



REBECA

*Regulation of
Biological Control Agents*



Risks and Regulation: are they in balance?

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Regulation of
Biological Control Agents



Risks and Regulation: are they in balance?

Asking the question already means that we think that they are not in balance

- What is the regulation?
- What are the risks?
- Are all the requirements needed to assess the risks?



Regulation as it exists

- **Directive 91/414** for plant protection products applies to chemicals and microorganisms
- Data requirements for microorganisms have been adapted in the **Directive 2001/36**
- Nothing specific for natural products and pheromones
- No regulation for macroorganisms



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Risk and regulation are
they in balance?



Aim of the directive

As presented by Patrizia Pitton DGSANCO the aim of the directive is to

- “ensure high level of protection of human, animal health and environment”
- “improve functioning of internal market and granting availability of ppp to farmers”

Regulation is needed, but often not accepted



How is regulation perceived by SMEs (*from Ralf's presentation*)

- Regulation is time consuming
- High financial cost
- BCAs are specific thus the market is small
- Market size does not allow expensive studies
- Regulation requires scientific knowledge
- The reasons for some requirements are not understood



How is regulation perceived by SMEs

(from Ralf's presentation)

- Registration seems to be a black box
- Barrier of entry for SMEs
- Regulation prevents the technical progress to be introduced into the market
- BCAs sit on the shelf of the scientists
- BCAs suffer from introduction of PP
- Registration keeps safe products off the market



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We know the regulation as it exists

91/414, 2001/36, 2005/25

I will review it in a few minutes

What are the risks?

much more difficult to identify



Risks for man

- **Natural products:**
 - **Toxicity**
- **μorganisms:**
 - **pathogenicity, infectiosity,**
 - **toxicity of secondary metabolites**

The exposure for workers, bystanders and consumers is different, but regulation applies to all of them



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Risks for the environment

- **Natural substances:**
 - toxicity for non target organisms
- **μorganisms:**
 - pathogenicity on non target organisms
 - toxicity for non target organisms
 - multiplication in the environment,
 - displacement of the natural balance



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Risk is defined as the probability of a hazard to occur

- The principle consists in
 - **characterizing the hazards**, ie, the deleterious, negative effects
 - **determining the probability for the hazard to occur**, this depends on exposure



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Risk being defined as the probability of a hazard to occur

- considerable efforts are dealing with **hazard identification** even without any evidence of risk; indeed if there is no stated hazard there is no risk
- but reverse is also true: when there is no exposure, there is no risk even if the hazard exists



What are the main requirements of the annex II of the directive

- 1/ Identity of the active substance / of the microorganism
- 2/ Physical and chemical properties of the AS / Biological properties of the μ organism
- 3/ Further information on the AS / on the μ organism
- 4/ Analytical methods



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What are the main requirements of the annex II of the directive

5/ Toxicological and metabolism studies / Effect
on human health

6/ Residues in or on treated products, food and
feed

7/ Fate and behavior in the environment

8/ Ecological studies / Effects on non target
organisms



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- **Are the requirements of the directive (annex II) all needed to assess the risks?**
- **How can we simplify the data requirements without compromising the level of safety?**



1/ Identity of the active substance

- **Aim:** to know precisely what will be put on the market
- **Question:**
 - How to be sure that the composition of the ppp will be constant without characterizing every molecule / μ organism present in the ppp?
- **Problems:**
 - with natural products which are mixture of molecules
 - with microorganism : which level of identification: genus, species, strain



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1/ Identity of the active substance

Why should a μ organism be characterized at the species and strain level?

Among *Trichoderma* species many are known for their biocontrol capacities but at least one *T. longibrachiatum* can be pathogenic for man

Trichoderma species are difficult to identify based on morphological characters

But the use of molecular tools enables a clear identification of the different species



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1/ Identity of the μ organism at the strain level

The best method today is to design a SCAR marker

Advantages:

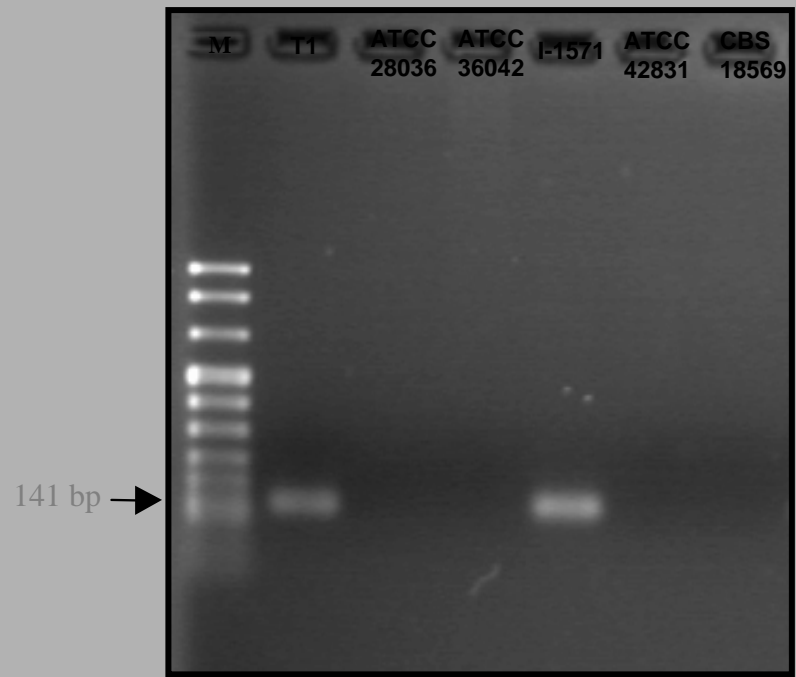
- Possibility to trace the μ organism on food and in the environment
- Protection of the strain

Example of strain T1 of *Trichoderma atroviride*

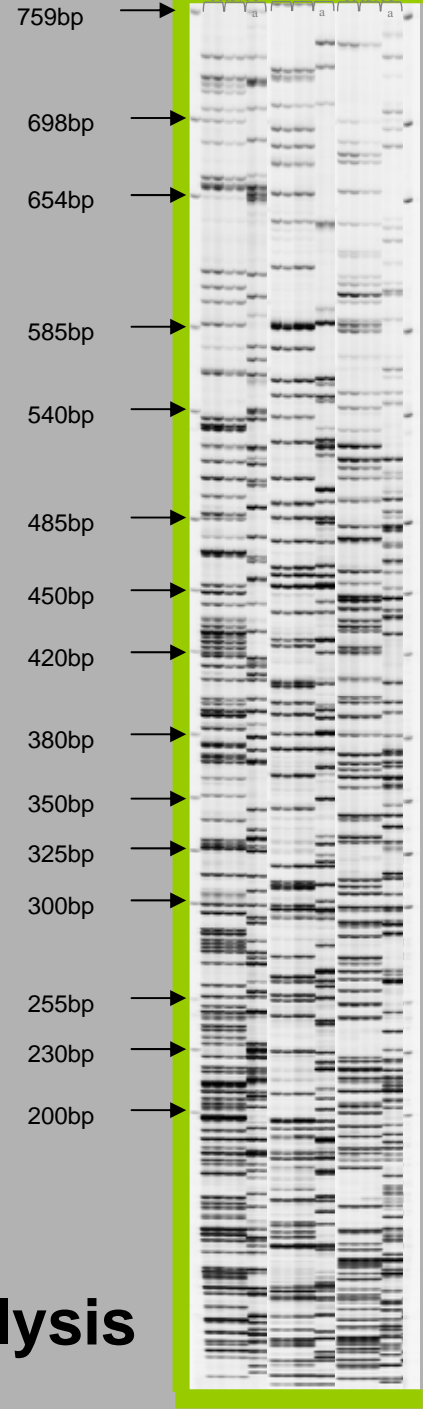
(Cordier et al., *Journal of Microbiological Methods*, 2006)

Identity of the μ organism at the strain level

T. atroviride strains



Specificity of the SCAR marker



AFLP analysis



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1/ Identity of the μ organism

- At the strain level: is that always necessary?
 - Baculoviruses viruses should be included at the family level : Rebeca proposal
 - *Bacillus thurengiensis*

Problems with the contaminants that might be at a high density and which are not characterized



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2/ Physical and chemical properties of the active substance

- Problem with botanical which are always mixtures of molecules
 - For natural products the proposal made by REBECA is very pertinent
 - We should develop methods enabling to ensure the constant composition of a mixture without characterizing each component



2/ Biological properties of the microorganisms

- **2.2.2 modes of action :**

- there are always several modes of action
- the modes of action described in vitro are not necessary the same as in nature
- when secondary metabolites are produced, their concentration is always very low (example of DDR)

we should admit that it is not possible to get a full knowledge of the modes of action



