

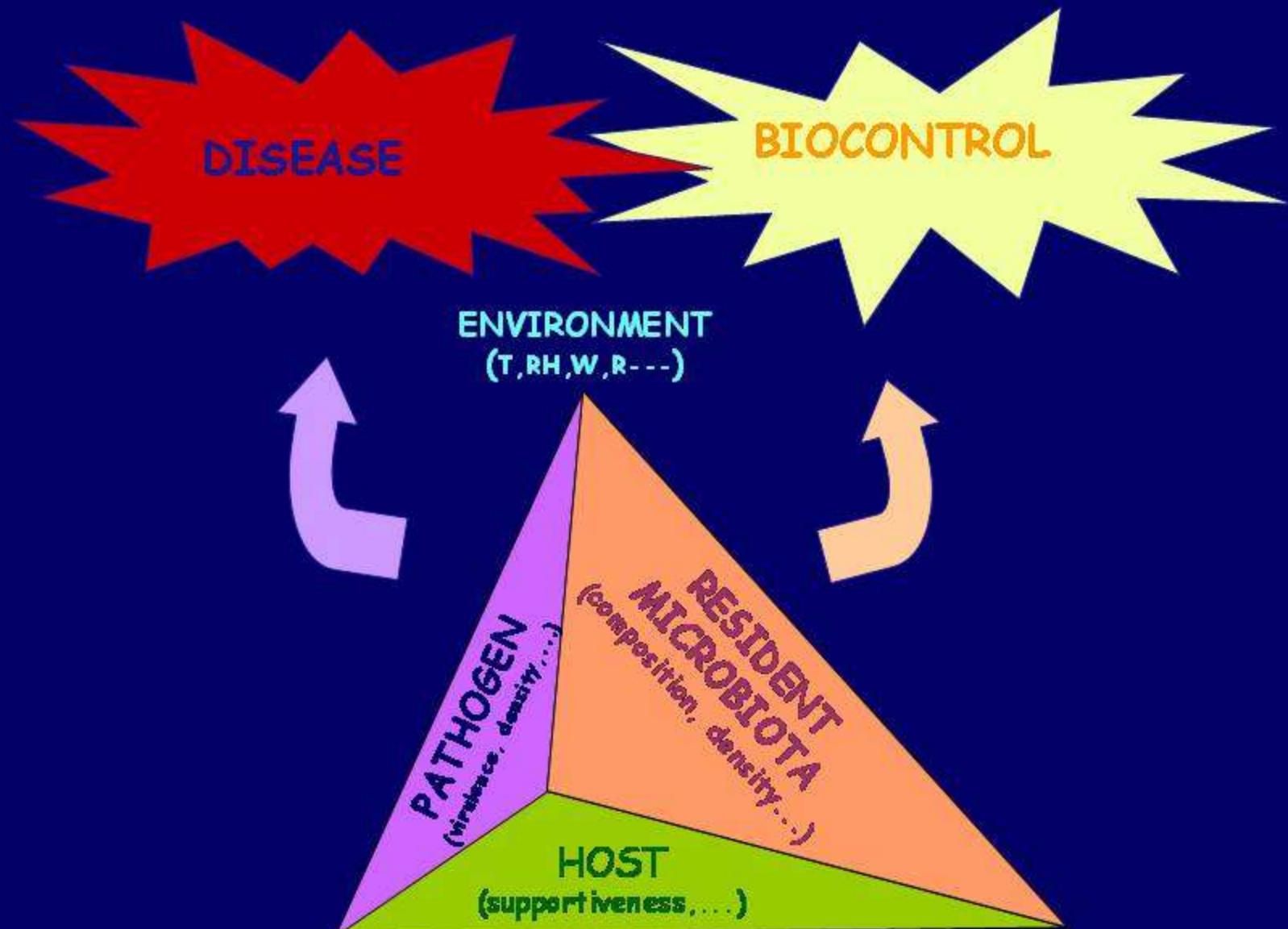


Achievements and future trends in biological control of fruit tree diseases

Emilio Montesinos

Plant Pathology
Institute of Food and Agricultural Technology
University of Girona
<http://www.udg.edu/cidsav>

Beneficial microorganisms and disease in plants



Microbial groups in which strains have been implicated in biological control of plant diseases

Bacteria	<i>Bacillaceae</i> <i>Cellulomonadaceae</i> <i>Enterobacteriaceae</i> <i>Lactobacillaceae</i> <i>Leuconostocaceae</i> <i>Pseudomonadaceae</i> <i>Rhizobiaceae</i> <i>Streptomycetaceae</i>	<i>Bacillus</i> <i>Brevibacillus</i> <i>Paenibacillus</i> <i>Cellulomonas</i> <i>Enterobacter</i> <i>Pantoea (Erwinia)</i> <i>Rahnella</i> <i>Serratia</i> <i>Lactobacillus</i> <i>Leuconostoc</i> <i>Burkholderia</i> <i>Pseudomonas</i> <i>Agrobacterium</i> <i>Streptomyces</i>
Yeast	<i>Ascomycota</i> <i>Basidiomycota</i>	<i>Aureobasidium</i> <i>Candida</i> <i>Conyothrium</i> <i>Debaryomyces</i> <i>Kloeckera</i> <i>Metschnikowia</i> <i>Pichia</i> <i>Cryptococcus</i> <i>Pseudozyma</i> <i>Rhodotorula</i> <i>Sporobolomyces</i>
Fungi (molds)	<i>Ascomycota</i> <i>Basidiomycota</i> <i>Oomycota</i>	<i>Ampelomyces</i> <i>Chaetomium</i> <i>Epicoccum</i> <i>Gliocladium</i> <i>Muscador</i> <i>Myrothecium</i> <i>Penicillium</i> <i>Trichoderma</i> <i>Ulocladium</i> <i>Phlebiopsis</i> <i>Pythium</i>

