Plant Protection and Plant Health in Europe:
Innovative biological techniques for IPM and their regulatory implications?
9th International Symposium, 19 - 20 November 2019, Braunschweig, Germany

# Products of bacterial agents in combination with soil conditioners and arbuscular mycorrhizal fungi:

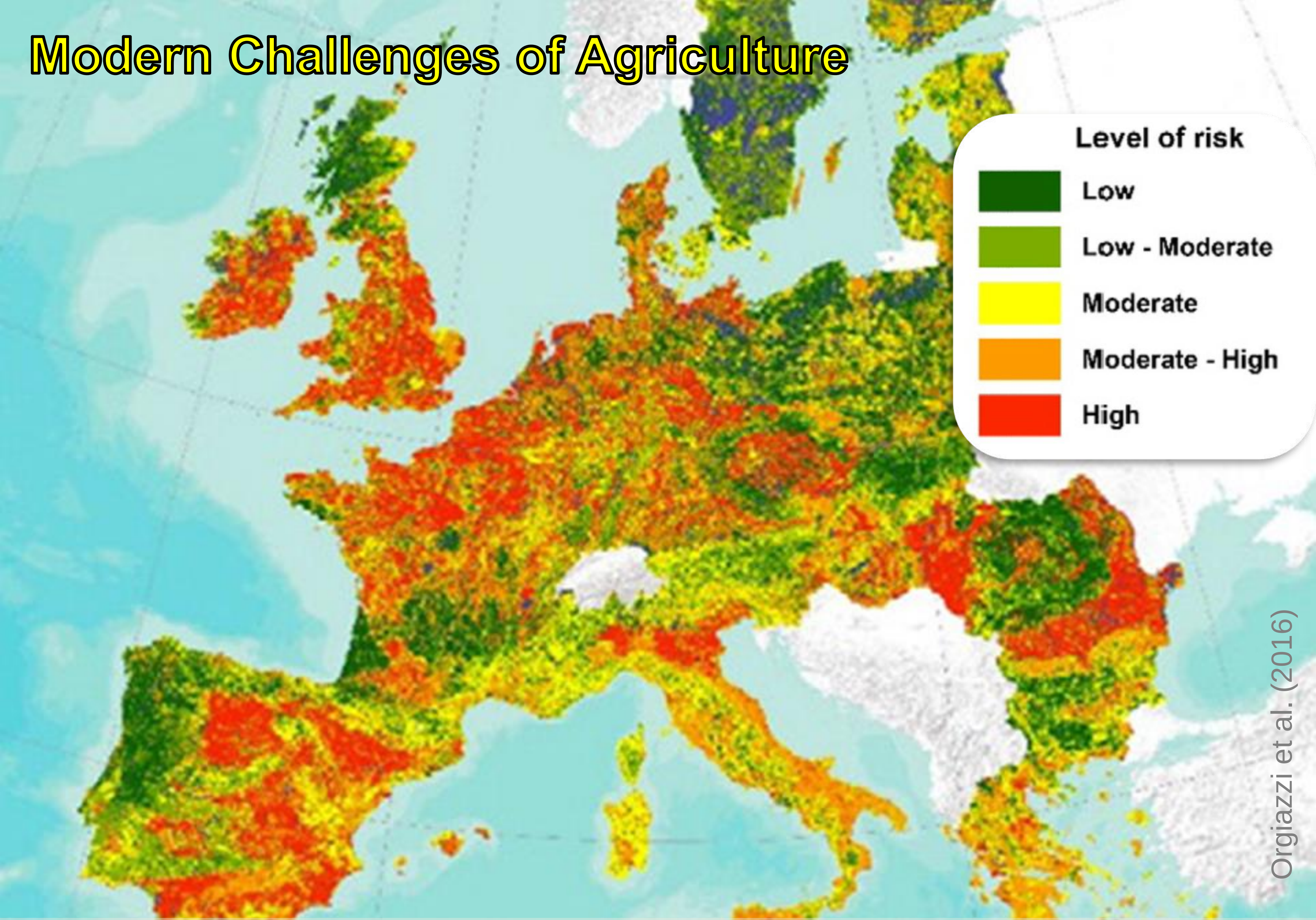
# how to register?

M. Weinmann, T. Tenbohlen, A.K. Christ,
A. A. Mamun, A. Aneesh, K. Bradáčová,
M. Raupp, T. Müller, U. Ludewig, G. Neumann
University of Hohenheim (340 h + i), Stuttgart

(Picture: www.valagro.com/en/)

SolACE is funded by the European Union within the Horizion 2020 Framework Programme, Grant Agreement No. 727247

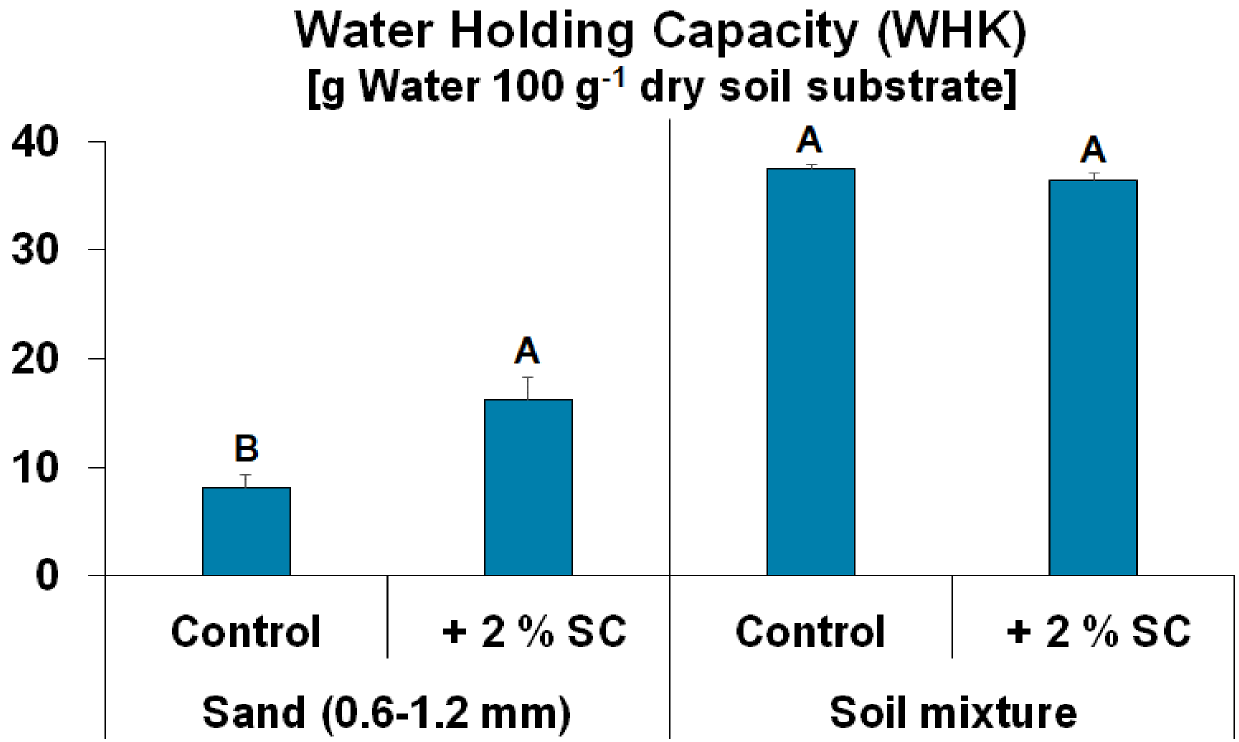
# Modern Challenges of Agriculture




**Risk to soil degradation by loss of soil biological functions.**

silty soil

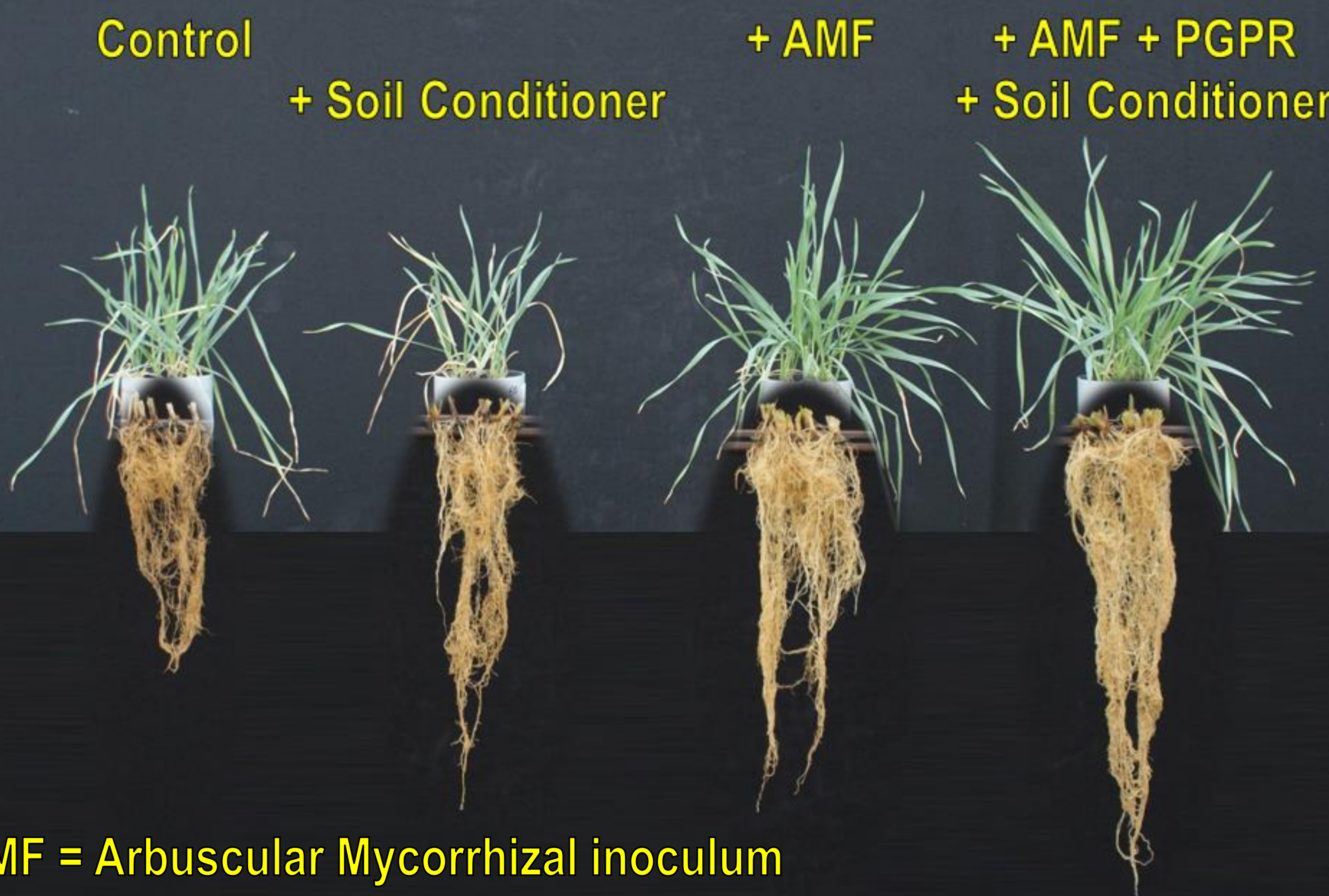
+ 5 % w/w hydrogel soil conditioner




- In sandy substrate, hydrogel soil conditioner (SC) increased the WHK
- In silty soil subtrate, SC improved the aggregate stability

SolACE is funded by the European Union within the Horizion 2020 Framework Programme, Grant Agreement No. 727247

