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Can plant pathogens develop resistance to microbial biocontrol agents?

Marc Bardin

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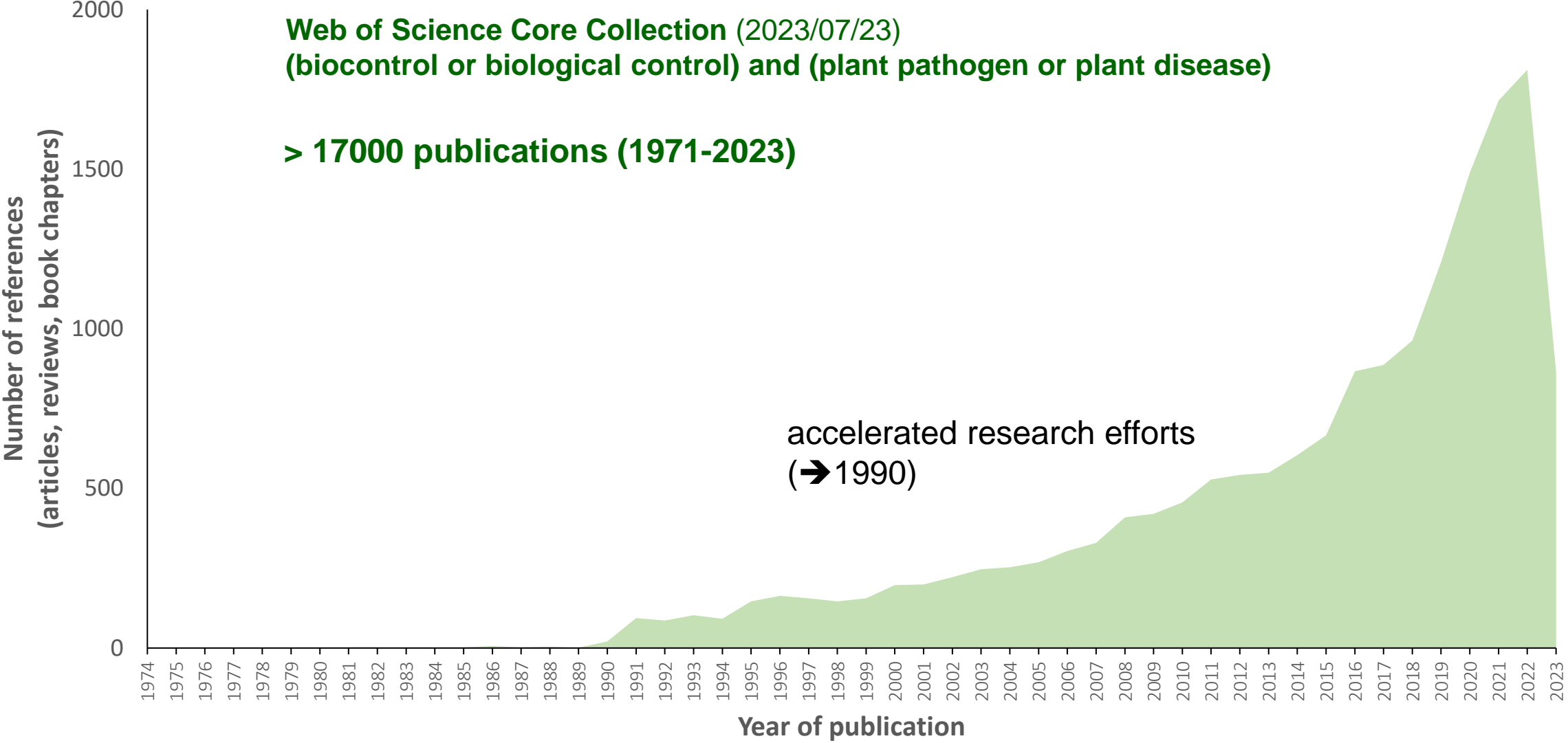


➤ Can plant pathogens develop resistance to microbial biocontrol agents?



Marc Bardin

Biocontrol of diseases: dynamic research field

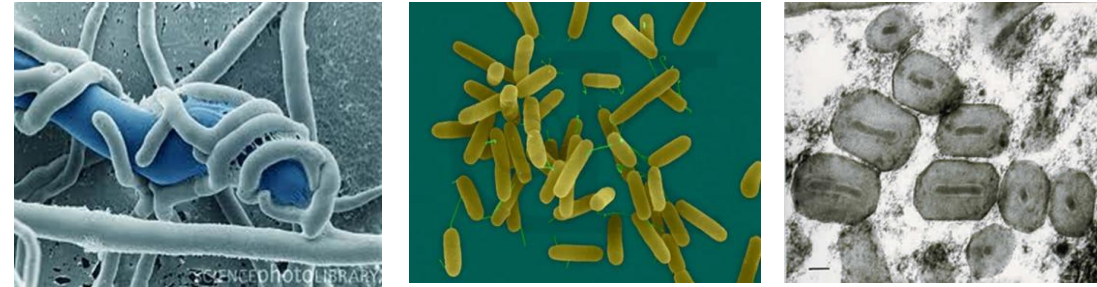


An increasing number of registered biocontrol products for disease management

At world level, against diseases of plants
(virus, bacteria, oomycetes, fungi)

91 microbial biocontrol strains

49 fungi/yeast/oomycetes
+ 37 bacteria/actinomycetes
+ 5 virus/phages



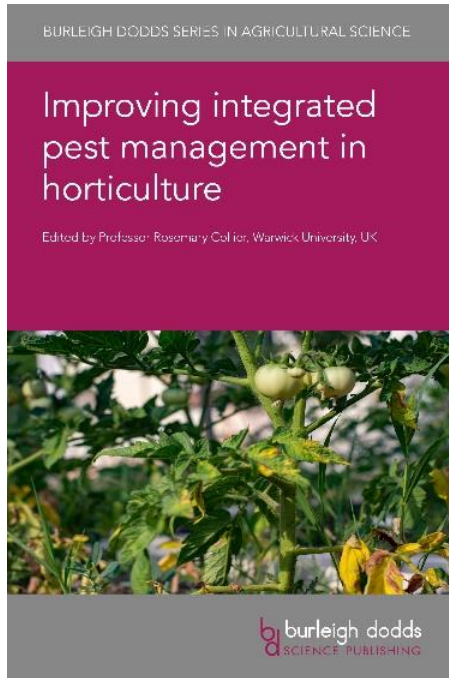
BioControl
DOI 10.1007/s10526-017-9801-4



**Biological control using invertebrates and microorganisms:
plenty of new opportunities**

Joop C. van Lenteren · Karel Bolckmans · Jürgen Köhl ·
Willem J. Ravensberg · Alberto Urbaneja

