ENDURE

European Network for Durable Exploitation of crop protection strategies

Project number: 031499

Network of Excellence
Sixth Framework Programme

Thematic Priority 5
FOOD and Quality and Safety

Deliverable DR4.7

Review of factors influencing the success or failure of biocontrol and recommended orientation for new R&D projects

Due date of deliverable: M33

Actual submission date: M36

Start date of the project: January 1st, 2007 **Duration**: 48 months

Organisation name of lead contractor: IBMA + CNR

Revision: V2

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)

Dissemination Level

PU Public	**PU**
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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Glossary

BCA: biological control agent
CBC: conservation biological control
GMO: genetically modified organism
IGP: intra guild predation
IOBC-wprs: International Organisation for Biological and integrated Control of noxious animals and plants – West Palaearctic Regional Section
IPM: integrated pest management

Summary

Research Activity 4.3 of ENDURE has brought together representatives of industry and scientists from several European countries with experience ranging from fundamental biology to applied field work on biological control against pests and diseases. The unique diversity of expertise and concerns allowed the group to set up very complementary approaches to tackle the issue of the factors of success of biocontrol.

The initial part of the work accomplished by this group consisted in a thorough review of scientific literature published on all types of biological control. Although it had to be focused on selected key European crops and their major pests and pathogens, this review is unique in the scope of the topics it covered and in the comprehensive inventories it allowed to gather on the potential of biocontrol and factors of success at field level. A large part of this study was dedicated to the increasingly promising field of research on conservation biological control.

In parallel with identifying knowledge gaps and key factors from published research, information was gathered on aspects linked to the production and commercialization of biocontrol agents.

These results, complemented by the views of experts in the field of biocontrol consulted at the occasion of meetings of IOBC-wprs, allowed the identification of majors gaps in knowledge and bottlenecks for the successful deployment of biocontrol and lead to the proposition of key issues for future work by the research community, the field of development and prospects for technological improvement by industry.

Definitions

Biological control / biocontrol
Many different definitions of biological control have been proposed. Most of them are clearly related to the control of pests, mainly insects and mites, and have been extrapolated to disease control. Eilenberg (2006) defined "biological control or biocontrol as the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be".

Augmentation / augmentative biological control
Augmentation biological control includes activities in which natural enemy populations are increased through mass culture, periodic release (either **inoculative** or **inundative**) and colonization, for suppression of native or non-native pests. (Orr, 2009[1])

Classical (also "importation") biological control
Classical biological control is defined in this study as the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control.

Conservation biological control
"Conservation biological control involves manipulation of the environment to enhance the survival, fecundity, longevity, and behaviour of natural enemies to increase their effectiveness" (Landis et al., 2000). See also chapter 3.1.

Microbials / Micro-organisms used for biological control.
The term micro-organism is defined in Council Directive 91/414/EEC (as amended by Commission Directive 2001/36/EC) as follows: A microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material. The definition applies to, but is not limited to, bacteria, fungi, protozoa, viruses and viroids. It does not include multicellular organisms, such as nematodes or insects.

Pest
Unless otherwise specified, the term "pest" in the present document is meant to cover the whole range of crop-damaging animals (invertebrate and vertebrate), agents of plant diseases and weeds.

[1] Orr, D. 2009. Biological Control and Integrated Pest Management, pp 207-239 IN: R. Peshin, A.K. Dhawan (eds.), *Integrated Pest Management: Innovation-Development*

1. Foreword

The present document results from the collective effort of researchers from five European research institutions (CNR, INRA, RRes, UdL and WUR), together with representatives of the International Biocontrol Manufacturers Association (IBMA)

The organisation and edition of this document was coordinated by P. NICOT (**INRA**-Avignon).

The specific contributors to the research work and their role / expertise were as follows:

ALABOUVETTE Claude, **INRA**, Plant Pathologist (review of scientific literature on biocontrol against *Fusarium*; analysis of regulatory issues; review of CBC of soil-borne pathogens)
ALOMAR Oscar, **UdL**, Entomologist (meta-review of scientific literature on conservation biological control against arthropod pests)
BARDIN Marc, **INRA**, Plant Pathologist (review of scientific literature on biocontrol against *Botrytis*, rusts)
BLUM Bernard, **IBMA** (economic survey, regulatory issues)
FERGUSON Andrew, **RRES**, Entomologist, (meta-review of scientific literature on conservation biological control against arthropod pests, with assistance from Stephen Moss and Jonathan Storkey for section on weed control)
GIORGINI Massimo, **CNR**, Entomologist (review of classical and augmentative/inundative biocontrol against arthropod pests)
HEILIG Ulf, **IBMA** (inventory of commercial biocontrol products, and their usage in 5 European countries; analysis of regulatory issues)
KÖHL Jürgen, **WUR,** Plant Pathologist (*Venturia, Ulocladium*)
MALAUSA Jean Claude, **INRA**, Entomologist (review of scientific literature on classical biocontrol and parasitoids against arthropod pests)
NICOT Philippe, **INRA**, Plant Pathologist, (review of scientific literature on biocontrol against downy mildews and late blight, *Monilia;* analysis of reviews concerning plant diseases; review of CBC of aerial pathogens)
RIS Nicolas, **INRA**, Entomologist (review of scientific literature on classical biocontrol and parasitoids against arthropod pests)
RUOCCO Michelina, **CNR**, Plant Pathologist (review of scientific literature on biocontrol against downy mildews; review of factors of efficacy of biocontrol agents against plant diseases)

2. Introduction

Biological control methods against pests and diseases constitute key elements for the development of integrated protection and integrated production of cultivated crops.

The objectives of the present study were to conduct a review of the current status of European research on the exploitation of natural biological processes for Biological control, to identify knowledge gaps and the main constraints for its implementation in the field and finally, to provide suggestions for possible improvements and needs for further research efforts.

In this study, both conservation biological control and the use of classical or augmentative biological control have been considered.

To optimize the available expertise and human resources gathered for this Research Activity, efforts were focused on (but not systematically limited to) situations relevant for Case Studies and Systemic Case Studies conducted elsewhere by the ENDURE network.

The targets of the biological control were mostly arthropod pests and plant pathogens. Biological control of weeds was only marginally considered due to the lack of weed experts participating in the group.

3. **Conservation biological control (CBC):** current status of research relevant to the major cropping systems in Europe and recommendations for multi-site experiments

Contributors:
Andrew Ferguson RRES, Entomologist, field crops, co-leader, with assistance from Stephen Moss and Jonathan Storkey for section on weed control
Oscar Alomar UdL, Entomologist, field vegetables, co-leader
Claude Alabouvette INRA, Pathologist, soil pathogens
Philippe Nicot INRA, Pathologist, aerial pathogens

3.1. Definitions related to Conservation Biological Control

"Conservation biological control involves manipulation of the environment to enhance the survival, fecundity, longevity, and behaviour of natural enemies to increase their effectiveness" (Landis et al., 2000)

"Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests" (Eilenberg et al., 2001)

This encompasses:

- protection and/or enhancement of natural enemies or other naturally-occurring organisms that reduce the effect of pests by manipulating their environment and providing resources to increase their effectiveness.

For the purpose of this review, this does *not* encompass:

- released biological control agents.
- cultural control measures that depend for success on their direct effects on pests rather than on protection or enhancement of natural enemies or other non-pest organisms.

In this review the term 'natural enemy' (NE) is preferred over 'biological control agent' for describing organisms with a potential role in CBC. 'Natural enemy' is a more inclusive term that can be applied to any organism with a trophic relationship with a pest that has potential value for the biocontrol of that pest. By contrast, the term 'biological control agent' implies a proven ability to control the pest.

3.2. Scope and aims of this chapter

Pest groups considered:

- Invertebrate pests, the main focus of the report
- Plant pathogens, airborne and soil-borne
- Weeds

The main focus of this report is on CBC for the management of invertebrate pests. This is the field for which the terminology was coined and in which it is most used. However, the authors believe that the principles of CBC could be applied equally to management of plant pathogens and weeds should the appropriate circumstances exist for conserving and promoting their natural enemies. Therefore separate sections of this report give consideration to research relevant to CBC of airborne plant pathogens, soil-borne plant pathogens and weeds.

Reviews of research literature:
Each pest group is the subject of review of research literature to:

- establish the status of CBC research relevant to the major cropping systems of Europe and
- identify gaps in the scientific knowledge-base that represent impediments to the implementation of CBC and require further research.

The review of invertebrate pests also reports on other challenges to CBC implementation identified by authors of review papers.

Recommendations for multi-site experiments:
Priorities for future research in European cropping systems that would benefit from a multi-site, supra-national approach are identified from the reviews (see summary). It is proposed that these priorities should be discussed at a joint workshop of ENDURE research sub-activities 4.3 (biocontrol), systems case studies and 2.3 (landscape), for consideration of gaps in research and joint recommendations for multi-site experiments. The ENDURE Annual Meeting in October 2009 in Wageningen would be a suitable occasion for this.

3.3. Limits of this report

- This report does not attempt to cover CBC in every crop and cropping system. Its focus is determined by what authors of the review literature consider to be the most important research results and knowledge gaps.
- This report does not review the value for CBC of chemical pesticides that are selective in their action, i.e. less injurious to biological control agents than to pests. This principle is well accepted.
- This report does not address regulatory or policy issues that influence the uptake of CBC except inasmuch as they are mentioned in the papers reviewed.
- This report does not attempt to survey the current extent of implementation of CBC.

3.4. Reviewing methods

- Invertebrate pests: a meta-review of the review literature published between 1989 and 2009.
- Plant pathogens } expert knowledge of the authors and their colleagues, citing the
- Weeds } most significant primary and review literature.

Invertebrate pest literature review

- A meta-review of peer-reviewed published research on CBC relevant to the major cropping systems in Europe since 1989.
- This is accomplished primarily by examination and analysis of review papers identified in a search of Web of Science, CAB Abstracts and BIOSIS databases.
- <u>Search terms</u>:
  Literature searches for reviews were based on the following search terms:
  - conservation or "habitat management" or "ecological infrastructure*"
  and
  - biocontrol or "biological control" or "natural enem*" or predator* or parasitoid*

A number of additional review references identified during the course of this study were added as the search did not identify all relevant review papers, particularly those published as book chapters.

- Spatial scale: this review focuses on field to landscape scales, i.e.:
    - o The sown crop
    - o The field margin (components and terminology as Greaves and Marshall, 1987 and Marshall and Moonen, 2002).
    - o Managed non-crop habitats within the field (e.g. beetle banks, flower margins etc)
    - o Landscape scales (within *c*. 2-3km radius).

  Note that landscape issues are also addressed in depth by ENDURE RA2.3 and the impact of landscape management on pest abundance is the subject of RA 2.3 Deliverable DR 2.9, 'Synthesis on impacts of landscape characteristics on densities of pests and their regulation by natural enemies'. As landscape management is of such great importance in the management of natural enemies, no comprehensive review of CBC would be complete without its inclusion. The RA 4.3 and RA 2.3 reviews will provide complimentary views of the role of the landscape in pest management. The present RA 4.3 review is a meta-review assessing landscape management in the wider context of all CBC techniques whereas the RA 2.3 review assesses the primary research literature and provides is focussed solely on landscape issues.
- The geographic coverage of the review is world-wide but is focussed on studies relevant to the major cropping systems in Europe
- The following are reviewed:
    - major research results, CBC techniques and issues discussed in the review literature whenever the subject is relevant to the European situation
    - CBC techniques or practices that have potential for practical application or are already practically applied
    - CBC research reported in relation to crop type, natural enemy taxon and pest taxon.
    - experimental systems used in CBC research
    - evidence for the success of different CBC techniques in supporting natural enemies and depressing pest populations
    - evidence for the success of CBC in different cropping systems
    - the strength of the contribution of European scientific institutions to the primary literature on CBC research reported in review papers and the relationship of that contribution with crop type and CBC technique.
    - the relative contributions of scientific institutions in Europe and elsewhere to the authorship of review literature concerning CBC
    - analysis of the number of review papers addressing CBC research by year, 1990-2009
    - gaps in the scientific knowledge-base that represent impediments to the implementation of CBC and require further research.
    - other challenges to CBC implementation identified by authors of review papers.
- Scoring of content of review papers

  Each review paper was scored on a spreadsheet using the following headings:

  A. Headings scored for each aspect of CBC research discussed by each review paper, including efficacy of CBC techniques (each combination of headings 1-13 that was unique within each review paper was separately scored; thus for the same review paper there could be several lines of data):
    1. crop type
    2. experimental system
    3. whether research includes modelling
    4. the country where experiments were done

5. whether experiments were done in Europe
6. CBC practice and techniques group
7. specific CBC practice or technique
8. pest species or group
9. class of natural enemy
10. natural enemy species or group
11. effect on abundance or fitness of natural enemy
12. effect on pest control
13. effect on intra-guild predation (IGP)

B. Headings for the views of review paper authors on gaps and challenges
14. research gaps identified
15. challenges to implementation discussed

C. Headings for details of authors and institutions
16. reference number
17. year published
18. first author
19. any authors based in Europe?
20. country or countries where authors' institution(s) located
21. names of authors' institution(s)

The content of the review paper was scored under each heading by allocation of one of a number of alternative terms. A complete table of headings and of the terms that could be used under each heading when scoring the papers is given in Appendix 1. Note that terms are scored under 'CBC practices and techniques' headings when research that supports the development of those techniques is reported. For example, a score under 'Landscape management' does not necessarily imply that the landscape was manipulated but that data were collected that would assist the future design and management of landscapes to optimise CBC.

- Data analysis and collation
  - Frequencies of occurrence of different terms under headings relating to aspects of the CBC research reported were summarised simply, using XL spreadsheets to draw up two-way tables and bar charts. Particular attention was paid to:
    - the relationship between choice of CBC techniques and practices and the target pest, the cropping system and the experimental system
    - the effectiveness of CBC in relation to CBC technique and cropping system
    - the contribution of Europe to CBC research in relation to CBC technique, cropping system and experimental system
  - The reporting of CBC research gaps and of challenges to CBC implementation was analysed by grouping them into categories to enable their collation and analysis of frequencies using bar charts.

    Research gaps identified were subjected to a two-part analysis:

    1. Analysis of gaps in relation to particular CBC practices and techniques groups: The aim of the first analysis was to enable a direct comparison of the weight given by reviews to the reporting of different CBC techniques (from past research papers) with the needs for future research identified by the reviews. Gaps were allocated (where possible) to one of six headings matching the categories of CBC techniques by which past research was analysed: 'limiting pesticide use', 'manipulation of behaviour', 'reduced disturbance', 'provision of refugia and resources', 'increased biodiversity' and 'landscape management'.
    2. Analysis of gaps in the science underpinning CBC:

The second analysis was more comprehensive and was designed to summarise the gaps in science that underpins CBC according to scientific discipline. It covered all gaps identified by review papers, not only those relating to specific CBC techniques. Each reported gap in the science-base was allocated to one of 11 research gap categories and 36 sub-categories that were defined *a posteriori* according to what was found in the review papers.

The challenges to CBC implementation identified by the review papers were summarised by allocating each to one of five categories of challenge: scientific practice, R&D costs, knowledge transfer, socio-economic, and policy. These categories were further divided into sub-categories.

- The authors, institutions and countries represented in the review literature, together with their dates of publication, were summarised simply using XL spreadsheets.

3.5. Results: Management of invertebrate pests

3.5.1. Summary of the source literature for the meta review

Ninety review papers covering CBC research were analysed, see bibliography in Appendix 2. Most of these were published from 1998 onwards (Figure 1). The majority (48) were published in peer-reviewed international journals; three were published in conference proceedings and 39 as book chapters. This large body of review literature reflects a large primary literature. A search of primary papers from 1967 to 2009 in the same databases and based on the same search terms delivered 2,675 references.

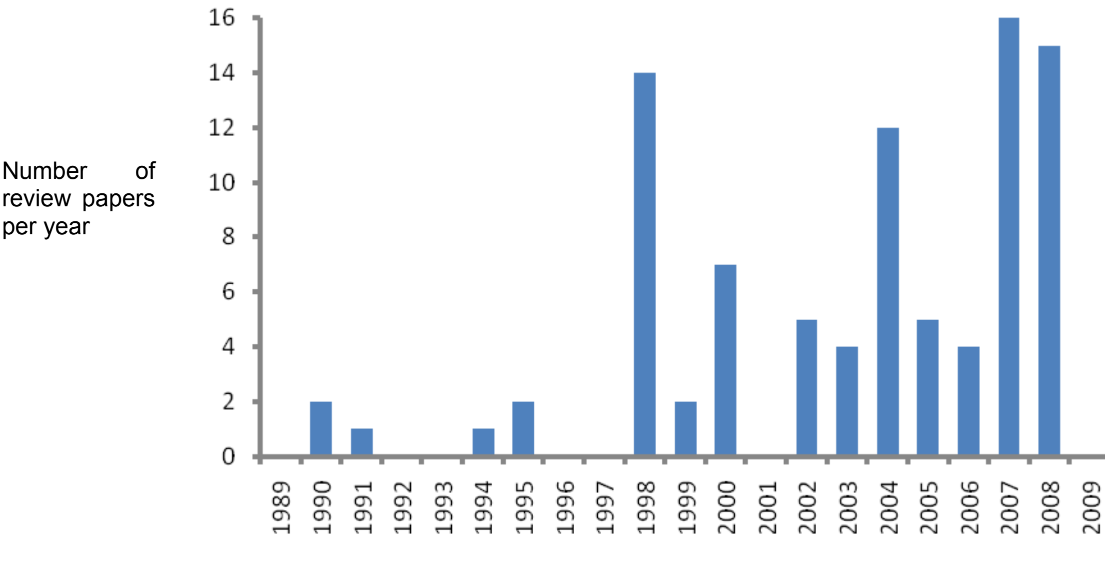

Figure 1: Year of publication of review papers analysed

3.5.2. Detailed analysis of CBC research reported by review papers

3.5.2.1. Reporting of CBC methods.

A total of 221 lines of data were scored concerning the reporting of CBC methods.

CBC techniques

- Ten categories and 48 sub-categories of CBC practices or techniques were identified (Table 1).

- The four CBC techniques most commonly reported to be the subject of research all involved the provision or management of resources and refugia in the agroecosystem for natural enemies, i.e.: the provision of refugia and resources in the field at concentrated locations (e.g. sown flower strips) or spread across the crop (e.g. weed management, ground-cover management and mulch), landscape management and reduced disturbance (reduced tillage) (Table 1). Reports concerning these techniques made up 177 of all 221 reports (80%).
- Limiting pesticide use through the use of pest resistant crop cultivars (GMO's or otherwise), IPM, buffer zones or spatial targeting (precision farming) was much less discussed (5% of reports).
- Manipulation of (invertebrate) behaviour, habitat manipulation and optimising plant morphology together comprised 6% of reports.
- Increased ecosystem or natural enemy biodiversity comprised 4% of reports (Table 1).

Crop type

- Arable crops were the subject of more reports of CBC techniques from single crop types (74%) than any other crop type (Table 2).
- Maize comprised 12% of the reports on arable crops and 9% of all reports from single crop types.
- Orchards, vines and field vegetables were each the subject of less than 10% of reports from single crop types (Table 2).
- Perennial crops were the subject of 19% of reports from single crop types.
- Greenhouse vegetables were the subject of only one report, probably because augmentative biological control is more commonly practised in this system than CBC.
- Provision of refugia and resources was the most commonly reported CBC technique in each crop type usually followed by landscape management.

Pest taxon

- Hemiptera (mostly aphids) were the target pests in 18% of reports of CBC (Table 3). Lepidoptera were the next most common targets (6% of reports).
- Thirty percent of the reports of CBC referred to more than one pest.
- A significant proportion (40%) of reports of CBC in the review papers did not specify the target pest and this was especially true when discussing predators (Table 3).

Natural enemy taxon

- Predators were much the most frequently discussed natural enemies in reports of CBC (42%; Table 3). Parasitoids were discussed in 17% of reports and entomopathogenic fungi in 6%.
- A synoptic list of the natural enemy taxa referred to in reviews, and the number of times they were reported, can be found in Appendix 3. No attempt has been made to compile a complete list of the natural enemy species referred to.
- Predators were the most commonly discussed natural enemies of hemipteran pests and parasitoids were the most commonly discussed natural enemies of lepidopteran and coleopteran pests (Table 3).
- A full analysis of the classes of natural enemy discussed in relation to all pest species or groups reported can be found in Appendix 4

Table 1: Number of times that review papers reported research on different CBC practices

CBC practice and techniques group	Specific CBC practice or technique	No. of times practice referred to	Total
Limiting pesticide use	GMO (pest resistant)	6	12
	IPM	1	
	buffer zones	1	
	pest resistant cv. & var.	2	
	spatial targeting	2	
Manipulation of behaviour	push-pull	2	5
	semiochemicals	2	
	unspecified	1	
Habitat manipulation	cultural methods that increase humidity	1	5
	irrigation	1	
	various	3	
Plant morphology	hairiness	1	4
	cuticular wax	1	
	plant architecture or canopy structure	2	
Reduced disturbance	reduced tillage	13	13
Provision of refugia / resources at concentrated locations	alternative prey	4	70
	artificial shelters	1	
	banker plant	1	
	beetle bank	6	
	conservation headlands	4	
	crop residue	1	
	field margins	1	
	flower(s) sown strips	17	
	grass sown weed strips	2	
	grassy margin	2	
	hedge	4	
	perennial margin	1	
	refuge crop strips	1	
	set-aside	1	
	sown weed strips	3	
	weed strips	4	
	various	15	
	unspecified	2	
Provision of refugia / resources spread across crop	alternative prey	2	52
	cover crop	2	
	flower(s) sown strips	3	
	food sprays	3	
	ground cover management	8	
	honeydew	2	
	intercropping	5	
	manure	2	
	mulch	7	
	nectar sources	2	
	pollen	1	
	soil surface architecture	1	
	undersowing	3	
	weed management	10	
	unspecified	1	
Landscape management	crop diversification & rotation in landscape	2	42
	diversification of landscape vegetation	19	
	movement facilitation landscape	6	
	quantified discussion of landscape influences	2	
	refugia in landscape	11	
	various	1	
	unspecified	1	
Increased ecosystem biodiversity	various	3	5
	unspecified	2	
Increased biodiversity of natural enemies	various	1	4
	unspecified	3	
Various	alternative prey	1	4
	various	3	
Unspecified	unspecified	5	5
All CBC practices			221

Table 2: Number of times that review papers reported research on different CBC practices in different crop types.

CBC practice and techniques group	Number of times CBC research in different crops was reported*								
	greenhouse vegetables	field vegetables	arable crops (incl. maize)	maize only	vines	orchards	various	unspecified	all crops
Limiting pesticide use			8				3	1	12
Manipulation of behaviour			2				2	1	5
Habitat manipulation			2				3		5
Optimizing plant morphology		1			1			2	4
Reduced disturbance			11	1			2		13
Provision of refugia / resources at concentrated locations	1	3	41	2	4	3	10	8	70
Provision of refugia / resources spread across crop		4	16	6	3	8	17	4	52
Landscape management			19	3	3	3	6	11	42
Increased ecosystem biodiversity							1	4	5
Increased biodiversity of natural enemies			1				1	2	4
Various			1				3		4
Unspecified						1	2	2	5
All CBC practices	1	8	101	12	11	15	50	35	221

*Note that zeros have been omitted from tables of frequencies in order to draw attention to the distribution of non-zero scores. All blank spaces in columns of numbers represent frequencies of zero.

Table 3: Relationship between pest order and the class of natural enemy addressed by the CBC research reported

Taxonomic order of pest	Number of times different classes of natural enemy referred to:						total number of times reported
	Parasitoid	Predator	Entomo-pathogenic nematodes	Entomo-pathogenic fungi	Various	Unspecified	
Acari		3					3
Coleoptera	5	2					7
Diptera		1		1			2
Hemiptera	8	20		6	6	1	41
Hymenoptera	1						1
Lepidoptera	8	4		1			13
Various	12	11	1	3	38	1	66
Unspecified	3	52	1	2	13	17	88
All	37	93	2	13	57	19	221

Experimental systems used in CBC research

- Field studies comprised the great majority (81%) of all reported CBC research and the field was the most commonly specified experimental system for every CBC techniques group (Table 4).
- Three percent of reported studies were conducted at scales no larger than semi-field scale. No reported studies were exclusively laboratory-based.
- Only three studies were conducted exclusively by modelling (Table 4) but 11 of the reported studies included the technique.

Table 4: Experimental systems used for the study of different CBC practices or techniques

CBC practice and techniques group	Number of times review papers discussed research in different experimental systems					
	field	lab-semifield	model only	various	unspecified	all systems
Limiting pesticide use	9			3		12
Manipulation of behaviour	2	1		1	1	5
Habitat manipulation	2			2	1	5
Optimizing plant morphology	1				3	4
Reduced disturbance	13					13
Provision of refugia / resources at concentrated locations	63	1	1	4	1	70
Provision of refugia / resources spread across crop	45	2		4	1	52
Landscape management	36	1	2	2	1	42
Increased ecosystem biodiversity	3	1		1		5
Increased biodiversity of NE	1			2	1	4
Various	1			2	1	4
Unspecified	2			3		5
All CBC practices	178	6	3	24	10	221

3.5.2.2. Evidence for the success of different CBC techniques

How the success of CBC was assessed in the literature

- The great majority of reports of CBC techniques were accompanied by an assessment of the effectiveness of the techniques (Table 5).
- The effect of CBC on natural enemies was discussed in over 90% of reports of CBC techniques.
- The effect of CBC on pests was discussed much less frequently (47% of reports) and it was rarely reported without also reporting the effect on natural enemies (Table 5).
- Only reports on the influence of increased ecosystem and natural enemy biodiversity discussed effects on pests as frequently as they discussed effects on natural enemies (Table 5).

Over-all effectiveness of CBC

- The implementation of CBC techniques was accompanied by increased abundance or fitness of natural enemies in the great majority of reports where the evidence was assessed (94%); the evidence was judged to be strong in 42% of these positive reports (Figure 2).
- CBC was accompanied by increased pest control in 80% of reports where the evidence was assessed. This evidence was judged to be strong in 25% of those positive reports.
- The effect of CBC on intra-guild predation (IGP) was rarely reported and so will not be considered further in the results section.

Table 5: The frequencies that reports of CBC techniques were accompanied by assessments of effects on natural enemies and pests.

Practice and techniques group	Frequency that effects of CBC were not reported	Frequency that effects of CBC were reported			Total number of reports
		on NE only	on pest control only	on both NE and pest	
Limiting pesticide use	1	4		7	12
Manipulation of behaviour	1	3		1	5
Habitat manipulation	1	1		3	5
Optimising plant morphology		2		2	4
Reduced disturbance		9		4	13
Provision of refugia / resources at concentrated locations	3	34		33	70
Provision of refugia / resources spread across crop	1	25	1	25	52
Landscape management	4	20	1	17	42
Increased ecosystem biodiversity				5	5
Increased biodiversity of natural enemies	1		1	2	4
Various	1	2		1	4
Unspecified	3	1		1	5
All	16	101	3	101	221

Effectiveness of different CBC techniques

- The strongest evidence for a positive effect of CBC on natural enemies was associated with the three techniques groups covering management of the landscape and provision of refugia and resources within it (Figure 3). In 99% of the 154 reports of these CBC techniques there was judged to be accompanying benefit to natural enemies and the evidence for this was strong in 46% of the reports.
- The other CBC techniques were less reported but most reports were accompanied by evidence of effects on natural enemies that were exclusively beneficial. There was usually a smaller proportion of reports where the evidence was judged to be strong except in the case of 'increased ecosystem biodiversity' for which the reported degree of benefit was similar to refugia and resource provision, probably for the same reasons.
- Evidence for the benefit to natural enemies of limiting pesticide use was surprisingly weak and this may reflect choice of search terms used rather than the literature published on the subject. The two reports of negative effects of limiting pesticide use are associated with the use of insect-resistant GM crops.
- There was no consistent evidence that increasing NE biodiversity of was either beneficial or detrimental to their abundance or fitness but this subject was reported only twice.
- The strongest evidence for a positive effect of CBC on pest control was again associated with the three techniques groups covering management of the landscape and provision of refugia and resources within it (Figure 4). Of the 77 reports discussing effects on pests, 82% reported increased pest control. However, there were fewer reports compared to natural enemies and the evidence for benefit was strong in 17% of them only.
- Although effects on pest control associated with other CBC techniques were more rarely discussed, for each technique there were reports of increased pest control and none of decreased pest control.
- The strong increase in pest control associated with limiting insecticide use was derived from six reports on the effect of insect-resistant GM crops.

- One review reported that there was strong evidence for increased pest control (aphids) associated with increased natural enemy biodiversity.
- A complete table summarising the reported effects of different CBC practices by category and sub-category and on pests and natural enemies is given in Appendix 5.

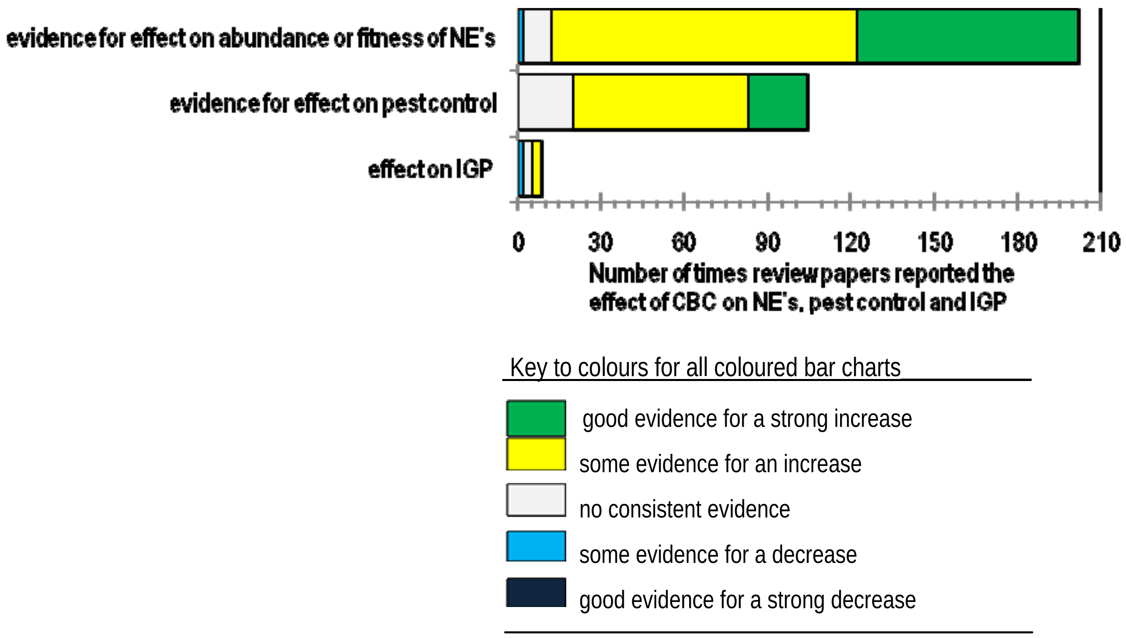

Figure 2: Reported influence of CBC on natural enemies, pest control and intra-guild predation (IGP).

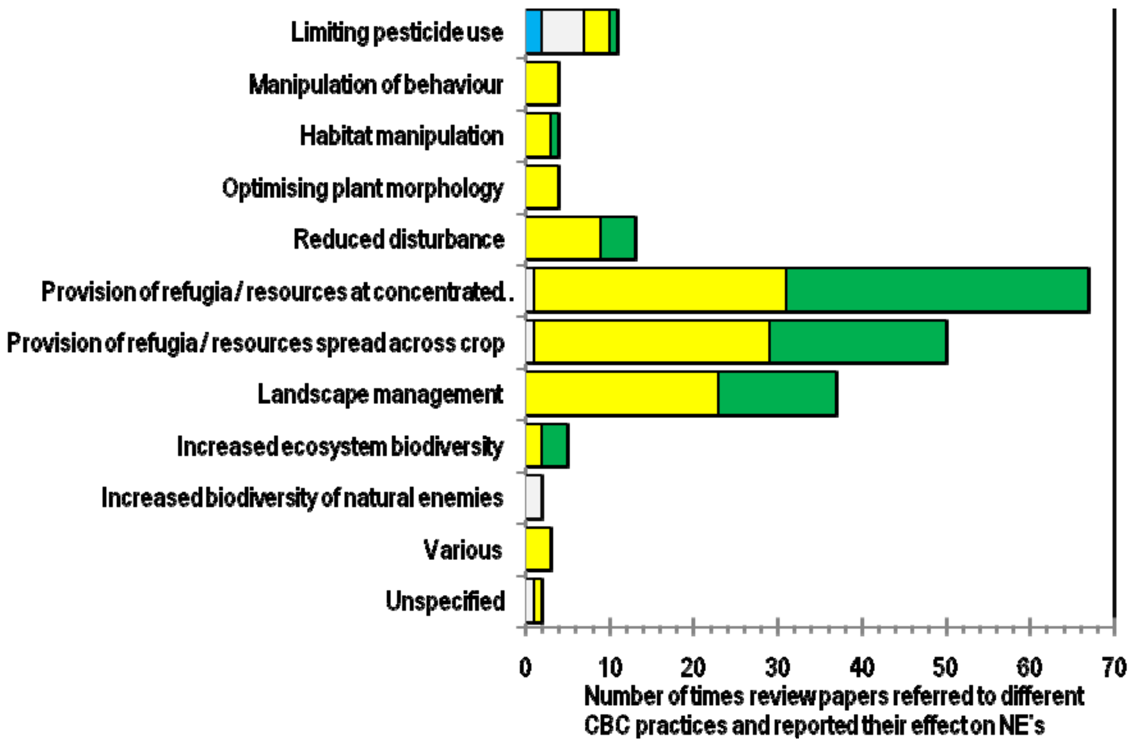

Figure 3: Reported influence of different CBC practice and technique groups on abundance or fitness of natural enemies. See Fig. 2 for key to colours.

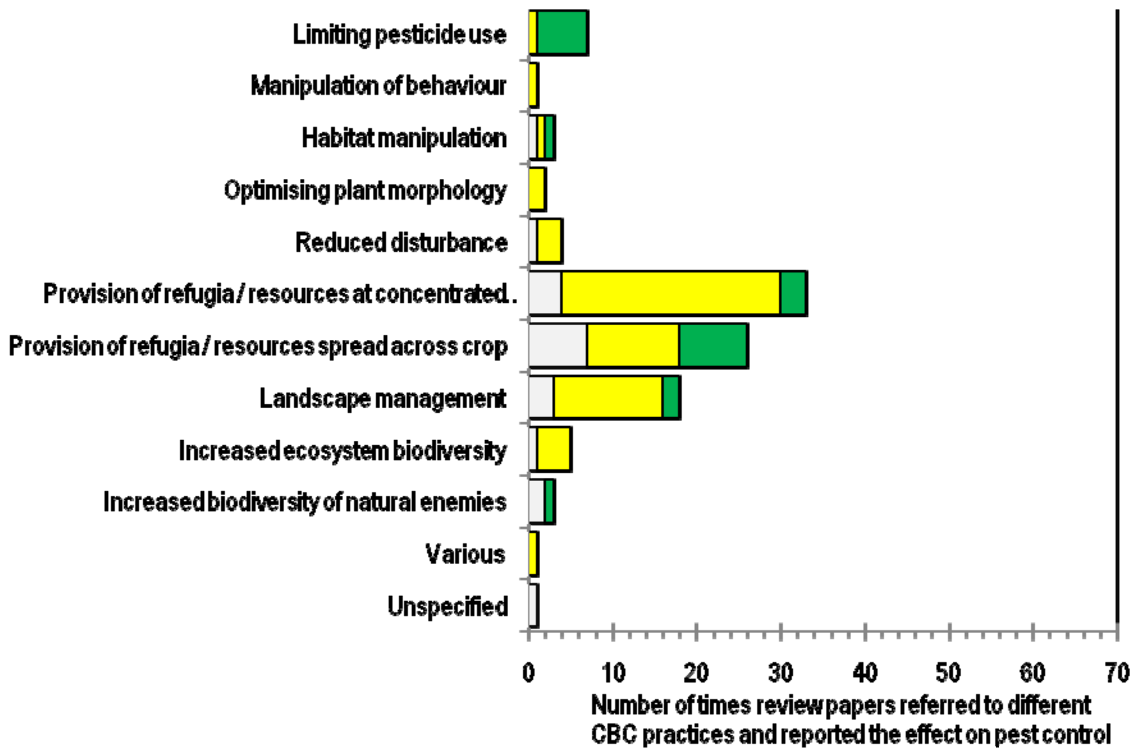

Figure 4: Reported influence of different CBC practice and technique groups on pest control. See Fig. 2 for key to colours.

Effectiveness of CBC in different crop types: natural enemies

- The largest proportion of reports that gave strong evidence that CBC promoted natural enemies were from field vegetables and vines (63% and 60%, respectively, of reports where it was assessed) (Figure 5). In 10 of these 12 reports, the strong benefit of CBC to natural enemies was associated with the provision of refugia or resources and in two reports (in vines) it was associated with landscape management.
- Many reports relating to arable crops (45%) also linked CBC with strong evidence of natural enemy promotion. Most such reports concerned the provision of refugia or resources (31 out of 44) or landscape management (8 out of 44).
- Smaller proportions of reports on orchards and maize cited strong evidence for effects of CBC on natural enemies (29% and 36%, respectively) but each of these reports was again associated with the provision of refugia or resources.

Effectiveness of CBC in different crop types: pests

- Reports relating to vines had the largest proportion (57%) that referred to strong evidence that CBC promoted pest control (Figure 6). The CBC techniques associated with this benefit were the provision of refugia or resources (three reports) and landscape management (one report).
- For all the other crops, the proportion of reports indicating strong effects of CBC on pest control was considerably smaller, amounting to a total of ten out of 59 reports (field vegetables 40%, orchards 33%, arable crops 17%, maize 0%). Five of these ten reports related to the provision of refugia or resources and one to landscape management. The remaining four were associated with the use of GM insect-resistant arable crops.

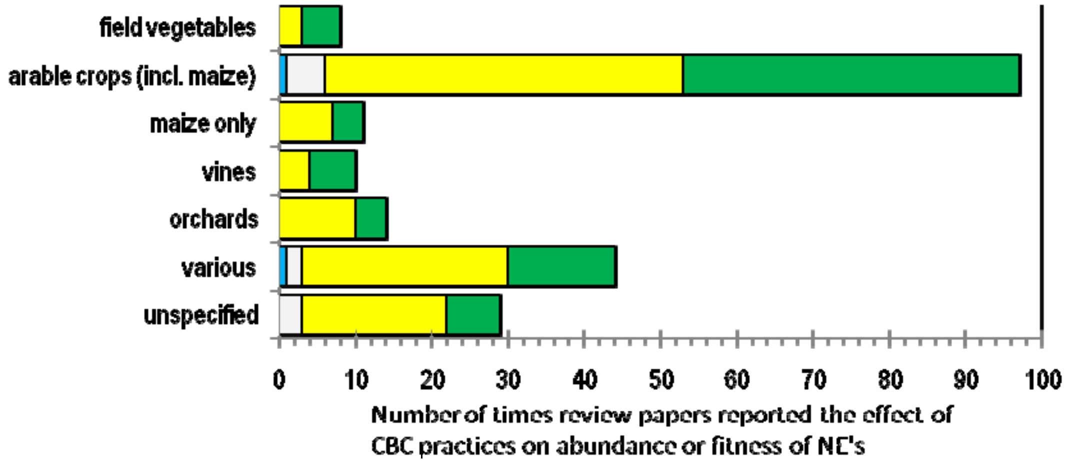

Figure 5: **Reported effectiveness of CBC in promoting the abundance or fitness of natural enemies in different crop types. See Fig. 2 for key to colours.**

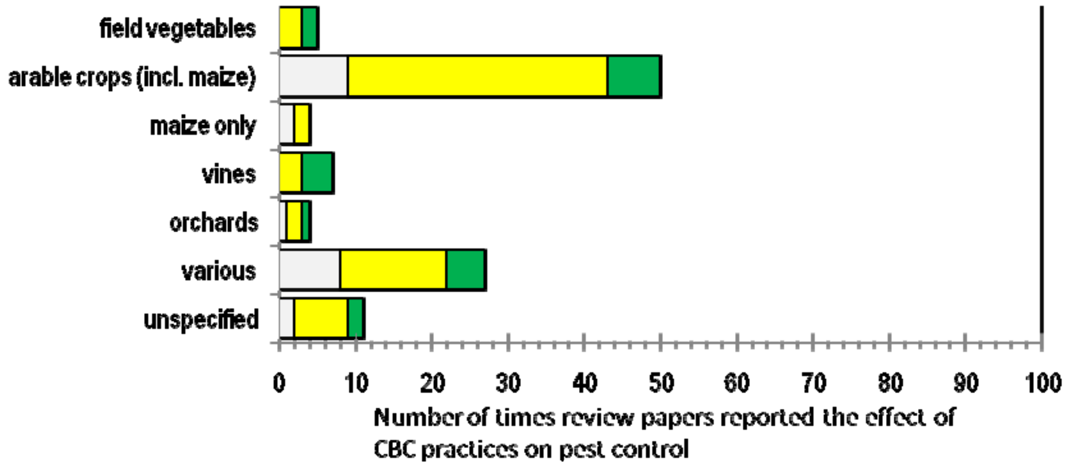

Figure 6: **Reported effectiveness of CBC in pest control in different crop types. See Fig. 2 for key to colours. See Fig. 2 for key to colours**

3.5.2.3. The involvement of European research institutions in CBC research

3.5.2.3.1 The involvement of European institutions in the primary research reported by review papers

Countries represented

- Countries in the European Union have a strong record of research on CBC, being involved with 63% of all reports of CBC techniques analysed here (Table 6).
- Those reports that derived from individual European countries were exclusively from northern and central Europe. The UK was particularly well represented in the reports, followed by Germany and Switzerland.
- The USA was the non-European country most strongly represented in reports of research derived from a single country, followed by New Zealand (Table 6; note that these countries were also represented in the 'EU and elsewhere' category).

Table 6: Representation of European and other countries in reported CBC research

Region(s) from where research activity was reported	Country	Number of times research activity was reported
Europe: EU	Czech Republic	1
	Finland	1
	Germany	5
	Hungary	1
	Netherlands	2
	Sweden	3
	UK	16
	various	14
EU and elsewhere		97
Europe: not EU	Switzerland	5
All Europe		145
Exclusively outside Europe	China	1
	New Zealand	6
	USA	48
	various	4
	All	59
Unspecified		17
All regions		221

CBC practices and techniques studied in Europe and elsewhere

- Research on CBC in Europe in the review period focussed strongly on the provision of refugia and resources (55% of reported research involving any European country), especially at concentrated locations, and to a lesser extent on landscape management (17% of reports) (Table 7).
- The focus of CBC research outside Europe was similar, 56% reports concerning the provision of refugia and resources (but especially those spread across the crop) and 27% concerning landscape management (Table 7).

Crops studied in Europe and elsewhere

- Arable crops were the subject of the great majority of CBC research in Europe that was reported by the reviews (85% of reports concerning single crop types) (Table 8).
- No other crop was the subject of more than 7% of such reports from Europe. Studies on maize in Europe were reported only once and on vines not at all.
- Outside Europe, arable crops were also the subject of the largest proportion of the reports of CBC research concerning single crop types (51%) but this proportion was smaller than in Europe (Table 8).
- Vines, maize and orchards were the subject of 24%, 22% and 20%, respectively, of such reports from outside Europe.

Table 7: Focus of CBC research in Europe and elsewhere: (a) CBC practice or technique

CBC practice and technique group	Number of times review papers reported research activity in:						
	European Union	EU and elsewhere	Europe but not EU	all Europe	exclusively outside Europe	unspecified	all regions
Limiting pesticide use	3	8		11		1	12
Manipulation of behaviour	2	1		3		2	5
Habitat manipulation		3		3		2	5
Optimizing plant morphology		1		1	3		4
Reduced disturbance	1	6		7	5	1	13
Provision of refugia / resources at concentrated locations	17	34	2	53	11	6	70
Provision of refugia / resources spread across crop	10	15	2	27	22	3	52
Landscape management	9	14	1	24	16	2	42
Increased ecosystem biodiversity	1	3		4	1		5
Increased biodiversity of NE		3		3	1		4
Various		4		4			4
Unspecified		5		5			5
All CBC practices	43	97	5	145	59	17	221

Table 8: Focus of CBC research in Europe and elsewhere: (b) crop type

Crop type	Number of times review papers reported research activity in:						
	European Union	EU and elsewhere	Europe but not EU	all Europe	exclusively outside Europe	un-specified	all regions
Greenhouse vegetables	1			1			1
Field vegetables	2	2	1	5	2	1	8
Arable crops (incl. maize)	32	39	1	72	23	6	101
Maize only	1			1	10	1	12
Vines					11		11
Orchard	1	2	3	6	9		15
Various	3	39		42	5	3	50
Unspecified	4	15		19	9	7	35
All crop types	43	97	5	145	59	17	221

Experimental system used in Europe and elsewhere

- Research on CBC in Europe was overwhelmingly field-based, with only small proportions relying exclusively on laboratory and semi-field scale studies or on modelling (Table 9).
- Outside Europe, CBC research was also overwhelmingly field-based (Table 9).
- Europe is represented strongly in the record of modelling for CBC research. Modelling was included in 11 out of 134 studies with European participation (where specified). Modelling was not reported from the 53 studies exclusively outside Europe where methods and countries were specified.

Table 9: Focus of CBC research in Europe and elsewhere: (c) experimental system

Experimental system	Number of times review papers reported research activity in:						
	European Union	EU and elsewhere	Europe but not EU	all Europe	exclusively outside Europe	un-specified	all regions
Field	39	71	5	115	52	11	178
Laboratory - semifield	2			2	4		6
Model only	1	1		2		1	3
Various	1	23		24			24
Unspecified		2		2	3	5	10
All systems	43	97	5	145	59	17	221

3.5.2.3.2 The contribution of European institutions to the authorship of the review papers used for this meta-review

- Institutions in European countries contributed strongly to the review literature on CBC. Half of all contributing institutions were European and 40% of all contributions were from European countries (Table 10). For complete lists of contributing institutions in Europe and elsewhere, together with their contributions to review paper authorship, see Appendices 6 and 7.
- The UK was particularly well represented and the Netherlands and Switzerland also made strong contributions.
- The USA was the single country most strongly represented in authorship of review papers (Table 10).
- Countries outside Europe and the USA were represented only by single contributions to review papers on CBC except for Australia, Kenya and New Zealand. Lincoln University, the only contributing institution in New Zealand, was involved in a remarkably large number of the reviews (Table 10; Appendix 7).

Table 10: Representation of European and other institutions in authorship of the reviews that were the source literature for this meta-review

Region(s) where institutions were located	Country	Number of institutions contributing to authorship of review papers	Total number of times institutions represented in authorship of review papers
Europe: EU	Austria	2	2
	Belgium	1	1
	Denmark	1	2
	Finland	1	1
	France	2	2
	Germany	2	6
	Hungary	2	2
	Italy	2	1
	Netherlands	5	10
	Spain	1	1
	Sweden	1	2
	UK	24	38
Europe: not EU	Switzerland	5	7
All Europe		49	75
Outside Europe	Australia	5	15
	Canada	1	1
	Indonesia	1	1
	Israel	1	1
	Japan	1	1
	Kenya	2	3
	México	1	1
	New Zealand	1	18
	USA	37	71
All outside Europe		50	112
All regions		99	187

3.5.3. Knowledge gaps that represent barriers to the implementation of CBC

3.5.3.1. Analysis of gaps in relation to the CBC techniques reviewed in section 2.5.2

- Landscape management was the CBC practice most frequently identified as a priority for future research, 39 of the 90 review papers recording this as a need (43%) (Figure 7). This is proportion is considerably larger than the proportion of reports of past research that were related to landscape management (19%; Table 5), indicating a view that this area of work should be expanded.
- The provision of refugia and resources was the CBC practice next most frequently identified as needing more research (36% of reviews; Figure 7). This topic was the subject of 55% of the reports of past CBC research in the reviews (Table 5).
- The impact of increased biodiversity on CBC was the subject of only 4% of reports of past research (Table 5) but was frequently stated as a priority for future research (30% of reviews; Figure 7).
- Manipulation of behaviour and limiting pesticide use were seen as priorities for further research by significant minorities of review papers. Reduced disturbance was much less frequently recommended (Figure 7).

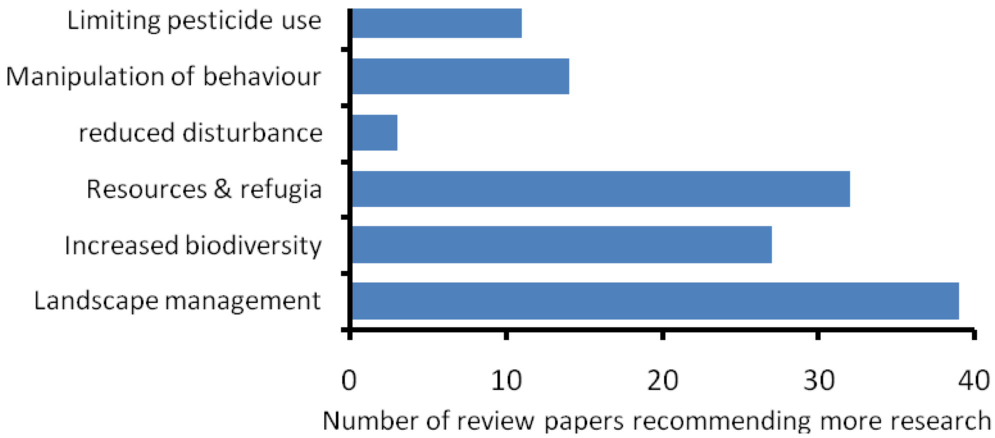

Figure 7: Numbers of review papers recommending further research in different 'practice or technique' groups.

3.5.3.2. Analysis of gaps in the science underpinning CBC

- The research gap categories identified by review papers were grouped into 11 categories (Figure 8) and 36 sub-categories (Figure 9). See Appendix 8 for more complete definitions of the sub-categories.
- 'Landscape scale interactions' was the category of gap most frequently identified as a priority for further research, 41 of 90 review papers recording this as a need (46%) (Figure 8). Within this category, studies of the appropriate spatial scale for landscape management for CBC and studies of the movement of natural enemies within the landscape were the topics most frequently recommended for further study (Figure 9).
- Community ecology, autecology and behavioural ecology were identified as priorities for further research by 35, 23 and 15 review papers (39%, 26% and 17%), respectively. In these categories the following were considered most important for future study: the impact of natural enemy diversity, interactions and community dynamics on CBC; the study of the traits and population dynamics of natural enemies and their responses to habitats; the manipulation of natural enemy behaviour (e.g. by exploiting chemical ecology, push-pull, mixed cropping) (Figure 9).
- Determination of plant or habitat characteristics that encourage CBC was recommended as a priority for further research by 29 out of the 90 review papers (32%) (Figure 8). In this category the topics considered most important for study included the comparative benefits of plants and habitats to natural enemies, their management for natural enemies, their role as sources or sinks for natural enemies, and their relative value to pests and to beneficial organisms (Figure 9). Only four reviews recommended studies of the influence of plant resistance to insects on CBC and four recommended studies of the risks and benefits to CBC of transgenic crops.
- Assessment of the impact of CBC was seen as a priority for future research by 22 review papers (24%) (Figure 8). It should focus on testing the effectiveness of CBC in relation to pest control, reduction in pesticide use, improved crop yield and cost-benefit analysis, as well as identification of natural enemies with the most impact on biological control (Figure 9; Appendix 6).
- The provision of resources and refugia was a subject considered to need more research by 13 of the reviews (Figure 8) and this should focus on means of managing resources for natural enemies (e.g. banker plants, food sprays, nectar and pollen sources, alternative prey) (Figure 9).
- Thirteen of the reviews recommended that CBC research should make more use of particular methodologies (Figure 8). Modelling was singled out by five of the reviews as a priority and long term studies by three (Figure 9).

- Nine reviews recommended more research on non-arthropod natural enemies, seven of these advocating that entomopathogens were worthy of more study and two advocating entomopathogenic nematodes (Figure 8, Figure 9).
- Only eight reviews specifically mentioned IPM or precision farming in their recommendations for further research (Figure 8). Five of those that did so recommended that more research effort should be applied to the integration of CBC into IPM (Figure 9).
- The socio-economic drivers of the uptake of CBC by farmers were mentioned as a priority for further research by only three of these science-based reviews (Figure 8).
- Spatial and temporal factors were considered important in relation to many of the research categories discussed in this section (above). Further study of the effect of spatial temporal factors on the potential effectiveness of CBC was recommended by 43 and 13 of the 90 review papers, respectively.

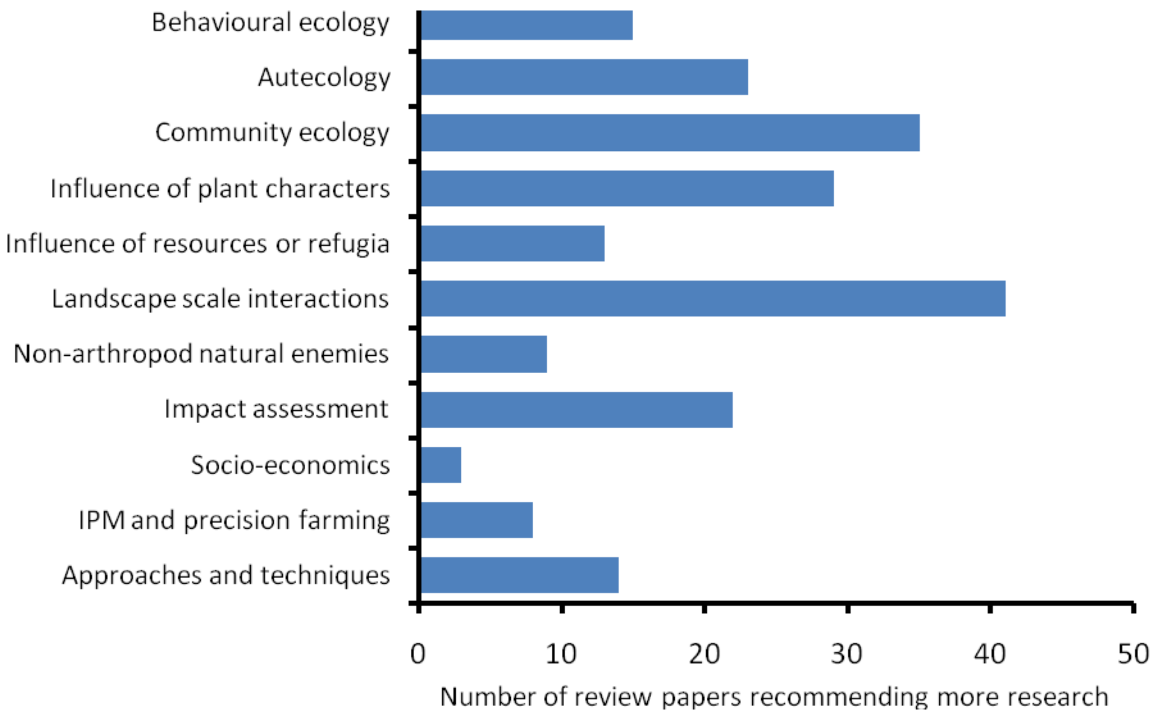

Figure 8: Categories of science underpinning CBC that were identified by review papers to need further research.

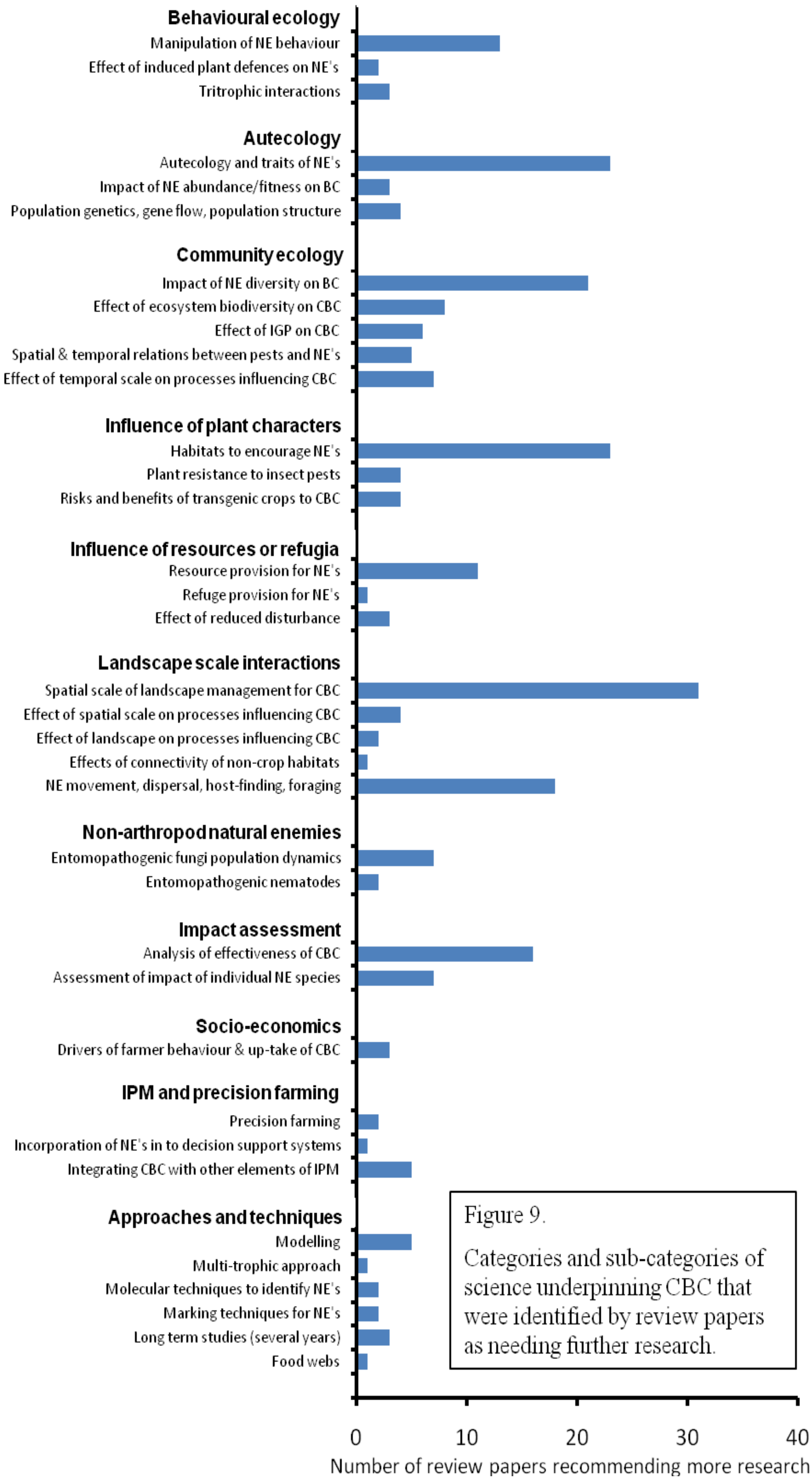

Figure 9: Categories and sub-categories of science underpinning CBC that were identified by review papers as needing further research.

3.5.4. Other challenges to the implementation of CBC discussed in the literature

- Challenges to CBC implementation identified in the review papers were allocated to five categories and 14 sub-categories (Figure 10, Figure 11; see Appendix 9 for more complete definitions of the sub-categories).
- These largely non-scientific challenges to CBC implementation were mentioned less frequently by the 90 scientific review papers than were scientific gaps in the knowledge-base (Figure 8, Figure 10).
- A lack of interdisciplinary research, e.g. the division of practitioners into different ecological, agronomic and socio-economic disciplines and sub-disciplines, was seen to hamper scientific advancement in support of CBC by five of the reviews (Figure 11).
- High research and development costs were seen to be a challenge to the implementation of CBC by 7 reviews (Figure 10), particularly in relation to the cost of large landscape-scale or long term studies, the cost of semiochemical registration and the difficulty of creating a saleable commodity to provide a return on research investment (Figure 11).
- Eight reviews cited knowledge transfer as a challenge to CBC implementation (Figure 10). They advocated the development of improved knowledge transfer methods and highlighted the complexity introduced by the effect of local ecological variation on CBC success (Figure 11). Two reviews regarded a shortage of taxonomic expertise and training materials for natural enemy identification as a problem.
- Socio-economic factors were considered to be potential impediments to CBC implementation by 7 reviews. The perceived risk of implementing CBC (lack of consistent evidence for success), its perceived complexity in comparison to conventional chemical-based control and the transitional costs of establishing CBC were cited. Cultural conservatism was considered a potential problem by two reviews.
- Six reviews discussed potential challenges attending policymakers in the development and implementation of agri-environment policy to support establishment of CBC (Figure 10). In particular they discussed the difficulty of designing policy to promote large-scale landscape changes that would be implemented through individual farmers who make their living at smaller spatial scales (Figure 11). One review mentioned that the multiple functions of agri-environment schemes led them to be complex and another highlighted the challenge facing policymakers of increasing both crop production and biodiversity.