# SolACE - Solutions for improving Agroecosystem and Crop Efficiency for water and nutrient use




AMF = Arbuscular Mycorrhizal inoculum
PGPR = Plant Growth Promoting Rhizobacteria (in tryptic soy medium)

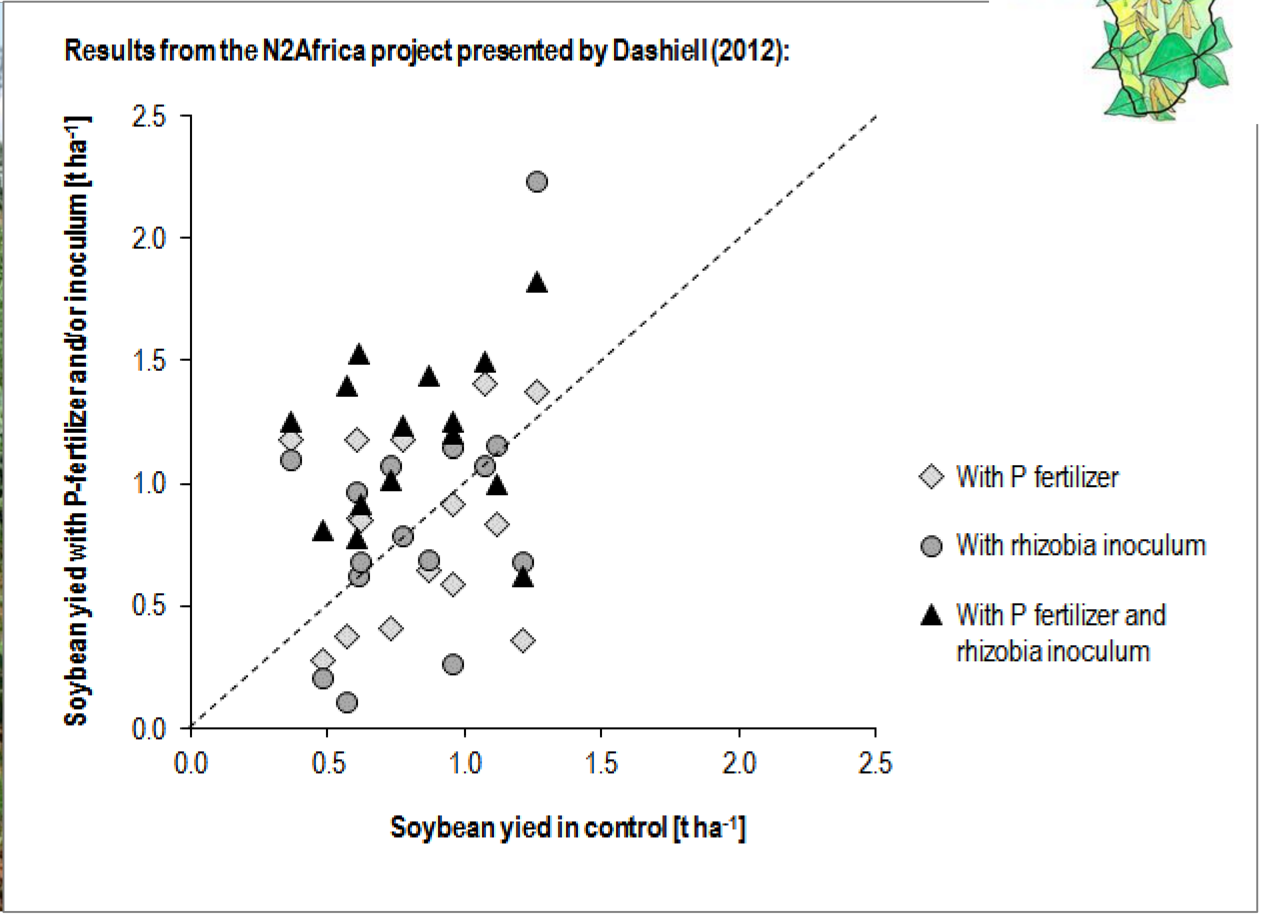
SolACE is funded by the European Union within the Horizion 2020 Framework Programme, Grant Agreement No. 727247

# Synergistic interaction: the whole is more and other than the sum of the parts


**Favorable conditions for AMF + PGPR activity**

SolACE is funded by the European Union within the Horizion 2020 Framework Programme, Grant Agreement No. 727247

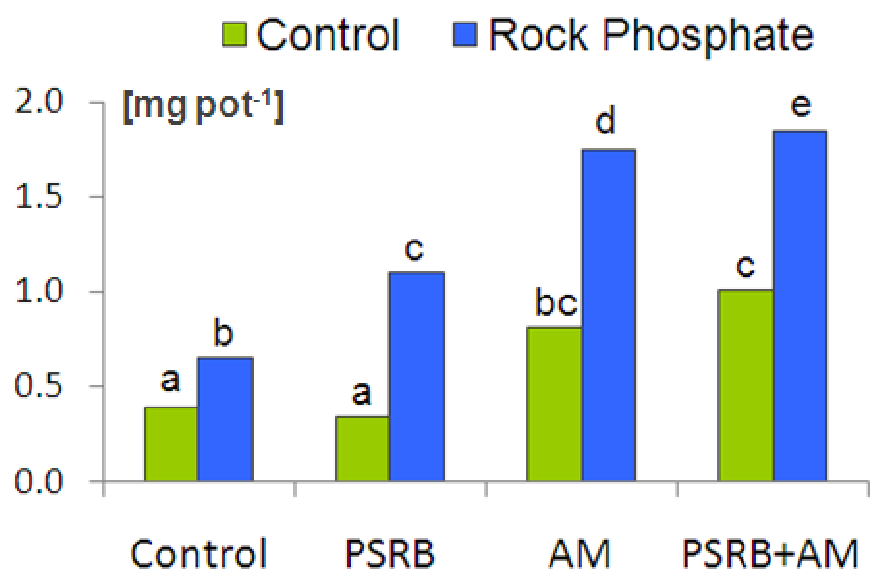
# $N_2$Africa demonstration trial results in Kenya 2010




Yield of soybeans in response to the application of P fertilizer, rhizobia inoculum, or P fertilizer and rhizobia inoculum versus the yield of soybeans in untreated control plots (Dashiell, 2012).

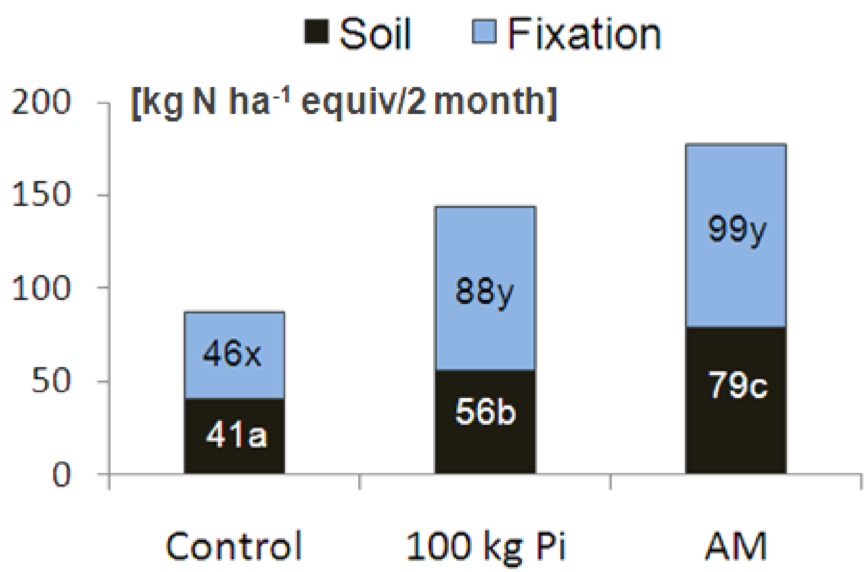
# Synergistic Action of PSRB, AMF and Rhizobia

### Effect on the P uptake of alfalfa (shoot P content)




Influence of microbial inoculants on the P-uptake of *Medicago sativa* L. on a Cambisol with and without rock phosphate supply.

### Effect on the N uptake of a legume crop from soil and by $N_2$-fixation



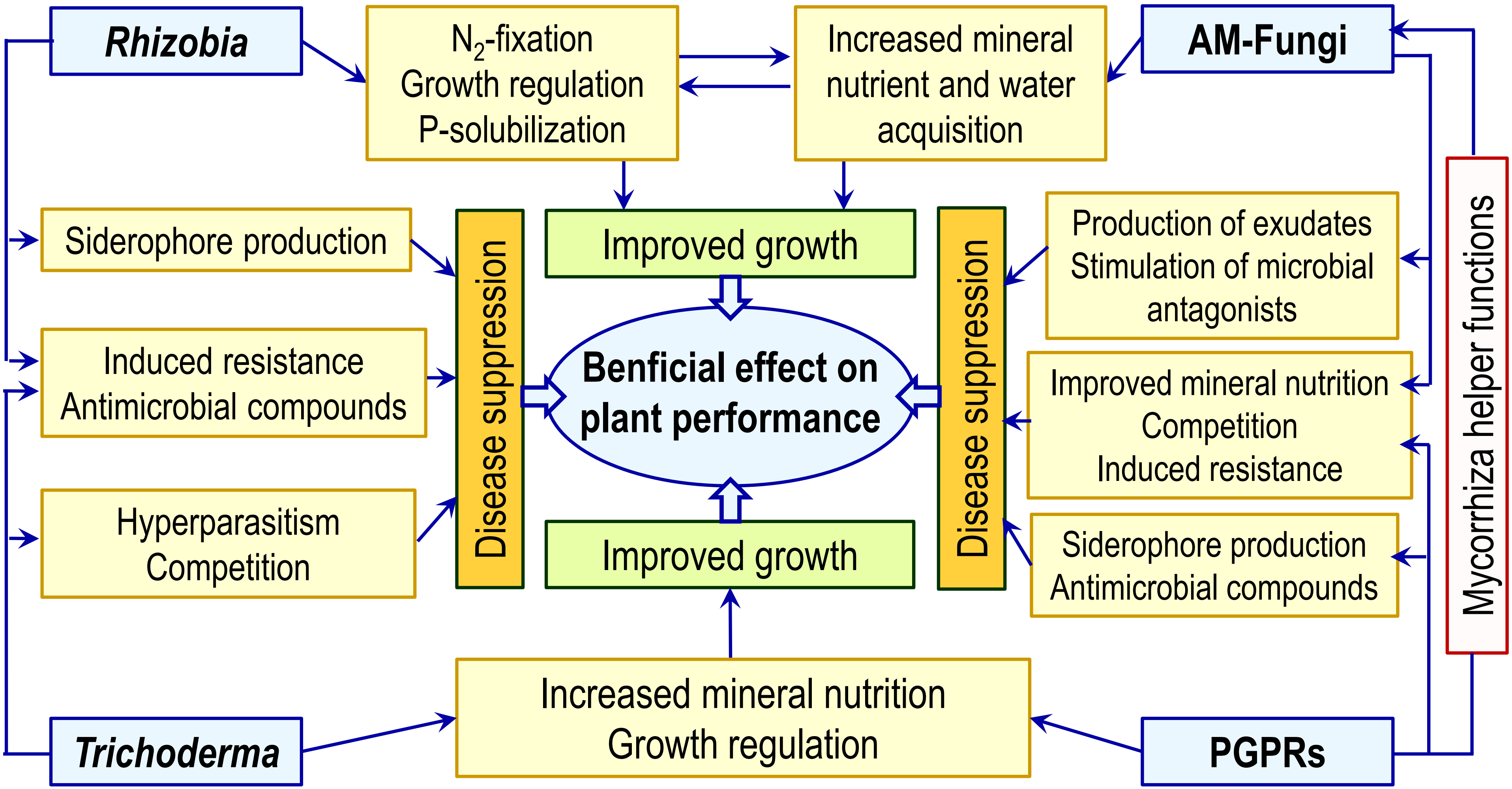

Influence of P-supply (100 kg Pi ha$^{-1}$) and AM fungi on the N uptake of *Hedysarum coronarium* L. from soil and by $N_2$-fixation on a soil inoculated with *Rhizobium* sp.