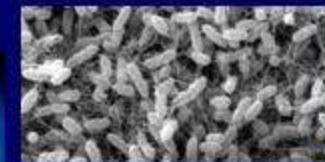
HOST MEDIATED RESISTANCE

- Induced systemic resistance (ISR)
- Systemic acquired resistance (SAR)



COMPETITIVE EXCLUSION

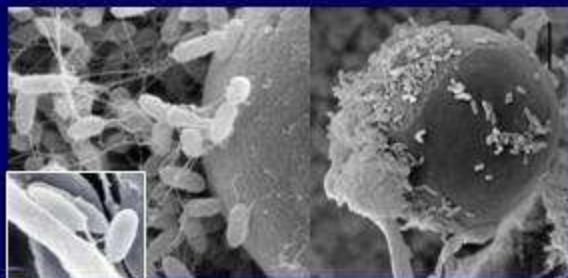
- Colonization of entry sites on host
- Competition for nutrients (C,N,P, iron)
- Physical barriers (biofilm)



MECHANISMS OF ACTION OF BIOCONTROL AGENTS

DIRECT CELL-TO-CELL INTERACTION

- Hyperparasitism
- Interference with cell/hyphal growth



ANTIBIOSIS

- Lytic enzymes
- Antimicrobial peptides and proteins
- Phenolic compounds
- Polyketides
- others



SIGNAL INTERFERENCE

- Quorum sensing



Registration (authorization for commercialization in the EU)

Regulatory issues for commercialization of microorganisms as biological control agents in the European Union



Directives for registration and authorization of Plant Protection Products (PPPs)

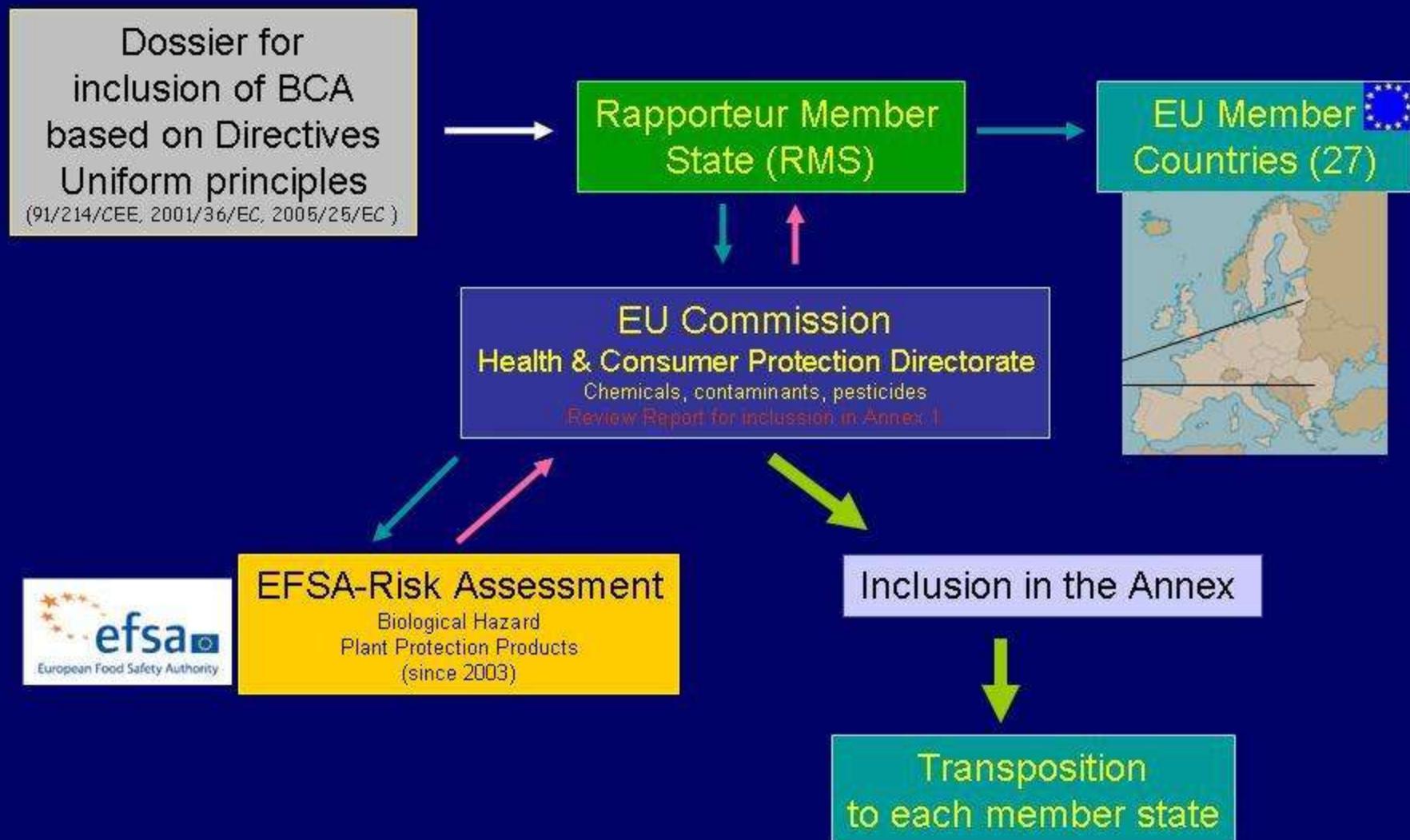
(91/214/CEE, 2001/36/EC, 2005/25/EC, Regulation (EC) No 1107/2009, 2009/128/EC)

- Species (strain) identification
- Biological properties
- Analytical methods
- Residues
- Traceability. Fate and behaviour in the environment
- Efficacy
- Adverse potential effects on human health and non-target organisms. Ecotoxicology and mammalian toxicology

Specific regulations at country level of Plant Strengtheners
(considered as "other means of control", not biocides, not fertilizers,
generally low risk profile)

(Spain: ORDEN APA/1470/2007, LEY 43/2002 DE SANIDAD VEGETAL)

DECISION SYSTEM FOR APPROVAL AND COMMERCIALIZATION OF MICROBIAL PESTICIDES IN THE EUROPEAN UNION



Commercial microbial biopesticide products



Authorized active ingredients of microbial type in the EU

As plant protection products Annex 1 (Directive 91/414/EEC and so...)

