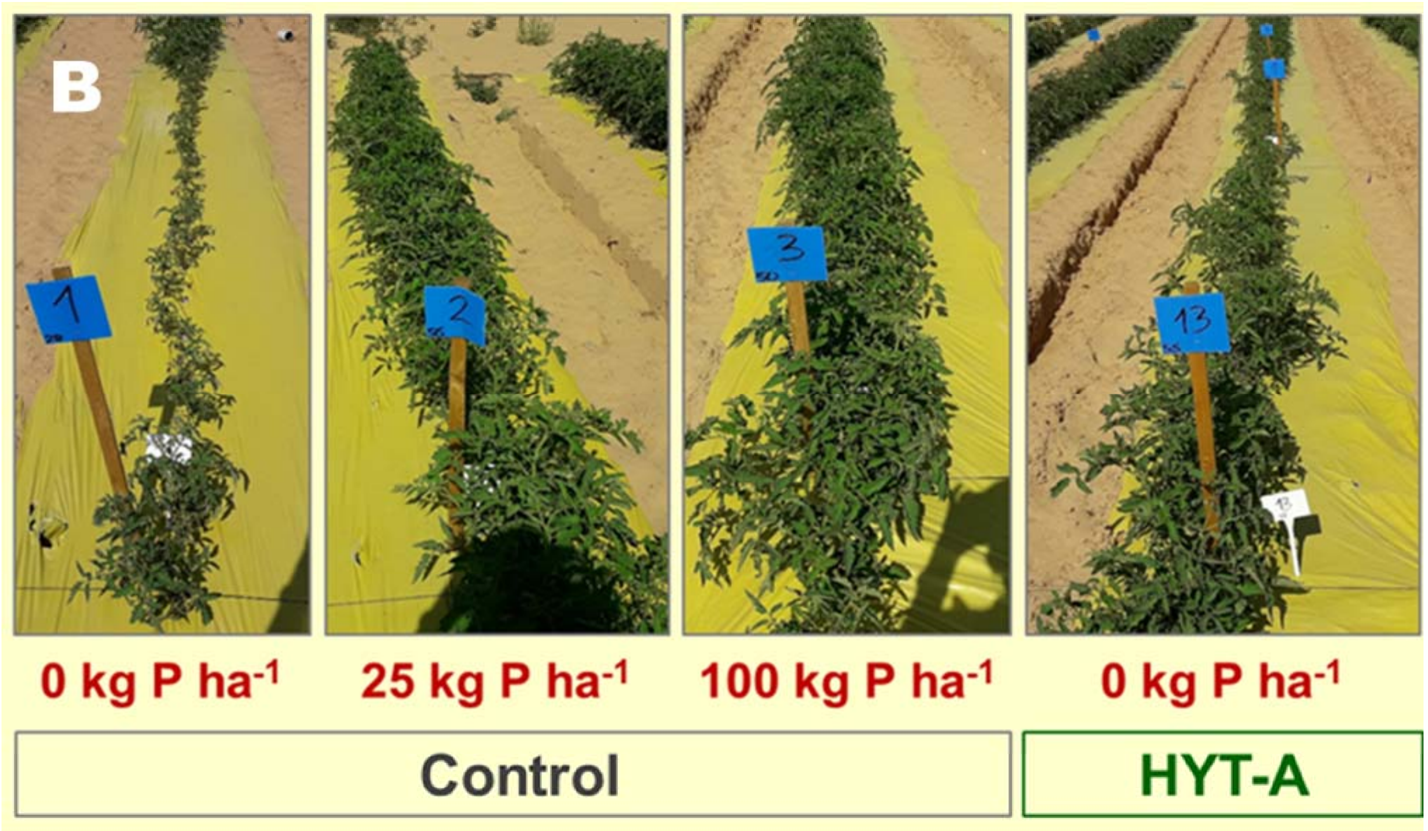
Differences in profiles of exudate samples (rhizosphere soil solution) are rather quantitative than qualitative.



Neumann et al., 2014

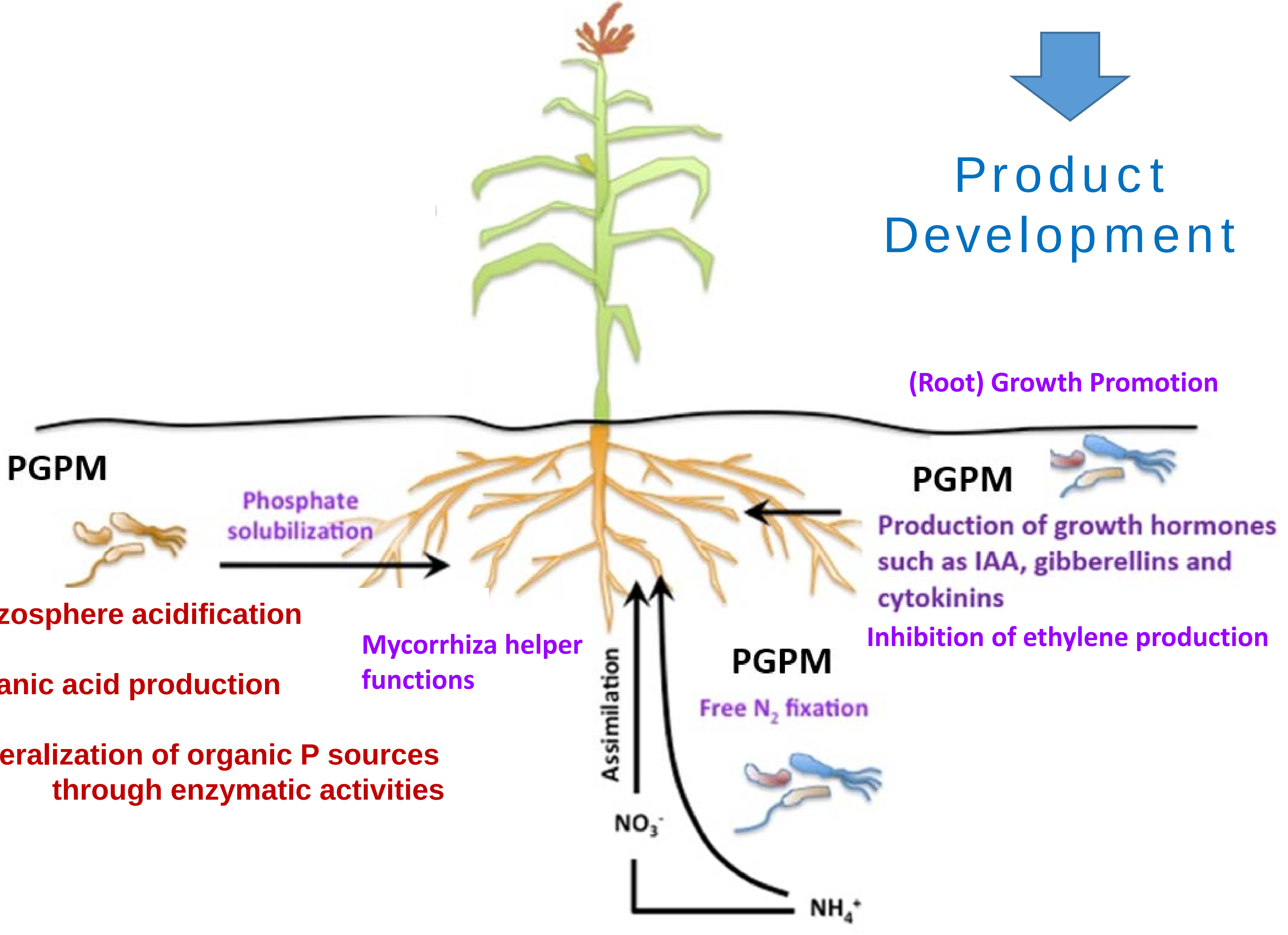
Field inoculation with the microbial consortium product HYT-A in drip-irrigated tomato production (Negev Israel)

Shoot FW [kg/plant]	0.30c	0.42 bc	0.62 ab	0.64 ab
Yield [kg/m^2]	1.44 c	3.61 b	5.67 a	3.32 bc



Bar Tal et al. 2017

Conceptual model for Plant-PGPM interactions in the rhizosphere to support plant nutrient acquisition



- ➢ **Rhizosphere acidification**
- ➢ **organic acid production**
- ➢ **Mineralization of organic P sources through enzymatic activities**

<u>Example:</u>

Consortium products for improved nutrient acquisition and stress resistance of crops

- **Associative or symbiontic N_2 fixation (BNF) promoted by inoculation with selected strains of BNF inoculants** (Azotobacter, Azosporillum, Clostridium, Rhizobium etc)

- **Carbon supply for BNF inoculants promoted by co-noculation with selected strains of C-decomposer**s (Bacillus, Trichoderma etc)

- **Phosphate supply for BNF microbes and host plants promoted by co-inoculation with selected strains of P-solubilising microorganisms (PSMs)** (Bacillus, Pseudomonas, Acetobacter, Micrococcus, Trichoderma etc)

- **Additional protective agents and co-factors** (microbial pathogen antagonists, micronutrients, seaweed extracts , starter C, etc)

- **Improved fertilizer use efficiency**
- **Reduction of N fertilizer demand 40-80%**
- **Improved stress resistance**

Poster Bradacova et al.

(information based on patent applications and product descriptions)

But: Inoculation with accociative N_2 fixers rarely leads to improved N acquisition and higher yields under field conditions in temperate climates

Parameter	N-fertilization level (kg N ha^{-1}) 0	40	80	120
Number of cobs per plant				
Control	1.069a	1.181a	1.233a	1.289ab
A. brasilense Sp245	1.039a	1.138a	1.177a	1.194b
A. irakense KBC1	1.065a	1.121a	1.194a	1.310a
Fresh cob yield (kg ha^{-1})				
Control	12409a	15225a	16350a	16924a
A. brasilense Sp245	12439a	15008a	15923a	16380a
A. irakense KBC1	13500a	15158a	16421a	17719a
N content in grains (%)				
Control	1.322a	1.367a	1.445a	1.496a
A. brasilense Sp245	1.343a	1.398a	1.481a	1.486a
A. irakense KBC1	1.346a	1.399a	1.419a	1.536a

Azotobacter field inoculation experiments with maize in Belgium

Similar results in three EU-funded projects During 2000 – 2010 (Micro N-Fix, RHIBAC, MicroMAIZE)

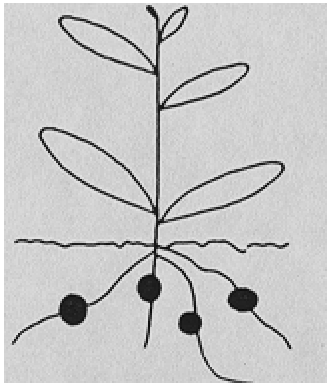
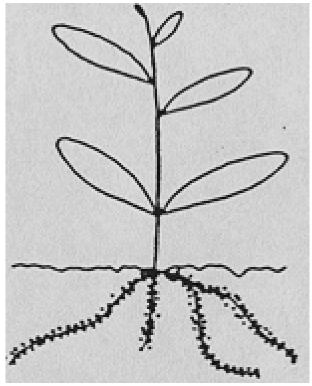
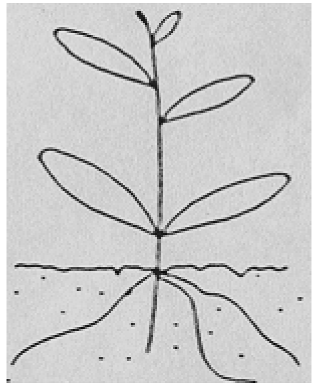
(Dobbelaere et al. 2001, Austr. J. Plant Physiol. 28: 871-879)

Inoculation with associative N_2 fixers more frequently leads to improved growth and higher yields in sub-/tropical climates

Field inoculation of cereals with Azospirillum in Mexico

Crop	No. of evaluated sites	Evaluated area (ha)	Positive effects (%)	Range of grain yield increase (%)	Average increase (%)
Maize: no N-fertilization or less than 90 kg N ha^{-1}	62	248	95	11–98	33
Maize: fertilized in the range of 100 to 150 kg N ha^{-1}	69	276	62	6–49	12
Sorghum: no N-fertilization or less than 90 kg N ha^{-1}	12	48	62	11–73	36
Sorghum: fertilized in the range of 100 to 150 kg N ha^{-1}	8	32	63	6–17	10
Wheat: no N-fertilization or less than 90 kg N ha^{-1}	7	35	83	10–58	22
Barley: no N-fertilization or less than 110 kg N ha^{-1}	13	39	86	17–65	42

N2 fixation potential in different BNF systems

system of N_2 fixation ($N_2 \rightarrow NH_3$) and microorganisms involved	symbiosis  (e.g. *Rhizobium*, *Actinomycetes*)	associations  (e.g. *Azospirillum*, *Azotobacter*)	free living  (e.g. *Azotobacter*, *Klebsiella*, *Rhodospirillum*)	
energy source (organic carbon)	sucrose metabolites (from the host plant)	root exudates from host plant	heterotroph. plant residues	autotroph. photosynt.
estimates of amounts fixed (kg N/ha*a)	Legumes: 50-400 Nodulated non-egumes: 20-300	30-40 (tropical C4 grasses) 10-30 (temperate climates)	<5 (temperate climates)	10-80

(adapted from Marschner, 1995 and Roper and Gupta, 2016 Open Agr. J. 10: 7-27)

Comparison of symbiotic and free living N_2 fixation

Symbiotic N_2 fixation:

about 7g carbohydrates /g reactive N

= 1 t carbohydrates /150 kg N

- direct and preferential access to host carbohydrates,
- low competition due to endophytic colonization;
- N release by microsymbionts

N_2 fixation by free living C-heterotrophic microorganisms:

= 1 t carbohydrates (crop residues) /1.5 kg N

- Substrate (C) limitation
- Competition with other C-heterotropghic microorgamnisms
- N release mainly related with turnover of microbial biomass

Aspects to be considered for efficiency assessment of associative BNF inoculants

- Benefits **largely determined by site-specific conditions**
  - Tropical climates with rapid and intense C turnover,
  - C_4 grasses with high C-input as root exudates and crop residues and preferential endophytic associations

- Global change: Increased temperatures ? Increased rhizosphere C alloation ?
- Conservation agriculture – reduced tillage, C accumulation in top soil ?

- More widespread PGPR effects by additional modes of action (root growth promotion, stress priming)

- Low input vs standard input systems

Wheat Production	Grain yield [t ha^{-1}]	N demand [kg ha^{-1}]	N_2 fixation [t ha^{-1}]	BNF share of N demand [%]
Germany	6-8 t/ha	160	10-30	6-19
Australia	1-2 t/ha	40	10-30	25-75

(adapted from Roper and Gupta, 2016 Open Agr. J. 10: 7-27)

Phosphate Solubilizing Microorganisms (PSMs)

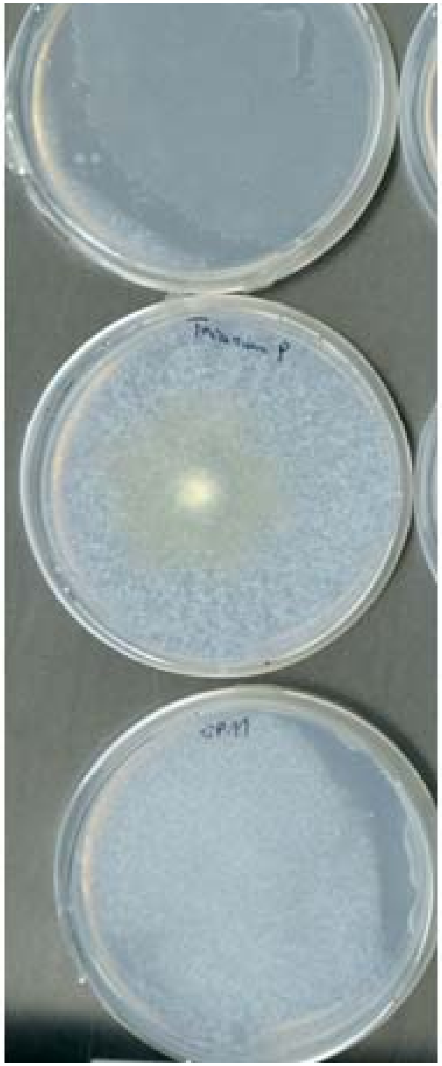
Proradix ✓

(*Pseudomonas DMSZ 13134*)

Paenibacillus m. ✓

Biol. Fertilizer OC ✓

(*Penicillium sp.*)



Kuhlmann & Nkebiwe, 2014

Rhizovital ✓

(*Bacillus amylo-liquefaciens*)

Trianum P ✗

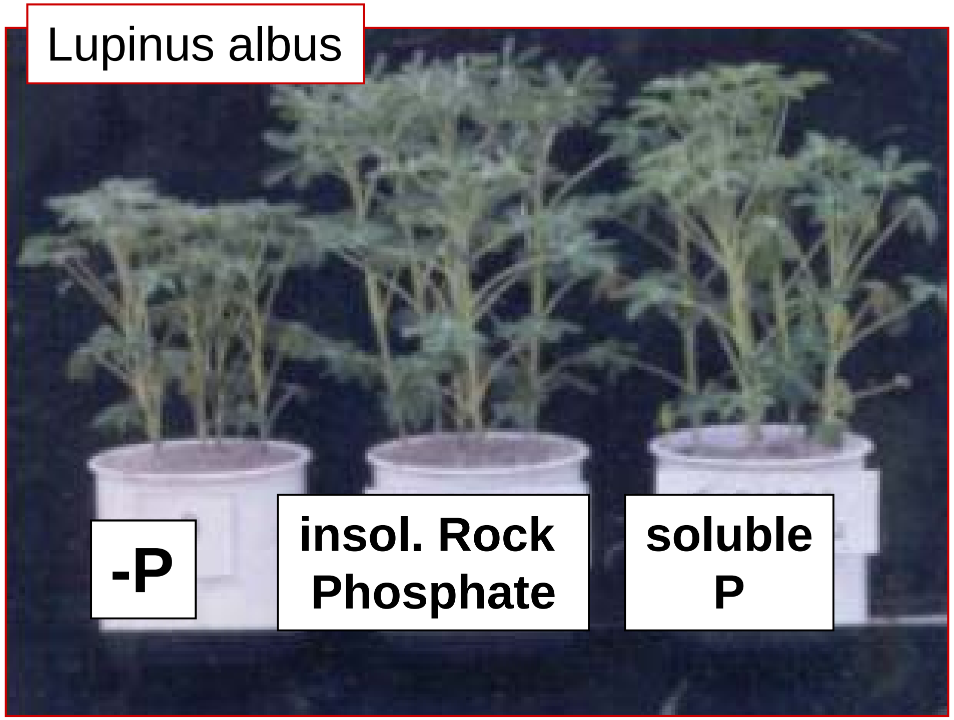
(*Trichiderma Harzianum T22*)

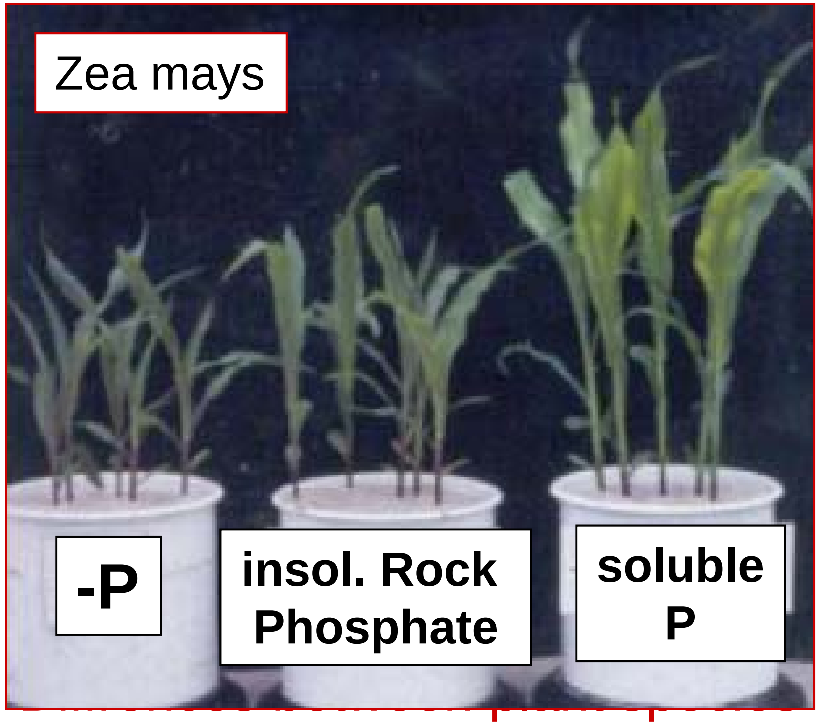
Vitalin SP11 ✓

(Combination product)

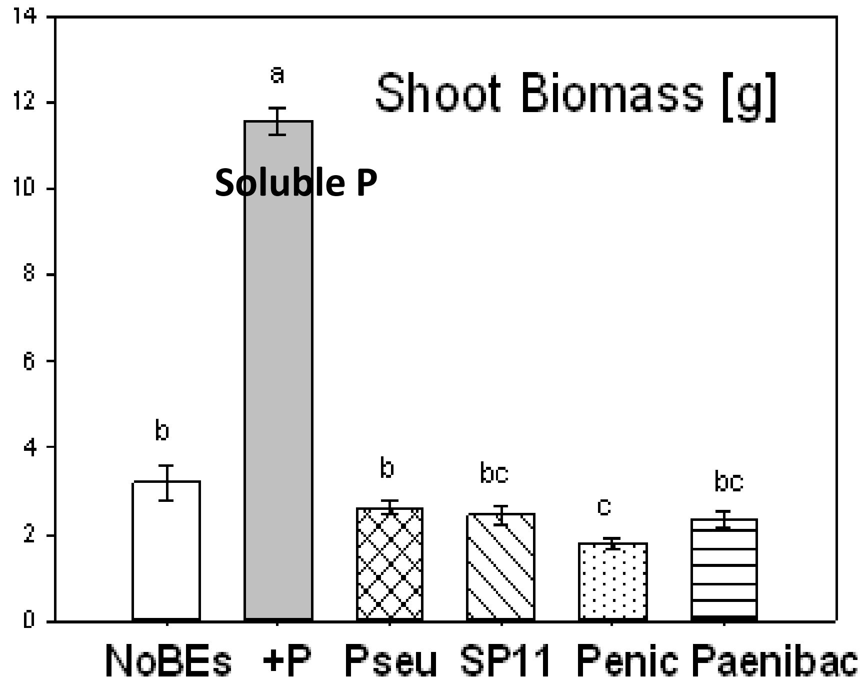
14 fungal and bacterial strains with phosphate-solubilizing potential **(PSMs**: *Trichoderma, Penicillium, Pseudomonas, Bacillus, Paenibacillus, Burkholderia and Streptomyces)* identified in the BIOFECTOR project

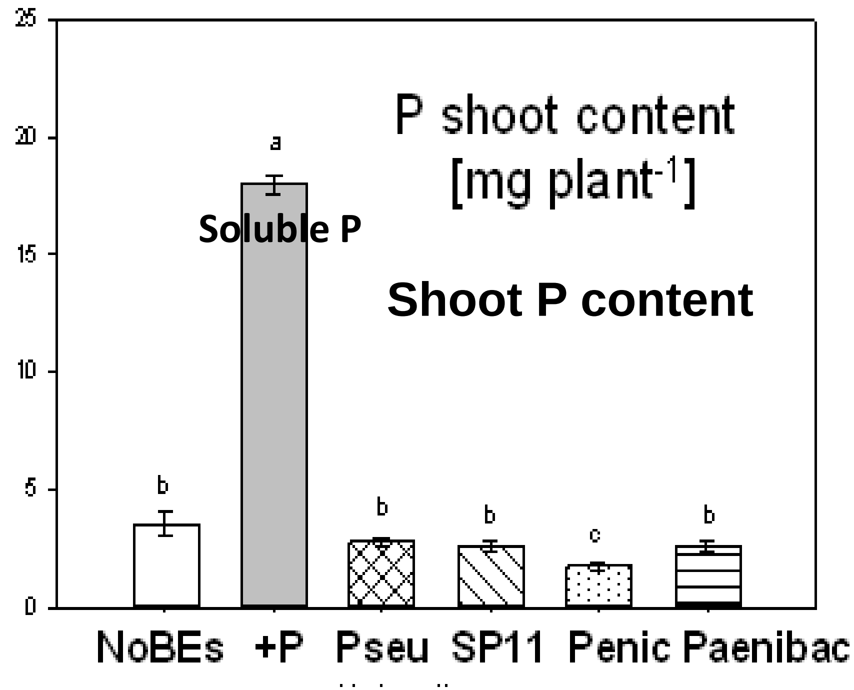
Requirements for testing PSM-mediated plant growth promotion





- Microbial strains with P-solubilising potential
- Test plant without P-solubilizing potential
- Soil with limited plant-available (soluble) P levels but rich in spatingly soluble P sources





(Probst, et al., 2014)

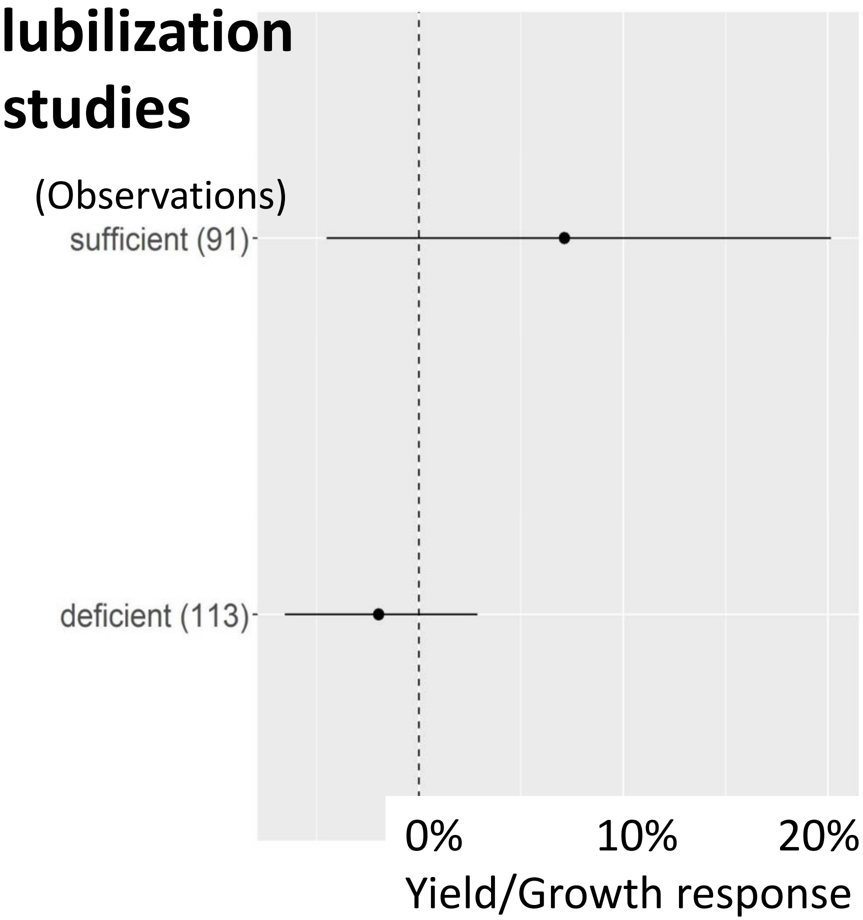
- **No plant growth promotion in maize via P mobilisation by selected PSMs on a low-P soil pH 7.5 with insoluble Ca-P as dominant P source**

- **Similar results in 10 experiments on low-P soils with 14 BEs in 8 countries and 4 crops** (Lekfeldt et al. 2016; Thonar et al. 2017)

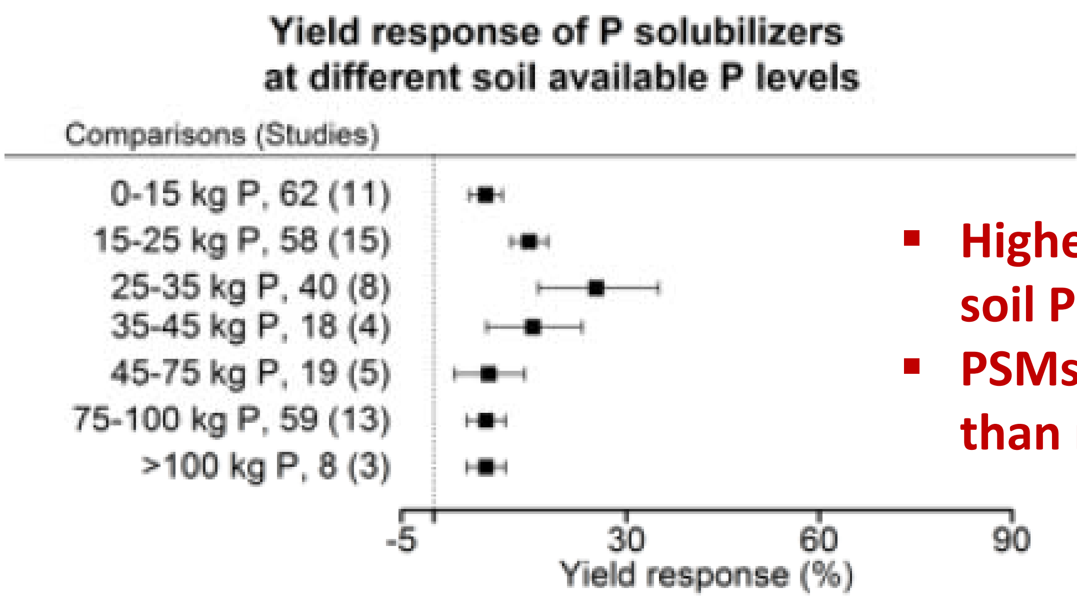
Low efficiency of PSM-assisted P solubilization in crops also confirmed in Metastudies

A) **<u>BIOFECTOR Metastudy</u>** (150 experiments 38 products, 3 crops)
Effect of the soil P status on growth/yield responses of microbial inoculants

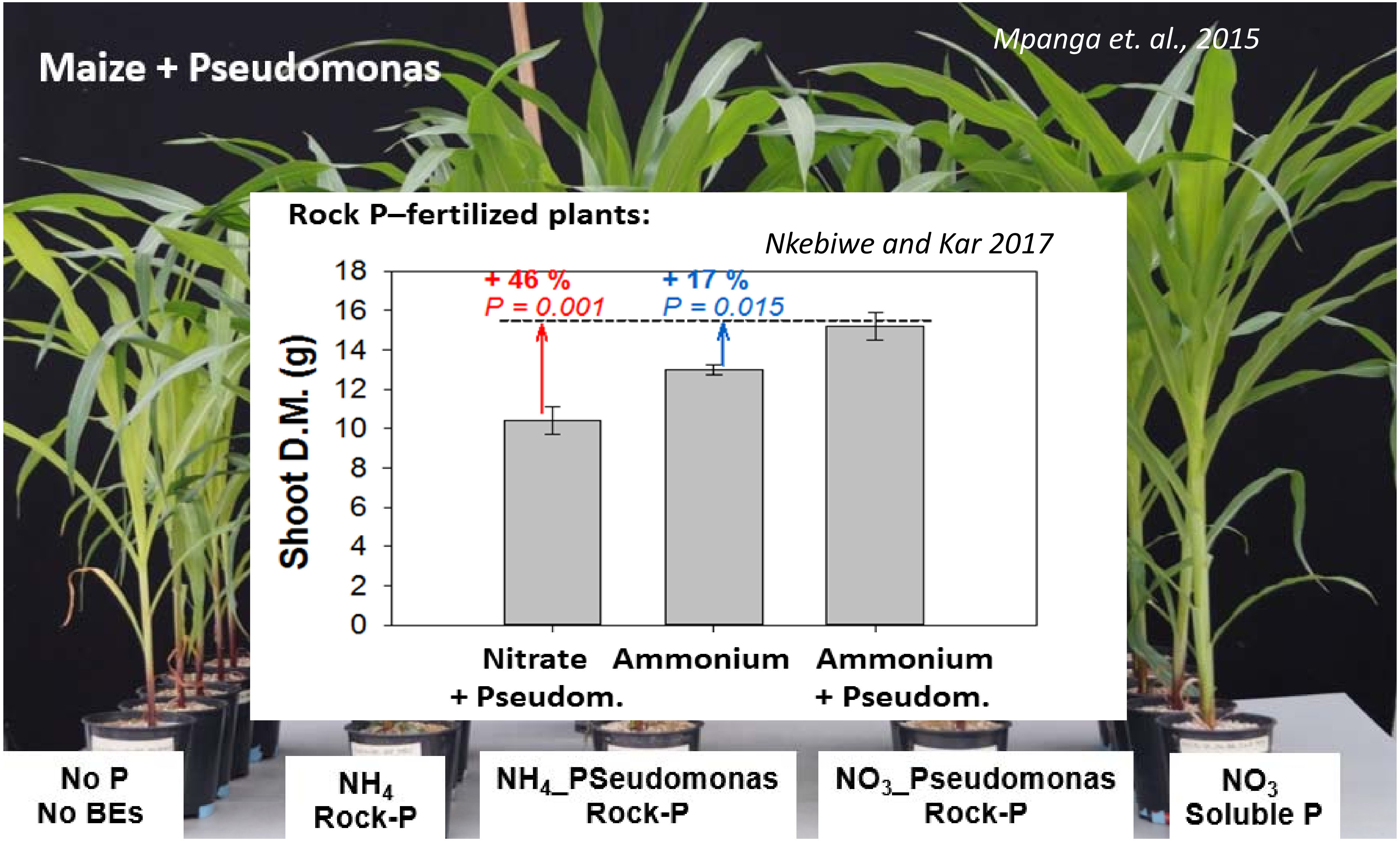
Lekfeldt et al. 2017



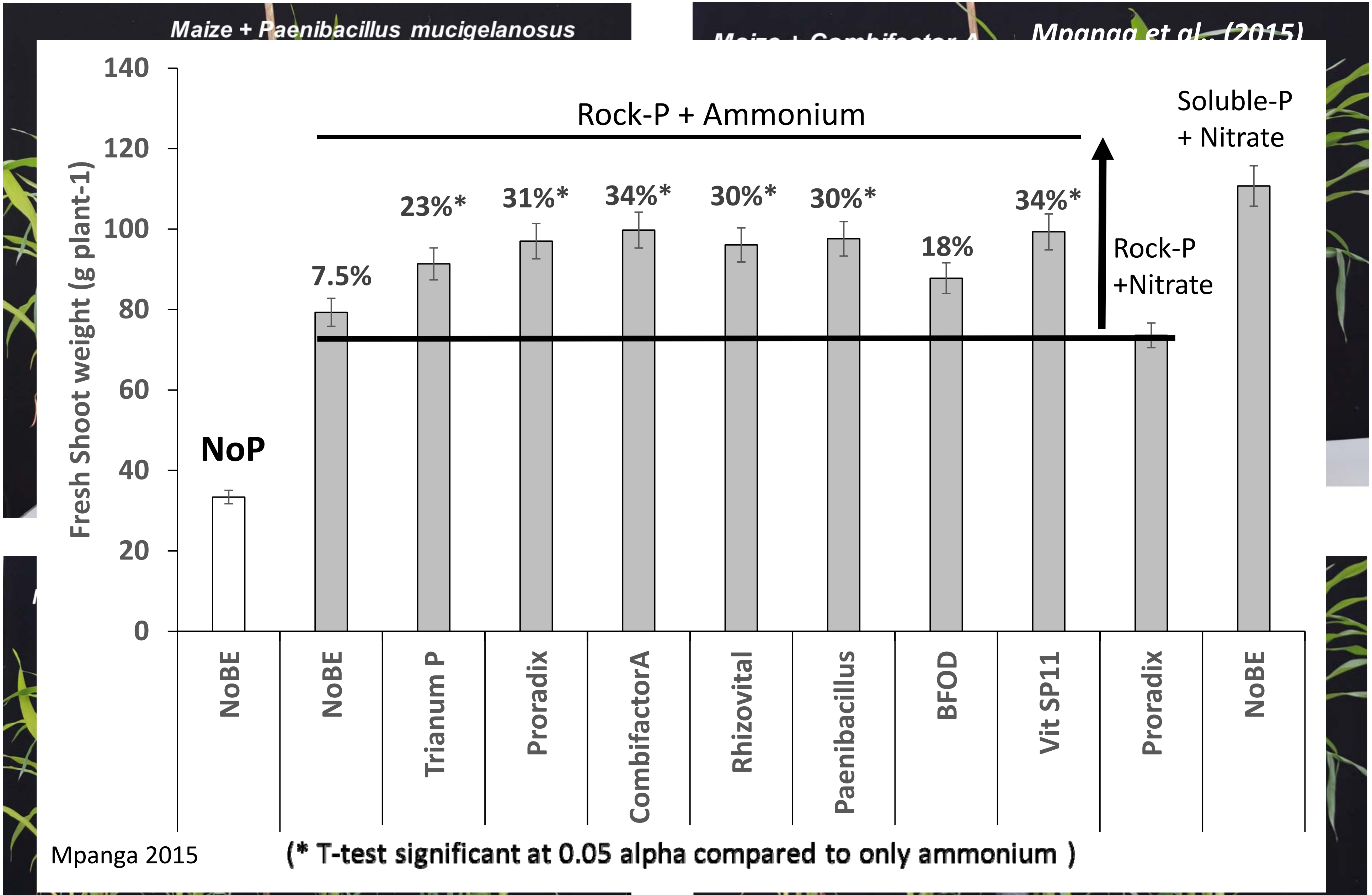
B) **<u>Literature survey</u>** (160 studies)



- **Highest effciency at moderate available soil P levels.**
- **PSMs rather support soluble P acquisition than mobilization of insoluble P**

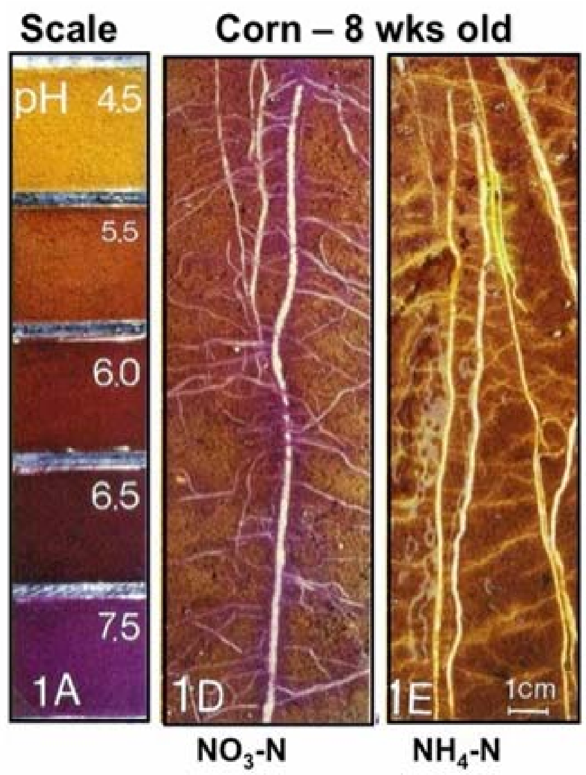


Stabilized Ammonium fertilization synergistically supports plant growth promotion in maize supplied with sparingly soluble Ca-P (Rock-P) after inoculation with *Pseudomonas sp. DMSZ13134 (Proradix)*

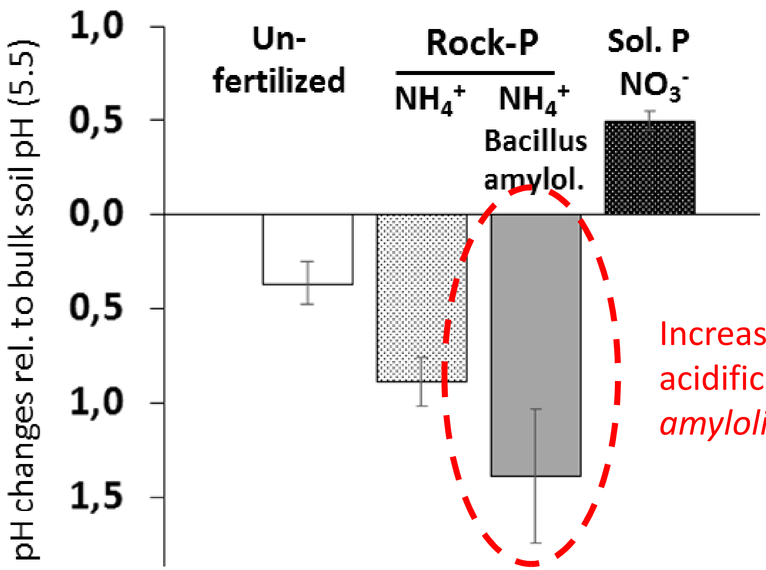


Similar synergistic ammonium effects also after inoculation with other bacteria and fungi belonging to the genera *Trichoderma, Penicillium, Pseudomonas, Bacillus, Paenibacillus, and Streptomyces*.

Benefits of ammonium fertilization for Plant- PGPM interactions

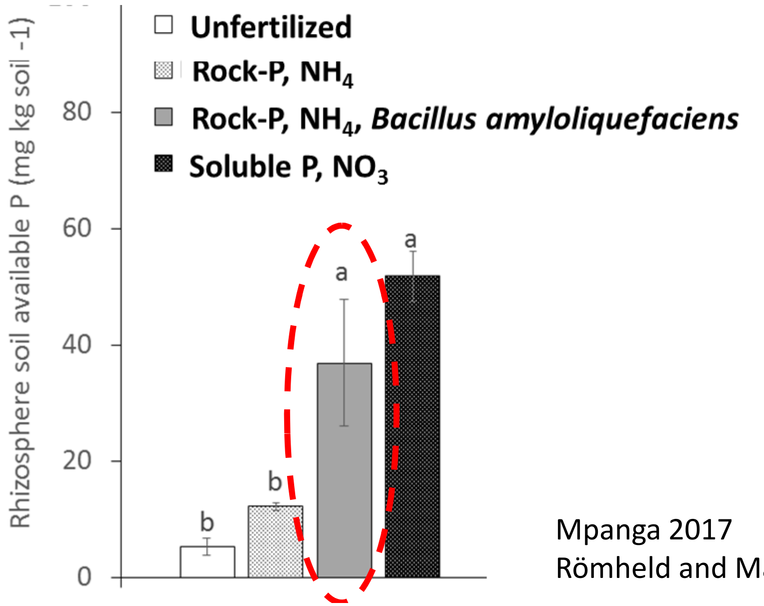


NH_4-induced rhizosphere pH changes in maize



Increased rhizosphere acidification by *Bacillus amyloliquefaciens*

Increased P availability in the rhizosphere

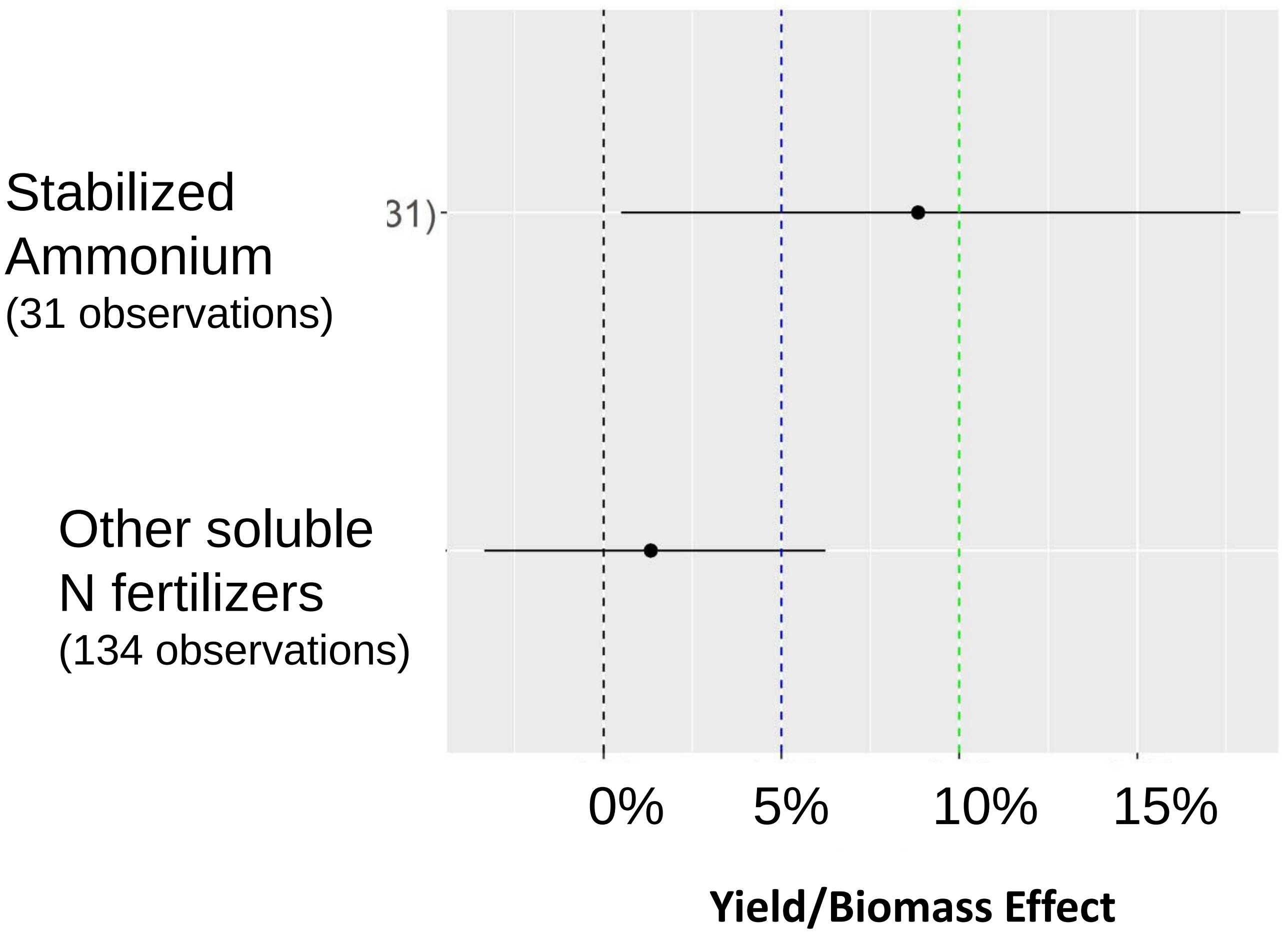


Ammonium uptake induces rhizo-sphere acidification which mediates Rock-P mobilization:

- supports activity of P solubilising BEs
- is supported also by root growth-promoting BEs due to formation of a larger acidifying root system

Mpanga 2017
Römheld and Maschner 1993

Synergistic effects of PSMs with stabilized Ammonium fertilization also confirmed by the BIOFECTOR Meta-study



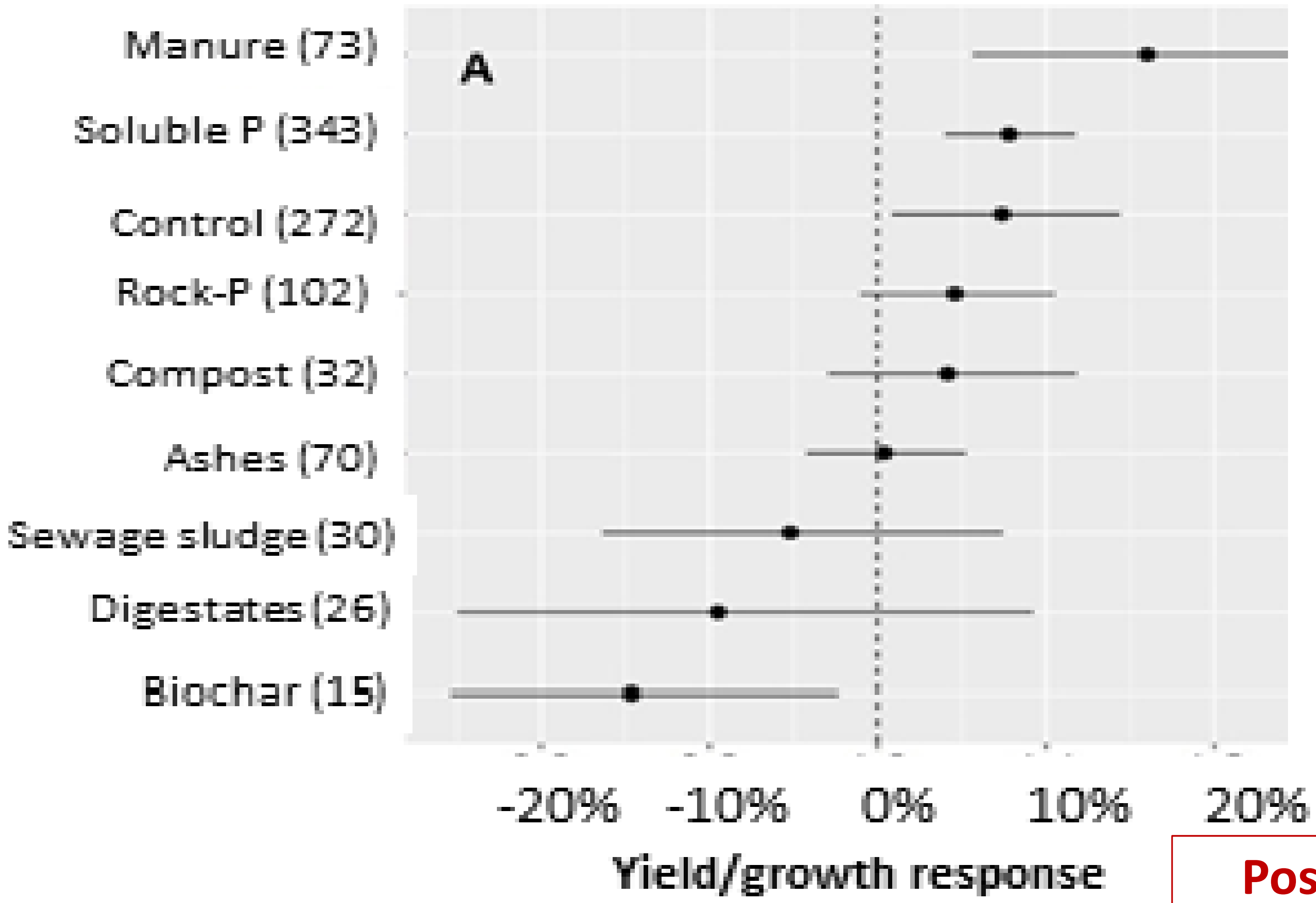
9 % effect of PSMs here

Only significant in combination with stabilized ammonium

Poster Mpanga et al.