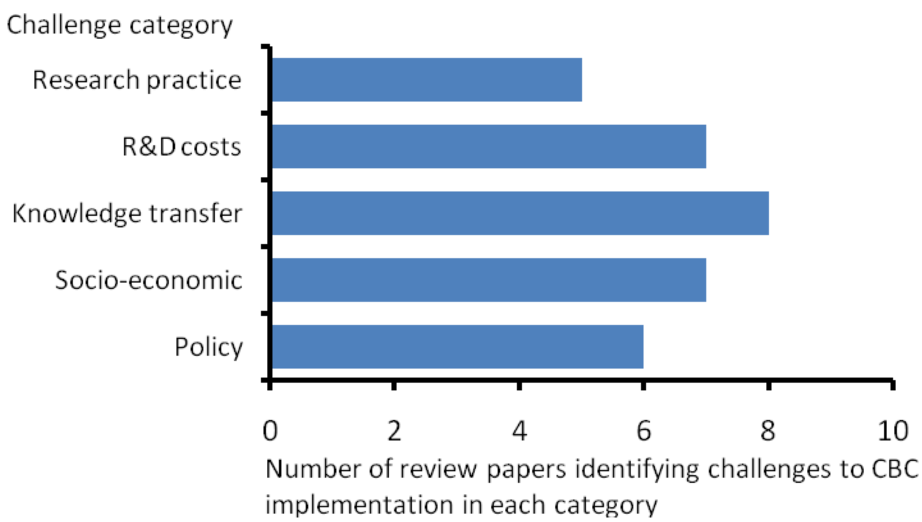

Figure 10: Categories of challenge to the implementation of CBC that were identified by review papers.

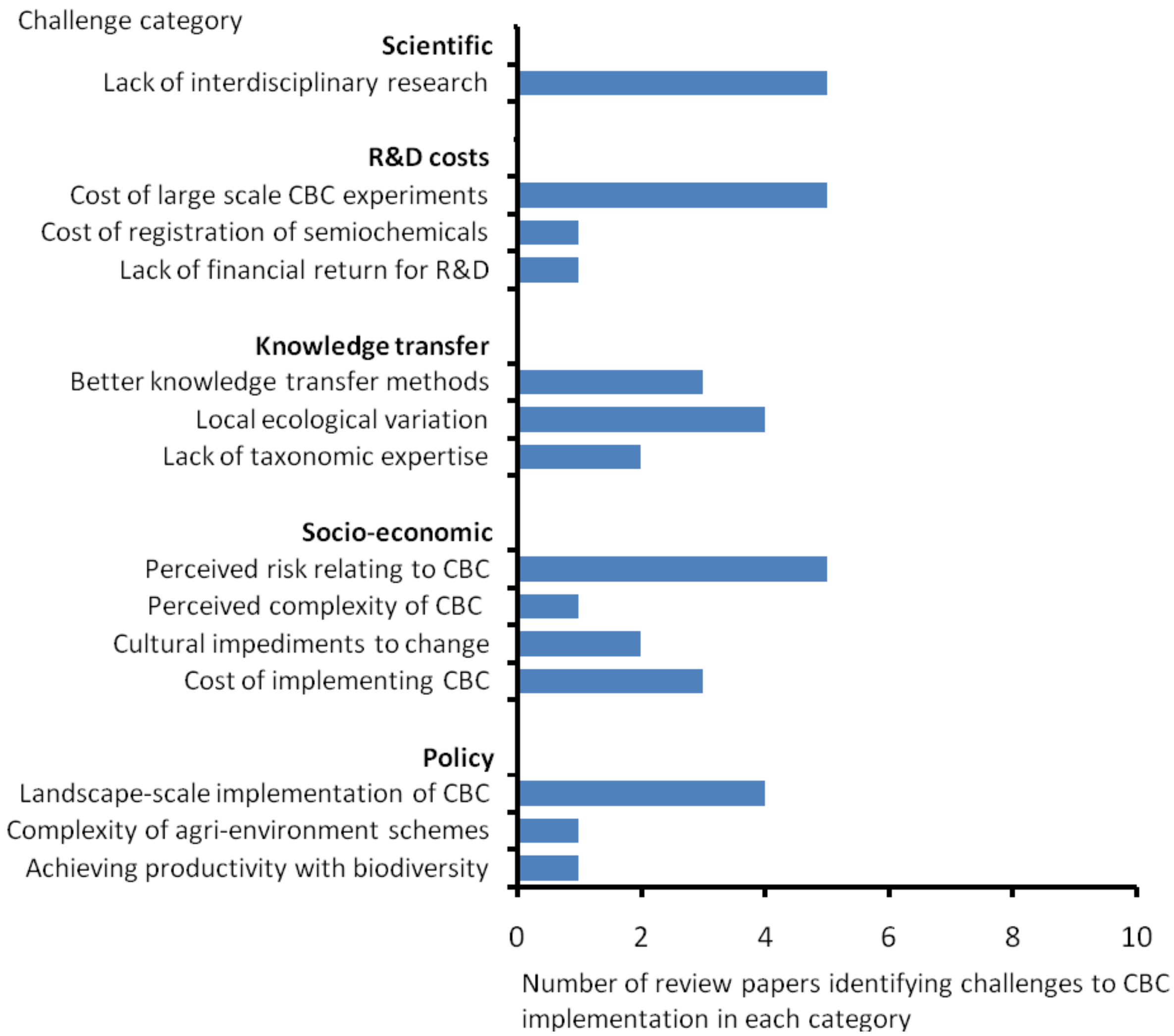

Figure 11: Categories and sub-categories of challenge to the implementation of CBC that were identified by review papers.

3.6. Results: Management of plant pathogens

For the management of plant diseases conservation biological control is achieved either by preserving the fraction of the micro-flora with antagonistic or competitive effects against the plant pathogens or by active measures aiming to foster their survival or their development at the expense of the plant pathogens (van Driesche & Bellows, 1996).

3.6.1. Biological control of air-borne pathogens

Biological control strategies against air-borne pathogens are seldom conceived as anything but deployment of inoculative/inundative releases (Hajek, 2004) and in the scientific literature, Conservation Biological Control is very rarely associated with the management of air-borne diseases, whether in terms of field use or even merely as a research topic.

Nevertheless, naturally present epiphytic micro-organisms with competitive or antagonistic effects against air-borne plant pathogens may play a greater than suspected role in protecting plant surfaces and an analysis of what little information is available suggests that the potential of CBC approaches in the canopy should not be overlooked. For example, Dik and VanPelt (1992) have concluded, from field studies on the control of Septoria leaf blotch, that naturally occurring saprophytes on the surface of wheat leaves should be protected (by eliminating harmful chemicals) to enhance protection. In the last 20 years, much progress has been made in the comprehension of microbial communities in the phyllosphere (see recent review by Whipps et al. 2008) and ways to manipulate their composition have been explored (Nix-Stohr et al. 2008) and could offer a potential for biological control.

Interestingly, conservation biological control strategies set in place for the control of arthropod pests may also have beneficial effects against air-borne diseases (Gurr et al. 2007). In New Zealand, manipulating the floor of the vineyards has provided much success and attracted interest from industry for the management of *Epiphyas postvittana*. In a similar way, application of mulch was used successfully to enhance soil microbial activity and degradation of vine debris, resulting in significant reduction in a major source of primary airborne inoculum of *Botrytis cinerea* (Jacometti *et al*., 2007).

Conservation Biological Control represents a virtually untapped resource for the management of air-borne diseases and much work will be necessary to fully explore this promising area. Possible avenues for future research include:

- manipulate the resident phylloplane microflora to foster the establishment of components with the highest effects against air-borne plant pathogens. Microbial ecology studies will be required to identify those beneficial components of the phyllosphere microflora and the key factors that regulate their populations. Models describing their interactions will be needed to allow the screening of large numbers of scenarii and evaluate the usefulness of various manipulative approaches.
- breed plant varieties able to harbour a larger fraction of the natural phylloplane microflora with antagonistic or competitive effect against selected major airborne plant pathogens.

3.6.2. Biological control of soil-borne pathogens

3.6.2.1. Context and definitions.

Biological control of soil-borne diseases was proposed more than forty years ago. Indeed, a symposium held in Berkley in 1965 was entitled: “Ecology of soil-borne plant pathogens; prelude to biological control” (Baker & Snyder, 1965). The two main approaches to biological control of soil-borne pathogens were already proposed: enhancement of the naturally occurring populations of antagonists and introduction of a selected biological control agent.

These two strategies can be respectively compared to “conservation biological control” and “inoculation biological control” as defined by Eilenberg (2006). “Inoculation biological control” is “the intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently”. On the other hand, “Conservation biological control” is the modification of the environment or existing practices to protect and enhance specific enemies or other organisms to reduce the effects of pests”.

In the case of soil-borne diseases, the pathogens are always included in the soil matrix; this lead to the concepts of “soil inoculum potential” and “soil suppressiveness to diseases”. The soil inoculum potential is the soil-dependent capacity of pathogens to incite disease, and it results from the interactions between inoculum density and soil suppressiveness. Soil suppressiveness corresponds to the global effects of the soil microbiota interacting with the pathogens. Two main mechanisms are responsible for soil suppressiveness: general suppression, which is correlated with the activity of the total microbial biomass at critical times for the pathogen, and specific suppression which is due to the activity of specific micro-organisms that are antagonistic to the pathogen (Cook & Baker, 1983). Soil suppressiveness being essentially biological, it is possible to increase soil suppressiveness by cultural practices that influence different aspects of soil biology. Managing cultural practices in order to increase soil suppressiveness to diseases corresponds to “conservation biological control” as defined above.

3.6.2.2. Cultural practices used for conservation biological control of soil-borne diseases.

Crop rotation

As a rule, continuous cropping of the same plant species will lead to an increase in incidence of soil-borne diseases, while rotation with non-hosts should lead to a decrease in incidence. There are few exceptions to this general law that mono-cropping will increase disease. The best known example is that of take-all decline: after increasing during a few years (4-5) disease severity will decrease, to such a level that the yield will not be affected by the disease (Hornby, 1998). This is a clear example of continuous cropping altering disease suppressiveness of soil through its effects on specific components of the soil microflora. On the contrary, most of the diseases induced by soil borne plant pathogens could be controlled by an appropriate crop rotation sequence. The main effect of crop rotation is to allow time for decrease, through natural mortality, of inoculum of pathogens that are poor saprophytic competitors. Clean fallows have the same mechanism. However, since mortality of pathogen propagules in soil is frequently due to the effects of other organisms, the stimulation of microbial activity by the growing rotational crop should make rotation more effective than fallowing. Crop rotation increases the diversity of plants within an agricultural system, which may have effects on the diversity of soil biota (Lupwayi et al., 1998).

Tillage

Soil disturbance by tillage has been shown to have a variety of effects on diseases. Root rots of many crop plants caused by R. solani are generally less severe after tillage than with direct drilling (Roget et al., 1985). On the other hand, common root rot of wheat caused by Cochliobolus sativus may be more severe in tilled soils (Mathieson et al., 1990). These well-characterized effects of tillage on disease seem to act directly on the pathogen, with no evidence yet of effects on other components of the soil biota. However, tillage is expected to have some influence on soil suppressiveness because it does alter the activity and diversity of soil microflora. Typically, tillage reduces bacterial biomass and diversity in soil, possibly through its effects on soil aggregation (Lupwayi et al., 2001). Reduced tillage systems should therefore have more diverse and active microflora, and greater general suppression of diseases.

Residue management

Residue management can have conflicting effects on disease. Retaining residues increases the inoculum potential of pathogens that survive in the residue. On the contrary, residue retention can boost the levels of general suppression in soils. Indeed, general suppression has been linked to high levels of microbial activity, which depend on high levels of OM input into soils. Moreover, residue retention may favour specific antagonists increasing the level of specific suppression. For example, populations of cellulolytic organisms tend to be higher in soils where crop residues are retained and high cellulolytic activity has been correlated with suppression of disease such as Fusarium seedling blight in barley (Papavizas, 1985).

Solarisation

Solarisation or solar heating is a method that uses the solar energy to enhance the soil temperature and reach levels at which many plant pathogens will be killed or sufficiently weakened to obtain significant control of the diseases. Solarisation does not destroy all the soil micro-organisms, but modifies the microbial balance in favour of the beneficial micro-organisms. Efficacy of soil solarisation is not only due to a decrease of the pathogenic populations but also to an increase of the density and activity of populations of micro-organisms antagonistic to the pathogens. Soil solarisation is really a conservation biological control practice. It possesses a very large spectrum of activity; it controls fungi, nematodes, bacteria, weeds, arthropod pests and some unidentified agents (Katan, 1996).

Biofumigation or biodisinfection

Biological soil disinfection is based on plastic tarping of the soil after incorporation of fresh organic matter. The mechanisms involved are not totally understood. Fermentation of organic matters results in the production of toxic metabolites and in anaerobic conditions which both contribute to the inactivation or destruction of the pathogenic fungi. Based on the dominant type of mechanisms involved, it was proposed to make the distinction between (i) biofumigation which corresponds to the use of specific plant species containing identified toxic molecules, and (ii) biodisinfection which refers to the use of high quantities of organic matter which results in anaerobic conditions mainly responsible for the destruction of the pathogens (Lamers et al., 2004).

Compost amendments
Addition of organic amendments such as animal manures and industrial by-products is the best-documented strategy for increasing disease suppression in soils. Manures and other amendments tend to increase microbial biomass and biological activity in soil, and thus to enhance general suppression. Composting organic matter is an interesting process enabling to transform wastes from different origin in composts which are beneficial for soil health. This is a biological process characterized by a heat peak which destroys the thermo-susceptible micro-organisms, resulting in compost free from most plant pathogens Most of these composts possess the capacity to increase soil suppressiveness to diseases However, there is no universal rule; the level of disease control obtained depends on many factors such as the chemical properties of the parent materials, the composting process, the types of micro-organisms which colonized the compost after the heat-peak and obviously the type of plant pathogens to be controlled (Termorshuizen et al., 2006).

3.6.3. Conclusions

These management practices that contribute to control soil-borne plant pathogens are not exclusive from other biological control methods. On the contrary they should be used in association with other biological methods such as the use of specific biological control agents. However it is our opinion that these conservation biological control methods have been neglected. Obviously there are much more difficult to apply than other control methods and their efficacy depends on many factors which are not easy to control. But the sustainable approach requires that all the available methods should be used in association in order to drastically reduce the use of chemical pesticides.

3.7. Results: Management of weeds

Biological control of weeds has to date largely focussed on the release of agents as ‘bioherbicides’ in augmentative release, inundative release or classical biological control programmes (Rao, 2000). The development of conservation strategies for weed biological control agents has been directed at these released agents (fungal pathogens, rhizobacteria, insects and nematodes). Considerably less research has been directed towards CBC strategies that optimise the impact of naturally-occurring populations of weed natural enemies than has been done for invertebrate pests. There is a view that, although naturally-occurring levels of biological control are typically high (particularly through seed predation), the control exerted is not easily manipulated and is thus of limited value as a management tool (Norris, 2007). Nevertheless, there are good examples of native agents that control weeds that might be successfully exploited should management strategies be established to conserve them (Newman et al., 1998).

Factors influencing the conservation of naturally-occurring biological control agents in CBC strategies are likely to be similar to those affecting released agents. These include the timing and nature of disturbances such as tillage, grazing, mowing, harvesting and pesticide applications, the choice of crop rotation and the provision of habitats and refugia (Newman et al., 1998, Rao, 2000).

The biological control agents that have received most attention in weed CBC research are deleterious rhizobacteria, and granivorous carabids, ants and small rodents. The techniques that appear to have most potential for weed CBC are the management of crop residues by conservation tillage and by manipulation of crop rotations, and the management of habitats (refugia and resources) for invertebrates. Both deleterious rhizobacteria and carabids can benefit from the accumulation of crop residues in the soil or at its surface.

Deleterious rhizobacteria in CBC strategies for weeds

Amongst the many cultural control methods used for weed management are the use of mulches and residues with allelopathic properties (Shennan, 2008). There is good evidence that such soil management practices lead to accumulation of organic matter and to increased soil enzyme activity that can be associated with suppression of both plant diseases and weeds (Kremer and Li, 2003). In soils thus managed, the diversity of microbial populations is increased and the potential to develop weed-inhibiting bacterial communities is enhanced (Kremer and Li, 2003). Among such bacterial communities are deleterious rhizobacteria that can be isolated from rhizosphere soils and can inhibit weed growth (Ibekwe and Kennedy, 1999, Kremer and Kennedy, 1996).

It has been proposed that crop residue management could be specifically designed for CBC strategies to encourage the strains of naturally-occurring deleterious rhizobacteria that are harmful to weeds (Kremer and Li, 2003, Rao, 2000). Moreover, it is possible that crop rotations could be redesigned to optimise the development of specific strains of rhizobacteria for weed suppression because particular rhizobacteria can be associated with the roots of particular plant species (e.g. maize; Turco et al., 1990, Rao, 2000).

Insects in CBC strategies for weeds

There is good evidence that native populations of phytophagous insects can be managed to exert substantial control on weed species. For example, snakeweeds and locoweeds, native weeds of rangeland in the south-western, USA can be substantially controlled by native grasshoppers and root-boring beetles if prescribed burns and insecticide applications are timed to avoid vulnerable points in their life-cycle (Newman et al., 1998). Furthermore, both experimental and modelling studies show that predation of weed seed by invertebrates has the potential to reduce the weed seed-bank (Menalled et al., 2005).

A significant number of species of carabid beetle are predominantly or in part phytophagous and some of these have a granivorous habit (especially in the genera *Amara*, *Harpalus*, *Ophonus* and *Zabrus*) that gives them particular potential in CBC strategies for weed control. In warm temperate areas such as the Mediterranean region, ants are more significant seed predators and in some crops (e.g. winter cereals) they are the dominant seed predators (Baraibar et al., 2009).

Intensive high-input agriculture has been accompanied by a decline in populations of carabids in farmland (Kromp, 1999) and so measures to conserve and promote them are needed if their influence on weed control is to be maximised. Ploughing reduces the survival of many carabid species (Holland, 2004). By contrast, populations of many invertebrates are enhanced in minimum tillage or conservation tillage regimes where soil disturbance is reduced and a richer habitat is provided by the presence of crop residues and greater weed diversity (Holland, 2004, Kromp, 1999). Weed seed predation by carabids, ants and mice is higher in no-tillage than in conventional systems (Brust and House, 1988, Baraibar et al., 2009) and it has been suggested that a significant proportion of the influence of crop residues in suppressing broadleaf weeds in low-input no-tillage systems is due to seed predation by carabids (Brust, 1994). These positive benefits of no-tillage need to be balanced against the effectiveness of ploughing in suppressing weed populations by burying weed seeds.

Habitat manipulation is believed to be an important means of promoting natural enemies of insect pests (see Section 5: Results: Management of invertebrate pests) and may have a similar potential for increasing weed seed predation (Landis et al., 2005). There is evidence that weed seed predation can be limited by a shortage of suitable habitats and refugia for herbivores (Diaz, 1994) and that weed seed predation (both pre-dispersal and post-dispersal) is greater in complex landscapes than in simple ones (Menalled et al., 2000, Steffan-Dewenter et al., 2001). Crop and landscape diversification by intercropping and the provision of enhanced boundary habitats, such as sown weed strips, beetle banks and conservation headlands, generally enhance carabid diversity and promote the populations of some species in farmland (Kromp, 1999). However, the impact of such measures will depend on the extent of the extent of ingress of margin beetle communities into the field centre (Collins et al., 2002) and the degree of synchrony of beetle activity with weed seed production. Management of boundary habitats and of cropped areas also infuences the activitiy of rodent seed predators. For example, cover crops may be valuable for maintaining activity of rodents in fields (Westerman et al., 2006).

Research gaps that represent barriers to the implementation of CBC strategies for weeds
The development of CBC strategies for weeds is in its infancy and considerable research effort is needed if strategies are to be developed and tested and if risks and benefits are to be explored to the satisfaction of both researchers and growers. Research should focus both on the ecology of relationships between weeds and their natural enemies and on assessment of the impact of CBC. Quantification of the effectiveness, reliability and cost of CBC strategies for weeds under realistic field conditions is particularly important if it is to be adopted into farm practice.

The following research needs were particularly highlighted in the literature reviewed:

- Studies of the influence of soil aggregate characteristics on soil enzyme activity to elucidate the relationship between the soil type and the microbial community supported, including deleterious rhizobacteria (Kremer and Li, 2003).
- In-depth research on the ecology of relationships between deleterious rhizobacteria and plants and on the mechanisms of action against weeds, including characterization of phytotoxins (Kremer and Kennedy, 1996).
- Design of crop rotations to optimise the development of specific strains of deleterious rhizobacteria for weed suppression (Rao, 2000).
- Integration of conservation strategies for natural enemies of weeds into weed management (Newman et al., 1998).
- Studies of pesticide targeting to conserve native biological control agents (Newman et al., 1998).
- A comprehensive study of the ecology of predation of weed seed by invertebrates and vertebrates and its impact on weed populations (Menalled et al., 2005).
- Research is needed on manipulation of the soil environment to encourage predators and pathogens of weed seed, e.g. by conservation tillage systems (Derksen et al., 1996).
- Research on habitat protection and plant community management (e.g. in field margins) to conserve critical habitats or refugia for weed seed predators (Newman et al., 1998).
- Demonstration of the extent that landscape diversification benefits carabid populations within cropped land (Kromp, 1999) and assessment of the impact on weed control.
- Studies to quantify the costs and benefits of promoting carabids and to assess their reliability in weed control (Kromp, 1999).
- Rigorous evaluation of the effectiveness of weed biological control projects and the reasons for success or failure (Newman et al., 1998).

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4. Classical and augmentative biocontrol: critical status analysis for selected crops

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4.1. Potential of biocontrol based on published research

4.1.1. Review of the scientific literature on biocontrol against the main plant pathogens of selected crops

4.1.1.1. Evolution of the scientific literature 1973-2008

The scientific literature published in the last 35 years comprises a wealth of studies on biological control against diseases and pests of agricultural crops. A survey of the CAB Abstracts® database shows a steady increase in the yearly number of these publications from 20 in 1973 to over 700 per year since 2004 (Figure 12).

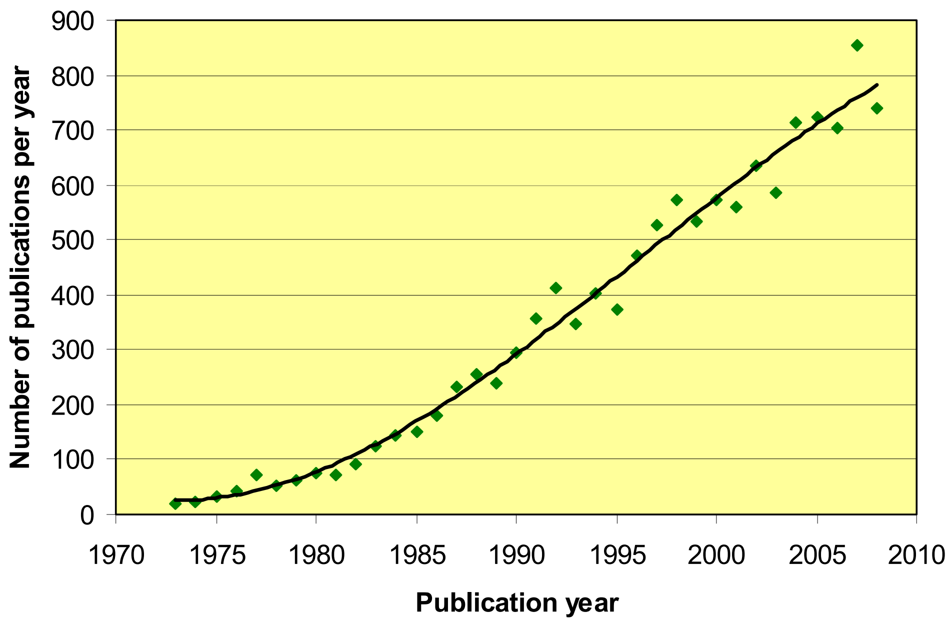

Figure 12: Evolution of the yearly number of publications dedicated to biological control of plant diseases based on a survey of the CAB Abstracts® database.

This survey was further refined by entering keywords describing some of the major plant pathogens/diseases of cultivated crops in Europe, alone or cross-referenced with keywords indicating biocontrol. Among studies published in the period between 1973 and 2008 on these plant pathogens and pests, the percentage dedicated to biological control was substantial, but unequally distributed (Table 11). It was notably higher for studies on soil-borne (9.5% ± 1.6% as average ± standard error) than for those on air-borne diseases (2.8% ± 0.7%).

Table 11: Scientific papers published between 1973 and 2008 on biological control against major plant diseases (from CAB Abstracts® database).

Disease or plant pathogen	Total number of references	References on biological control	
			%
Soil-borne:			
Fusarium	34 818	1 925	5.5
Rhizoctonia	10 744	1 278	11.9
Verticillium	7 585	592	7.8
Pythium	5 772	821	14.2
Sclerotinia	5 545	456	8.2
Air-borne:			
rusts	29 505	360	1.2
powdery mildews	18 026	251	1.4
Alternaria	12 766	415	3.3
anthracnose	12 390	351	2.8
Botrytis	9 295	705	7.5
downy mildews	8 456	80	1.0
Phytophthora infestans	5 303	61	1.1
Monilia rot	1 861	81	4.3
Venturia	3 870	104	2.7

4.1.1.2. Inventory of potential biocontrol agents (microbials, botanicals, other natural compounds)

The scientific literature described above was further examined to identify biocontrol compounds and microbial species reported to have a successful effect. Due to the great abundance of references, it was not possible to examine the complete body of literature. The study was thus focused on several key diseases selected for their general importance on cultivated crops, and in particular on those crops studied in ENDURE case studies.

- **Methodology:**

Three steps were followed.

The first step consisted in collecting the appropriate literature references for the selected key diseases/plant pathogens to be targeted by the study. The references were extracted from the CAB Abstracts® database and downloaded to separate files using version X1 of EndNote (one file for each target group). The files were then distributed among the contributors of this task for detailed analysis.

In the second step, every reference was examined and we recorded for each:

- the types of biocontrol agents (Microbial, Botanical or Other compounds) under study and their Latin name (for living organisms and plant extracts) or chemical name
- the Latin name of the specifically targeted pathogens,
- the crop species (unless tests were carried out exclusively *in vitro*),

- the outcome of efficacy tests.

Two types of efficacy tests were distinguished: Controlled environment tests (including tests on plants and *in vitro* tests), and field trials. The outcome of a test was rated (+) if significant effect was reported, (0) if no significant efficacy was shown and (-) if the biocontrol agent stimulated disease development.

To allow for the analysis of a large number of references, the abstracts were examined for the presence of the relevant data. The complete publications were acquired and examined only when the abstracts were not sufficiently precise.

The data were collected in separate tables for each type of key target pest. For each table, they were sorted (in decreasing order of priority) according to the type and name of the biocontrol agents, the specifically targeted pest, and the outcome of efficacy tests.

In the third step, synthetic summary tables were constructed to quantify the number of different biocontrol compounds and microbial species and strains reported to have successful effect against each type of key pathogen/disease or pest target.

- **Results**

A total number of 1791 references were examined for key airborne diseases including powdery mildews, rusts, downy mildews (+ late blight of Potato/Tomato) and *Botrytis* and *Monilia* rots, together with soilborne diseases caused by *Fusarium oxysporum* (Table 12). Based on the examination of these references, successful effect in controlled conditions was achieved for all targets under study with a variety of species and compounds (Appendices 10-15, Table 13).

Table 12: Numbers of references on biocontrol examined per group of disease/plant pathogen.

Target disease / plant pathogen	Relevance to ENDURE Case Studies	Number of references examined	Period of publication examined	Contributor
Botrytis	OR, FV, GR* (postharvest)	880	1998-2008	INRA
Powdery mildews	all	166	1998-2008	CNR
Rusts	AC, FV, OR	154	1973-2008	INRA
Downy mildews + *Phytophthora infestans*	FV, GR, PO, TO	349	1973-2008	INRA
Monilinia rot	OR	194	1973-2008	INRA
Fusarium oxysporum	FV, TO	48	2007-2009	INRA

*AC: Arable Crops; FV: Field Vegetables; GR: Grapes; OR: orchard; PO: Potato; TO: Tomato

Concerning **airborne diseases and pathogens**, the largest number of reported successes was achieved with microbials, but there is a growing body of literature on plant and microbial extracts, as well as other types of substances (Table 13). On average, reports of success were far more numerous for experiments in controlled conditions (*in vitro* or *in planta*) than for field trials.

Very contrasted situations were also observed depending on the type of target disease/pathogen, with rare reports on the biocontrol of rusts and mildews compared to *Botrytis*, despite the fact that the literature was examined over a 35 year period for the former diseases and only over the last 10 years for the latter.

In total in this review, 157 species of micro-organisms have been reported for significant biocontrol activity. They belong to 36 genera of fungi or oomycetes, 13 of yeasts and 25 of bacteria. Among them, 29 species of fungi/oomycetes and 18 bacteria were reported as successful in the field against at least one of the five key airborne diseases included in this review (Table 14).

Table 13: Numbers of different biocontrol compounds and microbial species reported as having successful effect against key airborne pathogens/diseases of selected crops. Detailed information and associated bibliographic references are presented in Appendices 10-14

Target plant pathogen / disease	Botanicals		Microbials[y]		Others[z]	
	laboratory tests[x]	field trials	laboratory tests[x]	field trials	laboratory tests[x]	field trials
Botrytis						
in vitro	26	**-**	31 b, 21 f	**-**	7	**-**
legumes	4	**2**	10 b, 12 f	**3b, 9 f**	0	**0**
protected vegetables	0	**1**	22 b, 24 f	**8 b, 9 f**	5	**1**
strawberry	0	**0**	14 b, 21 f	**2 b, 13 f**	7	**1**
field vegetables	0	**0**	5 b, 15 f	**2 f**	0	**0**
grapes	1	**3**	5 b, 27 f	**5 b, 13 f**	0	**1**
pome/stone fruits	1	**0**	12 b, 35 f	**2 b, 6 f**	4	**0**
others	3	**0**	15 b, 25 f	**6 b, 6 f**	0	**0**
Powdery mildews						
Grape	1	**1**	4b; 10f	**2b; 12f**	3	**2**
Arable crops	1	**0**	2b;9f	**1b**	5	**0**
Strawberry	0	**0**	4b; 6f	**0**	0	**0**
Cucurbitaceae	4	**0**	14b; 22f	**4b; 9f**	9	**1**
Pome/stone fruits	0	**0**	3f	**1f**	0	**0**
Pepper	1	**0**	4f	**0**	1	**0**
Tomato	5	**0**	4b; 5f	**1f; 1b**	0	**0**
Various	2	**0**	2b; 10f	**1b; 1f**	5	**0**
Rusts						
arable crops	0	**0**	5 b, 6 f	**2 b**	2	**0**
others	0	**0**	8 b, 13 f	**0**	1	**0**
Downy mildews + late blight						
grapes	2	**4**	2 f	**3 b, 2 f**	2	**3**
field vegetables	0	**0**	4	**0**	4	**6**
potato	9	**1**	8 b, 10 f	**5 b, 4 f**	3	**1**
tomato	2	**1**	5 b, 5 f	**4 b**	12	**1**
Monilia rot						
in vitro	0	**-**	8	**-**	1	**-**
pome fruit	0	**0**	7	**0**	0	**0**
stone fruit	0	**1**	23b, 19	**7b, 7f**	2	**2**
others	0	**0**	1b	**2b, 1f**	0	**0**

[x] tests conducted *in vitro* and/or *in planta* in controlled conditions
[y] b: bacteria; f: fungi / oomycetes / yeasts
[z] including culture filtrates and extracts from microorganisms

Table 14: **Microbial species of fungi/oomycetes, yeasts and bacteria reported to have a significant effect against five main types of airborne diseases or pathogens in laboratory conditions or in the field (yellow highlight).** Bibliographic references are presented in Appendices 10 to 14.

A. Fungi and oomycetes

Microbial species	**Target disease / pathogen**				
	Botrytis	**Powdery mildew**	**Rust**	**Downy mildew, late blight**	**Monillia rot**
Acremonium spp.			others		
Acremonium alternatum		cereals, **protected vegetables**			
A. cephalosporium	**grapes**				
A. obclavatum			others		
Alternaria spp.	grapes	cereals			
A. alternata			others	grapes	
Ampelomyces quisqualis		fruits, **grapes**, strawberry, **protected vegetables**, others,			
Aspergillus spp.			others	tomato	
A. flavus				others	
Beauveria sp	protected vegetables				
Botrytis cinerea non-aggressive strains	legumes				
Chaetomium cochlioides	**grapes**				
C. globosum	legumes				
Cladosporium spp.	flowers		others		
C. chlorocephalum				others	
C. cladosporioides	flowers, legumes	others			
C. oxysporum	flowers	others	others		
C. tenuissimum		strawberry	field vegetables, others		
Clonostachys rosea	**flowers, legumes**, others, strawberries, field vegetables, **protected vegetables**,				
Coniothyrium spp.	grapes				
C. minitans	field vegetables				
Cylindrocladium	others				
Drechslera hawaiinensis		others			
Epicoccum sp	flowers, grapes, field vegetables				
E. nigrum	legumes, **strawberries**				plum, **peach**
E. purpurascens					apple, **cherry**
Filobasidium floriforme	fruits				
Fusarium spp.	flowers		others		
F. acuminatum		cereals			
F. chlamydosporum			others		
F. oxysporum		cereals		tomato	
F. proliferatum				**grapes**	
Galactomyces geotrichum	fruits				
Gliocladium spp.	**grapes**, **protected vegetables**, **others**				
G. catenulatum	**protected vegetables**, **legumes**				
G. roseum	flowers, **grapes**, legumes, **others**	others			blueberry
G. virens	strawberries, field vegetables			**potato**, **others**	
G. viride	**protected vegetables**				

Lecanicillium spp.		protected vegetables			
L. longisporum		protected vegetables			
Meira geulakonigii		protected vegetables			
Microdochium dimerum	protected vegetables, **protected vegetables**				
Microsphaeropsis ochracea	**field vegetables**				
Muscodor albus	fruits, grapes				peach
Paecilomyces farinosus		cereals			
P. fumorosoroseus		protected vegetables			
Penicillium spp.	fruits, field vegetables		others	potato, tomato	
P. aurantiogriseum	**legumes**			potato	
P. brevicompactum	legumes				
P. frequentans					plum, **peach**
P. griseofulvum	**legumes**, field vegetables				
P. purpurogenum					**peach**
P. viridicatum				potato	
Phytophthora cryptogea				**potato**	
Pseudozyma floculosa		**grapes**, **protected vegetables**,			
Pythium oligandrum	protected vegetables				
P. paroecandrum	grapes				
P. periplocum	grapes				
Rhizoctonia	flowers			potato	
Scytalidium	grapes				
S. uredinicola			others		
Sordaria fimicola					apple
Tilletiopsis spp.		grapes			
T. minor		**others**			
Trichoderma spp.	flowers, **grapes**, legumes, strawberries, **protected vegetables**, others			**potato**	
T. asperellum	strawberries				
T. atroviride	**legumes**, strawberries				peach
T. hamatum	flowers, **legumes**				
T. harzianum	flowers, **grapes**, **legumes**, **strawberries**, field vegetables, **protected vegetables**, **others**	others, strawberry, **protected vegetables**,	others	**grapes**, potato, tomato, field vegetables, **others**	cherry, peach
T. inhamatum	flowers				
T. koningii	strawberries, field vegetables				peach
T. lignorum				**others**	
T. longibrachiatum	strawberries				
T. polysporum	**strawberries**				apple
T. taxi	protected vegetables				
T. virens	**grapes**				
T. viride	fruits, grapes, **legumes**, strawberries, field vegetables, **others**	others	others	**potato**, others	peach
Trichothecium	grapes				
T. roseum	grapes, **legumes**				
Ulocladium sp.	grapes, field vegetables				
U. atrum	**flowers**, **grapes**, **strawberries**, **field vegetables**, protected vegetables				
U. oudemansii	**grapes**				

Ustilago maydis	protected vegetables				
Verticillium	grapes		legumes		
V. chlamydosporium			cereals		
V. lecanii	strawberries	cereals, **protected vegetables**, **others**	legumes, others		

B. Yeasts

Microbial species	Target disease / pathogen				
	Botrytis	**Powdery mildew**	**Rust**	**Downy mildew, late blight**	**Monillia rot**
Aureobasidium pullulans	fruits, grapes, strawberries, protected vegetables				apple, cherry
Candida spp.				tomato	peach
C. butyri	fruits				
C. famata	fruits				
C. fructus	strawberries				
C. glabrata	strawberries				
C. guilliermondii	grapes, protected vegetables				cherry
C. melibiosica	fruits				
C. oleophila	fruits, grapes, strawberries, protected vegetables				
C. parapsilosis	fruits				
C. pelliculosa	protected vegetables				
C. pulcherrima	fruits, strawberries				
C. reukaufii	strawberries				
C. saitoana	fruits				
C. sake	fruits				
C. tenuis	fruits				
Cryptococcus albidus	fruits, strawberries, protected vegetables				
C. humicola	fruits				
C. infirmo-miniatus	fruits				cherry
C. laurentii	fruits, strawberries, protected vegetables				cherry, peach
Debaryomyces hansenii	fruits, grapes				cherry, peach
Hanseniaspora uvarum	grapes				
Kloeckera spp	grapes				
K. apiculata	fruits				cherry, peach
Metschnikowia fructicola	fruits, grapes, strawberries				
M. pulcherrima	fruits				apple, apricot
Pichia anomala	grapes, fruits				
P. guilermondii	fruits, strawberries, protected vegetables				
P. membranaefaciens	grapes				peach
P. onychis	field vegetables				
P. stipitis	fruits				
Rhodosporidium diobovatum	protected vegetables				
R. toruloides	fruits				
Rhodotorula					peach
R. glutinis	flowers, fruits, strawberries, protected vegetables	field vegetables,			
R. graminis	flowers				
R. mucilaginosa	flowers				
R. rubra	protected vegetables				
Saccharomyces cerevisiae	fruits	protected vegetables			
Sporobolomyces roseus	fruits				
Trichosporon sp.	fruits				

T. pullulans	fruits, grapes, protected vegetables				

C. Bacteria

Microbial species	Target disease / pathogen				
	Botrytis	**Powdery mildew**	**Rust**	**Downy mildew, late blight**	**Monillia rot**
Acinetobacter lwoffii	**grapes**				
Azotobacter				**other**	
Bacillus spp.	**grapes**, strawberry, protected vegetables**,** others	**protected vegetables**	**others**	**potato,** field vegetables	**apricot**
B. amyloliquefaciens	arable crops, **flowers,** fruits, field vegetables, **protected vegetables**				peach
B. cereus	**flowers,** legumes		others	**tomato**	
B. circulans	protected vegetables				
B. lentimorbus			others		
B. licheniformis	fruits, others, strawberry, **protected vegetables**				
B. macerans	legumes				
B. marismortui	strawberry				
B. megaterium	legumes, others				
B. pumilus	fruits, strawberry			tomato, **others**	
B. subtilis	flowers, fruits, **grapes, legumes**, strawberry, field vegetables, **protected vegetables**	**cereals, grapes,** strawberry, protected vegetables, **others**	**legumes**	**grapes, potato, others**	apricot, **blueberry, cherry, peach**
B. thuringiensis	strawberry				
Bakflor (consortium of valuable bacterial physiological groups)	**protected vegetables**				
Brevibacillus brevis	field vegetables, **protected vegetables**	grapes, **protected vegetables**		**grapes**	
Burkholderia spp.				**tomato**	
B. cepacia	protected vegetables				cherry
B. gladii					**apricot**
B. gladioli	**flowers**				
Cedecea dravisae			others		
Cellulomonas flavigena				tomato	
Cupriavidus campinensis	grapes, protected vegetables, others				
Enterobacter cloacae		protected vegetables		potato	
Enterobacteriaceae	strawberry				
Erwinia	fruits, others				
Halomonas sp.	strawberry, protected vegetables				
H. subglaciescola	protected vegetables				
Marinococcus halophilus	protected vegetables				
Salinococcus roseus	protected vegetables				
Halovibrio variabilis	protected vegetables				
Halobacillus halophilus	protected vegetables				
H. litoralis	protected vegetables				
H. trueperi	protected vegetables				
Micromonospora coerulea	protected vegetables				
Paenibacillus polymyxa	**strawberry, protected vegetables**				
Pantoea spp.	grapes, protected vegetables				

P. agglomerans	**fruits**, **grapes**, **legumes**, strawberry		legumes		apple, apricot, blueberry, cherry, peach, plum
Pseudomonas spp.	flowers, fruits, grapes, field vegetables		others	**potato**, **tomato**, field vegetables	**apricot**
P. aeruginosa	protected vegetables				
P. aureofasciens		**cereals**			cherry
P. cepacia	strawberry				**peach**
P. chlororaphis	strawberry				cherry
P. corrugata					**peach**
P. fluorescens	fruits, **grapes**, **legumes**, **strawberry**, protected vegetables, others	**cereals**, , **protected vegetables** others	**legumes**	**grapes**, **potato**, tomato, **others**	blueberry, cherry
P. putida	**flowers**, legumes, **protected vegetables**, others		cereals		
P. syringae	**fruits**, strawberry, field vegetables	grapes			apple, peach
P. reactans		strawberry			
P. viridiflava	fruits				
Rhanella spp.				potato	
R aquatilis	fruits				
Serratia spp.				potato	
S. marcescens	flowers				
S. plymuthica	protected vegetables				
Stenotrophomonas maltophilia			legumes		
Streptomyces spp.				**tomato**	
S. albaduncus	legumes				
S. ahygroscopicus	protected vegetables				
S. exfoliatus	**legumes**				
S. griseoplanus	legumes				
S. griseoviridis	**protected vegetables, others**				
S. lydicus	protected vegetables				
S. violaceus	legumes				
Virgibacillus marismortui	strawberry				
Xenorhabdus bovienii				potato	
X. nematophilus		protected vegetables			

One striking aspect of this inventory is that although the five target diseases / pathogens included in our review are airborne and affect mostly the plant canopy, the vast majority of cited biocontrol microorganisms are soil microorganisms. The scarcity of biocontrol agents originating from the phyllosphere could be due to actual lack of effectiveness, or it could be the result of a bias by research groups in favour of soil microbes when they gather candidate microorganisms to be screened for biocontrol activity. This question would merit further analysis as it may help to devise improved screening strategies. As "negative" results (the lack of effectiveness of tested microorganisms, for example) are seldom published, the completion of such an analysis would in turn necessitate direct information from research groups who have been implicated in screening for biocontrol agents, or the development of a specific screening experiment comparing equal numbers of phyllosphere and of soil microbial candidates.

Another striking aspect is that most of the beneficial micro-organisms inventoried in this study (49 fungi/oomycetes, 28 yeasts and 41 bacteria) are cited only for biocontrol of one of the five types of airborne diseases included in the survey (Figure 13). However, several species clearly stand out with a wide range of effectiveness, as they were successfully used against all five types of target diseases on a variety of crops. This includes the fungi *Trichoderma harzianum* and

Trichoderma viride (2 of 12 species of *Trichoderma* reported as biocontrol-effective in the reviewed literature) and the bacteria *Bacillus subtilis* and *Pseudomonas fluorescens*.

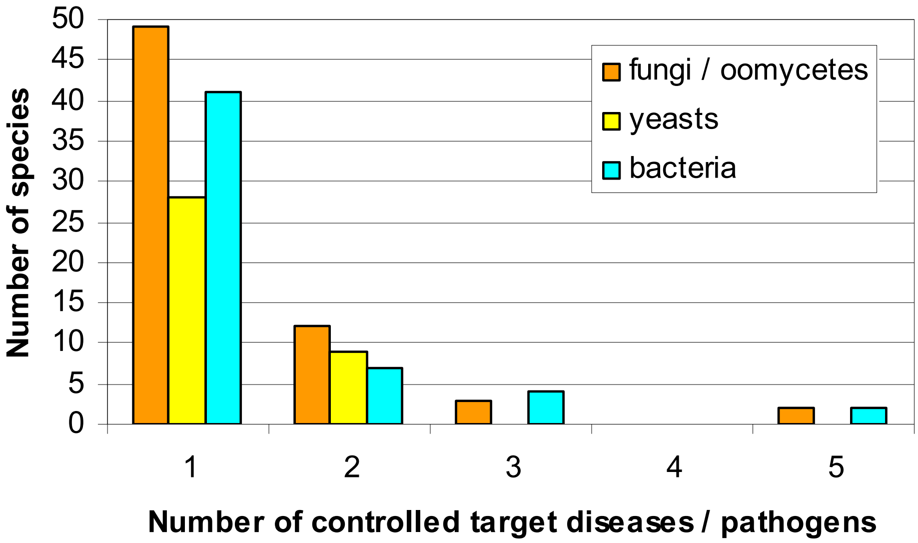

Figure 13: Range of efficacy of 157 microbial biocontrol agents against five main types of airborne diseases. Detailed data are presented in Table 14.

Concerning ***Fusarium oxysporum***. Data base interrogation was done on October 6, 2009. With the key words “Fusarium oxysporum AND biological control” the numbers of references were as follows:

1973-2009: 2266 references
1999-2009: 1426 references
2004-2009: 899 references
2007-2009: 502 references

Using these key words we did not select only papers regarding biological control of diseases induced by *F. oxysporum* but also all the paper dealing with the use of strains of *F. oxysporum* to control diseases and weeds. There are quite many papers dealing with the use of different strains of *F. oxysporum* to control Broom rape (orobanche) and also the use of *F. oxysporum* f. sp. *erythroxyli* to eradicate coca crops.

We decided to limit our review to the two last years and to concentrate on references for which full text was available on line.

Finally we reviewed 48 papers. All these papers were dealing with the selection and development of micro-biological control agents; only two were considering others methods. One was addressing the use of chemical elicitors to induce resistance in the plant; the other was aiming at identifying the beneficial influence of non-host plant species either used in rotation or in co-culture.

Based on this very limited number of papers we identified;

The formae speciales of *F. oxysporum* the most frequently studied:

F.o. f. sp. lycopersici is the model pathogen in 17 papers,

f. spp. melonis, ciceris, cubense, niveum and *cucumerinum* are used in 2, 3 or 4 studies.

The antagonists studied:

Bacillus spp and *Paenibacillus* are considered in 16 papers, *B. subtilis* being the most frequently used

Trichoderma spp. are considered in 14 papers

Fluorescent *Pseudomonas* spp in 7 papers

Actinomycetes in 5 papers

Non pathogenic strains of F. oxysporum in 5 papers

Mycorrhizal fungi in 3 papers

Penicillium in 2 publications

Most of the publications (28) are reporting in vitro studies. Among them a few are studying in vitro, or in planta the mechanisms of action of the antagonists, the others just relate screening studies using plate confrontation between the antagonists and the target pathogens. In most of these papers (22) the in vitro screening is followed by pots or greenhouses experiments aiming at demonstrating the capacity of the antagonist to reduce disease severity or disease incidence after artificial inoculation of the pathogen. Finally only 9 publications report results of field experiments.

Most of these papers conclude on the promising potential of the selected strains of antagonists able to decrease disease incidence or severity by 60 to 90 %.

In contrast to these optimistic results, there is still no preparation on the market targeting control of Fusarium wilts. The strains of *Trichoderma* on the market sometimes claim efficacy against *Fusarium oxysporum*, but they are mostly used to control damping-off and root-rot, not wilt. Similarly the strains of *Bacillus subtilis* already on the market are not targeting Fusarium wilts.

In the eighties, a strain of *Pseudomonas fluorescens* was developed in the Netherlands to control Fusarium wilt of radish, but it is no more on the market.

Only a very few teams are regularly publishing on biological control of Fusarium wilts. They are interested in all the aspects from the modes of action of the antagonists, the plant-fungal interactions, the fate of the antagonists in the environment and the processes of production and formulation of the biological control product.

The most promising results concern the use of non pathogenic strains of *F. oxysporum* and of a strain of *Penicillium oxalicum*.

Generally speaking, this limited literature review shows that most of the lab studies are not followed by field studies. There is a need for implementation of biological control in the fields.

- **Identified knowledge gaps**

Several types of knowledge gaps have been identified in this review. They include:

- the near absence of information on biocontrol against diseases of certain important European crops such as winter arable crops.
- the scarcity of reports on biocontrol against several diseases of major economic importance on numerous crops, such as those caused by obligate plant pathogens (rusts, powdery mildews, downy mildews)
- the still limited (but increasing) body of detailed knowledge on specific mechanisms of action and their genetic determinism. The little knowledge available at the molecular level is concentrated on few model biocontrol agents such as *Trichoderma* and *Pseudomonas*.
- the still very limited information on secondary metabolites produced by microbial biocontrol agents
- the lack of understanding for generally low field efficacy of resistance-inducing compounds
- the lack of knowledge on variability in the susceptibility of plants pathogens to the action of BCAs and on possible consequences for field efficacy and its durability.

4.1.2. Review of the scientific literature on beneficials for classical and augmentative/inundative biocontrol against insect pests

4.1.2.1. Bibliographic survey on augmentative biological control against arthropod pests in selected crops

We carried out a preliminary bibliographic survey to quantify the literature on augmentative biological control of pests published from 1973 to 2008. The survey was restricted to crops relevant to case studies of ENDURE. They included grapevine; orchards: apple and pear; arable crops: corn and wheat; field vegetables: carrot and onion. Augmentative biological control (Van Driesche & Bellows, 1996) comprises of inoculative augmentation (control being provided by the offspring of released organisms) and inundative augmentation (control expected to be performed by the organisms released, with little or no contribution by their offspring).

Our bibliographic survey was conducted by the CAB Abstracts database by entering the name of each crop and one key word selected from the following list in order to retrieve the maximum number of references. For each selected crop, the key words used for the bibliographic survey were: *a*) augmentative biological control; *b*) augmentation biological control; *c*) inoculative biological control**;** *d*) inundative biological control. The survey with these key words produced a very low number of results all of which were examined. For this reason we added two key words that were more general: *e*) insects biological control; *f*) mites biological control. For the searching criteria *a* to *d*, total records will be examined. In this case, given the extremely high number of records, only references within the period 1998-2008 were examined to select only the publications concerning the augmentative biological control. The results of this survey are reported in Appendix 16.

The analytical review of the scientific literature on augmentative biological control has been done only for grapevine.

4.1.2.2. Status of researches on augmentation of natural enemies to control arthropod pests in grapevine

The references extracted from the CAB Abstracts database, following the criteria described in the previous paragraph, were examined to identify those concerning the use of natural enemies in augmentation biological control in grapevine. The abstracts of 607 references were examined and only 70 papers reported data on application and efficiency of augmentative biocontrol (Table 15).

Table 15: References extracted from the CAB Abstracts database and examined for reviewing augmentation biological control in grapevine.

Key words	Total records (1973-2008)	1998-2008
Augmentative biological control	7	6
Augmentation biological control	10	6
Inoculative biological control	4	1
Inundative biological control	7	3
Insects biological control		373
Mites biological control		190
Total references examined	28	579
Total references showing data on augmentative biocontrol	70	

The survey includes records for grapevine, grape and vineyard.

- **Results**

Very few papers (62) on augmentative biocontrol in grapevine have been published during the period 1998-2008, with an average of 5.6 publications per year. Most references (93.5%) showed data on biological control of insects and only 4 papers on the biological control of mites were published (Figure 14).

The data extracted from the abstracts of the selected references were collected analytically in separate tables for each group of biocontrol agents (Appendix 17): references were sorted chronologically (starting from the eldest). For each species of biocontrol agent, target species of pest, Country, type of augmentation (inundative, inoculative), type of test (laboratory, field), efficacy of biocontrol, additional information and results were reported.

Data reported in Appendix 17 were summarized in Table 16, Table 17, Table 18, Figure 15 and Figure 16. A list of the biocontrol agents used in augmentative biological control in grapevine is reported in Table 16 and Figure 15. A list of groups and species of the targeted pests and the antagonists used for their control is reported in Table 17 and Figure 16; the efficacy of biocontrol agents is reported in Table 18.

The group of pests on which the highest number of augmentative biocontrol researches has been carried out is Lepidoptera (60% of total references) with the family Tortricidae representing the main target (55%) (Figure 16) including the grape berry moths key pests *Lobesia botrana* and *Eupecilia ambiguella* (Table 17). *Bacillus thuringiensis* has resulted the most frequently used biocontrol agent against Lepidoptera by achieving an effective control of different targets in different geographic areas (Table 17, Table 18, Appendix 17.7). We sorted 28 references (39% of the total citations) dealing with the use of *B. thuringiensis* of which 23 references were referred to the control of *L. botrana*. The augmentation of egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) resulted the alternative strategy to *B. thuringiensis* to control Lepidoptera Tortricidae (13 references, 16% of total citations) (Table 17, Table 18). Field evaluations indicated *T. evanescens* as a promising biocontrol agent of *L. botrana* (El-Wakeil et al., 2008 in Appendix 17.1).

Fewer researches were carried out on augmentative biocontrol of other group of pests. First in the list were mealybugs (Hemiptera: Pseudococcidae) (9 references, 13% of the total citations). In field evaluations (4 papers) parasitoid wasps of the family Encyrtidae have resulted extremely active and promising to be used in augmentative biocontrol of mealybugs (Appendix 17.2).

Antagonists used in augmentative biocontrol in grapevine were mainly represented by insect pathogens (59% of the total citations), including the bacterium *B. thuringiensis*, fungi and nematodes (Figure 15, Table 16). Beside the efficacy of *B. thuringiensis*, promising results were obtained from researches in the control of the grape phylloxera *Daktulosphaira vitifolie*, a gall-forming aphid, by soil treatments with the fungus *Metarhizium anisopliae* (Table 18, Appendix 17.5). Once controlled by grafting European grape cultivars onto resistant rootstocks, the grape phylloxera has gone to resurgence in commercial vineyards worldwide and new biological control strategy could be necessary to complement the use of resistant rootstocks and to avoid the distribution of chemical insecticides in the soil.

Entomophagous arthropods, including parasitoid wasps and predators represented 41% of the total citations (Figure 15, Table 16). Best results were obtained from researches on parasitoids (18 references), namely the use of Trichogrammatidae and Encyrtidae in augmentative biocontrol of grape moths (Tortricidae) and mealybugs (Pseudococcidae) respectively (Table 17, Table 18, Appendix 17.1 and 17.2). Among predators, augmentation of Phytoseiidae mites has produced some positive results in controlling spider mites and eriophyid mites on grape (Table 17, Table 18, Appendix 17.3).

Table 16: Biocontrol agents evaluated in researches on augmentative biological control of pests in grapevine.

Target pests and biocontrol agents		References before 1998	References 1998-2008	*Number of citations*
BIOLOGICAL CONTROL OF INSECTS				
Bacteria	**[1 species: 2 subspecies]**	**0**	**28**	
- *Bacillus thuringiensis* (subsp. *kurstaki*, subsp. *aizawai*)				*28*
Fungi	**[5 species]**	**0**	**10**	
- *Metarhizium anisopliae*				*7*
- *Beauveria bassiana*				*2*
- *Beauveria brongniartii*				*1*
- *Verticillium lecanii*				*1*
- *Clerodendron inerme*				*1*
Nematodes	**[5 species]**	**1**	**3**	
- *Steinernema* spp.	*2 spp.*			*2*
- *Heterorabditis* spp.	*3 spp.*			*3*
Parasitoid Hymenoptera	**[15 species]**	**2**	**16**	
- *Trichogramma* spp. (Trichogrammatidae)	*10 spp*			*13*
- *Coccidoxenoides* spp. (Encyrtidae)	*2 spp.*			*2*
- *Anagyrus* spp. (Encyrtidae)	*2 spp.*			*3*
- *Muscidifurax raptor* (Pteromalidae)	*1 spp.*			*1*
Predators	**[5 species]**	**2**	**4**	
- *Chrysoperla* (Neuroptera: Chrysopidae)	*3 spp.*			*3*
- *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae)				*2*
- *Nephus includens* (Coleoptera: Coccinellidae)				*1*
BIOLOGICAL CONTROL OF MITES				
Predators (Acari: Phytoseiidae)	**[4 species]**	**2**	**4**	
- *Typhlodromus pyri*				*5*
- *Kampimodromus aberrans*				*2*
- *Amblyseius andersoni*				*1*
- *Phytoseiulus persimilis*				*1*

Figure 14: Number of papers per year published during 1998-2008 concerning augmentative biological control of pests in grapevine.

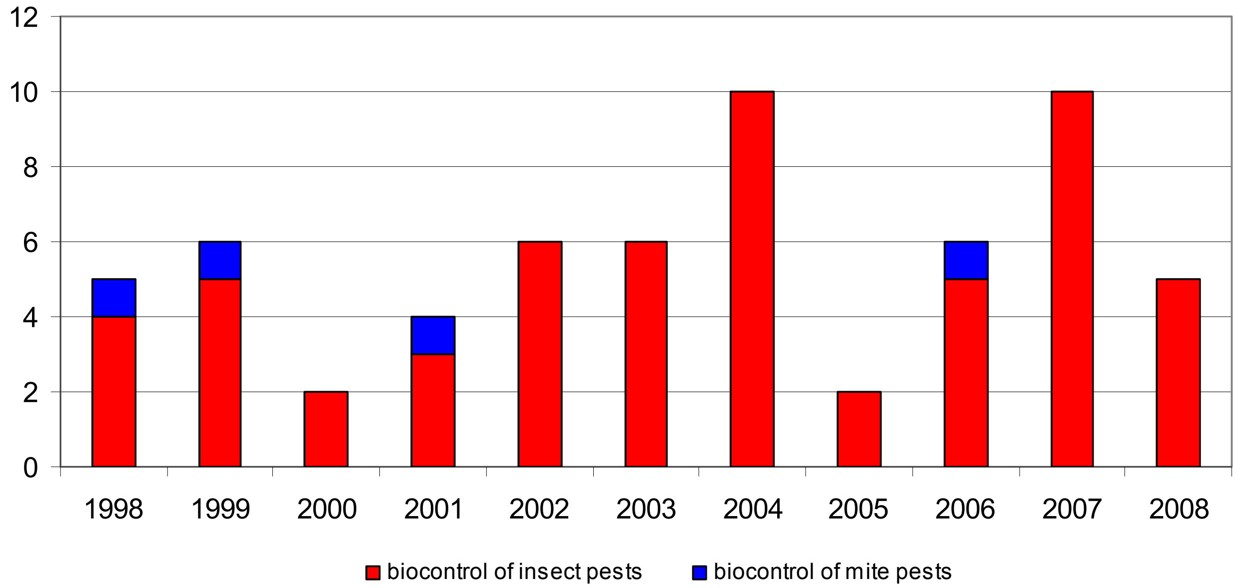

Table 17: Number of references on augmentative biocontrol agents per group and species of target pest in grapevine.

Pest	References	*Bacillus thuringiensis* (2 subspecies)	*Trichogramma* (10 species)	other parasitoids (5 species)	Predators of mites Acari: Phytoseidae (4 species)	Predators of insects Coleoptera: Coccinellidae (2 species)	Predators of insects Neuroptera: Chrysopidae (3 species)	Fungi (5 species)	Nematodes (5 species)
Lepidoptera: Tortricidae	**39**								
Lobesia botrana (grape berry moth)	28	23	5						
Eupoecilia ambiguella (grape berry moth)	6	3	3						
Epiphyas postvittana (light brown apple moth)	3		3						
Argyrotaenia sphaleropa (South American tortricid moth)	3	1	2						
Bonagota cranaodes (Brasilian apple leafroller)	2		2						
Endopiza viteana (grape berry moth)	2		2						
Sparganothis pilleriana (grape leafroller)	1	1							
Epichoristodes acerbella (South African carnation tortrix)	1	1							
Lepidoptera: Pyralidae	**1**								
Cryptoblabes gnidiella (honey moth)	1	1							
Lepidoptera: Arctiidae	**1**								
Hyphantria cunea (fall webworm)		1							
Lepidoptera: Sesiidae	**2**								
Vitacea polistiformis	2								2

Hemiptera: Pseudococcidae	**9**						
Planococcus ficus	6	4 Encyrtidae		2			
Pseudococcus maritimus	1			1			
Pseudococcus longispinus				1			
Maconellicoccus hirsutus		1 Encyrtidae				1	
Hemiptera: Cicadellidae	**3**						
Erythroneura variabilis	3				3		
Erythroneura elegantula	3				3		
Hemiptera: Phylloxeridae	**5**						
Daktulosphaira vitifoliae (grape phylloxera)						4	1
Diptera: Tephritidae	**1**						
Ceratitis capitata	1	1 Pteromalidae					
Coleoptera: Scarabeidae	**2**						
Melolontha melolontha	2					1	1
Thysanoptera: Thripidae	**3**						
Frankliniella occidentalis	2					2	
grape thrips	1					1	
Acari: Tetranichidae	**6**						
Panonychus ulmi	5		5				
Tetranychus urticae	1		1				
Tetranychus kanzawai	1		1				
Eotetranychus carpini	2		2				
Acari: Eriophyidae	**2**						
Calepitrimerus vitis	1		1				
Calomerus vitis	1		1				

Table 18: Number of references reporting data on the efficacy of augmentative biocontrol of pests in grapevine.

