Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

04-Nov-2015

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 06 August 2015

ANNEX TO THE REPORT OF THE 5TH BIOPESTICIDES STEERING GROUP SEMINAR ON APPLICATION TECHNIQUES FOR MICROBIAL PEST CONTROL PRODUCTS AND SEMIOCHEMICALS: USE SCENARIOS AND ASSOCIATED RISKS

Series on Pesticides
No. 80

Please note that this document accompanies the seminar report which is available on OLIS under reference ENV/JM/MONO(2015)38.

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ENV/JM/MONO(2015)38/ANN
Unclassified
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This document only contains Annex 4 of the report of the BPSG Seminar. Annex 4 includes slides of all presentations made during the seminar.

The main part of the seminar report, as well as Annexes 1-3, is published under the reference ENV/JM/MONO(2015)38.

ANNEX 4
PRESENTATIONS (SLIDES)

[PPT 1] Presentation on the OECD, the work of OECD-BPSG and general introduction to the seminar
Jeroen Meeussen, BPSG Chair, European Commission

[PPT 2a & 2b] Semiochemicals: different application techniques with focus on pheromones
Cristina Alfaro (Suterra Europe Biocontrol, Barvelona; Spain) & Ulf Heilig (IBMA)

[PPT 3] EFSA's experience in the assessment of semiochemicals and Microbial Pest Control Products
José Tarazona (Parma, Italy)

[PPT 4] Practical considerations for effective application and further development of microbial control agents
Roy Bateman (International Pesticide Application Research Consortium, Cornwall, UK)

[PPT 5] A review of studies on application techniques of MPCPs
Roma Gwynn (Rationale - Biopesticide Strategists, Duns, Scotland) with Willem Ravensberg (Koppert Biological Systems, Berkel & Rodenrijs, the Netherlands)

[PPT 6] Key factors for the success of different types of application techniques of entomopathogenic fungi
Dietrich Stephan (Institute for Biological Control [JKI], Darmstadt; Germany)

[PPT 7] Aerial application techniques with microbials used in plant protection and biocidal products
Denise Munday (Valent BioSciences, Nyon; Switzerland)

[PPT 8] The use of bumblebees for the application of biopesticides
Felix Wäckers (Biobest, Westerlo; Belgium)

[PPT 9] Practical experience with evaluation and assessment of microbial pesticides relating to use scenarios and associated risks
Kersti Gustafsson (Swedish Chemicals Agency - KemI; Sweden)

[PPT 10] Practical experience in the evaluation and assessment of different types of applications of MPCPs
Christine Vergnet (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)

[PPT 11] Horticultural workers' exposure to microbial bioaerosol components from MPCP and naturally occurring counterparts of MPCA
Anne Mette Madsen (National Research Centre for the Working Environment, Copenhagen; Denmark)

Presentation on the OECD, the work of OECD-BPSG and general introduction to the seminar
[PPT 1]

Jeroen Meeussen, BPSG Chair, European Commission


OECD
OECD: The Organisation for Economic Co-operation and Development
OECD


OECD
– Started after World War II;
– Transformed in 1961 into the Organisation for Economic Co-operation and Development with trans-Atlantic and then global reach;
– Today the OECD has 34 member countries;
– More than 70 developing and transition economies are engaged in working relationships with the OECD (Brazil, China and India).

OECD

What is OECD?

A forum in which governments work together to:

- Co-ordinate and harmonise policies;
- Discuss issues of mutual concern;
- Work together to respond to international problems.

A provider of comparative statistics and economic and social data with more than 250 publications per year.

OECD

How do pesticides fit in all this?

One of the fields in which OECD is actively involved is the sustainability of agriculture.

OECD-WGP

OECD - Working Group on Pesticides

The OECD work on agricultural pesticides aims to help member countries:

— improve the efficiency of pesticide control;

— share the work of pesticide registration and re-registration;

— minimise non-tariff trade barriers;

— reduce risks to human health and the environment.




OECD-WGP

OECD - Working Group on Pesticides

Working Group on Pesticides:

- Registration Steering Group (RSG)
- Risk Reduction Steering Group (RRSG)
- **BioPesticides Steering Group (BPSG)**

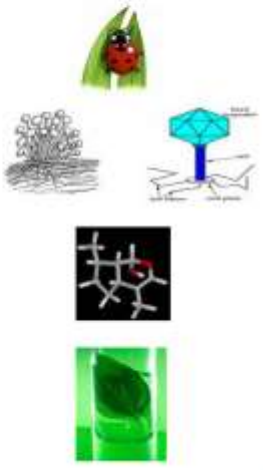
OECD-BPSG

- The **BioPesticides Steering Group** (BPSG) was established by the WGP in 1999 to help member countries to **harmonise** the methods and approaches used to **assess biological pesticides**.

OECD-BPSG

Biological Pesticides:

- Macro-organisms
- Microbial biopesticides
- Semiochemicals
- Plant extracts/Botanicals



OECD-BPSG

The first tasks of the BPSG consisted of:

(i) reviewing regulatory **data requirements** for three categories of biopesticides; and
(ii) developing **formats for dossiers and monographs** for microbials, and pheromones and other semio-chemicals.



OECD-Publications (I)

Registration requirements:

- for **pheromones** (Series on Pesticides, No. 12, 2001);
- for **microbial pesticides** (Series on Pesticides, No. 18, 2003);
- for **invertebrate biocontrol agents/IBCAs** (Series on Pesticides, No. 21, 2004).

OECD-Publications (III)

- OECD **Dossier** and **Monograph** Guidance for **Microbials**, 2003 rev. August 2006;
- OECD **Dossier** and **Monograph** Guidance for **Pheromones and other Semiochemicals**, 2003
- Working Document on the **Evaluation of Microbials** for Pest Control (Series on Pesticides No. 43, 2008).

OECD-BPSG

The BPSG then decided to concentrate its efforts on **science issues** that remain as barriers to harmonisation and work-sharing.

OECD-Publications (IV)

- Issue Paper on **Microbial Contaminant Limits** for Microbial Pest Control Products (Series on Pesticides No. 65, 2011);
- Guidance to the **Environmental Safety Evaluation** of Microbial Biocontrol Agents (Series on Pesticides No. 67, 2012).

OECD-BPSG workshops

- Workshop on the Regulation of Biopesticides: Registration and Communication Issues; 15-17 April 2008, EPA, Arlington, USA.
- Workshop on Microbial Pesticides: Risk Assessment and Risk Management; 17-19 June 2013, Saltsjöbaden, Sweden

OECD-Seminars (I)

- Report of Seminar on "Identity and Characterisation of micro-organisms", OECD Series on Pesticides No. 53, 2010);
- Report of Seminar on "The fate in the environment of microbial control agents and their effect on non-target organisms", OECD Series on Pesticides No. 64, 2011);



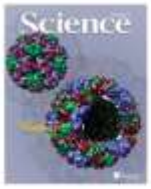
OECD-Seminars (II)

- Report of Seminar on "Characterisation and Analyses of Botanicals for the use in Plant protection Products", OECD Series on Pesticides No. 72, 2012);
- Report on Seminar on " *Trichoderma* spp. for the use in Plant Protection Products: similarities and differences" OECD Series on Pesticides No. 74, 2013).




Workplan 2013-2016

- Promote **communication** and **exchange of information** among regulatory authorities of participating countries.
- Organise **seminars** and **workshops** on topics of common interest.



Seminar on application techniques

Why was this topic selected?

- The application technique and/or use pattern for Microbial Pest Control Products (MPCPs) and semiochemicals can **differ significantly** from conventional chemicals and hence may result in a **different risk and/or risk assessment**.
- The seminar can contribute to a better understanding of *application techniques and exposure assessment in different scenarios* and **facilitate the registration** of MPCPs and semiochemicals.

Seminar - Scope

Discussion on:

- different use patterns in particular if different from conventional chemical spray applications;
- application techniques used (formulation types, application rates);
- human exposure and human risk assessment;
- residue issues (pheromones)
- environmental consequences;
- biocidal uses,
- ....etc.

Seminar - Structure

Presentations on:

- government, research and stakeholder experience and perspectives,

followed by discussion after each set of presentations.



Seminar - Results

With the focus on "application techniques for MPCPs and semiochemicals and associated risks", the goals of this seminar are

1. for participants to share information and to promote a dialogue, and

2. to suggest future work/issue papers and recommendations related to the approval of micro-organisms and semiochemicals in general.

Seminar on application techniques



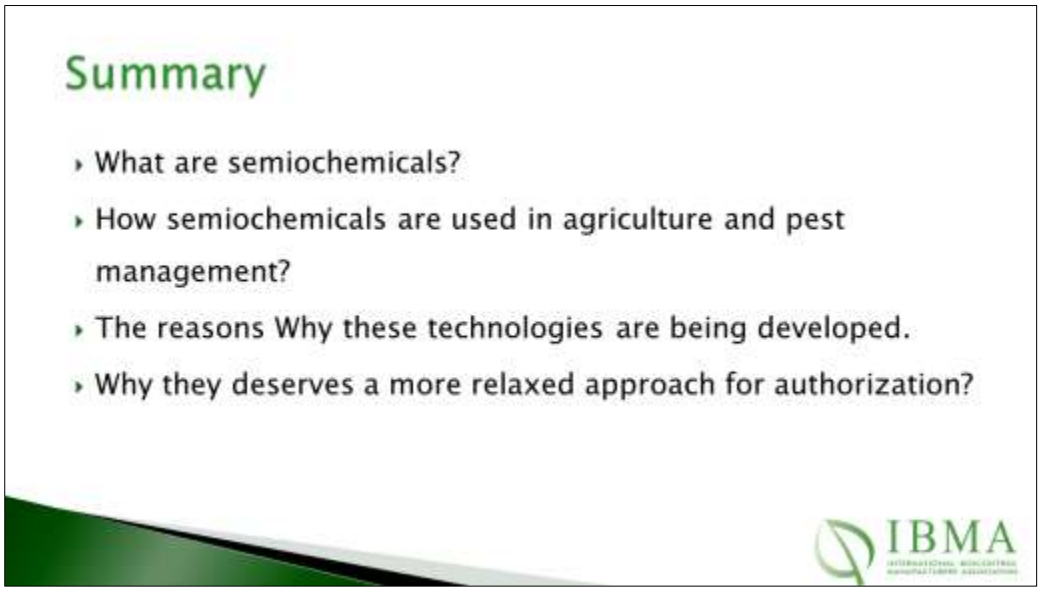
I wish you an interesting and useful seminar!

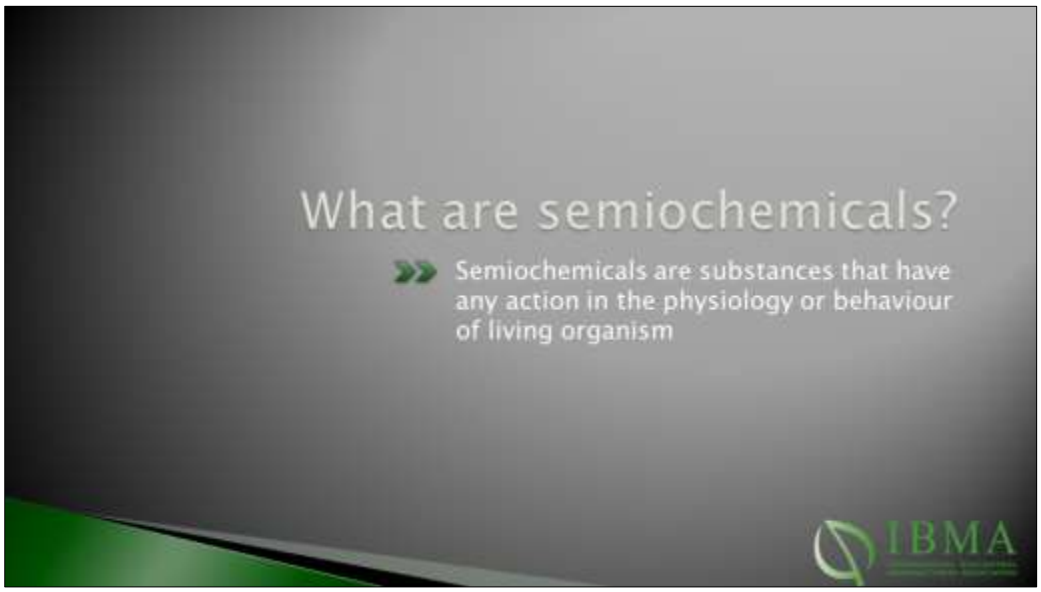
Thank you very much for your attention.

Semiochemicals: different application techniques with focus on pheromones

[PPT 2a & 2b]

Cristina Alfaro (Suterra Europe Biocontrol, Barcelona; Spain) & Ulf Heilig (IBMA)




What are semiochemicals?
Semiochemicals are substances that have any action in the physiology or behaviour of living organism
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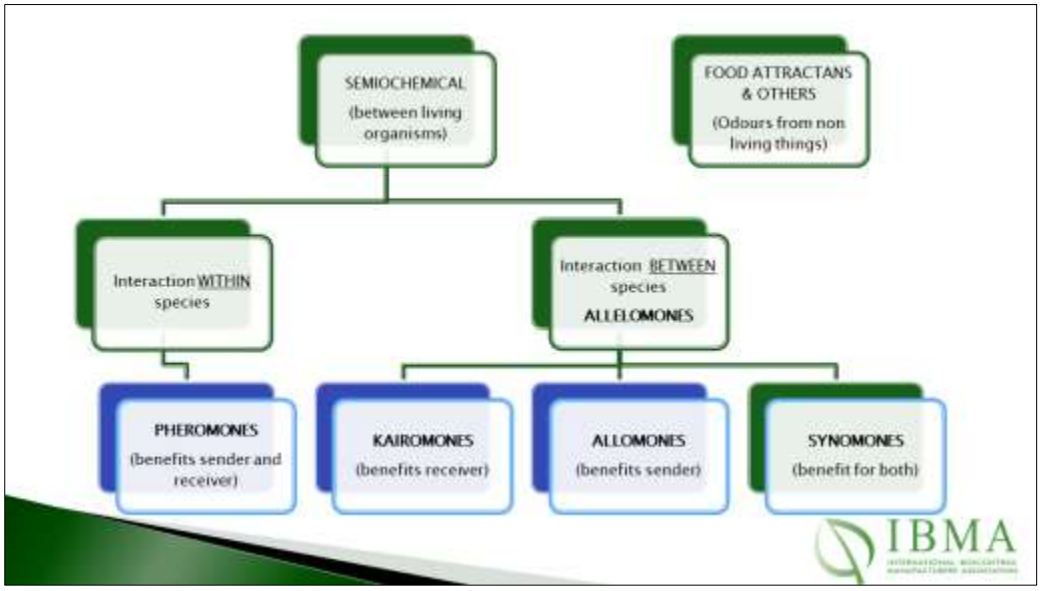
SEMIOCHEMICAL
(between living organisms)
FOOD ATTRACTANS & OTHERS
(Odours from non living things)
Interaction WITHIN species
Interaction BETWEEN species
ALLELOMONES
PHEROMONES
(benefits sender and receiver)
KAIROMONES
(benefits receiver)
ALLOMONES
(benefits sender)
SYNOMONES
(benefit for both)
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Pheromones, the most used in Agriculture

- What are they?
  - Chemicals produced by an individual to affect other individuals of the same species
- What types are they?

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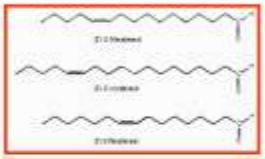
Some examples of pheromones



Chilo suppressalis

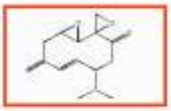
Cydia pomonella

Thaumetopoea pityocampa

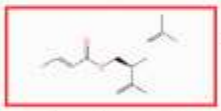
Lobesia botrana

SCLP pheromones

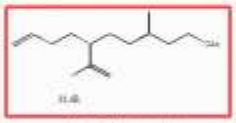
Bactrocera oleae



Periplaneta americana



S-lavandulyl senecioate



Aonidiella aurantii

Other phermones

Pheromone features

- Low polarity and medium molecular weight to reach suitable volatility
- Mostly they are **straight chain aliphatic compounds,** 90% contains 14, 16 or 18 C. (case of SCLP)
- Most of them have structures related chemically and biogenetically to common alcohols and fatty acids
- Often a pheromone is a blend of up to five compounds

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Pheromone features

- High specificity

Butenol: Pheromone of *Bombix mori*

Activity assay

10E, 12Z	10^{-12} µg/ml	
10Z, 12Z	1 µg/ml	

1 billion times more active

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How are SCs used in Pest Managment?

- **MONITORING**: To measure population and determine biological cycles. Improve the use of insecticides
- MASS TRAPPING: Catching as many individuals as possible
- ATTRACT & KILL: Bringing insects to the insecticide
- MATING DISRUPTION: Preventing mating

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SMELL PERCEPTION: THE INSECT NOSE

- Sex pheromone is the key factor for mating
- Well developed antennae
- Undeveloped eyes

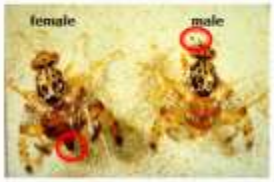
MATING DISRUPTION

- More factors involve in the mating process
- Small antennae
- Complex eyes

MASS TRAPPING/ATTRACT&KILL

Mass Trapping

- Trap + Lure + Killing agent
- Killing agent must be physical
- Key is to attract and trap females
- Kairomones becoming increasingly important



Sex pheromones. MAT (Lepidopteran)

Food attractans. Attracting both sexes (Fruit Flies)

Attract & Kill.

Mostly developed against tephritids like fruit flies *(Ceratitis sp. , B. oleae)*

- Bringing the insects to the killing agent
- Killing agent can be chemical, microbial, beneficial, sterile agent…
- To use low dose of insecticide
- To avoid the contact between insecticide and fruit
- Conventional traps retain the insects caught; A & K devices do not retain insects so, do not saturate and need less maintenance
- Key again is to attract females






Mating Disruption

- Most widely used pest control technique based on pheromones
- Employed against many pests worldwide (RSB, CM, LB, OFM…)
- Goal: To cover the field with a pheromone cloud in order to disrupt the sex-pheromone signal released from the female.

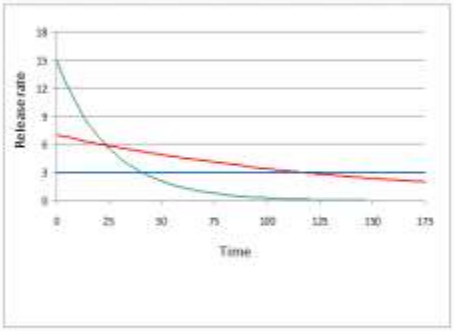

NON- MD

MD treatment

Objectives to be achieved with formulated product

- To release the pheromone as constant as possible over time
- To protect the substance from degradation
- To last the whole season
- To release the pheromone as similar to the female as possible

Imitating the NATURE



Hand applied dispensers

- Conventional application
- Different emission technology

Extruded Dispensers

Reservoir systems

Hand applied dispensers

- Passive emission
- High number of emission points (300–1000 dispensers/ha)
- Low emission rate per dispenser
- Small area of influence per dispenser
- Pheromone released during the whole day
- Dependent on weather conditions
- Logarithmic release rate

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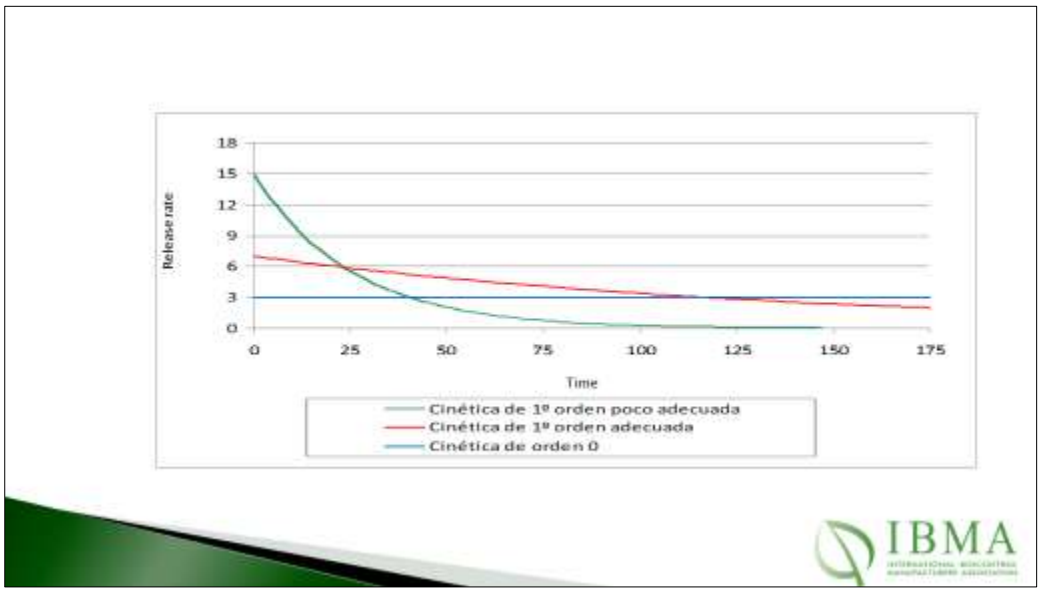
Release rate
18
15
12
9
6
3
0
0
25
50
75
100
125
150
175
Time
Cinética de 1º orden poco adecuada
Cinética de 1º orden adecuada
Cinética de orden 0
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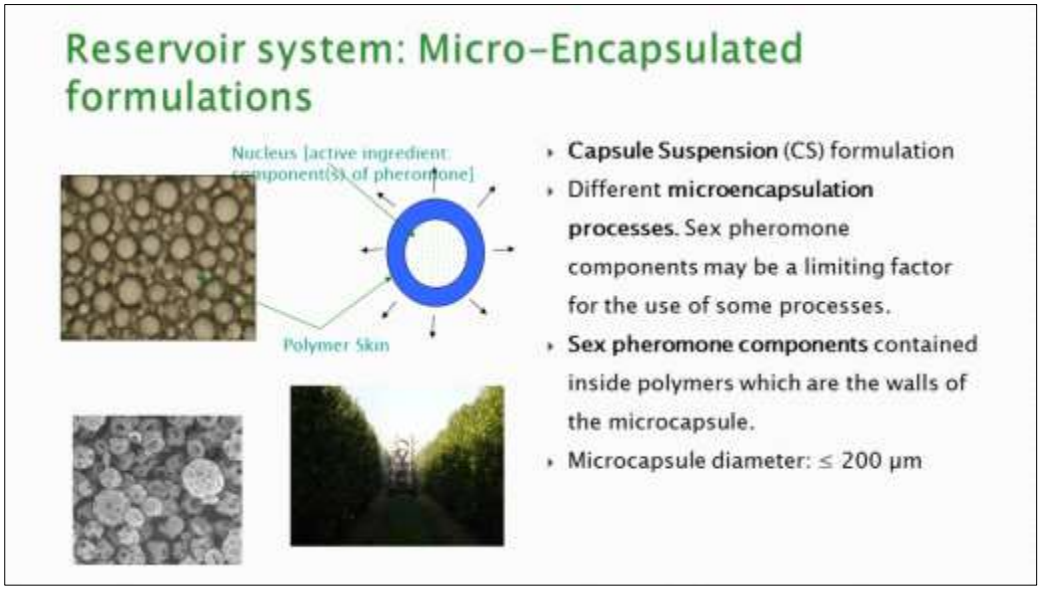
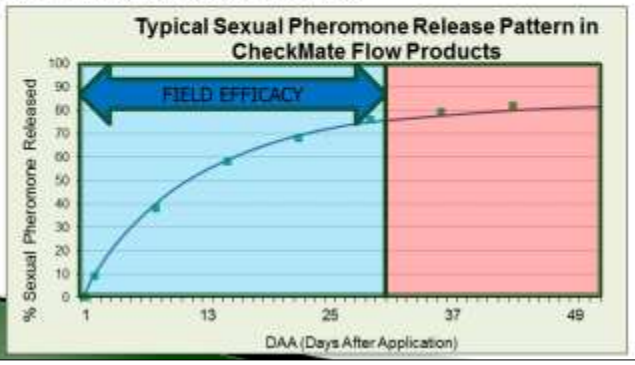

Reservoir system: Micro-Encapsulated formulations
Nucleus [active ingredient component(s) of pheromone]
Polymer Skin
Capsule Suspension (CS) formulation
Different microencapsulation processes. Sex pheromone components may be a limiting factor for the use of some processes.
Sex pheromone components contained inside polymers which are the walls of the microcapsule.
Microcapsule diameter: ≤ 200 µm

Reservoir system: Micro-Encapsulated formulations

- Microcapsule wall controls to some extent the release
- As in any other passive dispenser, microcapsule release rates also depends on weather conditions.



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Timed Release Aerosol Delivery

- Aerosol Formulation contains the sex pheromone.
- Active emission
- High emission rate per dispenser
- Large area of influence per dispenser
- Low number of emissions points (1,25 - 5 d/ha)
- Pheromone released during flight activity
- Constant release rate

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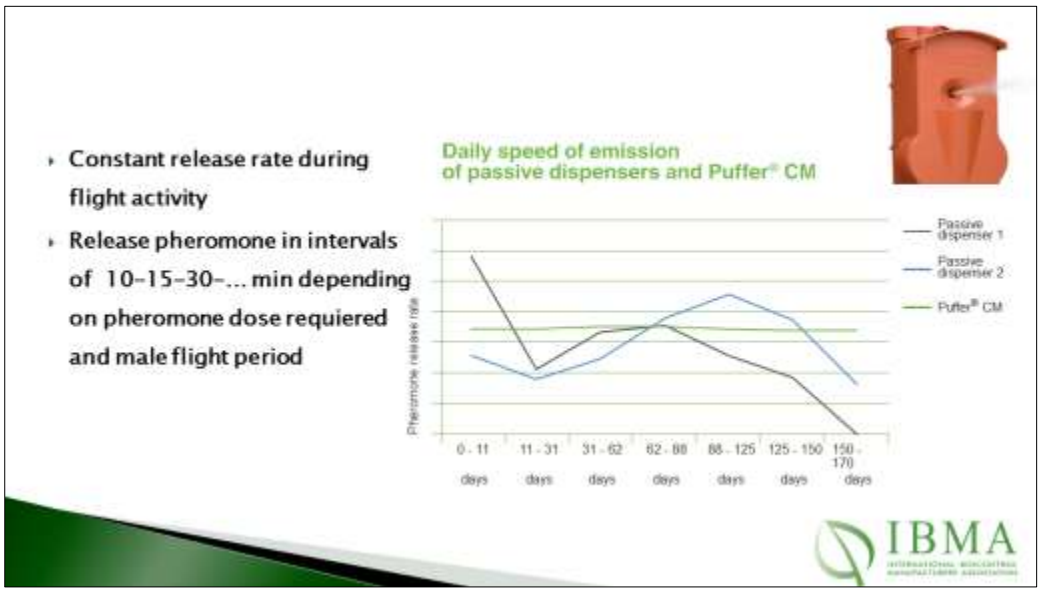
Constant release rate during flight activity
Release pheromone in intervals of 10–15–30–... min depending on pheromone dose requiered and male flight period
Daily speed of emission of passive dispensers and Puffer® CM
Passive dispenser 1
Passive dispenser 2
Puffer® CM
Pheromone release rate
0 - 11 days
11 - 31 days
31 - 62 days
62 - 88 days
88 - 125 days
125 - 150 days
150 - 170 days
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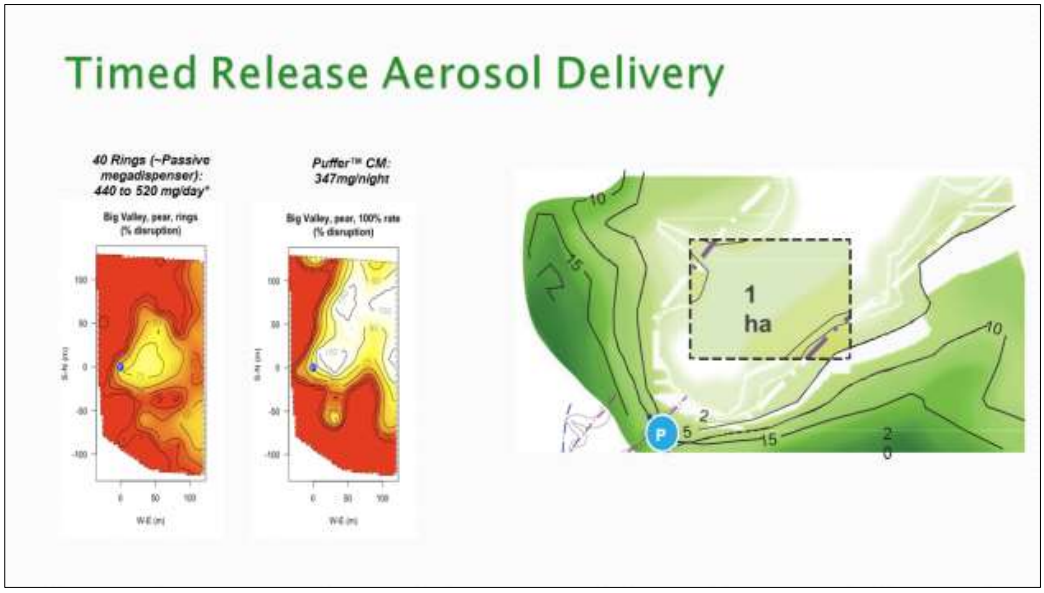
Timed Release Aerosol Delivery
40 Rings (~Passive megadispenser): 440 to 520 mg/day*
Puffer™ CM: 347mg/night
Big Valley, pear, rings (% disruption)
Big Valley, pear, 100% rate (% disruption)
1 ha
10
15
2
5
P

Timed Release Aerosol Delivery

- Same technique
- Same concept
- Same a.i.
- Different emission technology

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Timed Release Aerosol Delivery

Deployment strategies

Microencapsulated formulations

- 50 – 100 million caps / ha per application
- 350 ng pheromone / capsule

Hand-Applied Dispensers

- 250 to 1000 dispensers per ha
- **80 – 200 g A. I. per ha / season**

Aerosol dispensers

- 2 to 3 per ha
- **110 g A.I. per ha / season**

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How much pheromone is there in a MD treatment?

- Can that amount be considered a contaminant?

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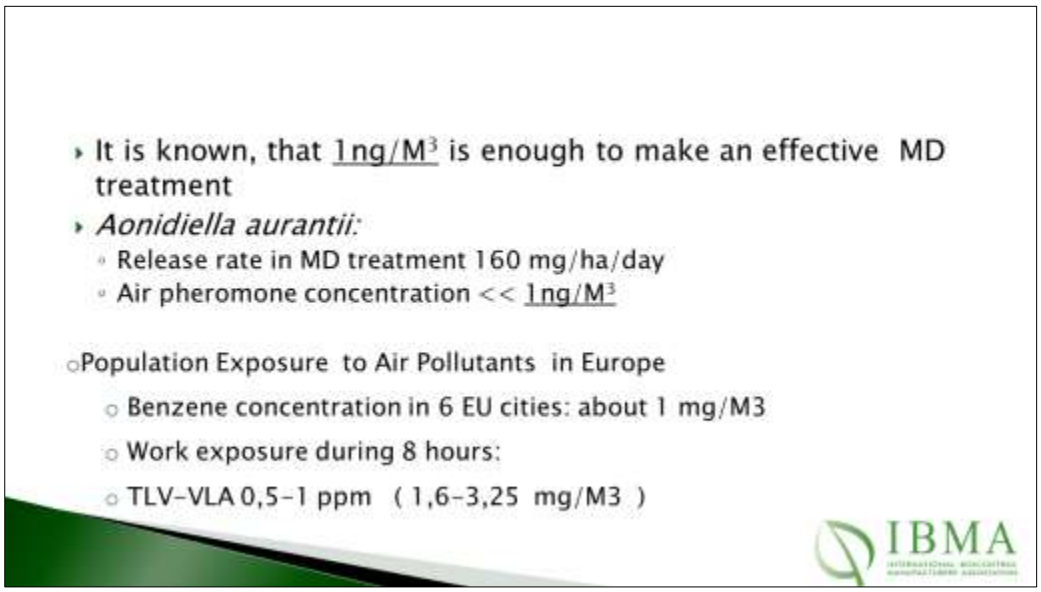
It is known, that 1ng/M3 is enough to make an effective MD treatment
Aonidiella aurantii:
Release rate in MD treatment 160 mg/ha/day
Air pheromone concentration << 1ng/M3
Population Exposure to Air Pollutants in Europe
Benzene concentration in 6 EU cities: about 1 mg/M3
Work exposure during 8 hours:
TLV-VLA 0,5-1 ppm (1,6-3,25 mg/M3)
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Why are these technologies being developed?
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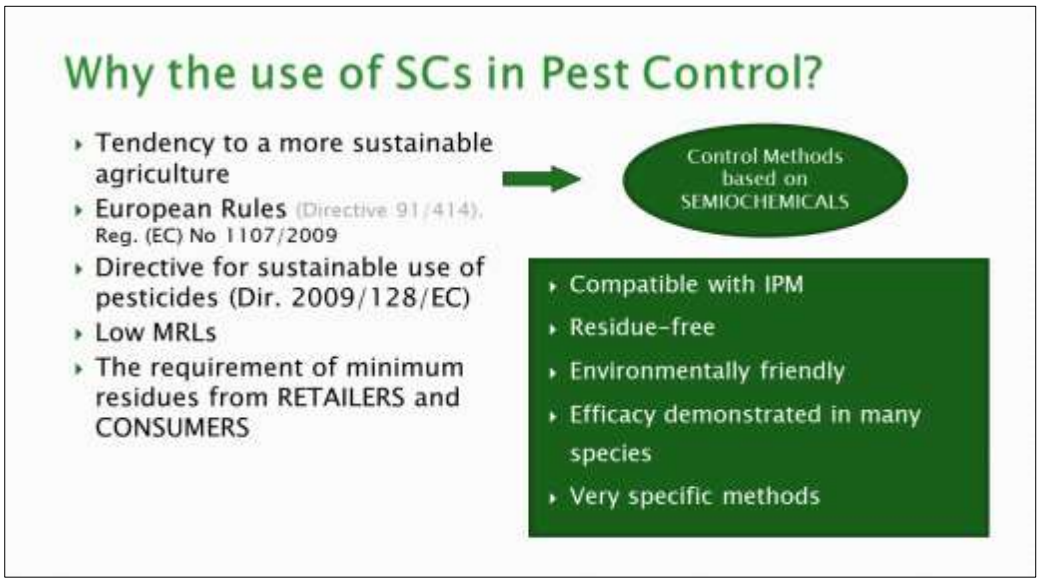
Why the use of SCs in Pest Control?
Tendency to a more sustainable agriculture
European Rules (Directive 91/414), Reg. (EC) No 1107/2009
Directive for sustainable use of pesticides (Dir. 2009/128/EC)
Low MRLs
The requirement of minimum residues from RETAILERS and CONSUMERS
Control Methods based on SEMIOCHEMICALS
Compatible with IPM
Residue-free
Environmentally friendly
Efficacy demonstrated in many species
Very specific methods


Pheromone history
Fabre Observes Insect Chemical Communication
Götz Hypothesis on EGVM MD
Butenandt Identifies Bombykol
Harry Shorey Conducts First Field Trial (T.ni)
P. gossypiella Registered by EPA
1870
1940
1959
1967
1978
1800s
1940
1980
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Evolution in pheromone research

1960	1965	1970	1975	1980	1986	1990
1	1	22	80	222	436	800

- Till the 1990's the field of semiochemicals was mostly of scientific interest
- During last 20 years, however there has been an enormous increase in the rate of discovery
- 2013: On Pherobase [www.pherobase.com] , 7000 insect species, 8000 chemical compounds of which 3500 are semiochemicals
- The sex pheromones of over 2,500 species of Lepidoptera have been published
- Mating disruption is already available for non lepidopteran species like CRS (*Aonidiella aurantii*) and VMB (*Planococcus ficus*)

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US EPA Pheromone Registrations

More than 3 Decades Have Passed!!