Lekfeldt et al. 2017

Highly selective effects of microbial biostimulants also in combination with organic P fertilizers

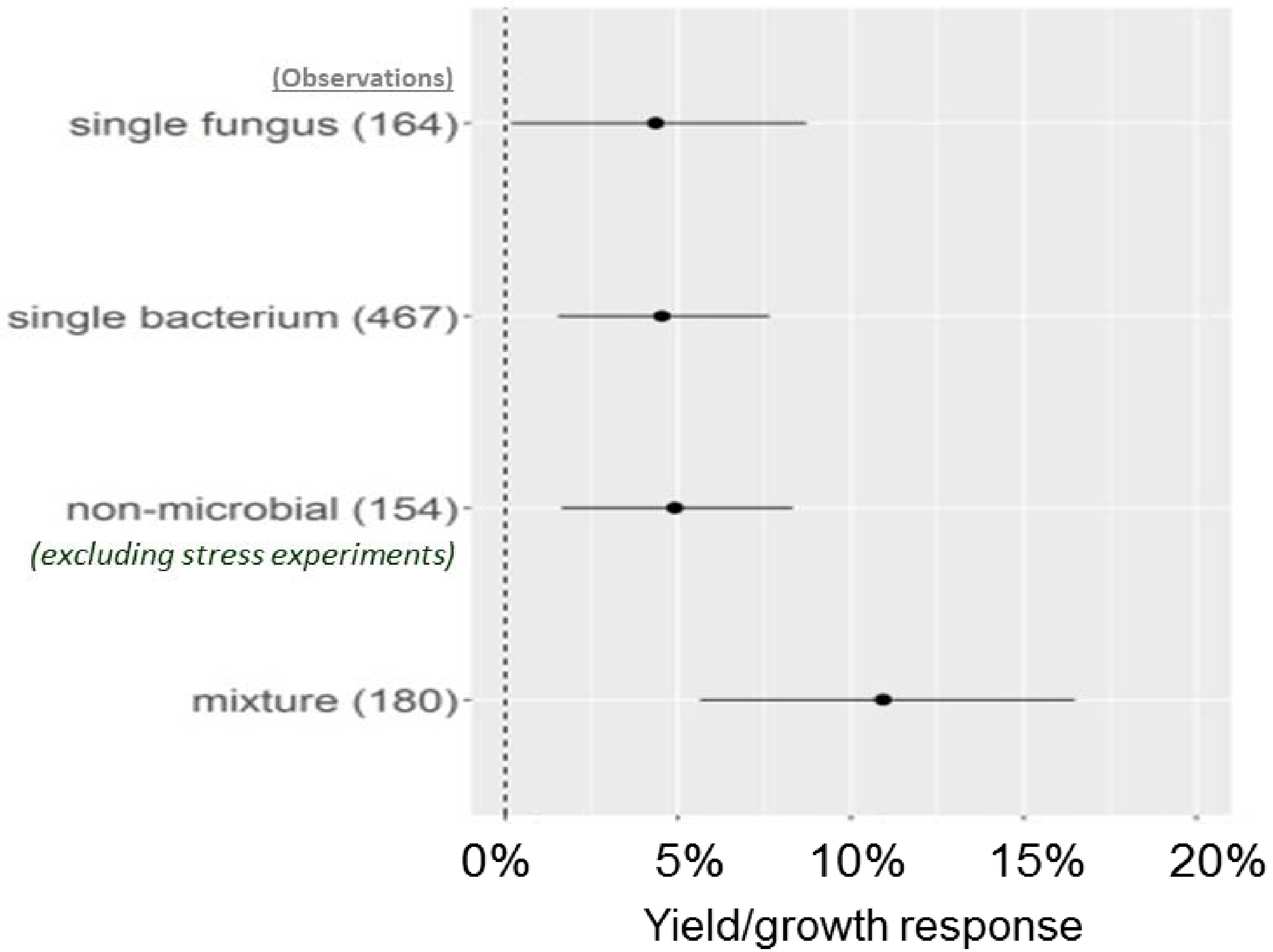


Lekfeldt et al. 2017

Poster Posta et al.

Assessment of Consortium Products

Better performance of combination (consortium products) as compared with single agents ?



The effect of single or combination product (mixture) application on the mean effect of microbial inoculants on plant growth/yield. A total of 965 observations from BIOFECTOR pot and field experiments were included into the analysis (Lekfeldt et al., 2017)

Example:

Bio-stimulants for improved cold stress tolerance during early growth in maize - Synergistic Effects -

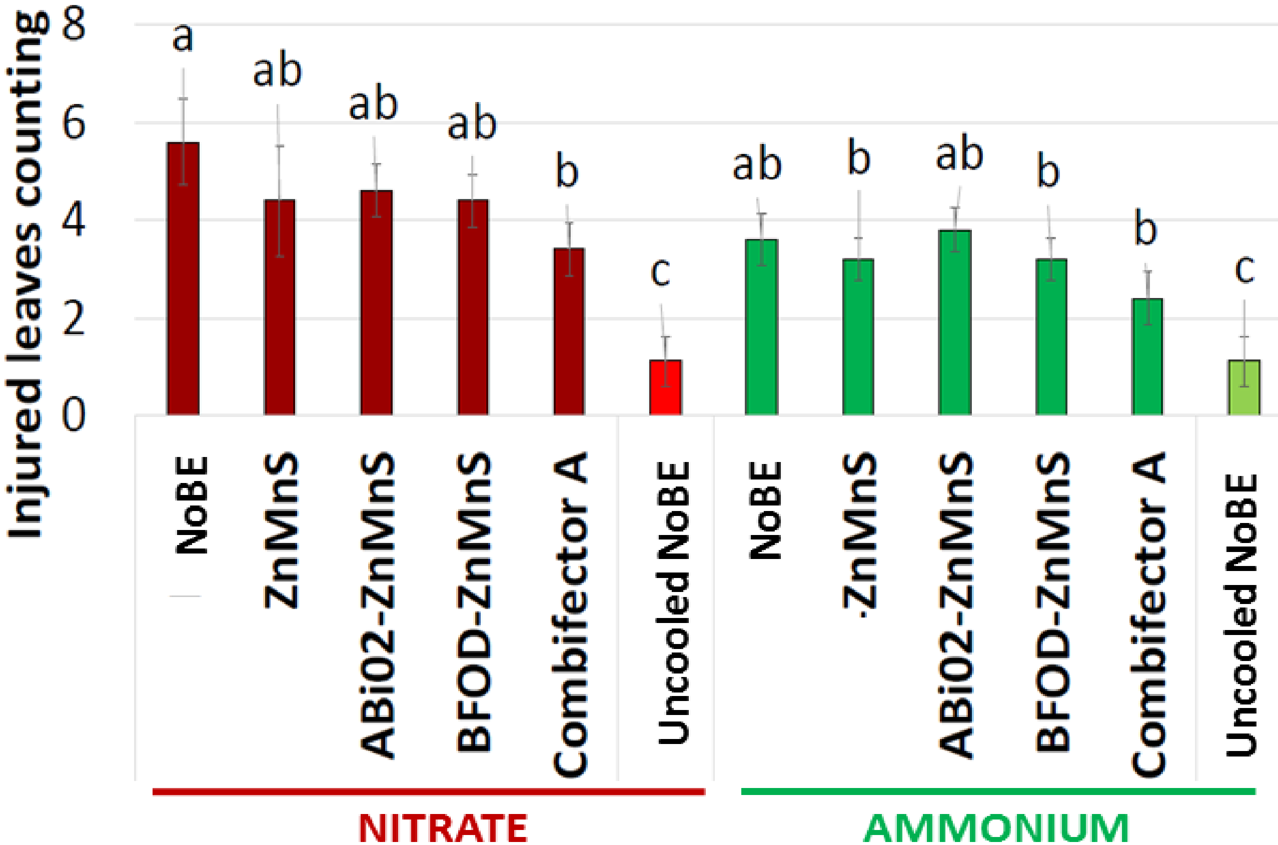
Poster Moradtalab et al.

Maize ;2 weeks
12°C soil temperature

Bradacova et al., 2016

Number of damaged leaves

(cold stress experiment maize – silty loam pH 6.8, 14d 12°C)

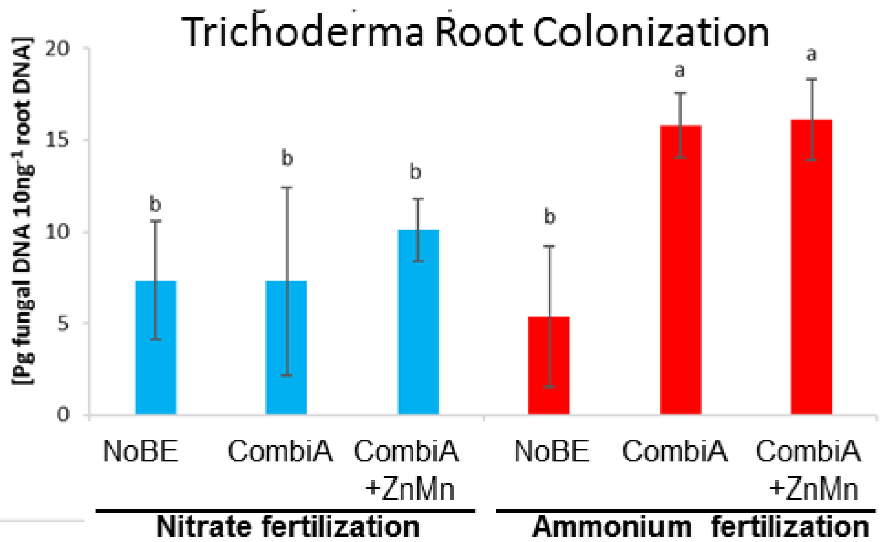


- Ammonium fertilization showed synergistic protective effects in combination with Zn/Mn seed dressing (ZnMnS) and microbial Inoculants:
  - **ABI02** (*Bacillus atrophaeus*) **BFOD** (Penicillium sp.),
  - **Combifector A:** Bacillus/Trichoderma/Pseudomonas + Micronutrients

- Leaf damage declined in the order :
  ABI02+ZnMn > ZnMn > BFOD+ZnMn > CombifectorA > uncooled control

Synergistic activation of metabolic stress defence lines by fertilizer effects and different components of the consortium product CombiA

% change relative to NO_3 fertilization	NH_4 only	CombiA	CombiA Zn/Mn
Root length	n.s	+ 101	+159
Oxidative stress			
Leaf damage	- 33	- 33	- 62
SOD	+23	+51	+ 66
POD	+29	+58	+ 58
Antioxidants	+15	+46	+ 46
Phenolics	+13	+38	+130
Cryo-Protectants			
Proline	n.s	+104	+102
Sugars	n.s	+ 72	+ 34
Nutrient status: Zn	+72	n.s.	+133
Stress Priming			
ABA	+36		
Salicylic acid	+28		
Jasmonic acid	+42		
Indole acetic acid	+48		



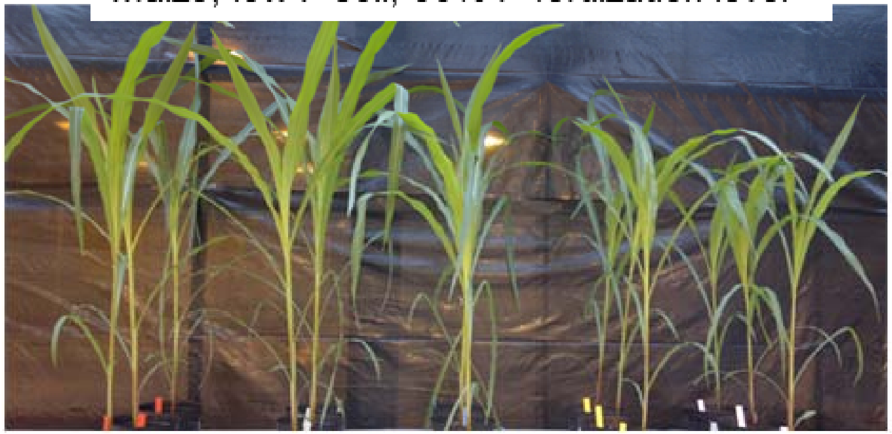
- Ammonium fertilization but not Zn/Mn promotes root colonization with the CombiA strain of Trichoderma

Moradtalb, Ahmed and Geistlinger 2017

Tomato, low P soil, 50% P fertlization level

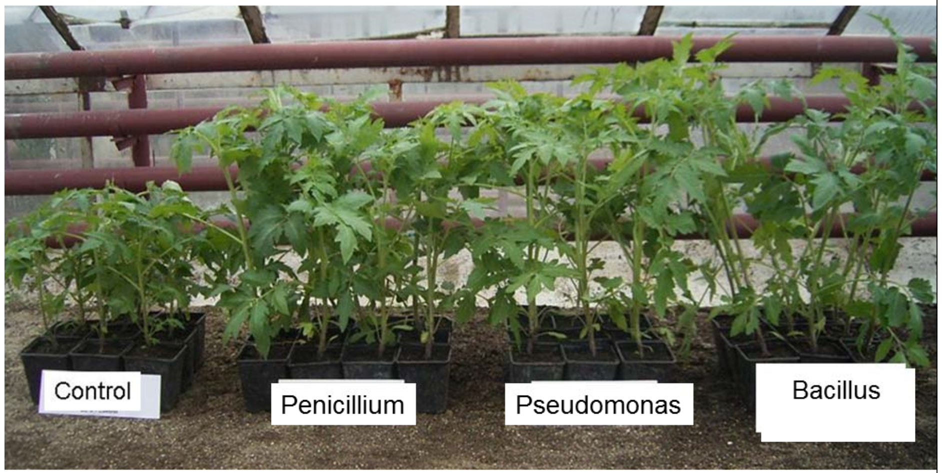
Control Trichoderma Proradix FZB42 P. jessenii

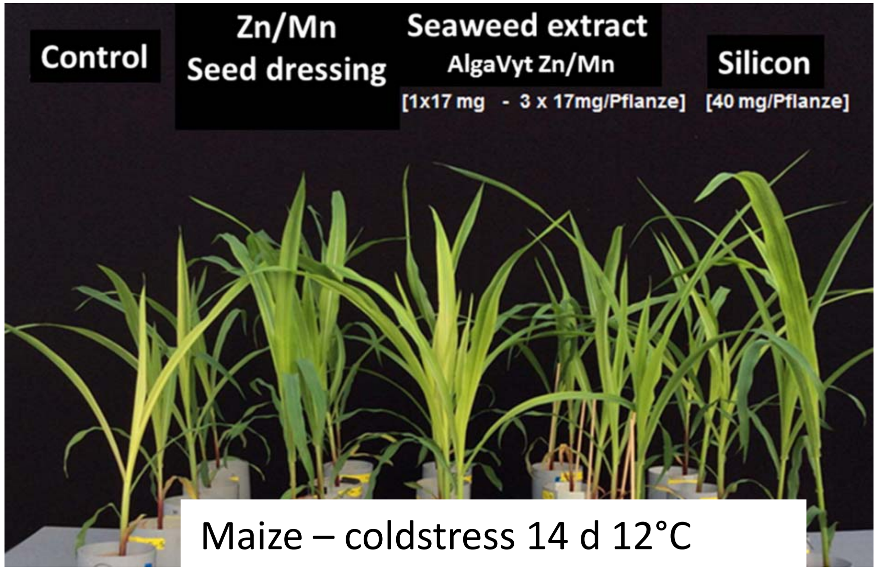
Maize, low P soil, 50% P fertlization level



P. jessenii FZB42 Proradix Trichoderma Control

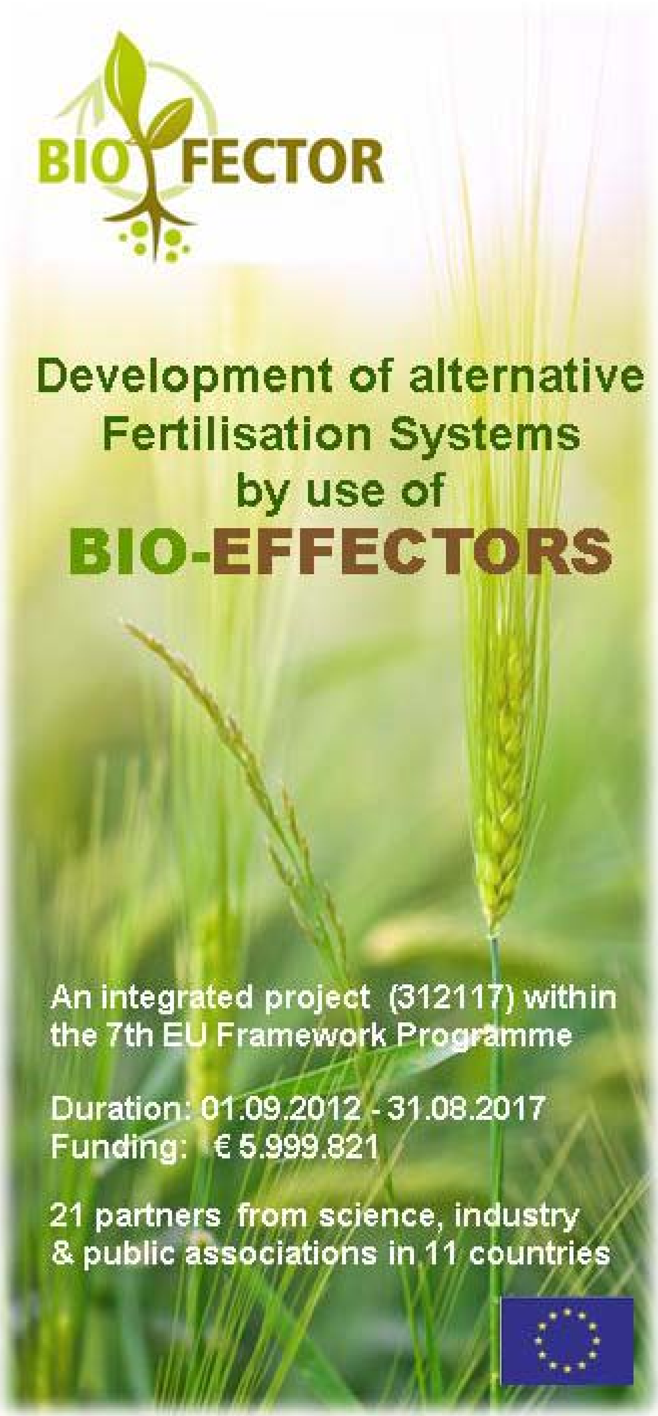
Organic Recycling Fertilisers – Composted Manures





The right set of circumstances (RSC)

Under suitable application conditions, Biostimulants of different origin exhibit similar effects → underlines importance of environmental factors



Conclusions

- The efficiency of **biostimulants** is largely determined **by specific application conditions**

„The right Set of Circumstances“(RSC**)**

No general responses !

- Under **RSC** conditions significant und reproducible effects can be expected for various BEs of different origin

- Our challenge is a clearer definition and under-standing of the **RSC** condtions, and to find management tools to meet these requirements for practice implementation

Do we need new application equipment for application of „biorationals“?

Efficacy and risks of biorational products in IPM strategies - acceptable?

13-14 December 2017 - Braunschweig, Germany

Ing. Petr Harašta, Ph.D., Czech Phytomedical Society

“Biorationals“

- “biorationals“ - a concept that affects a **very wide range** of products and preparations, the application is also a broad concept in this case,
- Microorganism, plant extracts, basic substances, semiochemicals, as well as non-pesticidal products like biostimulants, biological yield enhancers, plant health promoters, and soil conditioners,…
- some can be applied in a common way (spraying), others require attention

“Biorationals“

- The critical point using „biorationals“ is that, they are deemed to have low effectiveness.
- How can the right application technology can support their use, avoid losses and find the target precisely?

- Between the different equipment types we find approx. 30% differences in dose and application effectiveness.

- the quality of the carrier medium (e.g. for pesticides, mostly water - must be clean and its inappropriate characteristics may affect their effectiveness)

- Do we need new application equipment for applying „biorationals?”

Application equipment

- Quality of application plays a major role in all cases, whether they are pesticides, fertilizers or other products and resources including „biorationals“!
- The use of application techniques must be safe, effective and accurate in all areas of pest control and for use of all products

Drift reduction

application

Application equipment-questions

What about drift

What about nozzles: standard, antidrift, other, what is
a solution for some bioproducts?

What about filtration:

What about mixing (agitation)of liquid, ……..if spraying?

Incorrect use, settings, calibration, maintenance and control = increasing risks and losses, reduction of effects of products

Appl. of “Biorationals“

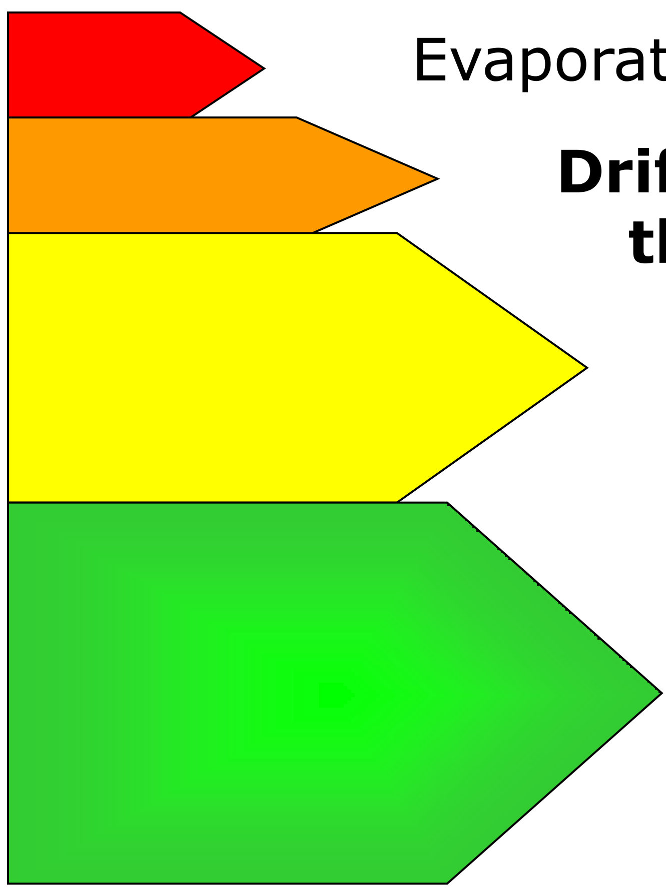
- Application equip. = spraying, manual application, aerial application, UAE application, seed treatment, many others….
- Pesticides application equipment = ISO EN standards (sprayers - 16119, 19934, 16122,….)

  only for pesticides (chemicals) application!!

- Spreaders – fertilizers application, basic requirements, equipment in use - no inspection

ESTIMATION OF SPRAY LOSSES IN ARBOREAL CROPS

(% of applied)



Evaporation losses (4 - 6%)

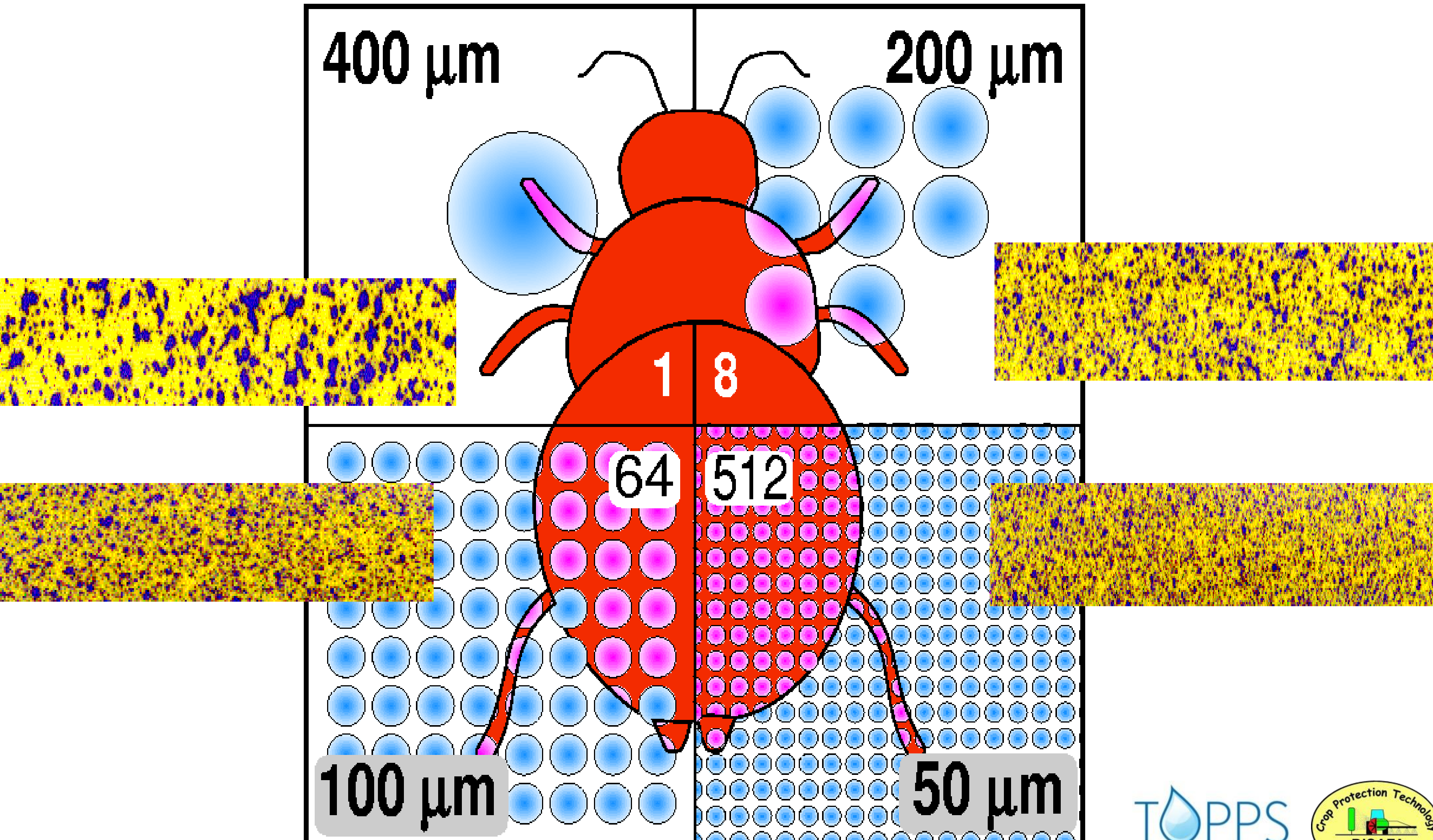
Drift losses in atmosphere and over the target (8 - 15%)

Ground losses (10 - 60%)

(They can originate run-off)

Useful for biological scopes (19 - 56%)

DROPLET SIZE INFLUENCES THE NUMBER OF AVAILABLE DROPLETS



Non-chemical products-CZ

- Non chemical products =biocontrol, physical, mechanical methods,….
- support from the officials

- registration/authorization of bioproducts - without some fees
- promotion through IPM, risks mitigation, replacement of pesticides by non-chemical products , ecofarming
- subsidy amounting of 50% for purchase of some products is discussed

no discussion about their application

Example: Consumption of product XX: kg/year – 140 kg = 1 400 ha (savings cca 1 400 l of chemical fungicides) . Tank-mix with herbicides, insecticides and fertilizers - is it OK?


Thank you for your attention

Microorganisms between Plant Protection and Biostimulation

Joerg Geistlinger
Hochschule Anhalt
Anhalt University of Applied Sciences
Institute of Bioanalytical Sciences

Focus of the Talk:

Vital Microbial Inoculants Applied in Agricultural Practice

Plant beneficial bacteria and fungi

Definition Biostimulant:

Plant biostimulants are any (substances or) microorganisms applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content.

Plant biostimulants stimulate natural processes *in planta*!

Biostimulation Features

- Growth promotion, incl. root growth (lateral root development)

  Enhanced nutrition efficiency

  Hormonal effects, hormone-like (bioactive) compounds
- Abiotic stress tolerance

  (drought/flood, salt/osmotic, heat/cold, heavy metal, photo, oxidative)
- Enhanced crop resilience/vigor
- Early flowering, enhanced flowering
- Crop quality maximization (value-adding ingredients)
- Post-harvest traits (extended shelf-life, decay delay, foraging capacity)

Definition Biocontrol Agents

The control of one organism by another.

Biocontrol agents are living (micro)organisms protecting plants against their enemies, i.e. reducing the population of pests or diseases to acceptable levels.

Biocontrol Features

- Antibiosis – anti-microbial metabolites (antagonism)
- Competition – for space (occupying ecological niches)

  for macro/micro nutrients

  for Iron (chelators, siderophores)
- Parasitism – myco-parasitism (antagonism)

  bacterial (*Actinomycetes*)

  fungal (*Trichoderma* spp.)
- Induced Systemic Resistance (ISR) – cell walls (callose, lignin, phenolics)

  enzymes (chitinase, peroxidase, polyphenoloxidase, PAL)

  small molecules (phytoalexines)

Conflicting Contents

Biostimulation

- Abiotic stress tolerance (<u>mediated by plant signaling cascades</u>)

    via perception of bioactive substances

    induction/release of signaling compounds

Biocontrol

- Induced Systemic Resistance (<u>mediated by plant signaling cascades</u>)

    via perception of bioactive substances

    induction/release of signaling compounds

Overlap

Term: **Broad-spectrum resistance**
pathogens, insects and/or nematodes but also against abiotic stresses

- Multiple-stress responsive genes
- Shared responses in major biotic and abiotic stress pathways
- Biotic and abiotic crosstalk in several gene networks

ELSEVIER

Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks

Miki Fujita[1,2,3], Yasunari Fujita[4], Yoshiteru Noutoshi[5,6], Fuminori Takahashi[1,3,7], Yoshihiro Narusaka[8], Kazuko Yamaguchi-Shinozaki[2,4,9] and Kazuo Shinozaki[1,2,3]

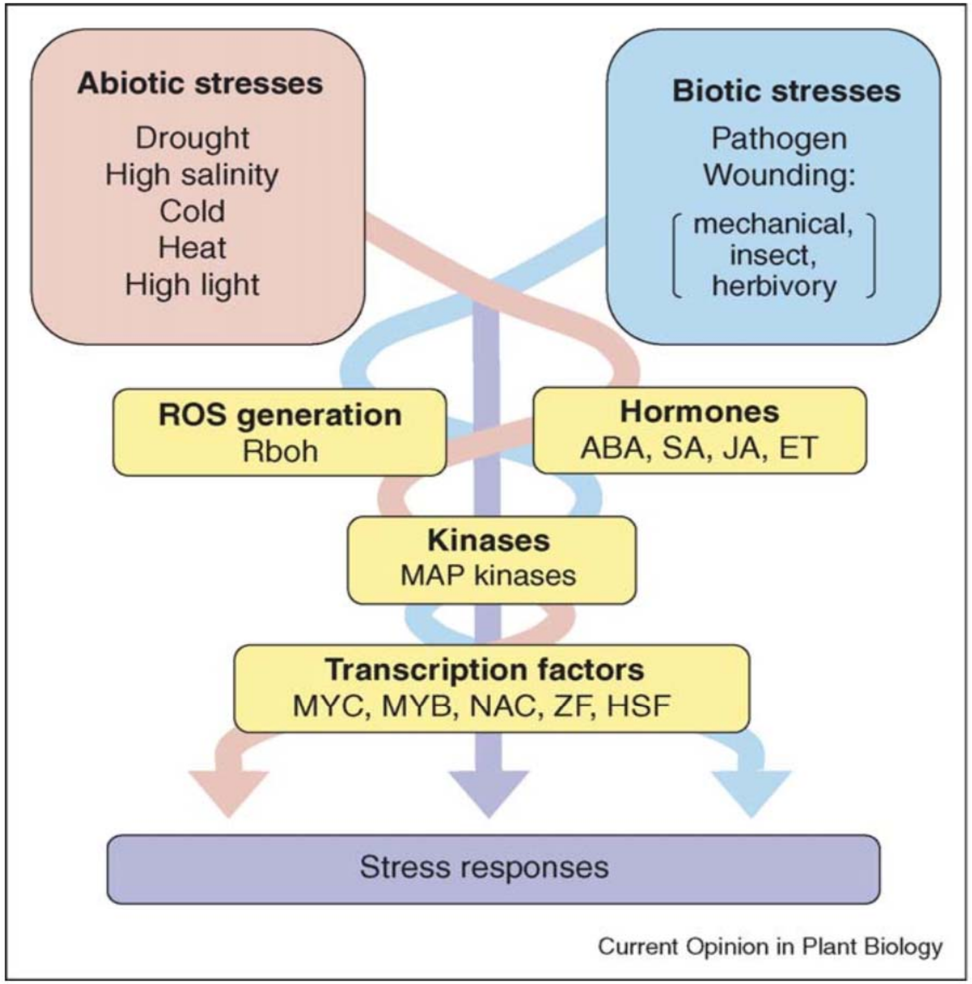
Plants have evolved a wide range of mechanisms to cope with biotic and abiotic stresses. To date, the molecular mechanisms that are involved in each stress has been revealed comparatively independently, and so our understanding of convergence points between biotic and abiotic stress signaling pathways remain rudimentary. However, recent studies have revealed several molecules, including transcription factors and kinases, as promising candidates for common players that are involved in crosstalk between stress signaling pathways. Emerging evidence suggests that hormone signaling pathways regulated by abscisic acid, salicylic acid, jasmonic acid and ethylene, as well as ROS signaling pathways, play key roles in the crosstalk between biotic and abiotic stress signaling.

Addresses
[1] Gene Discovery Research Group, RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Yokohama, Kanagawa 203-0045, Japan

Introduction

Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. To survive under such conditions, plants have evolved intricate mechanisms to perceive external signals, allowing optimal response to environmental conditions. Phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) are endogenous, low-molecular-weight molecules that primarily regulate the protective responses of plants against both biotic and abiotic stresses via synergistic and antagonistic actions, which are referred to as signaling crosstalk [1,2,3••]. Moreover, the generation of reactive oxygen species (ROS) has been proposed as a key process that is shared between biotic and abiotic stress responses [4,5•]. Rapidly accumulating data, resulting from large-scale transcrip-

Plant signaling pathways consist of complex networks with frequent crosstalk, allowing plants to regulate both abiotic stress tolerance AND disease resistance.



Convergence points in abiotic and biotic stress signaling networks.

Convergence Points

Abscisic Acid (**ABA**) – abiotic stress response
Jasmonic Acid (**JA**), Salicylic Acid (**SA**), Ethylene (**ET**), Hypersensitive Reaction (**HR**)
Reactive Oxigen Species (**ROS**) – biotic stress response

Respiratory burst oxidase homolog (*rboh*), required for ROS production
induces ABA and HR → crosstalk point

Enhanced **r**esponse to **A**BA 3 (*era3*) allelic to **e**thylene **in**sensitive 2 (*ein2*)
intersection in ABA and ET signaling → crosstalk point

Jasmonate **in**sensitive 1 (*jin1*) allelic to MYC2 transcription factor (tf)
Low MYC2 enhances JA/ET pathogen responses → crosstalk point

MYB2 (tf) highly homolog to *Botrytis* susceptible 1 (*bos1*), mediated by JA
bos1 mediates biotic and abiotic stress signaling via ROS → crosstalk point

NAC RD26 (tf) induced by JA, H_2O_2, pathogens, also by drought, salt, ABA
functions in pathogen defense, senescence, ABA signaling → crosstalk point

Convergence Points

MAPK (mitogen-activated protein kinase) cascades include multiple stress responses

MKK2 – MPK4/6 cascade involved in cold and salt stress signaling

MKK4/5 – MPK3/6 cascade regulates pathogen defense via WRKY22/29 (tf)

MPK3/6 also induced by abiotic stress (via ABA) → crosstalk point

MPK6 – induces ET production via ACC (aminocyclopropan 1 carbocylic acid) synthase

induces JA-dependent root growth and MYC2 expression → crosstalk point

activates ROS signaling via serine/threonine kinases → crosstalk point

inducible by H_2O_2 and ABA → crosstalk point

*Os*MPK5 ortholog to *At*MPK3, induced by ABA and infection in rice → crosstalk point

the plant journal

SEB Society for Experimental Biology

The Plant Journal (2017) **92**, 557–570 doi: 10.1111/tpj.13674

MAPK kinase 10.2 promotes disease resistance and drought tolerance by activating different MAPKs in rice

Haigang Ma, Jie Chen, Zhenzhen Zhang, Ling Ma, Zeyu Yang, Qinglu Zhang, Xianghua Li, Jinghua Xiao and Shiping Wang*
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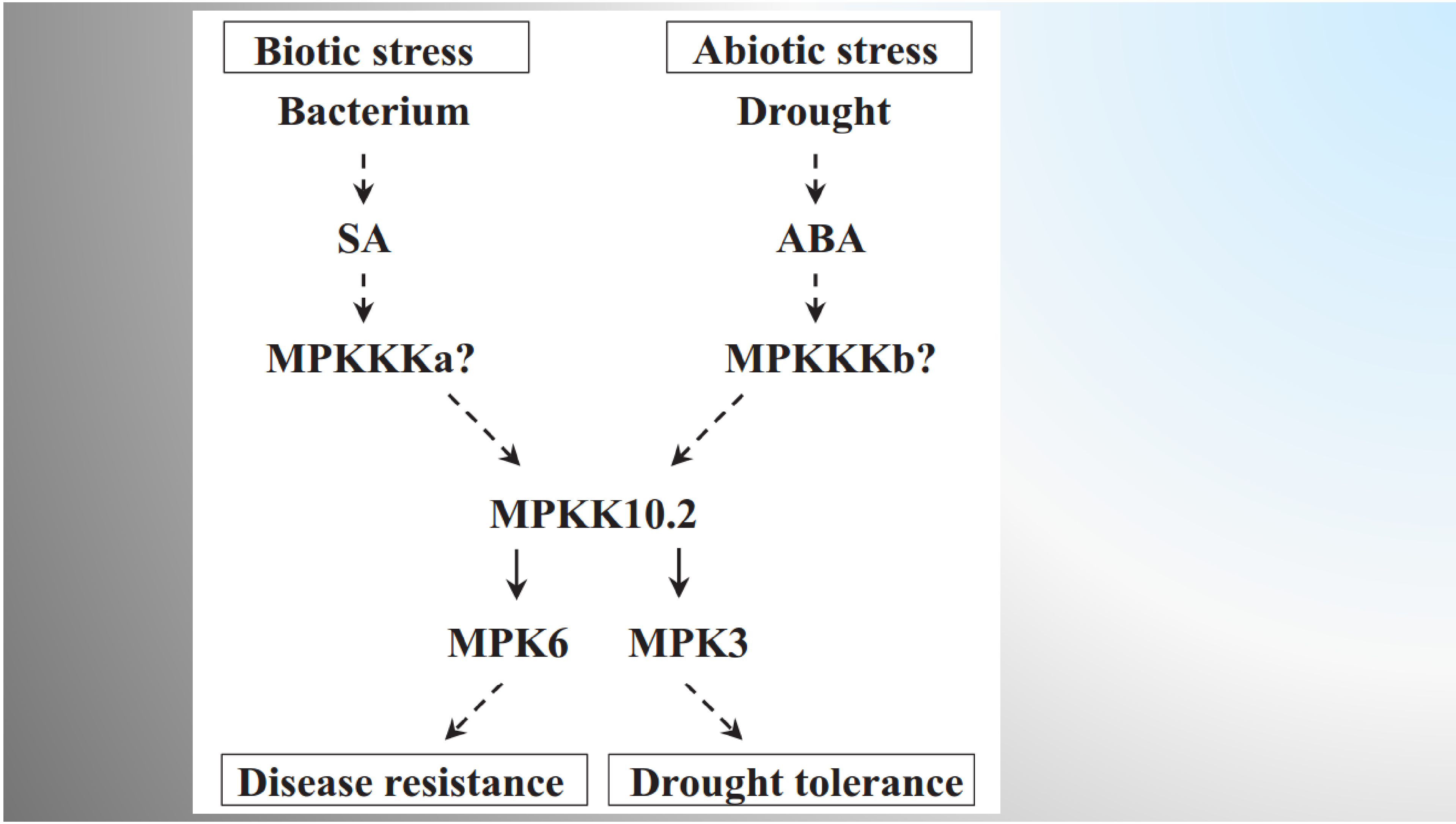
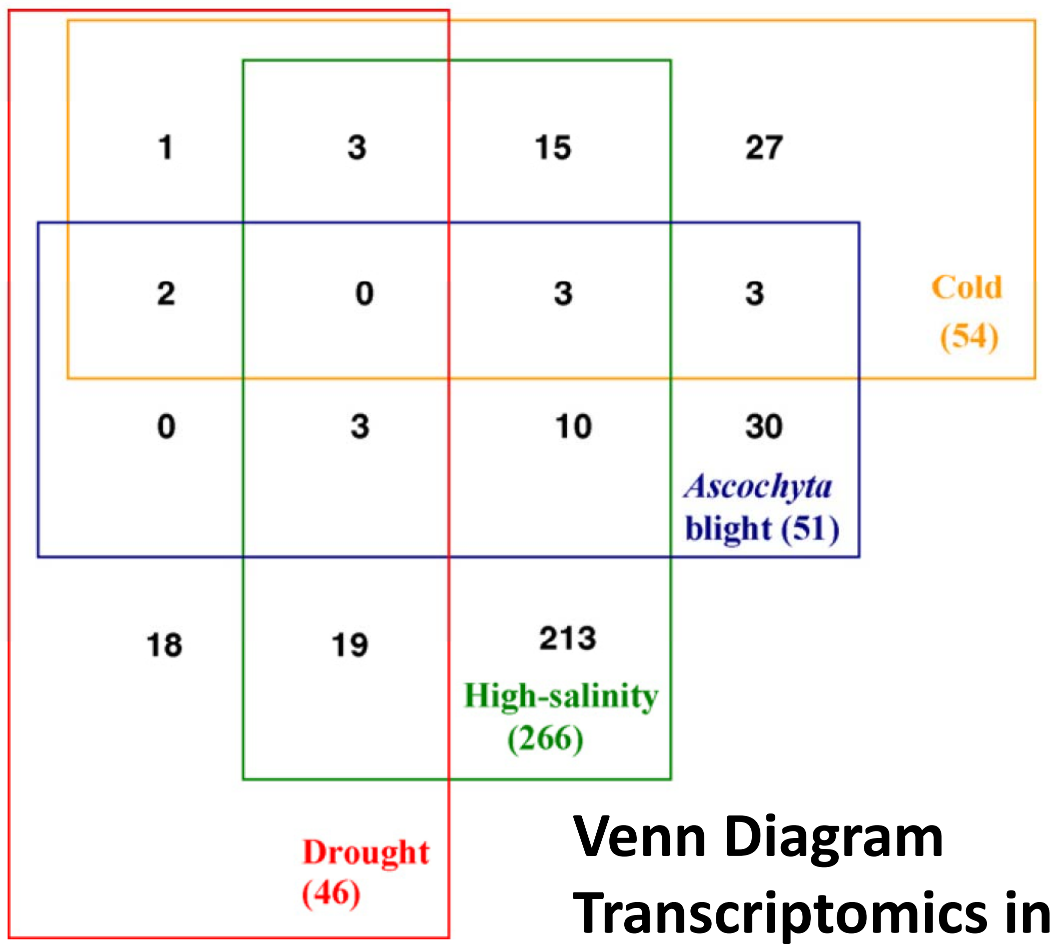
SUMMARY

Mitogen-activated protein kinase (MAPK) cascades, with each cascade consisting of a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK, have important roles in different biological processes. However, the signal transduction in rice MAPK cascades remains to be elucidated. We show that the structural non-canonical MAPKK, MPKK10.2, enhances rice resistance to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), which causes bacterial streak disease, and increases rice tolerance to drought stress by phosphorylating and activating two MAPKs, MPK6 and MPK3, respectively. *MPKK10.2*-overexpressing (oe) plants showed enhanced resistance to both *Xoc* and drought, whereas *MPKK10.2*-RNA interference (RNAi) plants had increased sensitivity to both *Xoc* and drought. MPKK10.2 physically interacted with MPK6 and MPK3, and phosphorylated the two MAPKs *in vivo*. Transcriptionally modulating *MPKK10.2* influenced MPK6 phosphorylation during rice-*Xoc* interaction, and *MPKK10.2*-oe/*MPK6*-RNAi double mutants showed increased sensitivity to *Xoc*. *MPKK10.2*-oe/*MPK3*-RNAi double mutants showed survival rates similar to those of control plants, although the survival rates of *MPKK10.2* transgenic plants changed after drought stress. These results suggest that MPKK10.2 is a node involved in rice response to biotic and abiotic responses by functioning in the cross-point of two MAPK cascades leading to *Xoc* resistance and drought tolerance.

Keywords: MAPK, bacterial streak, drought, phosphorylation, *Oryza sativa*.

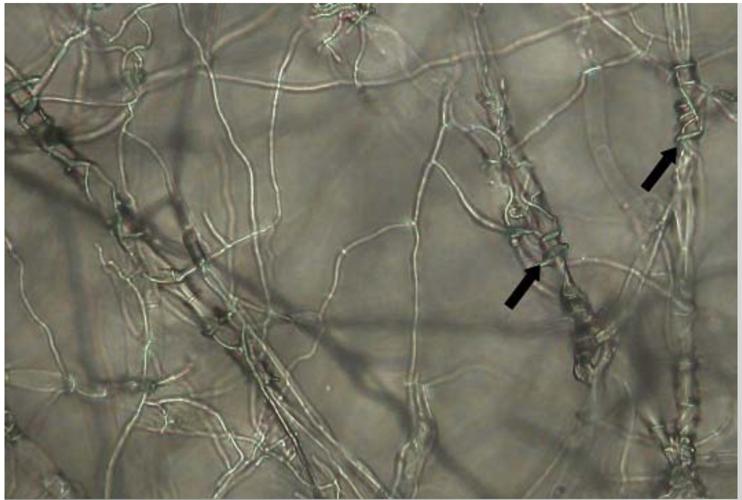

Biotic stress
Abiotic stress
Bacterium
Drought
SA
ABA
MPKKKa?
MPKKKb?
MPKK10.2
MPK6
MPK3
Disease resistance
Drought tolerance

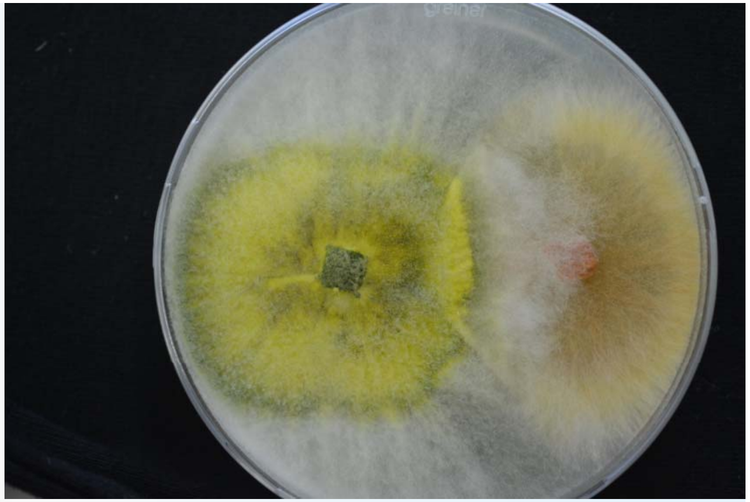
Total: 417 genes



Venn Diagram Transcriptomics in *Cicer arietinum*

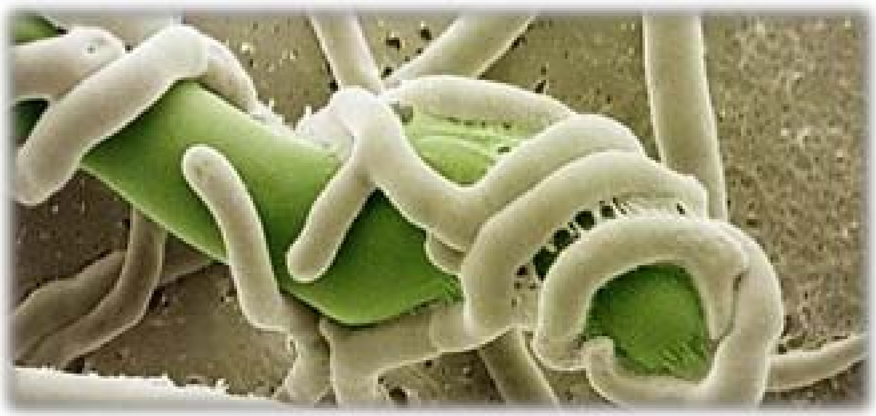
**Why should microbial biostimulants
that activate multiple biotic and abiotic stress pathways
for enhanced broad-spectrum crop resistance
be classified as (bio)pesticides ?**





Biocontrol

What we got…..