Groups of Pests	Biocontrol agents	Total number of references	Number of references reporting data on efficacy in pest and related damage control*	
			Laboratory assays	Field evaluation
Lepidoptera: Tortricidae	*Bacillus thuringiensis*	26	2 +	16 + 1 -
	Trichogramma spp. parasitoids	13	1 -	9 + 1 -
Lepidoptera: Pyralidae	*Bacillus thuringiensis*	1		1 +
Lepidoptera: Arctiidae	*Bacillus thuringiensis*	1		1 +
Lepidoptera: Sesiidae	Nematodes	2	2 +	1 + 1 + (greenhouse)
Hemiptera: Pseudococcidae	Encyrtidae parasitoids	5		4 +
	Coccinellidae	3		1 + (greenhouse)
	Fungi	1		1 +
Hemiptera: Cicadellidae	Chrysopidae	3		2 -
Hemiptera: Phylloxeridae	Nematodes	1	1 +	
	Fungi	5	1 +	2 + 1 -
Diptera: Tephritidae	Pteromalidae parasitoids	1	1 +	1 +
Acari: Tetranichidae	Phytoseidae	6		4 +
Acari: Eriophyidae	Phytoseidae	2		1 +
Coleoptera: Scarabeidae	Nematodes	1		1 +
	Fungi	1		1 +
Thysanoptera: Thripidae	Fungi	3	1 +	2 +

* + means effective, - means not effective biocontrol agent

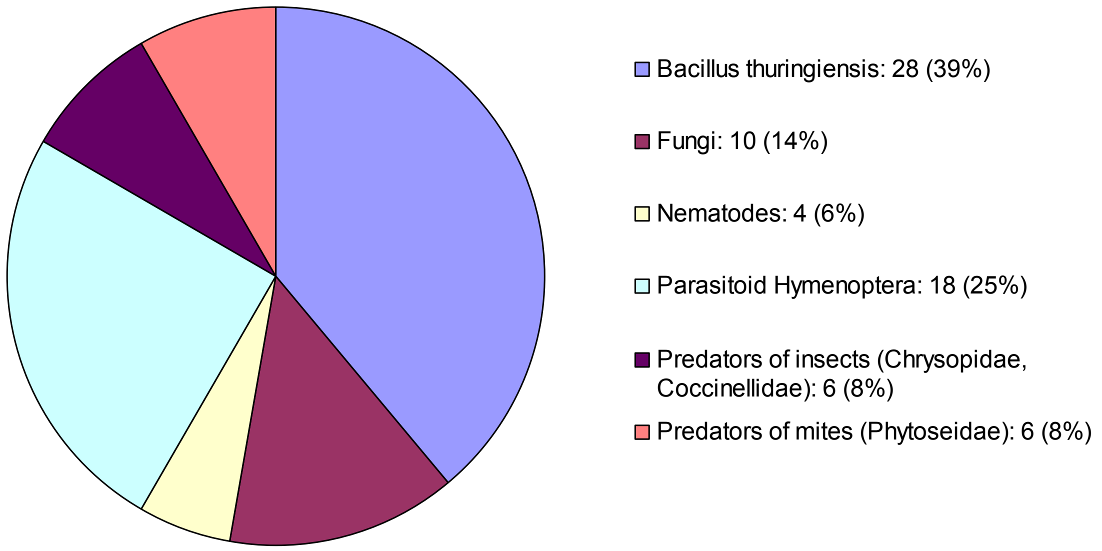

Figure 15: Groups of biocontrol agents investigated in augmentative biological control researches in grapevine. Number of references for each group is reported.

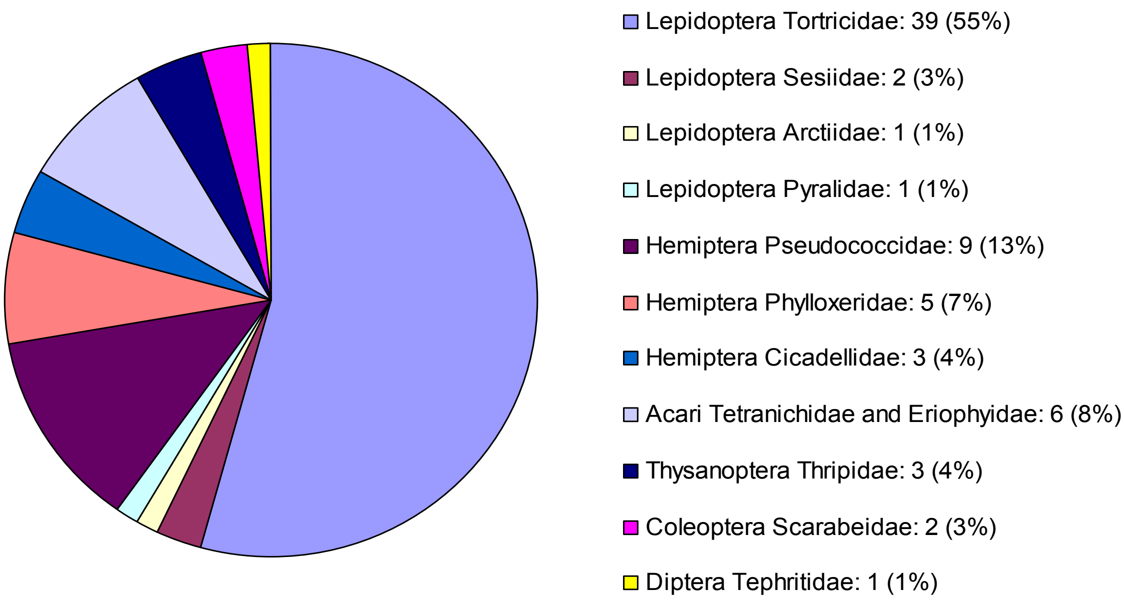

Figure 16: Groups of target pests investigated in augmentative biological control researches in grapevine. Number of references for each group is reported.

- **Brief considerations**

Key pests of grapevine like *L. botrana* and *E. ambiguella* can be controlled effectively with augmentative strategies that rely on the use of *B. thuringiensis*. To date, formulations of *B. thuringiensis* are currently used in IPM strategies. The specificity of *B. thuringiensis* could be a problem in those vineyards where other pasts can reach the status of economically importance, if not controlled by indigenous and/or introduced natural enemies. Researches on augmentative biocontrol should be implemented in order to develop new strategies to solve problems related to emerging pests and alternatives to *B. thuringiensis* if resistant strains should appear in target species.

4.1.3. Research and Development in classical biological control with emphasis on the recent introduction of insect parasitoids

4.1.3.1. *Scope of the review*

Defined as “the intentional introduction of an exotic, usually co-evolved, biological control agent [hereafter BCA] for permanent establishment and long-term pest control’, classical biological control [hereafter ClBC] is a pest control strategy that has crystallized numerous studies since more than one century and provided numerous efficient solutions for pest control. The main advantages and risks of this strategy can be summarized as follows. In a context of the globalisation of international trade and human mobility, an ever growing number of exotic pests emerge locally. Such species can rapidly pullulate and jeopardize cultural practices. This general trend can also be favoured by global climatic changes that may allow the development of agronomic pests beyond their initial distribution area and increase their demography. Within this context, ClBC appears often to be the first way to try to regulate such pest populations. Moreover, when successful, ClBC appears to be very economic insofar as financial costs are only associated with the identification, evaluation and initial releases of exotic BCA. Contrary to other pest control strategy, the implication of practitioners and other costs are not necessary after the establishment of the BCA. The overall financial costs of such operations are consequently rather limited with regard to the durability of the pest control, in particular when the local introduction of a new BCA benefits from the previous experiences in other countries. Nevertheless, at least two kinds of risks are usually associated with ClBC. First of all, the average success rate of ClBC varies between 10 and 30% according to the authors for a total of more than 5000 introductions worldwide during the last century. As consequence, such operations may also appear too risky to be funded. Another risk is those associated with the non-target effects. Although few cases have been reported, their echoes may have contributed to a more harmonized approach and in some countries to more or less stringent regulations.

As consequences, classical biological programmes are at the crossroad of several concerns:

- agronomic; insofar as each introduction of exotic BCA is obviously an hope for the producers ;
- scientific; ClBC namely questions both ecologist and evolutionist in order to optimize the probability of establishment while minimizing the non-target effects. Their implication on such issues nevertheless depends on their own interest (in term of scientific question and/or possibility or publishing);
- political; since the introduction of BCA may depend on regulation or homologation decided at national or international levels;
- financial; since the development of ClBC is relying on various sources of funding (agronomic partners, scientific partners, politic institutions) with various interests and rationale (more or less short-term results, scientific excellence versus applied objectives).

Within this context, global evaluations of CIBC programmes are necessary to better understand the evolution of this practice and try to improve its use and efficiency. This has been repeatedly achieved during the last years either through reviews or meta-analysis. Based on a large (but probably not exhaustive) bibliographic survey, the present work aims to give a complementary point of view with the willingness to portray a realistic "state of the art" of Research and Development programmes of CIBC against arthropods. This chapter also firstly gives a broad temporal survey of the publication and a more precise survey of the literature for the decade [1999; 2008]. Biocontrol programmes against arthropods were then more precisely detailed with the objectives to give qualitative cues about the main pests and the types of related studies. Finally, a particular emphasis has been put on recent introductions of exotic insect parasitoids.

Based on these data, we also address some more or less important subjective recommendations based on our own opinion.

4.1.3.2. *Method*

A large bibliographic survey has been conducted with the CAB abstracts. Several combinations of key-words were used with various successes. Too broad (e.g; cases for which discussion about CIBC are marginal) or unprecise (e.g. cases for which a pest is not precised) publications were excluded.

764 publications were found using the key-words "classical biological control" or "classical biocontrol". 452 papers were published during the period [1999-2008] but about 30% were not relevant with regard to the purpose of this survey and have been discarded.

329 CIBC-related publications were obtained using the more complex combinations ["biological control" AND "exotic" AND "introduction"] but only 253 dressed precisely questions related to classical biological control. 117 were published during the selected temporal frame but only 81 were relevant with regard to our objectives.

47 CIBC-related publications were obtained using the more key-words association ["biological control" AND "exotic" AND "importation"] with 17 papers for the last ten years. Most of this literature was dedicated to the risk or regulatory aspects associated with the importation of exotic BCA so that only 7 relevant publications with regard to our objectives.

Finally, 130 publications were found using "acclimatization" AND "biological control" for only one relevant publication for the targeted period.

A total of 358 publications were also obtained which is probably for far from being exhaustive. For instance, 37 new references about BCA introductions were found in addition to the first 35 references found with the previous key-words combinations (see Table 19).

Additional bibliographic research were also realised for some taxa (see §4.1.3.3)

[Recommendation 1 (Minor - Scientists): Although the terms "classical biological control" or "classical biocontrol" may be not as explicit as others ("introduction", "importation"), the generalization of their use in titles, key-words or abstracts should be nevertheless used in order to improve the efficiency of bibliographic survey]

4.1.3.3. *General trends*

The temporal survey shows a quite regular increase of CIBC related publications with a mean of about 45 hits / year for the last ten years (Figure 17). Within this period, we observe a relative stability between the different combinations of pests and BCA (Figure 18). The main part of the publications (56%) of the cases deals with the biocontrol of phytophagous arthropods on which we will focus here. 42% of the papers deal with the biocontrol of weed. In this case, BCA are for 57% of the cases phytophagous insects and for 41% fungi (data not shown).

More than 70 arthropod pests were listed which cover 7 orders and approximately 40 families. As shown in Figure 19, Hemiptera and Lepidoptera were the two main orders with a total of 66% of the pest species and 70% of the publications. If the citation rate / order is highly correlated with the number of pests / order, this trend hides a great variability at the infra-order level. Indeed, the citation rate highly differs with regard to the pest species with a median of 2 papers / pest species and a range from 1 to 13 citations. The 13 most cited pests are listed in Figure 20. Two main observations can be drawn from this short list.

⇨ Firstly, this list is quite equally composed of either very specialist pests like Phyllocnistis citrella (on Citrus species), *Mononychellus tanajoa* (on cassava) or *Toxoptera citricida* (on Citrus species) or more generalist taxa like *Homalodisca vitripennis*, *Lymantria dispar* or *Pseudococcus viburni*. All of them are phytophagous pests whose damage are linked either to their herbivory, consumption of sap or virus transmission except the particular case of the fire ant *Solenopsis invicta* which is responsible for direct nuisance on farmers or indirect ecological modifications in the agrosystems.

⇨ The second observation is that the percentage of CIBC related publications / pest is negatively correlated with the corresponding total number of references (including also studies on other pest control strategies and/or various biological topics). For instance, 22% of the 32 references focusing on *H. vitripennis* explicitly deal with classical biological control while this percentage falls down to only 1% to 3% for well documented species like L. dispar, *S. invicta* or *D. virgifera virgifera*. This may be explained by the fact that CIBC is mainly considered as a “pionneer” pest control strategy that are developed either soon after the emergence of a new invasive pest or on “non biological model” for which the investigations on other biological aspects are limited.

[Recommendation 2 (Major – Politics, Scientist): Although Classical Biological Control can be perceived as a “pioneer” pest control strategies on non “biological models”, substantial investments are required on several biological aspects (e.g. community ecology, population genetics)]

4.1.3.4. *Biocontrol agents used*

The biocontrol agents related to CIBC (hereafter CIBCA) against arthropod species were not detailed in only 12% of the papers. These are in most of the cases either prospective works (55%) such as faunistic inventories of natural enemies on “new” pests like *Diabrotica virgifera virgifera* or retrospective studies (35%) on advanced programmes that take into account several BCA (see Appendix 18.1). Among the documented cases, 76% of CIBC programmes were based on the use of insect parasitoids (see Section 3.1.3.5). Pathogens and nematodes on one side and predatory arthropods on the other side are equally represented with about 12% of the publications for each case.

- **Pathogens and Nematodes as candidate for CIBCA**

The particular cases of pathogens and nematodes have been recently reviewed by Hajek and co-workers (62, 63[2]). Our own survey indicates that half of the papers actually deal with entomopathogenic fungi. Six pest species were identified including two mites (*Aceria guerreronis* and *Mononychellus tanajoa*) and two insects (*Aphis gossypii* and *Coptotermes formosanus*). However, except for the evaluation of *Neozygites* species against *M. tanajoa* (14, 39, 42, 43), other attempts seem to be rather limited. With regard to the catalogue of Hajek et al.(62), two other cases of entomopathogen fungi were missed in our own survey. These are the introductions of Entomophaga maigmaiga and Metarhizium anisopliae, against respectively the *Lymantria dispar* and the *Curculionidae Otiorynchus nodosus* for which the sources of Hajek and coworkers were mainly personal communications. The rather limited use of entomopathogenic fungi in CIBC was also confirmed by the review of Shah and Pell(156). The use of viruses as biocontrol agent for CIBC against arthropod pests were only documented fort three cases that are the *Lepidoptera* species *Anticarsia gemmatalis* (48, 127) and Lymantria dispar (16) and the

[2] within this section (4.1.3) numbers in parentheses refer to references listed in Appendix 18.4

Coleoptera Oryctes rhinoceros (81). *Microspodia* as candidate for CIBC were reported in only two studies (25, 165). The sole case of the use of nematodes is the study of Hurley et al. (79) who studied the extension of the use of parasitic nematode *Deladenus siricidicola* against the woodwasp Sirex noctilio.

- **Predatory arthropods as candidate for CIBCA**

The literature about predatory arthropods is dominated by four case-studies. The first one is the classical biocontrol of the cassava green mites *M. tanajoa* by *Typhlodromalus aripo* and, to a lesser extent, *T. manihoti*. All these studies are the extension of a very large classical biocontrol programme at a continental scale; two main issues were addressed during the recent decade that are the introduction and field evaluation of *T. aripo* in Mozambique and Malawi (125, 194) and the ecological interactions with other species (14, 124, 193) or plants(55). The second case-study is those of the predatory ladybird *Harmonia axyridis* (19, 90, 91, 137). The main concern of these publications is nevertheless not the Research and Development in CIBC but rather the risks of non-intended effects and geographic spray of this insect that is now considered as a world-wide invasive species. Another case of the use of ladybird is those of *Cryptolaemus montrouzieri* and *Scymnus coccivora* which have been successfully used to control the hibiscus mealybug *Maconellicoccus hirsutus* (51, 86, 103) which is the extension of a worldwide use of these species. The fourth main case-study is the classical biocontrol programme of *Prostephanus truncatus*, a serious pest of stored maize beetle using *Teretrius* (formerly *Teretriosa*) nigrescens (73, 169, 170). The lasts reported uses of predatory arthropods as candidate for CIBC were those of the *Coleoptera Laricobius nigrinus* against the adelgid *Adelges tsugae* (197) and the phytoseid *Neoseiulus baraki* against the coconut mite *A. guerreronis* (119). Contrary to other cases which were the continuity of older programmes, these two studies are associated with new BCA inventories undertaken during the last ten years - see respectively (196) and(99).

4.1.3.5. *Insect parasitoids as BCA*

- **Related journals papers and categorization of the studies**

125 publications were used for this analysis. Only 14% were associated to proceedings of meetings or other supports than journals. 43 different journals were identified but 50% of the publications were published only by five: Biological Control (21%), BioControl (8%), Biocontrol Science and Technology (7%), Florida Entomologist (7%) and Bulletin of Entomological Research (7%). Impact Factors are respectively 1.805, 1.957, 0.874, 0.886 and 1.415.

The types of the works were categorized according to the simplified sequential steps in R&D of biological programmes: BCA Inventories BCA characterization (systematic, molecular tools) Pest or BCA rearing ⇨ BCA biology (life history traits, thermal biology, behavioural ecology) ⇨ Pre-release survey ⇨ BCA introduction ⇨ Post-release survey. Studies related to “non-target effects” (i.e. the direct or indirect impacts of the CIBCA on non-target species) as well as those related to the “biocontrol disruption” (i.e. the negative impacts of organisms on the CIBCA) (details in Appendix 18.3) were also categorized. As shown in Figure 21, most of the CIBC related publications logically deals either with BCA biology, BCA introductions or post-release surveys which are central steps of the CIBC programmes. A strong discrepancy nevertheless exists between the different types of work in term of scientific publication; highest Impact Factors are relied to studies linked to Non-intended effects, Biocontrol disruption or BCA Biology.

[Recommendation 3 (Minor – Politics, Scientists): The different steps of R&D in Classical Biological Control are currently unequally promoted with regard to “scientific criteria”, with a clear emphasis on community ecology including non target effects. Such

trend may be detrimental to the short-term development of less gratifying tasks and consequently on the whole dynamism of ClBC.]

- **BCA Introductions**

As shown in Table 19, 65 introductions were recorded during the period of 1991-2006. This list is probably not exhaustive insofar as "cryptic introductions" may have been missed. This list does not also cover all the R&D in classical biocontrol programmes since some programmes may have been interrupted before releases. A faunistic inventory of the natural enemies of the North American leafhopper *Scaphoideus titanus* has for instance been led by our lab in 2000-2002 but the rearing of BCA candidates (mainly dryinids and egg-parasitoids) were not successful.

⇨ All these releases involve 55 different biocontrol agents (all hymenopteran except the *Pseudacteon* species used against the fire ant *Solenopsis invicta*) and 35 pests. 57% of these pests were *Hemiptera*, other being quite equally distributed between *Lepidoptera*, *Diptera*, *Hymenoptera* and *Coleoptera*.

⇨ Most of these introductions were realized against pest found on orchards and in particular Citrus. Other targeted crops were mainly tropical productions, ornamental or forest.

⇨ Most of the BCA introductions (42%) were realized in Europe or neighbouring countries (including Mediterranean Basin) and in North America (26%). The percentages of introductions in other geographical areas were: Australia-New Zealand and neighbouring islands (12%), South America (8%), sub-Saharan Africa (8%), Pacific Islands (3%), Asia (1%).

⇨ The total number of released parasitoids and number of sites were highly variable ranging respectively from 456 to 660000 individuals and from 2 to 132 sites. The percentage of establishment was 83% and, when established, high parasitism was found in 42% of the cases. It is noteworthy that these values are relatively high compared to other estimates and we are currently unable to say if this is linked to an improvement of practices or methodological differences or biases.

[Recommendation 4 (Major – Politics): With regard to natural or other human-mediated introductions of exotic species, species flow associated with the ClBC seems to be rather limited. Although possible non-intended effects cannot be excluded (their studies having to be increased), we fear that too drastic regulations could severely disturbed R&D programmes]

[Recommendation 5 (Major – Scientists): Estimating the success of ClBC is difficult because of methodological several biases ("cryptic introductions", barriers linked to languages and/or publishing). Shared international database should be necessary for more accurate estimation as well as an increasing traceability.]

[Recommendation 6 (Minor –Scientist)]: In parallel with the geographical expansion of their related pests, some biocontrol agents have been repeatedly released and established worldwide. Population genetics studies in such pest-BCA interactions should be particularly interesting to understand local adaptations, co-evolutionary processes and ultimately, the durability of Classical Biological Control.]

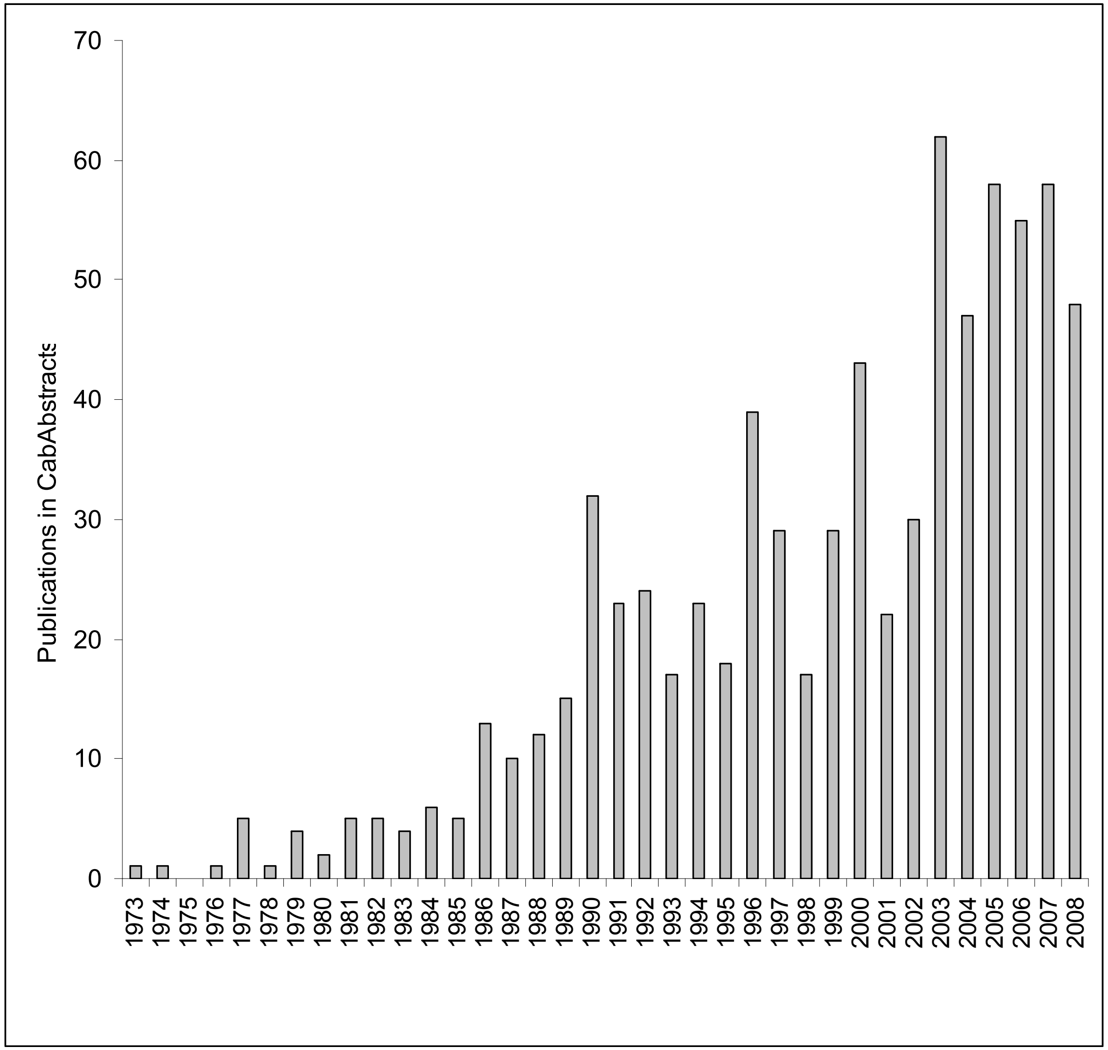

Figure 17: Large-scale temporal survey of the publications associated with classical biological control

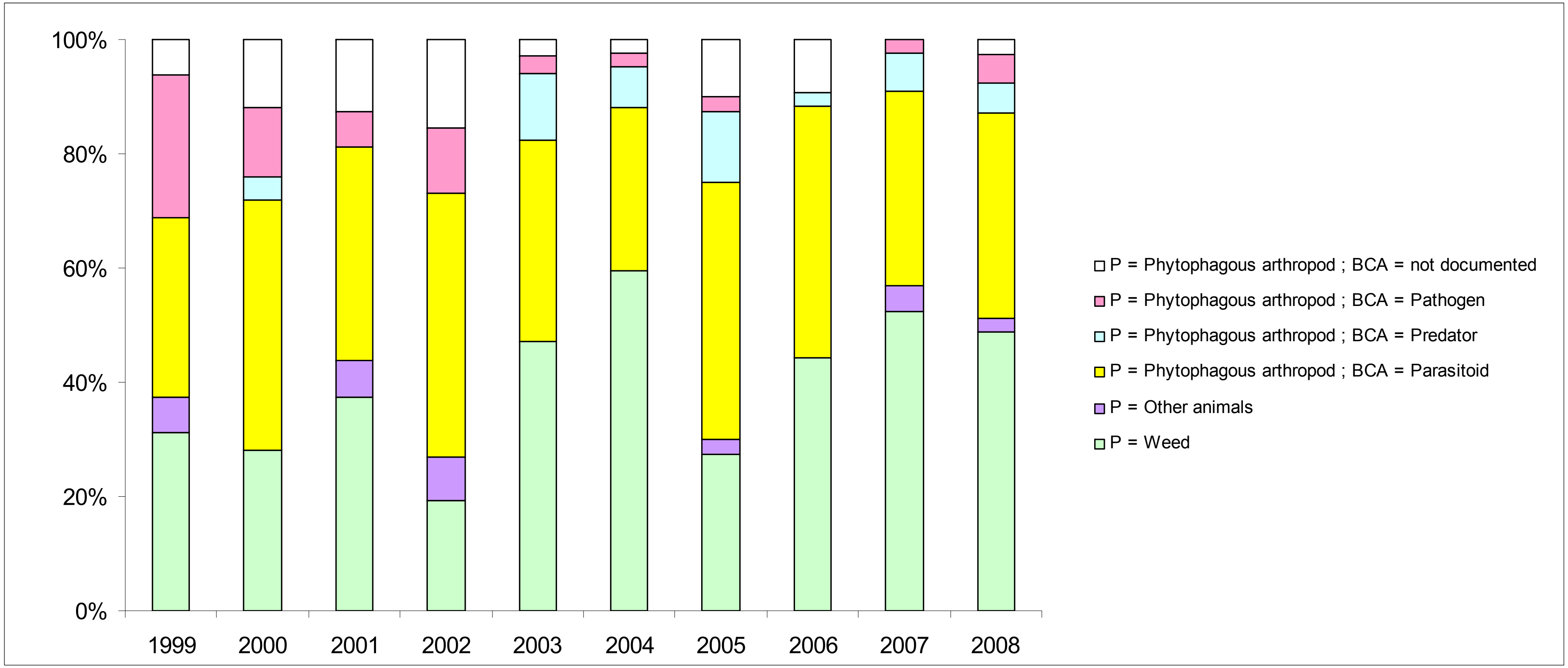

Figure 18: **Relative importance of the different types of biocontrol during the temporal frame [1999-2008]**

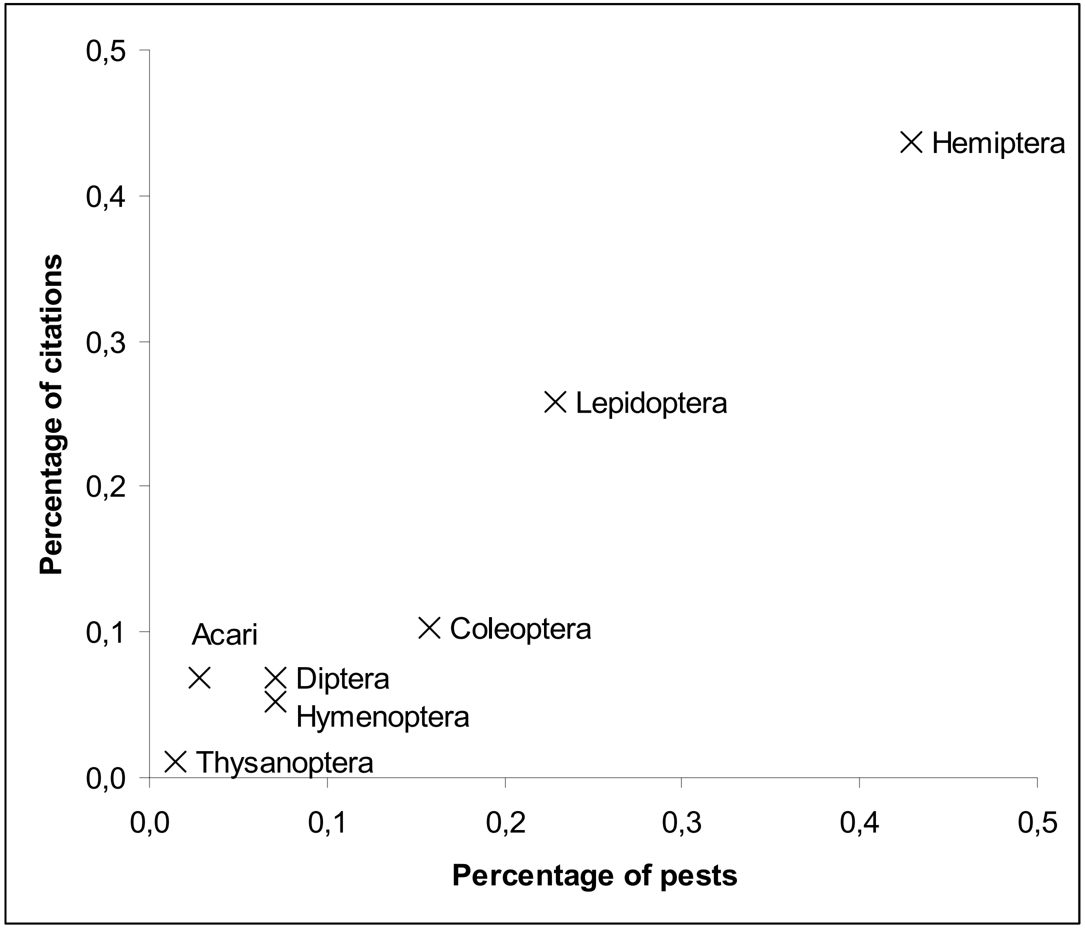

Figure 19: **Number of pest species and related citation rate by orders during the period [1999 ; 2008]**

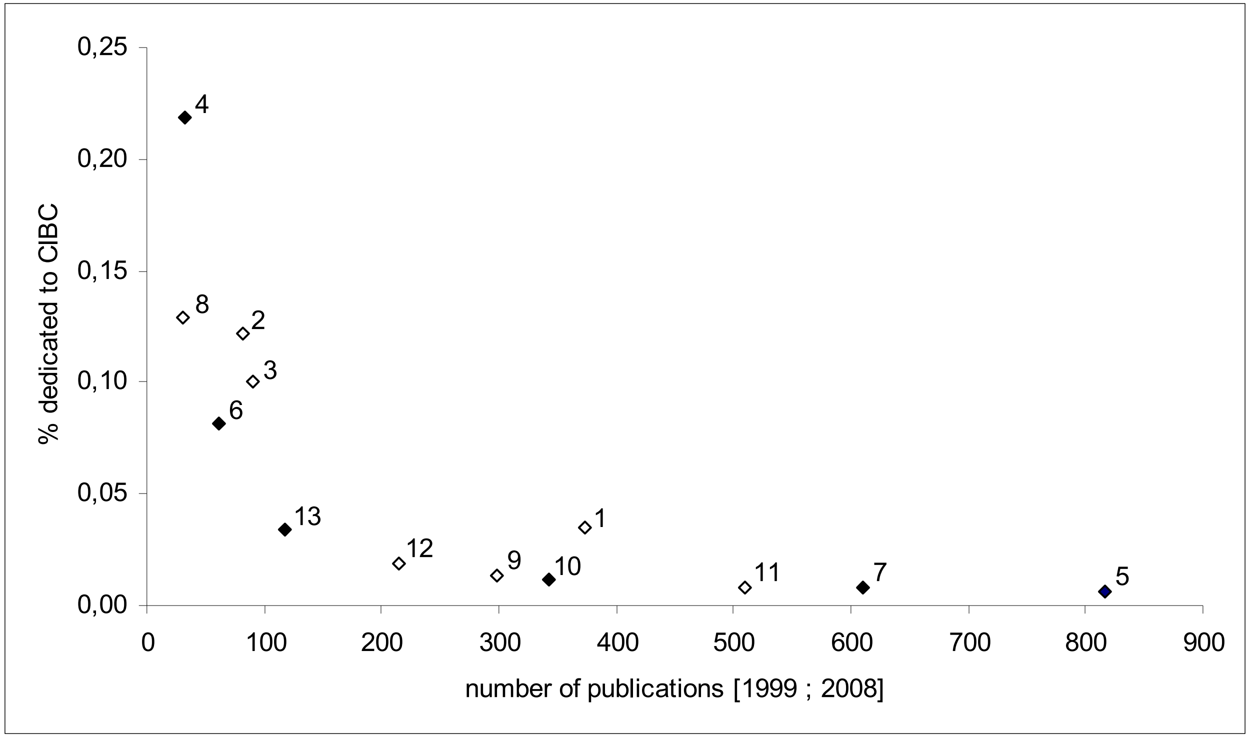

Figure 20: **Relationships between the number of publications associated to the main pests and the relative percentage of CIBC related studies.** Pest species are ranked in the decreasing order in number of publications : 1 : *Phyllocnistis citrella* ; 2 : *Mononychellus tanajoa* ; 3 : *Toxoptera citricida* ; 4 : *Homalodisca vitripennis* ; 5 : *Lymantria dispar* ; 6 : *Pseudococcus viburni* ; 7 : *Solenopsis invicta* ; 8 : *Aleurocanthus spiniferus* ; 9 : *Bactrocera oleae* ; 10 : *Chilo partellus* ; 11 : *Diabrotica virgifera virgifera* ; 12 : *Diatraea saccharalis* ; 13 : *Maconellicoccus hirsutus*.
Specialist and generalist pests are respectively indicated by white and dark diamonds.

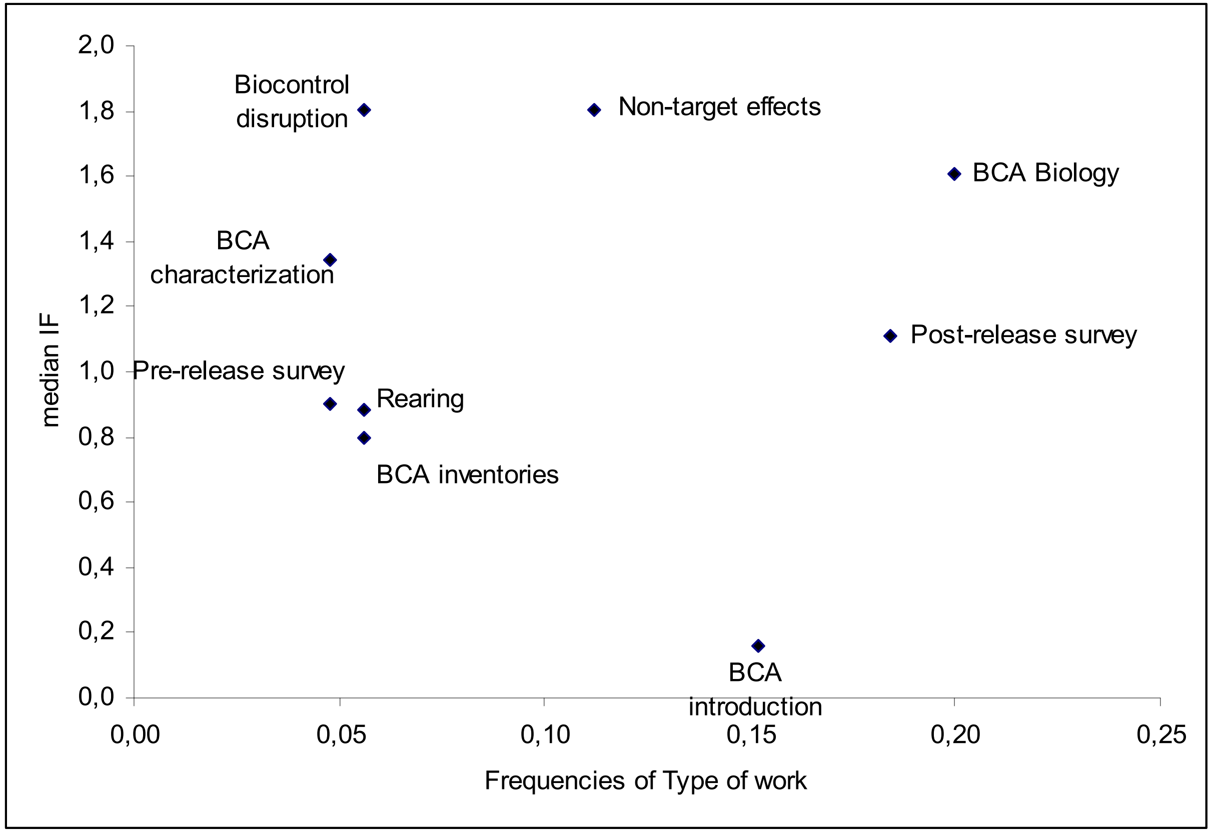

Figure 21: **Frequencies of papers and associated median IF related to the different categories of work**

Table 19: **Recent introductions of parasitoids as Classical Biocontrol agents**

Targeted pest	Crop	BCA Name	Introduction Area	Introduction Date	Individuals (sites)	Outcome	References
Aleurocanthus woglumi	*Citrus*	*Amitus hesperidum*	Trinidad	2000	1600 (3)	Establishment High parasitism	(White et al., 2005)
Aleurodicus dispersus	Banana	*Encarsia guadeloupae*	Spain (Tenerife)	_	_	_	(Nijhof et al., 2000)
		Lecanoideus floccissimus		_	_	_	
		Encarsia haitiensis	Australia	1992-1996	_	Establishment	(Lambkin, 2004)*
Aleurolobus niloticus	Orchard	*Eretmocerus siphonini*	Egypt	1998-1999	237000	Establishment High parasitism	(Abd-Rabou, 2002)
Aonidiella aurantii	*Citrus*	*Aphytis lingnanensis*	Spain	2000	_	Establishment	(Pina and Verdu, 2007)*
Aphis gossypii	Vegetable	*Lysiphlebus testaceipes*	Bulgaria	_	_	Establishment	*(Dimitrov et al., 2008)**
Bactrocera dorsalis	Orchard	*Fopius arisanus*	French Polynesia	2003		Establishment High parasitism	*(Vargas et al., 2007)**
Bemisia tabaci	Arable crops Vegetable	*Eretmocerus hayati*	Egypt	2000-2002	200700	Establishment	*(Abd-Rabou, 2004)**
Ceratitis capitata	Orchards	*Diachasmimorpha krausii*	Israel	2002-2004	75881	Establishment	*(Argov and Gazit, 2008)**
	(incl. *Citrus*)	*Fopius arisanus*		2002-2004	258750	?	
		Fopius ceratitivorus		2002-2004	58860	Establishment	
		Psyttalia concolor (complex)		2002-2004	75881	?	
Ceroplastes rubens	Orchard (incl. *Citrus*)	*Anicetus beneficus*	Papua New Guinea	2002	2200 (2)	Establishment	(Krull and Basedow, 2005)
Chilo sacchariphagus	Sugarcane	*Xanthopimpla stemmator*	Mozambique	2001	5000 (5)	?	(Conlong and Goebel, 2002)
Cinara cupressivora	Forest Ornamental	*Pauesia juniperorum*	Mauritius	2003-2004	1500	?_	(Alleck et al., 2006)
Coccus viridis	Citrus Coffee	*Diversinervus* sp. near *stramineus*	Australia	_	_ (4)	Establishment High parasitism	*(Smith et al., 2004)**

Ctenarytaina eucalypti	Forest Ornamental	*Psyllaephagus pilosus*	Chile	2001	_	Establishment High parasitsm	*(Rodriguez and Saiz, 2006)**
Diaphorina citri	*Citrus*	*Diaphorencyrtus aligarhensis*	USA	_	_	_	(Hoy, 2005)
		Tamarixia radiata	USA	_	_	_	
Diatraea saccharalis	Sugarcane	*Cotesia flavipes*	USA	2001-2002	_ (4)	Failure	*(White et al., 2004)**
Dryocosmus kuriphilus	Forest Ornamental	*Torymus sinensis*	Italy	2005-2006	1100 (14)	Establishment	(Aebi et al., 2007)

Legend : _ : data not available ; ? : long-term establishment not sure ; * : additional references

Table 19: Recent introductions of parasitoids as Classical Biocontrol agents (continued)

Hemiberlesia pitysophila	Forest	*Coccobius azumai*	China	2002	–	Establishment	(Wang et al., 2004)*
Homalodisca vitripennis	Wide range	*Gonatocerus ashmeadi*	Tahiti	2005	14000 (27)	Establishment High parasitism	(Grandgirard et al., 2007a) (Grandgirard et al., 2008) (Petit et al., 2008)
Hypothenemus hampei	Coffee	*Cephalonomia stephanoderis*	Cuba	–	– (2)	?	(Murguido Morales et al., 2008)*
		Phymastichus coffea	Colombia	–	– (41)	Establishment	(Aristizabal et al., 2004)*
Lilioceris lilii	Ornamental	*Diaparsis jucunda*	USA	–	–	–	(Casagrande and Tewksbury, 2005)*
		Lemophagus errabundus		–	–	–	
		Tetrastichus setifer		2001	1700 (21)	–	(Tewksbury et al., 2005)*
Liriomyza trifolii	Vegetables	*Dacnusa sibirica*	Egypt	–	90000	?	(Abd-Rabou, 2006)*
		Diglyphus isaea		–	90000	?	
Listronotus bonariensis	Pasture	*Microctonus hyperodae*	New Zealand	1991-1998	66000 (121)	–	(McNeill et al., 2002) (Phillips et al., 2008)
Maconellicoccus hirsutus	Wide range	*Anagyrus kamali*	North America			Establishment High parasitism	(Kairo et al., 2000)
Metcalfa pruinosa	Wide range	*Neodryinus typhlocybae*	Greece	2006	– –	Establishment	(Anagnou-Veroniki et al., 2008)
Ophelimus maskelli	Forest Ornamental	*Closterocerus chamaeleon*	Israel	2005-206	12000 (6)	Establishment High parasitism	*(Protasov et al., 2007)**

		Closterocerus sp.	Italy	–	_ (5)	Establishment High parasitism	*(Rizzo et al., 2006)**
Paracoccus marginatus	Wide range	*Acerophagus papayae*	Palau	2003-2004	–	Establishment High parasitism	(Muniappan et al., 2006)
		Anagyrus loecki		2003-2004	–	Establishment High parasitism	
		Pseudleptomastix mexicana		2003-2004	_	Failure	

Legend : _ : data not available ; ? : long-term establishment not sure ; * : additional references

Table 19: Recent introductions of parasitoids as Classical Biocontrol agents (continued)

Phyllocnistis citrella	Citrus	*Ageniaspis citricola*	Morocco	1995-1996	–	Failure	(Rizqi et al., 2003)
			USA	–	–	Establishment High parasitism	(Hoy, 2005)
			Italy	1995	–	Failure	(Siscaro et al., 2003)
			Italy	1996-1997	–	Establishment High parasitism	(Siscaro et al., 1999)
			USA	1999	25000 (132)		(Paiva et al., 2000)
			Argentina	2001-2004		?	(Zaia et al., 2006)
			Brazil	1999	25000	–	(Paiva et al., 2000)*
		Cirrospilus ingenuus	Morocco	–	–	–	(Rizqi et al., 2003)
		Cirrospilus quadristriatus [C. ingenuus]	USA	–	–	Establishement	(Hoy, 2005)
		Citrostichus phyllocnistoides	Italy	1995	–	Establishment	(Siscaro et al., 2003)
			Morocco	2000	–	Establishment	(Rizqi et al., 2003)
			Spain	1996-1999	–	Establishment High parasitism	(Garcia-Mari et al., 2004)*
		Quadrastichus sp	Morocco		–	–	(Rizqi et al., 2003)
			Italy	1995	–	Failure	(Siscaro et al., 2003)
			Italy	1996-1997	–	Failure	(Siscaro et al., 1999)
		Semielacher petiolatus	Morocco	1996-1997	–	Establishment	(Rizqi et al., 2003)
Pseudococcus viburni	Orchard	*Pseudaphycus maculipennis*	New Zealand	2001	–	–	(Charles, 2001)
Saissetia coffeae	Olive	*Coccophagus cowperi*	Egypt	2001-2003	300000	Establishment	*(Abd-Rabou, 2005)**
Siphoninus phillyreae	Orchard	*Eretmocerus siphonini*	Egypt	1998-1999	237000	Establishment High parasitism	(Abd-Rabou,

							2002)
Sirex noctilio	Forest	*Ibalia leucospoides*	South Africa	1998-2001	456	Establishment	*(Tribe and Cillie, 2004)**
Solenopsis invicta	_	*Pseudacteon curvatus*	USA	2003	10100 (2)	Establishment	(Vazquez et al., 2006)
		Pseudacteon obtusus	USA	2006		?	(Gilbert et al., 2008)
		Pseudacteon tricuspis	USA	1999-2001		Establishment	
Tephritidae sp.	Orchard (incl. *Citrus*)	*Diachasmimorpha longicaudata*	Brazil	2002	34000 (2)	Failure	(Alvarenga et al., 2005)
Toxoptera citricida	*Citrus*	*Lipolexis oregmae*	USA	2000-2002	33500	Establishment	(Hoy, 2005) (Persad et al., 2007)
Yponomeuta malinellus *	Orchard	*Ageniaspis fuscicollis*	Canada	1987-1997	_	Establishment	(Cossentine and Kuhlmann, 2007)*

Legend : _ : data not available ; ? : long-term establishment not sure ; * : additional references

4.2. Currently registered biocontrol products in the EU

4.2.1. Collection of information

A small team formed by ACTA and IBMA conducted a survey on biological active substances approved in the European Union and on Biological Control Products (BC products) authorised in a several countries. The investigation focused on crops covered by ENDURE RA1case studies. The frame of the present survey was defined in a meeting on 9th January 2009 in Basle, and the work was performed during the period from April to September 2009.

To compile a list of registered biocontrol products, the online EU Pesticides Database was consulted on 21st April 2009. Data were retrieved and the list was reorganised and the information about use categories complemented with the help of the inclusion directives where necessary. Substances deemed suitable for biocontrol were identified and it was decided to distinguish four major groups: micro-organisms, semiochemicals (attractants), botanicals and "other plant protection substances of natural origin".

This study was complemented by an analysis of specific uses of products commercialized in four countries of the EU (France, Germany, Spain and the United Kingdom). A fifth country, Switzerland was included in the study for comparison, because it has not been restricted by the implementation of Directive 91/414/EEC until recently. For each country, official national online databases on authorised plant protection products (Table 20) were screened for authorised biocontrol active substances:

Table 20: Consulted sources of information on authorized biocontrol plant protection products in five European countries:

Country	Official source / website	Reference date
France	e-phy database of the Ministry of Agriculture & Fisheries http://e-phy.agriculture.gouv.fr	31/8/2009
Germany	Online-Datenbank Pflanzenschutzmittel of the Federal Office of Consumer Protection and Food Safety (BVL) http://www.bvl.bund.de/cln_027/DE/04__Pflanzenschutzmittel/02__ZugelassenePflanzenschutzmittel/02__OnlineDatenbank/onlineDB__node.html__nnn=true	12 /8/2009
Spain	Registro de productos Fitosanitarios of the Ministerio de Ambiente y Medio Rural y Marino http://www.mapa.es/es/agricultura/pags/fitos/registro/menu.asp	
Switzerland	Plant protection index ("Pflanzenschutzmittelverzeichnis") of the Federal Office for Agriculture (BWL, Fachbereich Pflanzenschutzmittel) http://www.psa.blw.admin.ch/index_de_5_2_A.htm	31/7/2009
United Kingdom	Pesticides Register of UK approved products under the responsibility of the Chemicals Regulation Directorate Pesticides https://secure.pesticides.gov.uk/pestreg/ProdSearch.asp	4/2009

The survey was limited to uses concerning seven crops or cropping groups which are subject to ENDURE case studies: pomefruit (apples and pears), grapevine, cereals, rape, maize, potatoes and tomatoes (greenhouse and field), the latter being extended to other vegetables where deemed of interest. Country lists of representative products (generally up to two) were created and sorted according to uses in crops, target pests and pathogens were identified by English and scientific names wherever possible.

4.2.2. Substances suitable for biological control and registered on Annex 1 of the EU Pesticides Database

The complete list compiled from data retrieved in the EU Pesticides Database is presented in Appendix 19. Excerpts concerning the four categories of substances compatible with biological control are presented in Table 21.

4.2.2.1. Botanicals

Botanicals are plant-substances resulting from simple processing e.g. pressing or from extraction. By extension the definition applies to a small numbers of compounds or even single ones extracted from plants and purified e.g. laminarine.

Fourteen botanicals have been identified (Table 21) including two borderline cases for which single molecules identical to naturally occurring substances have been synthesised.

- Four botanicals are authorised as repellents only: Extract from the tea tree, garlic extract, clove oil (plant oils) and pepper.
- Six botanicals enter into the category of plant growth regulators.
- The phytohormones gibberellic acid and gibberelline are botanicals produced in fermenters acting on plant growth. Spearmint oil and sea-alga extract are listed for their effect on plant growth as well.
- The phytohormone ethylene is naturally present in plants and in soil and can be included here although it is typically produced in the petrochemical industry by steam cracking.
- Carvone is a terpene produced by aromatic plants in particular by the mint it can also be classified among the botanicals. To obtain a pure grade it is generally synthesised. In plant protection it is used as a growth regulator.
- Laminarin is extracted from sea weed and is classified as elicitor. Rape seed oil enters into the category of insecticides/acaroids. Citronella oil is the only BCA approved as a herbicide.
- Pyrethrins are extracted from Pyrethrum flowers, from cultivars of *Chrysanthemum cinerariaefolium.* By their origin they are botanicals but their structures are analogue and their properties are similar to those of synthetic pyrethroids. Due to their mode of action which is analogous to conventional insecticides and their toxicity for aquatic and other non target organisms, they are not typical biological substances although they are accepted in organic farming.

Table 21: Active substances suitable for biological control listed on Annex I of 91/414/EEC (EU Pesticide Database) - Status on 21st April 2009

Substance	Category[1, 2]	List[3]	Inclusion Date	Expiry Date	Legislation
Botanicals					
Extract from tea tree	RE	A 4	01/09/2009	31/08/2019	2008/127
Garlic extract	RE	A 4	01/09/2009	31/08/2019	2008/127
Gibberellic acid	PG	A 4	01/09/2009	31/08/2019	2008/127
Gibberellin	PG	A 4	01/09/2009	31/08/2019	2008/127
Laminarin	EL	C	01/04/2005	31/03/2015	05/3/EC
Pepper	RE	A 4	01/09/2009	31/08/2019	2008/127
Plant oils / Citronella oil	HB	A 4	01/09/2009	31/08/2019	2008/127
Plant oils / Clove oil	RE	A 4	01/09/2009	31/08/2019	2008/127
Plant oils / Rape seed oil	IN, AC	A 4	01/09/2009	31/08/2019	2008/127
Plant oils / Spearmint oil	PG	A 4	01/09/2009	31/08/2019	2008/127
Sea-algae extract (formerly sea-algae extract and	PG	A 4	01/09/2009	31/08/2019	2008/127

seaweeds)					
Botanicals copied by synthesis (s) or excluded (e)					
Carvone (s)	PG	C	01/08/2008	31/07/2018	2008/44/EC
Ethylene (s)	PG	A 4	01/09/2009	31/08/2019	2008/127
Pyrethrins (e)	IN	A 4	01/09/2009	31/08/2019	2008/127
Microbials					
Ampelomyces quisqualis strain AQ10	FU	C	01/04/2005	31/03/2015	05/2/EC
Bacillus subtilis str. QST 713	BA, FU	C	01/02/2007	31/01/2017	07/6/EC
Bacillus thuringiensis subsp. aizawai (ABTS-1857 and GC-91)	[IN]	A 4	01/01/2009	31/12/2018	2008/113
Bacillus thuringiensis subsp. israelensis (AM65-52)	[IN]	A 4	01/01/2009	31/12/2018	2008/113
Bacillus thuringiensis subsp. kurstaki (ABTS 351, PB 54, SA 11, SA12 and EG 2348)	[IN]	A 4	01/01/2009	31/12/2018	2008/113
Bacillus thuringiensis subsp. tenebrionis (NB 176)	[IN]	A 4	01/01/2009	31/12/2018	2008/113
Beauveria bassiana (ATCC 74040 and GHA)	IN	A 4	01/01/2009	31/12/2018	2008/113
Coniothyrium minitans	FU	C	01/01/2004	31/12/2013	03/79/EC
Cydia pomonella granulosis virus (CpGV)	[IN]	A 4	01/01/2009	31/12/2018	2008/113
Gliocladium catenulatum strain J1446	FU	C	01/04/2005	31/03/2015	05/2/EC
Lecanicillimum muscarium (Ve6) (former *Verticillium lecanii*)	IN	A 4	01/01/2009	31/12/2018	2008/113
Metarhizium anisopliae (BIPESCO 5F/52)	IN	A 4	01/01/2009	31/12/2018	2008/113
Paecilomyces fumosoroseus Apopka strain 97	[IN]	C	01/07/2001	30/06/2011	01/47/EC
Paecilomyces lilacinus	[IN]	C	01/08/2008	31/07/2018	2008/44/EC
Phlebiopsis gigantea (several strains)	FU	A 4	01/01/2009	31/12/2018	2008/113
Pseudomonas chlororaphis strain MA342	FU	C	01/10/2004	30/09/2014	04/71/EC
Pythium oligandrum (M1)	FU	A 4	01/01/2009	31/12/2018	2008/113
Spodoptera exigua nuclear polyhedrosis virus	FU	C	01/12/2007	30/11/2017	07/50/EC
Streptomyces K61 (K61) (formerly *Streptomyces griseoviridis*)	FU	A 4	01/01/2009	31/12/2018	2008/113
Trichoderma aspellerum (ICC012) (T11) (TV1) (formerly *T. harzianum*)	FU	A 4	01/01/2009	31/12/2018	2008/113
Trichoderma atroviride (IMI 206040) (T 11) (formerly *Trichoderma harzianum*)	FU	A 4	01/01/2009	31/12/2018	2008/113
Trichoderma gamsii (formerly *T. viride*) (ICC080)	FU	A 4	01/01/2009	31/12/2018	2008/113
Trichoderma harzianum Rifai (T-22) (ITEM 908)	FU	A 4	01/01/2009	31/12/2018	2008/113
Trichoderma polysporum (IMI 206039)	FU	A 4	01/01/2009	31/12/2018	2008/113
Verticillium albo-atrum (WCS850) (formerly *Verticillium dahliae*)	FU	A 4	01/01/2009	31/12/2018	2008/113
Other Natural					
Abamectin (aka avermectin)	AC, IN	A 3	01/01/2009	31/12/2018	2008/107
Acetic acid	HB	A 4	01/09/2009	31/08/2018	2008/127
Aluminium silicate (aka kaolin)	RE	A 4	01/09/2009	31/08/2019	2008/127
Blood meal	RE	A 4	01/09/2009	31/08/2019	2008/127
Carbon dioxide	IN, RO	A 4	01/09/2009	31/08/2019	2008/127
Fat distilation residues	RE	A 4	01/09/2009	31/08/2019	2008/127
Ferric phosphate	MO	C	01/11/2001	31/10/2011	01/87/EC
Kieselguhr (diatomaceous earth)	IN	A 4	01/09/2009	31/08/2019	2008/127
Milbemectin	IN, AC	C	01/12/2005	30/11/2015	05/58/EC
Quartz sand	RE	A 4	01/09/2009	31/08/2019	2008/127
Spinosad	IN	C	01/02/2007	31/01/2017	07/6/EC
Other Natural, produced by synthesis					
Benzoic acid	BA, FU, OT	C	01/06/2004	31/05/2014	04/30/EC
Potassium hydrogen carbonate	FU	A 4	01/09/2009	31/08/2019	2008/127
Urea	IN	A 4	01/09/2009	31/08/2019	2008/127
Other Natural, fatty acid					
Capric acid (CAS 334-48-5)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Caprylic acid (CAS 124-07-2)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Fatty acids C7 to C20	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Fatty acids C7-C18 and C18 unsaturated potassium salts (CAS 67701-09-1)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127

Fatty acids C8-C10 methyl esters (CAS 85566-26-3)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Lauric acid (CAS 143-07-7)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Methyl decanoate (CAS 110-42-9)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Methyl octanoate (CAS 111-11-5)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Oleic acid (CAS 112-80-1)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Pelargonic acid (CAS 112-05-0)	IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Other Natural, repellent					
Calcium carbonate	RE	A 4	01/09/2009	31/08/2019	2008/127
Limestone	RE	A 4	01/09/2009	31/08/2019	2008/127
Methyl nonyl ketone	RE	A 4	01/09/2009	31/08/2019	2008/127
Sodium aluminium silicate	RE	A 4	01/09/2009	31/08/2019	2008/127
Repellents by smell/Fish oil	RE	A 4	01/09/2009	31/08/2019	2008/127
Repellents by smell/Sheep fat	RE	A 4	01/09/2009	31/08/2019	2008/127
Semiochemical					
(Z)-13-Hexadecen-11yn-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z,Z,Z,Z)-7,13,16,19-Docosatetraen-1-yl isobutyrate	AT	A 4	01/09/2009	31/08/2019	2008/127
Ammonium acetate	AT	A 4	01/01/2009	31/12/2018	2008/127
Hydrolysed proteins	IN	A 4	01/09/2009	31/08/2019	2008/127
Putrescine (1,4-Diaminobutane)	AT	A 4	01/09/2009	31/08/2019	2008/127
Trimethylamine hydrochloride	AT	A 4	01/09/2009	31/08/2019	2008/127
Straight Chain Lepidoptera Pheromones	AT	A 4	01/09/2009	31/08/2019	2008/127
Semiochemical / SCLP					
(2E, 13Z)-Octadecadien-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(7E, 9E)-Dodecadien 1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(7E, 9Z)-Dodecadien 1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(7Z, 11E)-Hexadecadien-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(7Z, 11Z)-Hexadecdien-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(9Z, 12E)-Tetradecadien-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(E)-11-Tetradecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(E)-5-Decen-1-ol	AT	A 4	01/09/2009	31/08/2019	2008/127
(E)-5-Decen-1-yl-acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(E)-8-Dodecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(E,E)-8,10-Dodecadien-1-ol	AT	A 4	01/09/2009	31/08/2019	2008/127
(E/Z)-8-Dodecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-11-Hexadecen-1-ol	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-11-Hexadecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-11-Hexadecenal	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-11-Tetradecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-13-Octadecenal	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-7-Tetradecenal	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-8-Dodecen-1-ol	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-8-Dodecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-9-Dodecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-9-Hexadecenal	AT	A 4	01/09/2009	31/08/2019	2008/127
(Z)-9-Tetradecen-1-yl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
Dodecyl acetate	AT	A 4	01/09/2009	31/08/2019	2008/127
Tetradecan-1-ol	AT	A 4	01/09/2009	31/08/2019	2008/127

[1] AC=acaricide, AT= attractant, BA=bactericide, EL=elicitor, FU=fungicide, HB=herbicide, IN=insecticida, MO=molluscicide, NE=nematicide, PA=Plant Activator, PG=Plant Growth, RE=repellent, RO=rodenticide.
[2] Category in [ ] added by author
[3] A: Existing active substances divided into four lists for phased evaluations; C: New active substances

4.2.2.2. Micro-organisms

The term micro-organism is defined in Council Directive 91/414/EEC (as amended by Commission Directive 2001/36/EC) as follows: A microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material. The definition applies to, but is not limited to, bacteria, fungi, protozoa, viruses and viroids. It does not include multicellular organisms, such as nematodes or insects.

Twenty five microbial species are included in annex I, some of which are represented by several strains.

Six bacterial (sub)species (*Bacillus subtilis*, *Pseudomonas chlororaphis* and four subspecies of *Bacillus thuringiensis*) and two virus species (*Cydia pomonella* Granulose Virus and *Spodoptera exigua* NPV) are included. All B.t. subspecies and viral agents are approved for insect control. *Pseudomonas* is approved for fungicidal seed treatments and *Bacillus subtilis* can be used against plant pathogenic fungi and bacteria.

Seventeen fungal agents belonging to twelve genera are listed, *Trichoderma* being represented by five species. *Beauveria bassiana*, *Lecanicillimum muscarium* and *Metarhizium anisopliae* are approved for use as insecticides, the other fungal agents for use against fungal diseases.

4.2.2.3. Semiochemicals (attractants)

Semiochemicals are chemical substances such as pheromones, kairomones and allomones that act to modify the behaviour of pests or their natural enemies.

In the table based on the EU Pesticides Database, Straight Chain Lepidopteran Pheromones (SCLP) are highlighted in green, non SCLP-pheromones in light cyan and other attractants (including hydrolysed proteins) are highlighted in yellow. There is one repellent which is marked in light red.

SCLPs are included in annex I as a group but 25 compounds of this group are also listed individually. In the inclusion directive 2008/127/EC, some molecules are mentioned three times, as an individual substance, in a blend of the same type, e.g. acetates and in mixed blends, e.g. alcohols and acetates.

Often single SCLP compounds show attraction to one or more moth species and typically a combination of two or more of these compounds in a precise ratio enhances the attraction and the specificity. Thus SCLPs should be considered as a whole group and it must not be concluded that each compounds stands for one species.

The SCLPs listed individually are typical examples found in the pheromone blends of moth pest species currently of economic importance. A large variety of compounds and isomers, an estimated number of about 300 identified molecules, used by Lepidopterans are not listed here. They differ in carbon chain length, in the number of double bonds and/or their positions and in their chemical functional group (alcohol, acetate or aldehyde)

SCLPs can be used for mass trapping, mating disruption or in attract and kill devices (A&K) or formulations. When associated with an insecticide, i.e in A&K devices, attractants do not need to be included in annex I.

Two non SCLP pheromones as well as four semiochemicals other than pheromones attractive to different fly (Diptera) species are listed in the EU Pesticides Database: Ammonium acetate, hydrolysed proteins, putrescine (1,4-diaminobutane) and Trimethylamine hydrochloride.