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EU Pheromones Registration
In the 80's, first products in EU market from BASF and CBC/ShinEtsu
In the 90's, about 20 formulations in the market
The registration of pheromones (SCLPs) are from early-mid 1990s. Before, registration not required.
1990 was registered a OFM product by BASF
1995 was registered a RSB product in Spain by Agrisense
2000, about 20 formulations were registered for Peach/Apple/Grape (Dir. 91/414)
2010, Revisión of SCLP (still on going). Very slow advances
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Pheromone treated area worldwide
2006: Total: 641,000 ha
2011: Total: > 767,000 ha
2013: It is over 1.000.000 ha
Major Crops:
Forestry – Lymantria dispar
Apples – Cydia pomonella
Stone fruits – Grapholitha molesta
Grapes - Lobesia & Eupoecilia
Cotton – Pectinophora gossypiella
Surface
Europe 250,000 ha
NAFTA 320,000 ha
South America 100,000 ha
Australia and South Africa 50,000 ha
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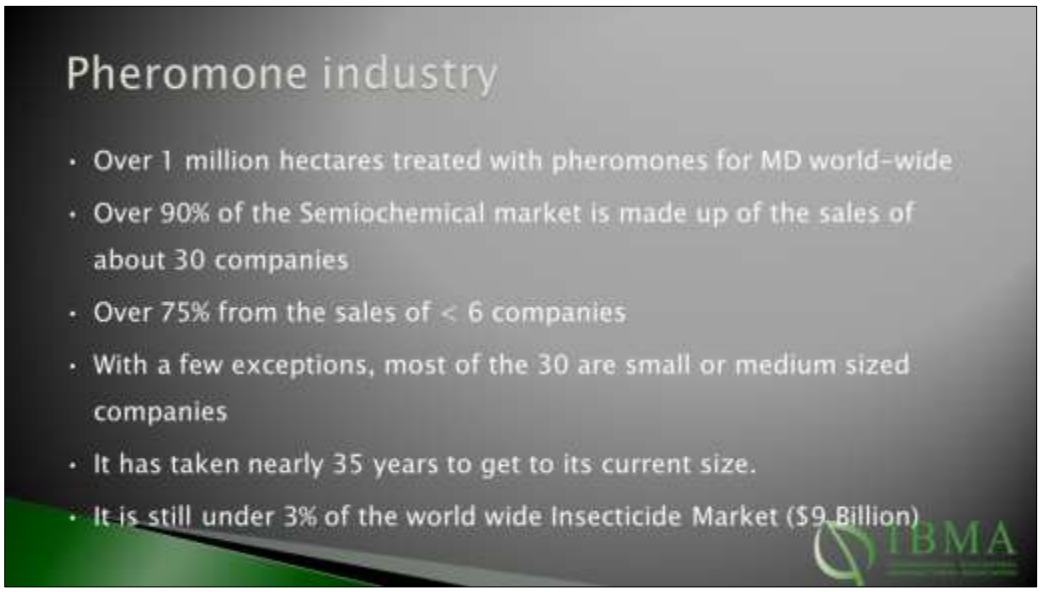
Pheromone industry
• Over 1 million hectares treated with pheromones for MD world-wide
• Over 90% of the Semiochemical market is made up of the sales of about 30 companies
• Over 75% from the sales of < 6 companies
• With a few exceptions, most of the 30 are small or medium sized companies
• It has taken nearly 35 years to get to its current size.
• It is still under 3% of the world wide Insecticide Market ($9 Billion)
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Advantages in the Use of Pheromones
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Pheromone Advantages
Low toxicity substances
Highly specific
Have no effect on beneficials
Low application dose (less than 375g/ha/year)
Residue free
No Resistance developed to date
Easily photo and biodegradable
Success as Pest Control Methods
A 20 year history of exposure to humans demonstrating no adverse effects
IBMA


Semiochemicals and their use in Agriculture (2)
OECD BioPesticides Steering Group Seminar
31st March 2014
Paris
IBMA


Semiochemicals: risk assessment and risk management:
a view of the EU approach
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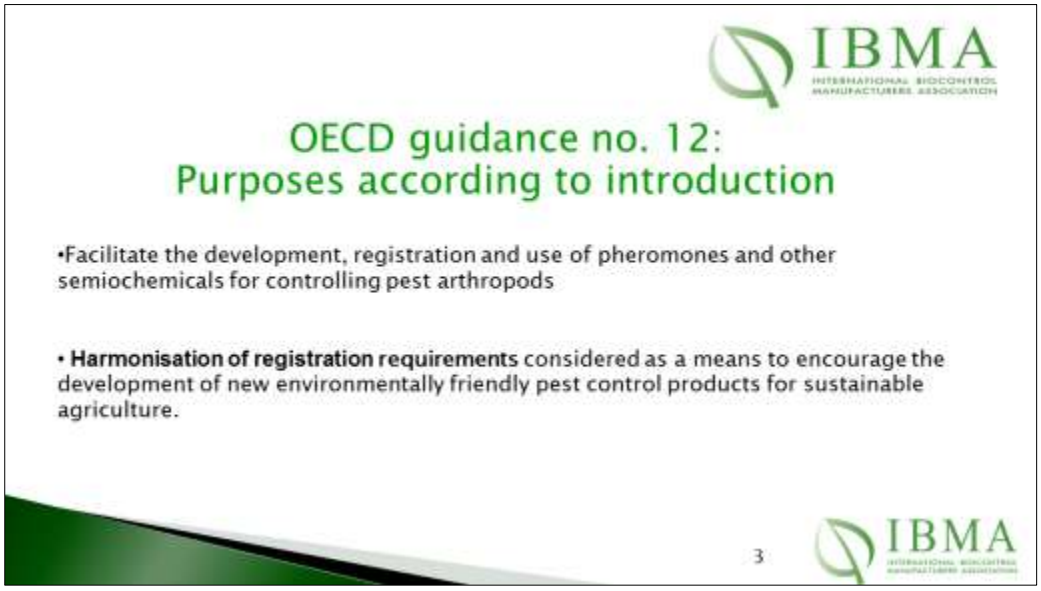
IBMA
INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION
OECD guidance no. 12:
Purposes according to introduction
•Facilitate the development, registration and use of pheromones and other semiochemicals for controlling pest arthropods
• Harmonisation of registration requirements considered as a means to encourage the development of new environmentally friendly pest control products for sustainable agriculture.
3
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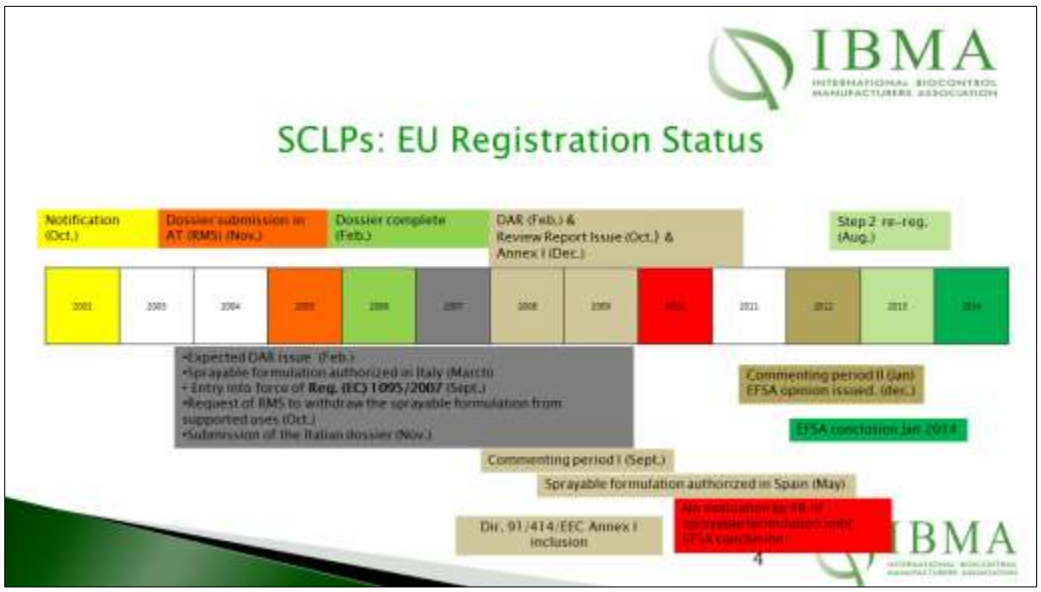
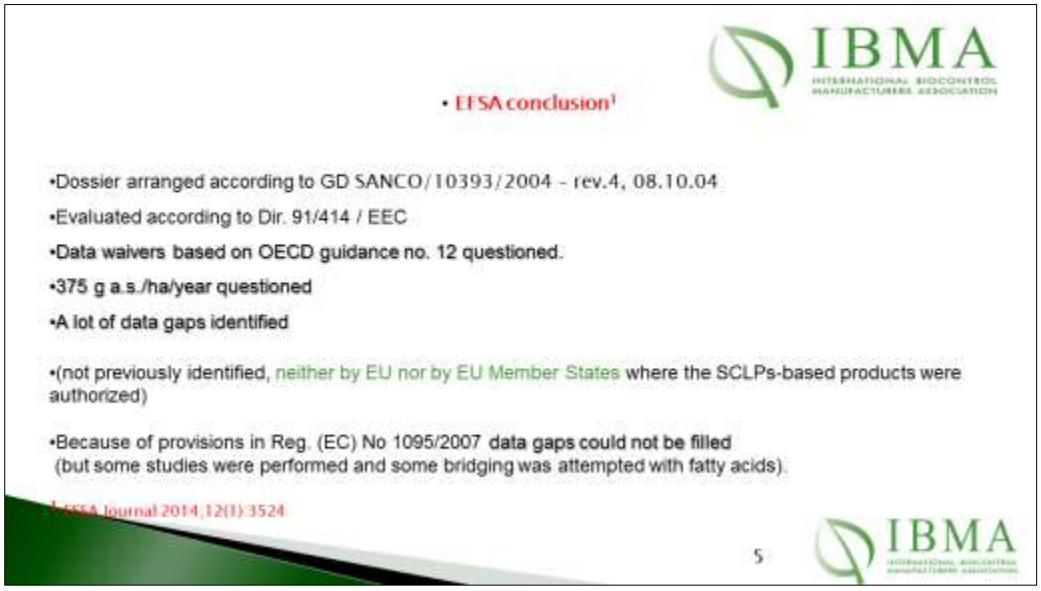

IBMA
INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION
SCLPs: EU Registration Status
Notification (Oct.)
Dossier submission in AT (RMS) (Nov.)
Dossier complete (Feb.)
DAR (Feb.) & Review Report Issue (Oct.) & Annex I (Dec.)
Step 2 re-reg. (Aug.)
2001
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
•Expected DAR issue (Feb.)
•Sprayable formulation authorized in Italy (March)
+ Entry into force of Reg. (EC) 1095/2007 (Sept.)
•Request of RMS to withdraw the sprayable formulation from supported uses (Oct.)
•Submission of the Italian dossier (Nov.)
Commenting period II (Jun)
EFSA opinion issued. (dec.)
EFSA conclusion Jan 2014
Commenting period I (Sept.)
Sprayable formulation authorized in Spain (May)
Dir. 91/414/EEC Annex I inclusion
4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• EFSA conclusion[1]
•Dossier arranged according to GD SANCO/10393/2004 - rev.4, 08.10.04
•Evaluated according to Dir. 91/414 / EEC
•Data waivers based on OECD guidance no. 12 questioned.
•375 g a.s./ha/year questioned
•A lot of data gaps identified
•(not previously identified, neither by EU nor by EU Member States where the SCLPs-based products were authorized)
•Because of provisions in Reg. (EC) No 1095/2007 data gaps could not be filled
(but some studies were performed and some bridging was attempted with fatty acids).
[1] EFSA Journal 2014;12(1):3524
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VP products (Vapour-releasing Products)

- **OECD guidance no. 12**. A reduced dossier is foreseen. 375 g a.s./ha/year as a trigger to avoid long term tox studies, residue trials, environmental fate and eco-toxicology.

- Italy, France, Spain, Portugal, Greece, Germany, U.S.A., Mexico, Argentina, Chile, Uruguay, Israel, Australia, New Zealand:

accepting waivers based on OECD guidance no. 12 and authorizations obtained.

- RMS [AT]: in agreement with OECD guidance no. 12. No major concerns.

VP products/2 in EFSA conclusion

Consumer risk assessment	Data gap due to the absence of robust natural **background levels**
Operator/worker/bystander	Data gap due to the absence of robust natural **background levels**
Environmental risk assessment	Data gap due to the absence of robust natural **background levels**
Ecotoxicological risk assessment	**Bridging** between and in the group **questioned.** Data gap due to the absence of robust natural **background levels**

As in 2001, robust natural background levels are not feasible.

The TF has charged independent experts to prepare a rationale and provide the available information on the background levels

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CS products (Capsule Suspension Products)

- OECD guidance no. 12. A reduced dossier is foreseen. 375 g a.s./ha/year as a trigger to avoid long term tox studies and other studies
- Italy, Spain, U.S.A., Mexico, Argentina, Chile, Uruguay, Australia, New Zealand: we have always presented waivers basing on OECD guidance no. 12 and obtained the authorization.
- RMS [AT]: not in agreement with OECD guidance no. 12

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CS products/2 in EFSA Conclusion

Consumer risk assessment	Data gap due to the absence of robust natural **background levels**
Operator/worker/bystander	Data gap due to the absence of robust natural **background levels**
Environmental risk assessment	Data gap due to the absence of robust natural **background levels**
Ecotoxicological risk assessment	Bridging between and in the group questioned. Data gap due to the absence of robust natural **background levels**

The company owner of the CS representative formulation performed studies and offered documentation, that could not be evaluated due to Reg. (EC) No 1095/2007

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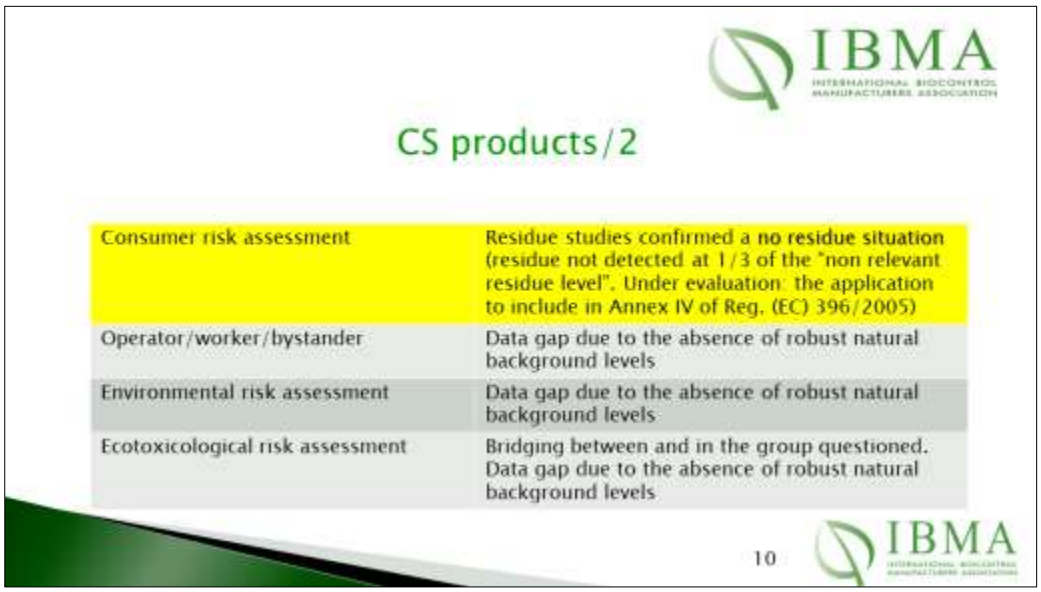
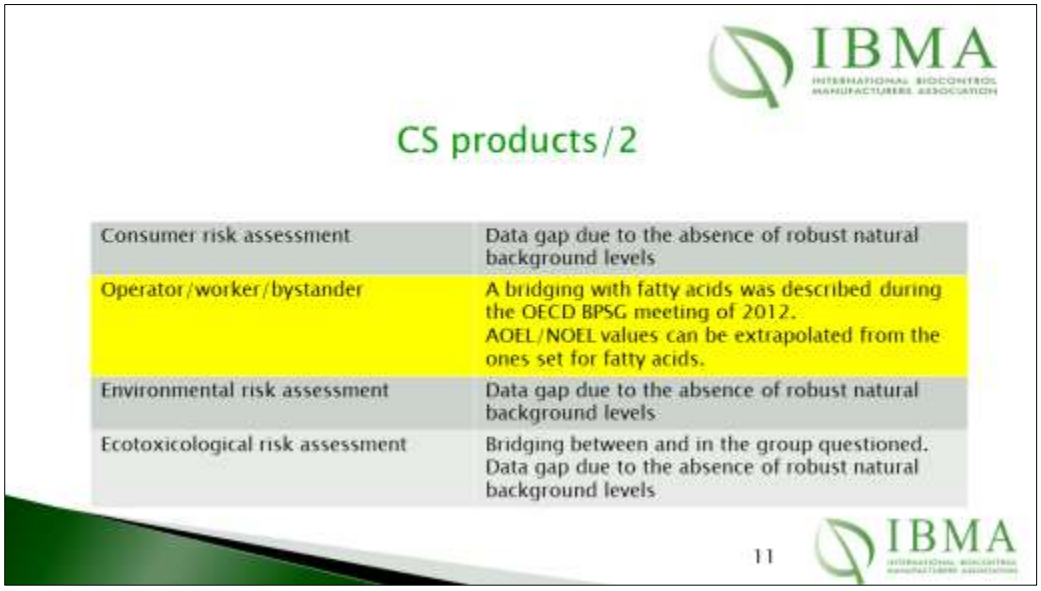
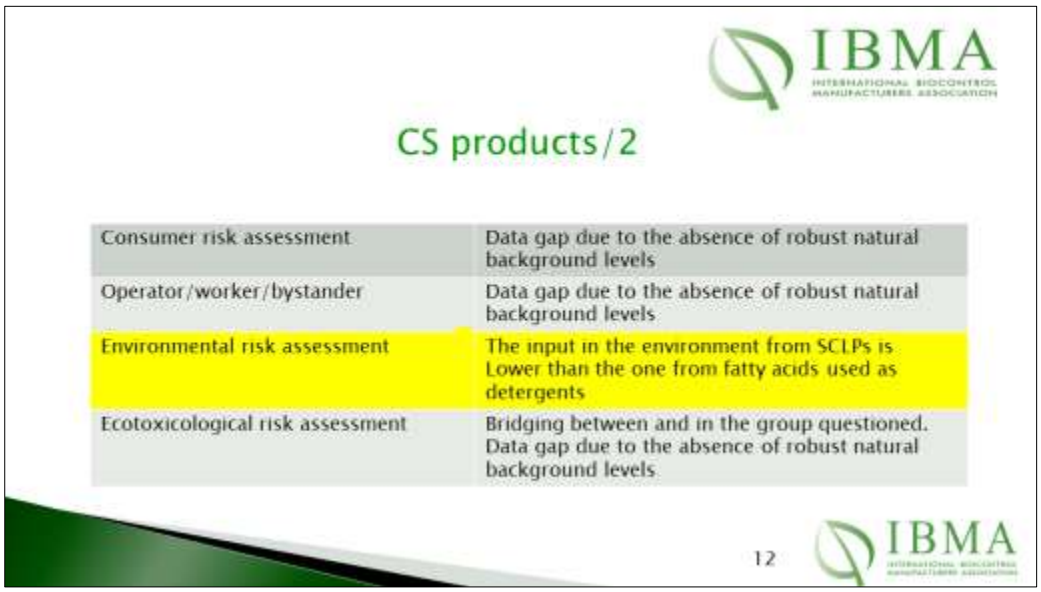

IBMA
CS products/2
Consumer risk assessment
Residue studies confirmed a no residue situation (residue not detected at 1/3 of the "non relevant residue level". Under evaluation: the application to include in Annex IV of Reg. (EC) 396/2005)
Operator/worker/bystander
Data gap due to the absence of robust natural background levels
Environmental risk assessment
Data gap due to the absence of robust natural background levels
Ecotoxicological risk assessment
Bridging between and in the group questioned. Data gap due to the absence of robust natural background levels
10
IBMA


IBMA
CS products/2
Consumer risk assessment
Data gap due to the absence of robust natural background levels
Operator/worker/bystander
A bridging with fatty acids was described during the OECD BPSG meeting of 2012. AOEL/NOEL values can be extrapolated from the ones set for fatty acids.
Environmental risk assessment
Data gap due to the absence of robust natural background levels
Ecotoxicological risk assessment
Bridging between and in the group questioned. Data gap due to the absence of robust natural background levels
11
IBMA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CS products/2
Consumer risk assessment
Data gap due to the absence of robust natural background levels
Operator/worker/bystander
Data gap due to the absence of robust natural background levels
Environmental risk assessment
The input in the environment from SCLPs is Lower than the one from fatty acids used as detergents
Ecotoxicological risk assessment
Bridging between and in the group questioned. Data gap due to the absence of robust natural background levels
12
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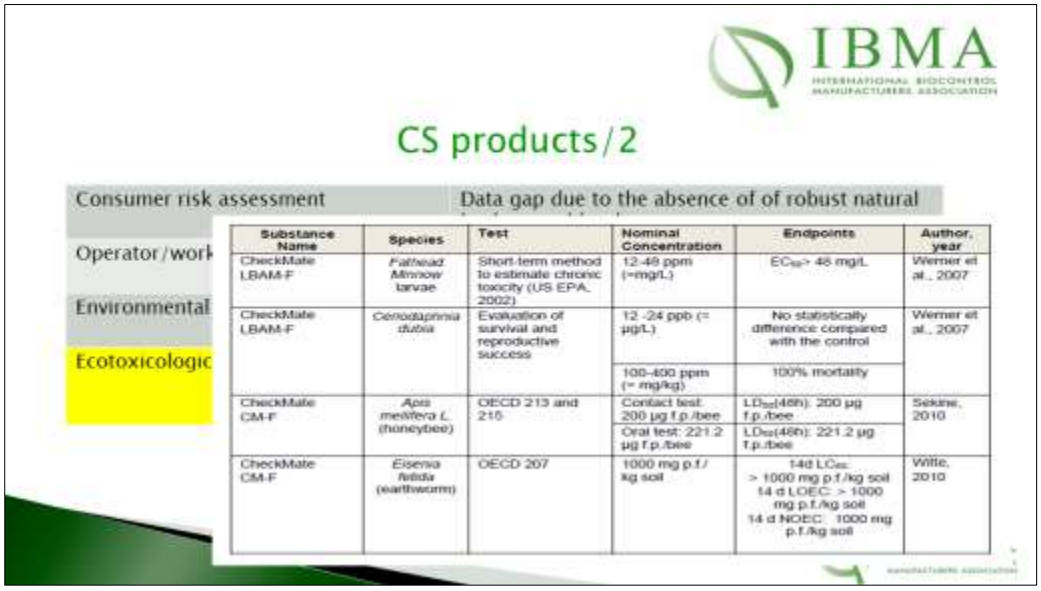
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CS products/2
Consumer risk assessment
Data gap due to the absence of of robust natural
Operator/work
Environmental
Ecotoxicologic
Substance Name
Species
Test
Nominal Concentration
Endpoints
Author, year
CheckMate LBAM-F
Fathead Minnow larvae
Short-term method to estimate chronic toxicity (US EPA, 2002)
12-48 ppm (=mg/L)
EC50 > 48 mg/L
Werner et al., 2007
CheckMate LBAM-F
Ceriodaphnia dubia
Evaluation of survival and reproductive success
12 -24 ppb (= µg/L)
No statistically difference compared with the control
Werner et al., 2007
100–400 ppm (= mg/kg)
100% mortality
CheckMate CM-F
Apis mellifera L. (honeybee)
OECD 213 and 215
Contact test: 200 µg f.p./bee
LD50(48h): 200 µg f.p./bee
Sekme, 2010
Oral test: 221.2 µg f.p./bee
LD50(48h): 221.2 µg f.p./bee
CheckMate CM-F
Eisenia fetida (earthworm)
OECD 207
1000 mg p.f./kg soil
14d LC50: > 1000 mg p.f./kg soil 14 d LOEC: > 1000 mg p.f./kg soil 14 d NOEC: 1000 mg p.f./kg soil
Wille, 2010

Threshold of 375 g a.s./ha/year for SCLPs

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This application rate would create a pheromone concentration in the air comparable to the natural one during severe insect infestation. It is **not the yearly pheromone amount released** by the lepidoptera.

Pheromone products are applied before the first flight of the insects. They spread out the **release** over a long period of time i. e. all over the day, **continuously for the whole season**, while the **moths release pheromones in the evening/night and the rate varies** with the population sizes and generations.

The **degradation** of pheromones is **very fast without any accumulation**. Thus it is deemed appropriate to **compare the conc. reached in treated fields with those in untreated heavily infested field** and not the cumulated annual release of pheromone dispensers with the one released by female moths.
The application threshold value of 375 g a s/ha/y was established with this in mind.

The application rate of 375 g a.s./ha/year is considered to be a valid threshold. It will facilitate the registration of SCLPs based products and thus be a major incentive for applicants.

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In conclusion ...

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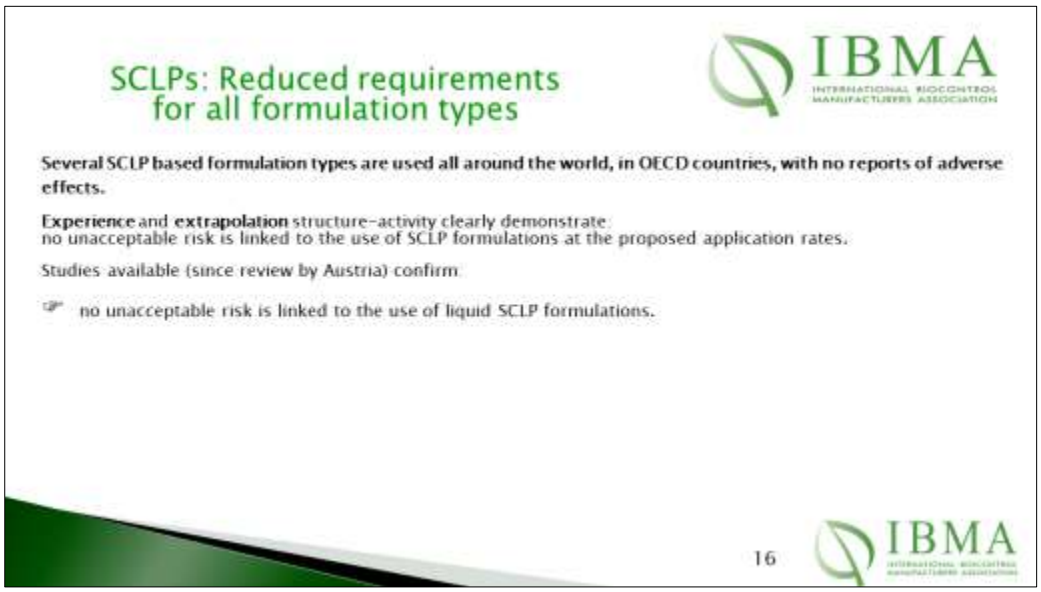
SCLPs: Reduced requirements for all formulation types
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Several SCLP based formulation types are used all around the world, in OECD countries, with no reports of adverse effects.
Experience and extrapolation structure-activity clearly demonstrate:
no unacceptable risk is linked to the use of SCLP formulations at the proposed application rates.
Studies available (since review by Austria) confirm:
no unacceptable risk is linked to the use of liquid SCLP formulations.
16
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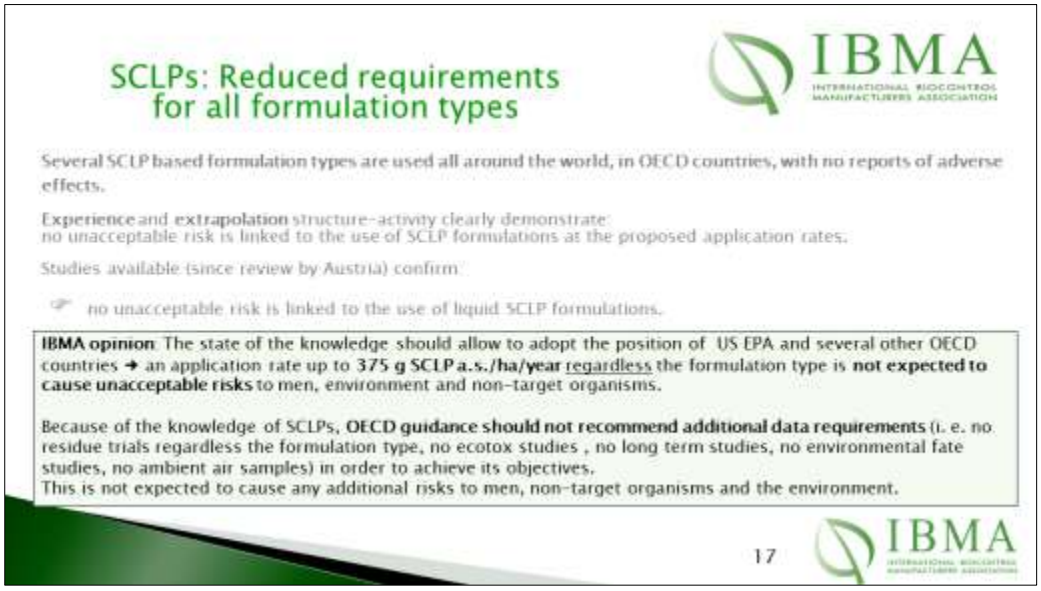
SCLPs: Reduced requirements for all formulation types
IBMA
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Several SCLP based formulation types are used all around the world, in OECD countries, with no reports of adverse effects.
Experience and extrapolation structure-activity clearly demonstrate:
no unacceptable risk is linked to the use of SCLP formulations at the proposed application rates.
Studies available (since review by Austria) confirm:
no unacceptable risk is linked to the use of liquid SCLP formulations.
IBMA opinion: The state of the knowledge should allow to adopt the position of US EPA and several other OECD countries → an application rate up to 375 g SCLP a.s./ha/year regardless the formulation type is not expected to cause unacceptable risks to men, environment and non-target organisms.
Because of the knowledge of SCLPs, OECD guidance should not recommend additional data requirements (i. e. no residue trials regardless the formulation type, no ecotox studies , no long term studies, no environmental fate studies, no ambient air samples) in order to achieve its objectives.
This is not expected to cause any additional risks to men, non-target organisms and the environment.
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SCLPs: Does it makes sense to continue evaluating 'new' ones?

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With the SCLPs approved there is basically nothing to evaluate for a new SCLPs except specification and analytical method. The phys/chem, toxicity and eco-toxicity properties can be extrapolated from the approved SCLPs.

Example: (Z)-8-14Ac

Already approved: (Z)-8-12Ac, (Z)-9-12Ac, (Z)-9-14Ac etc.

Does it make sense to 'evaluate' this 'new' one (since there is no additional data except specification)?

→ Limited public resources could be allocated to work on other issues!

→ SCLP-based products important for IPM in sustainable cropping systems could be introduced much faster!

After the final approval of the SCLP group why not simply extent this approval to all SCLPs complying with the OECD definitions?

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Thank you!