2.2.2 modes of action of *Trichoderma* spp

- Competition for nutrient, for space
- Hyperparasitism
- Antibiosis: production of many different types of secondary metabolites:
 - Cell wall degrading enzymes
 - Chitinases
 - Peptaboil
 - Anti-fungal substances, antibiotics
- Production of metabolites varies according to the plant and the target pathogen



3/ Further information on the active substance

- **3.1/Function:** useful to know the function, but is it needed to assess the risks? and what about the uses not yet recognized such as plant strenghteners?
- **3.5/Modes of action:** useful to know the modes of action , but we must admit that we do not know everything especially in relation to **the modes of action of natural substances** (elicitation of plant defense mechanisms)
 - *Don't ask question when science is not able to propose an answer*



3/ Further information on the μ organism

- 3.4/ method of production and quality control
cf problem of contaminants
- 3.6/ method to prevent loss of virulence :
we do not know how to address the
question of genetic stability but loss of efficacy
does not pose any new risk (see Rebeca
proposal)
- 3.7/ handling, storage etc...
we should remember that we are dealing
with living organisms not with chemical



5/ Toxicological and metabolism studies / effects on human health

- Everybody will agree that we have to protect man, but...
 - Are all the studies needed? Anytime?
 - Are the methods adapted to
 - Botanicals?
 - μ organisms ?



5/ Toxicological and metabolism studies / effects on human health

Methods designed for synthetic chemicals are not necessarily adapted to the study of natural substances and μ organisms (sensitisation genotoxicity)

The main route of exposure should be considered to chose the method of application (oral or...)

Is it necessary to follow the clearance of the μ organism?



6/ Residues (natural product) in or on treated products, food and feed

- Natural products differ from synthetic ppp
- They have always been present in natural environment
- Most of them are rapidly metabolized in the environment

thus this point has to be considered with the next one: fate in the environment



6/ Residues (microorganism) in or on treated products, food and feed

- Usually the residue is the microorganism itself

thus this point has to be considered with the next one: fate in the environment



7/ fate and behavior in the environment

- The fear is of an uncontrolled multiplication of the microbial BCA in the environment
- **Do we have examples justifying that fear?**
- The only example is the myxoma virus released in Australia to control rabbit .

Do we have examples justifying that fear?
In my opinion, this fear is not justified and we should ask for revised requirements



7/ Fate and behavior in the environment

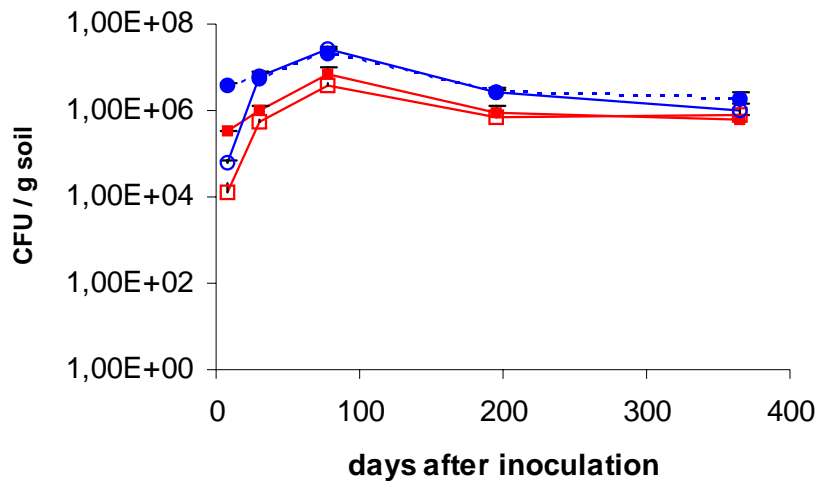
- If the microbial BCA (a native organism) is re-introduced in the environment from which it has been isolated
 - it will survive
 - it will not become dominant without a specific selection pressure
 - It will not durably modify the natural balance