4.2.2.4. Other Plant Protection substances of natural origin

This group has been created for the purpose of the survey. It includes mineral substances as well as substances produced by or derived from animals or from micro-organisms. Thus very diversified substances and products like limestone powder, kaolin as well as diatomaceous earth (Kieselguhr), fatty acids and their derivates (e.g. soaps) can be found in this group. Not all substances of this group do meet the expectation of low non-target toxicity and low environmental impact.

Some active substances included in annex I are produced by micro-organisms. Spinosad which is produced by the bacterium *Saccharopolyspora spinosa* finds its place here; it is accepted for organic farming. Milbemectin is a mixture of natural compounds (milbemycins) isolated from fermentation broth of the fungus *Streptomyces hygroscopicus* subsp. *aureolacrimosus*. The substance is active against insects of different families and a large range of mites. Abamectin contains avermectins which are biosynthesised by *Streptomyces avermitilis*. The substance shows very high toxicity in Mammals and in aquatic organisms. Milbemectin and abamectin are not authorised in organic crop protection.

Potassium hydrogen carbonate is a slightly basic substance used for its fungicidal properties. The US FDA considers this substance as GRAS (Generally Recognised as Safe).

Six natural substances are specifically marked in the EU List, they are used as animal repellents: three are minerals (Calcium carbonate, limestone, sodium aluminium silicate), two are of animal origin (fish oil and sheep fat) while methyl nonyle ketone is either produced by synthesis or extracted from plant oils (rue). The latter repellent acts by its strong odour. It is naturally present in some edible crops and spices.

- **Limit cases and exclusions**

With regards to their (eco)toxicological profile and environmental impact neither sulphur and its derivates (iron sulphate) nor cupric compounds i.e. Bordeaux mixture, copper hydroxide, copper oxichloride and cuprous oxide are considered here as typical biological substances although they might be accepted in organic agriculture.

Tall oils (crude or pitch) are a by-product in the Kraft process used in the paper industry. Thus they are substances resulting from a chemical process and are classified as chemicals here.

Calcium carbide is produced from lime and coke in electric arc furnaces. It is fitted among chemicals but is used as a repellent like some other minerals.

1-Methyl-cyclopropene is an inhibitor of the effects of the phytohormone ethylene and is mainly used to conserve cut flowers. It is placed among the chemicals.

4.2.3. Uses of biocontrol products in five European countries

4.2.3.1. Registered plant protection substances

In each country all BCAs authorised for uses in seven crops or cropping groups were identified. Lists of representative products (generally up to two) were created and sorted according to uses in crops: pomefruit (apples and pears), vine, cereals, rape, maize, potatoes and tomatoes (greenhouse and field), the latter was extended to other vegetables where deemed of interest.

In **France** twelve different microbial BCA species (or sub-species in the case of *Bacillus thuringiensis*) are authorised among which two species, *Beauveria tenella* and *Candida oleophila* are not yet included in 91/414 Annex I. Only four botanical active substances are authorised, including pyrethrum and rotenon which were excluded from our survey. Fenugreek extracts benefited from a specific French approach to plant extracts under former national rules, and EU approval for this active is still pending. Laminarin is included in Annex I. Five Straight Chain Lepidopteran Pheromones (SCLP) blends or associations (one just specifying minor components used for the single target codling moth) are registered for mating disruption in orchards or vineyard.

In **Germany** nine microbial BCAs are authorised in Plant Protection Products (all included). Only four botanical substances are listed for plant protection, two of which are included in Annex I (pyrethrins and rape seed oil), two are not (azadirachtin and lecithin). Three different SCLP associations are authorised for mating disruption against Codling Moth or Vine Moths.
For Germany only fully registered BC products according to the rules of the PPP directive were included in the survey. As a consequence, plant strengtheners authorised according to the Federal Plant Protection Act §§ 31ff were excluded. Plant Strengtheners can avoid the EU procedures and requiremments for plant protection products but they must not claim specific protective properties either.

In **Spain** ten microbial BCAs are authorised, all of which are included in EU Annex 1. Only three botanical substances could be identified: Pyrethrins and rotenon which are excluded from the survey and Azadirachtin (Neem extract) which is not included in EU Annex I. The plant growth regulators gibberellinic acid/gibberellin were not explored. Only four SCLP associations are authorised for mating disruption in vine and orchards including two for oriental fruit moth and peach twig borer typical for peach orchards.

In **Switzerland** twelve different microbial BCA species (or sub-species in the case of *Bacillus thuringiensis*) are authorised, among which is one species not included in 91/414 Annex I: *Beauveria brognartii*.
Eleven botanicals are approved, among which the insecticides Pyrethrum (included in EU Annex I) and rotenon (rejected from Annex I) have been excluded from the survey because of their toxicological profile. The plant growth regulators gibberellic acid and gibberellin were also excluded from the survey. Five substances not included in EU Annex I are authorised: Azadirachtin (Neem extract), fennel oil, lecithin, mustard powder and Quassia extract.
An impressive number of semiochemicals, eleven different SCLP associations are authorised for mating disruption allowing the control a large variety of moths in orchards (including one association of 8 compounds against five different species) and vineyards. This can be related to the facilitated approval of pheromone products in Switzerland.

In the **UK** eight microbial BCAs are approved but only a single botanical (Laminarin, EU approved) and a single pheromone blend (for codling moth). No biological plant protection products are available for use in grapevine, rape, maize or potatoes.

With regard to the global availability of biological control products in the different crops, pomefruit, vegetables and vine are generally in a better position than arable crops in the countries included in the survey. In the UK e.g. only laminarin is available on wheat and cereals, and no biological plant protection products are registered for rape, maize or potatoes.

Conclusion: None of the EU Member States covered in the present survey shows such a variety of BCAs as Switzerland where we find the largest numbers of microbials, botanicals and pheromone blends authorised in the crops subject of the inquiry. Only France reaches the number of twelve microbial BCAs in registered products. The privileged situation in the Helvetic Confederation can be explained by the flexible regulatory approach of the competent authorities in the past, until the progressive implementation of EU directive 91/414/EEC and the related framework, as well as the sustained support by experts in confederal agronomic institutes.

4.2.3.2. Invertebrate BCAs

Invertebrate BCAs were listed separately for each country.

In **France** invertebrate BCAs cannot be registered, they do not even need to be formally declared. The list provided in the survey is based on the voluntary declarations to ACTA by the producers wishing to have their beneficials published in the non-official Index Phytosanitaire.

They must be registered in **Germany**. An official list which is regularly updated is published by the Julius Kühn Institute.

In **Spain** companies which are responsible of commercialisation of IBCAs must give an information to the Ministry of Agriculture to allow the inscription into a register before commercialisation (Orden APA/1470/2007). This information given is about name of commercial product, identification of the organism, the manufacturer, the responsible of commercialisation. Another law (43/2002; 20th of November 2002) covers the introduction of exotic organisms (article 44).

In **Switzerland** invertebrate BCAs must be formally approved by the BLW (Bundesamt für Landwirtschaft) and they are listed together with the plant protection products.

In the **UK** no authorisation is required to release indigenous beneficials but the import (and release) of non indigenous species must be approved by the Advisory Committee for the release of exotics (ACRE acting under DEFRA).

4.3. Regulatory aspects

4.3.1. Objectives

The objective of the work was to identify typical hurdles for the placing of biological plant protection products on the market experienced by biocontrol industry or evaluators in the recent past under the European directive 91/414/EEC. In parallel, we examined the new regulation (No 1107/2009/EC of the European Parliament and of the Council of 21 October 2009) concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC and the new directive (N° 2009/128/EC of the European Parliament and of the Council of 21 October 2009) establishing a framework for Community action to achieve the sustainable use of pesticides. These two texts[1] were examined for provisions creating new opportunities for the approval biocontrol agents, their placing on the market and use. *In fine,* it was the intent to establish a dialogue with EU regulators and evaluators in European institutions, i.e. in the European Commission and in the European Food Safety Agency (EFSA) and to seek solutions in common for the problems encountered.

4.3.2. Working method

An *ad hoc* group of representatives from biocontrol industry and INRA called "Regulatory Review Team" was set up. Two full-day working sessions were organised on 12th March in Paris and on 18th May in Basle in which regulatory experts identified difficulties and questions but also described positive experience and perspectives.

The work of the Regulatory Review Team active under RA4.3 was summarised and reported in a meeting of a delegation of ENDURE partners (IBMA, INRA and ACTA) with representatives of the

[1] The text is available form the Official Journal of the European Union L 309, Volume 52 of 24th November and can be downloaded from the EUR-Lex website: http://eur-lex.europa.eu/JOHtml.do?uri=OJ%3AL%3A2009%3A309%3ASOM%3AEN%3AHTML .

European Commission (DG SANCO, DG Agriculture, DG Research) and the EFSA on 24th September in Brussels.

4.3.3. Results

PowerPoint files of the presentations in the working sessions on general regulatory issues (U. Heilig/IBMA), micro-organisms (C. Alabouvette/INRA & chair of the CES on Micro-organisms giving opinion to Afssa-DiVE), Straight Chain Lepidopteran Pheromones (R. Sheppard/ IBMA) and Botanicals (N. Walther/IBMA) are available in the restricted access section of the ENDURE website.

The PowerPoint presentation under the title "*Gaps - Problems - Opportunities for BCAs in E.U. Regulation - From Past to Future*" prepared for the ENDURE – Commission meeting can also be accessed there. In this final document, two key issues related to directive 2001/36/EC annex II B fixing requirements for **microbial active substances** were highlighted. Tests suggested by evaluation experts and intended to establish the genetic stability of a strain do not reflect practical conditions, while in the case of potential microbial contaminants no European reference list is available. The incidence of many pathogens can be excluded by production methods or the geographic location of production sites. Tolerance limits for contamination levels could take into consideration thresholds used in food industry, application levels for the microbial product and naturally occurring background levels. The two issues presented here but also other examples put forward to the Regulatory Review Team lead to the statement that "*not all the studies or tests that can be performed for microbials will necessarily yield relevant data*".

The most important experience with semiochemicals was made during the on-going re-assessment of **Straight Chain Lepidopteran Pheromones** (SCLPs), which were supported by an IBMA Task Force. Regulators and evaluators were flexible in accepting a single common dossier for all compounds notified but although an OECD guidance document recommends data waiving for numerous SCLP requirements, the Rapporteur Member State insisted that all existing data and study reports on all compounds be submitted on the grounds that the requirements of the directive are superior to the guidance document recommendations. So far, the re-assessment procedure resulted in the inclusion with postponed peer review of SCLPs as a group, but 25 substances are also listed individually. New substances can be included in a simplified procedure provided that the applicant has access to the existing dossier. Remaining questions include what industry input will be required during the peer review by EFSA, the E.U. status of a revised OECD guidance document for semiochemicals other than SCLPs, the decision if MRLs are required for sprayable SCLP formulations, and equivalence criteria for SCLP substances. It was also noted that under the Biocidal Product Directive, rules and fees applied to SCLPs created an economic hurdle which resulted in the submission of a dossier for only one compound.

Extracts from plants - as long as not purified - consist of mixtures of molecules while data requirements of directive 91/414/EEC are basically designed for defined single substances. Thus those requirements often do not fit for mixtures of several substances. It must be decided if the most “active” substance, the one with the highest content in the extract or the whole extract shall be used in studies required for different sections of a dossier i.e. for data on physical-chemical properties, metabolism, toxicology, residues, environmental fate and behaviour, and which data shall be used in risk assessment. While the whole extract can be recommended for use in toxicity studies, it is not convenient for residue, metabolism or environmental studies because in practice it is generally not possible to determine the fate of all compounds contained in an extract. Questions asked by evaluators from several Member States after the issuing of a draft assessment report for Neem extract and its lead substance Azadirachtin A illustrate the difficulties experienced by an applicant in the evaluation process for a botanical.

The **new regulation** concerning the placing of plant protection products on the market provides for a specific status for "low risk active substances" (article 22). Many biocontrol substances can be expected to qualify for this new category but one exclusion criterion, the half-life in soil, may cause problems for microbial active substances unless it is clearly limited to chemicals. A full set of data is required to gain the status of low risk active substance but products containing them exclusively and without co-formulants of concern will benefit from reduced dossier requirements and time lines for approval. Micro-organisms, plant extracts or other natural substances may also meet the criteria for "Basic substances" provided for in article 23 but the discussion in the ENDURE-Commission meeting made it clear that this category is without interest for manufacturers who intend to market their substances for plant protection. It was noted that the new regulation does not provide for generic waivers i.e. for justifications of non submission of data or exemptions from requirements for groups of substances or products.

In the **framework directive** a number of provisions in favour of biological pest control measures or non-chemical methods have been identified. The new regulation also mentions in recital 35 that priority should be given to "non-chemical and natural alternatives wherever possible" but since the definition of non-chemical methods refers to "physical, mechanical or biological pest control" and does not specifically mention microbials, semiochemicals, botanicals or other natural substances with non-toxic mode of action it must be clarified how those groups are covered by the definition.

4.3.4. Conclusion

In the **meeting between the ENDURE delegation and representatives of the European Commission,** the need for discussions between regulators, evaluators and industry about requirements especially those relevant for microbial and botanical substances was recognised. Article 77 of the new plant protection product regulation authorises the Commission to "*adopt or amend technical and other guidance documents e.g. explanatory notes or guidance documents on the content of the application concerning micro-organisms, pheromones and biological products*." Thus at least part of the problems experienced by applicants can be addressed in guidance documents. Industry representatives and companies directly concerned by evaluations or reviews of biocontrol agents should enter into discussions with evaluators (EFSA or Competent Authorities in Member States) without forgetting the leading role of the Commission. Industry should fix priorities, prepare rationales and make substantiated proposals dealing with data requirements considered inappropriate, unnecessary or unrealistic.

4.4. Identified difficulties and conditions for success at field level

4.4.1. Technical aspects: factors of efficacy

4.4.1.1. Quality of the BCAs formulations

Numerous investigations on the development of biopesticides have been initiated as legislation and government policy have demanded less reliance on chemical pesticides and greater adoption of IPM. In Europe, some countries have set goals of reducing pesticide use by 50%. Successes have been achieved through better timing of applications, so that lower dosages are effective and substituting less hazardous and more active materials, to reduce the number of applications.
Biopesticides are distinguished from conventional chemical pesticides as many are very selective and are non-toxic towards non-target organisms. While biopesticides are likely to be less harmful to the environment than the conventional ones, care needs to be taken that wastage is minimised, by selecting the most appropriate droplet spectrum. A disadvantage of biological agents relative to chemicals, is that many are not sufficiently persistent and are relatively slow acting; therefore, research has been directed at extending the period of activity. However, some such agents may

persist in the field or the forest for many months, and a risk–benefit analysis should be performed to establish their environmental acceptability.
Transition from the optimised conditions of a laboratory experiment to the harsh conditions experienced in the field has so far proved more difficult for application of biopesticides in contrast to chemicals. This has undoubtedly been due to lack of investment in the development of effective formulations and delivery systems, in order to commercialise more potential biopesticides. The relatively small effort invested in target-specific sprayers, compared with the investment in laboratory studies, has led to unbalanced development, and exemplifies the need for closer integration between formulation and engineering research. The challenge is to get effective formulations so that biological control agents can be easily applied by farmers.

4.4.1.2. A good example, the case of **Trichoderma***: direct and indirect mode of action against plant pathogens*

Trichoderma species have long been recognized as biological control agents (BCAs) for the control of plant disease and for their ability to increase plant growth and development. They are widely used in agriculture, and some of the most useful strains demonstrate a property known as 'rhizosphere competence', the ability to colonize and grow in association with plant roots (Harman, 2000[1]). Much of the known biology and many of the uses of these fungi have been documented recently (Kubicek et al., 1998, Harman et al., 2004c, Perello et al., 2009). The taxonomy of this fungal genus is continually being revised, and many new species are being described (Komon-Zelazowska et al., 2007); (Samuels, 2006, Overton et al., 2006, Kubicek et al., 2008, Samuels and Ismaiel, 2009). The mechanisms that *Trichoderma* uses to antagonize phytopathogenic fungi include competition, colonization, antibiosis and direct mycoparasitism (Howell, 2003, Harman, 2006). This antagonistic potential serves as the basis for effective biological control applications of different *Trichoderma* strains as an alternative method to chemicals for the control of a wide spectrum of plant pathogens (Harman et al., 1991).
The colonization of the root system by rhizosphere competent strains of *Trichoderma* results in increased development of root and/or aerial systems and crop yields (Chacon et al., 2007); (Kubicek et al., 1998); (Yedidia et al., 2003). *Trichoderma* has also been described as being involved in other biological activities such as the induction of plant systemic resistance and antagonistic effects on plant pathogenic nematodes (Sharon et al., 2001, Jegathambigai et al., 2008).Some strains of *Trichoderma* have also been noted to be aggressive biodegraders in their saprophytic phases, in addition to acting as competitors to fungal pathogens, particularly when nutrients are a limiting factor in the environment (Worasatit et al., 1994). These facts strongly suggest that in the plant root environment *Trichoderma* actively interacts with the components in the soil community, the plant, bacteria, fungi, other organisms, such as nematodes or insects, that share the same ecological niche.
Trichoderma spp. are important participants in the nutrient cycle. They aid in the decomposition of organic matter and make available to the plant many elements normally inaccessible. Yedidia et al. (Yedidia et al., 2001) noted that the presence of the fungus increased the uptake and concentration of a variety of nutrients (copper, phosphorus, iron, manganese and sodium) in the roots of plants grown in hydroponic culture, even under axenic conditions. These increased concentrations indicated an improvement in plant active-uptake mechanisms. Corn that developed from seeds treated with *T. harzianum* strain T-22 produced higher yields, even when a fertilizer containing 40% less nitrogen was applied, than the plants developed from seed that was not treated with T-22 (Harman, 2000). This ability to enhance production with less nitrate fertilizer, provides the opportunity to potentially reduce nitrate pollution of ground and surface water, a serious adverse consequence of large-scale maize culture. In addition to effects on the increase of nutrient uptake and the efficiency of nitrogen use, the beneficial fungi can also solubilize various nutrients in the soil, that would be otherwise unavailable for uptake by the plant (Altomare et al., 1999b).

[1] The references cited in Chapter 4.4.1 are listed in paragraph 4.4.1.5

The cross-talk that occurs between the fungal BCA and the plant is important both for identification of each component to one another and for obtaining beneficial effects. Somehow, the plant is able to sense, possibly by detection of the released fungal compounds, that *Trichoderma* is not a hostile presence, therefore the plant defence system is not activated as it is when there is pest attack and the BCA is recognized as a plant symbiont rather than a plant pathogen (Woo and Lorito, 2006). Molecules produced by *Trichoderma* and/or its metabolic activity also have potential for promoting plant growth (Chacón et al., 2007); (Vinale et al., 2008a, Vinale et al., 2008b);(Yedidia et al., 1999). Applications of *T. harzianum* to seed or the plant resulted in improved germination, increased plant size, augmented leaf area and weight, greater yields (Altomare et al., 1999a, Harman et al., 2004a, Harman et al., 2004b, Inbar and Chet, 1995, Vinale et al., 2008b).
Numerous studies indicated that metabolic changes occur in the root during colonization by *Trichoderma* spp., such as the activation of pathogenesis-related proteins (PR-proteins), which induce in the plant an increased resistance to subsequent attack by numerous microbial pathogens (Table 22).

Table 22: Evidence for, and effectiveness of, induced resistance in plants by *Trichoderma* species (Harman et al., 2004a).

Species and strain	Plant	Pathogens	Evidence or effects	Time after application	Efficacy
T. virens G-6, G-6-5 and G-11	Cotton	*Rhizoctonia solani*	Protection of plants; induction of fungitoxic terpenoid phytoalexins	4 days	78% reduction in disease; ability to induce phytoalexins required for maximum biocontrol activity
T. harzianum T-39	Bean	*Colletotrichum lindemuthianum*; *Botrytis cinerea*	Protection of leaves when T-39 was present only on roots	10 days	42% reduction in lesion area; number of spreading lesions reduced
T. harzianum T-39	Tomato, pepper, tobacco, lettuce, bean	*B. cinerea*	Protection of leaves when T-39 was present only on roots	7 days	25–100% reduction in grey-mould symptoms
T. asperellum T-203	Cucumber	*Pseudomonas syringae* pv. lachrymans	Protection of leaves when T-203 was present only on roots; production of antifungal compounds in leaves	5 days	Up to 80% reduction in disease on leaves; 100-fold reduction in level of pathogenic bacterial cells in leaves
T. harzianum T-22; *T. atroviride* P1	Bean	*B. cinerea* and *Xanthomonas campestris* pv. phaseoli	Protection of leaves when T-22 or P1 was present only on roots; production of antifungal compounds in leaves	7–10 days	69% reduction in grey-mould (*B. cinerea*) symptoms with T22; lower level of control with P1. 54% reduction in bacterial disease symptoms.
T. harzianum T-1 & T22; *T. virens* T3	Cucumber	Green-mottle mosaic virus	Protection of leaves when *Trichoderma* strains were present only on roots	7 days	Disease-induced reduction in growth eliminated
T. harzianum T-22	Tomato	*Alternaria solani*	Protection of leaves when T-22 was present only on roots	3 months	Up to 80% reduction in early blight symptoms from natural field infection
T. harzianum T-22	Maize	*Colletotrichum graminicola*	Protection of leaves when *Trichoderma* strains were present only on roots	14 days	44% reduction of lesion size on wounded leaves; no disease on non-wounded leaves
Trichoderma GT3-2	Cucumber	*C. orbiculare*, *P. syringae* pv. lachrymans	Protection of leaves when *Trichoderma* strains were present only on roots; induction of lignification and superoxide generation	1 day	59% and 52% protection from disease caused by *C. orbiculare* or *P. syringae*, respectively
T. harzianum	Pepper	*Phytophthora capsici*	Protection of stems when *Trichoderma* strains were present only on roots; enhanced production of the phytoalexin capsidiol	9 days	~40% reduction in lesion length
T. harzianum NF-9	Rice	*Magnaporthe grisea*; *Xanthomonas oryzae* pv. oryzae	Protection of leaves when NF-9 was present only on roots	14 days	34–50% reduction in disease

The induction of systemic resistance (ISR) observed *in planta* determines an improved control of different classes of pathogens (mainly fungi and bacteria), which are spatially and temporally distant from the Trichoderma inoculation site. This phenomenon has been observed in many plant species,

both dicotyledons (tomato, pepper, tobacco, cotton, bean, cucumber) and monocotyledions (corn, rice). For example, *Trichoderma* induces resistance towards *Botrytis* cinerea in tomato, tobacco, lettuce, pepper and bean plants, with a symptom reduction ranging from 25 to 100%. Moreover, *Trichoderma* determined an overall increased production of defence-related plant enzymes, including various peroxidases, chitinases, β-1,3-glucanases, and the lipoxygenase-pathway hydroperoxide lyase (Harman et al., 2004a, Howell et al., 2000, Yedidia et al., 1999) of *T. harzianum* strain T-39, the active ingredient of the commercial product TricodexTM.
Thus far, *Trichoderma* is able not only to produce toxic compounds with a direct antimicrobial activity against pathogens, but also to generate fungal substances that are able to stimulate the plant to produce its own defence metabolites. In fact, the ability of *T. virens* to induce phytoalexin accumulation and localized resistance in cotton has already been discussed (Hanson and Howell, 2004). In cucumber, root colonization by strain T-203 of *T. asperellum* caused an increase in phenolic glucoside levels in the leaves; the aglycones, which are phenolic glucosides with the carbohydrate moieties removed, are strongly inhibitory to a range of bacteria and fungi (Yedidia et al., 2003).
A fundamental part of the *Trichoderma* antifungal capability consists in the production and secretion of a great variety of extracellular cell wall degrading enzymes (CWDEs), including endochitinases, β-N-acetylhexosaminidase (N-acetyl-β-D-glucosaminidase), chitin-1,4-β-chitobiosidases, proteases, endo- and exo-β-1,3-glucanases, endo β-1,6-glucanases, lipases, xylanases, mananases, pectinases, pectin lyases, amylases, phospholipases, RNAses, DNAses, etc. (Benítez et al., 1998; Lorito, 1998). The chitinolytic and glucanolytic enzymes are especially valuable for their CWDE activity on fungal plant pathogens, hydrolyzing polymers not present in plant tissues (Woo et al., 1999). Each of these classes of enzymes contains diverse sets of proteins with distinct enzymatic activities. Some have been purified, characterized and their encoding genes cloned (Ait-Lahsen et al., 2001, de la Cruz et al., 1992, de la Cruz et al., 1995a, de la Cruz et al., 1995b, Garcia et al., 1994, Limon et al., 1995, Lora et al., 1995, Lorito et al., 1994a, Lorito et al., 1993, Montero et al., 2007, Peterbauer et al., 1996, Suarez et al., 2004, Viterbo et al., 2001, Viterbo et al., 2002). Once purified, many *Trichoderma* enzymes have shown to have strong antifungal activity against a wide variety of phytopathogens, and they are capable of hydrolyzing not only the tender young hyphal tips of the target fungal host, but they are also able to degrade the hard, resistant conservation structures such as sclerozi.
Trichoderma spp. have been widely studied, and are presently marketed as biopesticides, biofertilizers and soil amendments, due to their ability to protect plants, enhance vegetative growth and contain pathogen populations under numerous agricultural conditions (Harman, 2000, Harman, 2004, Vinale et al., 2008a). The commercial success of products containing these fungal antagonists can be attributed to the large volume of viable propagules that can be produced rapidly and readily on numerous substrates at a low cost in diverse fermentation systems. The living microorganisms, conserved as spores, can be incorporated into various formulations, liquid, granules or powder etc., and stored for months without losing their efficacy (Jin et al., 1996). To date more than 50 different *Trichoderma*-based preparations are commercialized and used to protect or increase the productivity of numerous horticultural and ornamental crops (Table 23; Lorito et al. 2006).

Table 23: Trichoderma-based preparations commercialized for biological control of plant diseases.

Commercial Product	Biocontrol Organism(s)	Product Type	Formulation, Application	Uses - Location, Crops	Uses, Pathogens controlled	Manufacturer/Supplier, Country, Internet Reference
Ago Biocontrol *Trichoderma* 50	*T. harzianum*	Biological fungicide	n/a	Flowers, vegetables, fruits, other crops	*Fusarium*, *Rhizoctonia*, *Alternaria*, *Rosellinia*, *Botrytis*, *Sclerotium*, *Phytophthora* spp	Ago Biocontrol, Colombia (http://www.sipweb.org/directorymcp/fungi.html)
Antagon	*Trichoderma* spp.	Biological fungicide	powder	Horticulture (commercial), parks, recreational areas, sports fields	damping-off diseases	De Ceuster Meststoffen N.V. (DCM), Belgium (http://www.agroBiologicals.com/products/P1609.htm)
Binab T	*T. harzianum*, *T. polysporum*	Biological fungicide	Pellets, wettable powder or granules; spray, drench, mixed in soil	Wood products; ornamental, shade, forest trees; greenhouse, nursery, field; cut flowers, potted plants, vegetables, mushrooms, flower bulbs	Wood rots causing internal decay, or originating from pruning wounds; *Didymella*, *Chondrostereum*, *Heterobasidion*, *Botrytis*, *Verticillium*, *Pythium*, *Fusarium*, *Phytophthora*, *Rhizoctonia*	BINAB Bio-Innovation AB, Sweden (http://www.algonet.se/~binab/index2.html); Henry Doubleday Research Association, United Kingdom; Svenska Predator AB, Sweden; E.R. Butts International, Inc., USA
BioFit	*T. viride*	Biological fungicide	Seed treatment, root/tuber dip, drench; Used alone or in combination with chemicals.	Gram, pepper, groundnut, wheat, potato, ginger, turmeric, peas, matki, mung, urid , tomato, bhindi, onion, other vegetables, grapes.	*Pythium*, *Rhizoctonia*, *Fusarium*, *Sclerotium*, other root rots; for *Botrytis* in combination with chemicals	Ajay Bio-tech (India) Ltd., India (http://www.ajaybio.com)
Bio-Fungus (formerly Anti-Fungus), Supresivit	*Trichoderma* spp.	Biological fungicide	granular, wettable powder, sticks, crumbles; soil incorporation; spray or injection	Flowers, strawberries, trees, vegetables	*Sclerotinia*, *Phytophthora*, *Rhizoctonia solani*, *Pythium* spp., *Fusarium*, *Verticillium*	BioPlant, Denmark (www.bioplant.dk); De Ceuster Meststoffen N.V. (DCM), Belgium

Commercial Product	Biocontrol Organism(s)	Product Type	Formulation, Application	Uses - Location, Crops	Uses, Pathogens controlled	Manufacturer/Supplier, Country, Internet Reference
Combat	*T. harzianum, T. virens (=T. lignorum G. virens), Bacillus subtilis*	Biological fungicide	Talc; seed treatment, broadcast, root dip, drench, foliar spray	Grapes, cotton, pulses, tea, potato, tomato, oil seeds, tobacco, spices, cereals, vegetables, horticultural crops	Downy mildew, powdery mildew, die back, *Verticillium, Fusarium,* Panama wilt; pod, seedling, late blight; root, collar, stem, red, soft, clump, dry, bean, fruit, pod rot; black leg, damping off, abnormal leaf fall, black thread, canker	BioAg Corporation USA (http://www.bioag.com/products.html)
Harzian 20 (under development)	*T. harzianum*	Biological fungicide	n/a	orchard crops, vineyards	*Armillaria* spp., *Pythium* spp., *Sclerotinia* spp.	Natural Plant Protection (NPP), France (http://www.agroBiologicals.com/products/P1362.htm)
PlantShield	*T. harzianum*	Biological fungicide	Granules, wettable powder; soil drench, foliar spray	Greenhouse, flowers, ornamentals, herbs, nursery, vegetable crops; hydroponic, orchard trees	*Pythium, Fusarium, Rhizoctonia, Cylindrocladium, Thielaviopsis*; suppresses *Botrytis*	BioWorks, Inc., USA (http://www.bioworksbiocontrol.com)
Primastop	*G. catenulatum*	Biological fungicide	Powder; drench, spray, irrigation	ornamental, vegetable, tree crops	pathogens causing seed, root, stem rot, wilt disease	Kemira Agro Oy, Finland (http://growhow.kemira-agro.com); AgBio Development Inc.USA
Root Pro, RootProtato	*T. harzianum, T. cornedia*	Biological fungicide	Powder; spores mixed with growing media	Seedling, rooting stage in nursery; Horticulture - flowers, vegetables, potatoes	*Rhizoctonia solani, Pythium spp., Fusarium spp., Sclerotium rolfsii*	Mycontrol Ltd., Israel; Efal Agri, Israel (http://www.efal.com/main.htm, http://www.agroBiologicals.com/company/C1096.htm)

4.4.1.3. *The case* **Trichoderma***: mode of application, persistence on the target and new formulations.*

Effectiveness under controlled conditions (even under field conditions) does not necessarily guarantee that the organism will perform successfully; proper formulation is a prime condition for meeting market requirements. For instance an efficient biocontrol agent of soilborne and airborne pathogens must first and foremost protect the young seedling against detrimental attack by infective inoculum. Therefore some factors may be considered:

(a) soil ecosystem factors such as moisture, pH, structure, and temperature; (b) root colonization capacity;

(c) reasonable shelf life;

(d) efficiency of application of the biocontrol agent in terms of its specific habitat and target (Spiegel and Chet, 1998)

Many preparations have been developed to ensure a good shelf life of the product based on *Trichoderma*. Some of that formulation are stable in terms of pH, that remains constant and low (5.5) during the entire growth period, thus preventing bacterial contamination. Moreover the shelf life of the fungus at 25 °C is 1 year and from 1 to 2 years, the number of colonies-forming-units (CFUs) decreases by one order of magnitude. Many of that formulation have been proven successful in several experiments in the greenhouse and field. The rapid mass production of promising antagonists in the form of spores, mycelia or mixtures of both, has been achieved by liquid-fermentation technology: mass production of biomasses of *T. hamatum*, *T. harzianum*, and *T. viride* was reached by utilizing commercially available, inexpensive ingredients such as molasses, brewer's yeast, cotton seed flour, or corn-steeped liquor. 1984). Other techniques have been employed to improve the delivery of the biocontrol agents. A lignite-stillage (a by-product of sorghum fermentation) carrier system was tested for applying a *T. harzianum* preparation to the soil. Encapsulation of the biocontrol agent in an alginate-clay matrix, using Pyrax as the clay material, improved yield and propagule viability over time.

Pelletized formulations of wheat bran or kaolin clay in an alginate gel containing conidia, chlamydospores or fermentex biomass of several *Trichoderma* isolates revealed increased viability of stored pellets, and the number of CFUs formed after adding these pellets to the soil was comparable to that formed from freshly prepared pellets. These growth media and delivery systems for formulations of biocontrol fungi show promise because they are able to introduce high levels (10^{6}-10^{10} CFU/g) of fungi into soils not steamed, fumigated, or treated with other biocides.

To enhance biocontrol efficacy, appropriate introduction of the antagonist into the microenvironment appears to be crucial: formulations have been applied to seedlings prior to planting or to seeds in furrows. Economic considerations have forced biotechnologists to improve the application techniques: seed-coating, a technique involving minimal amounts of inoculum was developed.

Increased biocontrol activity may be achieved by combining two types (or more, if possible) of biocontrol agents, for example combining *Trichoderma* with a bacterium, or another beneficial fungus. The combined activity of the antifungal compounds produced by both microorganisms could expand the spectrum of pathogens controlled. In fact, in field trials combining *T. koningii* with certain fluorescent pseudomonads, greater suppression of take-all disease and increased wheat yield were achieved relative to plants treated with *T. koningii* alone (Duffy et al., 1996).

Delivery systems must ensure that biocontrol agents will grow well and achieve their purpose. It is generally recognized that delivery and application processes must be developed on a crop by crop and application by application basis. No general solutions exist, and so biocontrol systems must be developed for each crop. It is very important to use the organism properly and to have appropriate expectations. Any biocontrol organism will be unable to protect seeds as well as chemical fungicides. However, it colonizes roots, increases root mass and health, and consequently frequently provides yield increases, which chemical fungicides applied at reasonable rates cannot do. An effective method of use is to use the biocontrol fungus in conjunction with chemical fungicides. The chemicals provide good short-term seed protection, and the biocontrol fungus provides long-term root

protection. As a consequence, yields frequently are increased over use of the chemical alone.
Some experiences evidence that *Trichoderma* spp. is also highly effective when applied to blossoms or fruits for control of *B. cinerea.* Even low levels of the organism applied to strawberry blossoms by bee delivery or by sprays of liquid formulations are effective. For maximum control of the *Botrytis* bunch rot of grape, this initial application needs to be augmented by sprays as fruits mature, and addition of iprodione as a tank mix to this late application appears to have synergistic activity over either the biocontrol agent or the chemical fungicide alone.

- **Novel applications of *Trichoderma* spp.**

Trichoderma produces a variety of lytic enzymes that have a high diversity of structural and kinetic properties, thus increasing the probability of this fungus to counteract the inhibitory mechanisms used by neighbouring microorganisms. Further, Trichoderma hydrolytic enzymes have been demonstrated to be synergistic, showing an augmented antifungal activity when combined with themselves, other microbial enzymes, PR proteins of plants and some xenobiotic compounds (Fogliano et al., 2002, Lorito et al., 1994a, Lorito et al., 1996, Lorito et al., 1994b, Lorito et al., 1994c, Lorito et al., 1998, Schirmbock et al., 1994, Woo et al., 2002). In fact, the inhibitory effect of chemical fungicides for the control of the foliar pathogen *B. cinerea* was substantially improved by the addition of minute quantities (10-20 ppm) of *Trichoderma* CWDEs to the treatment mixture (Lorito et al., 1994b).
Extensive testing of *T. harzianum* strain T22 conducted for the registration of this biocontrol agent in the USA by the Environmental Protection Agency (EPA) has found that the CWDEs do not have a toxic effect on humans and animals (ED50 and LD50), and that they do not leave residues, but degrade innocuously in the environment. Therefore, these *Trichoderma* hydrolytic enzymes present a novel product for plant disease control based on natural mycoparasitic compounds used by the antagonistic fungi. Single or mixed combinations of CWDEs with elevated antifungal effects, obtained from fermentation in inducing conditions, over-expression of the encoding genes in strains of *Trichoderma*, or heterologous expression of the encoding genes in other microbes are possible alternatives for pathogen control. These natural substances originating from the BCA are an improvement over the use of the living microorganism in the production of commercial formulations because they are easily characterized, resist desiccation, are stable at temperatures up to 60° C, and are active over a wide range of pH and temperatures in the agricultural environment.

- **Some experiments conducted to evaluate concentration and stability assessment of a new liquid Trichoderma bio-formulate.**

In order to develop a marketable formulate, part of a culture broth obtained by fermentation was concentrated by using spray drying and lyophilisation techniques. Glycerol was added to the samples (20% v/v) to better preserve the spore vitality. Results showed in showed no significant differences in terms of chitinolytic activity before after treatments. Moreover, spore vitality was not significantly affected by the lyophilisation when glycerol was added; without glycerol, the spore concentration reduced from 7.0×10^6 to 1.8×10^6 spores/ml after treatment. Conversely, the sample treated by spray drying lost completely its activity and no enzymatic activity was registered at all. To assess the stability of the novel formulate, the decreases of spore vitality and enzymatic activities were monitored, as well as the effect of different stabilizing compounds (ampicillin, mineral oil, glycerol, PMSF). The results showed no considerable reduction of both spore vitality and chitinolytic activities at 45 and 110 d after fermentation. Moreover, the different stabilizing treatments did not differ with each other significantly (Ruocco personal communication).
The important factors to consider in a commercial bio-formulation are product stability, the capacity to produce consistent results by preserving the characteristics producing the biological effects; the storability of the material, the ability to be conserved in unspecialized

conditions similar to those of chemical pesticides; and a reasonable shelf-life or time that the product can be stored and used without compromising the efficacy (Agosin and Aguilera, 1998; Agosin et al., 1997; Powell and Jutsum, 1993). When a formulation contains the living microorganism component, the treatment must consist of stabilizing the viability of the BCA. For liquid formulations this can be achieved by maintaining the product in refrigeration (<10° C) or by freezing in the presence of cryoprotectant substances. However, conservation of a commercial product in these conditions is not economic for maintaining low temperatures or efficient because the liquid is both bulky and heavy, plus it is difficult to sustain these conditions in storage and transportation. In comparison, it is preferable to obtain formulations that contain a dehydrated product, stored as a powder, granule, talc, etc. Some works (Ruocco et al. unp) demonstrated that lyophilisation did not reduce chitinolytic activity and spore vitality when the fermented cultures were treated with compounds that protect the osmotic integrity of the living material such as glycerol. Generally, lyophilisation is the method that best maintains viability, but its cost is very high. At the industrial level and in order to obtain a low-cost product, the methods preferred is spray- or fluidized bed- drying. Many products are obtained by spray-drying, but this method produces a high loss of viability in some microorganisms (observed also in this formulation), due to the thermal treatment. Moreover, different compounds (ampicillin, mineral oil, glycerol, PMSF) were added to determine if they aided in to maintaining the stability of the formulation. The enzyme activity in samples assayed over time were not affected neither positively nor negatively by the addition of the compounds in comparison to the untreated control. Obviously, it is very important to maintain good sanitary conditions throughout the fermentation process and during packaging in order to avoid possible contamination that will compromise the product during storage.

In spite of the relatively abundant number of patents filed for microbial pesticides, the number of commercial applications has not been as dramatic as expected (Montesinos, 2003). In Europe, the limiting factor for registration, apart from the cost, is undoubtedly the slow process of decision-taking. As an example, the first application for patenting a biopesticide, Paecilomyces fumosoroseus, was submitted to the European Union in 1994 and approved only in 2001. In most cases, excessive specificity is a problem difficult to solve because it is intrinsic to the biological control system. In fact, success depends on three living systems: the pathogen or pest, the BCA and the host plant. Biosafety and environmental concerns are also major limiting factors for microbial pesticide prospects. Furthermore, the registration procedure to approve a biopesticide formulation on the market has not been altered to consider the biological aspects of the product, criteria which are different than those considered for the testing of chemical based products.

4.4.1.4. Persistence, physiological stresses, timing and coverage of others biological agents

Others references have been screened for biocontrol agents considering the analysis of:

- persistence on the target,
- resistance to physiological stresses,
- timing and coverage.

➢ <u>*Cladosporium cladosporioides*</u>

The antagonist has been effective in reducing sporulation of *Venturia inaequalis* under orchard conditions. Furthermore, the results of the pre-screening
indicate that it is cold and drought tolerant and results of experiments on spore production in solid state fermentation show that mass production is economically feasible. These results have been obtained in a stepwise selection approach (Kohl, 2009).

➢ <u>*Ulocladium atrum* and *Gliocladium roseum*</u>

Köhl et al., 1998 described the effect of treatments with conidial suspensions of *Ulocladium atrum* and *Gliocladium roseum* on leaf rot of cyclamen caused by *Botrytis cinerea* was investigated under commercial greenhouse conditions. Spraying *U. atrum* (1 × 106 conidia per ml) or *G. roseum* (2 × 106 conidia per ml and 1 × 107 conidia per ml) at intervals of 2 to 3 weeks during the production period and spraying U. atrum (1 × 106 conidia per ml) at intervals of 4 to 6 weeks resulted in a significant reduction of natural infections of petioles by *B. cinerea. U. atrum* or *G. roseum* (1 × 107 conidia per ml) was as effective as the standard fungicide program. B. cinerea colonized senesced leaves within the plant canopy and infected adjacent petioles and leaves later. The antagonists colonized senesced leaves and reduced *B. cinerea* development on these leaves. Thus, the inoculum potential on petioles adjacent to necrotic leaf tissues was reduced. The fate of *U. atrum* conidia on surfaces of green cyclamen leaves during a 70-day period after application was studied. The number of conidia per square centimetre of leaf surface remained relatively constant during the entire experiment. Sixty percent of the conidia sampled during the experiments retained the ability to germinate. When green leaves were removed from the plants to induce senescence and subsequently were incubated in a moist chamber, *U. atrum* colonized the dead leaves. Senesced leaves also were colonized by other naturally occurring fungi including *B. cinerea*. On leaves treated with *U. atrum* from all sampling dates, sporulation of *B. cinerea* was significantly less as compared with the untreated control. Our results indicate that early applications of *U. atrum* before canopy closure may be sufficient to achieve commercially satisfactory control of *Botrytis* leaf rot in cyclamen.

Kessel et al., 2005 developed a spatially explicit model describing saprophytic colonization of dead cyclamen leaf tissue by the plant-pathogenic fungus *Botrytis cinerea* and the saprophytic fungal antagonist *Ulocladium atrum*. Both fungi explore the leaf and utilize the resources it provides. Leaf tissue is represented by a two-dimensional grid of square grid cells. Fungal competition within grid cells is modelled using Lotka-Volterra equations. Spatial expansion into neighbouring grid cells is assumed proportional to the mycelial density gradient between donor and receptor cell. Established fungal biomass is immobile. Radial growth rates of *B. cinerea* and *U. atrum* in dead cyclamen leaf tissue were measured to determine parameters describing the spatial dynamics of the fungi. At temperatures from 5 to 25°C, B. cinerea colonies expanded twice as rapidly as U. atrum colonies. In practical biological control, the slower colonization of space by *U. atrum* thus needs to be compensated by a sufficiently dense and even distribution of conidia on the leaf. Simulation results confirm the importance of spatial expansion to the outcome of the competitive interaction between *B. cinerea* and *U. atrum* at leaf scale. A sensitivity analysis further emphasized the importance of a uniform high density cover of vital *U. atrum* conidia on target leaves.

4.4.1.5. References cited in Chapter 4.4.1

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4.4.2. Economic aspects: cost analysis

The industrial and commercial development of biologicals, although needed as an alternative to chemical pesticides in both organic farming and IPM systems is facing different constraints which are particularly difficult to overcome due to the size of the involved companies and the early development stage of the market.

These constrains can be classified within 4 categories:

- size of the targeted market
- cost of production
- costs of registration
- business profitability

In this paper, in order to be more specific, we shall consider the situation regarding microbial biocontrol agents (MBCAs), using the real case of a well defined product that we cannot mention here due to proprietary rights.

4.4.2.1. Size of the targeted markets

In most of the situations MBCAs are being developed with rather small, if not niche markets. The total value of MBCAs sold worldwide amounted in 2008 to 620 Mio Euro (122 Mio Euro in Europe) including products with insecticidal or fungicidal effects. This value can be compared with the sales of chemical insecticides and fungicides amounting to a total of 21 000 Mio Euros.
MBCAs, with the exception of Bt products which can be used in larger crops such as grapes, forestry or even cereals, are presently still used in speciality crops, greenhouses and covered crops.
The size of these crops is not growing anymore or at a very reduced rate. The only optimistic perspective is the intention to develop organic faster farming (objective 20% of the production area in France in 2030) where MBCAs can find a good market.

Additionally the potential market is widely fragmented within a long list of crops such as carrots, petersillium, onions, etc, usually referred to as “Minor crops”. These markets are so small that even large chemical companies refrain from the investments that would cover the needs and the manufacturers of MBCAs, due to the specificity of their products, are obliged to invest and cover costs where scale economy can never be reached.

4.4.2.2. Cost of production

Contrary to the synthesis of chemicals, producing MBCAs requires a complicated and extremely expensive process of production which can be divided into 4 phases:
- fermentation
- extraction
- purification
- formulation and packaging

All these phases are difficult and require relatively heavy costs.

a) fermentation
   This first step has to be undertaken either with solid or with liquid phase technology. Although the liquid phase fermentation is usually simple and cost effective, the process is more risky because the produced spores are more fragile. In the contrary using solid fermentation substrates will produce stronger, but it becomes more difficult to increase the production volume.

b) extraction
   Here again, there is a very strong difference between the MBCAs produced in liquid or in solid fermenters.
   In a liquid, the extraction will be rather easy by filtration, but the product will need to be dried, which is a very long, energy-demanding and expensive process.
   From a solid fermentation process, the extraction will be mechanical. Such a process is rather harmful for the spores: It is again energy demanding and it is extremely difficult to extract more that 60% of the spores from a substrate. In such a case the productivity becomes rather poor.

c) purification
   This step is very important to ensure the stability of the MBCAs produced. The industrially produced MBCAs always contain impurities which, although biologically inactive, may become critical over time, potentially creating risks of degradation, inactivation etc.
   In all situations the purification step requires a high level of sophistication and expensive processes.

d) Formulation and packaging
Formulation and packaging of MBCAs, due to their living state (and the requirement that they remain alive for satisfactory effectiveness of the product), constitute an extremely difficult step and in any case more expensive than the equivalent process for chemicals. The choice of co-formulants, adjuvants and packaging material must secure the quality of the MBCAs and its vitality. This is again a source of problems and heavy costs.

Additionally to all the above mentioned hurtles, it has to be secured that no contamination will occur, during the fermentation process naturally, but also during the extraction, the purification, the formulation and the packaging. All the safety measures are very expensive to carry out, but they are necessary in order to ensure the quality of the product brought to the market

As a consequence of all these extra expenses and technical difficulties the MBCAs used for this analysis were more than 4 times more expensive to produce than an equivalent chemical pesticide (Table 24).

Table 24: Compared structure of the production costs for a microbial biocontrol agent (MBCA) and a chemical insecticide (source IBMA).

	Typical Insecticide	MBCA	Comments
Sales value	**100**	**100**	
Type of production cost			
Raw materials	%* 8	29	40% lost material for MBCA by solid fermentation process
Packaging	1	2	
Energy and miscellaneous	1	2	
Manpower	5	9	
Consumables	2	3	
Amortisation	4	11	
TOTAL	**21**	**56**	

* costs are expressed as percent of the sales value of the commercial product

4.4.2.3. Cost of registration

It has been already mentioned that biological control agents suffer from a highly unfavourable situation compared to chemical pesticides. The regulations for registration have initially been set up to reduce the risks attached to molecules and the regulator is trying to extrapolate these requirements for the registration of living organisms.

The estimated cost for registering a microbial biocontrol agent is currently lower than that for a chemical pesticide (Table 25). However, the size of this investment is still very high for a company in comparison with the market potential (

Table 26).This evaluation indicates that the introduction on the market of a MBCA is about 4 times less effective than its chemical equivalent.

Table 25: Compared potential costs of registration for a microbial biocontrol agent (MBCA) and a chemical pesticide (source IBMA)

Area	Study Type	Cost for Chemical (€)	Cost for MBCA (€)
Toxicity of the active substance	Acute studies (6 tests)	140 000	140 000
	Sub-acute (rat study)	140 000	120 000
	Mutagenicity	40 000	may be waived
	Toxicity on cultured cells	10 000	not required
Toxicity of the formulation	Acute studies	140 000	140 000
	Toxicity on cultured cells	10 000	not required
Environmental fate	Soil, water, air	200 000	70 000
Biology	Mode of action etc	150 000	*50 000
Ecotoxicology of active substance	Birds, fish, bees, algae, daphnia, earthworm	60 000	40 000
	Beneficials	20 000	may be waived
Ecotoxicology of formulation	Birds, fish, bees, algae,daphnia, earthworm	60 000	40 000
	Beneficials	20 000	
Residues	8 trials / crop	80 000	may be waived
	Development of analytical methods	100 000	**variable
Formulation	Physical properties, shelf life, etc.	200 000	220 000
Efficacy	8 field trials	40 000	40 000
TOTAL		**1 410 000**	**860 000**

* cost of strain identification
** e.g. development of strain-specific markers

Table 26: Compared estimated market potential for a microbial biocontrol agent (MBCA) and for a chemical pesticide (source: IBMA)

Year	Estimated sales value (Mio€)	
	Chemical pesticide	MBCA
1	0.1	0.05
2	1.2	0.15
3	6.0	0.90
4	15.0	1.50
5	35.0	3.50
Total early sales	**57.3**	**6.10**
Plateau sales	120.0	15.00
Registration costs	1.410	0.860
Ratio registration/ early sales	**2.4 %**	**14.0 %**
Ratio registration/ Plateau sales	**1.1 %**	**5.7 %**

4.4.2.4. Business profitability

Comparing estimated production and other costs, relative to the sales value at plateau level, points out large differences between chemical pesticides and microbial biocontrol agents

(Table 27). The gap between the two in terms of estimated profit is nearly 10-fold in favour of the chemical industry.

Table 27: Compared margin structure estimates for the production and sales of a microbial biocontrol agent (MBCA) and a chemical pesticide (source IBMA)

%*	Chemical pesticide	MBCA
Sales value at plateau level	100	100
Costs of production	**21**	56
Gross margin	79	44
Cost of sales	21	15
Cost of research	8	12
Cost of administration	4	3
Earnings before investments taxes and amortisation (EBITA)	46	14
Profit after taxes, provisions and amortisation	18 ou 10?	2

* costs and margins are expressed as percent of the sales value of the commercial product

4.4.2.5. Conclusion and outlook for industry

These data show clearly that the profitability of a biocontrol business is much less attractive than that of chemical pesticides and may explain why the large chemical companies decided in the 90's to retreat from this business. Although these companies show presently some new signs of interest, they seem to remain basically reluctant to re-enter despite the new attractiveness of a fast growing biocontrol market. Contrary to European and US-based companies, several Japanese firms, such as Sumitomo chemicals or Mitsui appear to have invested for a potential long term return. Taking advantage of the divestment by the chemical majors, they have been able to acquire a good business basis at very attractive conditions. This should enable them to consider optimistically the future development of the biocontrol industry and its positive trend.

The smaller companies which have invested in this business and try to overcome their financial problems have only two alternatives:

- Either develop, often at lost, into larger markets (grapevine, field crops etc), if they can. In order to sustain these efforts, they will need a strong support from venture capital companies;
- or enter into venture agreements with other manufacturers/suppliers, in order to build up a product portfolio which will make them successful in the future.

4.4.3. Socio-economic aspects: market analysis and outlook

With estimated sales amounting to only 200 Mio€ in Europe in 2008, the market for biological control agents appears to be extremely small compared with the 7 000 Mio€ turnover achieved with chemical pesticides. However, very important efforts have been undertaken for the development of biocontrol agents. The OECD estimated that 5 000 Mio$ have been spent worldwide in public research for biocontrol during the last 40 years. This amounts to a yearly average of 500Mio$, not far from the 600 Mio$ spent yearly in research by the agrochemical industry, but with a comparatively poor result!

In the Conference on biological control organised in 2003 by IBMA in Béziers, France, the major stakeholders (farmers, retailers, distributors, regulators etc.) have provided a list of gaps considered to play a role in preventing wide adoption of biocontrol products. This list

was meant to cover all potential explanations, but provided neither figures nor priority ranking, making it difficult to prioritize actions for improvement. It was however a general opinion that the complicated and costly system of registration was the major reason of the problem. As a result, important efforts have been undertaken to convince the regulators to adopt more facilitating procedures for the registration of biologicals. These efforts were not without effect and the newly adopted "Pesticides package" makes it easier, under certain conditions, to register biologicals. In the meantime, several EU member states have adopted easier registrations tracks, such as the Biopesticides Scheme in the UK,.for example.
In reality, the unique assumption that the current regulations in Europe significantly hamper the development and the use of biologicals does not seem to be proven by the facts. During a very long period, the biologicals were not subject to registration and very few products were brought successfully to the market. At the same time countries such as the USA, New Zealand or Japan have adopted very liberal registration procedures, but the sales of biologicals remain marginal.

In the frame of ENDURE, it has been therefore decided to get a detailed and quantified idea on the gaps which, in Europe, restrain the adoption of biologicals, especially at the users and commercial levels. In order to achieve this objective, a Pan-Europa survey was undertaken from 2007 until 2008, with the assistance of the public opinion organisation Agridata.

4.4.3.1. Methodological approach: survey of European farmers

Since no validated data were available about the real market and the use of biological control agents in Europe, it has been necessary to build up a form of electronic map of the European agriculture and of the distribution of the potential users.

A survey was carried out to evaluate the size of the biocontrol market in Europe and to identify key factors that could influence its future evolution. This study included four main steps:

- Localisation of the main crops and cropping systems.
  Using the data from EUROSTAT and national statistics a model of European agriculture was constructed.
- Randomised sampling of farmers and retailers.
  The model was used for the selection of 12 production systems (

Table 28) located on 25 sites in 9 countries (Table 29) where 2000 farmers and 21 retailers were identified.

- The selected sample was contacted by phone directly and a questionnaire (Table 30) was sent to those who agreed to participate in the survey. A total of 675 full responses were obtained and analysed.
- Complementary survey.
  In order to validate the process, more specific data was collected in a survey concerning the biological control of wood diseases of grapevine in France

Table 28: Production systems selected for a survey of factors influencing biocontrol use in Europe (source IBMA)

Type of cropping system	Geographical sub-categories
Large arable crops	North, South
Multicropping • arable crops dominant • animal production dominant	 Mountains, North, South Mountains, North South
Fruit production • orchards • grapes	
Tomato production • protected • field	

Table 29: Geographical distribution of sampling sites for a survey of factors influencing biocontrol use in Europe (source IBMA)

Country	number of sampling site
Austria	2
Denmark	1
Germany	4
Greece	2
France	4
Italy	4
Poland	3
Spain	3
United Kingdom	2

Table 30: Structure of the questionnaire used in a survey of European farmers and retailers of biological control products

Categories of questions	Nbr of Questions
Geographical identification	5
System of production concerned	12
Ownership and social related aspects	5
Crop protection issues / pest occurrence, etc	18
Economy of the farm, actual costs, revenues etc	12
Expectations for future, cropping systems, investments, etc	9
Relations with input suppliers	18
Relations with advisors	18
Relations with authorities	18
Relation with the food chain (coops, supermarkets etc.)	18
Relations with the consumers	18
Relations with the public	18
Expectations about innovations, role of science	12
Open comments	2

4.4.3.2. Survey Results: The estimated market of biocontrol in Europe

The questionnaire made it possible to estimate the total biological market in ha and in value (Figure 22) and its partition among different crops (Figure 23).

These data confirm that in 2008, the main use of biologicals was in protected crops, followed by grapevine and fruit production. Nearly 40% of the estimated biocontrol market consisted in sales of beneficial insects, compared to 25% for microorganisms and 21% for semiochemicals (Figure 22).

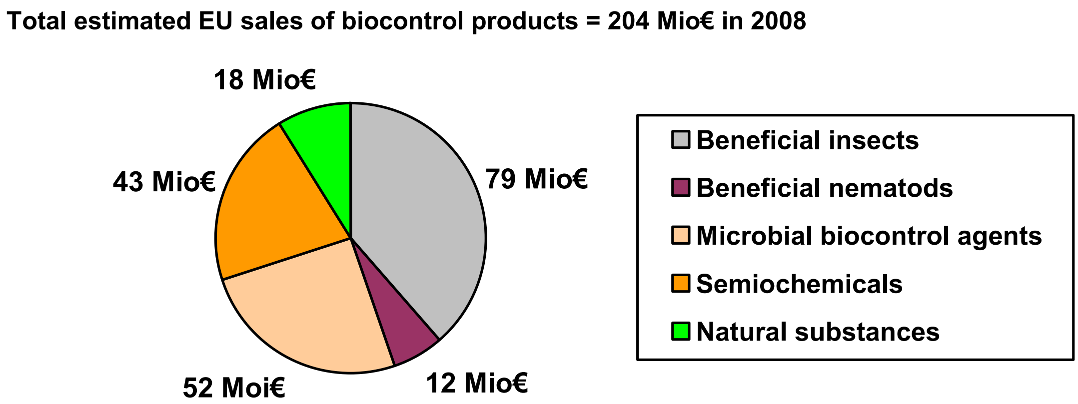

Figure 22: Estimated sales of biocontrol products in Europe in 2008 (in Million €). The estimates were obtained by extrapolating use patterns in a representative sample of EU farmers.

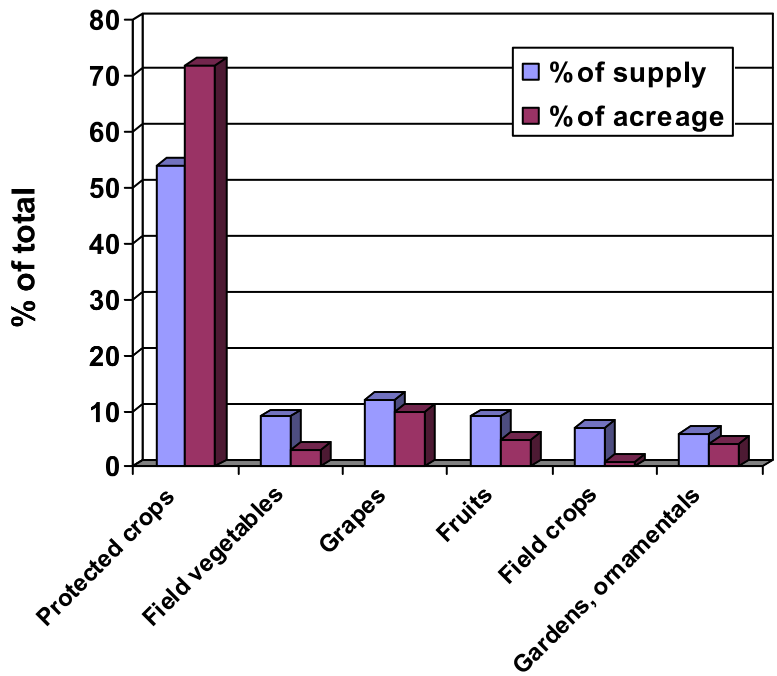

Figure 23: Estimated distribution of biocontrol use among types of crops in 2008 in Europe

4.4.3.3. Survey results: Factors of development of biocontrol

The exploitation of the questionnaires was somewhat difficult due to the large variety of farmers and situations. Additionally, several open ended questions were introduced to collect opinions on possible additional gaps and opportunities which were not mentioned in the form.

Qualitative analysis:

In a first step, the analysis of the responses led to the identification of 12 factors deemed to have a significant influence on the future development of biological control

9 factors with a positive influence:

- Ability of manufacturers to invest in R&D
- Financial strength of manufacturers
- Direct involvement of leading distributors
- Pull from the fresh food wholesalers and from the food industry
- Demand from consumers and NGOs
- Incentives given to growers
- Education of advisors and growers
- Availability of Decision Support Systems (DSS)
- Regulatory obstacles to chemical pesticides

3 factors with a negative influence:

- Regulations not adapted to Biological control
- Discovery of novel effective and safe chemicals
- Development of new resistant crops

Quantitative analysis

In a second step, a quantitative analysis was conducted to estimate the influence of the identified factors. For this, 320 contacts (50% of the sample) were requested to indicate which of the 12 factors they considered as important in terms of their potential impact on the evolution of future use of biological control agents. For those factors selected as important, the respondants were asked to weigh the expected impact positively or negatively on a scale from 0 to 20.

The data were used to compute for each of the 12 factors:

a) an **Influence Index**, calculated as the percentage of respondants who selected the factor as important
b) a **Weight Index**, calculated as the average of the weights attributed to the factor by those respondants who selected it as important
c) a **Growth Index**, combining the two other indices according to the following formula:

GI = (Influence Index)*(Weight Index)/10

This index represents the overall estimate of the influence of a factor on the future use of biological control agents by European farmers.

The scores computed for each of the 12 factors are presented in Table 31. Among the factors deemed to carry the most impact on future use of biological control by European farmers the action by far the most cited was the establishement of incentives for farmers (factor D).