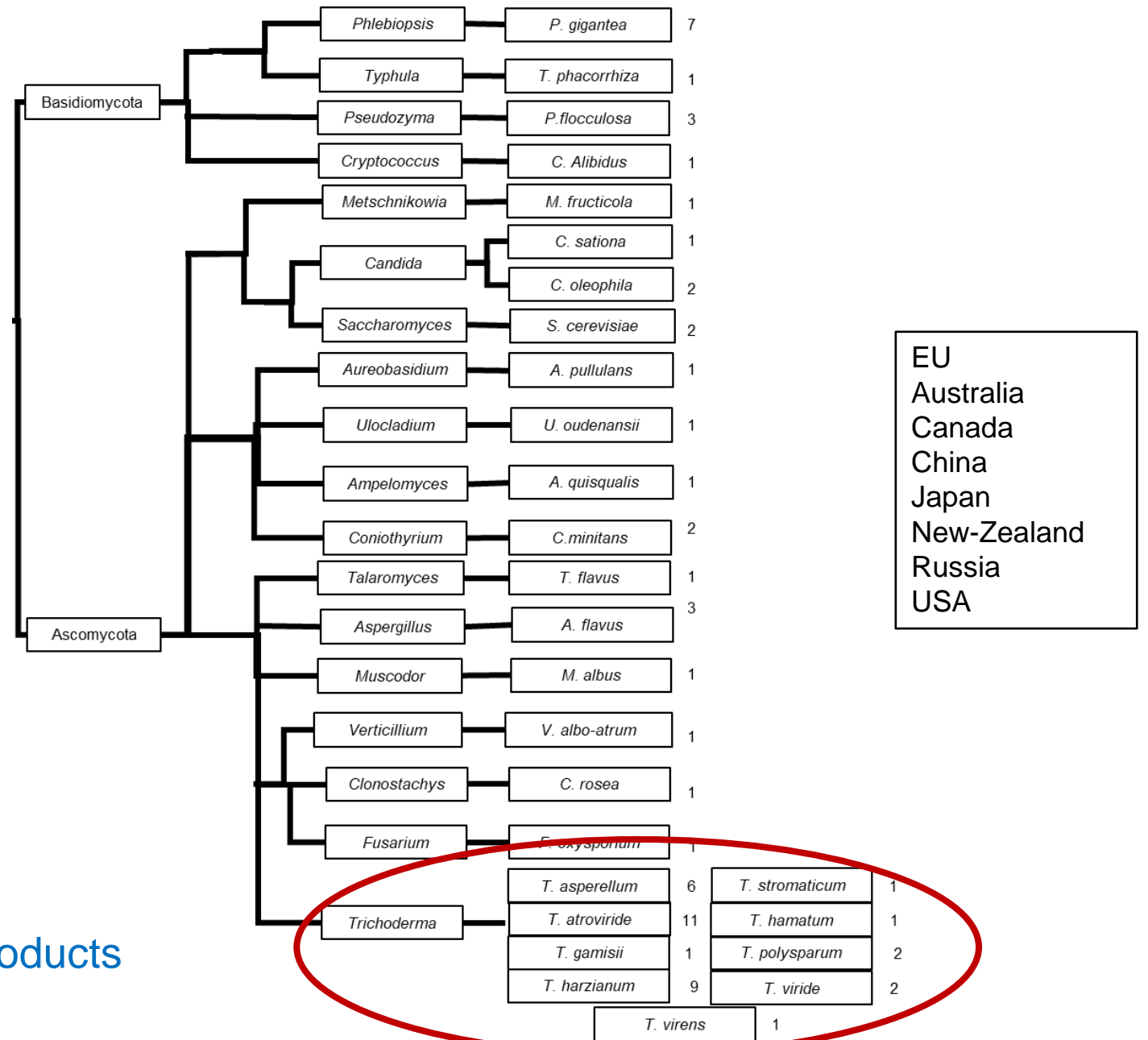
Various fungal species represented



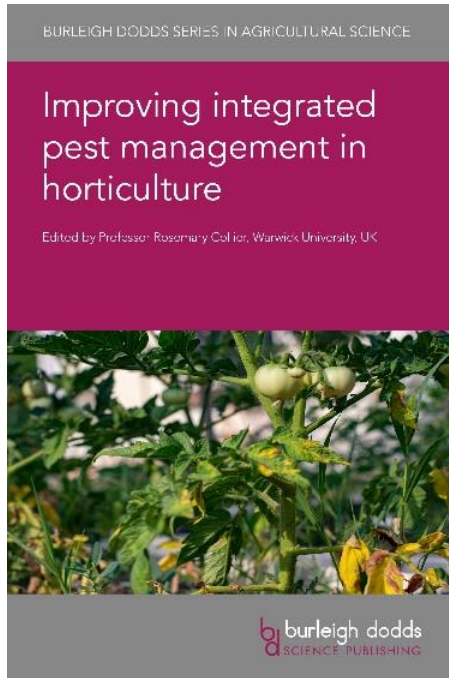
Nicot, Pressecq, Bardin. 2022

65 products (27 species)
including 34 *Trichoderma*-based products

Fungi



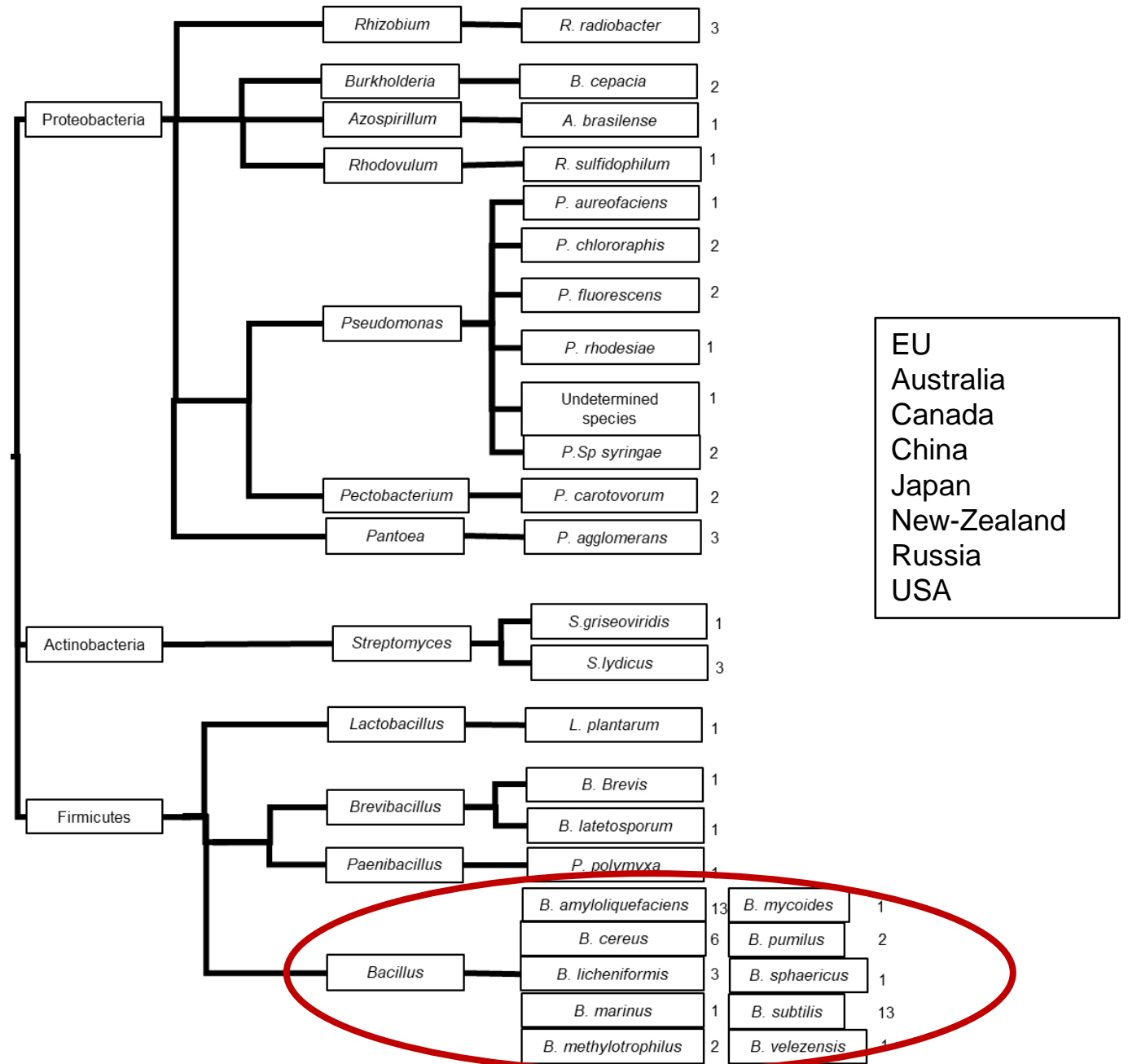
Various bacterial species represented



Nicot, Pressecq, Bardin. 2022

72 products (28 species)
including 43 *Bacillus*-based products

Bacteria



An increasing number of registered biocontrol products for disease management

At world level, against diseases of plants
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91 microbial biocontrol strains

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+ 37 bacteria/actinomycetes
+ 5 virus/phages

41 microbial strains approved in Europe

(EU pesticide database, 07/2023)



➔ **Various modes of action**



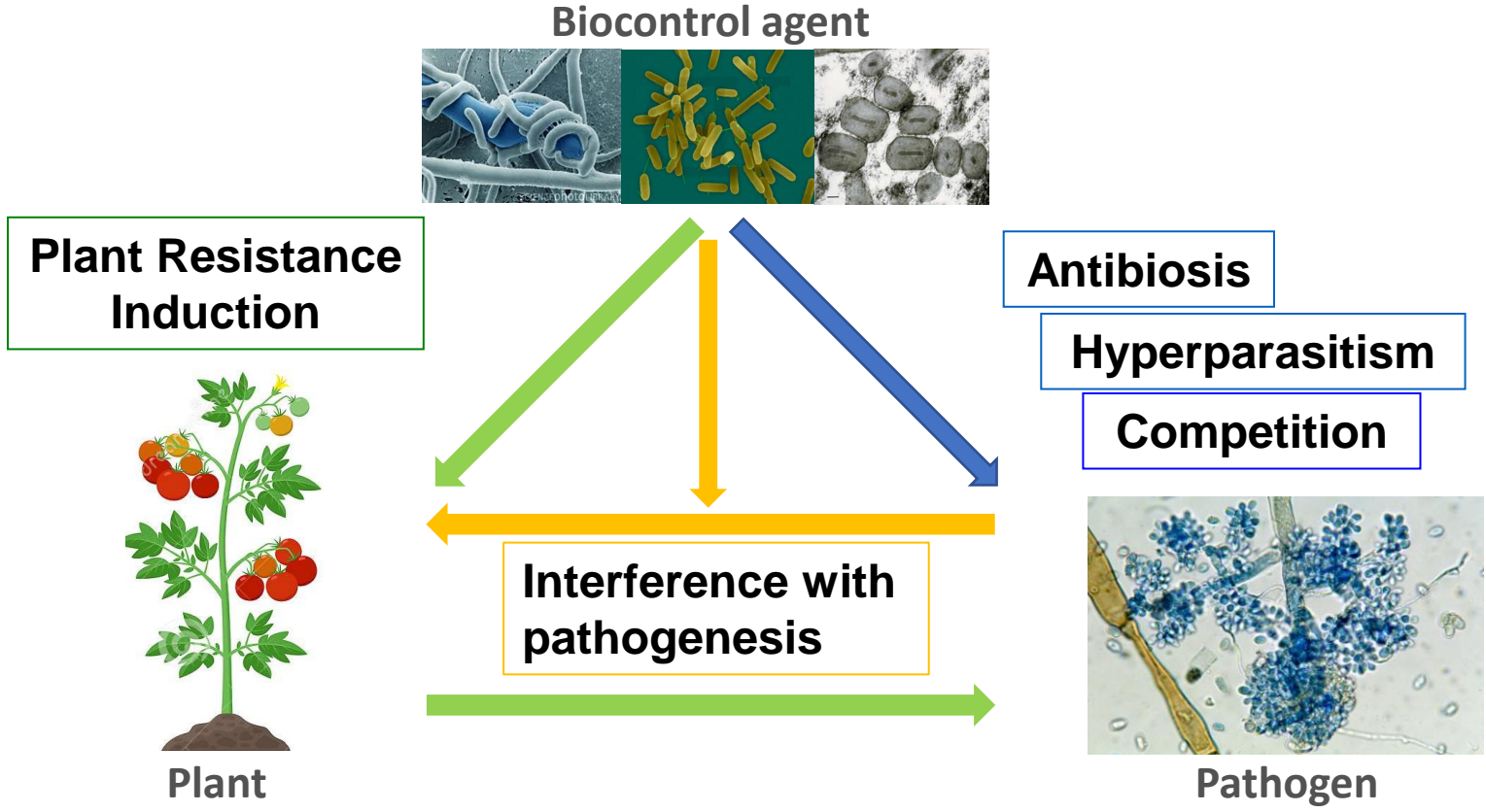
BioControl
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**Biological control using invertebrates and microorganisms:
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Main modes of action of biocontrol agents



Possible combinations

Common case for many biocontrol agents

Microbial Biocontrol Agents
Developing Effective Biopesticides
Edited by Gerardo Puopolo

Modes of Action of Microbial Biocontrol Agents against Plant Diseases

ÉSTELLE TURC^{1,2}, THOMAS PRESSECO^{1,3}, PHILIPPE C. NICOT¹ AND MARC BARDIN^{1*}

¹INRAE, Pathologie Végétale, Montfavet, France
²Groupe Eléphant Vert France, Serris, France
³INRAE, Ecodéveloppement, Avignon, France

CABI

An increasing number of registered biocontrol products for disease management

At world level, against diseases of plants
(virus, bacteria, oomycetes, fungi)

91 microbial biocontrol strains

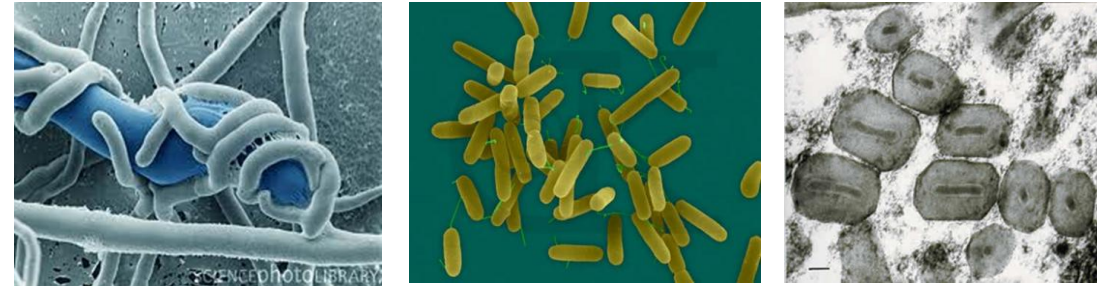
49 fungi/yeast/oomycetes
+ 37 bacteria/actinomycetes
+ 5 virus/phages

41 microbial strains approved in Europe

(EU pesticide database, 07/2023)

Mission accomplished ?

→ How are these products actually used by farmers?



BioControl
DOI 10.1007/s10526-017-9801-4



**Biological control using invertebrates and microorganisms:
plenty of new opportunities**

Joop C. van Lenteren · Karel Bolckmans · Jürgen Köhl ·
Willem J. Ravensberg · Alberto Urbaneja

Use of microbial biocontrol against diseases



Thomas
PRESSECC

Survey of French vegetable farmers and farm advisors

- **Online questionnaires disseminated** to more than 500 professionals (217 answers):

2 key lessons from the analysis of responses:

- ✓ Only few biocontrol products known (21/32) and used (15)
- ✓ **Efficacy perceived as low**

- **Face-to-face interviews performed** (42 / 217 respondents):

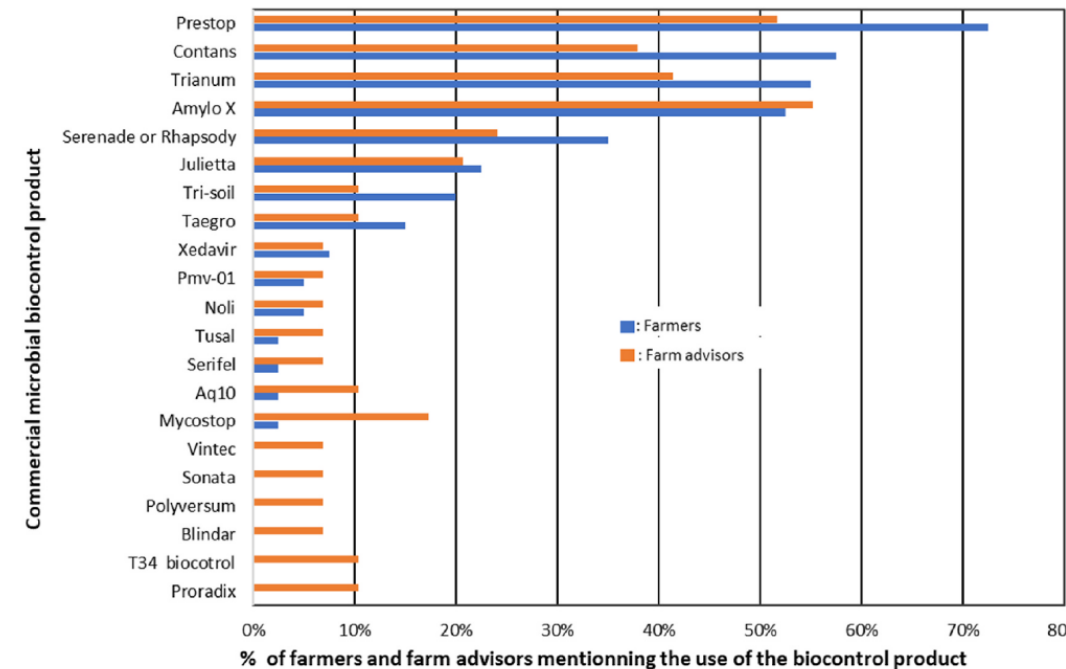
3 key lessons

- ✓ Aware of possible benefits of applying biocontrol products to manage plant diseases

But...

- ✓ Difficulties in using biocontrol (with other cultural practices) and need for guidelines
- ✓ **Unstable efficacy**

➔ **What are the possible causes for this instability in the efficacy of protection?**



Example of factors that can modulate the efficacy of biocontrol against plant diseases

Can available scientific information be mobilised to ensure the efficacy of microbial biocontrol agents against plant diseases in the field?

Thomas Pressecq^{1,2,3}, Philippe C. Nicot¹, Jean François Bourgeay¹, Aurélie Rousselin³, Claire Gouillon³, Marc Tchamitchian², Marc Bardin¹

Climatic conditions



Cultural practices

Agron. Sustain. Dev. (2014) 34:641–648
DOI 10.1007/s13593-013-0168-3

RESEARCH ARTICLE

Nitrogen fertilization impacts biocontrol of tomato gray mold

Manzoor A. Abro · François Lecompte · Marc Bardin · Philippe C. Nicot



Other phytosanitary treatments including biocontrol

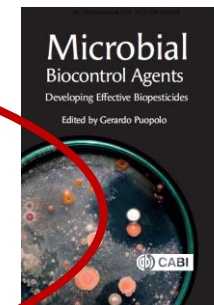
Biological Control 46 (2008) 476–483

Compatibility between biopesticides used to control grey mould, powdery mildew and whitefly on tomato

M. Bardin^{a,*}, J. Fargues^b, P.C. Nicot^a

^aINRA, Unité de Pathologie Végétale, UR 407, F-84140 Montfavet, France
^bINRA, Centre de Biologie et de Gestion des Populations, Campus international de Baillarguet, CS 30 016, 34988 Montpellier-sur-Léz cedex, France

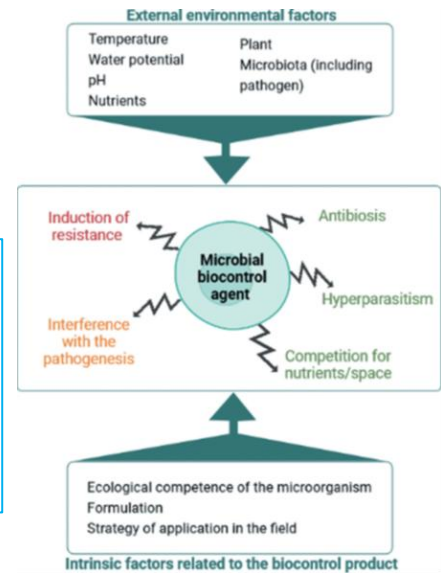
Mode(s) of action and their activation



Modes of Action of Microbial Biocontrol Agents against Plant Diseases

ESTELLE TURC^{1,2}, THOMAS PRESSECCQ^{1,3}, PHILIPPE C. NICOT¹ AND MARC BARDIN^{1*}

¹INRAE, Pathologie Végétale, Montfavet, France
²Groupe Eléphant Vert France, Serris, France
³INRAE, Ecodéveloppement, Avignon, France



Resistance of the target pathogen to biocontrol

frontiers in Plant Science

REVIEW published: 27 July 2015
doi: 10.3389/fpls.2015.00505

Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides?