Bacterial Biocontrol Agents

Genera: *Bacillus*, *Pseudomonas*, *Burkholderia*, *Serratia*, *Streptomyces*, *Actinoplanes*, *Stenotrophomonas*, *Paenibacillus*, *Klebsiella*, *Alcaligenes*, *Pantoea*, *Ralstonia*…

Competition for nutrients and space (root niches, root adhesion)
motility, chemotaxis, detox. antimicrobial root compounds
root exudates and mucilage (sugars, organic acids, amino acids)

Allelopathy/Allelochemicals:
Iron-chelating siderophores, antibiotics, biocidal volatiles, antifungals

Antibiosis: amphisin, oomycin, phenanzine, oligomycin, zwittermicin, tensin…..
Lytic enzymes, cell wall hydrolases

Detox/degradation: antibiotics, phytotoxine produced by pathogens

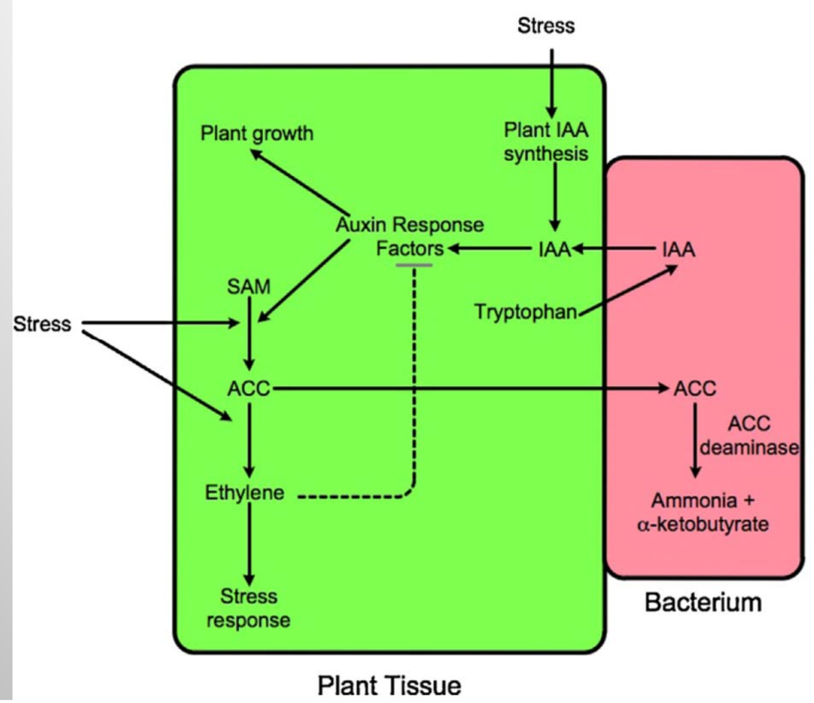
ISR/Priming: flagellin, siderophores, lipopolysaccharide, volatiles
induce plant defense chemicals (callose, phenolics, lignin, phytoalexins, lytic enzymes, PR-proteins)

Bacteria for Biostimulation

Genera: *Rhizobium*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Bredybacillus*, *Rhanella*, *Azotobacter, Azospirillum*, *Klebsiella*, *Acinetobacter*, *Stenotrophomonas*, *Serratia…*

Growth promo: N_2-fixation, nutrient solubilization (P), auxin and IAA production, ACC deaminase production, ammonia, HCN, siderophores, mycorrhiza helper functions

Abiotic stress tolerance:
Bacterial auxin, IAA and SA production
Decreased heavy metal toxicity
Micro-nutrient mobilization



Fungal Biocontrol Agents

Genera: *Trichoderma*, *Talaromyces*, *Cladorrhinum*, *Idriella*, *Penicillium*, *Chaetomium*, *Minimedusa*, *Coniothyrium*…
avirulent *Fusarium*, *Rhizoctonia* and *Phialophora* strains

Competition for nutrients and space (infection sites)

Antibiosis: Gliovirin, Gliotoxin, Glucose-Oxidase (H_2O_2 production)

Mycoparasites: *Trichoderma*, *Leucosporidiella*, *Platygloea*, *Rhodotorula*, *Sporidiobolus*

ISR: avirulent strains, *Trichoderma*, *Penicillium*

Effector molecules (elicitors): xylanase, oligogalacturonide, chitosan

Fungi for Biostimulation

Genera: *Trichoderma*, *Glomus* (AMF), endo/ekto-mycorrhizal fungi, endophytes...

Abiotic stress tolerance:
Sebacina, *Piriformospora*, *Neotyphodium*, *Trichoderma*, *Curvularia, Acremonium*
mainly salt, drought and cold stress
- triggering stress response systems in plants
- produce anti-stress chemicals (osmolyte, loline, sugar/alcohols)
oxidative stress: ROS / RNS detox., antioxidants

Plant growth promotion:
Neotyphodium positively influences photosynthetic CO_2 fixing in host plants
Piriformospora produces cytokinine
Paecilomyces produces IAA and gibberelline

Overlaps again

Bacteria in biocontrol and biostimulation
Bacillus, *Pseudomonas*, *Paenibacillus*, *Klebsiella*, *Serratia*, *Stenotrophomonas*

Fungi in biocontrol and biostimulation
Trichoderma, *Penicillium* – mycorrhiza and endophytes

Should competition really be a biocontrol trait ?

Regulation, Legislation

Microorganisms should be identified at the strain level (**not always easily done!**)
postulating that most biological activities are strain-specific (**right or wrong?**)

Biostimulants

should be defined according to the **intended agricultural outputs**
<u>nutrient efficiency</u> may cover nutrient mobilization, uptake, transport, storage, assimilation but also root development
<u>abiotic stress tolerance</u> physical or chemical stressors of non-biological origin
<u>quality traits</u> may be very diverse (from nutritional value to shelf-life)

Registration is possible
under national fertilizer legislations **or**
EU PPP regulation

Why not register under the EU fertilizer laws ?

Impossible, BIOSTIMULANTS cannot be fertilizers under EU legislation !
Here, fertilizers are clearly defined:

- Provide nutrients for plants
- **Primary nutrients**: NPK
- **Secondary nutrients**: Ca, Mg, Na, S
- **Micro-nutrients**: boron, cobalt, copper, iron, manganese, molybdenum, zinc
  excl. inorganic materials (exception: chelating or complexing agents)

All **microorganisms that interact with plant physiology** must be registered under the PPP regulation, even if they do not protect plants from pests or diseases.

Subject:	Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL laying down rules on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009

(4) Decision No 768/2008/EC of the European Parliament and of the Council[4] lays down common principles and reference provisions intended to apply across sectoral legislation in order to provide a coherent basis for revision or recasts of that legislation. Regulation (EC) No 2003/2003 should therefore be replaced by a Regulation drafted to the extent possible in accordance with that Decision.

(5) Contrary to most other product harmonisation measures in Union legislation, Regulation (EC) No 2003/2003 does not prevent non-harmonised fertilisers from being made available on the internal market in accordance with national law and the general free movement rules of the Treaty. In view of the very local nature of certain product markets, this possibility should remain. Compliance with harmonised rules should therefore remain optional, and should be required only for products, intended to provide plants with nutrient or improve plants' nutrition efficiency, which are CE marked when made available on the market. This Regulation should therefore not apply to products which are not CE marked when made available on the market.

Currently no harmonized frameworks are existing neither in the EU, nor in the US

EC regulation No 1107/2009 on plant protection products (‘PPPs’) is applicable to all categories of biostimulants and biocontrol agents, considering a very broad definition of PPPs.

Lengthy and costly procedure to place a PPP on the European market.

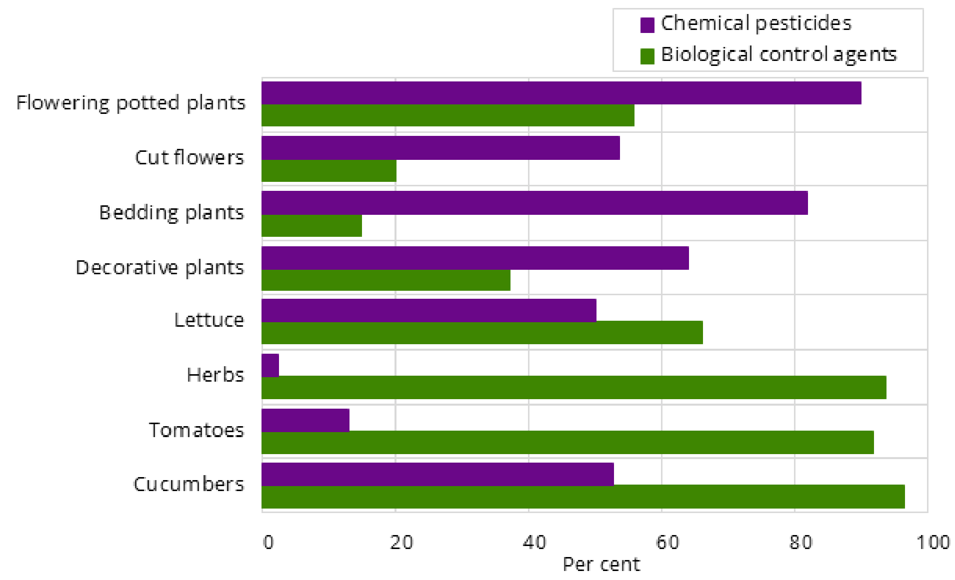
Alternative: The national fertilizer legislations !

BCA Market

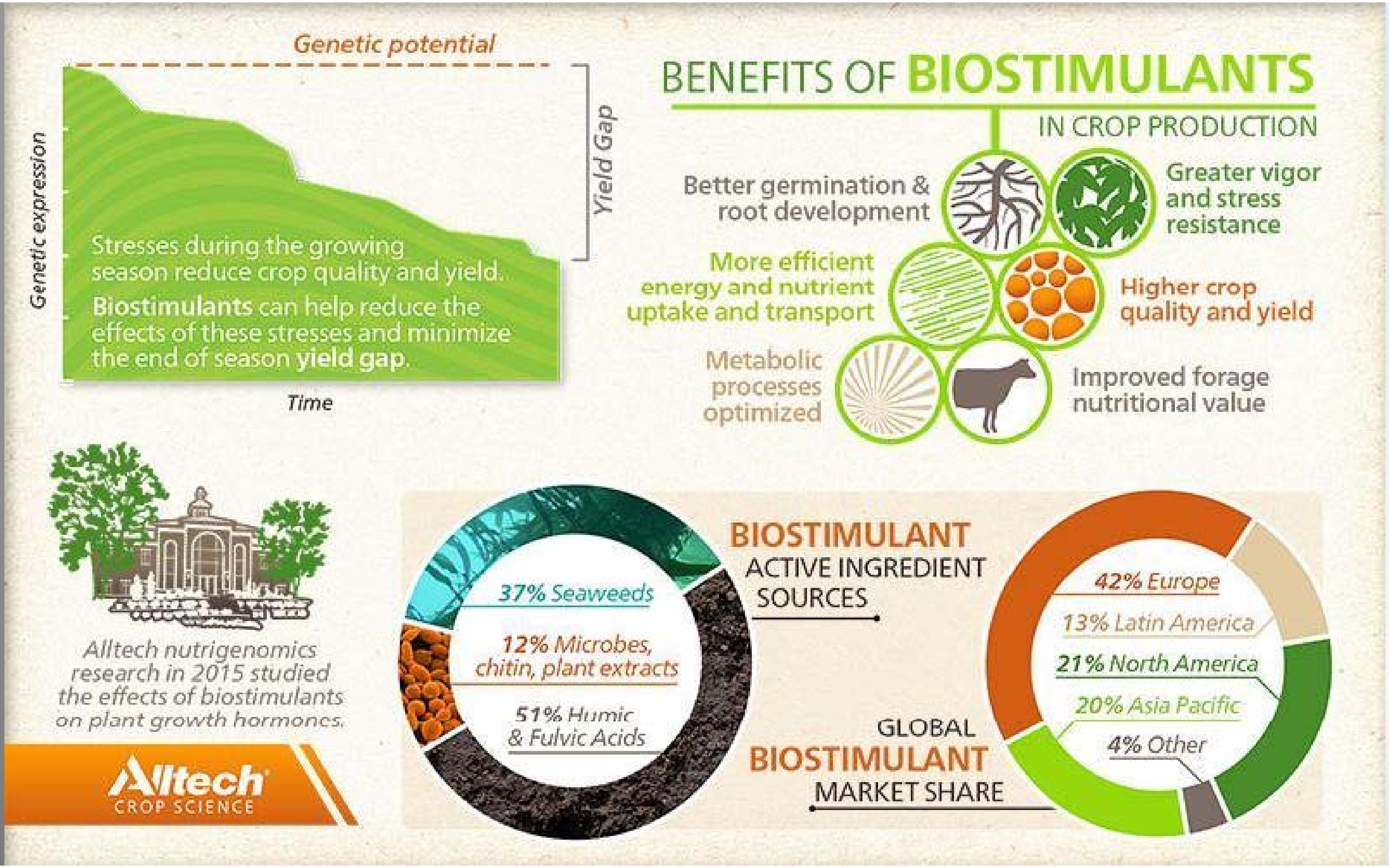
Top Players

BASF
Syngenta
Bayer CropScience Biologics
DuPont
Novozymes
Koppert Biological Systems
Monsanto Company
Marrone Bio Innovations
Biobest
Certis USA
Andermatt Biocontrol

Figure 1. Share of greenhouse area treated[1] with biological control agents and chemical pesticides in 2015

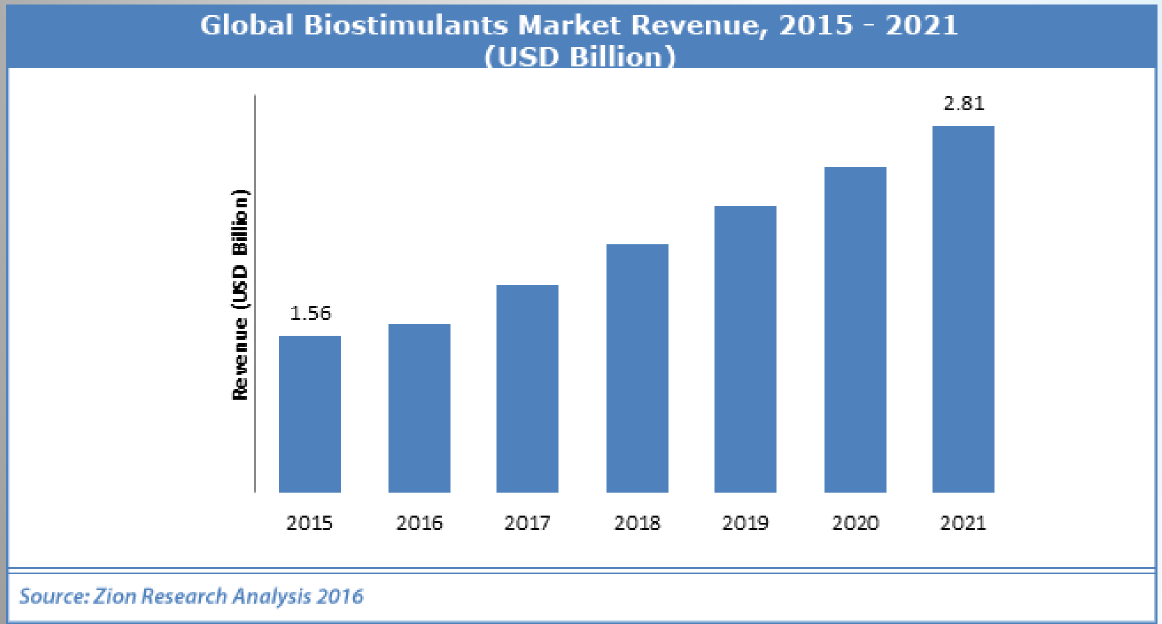


The BCA market is expected to reach 3 billion USD by 2020 (1.7 b. USD in 2015) growth rate > 10%, approx. 4% of the global pest control market



Genetic potential
Genetic expression
Yield Gap
Stresses during the growing season reduce crop quality and yield.
Biostimulants can help reduce the effects of these stresses and minimize the end of season yield gap.
Time
BENEFITS OF BIOSTIMULANTS
IN CROP PRODUCTION
Better germination & root development
Greater vigor and stress resistance
More efficient energy and nutrient uptake and transport
Higher crop quality and yield
Metabolic processes optimized
Improved forage nutritional value
Alltech nutrigenomics research in 2015 studied the effects of biostimulants on plant growth hormones.
Alltech
CROP SCIENCE
37% Seaweeds
12% Microbes, chitin, plant extracts
51% Humic & Fulvic Acids
BIOSTIMULANT
ACTIVE INGREDIENT SOURCES
GLOBAL
BIOSTIMULANT
MARKET SHARE
42% Europe
13% Latin America
21% North America
20% Asia Pacific
4% Other

Comparable to BCA market: 1.7 to 3.0 billion USD



Due to the **lack of acceptance** of biostimulant concepts, market data are of limited reliability. The **regulatory status** of biostimulants is diverse, depending on registration as fertilizer or pesticide, authorized or not in organic production. Biostimulants may be spread over many regulations, without being named as such.

Conclusions

- **Overlaps exist** between Biostimulation and Biocontrol
  - biotic/abiotic stress responses, ISR
  - microbial genera/species/strains applied
- **Traditional definition** of Biocontrol is narrow and precise (**implies direct interaction**)
  - definition for regulation/registration is very broad
  - mainly because indirect effects – **mediated by the crop** – were included
  - indirect effects **better apply to biostimulation** – stimulate natural processes *in planta*
- **Harmonized frameworks** for differentiation, regulation and registration **are lacking**
  - forcing SMEs to **register biostimulants under PPP** would outcompete most companies
  - only multinational enterprises could afford millions USD per registered product
  - **loophole escapes** using national laws are preferable
  - current EU proposal on fertilizers opens this route **on purpose (?)**
- **Markets are fast growing** – all could participate (as long as PPP is avoided ☺)
  - broad participation serves the good aim of “sustainable agriculture”

Latest Trends in Biostimulation and Biocontrol

- Strain combinations
- Complex microbial consortia

Mixtures are considered to be more effective than single strains!

- Synergistic effects
- Enhanced rhizosphere competence (mixtures of fungi and bacteria)

Route of EU regulation for **microbial combination products** not yet determined

Examples exist for substances:

- UVCB (unknown/variable composition biological) substances (EU-REACH regulation)
- Botanicals (one or more components found in plants) under EU PPP regulation

REACH: registration, evaluation, authorization of chemicals

CRISPR-Cas

Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats - **C**RISPR-**as**sociated nucleases

Genome editing occurs as a natural process
Programmable nucleases induce specific double-strand breaks and the cell's own mechanisms repair the breaks by natural processes.

GMO: Involves promotors (35S), transgenes and selection markers (kanamycin)

CRISPR-Cas: possible solely on the protein level (guide-RNAs and/or DNA Oligos)
Leaves no footprints in the edited genomes – CRISPR testing difficult

Discussion still pending – will CRISPR be classified as GMO technique or not?

<u>CRISPR-strains:</u> **to see, or not to see?** – That is the question!

Thank You !

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BioStimulants versus BioControl Agents- Two Sides of a Coin

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Institute for Biology and Biotechnology of Plants, University of Münster, Germany

According to the proposed draft of the fertilizer regulation EC 2003/2003[1], **biostimulants** will be defined as products (containing substances or microorganisms) that “are not as such nutrients, but nevertheless stimulate plants' nutrition processes”, and that“aim solely at improving the plants' nutrient use efficiency, tolerance to abiotic stress, or crop quality traits”. However, if they also increase plant disease resistance, either by stimulating the plant’s own ability to defend itself against a pathogen or by direct action against the pathogen, i.e. in the case of dual-function substances or microorganisms, they are to be treated as **plant protection products**to which EC regulation 1107/2009[2]applies. Here, the question arises whether a product that is marketed solely to support plant nutrition can be treated as a biostimulant even if plant protective activities have been described in the literature for the active ingredient (but are not claimed for the product). If that is not the case, i.e. if such a product would not be eligible as a biostimulant, problems will automatically arise. Treatments that will improve plant vigour by improving plant nutrition will not only increase plant stress tolerance, but at the same time also plant disease resistance. <u>An example microorganism would be mycorrhiza</u> fungi which are explicitly mentioned as biostimulants in Annex II of the draft fertilizer regulation[1], but which are known to also improve plant disease resistance.

<u>An example substance would be chitosan</u>. The term chitosan describes a family of biopolymers and oligomers consisting of varying numbers and ratios of glucosamine and *N*-acetylglucosamine residues. Depending on the number of residues in the molecule (its degree of polymerisation) and the ratio of the two monomeric units (its degree of acetylation), and possibly also depending on the sequence of the two units within the oligomer or polymer (its pattern of acetylation), chitosans can have different biological activities. Some chitosans can inhibit microbial growth (being funigstatic and bacteriostatic rather than being fungicidal or bactericidal), some chitosans can induce disease resistance in a plant (either by acting as an elicitor triggering resistance reactions, or by acting as a priming agent enabling plant cells to react more efficiently against pathogens), some chitosans can improve abiotic stress tolerance in plants (e.g. against drought or heat stress), and some chitosans can promote plant growth (e.g. root and/or shoot growth) and/or development (e.g. more fruit and/or earlier ripening).

Modern biotechnological methods of chitosan production and/or modification as well as new techniques for chitosan in depth structural analysis allow to produce well-defined (second generation) chitosans having defined and reliable, specific bioactivities, but lacking others. In this way, it is e.g. possible to produce chitosans which have no antimicrobial activities but which do have plant strengthening (i.e. growth promoting and stress tolerance and disease resistance inducing activities). It will probably also be possible to generate chitosans with more pronounced ability to induce stress tolerance and less marked ability to induce disease resistance, and *vice versa*. However, it is highly unlike that it will be possible to dissect these activities fully. The reason behind this is the intimate cross-talk of intracellular signal transduction pathways in plant cells. Even if it were possible to identify a specific chitosan oligomer (or any other compound) that is recognised by a specific receptor which triggers a signal transduction chain leading to metabolic answers increasing abiotic stress tolerance, and to identify another chitosan oligomer (or any other compound) that is recognised by a different receptor which triggers a different signal transduction chain leading to different metabolic answers increasing disease resistance - treatment of the plant cell with either of the two chitosan oligomers will invariably lead to a mixed response, possibly with stronger emphasis on one of the two reactions and less pronounced expression of the other, but not with just one answer(Fig. 1). Clearly, thus, there is a strong interconnection between abiotic stress tolerance and disease resistance.

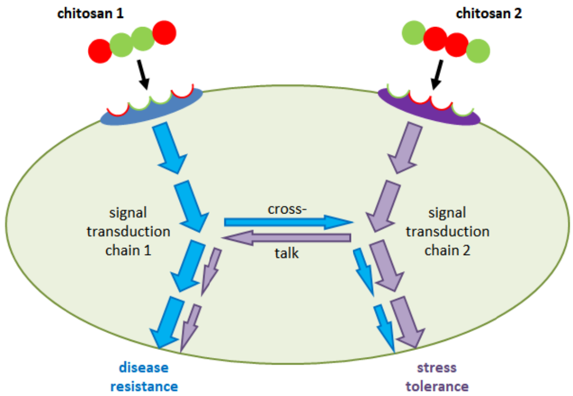


Figure 1: Cross-talk between intracellular signal transduction pathways establishes a signal transduction network, so that induction of abiotic stress tolerance automatically also induces disease resistance, and *vice versa*.

In fact, such a relationship is long known. Under optimum fertilisation conditions, plants grow best and are also healthiest, having the strongest ability to defend themselves against disease and to withstand abiotic stress. Both under-nourished and over-fertilized plants, however, are more prone to disease and stress induced damage. Thus, it is simply not possible to clearly distinguish between **biostimulant action** (as it is suggested to be defined) and biocontrol action (as it is defined now). It would appear more realistic to first distinguish two types of **biocontrol activity**, namely

- (i) direct action against the microbial pathogens and
- (ii) indirect action by strengthening the plant's own ability to defend itself.

(This would be similar to antibiotic treatment (i) *versus*vaccination (ii) in human medicine.) Products containing substances or microorganisms acting on the pathogenic microorganisms would fall under category (i), while products containing substances or microorganisms acting on the plant would fall under category (ii) (Table 1). The former would have to be treated as **plant protection products** under EC 1107/2009while the latter would be treated as **biostimulants**, falling under EC 2003/2003. In this way, consumer safety would also be maintained, as only category (i) products with direct antimicrobial mode of action have potentially toxic activities.

This categorisation would solve the problem that (almost) all products which induce tolerance to abiotic stress also induce resistance to disease - which would otherwise, if the categories defined in the current draft of the EU regulations would enter into force, lead to the situation that (almost) no biostimulants would exist. The alternative solution, namely to categorize products solely according to the claims made by the producers instead of what is known about the bioactivities of the active ingredients, would fail to protect the safety of consumers and the environment, as potentially toxic category (i) products could be registered as biostimulants under the fertiliser regulations which, reasonably, puts less emphasis on toxicity testing. (While the situation would be rather clear then for substances, it might still not be entirely clear for microorganisms which might act directly against pathogenic microbes but not via toxic principles but rather by competition in which case a categorisation as a plant protectant would not be appropriate.)

aim of treatment	disease protection		stress protection
target of treatment	pathogen	crop plant	
mode of action	inhibition of microbial growth	induction of disease resistance	induction of stress tolerance
toxicity	yes	no	
risk potential	high	low	
category	biocontrol (i)	biocontrol (ii)	biostimulant
regulatory framework	1107/2009	2003/2003	

Table1: Proposed categorisation of substances and microorganisms with biocontrol *versus*biostimulant activities depending on whether they act on the microbial pathogen or on the crop plant.

[1] http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52016PC0157&from=EN

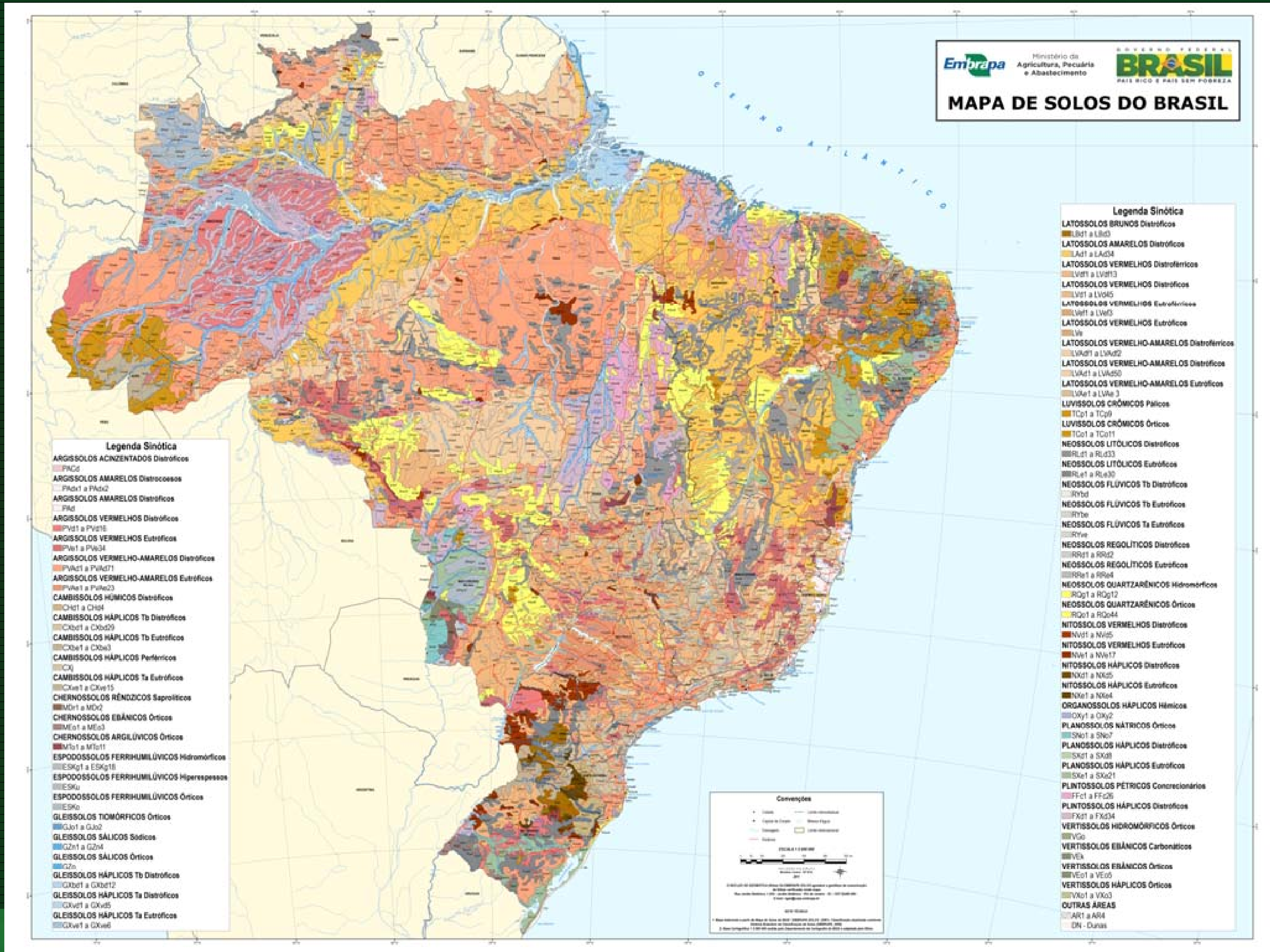
[2] http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02009R1107-20140630&from=EN

8th International Symposium on Plant Protection and Plant Health in Europe

Biorational Use in Brazilian Agriculture

José Pereira da Silva Júnior
Embrapa Wheat
Passo Fundo - Brasil

Embrapa
Trigo



Embrapa
Ministério da Agricultura, Pecuária e Abastecimento
GOVERNO FEDERAL
BRASIL
PAÍS RICO É PAÍS SEM POBREZA
MAPA DE SOLOS DO BRASIL
OCEANO ATLÂNTICO
Legenda Sinótica
ARGISSOLOS ACINZENTADOS Distróficos
PACd
ARGISSOLOS AMARELOS Distrocoesos
PAdx1 a PAdx2
ARGISSOLOS AMARELOS Distróficos
PAd
ARGISSOLOS VERMELHOS Distróficos
PVd1 a PVd16
ARGISSOLOS VERMELHOS Eutróficos
PVe1 a PVe34
ARGISSOLOS VERMELHO-AMARELOS Distróficos
PVAd1 a PVAd71
ARGISSOLOS VERMELHO-AMARELOS Eutróficos
PVAe1 a PVAe23
CAMBISSOLOS HÚMICOS Distróficos
CHd1 a CHd4
CAMBISSOLOS HÁPLICOS Tb Distróficos
CXbd1 a CXbd29
CAMBISSOLOS HÁPLICOS Tb Eutróficos
CXbe1 a CXbe3
CAMBISSOLOS HÁPLICOS Perférricos
CXj
CAMBISSOLOS HÁPLICOS Ta Eutróficos
CXve1 a CXve15
CHERNOSSOLOS RÊNDZICOS Saprolíticos
MDr1 a MDr2
CHERNOSSOLOS EBÂNICOS Órticos
MEo1 a MEo3
CHERNOSSOLOS ARGILÚVICOS Órticos
MTo1 a MTo11
ESPODOSSOLOS FERRIHUMILÚVICOS Hidromórficos
ESKg1 a ESKg18
ESPODOSSOLOS FERRIHUMILÚVICOS Hiperespessos
ESKu
ESPODOSSOLOS FERRIHUMILÚVICOS Órticos
ESKo
GLEISSOLOS TIOMÓRFICOS Órticos
GJo1 a GJo2
GLEISSOLOS SÁLICOS Sódicos
GZn1 a GZn4
GLEISSOLOS SÁLICOS Órticos
GZo
GLEISSOLOS HÁPLICOS Tb Distróficos
GXbd1 a GXbd12
GLEISSOLOS HÁPLICOS Ta Distróficos
GXvd1 a GXvd5
GLEISSOLOS HÁPLICOS Ta Eutróficos
GXve1 a GXve6
Legenda Sinótica
LATOSSOLOS BRUNOS Distróficos
LBd1 a LBd3
LATOSSOLOS AMARELOS Distróficos
LAd1 a LAd34
LATOSSOLOS VERMELHOS Distroférricos
LVdf1 a LVdf13
LATOSSOLOS VERMELHOS Distróficos
LVd1 a LVd45
LATOSSOLOS VERMELHOS Eutroférricos
LVef1 a LVef3
LATOSSOLOS VERMELHOS Eutróficos
LVe
LATOSSOLOS VERMELHO-AMARELOS Distroférricos
LVAdf1 a LVAdf2
LATOSSOLOS VERMELHO-AMARELOS Distróficos
LVAd1 a LVAd50
LATOSSOLOS VERMELHO-AMARELOS Eutróficos
LVAe1 a LVAe3
LUVISSOLOS CRÔMICOS Pálicos
TCp1 a TCp9
LUVISSOLOS CRÔMICOS Órticos
TCo1 a TCo11
NEOSSOLOS LITÓLICOS Distróficos
RLd1 a RLd33
NEOSSOLOS LITÓLICOS Eutróficos
RLe1 a RLe30
NEOSSOLOS FLÚVICOS Tb Distróficos
RYbd
NEOSSOLOS FLÚVICOS Tb Eutróficos
RYbe
NEOSSOLOS FLÚVICOS Ta Eutróficos
RYve
NEOSSOLOS REGOLÍTICOS Distróficos
RRd1 a RRd2
NEOSSOLOS REGOLÍTICOS Eutróficos
RRe1 a RRe4
NEOSSOLOS QUARTZARÊNICOS Hidromórficos
RQg1 a RQg12
NEOSSOLOS QUARTZARÊNICOS Órticos
RQo1 a RQo44
NITOSSOLOS VERMELHOS Distróficos
NVd1 a NVd5
NITOSSOLOS VERMELHOS Eutróficos
NVe1 a NVe17
NITOSSOLOS HÁPLICOS Distróficos
NXd1 a NXd5
NITOSSOLOS HÁPLICOS Eutróficos
NXe1 a NXe4
ORGANOSSOLOS HÁPLICOS Hêmicos
OXy1 a OXy2
PLANOSSOLOS NÁTRICOS Órticos
SNo1 a SNo7
PLANOSSOLOS HÁPLICOS Distróficos
SXd1 a SXd8
PLANOSSOLOS HÁPLICOS Eutróficos
SXe1 a SXe21
PLINTOSSOLOS PÉTRICOS Concrecionários
FFc1 a FFc26
PLINTOSSOLOS HÁPLICOS Distróficos
FXd1 a FXd34
VERTISSOLOS HIDROMÓRFICOS Órticos
VGo
VERTISSOLOS EBÂNICOS Carbonáticos
VEk
VERTISSOLOS EBÂNICOS Órticos
VEo1 a VEo5
VERTISSOLOS HÁPLICOS Órticos
VXo1 a VXo3
OUTRAS ÁREAS
AR1 a AR4
DN - Dunas
Convenções

Presentation Guidelines

1. Distribution of Agricultural Area by Crops
2. Use of Microorganisms in Brazilian Agriculture
3. Legal Framework
4. Selection and Recommendation of Inoculants

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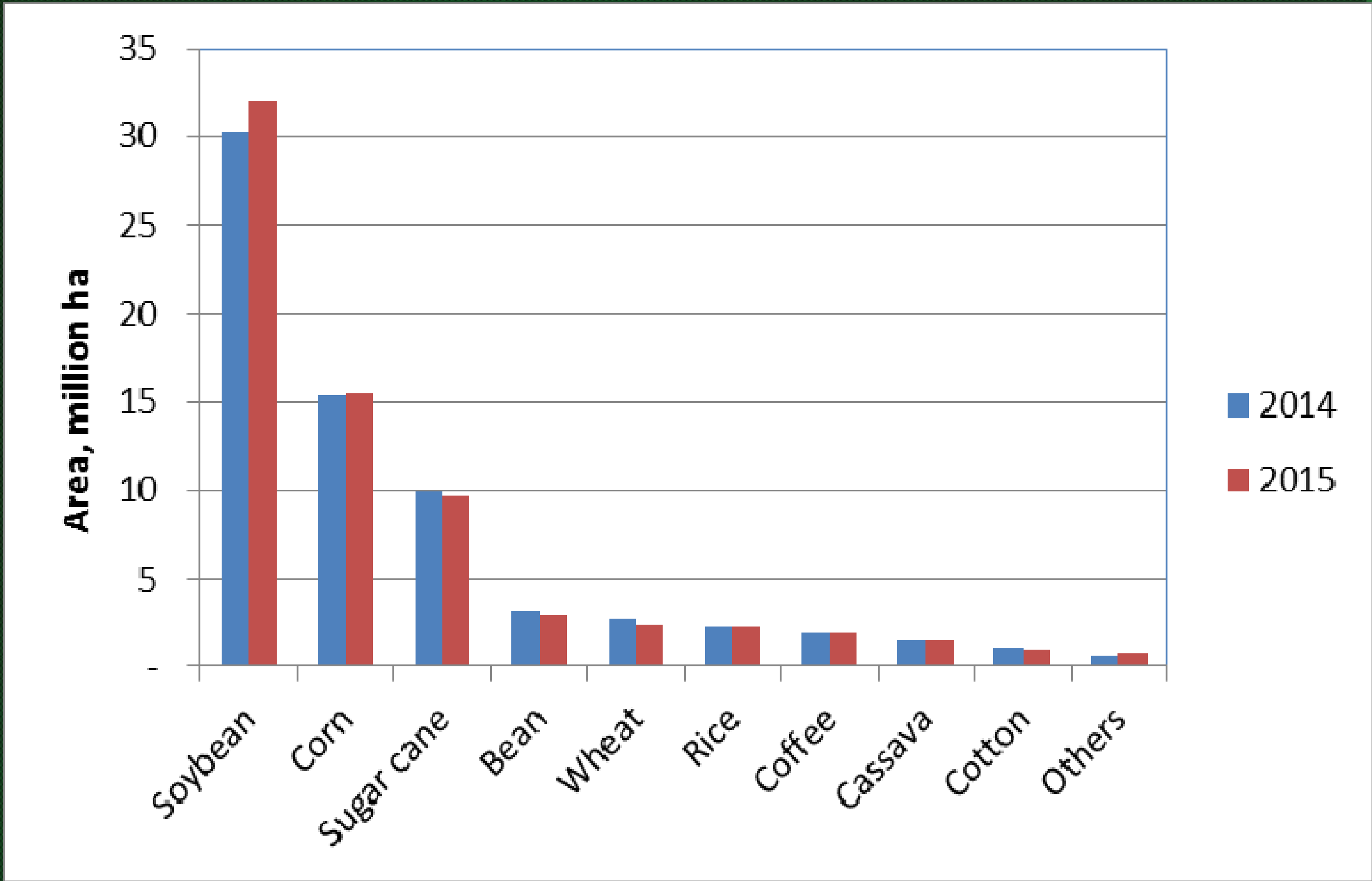
Distribution of Agricultural Area



Use of Microorganisms in Brazilian Agriculture

Possibilities of Microorganism Applications in Crop Production

Growth Plant Promoters

Biocontrol agents

Biofertilizers

Embrapa Trigo

Biocontrol agents

Soybean Lizard – *Anticarsia gemmatalis*

Control only with *Baculovirus* aplications

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Biocontrol agents

Sugarcane - spittlebug

Control with *Metarhizium anisopliae*

Biocontrol agents

Whitefly *Bemisia tabaci* – bean , cotton e tomate

Control with *Beauveria bassiana*

Biofertilizers

Rhizobia – Soybean and Bean

- The most common N input – > 22 million ha use rhizobia inoculation;
- Around 6.8 million ton of N mineral are saved each crop season;
- 5.3 billion Euro saved per year.

Embrapa
Trigo

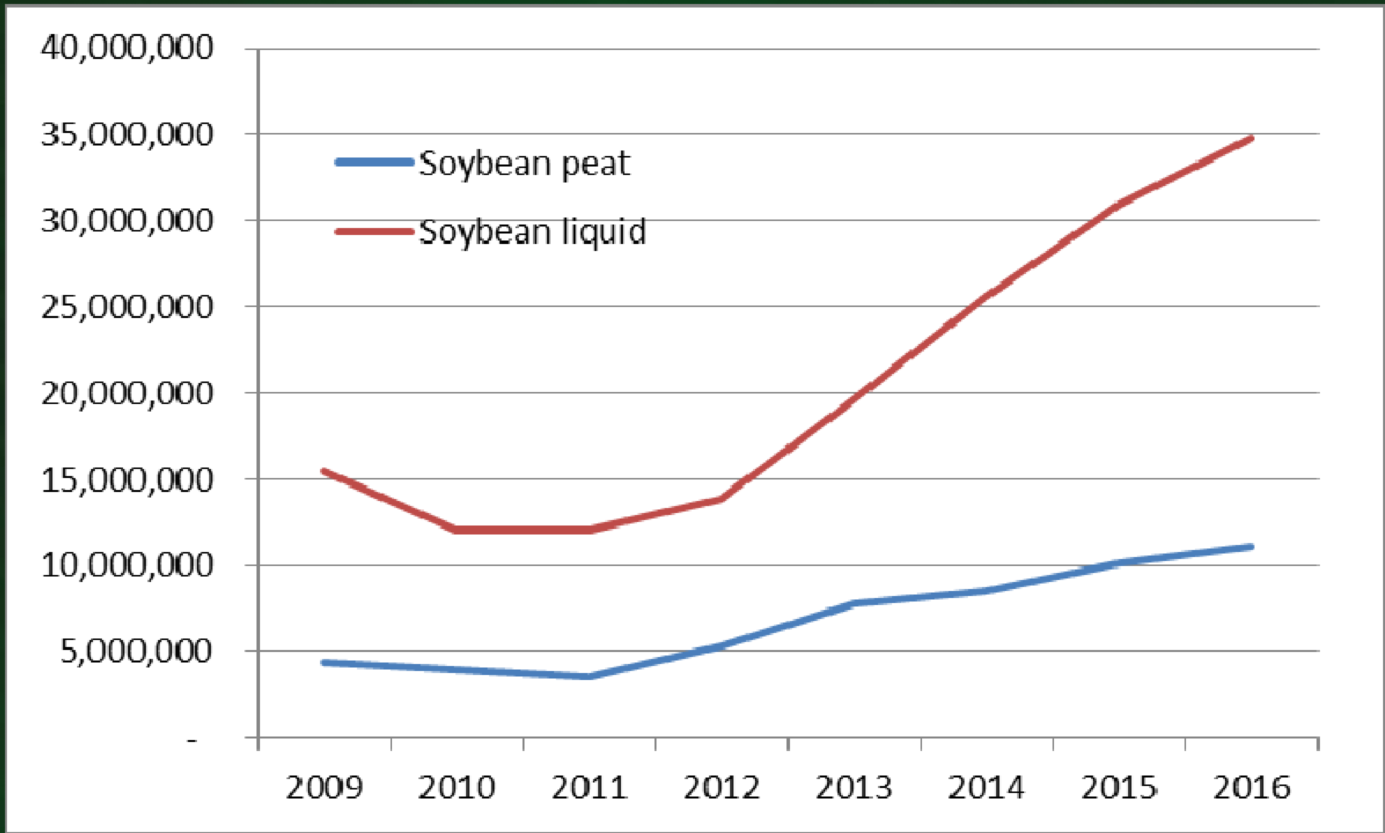
Inoculant Market in Brazil

ANPII

Laboratório Farroupilha / Lallemand
Bayer CropScience
BASF The Chemical Company
Biosoja Agrociência
GRAP Agrocete
novozymes Rethink Tomorrow
Microquimica
Bioagro Santa Maria - RS
Stoller
Rizobacter
Total Biotecnologia

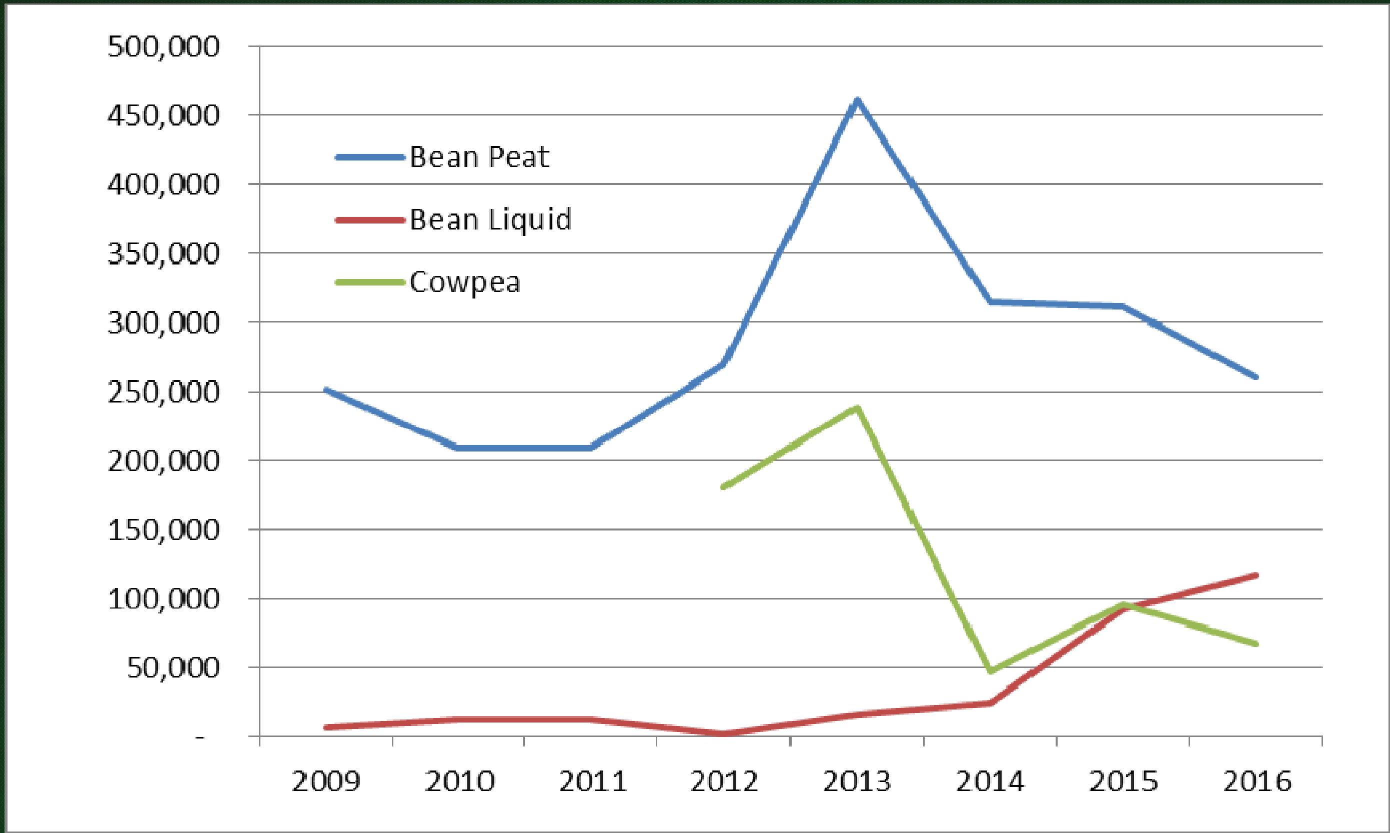
Inoculant Market in Brazil

Number of Doses per Year



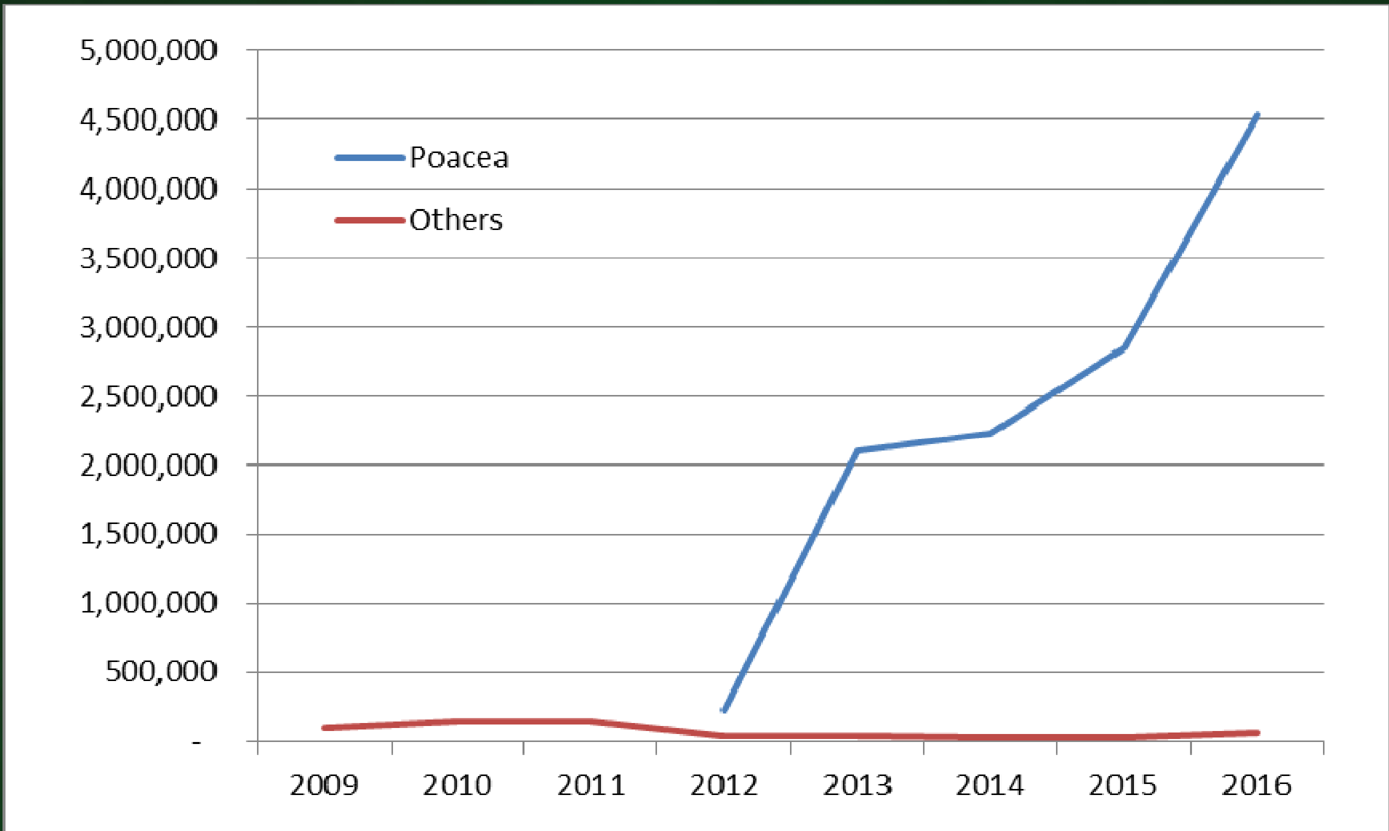
Inoculant Market in Brazil

Number of Doses per Year



Inoculant Market in Brazil

Number of Doses per Year



Embrapa Trigo

Legal Framework

Brazilian Agriculture Ministry
Normative Instruction 13/2011

Specificactions

Warranties

Registration

Packing

Labeling

Legal Framework

Brazilian Agriculture Ministry
Normative Instruction 13/2011

↕

RELARE
Laboratory Networking of Recommendation, Standardization and Transfer of Microbial Inoculants for Agriculture

RELARE

- Performed by the Agriculture Ministry, inoculant producers, different public and private institutions with researches on microbial inoculants for agriculture.