Table 31: Impact of twelve factors on the future use of biocontrol agents by European farmers according to a survey of 320 farmers

Factors		Influence Index (%)*	Weight Index* (scale from -20 to +20)	**Growth Index***	Rank of positive influence
A	Registration for biological control products remains as present	12	**- 15**	**- 18.0**	
B	Involvement of distribution	65	8	**52.0**	4
C	Size / strength of the manufacturers	55	12	**66.0**	3
D	Incentives to growers	87	18	**156.6**	1
E	Education of advisors and growers	27	8	**21.6**	5
F	Decision Support Systems available	12	7	**7.2**	9
G	Pull from wholesalers and food industry	43	16	**66.8**	2
H	Stringent registration of chemicals	16	14	**22.4**	6
I	New safe chemical pesticides	42	**- 12**	**- 3.0**	
J	Progress in R&D of Biocontrol	8	14	**11.2**	8
K	New resistant varieties	16	**- 4**	**- 6.4**	
L	Pull from Consumers	67	2	**13,4**	7

* see main text above for the specific definition of the indices

The second most important factors based on the Growth Index (G, C and B in Table 31) were linked to the influence of key economic actors (the wholesalers, the food industry, the distributors and manufacturers of biocontrol products).

The factors with the lowest scores were those related to scientific innovation (factors K, I, J). Interestingly, both factors linked to regulatory aspects (factors H and A) also had a relatively low Growth Index. The registration requirements are obviously more a concern for the industry than for the users of the plant protection products.

Surprisingly, the efficacy and the price of the biologicals, usually considered as two critical factors, were not mentioned as real constraints. This may be due to two reasons:
(1) It is anticipated that only "effective" solutions will be registered in the EU, showing the high confidence of the farmers and the retailers in the registration systems
(2) The selling price of the new solutions (biological control products) will necessarily cope with the current price levels. Too highly priced, the new solutions will simply be ignored.

4.4.3.4. Conclusions

The gaps and the opportunities for the development of biological crop protection products are extremely relative to people concerned. While the industry, due to the heavy factor time/cost to the market, considers the regulation requirements as a major obstacle, the users and the retailers are much more influenced by the pull and push actions exercised at the market level. Somewhat disappointing is the relative low concern about the technical progress offered by the biological solutions.

5. Conclusions and perspectives for future R&D projects

5.1. Improving classical and augmentative biological control

The review of published scientific literature on the biological control of selected pests and diseases has lead to the identification of clear knowledge gaps highlighted in previous chapters. Further bottlenecks were revealed by seeking the possible reasons for the striking discrepancy between the rich inventory of potential biocontrol agents described by scientists and a very small number of commercial products on the market.

To complement these analyses, consultations of experts (scientists, extension specialists and representatives of the Biocontrol industry) were organised at the occasion of scientific meetings of three Working Groups of IOBC-wprs[1].

- Working Group "Integrated Control of Plant Pathogens": meeting on "Molecular Tools for Understanding and Improving Biocontrol" in Interlaken (Switzerland) September 9-12, 2008. (attended by P. Nicot and B. Blum – discussion session about the outlook on biocontrol against plant diseases)
- Working Group “Multitrophic Interactions in Soil” meeting in Uppsala (Sweden), 10-13 June 2009. (attended by C. Alabouvette and C. Steinberg – roundtable about the outlook on biocontrol of soilborne pests and diseases)
- Working Group "Insect Pathogens and Insect Parasitic Nematodes": meeting on "Future Research and Development in the Use of Microbial Agents and Nematodes for Biological Insect Control" in Pamplona (Spain), 22-25 June, 2009. (attended by C. Alabouvette – his plenary presentations about the outlook on biocontrol of diseases and pests is provided in Appendix 20)

These consultations were further complemented by discussions at the occasion of various meetings of RA 4.3 participating partners to identify the most prominent issues that could be tackled by future research and development activities. The key elements are organised below in three categories, based on their relevance to the concern of the research community, development or industry.

5.1.1. Key research issues

Five key issues have been identified in term of research needs:

- Devise better strategies for the screening of biocontrol agents. The demand for new biocontrol agents is already high. It is expected to increase sharply in the EU, with the ongoing reduction of available chemical pesticides and the need for new non-chemical plant protection tools to comply with Directive 2009/128/EC (see chapter 4.3.1). Current methods need to be improved both in terms of logistics (high throughput to allow rapid mass screening of large numbers of candidates) and in terms of the pertinence of criteria for efficacy, production and commercialization. This topic is currently being tackled within ENDURE RA 4.3 for microbial biocontrol agents against diseases and the results will be presented as Deliverable 4.9.
- Improve knowledge on efficacy-related issues. The criteria traditionally used to asses the efficacy of biological control methods may be misleading because contrarily to conventional pesticides, biocontrol does not intend to eradicate pests and diseases but, rather, to install a biological balance which will enable the plants to

[1] International Organisation for Biological and integrated Control of noxious animals and plants – West Palaearctic Regional Section

grow more healthily. However the consistency of field efficacy remains one of the constraints for the large scale use of biological control of plant diseases. Despite much recent progress, research efforts are still necessary for (1) a better understanding of key parameters of field efficacy in relation to the type of biocontrol agent and their modes of action and (2) implementing the most promising methods for efficacy improvement. Promising avenues of research are to be sought both in terms of exploiting the biological properties of the biocontrol agents and enhancing their effectiveness through formulation of the products. Results obtained on these topics should provide key information both for the design of optimised production and application strategies, but also for improving the screening process of future biocontrol agents as mentioned in the point above.

- Promote multidisciplinary approaches to integrate better biocontrol with IPM and other production issues. Based on passed published experience, it is clear that levels of protection provided by a single biocontrol agent alone will seldom be sufficient, especially when faced with field conditions unfavourable to their effectiveness or with very high inoculum pressures of a pest or plant pathogen. More emphasis will need to be placed on the compatibility of biocontrol agents with the implementation of IPM, preferably in a systemic approach of integrated production. Among the many possible interactions to be considered, compatibility and combined used of biocontrol and plants genetically modified for improved resistance to pest or plant diseases should not be overlooked.
- Develop adapted delivery technologies. Much progress has been made in packaging technology and delivery for macrobial biocontrol agents (e.g. beneficial arthropods). In contrast, treatments with microbial biocontrol agents (against pests or diseases) still rely on sprayers developed for the application of pesticides. Research is needed to provide growers with low pressure spraying equipment to preserve the viability of the microbials. Technological improvements are also needed for optimal coverage of the target plant surfaces to be protected by the biocontrol agents.
- Safeguard the durability of biocontrol. Certain pests and pathogens are known for their capacity to develop resistance to chemical pesticides or to overcome varietal resistance. The durability of biological control has often been assumed to be higher than that of chemical control, but several examples of resistance of pests have already been reported. Much less is known about plant pathogens, probably in part because biological control against diseases is still very rare. Significant research efforts are needed to anticipate the potential hurtles in this domain and integrate durability concerns both in the screening of new biocontrol agents and in the careful management of their use once they become commercially available.

5.1.2. Issues for development

Three key issues have been identified in terms of development. They are directly related to improving the efficacy of crop protection but also to acceptability of biocontrol by farmers.

- Training of advisers and farmers. Compared to chemical control, the implementation of biological control presents an additional level of technical complexity when the "active substance" is a living organism or microorganism, whose liveliness and development on the target crop underpins the effectiveness of the protection. In many situations, achievement of successful biocontrol of pests has been linked to an active role of advisers in accompanying the farmers, at least during their initial phase of adoption and implementation. The success of large scale use of biological control in the future will require stepping up the technical training of farmers and of advisors. Such action will also positively influence the adoption issues mentioned below.
- Development and dissemination of Decision Support Systems (DSS). Growers routinely make decisions that take into account multiple constraints (both technical

and economic) of their activity. However, the complexity of biocontrol and its necessary integration in a systems approach of crop protection and crop production make DSS more and more indispensible, including in their function as easily consultable repositories of knowledge on available choices.

- Establishment of demonstration schemes and development of farmers' networks. This action is needed to stimulate the dissemination of information to and among farmers, but also to facilitate exchange between the end users of biocontrol and the other actors of research, development and commercialization of the products. Breaking up regional and national barriers and including a European dimension to such networks is desirable for optimal efficacy of multisite experimental trials.

5.1.3. Industrial issues

- Quality control. Ongoing efforts by the manufacturers of biological control agents to guarantee the quality of their products need to be stepped up. The definition of tests and their routine implementation is crucial to ensure reliable effectiveness and maintain confidence of farmers for biocontrol. Whenever possible, such tests should include not only an evaluation of viability of the biocontrol agent but also an evaluation of physiological parameters related to its efficacy, based on knowledge of its modes of action.
- Improve distribution systems.

5.2. Conservation Biological Control

The meta-review of Conservation Biological Control (CBC) research on invertebrate pests has analysed 90 review papers published from 1989 to 2009 that address a very significant body of primary literature. We identified and analysed 221 reports of research into CBC in the review papers. Few of the review papers were published before 1998.

Europe has made strong contributions to both the primary literature and the review literature concerning CBC and, together with the USA, has a leading position in this field of research. Countries in the European Union were involved with 63% of all reports of CBC research analysed and half of the institutions contributing to authorship of the reviews were European. The main elements of research efforts in Europe were the same as those found in all the papers reviewed but with a greater emphasis on arable crops.

Arable cropping systems with annual or biennial crops dominated the reported research, as is appropriate to reflect the land area that they cover and their status as dietary staples. Field vegetables, maize, vines and orchards, all the subjects of ENDURE Case Studies, also were well represented. However, little CBC research on maize was reported in Europe and none on vines and there was only a single report of CBC research in glasshouse vegetables. This highlights a need for primary research on CBC in Europe to extend to the full range of significant crops, for all research results to be published and for more crops to be included in reviews.

Our analysis identified ten categories and 48 sub-categories of CBC practices or techniques and revealed a clear emphasis in the literature on research into the management of resources and refugia to support and promote natural enemies. In addition, investigations into how these might best be managed at a landscape scale comprised 19% of reports in the reviews. The great majority of CBC research that was reported was conducted at the field scale. No studies were exclusively laboratory-based. Modelling was used in 5% of the research reported and was a field in which European institutions were particularly well represented.

The most commonly cited target pests for CBC were Hemiptera (mostly aphids) and Lepidoptera, in keeping with their status as agricultural pests. Predators and parasitoids were the most commonly discussed natural enemies of Hemiptera and Lepidoptera, respectively.

The great majority of reports provided evidence for the degree of success of CBC techniques, especially in promoting natural enemies. The provision of refugia and resources and landscape management were not only the topics that received most attention but also the CBC techniques for which the benefits were best demonstrated. Evidence of success was particularly strong in field vegetables, vines and arable crops. Evidence of improved pest control was less often provided and usually weaker, indicating an important gap in research.

- Considerably less research has been directed towards CBC strategies that optimise the impact of naturally-occurring populations of weed natural enemies than has been done for invertebrate pests. The two groups of biological control agents that have received most attention in weed CBC research are deleterious rhizobacteria and granivorous carabids. The techniques with most potential for weed CBC appear to be the management of crop residues by conservation tillage and by manipulation of crop rotations, and management of habitats (refugia and resources) for invertebrates. Management of crop residues could be used for CBC strategies to encourage both strains of naturally-occurring deleterious rhizobacteria that are specifically harmful to weeds and granivorous carabids that prey on weed seeds. Weed seed predation can be limited by a shortage of suitable habitats and refugia for herbivores and seed predation is greater in complex landscapes than in simple ones.

Major needs for further research identified by the review literature:

- ‘Landscape-scale interactions’ was the subject most frequently identified as a priority for further research. Studies of the appropriate spatial scale for landscape management for CBC and studies of the movement of natural enemies within the landscape are needed.
- The comparative benefits of plants and habitats to natural enemies, their management and their role as sources or sinks for natural enemies, and their relative value to pests and to beneficial organisms were considered priorities for further study.
- Community ecology, autecology and behavioural ecology were identified as high priorities for further research, especially: the impact of natural enemy diversity, trophic interactions and community dynamics on CBC; the study of traits and population dynamics of natural enemies and their responses to habitats; the manipulation of natural enemy behaviour (e.g. by exploiting chemical ecology, push-pull, mixed cropping).
- The impact of increased biodiversity on CBC was frequently stated as a priority for future research and was the subject of only 4% of reports of past research.
- Further study of spatial and temporal factors affecting CBC was recommended by 43 and 13 of the 90 review papers, respectively. Large scale and long term studies are needed.
- Impact assessment: there is a need for more studies that assess the effect of CBC practices on pest control. Most reports of CBC included an assessment of the benefits to natural enemies but fewer than half also assessed the effect on pest control. This probably reflects the difficulties inherent in proving a link between natural enemy promotion and the depression of pest populations. The complexity of trophic relationships and the scale over which they operate has practical implications for the scale and complexity of experimental design. However, a proper assessment of the impact of CBC is needed. It should focus on testing the effectiveness of CBC in relation to pest control, reduction in pesticide use, improved crop yield and cost-

benefit analysis. The impact in CBC of different natural enemy species should also be assessed.
- Modelling was singled out by some of the reviews as a priority for further use in CBC research. Provided that suitable parameters are available or can be estimated and that predictions can be tested in existing landscapes, modelling could provide means to tackle questions where scale and complexity make manipulative field experimentation difficult.
- Several reviews advocated that non-arthropod natural enemies, particularly entomopathogens, were worthy of more study for CBC.
- More research effort should be applied to the integration of CBC into IPM.
- The socio-economic drivers of the uptake of CBC by farmers were mentioned as a priority for further research by a minority of these science-based reviews.

Research gaps on CBC of weeds include:
- In-depth research on the ecology of relationships between deleterious rhizobacteria and plants and on the mechanisms of action against weeds.
- Design of crop rotations to optimise the development of specific strains of deleterious rhizobacteria for weed suppression.
- A comprehensive study of the ecology of predation of weed seed by invertebrates and vertebrates and its impact on weed populations.
- Research on manipulation of the soil environment to encourage predators of weed seed, e.g. by conservation tillage systems.
- Demonstration of the extent that landscape diversification benefits carabid populations within cropped land and assessment of the impact on weed control.
- Rigorous evaluation of the effectiveness of weed biological control projects and the reasons for success or failure

Other challenges to the implementation of CBC discussed in the literature
Other challenges to implementation of CBC in agricultural practice that were cited included:
- A lack of interdisciplinary research was seen to hamper scientific advancement in support of CBC.
- High research and development costs, particularly in relation to large landscape-scale or long term studies.
- Insufficient knowledge transfer (including a shortage of taxonomic expertise for natural enemy identification).
- Farmer reluctance due to: risk perception, perception that CBC is complex compared to chemical control, transitional costs, cultural conservatism.
- The difficulty of designing policy to promote large-scale landscape changes that would be implemented by individual farmers.

6. Technical annexes

6.1. Appendix 1: Scoring of content of review papers, headings and term lists

ENDURE RA4.3, Term list for entry of data for CBC review.

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	B14	B15
Crop type	**Experimental system**	**Research includes modelling?**	**Country where expt done**	**Expt. done in Europe?**	**Practice and techniques group**	**Specific practice or technique**	**Pest species or group**	**Class of NE**	**NE species or group**	**Evidence for effect on abundance or fitness of NE**	**Evidence for effect on pest control**	**Effect on IGP**	**Research gaps identified**	**Challenges to implementation of CBC discussed**
• arable • field veg • gh veg • vines • orchard • maize • various • unspecified	• model only • lab - semifield • field • various • unspecified	• yes • no • unspecified	• name of country* • various • unspecified	• unspecified • EU • extra-EU • no • EU+	• limiting pesticide use	• decision support systems (thresholds) • temporal targetting • spatial targetting • pest resistant cv. & var. • GMO (pest resistant) • IPM • buffer zones • cv & var mixtures	• fill in according to the paper • unspecified	• pa • pr • epn • epf • various • unspecified	• name of sp. /group • various • unspecified	• unspecified • 1 • 2 • 3 • 4 • 5	• unspecified • 1 • 2 • 3 • 4 • 5	• unspecified • 1 • 2 • 3 • 4 • 5	• fill in according to the paper	• fill in according to the paper
			* political unit	EU+ denotes at least one EU country plus a non-EU country anywhere in the world extra-EU denotes a European country outside the EU	• Manipulation of behaviour	• trap crop • semiochemicals • push-pull		NB 'various' here means predators and parasitoids						
					• Habitat manipulation	• irrigation • cultural methods that increase humidity								
					• Optimising plant morphology	• hairiness • cuticular wax • plant architecture or canopy structure								
					• reduced disturbance	• reduced tillage • delayed harvest								
					• Provision of refugia / resources at concentrated locations	• hedge • grass sown weed strips • flower(s) sown strips • beetle bank • refuge crop strip • banker plant • game cover • perennial margin • alternative prey • conservation headlands • weed strips • sown weed strips • grassy margin • crop residue • artificial shelters • set-aside • field margins								
					• Provision of refugia / resources spread across the crop	• mulch • intercropping • weed management • ground cover management • cover crop • food sprays • soil surface architecture • manure • honeydew • pollen • nectar sources • alternative prey • undersowing • flower(s) sown strips								
					Landscape management	• diversification of landscape vegetation • refugia in landscape • crop diversification & rotation in landscape • movement facilitation, landscape • quantified discussion of landscape influences								
					• Increased ecosystem biodiversity	• unspecified • various								
					• Increased biodiversity of NE	• unspecified • various								

EU countries		Non-EU European countries	
Austria	Latvia	Albania	Monaco
Belgium	Lithuania	Armenia	Norway
Bulgaria	Luxembourg	Azerbaijan	Serbia (and Montenegro)
Cyprus	Malta	Belarus	Switzerland
Czech Republic	Netherlands	Bosnia (Herzegovina)	Turkey
Denmark	Poland	Croatia	Ukraine
Estonia	Portugal	Faroe Islands	Wales
Finland	Romania	Iceland	Yugoslavia
France	Slovakia	Liechtenstein	
Germany	Slovenia	Macedonia	
Greece	Spain		
Hungary	Sweden		
Ireland	UK		
Italy			

C16	C17	C18	C19	C20	C21
reference number	**Year published**	**First author**	**any authors based in Europe?**	**country or countries where authors' institution(s) are located**	**names of authors' institutions(s)**
		• name	• EU • extra-EU • no • unspecified (one answer per paper)	• country name • unspecified (one line per institution)	• institution name • unspecified (one line per institution)

Key

unspecified	not assessed or data not available
1	- - good evidence for a strong decrease of the function assessed
2	- some evidence for a decrease of the function assessed
3	0 no evidence for any effect on the function assessed
4	+ some evidence for an increase of the function assessed
5	+ + good evidence of a strong increase of the function assessed
pa	parasitoid
pr	predator
epn	entomopathogenic nematode
epf	entomopathogenic fungus

Explanations of terms in landscape group

landscape	spatial scales bigger than the single field
diversification of landscape vegetation	Complexity of landscape; scale of landscape fragmentation; provision of resources that the crop does not provide or does not provide for long enough, e.g. pollen and nectar
refugia in landscape	Overwintering sites, estivating sites, alternative host habitats for when the crop is not there or when hosts have been killed by pesticide
movement facilitation	Provision of linear habitats or arrangment of refuges across the landscape so that it is connected for the NE's
crop diversification & rotation in landscape	e.g. polycultures, monocultures, multi-crops, mixed-crops, not related to within-field intercropping

Additional terms permissable for all columns:

- various
- unspecified

6.2. Appendix 2: Bibliography of review papers analyzed for the meta-review on Conservation Biological Control

Aebi, A., Schonrogge, K., Melika, G., Quacchia, A., Alma, A., Stone, G.N.C.r.f.r., 2007. Native and introduced parasitoids attacking the invasive chestnut gall wasp Dryocosmus kuriphilus. Bulletin OEPP 37, 166-171.

Alebeek, F.v., Visser, A., Broek, R.v.d., 2007. Field margins as (winter) refuge for natural enemies. Entomologische Berichten 67, 223-225.

Altieri, M.A., Gurr, G.M., Wratten, S.D., 2004. Genetic engineering and ecological engineering: a clash of paradigms or scope for synergy? In: Gurr, G.M., Wratten, S.D.,Altieri, M.A. (Eds.), Ecological Engineering for Pest Management

CABI Publishing, Wallingford, UK, pp. 11-31.

Andow, D.A., 1991. Vegetational diversity and arthropod population response. Annual Review of Entomology 36, 561-586.

Bale, J.S., van Lenteren, J.C., Bigler, F., 2008. Biological control and sustainable food production. Philosophical Transactions of the Royal Society B-Biological Sciences 363, 761-776.

Barbosa, P., 1998. Agroecosystems and conservation biological control. In: Barbosa, P. (Ed.), Conservation Biological Control, Academic Press, pp. 39-54.

Barbosa, P., Benrey, B., 1998. The influence of plants on insect parasitoids: implications for conservation biological control. In: Barbosa, P. (Ed.), Conservation Biological Control, Academic Press, pp. 55-82.

Barbosa, P., Wratten, S.D., 1998. The influence of plants on invertebrate predators: implications for conservation biological control

In: Barbosa, P. (Ed.), Conservation Biological Control, Academic Press, pp. 83-100.

Benton, T.G., Vickery, J.A., Wilson, J.D., 2003. Farmland biodiversity: is habitat heterogeneity the key? Trends in Ecology & Evolution 18, 182-188.

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Cronin, J.T., Reeve, J.D., 2005. Host-parasitoid spatial ecology: a plea for a landscape-level synthesis. Proceedings of the Royal Society B-Biological Sciences 272, 2225-2235.

Cullen, R., Warner, K.D., Jonsson, M., Wratten, S.D., 2008. Economics and adoption of conservation biological control. Biological Control 45, 272-280.

Dix, M.E., Johnson, R.J., Harrell, M.O., Case, R.M., Wright, R.J., Hodges, L., Brandle, J.R., Schoeneberger, M.M., Sunderman, N.J., Fitzmaurice, R.L., Young, L.J., Hubbard, K.G., 1995. Influence of trees on abundance of natural enemies of insect pests: a review. Agroforestry Systems 29, 303-311.

Emden, H.F.v., 1990. Plant diversity and natural enemy efficiency in agroecosystems

In: Mackauer, M., Ehler, L.E.,Roland, J. (Eds.), Critical Issues in Biological Control, Intercept Ltd., Andover, UK, pp. 63-80.

Evans, E.W., 2008. Multitrophic interactions among plants, aphids, alternate prey and shared natural enemies - a review. European Journal of Entomology 105, 369-380.

Ferro, D.N., McNeil, J.N., 1998. Habitat enhancement and conservation of natural enemies of insects. In: Barbosa, P. (Ed.), Conservation Biological Control, Academic Press, pp. 123-132.

Ferron, P., Deguine, J.P., 2005. Crop protection, biological control, habitat management and integrated farming. A review. Agron. Sustain. Dev. 25, 17-24.

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Fuxa, J.R., 1998. Environmental manipulation for microbial control of insects. In: Barbosa, P. (Ed.), Conservation Biological Control, Academic Press, pp. 255-268.

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Gurr, G.M., Wratten, S.D., Barbosa, P., 2000. Success in conservation biological control of arthropods. In: Gurr, G.,Wratten, S. (Eds.), Biological Control: Measures of Success, Kluwer, Dordrecht, pp. 105-132.

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Holland, J.M., Oaten, H., Southway, S., Moreby, S.C.r.f.r., 2008. The effectiveness of field margin enhancement for cereal aphid control by different natural enemy guilds. Biological Control 47, 71-76.
Hoy, C.W., Feldman, J., Gould, F., Kennedy, G.G., Reed, G., Wyman, J.A., 1998. Naturally occurring biological controls in genetically engineered crops. In: Barbosa, P. (Ed.), Conservation Biological Control, Academic Press, pp. 185-205.
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6.3. Appendix 3. Synoptic list of natural enemy taxa mentioned in 221 reports of CBC research in review papers, arranged in taxonomic order

Natural enemy taxon	Natural enemy species or group	number of reports
Araneae	*Tyrophagus*	1
Araneae	mites	4
Araneae	phytoseid mites	2
Araneae	spiders	15
Coleoptera	*Bembidion sp*	2
Coleoptera	*Coleomegilla*	1
Coleoptera	*Hipodamia*	1
Coleoptera	carabids	24
Coleoptera	carabids, staphylinids	1
Coleoptera	coccinellids	6
Diptera	hoverflies	5
Hymenoptera	*Eriborus*	1
Hymenoptera	*Anagrus sp., Anagrus epos*	4
Hymenoptera	*Aphidius ervi*	2
Hymenoptera	*Dolichogenidea tasmanica*	1
Hymenoptera	ichneumonids	3
Hymenoptera	*Colpoclypeus*	1
Neuroptera	chrysopids	1
Ascomycota, Hypocreales	*Beauveria, Metarhizium*	1
Entomophthorales	*Entomopthora muscae*	1
Entomophthorales	*Pandora, Pandora neoaphidis*	3
Generalists		1
Various		91
Unspecified		49
All		221

6.4. Appendix 4. Relationship between pest taxon and the class of natural enemy addressed by the CBC research reported.

Taxonomic order of pest	Pest species or group referred to	Number of times different classes of NE referred to: Parasitoid	Predator	pathogenic nematode	Entomo-pathogenic fungi	various	un-specified	Total number of times reported
Acari	mites, spider mites		3					3
Coleoptera	Colorado potato beetle		1					1
Coleoptera	*Diabrotica undecimpunctata*		1					1
Coleoptera	leaf beetle	1						1
Coleoptera	pollen beetle	4						4
Diptera	*Delia antiqua, D. radicum*		1		1			2
Hemiptera	*Bemisia tabaci*					1		1
Hemiptera	*Rhopalosiphum padi*		1					1
Hemiptera	aphids	4	18		6	5	1	34
Hemiptera	grape leafhopper	2						2
Hemiptera	leafhopper	2						2
Hemiptera	pear psylla		1					1
Hymenoptera	chestnut gall wasp	1						1
Lepidoptera	armyworm	2						2
Lepidoptera	black cutworm		1					1
Lepidoptera	corn borer	2						2
Lepidoptera	corn rootworm		3					3
Lepidoptera	leafroller	1						1
Lepidoptera	lepidoptera				1			1
Lepidoptera	tortricids	3						3
	Various	12	11	1	3	38	1	66
	Unspecified	3	52	1	2	13	17	88
	All	37	93	2	13	57	19	221

6.5. Appendix 5. Reported influence of different CBC practice and techniques categories and sub-categories on abundance or fitness of natural enemies and on pest control

CBC practice and techniques group	specific CBC practice or technique	Number of times review papers referred to different practices and reported the effect on natural enemies or pests												Total number of times practice referred to
		evidence for effect on abundance or fitness of NE						evidence for effect on pest control						
		good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	un-specified	good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	un-specified	
Limiting pesticide use														
	GMO (pest resistant)		1	4	1							6		6
	IPM					1							1	1
	buffer zones				1								1	1
	pest resistant cv. & var.		1	1							1		1	2
	spatial targeting				1		1						2	2
	All		2	5	3	1	1				1	6	5	12
Manipulation of behaviour														
	push-pull				2								2	2
	semiochemicals				2						1		1	2
	unspecified						1						1	1
	All				4		1				1		4	5
Habitat manipulation														
	cultural methods that increase humidity				1						1			1
	irrigation					1						1		1
	various				2		1			1			2	3
	All				3	1	1			1	1	1	2	5
Plant morphology														
	hairiness				1								1	1
	cuticular wax				1						1			1
	plant architecture or canopy structure				2						1		1	2
	All				4						2		2	4

Appendix 5 continued.

CBC practice and techniques group	specific CBC practice or technique	Number of times review papers referred to different practices and reported the effect on natural enemies or pests												total number of times practice referred to
		evidence for effect on abundance or fitness of NE						evidence for effect on pest control						
		good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	unspecified	good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	unspecified	
Reduced disturbance														
	reduced tillage				9	4				1	3		9	13
	All				9	4				1	3		9	13
Provision of refugia / resources at concentrated locations														
	alternative prey					4					3		1	4
	artificial shelters			1									1	1
	banker plant						1						1	1
	beetle bank				1	5				1	2		3	6
	conservation headlands				2	2				1	1		2	4
	crop residue				1								1	1
	field margins				1								1	1
	flower(s) sown strips				10	7				1	10	1	5	17
	grass sown weed strips					2							2	2
	grassy margin					2							2	2
	hedge				1	2	1						4	4
	perennial margin					1					1			1
	refuge crop strips				1								1	1
	set-aside				1								1	1
	sown weed strips				3								3	3
	weed strips				2	2				1	1		2	4
	various				6	9					7	2	6	15
	unspecified				1		1				1		1	2
	All			1	30	36	3			4	26	3	37	70

Appendix 5 continued.

CBC practice and techniques group	specific CBC practice or technique	Number of times review papers referred to different practices and reported the effect on natural enemies or pests												total number of times practice referred to
		evidence for effect on abundance or fitness of NE						evidence for effect on pest control						
		good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	unspecified	good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	unspecified	
Provision of refugia / resources spread across crop														
	mulch				2	4	1				4		3	7
	nectar sources				1	1				1		1		2
	pollen				1								1	1
	alternative prey					2				1	1			2
	cover crop				2							1		1
	flower(s) sown strips				2	1						2	1	3
	food sprays				2	1					2		1	3
	ground cover management				10		3				2		1	5
	honeydew													2
	intercropping			1	3	1				2		1	2	5
	manure				2					1	1			2
	soil surface architecture					1							1	1
	undersowing				2	1					1	1	1	3
	weed management				4	6					1	2	7	10
	unspecified						1						1	1
	All			1	28	21	2			7	11	8	26	52
Landscape management														
	crop diversification & rotation in landscape					2					1	1		2
	diversification of landscape vegetation				10	7	2			2	9		8	19
	movement facilitation landscape				3	3				1	1	1	3	6
	quantified discussion				1		1						2	2
	refugia in landscape				9	1	1				2		9	11
	various					1							1	1
	unspecified						1						1	1
	All				23	14	5			3	13	2	24	42
Increased ecosystem biodiversity														
	various				1	2					3			3
	unspecified				1	1				1	1			2
	All				2	3				1	4			5

Appendix 5 continued.

CBC practice and techniques group	specific practice technique	CBC or	Number of times review papers referred to different practices and reported the effect on natural enemies or pests												total number of times practice referred to
			evidence for effect on abundance or fitness of NE						evidence for effect on pest control						
			good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	unspecified	good evidence for a strong decrease	some evidence for a decrease	no consistent evidence for an increase or decrease	some evidence for an increase	good evidence for a strong increase	unspecified	
Increased biodiversity of NE															
	various				1								1		1
	unspecified				1			2			2			1	3
	All				2			2			2		1	1	4
Various	alternative prey					1								1	1
	various					2		1				1		2	3
	All					3		1				1		3	4
Unspecified					1	1		3			1			4	5
All CBC practices				2	10	110	80	19			20	63	21	117	221

6.6. Appendix 6. Representation of European countries and institutions in the authorship of the reviews that were the source literature for this meta-review

Country where institutions were located	Number of institutions	Names of institutions	Number of times institutions represented in authorship
Austria	2	Federal Office and Research Centre of Agriculture, Vienna	1
		Ludwig Boltzmann-Institute for Biological Agriculture and Applied Ecology, Vienna	1
Belgium	1	Université Catholique de Louvain	1
Denmark	1	University of Copenhagen	2
Finland	1	University of Helsinki	1
France	2	CIRAD Montpellier	1
		INRA Montpellier	1
Germany	2	Federal Biological Research Centre of Agriculture and Forestry (BBA), Kleinmachnow	1
		Georg-August University, Göttingen	5
Hungary	2	Plant Protection Institute, Hungarian Academy of Sciences, Budapest	1
		Vas County Plant Protection and Soil Conservation Service, Tanakajd	1
Italy	2	Turin University	1
Netherlands	5	Alterra, Wageningen University and Research Centre	2
		Applied Plant Research (PPO), Wageningen University and Research Centre	1
		Netherlands Institute of Ecology (NIOO - KNAW)	1
		Plant Research International, Wageningen University and Research Centre	1
		Wageningen University	5
Spain	1	University of Castilla-La Mancha	1
Sweden	1	Swedish University of Agricultural Sciences, Uppsala	2
Switzerland	5	Agroscope FAL Reckenholz	2
		CABI Bioscience, Delémont	1
		FiBL (Research Institute of Organic Agriculture)	2
		University of Berne	1
		University of Zurich	1
UK	24	ADAS UK Ltd., Wolverhampton	1
		British Trust for Ornithology, Thetford	1
		Cardiff University	4
		Centre for Ecology and Hydrology, Dorchester	1
		Edinburgh University	1
		Horticulture Research International, Wellesbourne	3
		IACR Long Ashton Research Station, Bristol	1
		Lancaster University	1
		Marshall Agroecology Limited	1
		NERC Centre for Ecology and Hydrology, Monks Wood	1
		NERC Centre for Population Biology, Imperial College London, Silwood Park	1
		Rothamsted Research, Harpenden	5
		Royal Society for the Protection of Birds, Edinburgh	1
		The Game Conservancy Trust, Fordingbridge	5
		The University of Kent, Wye Campus	1
		University of Stirling	1
		University of Birmingham	1
		University of Bristol	1
		University of East Anglia	1
		University of Newcastle upon Times	1
		University of Oxford	1
		University of Plymouth	1
		University of Reading	2
		University of Sussex	1
All			75

6.7. Appendix 7. Representation of non-European countries and institutions in the authorship of the reviews that were the source literature for this meta-review

Country where institutions were located	Number of institutions	Names of institutions	Total number of times institutions represented in authorship
Australia	5	CSIRO Entomology, Queensland	2
		Charles Sturt University, Orange, New South Wales	5
		La Trobe University, Melbourne	1
		University of Queensland	1
		University of Sydney	6
Canada	1	Laval University, Quebec	1
Indonesia	1	International Centre for Research in Agroforestry, Bogor	1
Israel	1	Hebrew University of Jerusalem	1
Japan	1	Institute of Biological Control	1
Kenya	2	ICIPE (International Centre of Insect Physiology and Ecology)	2
		Tropical Soil Biology and Fertility Institute of CIAT, Nairobi	1
México	1	Universidad Nacional Autónoma de México	1
New Zealand	1	Lincoln University, Canterbury	18
USA	37	Clemson University	1
		Cornell University, Ithaca, New York	1
		Iowa State University	1
		Lousiana State University	2
		Miami University, Oxford, Ohio	1
		Michigan State University	7
		Montana State University	2
		Nature Mark, Boise, Idaho	1
		New York State Agricultural Experimental Station	1
		North Carolina State University	3
		Ohio State University	3
		Oregon State University	2
		Pennsylvania State University	1
		Rutgers University, New Brunswick	1
		Santa Clara University, CA	1
		South Central Research and Extension Center, Clay Center; Nebraska	1
		Southern Illinois University	1
		USDA Appalachian Fruit Research Station	1
		USDA Forest Service, Center for Semiarid Forestry	1
		USDA Plant Science and Water Conservation Research Laboratory, Stillwater	1
		USDA Southeastern Fruit and Tree Nut Research Laboratory, Georgia	1
		USDA Western Cotton Research Laboratory, Phoenix, Arizona	2
		University of Arkansas	1
		University of California, Berkeley	5
		University of California, Davis	2
		University of California, Santa Cruz	1
		University of Florida	2
		University of Idaho	1
		University of Maryland	5
		University of Massachusetts	2
		University of Minnesota	6
		University of Missouri-Columbia	1
		University of Nebraska, Lincoln	2
		University of Tennessee	1
		University of Wisconsin	2
		Utah State University	1
		Washington State University	3
All			112

6.8. Appendix 8. Categorization of research gaps identified by authors of review papers

Research gap category	Gap sub-category: topic that requires more study or technique that needs further exploitation (long description)	Short description of gap sub-category
Behavioural ecology	Manipulation of NE behaviour: chemical ecology, push-pull, attract and reward, mixed cropping	Manipulation of NE behaviour
	Effect of induced plant defences on NE's (induced systemic resistance [ISR] or systemic acquired resistance [SAR])	Effect of induced plant defences on NE's
	Tritrophic interactions: the role of the host plant in mediating NE-pest interactions, including effects of plant semiochemicals and plant structure	Tritrophic interactions
Autecology	Autecology and traits of NE's, including population dynamics and responses to habitats	Autecology and traits of NE's
	Relationship between NE abundance and/or fitness and BC	Impact of NE abundance/fitness on BC
	Population genetics, gene flow, population structure	Population genetics, gene flow, population structure
Community ecology	Relationships between NE diversity, niche complementarity, IGP, competition and BC (community dynamics, food webs).	Impact of NE diversity on BC
	Relationship between biodiversity, ecosystem functioning, natural enemy activity and pest control	Effect of ecosystem biodiversity on CBC
	Effect of IGP on CBC	Effect of IGP on CBC
	Spatial and temporal relationships between predators and prey, food webs	Spatial & temporal relations between pests and NE's
	Effect of temporal scale on processes influencing CBC	Effect of temporal scale on processes influencing CBC
Influence of plant characters	Characterising habitats or plant species that encourage NE's: sources or sinks; movement to and from them; relative benefits to pests and NE's.	Habitats to encourage NE's
	Effects on CBC of plant resistance to insect pests, breeding for plant resistance	Plant resistance to insect pests
	Assessment of potential risks and benefits of transgenic crops and associated husbandry to CBC and IPM	Risks and benefits of transgenic crops to CBC
Management of resources or refugia	Resource provision for NE's: banker plants, food sprays, nectar and pollen sources, alternative prey	Resource provision for NE's
	Refuge provision for NE's: ground cover, field margins etc	Refuge provision for NE's
	Effect of reduced habitat disturbance on CBC (e.g. non-inversion tillage)	Effect of reduced disturbance

Appendix 8 cont'd.

Research gap category	Gap sub-category: topic that requires more study or technique that needs further exploitation (long description)	Short description of gap sub-category
Landscape scale interactions	Studies of the appropriate scale or spatial arrangement of crops, habitats or landscape management to optimise natural enemy activity	Spatial scale of landscape management for CBC
	Effect of spatial scale on processes influencing CBC	Effect of spatial scale on processes influencing CBC
	Effect of landscape on processes influencing CBC	Effect of landscape on processes influencing CBC
	Effects of connectivity of non-crop habitats on NE abundance and diversity	Effects of connectivity of non-crop habitats
	NE movement, dispersal dynamics, host-finding, foraging strategies	NE movement, dispersal, host-finding, foraging
Non-arthropod natural enemies	Entomopathogenic fungi, population dynamics, distribution and dispersal	Entomopathogenic fungi population dynamics
	Entomopathogenic nematodes	Entomopathogenic nematodes
Impact assessment	Demonstration of the effect of CBC on pest populations, pesticide use, crop damage, yield, financial profit (cost-benefit analysis)	Analysis of effectiveness of CBC
	Assessment of impact of individual NE species in biological control	Assessment of impact of individual NE species
Socio-economics	Socio-economic drivers of farmer behaviour and their influence on the up-take of CBC	Drivers of farmer behaviour & up-take of CBC
IPM and precision farming	Precision farming, GIS, GPS, remote sensing, spatial pesticide targeting, pesticide application technology	Precision farming
	Incorporation of NE's into DSS's	Incorporation of NE's in to decision support systems
	Integrating CBC with other elements of IPM	Integrating CBC with other elements of IPM
Approaches and techniques	Modelling approaches	Modelling
	Multi-trophic approach	Multi-trophic approach
	Development of molecular techniques for identifying NE's	Molecular techniques to identify NE's
	Marking techniques for NE's	Marking techniques for NE's
	Long term studies (several years)	Long term studies (several years)
	Food webs	Food webs

Abbreviations used in Appendix 8: CBC: conservation biological control; BC: biological control; NE: natural enemy; IGP: intra-guild predation; IPM: integrated pest management; GIS: geographic information system; GPS: global positioning system; DSS: decision support system

6.9. Appendix 9. Categorization of challenges to implementation of CBC identified by authors of review papers

Challenge category	Challenge sub-category: factors that impede progress toward the implementation of CBC (long description)	Short description of challenge sub-category
Scientific practice	Division of ecological, agronomic and socio-economic research amongst different disciplines and sub-disciplines hampers scientific advancement	Lack of interdisciplinary research
R&D costs	Experiments needed for CBC development may be costly, especially if they address landscape scales and long time periods	Cost of large scale CBC experiments
	Costs associated with registration of semiochemicals for field use are very high	Cost of registration of semiochemicals
	The difficulty in commodifying CBC makes it difficult to recoup costs of R&D and inhibits research investment by commercial companies	Lack of financial return for R&D
Knowledge transfer	Better knowledge transfer methods are needed to enable extension services to communicate CBC methods and skills	Better knowledge transfer methods
	Local ecological variation influences the success of CBC techniques and the knowledge necessary to support them	Local ecological variation
	Lack of taxonomic expertise to identify pests and NE's and lack of accessible yet authoritative identification guides or techniques	Lack of taxonomic expertise
Socio-economic	Perception of risk associated with CBC, lack of consistent evidence for success of CBC	Perceived risk relating to CBC
	Perceived complexity of implementation of CBC in comparison to conventional chemical-based control	Perceived complexity of CBC
	Cultural impediments to change in agricultural practice	Cultural impediments to change
	Cost of implementing CBC measures, including transitional costs	Cost of implementing CBC
Policy	Design of policy instruments promoting large-scale landscape changes to support CBC but depending upon actions on individual farms	Landscape-scale implementation of CBC
	Complexity of agri-environment schemes and their multiple functions	Complexity of agri-environment schemes
	Increasing both crop production and habitat diversity for CBC	Achieving productivity with biodiversity

Abbreviations used in Appendix 9	CBC: conservation biological control	NE: natural enemy	R&D: research and development

6.10. Appendix 10. Inventory of biocontrol agents (M: microbials; B: botanicals; O: others) described in primary literature (1998-2008) for successful effect against Botrytis sp. in laboratory experiments and field trials with selected crops

Tomato + Cucumber + Pepper (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** Bacillus amyloliquefaciens BL3, pepper (Park et al., 1999) Bacillus licheniformis > FG (Lee et al., 2006) Bacillus subtilis strain QST 713 (Serenade ASO) (Ingram and Meister, 2006), Quadra 136, preventive (Utkhede and Mathur, 2006) Brevibacillus brevis (Seddon et al., 2000) (McHugh et al., 2002) (Schmitt et al., 2001) Brevibacillus brevis WT + Milsana / cucumber (Konstantinidou-Doltsinis et al., 2002) Paenibacillus polymyxa BL4, pepper (Park et al., 1999) Pseudomonas putida Cha94, pepper (Park et al., 1999) Streptomyces (Mycostop(R), (Lahdenpera and Korteniemi, 2008)actinomyces (Yao et al., 2007), strains III-61 and A-21 (Pan et al., 2005) Bakflor (consortium of valuable bacterial physiological groups) (Kornilov et al., 2007) **Fungi + yeasts:** Clonostachys rosea (ADJ 710 OMRI), (Shipp et al., 2008) Gliocladium sp. (Georgieva, 2004) Gliocladium catenulatum Prestop(R), preventive (Utkhede and Mathur, 2006) (Utkhede and Mathur, 2002) (Lahdenpera and Korteniemi, 2008) Gliocladium viride (Lisboa et al., 2007) Microdochium dimerum (Nicot et al., 2003) (Trottin-Caudal et al., 2001) Rhodosporidium diobovatum S33 preventive (Utkhede and Mathur, 2006) curative (Utkhede and Mathur, 2002), /cucumber (Utkhede and Bogdanoff, 2003) Trichoderma sp. (Georgieva, 2004) Trichoderma harzianum (Lisboa et al., 2007), T39 (Trichodex) tomato (Apablaza and Jalil R, 1998) (Moreno Velandia et al., 2007), tomato + cucumber (Elad, 2000b) (Dik and Wubben, 2001) / cucumber (Elad, 2000a), TM / pepper (Park et al., 1999), RootShield curative (Utkhede and Mathur, 2002), T22 PlantShield(R) curative (Utkhede and Mathur, 2006)	**Bacteria** Bacillus antagonists (Tsomlexoglou et al., 2000) (Enya et al., 2007) (Tsomlexoglou et al., 2001) (Tsomlexoglou et al., 2002) Bacillus circulans (Wang et al., 2008b) Bacillus subtilis (Wang et al., 2008b) (Sadfi-Zouaoui et al., 2007a) (Gu et al., 2008) (Sadfi-Zouaoui et al., 2007b) Bacillus licheniformis (Lee et al., 2006) (Sadfi-Zouaoui et al., 2007a) Brevibacillus brevis (White et al., 2001) (Seddon and Schmitt, 1999) (Seddon et al., 2000) (Allan et al., 2003) Cupriavidus campinensis / cuc, tom (Schoonbeek et al., 2007) Halomonas subglaciescola, Halobacillus litoralis, Marinococcus halophilus, Salinococcus roseus, Halovibrio variabilis, Halobacillus halophilus, Halobacillus trueperi (Sadfi-Zouaoui et al., 2008) Halomonas sp. K2-5 (Sadfi-Zouaoui et al., 2007b) Micromonospora coerulea (Kim et al., 1999) Pantoea (Enya et al., 2007) Pseudomonas aeruginosa (Hernandez-Rodriguez et al., 2004) 7NSK2 (Audenaert et al., 2002) Pseudomonas fluorescens (Yildiz et al., 2007) (Hernandez-Rodriguez et al., 2004) Burkholderia cepacia (Hernandez-Rodriguez et al., 2004) Serratia plymuthica HRO-C48 (Ma et al., 2007), IC1270 (Meziane et al., 2006), IC14 / cucumber (Kamensky et al., 2002, Kamensky et al., 2003) Streptomyces ahygroscopicus var. wuyiensis (Sun et al., 2004) Streptomyces lydicus/ cucumber (Farrag, 2003) **Fungi + yeasts:** Aureobasidium pullulans (Dik et al., 1999) (Dik and Elad, 1999) Beauveria sp. (Diaz et al., 2007) Candida guilliermondii strains 101 and US 7 (Saligkarias et al., 2002) Candida oleophila strain I-182 (Saligkarias et al., 2002) Candida pelliculosa (Bello et al., 2008) Clonostachys rosea (Nobre et al., 2005) (Sutton et al., 2002) (Yohalem, 2001) Cryptococcus laurentii (Xi and Tian, 2005) Cryptococcus albidus (Dik et al., 1999) (Dik and Elad, 1999) Gliocladium (Hmouni et al., 2005) (Hmouni et al., 2006, Hmouni et al., 1999) Gliocadium viride (Bocchese et al., 2007) (Lisboa et al., 2007) Microdochium dimerum (Bardin et al., 2008) (Bardin et al., 2004b) (Bardin et al., 2004a) (Decognet and Nicot, 1999) (Decognet et al., 1999) (Trottin-Caudal et al., 2001) (Nicot et al., 2002) Pichia guilliermondii (Zhao et al., 2008)

	Variable little or no effect once in the field (good in lab): Brevibacillus brevis WT / cucumber (Konstantinidou-Doltsinis et al., 2002) Gliocladium catenulatum (Prestop). (Ingram and Meister, 2006) Trichoderma (tomato + pepper) (Salas Brenes and Sanchez Garita, 2006) Trichoderma harzianum T39 Trichodex with BOTMAN (Moyano et al., 2003)	Rhodosporidium diobovatum (S33), (Utkhede et al., 2001) Rhodotorula glutinis Y-44 (Kalogiannis et al., 2006) Rhodotorula rubra (Bello et al., 2008) Trichoderma (Hmouni et al., 2005) (Hmouni et al., 1999) Trichoderma harzianum (Hmouni et al., 2006) (Fiume et al., 2008) (Barakat and Al-Masri, 2005) (Lisboa et al., 2007) T115 (Meyer et al., 2001) Trichodex T39 (Elad et al., 1998) (Yohalem et al., 1998) (Meyer et al., 1998) (Jalil R et al., 1997) (Dik et al., 1999) (Dik and Elad, 1999), RootShield (Utkhede et al., 2001), Th-B /pepper (Li et al., 2004), Rifai (Gromovikh et al., 1998) Trichoderma taxi ZJUF0986 (Wang et al., 2008a) Trichosporon pullulans (Cook, 2002) Ulocladium atrum (Nicot et al., 2002) (Fruit and Nicot, 1999) (Yohalem, 2001) / cucumber (Yohalem, 1997) Ustilago maydis (Teichmann et al., 2007) **<u>Oomycetes</u>** Pythium oligandrum (Floch et al., 2001) (Wang et al., 2007a) **Little or no effect once in the field (good in lab):** Trichoderma spp. commercial preparations/ cucumber (Yohalem, 1997)
B	Milsana + Brevibacillus brevis WT / cucumber (Konstantinidou-Doltsinis et al., 2002) **Variable little or no effect once in the field:** Reynoutria sachalinensis extract (Milsana); (Ingram and Meister, 2006)	volatile substances produced by grape cv. Isabella (Vitis labrusca) (postharvest) (Kulakiotu et al., 2004) (Kulakiotu and Sfakiotakis, 2003)
O	calcium foliar fertilizers (CaH2O2, CaSO4, Ca(NO3)2, CaCl2 and CaO), (Mizrakci and Yildiz, 2002)	Compost water extracts prepared from animal sources (horse, sheep, and cattle) and a plant source (olive), (Hmouni et al., 2006) Adipic acid monoethyl ester (Vicedo et al., 2005) Calcium foliar fertilizers (CaH2O2, CaSO4, Ca(NO3)2, CaCl2 and CaO), (Mizrakci and Yildiz, 2002) Chitosan Elexa (Acar et al., 2008) Benzothiadiazole (BTH) (Hernandez-Rodriguez et al., 2004) **Variable little or no effect :** Vital pasta, Vital gel and Elot-Vis (Gielen et al., 2004)

Grapes (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** Acinetobacter lwoffii PTA-113, (Magnin-Robert et al., 2007) Pseudomonas fluorescens PTA-CT2, (Magnin-Robert et al., 2007) Pantoea agglomerans PTA-AF1 (Magnin-Robert et al., 2007) Bacillus (isolate UYBC38) (Rabosto et al., 2006) Bacillus subtilis strain QST 713 (serenade) (Benuzzi et al., 2006) Serenade, moderate to good control (Schilder et al., 2002) **Fungi + yeasts:** Acremonium cephalosporium, strain B11 (Zahavi et al., 2000) Candida guilliermondii, strain A42 (Zahavi et al., 2000) Chaetomium cochlioides (Lennartz et al., 1998) Gliocladium (Cherif and Boubaker, 1998) Gliocladium roseum (Holz and Volkmann, 2002) Hanseniaspora uvarum (isolate UYNS13) (Rabosto et al., 2006) Trichoderma (Cherif and Boubaker, 1998) Trichoderma harzianum (Holz and Volkmann, 2002), Rootshield(R) (Marco and Osti, 2007) Rifai, 1295-22, (Harman et al., 1996), Trichodex 25 WP (Turcanu, 1997) Trichoderma virens 31 (Harman et al., 1996) Trichosporon pullulans (Holz and Volkmann, 2002) Ulocladium atrum, low disease pressure (Metz et al., 2002) (Roudet and Dubos, 2001) (Schoene et al., 1999) (Holz and Volkmann, 2002) (Lennartz et al., 1998) (Schoene and Kohl, 1999), isolate 385 (Schoene et al., 2000) Ulocladium oudemansii + 5-chlorosalicylic acid in combination (Reglinski et al., 2005) **Variable little or no effect once in the field:** Trichoderma harzianum partial effect (Monchiero et al., 2005) Ulocladium oudemansii partial effect (Monchiero et al., 2005) Ulocladium atrum, high disease pressure (Metz et al., 2002) (Roudet and Dubos, 2001)	**Bacteria** Bacillus sp., (Paul et al., 1998) (Krol, 1998) (Trotel-Aziz et al., 2003), isolate UYBC38 (Rabosto et al., 2006) Cupriavidus campinensis (Schoonbeek et al., 2007) Pseudomonas sp. (Trotel-Aziz et al., 2003), strain PsJN (Barka et al., 2002) Pseudomonas fluorescens (Krol, 1998) Pantoea (Trotel-Aziz et al., 2003) **Fungi + yeasts:** Alternaria spp., (Walter et al., 2006) Aureobasidium pullulans, L47 postharvest (Lima et al., 1997), LS-30 postharvest (Castoria et al., 2001) Candida oleophila (Lima et al., 1997), postharvest (El-Neshawy and El-Morsy, 2003) Coniothyrium (Sesan et al., 2002) Debaryomyces hansenii (Santos et al., 2004) Epicoccum spp (Sesan et al., 2002) (Walter et al., 2006) (Fowler et al., 1999) Gliocladium, (Sesan et al., 2002) Hanseniaspora uvarum (isolate UYNS13) (Rabosto et al., 2006) Kloeckera spp. (Cirvilleri et al., 1999) Metschnikowia fructicola, postharvest (Karabulut et al., 2003), postharvest (Kurtzman and Droby, 2001) Muscodor albus, postharvest (Gabler et al., 2006) Pichia anomala (strain FY-102) (Masih et al., 2000) (Santos et al., 2004) Pichia membranaefaciens (Masih and Paul, 2002) (Masih et al., 2001) (Santos and Marquina, 2004) (Santos et al., 2004) Scytalidium, (Fowler et al., 1999) Trichoderma spp. (Walter et al., 2006) (Fowler et al., 1999) Trichoderma harzianum CECT 2413 – mutant (Rey et al., 2001), Rifai postharvest (Batta, 2007) Trichoderma viride, (Sesan et al., 2002) Trichothecium, (Sesan et al., 2002) Tricothecium roseum (Fowler et al., 1999) Ulodadium spp (Walter et al., 2006) (Fowler et al., 1999) Ulocladium atrum isolate 385 (Schoene et al., 2000) Verticillium, (Sesan et al., 2002) **Oomycetes** Pythium paroecandrum (Abdelghani et al., 2004) Pythium periplocum (Paul, 1999b)
B	Croplife (citrus and coconut extract) + Plantfood (foliar fertilizer), moderate to good control (Schilder et al., 2002) Milsana (giant knotweed [Fallopia sp.] extract), moderate control (Schilder et al., 2002)	volatile substances produced by grape cv. Isabella (Vitis labrusca) (postharvest) (Kulakiotu et al., 2004) (Kulakiotu and Sfakiotakis, 2003)
O	Chitosan (Amborabe et al., 2004)	

Strawberry (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** Paenibacillus polymyxa 18191 (Helbig, 2001b) Pseudomonas fluorescens (Abada et al., 2002) **Fungi + yeasts:** Aureobasidium pullulans (Stromeng et al., 2006) Candida fructus, (El-Neshawy and Shetaia, 2003) C. glabrata, (El-Neshawy and Shetaia, 2003) C. oleophila (El-Neshawy and Shetaia, 2003) Cryptococcus albidus (Helbig, 2002) Epicoccum nigrum, (Stromeng et al., 2006) Metschnikowia fructicola (=FG) (Karabulut et al., 2004) Pichia guilermondii + Bacillus mycoides **mixture** (Guetsky et al., 2001) (Guetsky et al., 2002) Rhodotorula glutinis (Helbig, 2001a) Trichoderma harzianum (Abada et al., 2002) (Antoniacci et al., 2000) (Maccagnani et al., 1999), 1295-22 (Kovach et al., 2000), (atroviride) P1 (Hjeljord et al., 2001), T39 (Shafir et al., 2006), Trichodex (Freeman et al., 2001) (Freeman et al., 2002) (Freeman et al., 2004) Trichoderma products (BINAB) (Ricard and Jorgensen, 2000) Ulocladium atrum (Boff, 2001) (Boff et al., 2002a) (Boff et al., 2002b) (Kohl et al., 2001) (Kohl et al., 2004) (Kohl and Fokkema, 1998) **Variable little or no effect once in the field:** Bacillus subtilis (Gengotti et al., 2002) Gliocladium roseum (Chaves and Wang, 2004) Gliocladium catenulatum, (Prokkola et al., 2003), but low disease incidence (Prokkola and Kivijarvi, 2007) Trichoderma sp (Stensvand, 1997), (Stensvand, 1998) (Hjeljord et al., 2000) (Prokkola et al., 2003), but low disease incidence (Prokkola and Kivijarvi, 2007) Trichoderma harzianum (atroviride) (Hjeljord, 2002) (Hjeljord et al., 2001), (Gengotti et al., 2002), Trichodex 40 WP (Meszka and Bielenin, 2004)	**Bacteria** Bacillus sp. (isolate 17141) (Helbig et al., 1998) Bacillus pumilus (Essghaier et al., 2007), NCIMB 13374 (Swadling and Jeffries, 1998) Bacillus subtilis, (Essghaier et al., 2007) (Sardi et al., 2008) (Helbig and Bochow, 2001) (Marquenie et al., 1999) (Zhao et al., 2007) (Abada et al., 2002) (Gengotti et al., 2000) Bacillus marismortui, (Essghaier et al., 2007) Bacillus licheniformis, (Essghaier et al., 2007) Bacillus thuringiensis (Bacikol) (Kandybin, 2003) Virgibacillus marismortui, (Essghaier et al., 2007) Enterobacteriaceae (10B1, 5B4) (Guinebretiere et al., 2000) Halomonas sp. (Essghaier et al., 2007) Pantoea agglomerans strain EPS125, postharvest (Bonaterra et al., 2004) Pseudomonas fluorescens (Abada et al., 2002), NCIMB 13373 (Swadling and Jeffries, 1998) Pseudomonas cepacia (Marquenie et al., 1999) Pseudomonas chlororaphis isolate I-112 (Gulati et al., 1999) Pseudomonas syringae but phytotox (Pellegrini et al., 2007) **Fungi + yeasts**: Aureo basidium pullulans (Adikaram et al., 2002) Candida reukaufii, (Guinebretiere et al., 2000) Candida pulcherrima, (Guinebretiere et al., 2000) Clonostachys rosea (Cota et al., 2008), IK726 (Mamarabadi et al., 2008) Cryptococcus albidus (Helbig, 2002) Cryptococcus laurentii (Zheng et al., 2003) Gliocladium virens (Tehrani and Alizadeh, 2000) Metschnikowia fructicola (Shemer(R) postharvest (Ferrari et al., 2007) Pichia guilermondii + Bacillus mycoides mixture (Guetsky et al., 2002b) (Guetsky et al., 2001b) (Guetsky et al., 2001a) (Guetsky et al., 2002a) Rhodotorula glutinis, postharvest (Zhang et al., 2007a), (Helbig, 2001a) Trichoderma sp (Santorum et al., 2002) Trichoderma harzianum (Abada et al., 2002) (Tehrani and Alizadeh, 2000) (Sanz et al., 2002), T39 (Bilu et al., 2004) (Levy et al., 2004a) (Levy et al., 2006) (Levy et al., 2004b), atroviride P1 (Hjeljord, Stensvand et al. 2001) Trichoderma asperellum (Sanz et al., 2005) (Sanz et al., 2002) Trichoderma longibrachiatum (Sanz et al., 2002) Trichoderma atroviride (Sanz et al., 2002) Trichoderma koningii, (Tehrani and Alizadeh, 2000) Trichoderma viride (Tehrani and Alizadeh, 2000) Ulocladium atrum (Boff, 2001) (Berto et al., 2001) (Boff et al., 2001) Verticillium lecanii (Koike et al., 2004) Variable little or no effect once in the field: Pichia guilermondii (Wszelaki and Mitcham, 2003)

B	Messenger (harpin), (Meszka and Bielenin, 2004) **Variable little or no effect once in the field:** Biosept 33 SL (grapefruit extract) (Meszka and Bielenin, 2004) seaweed, garlic, and compost extracts (Prokkola et al., 2003), but low disease incidence (Prokkola and Kivijarvi, 2007)	
O	sodium bicarbonate (Funaro, 1997) **Variable little or no effect once in the field:** Biochicol 020 PC (chitosan) (Meszka and Bielenin, 2004) silicon (Prokkola et al., 2003), but low disease incidence (Prokkola and Kivijarvi, 2007)	Natural volatile compounds : benzaldehyde, methyl benzoate, methyl salicylate, 2-nonanone, 2-hexenal diethyl acetal, hexanol, and E-2-hexen-1-ol (Archbold et al., 1997)

Field vegetables (lettuce, onion, cabbage, melon) (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	Microsphaeropsis ochracea / onion (Carisse et al., 2006) Ulocladium atrum 385, onion (Kohl and Fokkema, 1998) (Kohl et al., 1999)	**<u>Bacteria</u>** Bacillus subtilis / lettuce (Fiddaman et al., 2000), L-form / Chinese cabbage (Walker et al., 2002), / melon (Wang et al., 2008c) Brevibacillus brevis / lettuce (McHugh and Seddon, 2001) Bacillus amyloliquefaciens/ melon (Wang et al., 2008c) Pseudomonas spp. (LC8, PF13, PF14, PF15), /lettuce (Card et al., 2002) Pseudomonas syringae pv. phaseolicola / Chinese cabbage (Daulagala and Allan, 2003) **<u>Fungus + yeast:</u>** Clonostachys rosea / onion (Nielsen et al., 2000) (Yohalem et al., 2004) Coniothyrium minitans / lettuce (Fiume and Fiume, 2005) Epicoccum sp. (E21) /lettuce (Card et al., 2002) Gliocladium virens [Trichoderma virens], / lettuce (Lolas et al., 2005) Penicillium griseofulvum, / onion (Tylkowska and Szopinska, 1998) Penicillium sp. 90/22, / onion (Tylkowska and Szopinska, 1998) Pichia onychis /onion postharvest (German Garcia et al., 2001) (Cotes, 2001) Ulocladium sp. (U13), /lettuce (Card et al., 2002) Ulocladium atrum / onion (Kohl et al., 2003), 385 and 302 / onion (Nielsen et al., 2000) (Yohalem et al., 2004) Trichoderma harzianum, / onion (Tylkowska and Szopinska, 1998), T39 / lettuce (Meyer et al., 1998) (Lolas et al., 2005), 'Supresivit' / cress (Borregaard, 2000) Trichoderma koningii / onion (Tylkowska and Szopinska, 1998) T. viride / onion (Tylkowska and Szopinska, 1998) **Variable little or no effect :** Trichoderma-Promot / onion (El-Neshawy et al., 1999)
B		
O		