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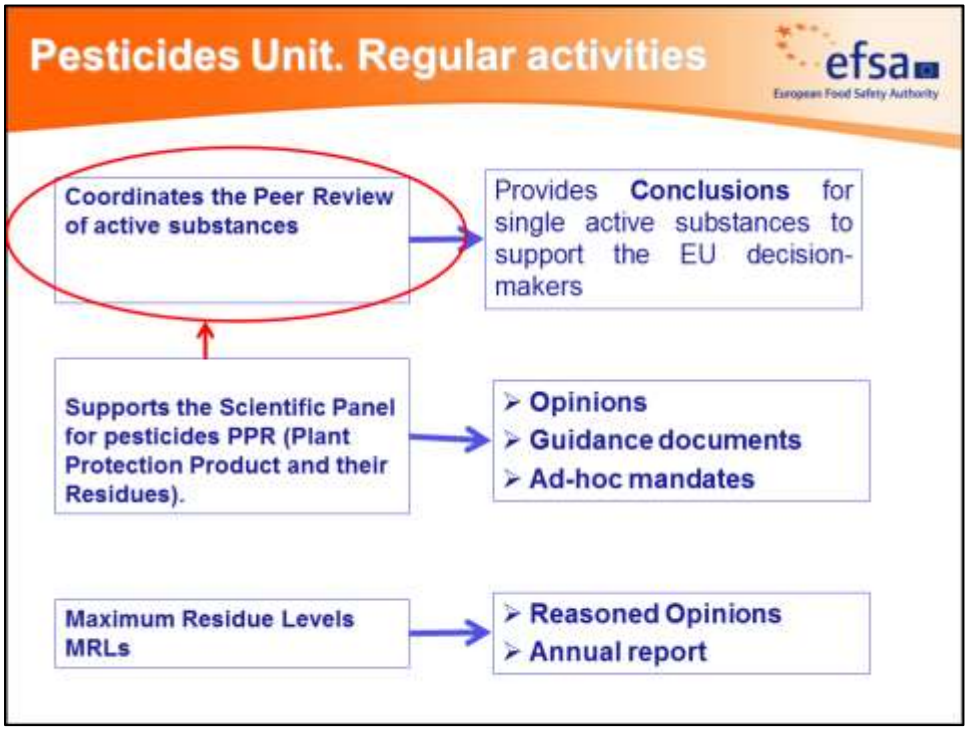
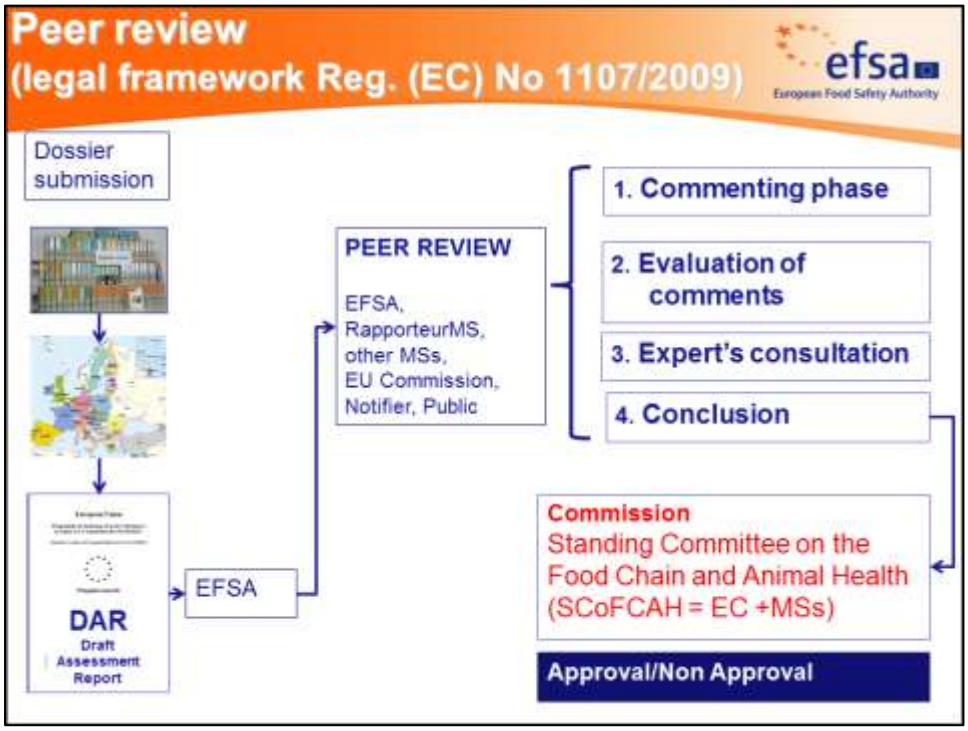
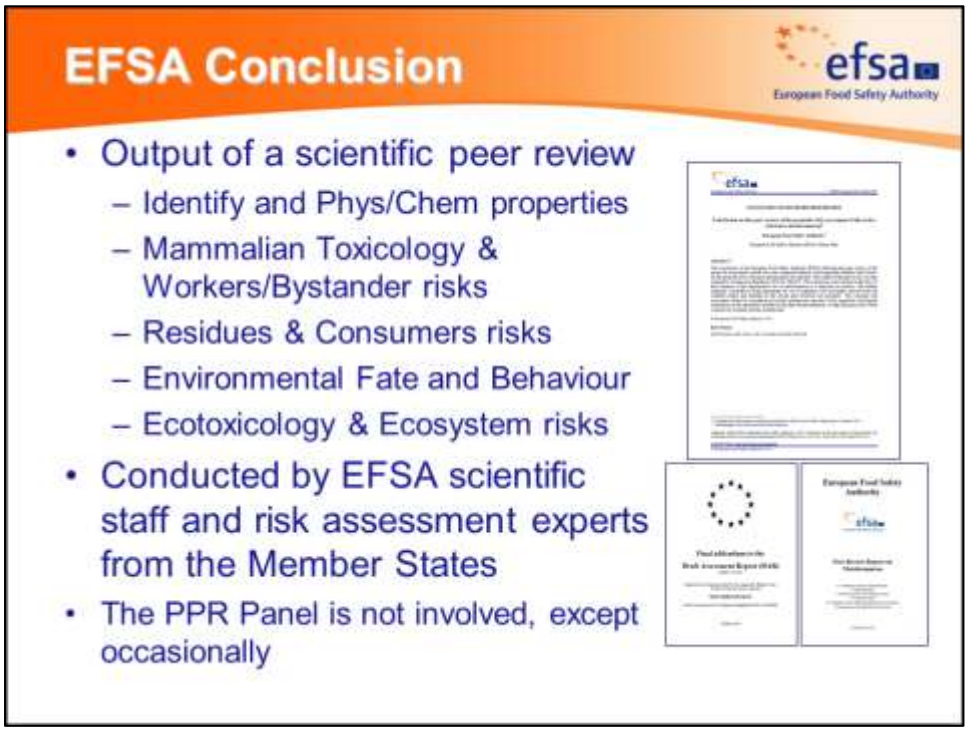
EFSA's experience in the assessment of semiochemicals and Microbial Pest Control Products

[PPT 3]

José Tarazona (Parma, Italy)




Peer review
(legal framework Reg. (EC) No 1107/2009)
efsa
European Food Safety Authority
Dossier submission
DAR
Draft Assessment Report
EFSA
PEER REVIEW
EFSA, RapporteurMS, other MSs, EU Commission, Notifier, Public
1. Commenting phase
2. Evaluation of comments
3. Expert's consultation
4. Conclusion
Commission
Standing Committee on the Food Chain and Animal Health (SCoFCAH = EC +MSs)
Approval/Non Approval


EFSA Conclusion
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European Food Safety Authority
• Output of a scientific peer review
– Identify and Phys/Chem properties
– Mammalian Toxicology & Workers/Bystander risks
– Residues & Consumers risks
– Environmental Fate and Behaviour
– Ecotoxicology & Ecosystem risks
• Conducted by EFSA scientific staff and risk assessment experts from the Member States
• The PPR Panel is not involved, except occasionally

Active substances

efsa European Food Safety Authority

- '**Active substances**': **substances, including micro-organisms** having general or specific action against harmful organisms or on plants, parts of plants or plant products.
  - 'substances': **chemical** elements and their **compounds**, as they **occur naturally** or **by manufacture**, including impurities from the manufacturing process
  - '**micro-organisms**': microbiological entity, including lower fungi and viruses, cellular or non-cellular, **capable of replication** or of **transferring genetic material**

Risk assessment for active substances

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- Approval criteria for "active substances" (e.g. Annex II Reg. 1107/2009
- Uniform principles (e.g. Reg. 546/2011)
  - Part 1. Chemical plant protection products
  - Part 2. plant protection products containing micro-organisms
- Data requirements (e.g. Reg. 283/2013)
  - Part A. Chemical active substances
  - Part B. Microorganisms including viruses

Chemicals
Semiochemicals
Microbial toxins
Inert microorganisms

Microbials

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Microorganisms

EFSA experience of concluding for each organism, using the information provided against the EU data requirements, with the existing guidance.

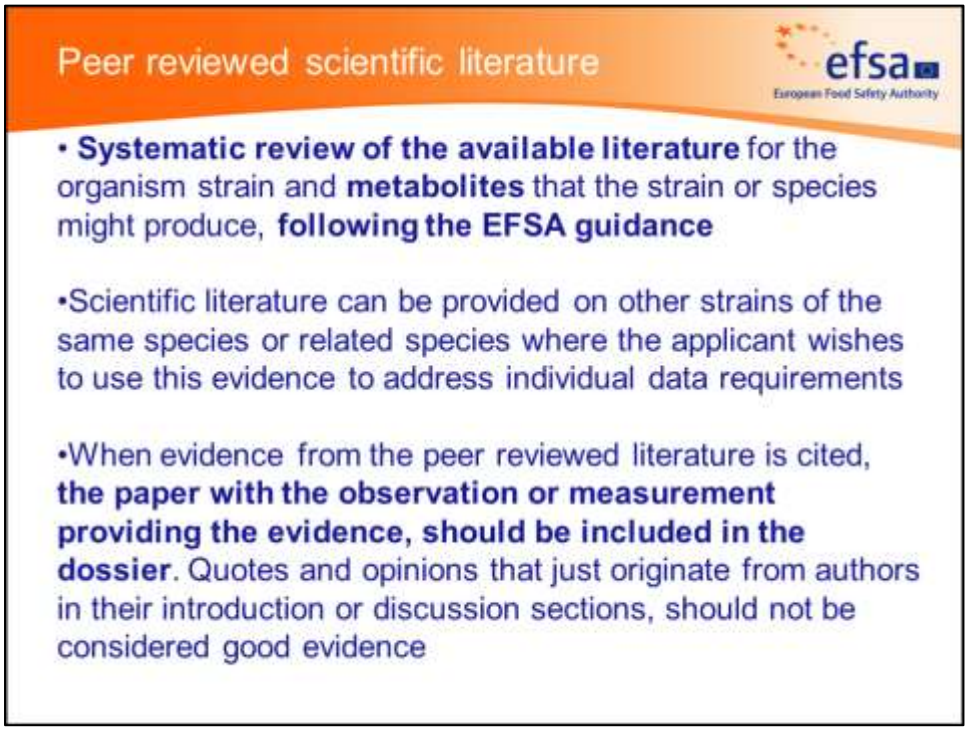
Guidance available that should be followed / is accepted:

- **OECD** issue paper on microbial contaminant limits for microbial pest control products **Series on pesticides No. 65** ENV/JM/MONO(2011)43 12-Oct-2011
- European Food Safety Authority; **Submission of scientific peer-reviewed open literature** for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092.

Microorganisms

Guidance available that gives pointers and approaches that can be followed / have been accepted by the peer review in selected cases:

- OECD guidance to the environmental safety evaluation of microbial biocontrol agents **Series on pesticides No. 67** ENV/JM/MONO(2012)1 17-Feb-2012


Peer reviewed scientific literature
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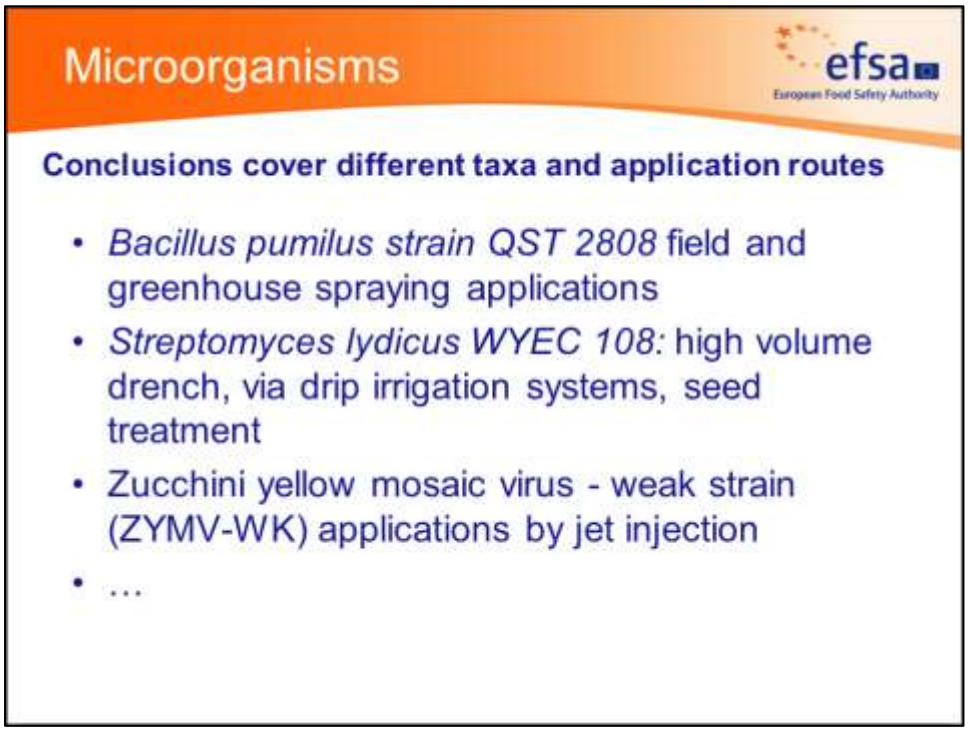
• Systematic review of the available literature for the organism strain and metabolites that the strain or species might produce, following the EFSA guidance
•Scientific literature can be provided on other strains of the same species or related species where the applicant wishes to use this evidence to address individual data requirements
•When evidence from the peer reviewed literature is cited, the paper with the observation or measurement providing the evidence, should be included in the dossier. Quotes and opinions that just originate from authors in their introduction or discussion sections, should not be considered good evidence


Microorganisms
efsa
European Food Safety Authority
Conclusions cover different taxa and application routes
• Bacillus pumilus strain QST 2808 field and greenhouse spraying applications
• Streptomyces lydicus WYEC 108: high volume drench, via drip irrigation systems, seed treatment
• Zucchini yellow mosaic virus - weak strain (ZYMV-WK) applications by jet injection
• …

Main basis for the conclusions

- Did not cause signs of toxicity, infectivity and pathogenicity
- Due to lack of data on the potential production of toxins/metabolites of concern risk assessment cannot be finalised.
- Potential risk of infecting and producing adverse effects in animal hosts and humans is low.
- Risk (effects and transmission) for non-target plants could not be finalise

Data gaps / issues identified in EFSA pesticide conclusions on microorganisms

- **Methods for identity of the strain**, best available technology, how to assess if the test procedures presented are appropriate
- Level of evidence to demonstrate **relationships** to / lack of relationships to **known pathogens** (systematic literature review is needed) and how to distinguish from related pathogens
- Information on **production of metabolites** including other strains of the species, conditions of production, evidence of absence of these from the product
- Characterization / evidence of **levels metabolites in environmental compartments** following application

Data gaps / issues identified in EFSA microorganism conclusions:

- Assessment of the potential for **transfer of genetic information to other species** (primary concern usually plasmids between bacteria species)
- Absence of **interference of organism** on prescribed methods of **analysis for pathogens in drinking water**
- Information on **competitiveness / multiplication** to conclude if the organism will decline to background levels within a year
- Effects studies provided for **characterising environmental risk** can be too short to conclude lack of pathogenicity or infectivity to non target species. Consider the biology of the active organism strain / species when designing tests conditions and duration

Key elements: microorganisms

- Theoretical broad coverage in terms of taxa and application routes
- But in reality current experience covers only assessments based on lack of hazard (application method not relevant)
- There is a need for developing guidance covering:
  - exposure assessment for the microorganisms
  - exposure assessment for metabolites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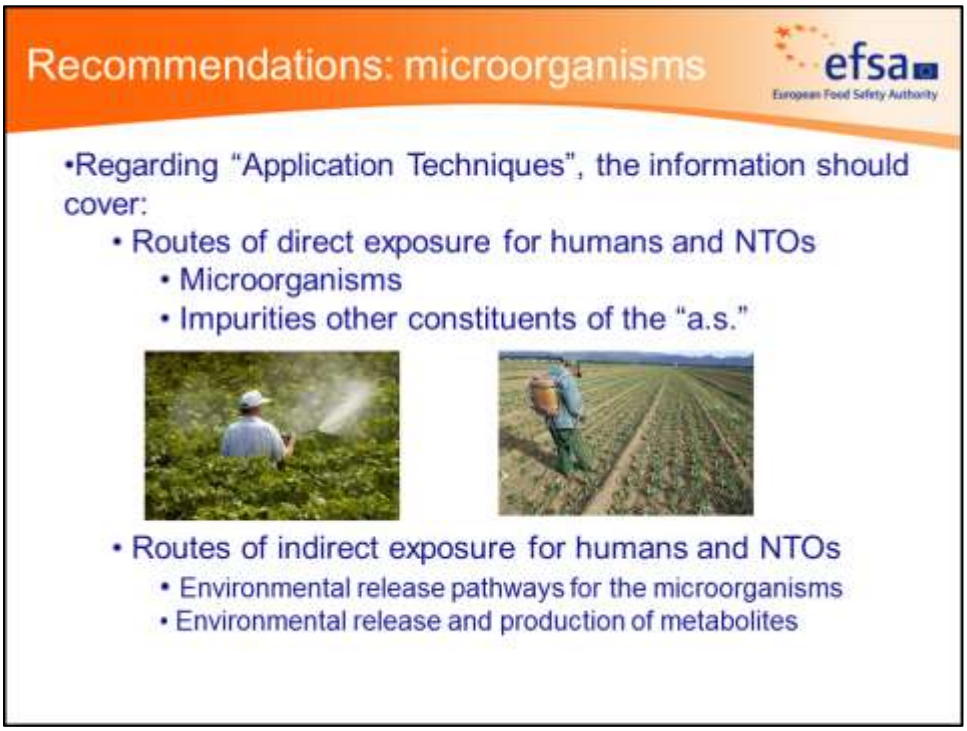
EFSA JOURNAL
NEW EFSA Guidance Protected Crops: 20 March 2014
Plant protection products (PPPs) of biological nature
•The guidance mainly concerns chemical PPPs, i.e. active substances and their metabolites.
•PPPs of biological nature, micro-organisms or microbial biological control agents (mBCAs) are also covered.
•However, no specific exposure models for mBCAs are available.
• Models proposed in this Guidance are not always capable of, or meant for, predicting fate and behaviour of such products in the environment, e.g.
• do not consider the potential growth of micro-organisms
• It is recommended that further guidance should be developed for mBCAs from protected crops and for open-field applications.


Recommendations: microorganisms
efsa
European Food Safety Authority
•Regarding "Application Techniques", the information should cover:
• Routes of direct exposure for humans and NTOs
• Microorganisms
• Impurities other constituents of the "a.s."
• Routes of indirect exposure for humans and NTOs
• Environmental release pathways for the microorganisms
• Environmental release and production of metabolites

EFSA external scientific report

11 December 2013

- Fate and behaviour of MPCAs in the environment
- Characteristics of produced metabolites and enzymes
- Field concentration and non-target effects observed for metabolites/toxins
- Influence of biotic and abiotic factors on MPCAs

Semio-chemicals

Straight Chain Lepidopteran Pheromones
As semio-chemical on stone fruit, pome fruit, tree nuts corn, vegetables, citrus, olives, rice, grapes, cotton and flowers
22 January 2014

- vapour releasing closed dispenser
- spraying a microencapsulated vapour releasing system

Z,Z,Z,Z-7,13,16,19-doco-satetraen-1-yl isobutyrate As semio-chemical on forests and oak forests
11 February 2014

No information, just data gaps

Pheromones

efsa European Food Safety Authority

Experience of concluding for straight chain lepidopteran pheromones

Guidance available that was followed / is accepted:

- OECD series on pesticides number 12 Guidance for Registration Requirements for Pheromones and Other Semiochemicals Used for Arthropod Pest Control. ENV/JM/MONO(2001)12 26-Feb-2002

SCL Pheromones

efsa European Food Safety Authority

- Products formulated as slow release →Emission at natural backgrounds.

Application rates of up to 375 g SCLP/ha/yr are generally understood to result in exposure levels which are comparable to natural emissions and safe for non-target species

- OECD guidance value of 375 g ai/ha/yr could not be traced back to published observations or measurements → does not satisfy the level of transparency expected by European regulation
- US EPA could arrange for the original observations and measurements
- Insufficient information to confirm exposure levels for spray applications

Final recommendations

Regarding "Application Techniques", not covered by those in current guidance, the development/information should cover:

- Routes of direct exposure for operators and workers, and if relevant, bystanders and residents
- Exposure routes for consumers
- Routes of direct exposure for NTOs
- Environmental release pathways
  - Conceptual justified description of the relevant pathways
  - Scenarios and parameterization (with supporting data) for quantitative release estimations
  - Connection of release estimations (e.g. units, timelines) with current environmental fate scenarios/models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Practical considerations for effective application and further development of microbial control agents

[PPT 4]

Roy Bateman (International Pesticide Application Research Consortium, Cornwall, UK)

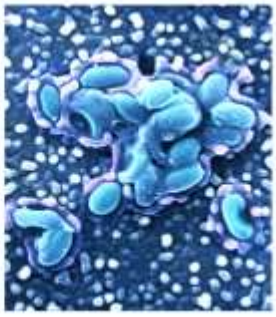
1. Using biodiversity

- Successful control starts with an efficacious microbial control agent (MCA)
- We are only now starting to understand the true nature of microbial bodiversity … and grossly underutilising potentially useful organisms.

IPARC

Effective inundative biological control

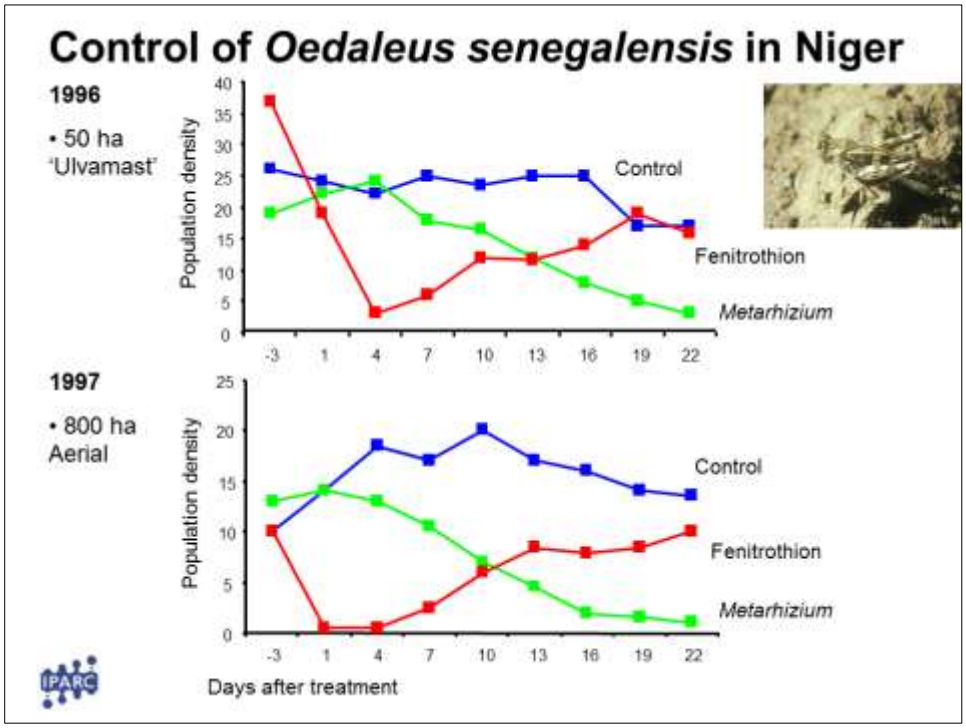
- Adapted, virulent isolate(s)
  - ➢ *Metarhizium acridum*
- Oil-based formulations; maintenance of viability
- Appropriate application



IPARC

GREEN MUSCLE


Aerial spraying
• Rotary atomisers (CDA)
• Oil-miscible flowable concentrate (OF) formulation

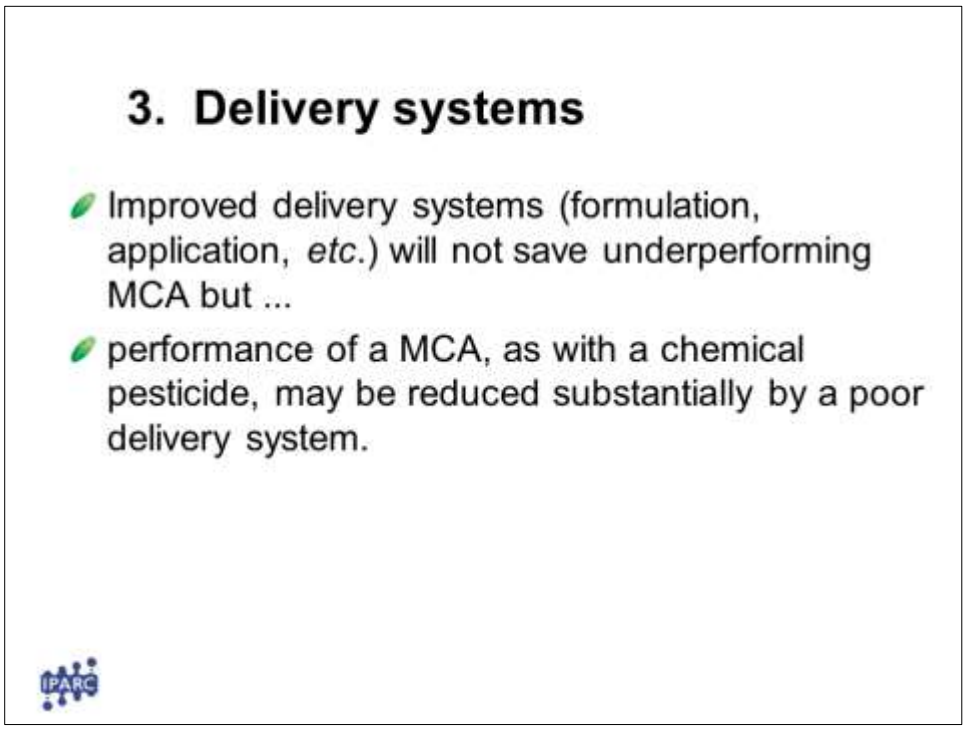

Control of Oedaleus senegalensis in Niger
1996
• 50 ha 'Ulvamast'
Population density
Control
Fenitrothion
Metarhizium
1997
• 800 ha Aerial
Days after treatment

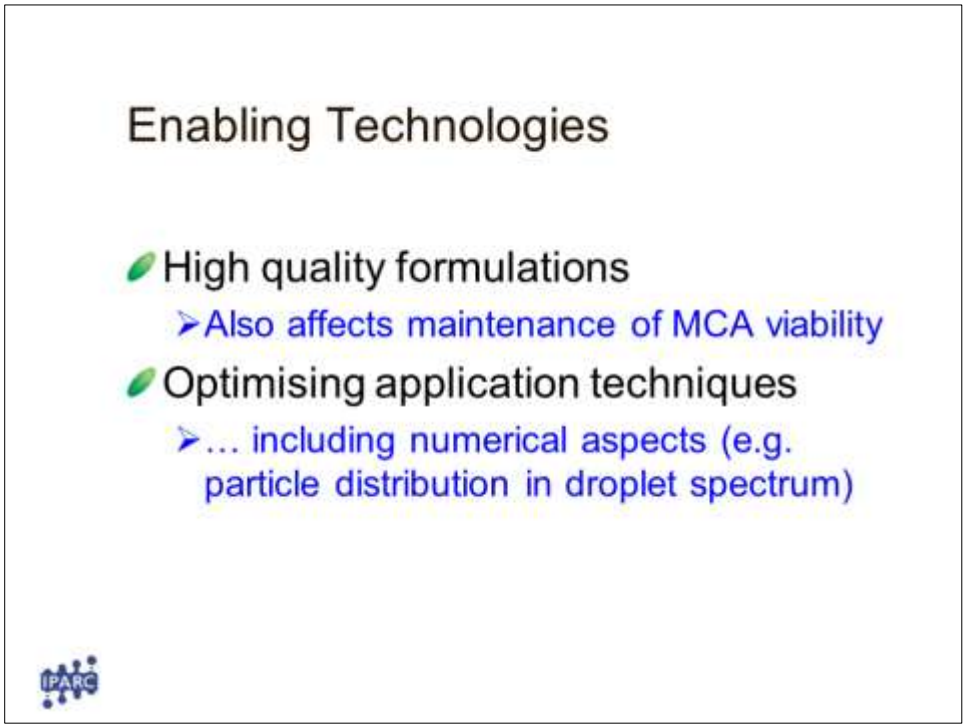
2. Biological agents and regulation

- In theory, the benign nature of biological agents, should liberate us from many of the restrictions associated with application of chemical pesticides.
- … especially wind drift …

IPARC

Oak processionary moth (*Thaumetopoea processionea*) Herridge's Copse, UK, 2013


3. Delivery systems
Improved delivery systems (formulation, application, etc.) will not save underperforming MCA but ...
performance of a MCA, as with a chemical pesticide, may be reduced substantially by a poor delivery system.
IPARC

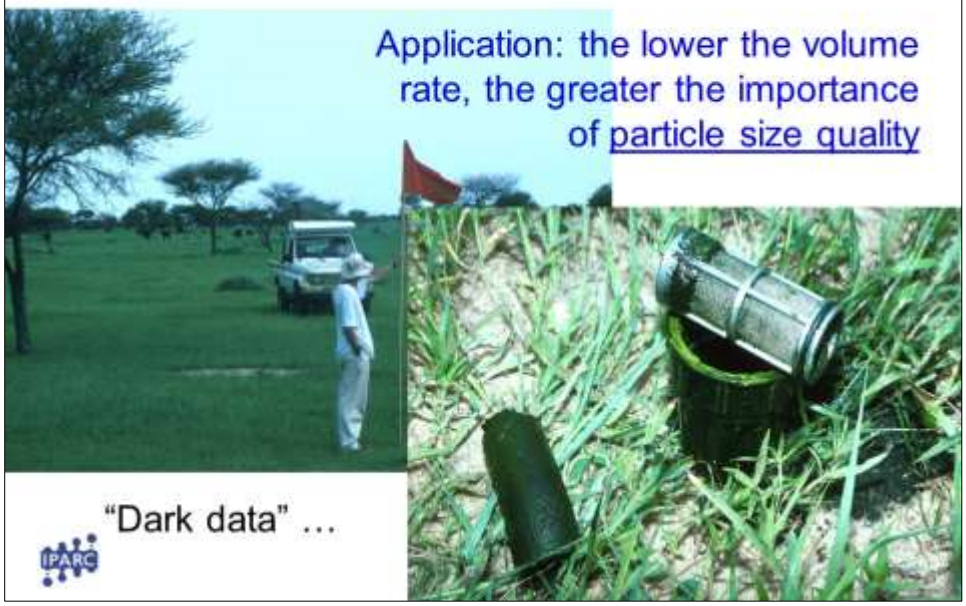

Enabling Technologies
High quality formulations
Also affects maintenance of MCA viability
Optimising application techniques
... including numerical aspects (e.g. particle distribution in droplet spectrum)
IPARC

Formulation linked to production: fungal spore types

- Aerial conidia

e.g. Metarhizium spp.

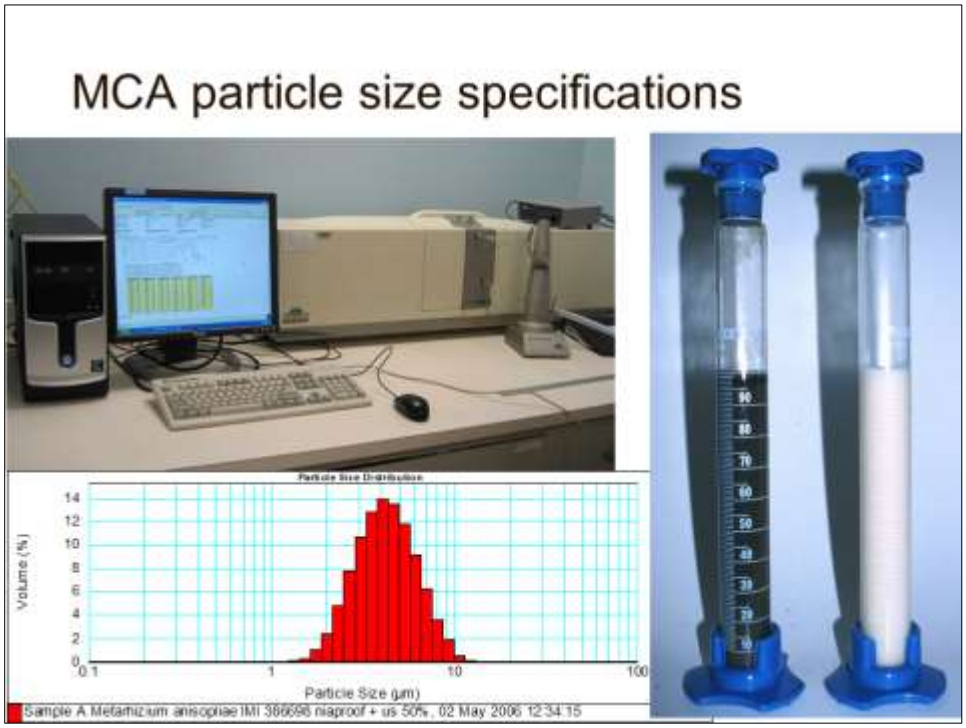
"Dark Data": early large scale ULV trials

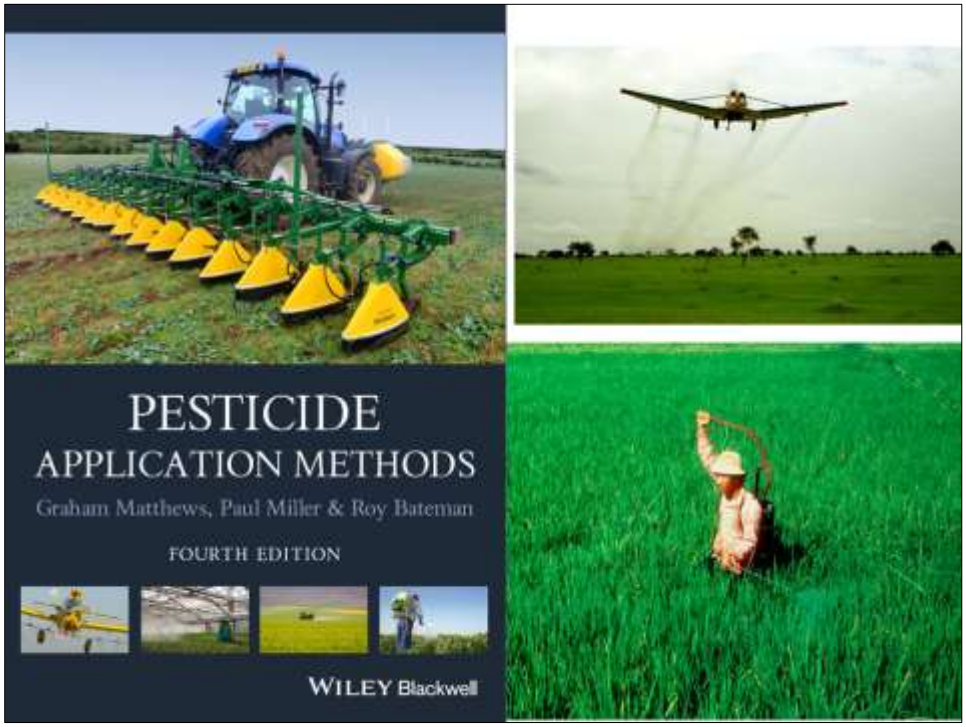


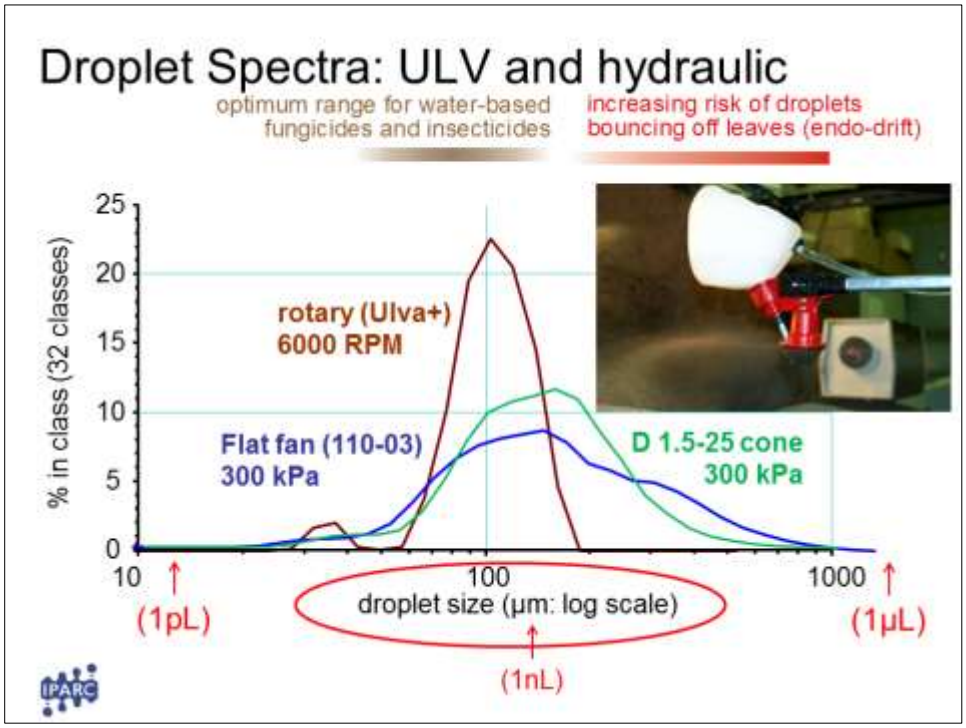
Application: the lower the volume rate, the greater the importance of particle size quality