**PSRB** = Phosphate Solubilizing Rhizobacteria (*Enterobacter* sp., Toro et al., 1998)
**AM** = Arbuscular Mycorrhiza (*Glomus mossae*)

(Barea et al. 1987 New Phytol 106:717-721; Barea et al. 2002 Nutr Cycl Agroecosyt 63:35-42)

# Multifunctional actions of microbial agents

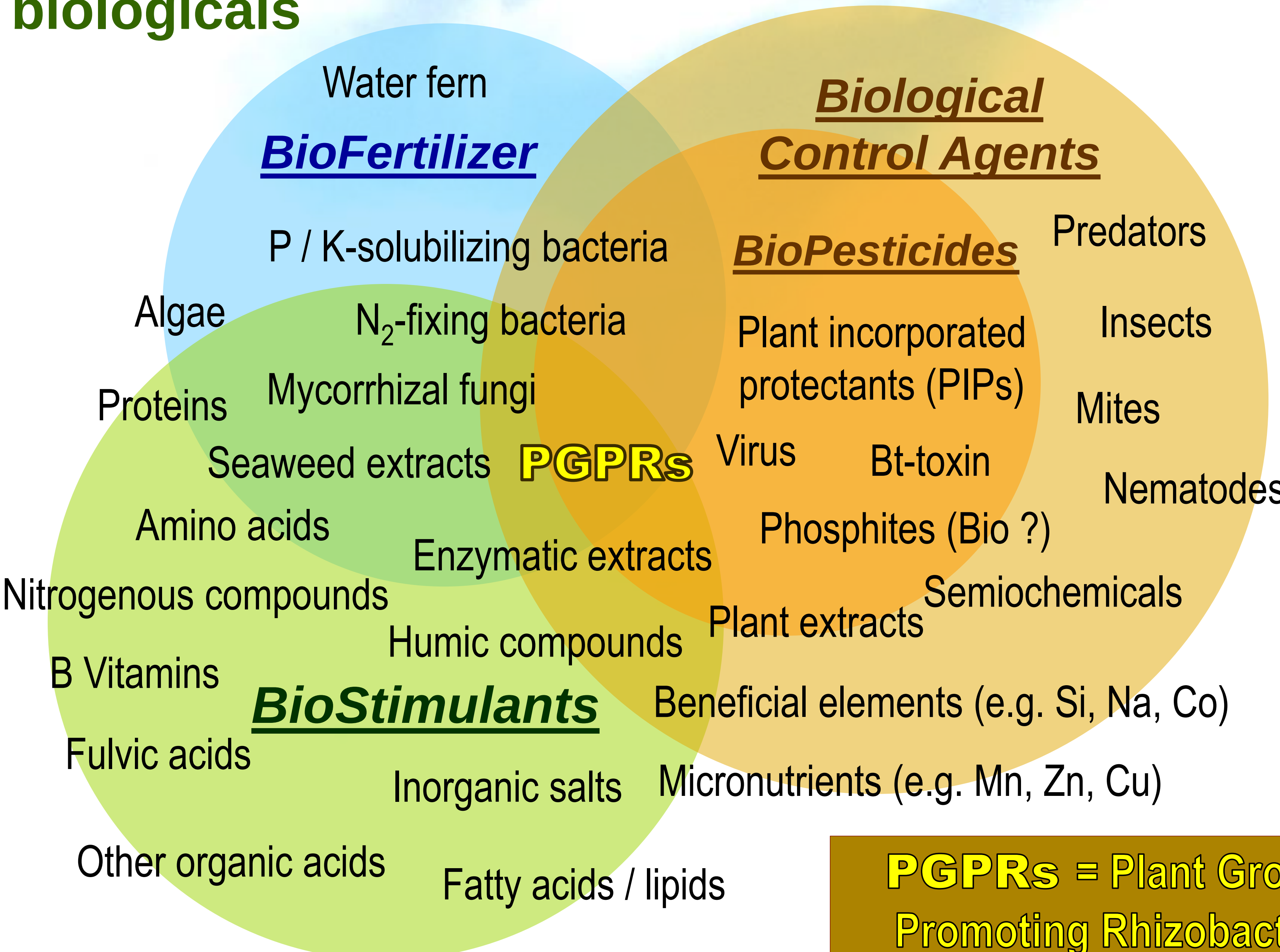
(adapted from Avis et al., 2008 )




Direct and indirect effects of microbial agents on plants

**But: How to register such product combinations legally ?**

# Product categories and active agents of agricultural biologicals




Adapted from: Agricen Sciences' analysis of market analysis, survey papers on Biostimulants, www.bpia.org

**PGPRs** = Plant Growth-Promoting Rhizobacteria

Food and Energy Security 2014, 3(1): 1-6
Open Access
aab Association of Applied Biologists

EDITORIAL

# Is the regulatory regime for the registration of plant protection products in the EU potentially compromising food security?

Peter Chapman, JSC International Limited, Harrogate, United Kingdom

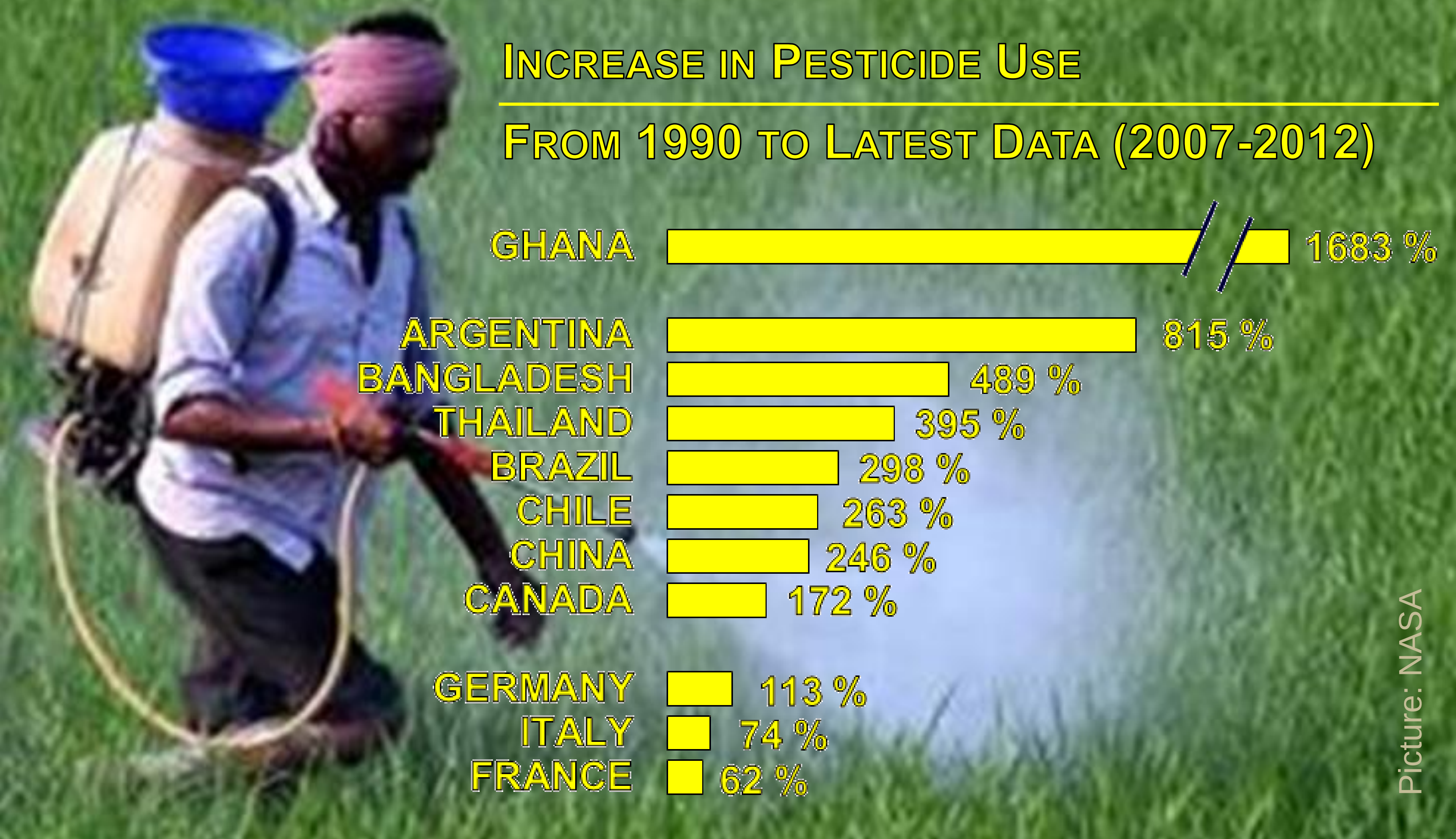
## Active substances in development and introduced to the market

| Region | 1980-1989 | 1990-1999 | 2005-2014 |
|---|---|---|---|
| Worldwide | 123 | 128 | 73 |
| Europe | 41 | 40 | 12 |
| Share Europe (%) | 33.3 | 31.3 | 16.4 |

Phillips McDougall (2013)

- Increasingly stringent regulatory regime and prohibitive costs
- **Decreasing** availability of old and **innovative** active substances
- Minor uses and niche applications are suffering in particular

# The Developing World Is Awash in Pesticides. Does It Have to Be?




Bajak (2016); Data: Pretty and Bharucha (2015)

1 | TASPO THEMA DER WOCHE 15. April 2011 | Nr. 15

# Sind in Zukunft weniger Pflanzenstärkungsmittel verfügbar?

Die neue EU-Pflanzenschutzmittelverordnung wird auch Auswirkungen auf die Listung von Stärkungsmitteln haben. Von **Katrin Klawitter**

## EU Regulation No 1107/2009: Plant Protection Products

§ „ ... substances, including micro-organisms having general or specific action against harmful organisms or on plants ...“