Fruits - postharvest (apple, pears, peach, sweet cherry, kiwi) (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** Pantoea agglomerans (CPA-2) (Nunes et al., 2002b) (Nunes et al., 2001b) Pseudomonas syringae, MA-4, MB-4, MD-3b and NSA-6 (=FG) (Zhou et al., 2001) **Fungi + yeasts**: Aureobasidium pullulans, Rhodotorula glutinis and Bacillus subtilis in combination (=FG) (Leibinger et al., 1997) Candida saitoana (El-Ghaouth et al., 2001a) , with chitosan (Bio-Coat) or lyric enzyme (Biocure) (El-Ghaouth et al., 2001b) Candida sake strain CPA-1 combined with diphenylamine (Zanella et al., 2003), CPA-1 + ammonium molybdate /pear (Nunes et al., 2002a) Metschnikowia pulcherrima (Migheli et al., 1997) Pichia anomala strain K beta -1,3-glucans and calcium chloride (Jijakli et al., 2002)	**Bacteria** Bacillus licheniformis (EN74-1) (Jamalizadeh et al., 2008) Bacillus subtilis (Ongena et al., 2005) , GA1 (Toure et al., 2004), Rizo-N (El-Sheikh Aly et al., 2000) Bacillus amyloliquefaciens 2TOE, /pears (Mari et al., 1996) Bacillus pumilus 3PPE, /pears (Mari et al., 1996) Erwinia sp (Floros et al., 1998) Pantoea agglomerans (Sobiczewski and Bryk, 1999) (Nunes et al., 2001a) Pseudomonas sp (Sobiczewski and Bryk, 1999) Pseudomonas syringae Strain ESC-11 BioSave (Janisiewicz and Jeffers, 1997), / pear (Sugar and Benbow, 2002) (Benhow and Sugar, 1997), MA-4 (Zhou et al., 2002), CPA5 (Nunes et al., 2007) Pseudomonas fluorescens (Mikani et al., 2007) (Mikani et al., 2008) Pseudomonas viridiflava (Bryk et al., 1999) Rahnella aquatilis (Calvo et al., 2007) **Fungi + yeasts**: Aureobasidium pullulans (Achbani et al., 2005) (Lima et al., 2005) (Schena et al., 1999), LS-30 (Lima et al., 1999) (Lima et al., 2003), + calcium chloride or sodium bicarbonate (Ippolito et al., 2005b) (Ippolito et al., 2005a) Candida butyri JCM 1501, (Wagner et al., 2006) Candida melibiosica 2515 (Wagner et al., 2006) Candida parapsilosis DSM 70125 (Wagner et al., 2006) Candida oleophila Aspire (Droby et al., 2003), Aspire/pear (Sugar and Benbow, 2002) (Benhow and Sugar, 1997), Aspire + 2% sodium bicarbonate (Wisniewski et al., 2001), strain O (Jijakli, 2000) (Bajji and Jijakli, 2007) (Jijakli et al., 2004) (Lahlali et al., 2007), /peach (Karabulut and Baykal, 2004), Candida saitoana (El-Ghaouth et al., 2001c) (El-Ghaouth et al., 2000a) (El-Ghaouth et al., 2000b) (El-Ghaouth et al., 2001b) Candida sake (Vinas et al., 1998) (Nunes et al., 2002d) (Giraud and Crouzet, 2004) (Cook, 2002b), CPA-1 + Pantoea agglomerans (Nunes et al., 2002c) Candida famata (21-D), (Lima et al., 1999) Candida tenuis, (Faten, 2005) Candida pulcherrima (Cook, 2002b) Cryptococcus laurentii (Benhow and Sugar, 1997) (Zhang et al., 2005) (Zhang et al., 2007b) (Sugar and Benbow, 2002) (Tian et al., 2004a) (Jing et al., 2008) (Colgan, 1997) (Lima et al., 2005) (Filonow, 1998) + Gibberellic acid (Yu and Zheng, 2007), +IAA (Yu et al., 2008), + salicilic acid (Yu et al., 2007), LS28 (Lima et al., 2006) (Lima et al., 1998) (Lima et al., 1999) (Lima et al., 2003) Cryptococcus albidus, (Fan et al., 2001a) (Fan and Tian, 2001) (Tian et al., 2002) Cryptococcus humicola (Anderson et al., 1997) (Filonow et al., 1996) Cryptococcus infirmo-miniatus (Benhow and Sugar, 1997) (Sugar and Benbow, 2002) Debaryomyces hansenii (strain 43E) / citrus (Arras and Arru, 1999)

		Filobasidium floriforme NRRLY7454, (Filonow et al., 1996) Galactomyces geotrichum (Cook, 2002b) Kloeckera apiculata / peach (Karabulut and Baykal, 2003) (Karabulut et al., 2005) Metschnikowia pulcherrima (Spadaro et al., 2002) (Piano et al., 1998) (Spadaro et al., 2004), MACH1 (Duraisamy et al., 2008) Metschnikowia fructicola (Karabulut et al., 2005) Muscodor albus (Mercier and Jimenez, 2004) (Ramin et al., 2008) (Schotsmans et al., 2008) Penicillium spp. (El-Sheikh Aly et al., 2000) Pichia stipitis CBS 5773 (Wagner et al., 2006) Pichia anomala strain K (Grevesse et al., 2003) (Jijakli, 2000) (Friel and Jijakli, 2007) (Friel et al., 2007) (Jijakli and Lepoivre, 1998) (Lahlali et al., 2007) Pichia guilliermondii (29-A), (Lima et al., 1999) Rhodotorula glutinis (Sugar and Benbow, 2002) (Benhow and Sugar, 1997) (Lima et al., 2005) (Lima et al., 1998) (Sansone et al., 2005), LS-11 (Lima et al., 1999) (Lima et al., 2003), Rhodosporidium toruloides NRRL Y1091, (Filonow et al., 1996) Sporobolomyces roseus FS-43-238 (Filonow et al., 1996) (Filonow, 1998) Saccharomyces cerevisiae, (Faten, 2005) Trichoderma harzianum Plant-guard (El-Sheikh Aly et al., 2000), Rifai (Batta, 2004) Trichoderma Viride (El-Sheikh Aly et al., 2000), Trichosporon sp., (Fan et al., 2001b) (Tian et al., 2002) Trichosporon pullulans (Cook, 2002b) R. glutinis SL 1 + C. laurentii SL 62 mixture (Calvo et al., 2003) Variable little or no effect : Candida oleophila (Aspire), (Colgan, 1997)
B		volatile substances produced by grape cv. Isabella (Vitis labrusca) (Kulakiotu and Sfakiotakis, 2003b) (Kulakiotu et al., 2004a)
O		Chitosan, (Faten, 2005) Calcium (Chardonnet et al., 2000) (Holmes et al., 1998) Phosphonate (Holmes et al., 1998) sodium bicarbonate (Karabulut et al., 2005)

Legumes (*Fabaceae*) (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** Bacillus subtilis K-3 / lupin (Kuptsov et al., 2004) Pantoea agglomerans / lentil (Huang and Erickson, 2002), LRC 954, / lentil (Huang and Erickson, 2005) Pseudomonas fluorescens, / lentil (Huang and Erickson, 2002) LRC 1788 / lentil (Huang and Erickson, 2005) **Fungi + yeasts**: Clonostachys rosea / alfalfa (Li et al., 2004a) Gliocladium catenulatum, / alfalfa (Li et al., 2004a) Penicillium aurantiogriseum LRC 2450 / lentil (Huang and Erickson, 2005) Penicillium griseofulvum / lentil (Huang and Erickson, 2002) Trichoderma hamatum / lentil (Huang and Erickson, 2002) Trichoderma harzianum LRC 2428 / lentil (Huang and Erickson, 2005) Trichoderma viride / chickpea (Abha et al., 1999) Trichoderma atroviride, / alfalfa (Li et al., 2004a) Trichothecium roseum / alfalfa (Li et al., 2004a) Mixture: Streptomyces exfoliatus + Trichoderma harzianum / faba bean (Mahmoud et al., 2004)	**Bacteria** Bacillus subtilis (Saad et al., 2005) Bacillus megaterium (Saad et al., 2005) Bacillus cereus (Kishore and Pande, 2007) Bacillus macerans BS 153 (Sharga, 1997) Pantoea agglomerans (Huang and Erickson, 2002), LRC 954, (Huang and Erickson, 2005) Pseudomonas fluorescens, (Huang and Erickson, 2002) LRC 1788 (Huang and Erickson, 2005) Pseudomonas putida BTP1 (Ongena et al., 2002) Streptomyces albaduncus (Razak et al., 2000) Streptomyces griseoplanus (Razak et al., 2000) Streptomyces violaceus T118 (Ahmad et al., 2002) **Fungi + yeasts**: Botrytis cinerea non-aggressive strains /bean leaves (Weeds et al., 2000) Chaetomium globosum (Pradeep et al., 2000) Cladosporium cladosporioides (Jackson et al., 1997) Epicoccum nigrum, (Szandala and Backhouse, 2001) Gliocladium roseum (Li et al., 2002) (Szandala and Backhouse, 2001) (Burgess and Keane, 1997) Penicillium brevicompactum (Jackson et al., 1997) Penicillium aurantiogriseum LRC 2450 (Huang and Erickson, 2005) Penicillium griseofulvum (Huang and Erickson, 2002) Trichoderma (Burgess and Keane, 1997) Trichoderma harzianum (Szandala and Backhouse, 2001), T39 (Bigirimana et al., 1997) (Kapat et al., 1998) (Elad et al., 2004), LRC 2428 (Huang and Erickson, 2005) Trichoderma viride /pigeon pea (Pradeep et al., 2000), / chickpea (Abha and Tripathi, 1999) (Mukherjee et al., 1997) Trichoderma hamatum (Huang and Erickson, 2002)
B	Eucalyptus citriodora + Ipomoea carnea extracts / faba bean (Mahmoud et al., 2004)	extracts from green parts of tomato, potato, rape (Smolinska and Kowalska, 2006) pterocarpan phytoalexin maackiain from chickpea (Stevenson and Haware, 1999)
O		

Flowers (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** Bacillus amyloliquefaciens B190 / lily (Chiou and Wu, 2003) Bacillus cereus / lily (Liu et al., 2008) Bacillus amyloliquefaciens / lily (Chiou and Wu, 2001) Burkholderia gladioli, / lily (Chiou and Wu, 2001) Pseudomonas putida / lily (Liu et al., 2008) **Fungi + yeasts**: Clonostachys rosea /rose (Morandi et al., 2003) Ulocladium atrum / cyclamen (Kohl et al., 2000) (Kohl et al., 1998) **Variable little or no effect** : Trichoderma harzianum / cyclamen (Minuto et al., 2002) (Minuto et al., 2004)	**Bacteria** Bacillus amyloliquefaciens / lily (Chiou and Wu, 2001) Bacillus subtilis / rose buds (Tatagiba et al., 1998) Burkholderia gladioli, / lily (Chiou and Wu, 2001) Pseudomonas sp. 677 /geraldton waxflower (Beasley et al., 2001) Serratia marcescens strain B2 / cyclamen (Someya et al., 2001) **Fungi + yeasts** Cladosporium spp. / rose (Morandi et al., 1999) Cladosporium oxysporum, / rose debris + buds (Tatagiba et al., 1998) Cladosporium cladosporioides / rose buds (Tatagiba et al., 1998) Clonostachys rosea /rose (Morandi et al., 1999) (Morandi et al., 2006) (Morandi et al., 2001) (Morandi et al., 2007) (Morandi et al., 2008) (Morandi et al., 2000b) (Morandi et al., 2000a) (Yohalem, 2004) (Yohalem, 2000) Epicoccum sp. / Geraldton waxflower (Beasley et al., 2001) Fusarium sp., / Geraldton waxflower (Beasley et al., 2001) Gliocladium roseum FR136 / rose debris (Tatagiba et al., 1998) Rhizoctonia (BNR), / geranium (Olson and Benson, 2007) Rhodotorula glutinis PM4 / geranium (Buck and Jeffers, 2004) (Buck, 2004) Rhodotorula graminis, / geranium (Buck, 2004) Rhodotorula Mucilaginosa / geranium (Buck, 2004) Trichoderma spp / Geraldton waxflower (Beasley et al., 2001) Trichoderma harzianum (Trichodex) / Geraldton waxflower (Beasley et al., 2005) Trichoderma hamatum / statice (Diaz et al., 1999), 382 / geranium (Olson and Benson, 2007) Trichoderma inhamatum, / rose debris (Tatagiba et al., 1998) Ulocladium atrum / cyclamen (Kessel, 1999) (Kessel et al., 2001) (Kessel et al., 2005) (Kohl and Molhoek, 2001) (Kessel et al., 2002) (Kessel et al., 1999), /lily (Kessel et al., 1999) (Elmer and Kohl, 1998) (Kessel et al., 2001), / geranium (Gerlagh et al., 2001), / rose (Yohalem and Kristensen, 2004) (Yohalem, 2004) (Kohl and Gerlagh, 1999) (Yohalem et al., 2007) (Yohalem, 2000), / pelargonium (Yohalem et al., 2007) **Variable little or no effect** : Trichoderma hamatum 382 in compost / begonia (Horst et al., 2005) Trichoderma harzianum preparations (Yohalem, 2000) (Trichodex and Supresivit) (Yohalem, 2004)
B		grapefruit [Citrus paradisi] extract / lily, peony and tulip (Orlikowski et al., 2002), / tulips, Gerbera jamesonii and carnations (Orlikowski and Skrzyoczak, 2003), Biosept 33 SL / tulip (Orlikowski and Skrzypczak, 2001) chitosan / tulips, Gerbera jamesonii and carnations (Orlikowski and Skrzyoczak, 2003)
O		

Miscellaneous crops (target pathogen = *B. cinerea*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** Streptomyces griseoviridis (Mycostop) / Pinus sylvestris (Capieau et al., 2001) (Capieau et al., 2004) **Fungi + yeasts** Gliocladium sp (GlioMix) / Pinus sylvestris (Capieau et al., 2001) (Capieau et al., 2004) Gliocladium roseum / Eucalyptus nurseries (Stowasser and Ferreira, 1997) Trichoderma harzianum and T. polysporum (Binab TF.WP), / Pinus sylvestris (Capieau et al., 2001) (Capieau et al., 2004) Trichoderma viride (Trichosemin 25 PTS (25% Tv), / sunflower (Eva, 2003) **Variable little or no effect** : Penicillium sp. / Eucalyptus nurseries (Stowasser and Ferreira, 1997) Trichoderma harzianum , Trichoderma viride / Eucalyptus nurseries (Stowasser and Ferreira, 1997)	**Bacteria** Bacillus spp./ Ginseng (Kim et al., 1997) (Chung et al., 1998) Bacillus subtilis Cot1 and CL27 / Astilbe hybrida, Aster hybrida, Daphne blayana, Photinia fraseri (Li et al., 1998) Bacillus amyloliquefaciens / oilseed rape (Danielsson et al., 2007) Bacillus licheniformis / Perilla (Son et al., 2002) B. megaterium / Perilla (Son et al., 2002) Cupriavidus campinensis / Arabidopsis thaliana (Schoonbeek et al., 2007) Erwinia / Ginseng (Kim et al., 1997) Pseudomonas fluorescens / castor crop (Raoof et al., 2003), WCS374r / Eucalyptus (Ran et al., 2005) Pseudomonas putida WCS358r / Eucalyptus (Ran et al., 2005) Streptomyces griseoviridis (Mycostop) / Pinus sylvestris (Capieau et al., 2001) (Capieau et al., 2004) **Fungi + yeasts** Clonostachys (A-10) / Pinus radiate, Eucalyptus globulus (Molina Mercader et al., 2006) Cylindrocladium spp. / Eucalyptus (Fortes et al., 2007) Gliocladium sp (GlioMix) / Pinus sylvestris (Capieau et al., 2001) (Capieau et al., 2004) Gliocladium roseum / Picea mariana (Zhang et al., 1996) Trichoderma spp. / Eucalyptus (Fortes et al., 2007) Trichoderma harzianum / Arabidopsis thaliana (Korolev and Elad, 2004) / castor crop (Tirupathi et al., 2006) (Raoof et al., 2003) (Bhattiprolu and Bhattiprolu, 2006), / hazelnut (Machowicz-Stefaniak et al., 2004) Trichoderma viride / castor crop (Tirupathi et al., 2006) (Raoof et al., 2003) (Bhattiprolu and Bhattiprolu, 2006), T 13-82 (Trichodermin-BL) / flax (Pristchepa et al., 2006), / hazelnut (Machowicz-Stefaniak et al., 2004) Trichoderma harzianum and T. polysporum (Binab TF.WP), / Pinus sylvestris (Capieau et al., 2001) (Capieau et al., 2004)
B		Mature leaf extract of Lantana camera / castor crop (Bhattiprolu and Bhattiprolu, 2006)
O		Cryptogein, elicitor secreted by Phytophthora cryptogea / tobacco (Blancard et al., 1998)

<table>
<tr><th colspan="2">Successful inhibition in vitro (target pathogen = B. cinerea)</th></tr>
<tr><td>M</td><td><u>Bacteria</u>
Alcaligenes faecalis (Honda et al., 1999)
Azotobacter (Khan et al., 2006)
Bacillus sp mutant strain (Bernal et al., 2002)
Bacillus amyloliquefaciens CCMI 1051 (Caldeira et al., 2007), BL-3 (Lee et al., 2001)
Bacillus brevis [Brevibacillus brevis](Gu et al., 2001) (Edwards and Seddon, 2001)
Bacillus cereus (Guven et al., 2008) (Huang and Chen, 2004)
Bacillus circulans (Paul et al., 1997)
Bacillus licheniformis W10 (Ji et al., 2007) (Gu et al., 2001)
Bacillus subtilis (Gu et al., 2001) (Chen et al., 2008) (Chen et al., 2004b) (Zhao et al., 2003) (Chen et al., 2004a) (Zakharchenko et al., 2007) (Gu et al., 2004) (Novikova et al., 2003) (Hsieh et al., 2003) (Feng et al., 2003) (Liu et al., 2007b)
Bacillus thuringiensis CMB26 (Kim et al., 2004)
Paenibacillus polymyxa BL-4 (Lee et al., 2001)
Photorhabdus luminescens ATCC 29999 (Hsieh et al., 2004)
Plutella xylostella (Indiragandhi et al., 2008)
Pseudomonas (Lian et al., 2007) (Cornea et al., 2007) (Kim et al., 2000) (Woo et al., 2002) (Bryk et al., 2004)
Pseudomonas aeruginosa PUPa3 (Kumar et al., 2005)
Pseudomonas antimicrobica (Walker et al., 2001)
Pseudomonas corrugata strain P94 (Guo et al., 2007)
Pseudomonas fluorescens (Nian et al., 2007) (Khan and Almas, 2002)
Pseudomonas putida (Cornea et al., 2007), Cha 94 (Lee et al., 2001)
Pseudomonas syringae pv. syringae strain B359 (Fogliano et al., 2002)
Lysobacter capsici sp. Nov (Park et al., 2008)
Serratia plymuthica C48 (Frankowski et al., 2001a) (Frankowski et al., 2001b)
Streptomyces + actinomycetes (Tian et al., 2004b) (Nadkarni et al., 1998) (Liang et al., 2007, Yan et al., 2004) (Han et al., 2004) (Liang et al., 2007) (Long et al., 2005) (Stoppacher et al., 2007) (Kim et al., 2007b)
Streptomyces ahygroscopicus (Sun et al., 2003) (Yang et al., 2007) (Zhao et al., 1998)
Streptomyces luteogriseus ECO 00001 (Li et al., 2008)
Streptomyces rimosus subsp. daheishanensis strain MY02 (Liu et al., 2004)
Streptomyces roseoflavus strain LS-A24 (Park et al., 2006)
Tripterygiun wilfordii (Shentu et al., 2006)
Xenorhabdus sp. strain CB43 (Xiao et al., 2005)
Xenorhabdus nematophilus YL001 (Liu et al., 2006)
marine bacteria (Nie et al., 2007)

<u>Fungi + yeasts</u>
Acremonium strictum (Kim et al., 2002)
Aspergillus fumigatus and A. terreus (El-Zayat, 2008)
Aspergillus clavatonanicus (Zhang et al., 2008)
Cryptococcus laurentii (isolate LS-28) (Castoria et al., 1997)
Fusarium lateritium extracts (Anitha, 2006)</td></tr>
</table>

	Fusarium semitectum (Altomare et al., 2000) Lecanicillium muscarium (Fenice and Gooday, 2006) Muscodor albus (Mercier and Jimenez, 2007) Rhodotorula (Calvente et al., 2001) Rhodotorula glutinis (Castoria et al., 1997) Trichoderma (Pezet et al., 1999) (Chen et al., 2005) (Liu et al., 2007a) Trichoderma viride (Machowicz-Stefaniak, 1998) T15 and T17 (Silva-Ribeiro et al., 2001) Trichoderma atroviride (Navazio et al., 2007) (Klemsdal et al., 2006) GMO (Brunner et al., 2005) Trichoderma harzianum (Dana et al., 2001) (Ding et al., 2002) (Limon et al., 2004) (Mach et al., 1999) T5A, T1 and T1A (Silva-Ribeiro et al., 2001) (Lee et al., 2001), T-33 (Witkowska and Maj, 2002) Trichoderma hamatum C-1 (Witkowska and Maj, 2002) Trichoderma reesei [T. longibractiatum] M7-1 (Witkowska and Maj, 2002) **Oomycetes** Pythium bifurcatum (Paul, 2003) Pythium citrinum (Paul, 2004) Pythium contiguanum (Paul, 2000) Pythium radiosum (Paul, 1999a)
B	Antifungal metabolites of endophytic fungus, A10 (Qian et al., 2006) antimicrobial peptide Ar-AMP from Amaranthus retroflexus L. (Lipkin, Anisimova et al. 2005) basic haem-peroxidase (WP1) from wheat (Triticum aestivum) kernels (Caruso, Chilosi et al. 2001) Extracts from Bazzania trilobata, Diplophyllum albicans, Sphagnum quinquefarium, Dicranodontium denudatum and Hylocomium splendens (Tadesse, Steiner et al. 2003) Extracts of Sophora flavescens (Zheng et al., 2000) (Zheng et al., 1999) Irpex lacteus (Fr.) Fr., Trametes versicolor (L.:Fr.) Pilat, and Chondrostereum purpureum (Pers.:Fr.) Pouzar (White and Traquair, 2006) Pyrrolnitrin, produced by several bacteria (Okada et al., 2005) Ten sesquiterpenes and six diterpenes from Pilgerodendron uviferum wood and bark (Solis et al., 2004)
O	chlorine dioxide (Zoffoli et al., 2005) earthworm (Eisenia fetida) polysaccharides (Wang et al., 2007b) chitosan derivatives (Rabea et al., 2003)

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6.11. Appendix 11. Inventory of biocontrol agents (M: microbials; B: botanicals; O: others) described in primary literature (1998-2008) for successful effect against powdery mildew in laboratory experiments and field trials on various crops.

Powdery mildew on cereals		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** *Pseudomonas aureofaciens ; Bacillus subtilis ; P. fluorescens* (Sanin et al., 2008) **Fungi + yeasts:**	**General paper:** Crop protection: management strategies (Prasad, 2005) **Bacteria** Rhizobacteria (Yigit, 2004) Bacteria, (Azarang, 2004) **Fungi + yeasts:** *Acremonium alternatum* (Kasselaki, 2006a, b) *Alternaria alternata, Aspergillus niger, Bipolaris spicifera, Cladosporium cladosporioides, Curvularia lunata, Fusarium acuminatum F. semitectum, Penicillium rubrum*, (Simian, 2008) BCAs mix (David, 2007) Fungi (Azarang, 2004) *Fusarium oxysporum f. sp. radicis-lycopersici* (Nelson, 2005) *Paecilomyces farinosus* (Szentivanyi, 2006) *Verticillium lecanii* (Koike, 2004)
B		Bryophyte extracts (Tadesse, 2003)
O		Aromatic substances (Koitabashi, 2002) Mycelial extracts (Haugaard, 2002) PAF from Penicillium chrysogenum (Barna, 2008) Secondary metabolic products of strain A19 of actinomycetes (Shen et al., 2008) Verlamelin (Kim, 2002)

Powdery mildew on pome/stone fruits		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** **Fungi + yeasts:** yeast (Y16) (Alaphilippe, 2007)	**General paper:** **Bacteria** **Fungi + yeasts:** *Ampelomyces quisqualis* (Harvey, 2006) *Ampelomyces quisqualis* (Sonali, 2005) yeast (Y16) (Alaphilippe, 2008)
B		
O		

Powdery mildew on grapes		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** *Bacillus subtilis* (Crisp, 2006) Photosynthetic bacteria (Robotic, 2002) **Fungi + yeasts:** *Ampelomyces hyperparasites* (Fuzi, 2003) *Ampelomyces quisqualis* (Angeli, 2006a, b, c, 2007a, b) *Ampelomyces quisqualis* (Hoffmann, 2007) *Ampelomyces quisqualis* 94013 (Lee, 2004) BCAs (Amaro, 2003) BCAs (Ari, 2004) BCAs (Kaine, 2003) BCAs (Linder et al., 2006) BCAs (Zulini, 2004) *Pseudozyma flocculosa* (Schmitt, 2001) Yeast (Robotic, 2002)	**General paper:** **Bacteria** *Brevibacillus brevis* (Schmitt, 2001, 2002) PGPR (Konstantinidou-Doltsinis, 2007) *Pseudomonas syringes* pv. *Syringe* (Kassemeyer, 1998) Serenade (*Bacillus subtilis*)(Schilder, 2002) **Fungi + yeasts:** *Ampelomyces quisqualis* (Angeli, 2006a, b, c, 2007a, b) *Ampelomyces quisqualis* 94013 (Lee, 2004) *Ampelomyces quisqualis* AQ10, (Schweigkofler, 2006) BCA mix (David, 2007) BCAs (Kaine, 2003) BCAs {Amaro, 2003 #177 *Pseudozyma flocculosa* (Schmitt, 2001) *Pseudozyma flocculosa* (SporodexReg. L) (Konstantinidou-Doltsinis, 2007) *Tilletiopsis* spp (Haggag, 2007)
B	Milsana (VP99) (Schmitt, 2001, 2002)	Milsana (VP99) (Konstantinidou-Doltsinis, 2001)
O	fresh or dried milk (10%),pinolene 1%, calcium chloride (2%), tripotassium phosphate (1%) and a mixture of mineral oil (1%),sodium bicarbonate/sodium silicate (0.5%) (Casulli, 2002) mycophagous mite (Melidossian, 2005)	Milk, whey, whey protein, *Bacillus subtilis*, yeast extract medium (Crisp, 2006) Mycophagous mite (Melidossian, 2005) *Orthotydeus lambi* mites (English-Loeb, 1999, 2006, 2007)

Powdery mildew on strawberry pathogen: Podosphaera aphanis f.sp. fragariae; Sphaerotheca macularis f.sp. fragariae		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** **Fungi + yeasts:**	**Bacteria** *B. subtilis* QST (Fiamingo, 2007a) *Bacillus subtilis* (Amsalem, 2004) *Bacillus subtilis* (Pertot, 2004) (Pertot, 2008) *Pseudomonas reactans* (Fiamingo, 2007b) **Fungi + yeasts:** *Ampelomyces quisqualis*, *Trichoderma harzianum* T39, *Bacillus* sp. F77, *Cladosporium tenuissimum* (Amsalem, 2004) BCAs mix (David, 2007)

		T. harzianum T39 (Fiamingo, 2007a) *Trichoderma harzianum* Rifai strain T-22 (Picton, 2003) *Trichoderma harzianum* T39 (Pertot, 2004) (Pertot, 2008)
B		
O		

Powdery mildew on tomato, pathogen: Leveillula taurica, Oidium neolycopersici, Oidium lycopersicum, Oidium spp.		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** *Pseudomonas fluorescens* (Shashi, 2007) **Fungi + yeasts:** *Trichoderma harzianum* (Shashi, 2007)	**General paper:** **Bacteria** *Bacillus brevis* (Seddon, 1999) *Bcillus subtilis* (Jacob, 2007) Rhizobacteria B101R, B212R, and A068R, (Silva, 2004) Serenade ; *Pseudomonas* strains (Laethauwer, 2006) **Fungi + yeasts:** *Acremonium alternatum* (Kasselaki, 2006a, b) *Lecanicillium lecanii* (Mycotal) (Bardin, 2004) *Lecanicillium muscarium* (Bardin, 2008) *Sporothrix flocculosa* (Jarvis, 2007) *Trichoderma* spp. (Moreno-Velandia, 2007) (Velandia, 2007)
B		Milsana (Seddon, 1999) MilsanaReg. (VP 1999)(Malathrakis, 2002) Milsana (Trottin-Caudal, 2003) Malsana (Bardin, 2004) (Bardin, 2008) Milsana ; (Laethauwer, 2006)
O		

Powdery mildew on pepper, pathogen: *Podosphaera leucotricha*		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** **Fungi + yeasts:**	**General paper:** **Bacteria** **Fungi + yeasts:** AQ10 (*Ampelomyces quisqualis*) (Tsror, 2004) *Trichoderma harzianum* (Gupta, 2005) *Trichoderma harzianum* T39; *Ampelomyces quisqualis* (Brand, 2002) *Verticillium lecanii*, *Tilletiopsis minor* (Haggag, 2008)
B		Milsana (Haggag, 2008)
O		Water extract of cattle manure compost, grape marc compost, , Kaligrin and Rifol (Tsror, 2004)

Powdery mildew on cucurbits, pathogen: *Podosphaera fusca*		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** *Bacillus brevis* (Schmitt, 1999) *Bacillus isolates* (Koumaki, 2001) *Brevibacillus brevis* (Abd-El-Moneim, 2004) **Fungi + yeasts:** *Acremonium alternatum* (Kasselaki, 2006a) Ampelomyces quisqualis (Kristkova, 2003) *Ampelomyces quisqualis* isolate M-10 (Benuzzi, 2006) *Ampelomyces quisqualis, Verticillium lecanii, Sporothrix flocculosa* (Dik, 1998) *Cryptococcus laurentii* and *Aureobasidium pullulans* (Lima, 2002) PlantShield *Trichoderma harzianum* (Utkhede, 2006) *Rhodotorula glutinis* (Lima, 2002) *T. harzianum* T39 (Levy, 2004) *Tilletiopsis washingtonensis* (yeast) (El-Hafiz-Mohamed, 1999) *Verticillium lecanii*; (Verhaar, 1999)	**Bacteria** *Bacillus spp* (Romero, 2004a) *Bacillus spp* (Romero, 2004b) *Bacillus subtilis* (Abd-El-Moneim, 2004) (Gilardi, 2008) (Keinath, 2004) (Romero, 2007b) (Romero, 2007d) BCAs mix (David, 2007) *Brevibacillus brevis* (Allan, 2007) (Konstantinidou-Doltsinis, 2002) (Schmitt, 2001) (White, 2001) *Enterobacter cloacae* (Georgieva, 2003) *Xenorhabdus nematophilus* (Shi, 2004) **Fungi + yeasts:** *Acremonium alternatum, Ampelomyces quisqualis* , *Lecanicillium lecanii* (Romero, 2003) *Acremonium alternatum, Verticillium lecanii* (Romero, 2007b) *Ampelomyces quisqualis* (Gilardi, 2008) (Rankovic, 1998) AQ10Reg. (*Ampelomyces quisqualis*) and MycotalReg. (*Lecanicillium lecanii*) (Romero, 2007b) BCAs mix (David, 2007) *Acremonium alternatum* and *Verticillium lecanii*, (Romero, 2001) *Lecanicillium longisporum* (Kim, 2008) *Lecanicillium* spp. (Goettel, 2008) *Meira geulakonigii* (Sztejnberg, 2004) *Paecilomyces fumosoroseus* (Kavkova, 2005) *Paecilomyces fumosoroseus; Verticillium lecanii* (Kavkova, 2001) *Pseudozyma flocculosa* (Konstantinidou-Doltsinis, 2002) (Schmitt, 2001) *Pseudozyma flocculosa, Ampelomyces quisqualis, Verticillium lecanii, Trichoderma harzianum* (Dik, 2002) *Saccharomyces cerevisiae* (El-Gamal, 2003) *Trichoderma harzianum* (Abd-El-Moneim, 2004) (Elad, 2000) *Trichoderma harzianum* T39; *Ampelomyces quisqualis* AQ10 (Elad, 1998) *Verticillium lecanii* (Askary, 1998) (Verhaar, 1998) Ampelomyces quisqualis isolate M-10 (Benuzzi, 2006)
B		Milsana (VP99) (Dik, 2002) (Schmitt, 2001) (White, 2001) Milsana (VP99) from Fallopia sachalinensis (Konstantinidou-Doltsinis, 2001)
O	fresh or dried milk (10%), pinolene 1%, calcium chloride (2%), tripotassium phosphate (1%) and a mixture of mineral oil (1%),sodium bicarbonate/sodium silicate (0.5%) (Casulli, 2002)	Fresh or dried milk (Casulli, 2002) gramicidin S; (Schmitt, 1999) lactoperoxidase system (Ravensberg, 2007) lipopeptide antibiotic neopeptins from Streptomyces sp. (Kim, 2007) lipopeptides (iturin and fengycin families of Bacillus subtilis) (Romero, 2007c) Lipopeptides of antagonistic strains of Bacillus subtilis (Romero, 2007a) oil formulations (Verhaar, 1999) Psyllobora bisoctonotata (Soylu, 2002) undiluted homogenised milk (Utkhede, 2006)

Powdery mildew on various crops, pathogen: *Oidium spp. Sphaerotheca spp., Erysiphe spp*		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bacteria** *Bacillus subtilis* (Nofal, 2006) **Fungi + yeasts:** *Verticillium lecanIi*, *Tilletiopsis minor* (Nofal, 2006)	**Bacteria** *Pseudomonas fluorescens* (Vimala, 2006) *P. fluorescens* (Hooda, 2006) **Fungi + yeasts:** *Acremonium* spp., *Ampelomyces* spp., *Penicillium* spp., *Cladosporium* spp., *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., *Bradyrhizobium* spp., *Brachybacterium* spp., *Curtobacterium* spp., *Cryptocoocus* spp., *Rhodosporidium* spp (Mmbaga, 2008) *Ampelomyces mycoparasites* (Kiss, 2004) BCAs (Dhananjoy, 2008) BCAs (Eken, 2005) BCAs(Casey, 2007) *Cladosporium cladosporioides*, *Cladosporium oxysporum*, *Drechslera hawaiensis*,*T richoderma viride* (Sankar, 2007b) *Cladosporium oxysporum* (Sankar, 2007a) *Gliocladium roseum* (Lahoz, 2004) Kyu-W63 (Koitabashi, 2005) *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, mixture of T. *harzianum P. fluorescens* (Hooda, 2006)
B		
O		Exudates from sclerotia of two *Sclerotium rolfsii* isolates (Pandey, 2007) *Mycophagous Ladybird* (Sutherland, 2005) *Phyllactinia corylea* (Krishnakumar, 2004) *Psyllobora bisoctonotata* (Muls.) (Soylu, 2002) *Psyllobora vigintimaculata*, (Sutherland, 2008; Sutherland, 2006)

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6.12. Appendix 12. Inventory of biocontrol agents (M: microbials; B: botanicals; O: others) described in prim*ary* literature (1973-2008) for successful effect against the rust pathogens in laboratory experiments and field trials on selected crops

Rust on wheat, oat, soybean, groundnut, bean		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Bean** – target pathogen = *Uromyces appendiculatus* Bacillus subtilis (Baker et al., 1985) **Groundnut** – target pathogen = *Puccinia arachidis* Pseudomonas fluorescens strain Pf1 (Meena et al., 2002)	**Bean** – target pathogen = *Uromyces appendiculatus* Pantoea agglomerans B1 (Yuen et al., 2001) Stenotrophomonas maltophilia C3 (Yuen et al., 2001) Cladosporium tenuissimum (Assante et al., 2004) **Groundnut** – target pathogen = *Puccinia arachidis* Bacillus subtilis AF 1 (Manjula et al., 2004) Pseudomonas fluorescens strain Pf1 (Meena et al., 2000) (Meena et al., 2002) Acremonium obclavatum (Gowdu and Balasubramanian, 1993) Fusarium chlamydosporum (Mathivanan and Murugesan, 2000) (Mathivanan et al., 1998) **Soybean** – target pathogen = *Phakopsora pachyrhizi* Verticillium psalliotae, Verticillium lecanii (Saksirirat and Hoppe, 1990) (Saksirirat and Hoppe, 1991) **Wheat, Oat** – target pathogens = *Puccinia recondite, P. coronata* Pseudomonas putida strain BK8661 (Flaishman et al., 1996) Chaetomium globosum strain F0142 (Park et al., 2005b) Verticillium chlamydosporium (Leinhos and Buchenauer, 1992) endophytic fungi (Dingle and McGee, 2003) Fusaric acid from Fusarium oxysporum EF119 (Son et al., 2008)
B		
O		**Bean** – target pathogen = *Uromyces appendiculatus* 2,6-dichloro-isonicotinic acid (CGA 41396) (Dann and Deverall, 1995)

Rust on other crops		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**Coffee** – target pathogens = *Hemileia vastatrix* *Bacillus* sp. (Haddad et al., 2006) *Pseudomonas* sp. (Maffia et al., 2005), **variable effect** (Haddad et al., 2006)	**Chrysanthemum** Verticillium lecanii (Whipps, 1993) **Coffee** – target pathogen = *Hemileia vastatrix* Bacillus lentimorbus (Shiomi et al., 2006) Bacillus cereus (Shiomi et al., 2006) Bacillus (Haddad et al., 2004) Cedecea davisae (Silva et al., 2008) Pseudomonas (Haddad et al., 2004) Acremonium (Haddad et al., 2004) Aspergillus (Haddad et al., 2004) Cladosporium (Haddad et al., 2004) Fusarium (Haddad et al., 2004) Penicillium (Haddad et al., 2004) **Geranium** – target pathogen = *Puccinia pelargonii-zonalis* Bacillus subtilis (Rytter et al., 1989) **Safflower** – target pathogen = *Puccinia carthami* Trichoderma viride and T. harzianum, Bacillus subtilis, B. cereus, B. thuringiensis, Pseudomonas fluorescens added alone and in combination (Tosi and Zazzerini, 1994) **Poplar** – target pathogen = *Melampsora ciliata* Alternaria alternata and Cladosporium oxysporum (Sharma et al., 2002) **Pine** – target pathogens = *Cronartium* and *Peridermium* Cladosporium tenuissimum (Moricca et al., 2001) Scytalidium uredinicola (Moltzan et al., 2001) Plant-growth-promoting rhizobacteria (Enebak and Carey, 2004)
B		
O	**Coffee** – target pathogens = *Hemileia vastatrix* acibenzolar-S-methyl (ASM) (Patricio et al., 2008)	

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6.13. Appendix 13. Inventory of biocontrol agents (M: microbials; B: botanicals; O: others) described in primary literature (1973-2008) for successful effect against the downy mildew / late blight pathogens in laboratory experiments and field trials on selected crops

Potato (target pathogen = *Phytophthora infestans*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	*Bacillus subtilis* (Basu et al., 2001) *Bacillus* sp. isolate PB2 (Atia, 2005) effect < fungicides *Pseudomonas fluorescens* (Basu et al., 2001) *Pseudomonas fluorescens* isolate PPfl (Atia, 2005) effect < fungicides *Pseudomonas* (El-Sheikh et al., 2002) *Gliocladium virens* (Basu et al., 2001) *Phytophthora cryptogea* (Quintanilla, 2002) *Trichoderma* spp (Saikia and Azad, 1999) *Trichoderma viride* (Basu et al., 2001) (Basu and Srikanta, 2003) but no effect in other studies (Singh et al., 2001) (Arora, 2000) (Arora et al., 2006) **little or no effect once in the field (good in lab):** *Acremonium strictum, Penicillium viridicatum* and *Penicillium aurantiogriseum* (Arora, 2000) (Arora et al., 2006) *Myrothecium verrucaria* and *Chaetomium brasiliense* (Arora et al., 2006)	Serenade (*Bacillus subtilis* strain QST 713) (Stephan et al., 2005) (Olanya and Larkin, 2006) *Bacillus subtilis* B5 (Ajay and Sunaina, 2005) *Bacillus, Pseudomonas, Rahnella*, and *Serratia* (Daayf et al., 2003) *Enterobacter cloacae* (Slininger et al., 2007) *Pseudomonas fluorescens* (Slininger et al., 2007) *Xenorhabdus bovienii* (Eibel et al., 2004) *Penicillium aurantiogriseum* (Jindal et al., 1988) *Penicillium viridicatum* (Hemant et al., 2004) Trichodex (Stephan et al., 2005) *Trichoderma viride* (Hemant et al., 2004) *Penicillium, Rhizoctonia* and *Trichoderma* spp (Phukan and Baruah, 1991) various microorganisms (Stephan and Koch, 2002)
B	carvone (Quintanilla, 2002)	carvone , thymol, pinochamphone, plumbagin (Quintanilla, 2002) extracts of *Rheum rhabarbarum* and *Solidago canadensis* (Stephan et al., 2005) oregano extract (Olanya and Larkin, 2006) Elot-Vis (Stephan et al., 2005) patatin J from potato tuber (Sharma et al., 2004)
O	culture filtrates from Streptomyces padanus (Huang et al., 2007) **negative effect:** salicylic acid (Quintanilla, 2002)	chitosan ElexaTM (Acar et al., 2008) cyclic lipopeptides from Pseudomonas: massetolide A (Tran Thi Thu, 2007) extracts from *Pseudomonas fluorescens* (Martinez and Osorio, 2007)

Tomato (target pathogen = *Phytophthora infestans***)**		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	*Bacillus cereus* (Silva et al., 2004) *Burkholderia* (Lozoya-Saldana et al., 2006), *Pseudomonas* (Lozoya-Saldana et al., 2006), *Streptomyces* (Lozoya-Saldana et al., 2006)	*Bacillus pumilus* (Yan et al., 2002) *Cellulomonas flavigena* (Lourenco Junior et al., 2006) *Pseudomonas fluorescens* (Yan et al., 2002) (Ha et al., 2007) (Tran Thi Thu, 2007) *Streptomyces* sp. AMG-P1 (Lee et al., 2005) *Aspergillus* sp., (Lourenco Junior et al., 2006) *Candida* sp. (Lourenco Junior et al., 2006) *Cryptococcus* sp. (Lourenco Junior et al., 2006) *Fusarium oxysporum* (Kim et al., 2007a) *Penicillium* sp. (Perez Mancia and Sanchez Garita, 2000) *Trichoderma harzianum* T39 (Ferrari et al., 2007)
B	Nochi leaf extract (Vanitha and Ramachandram, 1999)	capsidiol (El-Wazeri and El-Sayed, 1977) Elot-vis (Ferrari et al., 2007)
O	compost extracts (Zaller, 2006)	acibenzolar-S-methyl (Becktell et al., 2005) beta -amino butyric acid (Yan et al., 2002) Bion (benzothiadiazole) (Surviliene et al., 2003) bikaverin and fusaric acid (Son et al., 2008) cellulose (Perez Mancia and Sanchez Garita, 2000) chaetoviridin A (Park et al., 2005a) chitosan ElexaTM (Acar et al., 2008) Chitoplant (Ferrari et al., 2007) extracts from actinomycete isolates (Mutitu et al., 2008) extracts from *Bazzania trilobata* and *Diplophyllum albicans* (Tadesse et al., 2003) extract from *Gibberella zeae* (Kim et al., 1995) phosphate (Becktell et al., 2005)

Grapes (target pathogen = *Plasmopara viticola)*		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	Bacillus brevis (Schmitt et al., 2002) Bacillus subtilis (Serenade) (Schilder et al., 2002) Pseudomonas fluorescens (Rizoplan) (Kilimnik and Samoilov, 2000) (Rajeswari et al., 2008) Fusarium proliferatum (Falk et al., 1996) Trichoderma harzianum T39 (Vecchione et al., 2007) **little or no effect once in the field:** Bacillus licheniformis (Cravero et al., 2000) Biorange (Bacillus subtilis, Candida oleophila, Pseudomonas spp. and Streptomyces spp.) (Spera et al., 2003)	Alternaria alternata (Musetti et al., 2004) Fusarium proliferatum (Bakshi et al., 2001)
B	Croplife (citrus and coconut extract) (Schilder et al., 2002) Plantfood (foliar fertilizer) (Schilder et al., 2002) Milsana (giant knotweed extract) (Schilder et al., 2002) (Schmitt et al., 2002) neem (Rajeswari et al., 2008)	neem (Achimu and Schlosser, 1992) extract of giant knotweed (Schmitt, 1996)
O	acylbenzolar-s methyl (Dagostin et al., 2006) chitosan (Elexa) (Schilder et al., 2002) Mycosin (Angeli et al., 2006)	Alternaria alternata extracts (Musetti et al., 2006) EXP1, copper gluconate, salt of fatty acid, plant based alcohol extract (Dagostin et al., 2006)

Pearl millet *Pennisetum glaucum* **(**target pathogen = *Sclerospora graminicola***)**

	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	Bacillus pumilus strain INR7, strain SE34 (Raj et al., 2003) Bacillus subtilis (Raj et al., 2003) (Raj et al., 2005) Pseudomonas fluorescens (Umesha et al., 1998) (Latake and Kolase, 2007) Gliocladium virens (Arun et al., 2004) (Raj et al., 2005) Trichoderma harzianum (Raj et al., 2005) (Latake and Kolase, 2007) Trichoderma lignorum (Raj et al., 2005)	Pseudomonas fluorescens (Raj et al., 2004) Aspergillus flavus, Trichoderma harzianum and T. viride (Surender et al., 2005)
B		
O	milk (cow) (Arun et al., 2004)	

Other Vegetables and fruits

	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
Cauliflower and other crucifers (target pathogen = *Peronospora parasitica***)**		
M		Pseudomonas sp. XBC-PS (Li et al., 2007) Trichoderma harzianum (Pratibha et al., 2004)
B		
O	Bion (Pratibha et al., 2004) phosphonate (Kofoet and Fischer, 2007)	Bion (Gawande and Sharma, 2003)
Lettuce (*Bremia lactucae***)**		
M		
B		
O	phosphonate (Kofoet and Fischer, 2007) Trichodermin (Borovko, 2005) Pimonex, Timorex and also Alkalin potassium+silicon (Robak and Ostrowska, 2006)	
Melon / cucumber (target pathogen = *Pseudoperonospora cubensis***)**		
M		actinomycete (Shu and An, 2004) Bacillus strains, Z-X-3 and Z-X-10 (Li et al., 2003)
B		
O	phosphonate (Kofoet and Fischer, 2007)	attenuated cucumber mosaic cucumovirus (Qin et al., 1992) chitosan ElexaTM (Acar et al., 2008) compost extracts (Winterscheidt et al., 1990)

Miscellaneous

M	Azotobacter slight effect against Peronospora arborescens on opium poppy (Chakrabarti and Yadav, 1991)	Cladosporium chlorocephalum against Peronospora arborescens (Chaurasia and Dayal, 1985) (Nalini and Rai, 1988)
B		
O	phosphonate against Peronospora destructor on Allium (Kofoet and Fischer, 2007)	DL- beta -amino-n-butyric acid (BABA) against Plasmopara helianthi (Tosi and Zazzerini, 2000)

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6.14. Appendix 14. Inventory of biocontrol agents (M: microbials; B: botanicals; O: others) described in primary literature (1973-2008) for successful effect against Monillia in laboratory experiments and field trials on selected crops

Apple (target pathogens = *Monilinia fructigena; M. laxa*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M		Aureobasidium pullulans, Epicoccum purpurascens, Sordaria fimicola and Trichoderma polysporum (Falconi and Mendgen, 1994) Metschnikowia pulcherrima and (Spadaro et al., 2002), (Migheli et al., 1997) Pseudomonas syringae (Migheli et al., 1997) **(M laxa)** Pantoea agglomerans strain EPS125 (Bonaterra et al., 2004)

Apricot (target pathogen = *Monilinia laxa*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**bacteria** Burkholderia gladii OSU 7 (Altindag et al., 2006) (Esitken et al., 2005) Bacillus OSU-142 and Pseudomonas BA-8 (Esitken et al., 2005)	**bacteria** Pantoea agglomerans strain EPS125 (Bonaterra et al., 2003) (***M fructicola***) Bacillus subtillis strain B3 (Pusey and Wilson, 1984) **fungi, yeasts** Metschnikowia pulcherrima (Grebenisan et al., 2006) (Grebenisan et al., 2008)

Plum (target pathogen = *Monilinia laxa*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M		**bacteria** Pantoea agglomerans strain EPS125 (Bonaterra et al., 2004) Epicoccum nigrum (Larena et al., 2001) Penicillium frequentans (Cal et al., 2002) **(M fructicola)** Bacillus subtillis strain B3 (Pusey and Wilson, 1984)

remark: no B or O for any of the crops

Cherry (target pathogens = *Monilia fructicola, M. laxa, M. fructigena*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**bacteria** (***M laxa***) Serenade (Bacillus subtilis QRD137) (Haseli and Weibel, 2002), **fungi, yeasts** (***M fructicola***) Cryptococcus laurentii (Tian et al., 2004a) Epicoccum purpurascens (E. nigrum) and Gliocladium roseum (Wittig et al., 1997) (***M laxa***) Aureobasidium pullulans isolates 533 and 547 (Schena et al., 2003)	**bacteria** (***M fructicola***) Bacillus subtilis (15 isolates) (Utkhede and Sholberg, 1986) Burkholderia cepacia, Bacillus subtilis (Fan et al., 2001) (***M laxa***) Risoplan (Pseudomonas fluorescens), Gaupsin (Pseudomonas aureofaciens = P. chlororaphis) (Shevchuk, 2006) Pantoea agglomerans strain EPS125 (Bonaterra et al., 2004) **fungi, yeasts** (***M fructicola***) Candida guilliermondii, Kloeckera apiculata, Debaryomyces hansenii (Fan et al., 2001) Cryptococcus infirmo-miniatus (Spotts et al., 2002) Cryptococcus laurentii (Wang and Tian, 2007) (Qin and Tian, 2005) (Qin et al., 2006) (***M laxa*** + ***M fructigena***) Trichodex (Trichoderma harzianum) (Cardei, 2001)
B	(***M laxa***) Trilogy (azadirachtin-free Neemoil) (Haseli and Weibel, 2002)	
O	(***M laxa***) lime sulphur (calcium polysulfide) (Haseli and Weibel, 2002)	

Blueberry (target pathogen = *Monilinia vaccinii-corymbos*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**bacteria** Serenade (Bacillus subtilis QRD137) (Ngugi et al., 2005) (Dedej et al., 2004) (Scherm and Stanaland, 2001) (Schilder et al., 2006)	**bacteria** BlightBan (Pseudomonas fluorescens A506) (Scherm et al., 2004) Serenade (Bacillus subtilis QRD137) (Scherm et al., 2004) (Thornton et al., 2008) Pantoea agglomerans C9-1Sv (Thornton et al., 2008) **fungi, yeasts** Gliocladium roseum H47 (Thornton et al., 2008)
B		
O		

Peach / Nectarine (target pathogens = *Monilia fructicola, M. laxa, M. fructigena*)		
	Success in field trials	**Success in laboratory conditions** (*in vitro* and/or *in planta* in controlled conditions)
M	**bacteria** (***M fructicola***) Pseudomonas corrugata and P. cepacia; Bacillus subtilis strain B3 (Smilanick et al., 1993) **fungi, yeasts** Epicoccum nigrum (Mari et al., 2007) (***M laxa***) Epicoccum nigrum (Cal et al., 2004) (Foschi et al., 1995) (Larena et al., 2005) (Madrigal et al., 1994) (Melgarejo et al., 1986) Penicillium frequentans (Cal et al., 1990) (Melgarejo et al., 1986) (Pascual et al., 2000) Penicillium purpurogenum (Melgarejo et al., 1986)	**bacteria** (***M fructicola***) Rizo-N (Bacillus subtilis) (El-Sheikh Aly et al., 2000) Bacillus amyloliquefaciens C06 (Zhou et al., 2008) Bacillus subtillis (Gueldner et al., 1988) Bacillus subtillis strain B3 (Pusey et al., 1986) (Pusey et al., 1988) (Pusey, 1989) (Pusey and Wilson, 1984) Pantoea agglomerans strain IC1270 (Ritte et al., 2002) Pseudomonas syringae NSA-6 (Zhou et al., 1999) (***M laxa***) Pantoea agglomerans strain EPS125 (Bonaterra et al., 2003) (Bonaterra et al., 2004) **fungi, yeasts** (***M fructicola***) Candida sp(Karabulut et al., 2002) Cryptococcus laurentii (Yao and Tian, 2005) Debaryomyces hansenii (Stevens et al., 1997) (Stevens et al., 1998) Kloeckera apiculata yeast (Karabulut and Baykal, 2003) (McLaughlin et al., 1992) Muscodor albus (Mercier and Jimenez, 2004) (Schnabel and Mercier, 2006) Pichia membranaefaciens (Xu et al., 2008) Trichoderma atroviride (2 isolates), T viride & Rhodotorula sp (Hong et al., 1998) Plant-guard (T. harzianum) (El-Sheikh Aly et al., 2000) (***M laxa***) Penicillium purpurogenum (Foschi et al., 1995) (Larena and Melgarejo, 1996) Penicillium frequentans (Foschi et al., 1995) Trichoderma koningii (Foschi et al., 1995)
B	(	
O	Sodium bicarbonate enhances effect of Aspire (Candida oleophila) (Droby et al., 2003)	Extract from Bacillus subtillis (McKeen et al., 1986) Iturin peptides from Bacillus subtillis (Gueldner et al., 1988) Sodium bicarbonate (Wisniewski et al., 2001); enhances effect of Aspire (Candida oleophila) (Droby et al., 2003)

Successful inhibition *in vitro* (target pathogen = *B. cinerea*)	
M	**Bacteria** Pseudomonas syringae pv. morsprunorum BA35, Erwinia herbicola C9- (Voland et al., 1999) Serratia plymuthica, isolate EF-5 (Frommel et al., 1991) **Fungi + yeasts** Penicillium frequentans (Cal and Melgarejo, 1994) (Melgarejo et al., 1985) Aspergillus flavus, Epicoccum nigrum, Penicillium chrysogenum and P. purpurogenum (Melgarejo et al., 1985)
B	
O	*Thiolutin* from Streptomyces luteosporeus (Deb and Dutta, 1984)

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6.15. Appendix 15. Primary literature (2007-2009) on biological control against *Fusarium oxysporum*

Abo-Elyousr, K. A. M. and H. M. Mohamed (2009). "Biological Control of Fusarium Wilt in Tomato by Plant Growth-Promoting Yeasts and Rhizobacteria." Plant Pathology Journal 25(2): 199-204.
Three plant growth-promoting yeasts and two rhizobacteria were tested for controlling tomato wilt caused by Fusarium oxysporum L sp. lycopersici under greenhouse and field conditions. Under greenhouse and field conditions, all treatments were significantly reduced disease severity of tomato wilt relative to the infected control. The highest disease reductions in pots (75.0, 67.4%) and field (52.5, 42.4%) were achieved by Azospirillum brasilense and Bacillus subtilis compared to infected control. Under field condition all treatments produced the highest tomato yield compared to the control plants inoculated with the pathogen
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Al-Jedabi, A. A. (2009). "Biological control of Fusarium root-rot of sorghum." Research Journal of Agriculture and Biological Sciences 5(4): 465-473.
several crops including sorghum that result in low grain yield. All antagonists showed inhibition of mycelial growth of F. oxysporum and the maximum inhibition was recorded when Bacillus subtilis as biocontrol agent (67.7%). The in vitro root colonization study demonstrated that after four days of germination, the cell counts obtained from the roots have increased and the maximum count is achieved by B. subtilis (16.9*105 cfu/cm root). The greenhouse pot experiment demonstrated that T. viride and B. subtilis resulted in more than 80% suppression of root rot. The reduction in fresh weight of roots amounted to 93.6% in the control treatment inoculated with F. oxysporum alone, whereas 71.1% reduction in fresh root weight was recorded for the treatments inoculated with both the pathogen and B. subtilis; 66.8% reduction in fresh root weight was recorded for the treatments inoculated with both the pathogen and T. harzianum. Root dry weight of the control treatment inoculated with only F. oxysporum decreased by 94.5% in relation to the non-inoculated control. Among the potential biological control agents in this study, B. cereus resulted in 42.3 reduction in root dry weight compared to the 94.5% reduction recorded for the control inoculated with F. oxysporum alone. 100% of the roots from the control treatment (F. oxysporum only) rendered growth of F. oxysporum compared to an incidence ranging from 20 to 55% for plants treated with B. subtilis, B. lecheniformis, B. cereus, T. harzianum and T. viride. Both chlorophyll fractions increased when treated with antagonist and the maximum enhancement was recorded when Bacillus subtilis used as antagonist compared with that of control. The maximum values of the carbohydrate components were recorded when Bacillus subtilis used as antagonist relative to those of control.

Amini, J. (2009). "Induced Resistance in Tomato Plants Against Fusarium Wilt Invoked by Nonpathogenic Fusarium, Chitosan and Bion." Plant Pathology Journal 25(3): 256-262.
The potential of nonpathogenic Fusarium oxysporum strain Avr5, either alone or in combination with chitosan and Bion, for inducing defense reaction in tomato plants inoculated with E oxyysporum f. sp lycopersici, was studied in vitro and glasshouse conditions. Application Bion at concentration of 5, 50, 100 and 500 mu g/ml, and the highest concentration of chitosan reduced in vitro growth of the pathogen. Nonpathogenic F oxysporum Avr5 reduced the disease severity of Fusarium wilt of tomato in split plants, significantly. Bion and chitosan applied on tomato seedlings at concentration 100 mu g a.i./plant; 15, 10 and 5 days before inoculation of pathogen. All treatments significantly reduced disease severity of Fusarium wilt of tomato relative to the infected control. The biggest disease reduction and increasing tomato growth belong to combination of nonpathogenic Fusarium and Bion. Growth rate of shoot and root markedly inhibited in tomato plants in response to tomato Fusarium wilt as compared with healthy control. These results suggest that reduction in disease incidence and promotion in growth parameters in tomato plants inoculated with nonpathogenic Fusarium and sprayed with elicitors could be related to the synergistic and cooperative effect between them, which lead to the induction and regulation of disease resistance. Combination of elicitors and nonpathogenic Fusarium synergistically inhibit the growth of pathogen and provide the first experimental support to the hypothesis that such synergy can contribute to enhanced fungal resistance in tomato. This chemical could provide a new approach for suppression of tomato Fusarium wilt, but its practical use needs further investigation.

Anand, R., S. Kulothungan, et al. (2009). "Assay of chitinase and beta-1,3 glucanase in Gossypium hirsutum seedlings by Trichoderma spp. against Fusarium oxysporum." International Journal of Plant Sciences (Muzaffarnagar) 4(1): 255-258.
wilt in cotton. In this regard, the six species of Trichoderma, namely T. viride, T. virens [Gliocladium virens], T. hamatum, T. harzianum, T. koningii and T. reesi, were evaluated for its biocontrol properties and induction of defence-related enzymes, namely chitinase and beta1-3-glucanase in 30 days old cotton (G. hirsutum) seedlings. Trichoderma spp. could efficiently control the growth rate of F. oxysporum. In vitro assay of chitinase and beta-1,3-glucanase revealed the maximum production by T. harzianum (56 U/ml) and T. hamatum (80 U/ml), respectively. It also produced appreciable quantities of defence enzymes. The maximum induction of chitinase and beta1-3-glucanase in plants was found to be 80 U/ml when challenged with T. harzianum, in addition to the enhancement of defence mechanism in plants. Trichoderma spp. improved the germination rate of seedlings.

Anitha, A. and M. Rebeeth (2009). "Self-fusion of Streptomyces griseus enhances chitinase production and biocontrol activity against Fusarium oxysporum f. sp. lycopersici." Biosciences, Biotechnology Research Asia 6(1): 175-180.

Protoplasts were isolated from Streptomyces griseus (MTCC - *4734) strain using lysing enzymes and self-fusion of Streptomyces griseus protoplasts was carried out using 50% polyethylene glycol (MW 1000, Sigma Chemicals Co., USA) in protoplast buffer. The regenerated 8 self fused Streptomyces griseus were studied detailed for chitinase production and biocontrol activity. Parent strain (PSg) showed protein content of 2.7 mg/ml with chitinase activity of 120 IU/ml. High chitinase activity was measured in the culture filtrates of most of the self-fusants (87%) than the parent. Among the fusants, the strain SFSg 5 produced protein content of 7.8 mg/ml, maximum chitinase activity of 283.3 IU/ml with a two-fold increase as compared to the parent strain. All the self-fusants exhibited increased antagonistic activity against F. oxysporum f. sp. lycopersici than the parent. Maximum inhibition (82%, 80%) of mycelial growth of F. oxysporum was recorded with fusant of SFSg 5, SFSg 1 as against 61.1% with PSg. The result implies that, the self-fused Streptomyces griseus resulted in appreciable increase of chitinase production and biocontrol activity also the significance of the protoplast fusion technique, which could successfully be used to develop hybrid strains also for commercial formulation.

Baysal, O., M. Calskan, et al. (2008). "An inhibitory effect of a new Bacillus subtilis strain (EU07) against Fusarium oxysporum f. sp. Radicis-lycopersici." PMPP Physiological and Molecular Plant Pathology 73(1/3): 25-32.

destructive disease on tomato (Lycopersicon esculentum Mill.) transplant seedlings and the causal organism of crown and root rot of tomato plants growing in southern coast greenhouses of Turkey. An isolate of Bacillus subtilis (EU07) identified by the 16s RNA region code gene was selected as the best antagonist and evaluated against FORL in vitro studies. Strain EU07 at 106 CFU ml-1 was able to reduce disease incidence by 75%, when applied as an inoculant. It efficiently inhibited FORL compared to the control and QST 713 (AgraQuest, Davis, CA) whose inhibition ratio was only 52% in vivo. Random amplified polymorphic DNA analyses showed banding (genetic) differences between EU07 and QST 713 whereas there were no differences between DNAs of strains that have high homology to genes involved in the synthesis of antibiotics fengycin, bacillomycin and iturin when screened by oligonucleotide primers designed based on sequence information obtained from the NCBI database. Furthermore, one specific fragment in the EU07 genome showed the highest similarity to YrvN protein by 99% and AAA ATPase domain protein (72.2%) after amplifying oligonucleotide primers that are specific to the N-acyl-homoserine lactonase (HLS) gene as a biocontrol activity marker. These results suggested an effect of EU07 on control FORL by YrvN protein as subunit of protease enzyme. Furthermore, this fragment associated with HLS gene may be a potential molecular marker for selecting effective biological control agent belonging to Bacillus in order to control soilborne pathogens such as Fusarium, suggesting impairment in FORL invasion by signaling in the plant rhizosphere.