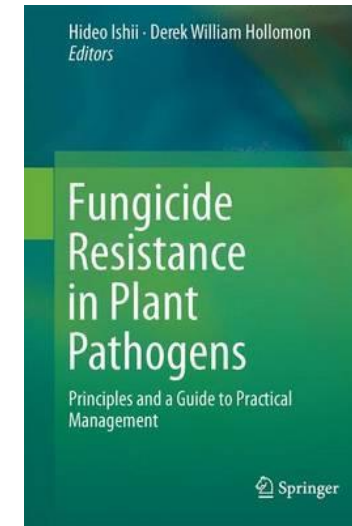
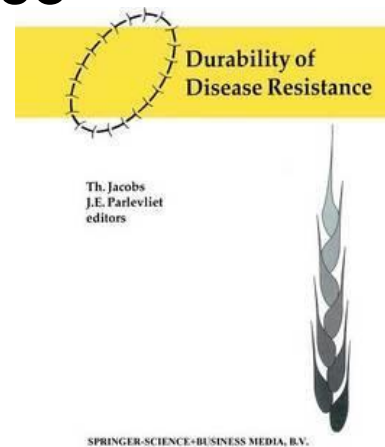
Marc Bardin^{1*}, Sakhr Ajouz¹, Morgane Comby¹, Miguel Lopez-Ferber², Benoit Graillet^{3,4}, Myriam Stegwart⁴ and Philippe C. Nicot¹

¹Plant Pathology Unit, Institut National de la Recherche Agronomique, UR407, Montfavet, France, ²Laboratoire de Climat, Environnement Industriel, Ecole des Mines d'Albi, Institut Mines-Télécom, Albi, France, ³Natural Plant Pathology, Arysta LifeScience Group, Pau, France, ⁴Plantes et Systèmes de Culture Horticoles Unit, Institut National de la Recherche Agronomique, UR1115, Avignon, France

Could resistance of the pathogens to protection tools be a factor explaining the variability of efficacy ?

well known in other contexts

- Development of resistance to fungicides
- Break-down of plant resistance



Durable resistance of crops to disease: A darwinian perspective
Annual Review of Phytopathology Brown, 2015

• Biological control ?

- ✓ comparatively limited deployment → little information
- ✓ often considered as highly durable by the biocontrol community



Durability of biocontrol against insect pests?

not always durable

Resistance to bio-insecticides or how to enhance their sustainability: a review

Myriam Siegwart^{1*}, Benoit Graillot^{2,3}, Christine Blachere Lopez⁴, Samantha Besse³, Marc Bardin⁵, Philippe C. Nicot⁵ and Miguel Lopez-Ferber²

• Field resistance to *Bacillus thuringiensis*



Indian meal moth
(*Plodia interpunctella*)

McGaughey, 1985



Diamondback moth
(*Plutella xylostella*)

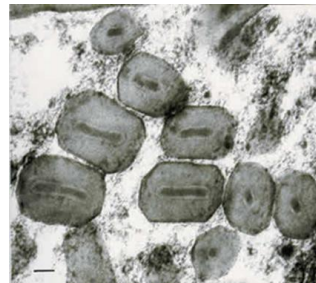
Tabashnik, 1994



Cabbage looper
(*Trichoplusia ni*)

Janmaat & Myers, 2003

• Field resistance to Codling moth granulovirus



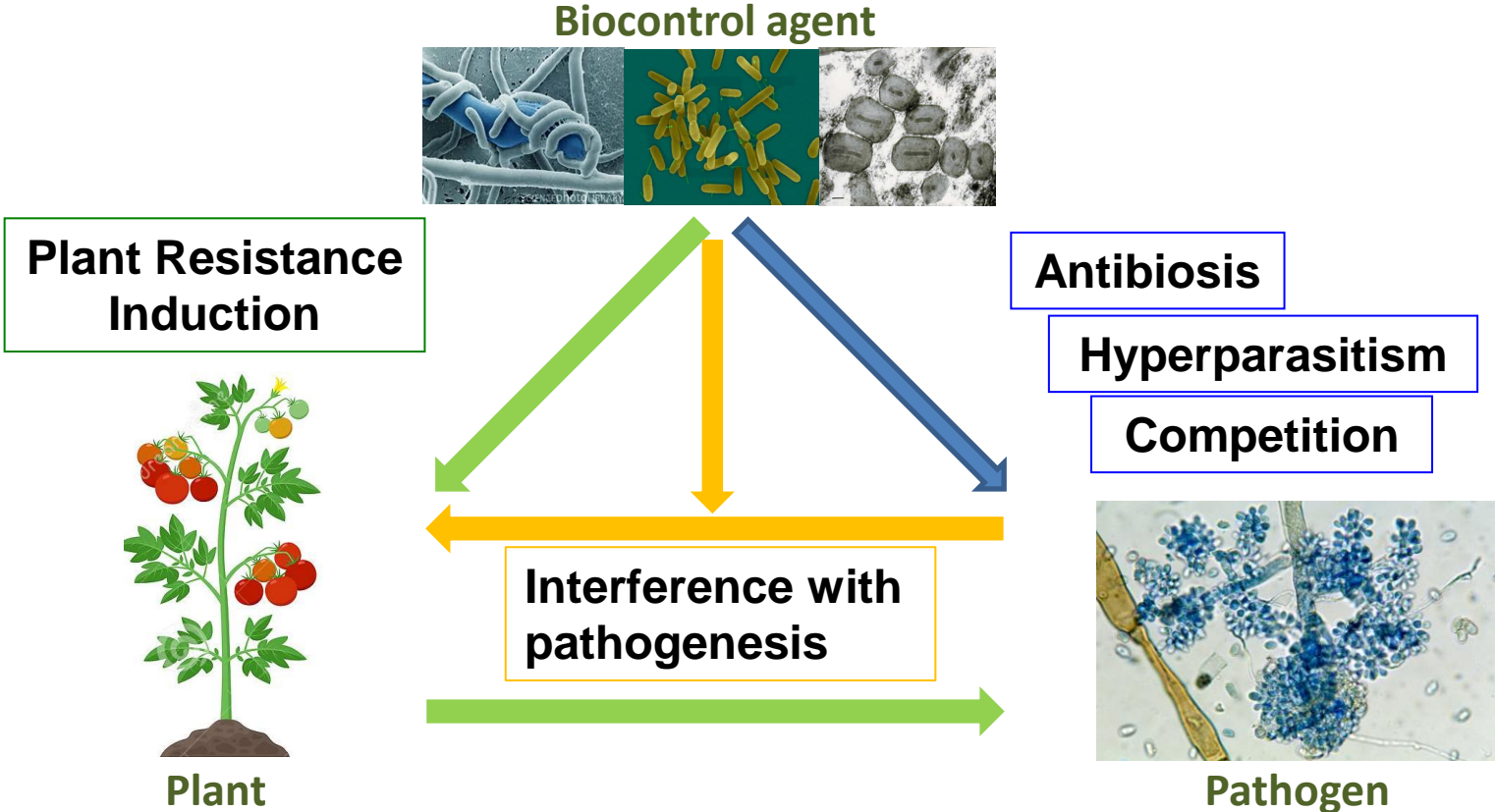
Codling moth
(*Cydia pomonella*)

Fritsch et al., 2005; Eberle and Jehle, 2006;
Sauphanor et al., 2006; Berling et al., 2009



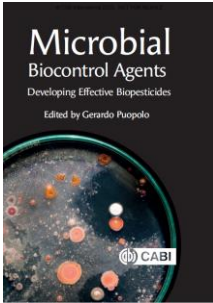
Durability of biocontrol against plant pathogens?

What is the diversity of sensitivity of pathogens to biocontrol ?



Possible combinations

Common case for many biocontrol agents
Situation most likely to show stability

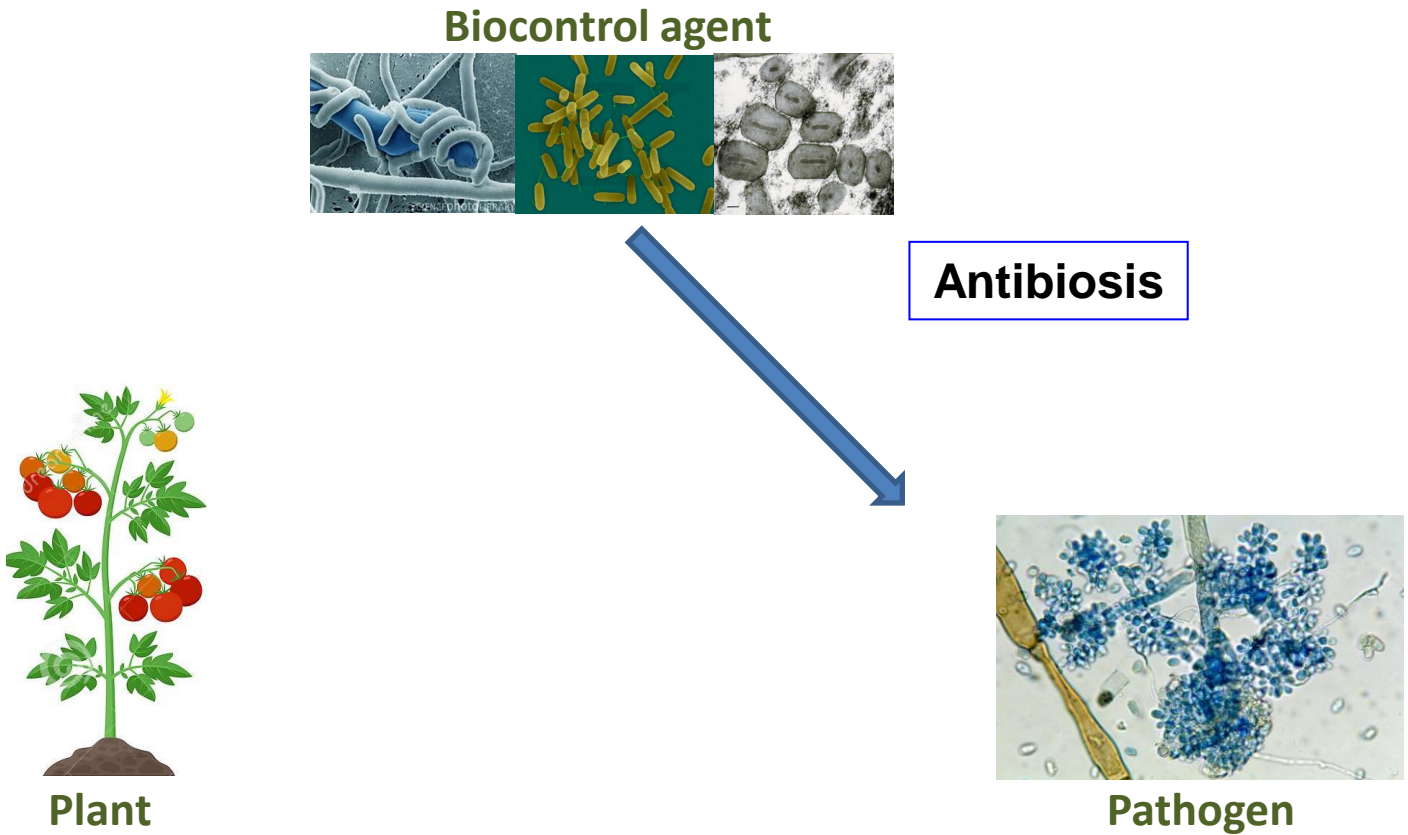


Modes of Action of Microbial Biocontrol Agents against Plant Diseases

ESTELLE TURC^{1,2}, THOMAS PRESSECO^{1,3}, PHILIPPE C. NICOT¹ AND MARC BARDIN^{1*}

¹INRAE, Pathologie Végétale, Montfavet, France
²Groupe Éléphant Vert France, Serris, France
³INRAE, Ecodéveloppement, Avignon, France

Diversity of sensitivity of plant pathogens to biocontrol agents ?



Diversity of sensitivity of plant pathogens to biocontrol agents ?