- Meeting biannually for discussing, presenting results and recommending strains, warranty level, standard methodology etc.

Brazilian Register Process

Company
- Submits Ministry new mo´s or formulation products
- Contracts the research institution for agronomic efficiency test

Relare Meeting
- Presentation of results
- Decision about recommendation

Agriculture Ministry
- Evaluation of report about agronomic efficiency
- Decision about Register

Viele Danke

jose.silva-junior@embrapa.br

MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO

SECRETARIA DE DEFESA AGROPECUÁRIA

INSTRUÇÃO NORMATIVA Nº 13, DE 24 DE MARÇO DE 2011

O SECRETÁRIO DE DEFESA AGROPECUÁRIA, DO MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO, no uso das atribuições que lhe conferem os arts. 10 e 42 do Anexo I do Decreto nº 7.127, de 4 de março de 2010, tendo em vista o disposto no Decreto nº 4.954, de 14 de janeiro de 2004, que regulamentou a Lei nº 6.894, de 16 de dezembro de 1980, e o que consta do Processo nº 21000.002705/2009-88, resolve:

Art 1º Aprovar as normas sobre especificações, garantias, registro, embalagem e rotulagem dos inoculantes destinados à agricultura, bem como as relações dos micro-organismos autorizados e recomendados para produção de inoculantes no brasil, na forma dos Anexos I, II e III, desta Instrução Normativa.

Art 2º Esta Instrução Normativa entra em vigor na data de sua publicação.

Art 3º Fica revogada a Instrução Normativa SARC no 05, de 6 de agosto de 2004.

FRANCISCO SÉRGIO FERREIRA JARDIM

ANEXO I

NORMAS SOBRE ESPECIFICAÇÕES, GARANTIAS, REGISTRO, EMBALAGEM E ROTULAGEM DOS INOCULANTES DESTINADOS À AGRICULTURA CAPÍTULO I DAS ESPECIFICAÇÕES, GARANTIAS MÍNIMAS E TOLERÂNCIAS DOS PRODUTOS

Art. 1º Os inoculantes produzidos, importados ou comercializados no país, de acordo com as suas características e para fins de registro, deverão observar as seguintes condições e especificações:

I - os produtos que contenham bactérias fixadoras de nitrogênio para simbiose com leguminosas deverão apresentar concentração mínima de 1,0 x 109 Unidades Formadoras de Colônias (UFC) por grama ou mililitro de produto, mantendo a garantia registrada até a data de seu vencimento;

II - para os demais inoculantes, formulados com bactérias associativas e micro-organismos promotores de crescimento de plantas, a concentração de micro-organismos será a informada no processo de registro do produto, de acordo com a recomendação específica emitida por órgão brasileiro de pesquisa científica oficial ou credenciado pelo MAPA;

III - serem elaborados em suporte esterilizado, e, quando sólido, livre de micro-organismos em fator de diluição 1 x 10-2;

IV - estarem livres de micro-organismos não especificados em fator de diluição 1 x 10-5;

V - serem elaborados em suporte que forneça todas as condições de sobrevivência ao micro-organismo;

VI - apresentarem prazo de validade de, no mínimo, seis meses a partir da data de fabricação; e

VII - serem elaborados somente com micro-organismos relacionados no Anexo II desta Instrução Normativa.

Parágrafo único. Os inoculantes produzidos com micro-organismos referenciados no Anexo III ou que não constem desta Instrução Normativa deverão observar o disposto no artigo 5º desta Instrução Normativa.

Art. 2º Para os resultados analíticos obtidos será admitida tolerância em relação à garantia do produto, limitada a 20% (vinte por cento) para concentração de unidades formadoras de colônias (UFC) por grama ou mililitro de produto.

CAPÍTULO II

DO REGISTRO DE PRODUTOS

Art. 3º Excetuados os casos previstos no regulamento aprovado pelo Decreto nº 4.954, de 2004, e legislação complementar, os inoculantes produzidos, importados e comercializados no território nacional deverão ser registrados no órgão competente do Ministério da Agricultura, Pecuária e Abastecimento.

Art. 4º Além do disposto na Seção II, do Capítulo II, do regulamento aprovado pelo Decreto nº 4.954, de 2004, na Seção II, do Capítulo II, da Instrução Normativa Ministerial nº 10, de 6 de maio de 2004, e em outros atos normativos próprios do MAPA, o pedido de registro ou de autorização para importação pelo consumidor final de inoculantes que contenham bactérias fixadoras de nitrogênio para simbiose com leguminosas deverá conter:

I - garantias mínimas de acordo com os incisos I, III, IV, V, VI e VII do art. 1º do Anexo I desta Instrução Normativa;

II - relação das matérias-primas utilizadas na fabricação do inoculante, bem como suas respectivas funções;

III - espécie de bactéria utilizada na fabricação do produto e número da cepa na coleção oficial, conforme Anexo II desta Instrução Normativa;

IV - natureza física; e

V - especificação da(s) cultura(s) a que se destina.

Parágrafo único. A natureza física a que se refere o inciso IV deste artigo classifica-se em (i) sólido, quando o suporte utilizado é composto fundamentalmente de partículas sólidas; e, (ii) líquido, quando o suporte utilizado é fundamentalmente um fluido com ou sem partículas sólidas.

Art. 5º Os processos de registro de produto novo, em qualquer um de seus aspectos técnicos, e de produto elaborado com cepa(s) do Anexo III, deverão ser instruídos com relatório técnicocientífico conclusivo emitido por órgão brasileiro de pesquisa oficial ou credenciado, que ateste a viabilidade e eficiência de seu uso agrícola.

§ 1º Os trabalhos de pesquisa com o produto deverão ser desenvolvidos de acordo com os requisitos mínimos e roteiros para avaliação da viabilidade e eficiência agronômica para seleção de micro-organismos e avaliação de viabilidade e eficiência agronômica de produtos e tecnologias, constantes na página eletrônica do MAPA na Internet, www.agricultura.gov.br.

§ 2º No processo deverão constar os métodos para a identificação e contagem dos micro-organismos declarados e para avaliação da pureza do produto.

§ 3º A critério do órgão de fiscalização poderá ser solicitado parecer técnico, emitido por especialista da área, quanto à inocuidade do(s) organismo(s) à saúde humana e animal e à sanidade vegetal.

§ 4º Deverão também ser atendidas as seguintes exigências para fins de registro:

I - garantias mínimas de acordo com o inciso II do art. 1º do Anexo I desta Instrução Normativa;

II - relação das matérias-primas utilizadas na fabricação do inoculante, bem como suas respectivas funções;

III - classificação taxonômica do(s) micro-organismo (s) utilizado (s) na fabricação do produto e, quando aplicável, número da cepa na coleção oficial, conforme Anexo III desta Instrução Normativa;

IV - natureza física; e

V - especificação da(s) cultura(s) a que se destina;

§ 5º A natureza física a que se refere o inciso IV deste artigo classifica-se em (i) sólido, quando o suporte utilizado é composto fundamentalmente de partículas sólidas; e, (ii) líquido, quando o suporte utilizado é fundamentalmente um fluido com ou sem partículas sólidas.

CAPÍTULO III

DA EMBALAGEM, ROTULAGEM E IDENTIFICAÇÃO DE PRODUTOS

Art. 6º Os inoculantes, para serem vendidos ou expostos à venda em todo o território nacional, ficam obrigados a exibir rótulos redigidos em português, em embalagens apropriadas, que contenham, além das informações e dados obrigatórios relacionados à identificação do fabricante ou importador e do produto, estabelecidas na Seção I, do Capítulo VI, do regulamento aprovado pelo Decreto nº 4.954, de 2004, e no Capítulo III, da Instrução Normativa Ministerial nº 10, de 2004, entre outras exigências, as seguintes informações:

I - denominação do produto, "inoculante", seguida da natureza física e da especificação da cultura a que se destina, conforme o seguinte exemplo: "inoculante líquido para soja", sendo facultado incorporar à denominação do produto, o tipo do suporte utilizado, como, por exemplo, "inoculante sólido turfoso para soja";

II - espécie(s) do(s) microrganismo(s) contido(s) no produto e número(s) na coleção oficial, conforme Anexo II ou III;

III - instruções sobre conservação, modo de aplicação e especificações de dosagens;

IV - prazo de validade acompanhado da data de fabricação, ou data de validade; e

V - número do lote a que se refere a unidade do produto.

Parágrafo único. Para os produtos importados, além do disposto no caput e nos incisos I, II, III, IV e V deste artigo, deverá ser informado o nome do país onde o produto foi fabricado.

Art. 7º Fica facultada a inscrição, nos rótulos, de dados não estabelecidos como obrigatórios, desde que:

I - não dificultem a visibilidade e a compreensão dos dados obrigatórios; e

II - não contenham:

a) afirmações ou imagens que possam induzir o usuário a erro quanto à natureza, composição, segurança e eficácia do produto, e sua adequação ao uso;

b) comparações falsas ou equívocas com outros produtos;

c) indicações que contradigam as informações obrigatórias;

e d) afirmações de que o produto é recomendado por qualquer órgão do Governo.

Art. 8º Quando, mediante aprovação do órgão de fiscalização, for juntado folheto complementar que amplie os dados do rótulo ou que contenha dados que obrigatoriamente deste devessem constar, mas que nele não couberam pelas dimensões reduzidas da embalagem ou pelo volume de informações, observar-se-á o seguinte:

I - deve-se incluir no rótulo frase que recomende a leitura do folheto anexo, antes da utilização do produto; e

II - devem constar, tanto do rótulo como do folheto, em qualquer hipótese, o nome, o endereço, o número de registro no MAPA do fabricante ou do importador, o número de registro do produto e suas garantias.

Art. 9º Quando o produto, em condições normais de uso, representar algum risco à saúde humana, animal e ao ambiente, o rótulo deverá trazer informações sobre precauções de uso e armazenagem, com as advertências e cuidados necessários, visando à prevenção de acidentes.

Art. 10. O rótulo, embalagem e etiqueta não poderão conter recomendação de uso com fertilizantes ou agrotóxicos, ressalvados os casos recomendados por instituições de pesquisa oficiais ou credenciadas mediante apresentação de relatório técnico-científico conclusivo ao Ministério da Agricultura, Pecuária e Abastecimento.

CAPÍTULO IV

DAS DISPOSIÇÕES FINAIS

Art. 11. O Ministério da Agricultura, Pecuária e Abastecimento - MAPA será responsável pela coleção oficial dos microorganismos para produção de inoculantes.

Parágrafo único. Entende-se por coleção oficial as relações de micro-organismos descritas nos Anexos II e III desta Instrução Normativa.

Art. 12. Outros micro-organismos com atuação favorável ao crescimento vegetal poderão ser incluídos nos Anexos II ou III, desde que recomendados por instituições de pesquisa oficiais ou credenciadas.

§ 1º A inclusão de que trata o caput deste artigo será feita mediante apresentação de relatório técnico-científico conclusivo, resultante de trabalho de pesquisa conduzido de acordo com os requisitos mínimos e roteiros para avaliação da viabilidade e eficiência agronômica.

§ 2º Os novos micro-organismos deverão ser depositados pela instituição responsável pela recomendação no(s) banco(s) de germoplasma indicado(s) pelo Ministério da Agricultura, Pecuária e Abastecimento e receberão designação específica.

Art. 13. As cepas constantes no Quadro 2 do Anexo II serão retiradas da relação de micro-organismos oficiais no prazo de dois anos a partir da data de publicação desta Instrução Normativa.

Parágrafo único. Instituições de pesquisa oficiais ou credenciadas, interessadas na manutenção das cepas, deverão apresentar publicação ou relatório técnico-científico conclusivo que ateste a sua viabilidade e eficiência agronômica.

Art. 14. Os bancos de germoplasma responsáveis pela guarda e manutenção das cepas constantes dos Anexos II e III desta Instrução Normativa serão homologados por ato do Secretário de Defesa Agropecuária.

Art. 15. Os estabelecimentos produtores e importadores deverão adquirir anualmente, de uma instituição responsável pela manutenção do banco de germoplasma, os micro-organismos correspondentes aos inoculantes que desejarem produzir.

Art. 16. Os estabelecimentos produtores e importadores de inoculantes terão prazo de 180 (cento e oitenta) dias, a partir da data de publicação desta Instrução Normativa, para se adaptarem às exigências relativas à embalagem e rotulagem previstas no Capítulo III.

Art. 17. O registro de produtos contendo micro-organismos resultantes de modificações por engenharia genética, bem como a inclusão destes organismos nos Anexos II e III desta Instrução Normativa somente poderão ocorrer após emissão de parecer favorável da Comissão Técnica Nacional de Biossegurança - CTNBIO.

ANEXO II

RELAÇÃO DOS MICRO-ORGANISMOS AUTORIZADOS PARA PRODUÇÃO DE INOCULANTES NO BRASIL.

Quadro 1

CULTURA LEGUMINOSA 1	NOME COMUM	CEPA AUTORIZADA (SEMIA)	GÊNERO/ESPÉCIE	Nº ACESSO GENBAN 2
LEGUMINOSAS DE GRÃOS				
Arachis hypogaea	Amendoim	6144	*Bradyrhizobium* sp. (*Arachis* sp.)	AY904750
Cicer arietinum	Grão de bico	396	*Mesorhizobium ciceri*	AY904731
Glycine max	Soja	5079	*Bradyrhizobium japonicum*	AF234888
		5080	*Bradyrhizobium japonicum*	AF234889
		587	*Bradyrhizobium elkanii*	AF234890
		5019	*Bradyrhizobium elkanii*	AF237422
Lens esculenta	Lentilha	344	*Rhizobium leguminosarum bv viciae*	FJ025087
		3025	*Rhizobium leguminosarum bv viceae*	FJ025091
		3026	*Rhizobium leguminosarum bv viceae*	FJ025093
Phaseolus vulgaris	Feijão	4077	*Rhizobium tropici*	EU488752
		4080	*Rhizobium tropici*	AF260274
		4088	*Rhizobium tropici*	EF054889
Pisum sativum	Ervilha	3007	*Rhizobium leguminosarum bv. viceae*	AY904742
		3033	*Rhizobium leguminosarum bv. viceae*	n.d.
Vigna unguiculata	Feijão de corda Feijão miúdo, caupi	6461	*Bradyrhizobium sp.*	EF158574
		6462	*Bradyrhizobium sp.*	AY649439
		6463	*Bradyrhizobium sp.*	EF158575

		6464	*Bradyrhizobium sp.*	AY649430
LEGUMINOSAS FORRAGEIRAS DE CLIMA TEMPERADO				
Lotus corniculatus	Cornichão	806	*Mesorhizobium amorphae*	FJ025125
		816	*Mesorhizobium sp.*	AY904737
Lotus penduculatus	Cornichão gigante	839	*Bradyrhizobium japonicum*	FJ390898
Medicago sativa	Alfafa	116	*Sinorhizobium meliloti*	FJ025128
		134	*Sinorhizobium meliloti*	AY904727
		135	*Sinorhizobium meliloti*	AY904728
Trifolium pratense	Trevo vermelho	222	*Rhizobium leguminosarum bv. trifolii*	AY904729
		265	*Rhizobium leguminosarum bv. trifolii*	FJ025088
		2081	*Rhizobium leguminosarum bv. trifolii*	AY904741
		2082	*Rhizobium leguminosarum bv. trifolii*	FJ025094
Trifolium repens	Trevo branco	222	*Rhizobium leguminosarum bv. trifolii*	AY904729
		235	*Rhizobium leguminosarum bv. trifolii*	FJ025090
		2082	*Rhizobium leguminosarum bv. trifolii*	FJ025094
		2083	*Rhizobium leguminosarum bv. trifolii*	FJ025096
Trifolium subterraneum	Trevo subterrâneo	222	*Rhizobium leguminosarum bv. trifolii*	AY904729
		265	*Rhizobium leguminosarum bv. trifolii*	FJ025088
Trifolium vesiculosum	Trevo vesiculoso	2050	*Rhizobium leguminosarum bv. trifolii*	FJ025095
		2051	*Rhizobium leguminosarum bv. trifolii*	AY904740
LEGUMINOSAS FORRAGEIRAS DE CLIMA TROPICAL				
Arachis pintoi	Amendoim forrageiro	6439	*Bradyrhizobium japonicum*	FJ025098
		6440	*Bradyrhizobium sp.*	AY904789
Cajanus cajan	Guandu	6156	*Bradyrhizobium sp.*	AY904758

Centrosema spp.	Centrosema	690	*Bradyrhizobium elkanii*	FJ025107
		6146	*Bradyrhizobium sp.*	AY904752
		6424	*Bradyrhizobium elkanii*	AY904787
		6425	*Bradyrhizobium elkanii*	AY904788
Desmodium ovalifolium (=D. heterocarpon)	Desmódio	6208	*Bradyrhizobium elkanii*	AY904773
		6209	*Bradyrhizobium japonicum*	n.d.
Indigofera hirsuta	Anileira, Indigófera	6156	*Bradyrhizobium sp.*	AY904758
		6158	*Bradyrhizobium elkanii*	AY904760
Lotononis bainesii	Lotononis	658	*Methylobacterium sp*	AY904733
Macroptilium atropurpureum	Siratro	656	*Bradyrhizobium sp.*	AY904732
Neonotonia wightii (=Glycine wightii)	Soja perene	656	*Bradyrhizobium sp.*	AY904732
Stylosanthes spp.	Estilosantes	6154	*Bradyrhizobium japonicum*	FJ025100
		6155	*Bradyrhizobium japonicum*	AY904757
LEGUMINOSAS PARA ADUBAÇÃO VERDE				
Calopogonium sp.	Calopogônio	6152	*Bradyrhizobium japonicum*	AY904756
Canavalia ensiformis	Feijão de porco	6156	*Bradyrhizobium sp.*	AY904758
		6158	*Bradyrhizobium elkanii*	AY904760
Crotalaria juncea	Crotalária	6156	*Bradyrhizobium sp.*	AY904758
Crotalaria spectabilis	Crotalária	6156	*Bradyrhizobium sp.*	AY904758
		6158	*Bradyrhizobium elkanii*	AY904760
Lupinus sp.	Tremoço	928	*Bradyrhizobium sp.*	FJ390904
		938	*Bradyrhizobium elkanii*	AY904739
Mucuna pruriens (=Stizolobium aterrimum)	Mucuna preta	6158	*Bradyrhizobium elkanii*	AY904760
Pueraria phaseoloides	Kudzu tropical	6175	*Bradyrhizobium elkanii*	AY904771
LEGUMINOSAS ARBÓREAS				
Acacia angustissima	Acácia	6430	*Mesorhizobium amorphae*	FJ025124
Acacia auriculiformis		6387	*Bradyrhizobium elkanii*	AY904778
		6391	*Bradyrhizobium elkanii*	AY904780
Acacia farnesiana		6430	*Mesorhizobium amorphae*	FJ025124
		6436	*Rhizobium sp.*	FJ025119
Acacia mangium	Acácia mangium	6387	*Bradyrhizobium elkanii*	AY904778

		6420	*Bradyrhizobium japonicum*	AY904786
Acacia salicina		6400	*Bradyrhizobium elkanii*	FJ025114
Acosmium nitens		6443	*Bradyrhizobium sp.*	FJ390932
Albizia lebbeck	Coração de negro, Pau preto	6160	*Bradyrhizobium elkanii.*	AY904762
		6432	*Bradyrhizobium elkanii*	FJ025110
Balizia pedicellaris		6396	*Bradyrhizobium japonicum*	FJ025099
		6408	*Bradyrhizobium elkanii*	FJ025103
Dalbergia nigra	Jacarandá	6101	*Bradyrhizobium elkanii*	AY904749
Enterolobium contortisiliquum	Timbaúva	6159	*Bradyrhizobium elkanii*	AY904761
Enterolobium cyclocarpum	Orelha-de-elefante	6159	*Bradyrhizobium elkanii*	AY904761
		6403	*Bradyrhizobium elkanii*	FJ025112
Enterolobium timbouva	Timbaúva	6159	*Bradyrhizobium elkanii*	AY904761
		6397	*Bradyrhizobium elkanii*	FJ025139
Erythrina verna	Suinã	6100	*Bradyrhizobium elkanii*	AY904748
Falcataria mollucanna (sin. Paraserianthes facataria, *Albi- zia falcataria)*	Albízia	6100	*Bradyrhizobium elkanii*	AY904748
		6169	*Bradyrhizobium elkanii*	AY904770
		6432	*Bradyrhizobium elkanii*	FJ025110
Gliricidia sepium	Glicidia	6168	*Rhizobium sp.*	AY904769
		6435	*Rhizobium sp.*	FJ025130
Leucaena diversifolia	Leucena	6162	*Sinorhizobium meliloti*	FJ025127
Leucaena leucocephala vK72, v.K8, v. Peru	Leucena	6153	*Bradyrhizobium japonicum sp.*	FJ025097
Leucaena leucocephala v. Cunnigha	Leucena	6069	*Bradyrhizobium elkanii*	AY904746
		6070	*Rhizobium sp.*	AY904747
Pithecellobium tortum		6406	*Rhizobium etli*	FJ025116
Prosopis juliflora	Algaroba	6161	*Sinorhizobium sp*	AY904763
		6162	*Sinorhizobium meliloti*	FJ025127
Pseudosamanea guachapele (=Albizia guachapele, =Acacia guachapele)		6403	*Bradyrhizobium elkanii*	FJ025112
Samanea saman (=Mimosa saman, Pithecellobium	Árvore da chuva	6403	*Bradyrhizobium*	FJ025112

saman, Enterolobium saman, Inga saman e Calliandra saman)			*elkanii*	
		6405	*Bradyrhizobium elkanii*	FJ025109
Sesbania virgata	Sesbania	6401	*Azorhizobium doebereinerae*	AY904783

1 Nomenclatura das leguminosas segundo o ILDIS (International Legume Database & Information Service). Disponível em http://www.ildis.org. Acesso em 10 dez 2008.

2 Número de acesso da sequência completa do gene ribossomal 16S no GenBank. Disponível em http://www.ncbi.nlm.nih.gov. N.d. se refere a acesso não disponível 3I, teste em tubos; II, teste sob condições estéreis; III, teste em solo; IV, teste a campo.

Quadro 2

CULTURA LEGUMINOSA 1	NOME COMUM	CEPA AUTORIZADA (SEMIA)	GÊNERO/ESPÉCIE	Nº ACESSO GENBANK 2
LEGUMINOSAS FORRAGEIRAS DE CLIMA TEMPERADO				
Adesmia latifolia	Adesmia	6437	*Rhizobium sp.*	FJ025118
		6438	*Rhizobium sp.*	FJ025120
Lathyrus odoratus	Ervilha de cheiro, sincho	388	*Rhizobium leguminosarum bv viceae*	FJ025089
		3018	*Rhizobium leguminosarum bv viceae*	FJ025092
Lotus glaber (=L. tenuis)	Cornichão	830	*Mesorhizobium sp.*	AY904738
Medicago polymorpha	Trevo carretilha	103	*Sinorhizobium meliloti*	AY904726
Ornithopus sativus	Serradela	905	*Bradyrhizobium japonicum*	FJ959100
		929	*Bradyrhizobium japonicum*	FJ390938
Trifolium semipilosum	Trevo do Quênia	2002	*Rhizobium leguminosarum bv. trifolii*	FJ025086
Vicia sativa	Ervilhaca	384	*Rhizobium etli*	AY904730
LEGUMINOSAS FORRAGEIRAS DE CLIMA TROPICAL				
Cajanus Cajan	Guandu	6157	*Bradyrhizobium elkanii*	AY904759
Desmodium incanum	Desmódio	6028	*Bradyrhizobium elkanii*	AY904744
Desmodium intortum	Desmódio	656	*Bradyrhizobium sp.*	AY904732
Galactia striata	Galáctia	6149	*Bradyrhizobium elkanii*	AY904754
		6150	*Bradyrhizobium elkanii*	AY904755

Lablab purpureus	Lablab	662	*Bradyrhizobium elkanii*	AY904734
		695	*Bradyrhizobium elkanii*	AY904735
Macrotyloma axillare	Macrotiloma	6149	*Bradyrhizobium elkanii*	AY904754
Neonotonia wightii (=Glycine	Soja perene	6148	*Bradyrhizobium sp.*	AY904753
wightii)				
LEGUMINOSAS PARA ADUBAÇÃO VERDE				
Crotalaria juncea	Crotalária	6145	*Bradyrhizobium sp.*	AY904751
Cyamoposis tetragonoloba	Feijão Guarda	6145	*Bradyrhizobium sp.*	AY904751
		6319	*Bradyrhizobium sp.*	AY904774
LEGUMINOSAS ARBÓREAS				
Acacia decurrens	Acácia da Austrália	6164	*Bradyrhizobium japonicum*	AY904765
Acacia mearnsii	Acácia negra	6163	*Bradyrhizobium japonicum*	AY904764
		6164	*Bradyrhizobium japonicum*	AY904765
Acacia podalyriaefolia	Acácia mimosa	6388	*Bradyrhizobium elkanii*	FJ959101
		6389	*Bradyrhizobium elkanii*	FJ025113
Acacia salicina		6392	*Mesorhizobium amorphae*	FJ025126
Acacia saligna		6096	*Bradyrhizobium elkanii*	FJ025115
		6428	*Bradyrhizobium elkanii*	FJ025106
Bowdichia virgilioides		6096	*Bradyrhizobium elkanii*	FJ025115
		6414	*Bradyrhizobium elkanii*	FJ025111
Calliandra houstoniana (=C. calothyrsus)	Caliandra	6395	*Bradyrhizobium sp.*	FJ025101
		6423	*Rhizobium sp.*	FJ025132
Calliandra surinamensis	Caliandra	6395	*Bradyrhizobium sp.*	FJ025101
		6423	*Rhizobium sp.*	FJ025132
Chamaecrista ensiformis		6392	*Mesorhizobium amorphae*	FJ025126
Dimorphandra jorgei		6099	*Bradyrhizobium sp.*	FJ3903941
		6400	*Bradyrhizobium elkanii*	FJ025114

Erythrina poeppigiana		6388	*Bradyrhizobium elkanii*	FJ959101
		6426	*Bradyrhizobium elkanii*	FJ959102
Erythrina speciosa		6395	*Bradyrhizobium sp.*	FJ025101
Inga marginata	Ingá	6433	*Bradyrhizobium elkanii*	FJ025105
		6434	*Bradyrhizobium sp.*	FJ390934
Lonchocarpus costatus		6399	*Bradyrhizobium elkanii*	FJ025102
		6404	*Bradyrhizobium elkanii*	FJ025104
Mimosa bimucronata		6386	*Bradyrhizobium sp.*	n.d.
Parapiptadenia rigida	Angico	6416	*Bradyrhizobium elkanii*	FJ025108
Poecilanthe parviflora		6403	*Bradyrhizobium elkanii*	FJ025112
Sclerolobium paniculatum (Tachigali vulgaris)	Taxi do campo	6160	*Bradyrhizobium elkanii*	AY904762
		6420	*Bradyrhizobium japonicum*	AY904786
Sesbania virgata		6402	*Azorhizobium sp.*	AY904784
Tipuana tipu	Tipuana	6192	*Bradyrhizobium japonicum*	AY904772

1 Nomenclatura das leguminosas segundo o ILDIS (International Legume Database & Information Service). Disponível em http://www.ildis.org. Acesso em 10 dez 2008.

2 Número de acesso da sequência completa do gene ribossomal 16S no GenBank. Disponível em http://www.ncbi.nlm.nih.gov. N.d. se refere a acesso não disponível 3 I, teste em tubos; II, teste sob condições estéreis; III, teste em solo; IV, teste a campo.

ANEXO III

RELAÇÃO DOS MICRO-ORGANISMOS RECOMENDADOS PARA PRODUÇÃO DE INOCULANTES NO BRASIL

CULTURA	NOME COMUM	GÊNERO/ESPÉCIE	DESIGNAÇÃO ORIGINAL	INSTITUIÇÃO QUE RECOMENDOU
Eucaliptus sp	Eucalipto	Bacillus subtilis	UFV 3918	Universidade Federal de Viçosa
Eucaliptus sp	Eucalipto	Frauteria aurantia	UFV R1	Universidade Federal de Viçosa
Eucaliptus sp	Eucalipto	Bacillus subtilis	UFV S1	Universidade Federal de Viçosa

Eucaliptus sp	Eucalipto	Bacillus subtilis	UFV S2	Universidade Federal de Viçosa
Triticum spp	Trigo	Azospirillum brasilense	Ab-V1	Embrapa Soja Universidade Federal do Paraná
Zea mays	Milho	Azospirillum brasilense	Ab-V4	Embrapa Soja Universidade Federal do Paraná
Zea mays e Triticum spp	Milho e Trigo	Azospirillum brasilense	Ab-V5	Embrapa Soja Universidade Federal do Paraná
Zea mays e Triticum spp	Milho e Trigo	Azospirillum brasilense	Ab-V6	Embrapa Soja Universidade Federal do Paraná
Zea mays	Milho	Azospirillum brasilense	Ab-V7	Embrapa Soja Universidade Federal do Paraná
Triticum spp	Trigo	Azospirillum brasilense	Ab-V8	Embrapa Soja Universidade Federal do Paraná
Oriza sativa	Arroz	Azospirillum brasilense	Ab-V5	Universidade Estadual de Maringá Universidade Estadual Paulista
Oriza sativa	Arroz	Azospirillum brasilense	Ab-V6	Universidade Estadual de Maringá Universidade Estadual Paulista

D.O.U., 25/03/2011 - Seção 1

Microbial consortia products

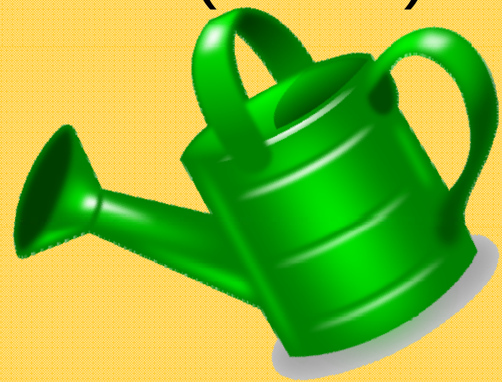
as biological inoculants for improved plant growth

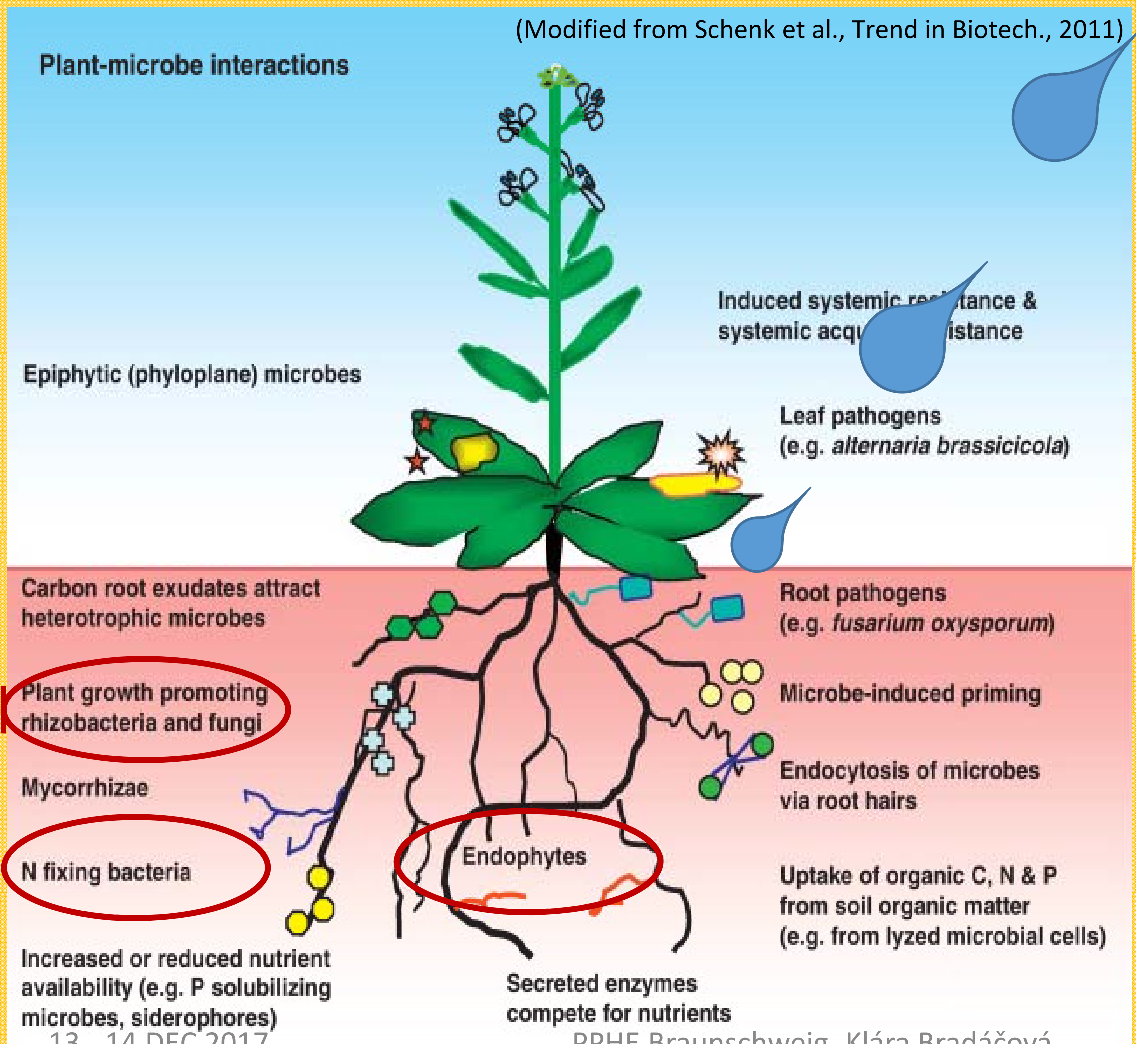
Klára Bradáčová

Max Sittinger, Dr. Markus Weinmann, Prof. Günter Neumann

University Hohenheim, Germany

Microbial Consortia Products (MCP)





Plant response

Growth promotion
Biotic and abiotic stress tolerance
Vigorous, strong, healthy plants
Yield increase

Beneficial microbes

Nutrient mobilization
Root exudates
Enzyme secretion
Activation of C, N, P turnover enzymes
Siderophores

Introduction of Microbial Consortia Product (MCP)

- description: dark brown liquid, should provide nutrient acquisition and enhancement of BNF

- bacterial composition: *Azotobacter vinlandii, Clostridium sp., Lactobacillus sp.,* ***Bacillus amyloliquefaciens****, B. subtilis* (SILoSil® BS), *B. thuringiensis,* ***Pseudomonas fluorescens****, Acetobacter, Enterococcus,* ***Rhizobium japonicum***

- funghal composition: *Saccharomyces, Penicillium roqueforti, Monascus, Aspergillus oryzae,* ***Trichoderma harzianum*** (TRICHOSIL)

- plant composition: *Arthrospira platensis (Spirulina), Ascophyllum nodosum*

Principal approaches for practical applications

Targeted Selection Hypothesis
(Concept of BIOFECTOR)

Beneficial effects on plants achieved by targed selection and inoculation with BEs particularly efficient for specific applications *(e.g. nutrient mobilisation, root growth promotion, pathogen antagonism)*

X

Auto-Selection Hypothesis
(MCP Concept, EM Concept)

Inoculation with huge consortia of plant growth-promoting bacteria with different functions. Different stress conditions will activate the most suitable populations (*also targeted pre-activation is possible prior to inoculation*)

Maize pot experiments

- **Pot experiment** (*3 kg substrate: 1:3 sand/ soil mix)* Maize cv. Jessy, 6 weeks)

- **Soil**: - **Horb field site pH 6.5, silty loam, CAL-P 52, N_{min} 11.3 (mg/kg soil)**
- - **Practical course, stored soil, pH 5.6, silty loam, CAL-P 18**

- **BE Treatments:** MCP (*fertigation fortnightly, dosage according to manufacturer´s recommendations)*

- **Fertilisation levels (**mg/kg soil)**:**

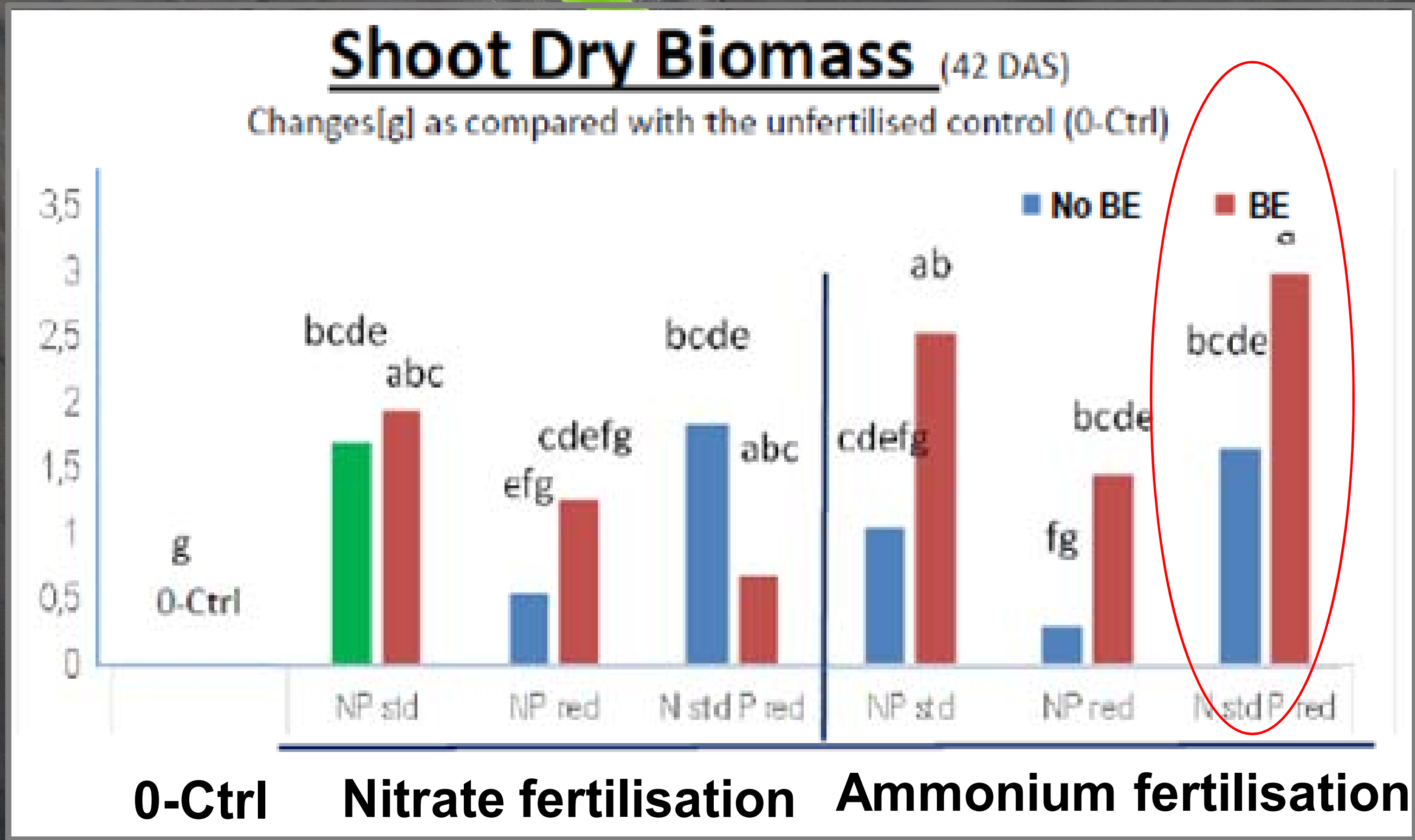
• 1.(N140, P130, K150, Mg50) Standard full nutrient supply

• 2. Reduced N/P fertilisation (N 70,P 50) or StdN and reduced P supply

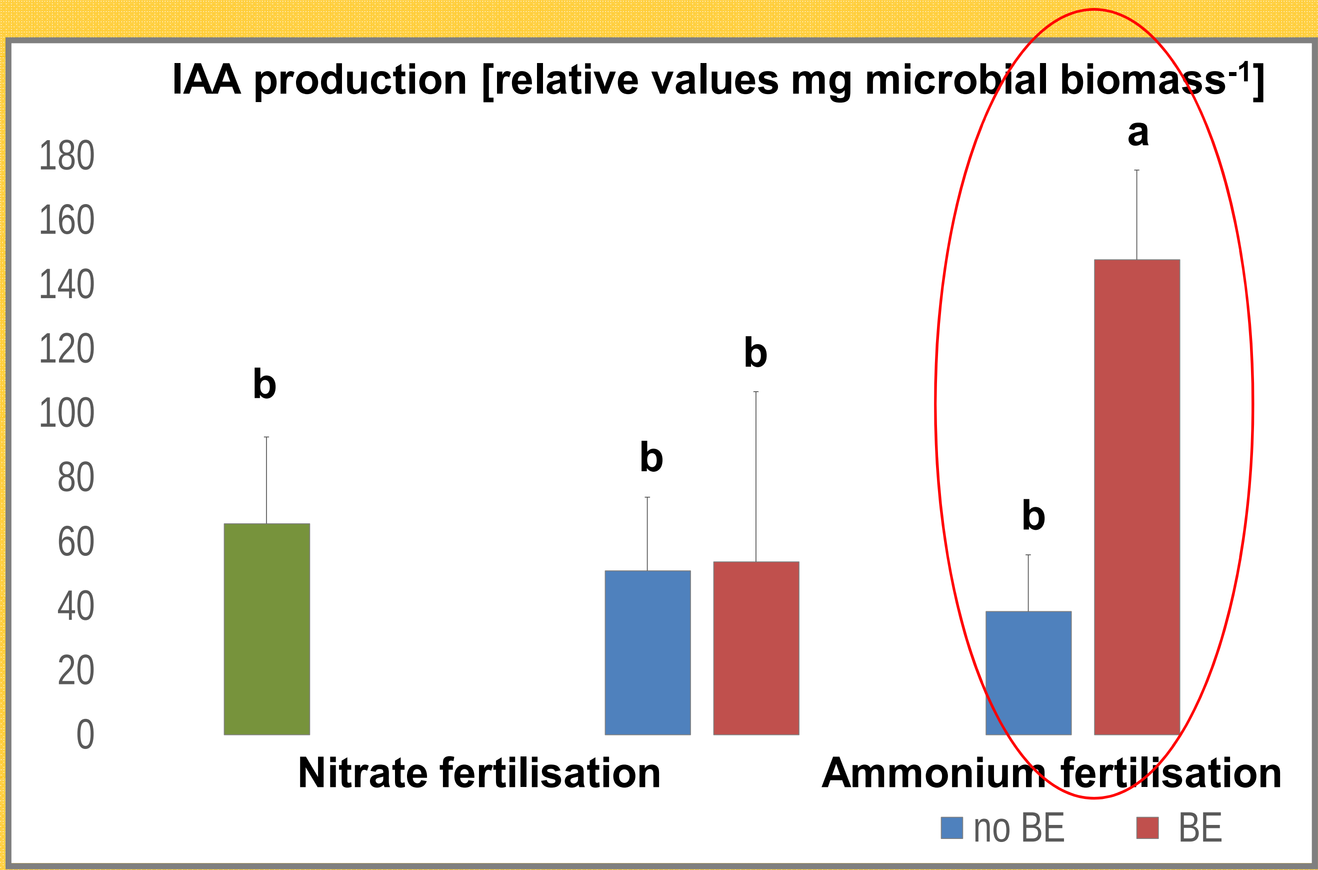
- **Different N forms:** Nitrate N vs Ammonium N
(ENTEC-stabilised Ammoniumsulfate, Novatec Solub, Compo)

- **General measurements:**
plant growth (height, stem diameter), shoot and root DM, root morphology (length diameter, fine roots), plant nutritional status

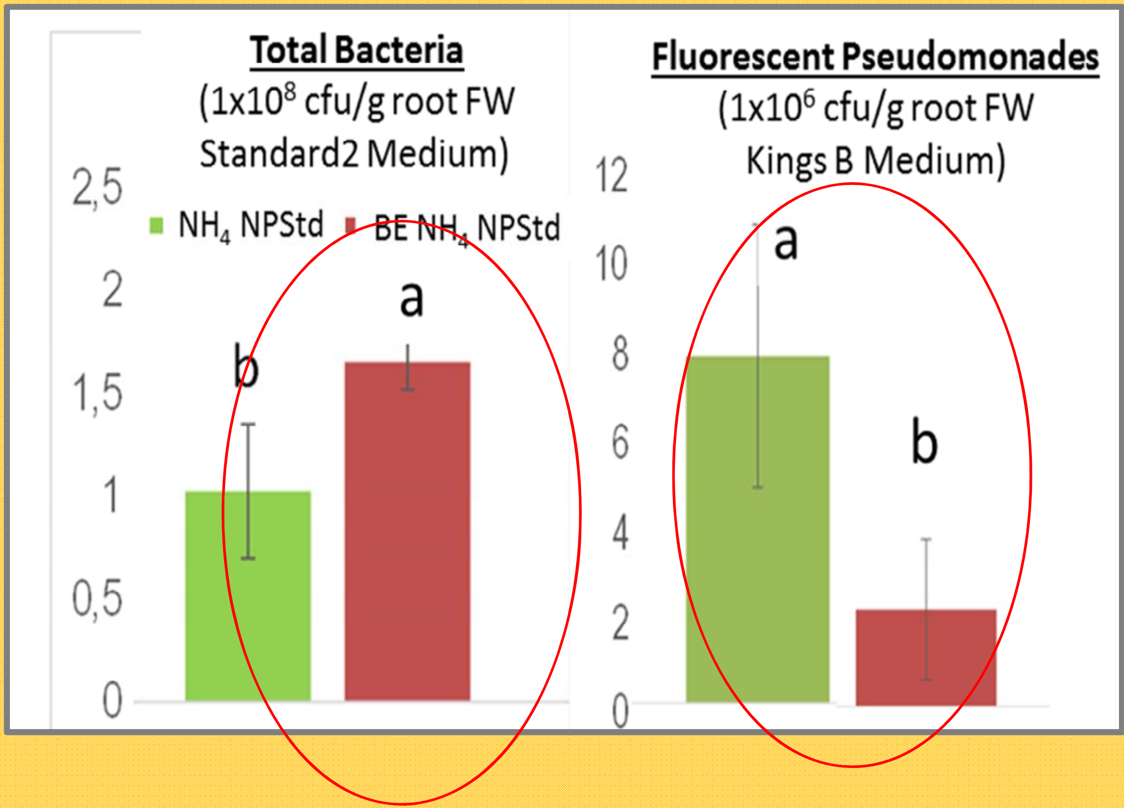
- **Specific measurements:**
Functional charcterisation of rhizosphere-microbial activities:
*(**Enzyme assays** in rhizosphere soil*
***Tracing for microorganisms**, **Auxin production potential**)*

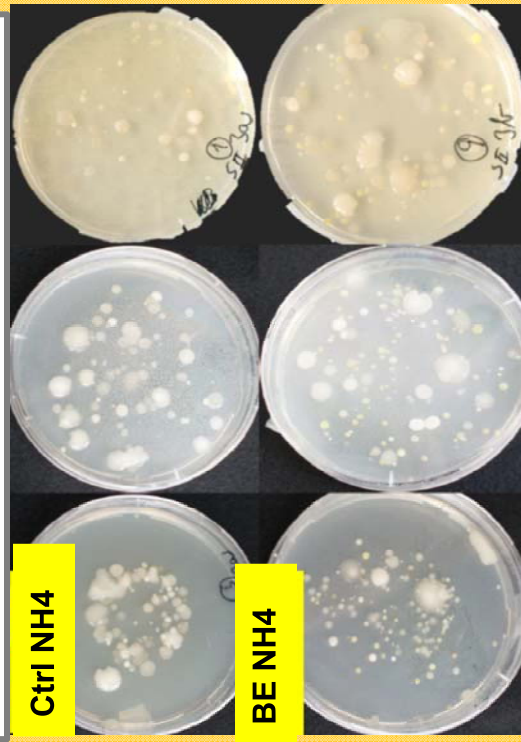


ECAG2895 shows significant stimulation of shoot biomass production in combination with stabilised ammonium fertilization. Effects detectable with standard fertilization an with reduced N/P input.