## Revision of the German Plant Protection Law (PfSchG 2012)

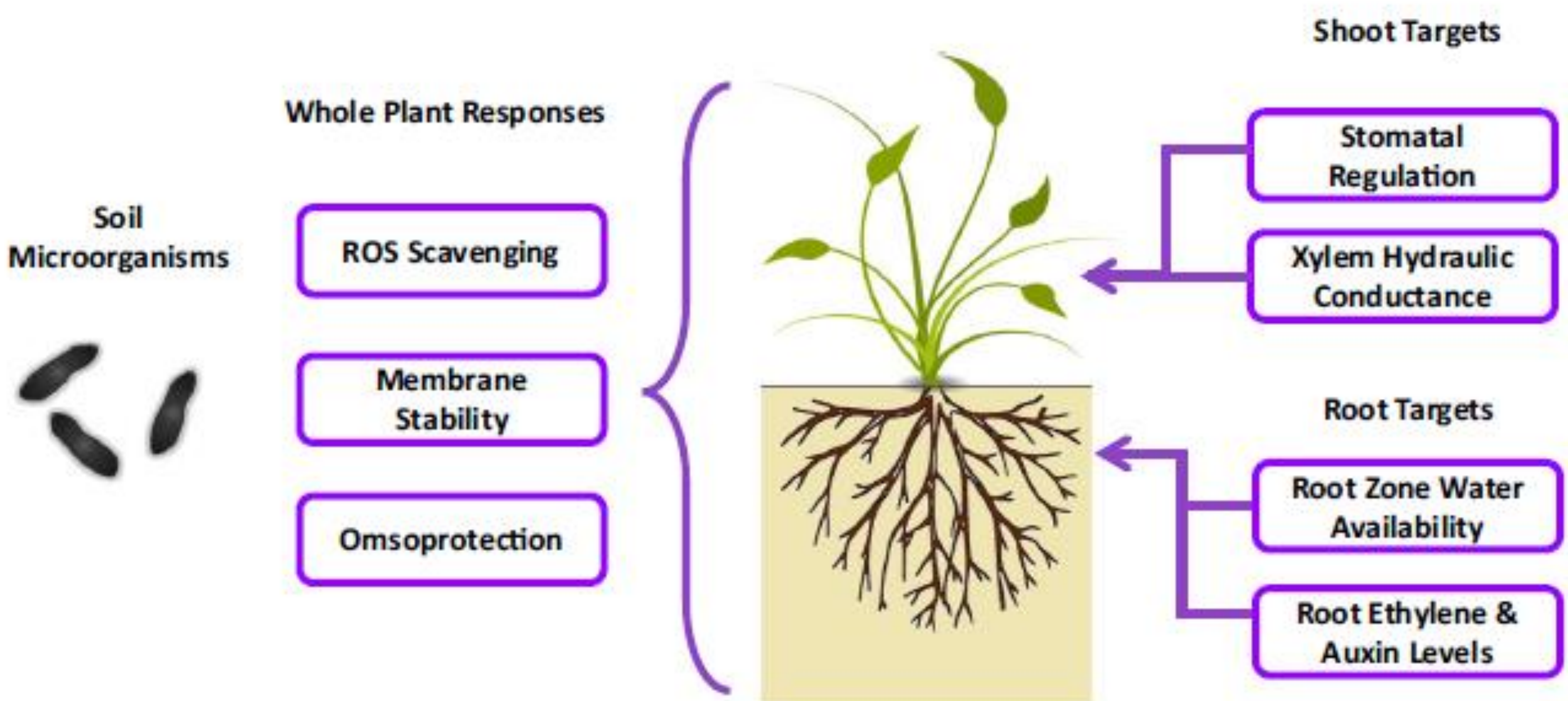
- Numerous biological agents cannot be registered as „Pflanzenstärkungsmittel“ furthermore
- Registration as „Pflanzenschutzmittel“ often too expensive

REVIEW Open Access

# The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants

Van Oosten et al. (2107) Chem. Biol. Technol. Agric. 4:5, 1-12.

## Key Mechanisms Targeted By Microbial Biostimulants



ELSEVIER

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti


## Plants biostimulants: Definition, concept, main categories and regulation

Patrick du Jardin (2015) Scientia Horticulturae 196, 3-14

„ … *any substance or microorganism* ***applied to plants with the aim*** *to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content.*"

Main categories:

- Humic and fulvic acids
- Protein hydrolysates & other N-containing compounds
- Seaweed extracts and botanicals
- Chitosan and biopolymers
- Inorganic compounds
- Beneficial fungi & bacteria

frontiers in Plant Science

REVIEW
published: 26 January 2017
doi: 10.3389/fpls.2016.02049

# Biostimulants in Plant Science: A Global Perspective

Yakhin O. I. et al. (2107) 7:2049, 1-32

“*a formulated product of biological origin*

*that improves plant productivity as a consequence of the novel,or* ***emergent properties*** *of the complex of constituents,*

*and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds.*”

⇨ Identification of a distinct mode of action would necessitate a legal classification according to this specific function

“The **E**uropean **B**iostimulants **I**ndustry **C**ouncil […] promotes the growth and development of the European Biostimulant Industry”

## EU Regulation 2019/1009: Fertilizing Products

§ Plant-Biostimulants means products with the sole aim of improving:

- nutrient use efficiency
- tolerance to abiotic stress
- quality traits
- availability of confined nutrients in soil or rhizosphere

EBIC

“The **E**uropean **B**iostimulants **I**ndustry **C**ouncil […] promotes the growth and development of the European Biostimulant Industry”

## EU Regulation 2019/1009: Fertilizing Products

### CMC 7: Positive list for microbial Biostimulants

| Micro-organisms | Plant Protection Effects |
|---|---|
| *Azotobacter* spp. | Chauhan et al. (2012) |
| Mykorrhizapilze | Woo and Pepe (2018) |
| *Rhizobium* spp. | Avis et al. (2008) |
| *Azospirillum* spp. | Tortora et al. (2011) |

**But: general or specific action against harmful organisms or on plants are well known !**

BASF We create chemistry

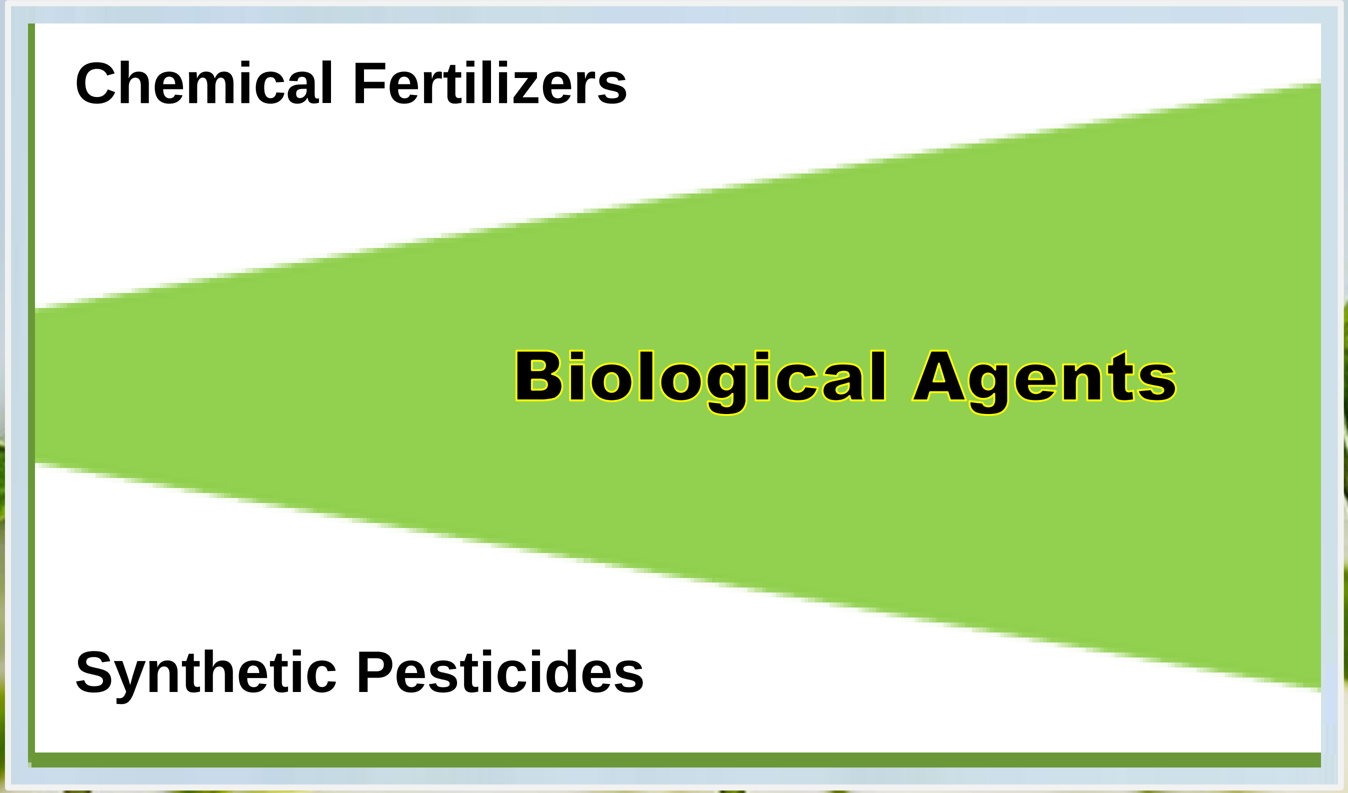
Bayer

syngenta

YARA

EuroChem Agro

# Growing interest in agricultural biologicals




- 100 x higher success rates in screening of biological agents
- 10 x lower costs for product development
- Market Forecast: 14 % Compound Annual Growth Rate
- Global-Market-Volume: 6.75 Billion US $ (2017)

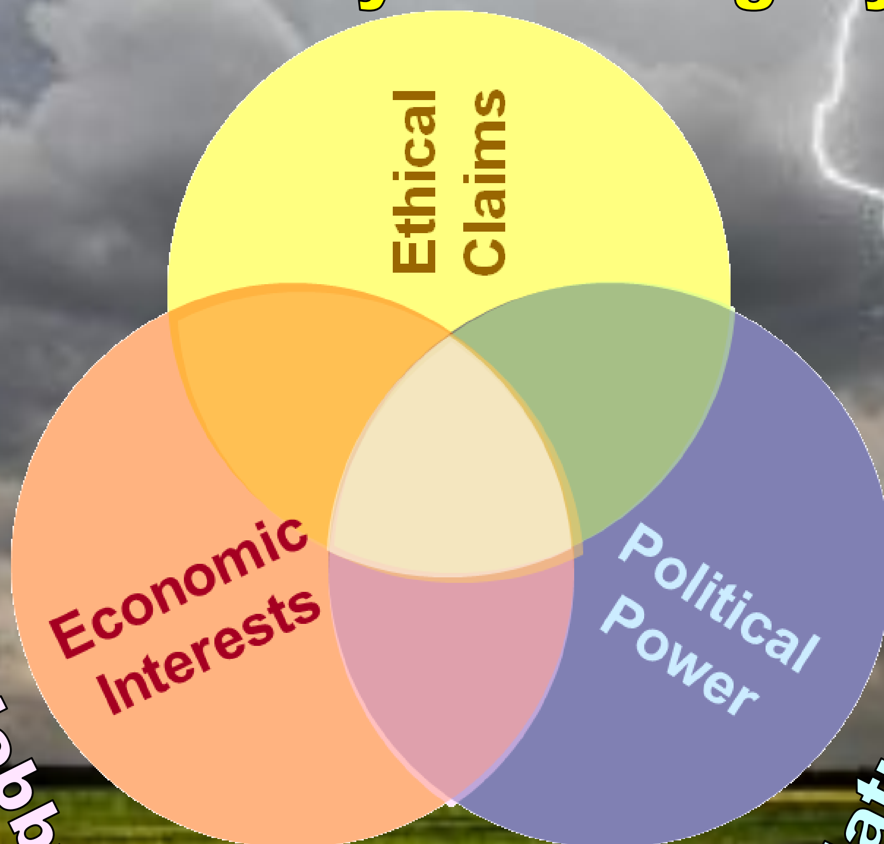
(Agra-Europe, 2017; Ravensberg, 2017; MarketsandMarkets, 2019)