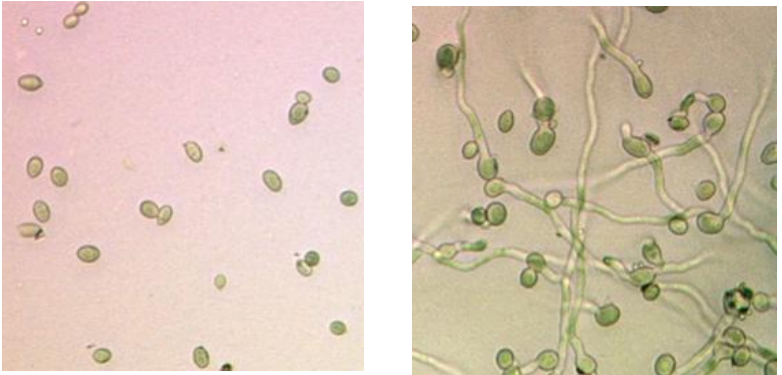
Ajouz et al. (2011)
BioControl 56:353

1. Antibiosis: *in vitro* inhibition by a single metabolite produced by biocontrol agents

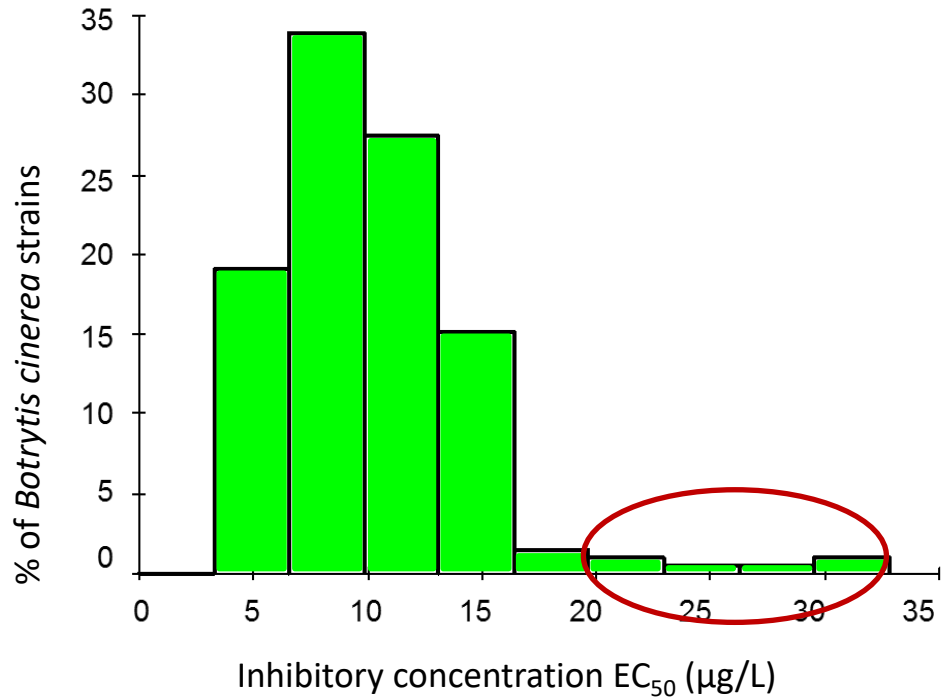
- **pyrrolnitrin** (*Serratia plymuthica*, Pseudomonads)

Structural analogue of phenylpyrrole fungicides

✓ *Botrytis cinerea* (204 strains)



Spore germination tests



- Wide range of sensitivity among 204 strains
- **existence of less sensitive strains:**
resistance factor ~8 fold between most and least sensitive strains

➔ **less sensitive to the biocontrol agents ?**

Diversity of sensitivity of plant pathogens to biocontrol agents ?

1. Antibiosis: *in vitro* inhibition by various compounds produced by biocontrol agents

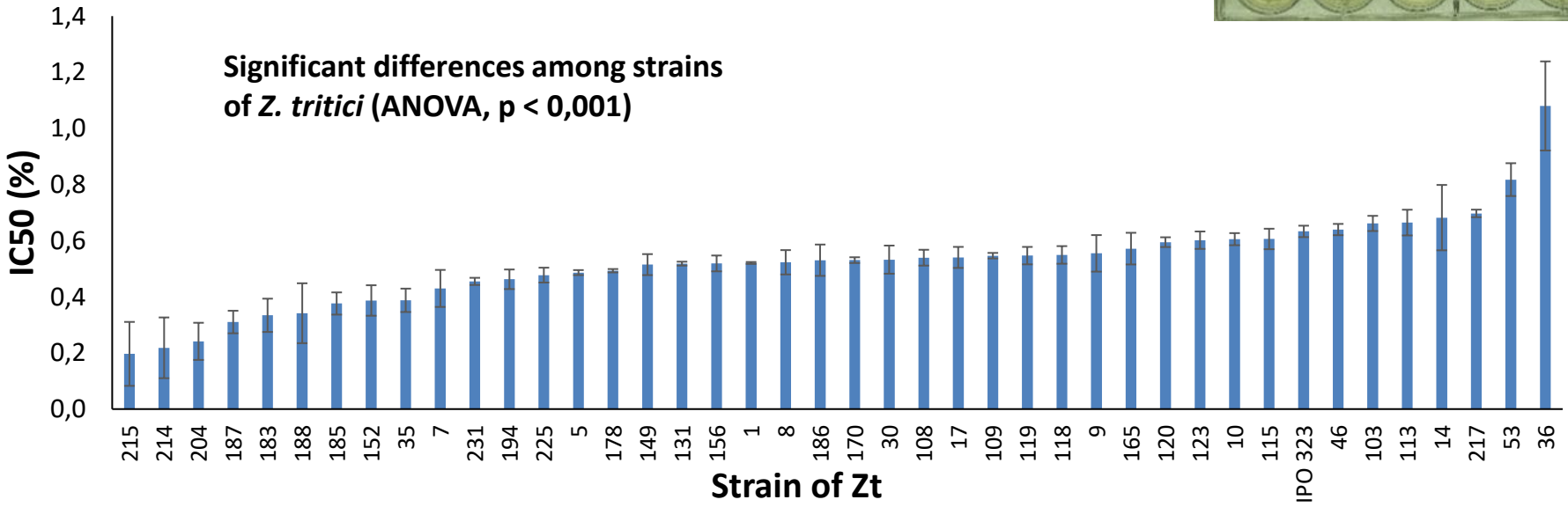
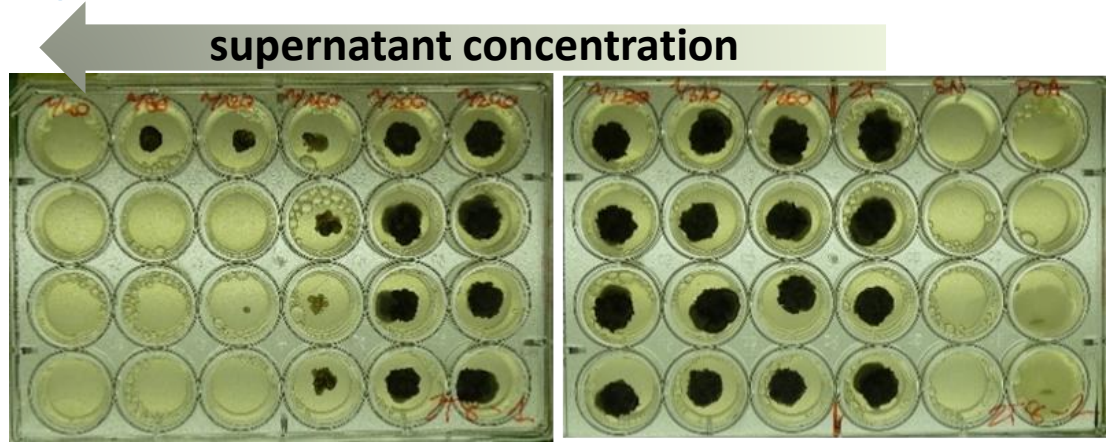
- *Pseudomonas* supernatant containing lipopeptides and other metabolites

✓ *Zymoseptoria tritici* (41 strains)



Musetta M (2022)
M1 internship

Mycelium growth tests
→ Measurement of IC50



→ One isolate less sensitive to the supernatant ?

Diversity of sensitivity of plant pathogens to biocontrol agents ?



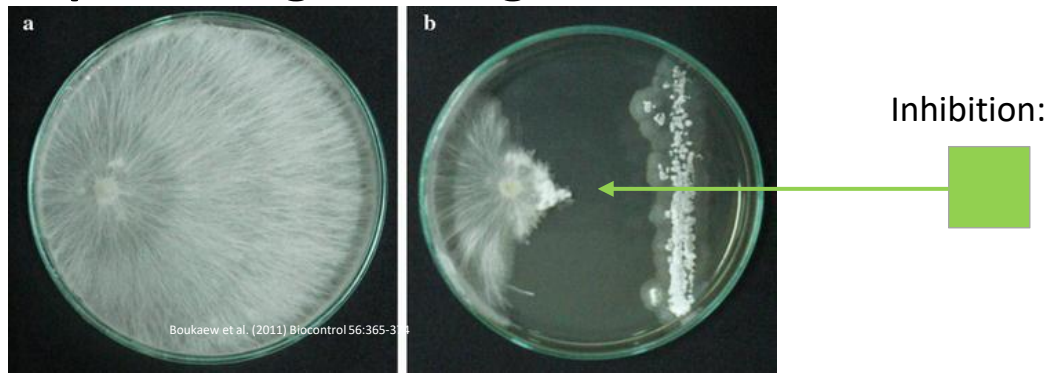
Boukaew et al. (2017)
BioControl 62:793

1. Antibiosis: inhibition by biocontrol agents producing antifungal metabolites

- *Streptomyces* spp.

Production of various antimicrobial compounds

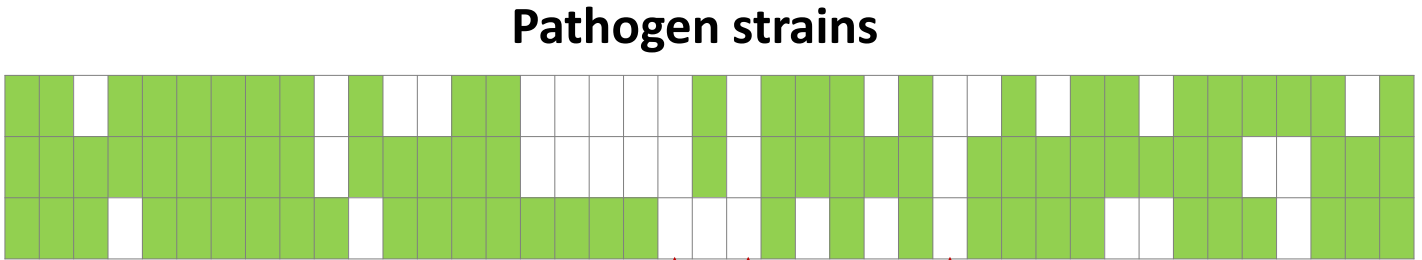
✓ *Botrytis cinerea* (41 strains)



Assay in Petri plates

3 antagonistic strains

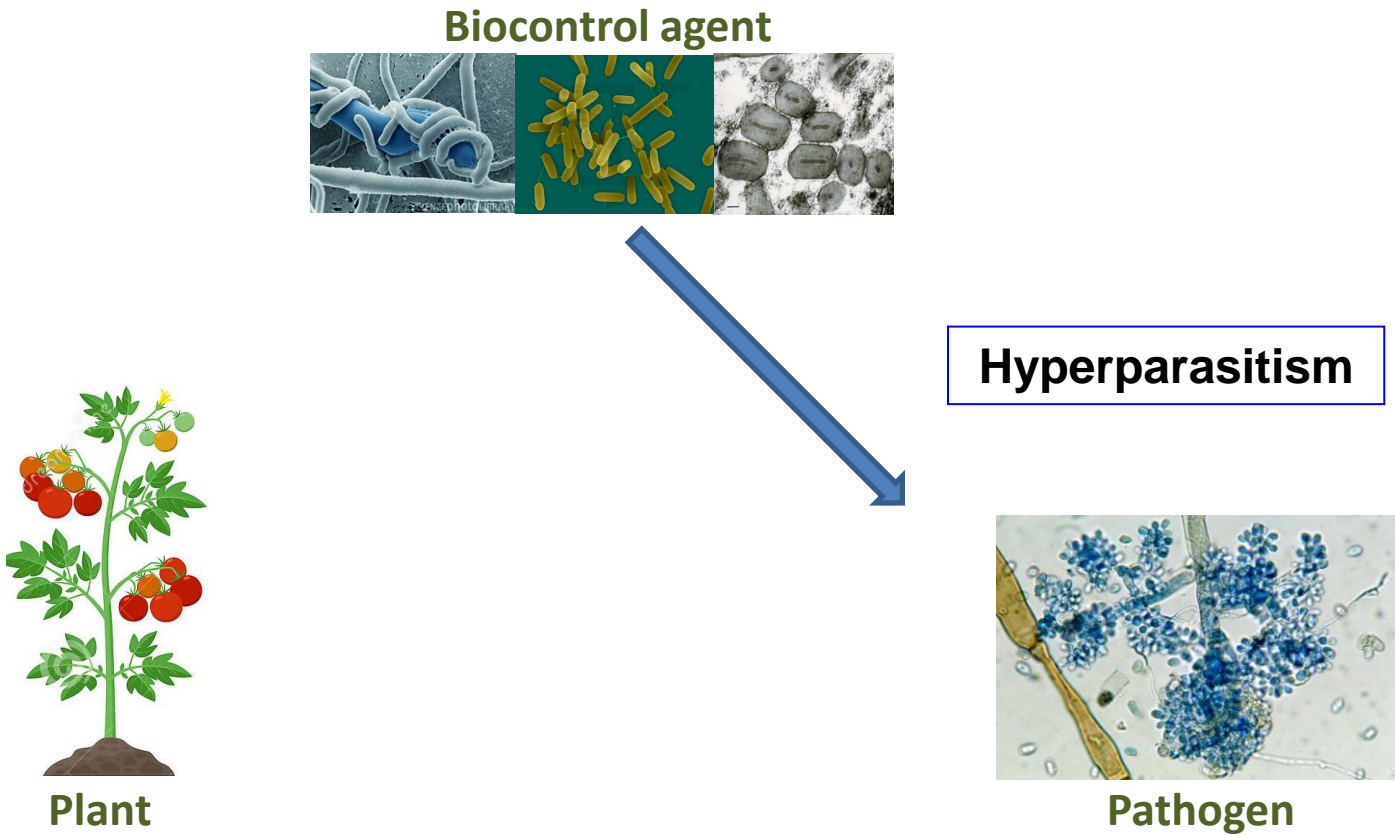
S. mycarofaciens SS-2-243
S. philanthi RL-1-178
S. philanthi RL-1-138



3 strains of *B. cinerea* not fully inhibited by any of the 3 bacteria

Wide spectrum of sensitivity to 3 antagonistic strains of *Streptomyces* sp.