Microorganism	Activity	Company
<i>Bacillus subtilis</i> QST713	F/B	AgraQuest (2006), now Bayer Cropscience
<i>Pseudomonas chlororaphis</i> MA342	F	BioAgri (2004)
<i>Streptomyces griseoviridis</i> K61	F	Verdera Oy (2009)
<i>Ampelomyces quisqualis</i> AQ10	F	Ecogen Europe (2005), now Intrachem
<i>Candida oleophila</i> O	F	Bionext sprl (provisional 2010)
<i>Coniothyrium minitans</i> (FU)	F	Prophyta GmbH (2003)
<i>Gliocladium catenulatum</i> J1446	F	Kemira Agro Oy (2005)
<i>Phlebiopsis gigantea</i>	F	Forectry Comm. (2)(2009)
<i>Pseudozyma flocculosa</i>	F	Maasmond-Westland (2007)
<i>Pythium oligandrum</i> M1	F	Biopreparaty Spol s.r.o. (2008)
<i>Streptomyces griseoviridis</i> K61	F	Kemira (2008)
<i>Trichoderma harzianum</i>	F	Binab BioInnovation AB (8) (2008)
<i>Trichoderma polysporum</i>	F	Binab-BioInnovation AB (2008)
<i>Trichoderma atroviride</i>	F	Agribiotec S.r.l. (2)(2008)
<i>Trichoderma asperellum</i>	F	Isagro, NBT, Agribiotec (2008)
<i>Trichoderma gamsii</i>	F	Isagro, NBT, Agribiotec (2008)
<i>Verticillium dahliae</i> albo-atrum	F	Arcadis Pl. B.V.(2008)

As plant strengtheners approved in different european countries

Microorganism	Activity	Company
<i>Candida sake</i> CPA-1	F Posth	Sipcam Inagra(Spain)
<i>Aureobasidium pululans</i>	F fruit	Bioprotect (Germany)
<i>Bacillus subtilis</i> BD170	B fruit	Andermatt Bioc. (Switzerland)
<i>B. subtilis</i> BS-F3	B fruit	Agribiotec (Italy)
<i>Pantoea agglomerans</i> P10c	B fruit	Agrifutur (Italy)

Our technology

Bacterial strains suitable for microbial biopesticide development

Microorganism	Origin	Disease/effect	Plant crop
<i>P. agglomerans</i> EPS125	fruit, 1993	postharvest rot	fruits
<i>P. fluorescens</i> EPS62e	fruit, 1997	fire blight	apple, pear, others
<i>P. fluorescens</i> EPS817	root, 1998	growth promotion	banana
<i>P. fluorescens</i> EPS894	root, 1999	root rot	strawberry, tomato
<i>P. fluorescens</i> EPS818	root, 1998	nematodes	<i>Prunus</i> , tomato
<i>L. mesenteroides</i> EPS160	fruit, 2004	food-borne pathogens	Vegetables, fruit
<i>W. cibaria</i> EPS128	fruit 2004	postharvest rot	fruits
<i>B. subtilis</i> EPS20	leaves, 2008	fungal diseases	Several



Key topics...

- Improvement of fitness, efficacy and consistency.
- Specific monitoring, fate and impact in the environment.
- New strains and mechanisms of action.
- Improvement of industrial production, preservation and formulation.
- Biosafety.

more topics to be added...

Biosafety

Biocontrol agents and biosafety in Europe

(Directive 2001/36/EC and 2000/54/EC)

DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 18 September 2000

on the protection of workers from risks related to exposure to biological agents at work
(seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC)