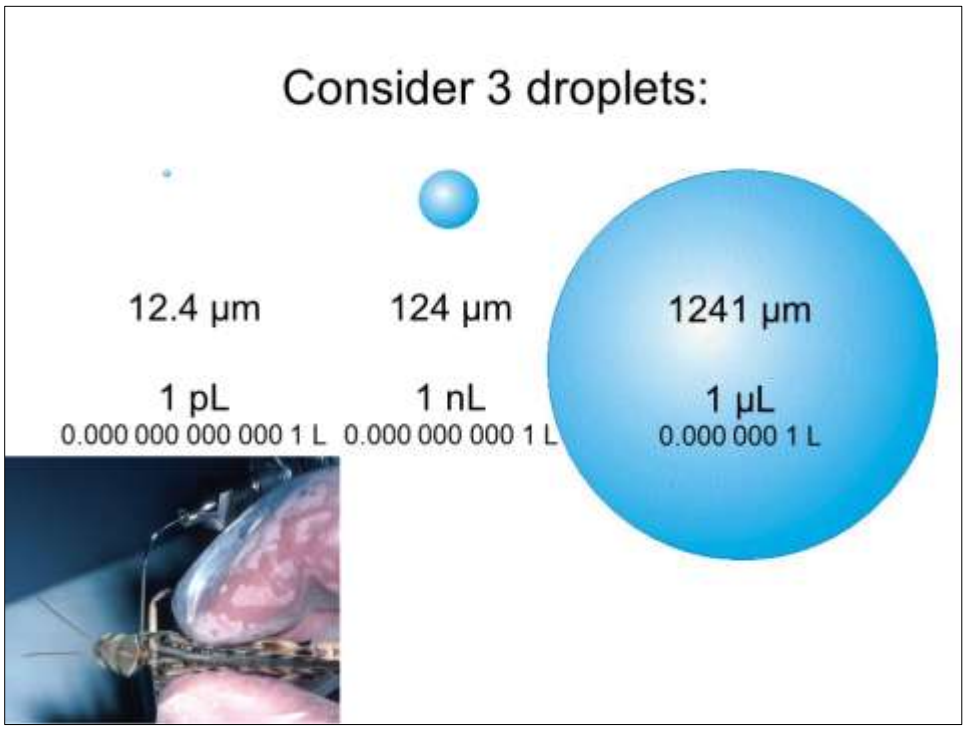
"Dark data" …

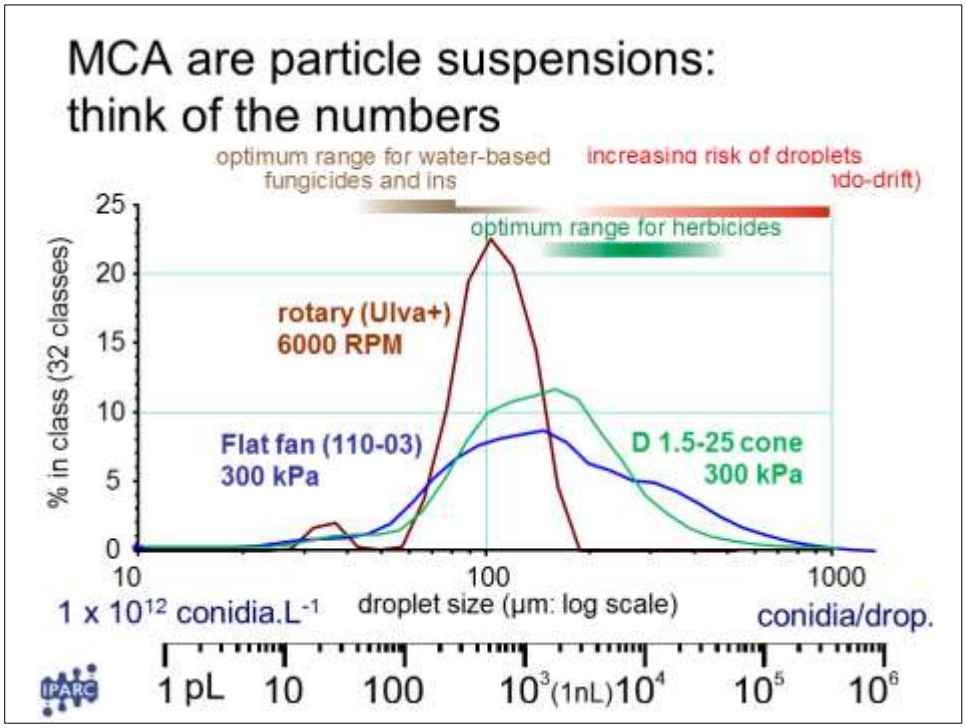

The 'MycoHarvester' a 'spin off'
MH 3
MH 5
Pure, single conidia, not substrate
stable suspensions
prevents blockages
facilitates product drying → storage stability
IPARC
www.mycoharvester.info


MCA particle size specifications
Particle Size Distribution
Volume (%)
Particle Size (µm)
Sample A Metarhizium anisopliae IMI 386696 niaproof + us 50%, 02 May 2006 12:34:15


PESTICIDE
APPLICATION METHODS
Graham Matthews, Paul Miller & Roy Bateman
FOURTH EDITION
WILEY Blackwell

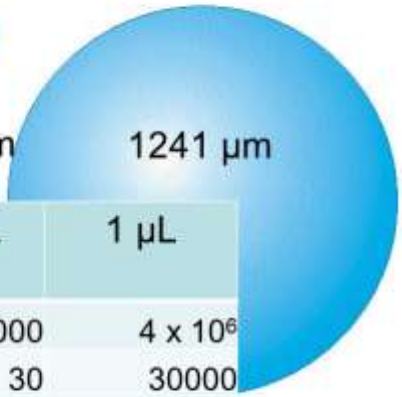

Droplet Spectra: ULV and hydraulic
optimum range for water-based fungicides and insecticides
increasing risk of droplets bouncing off leaves (endo-drift)
% in class (32 classes)
25
20
15
10
5
0
rotary (Ulva+)
6000 RPM
Flat fan (110-03)
300 kPa
D 1.5-25 cone
300 kPa
10
100
1000
droplet size (µm: log scale)
(1pL)
(1nL)
(1µL)
IPARC


Consider 3 droplets:
12.4 µm
1 pL
0.000 000 000 000 1 L
124 µm
1 nL
0.000 000 000 1 L
1241 µm
1 µL
0.000 000 1 L


MCA are particle suspensions:
think of the numbers
optimum range for water-based fungicides and ins
increasing risk of droplets
optimum range for herbicides
% in class (32 classes)
rotary (Ulva+)
6000 RPM
Flat fan (110-03)
300 kPa
D 1.5-25 cone
300 kPa
1 x 10¹² conidia.L⁻¹
droplet size (µm: log scale)
conidia/drop.
1 pL
10
100
10³(1nL)
10⁴
10⁵
10⁶

Theoretical particle loading per droplet

* '0' means <50% probability of droplet containing a particle



CFU / Ha	VAR (L/ha)	1 pL	1 nL	1 µL
2×10^{12}	0.5	4	4000	4×10^{6}
3×10^{12}	100	0*	30	30000
7×10^{13}	400	0*	175	175000
3×10^{11}	600	0*	1	500
3×10^{9}	1000	0*	0*	3

4. Major constraint

- Are we trying to apply 21st century control agents using application techniques that have changed little since the 19th century?

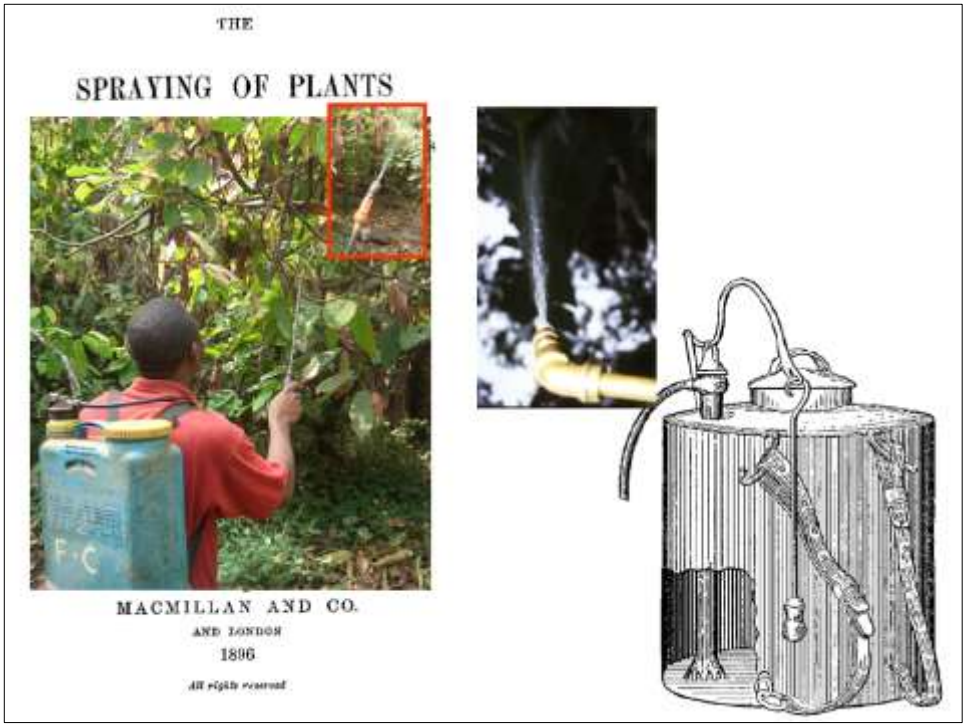
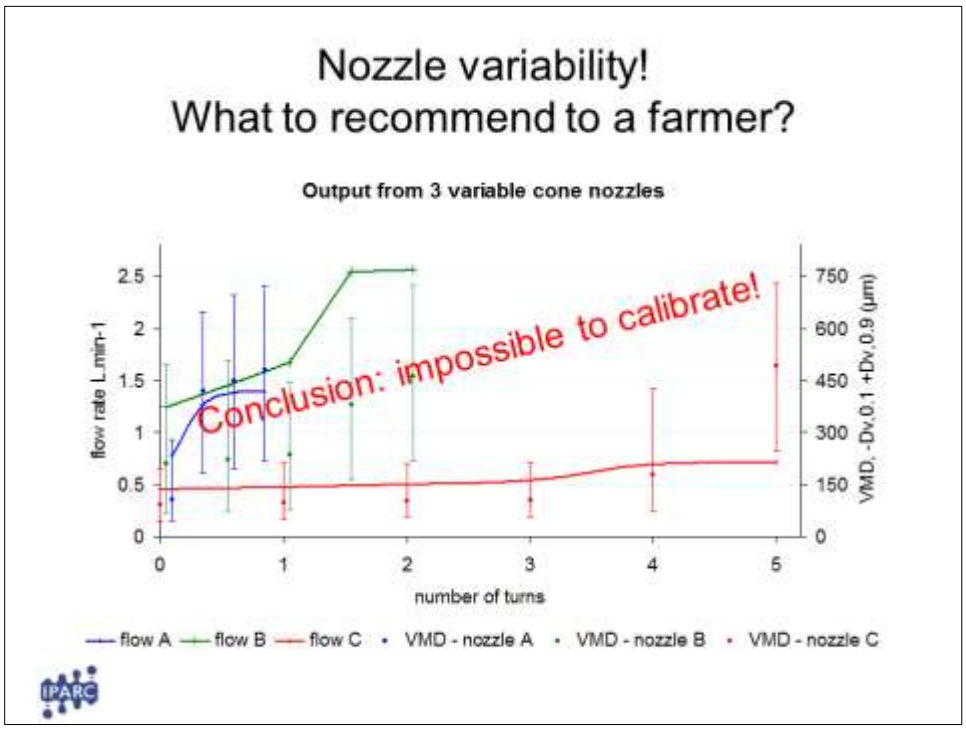
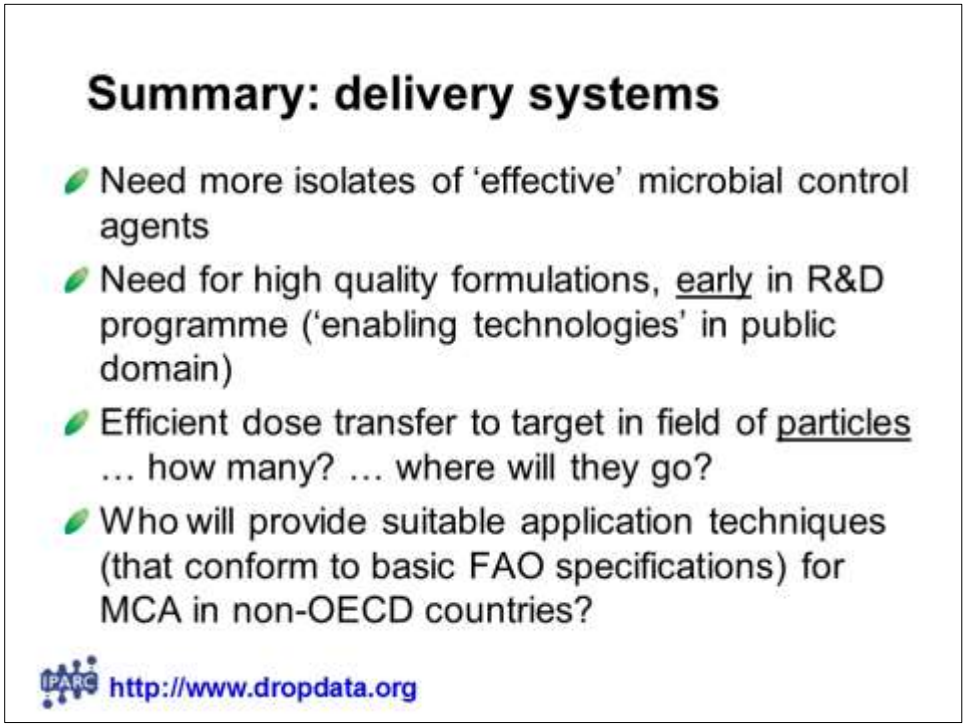


FIG. 18.—Spray nozzles. *a*, graduating spray "Gem"; *b*, "Boss"; *c*, "Bordeaux."


Nozzle variability!
What to recommend to a farmer?
Output from 3 variable cone nozzles
Conclusion: impossible to calibrate!
flow rate L.min-1
VMD, -Dv,0.1 +Dv,0.9 (µm)
number of turns
flow A
flow B
flow C
VMD - nozzle A
VMD - nozzle B
VMD - nozzle C
IPARC


The 'Cocoa Nozzle'
• Narrow cone
• Fixed setting
• Optimised droplet size
2x - 3x improvement in dose transfer efficiency
Bateman (2004) Crop Protection, 23: 989 - 999


Summary: delivery systems
Need more isolates of 'effective' microbial control agents
Need for high quality formulations, early in R&D programme ('enabling technologies' in public domain)
Efficient dose transfer to target in field of particles … how many? … where will they go?
Who will provide suitable application techniques (that conform to basic FAO specifications) for MCA in non-OECD countries?
IPARC
http://www.dropdata.org

A review of studies on application techniques of MPCPs

[PPT 5]

Roma Gwynn (Rationale - Biopesticide Strategists, Duns, Scotland) with Willem Ravensberg (Koppert Biological Systems, Berkel & Rodenrijs, the Netherlands)

Application methods – exposure routes

Growing media/soil application

- Drenches – hand held, irrigation equipment
- Growing media incorporated liquids and solids (powders, granules etc.)

Specialist application

- Seed treatment – coated onto seeds
- Injection

Foliar application

- Equipment: hand-held, tractor/machinery or aerial equipment fitted with appropriate single nozzles on multiple nozzles on a boom
- Applications range from high to low volume water volumes
- Some specialist equipment e.g. spinning discs, hooded sprayers.

Micro-organism biopesticide application

Conventional pesticide application know-how

From: TeeJet Technical Bulletin

Literature review – R Gwynn and R Glass

Review of literature and existing state of the art with respect to the application of microbial biopesticides in agriculture.*

- Micro-organism based products are most widely used in crop protection as inundative control agents.
- As for synthetic chemical pesticides, the dose delivery system in foliar use can have a profound impact on the in-field efficacy of a biopesticide as can the formulation type, use of adjuvants, protectants and other in-tank additives (Chapple *et al.)*.
- The essence of biopesticide application is the methodology of dose transfer relative to the place of delivery.

RATIONALE

* Work funded by DEFRA, UK

The logic and outputs - search of CAB abstracts 1973-onwards

Number	Searches	Results
1	Plant Pests [FF620]. Biological Control [HH100]	118620
2	Biological Control [HH100]	131034
3	Application methods.sh.	13128
4	Application techniques*.mp.	1493
5	(spraying equipment or sprayers or nozzles or application equipment).sh.	6999
6	Biopesticide*.mp. or entomopathogenic bacteria.sh. or natural enemies.sh. or microbial pesticides.sh. or bacterial insecticides.sh. or fungal insecticides.sh. or biological control.sh. or bacillus thuringiensis.od. or biological control agents.sh. or entomopathogens.sh.	137898
7	Biocontrol*.mp. or biological control agents.sh. or biological control.sh.	82418
8	2 or 6 or 7	150150
9	3 or 4 or 5	20533
10	8 and 9	**692**

* sh (subject header), mp (multi-purpose) and od (organism descriptors)

Number of references for micro-organism based biopesticides

Microbial	Number of references
Beauveria spp.	133
Trichoderma spp.	132
Metarhizium spp.	76
Bacillus spp. (excluding Bt)	100
Bacillus thuringiensis only	158
Verticillium and *Lecanicillium* spp.	26
Baculovirus	26
Virus	65
Coniothyrium spp.	5
Gliocladium spp.	15
Total	**736**

Literature review - method

- 692 abstracts reviewed
- 737 papers refer to one or more micro-organism based biopesticides
- 247 documents identified as potentially relevant
- 127 documents fully reviewed (scientific papers, books, reviews)
- Documents obtained from: EU member states, USA, Canada, China, Australia, New Zealand, Japan and India
- Consultation: HDC, IBMA, pesticide and biopesticide manufacturers, application equipment companies and scientific experts.
- All application methods considered: foliar, soil, seed,

Publications on principles of biopesticide application

Publications on principles of biopesticide application:

- Microbials: not mobile or translocated - without persistence and redistribution a good amount of microbials are needed on foliage.
- Timing is more critical - specificity of microbial activity.
- Microbials are often particulate - limit to dilution to maintain sufficient dose in each droplet.
- At higher volume care needed to keep particulate material in suspension
- Hydraulic spraying – to avoid waste use of a narrower droplet size spectrum and small droplets should improve efficiency of spraying.

The paper concludes further research on application of microbial insecticides is required.

From: Matthews (1983)

Publications on principles of biopesticide application

CHAPTER I-2

Theory and Practice of Microbial Insecticide Application.

ANDREW C. CHAPPLE[1], ROGER A. DOWNER[2], AND ROY P. BATEMAN[3]

[1] Environmental Health Biology, Hoechst Schering AgrEvo GmbH, H872, Hoechst Works, Frankfurt, Germany. [2] Laboratory for Pest Control Application Technology, OARDC, 1680 Madison Ave., Wooster Ohio 44691 USA. [3] CABI Bioscience, Silwood Park, Buckhurst Rd., Sunningbill, Ascot, Berkshire SL5 7TA, UK

L.A. Lacey and H.K. Kaya (eds.), Field Manual of Techniques in Invertebrate Pathology, 5–37.

APPLICATION

CAN BIOLOGICAL AGENTS BE SPRAYED LIKE CHEMICAL PESTICIDES?

Graham Matthews from the International Pesticide Application Research Centre (IPARC) at Silwood Park, UK, considers the application problems of biopesticides

 DOI: 10.1039/b102659n

Application principles for dose transfer

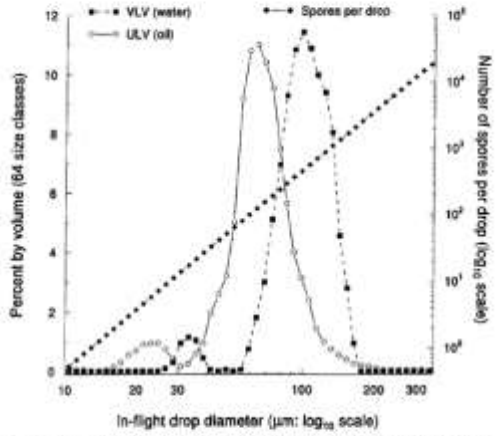


Figure 2. A comparison of drop spectra for two CDA formulations of *M. anisopliae*, oil v. water (NB: $\log_{10}$ scales). Oil based: 8000 rpm, 60 ml/min, 1 l/ha, 10 m swath, 5×10^{12} conidia / ha; and water based: 6000 rpm, 77 ml/min, 6.4 l/ha, 2 m swath, 7.8×10^{11} conidia / ha. Also shown, the relationship between drop size and spores per drop for a formulation. [VLV = very low volume; ULV = ultra low volume.]

From: Chapple, Downer and Bateman 2000

•*Bacillus thuringiensis* (Bt) bioinsecticides - application

- Papers on the application of Bt were the most common (158)
- Sundaram and Retnakaran (1987): Several papers that analyse in great detail properties of Bt suspensions and how they behave with rotary atomisers this formed the basis of the Canadian forestry biocontrol programme.
- Review by Steinke and Akesson (1993):
  - Authors looked at atomisation and application of Bt and baculoviruses.
  - Noted that physical properties of these micro-organisms and the equipment for applying them would not meet the desired droplet size of 80 to 100 micrometers.
  - Conducted studies on atomisation of Bt and a baculovirus to determine their atomisation characteristics.
  - Concluded: for Bt the high, and often variable, viscosity may adversely affect the efficiency of atomisation, particularly rotary screen atomisers, and further research is required to develop appropriate atomisers for these biopesticides.

Publications on principles of biopesticide application

- 127 papers on dose transfer reviewed - only 24 for non-Bt micro-organism application.
- Included work on micro-organism based insecticides, fungicides and herbicides.

Examples of findings:

- ULV versus hydraulic spray of EPF: ULV = better distributed deposits = better control.
- For granuloviruses: mist blower versus ULV spinning disc for codling moth (*Cydia pomonella*) control = deposition rates was similar but control better with mist blower.
- There are papers that indicate there have been problems with high heat and pressure so this should be checked. But equally there are examples of surprising good survivability of conidia (under heat and sheer stress).

Publications on Entomopathogenic nematodes (EPN) application – lessons for microbials?

- EPN are not MPCP. However, they are recommended to be applied as foliar sprays. Several papers considered the effect of spray pressure, nozzle type and pump type.
- Lello *et al.* (1996), compared different spray equipment for application of *Steinernema carpocapsae* versus diamond back moth (*Plutella xylostella*) on Chinese cabbage:
  - hydraulic nozzles deposited the greatest number of EPN and highest DBM mortality (98%).
  - Spinning disc: 50% DBM mortality while applying less that 9% of the EPN compared to the most effective hydraulic system.

Conclusions:

- Interaction of the application technique with biotic and abiotic factors important.
- Sheer forces in spray tanks may harm EPN – filters in equipment
- Measured doses can be delivered by irrigation equipment

Conclusions on MPCP application research

- Application considered for timing and dose rather than dose-transfer techniques; effects of parameters such as nozzle size, operating pressure, tank system little considered.
- Where dose-transfer considered: delivery techniques such as drenches to surfaces; soil, rootstock or tree bark, rather than for example, foliar canopies.
- Rarely resolved which aspects of the delivery were critical to improving dose-transfer performance.
- Where comparisons to foliar application were mentioned there was often little or no detail of the technique reported.
- Definitive empirical research absent on the droplet spectra or coverage achieved with different application techniques.
- Biopesticide application often different to chemical pesticides, particularly for foliar application of micro-organism based products. However, few experimental data available.

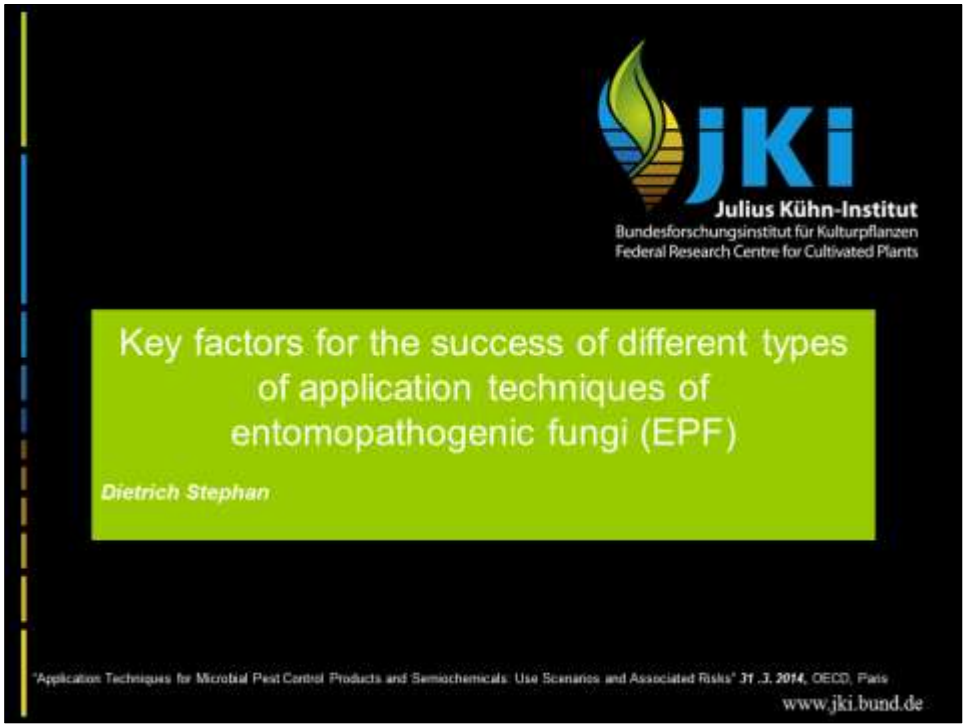
Microbial application versus exposure - conclusions

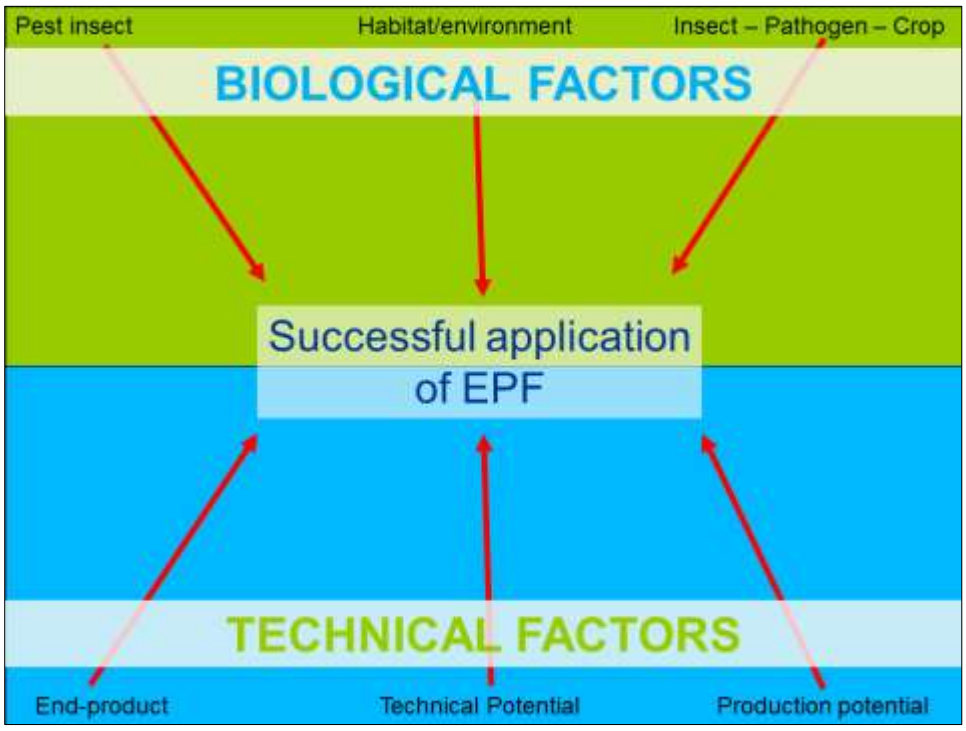
- Application for microbials is still poorly understood
- There is a need for real data to establish, for example:
  - Where the dose goes in the crop – LAI intercept and canopy penetration.
  - Relationship between efficacy and dose and droplet spectra – what is the optimum?
  - Droplet delivery – how many droplets contain an effective dose ?
  - Effect of total fermentation products containing spent media.
  - For products with multiple modes of action what is the best dose delivery mechanism
  - What is the effect of formulation, adjuvants, synergists etc.
- From this experimental work could we identify trends that would avoid lengthy examination for each product (one by one).


Thanks to Richard Glass*, FERA, UK who was co-author of the literature review and DEFRA for the funding of project PS2027.
*Current employer Eurofins Agroscience Services
RATIONALE
BIOPESTICIDE STRATEGISTS
rgwynn@biorationale.co.uk
IBMA
INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION

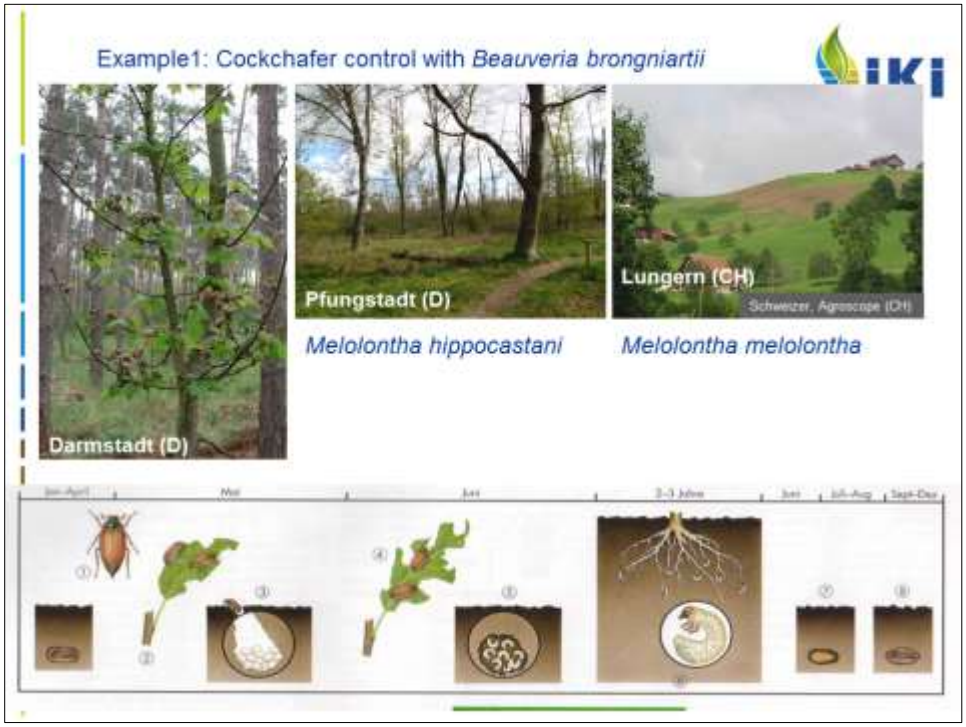
Key factors for the success of different types of application techniques of entomopathogenic fungi [PPT 6]

Dietrich Stephan (Institute for Biological Control [JKI], Darmstadt; Germany)




Pest insect
Habitat/environment
Insect – Pathogen – Crop
BIOLOGICAL FACTORS
Successful application of EPF
TECHNICAL FACTORS
End-product
Technical Potential
Production potential


Pest insect
Habitat/Environment
Insect – Pathogen – Crop
Example1: Cockchafer control with B. brongniartii
Example 2: Catch and infect
Attract and kill
CO2
CO2
CO2
Successful application of EPF
Example 3: Production of Metarhizium brunneum
End-product
Technical Potential
Production potential


Example1: Cockchafer control with Beauveria brongniartii
JKI
Darmstadt (D)
Pfungstadt (D)
Lungern (CH)
Schweizer, Agroscope (CH)
Melolontha hippocastani
Melolontha melolontha


Example1: May beetle/White grub control with Beauveria brongniartii
JKI
Schweizer, Grabenweber
Agroscope (CH)


Example1: May beetle/Cockchafer control with *Beauveria brongniartii*
JKI
Conidia
Submerged spores
But: No product available


Grasslands
Trials in Switzerland:
Flumserberge 2004 / 2005
JKI
Before
and one year after application


Orchard:
IKI
Fried. LRA Karlsruhe

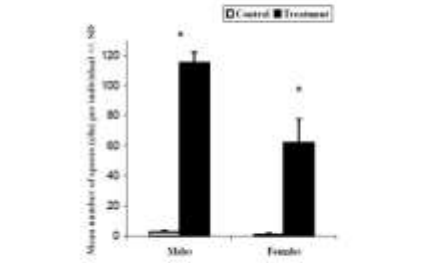

Fried. LRA Karlsruhe


Fried. LRA Karlsruhe


Fried. LRA Karlsruhe


Forest:
Soil Application
JKI
Planting hole:
in afforestation fields
After application of 20g/hole or 50 kg/ha Melocont® after two years 10^4-10^5 CFU/g soil were reisolated.
Band application
between in Pine forest

„Catch and Infect“ technique



Transfer from Trap to Male **YES**

Transfer from Male to Female **YES**

Transfer from Female to soil **NO**

Koller & Jung, JKI, HU

Autoinoculative device for field application of fungal conidia for tephritid fruit fly control

Table 6. Percent mortality and LT_{50} in *C. capitata* and *C.* r. var. *fasciventris* adult flies treated with *M. anisopliae* in cage experiments

Treatment	Mortality (%)	$LT_{50} \pm$ S.E	Slope ± S.E
C. capitata			
Cheesecloth	87.5 ± 4.7a	4.29 ± 0.6a	0.18 ± 0.02***
Cob/Cheesecloth	80.0 ± 2.1ab	5.10 ± 0.11a	0.20 ± 0.02***
Petri dish	70.0 ± 4.0b	6.49 ± 0.59a	0.15 ± 0.01***
Control	17.5 ± 4.7c		

Dimbi et al. (2003) *Mycopathologia* 156, 375-382.