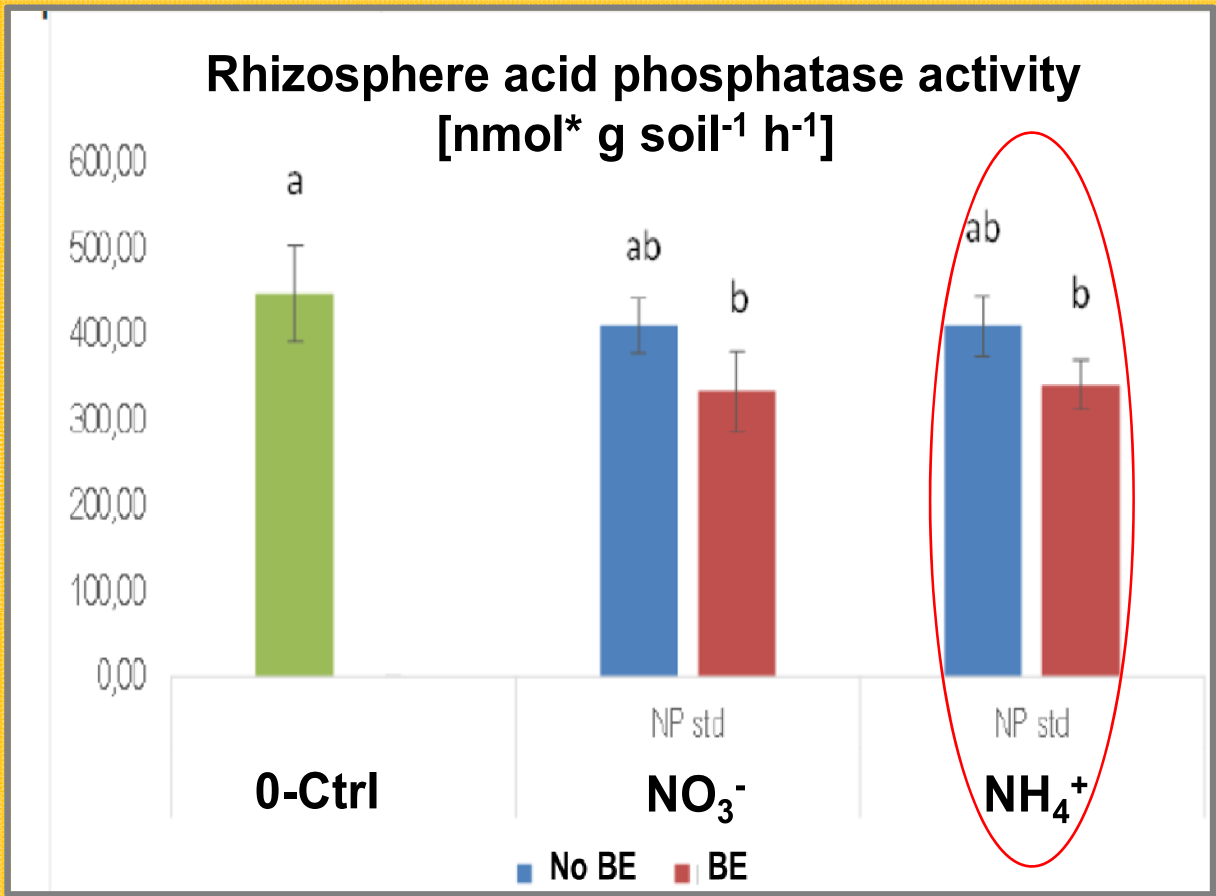


NH_{4+} fertilization increased the auxin production potential of rhizosphere bacteria in the ECAG2895 variants.



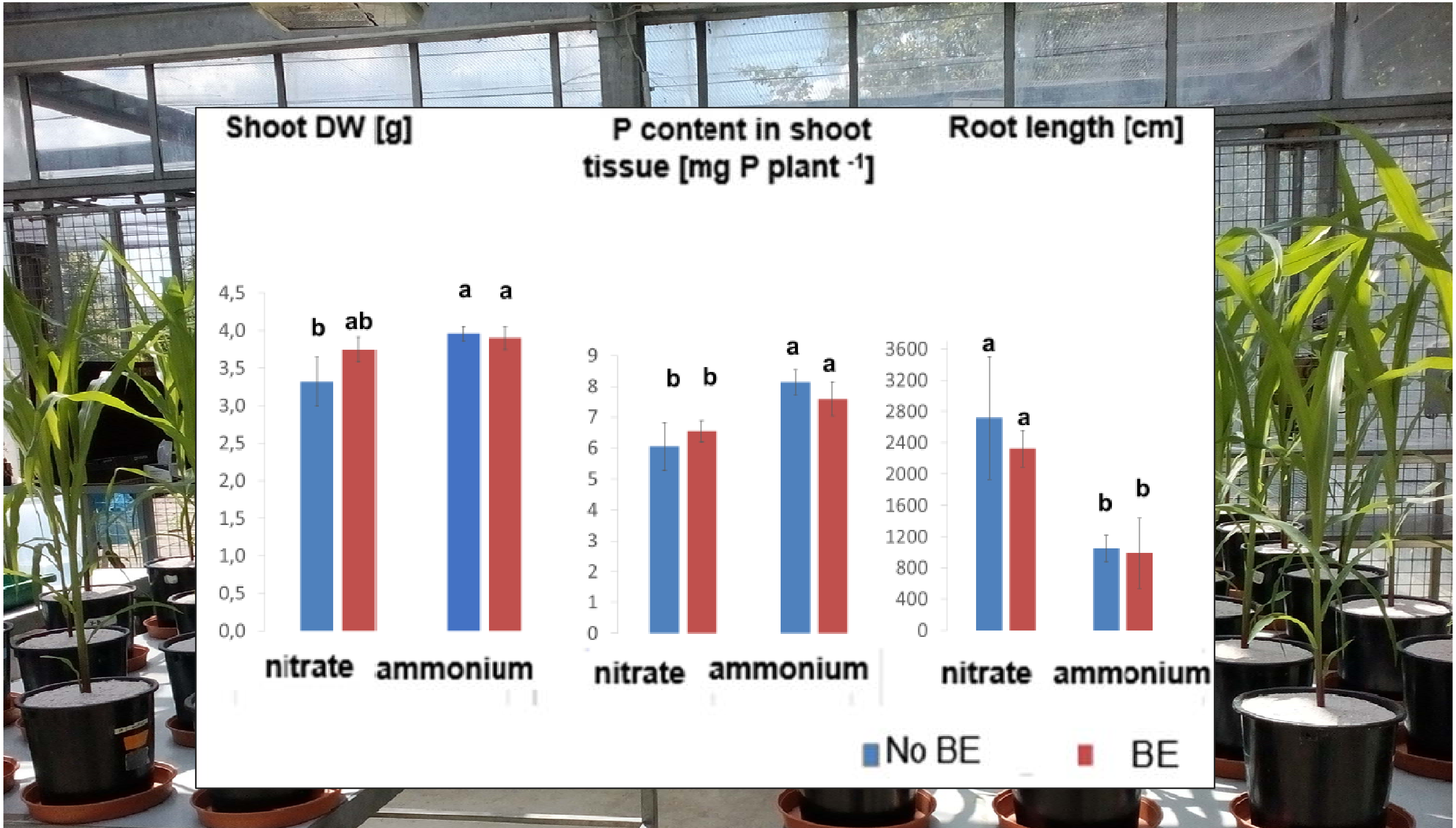


Total cultivable bacteria but not *Pseudomonas*-like bacteria were increased in ECAG2895 treated plants.



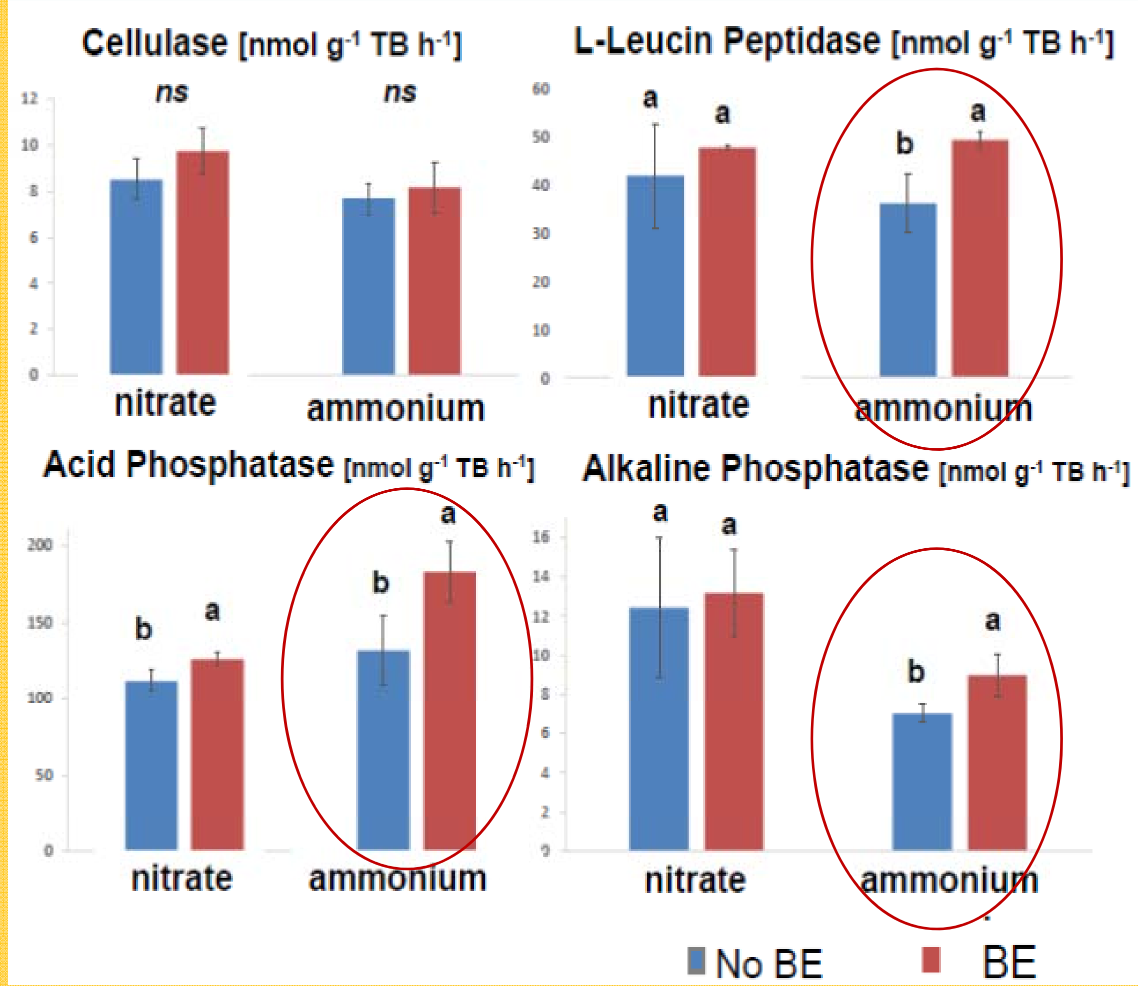
Lower rhizosphere acid phosphatase activity in MCP variants as compared with the unfertilized control.

No significant differences for alkaline phosphatase, glucosidase, xylosidase, cellulase and peptidase.

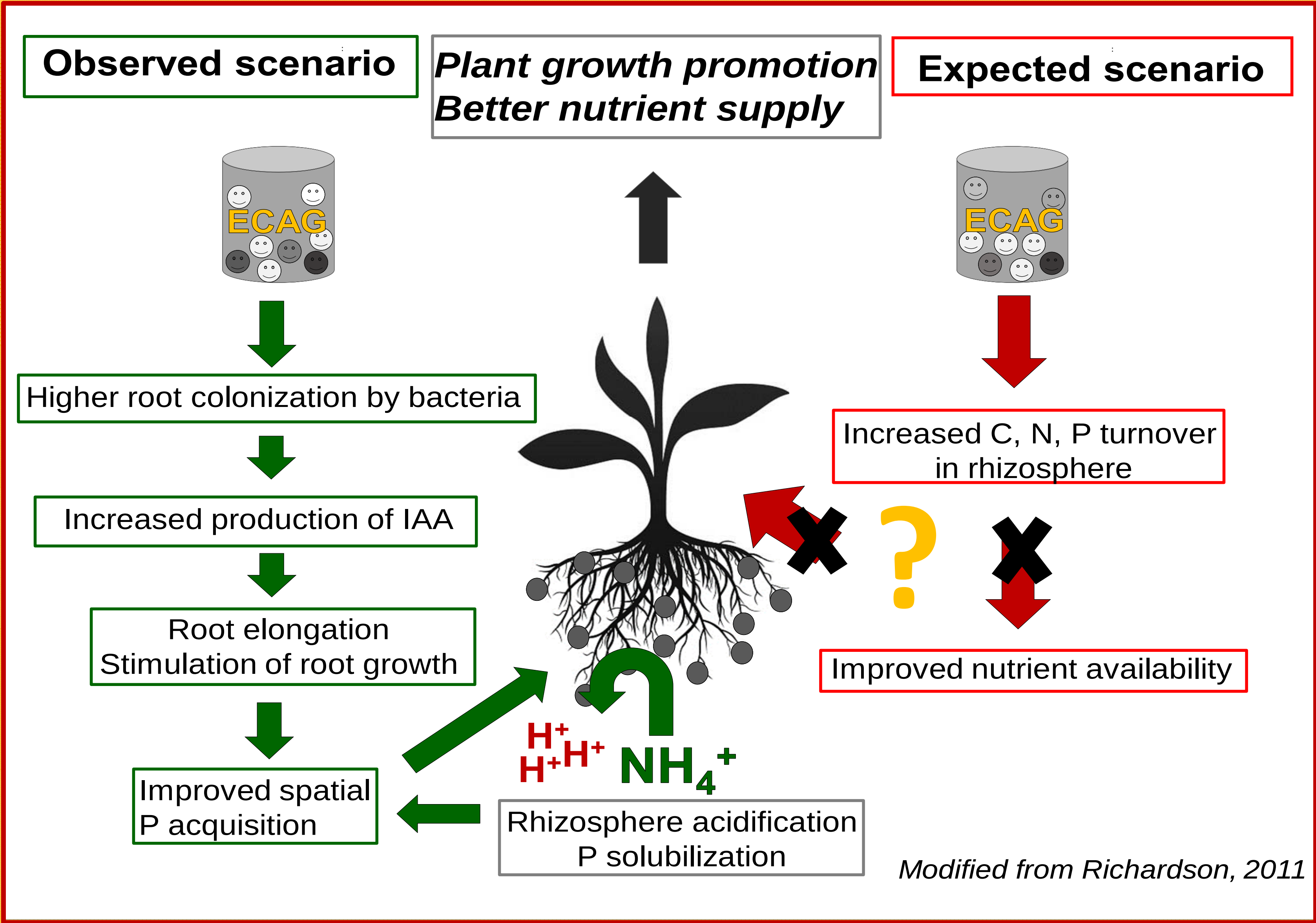


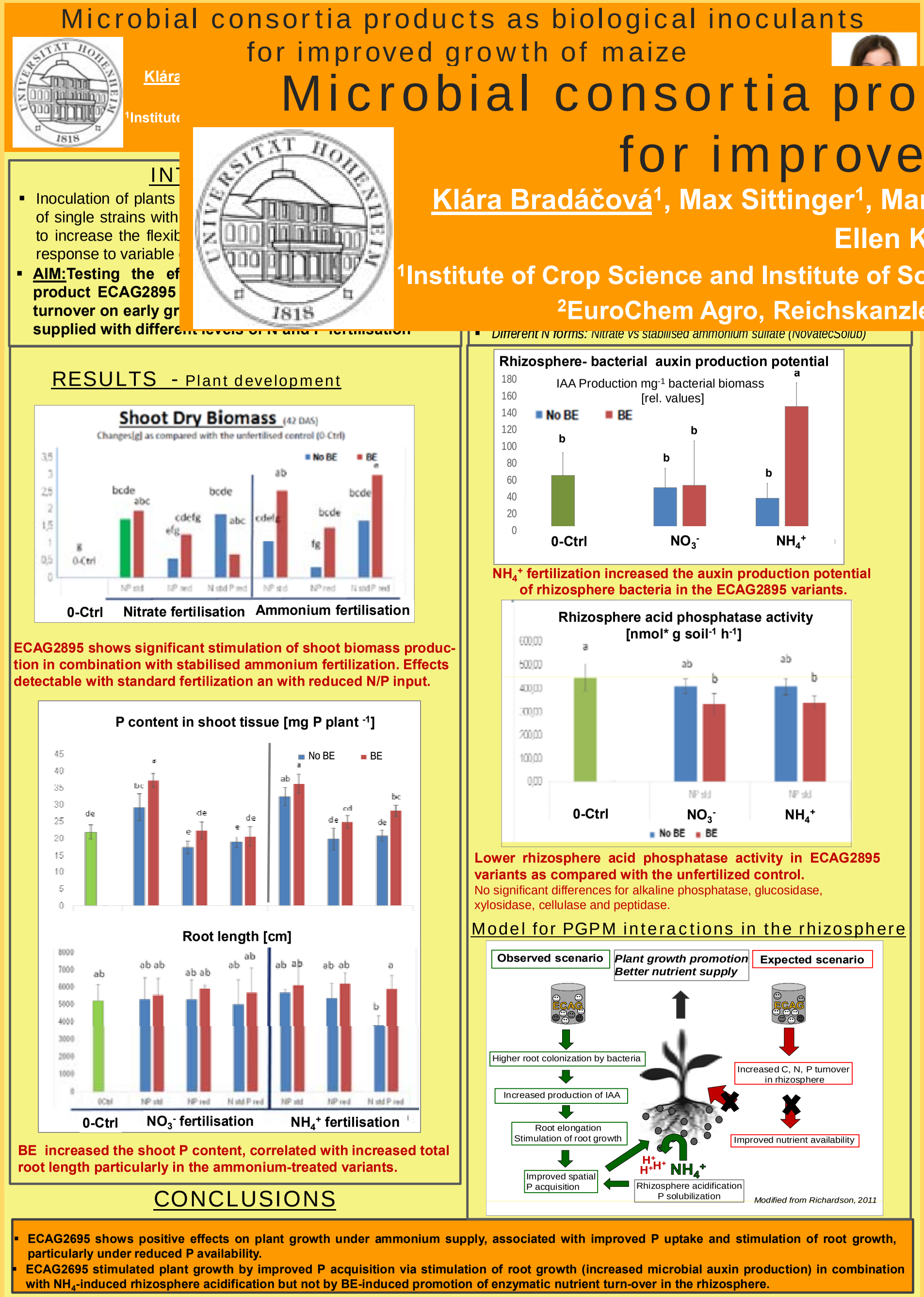
No significant increase in maize shoot biomass, root growth and P acquisition after MCP application

Marker enzymes in rhizosphere soil



Rhizosphere enzyme activites involved in N (leucine peptidase) and P turnover (phosphatases) were significantly increased by BE application.


Observed scenario
Plant growth promotion
Better nutrient supply
Expected scenario
ECAG
ECAG
Higher root colonization by bacteria
Increased production of IAA
Root elongation
Stimulation of root growth
Improved spatial
P acquisition
H^+ H^+ H^+ NH_4^+
Rhizosphere acidification
P solubilization
Increased C, N, P turnover
in rhizosphere
?
Improved nutrient availability
Modified from Richardson, 2011



Thank you for your attention!

Klára Bradáčová

University Hohenheim

Germany

Quality control of biostimulant inoculum

Carolin Schneider, December 13, 2017

PPPHE 2017

Efficacy and risks of „biorationals“ in organic and integrated
pest management - acceptable?

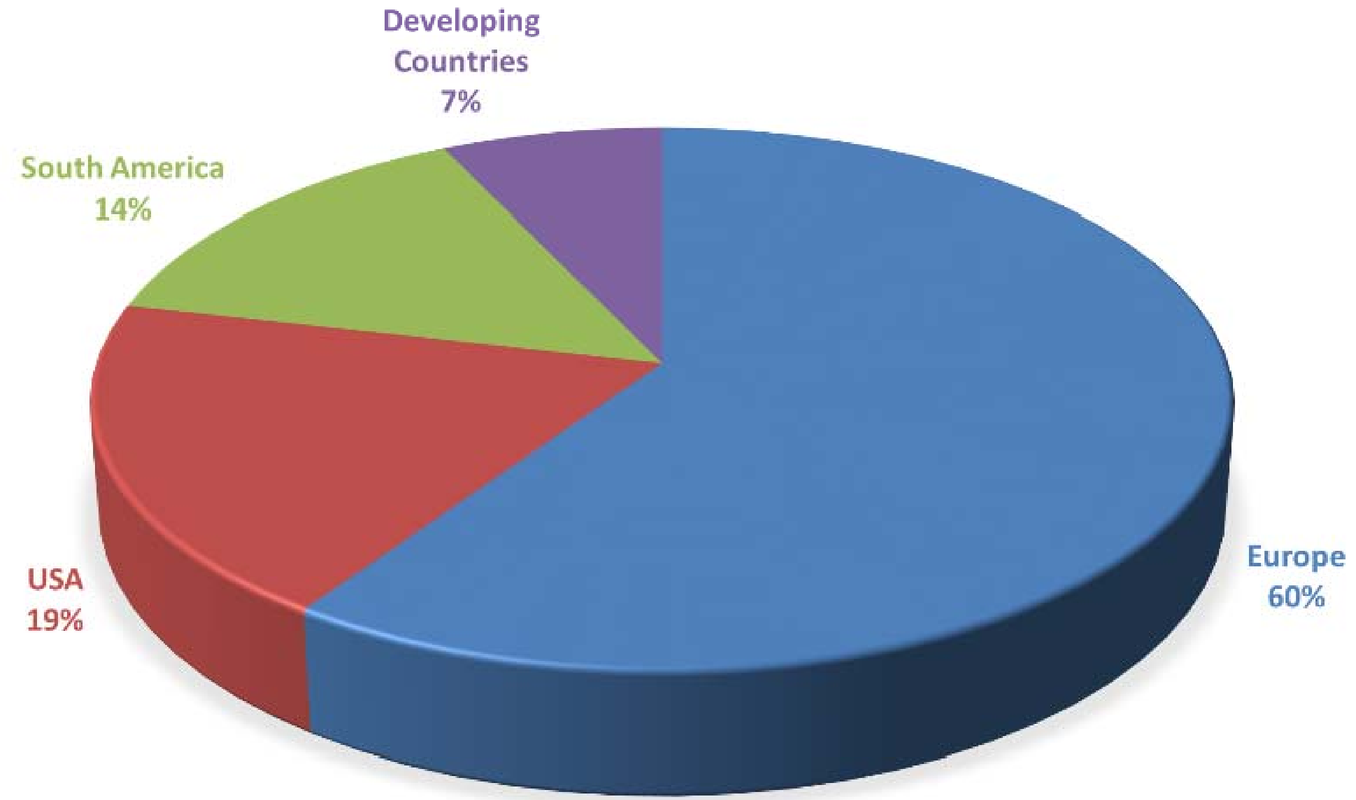
Example: Mycorrhiza

Mycorrhizal products are still a niche market. But the number of companies dealing with mycorrhizal products (producing and/or retailing) **increases** in Europe:

Number of companies (Arato, Inoq 2017)

2003	2014	2017
9	25	66

Worldwide distribution of mycorrhiza companies



Mycorrhizal products are sold as, i.e.

- Propagule powder/carrier
- Spores bound to carrier
- Pure *in vitro* spores
- Mix with organic fertilizer, seed, etc.

European IPM regulation under major revision

- Fertilizer act EC 2003/2003 and Plant Protection Act 1107/2009 are under revision
- Revised fertilizer act sets standards for (microbial) plant biostimulants <u>for the first time</u>
- The difference between biostimulation and plant protection is defined by *Mode of Action* of the organism:

Plant Biostimulant against **Abiotic stress (Fertilizer Act)**
Plant Protection Product against **Biotic stress (Plant Protection Act)**

Future definitions in draft fertilizer act, Annex I, ***Product Function Categories (PFC)*****:**

6 Plant biostimulant: stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant: (a) nutrient use efficiency; (b) tolerance to abiotic stress; (c) quality traits.

6(A) Microbial plant biostimulant: A microbial plant biostimulant shall consist of a micro-organism or a consortium of micro-organisms referred to in *Component Material Category (CMC)* 7 of Annex II.

Future definitions in draft fertilizer act, Annex I, *Product Function Categories (PFC)*:

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6(A) Microbial plant biostin[…][…]bial plant biostimulant shall consist of a micro-organism […]m of micro-organisms referred to in *Component Material C[…]* 7 of Annex II.

Challenge: positive list

Annex II, CMC 7: Micro-organisms

"A EU fertilising product belonging to PFC 6(A) may contain micro-organisms, including dead or empty-cell micro-organisms and non-harmful residual elements of the media on which they were produced, which have undergone no other processing than drying or freeze-drying and are listed below:

Mycorrhizal fungi, *Rhizobium* spp., *Azospirillum* spp. *Azotobacter* spp."

What about other species or genera? What will be the process to list new genotypes?

Challenge: positive list

Annex II, CMC 7: Micro-organisms

"A EU fertilising product belo ? A) may contain micro-organisms, including dead or empty-cell s and non-harmful residual elements of the media on wh roduced, which have undergone no other processing than dry ying and are listed below:

Mycorrhizal fungi, *Rhizobium* *um* spp. *Azotobacter* spp."

What about other species or genera? What will be the process to list new genotypes?

Challenge: double function

- if double function: plant protection product
- claim decides, not biology?

What will happen when plant protection function of a biostimulant is published, but not claimed?

Challenge: double function

- if double function: ?tion product
- claim decides, not b

What will happen wh tection function of a biostimulant is publis claimed?

Challenge: conformity assessment

- The new EU Fertilizer Act will harmonize regulation and registration in EU countries. At present, the legal regulation of biostimulation is organised on national level, often with high regulatory burden through national laws.
- In future, a conformity assessment will ease market entry EU wide and promote a single market.

How is conformity assessment proceeded?

Challenge: conformity assessment

- The new EU Fertilizer Act will harmonize regulation and registration in EU countries. At present … lation of biostimulation is organised on national le… high regulatory burden through national laws.
- In future, a conformity a… ease market entry EU wide and promote a single market…

?

How is conformity assessment proceeded?

Challenge: thresholds

Thresholds for anorganic contaminants	**Cadmium (Cd), Hexavalent chromium (Cr VI), Lead (Pb), Mercury (Hg), Nickel (Ni), Arsenic (As), Copper (Cu) and Zinc (Zn)**
Thresholds for pathogenic microorganisms	*Salmonella* spp., *Escherichia coli, Listeria monocytogenes, Vibrio* spp., *Shigella* spp., *Staphylococcus aureus, Enterococcaceae.* Anaerobic plate count unless the microbial biostimulant is an aerobic bacterium, yeast and mould count unless the microbial biostimulant is a fungus

Thresholds:

- f.e. phosphorus (P), lead (Pb), copper (Cu), magnesium (Mg), nickel (Ni) and calcium (Ca) are often "high" in mycorrhizal inoculum as a result of the production method
- But dosage is low, f.e. 100 mg inoculum/L substrate

Risk assessment:

- f.e. lead (Pb), mercury (Hg), arsenic (As) should be below threshold
- pathogenic microorganisms below threshold

Thresholds:

- f.e. phosphorus (P), lead (Pb), copper (Cu), magnesium (Mg), nickel (Ni) and calcium (Ca) are often high in mycorrhizal inoculum as a result of the production method ?
- But dosage is low, f.e. 100 mg inoculum/L substrate
- f.e. lead (Pb), mercury (Hg), arsenic (As) should be below threshold ✓
- pathogenic microorganisms below threshold ✓

Challenge: Identification and concentration

Additional requirements for Microbial plant biostimulants	all intentionally added **microorganisms shall be indicated**. Where the microorganism has several strains, the intentionally added **strains shall be indicated**. Their concentration shall be expressed as the **number of active units per volume or weight**, or in any other manner that is relevant to the micro-organism, e.g. colony forming units per gram (cfu/g).

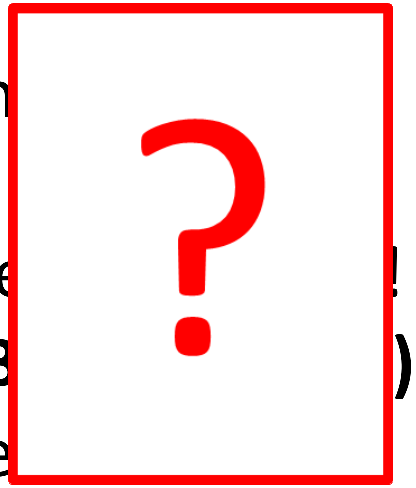
Identification + Concentration

- Strain = Species (easy)? Mycorrhiza isolate?

- Mycorrhiza propagules: never cfu! spores + vesicles? Propagule viability? **Biotest (MPN)?**
- Viability after storage? Shelf life?
- no strong correlation between propagules and colonization potential...

Identification + Concentration

- Species (easy)? Strain
- Mycorrhiza propagule ! spores + vesicles?
  Propagule viability? **B)?**
- Viability after storage
- no strong correlation between propagules and colonization potential...



Science-based:

MiRA, Microbe-induced resistance to agricultural pests. Marie Skłodowska-Curie Actions Innovative Training Network (ITN), 2017-2021

MycoSign, Novel plant biostimulants: Joint action of signal molecules & mycorrhiza for sustainable agriculture. EU Eurostars, 2017- 2019

MycoTom, Einsatz arbuskulärer Mykorrhizapilze als Bodenhilfsstoff zur Produktion qualitativ hochwertiger Tomaten im Gewächshaus, KMU-innovativ, 2017-2020

MICROMETABOLITE, Research Training Network on the microbial enhancement of bioactive secondary metabolite production in plants, Marie Skłodowska-Curie Actions Innovative Training Network (ITN). 2017-2021

INTERFUTURE, From microbial interactions to new-concept biopesticides and biofertilizers. Marie Skłodowska-Curie Actions Innovative Training Network (ITN), 2016-2020

BestPass, Boosting plant-Endophyte STability, compatibility and Performance Across ScaleS, Marie Skłodowska-Curie Actions Innovative Training Network (ITN), 2015-2019

Science-based:

PhD-position available, deadline 14.1.2018, see www.inoq.de

MiRA, Microbe-induced resistance to agricultural Innovative Training Network (ITN), 2017-2021

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Summary:

- ☑ definition of biostimulants and differentiation to plant protection products
- ? positive list: producers propose criteria-based evaluation and harmonized standards
- ? thresholds = safety criteria
- ? strain identification and microorganism concentration

Timeline for developing a European regulation recognizing biostimulants:

Ravensberg 2017, IBMA, EBIC

Thank you for your attention!

J Plant Dis Prot (2017) 124:413–419
DOI 10.1007/s41348-017-0109-5

OPINION PAPER

The unpredictable risk imposed by microbial secondary metabolites: how safe is biological control of plant diseases?

Holger B. Deising[1] · Iris Gase[1] · Yasuyuki Kubo[2]

Received: 18 May 2017 / Accepted: 7 June 2017 / Published online: 27 June 2017

Abstract Currently, a strong public and political demand for reducing chemical pesticides exists, supporting strengthening of biological plant protection programs in agriculture. Biological plant protection strategies largely depend on introducing antagonistic microorganisms to agro-ecosystems. This approach would indeed reduce the impact of synthetic chemistries and may help avoiding development of fungicide-resistant pathogen populations, but on the other hand may strongly increase the load of microbial toxins. In crops treated with biological control agents, neither the newly forming (confronting) microbial communities nor the secondary metabolites synthesized by these are known. Many microbial secondary metabolites may not only exhibit acute toxicities significantly exceeding those of fungicides, but even act as strong carcinogens. This paper discusses the risks imposed by the introduction of biological control agents and strongly suggests including secondary metabolite gene cluster expression data into the list of information required for approving any plant protection agents and releasing them to the field. Secondary metabolism gene expression data could strongly support assessing the risk imposed by microorganisms used in disease and pest control.

✉ Holger B. Deising
holger.deising@landw.uni-halle.de

[1] Chair of Phytopathology and Plant Protection, Institute for Agricultural and Nutritional Sciences, Faculty of Natural Sciences III, Martin-Luther-University Halle-Wittenberg, Betty-Heimann-Str. 3, 06120 Halle (Saale), Germany

[2] Laboratory of Plant Pathology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan

Keywords Biologics · Biologicals · Biological control · Microbiological control agents · Plant protection · Secondary metabolites · Toxins

Forgotten are severe epidemic disease outbreaks in crops, forgotten is hunger due to yield losses caused by plant pathogens, forgotten are intoxications of man by microbial secondary metabolites occurring at times when synthetic chemistries reliably controlling diseases were not available. In 1844/1845, for example, the Irish potato famine caused by the oomycete *Phytophthora infestans* caused the death of one million Irish, lives that could have been saved if appropriate fungicides had been available. In the mid-ages, infection of cereals by the ergot fungus *Claviceps purpurea* led to alkaloid poisoning, i.e., hallucinations and "burning" limbs, as documented by the painter Grünewald in 1512–1516 (Agression de saint Antoine par les démons; musée d'Unterlinden, Colmar, France) [for review, see Haarmann et al. (2009)], and many more examples of severe epidemics exist (Perlman 1977).

The first fungicides applied to control diseases in crops were inorganic compounds such as elementary sulfur and copper salts. Improved synthetic chemistry helped in designing highly specific single target compounds exhibiting very low toxicity in mammals. In 1968, the first systemic fungicides introduced to the market were the benzimidazoles, and the complex group of systemic azole fungicides has been extensively used from 1977 onward. Today the azoles still represent one of the most important fungicide classes, with low LD_{50} (rat, oral) values in the g/kg (not mg/kg!) range (Table 1). The widely used azole fungicide tebuconazole, for example, has an LD_{50} of 1.7–4.0 g/kg, depending on the toxicity assay used (Paranjape et al. 2015), and the strobilurin fungicides derived from an antifungal

Table 1 Acute toxicity of selected examples of microbial secondary metabolites, pesticides, and other substances

Compound	LD_{50} (mg/kg)	References
Secondary metabolites		
Aflatoxin B1	7.2^{m}–17.9^{f}	Dhanasekaran et al. (2011)
Ochratoxin A	20^{f}–22^{m}	Purchase and Theron (1968)
Patulin	6.8	Hayes et al. (1979)
T2 mycotoxin	3.8	Norppa et al. (1980)
BoNT-toxin	0.000001*	Bitz (2010)
Pesticides		
Neurotoxic insecticides		
Dichlor-diphenyl-trichloroethane (DDT)	300	Cameron and Cheng (1951)
Deltamethrin	128	Perger and Szadkowski (1994)
Permethrin	>4000	Perger and Szadkowski (1994)
Parathion-ethyl	3–8	Pasquet et al. (1976)
Imidacloprid	450	Sheets et al. (2016)
Fungicides		
Tebuconazole	1700–4000	Paranjape et al. (2015)
Kresoxim-methyl	>5000	Bartlett et al. (2002)
Azoxystrobin	>2000	Bartlett et al. (2002)
Others		
NaCl	3890^{m}–3620^{f}	Boyd and Shanas (1963)
Ethanol	7060^{o}–10600^{y}	Wiberg et al. (1970)

[a] Human, intravenously or subcutaneous application

m male, *f* female, *o* old, *y* young rats

compound secreted by the pinecone-colonizing fungus *Strobilurus tenacellus* (Anke et al. 1977) likewise exhibit LD_{50} values between >2 (azoxystrobin) and >5 g/kg (kresoxim-methyl) (Bartlett et al. 2002). For comparison, sodium chloride (NaCl), which we consume on a daily basis, has an LD_{50} of 4 g/kg (Boyd and Shanas 1963) and may thus be considered more toxic than kresoxim-methyl. Ethanol has a toxicity comparable to this fungicide (Wiberg et al. 1970) (see Table 1). Consumption of a 200-ml glass of wine containing 12% ethanol corresponds to an ethanol intake of 19.2 g, leaving most consumers without any concern. As a matter of fact, the World Health Organization (WHO) global status report on alcohol and health 2014 indicated a recorded annual per capita consumption of an average adult (15 years and older) corresponding to approx. 11.3 L of pure alcohol (WHO) in Germany (http://www.who.int/substance_abuse/publications/global_alcohol_report/msb_gsr_2014_3.pdf). While gram amounts of ethanol are consumed on a daily basis, the European legislation typically tolerates a concentration of only 0.01 mg of a modern fungicide per kg of food. The irrational thus is that the toxicity of ethanol is tolerated or ignored, but minute amounts of low-toxicity fungicides in our food cause emotional debates.

Since the 1970s, several classes of modern chemistries have been developed, allowing effective disease control in the field (Deising et al. 2017) and control of pre- and post-harvest colonization of crops by mycotoxin-forming fungi. However, in spite of the availability of modern fungicides fruit juices contaminated with *Penicillium* and *Aspergillus* species or produced from fruits infected by these fungi frequently contain the mycotoxins patulin or ochratoxin A, which exhibit worrying acute toxicities, as indicated by LD_{50} values of 7 and 22 mg/kg body weight, respectively, when orally administered to rats (Hayes et al. 1979; Purchase and Theron 1968). Aflatoxin B1 produced by the fungi *Aspergillus flavus* and *A. parasiticus* has an LD_{50} of 7 and 12 mg/kg in male and female rats, respectively. The acute toxicity of aflatoxin is caused by inhibition of RNA polymerase, as demonstrated more than 50 years ago by in vitro studies with ^{14}C-labeled orotic acid (Rees 1966).

Aspergillus flavus infects hazelnuts and other seeds rich in lipids, and the lipophilicity of aflatoxin results in the accumulation of the toxin. Alarmingly, a foundation concerned with consumer safety in Germany has recently published analyses showing that Aflatoxin was found in 16 of 21 tested hazelnut cocoa spread samples (Stiftung Warentest, “Test,” issue 4, 2016), clearly demonstrating the omnipresence of toxic microbial compounds in food and fodder. Furthermore, cereal grains may contain trichothecene toxins produced by species belonging to various fungal genera, including *Fusarium* (Yazar and Omurtag 2008). The trichothecene T2 mycotoxin has an acute toxicity (LD_{50}) of 3.8 mg/kg body weight (Norppa et al. 1980) and exhibits a rather non-specific mode of toxicity. The toxin reacts with thiol groups of proteins and therefore is a potent inhibitor of DNA and protein biosynthesis. Moreover, T-2 toxin impairs antibody production, membrane function, reduces lymphocyte proliferation, alters the maturation process of dendritic cells, introduces DNA strand breaks in vivo and in vitro, and causes apoptosis in various cell types [for review, see Kalantari and Moosavi (2010)]. Extremely high acute toxicity, however, has been reported for the proteinaceous botulinum-neurotoxin (BoNT) synthesized by the bacterium *Clostridium botulinum*, exhibiting an LD_{50} of 3.2^{-7} mg/kg body weight. This is in the nanogram per kilogram range! (Bitz 2010; Table 1).

The acute toxicity data discussed here for some selected examples of mycotoxins (Table 1) are indicative of only a part—and one must say: of a minor part—of the risk associated with these compounds. Of significantly greater concern is the mutagenic and, in particular, the carcinogenic potential of mycotoxins (Barkai-Golan and Paster 2008; Perlman 1977). The carcinogenic activity of aflatoxins depends on activation of these compounds by human P450 enzymes and is due to binding of the activated aflatoxins to DNA and adduct formation with guanosine bases. In fact, aflatoxins belong to the compounds with the highest carcinogenic potential occurring in nature (He et al. 2006; Pottenger et al. 2014).

Collectively, in the public view the toxicity of microbial secondary metabolites, including their carcinogenic activity, is strongly underestimated. In contrast, the risk imposed by synthetic pesticides is dramatically overestimated. In spite of some differences of the toxicity assays used for the compounds discussed here, comparison of the acute toxicity of BoNT with that of first generation insecticides, i.e., the neuronal Na^+-pump inhibitor dichlorodiphenyl-trichloroethane (DDT) banned in Germany since 1972, indicates approx. 300,000,000 (in words: three hundred million!) times higher toxicity of the natural toxin!

Given the low toxicity of modern pesticides and—in comparison—the unpredictable risk associated with the consumption of microbial toxins, the public rejection of pesticides appears irrational. Hence, it appears naive to believe that plant disease control by antagonistic microorganisms, as currently propagated, would not be harmful, simply because the microbial compounds they produce are natural. The preceding paragraphs show that the strongest poisons are biologically produced agents. Irritatingly, the public misperception of the toxicity of natural compounds and the strong public and political demand for applying biologics/biologicals, i.e., antagonistic microorganisms in disease control, may make biologics/biologicals the prime disease control measures in first world agriculture in the near future. Indeed, Marcus Meadows-Smith, Head of Biologics at Bayer CropScience, expects the biologics market to triple to almost US$ 4 billion by 2020 (www.seedgrowth.bayer.com/products).

Microorganisms, either bacteria or fungi, and irrespective of whether they act as plant pathogens or as biocontrol agents, exhibit an enormous potential of forming functionally and structurally distinct chemicals referred to as secondary metabolites. Mapping of fungal secondary metabolite gene clusters revealed that genomes of several *Aspergillus* species, i.e., *A. terreus*, *A. flavus*, *A. oryzae*, and *A. niger,* harbor more than 50 of such clusters (Cuomo et al. 2007; Khaldi et al. 2010). Remarkably, the model fungus *A. nidulans* is thought to be able to produce a minimum of 32 polyketides, 14 non-ribosomal peptides, and two indole alkaloids (Brakhage et al. 2008; Rank et al. 2010). The genome of the wheat head blight fungus, *Fusarium graminearum*, contains 20 non-ribosomal peptide synthetase, 15 polyketide synthase, and 17 terpenoid synthase genes. Again, *Fusarium* trichothecene toxins are known as highly toxic agents and may, in addition to their acute toxicities, be causal to esophageal cancer (Chang et al. 1992; Chu and Li 1994; Luo et al. 1990). Importantly, not only pathogens but also fungi used as biocontrol agents harbor secondary metabolite gene clusters. For example, the genomes of the entomopathogens *Metarhizium anisopliae*, *Beauveria bassiana,* and *Cordyceps militaris* harbor as many as 73, 45, and 37 secondary metabolite gene clusters (Xiao et al. 2012; Sbaraini et al. 2016), and the mycoparasitic *Trichoderma* species *T. reesei*, *T. atroviridae*, and *T. virens* harbor 27, 32, and 55 genes encoding secondary metabolite-synthesizing enzymes (Zeilinger et al. 2016). These numbers highlight the fact that microbes assumed to be “harmless” biological control agents also do have the potential to produce diverse and putatively harmful patterns of secondary metabolites.

Formation of secondary metabolites strongly depends on environmental factors fungi are exposed to. Intriguingly, in *A. niger* more than 70% of the gene clusters encoding PKS, NRPS, and PKS-NRPS hybrids remain transcriptionally repressed under standard laboratory conditions (Fisch et al. 2009). Regulatory factors controlling the expression of secondary metabolite gene clusters include not only global and specific transcriptional regulators but, in addition, epigenetic factors. The complexity and hierarchy of regulation of secondary metabolite formation strongly suggests that these compounds may play a role in fungal stress response or adaptation (Brakhage 2013; Macheleidt et al. 2016). In this context, it appears plausible that mycotoxins are thought to represent components of the fungal stress-response system (Ponts 2015). It is doubtless that natural habitats such as crop leaf surfaces impose significant stress to microorganisms, most of which resulting from interactions within competitive microbial communities. However, neither the number of secondary metabolite gene clusters expressed, nor the complexity of their products is known in individual species contributing to these microbial consortia. And this scenario is likely to become much more dramatic when biocontrol microorganisms are introduced into agro-ecosystems, creating a multitude of confronting interactions with many secondary metabolite gene clusters activated and thousands of so far unknown secondary metabolites formed. Thus, biocontrol organisms, may they be called biologicals or biologics, may not be consumer-friendly. Debating the issue of releasing biocontrol organisms, we need to keep in mind that we dramatically alter microbial consortia, with the number of secondary metabolites formed, the toxicity of these and their mode of action remaining unknown. Even with highly sophisticated analytical tools in hands, analyses aiming at compound identification will be more than challenging, as the amounts of the compounds may be far below the limit required for structural analyses. Among the greatest difficulties in risk assessment, however, is the fact that microbes do not only efficiently secrete but also take up many secondary metabolites secreted by other microbes and, depending on their enzymatic capacity, channel these metabolites into metabolic pathways yielding novel compounds with unknown toxicology.

The exchange of secondary metabolites between microorganisms belonging to different species can be demonstrated by a simple experiment. The *Colletotrichum* species, *C. graminicola* and *C. lagenarium*, infecting maize and cucumber, synthesize the polyketide melanin and incorporate this polymer into cell walls of vegetative hyphae and appressoria (Deising et al. 2000; Kubo and Furusawa 1986). In *C. graminicola* and *C. lagenarium,* melanin is a pathogenicity factor (Kubo et al. 1982; Ludwig et al. 2014), and mutants with defects in the melanin biosynthetic pathway are unable to penetrate the intact epidermal cell wall of their host plants and are unable to cause disease (Kubo et al. 1996; Ludwig et al. 2014).

Melanin biosynthesis (Fig. 1a; Kubo and Furusawa 1991) is initiated by the polyketide synthase Pks1, forming a pentaketide, which, after cyclization, yields a naphthalene ring with four hydroxyl groups, i.e., 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN). Reduction of this compound yields scytalone, which is dehydrated by an enzyme called scytalone dehydratase (Scd1) to form 1,3,8-trihydroxynaphthalene (1,3,8-THN). Repeated reduction and dehydration reactions lead to formation of vermelone and 1,8-dihydroxynaphthalene (1,8-DHN), the latter being polymerized in a radical reaction to yield the cell wall incrusting black pigment melanin (Fig. 1a).