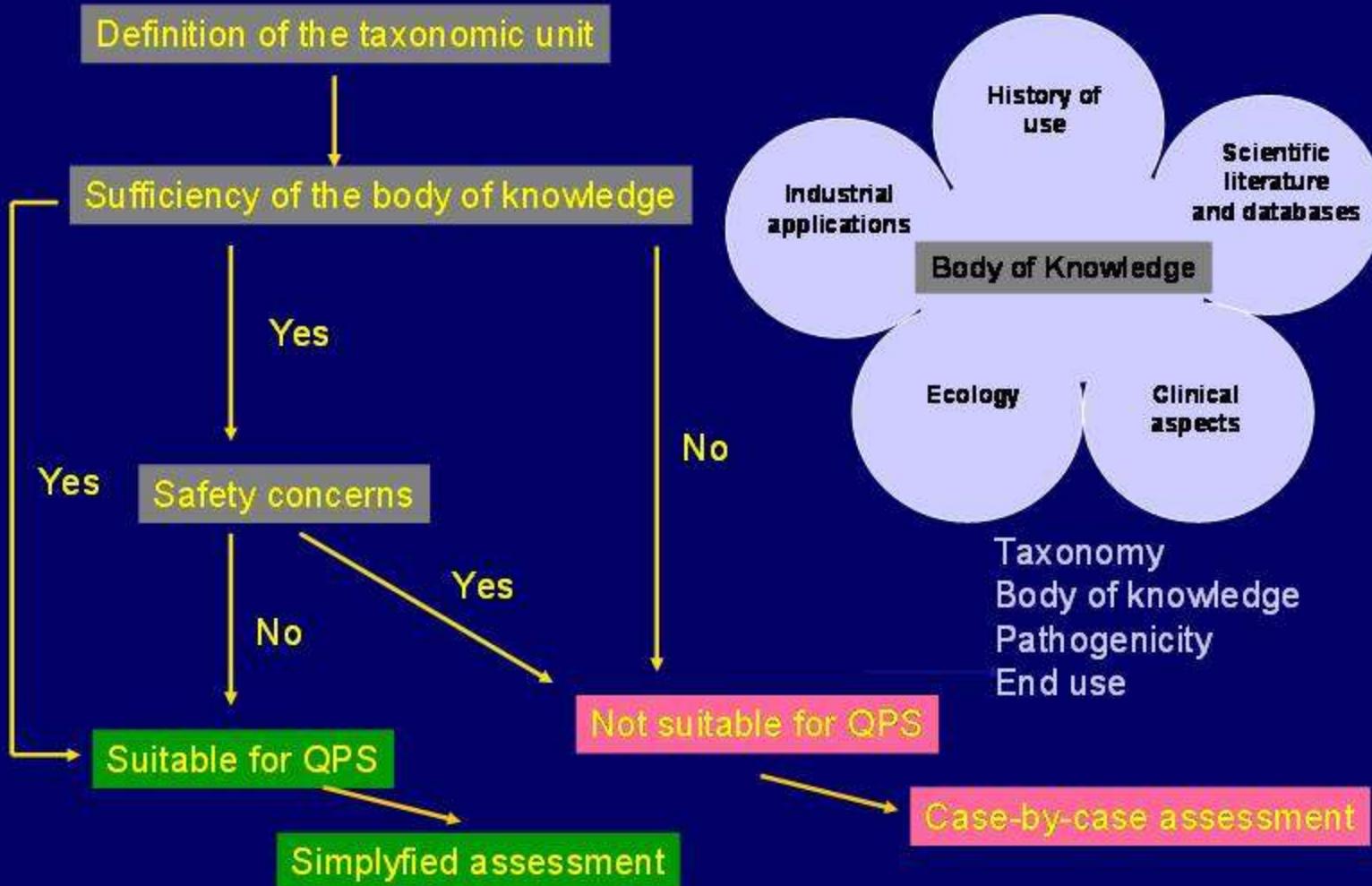


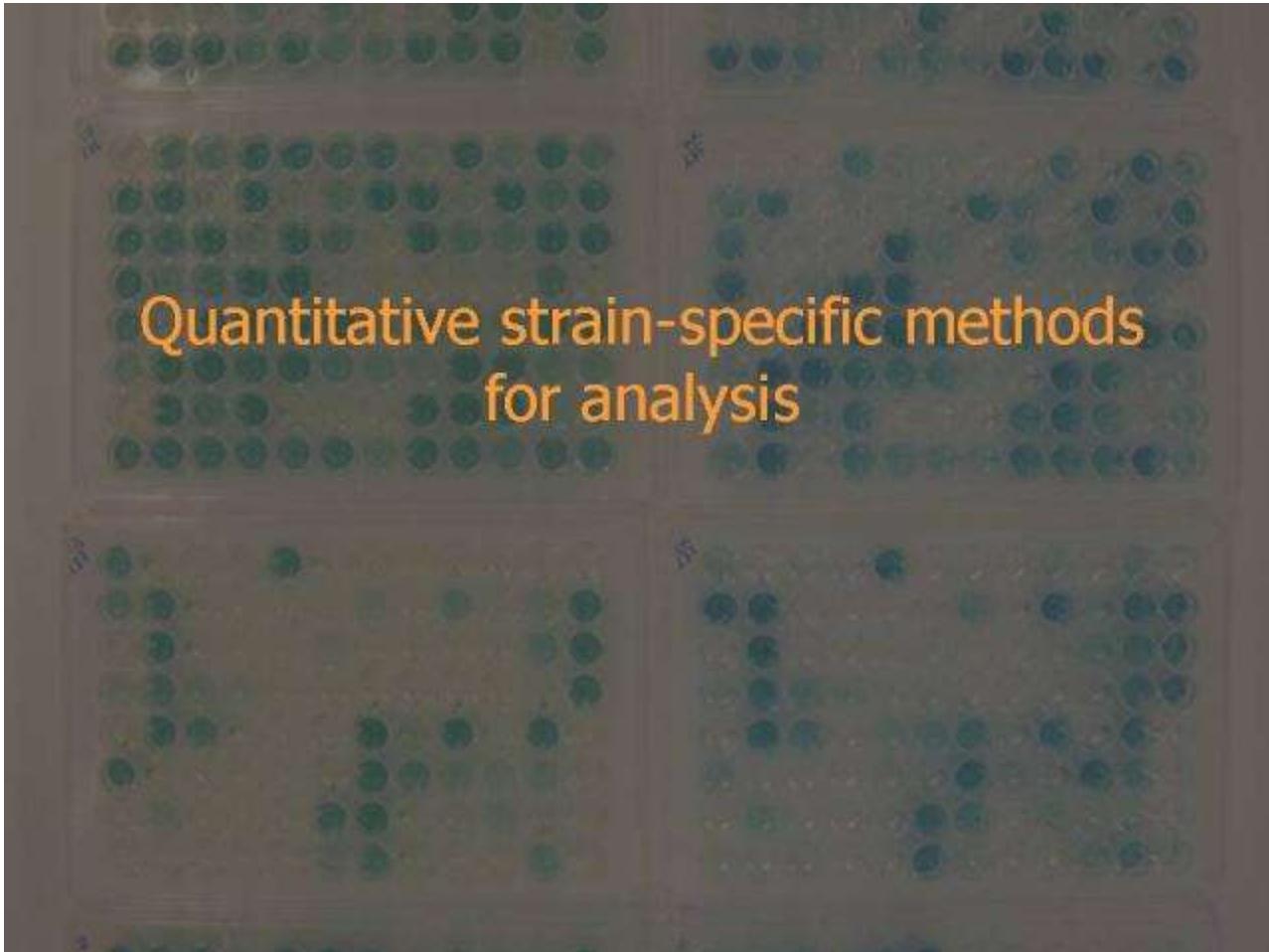
Pathogenicity to plants



Toxicology, pathogenicity in mammals and
other non-target organisms

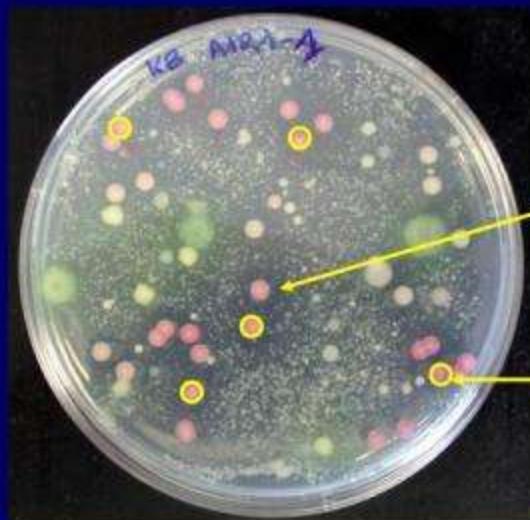
The EFSA QPS approach (Qualified Presumption of Safety)





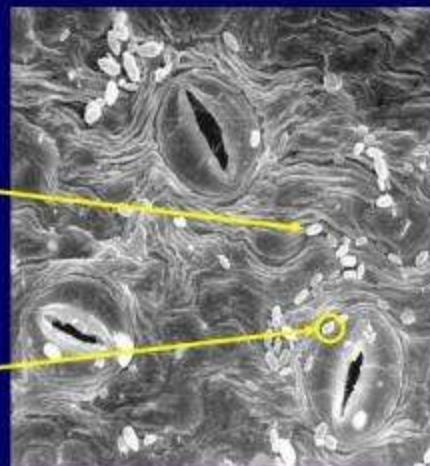