Diversity of sensitivity of plant pathogens to biocontrol agents ?


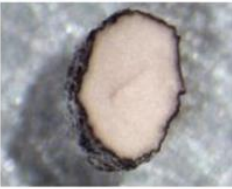




Diversity of sensitivity of plant pathogens to biocontrol agents ?

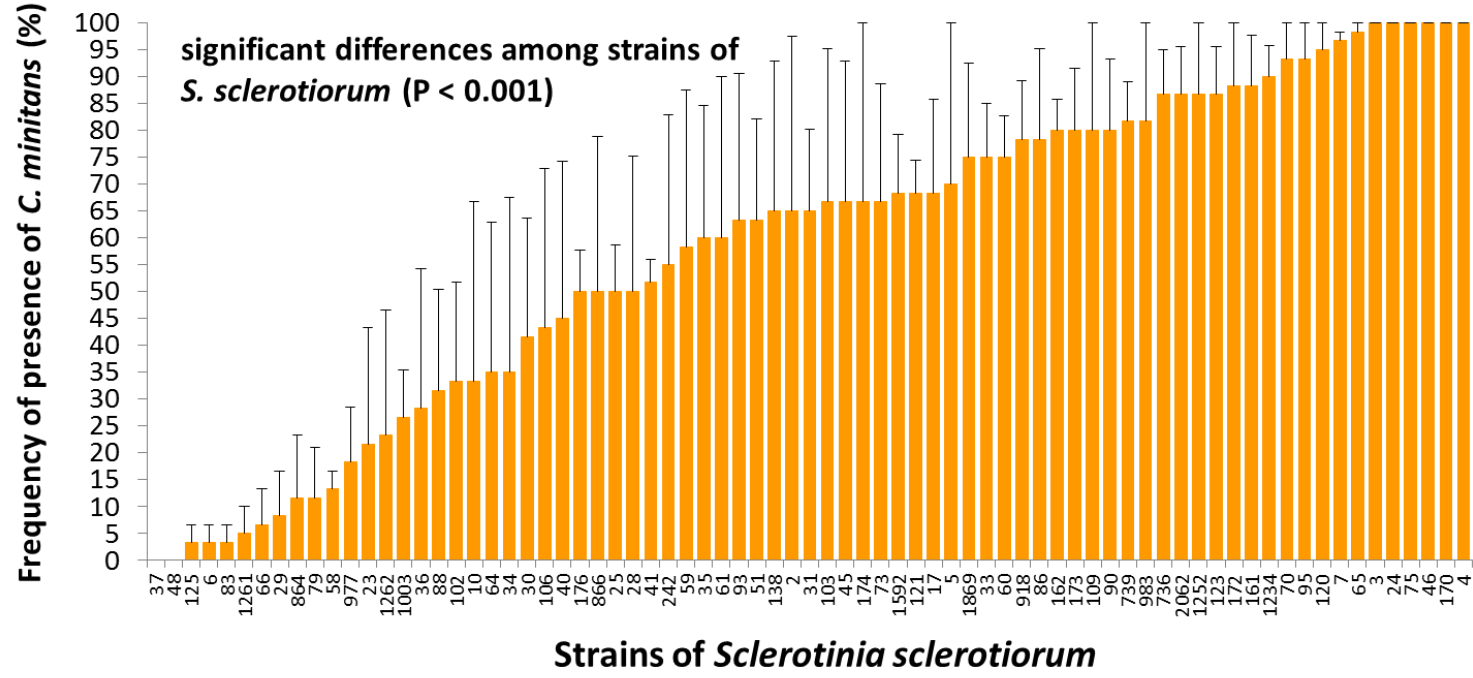
2. Hyperparasitism

- *Coniothyrium minitans*
- ✓ *Sclerotinia sclerotiorum* (75 strains)

Colonization and destruction of sclerotia of *Sclerotinia sclerotiorum*

Time after inoculation (days)	External appearance	Internal appearance
0 – healthy sclerotia		
28 – presence of pycnidia (arrows) externally and internally		

➔ Ability of the **mycoparasite** to penetrate sclerotia of *S. sclerotiorum*

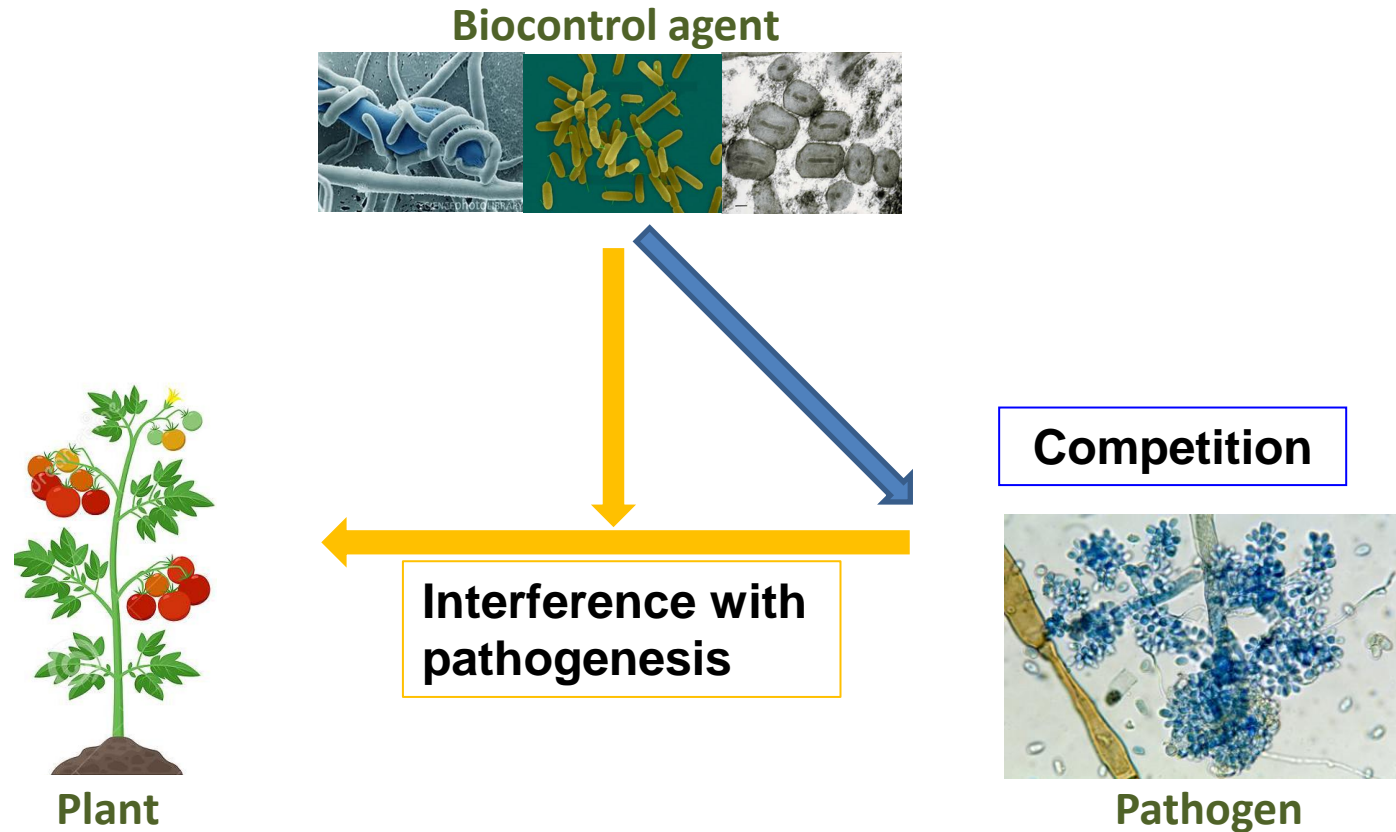


Nicot et al. (2019) Trop. plant pathol. 44:82

Assay in sterilized sand

Very high diversity in the susceptibility of *S. sclerotiorum* to the action of the mycoparasite

Diversity of sensitivity of plant pathogens to biocontrol agents ?



Combinations of several modes of action

Common case for many biocontrol agents
Situation most likely to show stability

Diversity of sensitivity of plant pathogens to biocontrol agents ?

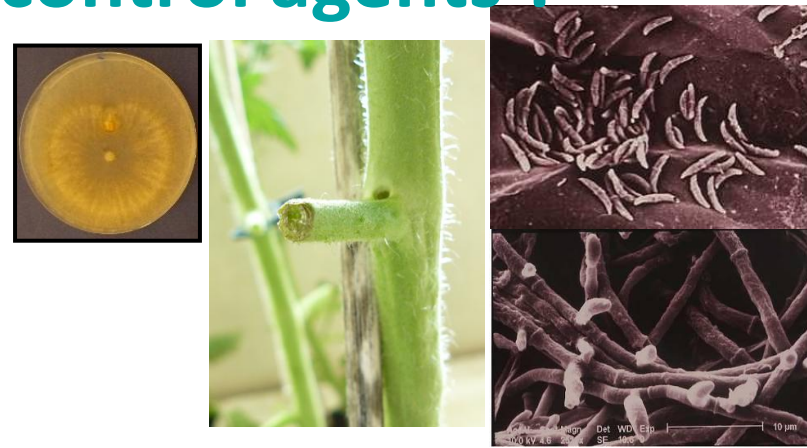
3. Combination of several modes of action

- Fusarium sp.* strain L13

- ✓ *Botrytis cinerea* (41 strains)

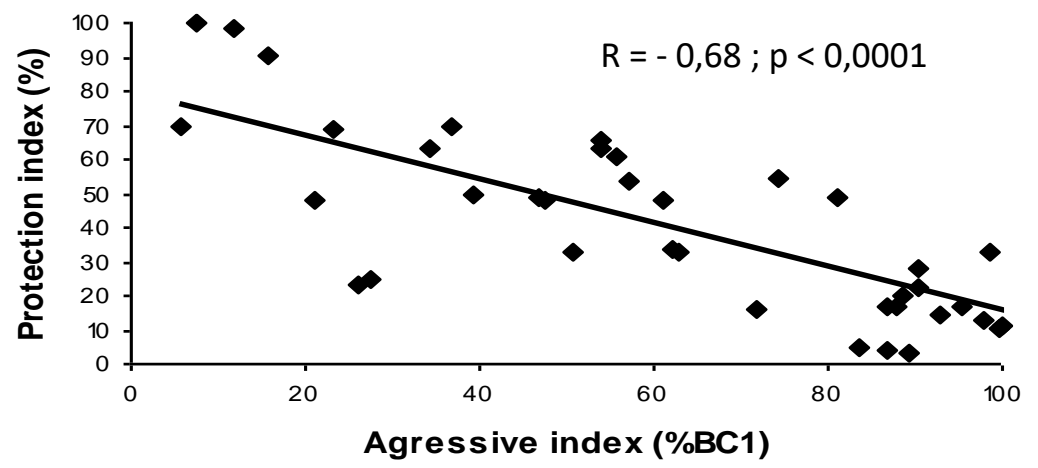
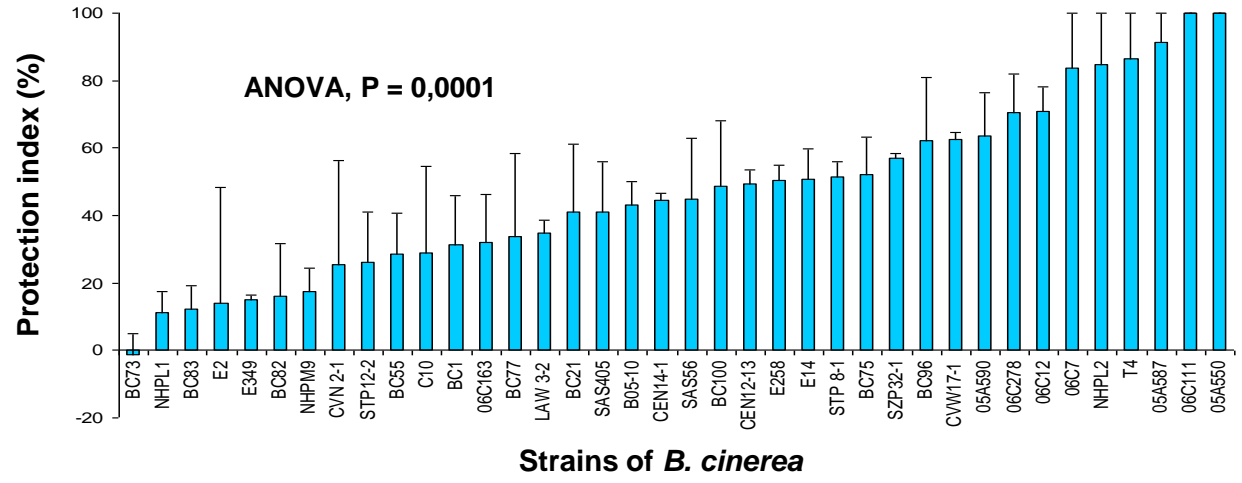
Bardin et al. (2013)
IOBC Bulletin 86:163