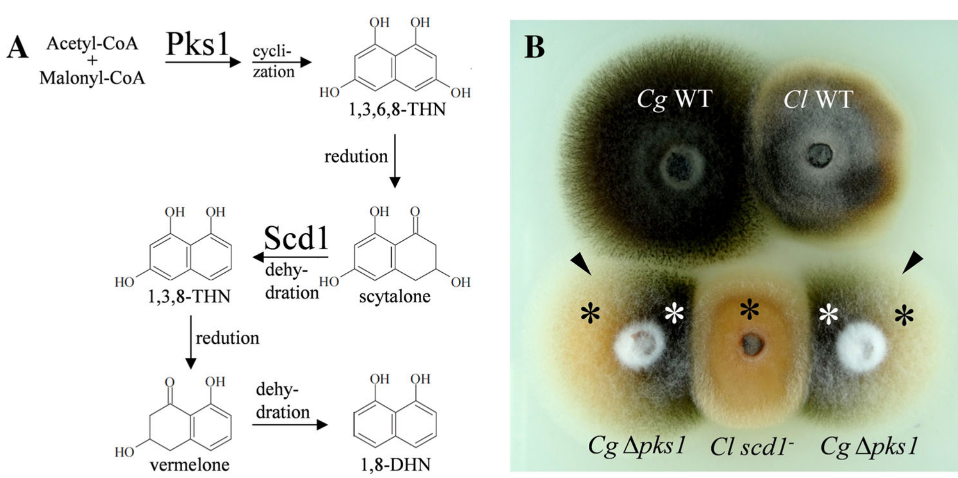

Fig. 1 Cross-species exchange of secondary metabolites in melanin biosynthesis. **a** The melanin biosynthesis pathway is initiated by a polyketide synthase (Pks1)-catalyzed reaction and cyclization, yielding 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN). Reduction of 1,3,6,8-THN gives rise to scytalone, which is subsequently dehydrated to 1,3, 8-trihydroxynaphthalene (1,3,8-THN). Two additional reduction and dehydration reactions lead to 1,8-dihydroxynaphthalene (1,8-DHN), which, in a radical reaction, is oxidatively polymerized to yield melanin. **b** Co-cultivation experiment demonstrating exchange of secondary metabolites across species. The scytalone dehydratase disruption mutant *Cl scd1*$^{-}$ of *C. lagenarium* is unable to further metabolize and therefore secretes scytalone. This compound is then taken up by the polyketide synthase-deficient mutant *Cg* Δ*pks1* of *C. graminicola*, chemically complementing and circumventing the genetic block of *PKS1*. Thus, while *Cg* Δ*pks1* and *Cl scd1*$^{-}$ mutant strains cannot synthesize melanin and remain salmon colored (black asterisks), the *Cg* Δ*pks1* mutant can form melanin at the interface with the *Cl scd1*$^{-}$ mutant of *C. lagenarium* (*white asterisks*) due to scytalone secretion by this latter strain. Those parts of the colony in contact with the WT strains do not get complemented (*arrowheads*) as that the WT strains do not release melanin biosynthesis intermediates. The *C. lagenarium* WT strain and the *Cl scd1*$^{-}$ mutant 9201Y are from the Kubo laboratory, the *C. graminicola* WT strain CgM2 was a gift of R.L. Nicholson, Purdue University, IN, USA, and the *Cg* Δ*pks1* mutant has been generated by R. Horbach, Martin-Luther-University Halle-Wittenberg, Germany (Ludwig et al. 2014). Colonies were grown on potato dextrose agar.

Perceivably, while the *C. graminicola* wild-type strain CgM2 (*Cg* WT) is able to synthesize melanin and forms black hyphae on potato dextrose agar (PDA), a mutant deficient in the polyketide synthase gene (*Cg* Δ*pks1*) does not, due to early disruption of the melanin biosynthesis pathway (Fig. 1b, *Cg* Δ*pks1*, black asterisk). Likewise, while the *C. lagenarium* WT strain 104-T (*Cl* WT) forms melanin, a scytalone dehydratase disruption mutant of this fungus (*Cl scd1*$^{-}$; Kubo et al. 1996) remains salmon colored (Fig. 1b, *Cl scd1*$^{-}$). Importantly, co-cultivation of *Cg* Δ*pks1* and *Cl scd1*$^{-}$ shows that scytalone is secreted by the *Cl scd1*$^{-}$ strain and complements the melanization defect of the *Cg* Δ*pks1* strain (Fig. 1b, *Cg* Δ*pks1*, white asterisk). This complementation experiment clearly demonstrates that metabolites are not only exchanged between mutants of the same species (Kubo et al. 1983), but also across species' borders (Fig. 1b).

Intriguingly, *trans*-kingdom secondary metabolite exchanges have recently also been shown between bacteria and fungi. Brakhage and co-workers have demonstrated a trans-kingdom "cross talk" between the filamentous fungi *Aspergillus nidulans* and *Aspergillus fumigatus* and the soil bacterium *Streptomyces rapamycinicus*. The bacterium triggered the activation of silent PKS and NRPS gene clusters and formation of novel secondary metabolites in the fungal species, due to the specific activation of chromatin remodelers (Netzker et al. 2015). Interestingly, the filamentous bacterium *Streptomyces platensis* produces and secretes the natural derivative of dienohydroxamic acid, Trichostatin A, a potent inhibitor of Zn^{2+}-dependent class I and class II histone deacetylases (HDACs), and may thus affect global gene expression, including the expression of secondary metabolite genes, in competing microorganisms (Izawa et al. 2009; Vanhaecke et al. 2004).

In order to understand the risk imposed by microbial secondary metabolites, not only the metabolites of individual species but rather of large microbial communities need to be analyzed. In an excellent overview, Vorholt highlights the enormous complexity of bacteria of the phyllosphere, forming populations consisting of an average of 10^6–10^7 bacteria per cm^2 of leaf surface; the global bacterial phyllosphere population is estimated to correspond to as many as 10^{26} cells (Vorholt 2012). The complexity of the fungal propagules on the leaf surface is expected to be lower than that of bacteria [see also Lindow and Brandl (2003)]. However, given the tremendous numbers of species, their capability of producing millions of compounds with unknown toxicity, potentiated by an exchange and enzymatic modification of these metabolites, it is questionable whether or not consumers' safety can be improved by replacing defined agrochemicals by microbial biological control agents. Clearly, based on the risks imposed by releasing antagonistic microorganisms to agroecosystems, as outlined above, politics-driven public false perception of the toxicity of chemically synthesized compounds and of microorganisms may lead to severe health problems caused by highly toxic secondary metabolites produced by microbial consortia.

However, as secondary metabolites, including mycotoxins, are components of the fungal general stress-response system (Ponts 2015), one may need to consider that stresses other than competing microorganisms, e.g., fungicides applied at sublethal doses, may likewise lead to activating secondary metabolite gene clusters and toxin formation. Indeed, long-term field trials have shown that application of strobilurin-containing fungicides increased trichothecene mycotoxin contents in winter wheat (Ellner 2005). Analyses of mycotoxin formation in *F. graminearum* after azole fungicide treatment, however, gave inconclusive results (Becher et al. 2010; Kulik et al. 2012).

Currently, the European Union (EU) Commission Regulations Nos. 283/2013 and 284/2013 define the data requirements for chemical active substances as well as for microorganisms, including viruses, to be used in plant protection. Unfortunately, while large amounts of direct toxicity and eco-toxicity data are required for substances to be released to the market, analyses of indirect effects such as activation of secondary metabolite gene clusters possibly leading to formation of highly toxic compounds are not required.

Today's DNA sequencing technologies have allowed sequencing the genomes of almost 1000 fungal species, allowing simple access to secondary metabolite genes by bioinformatics tools. Thus, RT-qPCR or large-scale microarray analyses could be employed to provide clear information on the risk of activation of secondary metabolite gene clusters not only imposed by biologics or biologicals confronting model fungi, but also imposed by pesticides. As these types of experiments can be performed with basically any microorganism the genome of which has been sequenced, it is a must for modern EU Commission Regulations to require such data prior to releasing biological or chemical plant protection agents to the field. Gene expression data would not allow to identify potentially toxic chemicals formed by microorganisms, but would provide clear evidence of the risk potential associated with novel and existing disease control measures. Consumers would significantly benefit from using modern molecular tools to predict the risk of formation of highly toxic secondary metabolites and from science-based decisions on strategies employed in plant protection.

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Plant protection and plant health in Europe Symposium

Registration of Biorationals in practice

G.Meunier – EMEA Regulatory Policy Manager

Bayer, CropScience Division
France.

Forward-Looking Statements

This presentation may contain forward-looking statements based on current assumptions and forecasts made by Bayer management.

Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer's public reports which are available on the Bayer website at http://www.bayer.com/.

The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.


Microbials
Plant-extracts
Yield-enhancer
Natural-Mimics
Macrobials
Bio-pesticides
Inoculants
basic-substance
Botanicals
Plant-strengthner
Biologically-similar
Low-risk-substances
Biocontrol
Bio-stimulants
Biologicals
Biorationals
Natural
Biologics

Agenda

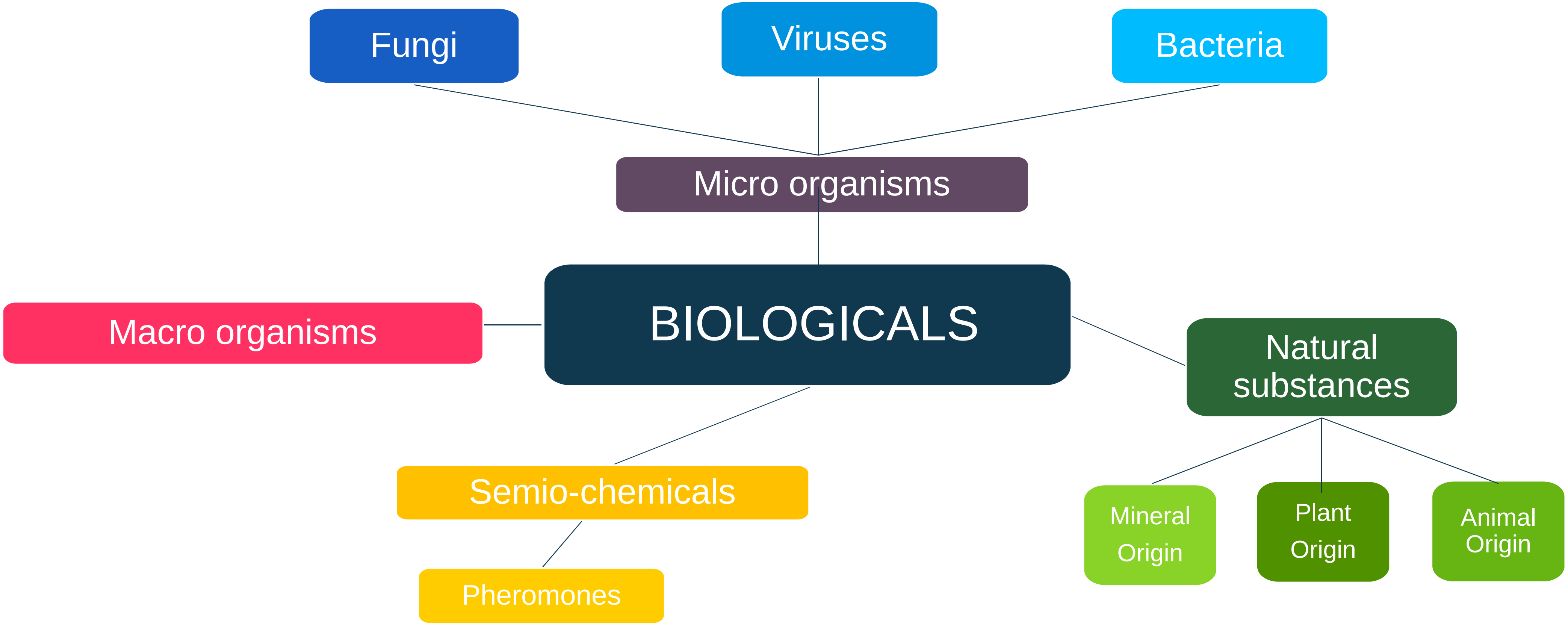
- What is a Biorational from a regulatory perspective?
- The Regulatory framework
  - What is the same ?
  - What is different ?
- The Requirements
  - What is the same ?
  - What is different ?
- Conclusion

WHAT IS A BIORATIONAL ? – SYMPOSIUM DEFINITION

Materials that are biologically-derived or, if synthetic, structurally similar and functionally identical to a biologically occurring material. Micro-organisms, plant extracts, basic substances, semiochemicals, […]

Invitation to the symposium « Plant protection and Plant health in Europe 13-14/12/2017 Braunschweig Germany

WHAT ARE BIO-CONTROL PRODUCTS IN FRANCE



From : « Le bio-contrôle pour la protection des cultures 15 recommandations pour soutenir les technologies vertes. Synthèse du rapport au premier ministrie. Mission parlementaire confiée à Antoine Herth. 2011

WHAT ARE BIOLOGICALS – A BAYER DEFINITION

Biologicals consist of **microorganisms** such as bacteria and fungi; **beneficial macroorganisms** (e.g. predatory mites); **semiochemicals** (e.g. pheromones); or **natural compounds** (e.g. plant extracts).

The Regulatory framework for biorationals

Proposal classification of Biological products by function

Biological Products

- **Biofertilizers**
- **BioStimulants**
- **Biological Control Products**
  - **BioPesticides**
  - **Macroorganisms**
    - Insects
    - Mites
    - Nema-todes

Revision of 2003/2003 :

Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL laying down rules on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009

Regulation 1107/2009

concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

Macroorganisms

- Insects; Mites; Nematodes
- Insects followed by mites makeup the largest groups
- Unique in that the live organism in the form of eggs, larvae, pupae or adult is used.
- Most important challenge for Macros is logistics—shipping live organisms that have to have special care to survive
- Normally not classified as a Biopesticide—only as Biological Control Products

Regulatory frameworks are based on the Function of the final product

1107/2009

1. This Regulation shall apply to products, in the form in which they are supplied to the user, consisting of or containing active substances, safeners or synergists, and intended for one of the following uses:
(a) **protecting plants or plant products against all harmful organisms** or preventing the action of such organisms, unless the main purpose of these products is considered to be for reasons of hygiene rather than for the protection of plants or plant products;
(b) **influencing the life processes of plants**, such as substances influencing their growth, other than as a nutrient;
(c) preserving plant products, in so far as such substances or products are not subject to special Community provisions on preservatives;
(d) **destroying undesired plants or parts of plants**, except algae unless the products are applied on soil or water to protect plants;
(e) **checking or preventing undesired growth of plants**, except algae unless the products are applied on soil or water to protect plants.

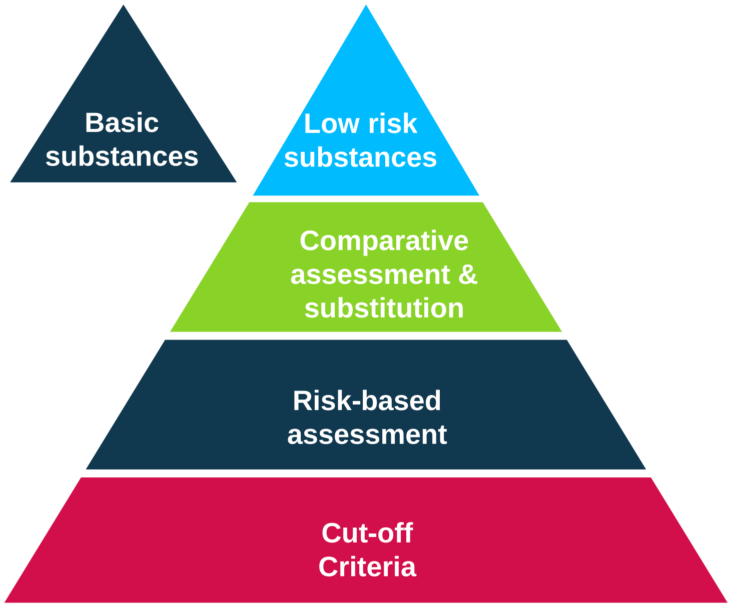
2003/2003 (revised)

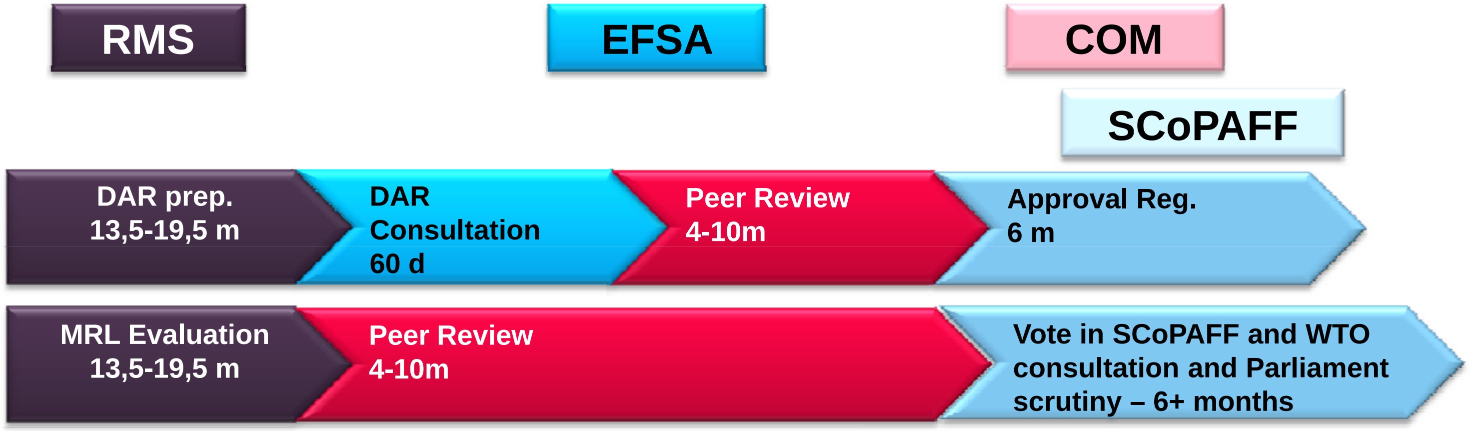
Text presented for Parliament first reading.

(1) '***plant nutrition*** product' means a substance, mixture, micro-organism or any other material, applied or intended to be applied, either on its own or mixed with another material, *on fungi or their mycosphere or* on plants *at any growth stage, including seeds, and/*or *rhizosphere*, **for the purpose of providing plants *or fungi* with nutrients or *of* improving their *physical or biological growth conditions or their general vigour, yields and quality, including by increasing the ability of the plant to take up nutrients*** *(with the exception of plant protection products covered by Regulation (EC) No 1107/2009)*;

Regulatory framework – What is the same ?

Active substance Approval



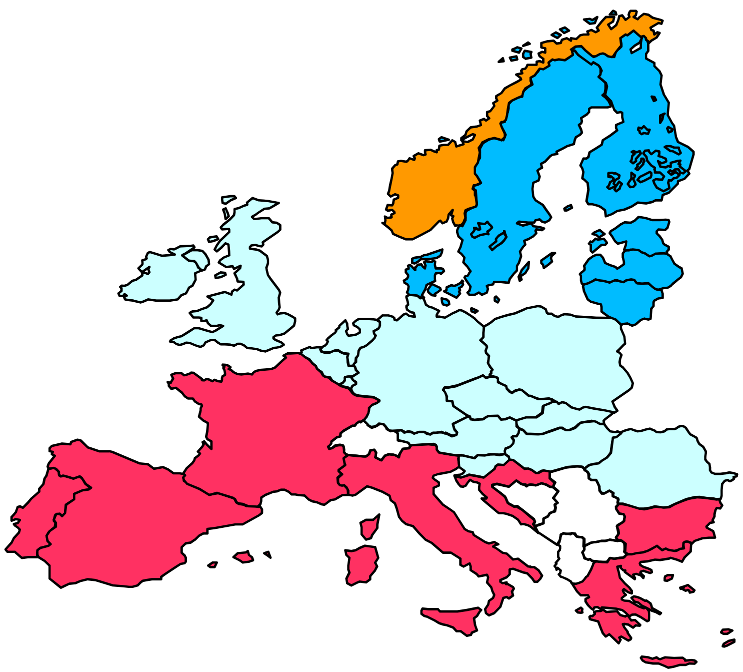


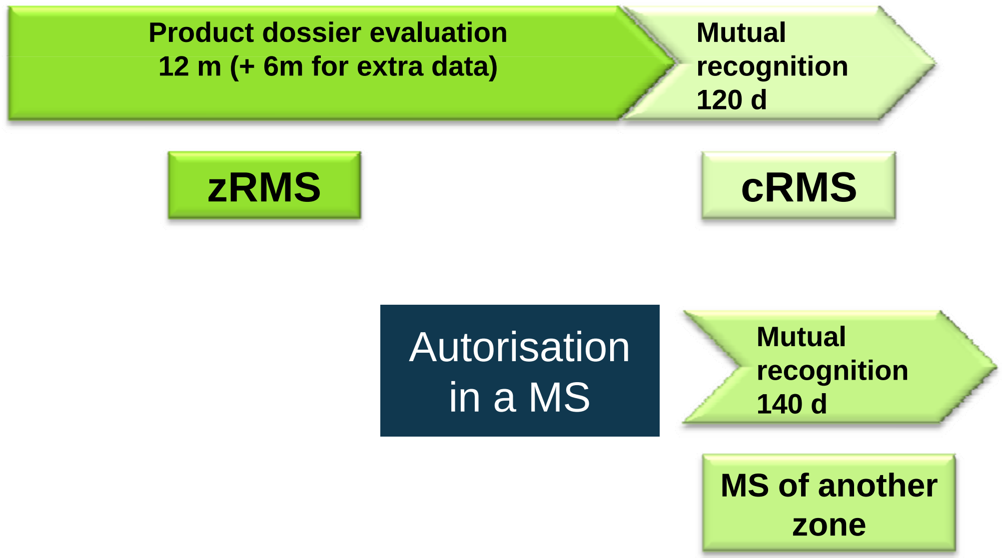
RMS: rapporteur member state
EFSA: European Food Safety Agency
COM: EU-Commission
DAR: Draft Assessment Report
MRL: maximum residue limit

Biopesticides are subject to 1107/2009 like all other PPPs.

Regulatory framework – What is the same ?

Product Authorisation

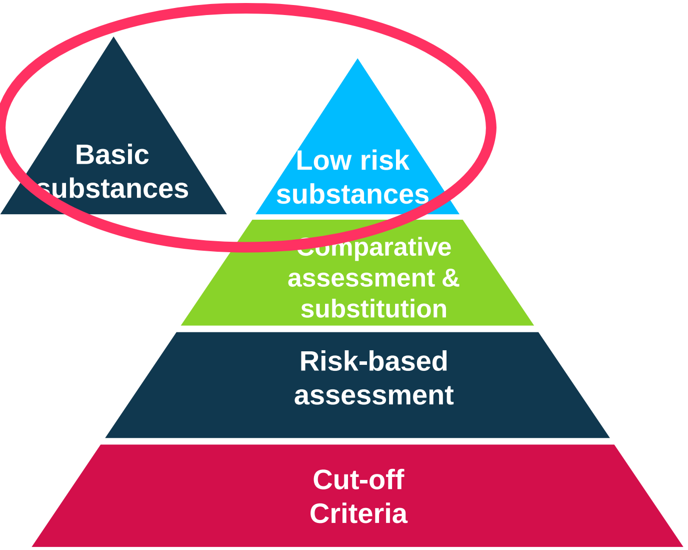




Biopesticides follow the Zonal approach.

Regulatory framework - what is different ?

Active substance Approval

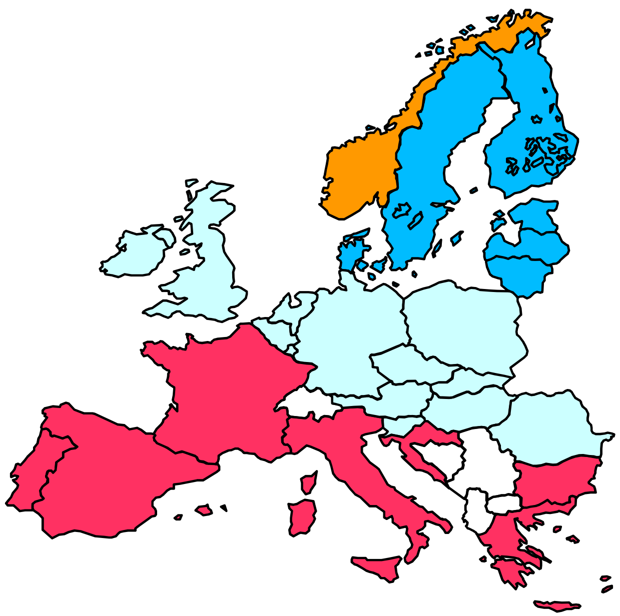


- **10 approved Low-risk substances**
  - 4 micro-organisms
  - 3 viruses
  - 1 yeast extract
- **18 approved Basic-substances**
  - Vinegar, beer
  - Mustard seed, salix spp. cortex etc…
- New Low risk criteria voted August 2017

Low-risk substances : longer approval, quicker approval of products.

Regulatory framework - what is different ?

Product Authorisation



- **Less difficult to find ZRMS and shorter “queue”**
- **Timelines better than for chemical products**
- **Inter-zonal mutual recognition works well for microbial products**

- Most of the questions during evaluation on :
  - Identity of the substance/organism
  - Contaminants and storage stability
  - Efficacy of the product

The Requirements

The Requirements – what is the same ?

COMMISSION REGULATION (EU) No 283/2013

of 1 March 2013

setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

(Text with EEA relevance)

PART A

CHEMICAL ACTIVE SUBSTANCES

PART B

MICRO-ORGANISMS INCLUDING VIRUSES

COMMISSION REGULATION (EU) No 284/2013

of 1 March 2013

setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

(Text with EEA relevance)

PART A

CHEMICAL PLANT PROTECTION PRODUCTS

PART B

PREPARATIONS OF MICRO-ORGANISMS INCLUDING VIRUSES

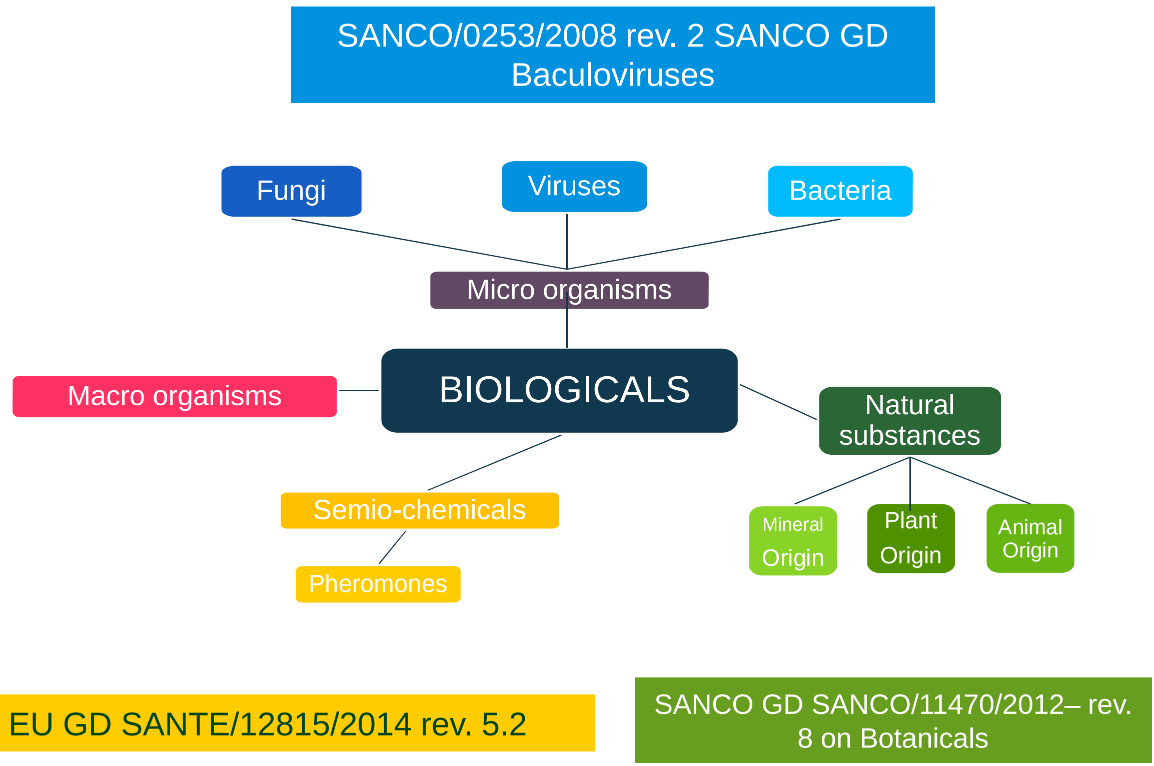
In theory only specific requirements for Microbials. In practice several guidance documents provide specific data-requirements for other types of biorationals.

The Requirements – what is different ?

Guidelines

EU

- EFSA RA on microbials
- <u>SANCO docs:</u>
  - SANCO/12823/2012 –rev. 4 **Equivalence of technical grade a.i.**
  - SANCO/12116/2012 –rev. 0 - **microbial contaminant limits**
  - Sanco/10754/2005 rev.5 - **Taxonomic level of microorganisms**
  - SANCO/12117/2012 –rev. 0 - **Environmental safety**
  - SANCO/11188/2013 - **inclusion of active substances into Annex IV**



The Requirements – what is different ?

A concrete example

For the a.i. (283/2013)

1.3. **Name and species description, strain characterisation**

(i) The micro-organism should be deposited at an internationally recognised culture collection and given an accession number and these details must be submitted.

(ii) Each micro-organism that is subject to the application shall be identified and named at the species level. The scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism, must be stated.

For the product (284/2013)

1.4. **Detailed quantitative and qualitative information on the composition of the preparation**

(i) Each micro-organism that is subject to the application shall be identified and named at the species level. The micro-organism shall be deposited at a recognised culture collection and given an accession number. The scientific name must be stated, as well as the group assignment (bacteria, virus, etc.) and any other denomination relevant to the micro-organism (e.g. strain, serotype). In addition, the development phase of the micro-

(ii) For preparations the following information must be reported:

— the content of the micro-organism(s) in the plant protection product and the content of the micro-organism in the material used for manufacturing of plant protection products. These must include the maximum, minimum and nominal content of the viable and non-viable material,

— the content of co-formulants,

— the content of other components (such as by-products, condensates, culture medium, etc.) and contaminating micro-organisms, derived from production process.

The contents shall be expressed in terms as provided for in Directive 1999/45/EC of the European Parliament and of the Council ([1]) for chemicals and appropriate terms for micro-organisms (number of active units per volume or weight or any other manner that is relevant to the micro-organism).

The Requirements – what is different ?

A concrete example

// Micro-organisms need to be :

- // Identified at strain level for active ingredient approval and product authorization.
- // Quantified in the product.

The Requirements – what is different ?

A concrete example : SERENADE® ASO

Example:
Serenade® ASO

IIIM 1.7.1.1 Also indicate: scientific name and strain/serotype of MPCA, its accession number in a recognised culture collection

Strain	QST 713
Species	*Bacillus subtilis*
Genus	*Bacillus*
Family	Bacilliaceae
Order	Bacilliales
Class	Bacilli
Group	Firmicutes
Division	Bacteria

The strain QST 713 has been added to the internationally accepted Agriculture Research Culture Collection (NRRL), Illinois, USA, code number NRRL B-21661.

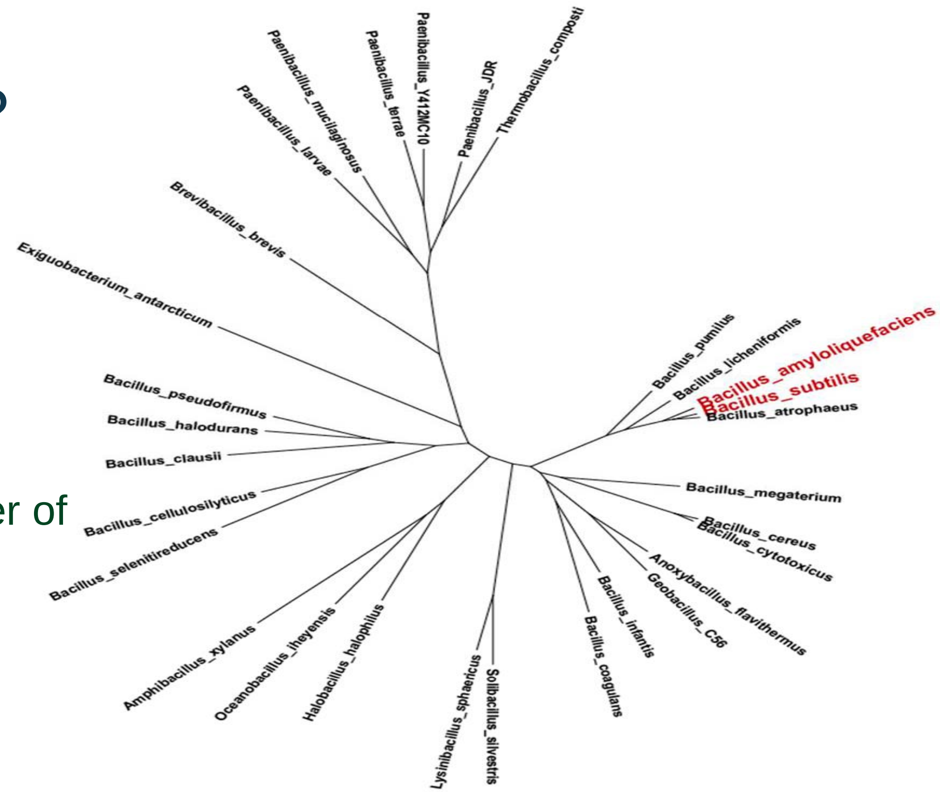
Why strain characterization is a critical point?

The Requirements – what is different ?

Identification of a microbial

- **Bacterial taxonomic classification is not a frozen picture.**

<u>Exemple</u>
The strain *Bacillus subtilis* QST 713 is now classified as a member of the closely related species *Bacillus amyloliquefaciens*



- **This could bring difficulties for :**
  - **other geographies,**
  - **internal databases and masterdata management**
  - **other legislations/standard based on specie name**

The Requirements – what is different ?

Quantification of a microbial

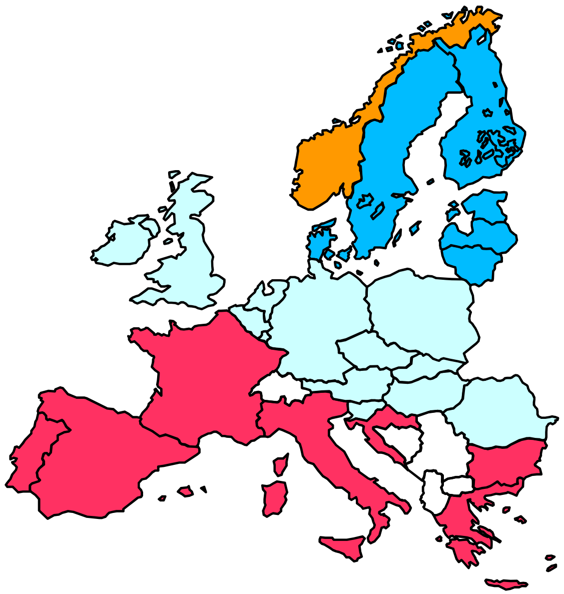
- **CFU method**
  - Best method to measure quantity of viable micro-organisms
  - But : some variability and issue with micro-organisms that tend to “cluster” on petri dishes.

- **Integrated process versus non-integrated process**
  - Certain micro-organisms cannot be easily isolated => integrated process
  - Only weight of biomass can be measured including residues and water
  - Spore weight obtained by calculation.

- **Specification based on Minimum versus Minimum and Maximum**
  - Differs from one country to the other

Requirements - what is different ?

Product Authorisation



- **Wide acceptability of public literature**
- **No/ a few higher tier studies**
- **Simple modelling calculations**

- Some technical guidance documents not adapted
  - Sensitization GD (microbial products)
  - Storage stability GD

CONCLUSION

CONCLUSION

- **Biorationals are very diverse by Nature.**
  - Same regulatory framework for all Plant Protection Products but necessity to have specificities of Biorationals to be taken into account.
    - Harmonisation of definition of Biocontrol/biorational.
    - Guidance document for specific substances/organisms.
  - A clear and precise “specification” is key.
    - Plant extracts
    - Micro-organisms

- **Biorationals can have diverse Functions.**
  - Plant Protection Products versus Biostimulants/Fertilizers

There is no regulatory “one size fits all” for Biorationals.

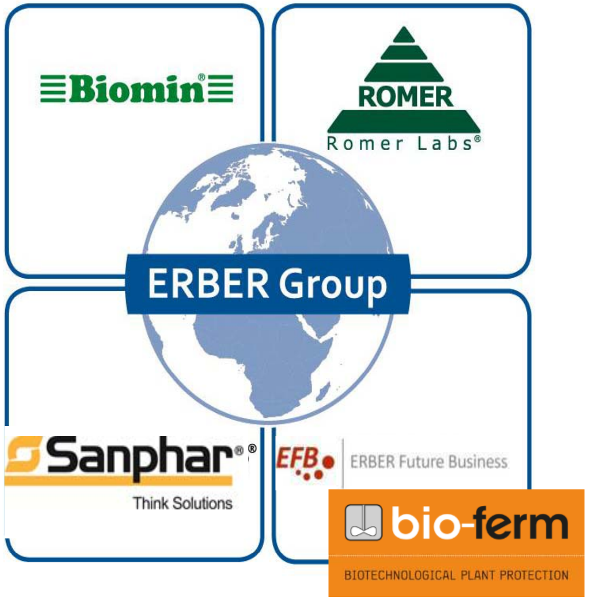
Thank you!

Rose is a rose is a rose…

bio-ferm
BIOTECHNOLOGICAL PLANT PROTECTION

Christina Donat,
Technical Director

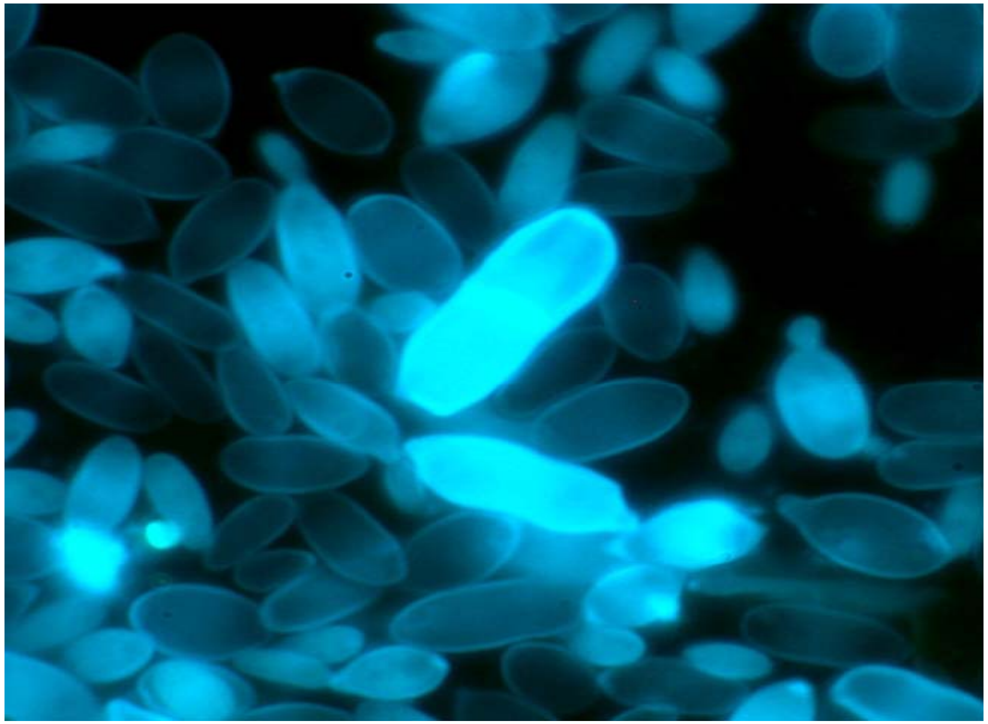
bio-ferm GmbH
Member of the Erber Group



bio-ferm is part of the Austrian family-owned Erber Group with more than 50 branches, active in almost 100 countries worldwide, has more than 1.600 employees and a turnover of 250 mio EUR.

Science cluster Tulln:

The main players are…
***Aureobasidium pullulans,* DSM 14940 and DSM 14941**



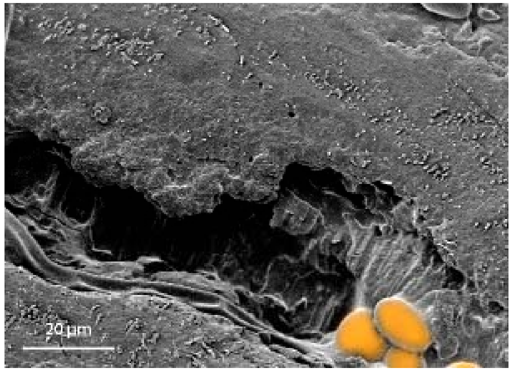
Aureobasidium pullulans

- Ascomycete with asexual, yeast-like reproducing cells (blastospores)
- Natural occurrence in the environment (soil, plant surface)
- Well adapted, resistant to drought and UV radiation
- Shelf life from date of manufacture:
- at room temperature (≤20ºC) 18 months
- at cold storage (≤8ºC) 30 months

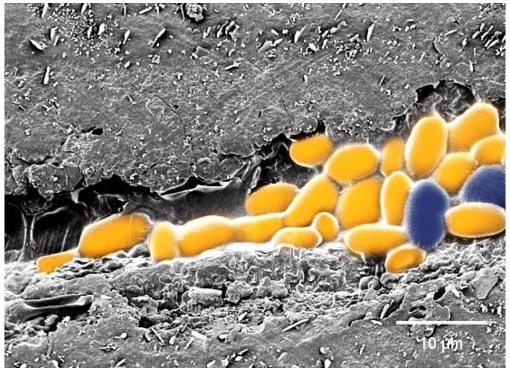


Aureobasidium pullulans, under the microscope, on an agar plate and dried to granules

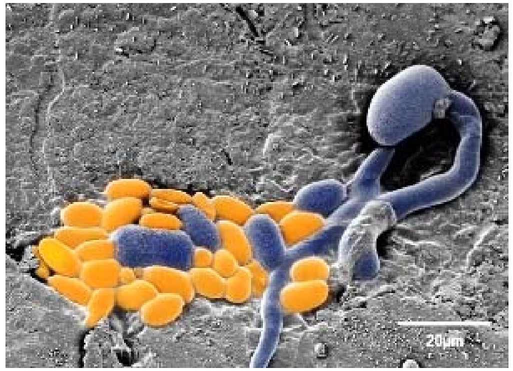
Mode of Action: Antagonism – competition for space and nutrients



1. Micro scratches on the fruit surface represent natural entrances for the pathogen. The scratches are colonized by *Aureobasidium pullulans* immediately after application of Botector® . (Picture: Mendgen).



2. Due to the high proliferation rate of *Aureobasidium pullulans* the pathogen cannot infect the plant . (Picture: Mendgen).



3. The micro scratch is completely colonized with *Aureobasidium pullulans*. Botector® acts as a natural shield which protects grape bunches from infection with *Botrytis cinerea* (Picture: Mendgen).

- *Aureobasidium pullulans*
- pathogen

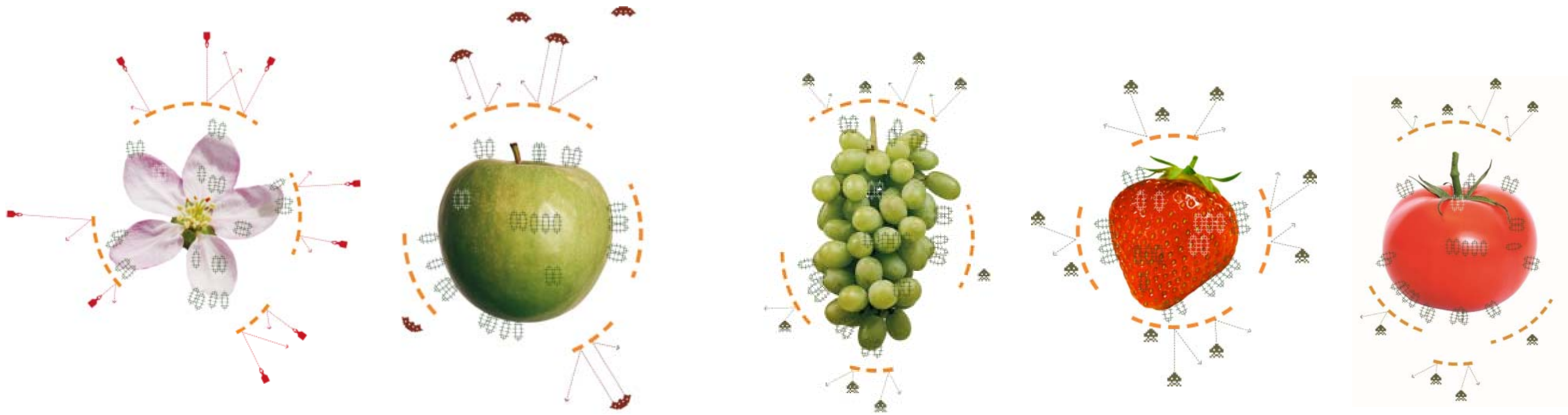
Scale up the production:




Marketing:

Blossom Protect™ ™

Botector®®



1989 University Konstanz, Chair of Phytopathologie: about 500 microbial isolates:
Schiewe und Mendgen 1992; Falconi und Mendgen 1994, …

1996: End of the research projects at Uni-Konstanz

2001: Bio-Protect GmbH founded, Konstanz

2005: bio-ferm GmbH founded, Start with the dossier for Annex I
Emergency use permits in several EU countries
Tox, Ecotox, Fate, Field trials

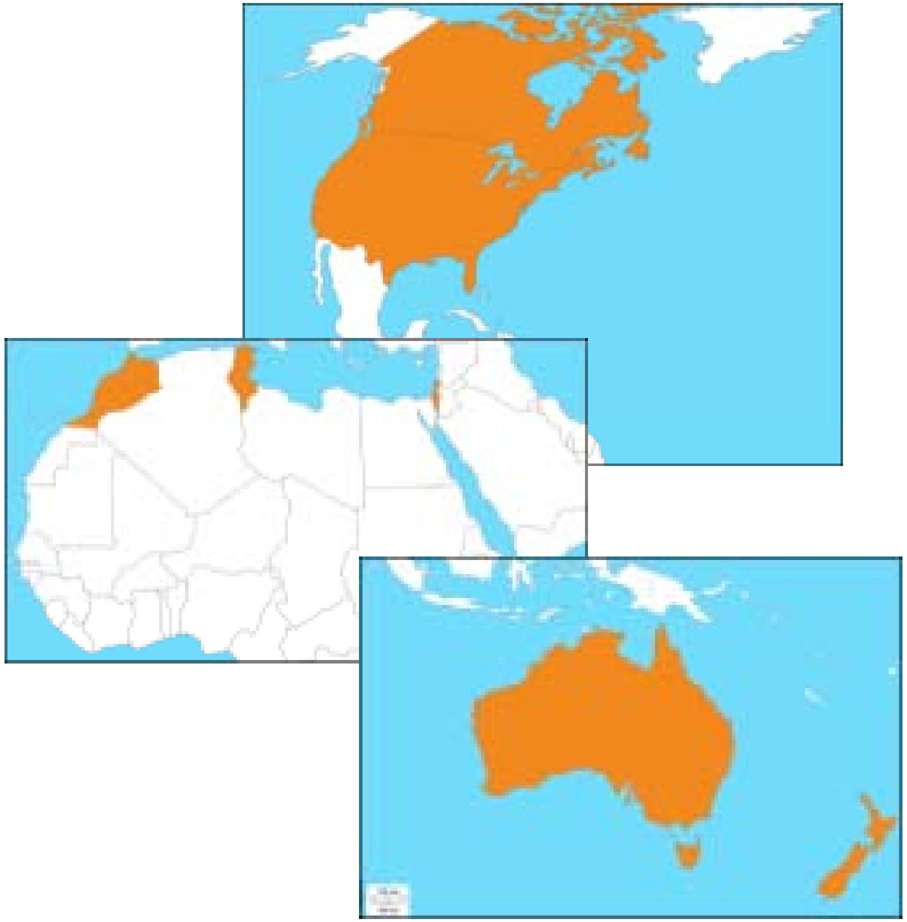
2007: EU submission of the dossier for *Aureobasidium pullulans* / Blossom Protect™

2009: Provisional registration of Blossom Protect™ in some EU countries

2010: Submission U.S.