**Quantitative strain-specific methods
for analysis**

Who is who, how much there is
and which is the performance ?



Natural
autochthonous
non-BCA strain

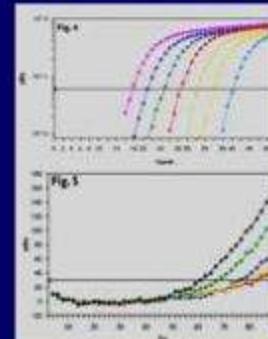
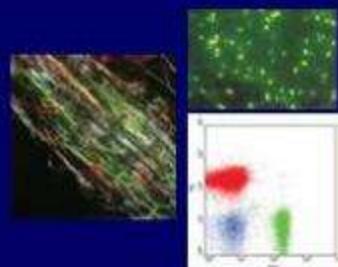
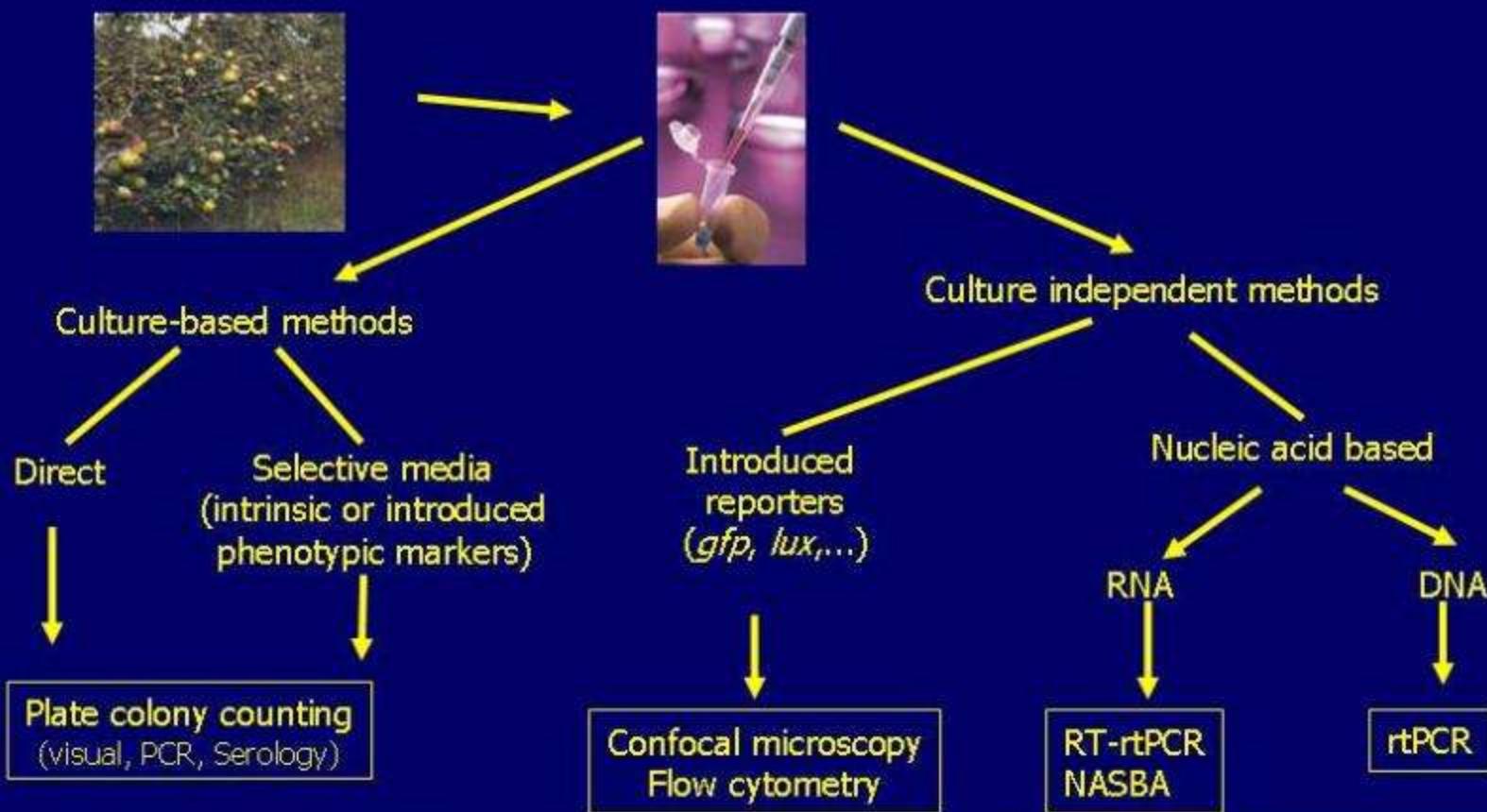
BCA strain