Competition,
Basification of the target



Assay on detached stem

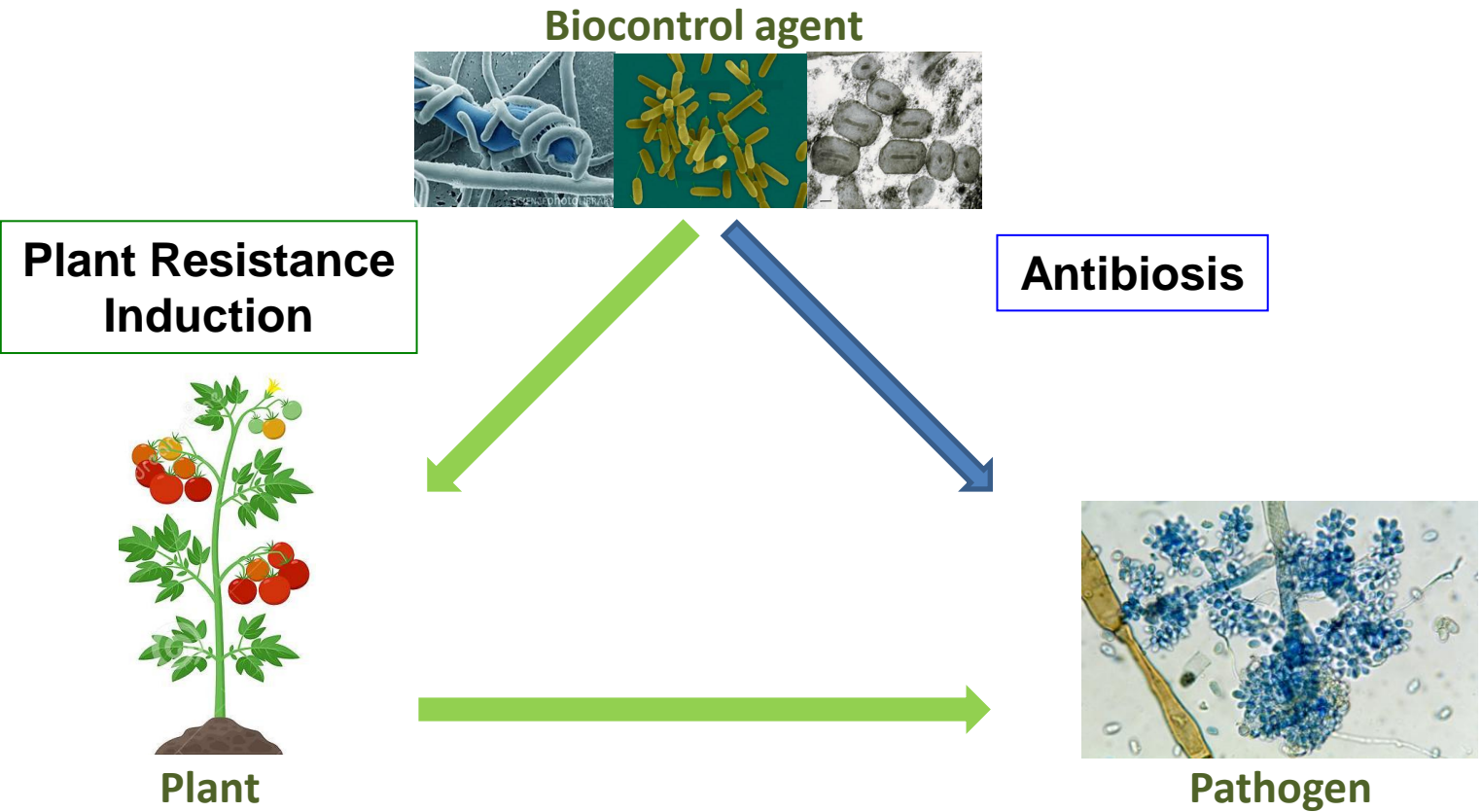
B. cinerea 10⁶ spores/ml + L13 10⁶ spores/ml



Very high diversity in the susceptibility of *B. cinerea* at a low dose of the biocontrol agent

Aggressiveness ++ → Protection --

Diversity of sensitivity of plant pathogens to biocontrol agents ?



Combinations of several modes of action

Common case for many biocontrol agents
Situation most likely to show stability

Diversity of sensitivity of plant pathogens to biocontrol agents ?

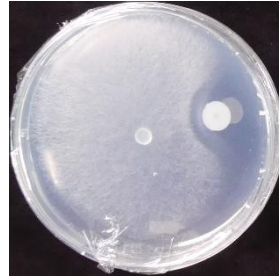
4. Combination of several modes of action

- a bacterial strain

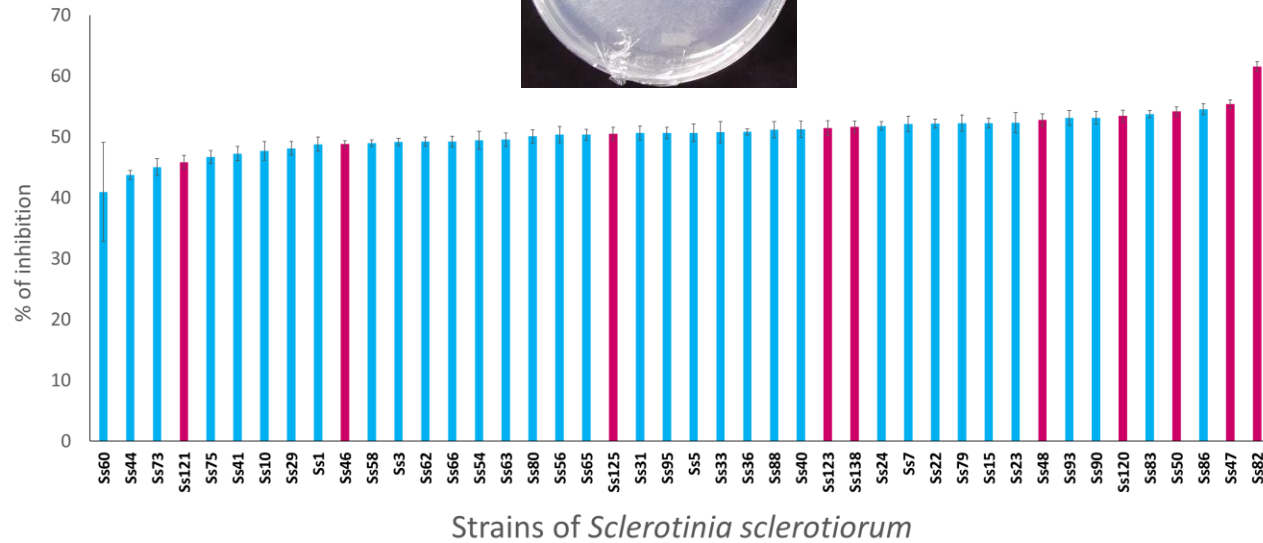
Combination of antibiosis and induced resistance



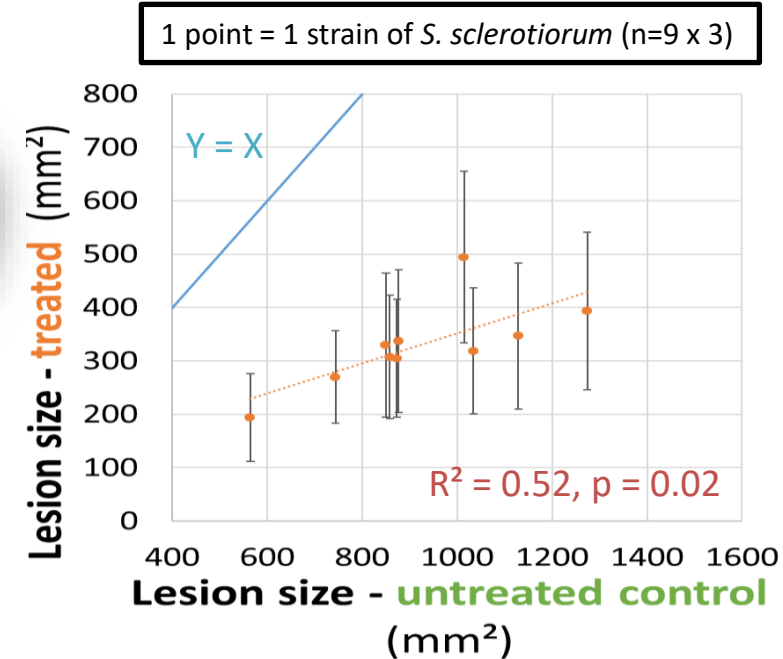
✓ *Sclerotinia sclerotiorum* (45 strains)



In vitro assay



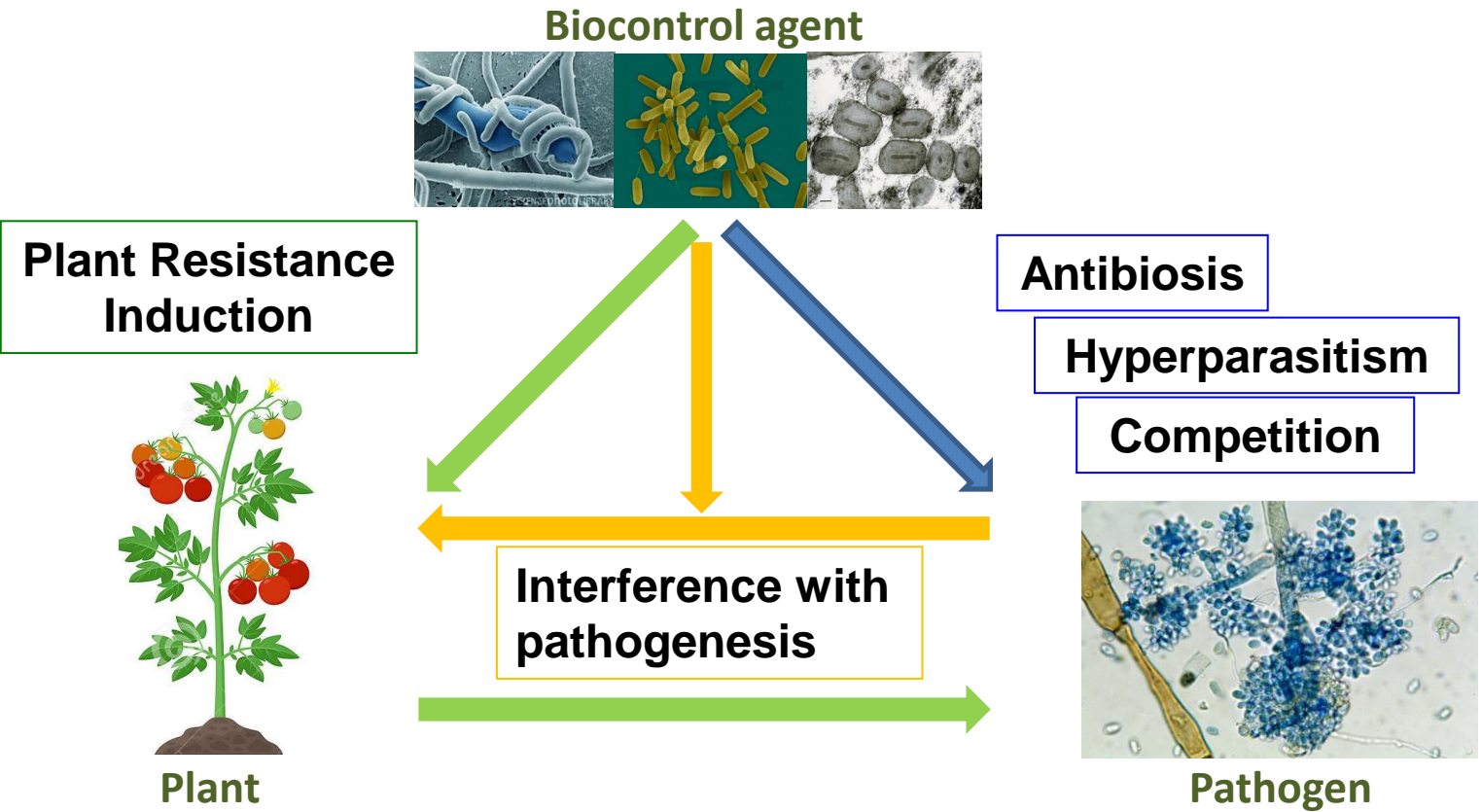
Assay on detached leaves – 10 strains



Significant differences between strains of *S. sclerotiorum*

Significant decrease in biocontrol efficacy with increasing strain aggressiveness

Diversity of sensitivity of plant pathogens to biocontrol agents ?



Possible combinations

Common case for many biocontrol agents
Situation most likely to show stability

Diversity of sensitivity of plant pathogens to biocontrol agents ?