Bernal-Vicente, A., M. Ros, et al. (2009). "Increased effectiveness of the Trichoderma harzianum isolate T-78 against Fusarium wilt on melon plants under nursery conditions." Journal of the Science of Food and Agriculture 89(5): 827-833.

BACKGROUND: The use of isolates of the genus Trichoderma to control Fusarium wilt in melon plants is one of the most recent and effective alternatives to chemical treatments. In this work we have studied the immobilization of the isolate Trichoderma harzianum T-78 on different carriers as an efficient method to control vascular Fusarium wilt of melon in nurseries. Different formulations were developed: liquids (spore suspension, guar gum and carboxymethylcellulose) and solids (bentonite, vermiculite and wheat bran). RESULTS: The introduction of F. oxysporum resulted in a significant decrease in seedling fresh weight. The treatments which gave a lesser reduction in weight and showing a greater biocontrol effect were the liquid conidial suspension and the solid treatments with bentonite and superficial vermiculite. Microbiological analyses revealed that the conidial suspension and all the solid treatments, except wheat bran, significantly decreased F. oxysporum populations. Of all the treatments assayed, bentonite produced the greatest decline in the F. oxysporum population. CONCLUSIONS: The most effective treatments against Fusarium wilt on melon plants were the solid treatments bentonite and superficial vermiculite. These two treatments gave the greatest plant weight, the lowest percentage of infected plants and the greatest T. harzianum population throughout the assay. (C) 2009 Society of Chemical Industry

Boureghda, H. and Z. Bouznad (2009). "Biological control of Fusarium wilt of chickpea using isolates of Trichoderma atroviride, T. harzianum and T. longibrachiatum." Acta Phytopathologica et Entomologica Hungarica 44(1): 25-38.

The efficiency of the antagonist species Trichoderma atroviride (strains Ta.3, Ta.7 and Ta.13), T. harzianum (Th.6, Th.12, Th.15, Th.16 and Th.18) and T. longibrachiatum (TL.1, TL.2, TL.4, TL.5, TL.8, TL.9, TL.10, TL.11, TL.14 and TL17) against Fusarium wilt (caused by Fusarium oxysporum f.sp. ciceris) was compared using in vitro- and in vivo-based bioassay. A significant decrease of both growth and conidia production of the pathogen was obtained compared to the control. The highest percentages of diameter colony reduction and conidial production were obtained with Ta.13, causing 65.64% reduction in colony diameter (direct confrontation), 48.71% reduction in colony diameter (indirect confrontation), and a complete

inhibition of conidial production. Once more in direct confrontation, T. atroviride overgrowth the pathogen colony and sporulate above. The seed treatment by Trichoderma spp. isolates before sowing in a soil already infested by the pathogen led to a significant decrease of disease severity compared to the untreated control. The weakest index of disease severity was obtained with Ta.13, which caused 83.92% reduction compared to the control. The most effective isolates in protecting chickpea seedlings against the disease were Ta.3, Ta.7 and Ta.13 as well as Th.16. The reduction of disease severity was associated with an increase of the vegetal growth including the stem height as well as the plant fresh and dry weights.

Casimiro Michel-Aceves, A., M. Antonio Otero-Sanchez, et al. (2009). "In vitro biocontrol of Fusarium subglutinans (Wollenweb. and Reinking) Nelson, Toussoun and Marasas and F. oxysporum Schlecht., causal agents of "Witches' broom" of mango (Mangifera indica L.) by Trichoderma spp." Revista Mexicana de Fitopatologia 27(1): 18-26.
The antagonistic effect of native strains of Trichoderma spp. was evaluated in vitro against Fusarium oxysporum (Fo) and Fusarium subglutinans (Fs), causal agents of mango "witches' broom". Ten strains of the antagonistic fungus were isolated, one of which was selected and identified to the species level (T. harzianum); this species showed the highest percentage of antagonism inhibiting mycelial growth of Fo by 62.9% and 42.0% of Fs. In dual Cultures between Fo and/or Fs with the selected strains of Trichoderma, the time for the first contact for Fo was between 3 and 4 days, and between 2 and 3 for Fs. The greatest intersection area (0.87 cm) was observed in T. lignorum against Fo, while the intersection area in Fs with the native strain Thzn-2 was 0.85 cm. Native strains Thzn-2 and Thzcf-12, and the commercial one showed antagonism class 2, being able to stop growth of both plant pathogens. Strain Thzn-2 is promising as an alternative for biocontrol of Fo and Fs; however, it is necessary to evaluate it under field conditions.

Chebotar, V. K., N. M. Makarova, et al. (2009). "Antifungal and phytostimulating characteristics of Bacillus subtilis Ch-13 rhizospheric strain, producer of bioprepations." Applied Biochemistry and Microbiology 45(4): 419-423.
Bacillus subtilis Ch-13 industrial strain was shown to have a wide spectrum of antagonistic activities against different species of phytopathogenic fungi and bacteria. The B. subtilis Ch-13 strain produces lytic enzymes; cyanide and other antifungal metabolites; stimulates plant growth, producing phytohormones-auxin derivatives. This strain by 2.5 times reduced the quantity of tomato plants infected with phytopathogenic fungus Fusarium oxysporum during inoculation. Fungi abundance on roots with bacterial inoculation was 6.9 times less than in the absence of inoculation. The application of detected antifungal metabolites as biochemical markers for the strain enables to control the stability of physiologic and biochemical characteristics of the producer, and ensures a rapid quality assay of biopreparations with high performance liquid chromatography (HPLC).

Chen, L. and W. Chen (2009). "Genome shuffling enhanced antagonistic activity against Fusarium oxysporum f. sp. melonis and tolerance to chemical fungicides in Bacillus subtilis BS14." Journal of Food, Agriculture & Environment 7(2): 856-860.
enhance antagonistic activity against Fusarium oxysporum f. sp. melonis (FOM) and tolerance to two chemical fungicides. Strain BS14 was identified as a strain of Bacillus subtilis by the analysis of 16S rDNA sequences. A stable recombinant F35 was obtained after three rounds of shuffling. Antagonistic activity of recombinant F35 against FOM was increased by 34.52% and 65.48% compared to that of the parent strain HN8-7 with highest activity and another parent strain utilized, BS14. The tolerance to chemical fungicides was also significantly improved (p0.05) compared to that of strain BS14. Reduction of FOM of 94% was observed by using recombinant F35, which was increased by 45% compared to that of strain BS14 (p0.05) and no significant differences (p>0.05) compared to that of thiophanate methyl (MRL). Reduction of FOM of 100% was dramatically observed by using an integrated treatment combining MRL (50% of usual dosage) with recombinant F35. Strain F35 with these improved traits would be a promising biocontrol agent in the control of FOM. Here genome shuffling was proved to be a practical methodology for strain improvement of antagonistic microorganism Bacillus subtilis BS14 for enhancing antagonistic activity against FOM and tolerance to chemical fungicides.

Clematis, F., M. L. Gullino, et al. (2009). "Antagonistic activity of microorganisms isolated from recycled soilless substrates against Fusarium crow rot." Protezione delle Colture(3): 29-33.
We report the results obtained in biological control trials against crown and root rot of tomato incited by Fusarium oxysporum f. sp. radicis lycopersici by using microorganisms isolated from soilless cultivation systems that showed suppressiveness against this disease. Among the tested microorganisms belonging to fluorescent bacteria (32 isolates) and to fungi belonging to Trichoderma (39 isolates) and Fusarium (38 isolated), 5 bacteria and 6 fungi showed a good activity against the pathogen. Such strains will be used in greenhouse trials, under situations closer to the field, in order to evaluate their potential to be adopted under practical conditions.

Eden Paredes-Escalante, J., J. Armando Carrillo-Fasio, et al. (2009). "Antagonistic microorganismos for control of the fungal complex that cause wilt in chickpea (Cicer arietinum L.) in the state of Sinaloa, Mexico." Revista Mexicana de Fitopatologia 27(1): 27-35.

The antagonistic activity in vitro of microorganisms isolated from chickpea rhizosphere, was evaluated against Fusarium oxysporum, Sclerotium rolfsii, and Rhizoctonia solani, causal agents of chickpea wilt. The native strains with the higher percentage of pathogen mycelial growth inhibition were selected and identified as Trichoderma lignorum (CIAD 06-540903), T. harzianum (CIAD 05-550903), Bacillus subtilis (CIAD-940111), and Pseudomonas fluorescens (CIAD-990111). These strains and a commercial strain of T. harzianum (T-22) were mixed with Glomus intraradices and their effectiveness to reduce chickpea wilt was compared against a chemical treatment (PCNB) and all absolute control in the field. The seed was treated with the microorganisms before sowing and evaluations of disease severity were conducted each 15 days, while root colonization by the antagonistic microorganisms was assessed 45 days after sowing. Colonization of T, harzianum CIAD 05-550903 + G. infraradices was 33 x 10(3) ufc/g fresh root-75% and B. subtilis + G. intraradices was 1.3 x 10(8) Ufc/g fresh root-75%; while the combination P.fluorescens + G. intraradices was 1.4 x 10(7) Ufc/g fresh root-88%. These treatments also showed a reduction of disease severity in 64, 57, and 51%, respectively in comparison with the control.

El-Khallal, S. M. (2007). "Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid & salicylic acid): 2 - changes in the antioxident enzymes, phenolic compounds and pathogen related-proteins." Australian Journal of Basic and Applied Sciences 1(4): 717-732.

Induction of plant defense against pathogen attack is regulated by a complex network of different signals. In the present study interaction between hormonal signals [jasmonic acid (JA) or salicylic acid (SA)] and bioagent [arbuscular mychorrhiza (AM) fungi] was used as new strategy to enhance tomato defense responses against wilt disease caused by Fusarium oxysporum (Fo). Thus changes in various physiological defenses including antioxidant enzymes, phenolic compounds and pathogenesis related (PR) proteins were investigated in leaves of tomato plants. Results appeared that production of reactive oxygen species (ROS), mainly H2O2 and O2 increasing the time of infection. Application with bioagent AM fungi and/or hormonal elicitors (JA & SA) markedly decreased these levels, while LOX activity greatly increased as compared with infected control. SA - treated plants had the highest MDA level but JA+AM fungi treated plants recorded the highest LOX activity. Infection by Fusarium oxysporm significantly increased activity of antioxidant enzymes (SOD, APX and CAT) in tomato leaves at different stages of growth. The highest activity was recorded in leaves of AM fungi+JA-treated plants, while treatments with SA especially when applied alone markedly decreased H2O2 scavenging enzymes (APX and CAT) and greatly increased SOD activity. Thus, imbalance between H2O2 - generation and scavenging enzymes in leaves may reflect a defense mechanism in tomato or a pathogenicity strategy of the fungus. Levels of certain phenolic acids greatly changed in tomato leaves in response to Fusarium oxysporum, AM fungi and hormonal elicitors. Benzoic and Galleic acids contents markedly decreased, however, contents of coumaric, cinnamic, chlorogenic and ferulic acids increased in leaves of all treatments. Also, activity of lignification enzymes POX, PPX and PAL significantly increased in leaves of infected tomato plants. JA-treated plants caused the highest POX and PPX activities, while SA-treated plants having the highest PAL activities. High accumulation of phenolic compounds and activity POX, PPX and PAL in these plants may reflect a component of many defense signals activated by bioagent and hormonal inducers which leading to the activation of power defense system in tomato against attack. Analysis of protein electrophoresis revealed that interaction between hormone signal (JA & SA) and bioagent AM fungi mediating the expression of the majority of different PR-proteins leading to increasing defense mechanism against Fusarium oxysporum infection. Thus, induction of protein bands of molecular weights 35, 33, 32, 31 (PR-2, beta-1, 3 glucanase), 30.5 and 27 (PR-3,-4, chitinase) in infected leaves indicated the important role which played in disease resistance. Finally, the new mechanism of the combination strategy between bioagent and hormonal signals (either synergistically or antagonistically) played important roles for increasing various defense systems and altering expression of defense genes which leading to different PR-proteins working together to increased resistance in tomato plants against wilt disease caused by Fusarium oxysporum. In addition, results revealed that defense mechanism in plants treated with AM fungi and JA are more effective than AM fungi plus SA-treated plants.

Floch, G. l., J. Vallance, et al. (2009). "Combining the oomycete Pythium oligandrum with two other antagonistic fungi: root relationships and tomato grey mold biocontrol." Biological Control 50(3): 288-298.

To reduce Pythium oligandrum biocontrol variability and improve its efficacy, experiments were performed by combining the oomycete with two other antagonistic fungi, Fusarium dishes, Fo47 or T. harzianum hyphae destroyed P. oligandrum cells by antibiosis and mycoparasitism processes; in the rhizosphere of tomato plants (Lycopersicon esculentum), the same antagonistic features were observed. However, in the rhizosphere, hyphae are frequently separated by a certain distance; this allows the coexistence and the persistence of the three microorganisms on the root systems. When introduced in the rhizosphere, Fo47 and P. oligandrum were able to penetrate the root tissues with Fo47 limited to the epidermal and upper layers of cortical cells while P. oligandrum colonized deeper tissue at a faster rate. The two antagonists were killed in few days within roots following elicited plant-defense reactions. T. harzianum was not able to penetrate root tissues. Root colonization with either P.oligandrum alone or in combination with Fo47 and/or T. harzianum resulted in systemic plant resistance which provided plant protection against Botrytis cinerea infection of leaves. The level of control and the expression of pathogenesis-related proteins (PR-proteins) in leaves were similar whatever the antagonistic microbial treatment applied to roots.

Gay, M. I. T., Anonymous, et al. (2009). Substrates containing a Trichoderma asperellum strain for biological control of Fusarium and Rhizoctonia, Universidad de Barcelona.
The strain of Trichoderma asperellum T34(2) CECT No. 20417 is useful for preparing substrates for biological control of vascular fusariose and death of plants caused by Rhizoctonia solani. The substrates can be peats, composts (hardwood compost, pine bark compost, cork compost, sludge compost from sewage treatment plants, garden residues, etc.) or formulations based on CPV-type compost (compost+peat+vermiculite). The fact that the substrates suppress both Fusarium oxysporum f. sp. lycopersici and Rhizoctonia solani provides an advantage in comparison with other substrates known in prior art. Another advantage is that the use of methyl bromide, a highly harmful product for the environment, in the control of vascular fusariose is avoided.

Huang, X., J. Luo, et al. (2009). "Isolation and bioactivity of endophytic fungi in Derris hancei." Journal of South China Agricultural University 30(2): 44-47.
Derris hancei Hemsl. The antagonism of endophytic fungi against fungal pathogens was tested in vitro. Penicillium sp. Q1, Rhizoctonia sp. S1, Phomopsis sp. N2, and Corticium sp. F1 isolated from the caudex of D. hancei, and Penicillium sp. Q2 isolated from the leaf, inhibited the hyphal growth of Colletotrichum gloeosporioides Penz, Fusarium oxysporum f. niveum (E. F. Smith) Snyber et Hansen, Rhizoctonia sp. S1 against Colletotrichum orbiculare Arx, and Phomopsis sp. N2 against Colletotrichum musae (Berk1 & Curt1) Arx1 on dual culture with inhibition index II. It was reported that endophytic fungus in D.hancei could produced antibacterial substances in this paper. The culture filtrates of Penicillium sp. Q2 treated in 48 h after treatment possessed 100.00% of adjusted mortality against the 2nd larvae of Spodoptera litura by leaves disc feeding bioassays, and 75.10% against Lipaphis erysimi Kaltenbach (apterous adult) by insect-soaking method, respectively, which showed that the activity of Penicillium sp. Q2 was higher than that of other endophytic fungi.

Jadeja, K. B. and D. M. Nandoliya (2008). "Integrated management of wilt of cumin (Cuminum cyminum L.)." Journal of Spices and Aromatic Crops 17(3): 223-229.
Four components of integrated management namely, soil solarization, crop rotation, chemicals and biocontrol agents were tested under field condition at Junagadh (Gujarat) for the management of wilt of cumin (Cuminum cyminum) caused by Fusarium oxysporum f. sp. cumini. Growing of sorghum (Sorghum bicolor) or maize (Zea mays) during kharif season did not reduce wilt incidence during the following rabi season. Soil solarization with 25 m LLDPE plastic cover for 15 days in summer proved most effective in reducing wilt incidence to 26.27% as against 44.90% in non-solarization and increasing yield to 396 kg ha-1 as against 286 kg ha-1 in non-solarized plots. Application of carbendazim granules @ 10 kg ha-1 one month after sowing or Trichoderma viride in organic carrier @ 62.5 kg ha-1 at sowing time were also effective. Integrating soil solarization followed by growing of sorghum in kharif and application of either carbendazim granules @ 10 kg ha-1 one month after sowing or application of T.viride in organic carrier @ 62.5 kg ha-1 was effective for the management of cumin wilt.

Kamilova, F., S. Validov, et al. (2009). Biological control of tomato foot and root rot caused by Fusarium oxysporum f.sp. radicis-lycopersici by Pseudomonas bacteria. Proceedings of the Second International Symposium on Tomato Diseases, Kusadasi, Turkey, 8-12 October 2007.
Rhizobacteria are a natural and most suitable source for the isolation of potential microbiological control agents that can protect plants from soilborne pathogens and consequently improve crop quality and yield. The beneficial effect of such bacteria on plant health depends in many cases on their ability to aggressively colonize the rhizosphere and compete with the indigenous, including pathogenic, microflora for nutrients and niches on the plant root. Bacterial strains Pseudomonas chlororaphis PCL1391 and P. fluorescens WCS365 employ antibiosis and induced systemic resistance, respectively, to control tomato foot and root rot (TFRR) caused by phytopathogenic fungus Fusarium oxysporum f.sp. radicis-lycopersici (Forl). For the selection of biocontrol bacteria acting via the mechanism "competition for nutrients and niches" we have developed an enrichment method for enhanced tomato root tip colonizers, starting from a crude mixture of rhizobacteria coated on the seed, using a sterile quartz sand/plant nutrient solution gnotobiotic system. As a result of this enrichment procedure, and subsequent tests on competitive tomato root tip colonization, the strongly competitive biocontrol strains P. fluorescens PCL1751 and P. putida PCL1760 were isolated. Both strains effectively suppress TFRR under soil and hydroponic cultivation conditions.

Kamilova, F., S. Validov, et al. (2009). "Biological control of tomato foot and root rot caused by Fusarium oxysporum f.sp. radicis-lycopersici by Pseudomonas bacteria." Acta Horticulturae(808): 317-320.
isolation of potential microbiological control agents that can protect plants from soilborne pathogens and consequently improve crop quality and yield. The beneficial effect of such bacteria on plant health depends in many cases on their ability to aggressively colonize the rhizosphere and compete with the indigenous, including pathogenic, microflora for nutrients and niches on the plant root. Bacterial strains Pseudomonas chlororaphis PCL1391 and P. fluorescens WCS365 employ antibiosis and induced systemic resistance, respectively, to control tomato foot and root rot (TFRR) caused by phytopathogenic fungus Fusarium oxysporum f.sp. radicis-lycopersici (Forl). For the selection of biocontrol bacteria acting via the mechanism "competition for nutrients and niches" we have developed an enrichment method for enhanced tomato root tip colonizers, starting from a crude mixture of rhizobacteria coated on the seed, using a sterile quartz sand/plant nutrient solution gnotobiotic system. As a result of this enrichment procedure, and subsequent tests on competitive tomato root tip

colonization, the strongly competitive biocontrol strains P. fluorescens PCL1751 and P. putida PCL1760 were isolated. Both strains effectively suppress TFRR under soil and hydroponic cultivation conditions.

Kannan, V. and R. Sureendar (2009). "Synergistic effect of beneficial rhizosphere microflora in biocontrol and plant growth promotion." Journal of Basic Microbiology 49(2): 158-164.
Biological systems are getting more relevance than chemical control of plant pathogens as they are not only eco-friendly and economic in approach but are also involved in improving the soil consistency and maintenance of natural soil flora. Plant growth promoting rhizosphere microorganisms were isolated from three different tree rhizospheres using selective culture media. Five microorganisms were selected from each rhizosphere soil based on their efficiency and screened for their ability to promote plant growth as a consortium. Each of the developed consortium has a phosphate solubilizer, nitrogen fixer, growth hormone producer, heterotrophic member and an antagonist. The plant growth promoting ability of the microbial members present in the consortium was observed by estimating the IAA production level and also by the nitrogenase activity of the nitrogen fixers. The biocontrol potentiality of the consortium and the antagonist present in the consortium were checked by both dual plate assay and cross-streaking technique. Consortial treatments effected very good growth promotion in Lycopersicon esculentum Mill and the treated plants also developed resistance against wilt pathogen, Fusarium oxysporum f. sp. lycopersici though the effect was well pronounced with consortium developed from Santalum album.

Li, J., Q. Yang, et al. (2009). "Evaluation of biocontrol efficiency and security of A Bacillus subtilis strain B29 against cucumber Fusarium wilt in field." China Vegetables(2): 30-33.
cucumerinum, was isolated from cucumber rhizosphere. After twice of 4-field-plot experiments, the control efficiencies of 100, 250 and 500 dilution times to cucumber Fusarium wilt were 70.3-88.2%, 62.3-85.9%, and 54.7-80.6%, respectively. The average efficiency of field trials with B29 was 84.9% during 2 years and the yield of cucumber increased by 12.57%. The acute toxicity of Bacillus subtilis strain B29 to big mouse through its mouth and skin was examined, and the LD50 was more than 5000 mg/kg. The application of strain B29 on cucumber, tomato, bean and seed pumpkin was safe based on the observed seedling rate, growth and development.

Liu, Q., J. C. Yu, et al. (2009). "Antagonism and Action Mechanism of Antifungal Metabolites from Streptomyces rimosus MY02." Journal of Phytopathology 157(5): 306-310.
The genus of Streptomyces, a saprophytic Gram-positive bacterium, has properties, which make them useful as pharmaceutical and biocontrol agents. A streptomyces strain MY02 from soil samples showed significant antagonism against 14 plant pathogenic fungi including Fusarium oxysporum f. sp. cucumarinum. Antifungal metabolite(s) SN06 from the culture of the strain MY02 were extracted with n-butanol and purified by silica gel column chromatography. The minimum concentration of SN06 inhibiting any visible fungal growth of F. oxysporum f. sp. cucumarinum is 12.5 mu g/ml by twofold serial dilutions method. The mycelia of F. oxysporum f. sp. cucumarinum treated with SN06 were observed under the normal optics microscope. The results showed that some cells of hyphae began to dilate and formed some strings of beads. The cytoplasm oozed out of the cells with the culture time and so most of the cells became empty. The hyphae broke into many segments and then collapsed after 48 h. After inoculated in potato dextrose medium for 48 h, the filtrate of mycelia treated with 1% NaCl containing 12.5 mu g/ml SN06 was scanned using ultraviolet spectrophotometer and absorption peak at 260 nm showed that the mycelia cell membrane of F. oxysporum f. sp. cucumarinum was broken and that nucleic acid oozed out of the cell.

Maina, M., R. Hauschild, et al. (2008). "Protection of tomato plants against fusaric acid by resistance induction." Journal of Applied Biosciences(JABs) 1: 18-31.
Objectives: The rhizobacteria Bacillus sphaericus B43, Pseudomonas fluorescens T58, and P. putida 53 are able to induce systemic resistance (ISR) against Fusarium oxysporum f.sp. lycopersici (FOL) in tomato. This study investigated if the ISR reduced the damage by the toxin fusaric acid (FA) produced by FOL. Methodology and Results: The bacteria were applied to the rhizosphere of tomato plants. Chlorophyll content and ion leakage were determined after placing the leaf discs in FA. Active oxygen species (AOS), superoxide and hydrogen peroxide levels were determined in leaves of plants injected with FA. Activities of superoxide dismutase (SOD), ascorbate (AS) and guaiacol peroxidases (GPX) involved in AOS metabolism were quantified. In untreated plants, FA led to high ion leakage and chlorophyll degradation caused by H_2O_2 accumulation. All the bacteria treatments decreased the chlorophyll degradation. Ion leakage was reduced by treatment with P. fluorescens T58 and B. sphaericus B43, while P. putida 53 was less effective. Treatment of plants with bacteria resulted in increased superoxide contents, but varying over time. Increased SOD and GPX activities in untreated plants were suppressed after bacteria treatment. Plants treated with P. fluorescens T58 showed only a transient increase in superoxide. P. putida 53-treated plants removed AOS, but high initial superoxide levels led to membrane damages. Treatment with B. sphaericus B43 suppressed the effects of FA, but AOS metabolism showed only slight alterations. Conclusions and potential applications of findings: ISR could also protect plant tissues from damage by pathogen toxins, which is a potential new dimension to the known mechanisms of action of biological control agents.

Martinez-Medina, A., J. A. Pascual, et al. (2009). "Interactions between arbuscular mycorrhizal fungi and Trichoderma harzianum and their effects on Fusarium wilt in melon plants grown in seedling nurseries." Journal of the Science of Food and Agriculture 89(11): 1843-1850.

BACKGROUND: Biological control through the use of Trichoderma spp. and arbuscular mycorrhizal fungi (AMF) could contribute to a reduction of the inputs of environmentally damaging agrochemical products. The objective of this study was to evaluate the interactions between four AMF (Glomus intraradices, Glomus mosseae, Glomus claroideum and Glomus constrictum) and Trichoderma harzianum for their effects on melon plant growth and biocontrol of Fusarium wilt in seedling nurseries. RESULTS: AMF colonisation decreased fresh plant weight, which was unaffected by the presence of T. harzianum. Dual inoculation resulted in a decrease in fresh weight compared with AMF-inoculated plants, except for G. intraradices. AMF colonisation level varied with the AM endophyte and was increased by T. harzianum, except in G. mosseae-inoculated plants. Negative effects of AMF on T. harzianum colony-forming units were found, except with G. intraradices. AMF alone were less effective than T. harzianum in suppressing disease development. Combined inoculation resulted in a general synergistic effect on disease control. CONCLUSION: Selection of the appropriate AMF species and its combination with T. harzioanum were significant both in the formation and effectiveness of AM symbiosis and the reduction of Fusarium wilt incidence in melon plants. The combination of G. intraradices and T. harzianum provided better results than any other tested. (C) 2009 Society of Chemical Industry

Matar, S. M., S. A. El-Kazzaz, et al. (2009). "Antagonistic and inhibitory effect of Bacillus subtilis against certain plant pathogenic fungi, I." Biotechnology 8(1): 53-61.
subtilis isolates (B1 to B14), obtained from different Egyptian sites, were tested against six fungal isolates belonging to four different genera, Rhizoctonia solani, Helminthosporium spp., Alternaria spp. and Fusarium oxysporum. Cultural, morphological and physiological characteristics of these isolates were found to be identical to B. subtilis. Four B. subtilis isolates (B1, B4, B7, B8) had more antagonistic effect on all fungal isolates. Supernatant of B. subtilis isolate B7 had antagonistic effect on 6 fungal isolates but it was more effective on Helminthosporium spp., Alternaria spp. and F. oxysporum. B. subtilis as well as isolate B7 showed effectiveness in reducing disease incidence and severity levels of tomato plants when added to the F. oxysporum and R. solani-infested soil. Also, it stimulated the growth of tomato plants compared to the other. HPLC analysis of the HCl precipitate of B.subtilis isolate B7 culture supernatant revealed that an identical pattern of five peaks to that of a purified preparation of iturin A was obtained.

Matar, S. M., S. A. El-Kazzaz, et al. (2009). "Bioprocessing and scaling-up cultivation of Bacillus subtilis as a potential antagonist to certain plant pathogenic fungi, III." Biotechnology 8(1): 138-143.
isolate G-GANA7 (GenBank accession No. EF583053), collected from Abo-Homos in Egypt, was tested against six fungal isolates belonging to four different genera, i.e. Rhizoctonia solani, Helminthosporium sp., Alternaria sp. and Fusarium oxysporum. B. subtilis isolate G-GANA7 was cultured in 3 litre bench-top New Brunswick Scientific BioFlow III bioreactor for producing the maximum yield of biomass and antifungal compound. Fed-batch processes were automated through a computer aided data bioprocessing system AFS-BioCommand multi-process management program to regulate the cell growth rate by controlling interactively the nutrient feed rate, temperature, pH and agitation speed based on dissolved oxygen. In batch cultivation, the process suffered from low yield of cell mass (3.2 g litre-1) and antifungal activity because of high initial glucose concentration followed by acetate formation which the causal agent for inhibition of cell growth. Constant and exponential fed-batch strategies were adopted to circumvent this potential problem. Fed-batch cultivation of B. subtilis was conducted at the specific growth rate of 0.13 and 0.1 h-1 for constant and exponential strategies, respectively. High cell density of 12.8 and 14.6 g litre-1 for both operations, with an overall biomass yield of 0.45 g g-1 was achieved. The inhibitory activity of antifungal in supernatant reached its maximum value of 2 and 2.2 cm for constant and exponential fed-batch cultivations.

Mazurier, S., T. Corberand, et al. (2009). "Phenazine antibiotics produced by fluorescent pseudomonads contribute to natural soil suppressiveness to Fusarium wilt." ISME Journal 3(8): 977-991.
Natural disease-suppressive soils provide an untapped resource for the discovery of novel beneficial microorganisms and traits. For most suppressive soils, however, the consortia of microorganisms and mechanisms involved in pathogen control are unknown. To date, soil suppressiveness to Fusarium wilt disease has been ascribed to carbon and iron competition between pathogenic Fusarium oxysporum and resident non-pathogenic F. oxysporum and fluorescent pseudomonads. In this study, the role of bacterial antibiosis in Fusarium wilt suppressiveness was assessed by comparing the densities, diversity and activity of fluorescent Pseudomonas species producing 2,4-diacetylphloroglucinol (DAPG) (phlD+) or phenazine (phzC+) antibiotics. The frequencies of phlD+ populations were similar in the suppressive and conducive soils but their genotypic diversity differed significantly. However, phlD genotypes from the two soils were equally effective in suppressing Fusarium wilt, either alone or in combination with non-pathogenic F. oxysporum strain Fo47. A mutant deficient in DAPG production provided a similar level of control as its parental strain, suggesting that this antibiotic does not play a major role. In contrast, phzC+ pseudomonads were only detected in the suppressive soil. Representative phzC+ isolates of five distinct genotypes did not suppress Fusarium wilt on their own, but acted synergistically in combination with strain Fo47. This increased level of disease suppression was ascribed to phenazine production as the phenazine-deficient mutant was not effective. These results suggest, for the first time, that redox-active phenazines produced by fluorescent pseudomonads contribute to the natural soil suppressiveness to Fusarium wilt disease and may act in synergy with carbon competition by resident non-pathogenic F. oxysporum.

Minerdi, D., S. Bossi, et al. (2009). "Volatile organic compounds: a potential direct long-distance mechanism for antagonistic action of Fusarium oxysporum strain MSA 35." Environmental Microbiology 11(4): 844-854.
Fusarium oxysporum MSA 35 [wild-type (WT) strain] is an antagonistic Fusarium that lives in association with a consortium of bacteria belonging to the genera Serratia, Achromobacter, Bacillus and Stenotrophomonas in an Italian soil suppressive to Fusarium wilt. Typing experiments and virulence tests provided evidence that the F. oxysporum isolate when cured of the bacterial symbionts [the cured (CU) form], is pathogenic, causing wilt symptoms identical to those caused by F. oxysporum f. sp. lactucae. Here, we demonstrate that small volatile organic compounds (VOCs) emitted from the WT strain negatively influence the mycelial growth of different formae speciales of F. oxysporum. Furthermore, these VOCs repress gene expression of two putative virulence genes in F. oxysporum lactucae strain Fuslat10, a fungus against which the WT strain MSA 35 has antagonistic activity. The VOC profile of the WT and CU fungus shows different compositions. Sesquiterpenes, mainly caryophyllene, were present in the headspace only of WT MSA 35. No sesquiterpenes were found in the volatiles of ectosymbiotic Serratia sp. strain DM1 and Achromobacter sp. strain MM1. Bacterial volatiles had no effects on the growth of the different ff. spp. of F. oxysporum examined. Hyphae grown with VOC from WT F. oxysporum f. sp. lactucae strain MSA 35 were hydrophobic whereas those grown without VOCs were not, suggesting a correlation between the presence of volatiles in the atmosphere and the phenotype of the mycelium. This is the first report of VOC production by antagonistic F. oxysporum MSA 35 and their effects on pathogenic F. oxysporum. The results obtained in this work led us to propose a new potential direct long-distance mechanism for antagonism by F. oxysporum MSA 35 mediated by VOCs. Antagonism could be the consequence of both reduction of pathogen mycelial growth and inhibition of pathogen virulence gene expression.

Nam, M. H., M. S. Park, et al. (2009). "Biological Control of Strawberry Fusarium Wilt Caused by Fusarium oxysporum f. sp fragariae Using Bacillus velezensis BS87 and RK1 Formulation." Journal of Microbiology and Biotechnology 19(5): 520-524.
Two isolates, Bacillus sp. BS87 and RK1, selected from soil in strawberry fields in Korea, showed high levels of antagonism towards Fusarium oxysporum f. sp. fragariae in vitro. The isolates were identified as B. velezensis based on the homology of their gyrA sequences to reference strains. BS87 and RK1 were evaluated for control of Fusarium wilt in strawberries in pot trials and field trials conducted in Nonsan, Korea. In the pot trials, the optimum applied concentration of BS87 and RK1 for pre-plant root-dip application to control Fusarium wilt was 10(5) and 10(6) colony-forming units (CFU)/ml, respectively. Meanwhile, in the 2003 and 2005 field trials, the biological control efficacies of formulations of RK1 were similar to that of a conventional fungicide (copper hydroxide) when compared with a non-treated control. The RK1 formulation was also more effective than BS87 in suppressing Fusarium wilt under field conditions. Therefore, the results indicated that formulations of B. velezensis BS87 and RK1 may have potential to control Fusarium wilt in strawberries.

Narayan, M., P. Tini, et al. (2009). "Biological and chemical management of tomato wilt caused by Fusarium oxysporum f.sp. lycopersici." Journal of Soils and Crops 19(1): 118-121.
Wilt of tomato is one of the most important known disease caused by Fusarium oxysporum f. sp. lycopersici. In the present study four bioagents (Trichoderma harzianum, T. viride, Bacillus subtilis and Pseudomonas fluorescens) and two fungicides (Carbendazim and Thiram) were evaluated both in vitro and in vivo conditions. In vitro evaluation, of Carbendazim (0.1%) completely inhibited the growth of tomato wilt pathogen Fusarium oxysporum f.sp. lycopersici and was found significantly superior over the rest of fungicides. While, among the biological agents Trichoderma viride was found significantly superior to the rest in checking the growth of pathogens and showed 85.69 per cent inhibition. In vivo under field condition, seedling dip treatment of Carbendazim (1 gl-1 water) was found most significant followed by Carbendazim+ T.viride (1+100 gl-1 water) and T. viride (100 gl-1 water) significantly reduced wilt incidence by 73.91, 69.56 and 68.11 per cent respectively as against 71.88 per cent wilting in control (under epiphytotic condition i.e. wilt sick soil).

Ortega-Morales, B. O., F. N. Ortega-Morales, et al. (2009). "Antagonism of Bacillus spp. Isolated from Marine Biofilms Against Terrestrial Phytopathogenic Fungi." Marine Biotechnology 11(3): 375-383.
We aimed at determining the antagonistic behavior of bacteria derived from marine biofilms against terrestrial phytopathogenic fungi. Some bacteria closely related to Bacillus mojavensis (three isolates) and Bacillus firmus (one isolate) displayed antagonistic activity against Colletotrichum gloeosporioides ATCC 42374, selected as first screen organism. The four isolates were further quantitatively tested against C. gloeosporioides, Colletotrichum fragariae, and Fusarium oxysporum on two culture media, potato dextrose agar (PDA) and a marine medium-based agar [yeast extract agar (YEA)] at different times of growth of the antagonists (early, co-inoculation with the pathogen and late). Overall antagonistic assays showed differential susceptibility among the pathogens as a function of the type of culture media and time of colonization ($P < 0.05$). In general, higher suppressive activities were recorded for assays performed on YEA than on PDA; and also when the antagonists were allowed to grow 24 h earlier than the pathogen. F. oxysporum was the most resistant fungus while the most sensitive was C. gloeosporioides ATCC 42374. Significant differences in antagonistic activity ($P < 0.05$) were found between the different isolates. In general, Bacillus sp. MC3B-22 displayed a greater antagonistic effect than the commercial biocontrol strain Bacillus subtilis G03 (KodiakA (R)). Further incubation studies and scanning

electronic microscopy revealed that Bacillus sp. MC3B-22 was able to colonize, multiply, and inhibit C. gloeosporioides ATCC 42374 when tested in a mango leaf assay, showing its potential for fungal biocontrol. Additional studies are required to definitively identify the active isolates and to determine their mode of antifungal action, safety, and biocompatibility.

Padghan, P. R. and M. M. Baviskar (2009). "Efficacy of bioagent and different root extracts for supression of chickpea wilt in vitro." Asian Journal of Bio Science 4(1): 56-58.
udid, sorghum (Sorghum bicolor), groundnut and mung bean and biological control agents (Trichoderma viride, T. harzianum, T lignorum and T. koningii) against the chickpea wilt pathogen, Fusarium oxysporum f.sp. ciceris (FOC), was studied in the laboratory. A lower radial mycelial growth and a higher inhibitory effect were recorded in sorghum root extract medium (28.00 mm and 54.34%), respectively, however, it was at par with groundnut root extract medium (30.00 mm and 51.08%), compared to the control (61.33 mm). In dual culture technique, the growth of FOC was restricted by T. viride (56.16%), followed by T. harzianum (50.57%). T. lignorum recorded the minimum zone of inhibition (40.45%).

Qiu, W., H. Huang, et al. (2009). "Screening of actinomycete against Fusarium oxysporum f. sp. cubense and identification of strain DA07408." Research of Agricultural Modernization 30(1): 126-128.
samples, and 8 of these strains showed significant activities against F. oxysporum f.sp. cubense. One actinomycete (DA07408) isolated from an arboretum in Danzhou, Hainan, China, exhibited marked antagonism towards F. oxysporum f.sp. cubense. The conditions for the fermentation of the actinomycete were optimized. Based on the morphological, physiological and biochemical characteristics of the strain, and on the analysis of 16S rDNA and phylogenetic tree, DA07408 was identified as Streptomyces olivochromogenes.

Raddadi, N., A. Belaouis, et al. (2009). "Characterization of polyvalent and safe Bacillus thuringiensis strains with potential use for biocontrol." Journal of Basic Microbiology 49(3): 293-303.
Sixteen Bacillus thuringiensis (Bt) strains were screened for their anti-insect, antibacterial and antifungal determinants by phenotypic tests and PCR targeting major insecticidal proteins and complements, chitinases, lactonases, beta-1,3-glucanases and zwittermicin A. Six strains had genes of at least two major insecticidal toxins and of insecticidal complements. With regard to fungal biocontrol, all the strains inhibited Fusarium oxysporum and Aspergillus flavus growth and four strains had all or most of the antifungal determinants examined, with strain Bt HD932 showing the widest antifungal activity spectrum. Autolysins, bacteriocin and AHL-lactonases were produced by all or most of the tested strains with different activity spectra including pathogens like Listeria monocytogenes. Safety evaluation was carried out via PCR by screening the B. cereus psychrotolerance-related genes, toxin genes and the virulence pleiotropic regulator plcR. Diarrheal enterotoxins and other toxin genes were widespread among the collection with strains Bt HD9 and H45 lacking psychrotolerance-related genes, while five strains were positive. Only three strains (BMG1.7, H172, H156) resulted positive with primer sets targeting partial or complete plcR gene. By Vero Cell Assays, Bt HD868 followed by Bt HD9 were shown to be the safest strains. These polyvalent and safe Bt strains could be very promising in field application.

Rasal, P. H., J. R. Shelar, et al. (2009). "Effect of endophytic antagonist on pigeonpea." Journal of Maharashtra Agricultural Universities 34(1): 52-53.
resistant (ICP 8863) and resistant (BDN2) cultivars of pigeon pea were screened against Fusarium oxysporum f. udum [F. udum]. The inoculation of endophytic antagonists into different cultivars of pigeon pea improved germination, plant height, branching, nodulation, root length and biomass production, and reduced wilt intensity significantly over the un-inoculated control. Among the inoculants, Pseudomonas-2 was the most beneficial, followed by Pseudomonas-3, Bacillus-3, Pseudomonas-1, and Bacillus-1 and -2. Antagonists isolated from resistant cultivar were the most beneficial, followed by antagonists from the moderately resistant cultivar, and antagonists isolated from the susceptible cultivar.

Recep, K., S. Fikrettin, et al. (2009). "Biological control of the potato dry rot caused by Fusarium species using PGPR strains." Biological Control 50(2): 194-198.
In this study, a total of 17 Plant Growth Promoting Rhizobacteria (PGPR) strains, consisting of eight different species (Bacillus subtilis, Bacillus pumilus, Burkholderia cepacia, Pseudomonas putida, Bacillus amyloliquefaciens, Bacillus atrophaeus, Bacillus macerans and Flavobacter balastinium), were tested for antifungal activity in in vitro (on Petri plate) and in vivo (on potato tuber) conditions against Fusarium sambucinum, Fusarium oxysporum and Fusarium culmorum cause of dry rot disease of potato. All PGPR strains had inhibitory effects on the development of at least one or more fungal species on Petri plates. The strongest antagonism was observed in B. cepacia strain OSU-7 with inhibition zones ranging from 35.33 to 47.37 mm. All PGPR strains were also tested on tubers of two potato cultivars 'Agria' and 'Granola' under storage conditions. Only B. cepacia strain OSU-7 had significant effects on controlling potato dry rot caused by three different fungi species on the two potato cultivars. There were no significant differences in rot diameters among the treatments in comparison to the negative control (with water). This is the first study showing that B. cepacia has great potential to be used as effective biocontrol agent of Fusanium dry rot of potatoes (F. oxysporum and F culmorum) under storage conditions. (C) 2009 Elsevier Inc. All rights reserved.

Riaz, T., S. N. Khan, et al. (2009). "Effect of co-cultivation and crop rotation on corm rot disease of Gladiolus." Scientia Horticulturae 121(2): 218-222.

Field and pot experiments were conducted to evaluate the effect of co-cultivation and crop rotation on the growth and corm rot disease of gladiolus (Gladiolus grandiflorus sect. Blandus) cv. Aarti caused by Fusarium oxysporum f.sp. gladioli (Massey) Snyd. and Hans. In the field experiment, gladiolus was co-cultivated with 10 agricultural/horticultural crops viz. Allium cepa L., Brassica campestris L., Capsicum annuum L., Eruca sativa Mill., Helianthus annuus L., Tagetes erectus L., Zea mays L., Vinca rosea L. and Rosa indica L., in a soil infested with F. oxysporum. All the crops except V. rosea and R. indica reduced disease incidence. The effect of H. annuus and T. erectus was significant and more pronounced than other co-cultivated crops. In general, root and shoot dry biomass, corm fresh weight, number of cormlets and number of flowers per spike decreased as compared to the un-inoculated monoculture gladiolus treatment (negative control) but these parameters enhanced as compared to the F. oxysporum inoculated monoculture gladiolus treatment (positive control). In a pot experiment, all the crops of the field experiment except V. rosea and R. indica were sown in rotation with gladiolus. Pot grown plants of different species were harvested at maturity and the soil was inoculated with F oxysporum. Gladiolus was cultivated I week after inoculation. Disease incidence was significantly suppressed in all the treatments ranging from 29% to 53%. The highest suppression of disease incidence was recorded in T erectus (53%) followed by B. campestris (49%). The effect of preceding crops on various vegetative parameters was similar in the pot experiment to that of the field experiment. The present study suggests that corm rot disease of gladiolus can be managed by mixed cropping of H. annuus and T erectus or cultivation of T. erectus and B. campestris in rotation. (c) 2009 Elsevier B.V. All rights reserved.

Saidi, N., S. Kouki, et al. (2009). "Characterization and selection of Bacillus sp strains, effective biocontrol agents against Fusarium oxysporum f. sp radicis-lycopersici, the causal agent of Fusarium crown and root rot in tomato." Annals of Microbiology 59(2): 191-198.
The antagonistic activities of 20 Bacillus isolates were tested with dual culture and greenhouse conditions against Fusarium oxysporum f. sp. radicis-lycopersici (FORL) race 0, the causal agent of Fusarium crown and root rot of tomato. Under dual culture, 10 isolates inhibited mycelial growth > 38% and the most effective inhibited fungal growth > 50%. The 20 Bacillus isolates were tested for production of volatiles, cyanide, antibiotics, and phosphorus solubilisation; 15 isolates produced volatiles that inhibited growth of pathogens, 9 isolates produced cyanide, 10 produced antibiotics, and five solubilised phosphorus. Greenhouse experiments with the same 20 isolates revealed the effectiveness of 12 strains, which increased the percentage of healthy plants in the tested cultivar from 66 to 96%. The best disease control was achieved by isolates B11, B5, B17, and B18. However, B11 and B17 were the only isolates that produced cyanide, antibiotics, solubilised phosphate and showed 44% inhibition of fungal growth. The selected strains could be considered in plant growth promotion and biological disease control.

Shi, Y. W., K. Lou, et al. (2009). "Isolation, quantity distribution and characterization of endophytic microorganisms within sugar beet." African Journal of Biotechnology 8(5): 835-840.
The present investigation was undertaken in order to document the spectrum of endophytes colonizing healthy leaves of sugar beet cultivars in Xinjiang Province (China) and to determine the degree of colonization at three growth stages. From the 360 sugar beet leaf and root segments incubated, 221 bacterial isolates, 34 fungal isolates and 5 actinomycete isolates were obtained. Of all the isolates, 7 bacterial species and 6 fungal species were identified. The actinomycete isolates were characterized as Streptomyces griseofuscus and Streptomyces globisporus. There were significant differences between microorganisms, stages of growth, and stages of microorganism interaction. The number of microorganisms isolated increased during the growth period of the sugar beet. At the same time, the number of microorganisms affecting different parts of the sugar beet tissue was quite different. The greatest number of microorganisms was found in the secondary root emergence zone of the sugar beet tissue. Endophytic microorganisms in sugar beet promote growth and increase the yield of the beet.

Son, S. H., Z. Khan, et al. (2009). "Plant growth-promoting rhizobacteria, Paenibacillus polymyxa and Paenibacillus lentimorbus suppress disease complex caused by root-knot nematode and fusarium wilt fungus." Journal of Applied Microbiology 107(2): 524-532.
Paenibacillus strains against disease complex caused by Meloidogyne incognita and Fusarium oxysporum f. sp. lycopersici interactions. Methods and Results: Paenibacillus strains were collected from rotten ginseng roots. The strains were tested under in vitro and pots for their inhibitory activities, and biocontrol potential against disease complex caused by M. incognita and F. oxysporum f. sp. lycopersici on tomato. In in vitro experiments, among 40 tested strains of Paenibacillus spp., 11 strains showed antifungal and nematicidal activities against F. oxysporum f. sp. lycopersici and M. incognita, respectively. Paenibacilluspolymyxa GBR-462; GBR-508 and P. lentimorbus GBR-158 showed the strongest antifungal and nematicidal activities. These three strains used in pot experiment reduced the symptom development of the disease complex (wilting and plant death), and increased plant growth. The control effects were estimated to be 90-98%, and also reduced root gall formation by 64-88% compared to the untreated control. Conclusion: The protective properties of selected Paenibacillus strains make them as potential tool to reduce deleterious impact of disease complex plants. Significance and Impact of the Study: The study highlights biocontrol potential of Paenibacillus strains in management of disease complex caused by nematode-fungus interaction.

Srinivasan, K., G. Gilardi, et al. (2009). "BACTERIAL ANTAGONISTS FROM USED ROCKWOOL SOILLESS SUBSTRATES SUPPRESS FUSARIUM WILT OF TOMATO." Journal of Plant Pathology 91(1): 147-154.

Five bacterial E,trains (FC-6B, FC-7B, FC-8B, FC-9B and FC-24B) isolated from used rockwool soilless substrates were identified using 16S ribosomal DNA (16S rDNA) sequence analysis as belonging to the Pseudomonas genus. Seven glasshouse trials were conducted in order to evaluate the efficacy of these bacteria strains (Pseudomonas putida FC-6B, Pseudomonas sp. FC-7B, Pseudomonas putida FC-8B, Pseudomonas sp. FC-9B and Pseudomonas sp. FC-24B) together with Achromobacter sp. AM1 and Serratia sp. DM1 obtained from suppressive sod, against Fusarium wilt of tomato. Two commercial bioproducts, Trichoderma harzianum T22 (RootShield) and Pseudomonas chlororaphis MA 342 (Cedomon) were also evaluated. Different treatment strategies including soil application (10(7) and 10(8) cfu ml(-1)) were adopted in different glasshouse trials (Trial I to VI) to test the efficacy of the bacterial strains against Fusarium wilt. Root dipping was used in Trial VII (10(8) and 10(9) cfu ml(-1)). The lowest: disease incidence (3.3) was recorded with a single application of P. putida FC-6B at 10(8) cfu ml(-1). Similar results were obtained with the same bacteria when the concentration was decreased to 10(7) cfu ml(-1) but an increasing number of applications was required. The highest plant biomass (50.3 g/plant) was recorded in the P. putida FC-8B treatment (Trial III). In conclusion, the current study showed the potential biocontrol activity of bacterial strains FC-6B, FC-7B, FC-8B, FC-9B and FC-24B isolated from re-used rockwool soilless substrates against Fusarium wilt disease, and the growth promoting activity of these strains on tomato plants.

Srivastava, D. K., A. K. Singh, et al. (2009). "Efficacy of bio-control agents and seed dressing fungicides against damping off of tomato." Annals of Plant Protection Sciences 17(1): 257-258.

in Unao, Madhya Pradesh, India, during 2005-06 yielded associated pathogen on PDA medium. The antagonistic activity of biological control agents against Fusarium oxysporum f.sp. lycopersici was determined using dual culture method. All the antagonists and fungicide inhibited the mycelial growth of Fusarium, however, Trichoderma viride caused maximum inhibition of mycelial growth. Trichoderma viride, Trichoderma harzianum, Gliocladium virens, carbendazim and thiram, which showed significant in vitro inhibition of Fusarium were tested in the field. Maximum increase in seed germination (83.4%), seedling survival (79.0) and plant height (6.32 cm) over the control was observed when treated with Trichoderma viride followed by Trichoderma harzianum, carbendazim, thiram, and Gliocladium virens.

Thanh, D. T., L. T. T. Tarn, et al. (2009). "Biological Control of Soilborne Diseases on Tomato, Potato and Black Pepper by Selected PGPR in the Greenhouse and Field in Vietnam." Plant Pathology Journal 25(3): 263-269.

Bacterial wilt, Fusarium wilt and Foot rot caused by Ralstonia solanacearum, Fusarium oxysporum, and Phytophthora capsici respectively, continue to be severe problems to tomato, potato and black pepper growers in Vietnam. Three bio-products, Bacillus vallismortis EXTN-1 (EXTN-1), Bacillus sp. and Puenibacillus sp. (ESSC) and Bacillus substilis (MFMF) were examined in greenhouse bioassay for the ability to reduce bacterial wilt, fusarium wilt and foot rot disease severity. While these bio-products significantly reduced disease severities, EXTN-1 was the most effective, providing a mean level of disease reduction 80.0 to 90.0% against bacterial wilt, fusarium wilt and foot rot diseases under greenhouse conditions. ESSC and MFMF also significantly reduced fusarium wilt, bacterial wilt and foot rot severity under greenhouse conditions. Bio-product, EXTN-1 with the greatest efficacy under greenhouse condition was tested for the ability to reduce bacterial wilt, fusarium wilt and foot rot under field condition at Song Phuong and Thuong Tin locations in Ha Tay province, Vietnam. Under field condition, EXTN-1 provided a mean level of disease reduction more than 45.0% against all three diseases compared to water treated control. Besides, EXTN-1 treatment increased the yield in tomato fruits 17.3% than water treated control plants.

Wu, H., X. Yang, et al. (2009). "Suppression of Fusarium wilt of watermelon by a bio-organic fertilizer containing combinations of antagonistic microorganisms." BioControl 54(2): 287-300.

the crop has been grown for many seasons. Its occurrence results in a severely decreased watermelon crop. The goal of this study was to assess the capability of a new product (bio-organic fertilizer) to control the wilt in Fusarium-infested soil. Pot experiments were conducted under growth chamber and greenhouse conditions. The results showed that the fertilizer controlled the wilt disease. Compared with control pots, the incidence rates of Fusarium wilt at 27 and 63 days following treatment of the plants with the bio-organic fertilizer at a rate of 0.5% (organic fertilizer+antagonistic microorganisms, including 3*109 CFU g-1 respectively, in both the growth chamber and greenhouse settings. The activities of antioxidases (catalase, superoxide dismutase and peroxidase) in watermelon leaves increased by 38.9, 150 and 250%, respectively. In the roots, stems and leaves, the activity of beta-1,3-glucanase (pathogenesis-related proteins) increased by 80, 1140 and 100% and that of chitinase increased by 240, 80, and 20%, respectively, while the contents of malondialdehyde fell by 56.8, 42.1 and 45.9%, respectively. These results indicate that this new fertilizer formula is capable of protecting watermelon from Fusarium oxysporum f.sp. niveum. The elevated levels of defense-related enzymes are consistent with the induction and enhancement of systemic acquired resistance of plant.

Wu, Q., H. Zeng, et al. (2009). "Stability of fermentation broth of actinomycete strain WZ162 resistance to Fusarium oxysporum f.sp. cubense of banana." Guangxi Agricultural Sciences 40(4): 366-369.

The fermentation broth of actinomycete strain WZ162 has strong inhibiting effect against Fusarium oxysporum f.sp. cubense of banana. Under different conditions, the stabilities of fermentation broth of WZ162 were detected. The results showed that the fermentation broth of WZ162 had better heat stability when temperature of water bath was below 80C. The antibiotics ingredient of fermentation broth would not be changed and can maintain the antifungal activity under conditions of sun light and ultraviolet rays. Under acid and neutrality conditions, the inhibition rate of fermentation broth against Focr4 was 24.92%-34.73% and 11.21%-25.39%, respectively. Therefore, the stability of fermentation broth in acid was better than that of neutrality. When the fermentation broth with pH 1-12 were treated with different time in 100C water bath, the inhibition rate was obviously lower than that of the treatments without water bath, and the stability of fermentation broth with pH 1 was the best.

Yin, X., D. Chen, et al. (2009). "An endophytic Erwinia chrysanthemi strain antagonistic against banana fusarium wilt disease." Chinese Journal of Biological Control 25(1): 60-65.

An endophytic strain E353 was obtained from the pseudostem of healthy banana plant in a field heavily infected with Fusarium oxysporum f. sp. cubense (FOC). Antagonism of the strain against FOC was tested via dual-culture, inhibition test on conidia germination, and pot trials. Results showed that E353 effectively inhibited mycelium growth and conidia germination. Efficacy of strain E353 to control the wilt disease was 60.67% in pot trials. Strain E353 was identified as Erwinia chrysanthemi according to its characteristics in morphology, physiology, biochemistry and 16S rDNA sequence.

Zhong, X., M. Liang, et al. (2009). "Study on the inhibition of Trichoderma sp. against Fusarium oxysporum f. sp. cubense in banana." Journal of Fruit Science 26(2): 186-189.

effective antagonist against Fusarium oxysporum f. sp. cubens, was isolated and identified as Trichoderma sp. based upon 18S rDNA gene analysis. With solid and liquid cultures, the inhibitive efficacy to the growth of Fusarium oxysporum f. sp. cubens was primarily studied. The experimental results showed that the cells of Fusarium oxysporum f. sp. cubens were completely covered by short fiber-like hyphace and spore stem of G2 within 7 days in the dual culture plate, and in the antagonist plate, the average rate of inhibitory by the culture solution of G2 was about 90.4%, the average rate of the inhibitory by volatile substance reached 68.3%. After 10 days' incubation with 20% (v/v) fungal strain G2, the melt of the pathogenic mycel and spore were observed in the liquid culture containing 1.0*107 cfu . L-1 G2 can strongly inhibit the growth of Fusarium oxysporum f. sp. cubens.

Zhu, H., Y. Ma, et al. (2009). "Control effect of combining biocontrol strains against Fusarium oxysporium f. sp. niveum and Verticillium dahliae." Journal of Northwest A & F University - Natural Science Edition 37(7): 152-156.

Objective: Five actinomycetes strains having certain inhibiting capability were screened as material to study the control effect of the actinomycetes and five combinations on watermelon Fusarium wilt and Eggplant Verticillium wilt, and to filter the combining biocontrol strains which have better biocontrol efficacy and growth promotion. Method: The biocontrol efficacy and growth promotion of single and combining strains were analyzed by antagonistic activity in vitro and manual inoculation in vivo. Result: Strain SC11 and SE2 had significant inhibiting effect on Fusarium oxysporium f. sp. niveum and Verticillium dahliae in vitro. Inhibiting rate on conidia germination was also high; in greenhouse experiment, 84.93% control ratio to Fusarium oxysporium f. sp. niveum and 71. 48% to Verticillium dahliae were found by C2; The fermentation broth of C3 had the most significant effect for every index of watermelon. The effect on reduction intensity of watermelon rootage was obvious. For eggplant, the growth promotion was only inferior to strain SF6. Conclusion: These results suggested that the control effect and growth promotion of combining biocontrol strains are significantly higher than individual, and combining strains express complementary biocontrol activities by collaboration. There is no correlation between the number of strains and control effect, only proper combinations of biocontrol strains can enhance disease control effect.

6.16. Appendix 16. Number of references retrieved by using the CAB Abstracts database in order to review scientific literatures on augmentation biological control in selected crops.

GRAPEVINE*

Key words	Total records	1998-2008
Biological control	1644	-
Augmentative biological control	7	6
Augmentation biological control	10	6
Inoculative biological control	4	1
Inundative biological control	7	3
Insects biological control	773	373
Mites biological control	320	190
Total references to be examined	**28**	**579**

* Survey include records for **grapevine**, **grape** and **vineyard**.

APPLE

Key words	Total records	1998-2008
Biological control	3971	-
Augmentative biological control	13	10
Augmentation biological control	18	9
Inoculative biological control	5	3
Inundative biological control	10	2
Insects biological control	2310	817
Mites biological control	981	258
Total references to be examined	**46**	**1099**

PEAR

Key words	Total records	1998-2008
Biological control	1270	-
Augmentative biological control	3	2
Augmentation biological control	2	1
Inoculative biological control	1	1
Inundative biological control	3	1
Insects biological control	756	325
Mites biological control	174	61
Total references to be examined	**9**	**391**

CORN*

Key words	Total records	1998-2008
Biological control	6828	-
Augmentative biological control	19	14
Augmentation biological control	38	18
Inoculative biological control	18	8
Inundative biological control	39	17
Insects biological control	4293	1682
Mites biological control	250	66
Total references to be examined	**114**	**1805**

* Survey include records for **corn** and **maize**.

WHEAT

Key words	Total records	1998-2008
Biological control	5250	-
Augmentative biological control	9	7
Augmentation biological control	13	6
Inoculative biological control	1	1
Inundative biological control	8	3
Insects biological control	2307	866
Mites biological control	157	66
Total references to be examined	**31**	**949**

CARROT

Key words	Total records	1998-2008
Biological control	360	-
Augmentative biological control	1	1
Augmentation biological control	1	1
Inoculative biological control	1	1
Inundative biological control	0	0
Insects biological control	179	62
Mites biological control	20	8
Total references to be examined	**3**	**73**

ONION

Key words	Total records	1998-2008
Biological control	810	-
Augmentative biological control	2	2
Augmentation biological control	3	3
Inoculative biological control	3	3
Inundative biological control	1	1
Insects biological control	532	233
Mites biological control	187	62
Total references to be examined	**9**	**304**

6.17. Appendix 17. Collection of data on augmentative biological control of pests in grapevine. Each table refers to a group of biocontrol agents.