2012: Registration and entry into the U.S. market

2014: EU Annex I inclusion, USA: Blossom Protect™ on 6.000 ha in use



Canada
- ✓ Blossom Protect™ since 2012
- ✓ Botector® since 2014

United States
- ✓ Blossom Protect™ since 2012
- ✓ Botector® since 2012

Morocco
- ✓ Botector® since 2009

Tunisia
- ✓ Blossom Protect™ since 2016
- Botector® *pending* since 2016

Turkey
- ✓ Blossom Protect™ since 2012

Israel
- ✓ Blossom Protect™ since 2017

Australia
- Botector® expected 2017

New Zealand
- Blossom Protect™ expected 2017

Europe

< 2014	**Provisional Registrations** (Art. 8(1) | Dir. 91/414) and **Emergency Use Permits** (Art. 53 | Reg. 1107/2009) **Final registrations** (not EU)
2014-01	**EU Approval of *A. pullulans***
2014-07	**Final Registrations** (zonal procedure, pending in C-EU and S-EU)

Transition period:
Provisional Registrations
remain valid until final registration (e.g. Austria, Belgium, Netherlands, Poland, Portugal…)

Emergency Use Permits

2007 EU submission of the dossier

2014-02 EU Approval of *A. pullulans*

2014-02 Submission of the renewal as a voluntary zonal work sharing project:

Central zone: zRMS: Austria, cMS: Belgium, Netherlands, Hungary, Slovakia, Poland

Southern zone: zRMS France, cMS: Italy, Spain , Greece, Portugal

dRR issued by FR 2016-10

Commenting 2016-12

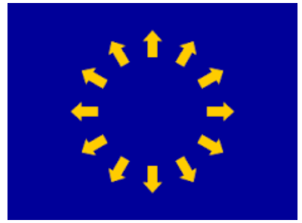
No RR till now, cMS not started up to now,

2017:

-> 2 final registrations, AT and HU

-> no final registrations

WHY?



HOT TOPICS

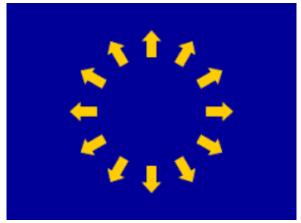
- **Classification:**

GHS 07 + H317

agreed on EU level (outcome of the PRAPeR meeting in 2009):

- the Regulation 1272/2008 is not applicable to microorganisms.
- no classification and labelling is required for the environment and mammalian toxicology for a microorganism, like *Aureobasidium pullulans*.
- because microorganisms have a sensitizing potential, a hazard phrase (but not a classification) will be proposed for the microorganisms in each EFSA conclusion in relation to sensitisation.
- the agreed warning phrase for microbials is:
  'Microorganisms may have the potential to provoke sensitising reactions.'
  EUH 208 – Contains (name of sensitising substance). May produce an allergic reaction.

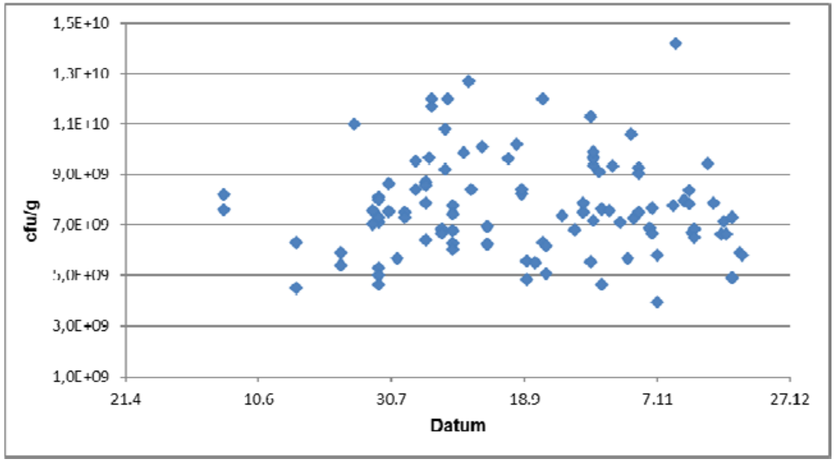
HOT TOPICS

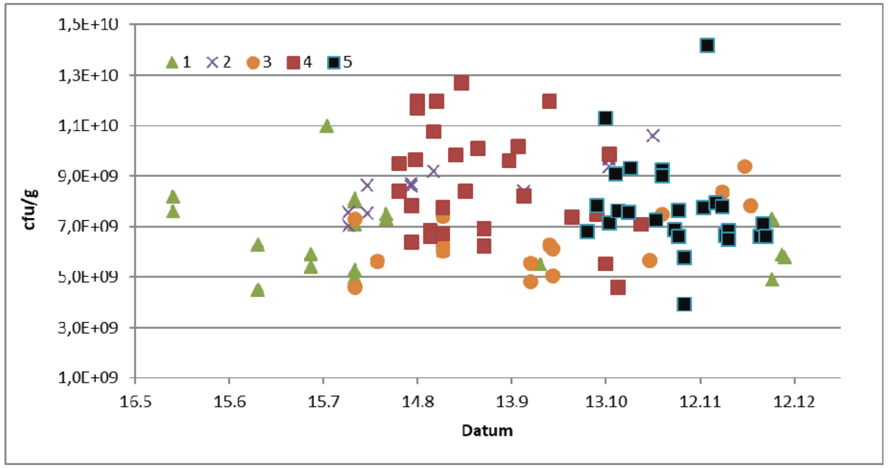


- **Identidy: Storage Stability**

Nomminal value in CFU/g, exponential growth possible

Interlaboratory study according to ISO 5725-2





HOT TOPICS

- **Environmental Fate and ecotoxicity of microbial plant protection products**

Calculations, Models?

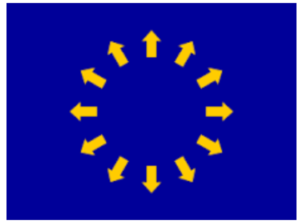
FOCUS et.al?

Dose response in Ecotoxtests?

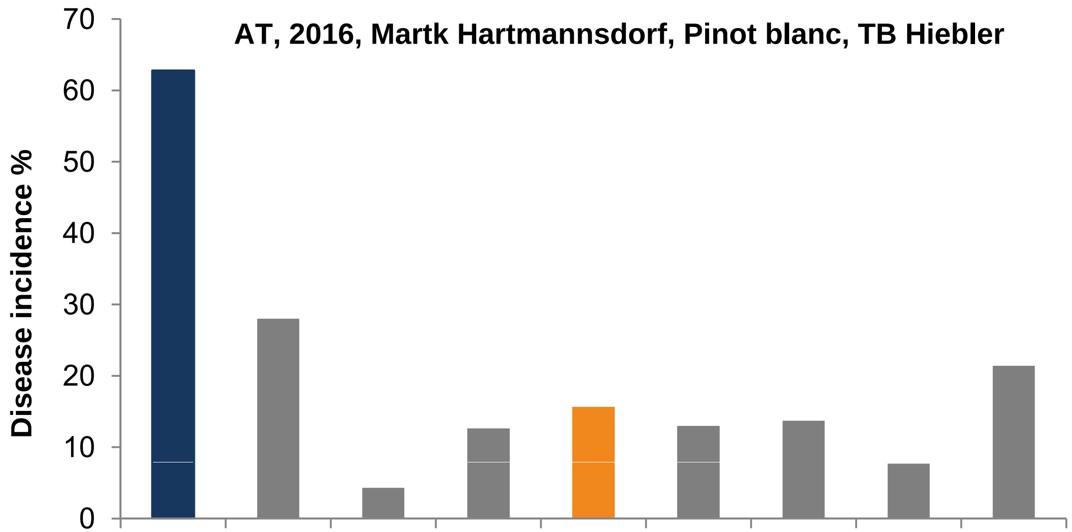
Buffer zones?

Qualitativ vs. quantitative risk assessment?

HOT TOPICS



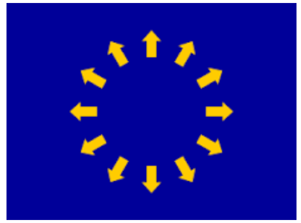
- **Efficacy Evaluation**



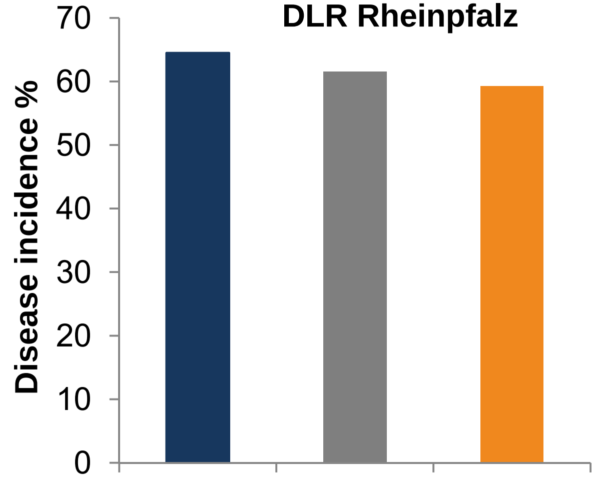
Efficacy	-	55	93	80	75	79	78	88	66
Significance	a	b	e	cde	cd	cde	cde	de	bc
BBCH 68	-	STD1	STD2	STD3	BOT	STD4	STD5	STD6	STD7
BBCH 77	-	STD1	STD2	STD3	BOT	STD4	STD5	STD6	STD7
BBCH 83	-	STD1	STD2	STD3	BOT	STD4	STD5	STD6	STD7
BBCH 85-89	-	-	-	-	-	-	-	-	-

	active substance
STD1	Cyprodinil/Fludioxonil
STD2	Fenhexamid
STD3	$KHCO_3$
BOT	*A. pullulans*
STD4	Boscalid
STD5	Pyrimethanil
STD6	Fenpyrazamine
STD7	Folpet

HOT TOPICS



- **Efficacy Evaluation**

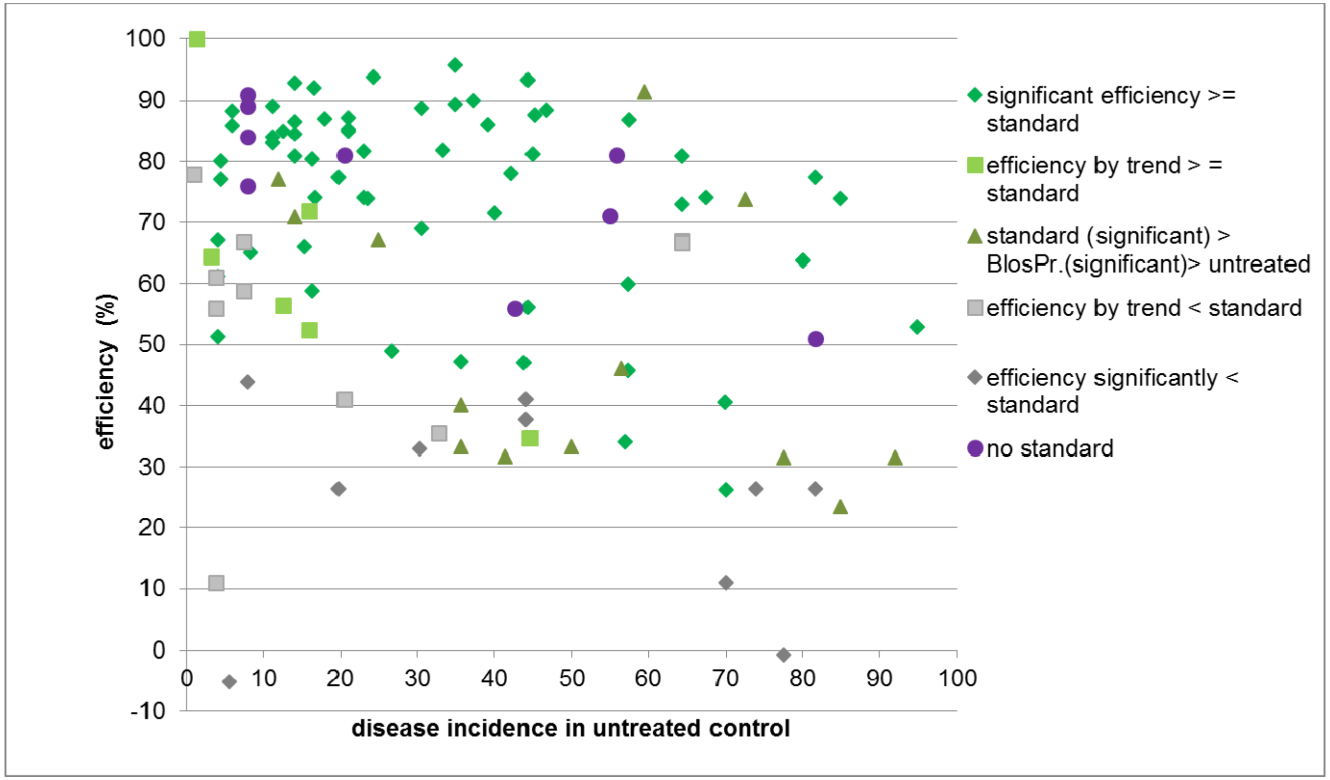


Efficacy	-	5	8
Significance	x	x	x
BBCH 68	-	-	-
BBCH 77	-	CH1	BOT
BBCH 85	-	CH2	BOT
BBCH 85-89	-	-	BOT

	application rate	active substance
CH1	0,96 kg/ha	Cyprodinil/Fludioxonil
CH2	1,6 kg/ha	Fenhexamid
BOT	0,8 kg/ha	*A. pullulans*

HOT TOPICS

- **Efficacy Evaluation**



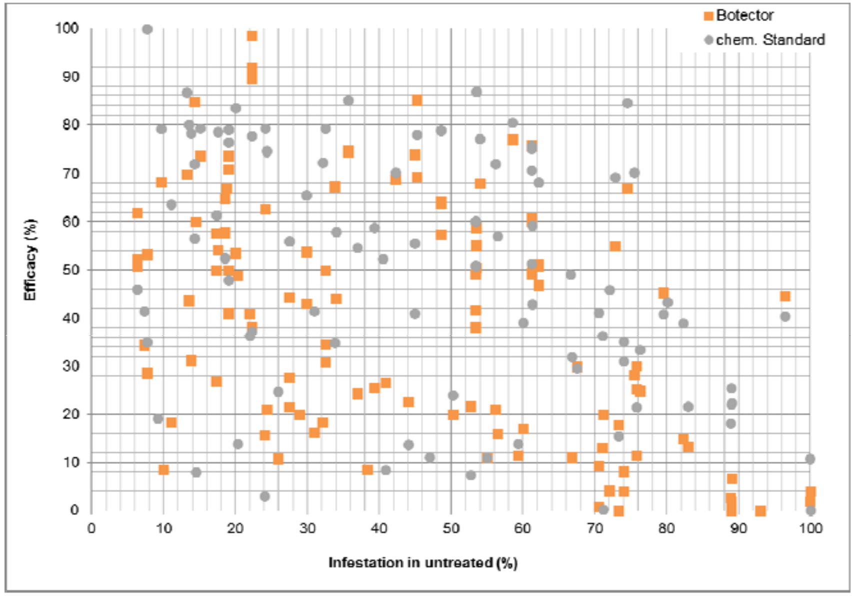
HOT TOPICS

- **Efficacy Evaluation**

Suppression vs control:

Label claims do influence marketing and sales!

Competitors showing resistance problems without having claims on the label



Changes in the GAP (2 m crown height -> 3 m crown height), LWA?

Dose Respons?

CONCLUSIONS:

30 years after investigation, 10 years after submission in EU

-> timelines in registration, especially EU

-> market potential:

trust of advisers and farmers in new techniques, that the efficacy of a biological product can be equivalent to chemical pesticides.

Rose is a rose is a rose…

… please make no difference!

- **In safety evaluation**
- **In efficacy evaluation**

products to be used in agriculture

not living living

risk evaluation, efficacy evaluation

- not low risk
- low risk

- control
- supression
- stimulation


Thank you for your attention!
bio-ferm
BIOTECHNOLOGISCHER PFLANZENSCHUTZ

2014-02 EU Approval of *A. pullulans*

2014-02 Submission of the renewal as a voluntary zonal work sharing project:

Central zone: zRMS: Austria, cMS: Belgium, Netherlands, Hungary, Slovakia, Poland

dRR issued by AT 2015-05

Commenting 2015-11

RR ready 2016-09 (+2,5 years after AI inclusion)

cMS status today (+3,5 years after AI inclusion, +1 year after RR):

- Belgium: postponed several times, now pending,
- Netherlands: pending, expected 2017
- Hungary: clear time schedule, **registration granted 2017-07**
- Slovakia: pending, unclear situation, mixture of old and new procedure
- Poland: pending, expected May 2018

Southern zone: zRMS France, cMS: Italy, Spain , Greece, Portugal

dRR issued by FR 2016-10

Commenting 2016-12

No RR till now, cMS not started up to now, no final registrations

Federal Ministry
of Food
and Agriculture

Plant Protection and / or Biostimulation − a Regulatory Point of View

bmel.de

PPP – Plant Protection Products

Regulation (EC) No 1107/2009 defines PPP (Article 2):

→ products, in the form in which they are supplied to the user

→ consisting of or containing active substances, safeners or synergists

→ and intended for one of the following uses:

... PPP...

→ protecting plants or plant products against all harmful organisms or preventing the action of such organisms,

unless the main purpose of these products is considered to be for reasons of hygiene rather than for the protection of plants or plant products

→ influencing the life processes of plants, such as substances influencing their growth, other than as a nutrient

... PPP ...

→ preserving plant products, in so far as such substances or products are not subject to special Community provisions on preservatives

→ destroying undesired plants or parts of plants, except algae unless the products are applied on soil or water to protect plants

→ checking or preventing undesired growth of plants, except algae unless the products are applied on soil or water to protect plants

Biostimulants – at present, substances or products with various definitions

→ European Commission
→ EBIC
→ Industry
→ Scientific community
→ …

→ but …

Federal Ministry of Food and Agriculture

Proposal

for a regulation of the European Parliament and of the Council laying down rules on the making available on the market of EU marked fertilising products amending Regulations (EC) No 169/2009 and (EC) No 1107/2009

→ Interinstitutional file 2016/0084 (COD), Brussels 7th November 2016

Federal Ministry
of Food
and Agriculture

Biostimulants – related to fertilisation

→ Certain substances, mixtures and micro-organisms, commonly referred to as plant biostimulants, are not as such nutrients, but nevertheless stimulate plants' nutrition processes.

→ Where such products aim solely at improving the plants' nutrient use efficiency, tolerance to abiotic stress, or crop quality traits, they are by nature more similar to fertilising products than to most categories of plant protection products.

→ Such products should therefore be eligible for CE marking under this Regulation and excluded from the scope of Regulation (EC) No 1107/2009.

Biostimulants – definition

→ Regulation (EC) No 1107/2009 is amended as follows:
→ (1) Article 2(1) (b) is replaced by the following:

→ "(b) influencing the life processes of plants, such as substances influencing their growth, other than as a nutrient or a plant biostimulant;"

→ (2) in Article 3, the following point is added:

→ "34. "plant biostimulant" means a product stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant:

(a) nutrient use efficiency;
(b) tolerance to abiotic stress;
(c) quality traits."

→**"plant biostimulant"** means a **product** **stimulating plant nutrition processes** independently of the product's nutrient content *with the sole aim of improving* one or more of the following *characteristics of the plant:*

(a) **nutrient use efficiency**
(b) **tolerance to abiotic stress**
(c) **quality traits**

Product Function Category (**PFC**) **6** – plant biostimulants

A microbial plant biostimulant

B non-microbial plant biostimulant

→ When the new regulation will enter into force, national regulations will not be replaced, however full harmonisation is still under discussion and could be prepared.

(5) *Contrary to most other product harmonisation measures in Union legislation, Regulation (EC) No 2003/2003 does not prevent non-harmonised fertilisers from being made available on the internal market in accordance with national law and the general free movement rules of the Treaty. In view of the very local nature of certain product markets, this possibility should remain.*

→ Will it be possible that products can be both PPP and biostimulant?

(15) *Certain substances, mixtures and micro-organisms, commonly referred to as plant biostimulants, are not as such nutrients, but nevertheless stimulate plants' nutrition processes. Where such products aim solely at improving the plants' nutrient use efficiency, tolerance to abiotic stress, or crop quality traits, they are by nature more similar to fertilising products than to most categories of plant protection products.*

Such products should therefore be eligible for CE marking under this Regulation and excluded from the scope of Regulation (EC) No 1107/2009 of the European Parliament and of the Council. Regulation (EC) No 1107/2009 should therefore be amended accordingly.

→ Will it be possible that products can be both PPP and biostimulant?

→ **NO**

(16) *Products with one or more functions, one of which is covered by the scope of Regulation (EC) No 1107/2009, are plant protection products covered by the scope of that Regulation. Those products should remain under the control tailored for such products and provided for by that Regulation. Where such products also have the function of a fertilising product, it would be misleading to provide for their CE marking under this Regulation, since the making available on the market of a plant protection product is contingent on a product authorisation valid in the Member State in question. Therefore, such products should be excluded from the scope of this Regulation.*

Article 4 – Product requirements

1. A EU fertilising product shall

→ (a) meet the requirements set out in **Annex I** for the relevant product *function category*;

→ (b) meet the requirements set out in **Annex II** for the relevant *component material category* or categories;

→ (c) be labelled in accordance with the *labelling requirements* set out in **Annex III**.

Article 42 – Amendments of Annexes

(2) Micro-organisms

Where the Commission amends Annex II in order to add new micro-organisms or strains of micro-organisms to the component material category for such organisms, it shall do so, after having verified which strains of the additional micro-organisms are fulfilling the provisions […], on the basis of the following data:

(a) name of the micro-organism
(b) taxonomic classification of the micro-organism: genus, species, strain and procurement method
(c) …

…

(d) scientific literature reporting about safe production, conservation and safe use of the micro-organism
(e) taxonomic relation to micro-organisms species fulfilling the requirements for a Qualifies Presumption of Safety as established by the European Food Safety Authority
(f) information on the production process
(g) information on the identity and residue levels of residual intermediates, toxins or microbial metabolites in the component material
(h) natural occurrence, survival an mobility in the environment

Component Material Category 7: Micro-organisms

An EU-fertilising product belonging to product function category 6(A) [PFC 6(A): microbial plant biostimulants] may contain micro-organisms, including dead or empty-cell micro-organisms and non-harmful residual elements of the media on which they where produced, which

- have undergone no other processing than drying or freeze-drying and
- are listed in the table below.

Azotobacter spp.

Mycorrhizal fungi

Rhizobium spp.

Azospirillum spp.

Julius Kühn-Institut
Federal Research Centre for Cultivated Plants

The Diversity of „Biorationals“ – learning from Biocontrol Agents

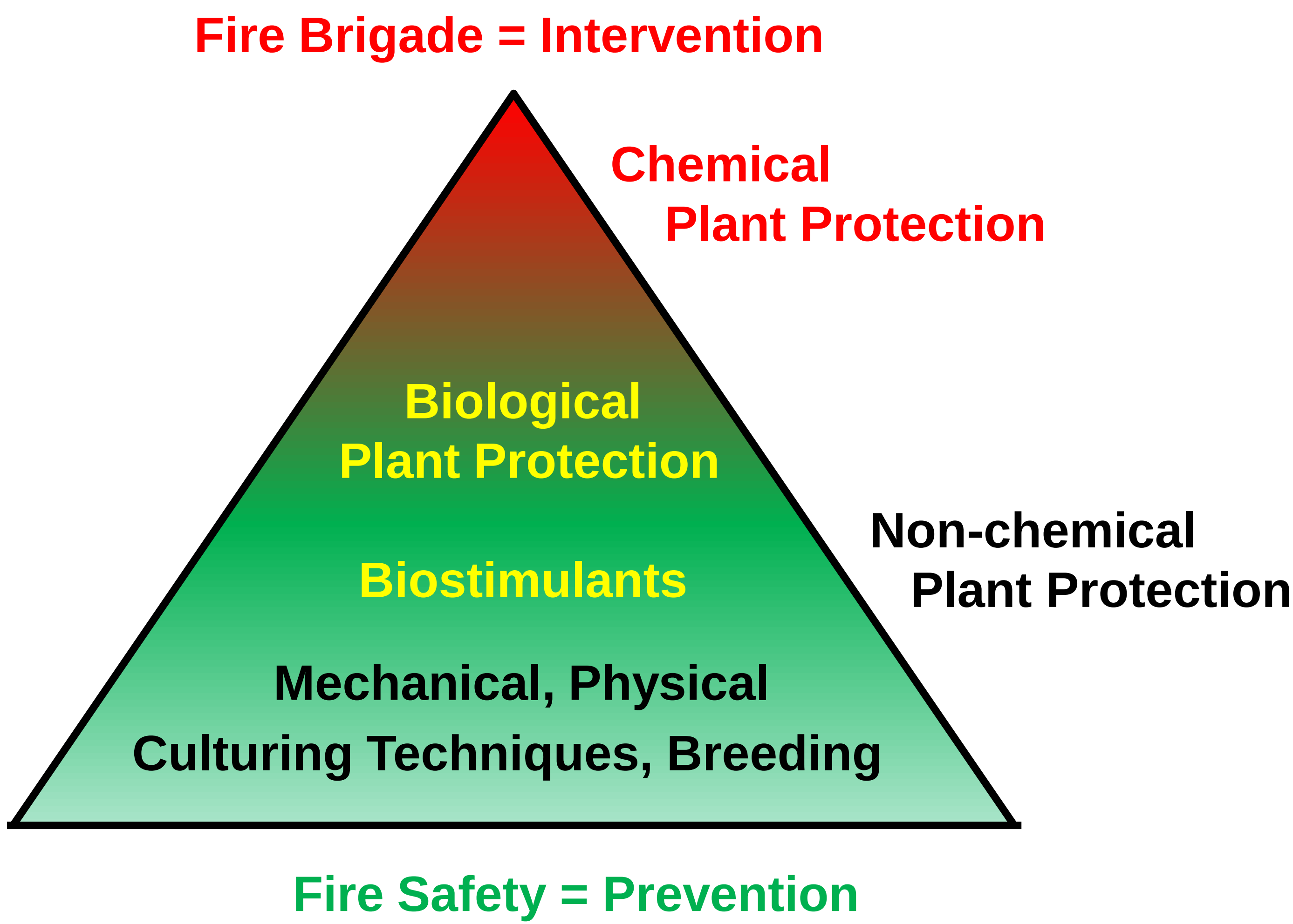
Johannes Jehle

Julius Kühn-Institut, Darmstadt, Germany

NAP-Forum 13./14.12.2017 Braunschweig

1. Concept of IPM
2. Biocontrol
3. Registration of Biocontrol Agents
4. Biocontrol in IPM
5. Conclusions

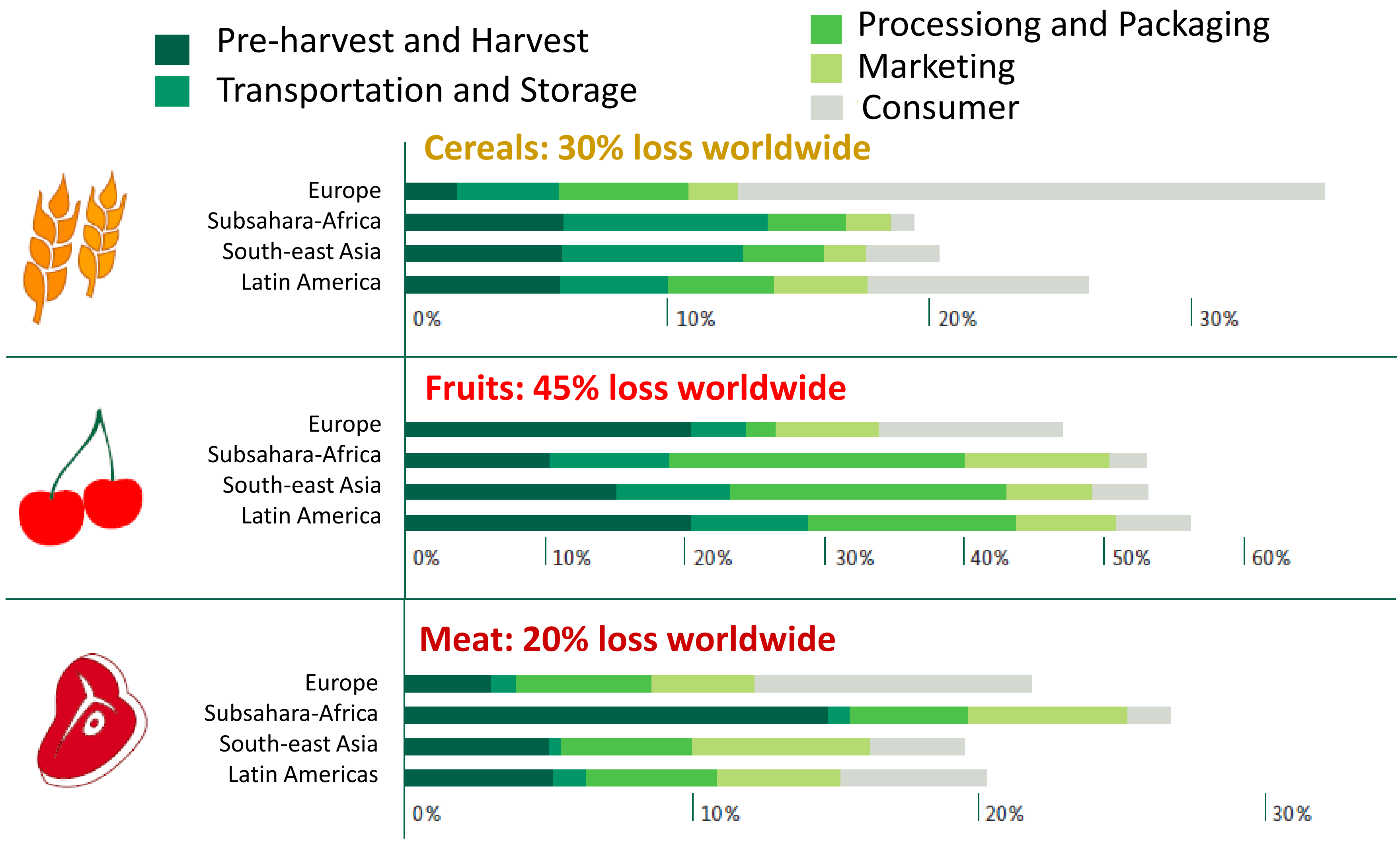
The Concept of IPM



Neglection of Fire Safety in Plant Protection

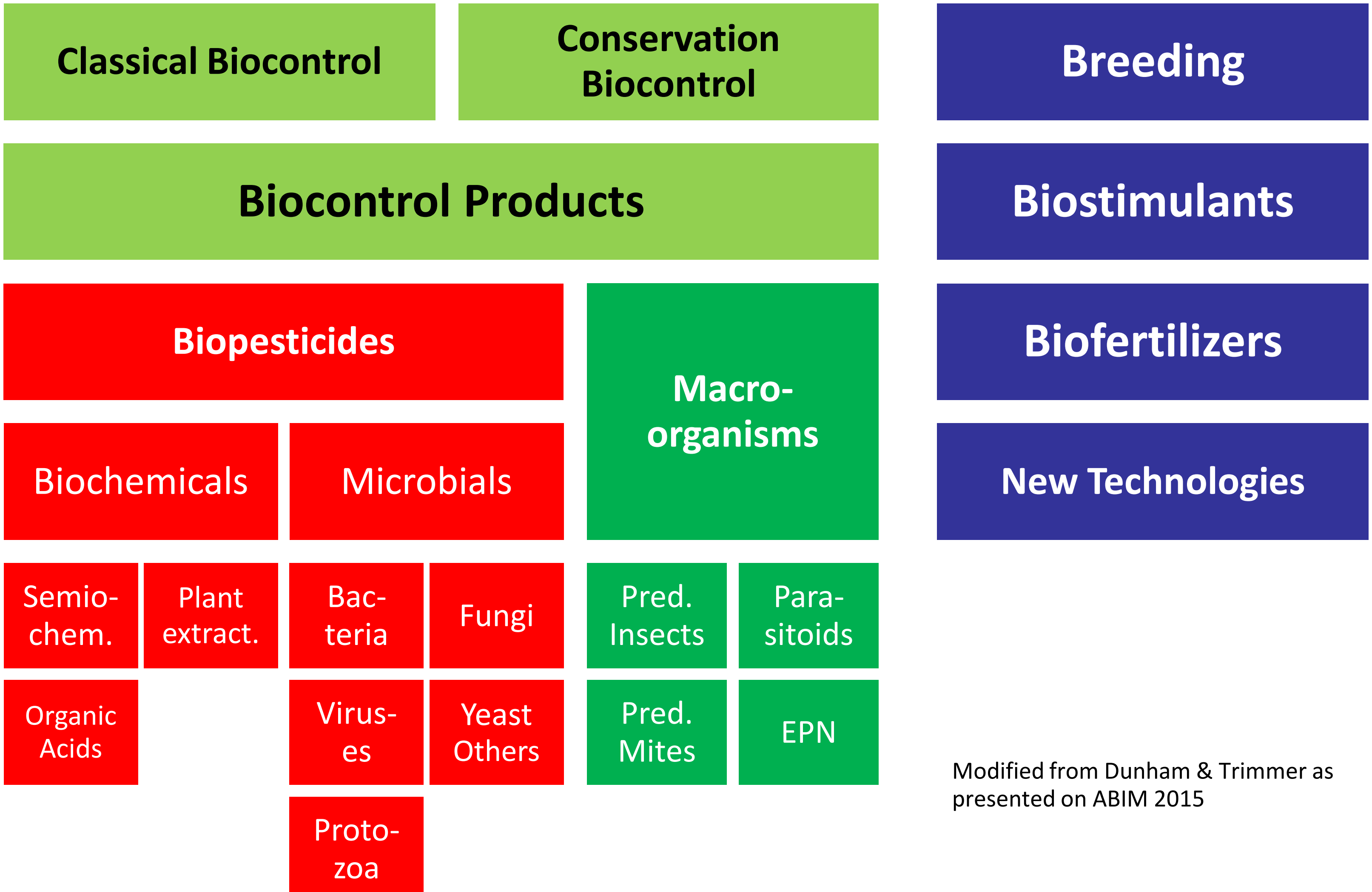
- Application of IPM is mandatory in Europe since 2014: **Directive 2009/128/EC**
- Despite 30 years of IPM in German Plant Protection Law: Plant protection mainly based on chemicals
  - Unintended non-target effects
  - Residues and (unrealistic) consumer expectations
  - Rapid development of resistance
- „Diesease spread is like a forest fire“: because of lack of fire safety
- 90-98 % of sprays do not reach target: Damage caused by fire figthing
- Decline of biodiversity: Agricultural intensification is also the consequence of low prizes and food waste

Loss of Foodstuff



Modified from BMEL/Gustavsson et al. 2011

The Bounty of Biological Tools



Modified from Dunham & Trimmer as presented on ABIM 2015

The Registration Process of Microbial Biological Control Agents (MBCAs)

Before 1993 in the national responsibility	• Many national registration • Individual registration in each country • No common leagal frame work
1993 – 2011 Directive 91/414/EC	• uniform rules on the evaluation, authorisation, placing on the market • control of plant protection products and the active substances they contain
Since 2011 Regulation (EC) No. 1109/2009	• Harmonization of plant protection registration • Reduce risk for human and animal health • Reduce risk for environment • Safeguarding the competitiveness of European Agriculture

414/91 based harmonization caused loss of MBCAs

- **Pre-91/414**: 22 species of micro-organisms in Europe registered

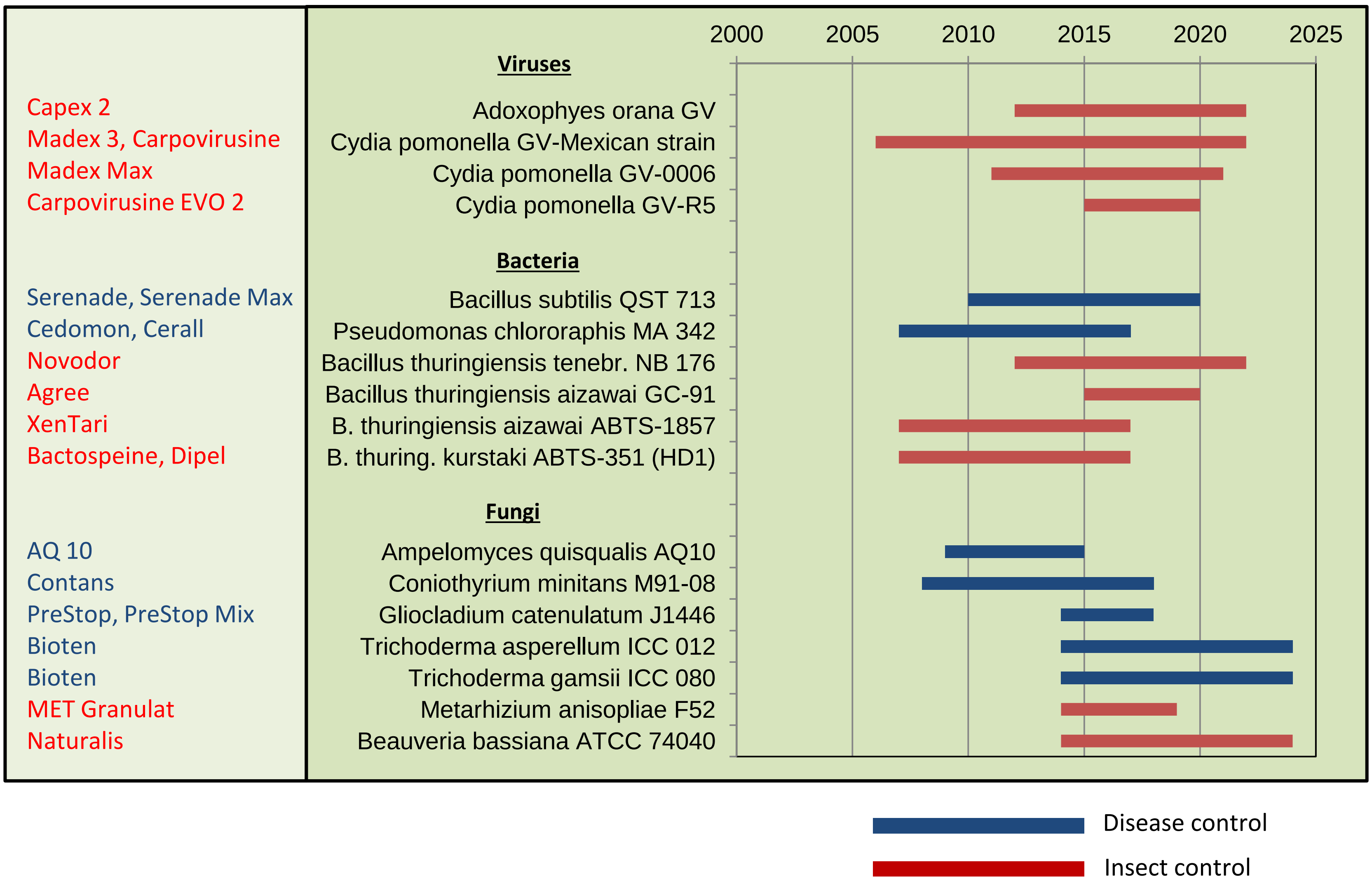
- **91/414 Transition**: only16 MOs defended as pre-exisiting, **thereof 8 Entomopathogens**

  6 MOs got lost (e.g. *Beauveria brongniartii*, three baculoviruses and others)

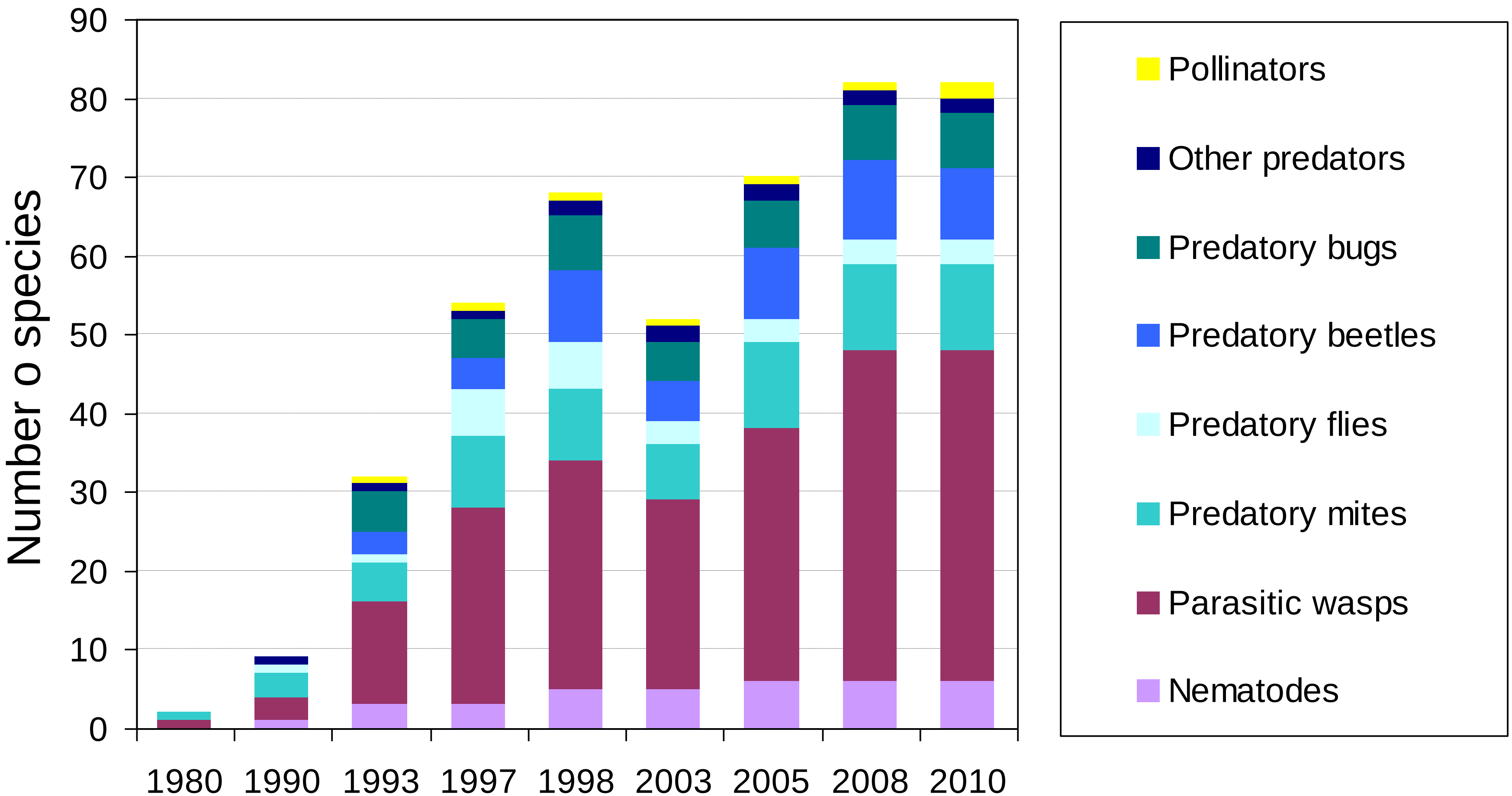
- **91/414:** Initially only 8 MBCAs included into Annex I, several were pending for seven and more years

Source: Hauschild, 2011

Registered Microbial Biocontrol Agents in Germany



Commercial Beneficials in Germany



Release area of Beneficials (ha/year)

Most important commercially available beneficials:

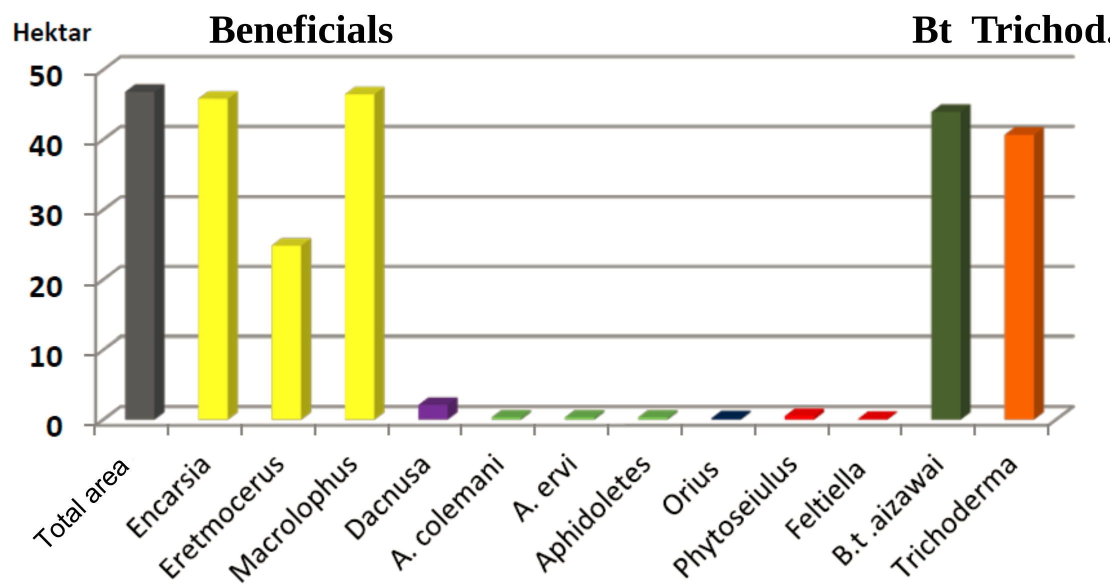
Beneficial species	1993	1996	2001	2009	2010	Crop	Target pests
Trichogramma brassicae	5900	5600	9443	19414	22484	Corn	*Ostrinia*
Encarsia formosa	196	403	273	198	1266	Veg & Orn	White fly
Aphidius - species	65	174	174	203	1042	Veg & Orn	aphids
Lysiphlebus testaceipes		0.5	7.8	20	315	Veg & Orn	aphids
Diglyphus isaea	19	73	95	27	119	Veg & Orn	Leaf miners
Aphidoletes aphidimyza	66	131	134	54	48	Veg & Orn	aphids
Chrysoperla carnea	10	55	40	4	62	Veg & Orn	aphids & more
Phytoseiulus persimilis	123	125	126	85	332	Veg & Orn	spider mites
Amblyseius sp.	104	174	201	25	1470	Veg & Orn	mites & thrips
Entomopath. Nematodes	47	413	200	1272	247	Veg & Orn	various

IPM is a System!

- ✓ Multic-component system by definition!
- ✓ Components are not inter-changeable!
- ✓ Components need to work together!
- ✓ Prevention should prevail intervention!
- ✓ Systemic approach of differently efficacous tools!
- ✓ Clear development: Fewer chemicals a.i. will be available!