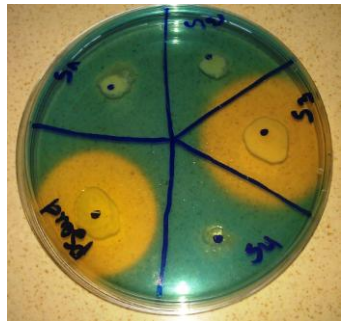
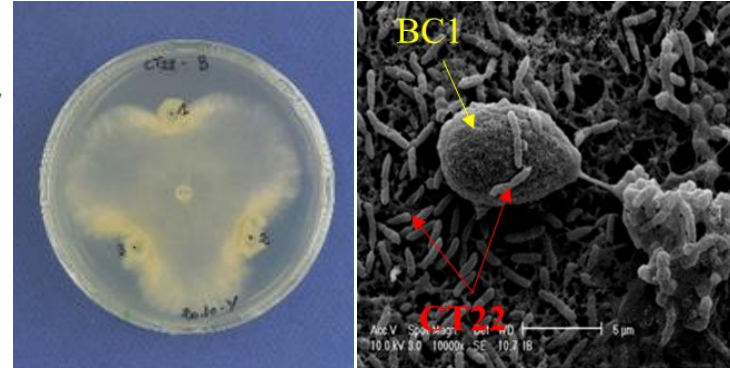
5. Combination of several modes of action

- Pseudomonas helmanticensis* CT22

- ✓ *Botrytis cinerea* (62 strains)

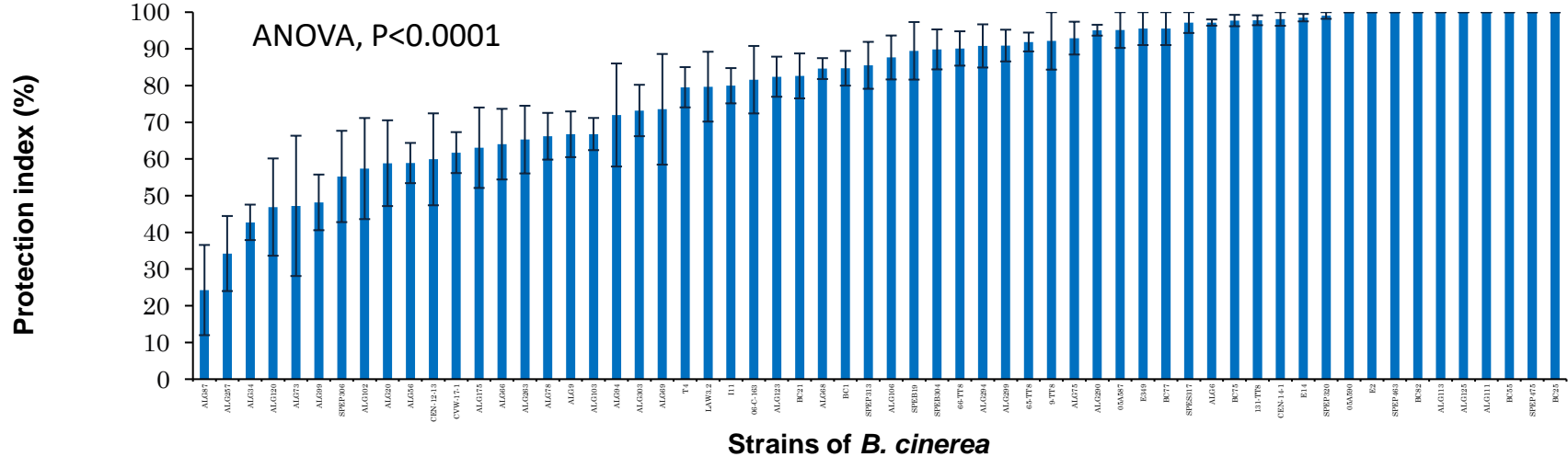
Mode of action under study but probably multiple

Assay on detached stem



Bouaoud et al. (2018) BioControl 63:299

Bouaoud et al. (2018). IOBC-WPRS Bulletin 133:100

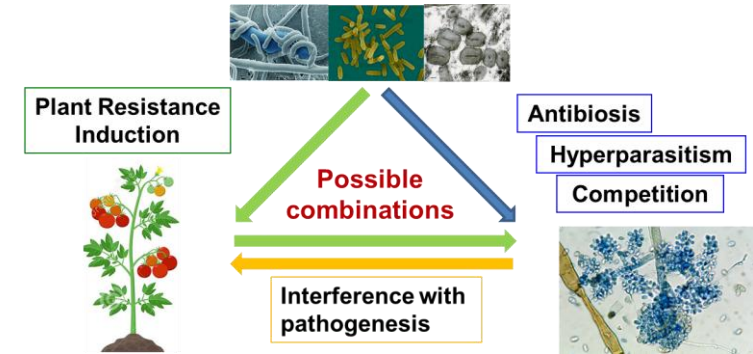


Significant differences of sensitivity of *B. cinerea* strains to CT22

Durability of biocontrol against plant pathogens?

Evidence of a wide diversity in the sensitivity of plant pathogens to biocontrol agents

- despite small number of published studies
- whatever the mode(s) of action of biocontrol agents



Could resistance to biocontrol occur through adaptation of the pathogens to the biocontrol agents?

Could plant pathogens adapt to biocontrol ?

Very few studies

- **One reported case of "field resistance" following repeated use of a biocontrol agent: *Bacillus subtilis* CL27**

Li & Leifert (1994) Z. Pflanzenkr. Pflanzenschutz 101:414–418

- ✓ **Production of 3 antifungal compounds**
 - 2 compounds effective *in vitro* against *Botrytis cinerea*
- ✓ **Protection of *Astilbe* microplants in glasshouse**
 - 8 treatments => decrease in protection efficacy
 - 10 treatments => **complete loss of protection**

- **Evaluation of risk through experimental evolution studies under selection pressure**

- durability of antibiotics in medicine
- adaptation of plant pathogens to fungicides

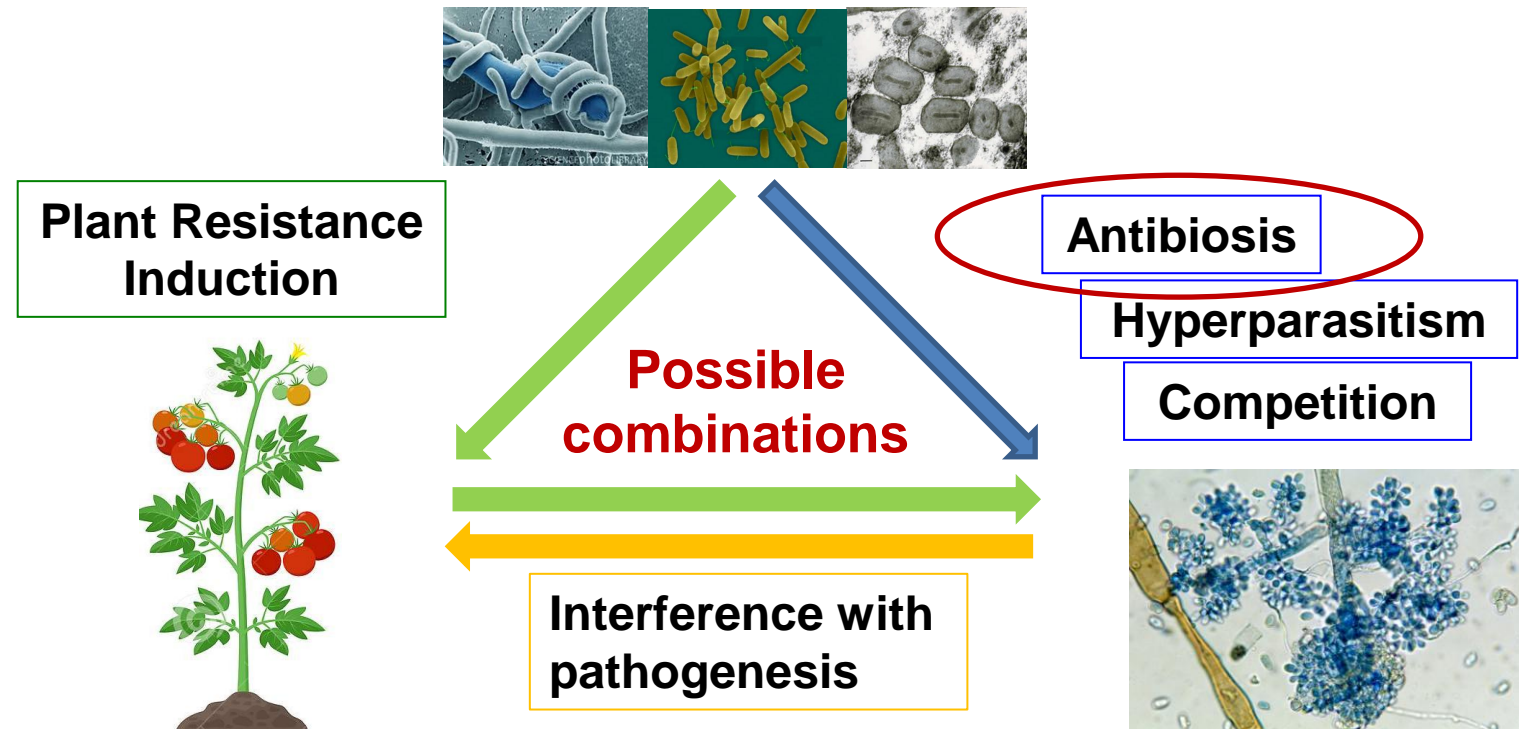


Astilbe hybrida

Could plant pathogens adapt to biocontrol ?

Experimental evolution

Mode of action of biocontrol agents



Could plant pathogens adapt to biocontrol ?



Ajouz et al. (2010)
Plant Pathol. 59, 556
Fillinger et al. (2012)
PLOS One

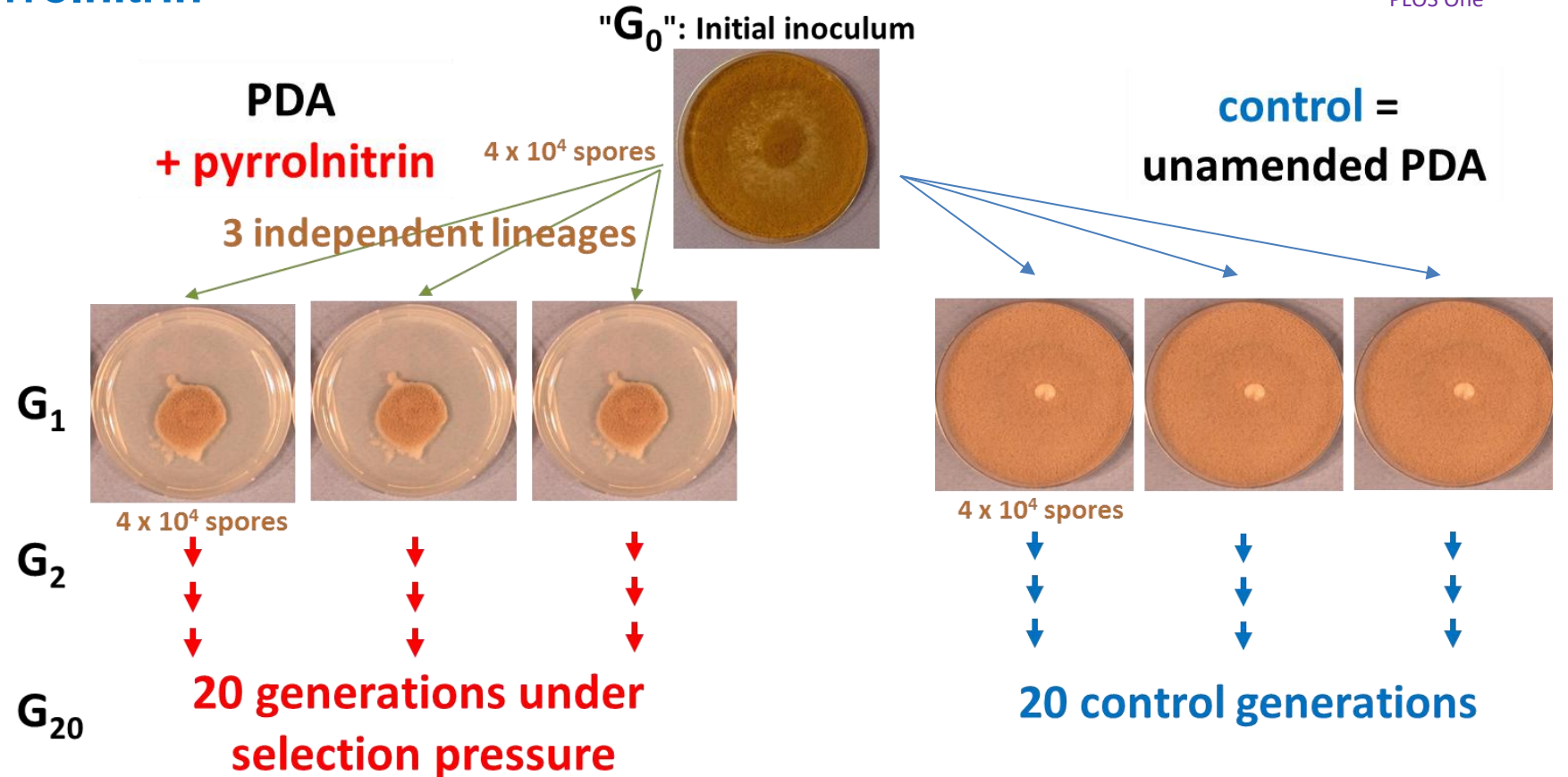
- Experimental evolution study of *Botrytis cinerea* in the presence of pyrrolnitrin