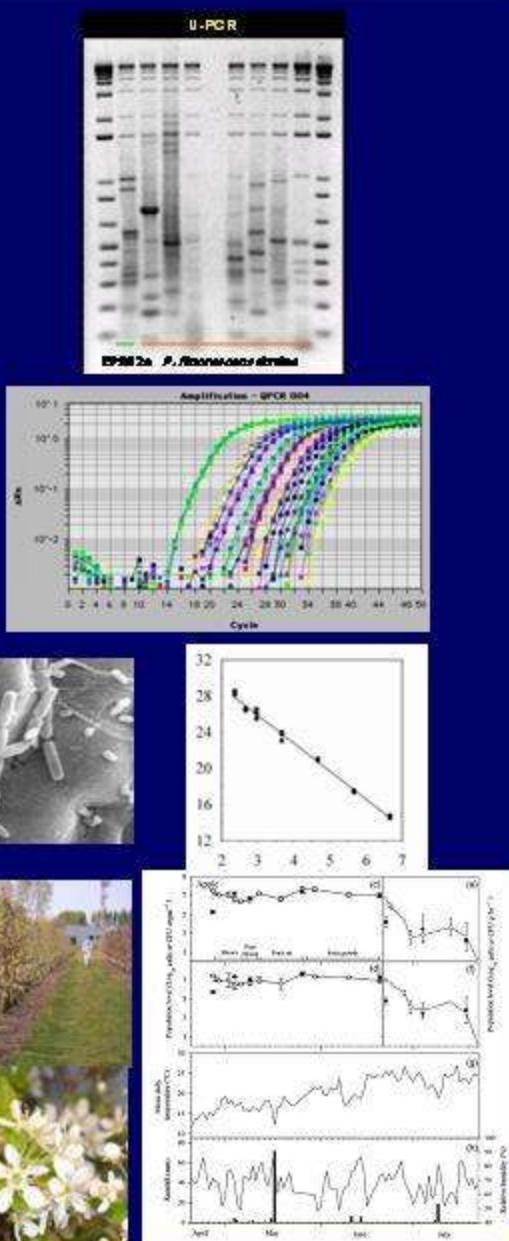
Need tools for specific and quantitative analysis to perform:

- plant, pathogen interaction studies
- ecological studies
- improving formulation and application methods
- traceability in food and environment

Quantitative analysis of BCAs at strain level



SPECIFIC MONITORING AND QUANTIFICATION OF BIOLOGICAL CONTROL AGENTS BY REAL TIME PCR



FEMS Microbiology Letters xxx (2005) xxx-xxx

FEMS
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www.fems-microbiology.org

Development of a strain-specific quantitative method for monitoring *Pseudomonas fluorescens* EPS62e, a novel biocontrol agent of fire blight

Marta Pujol, Esther Badosa, Jordi Cabrefiga, Emilio Montesinos *

Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, Campus Montilivi, 17071 Girona, Spain

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0099-2240/06/\$08.00 + 0 doi:10.1128/AEM.72.4.2421-2427.2006
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Assessment of the Environmental Fate of the Biological Control Agent of Fire Blight, *Pseudomonas fluorescens* EPS62e, on Apple by Culture and Real-Time PCR Methods

Marta Pujol,¹ Esther Badosa,¹ Charles Manceau,² and Emilio Montesinos^{1*}

¹Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, 17071 Girona, Spain,² and UMR 077 PaVe, Cenar INRA, 49071 Beauvois, France²



Epiphytic fitness of a biological control agent of fire blight in apple and pear orchards under Mediterranean weather conditions