Foto: www.bioestimulantesagricolas.net

# Interaction of Science, Economy and Politics – also Synergistic?

**value freedom of science**

**⟷ honesty and integrity**




Stakeholder lobby ⟷ social responsiblity

Natural laws ⟷ positive laws

Foto: tippsout.de

# Integrated scientific Concept of Bio-Effectors

**Utilization Groups / Intension**

Bio-stimulants

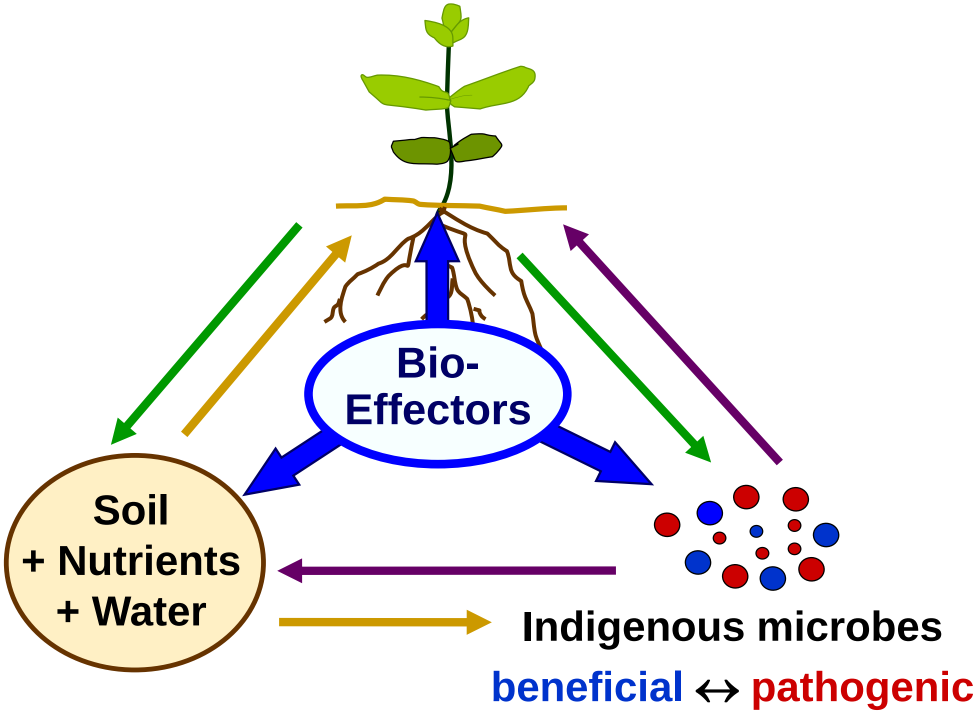
Bio-fertilizers

Bio-pesticides

Bio-control agents

**Bio-logicals**

**Active Ingredients / Mode of Action**

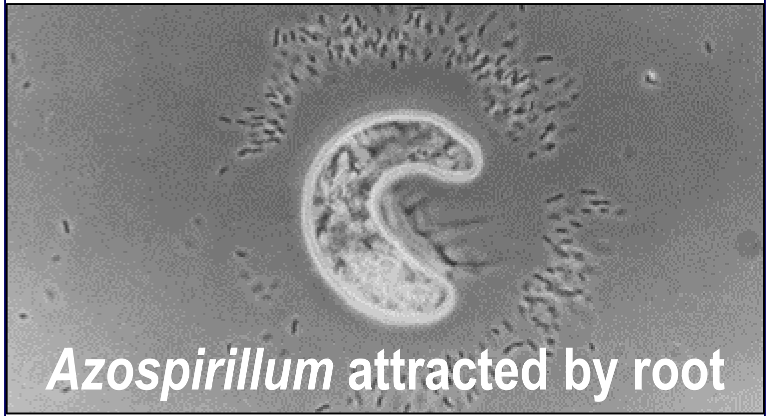



**Functional implementation or activation of biological mechanisms, especially those interfering with soil-plant-microbial interactions.**

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

# Bio-Stimulants and Bio-Fertilizers

## Major strategies to improve the mineral nutrition of plants:

| Spatial acquisition | Chemical acquisition | |
|---|---|---|
| Symbiotic | Associative / free living | Symbiotic |
|  |   |  |
| → soil exploration by external hyphae of arbuscular mycorrhiza | → Root growth stimulation<br>→ Dinitrogen ($N_2$)-fixation ?<br>→ Phosphorus (P) solubilization ? | → $N_2$-fixing rhizobia in root nodules of leguminous plants |

(Pictures: soil-environment.blogspot.de; Villecourt et al., 1999; boiled-peanut-world.com)

# ▪ Bio-Stimulants and Bio-Fertilizers

## Major strategies to improve the mineral nutrition of plants:

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| → soil exploration by external hyphae of arbuscular mycorrhiza | → Root growth stimulation<br>→ Dinitrogen ($N_2$)-fixation ?<br>→ Phosphorus (P) solubilization ? | → $N_2$-fixing rhizobia in root nodules of leguminous plants |

(Pictures: soil-environment.blogspot.de; Dobbelaere et al., 1999; boiled-peanut-world.com)

## ▪ Bacteria as Bio-Stimulants and Bio-Fertilizers

### Screening of microorganisms for their P-solubilizing capability

**Poor medium**

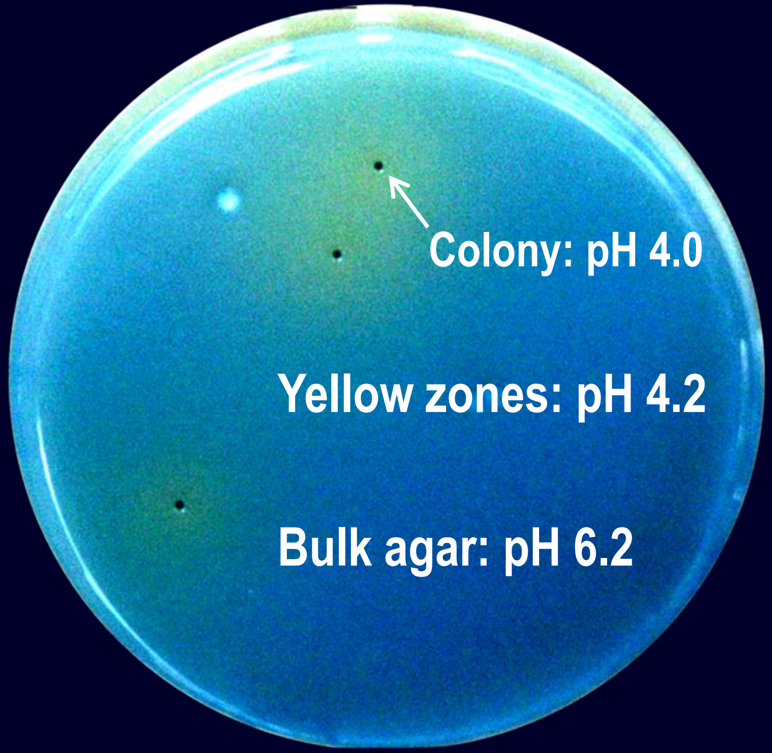
**Rich medium**

*Pseudomonas* contained in Proradix® (Sourcon Padena, Tübingen) formed clear zones of Ca-P solubilization on a nutrient poor, but not on a rich medium.

## ▪ Bacteria as Bio-Stimulants and Bio-Fertilizers

### Screening of microorganisms for their P-solubilizing capability

High density

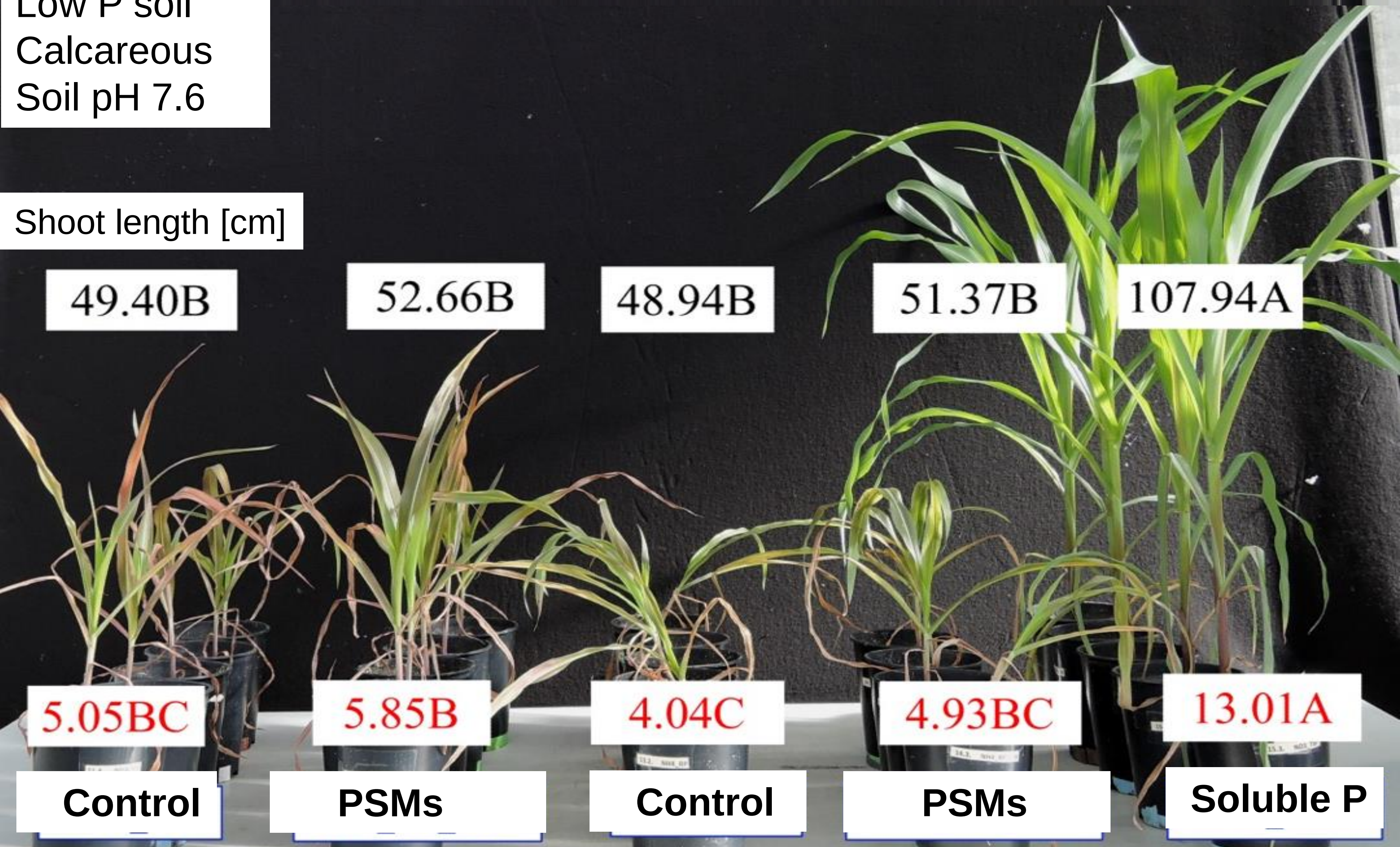



Low density

*Bacteria* contained in Phylazonit ® MC (Corax-Bioner, Budapest) based on *Bacillus* & *Azotobacter* species induced strong pH decreases at low, but not at high colony densities, when grown on a rich agar medium with Al-P.