Mariana, ICIPE

Contamination device (Cd) for tsetse control

Advantages:

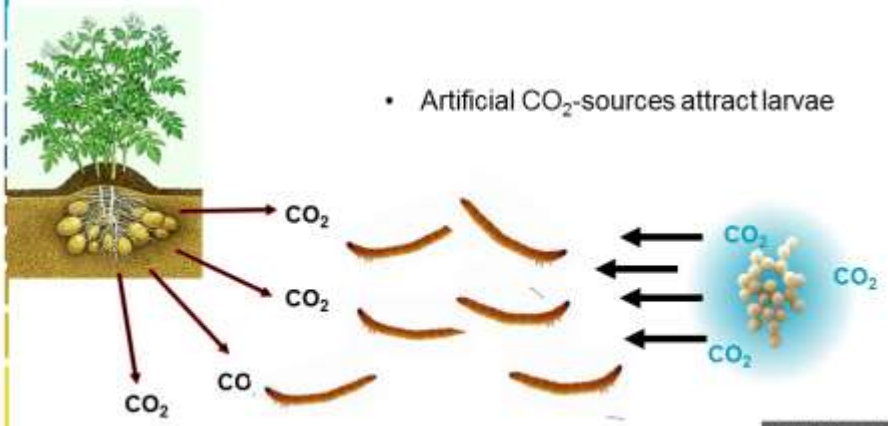
- Spores protected against UV radiation
- Long persistence in the field: up to 30 days
- Small amount of inoculum used (1-1.5g/trap)
- "Guided missile"!! (40% of flies that come to trap do not enter)

Maniania (2002) *Biocontrol Science and Technology* 12, 59-66

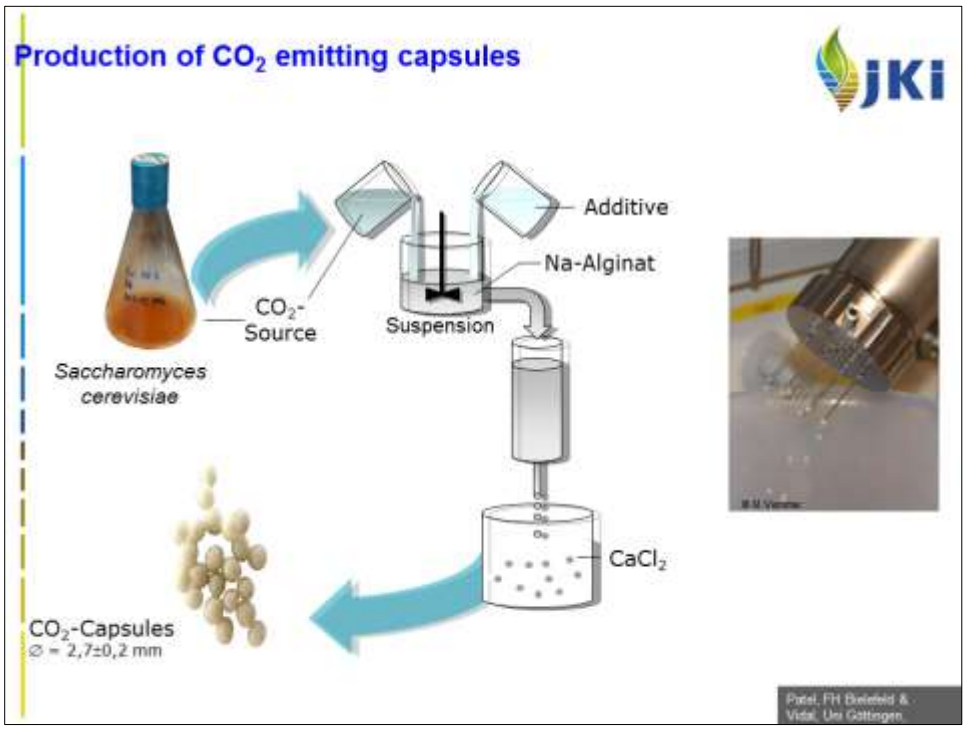
Maniania, ICIPE

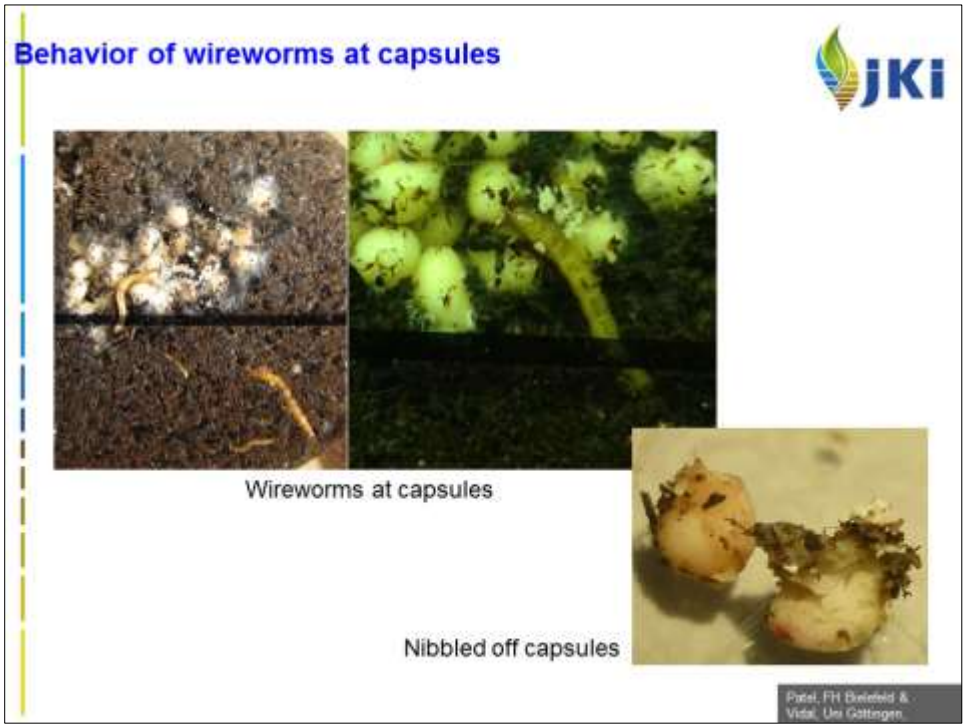
„Attract & Kill" strategy

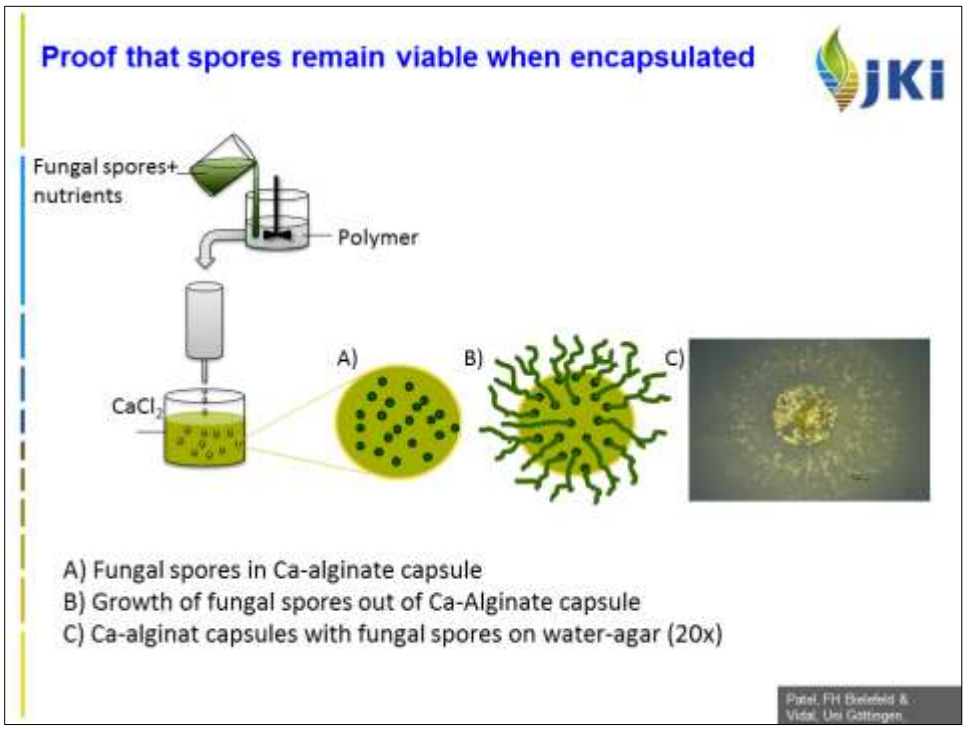
- Growing roots produce CO_2
- Soil dwelling pests are attracted towards CO_2-sources

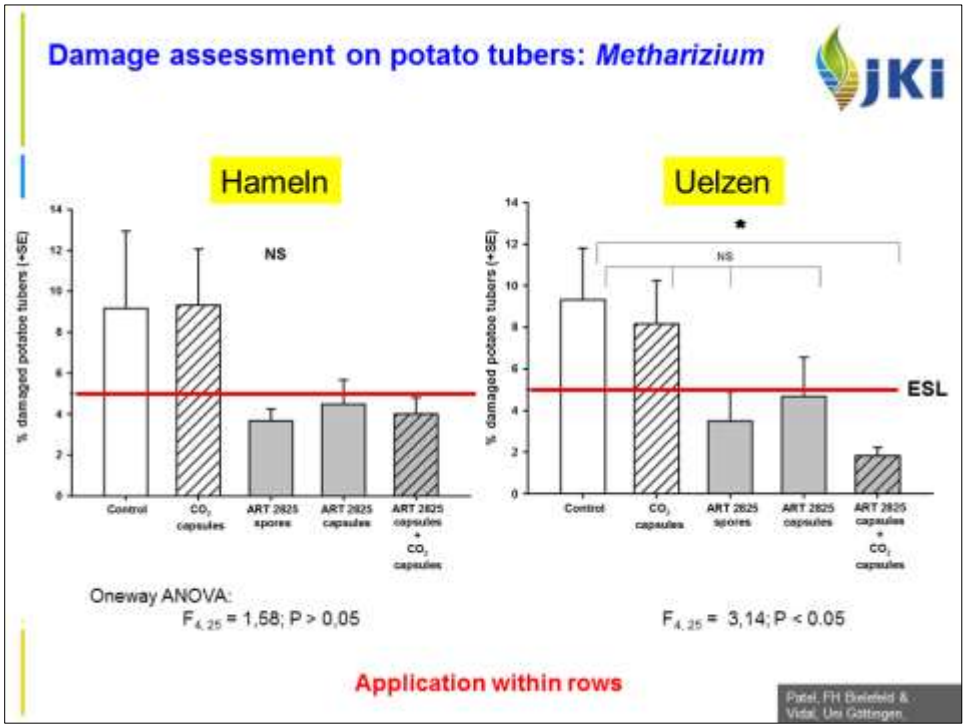


Patel, FH Bielefeld & Vidal, Uni Göttingen


Production of CO_2 emitting capsules
JKI
Additive
Na-Alginat
CO_2-Source
Suspension
Saccharomyces cerevisiae
$CaCl_2$
CO_2-Capsules
∅ = 2,7±0,2 mm
Patel, FH Bielefeld & Vidal, Uni Göttingen


Behavior of wireworms at capsules
JKI
Wireworms at capsules
Nibbled off capsules
Patel, FH Bielefeld & Vidal, Uni Göttingen


Proof that spores remain viable when encapsulated
JKI
Fungal spores+ nutrients
Polymer
CaCl2
A)
B)
C)
A) Fungal spores in Ca-alginate capsule
B) Growth of fungal spores out of Ca-Alginate capsule
C) Ca-alginat capsules with fungal spores on water-agar (20x)
Patel, FH Bielefeld & Vidal, Uni Göttingen


Damage assessment on potato tubers: Metharizium
JKI
Hameln
Uelzen
% damaged potatoe tubers (+SE)
NS
*
ESL
Control
CO2 capsules
ART 2825 spores
ART 2825 capsules
ART 2825 capsules + CO2 capsules
Oneway ANOVA:
F4, 25 = 1,58; P > 0,05
F4, 25 = 3,14; P < 0.05
Application within rows
Patel, FH Bielefeld & Vidal, Uni Göttingen

Example 3 Prduction of entomopathogenic fungi

Metarhizium brunneum (Strain Ma43=F52=BIPESCO5)

isolated in 1971 from *Cydia pomonella* by Müller-Kögler (JKI, formarly BBA)

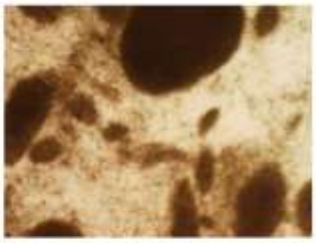
First Product-Generation
(liquid produced mycelial pellets)

Second Product-Generation
(solid state fermented grain with mycelium/conidia)

Third Product-Generation
(solid state fermented conidia)

Microsclerotial Production by *Metarhizium*

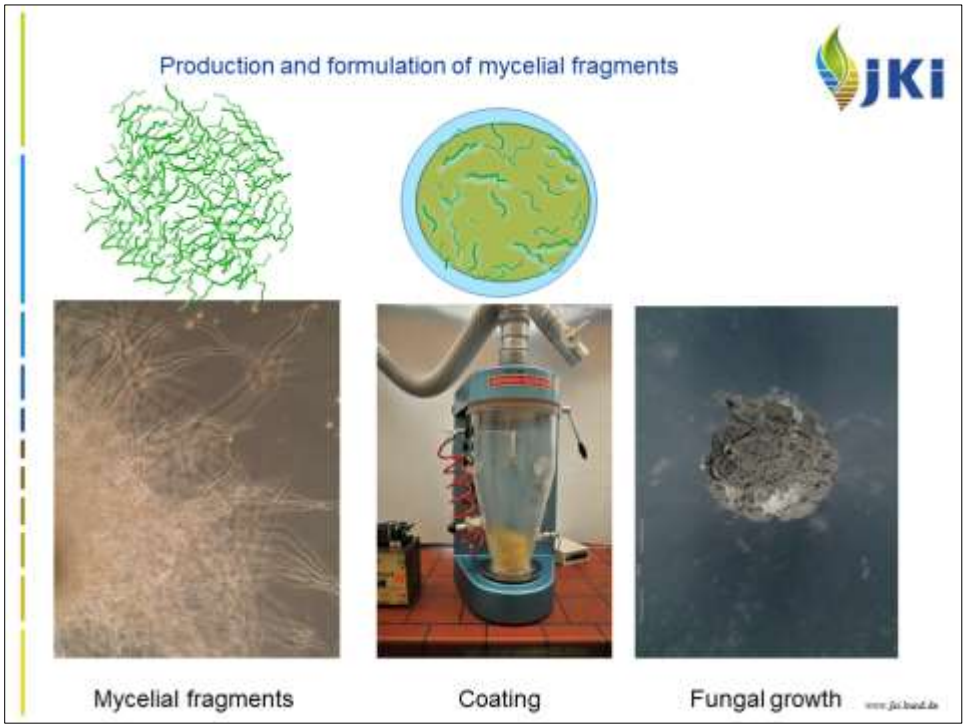
Day 4: Microsclerotium formation



Day 7-9; Microsclerotia heavily melanized

Jackson &Jaronski, USDA


Scale-up of microsclerotia fermentation
JKI
Addition of fermentation broth to holding vessel with diatomaceous earth
Harvest of sclerotia biomass from fermentation
• Flexible sizing, e.g., 0.5-1.5 mm diam.
•For at-planting application
•100 grains / g
• Free flowing
•Shelf stable:
1+ year @ 24-26° C


Production and formulation of mycelial fragments
JKI
Mycelial fragments
Coating
Fungal growth

Blastospore Production in liquid culture

Hemolymphe of larvae of Galleria melonellaLarve injected with Ma43








New formulations with new application strategies are under development !!

Ad

- Spores protected against UV radiation
- istence in the

Disadvantage

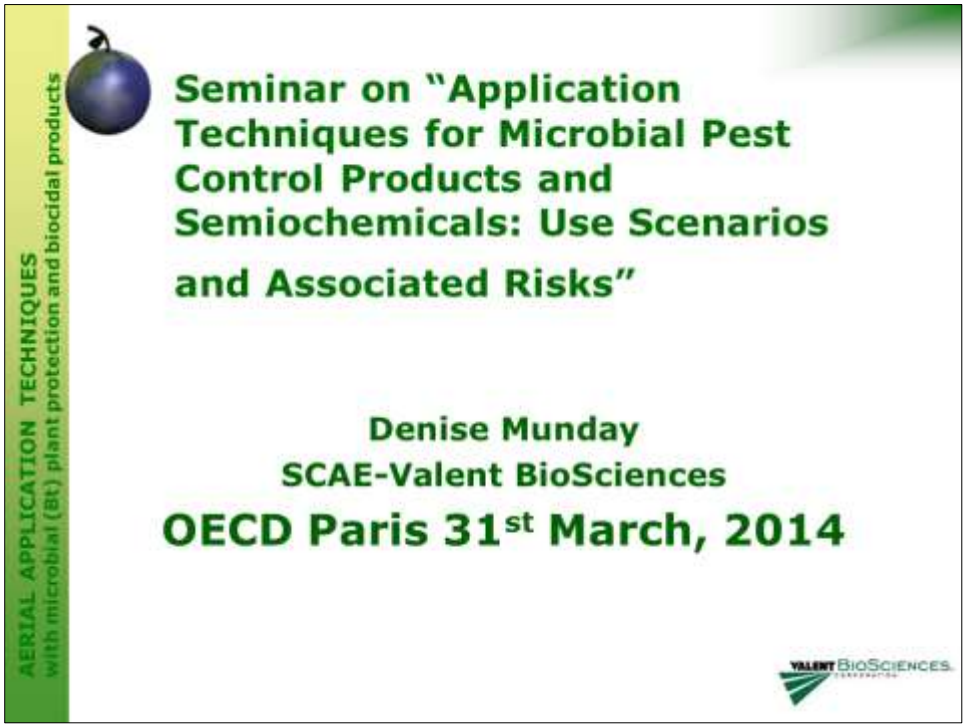
- Product is not always uniform
- Problems with contaminations are reported
- Particles too large for standard application technology
- Up to now population of cockchafers can be reduced to 20 larves/m² which is too high for orchards
- Metabolites (?)
- Technic... feasable.


JKI
Thanks for your attention!!

Aerial application techniques with microbials used in plant protection and biocidal products

[PPT 7]

Denise Munday (Valent BioSciences, Nyon; Switzerland)



AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Flying it on since 1929

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

History of aerial use

- Worldwide aerial application of Bt over decades, in particular for
  - Forests
  - Mosquito control over water
  - Extensive and/or high crops
  - Urban eradication of invasive pests

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with microbial (Bt) plant protection and biocidal products

Forest treated with Btk

GYPSY MOTH DEFOLIATION
FORAY SPRAY BLOCK, USA

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

BTI – BLACK FLY CONTROL BY AERIAL APPLICATIONS

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with microbial (Bt) plant protection and biocidal products

Btk – urban aerial applications

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Relative safety of Bt

- *Btk* is one of the few pesticides registered for widespread aerial application in many countries
- Even broadcast over urban populations to eradicate invasive pests soon after discovery
  - Asian gypsy moth in Vancouver, BC (1988)
  - Asian gypsy moth in North Carolina, USA (1993)
  - *Orgyia thyellina* in Auckland, NZ (1996)
  - *Teia anartoides* in Auckland, NZ (2003)

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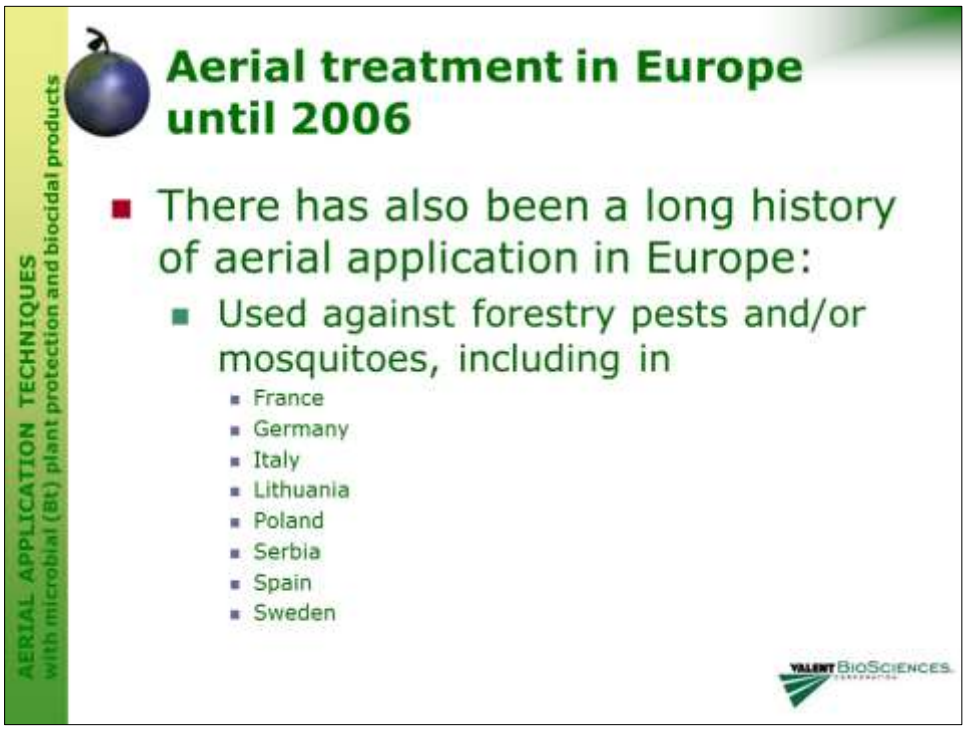
AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
Aerial treatment in Europe until 2006
There has also been a long history of aerial application in Europe:
Used against forestry pests and/or mosquitoes, including in
France
Germany
Italy
Lithuania
Poland
Serbia
Spain
Sweden
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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
Until... the SUD
Dir. 2009/128/EC Article 9
Aerial spraying is prohibited, unless...
No viable alternative
Human health and environmental advantages
Pesticides must be explicitly approved
Operators and enterprise must be certified
Risk management measures taken
Ensure no adverse effects on bystanders
No spray in proximity to residential areas
Use of best technology to reduce drift
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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
No viable alternative - forestry
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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
No viable alternative - mosquito
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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Human health impact

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Explicitly approved

- This has been done in FR, ES and IT
  - Foray 48B against processionary caterpillars
    - France:
      - Aerial application RA submitted
      - Registration granted, but with restrictions
    - Spain:
      - Mutual recognition granted based on France
  - Italy:
    - 1-2/yearly submission of emergency applications
    - Conditional on monitoring of treatment areas
  - Germany:
    - Voted use of Btk by aerial application in parliament

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with microbial (Bt) plant protection and biocidal products

Agricultural aircrafts

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

TWO TYPES OF ATOMIZERS

Flat fan hydraulic nozzles
Micronair rotary atomizers

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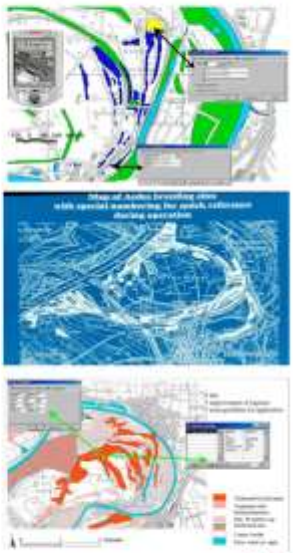
AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Survey / Mapping Using GPS and GIS



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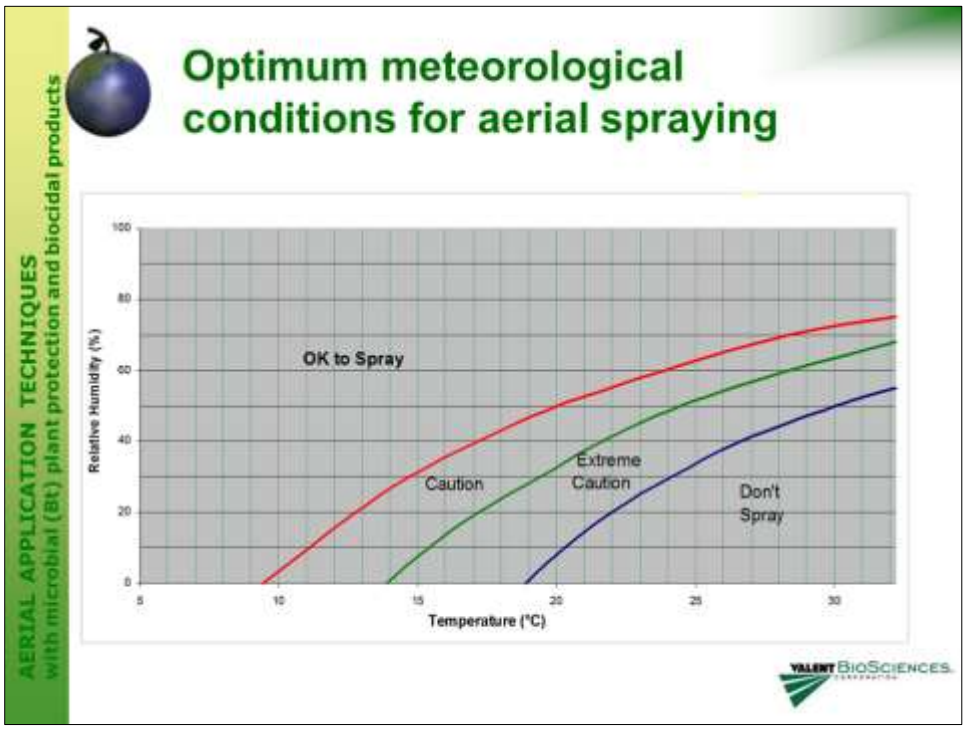
AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Variable for consideration for spray (liquid)

- Temperature and relative humidity has profound effect on droplet size and thus coverage on the foliage
  - Rising temperatures = lower RH's = increased evaporation
  - Evaporation reduces size of droplets and increases potential for drift
  - Very fine droplets may never be deposited on target

YALENT BIOSCIENCES


AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
Optimum meteorological conditions for aerial spraying
Relative Humidity (%)
Temperature (°C)
OK to Spray
Caution
Extreme Caution
Don't Spray
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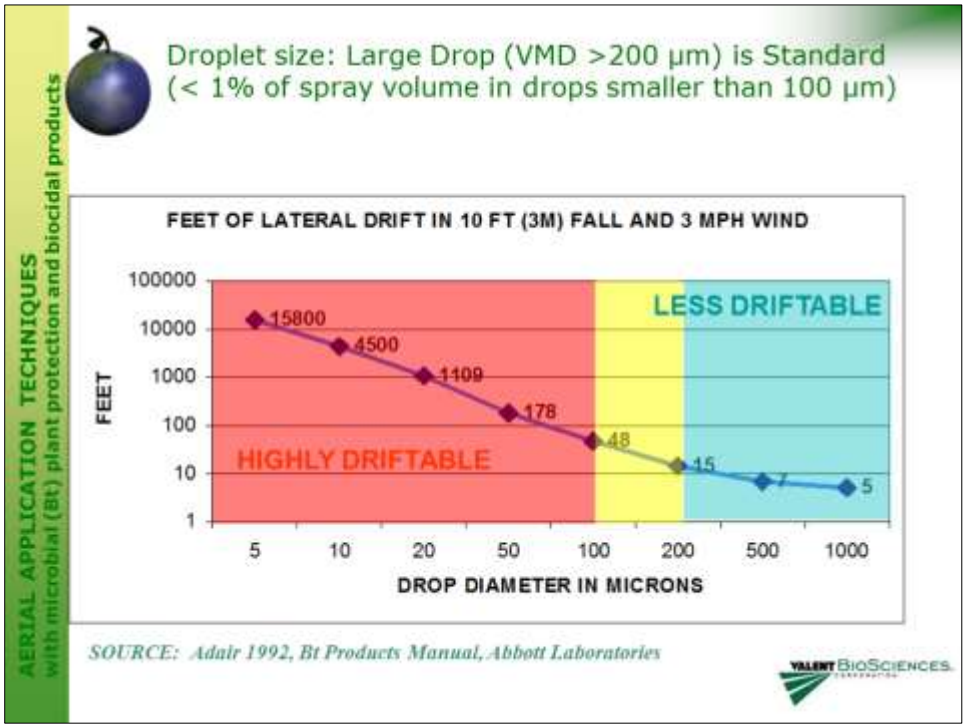
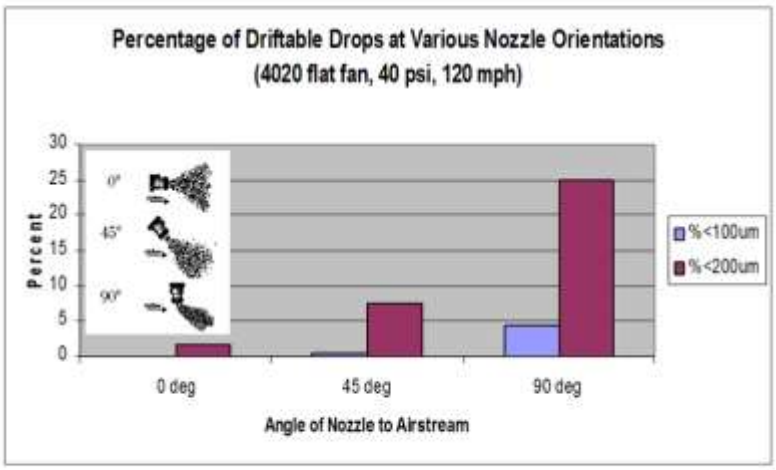

AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
Droplet size: Large Drop (VMD >200 µm) is Standard
(< 1% of spray volume in drops smaller than 100 µm)
FEET OF LATERAL DRIFT IN 10 FT (3M) FALL AND 3 MPH WIND
FEET
DROP DIAMETER IN MICRONS
LESS DRIFTABLE
HIGHLY DRIFTABLE
15800
4500
1109
178
48
15
7
5
SOURCE: Adair 1992, Bt Products Manual, Abbott Laboratories
VALENT BIOSCIENCES

AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

EFFECT OF NOZZLE ORIENTATION ON PERCENT DRIFTABLE DROPS



SOURCE: Kirk, I.W., AERIAL APPLICATORS SPRAY NOZZLE HANDBOOK, USDA-ARS AH-726

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with microbial (Bt) plant protection and biocidal products

Good conditions

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
Not so good conditions
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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products
Targeted applications:
Granular formulations
VECTOBAC GRANULATE
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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

CALIBRATION

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Systems are characterized to determine the most efficient swath (lane separation) and verify drop sizes.

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Configuration Developed for mosquito control in Italy

- Low volume of 15 litres per hectare
- 16 – Teejet flat fan TP4004 nozzles
- Large drops 200–800 µm
- Low drift
- Good coverage
- Good efficacy
- High efficiency

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

Field Characterization of LV Configuration for mosquito control in Italy

Average VMD = 566 µm
3% of volume <200 µm
0.4% of volume <100 µm
CV = 21%
14.6 Litre sprayed, 13.9 detected
95% Recovered on cards

- Test system:
  - Card line swath test
    - 2 reps of 40 meter card lines
    - 3 x 4 inch kromekote cards on CD cases
    - 1 meter card separation
    - Red dye in spray tank
    - Flight perpendicular to card line
  - 30 degree cross wind from right (5 kph)
  - Cards analyzed with scanner & Stainanlysis
- Application System
  - Schwiezer 300C
  - 15 LPH – 16 TP 4004 Nozzles

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AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

In summary: Optimum aerial application considerations

- Surveillance driven treatment decisions
- Targeted spray:
  - GPS planned area / monitored applications
  - Drift reduction techniques
    - Type of Nozzles/Atomizer
    - Weather conditions
    - Time of day
- Formulation characteristics
- Pilot skill
- Type of Aircraft

VALENT BioSciences

AERIAL APPLICATION TECHNIQUES
with microbial (Bt) plant protection and biocidal products

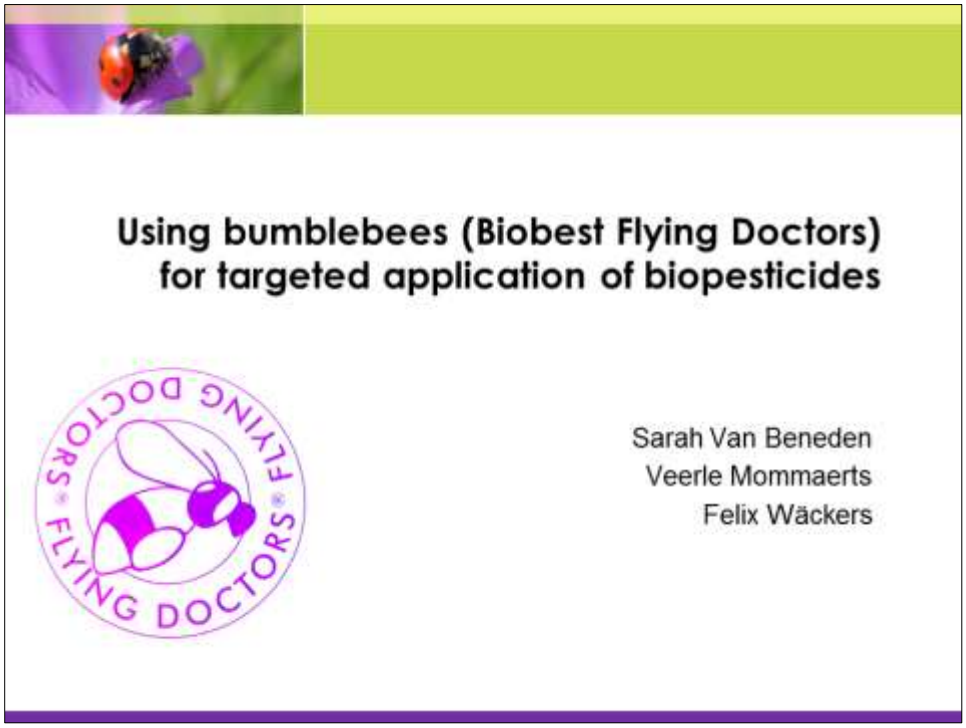
Acknowledgements:
Peter DeChant
Jacques Dugal
Steve Nicholson