An Example from Practice

Tomato in Greenhouse

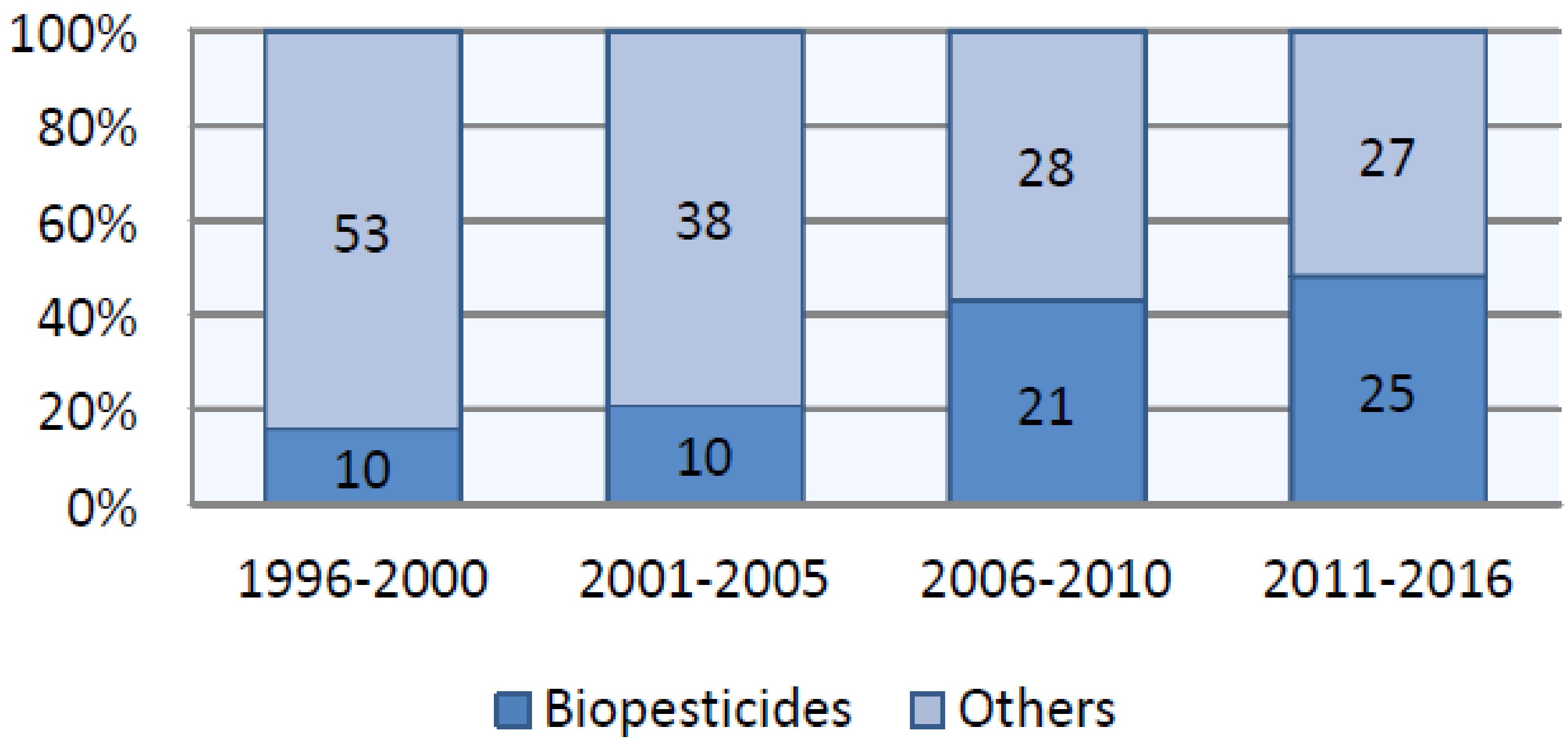


Tomato in Greenhouse in Niederrhein area:

- **Control of insect pests only with biocontrol**
- **Use on ~98% of growing area**

Source: LWK NW, Heike Scholz-Döbelin
Statusbericht Biolog. Pflanzenschutz 2013

Application of new Active Substances since 1996



Source: EU Comm. 2016

What are the consequences?

✓ Biological Control Agents
  - *are highly specific,*
  - *have a narrow application segment,*
  - *application profile have an* ***intrinisic reduced risk***

✓ „Replacing“ a single chemical a.i. will require many biologicals
  - *Registration of biological a.i. is strain-specific:*
    Different strains have similar properties!

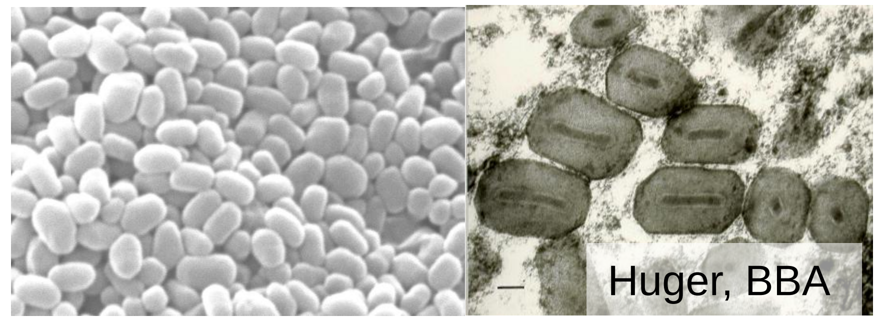
✓ Many potential biologicals not registered because of high registration costs compared to market volume (no ROI)

✓ Increasing gap of indications will require new approchaes in the registration of biologicals

✓ Registration needs to be SMART and SLIM and RISK-RELATED

Example: Baculoviruses

- dsDNA-Virus,
- largest insect virus group,
- specific for Lepidoptera, Hymenoptera, Diptera
- Highly specific for few targets



- OECD Consensus document No. 20 (2002): „baculoviruses are safe for humans, animals and environment“
- REBECA Proposal to **approve baculovirus a.i. on species level** (2006)
- **Realized** in SANCO/0253/2008 rev. 2. (2008)

CONSEQUENCE: fast registration of resistance-breaking CpGV isolates

Referring to QPS status (EFSA BIOHAZ panel) of baculoviruses, they are considered as **low risk on family level** (proposed amendment of (EC) No.1107/2009)

Microbial Biocontrol Agents:
Most will be Low Risk Substances

Substance	Category	Status under Reg. (EC) No 1107/2009	Date of approval
Bacillus amyloliquefaciens strain FZB24	Fungicide	Approved	01/06/2017
Cerevisane	Plant activator	Approved	23/04/2015
COS-OGA	Elicit./Fung.	Approved	22/04/2015
Ferric phosphate	Molluscicide	Approved	01/01/2016
Isaria fumosorosea Apopka strain 97 (formely *Paecilomyces fumosoroseus*)	Insecticide	Approved	01/01/2016
Mild Pepino Mosaic Virus isolate VC 1	Elicitor	Approved	29/03/2017
Mild Pepino Mosaic Virus isolate VX 1	Elicitor	Approved	29/03/2017
Pepino mosaic virus strain CH2 isolate 1906	Elicitor, Virus inoculation	Approved	07/08/2015
Saccharomyces cerevisiae strain LAS02	Fungicide	Approved	06/07/2016
Trichoderma atroviride strain SC1	Fungicide	Approved	06/07/2016

Lessons learned

- ✓ Not only fire fighting but improved fire safety is needed
- ✓ Re-thinking the paradigmas: IPM is not a tool box but a multicomponent system!
- ✓ Harmonization of registration should facilitate regulatory process in general BUT it caused loss of agents in the past (some never came back)
- ✓ Because of specificity, single biological solutions have inherent restrictions of applications (market)
- ✓ Be aware of strength and weakness of regulatory process
  - *Essential legal framework for producers farmers, and consumers*
  - *Regulatory process needs to be risk-related*
  - *Current conditions hampers registration of small products*
- ✓ New pathes needed, not running faster in the treadmill

The BIOFECTOR PROJECT database
Plant Protection and Plant Health in Europe
8th International Symposium jointly organised by DPG, JKI and HU-Berlin

Manfred G. Raupp
madora gmbh

BIOFECTOR is funded by the European Union within the 7th Framework Programme
Grant Agreement No. 312117

BIOFECTOR 2012-2017

- Internet und Homepage BIOFECTOR
- http://www.biofector.info/
- https://www.youtube.com/watch?v=Nd4uwx0ShPc
- http://www.biofector.info/mediapool/137/1375686/data/IPNC2017_BIOFECTOR_Presentation.pdf
- Publication of Results by Springer, SAB and others
- Summer Schools, Frankenhausen (D), Prague (CZ), Budapest (HU), Tomisoara (RO), Naples (I) etc.
- Collaboration with Partner Projects

BIOFECTOR is funded by the European Union within the 7th Framework Programme
Grant Agreement No. 312117

Situation Biofector Data Base 2017

- Data Base: BioControl, BioFertiliser & BioStimulants

  http://www.biofector-database.eu/en/biofectors-homepage.html
- Active ingredients
- Companies
- Purpose of application
- Crops
- Database managet by Uni-Hohenheim and Fibl-Projekte Frankfurt a.M.

BIOFECTOR is funded by the European Union within the 7th Framework Programme
Grant Agreement No. 312117

Situation in Europe

- Talks with Manufacturer Associations
- ABIM http://www.abim.ch/home.html
- ABISTA http://www.abista.eu/
- EBIC http://www.biostimulants.eu/
- IBMA http://www.ibma-global.org/
- Workshops together with DPG, JKI und BVL
- Datatransfer from JKI, Fibl, Universities etc.

BIOFECTOR is funded by the European Union within the 7th Framework Programme
Grant Agreement No. 312117

Lasting Legacy

- Harmonisation of Registration in the EU is a Problem
- Transfer from Laboratory to Practice
- Confirmation of the Positive Ecological Effects
- Publications in Agricultural Journals
- Integration of Government Trials with Bioeffector Trials and other scientific results in a open databank

BIOFECTOR is funded by the European Union within the 7th Framework Programme
Grant Agreement No. 312117

Outlook & Questions

- Demands of Bio-Stimulant Requirements in EU-Harmonisation
- Price and Cost for Development of Bio-Stimulants in the Market Place, including Conventional Agriculture
- Cost-Effective Application Methods for Bio-Stimulants

BIOFECTOR is funded by the European Union within the 7th Framework Programme
Grant Agreement No. 312117

Bioeffectors & Biorationals to Ensure Food Supply and Protect the Environment

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Defense responses against Rhizoctonia solani, interactions with bacterial inoculants and root exudation of antifungal compounds in lettuce are differentially expressed on different soils

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Previous studies conducted on a unique field site, comprising three contrasting soils (diluvial sand DS, alluvial loam AL, loess loam LL) under identical cropping history, demonstrated soil type-dependent differences in biocontrol efficiency against Rhizoctonia solani-induced bottom rot disease in lettuce by two bacterial inoculants (Pseudomonas jessenii RU47 and Serratia plymuthica 3Re-4-18). Disease severity declined in the order DS > AL > LL. These differences were confirmed under controlled conditions, using the same soils in minirhizotrone experiments. GC-MS profiling of rhizosphere soil solutions revealed benzoic and lauric acids as antifungal compounds, previously identified in root exudates of lettuce. Pathogen inoculation and pre-inoculation with bacterial inoculants significantly increased the release of antifungal root exudates in a soil type-specific manner, with the highest absolute levels detected on the least-affected LL soil. Soil type-dependent differences were also recorded for the biocontrol effects of the two bacterial inoculants, showing the highest efficiency after double-inoculation on the AL soil. However, this was associated with a reduction of shoot growth and root hair development and a limited micronutrient status of the host plants. Obviously, disease severity and the expression of biocontrol effects are influenced by soil properties with potential impact on reproducibility of practical applications.

Bioeffector-assisted P nutrition of wheat supplied with rock phosphate & placed NH4+

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Mehdi.Nkebiwe@uni-hohenheim.de

Introduction

Rock phosphate is a cheap P-fertilizer, however, it is less plant-available in neutral to alkaline soils than conventional soluble ammonium phosphate or superphosphate fertilizers. Plant growth-promoting microorganisms (PGPMs, here referred to as microbial bio-effectors or BEs) can increase the availability of sparingly soluble P-fertilizers by improved root and root hair growth via phytorhormones, thus promoting spatial exploitation of P in soil (Lugtenberg and Kamilova 2009; Mohite 2013); and by enhanced solubility of fertilizers by acidification through release of protons and/or low molecular weight organic acids (Altomare et al. 1999; Bashan et al. 2013). The aim of this study was to improve P acquisition and growth of wheat plants supplied with rock phosphate and placed NH4+-fertilizer by inoculating the substrate with various BEs. The effects N-form (NH4+ vs. NO3-) was further investigated for the most promising BE.

Methods

Spring wheat (Triticum aestivum L. Schirocco, KWS, Germany) was grown in a substrate based on 70 % low-P silt loam luvisol (pHCaCL2 6.4; CAL-P, 7mg kg-1; and 30 % sand (moisture: 24 % or70 % Max. WHC; 5.7 kg substrate (5 L pot)-1; 20 plants pot-1). P (150 mg kg-1 soil) was supplied as rock phosphate (RP, 7.6 % P) or as Ca(H2PO4)2 for a positive control (+P). 100 mg N kg-1 soil was placed as stabilized (NH4)2SO4+DMPP or mixed as Ca(NO3)2), ½ at sowing and ½ at 24 days after sowing (DAS). The first set of treatments included five BEs under N supply as NH4+: Control without bio-effector (NoBE), Pseudomonas sp DSMZ13134 Proradix (Pro), Bacillus amayloliquefaciens FZB42 Rhizovital (Rhiz), Paenibacillus mucilagenosus (Paeni) (at 1x109 CFUs or Spores kg-1); Trichoderma harzianum T-22 Trianum-P (T-22, 1x108 spores kg-1). Secondly, NH4+ or NO3- was combined with NoBE or Pro. Three inoculations were conducted at 0, 24 and 34 DAS. There were 5 replicates per treatment arranged in a randomized block design. Plants were grown in greenhouse/outdoors (02. Jun – 04. Sep. 2015; Av. daily temp.: 24°C (min: 8 °C, Max: 51 °C, Light intensity: 400-1200 µmol. photons m-2 s –1). Shoot growth and nutrient status was measured at booting and senescence stage. Root growth and rhozosphere available P level, grain and straw nutrient status was also measured.

Results and Discussion

Inoculation of either Pro or Paeni led to improved grain yield. Application of BE tended to improve root and shoot DM. Under N supply as NH4+, inoculation of Pseudomonas sp. DSMZ 1314 let to higher grain P concentration and each BE inoculation resulted in increased number of grains per pot. Overall, there was a strong negative relationship between residual CAL-extractable P in rhizosphere soil (mg P (100 g soil-1) and shoot P content (mg P plant-1) ($P< 0.0001$, $R2=-0.43$, $y = -0.0508x + 10.798$), indicating P uptake. For biomass formation and biomass nutrient status in general, NH4+ led to higher values than NO3-, and Pro also led to higher values than NoBE. The effect of Pro on P nutrition of wheat was stronger with NH4+ nutrition than NO3-.

Conclusions

Inoculation of Pseudomonas sp. DSMZ 1314 or Paenibacillus mucilagenosus led to increased grain yield. Generally, contents and/or concentrations of nutrients in shoot or grain were higher with NH4+ than with NO3-, and also higher with BE than without. Improvement of nutrient status and yield of wheat plants may be explained by enhanced chemical mobilization/solubilization of nutrients in the rhizosphere and by improved root interception of nutrients in soil.

Acknowledgements

Resource Preservation by Application of BIOefFECTORs in European Crop Production). Grant Agreement Number 312117 under the Seventh Framework Program (FP7), European Commission

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POSTER

Effects of self-drying plant oils in biological plant protection

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The fungicidal effect of various plant oils was demonstrated in the 1940s (Clayton et al., 1943). Plant oils could be advantageous because of their low ecotoxicological side effects, high biodegradability, and applicability in organic farming. However, despite their efficacy against fungi and pests, the role in plant protection has been somewhat limited until now. In the discussion about plant protection and the environment, biorationals, like natural plant oils, might be promising candidates for a more sustainable approach. Especially self-drying plant oils (e.g. linseed oil, tung oil), rich in unsaturated fatty acids, could be used in plant protection or plant strengthening. Based on previous knowledge, we hypothesized that self-drying plant oils could be effective against pathogens. In a greenhouse experiment on green beans (*Phaseolus vulgaris*), as well as on potato in the field, we tested several plant oils and mixtures. Self-drying oils showed a protective and a curative effect against the fungus *Uromyces appendiculatus* (bean rust) on green beans. A protective application (two days before inoculation (2dbi)) of six tested plant oils reduced bean rust infestation between 23% and 72%. This wide range in disease control of biorationals must be discussed and further evaluated. Also effects on the plant itself have to be considered, because they could possible lead to plant strengthening but also to plant stress. In future experiments, the effect of self-drying oils in different host-parasite interactions should be studied as well as effects on plant traits.

Clayton, E.E., Smith, T.E., Shaw, K.J., Gaines, J.G., Graham, T.W., Yeager, C.C. (1943). Fungicidal tests on blue mold (Peronospora tabacina) of tobacco. Journal of Agricultural Research 66:261–276.

Microbial consortia products as biological inoculants for improved growth of maize

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In contrast to selection of single microbial (Plant Growth-Promoting Microorganisms, PGPMs) strains with high efficiency for plant growth promotion, the use of large consortia of different PGPMs may be an alternative strategy to induce beneficial effects on plant performance, with the advantage of higher flexibility towards variability of environmental conditions due to selective promotion of the best-adapted strains, depending on the respective rhizosphere conditions. In this study, the effects of a commercial microbial consortium product (MCP) with 20 different fungal and bacterial PGPMs on early growth of maize were tested in pot experiments with different levels of N and P supply, including a functional characterization of inoculant effects on marker enzymes for N, P and C cycling in the rhizosphere. Auxin production potential of the re-isolated populations was evaluated spectrophotometrically. The application of the MCP induced a significant stimulation of shoot and root biomass production and could even compensate for reduced input of N and/or P. This was associated with increased fine root production and improved P-nutritional status, particularly in the inoculated variants with reduced nutrient input. The results suggest that the MCP-induced beneficial effects on plant growth and nutrient acquisition in maize could be mainly attributed to root growth stimulation. This rhizosphere effect of the inoculants deserves a more detailed future investigation.

Rose is a rose is a rose is a rose – double standards to be applied for biorationals?

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Preparing the efficacy package for a pesticide for the registration as well as for the marketing requires many resources. It can be stated that this requires usually at least a multiple investment compared to the rest of the data requirements to be addressed. Time is money - the field trials need to be performed over several seasons, and as long as the realization is done only on one hemisphere, it is even more time consuming. This shows how important this issue is for the whole production chain: at the end it is an economical question for the farmer to get the best result for his investment.

Plant protection tools are chosen with the aim to reduce the loss in agricultural production and it is important for the user to know what he can expect. In some cases less efficacy may be balanced by other benefits like no preharvest interval, a clear decision for the user should be possible – carefully comparing his expectations with the claims of the product on the label. In Europe plant protection products are evaluated by the competent authorities for their efficacy against the claimed disease or pest, but one has to keep in mind that an evaluation takes place only every ten years. Resistances can occur e.g. of fungal pathogens against chemical fungicides and it will take several years before this fact might be reflected on the label. Even if it is possible for biorational products to show the same efficacy as the chemical reference product, the communication of this fact is much more extensive. Biorationals are under general suspicion to be not as efficient as chemical products; a science based approach – for the risk as well as for the efficacy evaluation - could help to expand the application area of such rational products.

Second Generation ChitosanSas Reliable, Dual-Use Plant Biostimulants

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The term chitosan describes a family of biopolymers and oligomers consisting of varying numbers and ratios of glucosamine and N-acetylglucosamine residues. Depending on the number of residues in the molecule (its degree of polymerisation) and the ratio of the two monomeric units (its degree of acetylation), and possibly also depending on the sequence of the two units within the oligomer or polymer (its pattern of acetylation), chitosans can have different biological activities. Some chitosans can inhibit microbial growth (without being fungicidal or bactericidal, rather being funigstatic and bacteriostatic), some chitosans can induce disease resistance in a plant (either by acting as an elicitor triggering resistance reactions, or by acting as a priming agent enabling plant cells to react more efficiently against pathogens), some chitosans can improve abiotic stress tolerance in plants (e.g. against drought or heat stress), and some chitosans can promote plant growth (e.g. root and/or shoot growth) and/or development (e.g. more fruit and/or earlier ripening).

Modern biotechnological methods of chitosan production and/or modification as well as new techniques for chitosan in depth structural analysis allow to produce well-defined (second generation) chitosans having defined and reliable, specific bioactivities, but lacking others. In this way, it is e.g. possible to produce chitosans which have no antimicrobial activities but which do have plant strengthening (i.e. growth promoting and stress tolerance and disease resistance inducing activities). It will probably also be possible to generate chitosans with more pronounced ability to induce stress tolerance and less marked ability to induce disease resistance, and vice versa. However, it is highly unlike that it will be possible to dissect these activities fully. The reason behind this is the intimate cross-talk of intracellular signal transduction pathways in plant cells. Even if it were possible to identify a specific chitosan oligomer (or any other compound) that is recognised by a specific receptor which triggers a signal transduction chain leading to metabolic answers increasing abiotic stress tolerance, and to identify another chitosan oligomer (or any other compound) that is recognised by a different receptor which triggers a different signal transduction chain leading to different metabolic answers increasing disease resistance - treatment of the plant cell with either of the two chitosan oligomers will invariably lead to a mixed response, possibly with stronger emphasis on one of the two reactions and less pronounced expression of the other, but not with just one answer. Clearly, thus, there is a strong interconnection between abiotic stress tolerance and disease resistance. Consequently, we propose to categorize such compounds, like specific chitosans, which act on the plant cell rather than on the pathogen as plant strengthening agents or dual-use plant biostimulants.

Do wee need a new application equipment for application of “biorationals”?

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The quality of application plays a major role in all cases, whether they are pesticides, fertilizers or other products and resources, including biorationals. The use of application equipment must be safe, effective and accurate in all areas of pest control and for use of all products. Biorationals, it is a concept that affects a very wide range of products and preparations. Microorganisms, plant extracts, basic substances, semiochemicals, as well as non-pesticidal products like biostimulants, biological yield enhancers, plant health promoters, and soil conditioners - this is a brief list of products representatives that are included under the name „biorationals“. Their application is also a broad concept, some of them can be applied in a common way (spraying), other equipments require more or less attention.

The critical point using biorationals is that they are deemed to have low effectiveness. Can the right application technology supports their use, avoids losses and finds the target precisely? We find approx. 30% differences in dose and application effectiveness between the different equipment types, which depend not only on technology, but also on the way of application and conditions (meteorological conditions, etc.). Other risks may be a losses due to drift; a quality of the carrier medium (e.g. for pesticides, mostly water must be clean and its inappropriate characteristics may affect their effectiveness too); a quality of the application equipment and some of its components may also affect the application and its outcome. The application machinery currently placed on the market is designed especially for all kinds of pesticides application. Horizontal boom sprayers, orchard sprayers, hand-operated equipment, fixed and semi-mobile equipment, dusters, applicators of granulates and many others. These machinery must follow the requirements of international standards, which are focused on the design and operation of that machinery especially for pesticides application.

Do we need new application equipment for applying biorationals? Probably not for common applications but we will need to consider these issues when using certain products: suitable nozzles, adapted filtration system or agitation of liquid in the tank and etc. There will need to be discussed new requirements for some other devices that are not commonly used for pesticide application (inoculation, dressing, spreading, etc.) and find a solution for biorationals application.

References

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Bio-rational or bio-logical: different concept, different impact

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"Rational" from Latin ratio, 'reason' literally is a synonym to "logical" from Greek logos, 'reason', but contrary to the universal sense of 'reason' modern parlance tends to restrict the meaning of 'rational' to the calculated most efficient way to achieve a goal. In this mind, the term "bio-rational" has been proposed to define bio-agents with specific action against harmful organisms, but limited or no effect on non-target organisms. Recently the interest to commercialize bio-agents as "bio-pesticides" or "bio-fertilizers" has been growing. However, an increasingly stringent legislation in the EU concerning the registration of agents that are regulated under the plant protection or fertilizer law led to a far-reaching loss of products from the market. According to Regulation (EC) No 1107/2009, agents having general or specific action against harmful organisms are "plant protection products", which may not be registered as fertilizing products. For many bio-agents, such microbial strains, nevertheless, direct mobilization of mineral nutrients, stimulation of root growth, interactions with other helpful microbes, as well as general or specific action against pathogens are among the multifaceted modes of action how they can promote plant growth. A legal restriction to register these agents only as plant protection products would disregard many of their other beneficial traits that could be reasonably used in biological fertilization strategies for sustainable plant nutrition.

BioStimulants versus BioControl Agents- Two Sides of a Coin

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According to the proposed draft of the fertilizer regulation EC 2003/2003[1], **biostimulants** will be defined as products (containing substances or microorganisms) that “are not as such nutrients, but nevertheless stimulate plants' nutrition processes”, and that“aim solely at improving the plants' nutrient use efficiency, tolerance to abiotic stress, or crop quality traits”. However, if they also increase plant disease resistance, either by stimulating the plant’s own ability to defend itself against a pathogen or by direct action against the pathogen, i.e. in the case of dual-function substances or microorganisms, they are to be treated as **plant protection products**to which EC regulation 1107/2009[2]applies. Here, the question arises whether a product that is marketed solely to support plant nutrition can be treated as a biostimulant even if plant protective activities have been described in the literature for the active ingredient (but are not claimed for the product). If that is not the case, i.e. if such a product would not be eligible as a biostimulant, problems will automatically arise. Treatments that will improve plant vigour by improving plant nutrition will not only increase plant stress tolerance, but at the same time also plant disease resistance. An example microorganism would be mycorrhiza fungi which are explicitly mentioned as biostimulants in Annex II of the draft fertilizer regulation[1], but which are known to also improve plant disease resistance.

An example substance would be chitosan. The term chitosan describes a family of biopolymers and oligomers consisting of varying numbers and ratios of glucosamine and *N*-acetylglucosamine residues. Depending on the number of residues in the molecule (its degree of polymerisation) and the ratio of the two monomeric units (its degree of acetylation), and possibly also depending on the sequence of the two units within the oligomer or polymer (its pattern of acetylation), chitosans can have different biological activities. Some chitosans can inhibit microbial growth (being funigstatic and bacteriostatic rather than being fungicidal or bactericidal), some chitosans can induce disease resistance in a plant (either by acting as an elicitor triggering resistance reactions, or by acting as a priming agent enabling plant cells to react more efficiently against pathogens), some chitosans can improve abiotic stress tolerance in plants (e.g. against drought or heat stress), and some chitosans can promote plant growth (e.g. root and/or shoot growth) and/or development (e.g. more fruit and/or earlier ripening).

Modern biotechnological methods of chitosan production and/or modification as well as new techniques for chitosan in depth structural analysis allow to produce well-defined (second generation) chitosans having defined and reliable, specific bioactivities, but lacking others. In this way, it is e.g. possible to produce chitosans which have no antimicrobial activities but which do have plant strengthening (i.e. growth promoting and stress tolerance and disease resistance inducing activities). It will probably also be possible to generate chitosans with more pronounced ability to induce stress tolerance and less marked ability to induce disease resistance, and *vice versa*. However, it is highly unlike that it will be possible to dissect these activities fully. The reason behind this is the intimate cross-talk of intracellular signal transduction pathways in plant cells. Even if it were possible to identify a specific chitosan oligomer (or any other compound) that is recognised by a specific receptor which triggers a signal transduction chain leading to metabolic answers increasing abiotic stress tolerance, and to identify another chitosan oligomer (or any other compound) that is recognised by a different receptor which triggers a different signal transduction chain leading to different metabolic answers increasing disease resistance - treatment of the plant cell with either of the two chitosan oligomers will invariably lead to a mixed response, possibly with stronger emphasis on one of the two reactions and less pronounced expression of the other, but not with just one answer(Fig. 1). Clearly, thus, there is a strong interconnection between abiotic stress tolerance and disease resistance.

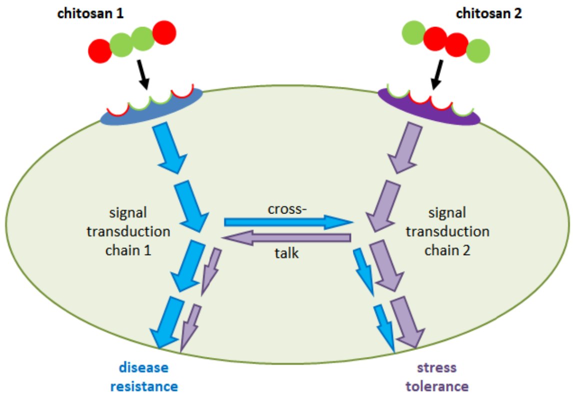

Figure 1: Cross-talk between intracellular signal transduction pathways establishes a signal transduction network, so that induction of abiotic stress tolerance automatically also induces disease resistance, and *vice versa*.

In fact, such a relationship is long known. Under optimum fertilisation conditions, plants grow best and are also healthiest, having the strongest ability to defend themselves against disease and to withstand abiotic stress. Both under-nourished and over-fertilized plants, however, are more prone to disease and stress induced damage. Thus, it is simply not possible to clearly distinguish between **biostimulant action** (as it is suggested to be defined) and biocontrol action (as it is defined now). It would appear more realistic to first distinguish two types of **biocontrol activity**, namely

- <u>(i) direct action against the microbial pathogens</u> and
- <u>(ii) indirect action by strengthening the plant's own ability to defend itself</u>.

(This would be similar to antibiotic treatment (i) *versus*vaccination (ii) in human medicine.) Products containing substances or microorganisms <u>acting on the pathogenic microorganisms</u> would fall under category (i), while products containing substances or microorganisms <u>acting on the plant</u> would fall under category (ii) (Table 1). The former would have to be treated as **plant protection products** under EC 1107/2009while the latter would be treated as **biostimulants**, falling under EC 2003/2003. In this way, consumer safety would also be maintained, as only category (i) products with direct antimicrobial mode of action have potentially toxic activities.

This categorisation would solve the problem that (almost) all products which induce tolerance to abiotic stress also induce resistance to disease - which would otherwise, if the categories defined in the current draft of the EU regulations would enter into force, lead to the situation that (almost) no biostimulants would exist. The alternative solution, namely to categorize products solely according to the claims made by the producers instead of what is known about the bioactivities of the active ingredients, would fail to protect the safety of consumers and the environment, as potentially toxic category (i) products could be registered as biostimulants under the fertiliser regulations which, reasonably, puts less emphasis on toxicity testing. (While the situation would be rather clear then for substances, it might still not be entirely clear for microorganisms which might act directly against pathogenic microbes but not via toxic principles but rather by competition in which case a categorisation as a plant protectant would not be appropriate.)

aim of treatment	disease protection		stress protection
target of treatment	pathogen	crop plant	
mode of action	inhibition of microbial growth	induction of disease resistance	induction of stress tolerance
toxicity	yes	no	
risk potential	high	low	
category	biocontrol (i)	biocontrol (ii)	biostimulant
regulatory framework	1107/2009	2003/2003	

Table1: Proposed categorisation of substances and microorganisms with biocontrol *versus*biostimulant activities depending on whether they act on the microbial pathogen or on the crop plant.

[1] http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52016PC0157&from=EN

[2] http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02009R1107-20140630&from=EN

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"C-IPM-fit": A new proposal for enhanced efficacy labeling of plant protection products[1]

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Background

Plant Protection Products (PPP) in Europe undergo an intensive two-step evaluation procedure as laid down in Regulation (EC) 1107/2009 (EC, 2009a), including the evaluation of toxicology, ecotoxicology, fate and residues in the approval of active substances as a first step. The data requirements follow the Regulation (EC) 283/2013 (EU, 2013a). The second step is the approval on product level. It additionally includes the evaluation of adverse effects on non-target organisms and on the cultivated plant itself, and the effectiveness ("direct efficacy") of the product towards the target organism. The positive and negative effects are here summarized to the overall efficacy according to the EPPO Standard 1/214(4) (OEPP/EPPO, 2017a). The data requirements follow Regulation (EC) 284/2013 (EU, 2013b).

Following Regulation (EC) 1107/2009 all approved PPP have to be "sufficiently effective" – without defining what "sufficient" means. The cited EPPO Standard 1/214(4) clarifies that "a sufficient overall agricultural benefit" should "justify the use of the plant protection product". "Sufficient efficacy" should be a level of efficacy "acceptable" ("satisfactory") in relation to its aim. Acceptability is ascertained if the PPP shows results "significantly superior to ...

[1] This opinion Paper does not reflect the official opinion of the Julius Kühn-Institute but the opinion of the cited authors.

untreated control” and/or “compares with ... a reference product” (OEPP/EPPO, 2017a).

The efficacy might be still acceptable, even if it is less effective than a reference product. “Furthermore, when direct efficacy[2] has not been shown to be acceptable, it may be possible to envisage management options (e.g. use limitations) that would improve it to a sufficient level” (OEPP/EPPO, 2017a). Clearly it must still enable growers to fulfil the 8 principals of IPM (Barzman et al., 2015).

Although efficacy is a key parameter of grower’s choice in plant protection, the information that is generated during the registration process is only partly visible for the user (e.g. the grower). In the case of the United Kingdom, the regulatory bodies label the product´s direct efficacy already (HSE, 2017). Furthermore, OEPP/EPPO recommends to label “clear warnings of the balance between positive and negative aspects... on the label” (OEPP/EPPO, 2017a). This is to a certain extent carried out as well on a national level.

The concept of “efficacy acceptability” cited above takes variability of effects into account including variability of effectiveness (direct efficacy). Currently there is a development towards a two class system, where, on the one hand, products which have advantages in the risk categories (environmental fate, human toxicity and adverse non target effects, so called “low risk products”), might be approved with relatively low effectiveness which is from the viewpoint of OEPP/EPPO still acceptable (compare standard PP 1/296, OEPP/EPPO 2017b). On the other hand PPP with higher risk and higher efficacy are registered at the same time. The reasoning behind is linked to the political will for enhanced marked share of low risk products in Europe (EU Council, 2016, EU Commission, 2016). This is also in line with European National Action Plans (NAP) to reduce risks in crop protection and to support organic and integrated crop production (e.g. BMEL, 2015, EC, 2009b). Such a strategy is comprehensible in general and will lead to a strong pressure to apply IPM strategies to a much

[2] We assume that not „direct efficacy“ but „efficacy“ in the sense of OEPP/EPPO, 2017a, is meant.

larger extent than in the past. PPPs with relatively low effectiveness – whether of low risk or not – could get their chance if all components of IPM strategies are evaluated altogether. However, without having for all products all information at hand, low risk products might automatically get the user perception of a product with low direct efficacy.

At this moment we recognize a communication problem: to plan IPM strategies information about the effectiveness and adverse effects of a PPP with special emphasis, for instance, to specific natural enemies is required by the grower as soon as the product is introduced to the market. In case of the product´s effectiveness, the information is available for the producer of a PPP, for the authority approving the PPP but initially not for the grower. Only after the first experiences of practice supported by advisory services estimations about the variability of effectiveness are published (compare LWK-NRW, 2018). This impedes the introduction of potentially useful IPM components in practice. These experiences should to our opinion be routinely reported Europe wide.

There is a similar lack of information given within the adverse effects. In practice, adverse effects of a PPP on natural enemies relevant at the actual growing situation are crucial for product choice by users. But during the evaluation process of competent authorities during the registration process, information on these effects are often restricted to standard indicator organisms as required by Regulation (EC) 284/2013 (Chapter 10.3.2; indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi*). Consequently and in line with current standards (e.g. the ESCORT2 document), this basic evaluation results in only a minor number of cases were information about additional relevant natural enemies is provided by the applicant. In Germany, an overview of this extended data is currently not online available for growers and advisors. A related German web-platform is planned organized by the Julius Kuehn-Institute (Hommel, personnel communication). To date, information on a broader and for growers often more relevant species spectrum is mainly provided by private producers of such organisms (e.g. Koppert side effects data base; Koppert, 2017). Unfortunately, very often it is not transparent for users on which observations and experiments these ratings base on. Our proposal is aimed to overcome these information deficits.

As outlined different PPPs may have variable effectiveness controlling pest organisms[3] even if they have been approved under the same European guidelines. Furthermore, a single PPP might have variable effectiveness due to e.g. variable environmental conditions, cultivars and so on. The topic we are stressing here is that the result of the official evaluation should provide information about the mean effectiveness and the deviation from the mean value. This should be finally published for each PPP in an easy and comprehensible way as it is e.g. partly carried out for herbicides in Germany. Accordingly, also other factors influencing the overall efficacy, mainly the adverse effects of the PPP on agronomical relevant factors (in our example natural enemies, including a broad range of species besides of the main indicator species for risk evaluation) should be considered and displayed.

The "C-IPM-fit" concept

The concept picks up the idea as described by OEPP/EPPO (2017a) and HSE (2017). It presents a description of "acceptable efficacy" with the focus on a) the effects of a certain PPP against the target pest organism in combination with b) the effects of this PPP on all other relevant factors, that naturally or due to active measures limit or reduce the target organism or its effects on the crop. Important information for growers and therefore relevant factors for the system proposed here are the effectiveness of a PPP on a target organism and – following our example - its effect on introduced or naturally occurring antagonists. All other

[3] We use the expression „pest organism" as an umbrella term for all organisms detrimental for cultivated plants including (arthropod) pests, pathogens, weeds and others.

principles of the IPM-strategy, especially an effective anti resistance strategy remain valid in the product choice (Barzman et al., 2015).

In this approach, effectiveness and adverse effects on relevant natural enemies are first measured in experiments, then evaluated and finally highlighted optically by use of "Colored Light Indicators" (CLI). The factors "effectiveness against the pest organism" and "adverse effect on natural enemies" receive different, independent CLIs (Table 1). Such CLIs can be weighted by the grower himself because each factor is evaluated and shown separately. The simple visualization of relevant plant protection factors allows growers and advisors a simpler and more comprehensive decision about the suitability of the PPP within the current IPM strategy to control a certain pest organism. In accordance to this weighting process, the definition of the current integrated pest management fit (C-IPM-fit) of any plant protection measure can be defined as the relation of its direct efficacy on pest organism population(s) and its impacts on all factors that limit or support the growth of that/these pest organism population(s). Natural enemies are one very relevant factor that we will focus on exemplarily. In case of PPP, also residual effects on future populations should be considered. Consequently it should be noted that the same measure can show a high C-IPM-fit in one crop situation and a low C-IPM-fit in another. For instance, a strong negative effect of a PPP1 on relevant natural enemies limiting the target pest population growth in the actual crop situation reduces the suitability of the respective product, even if its direct efficacy is high (i.e. low C-IPM-fit). A PPP2 with medium effectiveness and low impact on relevant natural enemies would be the better choice (i.e. high C-IPM-fit). On the other hand, if that natural enemy is actually not present, PPP1 with higher effectiveness (and now irrelevant adverse effects) would be first choice (i.e. high C-IPM-fit). By translating these parameters in easy comprehensible CLI, growers are enabled to decide for the most suitable PPP (i.e. the one with the highest C-IPM-fit) for their specific crop situation, depending on the population status of the pest and the natural enemies in the specific system.

Table 1 Proposed Colored Light Indicators for assessment of the C-IPM-fit after evaluation of effectiveness and adverse effects on natural enemies during the evaluation process of PPPs within the registration process

Colored Light Indicator	Effectiveness	Adverse effect[4]
Green	Constantly high effectiveness, ≥80% reduction	Constantly low, <30% - 0% reduction
Yellow	Constantly medium effectiveness, <80% - ≥50% reduction	Constantly reasonable, <50% - ≥30% reduction
Orange	Constantly reasonable effectiveness, <50% - ≥30% reduction	Constantly medium, <80% - ≥50% reduction
Red	Constantly low effectiveness, <30% - 0% reduction	Constantly high effects, ≥80% reduction
Grey	No data / data not sufficient for evaluation	No data / data not sufficient for evaluation
Colour range	If trials of a PPP are to less than 90% within one category, every other category that represents at least 10% of the trial results is added. Every gap category in-between the relevant categories is added additionally, resulting in a colour stream from the first to the last relevant category.	

The C-IPM-fit labelling of a PPP should includ as a first step the effectiveness against given specific pest organisms (e.g. *Myzus persicae*) and the adverse effect on natural enemies (e.g. *Aphidius colemani).* Both is labelled in a specific colour given that sufficient data is available; where data is not sufficient, a grey CLI is assigned (Table 1). In this concept, the species, the family level (e.g. Aphididae/Braconidae), or even a functional groups (e.g. "sucking insects"/"parasitic wasps") can be chosen for the labels, again depending on the reliability and broadness of the available data set. A proposal for a reliable data sets (i.e. the number of trials/observations) is ≥ 5 for a specific species, ≥ 15 and covering at least 50% of relevant species for a family level and ≥ 30 covering at least 50% of the relevant functional group for a functional group level. Variability of data sets within the chosen level can be represented by a colour range (Table 1). In any case, species of pest organisms and natural enemies which are not in line with the other assessments (because a product is not effective on a specific pest organism or has higher impact on a specific natural enemy) should be listed separately, as

[4] here: reduction of a given natural enemy species or group

done currently in the efficacy evaluation process. For products which show generally inconsistent effects for species/groups within the same level, the assessment should be done on the next lower level. As a result, a number of C-IPM-fit category combinations should be assigned and visualized with respective CLIs (Table 1). An example for a concrete labeling is shown in Figure 1.

Figure 1 Example of a product labeling for some possible categories of relevant pests and natural enemies with the C-IPM-fit categories defined in Table1

Effectiveness against pest organism		**Adverse Effect on natural enemies**	
Aphids (excluded: *Myzus persicae*)		*Aphidius colemani*	
Myzus persicae		*Aphidoletes aphidimyza*	
Trialeurodes vaporariorum		*Encarsia formosa*	
Bemisia tabaci		Predatory bugs	
		Coccinellidae	
		Syrphidae	

This practical approach can enhance data basis for evaluation and visibility for users at the same time: It promotes the generation of reliable data by companies for the evaluation by means of the subsequent labelling (e.g. on the label of PPP). The subsequent label then serves as a decision support tool for users and fosters IPM. To make this approach successful, the following aspects should be respected for delivered data complementing the legally required data package:

1) Voluntariness for companies in order to not hamper registration of new PPP for the European market.
2) Clearly defined guidelines for the additional data sets that need to be delivered by companies to claim a specific C-IPM-fit category for their products.
3) Easily comprehensible reports of the C-IPM-fit categories on the product label and / or in a central European database.

4) No impact from additional data concerning adverse effects on the official risk analysis.[5]

We are sure that this easy decision support system will favour the use of PPP with favourable C-IPM-fit characterization whether of low risk or not. It could be extended to basic substances easily. Furthermore, it provides the companies with an instrument to point out the special positive characteristics of their products related to the use in specific crop situations. This can become an effective driver for submission of enhanced data sets during the registration process of PPP independent from the actual data requirements.

Outlook

It is clear that the presented proposal still has to overcome obstacles until the concept is ready for use. The next steps could be to discuss the general concept within German official bodies and on specific symposia with plant protection advisors, efficacy evaluators of other European countries, and companies. If a consensus can be reached, an expert working group could be formed to further develop and define the guidance for the required data and the concrete implementation into product labelling and/or a web-database. The mentioned database on natural enemies already planned in DE at the Julius Kühn-Institut could form a basis for these further developments.

It is important to be clear in the point that the voluntary given additional information should not interfere with the risk analysis based on legal requirements. Both evaluations must be uncoupled, meaning that additional information cannot condition a re-evaluation process. To ensure this is clearly a baseline in order to get a more comprehensive picture of the C-IPM-fit of products during the registration process. Another but more far-reaching approach with

[5]This aspect concerns only effects on agriculturally important supporting factors such as natural enemies.

regard to natural enemies could be to change in general from a worst-case risk evaluation to a more broad evaluation, and to evaluate products rather from their average impact instead of extrapolation from few indicator species to the overall impact.

The described labelling can certainly be used for all types of PPP. At least effectiveness on pest organisms and adverse effects on natural enemies could be labelled in consistence with the C-IPM-fit concept for all pest organisms. On the long run other factors such as cultivar, effects on pollinators, or pest organism resistance could be incorporated into the C-IPM-fit concept as well. It remains clear that labelling is always a simplification and that after all IPM-solutions must be developed in a systems approach. Nevertheless, to our opinion labelling can serve as first information in decision making to estimate under which circumstances a specific PPP can promote pest control within a specific cropping system.

The endeavour to develop and implement this additional evaluation and labelling is high, but just as well is its benefit. Labelling can become an important incentive for industry to produce even more reliable, broader data sets to show in detail the specific C-IPM-fit characteristics of their products. This way a win-win situation for all sides is created: efficacy evaluators expectably receive additional data for a more concise evaluation, industry can decide to give good products an additional certificate which can become a very strong marketing tool, growers can rely on objective official results to choose the most suitable product for their actual crop situation, advisors get more prompt information about new products on the market and products with low adverse effects and proven effectiveness can increase market share in line with the current political agenda.

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Efficacy and risks of „Biorationals" in organic and integrated pest management – acceptable??

Comments of IBMA on the results of 8th International Symposium 13./14.12.2017 in Braunschweig

During the Symposium some issues were concluded (bullet points). From referents and participants (incl. IBMA-members) perspective the following recommendations could derive (green arrows):

- **Definition**
  There is no generally accepted definition of Biorationals yet. As a working definition the term was used for Biocontrol, Biostimulants and Biofertilizer agents, including microorganisms, natural occurring substances (botanical extracts, minerals, chitosan etc) and semichemicals. Macrobials were not in focus also they are part of the biocontrol concept.
- ➢ The word 'biorational' is not commonly used, and is not part of any regulations; therefore IBMA does not support the use of that term. For the conference however it created an interesting approach, showing regulative shortcomings due to dual functions of different products (see below). Thus the approach could lead to constructive solutions and serve sustainable plant protection strategies (see below).
- **Efficacy trails under natural conditions**
  The experienced efficacy of Biorationals in lab and greenhouse is not always reproducible under natural conditions, since it depends on environmental conditions (e.g. under nitrate application microbial consortia increase phosphorous availability inefficiently, while under ammonia application they deliver P reliably).
- ➢ Define flexible models for efficacy trails under field conditions. They should refer to the latest EPPO Guideline on efficacy for low risk PPPs, which has been accepted by EPPO and the EU MSs, including Germany. PP 1/296 (1) Principles of efficacy evaluation for low-risk plant protection products Bulletin OEPP/EPPO Bulletin (2017)0(0), 1–8
- **Efficacy in IPM**
  Biorationals are part of IPM and organic farming. Therefore they have to be considered as part of a management concept but no as a tool box for conventional farming. Low efficacy is tolerable under these circumstances, since the general principle of IPM is prevention, not intervention. Also in conventional programmes Biocontrol Agents and Biostimulants are valuable alternatives and often crucial for resistance management.
- ➢ Promote "revitalised" IPM strategies within the national NAP and SUD strategies to achieve sustainable plant protection. E.g. foster suppression of harmful organisms and resilience of crops through promotion of cultural methods and non-chemical alternatives. .
- ➢ Hence communicate the recommendations of the Symposium to competent authorities on EU and member states.
- ➢ Elaborate and promote local extension services, since the success of IPM depend on farmer skills.
- **Legislation, registration and innovation**
  Biorationals work with complex but effective mechanisms. In reality there is an overlap of stimulating and biocontrol effects. – The molecular response pattern to biotic and abiotic stress can be characterized as overlapping gene responses, were in total 417 genes are

involved. In consequence the flowing transition between Biostimulants and Biocontrol lead to a Dual Function. In registration this Dual Function is causing conflicts since Biocontrol falls under legislation ((EG)2009/1107 while Biostimulants falls under (EG)2013/2013).

- Manufacturers suffer from long and expensive registration procedures under ((EG)2009/1107. The development costs of PPP are estimated to amount up 5 to 10 Mill Euro, including registration costs of 2-3 Mill. Euro. The biggest hurdle is the very long registration process like about 5 years. In consequences the companies are hesitant and many innovative substances and products don't enter markets. In several cases legislative conditions (e.g. cut off criteria and low efficacy) even hamper innovative solutions and holistic approaches (e.g. chitosan). In consequence a fast track system is needed.
- Policies, consumer's preference as well as the regulation 2009/128/EG foster specific plant protection solutions. This has economic implications especially for minor crops, where the return of investment of PPP is low.
- Uniform rules in the EU do not mean that PPP are uniformly available.
- The interaction of registration and innovation is critical for the resistance management, if less than 3 PPP per crop and indication are available. –
- Generally the principles of the (EG)2009/1107 (e.g. efficacy approach and risk assessment) were supported and efforts of authorities (EU and National) to smoothen the process were perceived. Still …

➢ Push for a faster, reliable and cheaper registration process.
➢ Realize a fast track for minor uses, with minimum data requirements and thus costs.
➢ Create – for a minority of products (e.g. naturally occurring substances like copper, botanical extracts and microorganisms with dual function) flexible and pragmatic solutions (e.g. guidelines, specialised personal in the CA, accepted Data gaps, if requests are not applicable under explained conditions , QPS for Bacolo viruses, registration of strains).
➢ A reasonable solution could even be an alternative regulation in addition to (EG)2009/1107, including a consistent risk assessment for naturally occurring biologicals (including microbials) applied in agriculture with real expert evaluators at EFSA and MS level (e.g. USA EPA biopesticides stream). In respect the term "Biorational" act as a valuable forerunner model as mentioned under the first bulled point.
➢ Provide "low risk" label at the beginning and not at the end of registration procedure, to avoid that the 'non-approval' criteria 'low efficacy' will act as an innovation killer for low risk products.

- The approach of the fertilizer legislations (EG)2013/2013 seems promising but many questions are still unsolved (e.g. Complementation of Positive List, CE Label). A transparent and well reflected approach is recommended based on the expert consultations. It was stated that the communication between authorities and experts is insufficient so far.

➢ Establish an expert network to foster exchange, information flow and consultation.

- **Strain level requirement**
  For microbial products there is the requirement of providing information on the identity of the microorganism. Usually this is provided on strain level and is well accepted; as specific features are strain specific. However, it was raised that for some technology like Mycorrhiza it might not be possible, but since Mycorrhiza is already on the Positive List it is exempted from strain specific notifications.

- Include in the fertilizer legislations (EG)2013/2013 an amendment to the strain specific requirement by adding ", where possible." This amendment would allow providing technology relevant information on the identity different from information on the strain.
- Encourage applicants and governmental evaluation bodies to be pragmatic and flexible to provide/accept alternative data. The data should serve the purpose of the data requirement, like overall safety and prove of label claims. Governmental evaluation bodies should not insist on the formal availability of specific data that cannot be provided due to the nature of the technology. This would suppress innovation.