# Weak Effectiveness of Microbial Agents on low P soils

Low P soil
Calcareous
Soil pH 7.6




**Limited plant capacity to support P-solubilizing microbes in the rhizosphere?**

# ▪ Bio-control agents

Healthy soils are characterized by optimal microbial activity, proper root development and delivery of plant available nutrients:

## Healthy root

Large populations of beneficial organisms

Establishment of efficient symbioses with mycorrhizal fungi and rhizobia as aid for water and nutrient acquisition

## Diseased root

Low populations of beneficial organisms

Pathogen attack

Soil sickness is a sign of imbalance between natural antagonists and pathogenic organisms

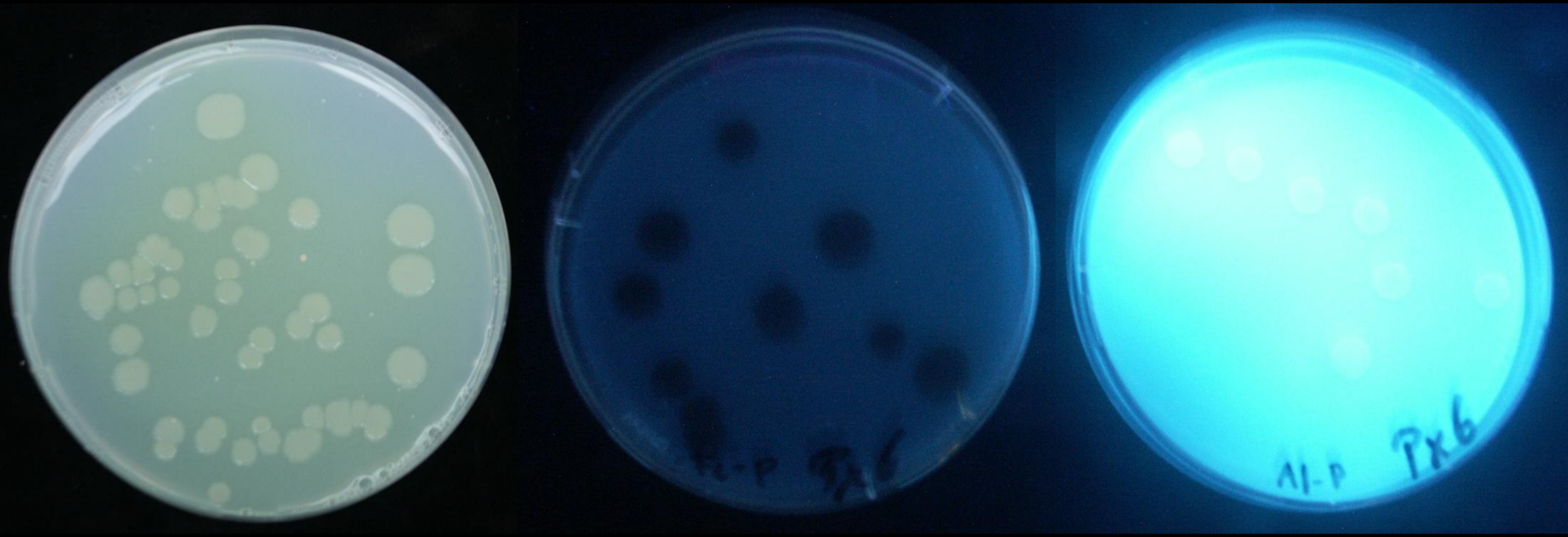
(Gunawardena et al., 2006)

➡ adequate management can result in suppression of distinct diseases

# Bacteria as Bio-control Agents

Pyoverdines: fluorescent pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas* spp. (Meyer, 2000)

**- Fe** **+ Fe** **- Fe**



day light

no fluorescence under UV light

fluorescence under UV light

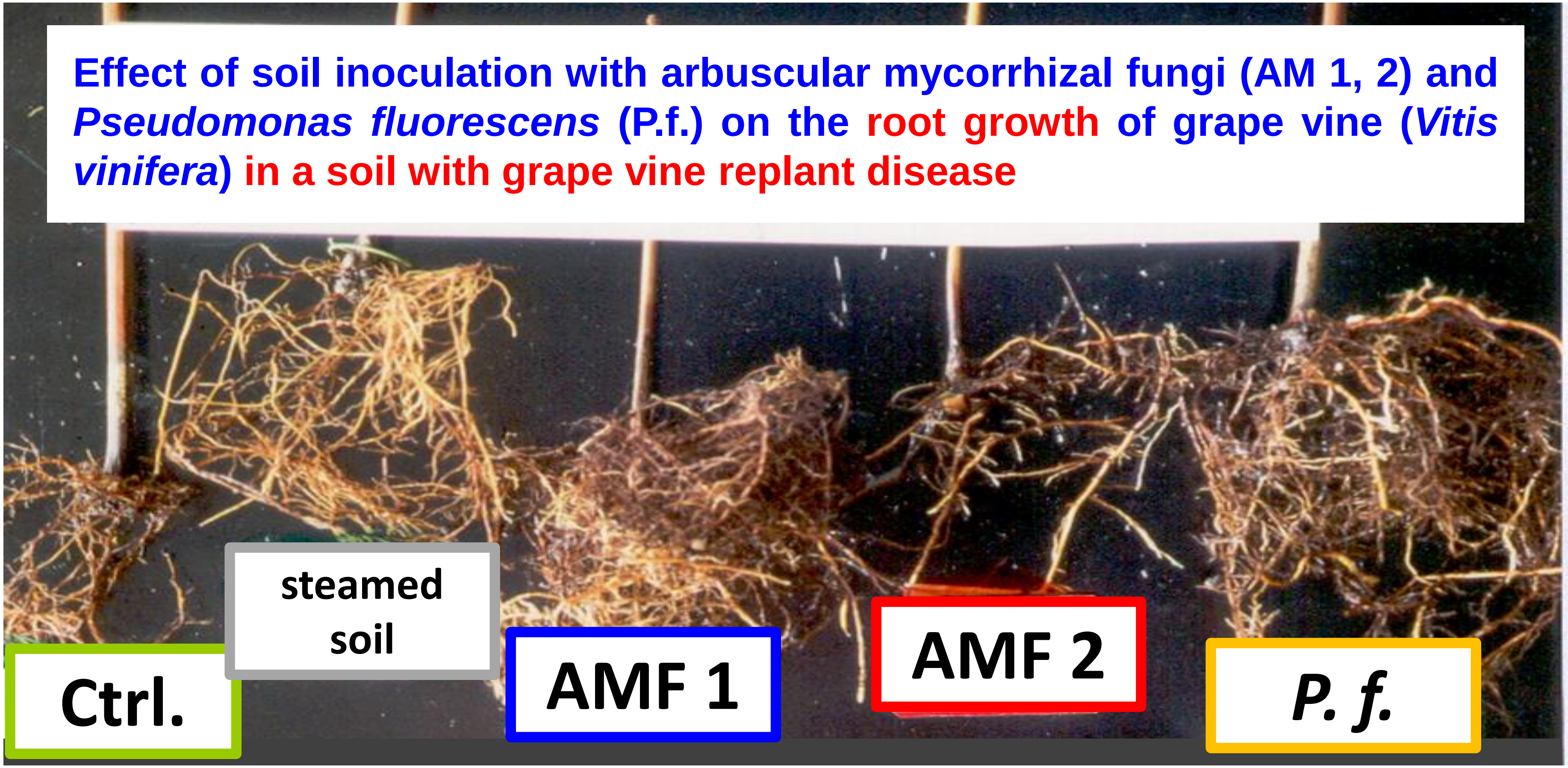
Production of fluorescent siderophores by *Pseudomonas* spp. can be assesed on iron free (- Fe) growth media. No siderophore production under Fe- supply (+ Fe).

# Case study: Replant Disease in Grape Vines



Grape vine nursery at Neustadt an der Weinstraße

# Interaction between PGPR and AMF in Bio-control




Weinmann, 2000

Ctrl = Control

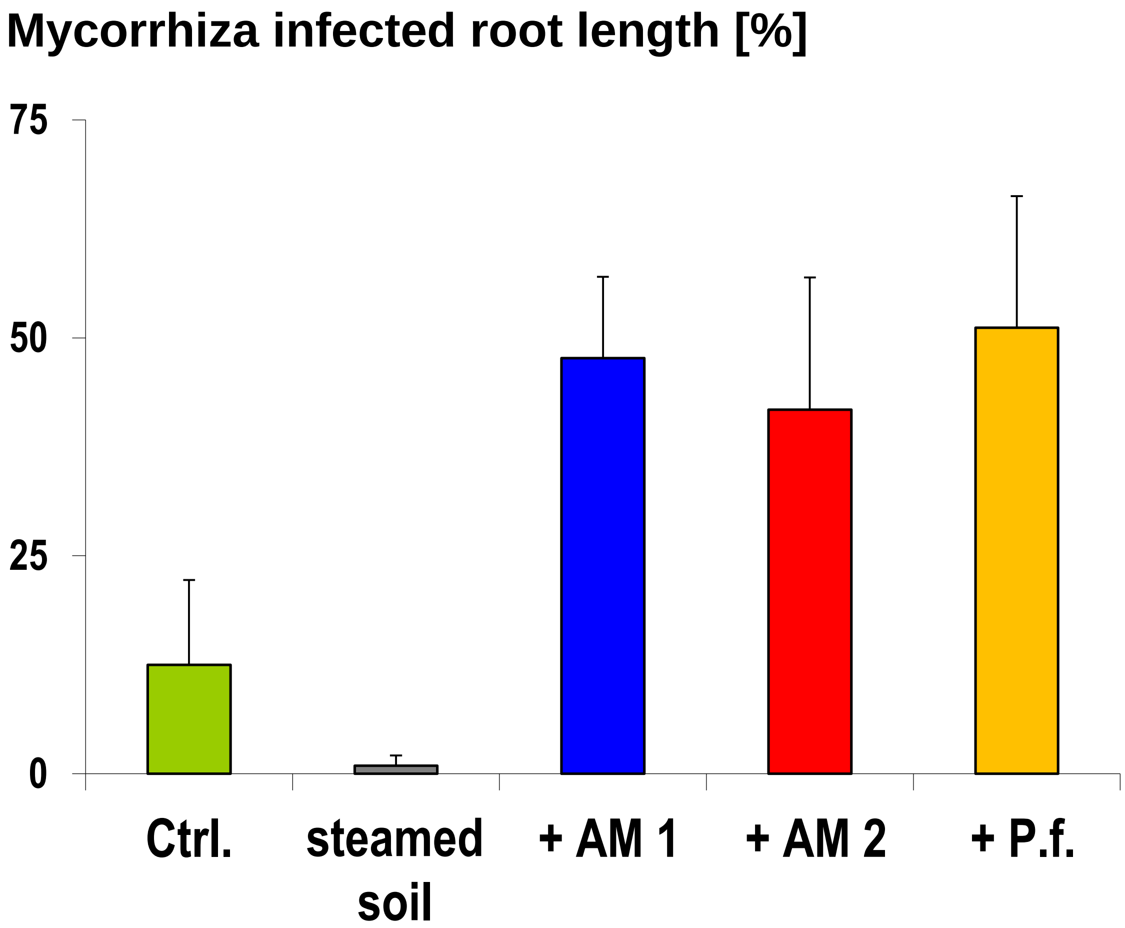
AMF1 = Mykotek® (*Glomus intraradices*)

AMF2 = Triton® (*Glomus etunicatum, G. intraradices, G. fasciculatus*)

*P. f.* = Phytobacter RA 56® (*Pseudomonas fluorescens* strain RA 56)

# Interaction between PGPR and AMF in Bio-control

**Effect of soil inoculation with arbuscular mycorrhizal fungi (AM 1, 2) and *Pseudomonas fluorescens* (P.f.) on the mycorrhization of grape vine (*Vitis vinifera*) roots in a soil with grape vine replant disease**