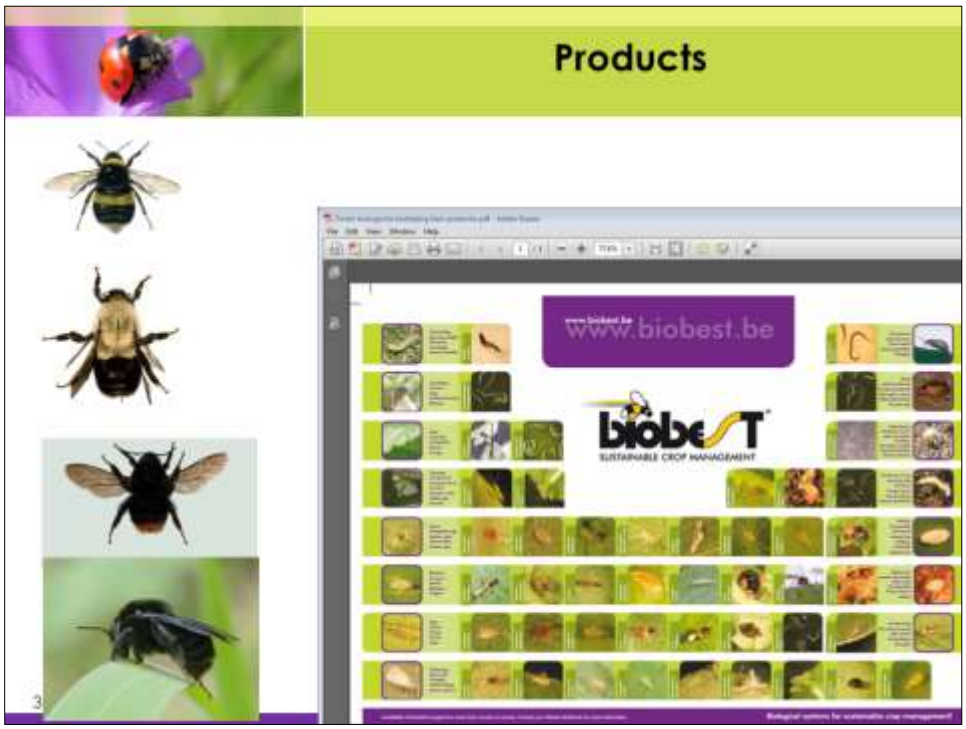
VALENT BioSciences

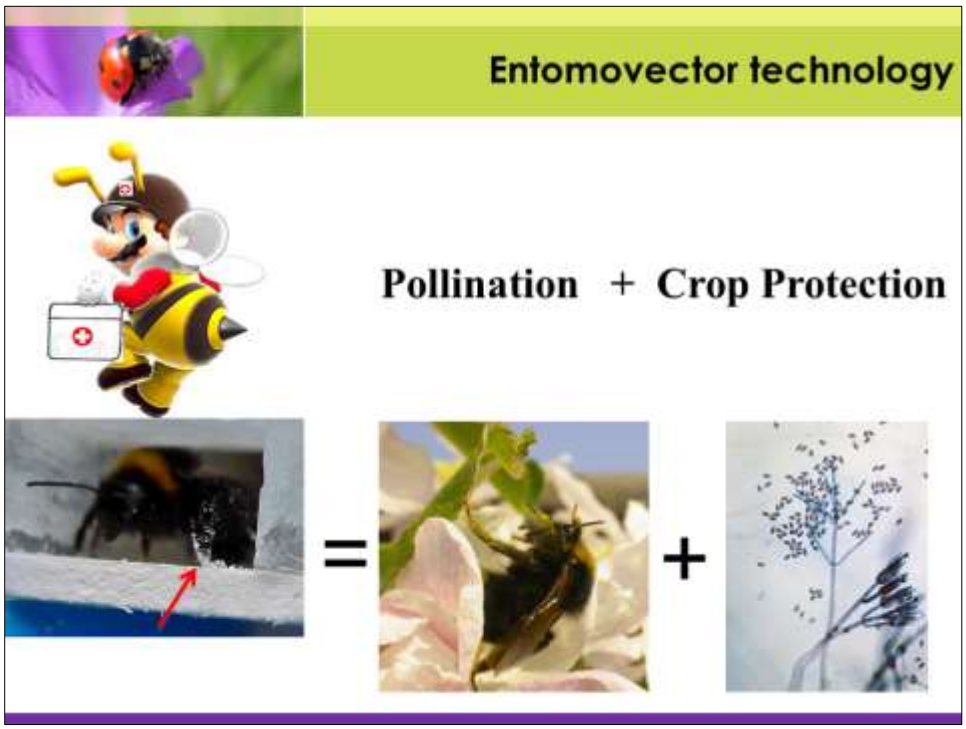
The use of bumblebees for the application of biopesticides

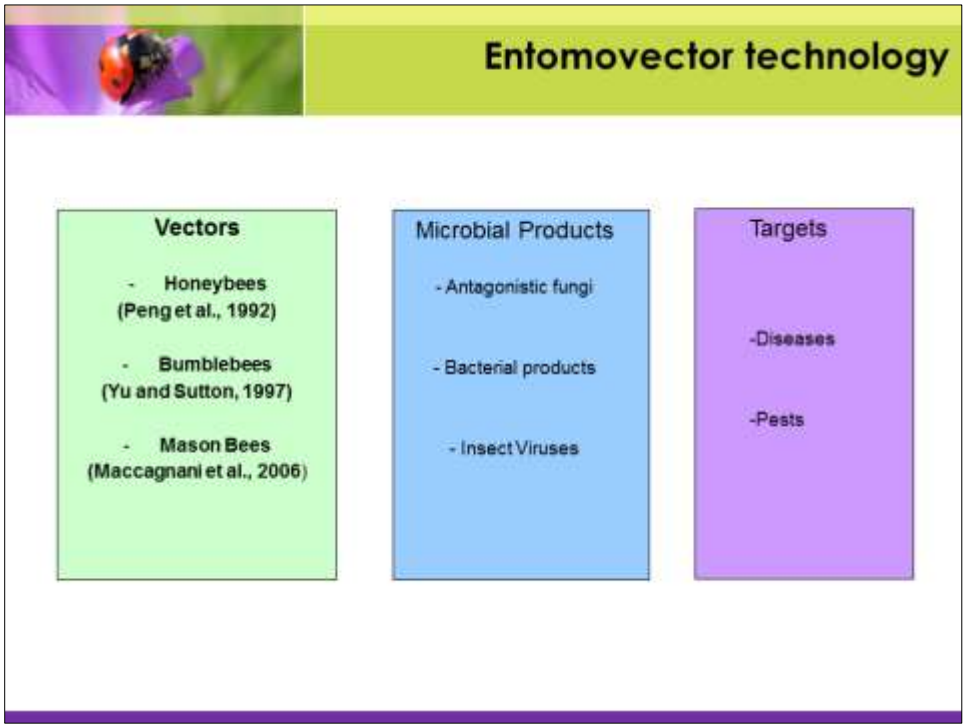
[PPT 8]

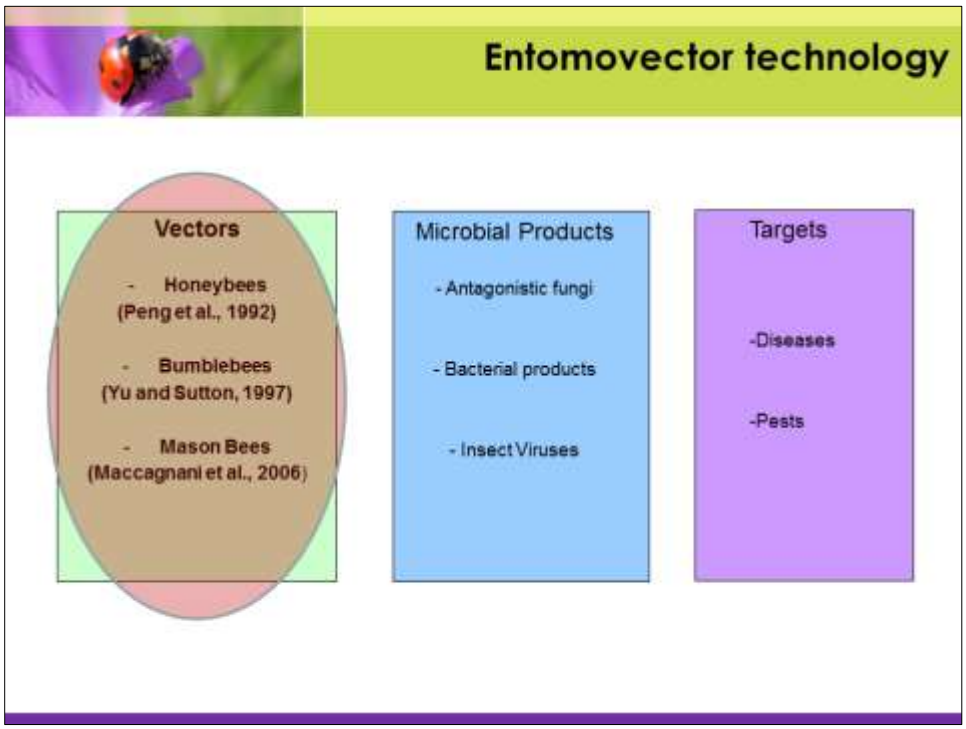
Felix Wäckers (Biobest, Westerlo; Belgium)






Entomovector technology
Pollination + Crop Protection
=
+


Entomovector technology
Vectors
- Honeybees (Peng et al., 1992)
- Bumblebees (Yu and Sutton, 1997)
- Mason Bees (Maccagnani et al., 2006)
Microbial Products
- Antagonistic fungi
- Bacterial products
- Insect Viruses
Targets
-Diseases
-Pests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Microbial Products
- Antagonistic fungi
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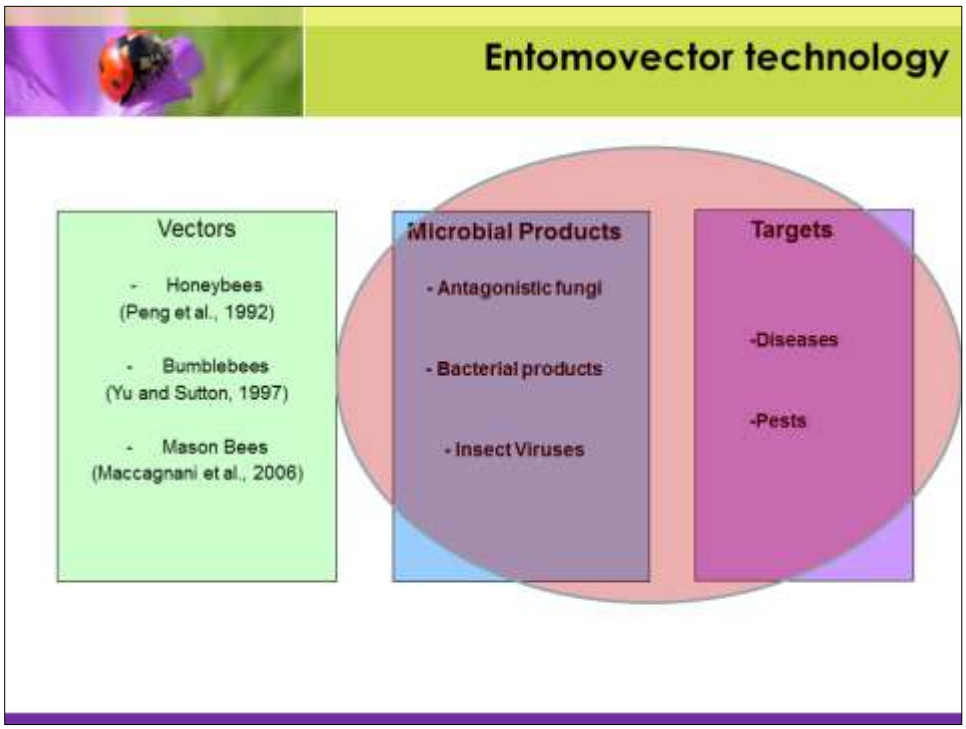
Targets
-Diseases
-Pests


Vector
Select the most efficient vector
– Crop dependent !
– Honeybees & bumblebees
→ available year around
Greenhouse crops: bumblebees only
– Widely used
– More effective
Open Field: bumblebees vs honeybees
– Better flight activity at cloudy/rainy/cold days
– Stay in closer proximity to their nest
– Larger loading capacity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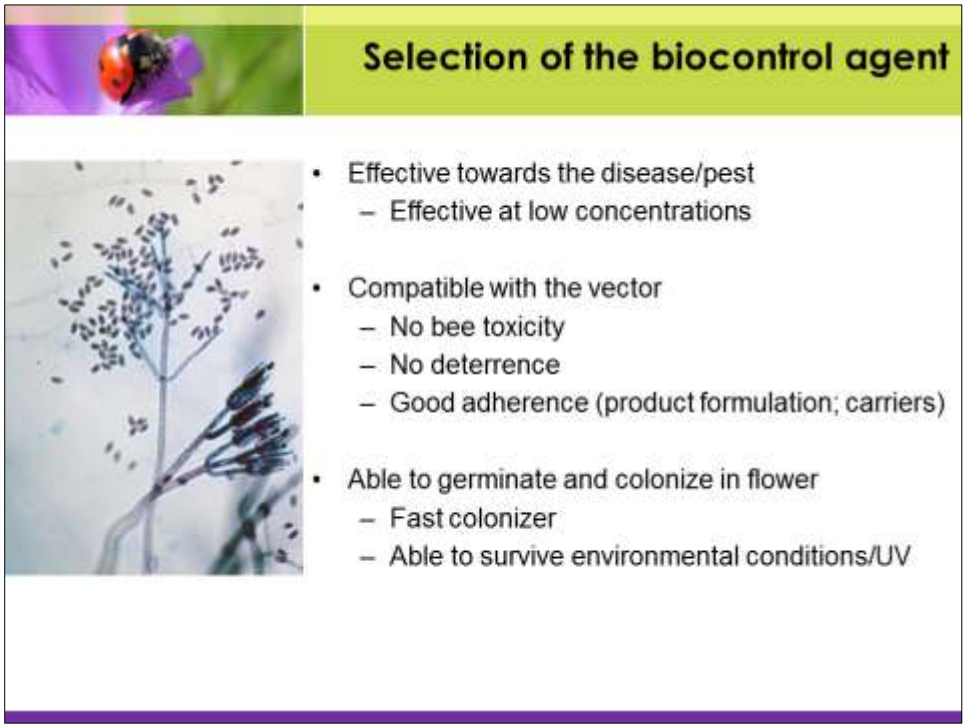
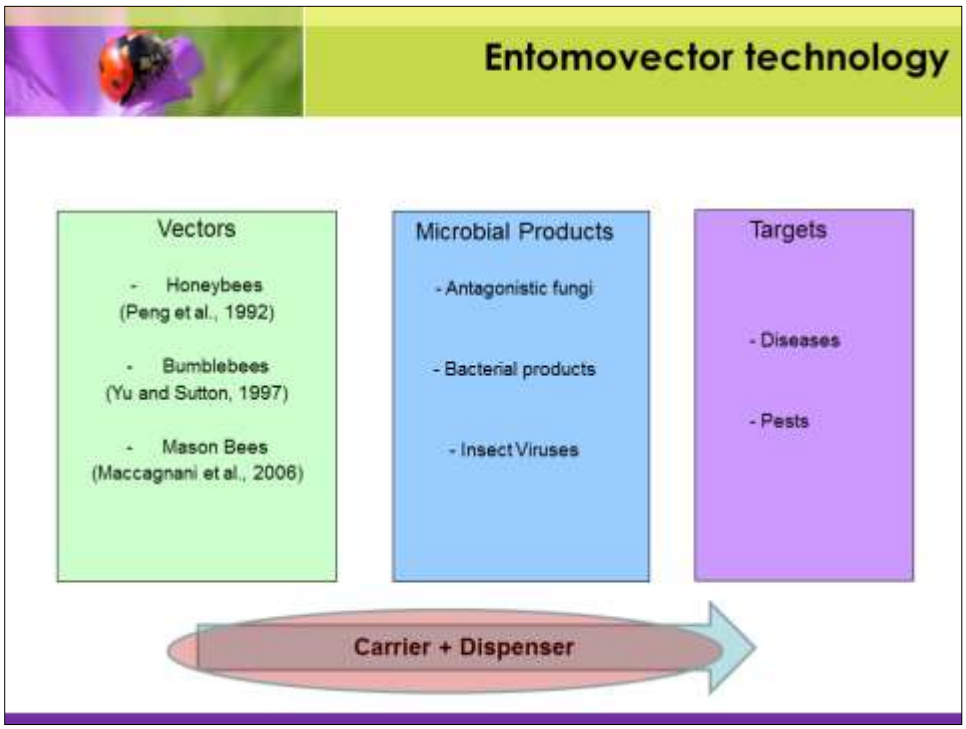

Selection of the biocontrol agent
• Effective towards the disease/pest
– Effective at low concentrations
• Compatible with the vector
– No bee toxicity
– No deterrence
– Good adherence (product formulation; carriers)
• Able to germinate and colonize in flower
– Fast colonizer
– Able to survive environmental conditions/UV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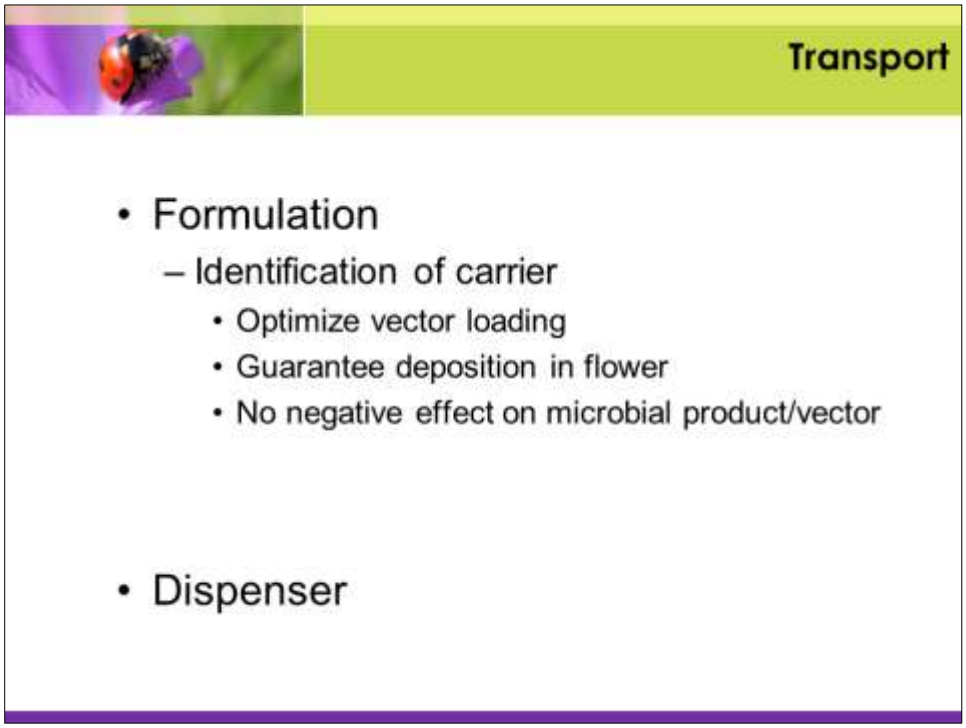
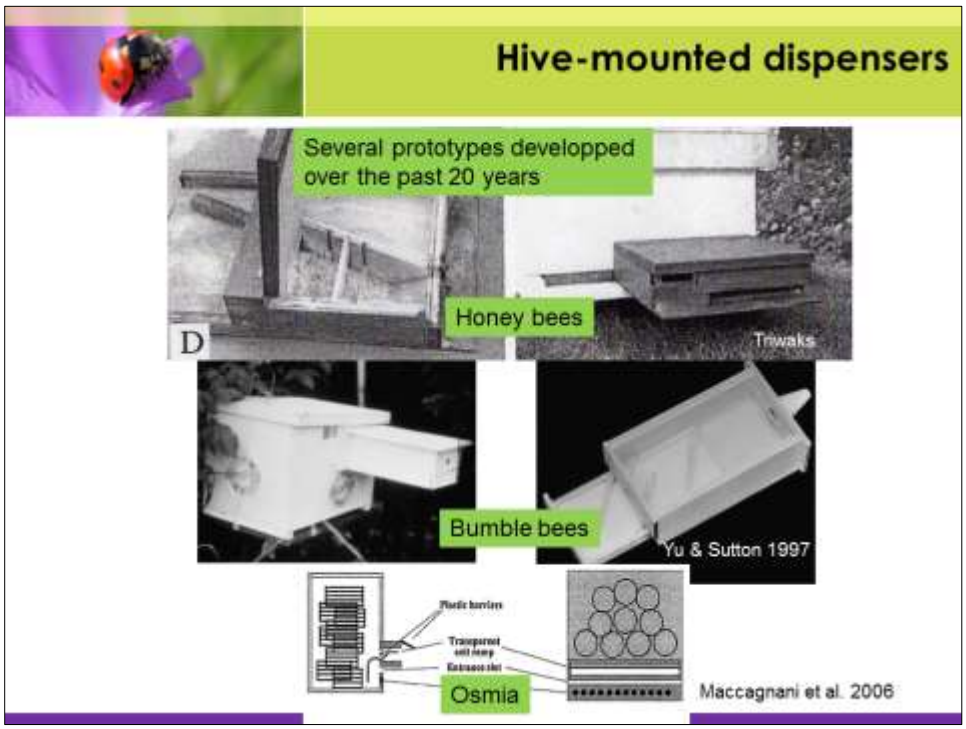
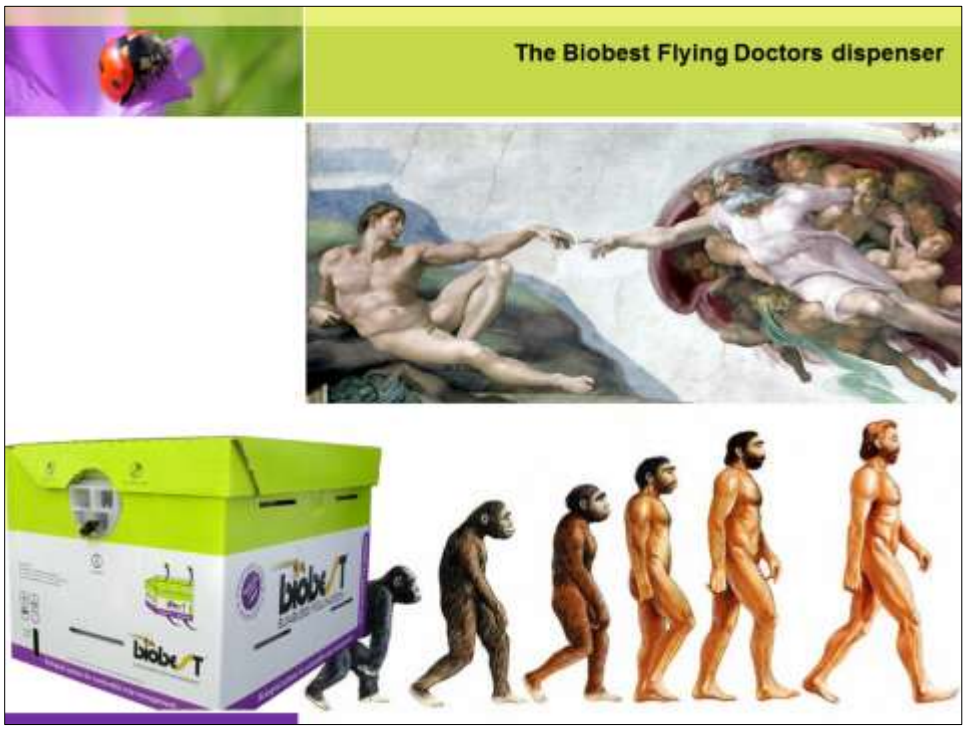
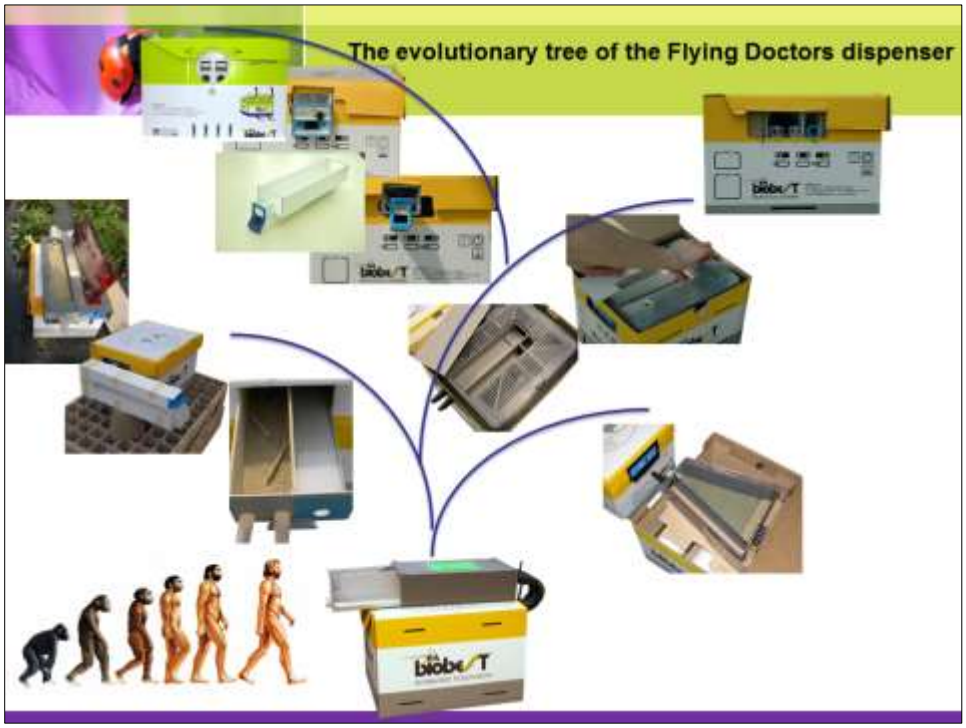
Microbial Products
- Antagonistic fungi
- Bacterial products
- Insect Viruses
Targets
- Diseases
- Pests
Carrier + Dispenser


Transport
• Formulation
– Identification of carrier
• Optimize vector loading
• Guarantee deposition in flower
• No negative effect on microbial product/vector
• Dispenser

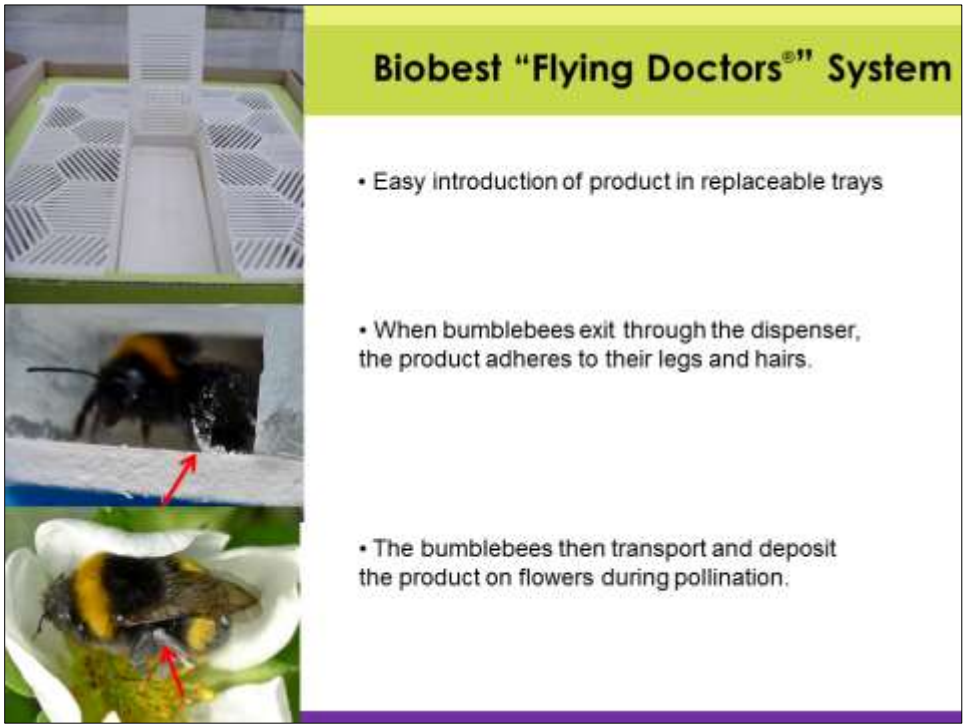

Hive-mounted dispensers
Several prototypes developped over the past 20 years
Honey bees
Tnwaks
D
Bumble bees
Yu & Sutton 1997
Osmia
Maccagnani et al. 2006


Bottlenecks
Impact on bee flight/pollination activity
Sufficient loading of outgoing bees
Prevent brood contamination

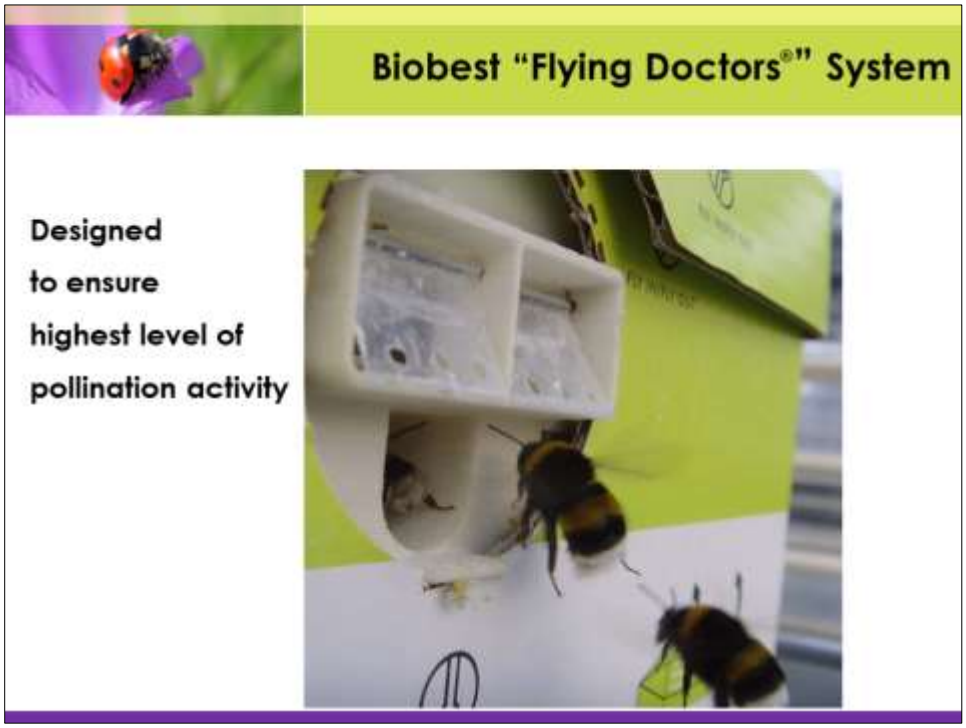

The Biobest Flying Doctors dispenser


The evolutionary tree of the Flying Doctors dispenser


2013 Milestone:
First commercially available dispenser system
BB
biobest
BUMBLEBEE POLLINATION


Biobest "Flying Doctors®" System
• Easy introduction of product in replaceable trays
• When bumblebees exit through the dispenser, the product adheres to their legs and hairs.
• The bumblebees then transport and deposit the product on flowers during pollination.

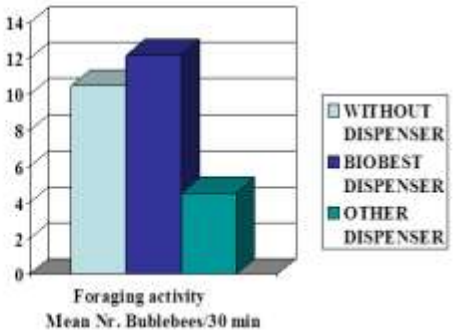

Biobest "Flying Doctors®" System
Advantages
• Targeted product delivery
• Strong reduction in product use
• Continuous application
• Considerable savings in labour


Biobest "Flying Doctors®" System
Designed
to ensure
highest level of
pollination activity


Flying Doctors® bumblebee dispenser hive
Patented one-way traffic design...
...ensures product pick-up only when bumblebees leave the hive
ONE WAY
DO NOT ENTER


Flying Doctors® bumblebee dispenser hive
Biobest Flying Doctors
view inside the dispenser

Bumblebee flight activity



Flying Doctors dispenser does not inhibit foraging

Bumblebee loading

Average product load (upon exit)
$3.7 * 10^5$ CFU

Head:	2%
Thorax:	56%
Abdomen:	36%
Legs:	6%

(Mommaerts, 2009)

Biobest "Flying Doctors®" System

Verdera B4: authorized in Belgium for use with FD system for control of *Botrytis cinerea* in Strawberry & Raspberry

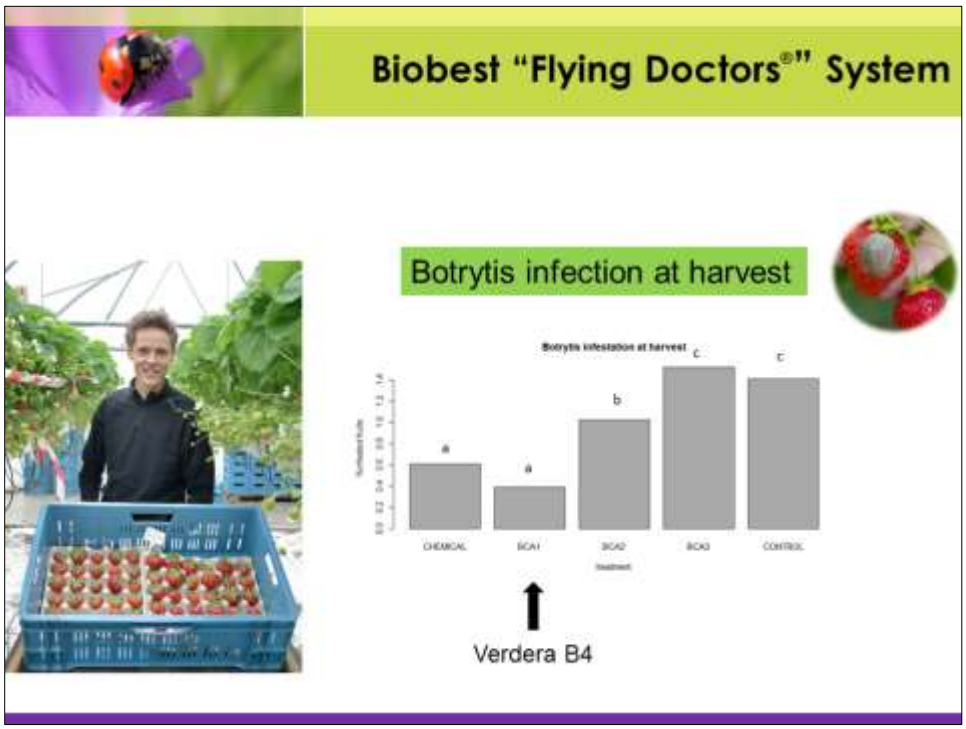
Gliocladium catenulatum J1446

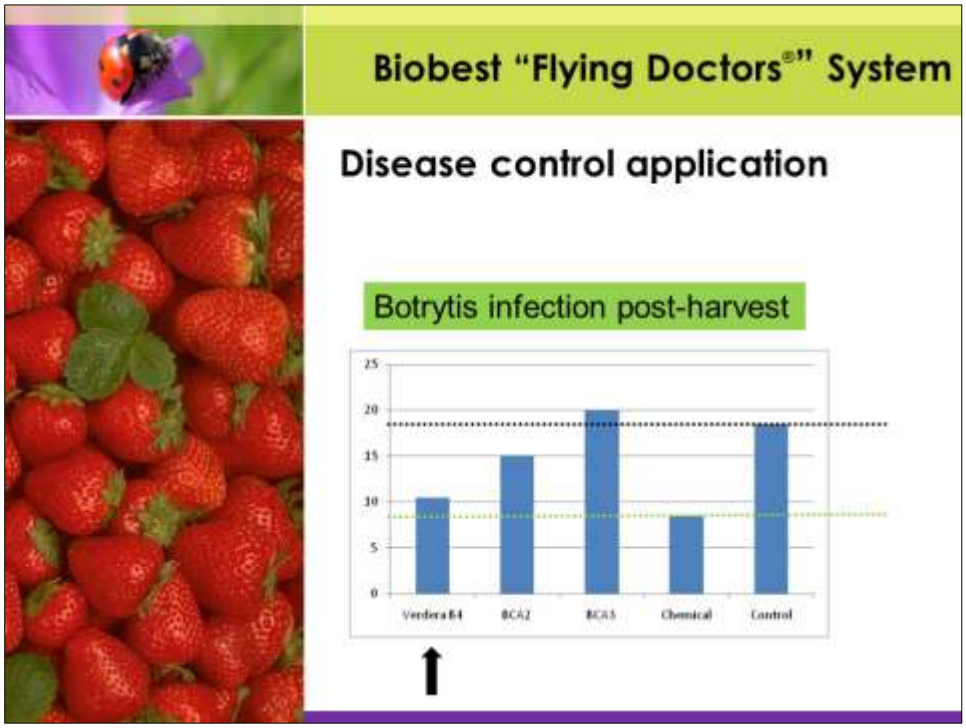
- Competition for nutrients and space
- Mycoparasitism