- ✓ Production of successive generations of *B. cinerea* on nutritive medium amended with pyrrolnitrin

Doses of pyrrolnitrin

G_1 to G_{10} : 10 $\mu\text{g/L}$

after G_{11} : gradual increase
from 100 $\mu\text{g/L}$ to 4000 $\mu\text{g/L}$

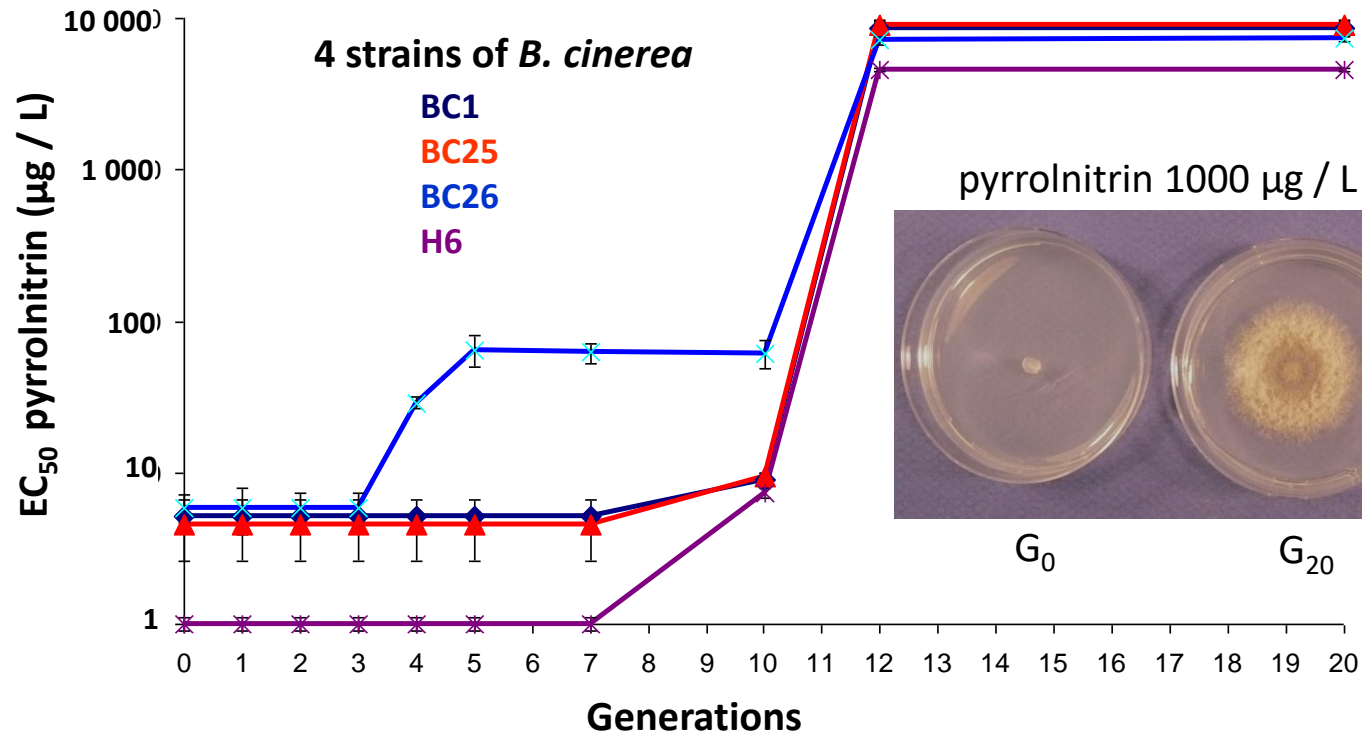


4 strains of *B. cinerea*

Could plant pathogens adapt to biocontrol ?

- Experimental evolution study of *Botrytis cinerea* in the presence of pyrrolnitrin

✓ Evaluation of generations for their sensitivity to pyrrolnitrin => EC₅₀



G₁ to G₁₀ : 10 µg/L

after G₁₁ : gradual increase
from 100 µg/L to 4000 µg/L

Decrease in sensitivity for one strain after only 4 generations (RF>10)

High level of resistance achieved for all strains after increasing selection pressure

OPEN ACCESS Freely available online

PLOS ONE

Functional and Structural Comparison of Pyrrolnitrin- and Iprodione-Induced Modifications in the Class III Histidine-Kinase Bos1 of *Botrytis cinerea*

Sabine Fillinger^{1*}, Sakhr Ajouz², Philippe C. Nicot², Pierre Leroux¹, Marc Bardin^{2*}

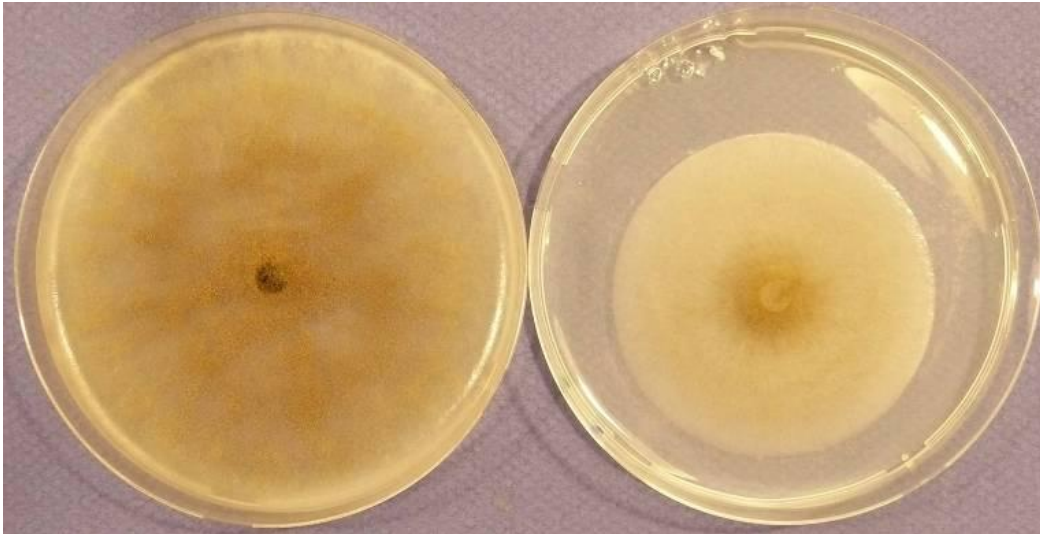
¹ INRA UR1290, BIOGER CPP, Thiverval-Grignon, France, ² INRA, UR407, Plant Pathology Unit, Montfavet, France

- ➔ High level of resistance to Iprodione (phenylpyrrole fungicide)
- ➔ Hypersensitivity to high osmolarity
- ➔ Affect the HAMP domains of class III histidine kinase

Could plant pathogens adapt to biocontrol ?

- Experimental evolution study of *Botrytis cinerea* in the presence of pyrrolnitrin
 - ✓ High fitness cost of resistance

reduced mycelial growth and sporulation

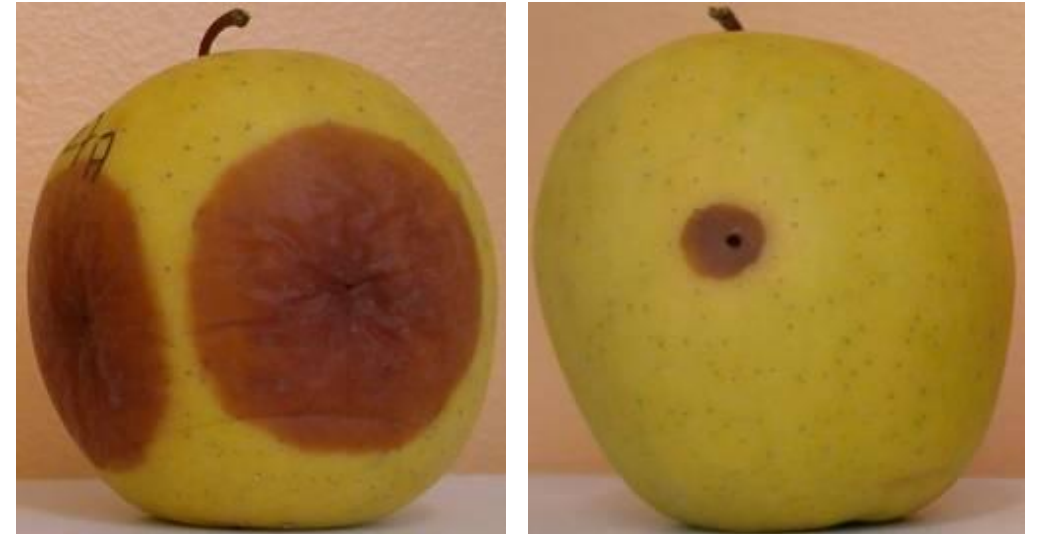


G₂₀
Pyrrolnitrin-sensitive

G₂₀
Pyrrolnitrin-resistant

Ajouz et al. (2011)
Eur. J. Plant Pathol. 129:31–42.

reduced aggressiveness



G₂₀
Pyrrolnitrin-sensitive

G₂₀
Pyrrolnitrin-resistant

➔ Reduced risk of propagation of the pyrrolnitrin-resistant mutants?

Could plant pathogens adapt to biocontrol ?

- Experimental evolution study of *Botrytis cinerea* in the presence of pyrrolnitrin
- **Experimental evolution study of *Zymoseptoria tritici* in the presence of an extract containing lipopeptides from a strain of *Pseudomonas* sp.**



Jacquin M (2024)
M1 internship



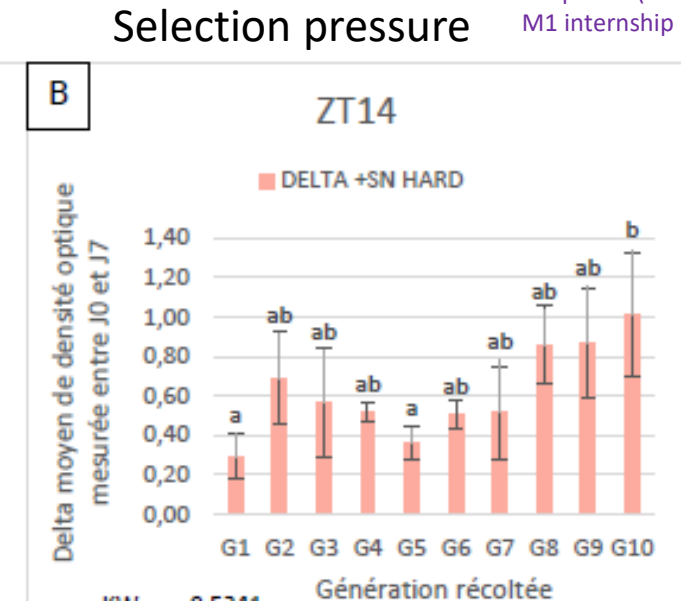
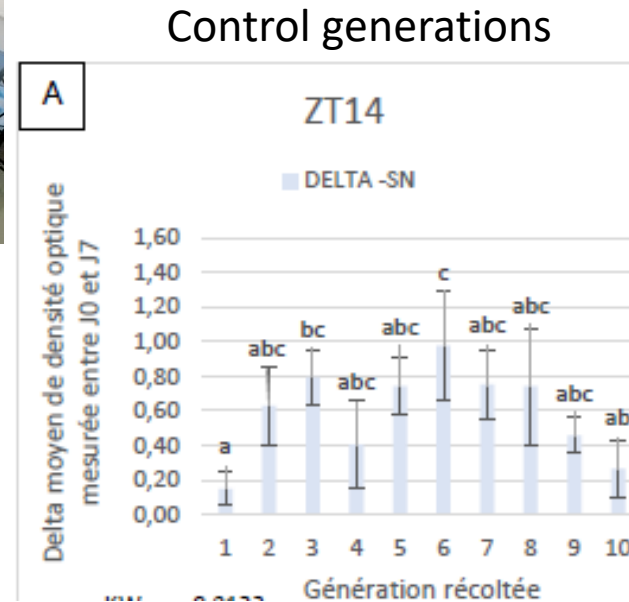
3 strains of Zt



Production of 10 successive generations in liquid media amended with various concentrations of supernatant



Fungal growth measurement (OD)



Evolution towards resistance ?
Additional generations?

Conclusions & perspectives

1. Evidence for high ranges of diversity in the sensitivity of several plant pathogens to different biocontrol agents

Much need for additional knowledge

- Relation with the irregularity of field efficacy?
- Probability for least sensitive strains to become selected?

2. Potential of adaption to biocontrol exists for certain pathogens

Huge need for additional knowledge

Possible points of vigilance and issues:

Deployment of biocontrol agents:

need to apply the recommendations of FRAC ? (alternation, different modes of action...)

Selection of new biocontrol agents:

integrate durability issues in the screening procedure of new biocontrol agents

(> 1 strain of the pathogen for screening, avoid certain mode of action, mixture of BCA strains...)



Thank you !

INRAE

AVIGNON
PATHOLOGIE
VEGETALE



Philippe
NICOT



Claire
TROULET



Jean-François
BOURGEAY



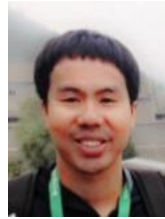
Magali
DUFFAUD



Sakhr
AJOUZ



Yousra
BOUAOUD



Sawai
BOUKAEW



Thomas
PRESSECQ



Estelle
TURC



Margot
GRIMONPONT



IOBC-WPRS



EU-China joint action
to increase the development
and adoption of IPM tools



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DE L'AGRICULTURE
ET DE L'ALIMENTATION