Ctrl = Control

AM 1 = Mykotek®

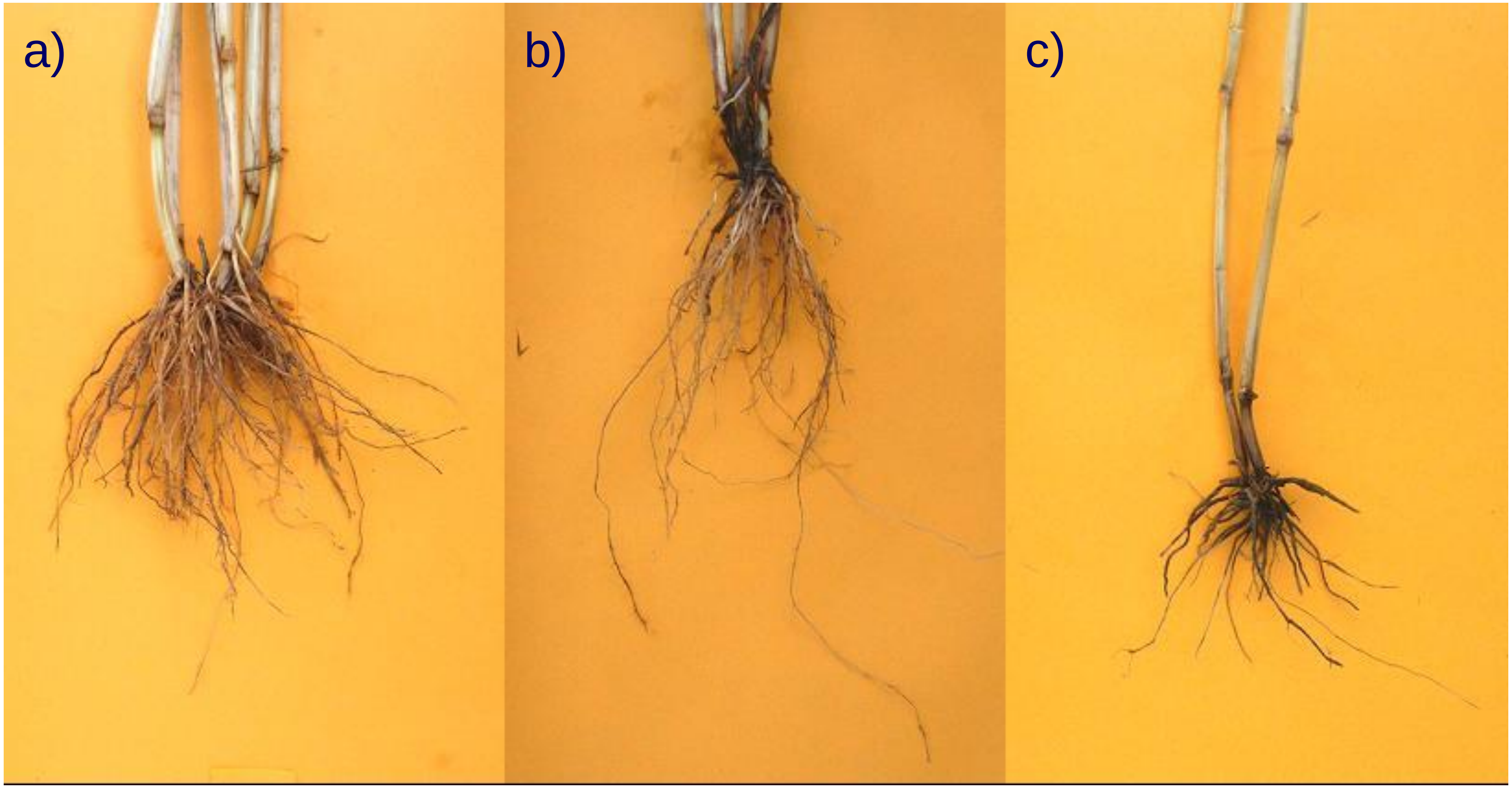
AM 2 = Triton®

P.f. = Phytobacter RA 56®

Weinmann, 2000

# ▪ Case-Study: Take-all in Wheat

## Root symptoms of take-all disease



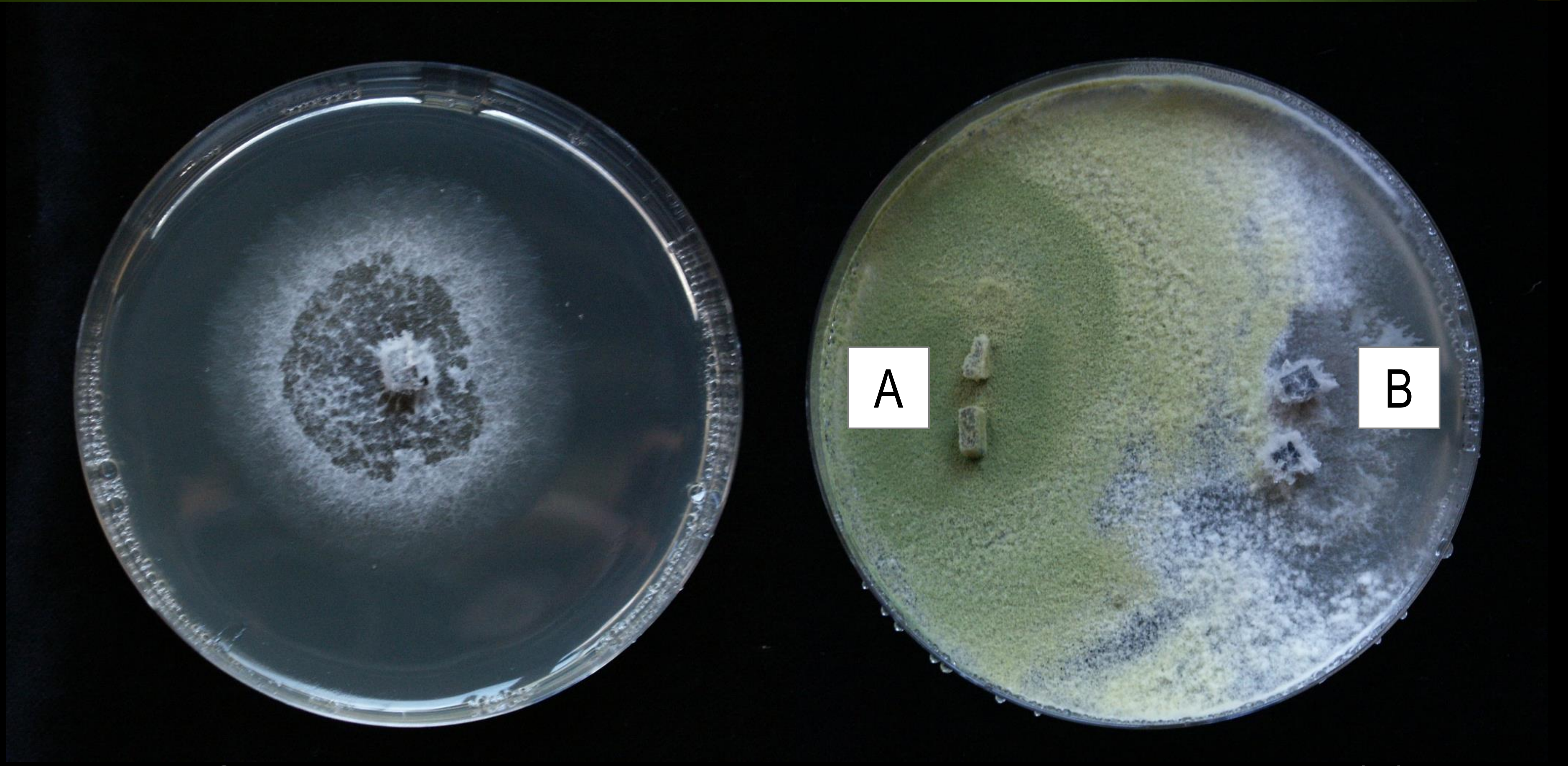

(Huber et. al., 1993, Hornby et. al., 1998)

Pictures (a) to (c) show increasing severity of root-rot correlated with decreased tillering.

➡ No economically effective fungicides or resistant wheat varieties available.

➡ Control by crop rotation, pH adjustment and fertilization.

# *Trichoderma* fungi as Bio-control Agents



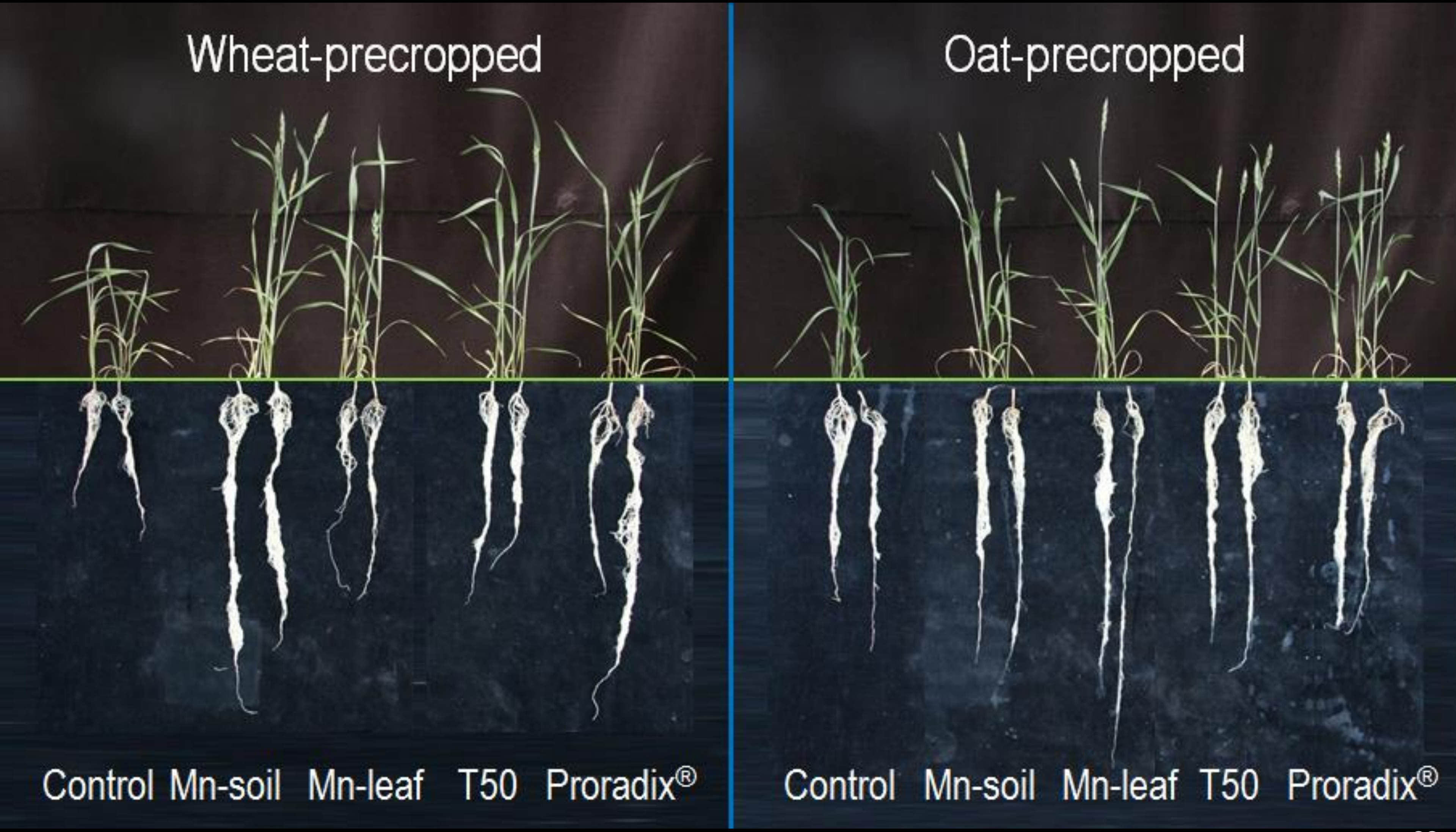

*G. graminis* var. *tritici*

*Trichoderma harzianum* (A)
*G. graminis* var. *tritici* (B)

**<u>Myco-parasitism:</u> *Trichoderma* spp. („Vitalin-T50“) grows tropically towards the hyphae of other fungi (*G. graminis*) and degrades them**