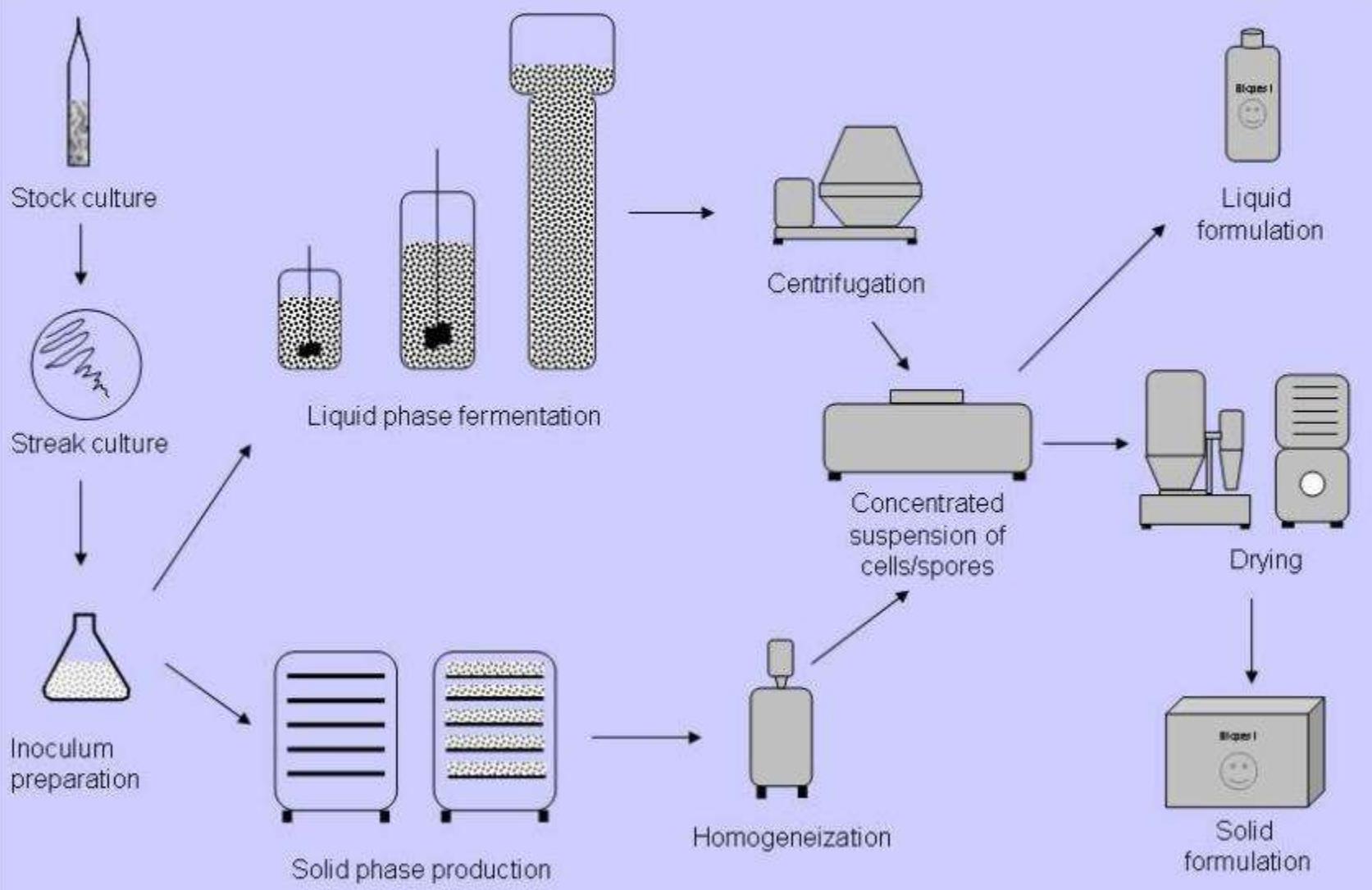
Marta Pujol, Esther Badosa & Emilio Montesinos

Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, Girona, Spain

Production, preservation and formulation



PRODUCTION OF A MICROBIAL PESTICIDE



from the lab to the field ...



High water availability and very reach culture media



Osmotic and nutrient stress
and poor growth conditions

Application of biocontrol agents



IMPROVEMENT OF FITNESS OF BIOLOGICAL CONTROL AGENTS BY NUTRITIONAL ENHANCEMENT AND OSMOADAPTATION



FEMS Microbiology Letters 250 (2005) 1-8

FEMS
MICROBIOLOGY
Letters

www.fems-microbiology.org

Osmotically induced trehalose and glycine betaine accumulation improves tolerance to desiccation, survival and efficacy of the postharvest biocontrol agent *Pantoea agglomerans* EPS125

Anna Bonaterra, Jaume Camps, Emilio Montesinos *



Increasing survival and efficacy of a bacterial biocontrol agent of fire blight of rosaceous plants by means of osmoadaptation

Anna Bonaterra, Jordi Cabrefiga, Jaume Camps & Emilio Montesinos

Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, Girona, Spain

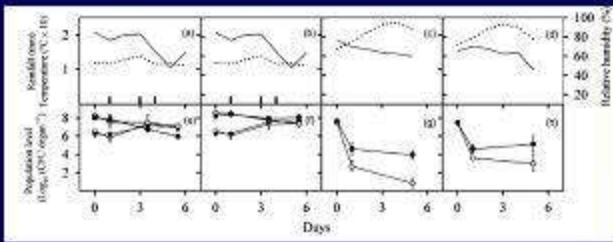
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2011, p. 3174-3181
0099-2240/11/\$12.00 doi:10.1128/AEM.02760-10
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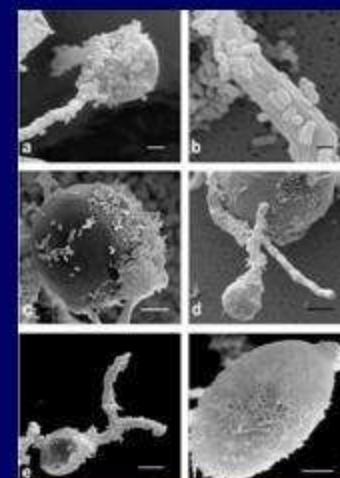
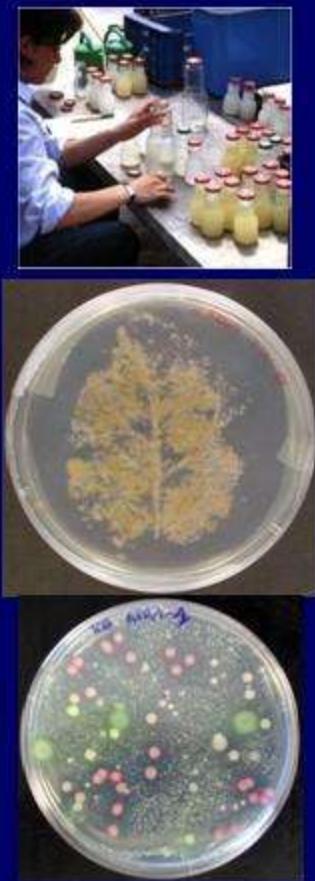
Improvement of Fitness and Efficacy of a Fire Blight Biocontrol Agent via Nutritional Enhancement Combined with Osmoadaptation[†]