17.1 Parasitoid Hymenoptera: *Trichogramma* spp. (Trichogrammatidae) [10 species]

References	**Species of biocontrol agent**	**Species of insect pest**	**Taxonomic category of pests**	**Country**	**Type of augmentation**	**Type of test**	**Efficacy of biocontrol agents***	**Additional information and results**
Remund & Bigler, 1986	*T. dendrolimi*	*Eupoecilia ambiguella* (grape berry moth)	Lepidoptera: Tortricidae			Lab		Evaluation of biological parameters
				Switzerland		Field		Evaluation of biological parameters
	T. maidis			Switzerland	Inundative	Field	+	
Segonca & Leisse, 1989	*T. semblidis*	*Eupoecilia ambiguella* and *Lobesia botrana*	Lepidoptera: Tortricidae	Ahr Valley, Germany	Inundative	Field	+	
Glenn & Hoffmann, 1997	*T. carverae*	*Epiphyas postvittana* (light brown apple moth)	Lepidoptera: Tortricidae	Victoria, Australia	Inundative	Field (small blocks)	+	
Basso et al., 1998	*T. pretiosum* *T. exiguum*	*Argyrotaenia sphaleropa* (South American tortricid moth), *Bonagota cranaodes* (Brasilian apple leafroller)	Lepidoptera: Tortricidae	Uruguay		Lab		Evaluation of biological parameters
Basso et al., 1999	*T. pretiosum* *T. exiguum*	*A. sphaleropa* *B. cranaodes*	Lepidoptera: Tortricidae	Uruguay	Inundative	Field	+	
Garnier-Geoffroy et al., 1999	*T. brassicae*	*Lobesia botrana*	Lepidoptera: Tortricidae			Lab	-	Evaluation of allelocemical relations
Hommay et al., 2002	*T. evanescens* and *T. cacoeciae* (two strains)	*Lobesia botrana*	Lepidoptera: Tortricidae	France	Inundative	Field	+ -	+ as % parasitization. - as % grapes attacked.
Nagargatti et al., 2002	*T. minutum*	*Endopiza viteana* (grape berry moth)	Lepidoptera: Tortricidae	Pennsylvania, USA		Field	+	+ as natural parasitism. Inundative releases of *T. minutum* in border rows is suggested
Thomson & Hoffmann, 2002	*T. carverae*	*Epiphyas postvittana* (light brown apple moth)	Lepidoptera: Tortricidae	Victoria, Australia		Lab Field		Assessment of quality indicators
Nagargatti et al., 2003	*T. minutum*	*Endopiza viteana*	Lepidoptera: Tortricidae	Pennsylvania, USA	Inundative	Field	+	Parasitoids released in border rows
Zimmermann, 2004	*Trichogramma* spp.	*Lobesia botrana* and *Eupoecilia ambiguella*	Lepidoptera: Tortricidae	Germany	Inundative	Field		Commercialized to be used in home garden
Begum et al., 2006	*T. carverae*	*Epiphyas postvittana*	Lepidoptera: Tortricidae	Australia	Inundative	Greenhouse/ Field	+	Ground-cover plant species identified to improve performance of mass released parasitoids.

El-Wakeil et al., 2008	*T. evanescens*	*Lobesia botrana* (European grape berry moth)	Lepidoptera: Tortricidae	Egypt	Inundative	Field	+	Parasitism > 97% and reduction percents of infestation reached 96.8%

* + means effective, - means not effective biocontrol agent.

17.2 Parasitoid Hymenoptera: Encyrtidae [4 species], Pteromalidae [1 species]

Reference	Species of biocontrol agent	Species of insect pest	Taxonomic category of pests	Cuontry	Type of augmentation	Type of test	Efficacy of biocontrol agents*	Additional information and results
Walton & Pringle, 1999	*Coccidoxenoides peregrinus* (Encyrtidae)	*Planococcus ficus* (vine mealybug)	Hemiptera: Pseudococcidae	South Africa		Lab		Compatibility of fungicides and incompatibility of insecticides with augmentative releases
Walton & Pringle, 2004	*Coccidoxenoides perminutus* (Encyrtidae)	*Planococcus ficus* (vine mealybug)	Hemiptera: Pseudococcidae	South Africa	Inundative	Field	+	Mass release was at least as effective as the chemical control
Abd-Rabou, 2005	*Anagyrus kamali* (Encyrtidae)	*Maconellicoccus hirsutus*	Hemiptera: Pseudococcidae	Egypt	Inundative	Field	+	It is concluded that the releases of parasitoids were suitable for control.
Daane et al., 2006	*Anagyrus pseudococci* (Encyrtidae)	*Planococcus ficus*	Hemiptera: Pseudococcidae	California	Inoculative	Field	+	Promising results. Commercial products are not yet available.
Daane et al., 2008	*Anagyrus pseudococci* (Encyrtidae)	*Planococcus ficus*	Hemiptera: Pseudococcidae	Israel	Inoculative	Field	+	Promising results. Commercial products are not yet available.
Kapongo et al., 2007	*Muscidifurax raptor* (Pteromalidae)	*Ceratitis capitata* (Mediterranean fruit fly)	Diptera: Tephritidae	Canada	Inundative	Field Lab cages	+	*M. raptor* constitutes a promising biocontrol agent in vineyards.

* + means effective, - means not effective biocontrol agent.

17.3 Predators of mites. Acari: Phytoseidae.

References	Species of biocontrol agent	Species of mite pest	Taxonomic category of pests	Country	Type of augmentation	Type of test	Efficacy of biocontrol agents*	Additional information and results
Boller et al., 1988	*Typhlodromus pyri*	*Panonychus ulmi*, *Tetranychus urticae*	Acari: Tetranychidae	Switzerland	Inoculative	Field		Inoculative release of *T. pyri* along with the increase of the internal ecological diversity achieved by proper management of the green cover plants will have a strong influence on predator densities.

Camporese & Duso, 1996	*Typhlodromus pyri*, *Amblyseius andersoni*, *Kampimodromus aberrans*	*Panonychus ulmi*	Acari: Tetranychidae	Italy	Inoculative	Field	+	Different colonization patterns on three grape varieties (with different pubescent leaf undersurfaces). The high competitiveness of *K. aberrans* over the other 2 phytoseid species is a major factor in selecting predatory species for inoculative releases.
Takahashi et al., 1998	*Phytoseiulus persimilis*	*Tetranychus kanzawai*	Acari: Tetranychidae	Japan	Inundative	Field (grape in green house)	+	Release of *P. persimilis* onto the grass ground cover in the spring. No chemical control was required.
Duso & Vettorazzo, 1999	*Kampimodromus aberrans*, *Typhlodromus pyri*	*Panonychus ulmi*, *Eotetranychus carpini* *Calepitrimerus vitis*	Acari: Tetranychidae Acari: Eriophyidae	Veneto, Italy	Inoculative	Field (A)	+	Releases were successful and the predators became more abundant on the variety with pubescent leaf under-surface. Native *A. andersoni* were displaced by *T. pyri*.
						Field (B)	+	Two grape varieties with different leaf hair density. *T. pyri* colonization failed; *K. aberrans* was more successful on glabrous varieties. *K. aberrans* displaced native *P. finitimus*.
Marshall & Lester, 2001	*Typhlodromus pyri*	*Panonychus ulmi*	Acari: Tetranychidae	Ontario, Canada	Inoculative	Field	+	*T. pyri* out-competed native *Amblyseius fallacies*. *T. pyri* is an effective biocontrol agent and may be introduced by transferring leaves.
Duso et al., 2006	*Typhlodromus pyri* strain resistant to organophosphates	*Panonychus ulmi*, *Eotetranychus carpini* *Calomerus vitis*	Acari: Tetranychidae Acari: Eriophyidae	North-eastern Italy	Inoculative	Field		15-years observations. The predator colonized the vineyard and competed successfully with other species. Role of alternative foods, leaf morphology and selective pesticides.

* + means effective, - means not effective biocontrol agent.

17.4 Predators of insects. Neuroptera: Chrysopidae [3 species] and Coleoptera: Coccinellidae [2 species]

Reference	Species of biocontrol agent	Species of insect pest	Taxonomic category of pests	Country	Type of augmentation	Type of test	Efficacy of biocontrol agents*	Additional information and results
	NEUROPTERA: CHRYSOPIDAE							
Daane et al., 1996	*Chrysoperla carnea* (common green lacewing)	*Erythroneura variabilis*, *E. elegantula* (leafhoppers)	Hemiptera: Cicadellidae	California	Inundative	Field (caged small-plot)	-	Average leafhopper density reduction 29.5%.
						Field (uncaged small-plot)	-	Release rates reflecting commercial recommendations. Average reduction 15.5%.
						Field (on-farm trials)	-	Average reduction 9.6% Not sufficient to lower the leafhopper density below the economic injury threshold.
Daane & Yokota, 1997	*Chrysoperla carnea*, *C. comanche*, *C. rufilabris*	*Erythroneura variabilis*, *E. elegantula* (leafhoppers)	Hemiptera: Cicadellidae	California	Inundative	Field	-	Aspects of release strategies evaluated. High mortality of lacewing eggs and neonate larvae.
Wunderlich & Giles, 1999	*Chrysoperla rufilabris*	*Erythroneura variabilis*, *E. elegantula* (leafhoppers)	Hemiptera: Cicadellidae	California	Inundative	Field		A mechanical technique was assessed for releasing eggs in liquid suspensions. Adhesion of eggs to the canopy was an issue.
	COLEOPTERA: COCCINELLIDAE							
Anagnou et al., 2003	*Nephus includens*	*Planococcus citri*	Hemiptera: Pseudococcidae	Greece		Field		It is suggested, for combined infestation by *L. botrana* and mealybugs, the application of *B. thuringiensis* and the releases of the effective predator *N. includens*.
Daane et al., 2008	*Cryptolaemus montrouzieri*	*Pseudococcus maritimus*, *P. longispinus* (mealybugs)	Hemiptera: Pseudococcidae	California	Inoculative	Field		Commonly released in vineyards, but release rates, timing, and expected outcomes have not been scientifically evaluated. It may be best used by releasing at hot spots where the mealybug density is high.
Mani, 2008	*Cryptolaemus montrouzieri*	*Planococcus citri*	Hemiptera: Pseudococcidae	India	Inundative	Green house	+	

* + means effective, - means not effective biocontrol agent.

17.5 Fungi [5 species]

Reference	Species of biocontrol agent	Species of insect pest	Taxonomic category of pests	Country	Type of augmentation	Type of test	Efficacy of biocontrol agents*	Additional information and results
Berner & Schnetter, 2002	*Beauveria brongniartii* (in combination with the nematode *H. bacteriophora*)	*Melolontha melolontha* (European cockchafer)	Coleoptera: Scarabeidae	Germany	Inundative	Field (soil)	+	Only under optimum conditions and with high doses control of the white grubs could be reached.
Tsitsipis et al., 2003	*Beauveria bassiana*	*Frankliniella occidentalis* (western flower thrips)	Thysanoptera: Thripidae	Greece	Inundative	Field	+ - 	*B. bassiana* in combination with mass trapping was compared to mass trapping or insecticides. Less efficient in the control of insect population if compared to some chemicals.
Al-Jboory et al., 2006	*Beauveria bassiana*	grape thrips	Thysanoptera: Thripidae	Iraq		Lab	+	Two isolates of *B. bassiana* showed 100% mortality after 5 days
Lopes et al., 2002	*Metarhizium anisopliae*	*Frankliniella occidentalis*	Thysanoptera: Thripidae	Brazil	Inundative	Field	+	The effect of chemicals (thiacloprid and methiocarb) with or without *M.a.* was tested. *M.a.* in combination with methiocarb was the best strategy.
Laengle et al., 2004	*Metarhizium anisopliae*	*Daktulosphaira vitifoliae* (grape phylloxera)	Hemiptera: Phylloxeridae	Austria	Inundative	Field		Non-target effects on soil fauna: no negative effects detected.
Kirchmair et al., 2004	*Metarhizium anisopliae*	*Daktulosphaira vitifoliae* (grape phylloxera)	Hemiptera: Phylloxeridae	Austria	Inundative	Lab	+	*M.a.* was effective in pot experiments. Potential role of *M.a.* in grape phylloxera control.
Kirchmair et al., 2005	*Metarhizium anisopliae*	*Daktulosphaira vitifoliae* (grape phylloxera)	Hemiptera: Phylloxeridae	Germany	Inundative	Field	+	*M.a.* was effective. No target effects on soil fauna (Acari, Collembola, Lumbricida and the Carabidae *Harpalus affinis*) and fungi.
Huber & Kirchmair, 2007	*Metarhizium anisopliae*	*Daktulosphaira vitifoliae* (grape phylloxera)	Hemiptera: Phylloxeridae	Germany	Inundative	Field	-	Evaluation of efficacy: more difficulties arise in testing the efficacy of *M.a.* under field conditions because of the uneven distribution of roots and pest insects in the soil.

Kirchmair et al., 2007	*Metarhizium anisopliae*	*Daktulosphaira vitifoliae* (grape phylloxera)	Hemiptera: Phylloxeridae	Germany	Inundative	Field	+	3 months after application an increase of the *M.a.* density in soil was observed. Compared with untreated plots a lower infestation was observed in the *M.a.*-treated plots. Two years after treatment a control effect was still observed whereas the density of *M.a.* in soil decreased. Three years after treatment no effect on the pest was detectable and the *M.a.* density had decreased to a value similar to that in the control . A periodically application is necessary.
Maheshkumar-Katke & Balikai, 2008	*Metarhizium anisopliae*, *Verticillium lecanii*, *Clerodendron inerme*	*Maconellicoccus hirsutus* (grape mealybug)	Hemiptera: Pseudococcidae	India	Inundative	Field	+	

* + means effective, - means not effective biocontrol agent.

17.6 Nematodes [5 species]

Reference	Species of biocontrol agent	Species of insect pest	Taxonomic category of pests	Country	Type of augmentation	Type of test	Efficacy of biocontrol agents*	Additional information and results
Saunders & All, 1985	*Steinernema carpocapsae*	*Vitacea polistiformis* (grape root borer)	Lepidoptera: Sesiidae	Georgia, USA	Inundative (soil)	Lab, Field	+	Susceptibility of *V.p.* 1st-instar larvae. Augmentation of nematode populations during the critical period of *V.p.* oviposition and eclosion is suggested as a control technique.
English-Loeb et al., 1999	*Heterorhabditis bacteriophora* (Oswego strain), *Steinernema glaseri* (isolate 326)	*Daktulosphaira vitifolia* (grape phylloxera - root form)	Hemiptera: Phylloxeridae	NY, USA		Lab	+ - -	*H. bacteriophora*: reduced survival of attached phylloxera by up to 80%. *S. glaseri* had no measurable impact. No evidence that *H.b.* could successfully reproduce within the bodies of the hosts. Augmentative use in the field in an release programme may be constrained by the need to use high densities, their dependence on moist soils, and their inability to propagate themselves within hosts.

Berner & Schnetter, 2002	*Heterorhabditis bacteriophora*, *H.bacteriophora* + *Beauveria brongniartii* (fungus)	*Melolontha melolontha* (European cockchafer)	Coleoptera: Scarabeidae	Germany	Inundative (soil)	Field	+	Only under optimum conditions and with high doses of nematodes control of grubs could be reached. New variant for the application of nematodes proposed.
Williams et al., 2002	*Heterorhabditis bacteriophora*, *H. zealandica*, *H. marelata*, and *Steinernema carpocapsae*	*Vitacea polistiformis* (grape root borer)	Lepidoptera: Sesiidae	Ohio, USA	Inundative	Lab Green house	+ +	*H. bacteriophora* strains GPS11 and Oswego, *H. zealandica* strain X1, and *H. marelata*. *S. carpocapsae* strain All less effective *H. zealandica* strain X1 *H. bacteriophora* strain GPS11

* + means effective, - means not effective biocontrol agent.

17.7 *Bacillus thuringiensis*

Reference	***B. thuringiensis* subspecies**	**Species of Insect pest**	**Taxonomic category of pests**	**Country**	**Type of test**	**Efficacy**	**Additional results and information**
Caroli et al., 1998	subsp. *aizawai*	*Lobesia botrana* (grape berry moth)	Lepidoptera: Tortricidae	Emilia-Romagna, Italy	Field	+	90-95% reduction in damage against severe pest infestations comparable to the standard chemical products.
Keil & Schruft, 1998		*L. botrana*, *Eupoecilia ambiguella* (grape berry moths)	Lepidoptera: Tortricidae		Lab		4 Bt products (0.2% Bactospeine FC, 0.1 % Delfin, 0.1% Dipel ES and 0.1% Thuricide HP) were compared. The influence of temperature on the efficacy is discussed.
Morando et al., 1998		*L. botrana*, *E. ambiguella*	Lepidoptera: Tortricidae	Piemonte, Italy	Field	+	The efficacy of Bt was compared to 7 insecticides. All the tested insecticides had a significantly good efficacy.
Boselli et al., 2000		*L. botrana*	Lepidoptera: Tortricidae	Emilia-Romagna, Italy	Field		Bt compared to insecticides.
Fretay & Quenin, 2000		*L. botrana*	Lepidoptera: Tortricidae	France	Field		Evaluation of new formularions.
Bagnoli & Lucchi, 2001	subsp. *kurstaki*	*Cryptoblabes gnidiella* (honey moth)	Lepidoptera : Pyralidae	Toscana, Italy	Field	+	
Boselli & Scannavini, 2001	subsp. *kurstaki* subsp. *aizawai*	*L. botrana*	Lepidoptera: Tortricidae	Emilia-Romagna, Italy	Field		Treatments included Agree (Bt kurstaki and aizawi), flufenoxuron, chlorpyrifos, lufenuron, tebufenozide, methoxyfenozide, indoxacarb and spinosad. The best **control** was obtained with methoxyfenozide, indoxacarb, and spinosad.
Neves & Frescata, 2001	*kurstaki* x *aizawai*	*L. botrana*	Lepidoptera: Tortricidae	Bairrada, Portugal	Field	+	TUREX was tested to control the *L. botrana* third generation. Great interest of this Bt product regarding its efficiency and persistence based in a correct spray moment determination.

Anagnou et al., 2003	subsp. *kurstaki* subsp. *aizawai*	*L. botrana*	Lepidoptera: Tortricidae		Lab	+	Several products incorporated into an artificial diet resulted in >90% larval mortality. The same formulations did not significantly affect the survival of *Nephus includens*.
Ifoulis & Savopoulou-Soultani, 2003		*L. botrana*	Lepidoptera: Tortricidae	Greece	Field	+	Two formulations of Bt are significantly more effective than the control, the dusting being more effective in most cultivars and the spraying in a few cultivars.
Roditakis, 2003		*L. botrana*	Lepidoptera: Tortricidae	Greece	Field		Pest control strategy involves *B.t.* application, mating disruption, botanical insecticides and minimal use of insecticides
Samoilov, 2003		*Sparganothis pilleriana* (grape leafroller)	Lepidoptera: Tortricidae	Odessa, Ukraine	Field	+	
Bakr, 2004	subsp. *kurstaki*	*Lobesia botrana*	Lepidoptera: Tortricidae	Egypt	Field	+	The addition of sugar as a feeding stimulant to a 50% reduced rate of Dipel-2X resulted in higher control rates (80%) compared to using the recommended field rates of Dipel-2X alone or Actellic [pirimiphos-methyl].
Besnard et al., 2004	subsp. *aizawai*	*Lobesia botrana*	Lepidoptera: Tortricidae	France	Field	+	Xen Tari commercial product.
Hera et al., 2004	subsp. *kurstaki*	*Hyphantria cunea* (fall webworm)	Lepidoptera: Arctiidae	Romania	Field	+	Dipel 2x WP at 0.075% also showed good protection. The synergenism of mixtures (50:50) of chemical and biological insecticides was effective in controlling the pest.
Laccone et al., 2004	subsp. *kurstaki*	*Lobesia botrana*	Lepidoptera: Tortricidae	Calabria, Italy	Field	+	Bt gave satisfactory control if applied at the onset of ovideposition and provided the canopy was managed in such a way as to expose the berries.
Mazzocchetti et al., 2004		*Lobesia botrana*	Lepidoptera: Tortricidae	Abruzzo, Italy	Field		Mating disruption was compared with the traditional methods generally used in the area: chemicals (phosphorganic molecules) and *B. thuringiensis*.
Moiraghi et al., 2004		*L. botrana* *E. ambiguella*	Lepidoptera: Tortricidae	Italy	Field	-	In four years, trials were carried out using several commercial products (9 insecticides and Bt). The best control was obtained using insecticides. Control was lower for azadirachtin and less constant for etofenprox and B. thuringiensis.
Delbac et al., 2006		*Lobesia botrana*	Lepidoptera: Tortricidae	France	Field	+	*L. botrana* was well-controlled by the use of *B.t.* or IGR's, without mating disruption justification
Marchesini et al., 2006	subsp. *aizawai* subsp. *kurstaki*	*Lobesia botrana*	Lepidoptera: Tortricidae	Veneto, Italy	Field	+	*Bta* compared to *Btk* and chemicals. High efficacy of *B.t. aizawai.*
Laccone, 2007		*Lobesia botrana*	Lepidoptera: Tortricidae	Molise and Calabria, Italy	Field		Pest control with indoxacarb, spinosad and *B. thuringiensis* applied against the 2nd generation of insects parasitizing fruit is also outlined
Mescalchin, 2007		*Lobesia botrana*	Lepidoptera: Tortricidae	Trentino, Italy	Field	+	5-years study (2000-2005). Formulations based Bt can be used for controlling tortricids such as *L. botrana*.
Mitrea et al., 2007	subsp. *kurstaki*	*Lobesia botrana*	Lepidoptera: Tortricidae	Romania	Field	+	Chemical insecticides followed by *Btk* to control the second or the third generation. Efficiency of the control treatments ranged between 89.4% and 91.4%.

Morandi-Filho et al., 2007		*Argyrotaenia sphaleropa* (South American tortricid moth)	Lepidoptera: Tortricidae	Brazil	Lab Field	+ +	Lab: reducition of the insect population by more than 90%. Field: reduced damage between 83.3 and 94.4%. The control efficacy of B.t was equal to that of chemicals.
Pryke & Samways, 2007	subsp. *kurstaki*	*Epichoristodes acerbella* **(**South African carnation tortrix)	Lepidoptera: Tortricidae	South Africa	Field	+	DiPelReg commercial formulation
Ruiz-de-Escudero et al., 2007		*Lobesia botrana*	Lepidoptera: Tortricidae		Lab	+	The potential of Bt Cry proteins to control L. botrana was explored. Either Cry1Ia or Cry9C could be used in combination with Cry1Ab to control this pest, either as the active components of Bt sprays or expressed together in transgenic plants.
Subic, 2007	subsp. *kurstaki*	*Lobesia botrana*	Lepidoptera: Tortricidae	Croatia	Field	+	Over 90% control was achieved.
Dongiovanni et al., 2008	subsp. *kurstaki*	*Lobesia botrana*	Lepidoptera: Tortricidae	Puglia, Italy	Field	+	

17.8 References

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6.18. Appendix 18. References on classical biological control against insect pests (cited in section 3.1.3.

18.1. Biocontrol agents not precisely known *(cf §3.1.3.4)*

Type of work	Pest (genus level)	References*
Prospective studies (55%)		(88)
	Aproaerema	(89)
	Cameraria	(61)
	Cryptococcus	(175) (94)
	Diabrotica	(154)
	Hypsipyla	(141)
	Liriomyza	(87)
	Lymanthria	(70)(72)
	Scirtothrips	(45)
	Tetranychus	
Retrospective studies (35%)		(166)
	Chilo	(128)
	Cinara	(56)
	Cosmopolites	(103)
	Maconellicoccus	(47)
	mealybugs	(191)
	Mononychellus	(97)
	Phenacoccus	
Other studies (10%)		(82)
Pest biology	*Enarmonia*	(88)

* Numbers correspond to refernces presented in section 18.4

18.2. Details on the use of pathogens, nematodes and predators as agents of Classical Biological Control

Pest	BCA lifestyle	BCA	References*
Aceria	Fungus Predatory mite	Hirsutella *Neoseiulus*	(114)
Adelges	Predatory Insect	*Laricobius*	(119)□
Anticarsia	Virus	Nucleopolyhedrovirus	(197)□
Aphids	Predatory Insect	*Harmonia*	(48) (127)□
Aphis	Fungus	*Neozygites*	(19) (90) (91) (137)□
Coptotermes	Fungus	*Beauvaria & Metarhizium*	(168)□
Lymantria	Fungus Virus	*Microspora* Nucleopolyhedrovirus	(35)□
Maconellicoccus	Predatory Insect	*Cryptolaemus* *Scymnus*	(165)
Mononychellus	Fungus Predatory mite	*Neozygites* *Neosiulus &Typhlodromalus*	(16)□
Oryctes	Virus	_	(51) (86)
Prostephanus	Predatory Insect	*Teretrius*	(51)□
Review	Fungus	_	(14) (39) (42) (43)
Review	Nematode	_	(14) (55) (124) (125) (193) (194)□
Sirex	Nematode	*Deladenus*	(81)□
Solenopsis	Fungus	*Vairimorpha*	(73) (169) (170)□

* Numbers correspond to refernces presented in section 18.4

18.3 Categorization of publications related to Insect parasitoids as CIBCA according to the type of work

Pest Biology

Pest rearing : (83, 183)

BCA Biology

BCA inventories : (30, 34, 65) (67) (88) (157) (178)
BCA systematics: (18, 52, 123) (36) (186)
BCA molecular characterization: (121, 132)
BCA rearing: (21, 58, 92, 163) (171)
BCA biology: (6, 10, 37) (74) (77) (85) (98) (100) (102) (104) (105) (158) (159) (160) (172) (190) (195)
BCA Evaluation: (12, 44, 46) (57) (80) (108) (151)

BCA Field Implications

Pre-release survey: (9, 60, 66) (122) (140) (166)
BCA introduction : see table 1
Post-release survey : (20, 22, 32) (33) (36) (50) (54) (64) (68) (76) (78) (106) (107) (113) (109) (135) (142) (145) (146) (148) (150) (162) (179)

Non-intended effects

(24, 29, 38) (58) (71) (84) (92) (65) (101) (129) (149) (155) (184) (189)

Biocontrol disruption

(17, 27, 69) (95) (130) (147) (180)

Miscellaneous

Economic valuation: (23)
Review: (75, 112, 152) (153)
Miscellaneous: (111, 115, 116) (139) (176)
“Conservation BC-like” : (173)

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6.19. Appendix 19. Substances included in the "EU Pesticides Database" as of April 21 2009.

	Substance	Cipac & incl 2008/ 127 √	Category	List (*)	Inclusion Date	Expiry Date	Legislation
Botanical	Extract from tea tree		RE	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Garlic extract		RE	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Gibberellic acid	'307	PG	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Gibberellin		PG	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Laminarin		EL	C	01/04/2005	31/03/2015	05/3/EC
Botanical	Pepper		RE	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Plant oils / Citronella oil		HB	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Plant oils / Clove oil		RE	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Plant oils / Rape seed oil		IN, AC	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Plant oils / Spearmint oil		PG	A 4	01/09/2009	31/08/2019	2008/127
Botanical	Sea-algae extract (formerly sea-algae extract and seaweeds)		PG	A 4	01/09/2009	31/08/2019	2008/127
Botanical copied by synthesis	Carvone		PG	C	01/08/2008	31/07/2018	2008/44/EC
Botanical copied by synthesis	Ethylene		PG	A 4	01/09/2009	31/08/2019	2008/127
Botanical but excluded	Pyrethrins	'32	IN	A 4	01/09/2009	31/08/2019	2008/127
Chemical	2,4-D	'1	HB, PG	A 1	01/10/2002	30/09/2012	01/103/EC
Chemical	2,4-DB	'83	HB	A 1	01/01/2004	31/12/2013	03/31/EC
Chemical	1-Methyl-cyclopropene		PG	C	01/04/2006	31/03/2016	06/19/EC
Chemical	Acetamiprid		IN	C	01/01/2005	31/12/2014	04/99/EC
Chemical	Acibenzolar-S-methyl (benzothiadiazole)		PA	C	01/11/2001	31/10/2011	01/87/EC
Chemical	Aclonifen	'498	HB	A 3	01/08/2009	31/07/2019	2008/116
Chemical	Alpha-Cypermethrin (aka alphamethrin)	'454	IN	A 1	01/03/2005	28/02/2015	04/58/EC
Chemical	Aluminium ammonium sulfate		RE	A 4	01/09/2009	31/08/2019	2008/127
Chemical	Aluminium phosphide	'227	IN, RO	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Amidosulfuron	'515	HB	A 3	01/01/2009	31/12/2018	2008/40
Chemical	Amitrole (aminotriazole)	'90	HB	A 1	01/01/2002	31/12/2012	01/21/EC
Chemical	Azimsulfuron		HB	C	01/10/1999	01/10/2019	99/80/EC
Chemical	Azoxystrobin		FU	C	01/07/1998	01/07/2008	98/47/EC
Chemical	Beflubutamid		HB	C	01/12/2007	30/11/2017	07/50/EC
Chemical	Benalaxyl	'416	FU	A 1	01/03/2005	28/02/2015	04/58/EC
Chemical	Benfluralin	'285	HB	A 3	01/01/2009	31/12/2018	2008/108
Chemical	Bensulfuron	'502	HB	A 3	01/11/2009	31/10/2019	2009/11
Chemical	Bentazone	'366	HB	A 1	01/08/2001	31/07/2011	00/68/EC
Chemical	Benthiavalicarb		FU	C	01/08/2008	31/07/2018	08/44/EC
Chemical	Beta-Cyfluthrin	'482	IN	A 1	01/01/2004	31/12/2013	03/31/EC
Chemical	Bifenazate		AC	C	01/12/2005	30/11/2015	05/58/EC
Chemical	Bifenox	'413	HB	A 3	01/01/2009	31/12/2018	2008/66
Chemical	Bordeaux mixture		FU	A 3	01/11/2009	30/11/2016	SCoFCAH voted 01.2009

Chemical	Boscalid		FU	C	01/08/2008	31/07/2018	08/44/EC
Chemical	Bromoxynil	'87	HB	A 1	01/03/2005	28/02/2015	04/58/EC
Chemical	Calcium carbide		RE	A 4	01/09/2009	31/08/2019	2008/127
Chemical	Calcium phosphide	'505	RO	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Captan	'40	FU	A 2	01/10/2007	30/09/2017	07/5/EC
Chemical	Carbendazim	'263	FU	A 1	01/01/2007	31/12/2009	06/135/EC
Chemical	Carfentrazone-ethyl		HB	C	01/10/2003	30/09/2013	03/68/EC
Chemical	Chloridazon (aka pyrazone)	'111	HB	A 3	01/01/2009	31/12/2018	2008/41
Chemical	Chlormequat (chloride)	'143	PG	A 3	01/12/2009	30/11/2019	
Chemical	Chlorothalonil	'288	FU	A 1	01/03/2006	28/02/2016	05/53/EC
Chemical	Chlorotoluron	'217	HB	A 1	01/03/2006	28/02/2016	05/53/EC
Chemical	Chlorpropham	'43	PG, HB	A 1	01/02/2005	31/01/2015	04/20/EC
Chemical	Chlorpyrifos	'221	IN, AC	A 1	01/07/2006	30/06/2016	05/72/EC
Chemical	Chlorpyrifos-methyl	'486	IN, AC	A 1	01/07/2006	30/06/2016	05/72/EC
Chemical	Chlorsulfuron	'391	HB	A 3	01/09/2009	31/08/2019	
Chemical	Cinidon ethyl		HB	C	01/10/2002	30/09/2012	02/64/EC
Chemical	Clodinafop		HB	A 2	01/02/2007	31/01/2017	06/39/EC
Chemical	Clofentezine	'418	AC	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Clomazone	'509	HB	A 3	01/11/2008	01/11/2018	2007/76
Chemical	Clopyralid	'455	HB	A 2	01/01/2007	30/04/2017	06/64/EC
Chemical	Clothianidin		IN	C	01/08/2006	31/07/2016	06/41/EC
Chemical	Copper compounds		FU	A 3	01/11/2009	30/11/2016	SCoFCAH voted 01.2009
Chemical	Copper hydroxide		FU	A 3	01/11/2009	30/11/2016	SCoFCAH voted 01.2009
Chemical	Copper oxychloride		FU	A 3	01/11/2009	30/11/2016	SCoFCAH voted 01.2009
Chemical	Cuprous oxide		FU	A 3	01/11/2009	30/11/2016	SCoFCAH voted 01.2009
Chemical	Cyazofamid		FU	C	01/07/2003	30/06/2013	03/23/EC
Chemical	Cyclanilide		PG	C	01/11/2001	31/10/2011	01/87/EC
Chemical	Cyfluthrin	'385	IN, AC	A 1	01/01/2004	31/12/2013	03/31/EC
Chemical	Cyhalofop-butyl		HB	C	01/10/2002	30/09/2012	02/64/EC
Chemical	Cymoxanil	'419	FU	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Cypermethrin	'332	IN, AC	A 1	01/03/2006	28/02/2016	05/53/EC
Chemical	Cyprodinil	'511	FU	A 2	01/05/2007	30/04/2017	06/64/EC
Chemical	Cyromazine	'420	IN	A 3	01/01/2010	31/08/2019	
Chemical	Daminozide	'330	PG	A 1	01/03/2006	28/02/2016	05/53/EC
Chemical	Deltamethrin	'333	IN	A 1	01/11/2003	31/10/2013	03/5/EC
Chemical	Desmedipham	'477	HB	A 1	01/11/2003	31/10/2013	04/58/EC
Chemical	Dicamba	'85	HB	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Dichlorobenzoic acid methylester		FU, PGR	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Dichlorprop-P	'476	HB	A 2	01/06/2007	31/05/2017	06/74/EC
Chemical	Didecyldimethylammonium chloride		FU	A 4			
Chemical	Difenacoum	'514	RO	A 4			
Chemical	Difenoconazole	'687	FU	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Diflubenzuron	'339	IN	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Diflufenican	'462	HB	A 3	01/01/2009	31/12/2018	2008/66
Chemical	Dimethachlor		HB	A 3	01/01/2010	31/08/2019	
Chemical	Dimethenamid ? P		HB	C	01/01/2004	31/12/2013	03/84/EC

Chemical	Dimethoate	'59	IN, AC	A 2	01/10/2007	30/09/2017	07/25/EC
Chemical	Dimethomorph	'483	FU	A 2	01/10/2007	30/09/2017	07/25/EC
Chemical	Dimoxystrobin		FU	C	01/10/2006	30/09/2016	06/75/EC
Chemical	Dinocap	'98	FU, AC	A 1	01/01/2007	31/12/2009	06/136/EC
Chemical	Diquat (dibromide)	'55	HB	A 1	01/01/2002	31/12/2011	01/21/EC
Chemical	Diuron	'100	HB	A 2	01/10/2008	30/09/2018	08/91/EC
Chemical	Dodemorph	'300	FU	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Epoxiconazole	'609	FU	A 3	01/01/2009	31/12/2018	2008/107
Chemical	Esfenvalerate	'481	IN	A 1	01/08/2001	31/07/2011	00/67/EC
Chemical	Ethephon	'373	PG	A 2	01/08/2007	31/07/2017	06/85/EC
Chemical	Ethofumesate	'233	HB	A 1	01/03/2003	28/02/2013	02/37/EC
Chemical	Ethoprophos	'218	NE, IN	A 2	01/10/2007	30/09/2017	07/52/EC
Chemical	Ethoxysulfuron		HB	C	01/07/2003	30/06/2013	03/23/EC
Chemical	Etofenprox	'471	IN	A 3	01/01/2010	31/12/2019	
Chemical	Etoxazole		IN	C	01/06/2005	31/05/2015	05/34/EC
Chemical	Famoxadone		FU	C	01/10/2002	30/09/2012	02/64/EC
Chemical	Fenamidone		FU	C	01/10/2003	30/09/2013	03/68/EC
Chemical	Fenamiphos (aka phenamiphos)		NE	A 2	01/08/2007	31/07/2017	06/85/EC
Chemical	Fenhexamid		FU	C	01/06/2001	31/05/2011	01/28/EC
Chemical	Fenoxaprop-P	'484	HB	A 3	01/01/2009	31/12/2018	2008/66
Chemical	Fenpropidin	'520	FU	A 3	01/01/2009	31/12/2018	2008/66
Chemical	Fenpropimorph	'427	FU	A 3	01/01/2009	31/12/2018	2008/107
Chemical	Fenpyroximate		AC	A 3	01/01/2009	31/12/2018	2008/107
Chemical	Fipronil	'581	IN	A 2	01/10/2007	30/09/2017	07/52/EC
Chemical	Flazasulfuron		HB	C	01/06/2004	31/05/2014	04/30/EC
Chemical	Florasulam		HB	C	01/10/2002	30/09/2012	02/64/EC
Chemical	Fluazinam	'521	FU	A 3	01/01/2009	31/12/2018	2008/108
Chemical	Fludioxonil	'522	FU	A 3	01/11/2008	01/11/2018	2007/76
Chemical	Flufenacet (formerly fluthiamide)		HB	C	01/01/2004	31/12/2013	03/84/EC
Chemical	Flumioxazin		HB	C	01/01/2003	31/12/2012	02/81/EC
Chemical	Fluoxastrobin		FU	C	01/08/2008	31/07/2018	08/44/EC
Chemical	Flupyrsulfuron methyl		HB	C	01/07/2001	30/06/2011	01/49/EC
Chemical	Fluroxypyr	'431	HB	A 1	01/12/2000	30/11/2010	00/10/EC
Chemical	Flurtamone		HB	C	01/01/2004	31/12/2013	03/84/EC
Chemical	Flusilazole	'435	FU	A 1	01/01/2007	30/06/2008	06/133/EC
Chemical	Flutolanil	'524	FU	A 3	01/01/2009	31/12/2018	2008/108
Chemical	Folpet	'75	FU	A 2	01/10/2007	30/09/2017	07/5/EC
Chemical	Foramsulfuron		HB	C	01/07/2003	30/06/2013	03/23/EC
Chemical	Forchlorfenuron		PG	C	01/04/2006	31/03/2016	06/10/EC
Chemical	Formetanate		IN, AC	A 2	01/10/2007	30/09/2017	07/5/EC
Chemical	Fosetyl	'384	FU	A 2	01/05/2007	30/04/2017	06/64/EC
Chemical	Fosthiazate		NE	C	01/01/2004	31/12/2013	03/84/EC
Chemical	Fuberidazole	'525	FU	A 3	01/01/2009	31/12/2018	2008/108
Chemical	Glufosinate	'437	HB	A 2	01/10/2007	30/09/2017	07/25/EC
Chemical	Glyphosate (incl trimesium aka sulfosate)	'284	HB	A 1	01/07/2002	30/06/2012	01/99/EC
Chemical	Imazalil (aka enilconazole)	'335	FU	A 1	01/01/1999	31/12/2008	97/73/EC
Chemical	Imazamox		HB	C	01/07/2003	30/06/2013	03/23/EC
Chemical	Imazaquin	'699	PG	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Imazosulfuron		HB	C	01/04/2005	31/03/2015	05/3/EC
Chemical	Imidacloprid		IN	A 3	01/08/2009	31/07/2019	2008/116
Chemical	Indoxacarb		IN	C	01/04/2006	31/03/2016	06/10/EC

Chemical	Iodosulfuron-methyl-sodium		HB	C	01/01/2004	31/12/2013	03/84/EC
Chemical	Ioxynil	'86	HB	A 1	01/03/2005	28/02/2015	04/58/EC
Chemical	Iprodione	'278	FU	A 1	01/01/2004	31/12/2013	03/31/EC
Chemical	Iprovalicarb		FU	C	01/07/2002	30/06/2011	02/48/EC
Chemical	Iron sulphate		HB	A 4	01/09/2009	31/08/2019	2008/127
Chemical	Isoproturon	'336	HB	A 1	01/01/2003	31/12/2012	02/18/EC
Chemical	Isoxaflutole		HB	C	01/01/2003	31/12/2012	03/68/EC
Chemical	Kresoxim-methyl		FU	C	01/02/1999	31/01/2009	99/01/EC
Chemical	lambda-Cyhalothrin	'463	IN	A 1	01/01/2002	31/12/2011	00/80/EC
Chemical	Lenacil	'163	HB	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Linuron	'76	HB	A 1	01/01/2004	31/12/2013	03/31/EC
Chemical	Lufenuron		IN	A 3	01/01/2010	31/12/2019	
Chemical	Magnesium phosphide	'228	IN, RO	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Maleic hydrazide	'310	PG	A 1	01/01/2004	31/12/2013	03/31/EC
Chemical	Mancozeb	'34	FU	A 1	01/07/2006	30/06/2016	05/72/EC
Chemical	Maneb	'61	FU	A 1	01/07/2006	30/06/2016	05/72/EC
Chemical	MCPA	'2	HB	A 1	01/05/2006	30/04/2016	05/57/EC
Chemical	MCPB	'50	HB	A 1	01/05/2006	30/04/2016	05/57/EC
Chemical	Mecoprop	'51	HB	A 1	01/06/2004	31/05/2014	03/70/EC
Chemical	Mecoprop-P	'475	HB	A 1	01/06/2004	31/05/2014	03/70/EC
Chemical	Mepanipyrim		FU	C	01/10/2004	30/09/2014	04/62/EC
Chemical	Mepiquat	'440	PG	A 3	01/01/2009	31/12/2018	2008/108
Chemical	Mesosulfuron		HB	C	01/04/2004	31/03/2014	03/119/EC
Chemical	Mesotrione		HB	C	01/10/2003	30/09/2013	03/68/EC
Chemical	Metalaxyl-M		FU	C	01/10/2002	30/09/2012	02/64/EC
Chemical	Metamitron	'381	HB	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Metazachlor	'411	HB	A 3	01/08/2009	31/07/2019	2008/116
Chemical	Metconazole		FU	A 2	01/06/2007	31/05/2017	06/74/EC
Chemical	Methiocarb (aka mercaptodimethur)	'165	IN, MO, RE	A 2	01/10/2007	30/09/2017	07/5/EC
Chemical	Methoxyfenozide		IN	C	01/04/2005	31/03/2015	05/3/EC
Chemical	Metiram	'478	FU	A 1	01/07/2006	30/06/2016	05/72/EC
Chemical	Metrafenone		FU	C	01/02/2007	31/01/2017	07/6/EC
Chemical	Metribuzin	'283	HB	A 2	01/10/2007	30/09/2017	07/25/EC
Chemical	Metsulfuron	'441	HB	A 1	01/07/2001	30/06/2011	00/49/EC
Chemical	Molinate	'235	HB	A 1	01/08/2004	31/07/2014	03/81/EC
Chemical	Nicosulfuron	'709	HB	A 3	01/01/2009	31/12/2018	2008/40
Chemical	Oxadiargyl		HB	C	01/07/2003	30/06/2013	03/23/EC
Chemical	Oxadiazon	'213	HB	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Oxamyl	'342	IN, NE	A 2	01/08/2006	31/07/2016	06/16/EC
Chemical	Oxasulfuron		HB	C	01/07/2003	30/06/2013	03/23/EC
Chemical	Penconazole	'446	FU	A 3	01/01/2010	31/08/2019	
Chemical	Pendimethalin	'357	HB	A 1	01/01/2004	31/12/2013	03/31/EC
Chemical	Pethoxamid		HB	C	01/08/2006	31/07/2016	06/41/EC
Chemical	Phenmedipham	'77	HB	A 1	01/03/2005	28/02/2015	04/58/EC
Chemical	Phosmet	'318	IN	A 2	01/10/2007	30/09/2017	07/25/EC
Chemical	Picloram	'174	HB	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Picolinafen		HB	C	01/10/2002	30/09/2012	02/64/EC
Chemical	Picoxystrobin		FU	C	01/01/2004	31/12/2013	03/84/EC
Chemical	Pirimicarb	'231	IN	A 2	01/02/2007	31/01/2017	06/39/EC
Chemical	Pirimiphos-methyl	'239	IN	A 2	01/10/2007	30/09/2017	07/52/EC
Chemical	Prohexadione-calcium		PG	C	01/10/2000	01/10/2010	00/50/EC
Chemical	Propamocarb	'399	FU	A 2	01/10/2007	30/09/2017	07/25/EC

Chemical	Propaquizafop		HB	A 3	01/12/2009	30/11/2019	
Chemical	Propiconazole	'408	FU	A 1	01/06/2004	31/05/2014	03/70/EC
Chemical	Propineb	'177	FU	A 1	01/04/2004	30/03/2014	03/39/EC
Chemical	Propoxycarbazone		HB	C	01/04/2004	31/03/2014	03/119/EC
Chemical	Propyzamide	'315	HB	A 1	01/04/2004	30/03/2014	03/39/EC
Chemical	Prosulfocarb	'539	HB	A 3	01/01/2009	31/12/2018	2007/76
Chemical	Prosulfuron		HB	C	01/07/2002	30/06/2011	02/48/EC
Chemical	Prothioconazole		FU	C	01/08/2008	31/07/2018	08/44/EC
Chemical	Pymetrozine		IN	C	01/11/2001	31/10/2011	01/87/EC
Chemical	Pyraclostrobin		FU, PG	C	01/06/2004	31/05/2014	04/30/EC
Chemical	Pyraflufen-ethyl		HB	C	01/11/2001	31/10/2011	01/87/EC
Chemical	Pyridate	'447	HB	A 1	01/01/2002	31/12/2011	01/21/EC
Chemical	Pyrimethanil		FU	A 2	01/06/2007	31/05/2017	06/74/EC
Chemical	Pyriproxyfen	'715	IN	A 3	01/01/2009	31/12/2018	2008/69
Chemical	Quinoclamine	'648	HB, AL	A 3	01/01/2009	31/12/2018	2008/66
Chemical	Quinoxyfen		FU	C	01/09/2004	31/08/2014	04/60/EC
Chemical	Quizalofop-P	'641	HB	A 3	01/12/2009	30/11/2019	SCoFCAH voted 01.2009
Chemical	Quizalofop-P-ethyl	'641	HB	A 3	01/12/2009	30/11/2019	
Chemical	Quizalofop-P-tefuryl	'641	HB	A 3	01/12/2009	30/11/2019	
Chemical	Rimsulfuron (aka renriduron)		HB	A 2	01/02/2007	31/01/2017	06/39/EC
Chemical	Silthiofam		FU	C	01/01/2004	31/12/2013	03/84/EC
Chemical	S-Metholachlor		HB	C	01/04/2005	31/03/2015	05/3/EC
Chemical	Sodium 5-nitroguaiacolate		PG	A 3	01/11/2009	31/10/2019	2009/11
chemical	Sodium hypochlorite		BA	A 4	01/09/2009	31/08/2019	2008/127
Chemical	Sodium o-nitrophenolate		PG	A 3	01/11/2009	31/10/2019	2009/11
Chemical	Sodium p-nitrophenolate		PG	A 3	01/11/2009	31/10/2019	2009/11
Chemical	Spiroxamine		FU	C	01/09/1999	01/09/2009	99/73/EC
Chemical	Sulcotrione		HB	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Sulfosulfuron		HB	C	01/07/2002	30/06/2011	02/48/EC
Chemical	Sulphur	'0018	FU, AC, RE	A 4			SCoFCAH voted 03.2009
Chemical	Tebuconazole	'494	FU	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Tebufenpyrad		AC	A 3	01/11/2009	31/10/2019	2009/11
Chemical	Teflubenzuron	'450	IN	A 3	01/12/2009	30/11/2019	
Chemical	Tepraloxydim		HB	C	01/06/2005	31/05/2015	05/34/EC
Chemical	Thiabendazole	'323	FU	A 1	01/01/2002	31/12/2011	01/21/EC
Chemical	Thiacloprid		IN	C	01/01/2005	31/12/2014	04/99/EC
Chemical	Thiamethoxam		IN	C	01/02/2007	31/01/2017	07/6/EC
Chemical	Thifensulfuron-methyl	'452	HB	A 1	01/07/2002	30/06/2012	01/99/EC
Chemical	Thiophanate-methyl	'262	FU	A 1	01/03/2006	28/02/2016	05/53/EC
Chemical	Thiram	'24	FU	A 1	01/08/2004	31/07/2014	03/81/EC
Chemical	Tolclofos-methyl	'479	FU	A 2	01/02/2007	31/01/2017	06/39/EC
Chemical	Tolylfluanid	'275	FU, AC	A 2	01/10/2006	30/09/2016	06/06/EC
Chemical	Tralkoxydim	'544	HB	A 3	01/01/2009	31/12/2018	2008/107
Chemical	Triadimenol	'398	FU	A 3	01/09/2009	31/08/2019	2008/125
Chemical	Tri-allate	'97	HB	A 3	01/01/2010	31/12/2019	
Chemical	Triasulfuron	'480	HB	A 1	01/08/2001	31/07/2011	00/66/EC
Chemical	Tribasic copper sulfate		FU	A 3			
Chemical	Tribenuron (aka metometuron)	'546	HB	A 2	01/03/2006	28/02/2016	05/54/EC
Chemical	Triclopyr	'376	HB	A 2	01/06/2007	31/05/2017	06/74/EC
Chemical	Trifloxystrobin		FU	C	01/10/2003	30/09/2013	03/68/EC

Chemical	Triflusulfuron		HB	A 3	01/01/2010	31/12/2019	
Chemical	Trinexapac (aka cimetacarb ethyl)		PG	A 2	01/05/2007	30/04/2017	06/64/EC
Chemical	Triticonazole	'652	FU	A 2	01/02/2007	31/01/2017	06/39/EC
Chemical	Tritosulfuron		HB	C	01/12/2008	30/11/2018	08/70/EC
Chemical	Warfarin (aka coumaphene)	'70	RO	A 1	01/10/2006	30/09/2013	06/05/EC
Chemical	zeta-Cypermethrin		IN	A 3	01/12/2009	30/11/2019	
Chemical	Ziram	'31	FU, RE	A 1	01/08/2004	31/07/2014	03/81/EC
Chemical	Zoxamide		FU	C	01/04/2004	31/03/2014	03/119/EC
Chemical repellent	Denathonium benzoate		RE	A 4	01/09/2009	31/08/2019	2008/127
Chemical repellent	Repellents by smell/ Tall oil crude (CAS 8002-26-4)			A 4	01/09/2009	31/08/2019	2008/127
Chemical repellent	Repellents by smell/Tall oil pitch (CAS 8016-81-7)			A 4	01/09/2009	31/08/2019	2008/127
Microbial	*Ampelomyces quisqualis* strain AQ10		FU	C	01/04/2005	31/03/2015	05/2/EC
Microbial	*Bacillus subtilis* str. QST 713		BA, FU	C	01/02/2007	31/01/2017	07/6/EC
Microbial	*Bacillus thuringiensis subsp. aizawai* (ABTS-1857 and GC-91)		[IN]	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Bacillus thuringiensis subsp. israelensis* (AM65-52)		[IN]	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Bacillus thuringiensis subsp. kurstaki* (ABTS 351, PB 54, SA 11, SA12 and EG 2348)		[IN]	A 4	01/01/2009	31/12/2018	2008/113
Microbial	Bacillus thuringiensis subsp. tenebrionis (NB 176)		[IN]	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Beauveria bassiana* (ATCC 74040 and GHA)		IN	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Coniothyrium minitans*		FU	C	01/01/2004	31/12/2013	03/79/EC
Microbial	*Cydia pomonella* granulosis virus (CpGV)		IN	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Gliocladium catenulatum* strain J1446		FU	C	01/04/2005	31/03/2015	05/2/EC
Microbial	*Lecanicillimum muscarium* (Ve6) (former *Verticillium lecanii*)		IN	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Metarhizium anisopliae* (BIPESCO 5F/52)		IN	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Paecilomyces fumosoroseus* Apopka strain 97		FU	C	01/07/2001	30/06/2011	01/47/EC
Microbial	*Paecilomyces lilacinus*		FU	C	01/08/2008	31/07/2018	2008/44/EC
Microbial	*Phlebiopsis gigantea* (several strains)		FU	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Pseudomonas chlororaphis* strain MA342		FU	C	01/10/2004	30/09/2014	04/71/EC
Microbial	*Pythium oligandrum* (M1)		FU	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Spodoptera exigua* nuclear polyhedrosis virus		FU	C	01/12/2007	30/11/2017	07/50/EC
Microbial	*Streptomyces* K61 (K61) (formerly *Streptomyces griseoviridis*)		FU	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Trichoderma aspellerum* (ICC012) (T11) (TV1) (formerly *T. harzianum*)		FU	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Trichoderma atroviride* (IMI 206040) (T 11) (formerly *Trichoderma harzianum*)		FU	A 4	01/01/2009	31/12/2018	2008/113

Microbial	*Trichoderma gamsii* (formerly *T. viride*) (ICC080)		FU	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Trichoderma harzianum* Rifai (T-22) (ITEM 908)		FU	A 4	01/01/2009	31/12/2018	2008/113
Microbial	Trichoderma polysporum (IMI 206039)		FU	A 4	01/01/2009	31/12/2018	2008/113
Microbial	*Verticillium albo-atrum* (WCS850) (formerly *Verticillium dahliae*)		FU	A 4	01/01/2009	31/12/2018	2008/113
Natural other	Abamectin (aka avermectin)	'495	AC, IN	A 3	01/01/2009	31/12/2018	2008/107
Natural other	Acetic acid		HB	A 4	01/09/2009	31/08/2018	2008/127
Natural other	Aluminium silicate (aka kaolin)		RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other	Blood meal		RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other	Carbon dioxide		IN, RO	A 4	01/09/2009	31/08/2019	2008/127
Natural other	Fat distilation residues		RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other	Ferric phosphate		MO	C	01/11/2001	31/10/2011	01/87/EC
Natural other	Kieselguhr (diatomaceous earth)		IN	A 4	01/09/2009	31/08/2019	2008/127
Natural other	Milbemectin		IN, AC	C	01/12/2005	30/11/2015	05/58/EC
Natural other	Quartz sand		RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other	Spinosad		IN	C	01/02/2007	31/01/2017	07/6/EC
Natural other by synthesis	Benzoic acid		BA, FU, OT	C	01/06/2004	31/05/2014	04/30/EC
Natural other by synthesis	Potassium hydrogen carbonate		FU	A 4	01/09/2009	31/08/2019	2008/127
Natural other by synthesis	Urea		IN	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Capric acid (CAS 334-48-5)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Caprylic acid (CAS 124-07-2)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Fatty acids C7 to C20		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Fatty acids C7-C18 and C18 unsaturated potassium salts (CAS 67701-09-1)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Fatty acids C8-C10 methyl esters (CAS 85566-26-3)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Lauric acid (CAS 143-07-7)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Methyl decanoate (CAS 110-42-9)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Methyl octaonate (CAS 111-11-5)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Oleic acid (CAS 112-80-1)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other fatty acid	Pelargonic acid (CAS 112-05-0)		IN, AC, HB, PG	A 4	01/09/2009	31/08/2019	2008/127
Natural other repellent	Calcium carbonate		RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other repellent	Limestone		RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other Repellent	Methyl nonyl ketone	√	RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other repellent	Sodium aluminium silicate		RE	A 4	01/09/2009	31/08/2019	2008/127

Natural other repellent	Repellents by smell/Fish oil		RE	A 4	01/09/2009	31/08/2019	2008/127
Natural other repellent	Repellents by smell/Sheep fat		RE	A 4	01/09/2009	31/08/2019	2008/127
Semio	(Z)-13-Hexadecen-11yn-1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio	(Z,Z,Z,Z)-7,13,16,19-Docosatetraen-1-yl isobutyrate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio	Ammonium acetate	√	AT	A 4	01/01/2009	31/12/2018	2008/127
Semio	Hydrolysed proteins	√	IN	A 4	01/09/2009	31/08/2019	2008/127
Semio	Putrescine (1,4-Diaminobutane)	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio	Trimethylamine hydrochloride	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio	Straight Chain Lepidoptera Pheromones	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(2E, 13Z)-Octadecadien-1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(7E, 9E)-Dodecadien 1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(7E, 9Z)-Dodecadien 1-yl acetate	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(7Z, 11E)-Hexadecadien-1-yl acetate	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(7Z, 11Z)-Hexadecdien-1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(9Z, 12E)-Tetradecadien-1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(E)-11-Tetradecen-1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(E)-5-Decen-1-ol	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(E)-5-Decen-1-yl-acetate	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(E)-8-Dodecen-1-yl acetate	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(E,E)-8,10-Dodecadien-1-ol	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(E/Z)-8-Dodecen-1-yl acetate	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-11-Hexadecen-1-ol	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-11-Hexadecen-1-yl acetate	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-11-Hexadecenal	√ √ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-11-Tetradecen-1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-13-Octadecenal	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-7-Tetradecenal	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-8-Dodecen-1-ol	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-8-Dodecen-1-yl acetate	√ √ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-9-Dodecen-1-yl acetate	422 √ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-9-Hexadecenal	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	(Z)-9-Tetradecen-1-yl acetate	√	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	Dodecyl acetate	√ √	AT	A 4	01/09/2009	31/08/2019	2008/127
Semio/SCLP	Tetradecan-1-ol	√	AT	A 4	01/09/2009	31/08/2019	2008/127
	Official Total Included:	334			A: Existing active substances divided into four lists for phased evaluations C: New active substances		

6.20. Appendix 20. Communication at the 12th meeting of the Working Group " Insect Pathogens and Insect Parasitic Nematodes" of IOBC-wprs, in Pamplona (Spain) 22-25 June 2009.

Insect Pathogens and Insect Parasitic Nematodes
IOBC/wprs Bulletin Vol. 45, 2009
pp. 3-5

Biological control of plant diseases: Future research goals to make it successful.

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Introduction

In this beginning of the XXI century, there is a need to increase the agricultural production for both food and energy and at the same time decrease the use of fertilizers and pesticides from chemical origin. In this context there is a renewed interest for alternative methods of control. This expression "alternative control methods", which means alternative to chemical methods, covers a lot of different approaches based on agricultural practices, use of "natural products", and beneficial organisms. In this presentation I will only consider biological control. Many different definitions of biological control have been proposed. Eilenberg (2006) defined "biological control as the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be". It was clearly stated that the term "pest applies for insect, mites and vertebrate pests, plant diseases, and weeds". In their book, "The nature and practice of biological control of plant pathogens" Cook and Baker (1983) reviewed the different components of biological control of plant diseases. The organisms which can be used to achieve biological control include (i) avirulent or hypo-virulent individuals or populations within the pathogenic species, (2) antagonistic micro-organisms, and (3) the host-plant manipulated toward greater or more effective resistance to the pathogen. In this presentation I will mainly focus on "microbiological control" of plant diseases, based on the use of living populations of non pathogenic or antagonistic micro organisms. Indeed, the main difference between biological control and other control methods is the use of living populations of beneficial organisms, having several modes of action, involving not only interactions with the target pathogen, but also interactions with the rest of the microbiota and the plant.

Despite the increasing number of scientific papers dealing with biological control, there are still a very limited number of products on the market. In the European Union, only a limited number of micro organisms have been included on Annex I of the directive 9 1/414. Most of them are bacteria and fungi targeting insects, there are only a few preparations targeting diseases. One can cite a strain of Coniothyrium minitans parasitizing the sclerotia of Sclerotinia spp., a strain of Gliocladium catenulatum targeting various soil-borne fungi, and several strains of Trichoderma spp. A few bacteria are also listed on Annex I, a strain of Bacillus subtilis aiming at controlling mostly aerial diseases and a strain of Pseudomonas chlororaphis for seed treatment against soil-borne pathogens of wheat and barley. It is therefore interesting to review progress and failures in biological control research, to identify the bottle necks which prevent faster success in application of microbial control. One can distinguish several domains in which research is needed: basic research aiming at a better understanding of the modes of action of the biological control agents, technological research aiming at improving the processes of production, formulation and application of biological control agents and also applied research in order to satisfy requirements of the regulation and finally much more field experiments to integrate biological control practices in cropping systems.

Identification of the biological control agent

Many people are complaining about the Directive 9 1/414, which imposes strong constraints. However, it provides a good framework to write the research plan needed to develop a biological control agent. The first requirement is an accurate identification of the biological control agent. In many cases the identification of a strain at the species level is not easy. Today, in complement to the traditional methods based on morphology, molecular tools are available to place the biological control strains in a phylogenetic tree among strains of known species. It is then useful to develop a method enabling to identify the biocontrol strain itself among other strains belonging to the same species. This is necessary for regulation procedures, and also to track the strain after release in the environment. Research in molecular biology will provide new tools to achieve this goal of perfect identification of biological control agents at the strain level.

Modes of action of the biological control agent

The second requirement is the study of the modes of action of the biological control agent. This is not an easy task even if the strain belongs to a well studied species. As stated above there are always several modes of action based on parasitism, antibiosis and competition. The secondary metabolites potentially of concern have to be identified and their toxicity has to be studied as it is required for a chemical pesticide. This point is one of the most controversial since micro-organisms are able to produce many different secondary metabolites which properties are not known. Moreover the production of these secondary metabolites depends on many factors such as the age of the culture, the growth medium or the plant organ on which the biological control agent is applied. It is quite impossible to predict, which metabolite will be produced, in which quantity and it is economically not possible to analyze all the metabolites present in a culture at trace levels. Thus the production of secondary metabolites is a domain in which research should be developed. Research is also needed in connection to the method of production of the active substance and on quality control. These important aspects are too often neglected. Working with living organisms it is important to develop processes enabling to grow the biological control agent in pure culture without contaminants, to formulate it to ensure a sufficient shelf life and finally to get a commercial product having the requested efficacy. This is necessary to develop quality control procedures and, in most cases, bio-assays have to be designed for this purpose. Specific research efforts should be made in that field.

Effects on human health

To satisfy the regulation requirements, effects on human health have to be studied. In that domain, research is needed to develop methods adapted to micro organisms. Most of the recommended protocols developed for chemical molecules can not be used to study toxicity of micro organisms. For example, all biological control agents are classified as potentially sensitizers since there is no proper method available to test for this risk; similarly, the Ames test aiming at studying the mutagenic activity is not adapted to micro organisms.

In relation to residues, the biological control agent itself has to be considered as the main residue. But again the question rises when the micro-organism produces secondary metabolites that are susceptible to be toxic. As stated above there is a need of research to develop easy procedures to determine if secondary metabolites are produced in situ, and more generally to propose methods adapted to the study of microbials and addressing important questions in relation to human health.

Behaviour in the environment and effect on non target species

Study of fate and behaviour of the plant protection product in the environment poses quite different questions whether the plant protection product is a chemical or a living micro¬organism. There is an unjustified fear that an introduced micro-organism can multiply in the environment and become a pest. This fear is not justified by facts. In the absence of any selection pressure and introduced bacteria or fungus originating from the natural environment will not become dominant when reintroduced in the same environment. To study the behaviour of a BCA in the environment, one must be able to distinguish the introduced strain from the naturally occurring strains belonging to the same species. Thus a molecular marker such as a SCAR which could have been developed to identify the biological control agent at the strain level is required. This type of procedure is time consuming, but we do have the methodology to address this question.

Finally it is also required to study the effect of the biological control agent on the non target organisms. Again the methodology developed to study the non target effects of chemical pesticides is not adapted to microbials. Considering that most of the actual methods were developed by IOBC working groups, should we ask IOBC colleagues to work together to propose methods adapted to test the non target effects of microbials?

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