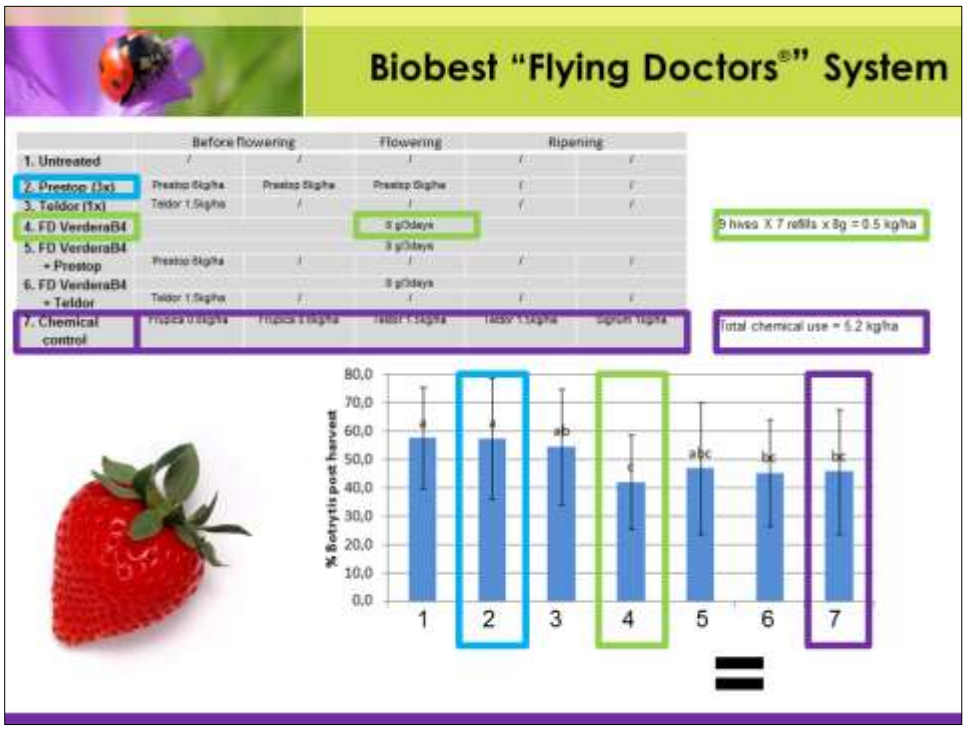
Biobest "Flying Doctors®" System

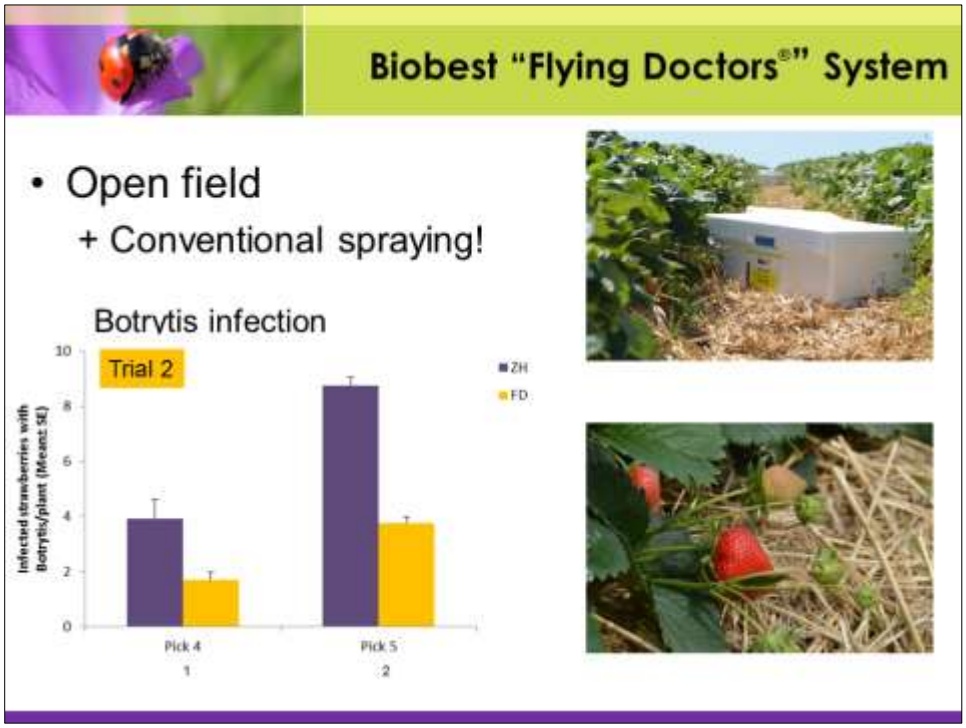
Disease control application

In combination with Verdera B4:
Proven efficacy for *Botrytis* control


Biobest "Flying Doctors®" System
Botrytis infection at harvest
Botrytis infestation at harvest
a
a
b
c
c
CHEMICAL
BCA1
BCA2
BCA3
CONTROL
Verdera B4


Biobest "Flying Doctors®" System
Disease control application
Botrytis infection post-harvest
25
20
15
10
5
0
Verdera B4
BCA2
BCA3
Chemical
Control


Biobest "Flying Doctors®" System
Before flowering
Flowering
Ripening
1. Untreated
2. Prestop (1x)
3. Teldor (1x)
4. FD VerderaB4
5. FD VerderaB4 + Prestop
6. FD VerderaB4 + Teldor
7. Chemical control
9 hives X 7 refills x 8g = 0.5 kg/ha
Total chemical use = 5.2 kg/ha
% Botrytis post harvest
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
a
a
ab
c
abc
bc
bc
1
2
3
4
5
6
7


Biobest "Flying Doctors®" System
• Open field
+ Conventional spraying!
Botrytis infection
Trial 2
ZH
FD
Infected strawberries with Botrytis/plant (Means± SE)
10
8
6
4
2
0
Pick 4
1
Pick 5
2

Other opportunities

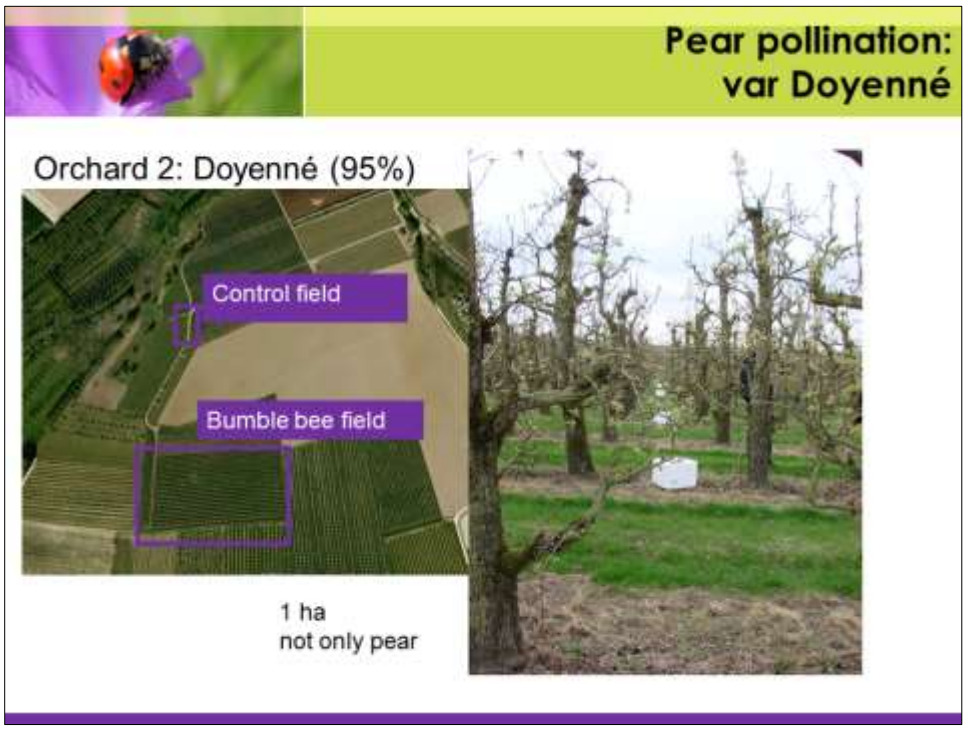
- Flower-transmitted diseases & pests

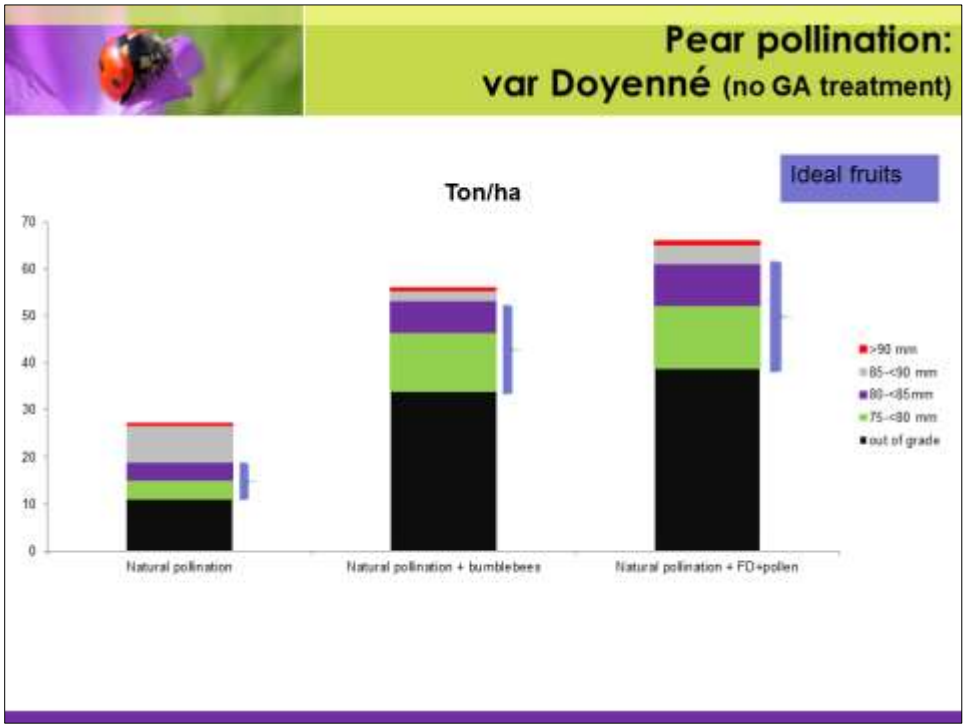
...

- Pollen vectoring

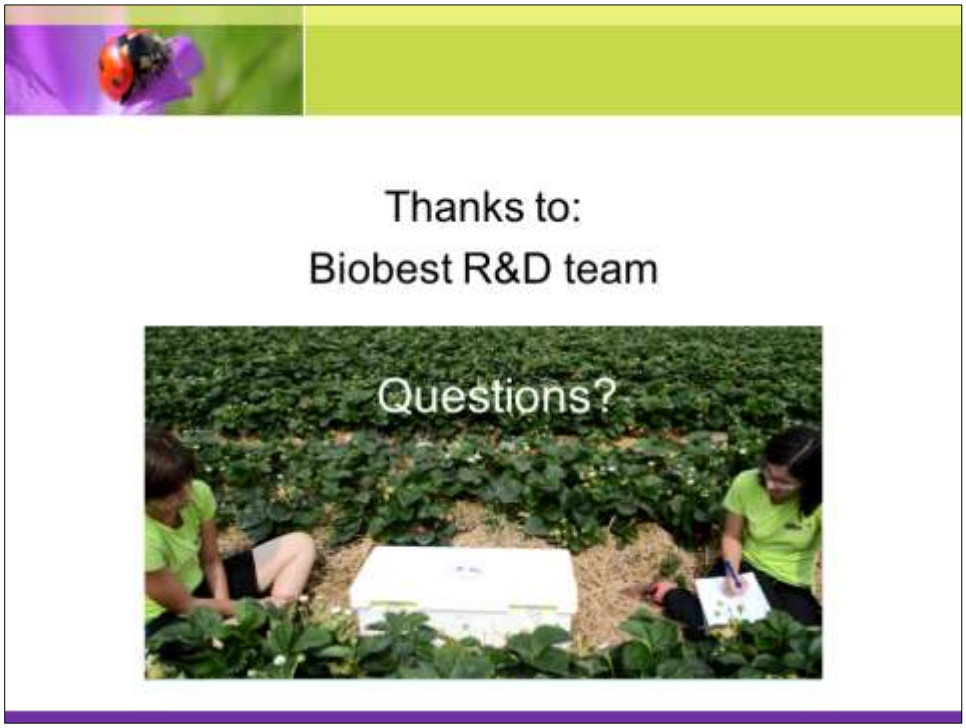
Regulatory issues

- New application. Requirements for registration extension?
- GEP efficacy trial requirements are based on small plot trials: Entomovectoring involves big trials. Replicates within plots?
- Registration requirements for basic substances as carriers/co-formulants unclear


Pear pollination:
var Doyenné
Orchard 2: Doyenné (95%)
Control field
Bumble bee field
1 ha
not only pear


Pear pollination:
var Doyenné (no GA treatment)
Ton/ha
Ideal fruits
70
60
50
40
30
20
10
0
>90 mm
85-<90 mm
80-<85mm
75-<80 mm
out of grade
Natural pollination
Natural pollination + bumblebees
Natural pollination + FD+pollen

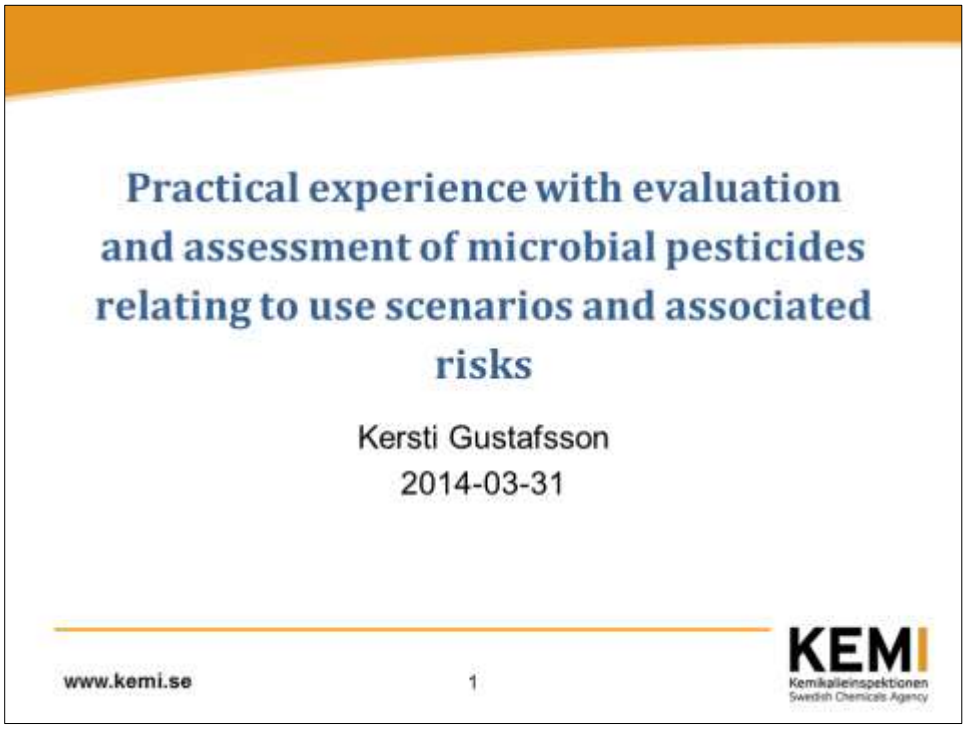

Summary
Advantages
• Targeted product delivery
• Strong reduction in product use
• Continuous application
• Considerable savings in labour
Succesful Applications
-Disease Control
-Pest Control
-Pollination
FLYING DOCTORS

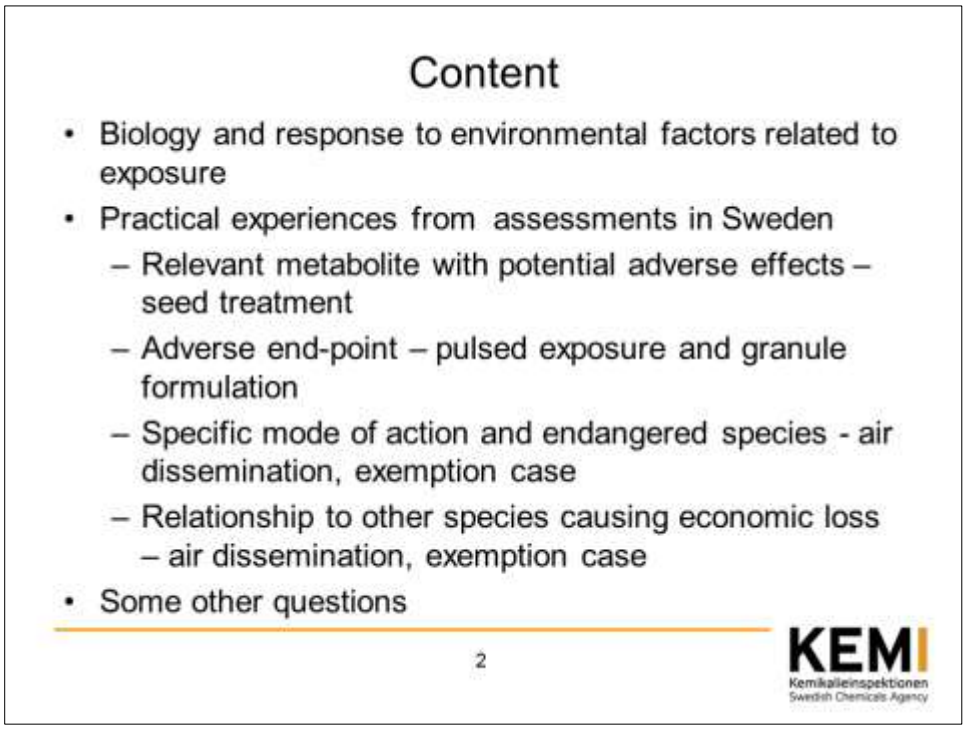

Thanks to:
Biobest R&D team
Questions?

Practical experience with evaluation and assessment of microbial pesticides relating to use scenarios and associated risks

[PPT 9]

Kersti Gustafsson (Swedish Chemicals Agency - KemI; Sweden)





OECD GUIDANCE TO THE ENVIRONMENTAL SAFETY EVALUATION OF MICROBIAL BIOCONTROL AGENTS
Series on Pesticides No. 67

- Application technique mentioned
  - Types of application and expected exposure of NTOs
  - Box 2 Consider the use pattern/application type and pattern
- Further guidance could be developed based on Box 2 and practical experiences

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Suggestion from the June 2013 OECD/Kemi/EU workshop on Microbial Pesticides

- "Develop methodology/models for an exposure assessment for operator, bystander, worker and resident that are specific for application methods for microbials differing from chemical techniques."
- Further guidance could be developed based on practical experiences

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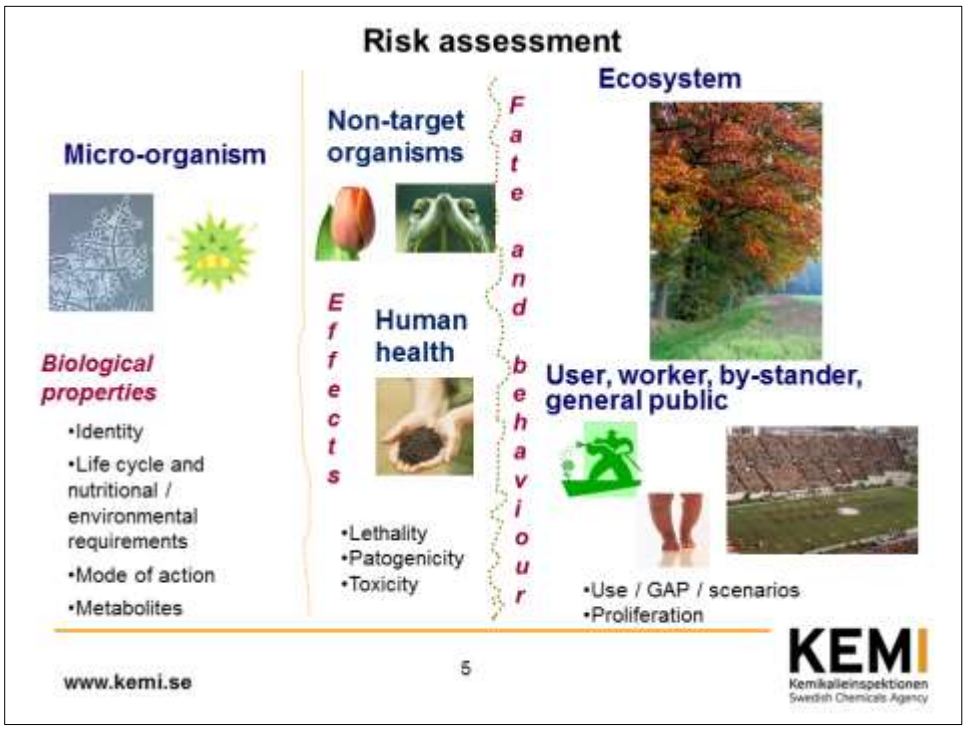
Risk assessment
Micro-organism
Non-target organisms
Ecosystem
Fate and behaviour
Effects
Human health
Biological properties
•Identity
•Life cycle and nutritional / environmental requirements
•Mode of action
•Metabolites
•Lethality
•Patogenicity
•Toxicity
User, worker, by-stander, general public
•Use / GAP / scenarios
•Proliferation
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Relevant metabolite with potential adverse effects
–
seed treatment
6
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Relevant metabolite

Suggestion for new definition (from work with guidance on biocidal microorganisms):

- metabolites that form a major part of the mode of action and/or are present in significant amounts and/or produce an adverse effect on humans or the environment under practical conditions of use

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Pseudomonas chlororaphis

- Sweden RMS around 2000 for a new active substance for seed treatment against fungal diseases
- *P. chlororaphis* common soil bacterium
- SE assessed the metabolite DDR as genotoxic and very potent
- DDR one part of mode of action
  - ➡ relevant metabolite

✓ *Biology is the difference to chemical pesticides*

RMS = Rapporteur Member State

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DDR protective action

- DDR is produced only during active bacterial growth
- Protective action takes place *in situ*, on the seed in the soil, where it is needed

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Questions on DDR

- When and where is the metabolite produced?
- How much is produced?
- Is it persistent?
- Who will be exposed?

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DDR production

- Produced during growth
  - during fermentation (production of bacteria)
  - on the seed in the soil after sowing
- Limit of quantification (LOQ) 2µg DDR/ml
- DDR is degrading with time (t ½ is 25h at 8°C)

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DDR in the product

- Formulated product
  - DDR an impurity since it is not an essential ingredient of the product
  - DDR only a residue from the fermentation process

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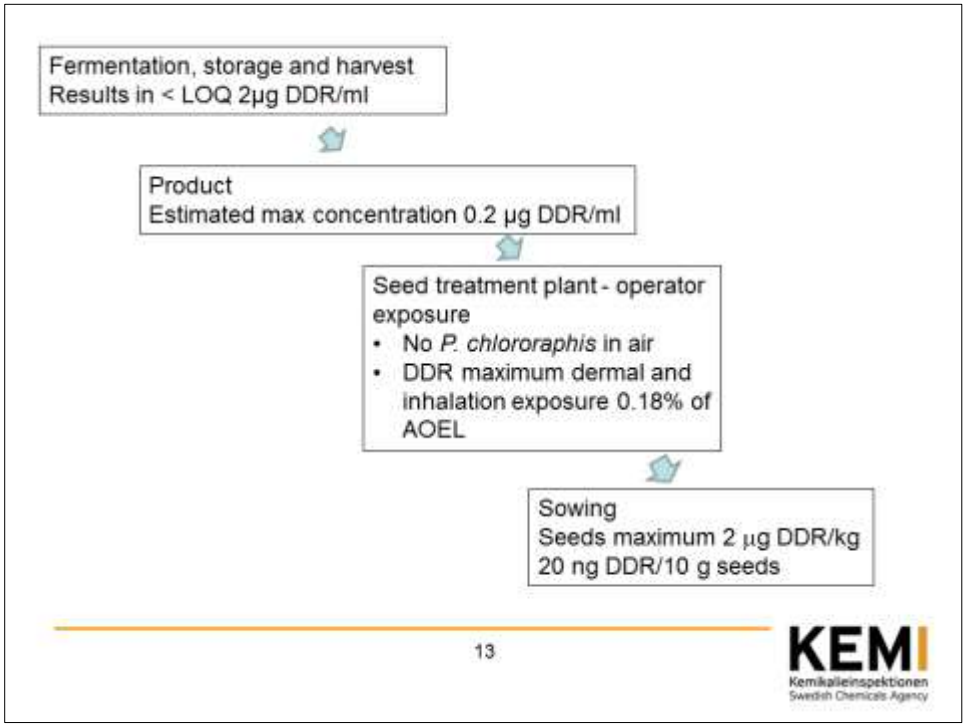
Fermentation, storage and harvest
Results in < LOQ 2µg DDR/ml
Product
Estimated max concentration 0.2 µg DDR/ml
Seed treatment plant - operator exposure
• No P. chlororaphis in air
• DDR maximum dermal and inhalation exposure 0.18% of AOEL
Sowing
Seeds maximum 2 µg DDR/kg
20 ng DDR/10 g seeds
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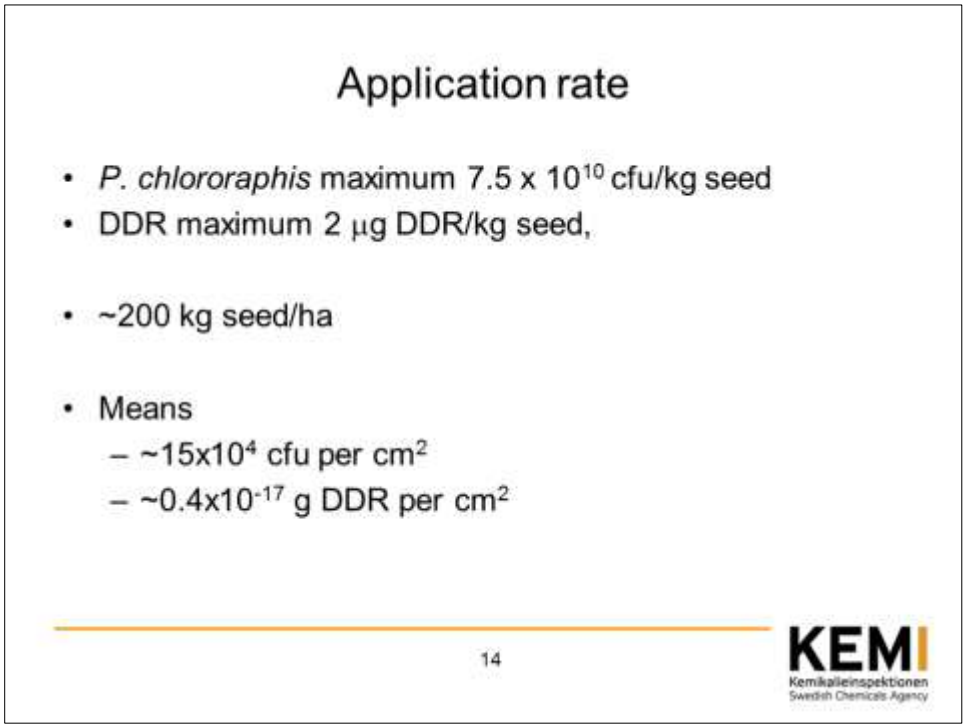
Application rate
• P. chlororaphis maximum 7.5 x 10^10 cfu/kg seed
• DDR maximum 2 µg DDR/kg seed,
• ~200 kg seed/ha
• Means
– ~15x10^4 cfu per cm^2
– ~0.4x10^-17 g DDR per cm^2
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Risk to seed-eating birds
Yellowhammer *Emberiza citrinella*

- Hatching in ditches around cultivated land
- Bird weight ~30 g
- Eat aroynd 50 seeds or ~20 g per day during spring
- 0.8 ng DDR/seed
  - ~40 ng DDR/20 g seeds per bird per day
- Potential effect on formation of blood vessels

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Environmental risk assessment

- Late spring yellowhammer mostly feed on insects
  - If birds eat seeds
    - DDR probably degraded quickly low pH in stomach
    - Amount of DDR on seeds ~detection limit
- *P. chloraphis* naturally occurring in the soil-ecosystem,
  - probably certain background level of DDR
- Conclusion
  - Environment and seed-eating birds in particular, will not be exposed to an unacceptable risk caused by the recommended use of the product.

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Consumer assessment

- Analysis of DDR in
  - plant parts grown from treated barley seeds (germinated shoot, seeds, roots)
  - grain harvest from plants grown from treated barley seeds
- Results < detection limit

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Adverse end-point
–
pulsed exposure and granule formulation

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Bacillus thuringiensis israelensis - Bti

- For use in Sweden against flood water mosquito larvae in a granular formulation
- Dissemination by air is not allowed in Sweden
- Use of pesticides in general not allowed in nature conservation areas/nature reserves/national parks in Sweden
  - When use is necessary - tricky assessment
- IT has developed an initial quantitative model for exposure to surface water
- Granule formulation – how is the loss from granules into water? Necessary information for calculations

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Qualitative assessment

- Effect
  - 21-day reproductive Daphnia test low effect dose close to application rate
    - Not known in detail what caused the observed effects in the study, could be Bti toxins, turbidity and/or toxic residues from the production step (growth media or toxic metabolites)
    - *Daphnia magna* is regarded as a focal species for the aquatic compartment
    - *D. magna* most sensitive non-target species to Bti technical powder
- Exposure
  - Used in flooded areas
    - No dissemination over the whole water surface in permanent waters
  - Application with pulsed exposure
    - Depending on flooding and occurrence of mosquito larvae
  - Formulation of adhered technical powder to inert granules with oil
    - Release into water not instantaneous, exposure likely lower than actual dose rate
- Conclusion
  - Safety level for the aquatic environment could be considered acceptable, even though it was not possible to calculate a risk characterisation quotient

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Specific mode of action and endangered species
-
air dissemination, exemption case

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Qualitative assessment of Bti in nature reserves

- *Platynus longiventris*
  - Endangered species of ground beetles
  - Terrestrial, flying organism
  - Prefer feeding collembola
- Bti specific mode of action
- Conclusion
  - Even though exposure this leads to no effect

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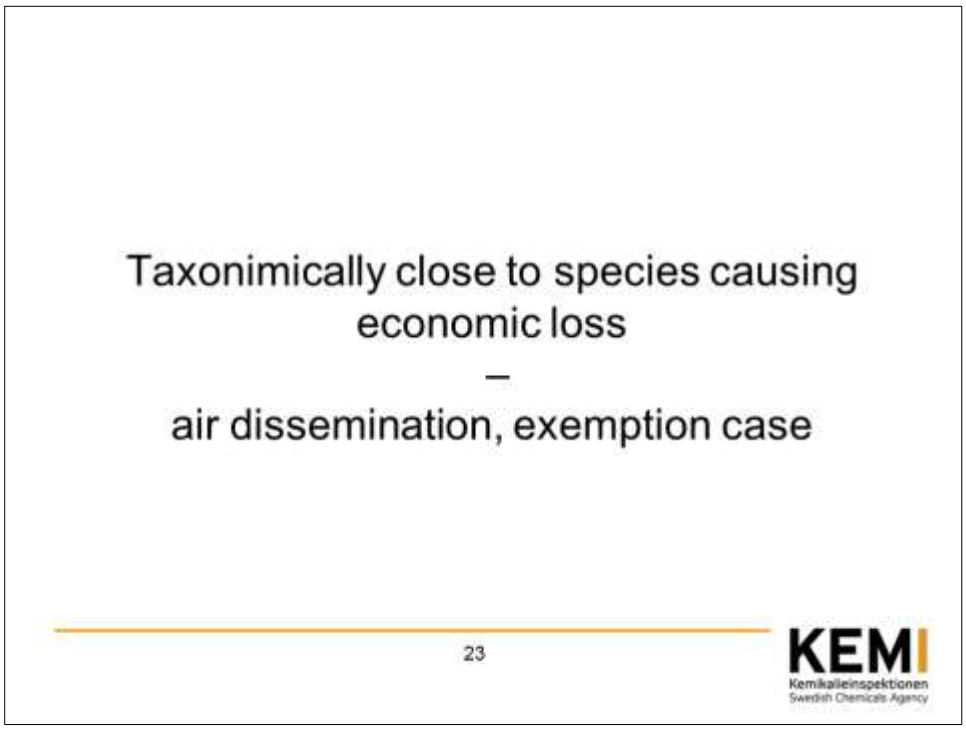
Taxonimically close to species causing economic loss
–
air dissemination, exemption case
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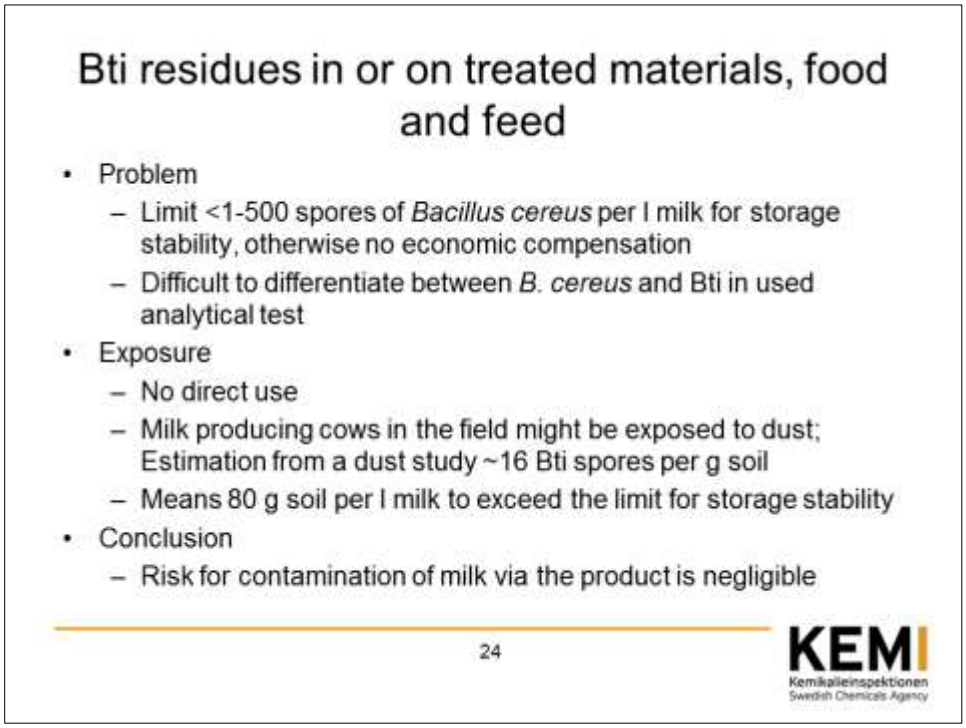
Bti residues in or on treated materials, food and feed
• Problem
– Limit <1-500 spores of Bacillus cereus per l milk for storage stability, otherwise no economic compensation
– Difficult to differentiate between B. cereus and Bti in used analytical test
• Exposure
– No direct use
– Milk producing cows in the field might be exposed to dust; Estimation from a dust study ~16 Bti spores per g soil
– Means 80 g soil per l milk to exceed the limit for storage stability
• Conclusion
– Risk for contamination of milk via the product is negligible
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Other application methods and exposure questions for possible discussions

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Some other questions on exposure scenarios

- Fungal spores on golf courses – wet dissemination directly down into the soil means less spore dusting
- Spores on strawberries close to harvest – inhalation of spores by consumers?
- Wound painting; wall painting after flooding; spraying of freshly cut stumps of pine and spruce – inhalation of spores by someone?
- Tree injection – any longterm problems with resting spores?
- Bumble bees as vectors – any problems?