J. Cabrefiga, J. Francés, E. Montesinos,^a and A. Bonaterra

Institute of Food and Agricultural Technology-CIDSAV-XaRTA, University of Girona, 17071 Girona, Spain



INCREASING SPECTRUM OF ACTION AND EFFICACY OF BIOLOGICAL CONTROL AGENTS BY USING MIXTURES OF STRAINS WITH DIFFERENT MECHANISM OF ACTION

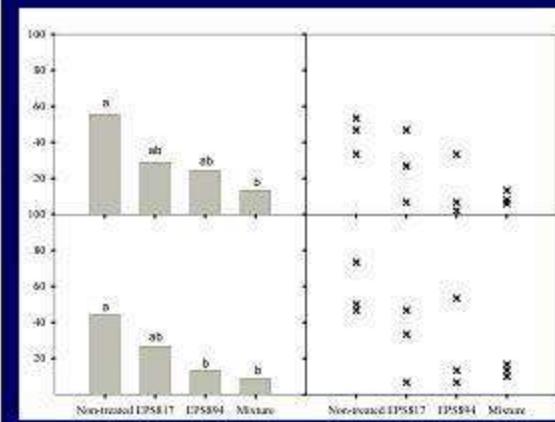
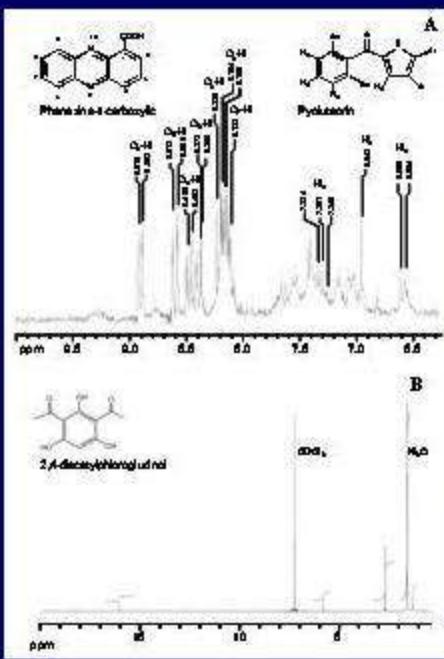


Journal of Plant Pathology (2011), 93 (2), 363-372 © Edizioni ETS Pisa, 2011

BIOCONTROL OF ROOT ROT OF STRAWBERRY CAUSED BY *PHYTOPHTHORA CACTORUM* WITH A COMBINATION OF TWO *PSEUDOMONAS FLUORESCENS* STRAINS

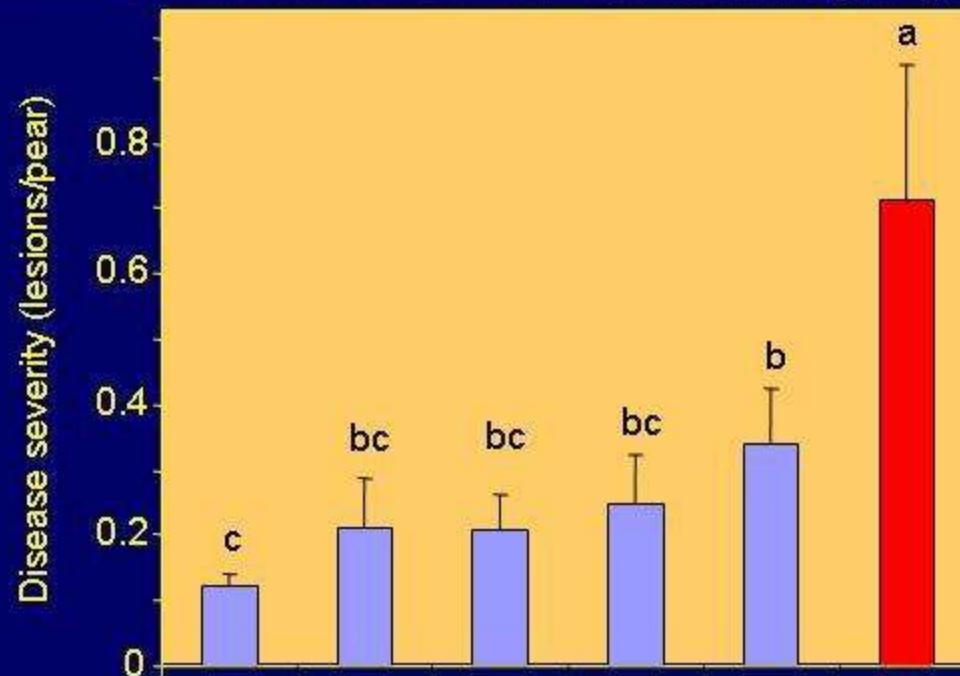
L. Agustí, A. Bonasterra, C. Moragrega, J. Camps and E. Montesinos

Institute of Food and Agricultural Technology-CIDSAV-XaRTA, University of Girona,
Campus Montilivi 17071 Girona, Spain



An example of use
under IFP rules

AN INTEGRATED CONTROL STRATEGY OF BROWN SPOT OF PEAR



LR	Yes	No	Yes	Yes	Yes	No
BC-T	Yes	No	Yes	No	No	No
BSPcast	Yes	Yes	No	Yes	No	No

THANKS!