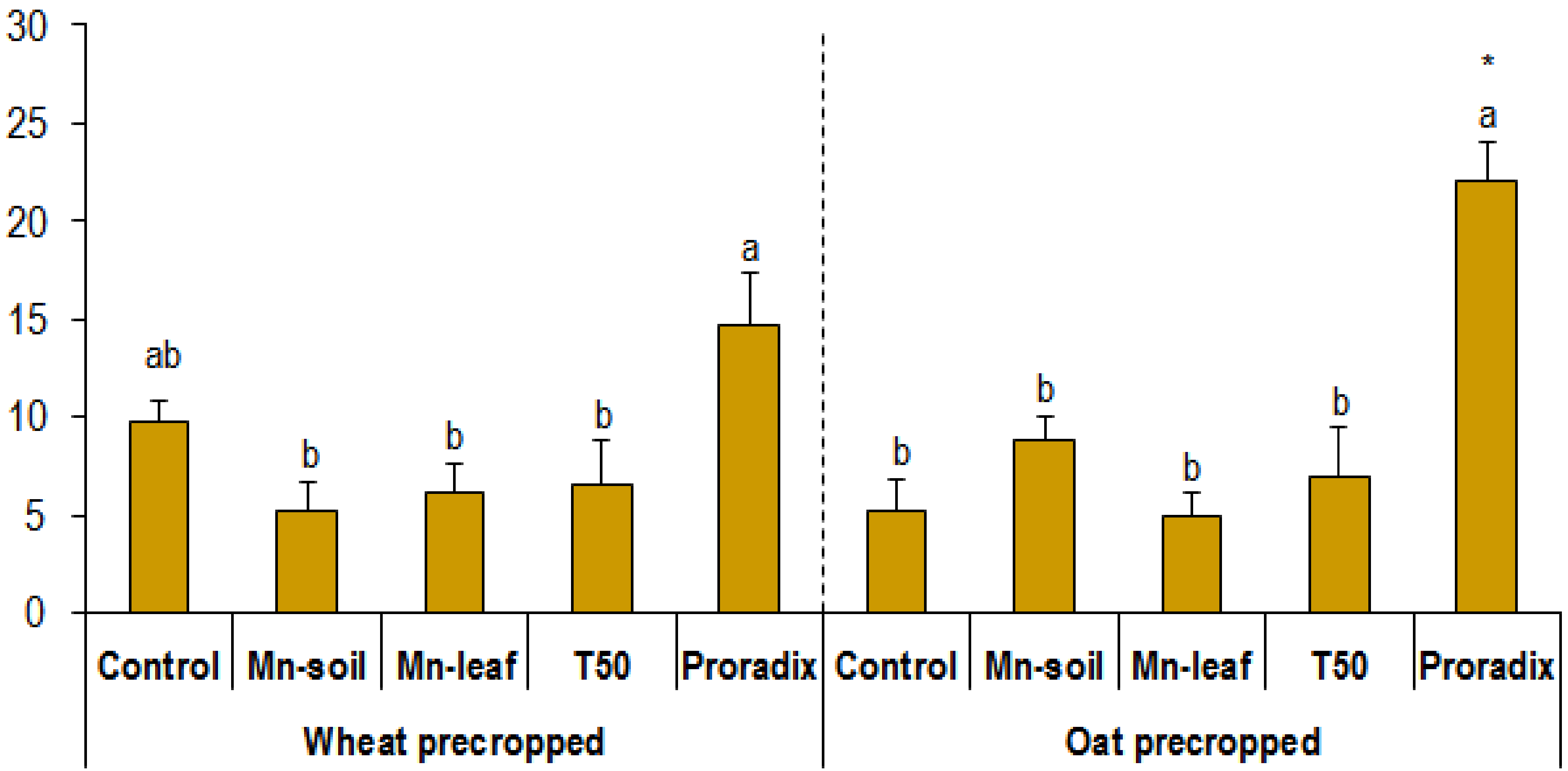
# Interaction between PGPR and AMF in Bio-control

## Root and shoot growth of wheat var. Paragon



# Interaction between PGPR and AMF in Bio-control

**Proportion of arbuscular mycorrhizal infected root length [%]**




Proradix® (*Pseudomonas*), but not Vitalin T50 (*Trichoderma*), increased the root colonization by AMF in wheat grown on a Take-all disease infested soil

# The BioFector Project: General Objectives

Development and implementation of viable alternatives to the current practice of mineral fertilization by use of bio-effectors.

- **Improvement and adaptation** of existing and development of novel bio-effector products
- Conduction of **field experiments** to validate the effectiveness of under practice conditions
- Economic **sustainability** and environmental friendliness

Approach to reach these objectives:

- Combination of existing alternatives for mineral fertilization with adapted bio-effectors


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

# Meta Results International Field Testing Network

BIO FECTOR

**38 biological products with proven effectiveness under controlled conditions were tested**

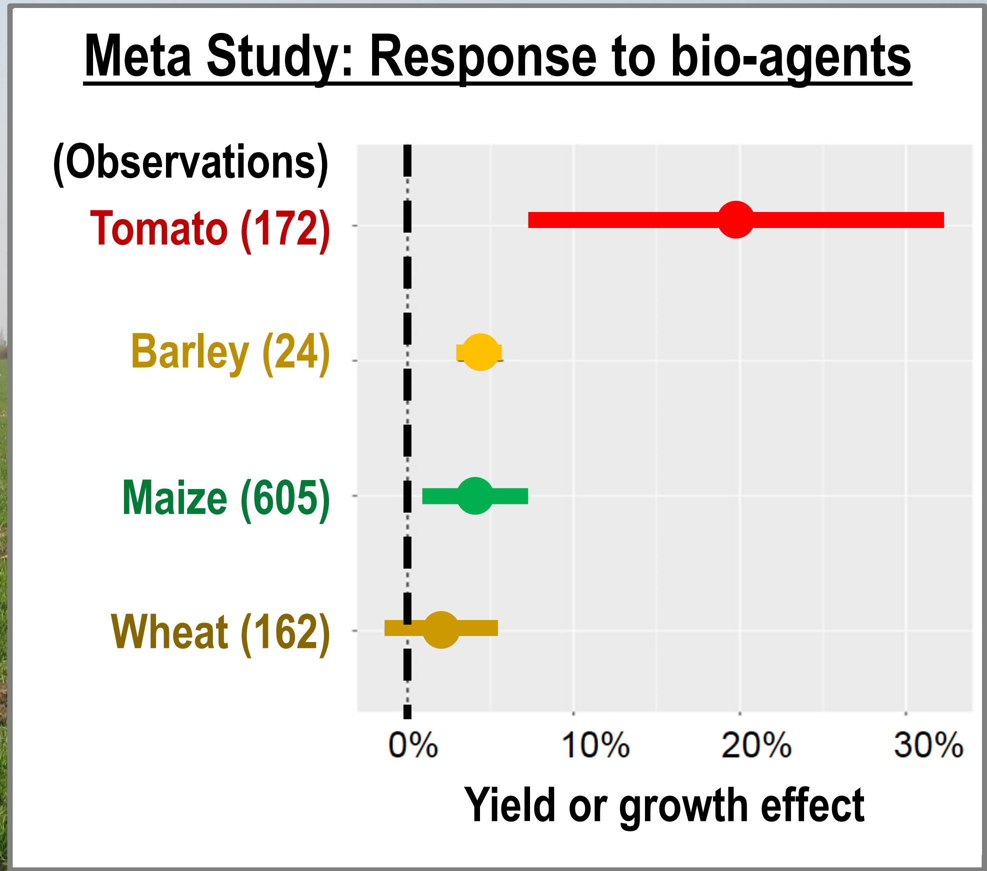
13 commercial products

25 new products

➡ Selected bacterial and fungal strains e.g.:

- *Bacillus, Pseudomonas, Azotobacter, …*
- *Trichoderma, Penicillium, …*

➡ Algae- , plant-, compostextracts




**But:** **a Meta Study (> 1100 data sets) shows strong effects in Tomato (mainly greenhouse) rather then agricultural crops.**

# A narrow window for effectiveness

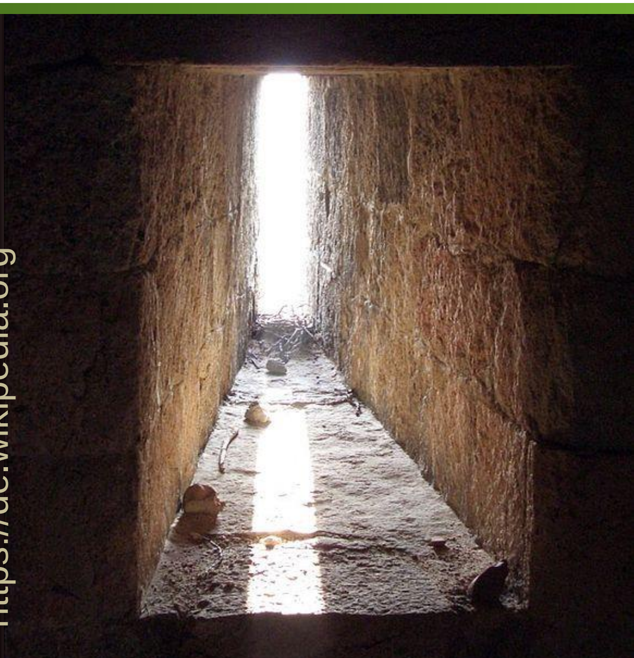


Foto: Michael Vogel,
https://de.wikipedia.org

**A NARROW WINDOW**

A narrow window may let in the light,
A tiny star dispel the gloom of night,
A little deed a mighty wrong set right.

A rose, abloom, may make a desert fair,
A single cloud may darken all the air,
A spark may kindle ruin and despair.

A smile, and there may be an end to strife;
A look of love, and Hate may sheathe the knife;
A word—ah, it may be a word of life!

(Florence Earle Coates, 1850-1927)

## What restricts the effectivenes of bio-effectors under field conditions?

### Critical Factors:

- **Inefficient Root colonization**
  (by microbial bio-effectors)
- **Stress factors hampering root growth and activity**
  (drought, heat, cold, water logging, oxygene deficiency, pathogens …)
- **Sufficient or luxury supply of mineral nutrients**

# Biological Agents in the scientific Literature

## Recent science is focussing on genetic and molecular aspects

*Table: Survey of articles published from 1980 to 2014 on micobial species*

| Database | Descriptors mentioned in titles: *Azospirillum*, *Bacillus*, *Pseudomonas*, *Rhizobium*, and/or *Trichoderma* | | | | |
|---|---|---|---|---|---|
| | Total number | Genetic / molecular | Physiology | Formulation | Field/on-farm application |
| CAB Abstract | 50 000 | 1 500 | 300 | 600 | 200 |
| SCOPUS | 89 000 | 2 900 | 400 | 400 | 100 |
| Google | 125 000 | 6 000 | 1 000 | 500 | 300 |
| **Mean** | **100 %** | **4.1 %** | **0.6 %** | **0.7 %** | **0.2 %** |

**But: Limited attention for formulation, application and agronomic aspects, which are critical for successful practice implementation !**

# Conclusions and Outlook

- **Integral rather than reductionist approaches** in science are necessary to better understand the complexity of soil-plant-microbe interactions

- Integrated **application strategies** are required to support the expression of benefical bio-effector traits under field conditions.

- **One sided legal categorization** as bio-pesticides or bio-stimulants (bio-fertilizers) hampers a holistic view of plant ecology and agricultural problem-solving

- Scientifically sound legal regulations need to be **“all inclusive”** of bio-stimulant, bio-fertilizer, bio-control traits to adequately judge on the multifaceted nature of microbial agents

SolACE is funded by the European Union within the Horizion 2020 Framework Programme, Grant Agreement No. 727247

UNIVERSITY OF HOHENHEIM

# Cordial Thanks !

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SolACE is funded by the European Union within the Horizion 2020 Framework Programme, Grant Agreement No. 727247

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