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Risk assessment - exposure scenarios

What will happen in different exposure scenarios with fate and behaviour of the microorganism?

- Where will it end up?
- Can it be an opportunistic pathogen – who will be affected?
- Is it a spore former – can it recover and affect?
- Does it produce metabolites with adverse effects – in what amounts and who will be affected?
- Can there be some residues – who will be affected?

Fate and behaviour

Ecosystem

User, worker, by-stander, general public

•Use / GAP / scenarios
•Proliferation

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Further guidance development

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To take into account for further guidance development

- Rather reasoning than further testing
- Analyse the exposure situation before further testing

- Develop with *OECD guidance to the environmental safety evaluation of microbial biocontrol agents* as a basis
  - Maybe a checklist with questions based on practical experiences

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Thanks for your attention

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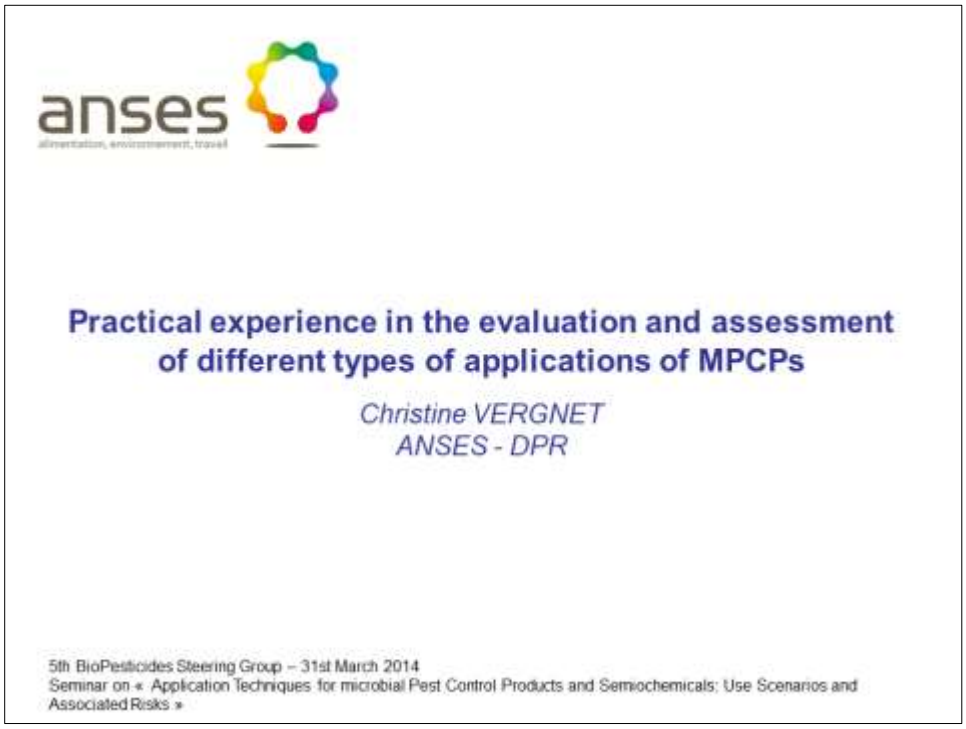
Practical experience in the evaluation and assessment of different types of applications of MPCPs

[PPT 10]

Christine Vergnet (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)



Different types of applications of MPCP

- Foliar treatments (**spray** – from ground or by aeronef)
- Soil treatments (drip irrigation, drench/dip, **spray**, incorporation)
- Localised treatment (granules in palm, seed treatment, wound painting)
- Dissemination by pollinators

3 anses

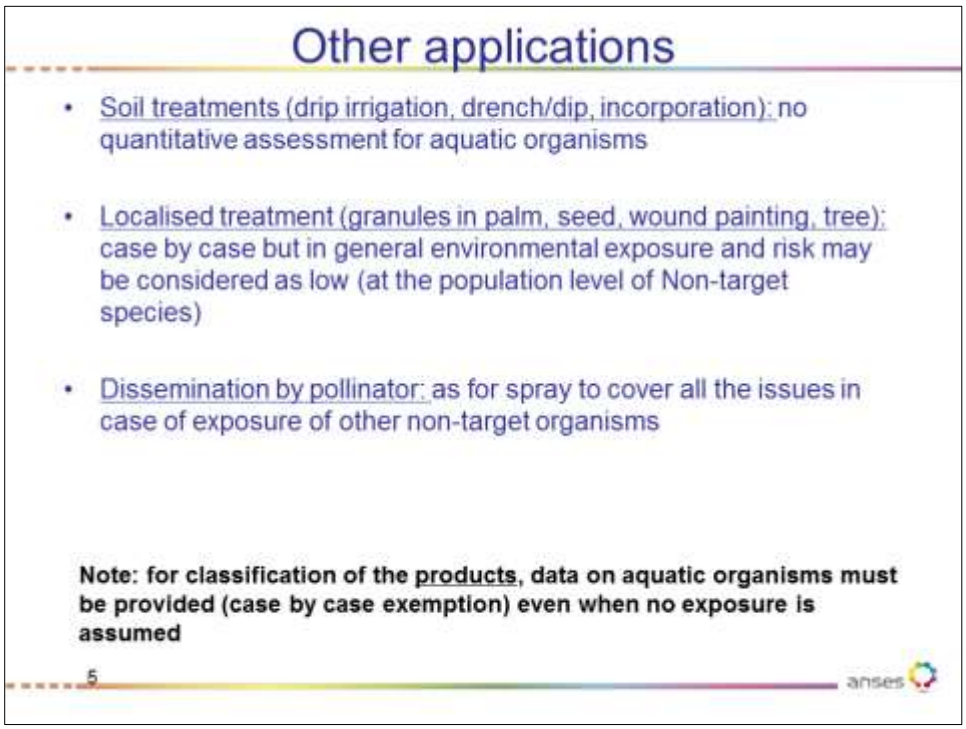
Spray applications (foliar or soil)

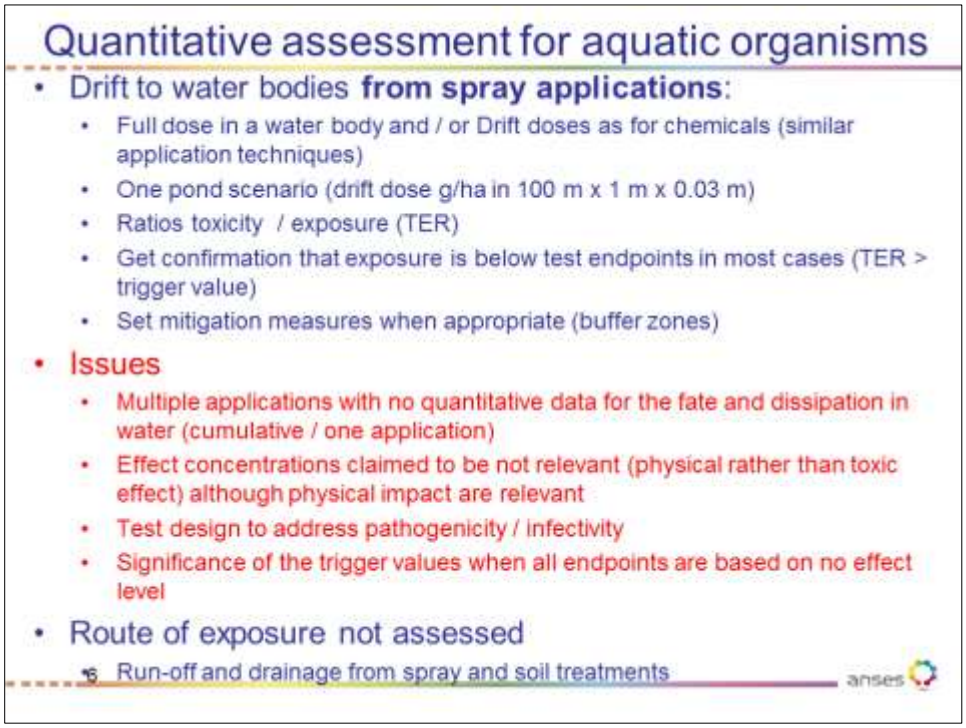
OUTDOOR USES	INDOOR USES
All groups of non target organisms	Covered by outdoor uses or Focus on: Pollinators Non target arthropods Soil organisms

Qualitative assessment (pathogenicity/infectiosity)
Quantitative assessment (toxicity or exposure endpoints available)

IN-FIELD EXPOSURE	OFF-FIELD EXPOSURE
Birds and mammals (outdoor)	**Aquatic organisms (outdoor)**
Bees and arthropods	Arthropods (may be waived ?)
Soil organisms	Terrestrial plants (outdoor)

4 anses


Other applications
• Soil treatments (drip irrigation, drench/dip, incorporation): no quantitative assessment for aquatic organisms
• Localised treatment (granules in palm, seed, wound painting, tree): case by case but in general environmental exposure and risk may be considered as low (at the population level of Non-target species)
• Dissemination by pollinator: as for spray to cover all the issues in case of exposure of other non-target organisms
Note: for classification of the products, data on aquatic organisms must be provided (case by case exemption) even when no exposure is assumed
5
anses


Quantitative assessment for aquatic organisms
• Drift to water bodies from spray applications:
• Full dose in a water body and / or Drift doses as for chemicals (similar application techniques)
• One pond scenario (drift dose g/ha in 100 m x 1 m x 0.03 m)
• Ratios toxicity / exposure (TER)
• Get confirmation that exposure is below test endpoints in most cases (TER > trigger value)
• Set mitigation measures when appropriate (buffer zones)
• Issues
• Multiple applications with no quantitative data for the fate and dissipation in water (cumulative / one application)
• Effect concentrations claimed to be not relevant (physical rather than toxic effect) although physical impact are relevant
• Test design to address pathogenicity / infectivity
• Significance of the trigger values when all endpoints are based on no effect level
• Route of exposure not assessed
• Run-off and drainage from spray and soil treatments
6
anses

Quantitative assessment for vertebrates

- Consumption of treated food items
  - Dose converted to a daily ingestion rate
  - chemical screening-tier 1 exposure scenarios (EFSA 2009)
  - Ratios toxicity / exposure (TER)
  - Get confirmation that exposure is below test endpoints in most cases (TER > trigger value)
- Issues
  - Multiple applications (cumulative/one application) with no quantitative data for the fate and dissipation in food item
  - Limit test dose of 2000 mg/kg bw might be insufficient
- Exposure not assessed
  - Secondary poisoning (not relevant)

7 anses

Quantitative assessment for soil organisms

- Soil concentrations
  - Full dose converted to a soil concentration
  - chemical scenario (g/ha in 750 tons soil i.e. 5 cm depth with density 1.5)
  - Ratios toxicity / exposure (TER)
  - Get confirmation that exposure is below test endpoints in most cases (TER > trigger value)
- Issues
  - Relevance of the depth of the soil depending on application techniques (5 cm)
  - Multiple applications (cumulative/one application) with no quantitative data for the fate and dissipation in soil
  - Test design to address pathogenicity / infectivity
- Not assessed
  - Accumulation (covered by the uniform principle for approbation)

8 anses

Quantitative assessment for arthropods

- Hazard quotient ratios
  - Dose / toxicity
  - Required in regulation as for chemical however not considered relevant
- Issues
  - Relevance of the tier 1 toxicity data (acute bees, Typhlodromus pyri and Aphidius rhopalosiphi laboratory tests) and the HQ calculations
- More relevant assessment to be encouraged
  - 10 day feeding test for bees instead of acute tests ?
  - Bumble bees in tunnel trials
  - Assessment in practical condition of use

9 anses

Conclusion

- Mandatory requirement for a quantitative assessment
  - In practice, simple calculations could be done when a quantitative endpoint is available
  - In most cases, these calculations demonstrate that test endpoints cover estimated exposure
- Issues
  - Multiple applications (cumulative / one application)
  - Test design and relevance of endpoints
  - Significance of the trigger values issues from chemical
- A more relevant assessment to be encouraged
- There is a need to "improve" the data requirement to give place to ecology relevance
  - flexibility of the core data set ?
  - scientific research study versus regulatory test results

10 anses

Horticultural workers' exposure to microbial bioaerosol components from MPCP and naturally occurring counterparts of MPCA

[PPT 11]

Anne Mette Madsen (National Research Centre for the Working Environment, Copenhagen; Denmark)

Horticultural workers' exposure to microbial bioaerosol components from MPCPs and naturally occurring counterparts of MPCA s
-Risk assessment of the use of MPCAs

Anne Mette Madsen

OECD The 5th BioPesticides Steering Group

NATIONAL RESEARCH CENTRE FOR THE WORKING ENVIRONMENT

Risk assessment of the use of MPCA

Background:

High exposure to microorganisms in horticultural environments has been found– then does the contribution from MPCPs matter?

Approach:

Exposure to MPCAs relative to exposure to other microorganisms

E.g. Exposure to *Bacillus thuringiensis* from MPCPs versus natural occurring *B. thuringiensis* and versus total number of bacteria.

Or

E.g. Exposure to *Trichoderma harzianum* from MPCPs versus natural occurring *T. harzianum* and versus total number of fungi.

Methods

- Aerosol sampling during normal work days; growers were instructed not to change their routines during the exposure measurements.
- Cultivation methods
- Total counts by microscopy
- PCR analysis have been performed to confirm the identity of MPCAs.

Literature study

Studied exposures:

MPCA	MPCP
Bacillus thuringiensis subsp. kurstaki subsp. israelensis	Dipel ES Bactimos L
Trichoderma harzianum *T. polysporum*	Supresivit Binab
Lecanicillium muscarium (Verticillium lecanii)	Mycotal
Streptomyces griseoviridis	Mycostop
Beauveria bassiana	BotaniGard 22WP BotaniGard ES

Results:
Exposure to *Trichoderma*

- When a powder with *T. harzianum* (Supresivit) was prepared for application in a greenhouse it was found in air samples (100,000 cfu m^{-3}) which was higher than the exposure to other fungi (1200 cfu/m^3).
- During the day of application 2 of 4 workers were exposed to *T. harzianum* in a median concentration of 51 cfu m^{-3} – the median exposure to other fungi was 5000 cfu/m^3.
- Exposure to airborne *T. harzianum* was not found in the greenhouse weeks post application – in these weeks workers were exposed to $7x10^5$ cfu fungi/m^3. (V.M. Hansen, A. Winding, & A.M. Madsen , 2010).

Exposure to *Trichoderma harzianum*

- Stationary measured exposure to *T. harzianum* applied as an MPCA has also been measured in greenhouses in USA.
- High concentrations of *T. harzianum* were found: up to 36,000 cfu m^{-3} and it constituted on average 38% of the total number of fungi

(Li D-W, LaMondia J. Aerobiologia. 2010;26:15-28).

- Time from application of *T. harzianum* to measurement was not mentioned

Trichoderma in an open field

- When bees were used for application of *Trichoderma* in an outdoor field for 3 months, *Trichoderma* was not found in the air.

(K. Tendal & A.M. Madsen, 2010)

- The concentration of airborne fungi was 5000 cfu/m^3 .

Background exposure to *Trichoderma harzianum*

- In greenhouses and open fields where *T. harzianum* had not been applied it was only very seldom found and constituted less than 0.1% of the total number of fungi.

Streptomyces griseoviridis (Mycostop)

- *S. griseoviridis* has been applied in an automatic watering system for watering of tomato plants.
- No exposure to *S. griseoviridis* (Mycostop) was found when it was prepared in a greenhouse
- No exposure of workers was seen post application (V.M. Hansen, A. Winding, & A.M. Madsen , 2010)

Personal exposure to mesofilic actinobacteria

Greenhouse	n	% positive samples	Median (cfu m^{-3})	Range (cfu m^{-3})
Tomato greenhouse, preparing Mycostop	1	100	190	190
Tomato greenhouse, nurturing plants	4	75	189	Bd-333
Open field broccoli, white cabbage, harvest	5	100	161	77 – 292
Open field, white cabbage, harvest	4	50	75	bd – 319
Greenhouse, cucumber harvest	7	100	3.9×10^{4}	$5.4 – 2.5\times10^{5}$
Greenhouse, cucumber, clearing	6	50	43	bd – 2.9×10^{4}
Indoor packaging of broccoli,	3	66	273	bd – 1417
Open field broccoli, celery, harvest	8	100	828	291 – 5659
Open field white cabbage, harvest	4	75	1228	bd – 2484

Beauveria bassiana

- *B. bassiana* has been found in the air in a forest when it was applied to the environment as an MPCA by releasing pine bark beetles contaminated with conidia of *B. bassiana* (M. Shimazu, H. Sato, & N. Maehara, 2002).
- The concentration other fungi was not measured.

Background exposure to *B. bassiana*

- We have measured personal exposure to *B. bassiana* during 26 days for 9 vegetable producers, 4-10 workers each time:

Environment	n	% positive samples	Median (cfu m^{-3})	Range (cfu m^{-3})
Cucumber green house, harvest	10	10	bd	bd-80
Cucumber green house, harvest	7	43	bd	bd-34
Cucumber green house, clearing	4	50	20	bd-44
Cucumber green house, harvest	7	29	bd	bd-96

Occupational exposure to MPCAs - *Bacillus*

- Exposure to *B. thuringiensis kurstaki* in the concentration 5,300 cfu m^{-3} has been found for a grower applying the product Dipel® with a hand pump.
- The person was exposed to higher concentrations of *B. thuringiensis kurstaki* than to other culturable bacteria. (V.M. Hansen, J. Eilenberg, & A.M. Madsen, 2010).
- Seven of the eight other growers performing other tasks in the greenhouse were also exposed to *B. thuringiensis*.
- Their exposure to *B. thuringiensis* was lower than the exposure to bacteria in general.

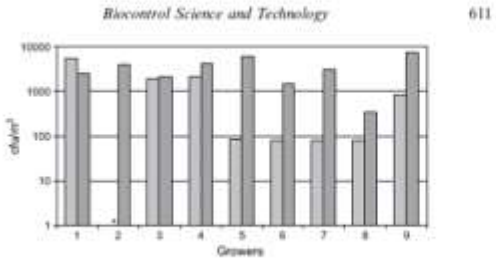


Figure 1. Personal exposure of nine growers on a day of Dipel® treatment in a tomato greenhouse.

Exposure to *Btk*: days post application

Table 2. Personal exposure in environments with Dipel® treated crops.

Environment (producer, crop, number of samplers)	Work task	Cfu/m³ air: HDI[a] Median (range)	Cfu/m³ air: Total mesophilic bacteria[a] Median (range)
T-2, tomato, n = 9	Harvest	4.7×10^2 (Bd–5.3×10^3)	3.1×10^3 (3.6×10^2–7.5×10^3)
T-2, tomato, n = 7	Clearing[b]	Bd (Bd–1.4×10^3)	5.3×10^4 (Bd–5.8×10^5)
F-1, broccoli & cabbage, n = 5	Harvest	Bd (Bd–4.1×10^2)	8.3×10^3 (5.6×10^2–1.2×10^4)
F-1, cabbage, n = 3	Harvest	Bd	4.7×10^2 (2.4×10^2–8.3×10^4)
F-3, celery, n = 3	Harvest	Bd (Bd–1.6×10^2)	1.9×10^4 (1.6×10^3–2.1×10^4)

(V.M. Hansen, J. Eilenberg, & A.M. Madsen, 2010).

- In a open celery field with *B. thuringiensis* (Dipel) treated plants the growers were exposed to 53 cfu m^{-3} 52 days post application and 1.9×10^4 cfu m^{-3} other bacteria (constitute 0.3%) (V.M. Hansen, J. Eilenberg, & A.M. Madsen, 2010).
- In areas with Dipel treated plants but with no work activity airborne *Ba. thuringiensis* was not found. (V.M. Hansen, J. Eilenberg, & A.M. Madsen, 2010).
- In a strawberry field with plants treated with *B. thuringiensis* the previous growth season airborne *B. thuringiensis* was not found. (K. Tendal & A.M. Madsen, 2010).

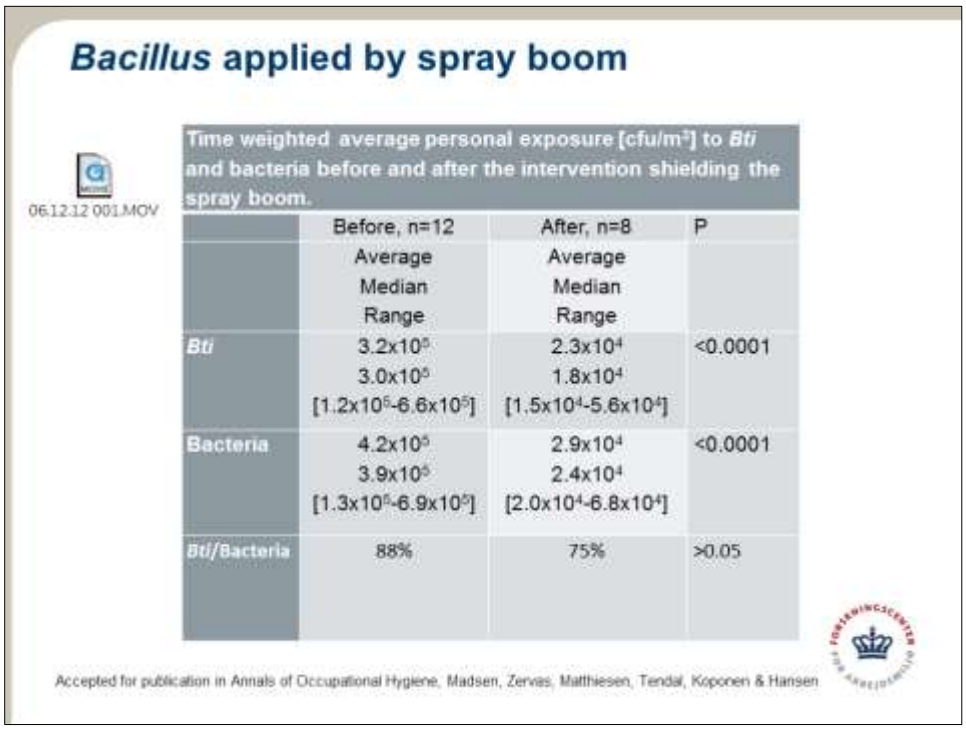
Bacillus (*Bti*) in potted plant production

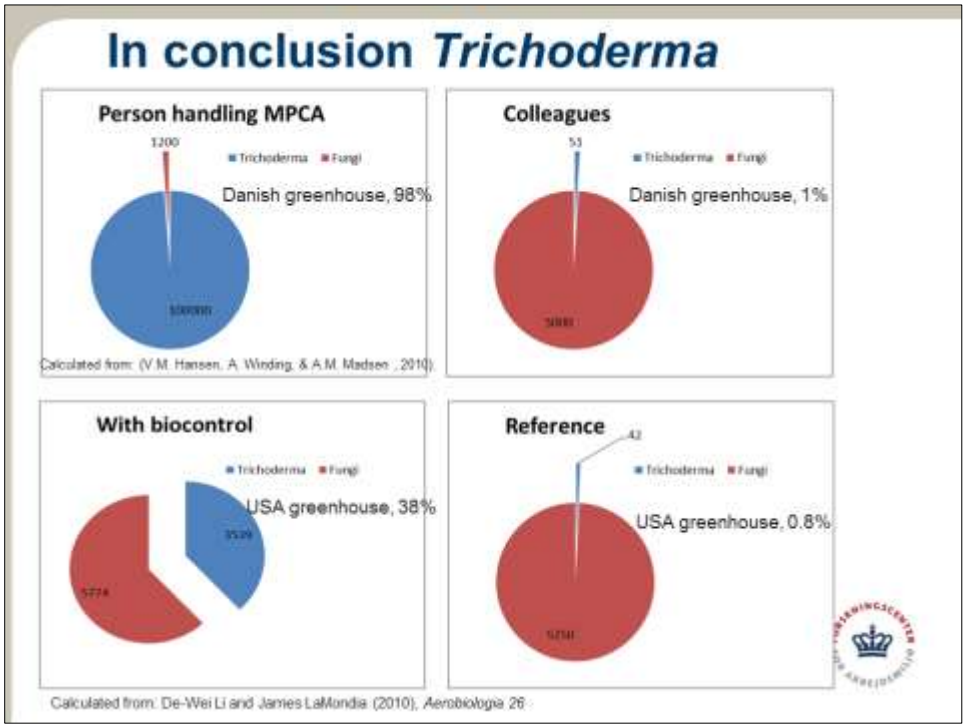
Time weighted average exposure [cfu/m³] to *Bacillus thuringiensis israelensis* (*Bti*) and bacteria in the morning and in the afternoon in the propagation hall and in the greenhouse.

	Personal measurement				Stationary measurement		
	Propagation hall		Greenhouse		Propagation hall		
	Morning, n=5	Afternoon, n=5	P[1]	n=17	Morning, n=8	Afternoon, n=8	P
	Median Range	Median Range		Median Range	Median Range	Median Range	
Bti[2]	1.7×10^5 [2.0×10^4-2.4×10^5]	3.9×10^5 [4.3×10^4-6.9×10^5]	0.045	3200 [78-3.0×10^4]	8.0×10^4 [2.0×10^4-1.0×10^5]	9.1×10^4 [1.5×10^4-4.0×10^5]	0.12
Bacteria[3]	3.4×10^5 [3.2×10^4-4.3×10^5]	4.3×10^5 [4.4×10^4-9.5×10^5]	0.052	5500 [130-1.6×10^5]	9.4×10^4 [2.1×10^4-2.2×10^5]	1.8×10^5 [2.7×10^4-6.1×10^5]	0.084

[1] Bti on Bacillus cereus selective agar was identified by PCR by its CRY-11 genes. [2] Bacteria able to grow on nutrient agar.

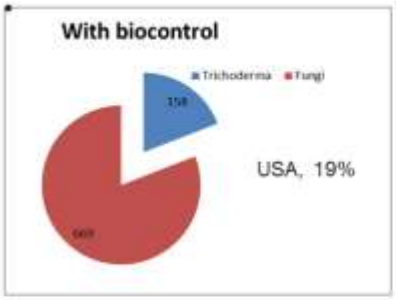
Accepted for publication in Annals of Occupational Hygiene. Madsen, Zervas, Mathiessen, Tendal, Koponen & Hansen


Bacillus applied by spray boom
06.12.12 001.MOV
Time weighted average personal exposure [cfu/m³] to Bti and bacteria before and after the intervention shielding the spray boom.
Before, n=12
After, n=8
P
Average
Median
Range
Bti
3.2x10⁵
3.0x10⁵
[1.2x10⁵-6.6x10⁵]
2.3x10⁴
1.8x10⁴
[1.5x10⁴-5.6x10⁴]
<0.0001
Bacteria
4.2x10⁵
3.9x10⁵
[1.3x10⁵-6.9x10⁵]
2.9x10⁴
2.4x10⁴
[2.0x10⁴-6.8x10⁴]
<0.0001
Bti/Bacteria
88%
75%
>0.05
Accepted for publication in Annals of Occupational Hygiene, Madsen, Zervas, Matthiesen, Tendal, Koponen & Hansen


In conclusion Trichoderma
Person handling MPCA
1200
Trichoderma
Fungi
Danish greenhouse, 98%
Calculated from: (V.M. Hansen, A. Winding, & A.M. Madsen , 2010)
Colleagues
53
Trichoderma
Fungi
Danish greenhouse, 1%
With biocontrol
Trichoderma
Fungi
USA greenhouse, 38%
Reference
42
Trichoderma
Fungi
USA greenhouse, 0.8%
Calculated from: De-Wei Li and James LaMondia (2010), Aerobiologia 26

Conclusion *Trichoderma* exposure - outdoor

- No, not when applied by bees (DK)
- Not outside greenhouses, when it was handled indoor (DK)



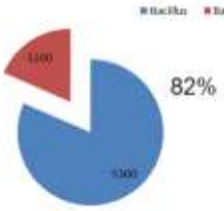
Calculated from: De-Wei Li and James LaMondia (2010), *Aerobiologia 26*

In conclusion Streptomyces griseoviridis (Mycostop)

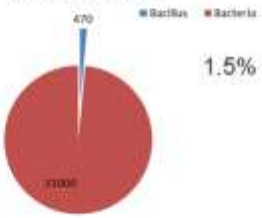
- Was handled in a greenhouse – but no exposure was seen.

In conclusion: *Bacillus* where it is used as spot treatment in greenhouses

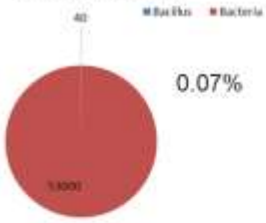
Person handling MPCA- Dipel



Harvest tomato



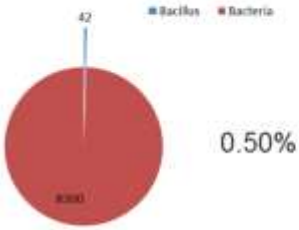
Clearing, Tomato



Btk not found outside the greenhouses

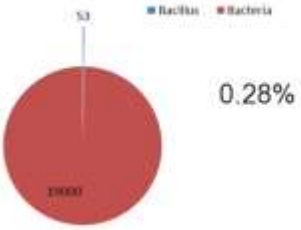
In conclusion: *Bacillus* where it has been used in open fields

Harvest cabbage outdoor

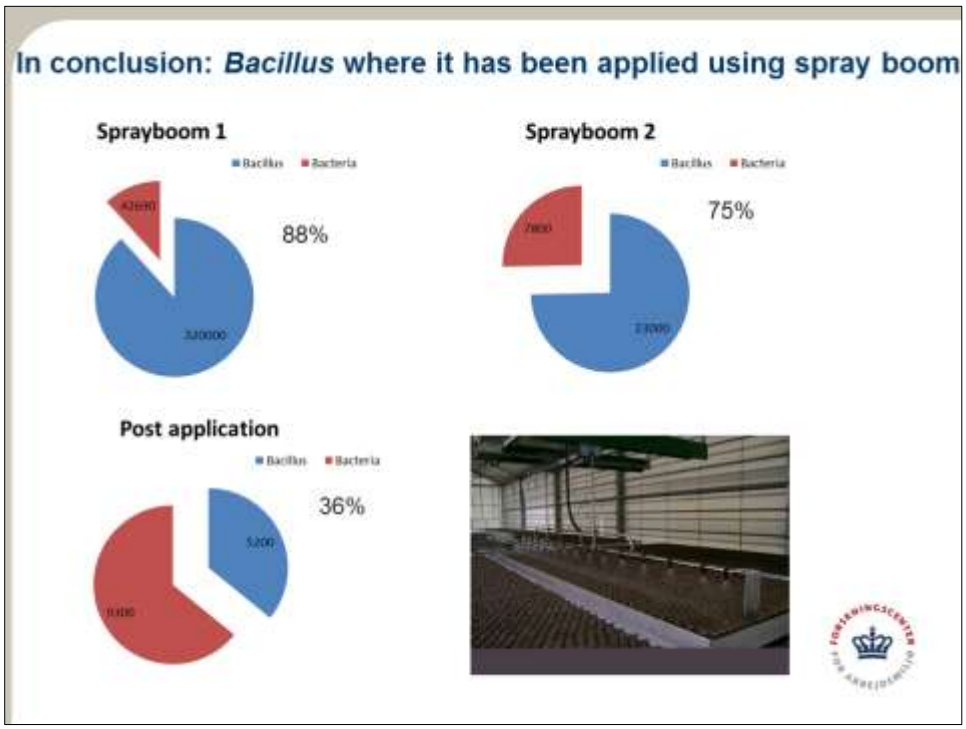


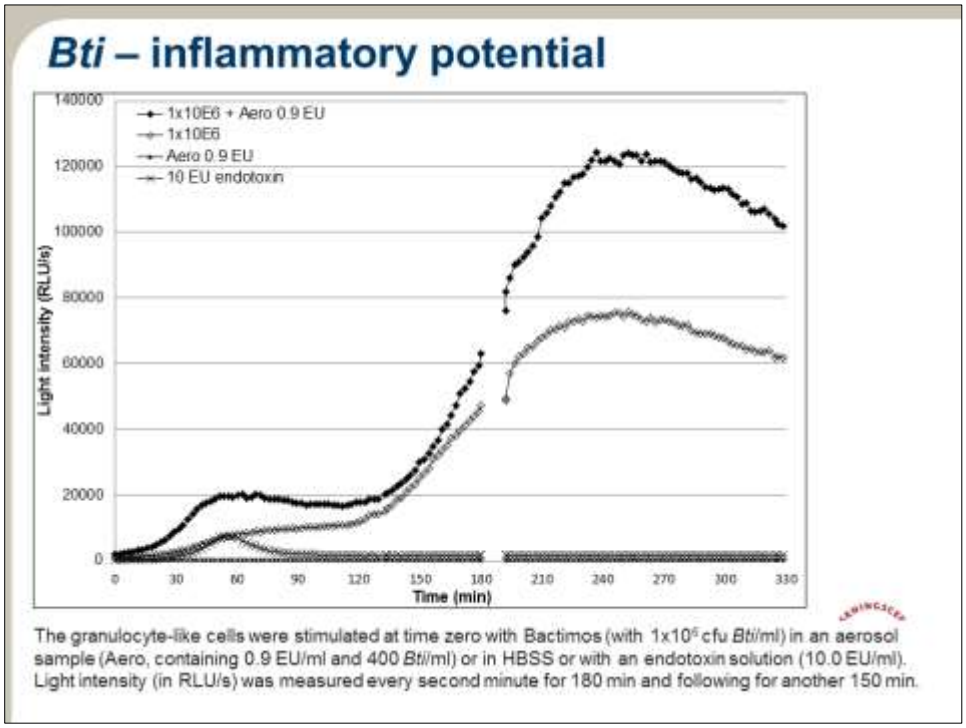
5-59 days post application in cabbage and celery, DK

Harvest celery outdoor



In strawberry it did not contribute to the exposure 1 year post application.





The granulocyte-like cells were stimulated at time zero with Bactimos (with $1x10^6$ cfu *Bti*/ml) in an aerosol sample (Aero, containing 0.9 EU/ml and 400 *Bti*/ml) or in HBSS or with an endotoxin solution (10.0 EU/ml). Light intensity (in RLU/s) was measured every second minute for 180 min and following for another 150 min.


Thank you
• Thank you for the attention
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