We have good demonstration of these assessments for soil microorganisms

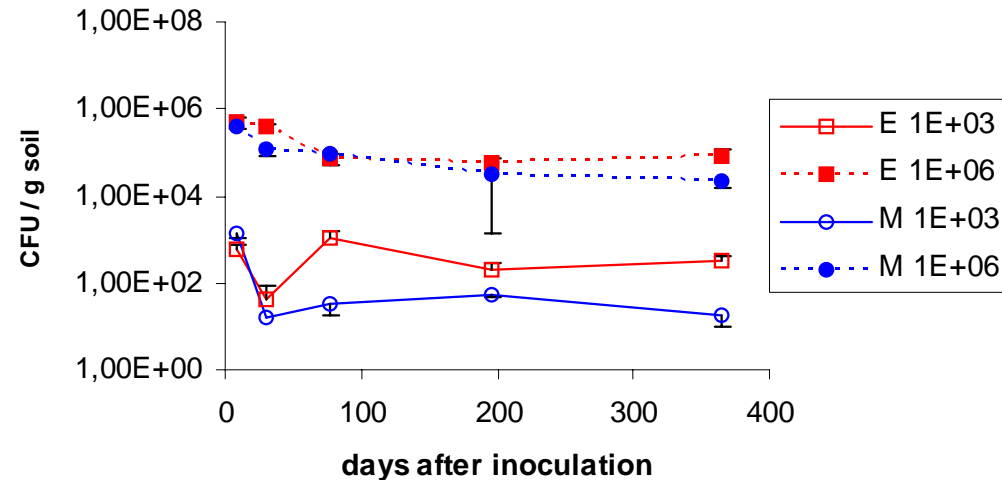


7/ Fate and behavior in the environment

Sterilized soils



Raw soils



Population kinetics of a fungal bca (Fo47) in two soils



8/ Effects on non target organisms

- μ organisms are usually highly host-specific:
why should we study their effects on non target organisms such as birds, fish, bees when host specificity has been proven?
- The tests proposed for chemical products are often not adapted to μ organisms (effects on aquatic organisms)



8/ Effects on non target organisms

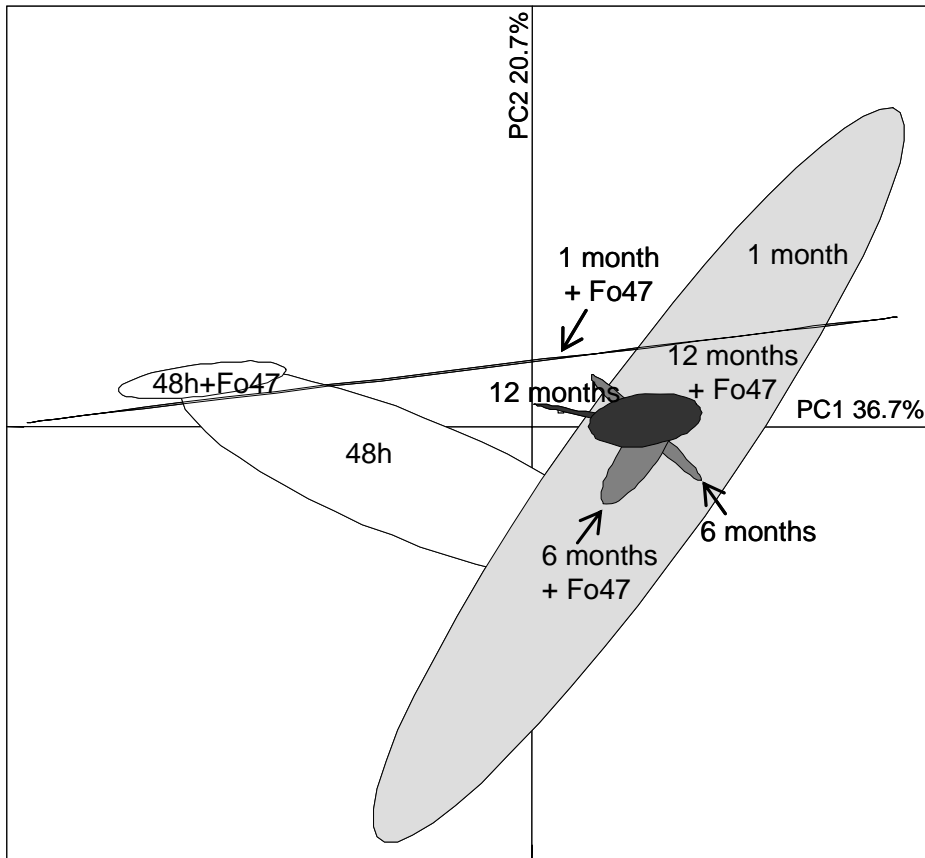
- Regarding effects on the soil microbial balance, functions are more important than species numbers and microbial density. The directive insists on N and C cycles, but addition of a single population of BCA will not affect these cycles.
- **New methods based on the characterization of physiological and molecular profiles of soil communities give a better view of the changes affecting the whole soil communities**



8/ Effects on non target organisms

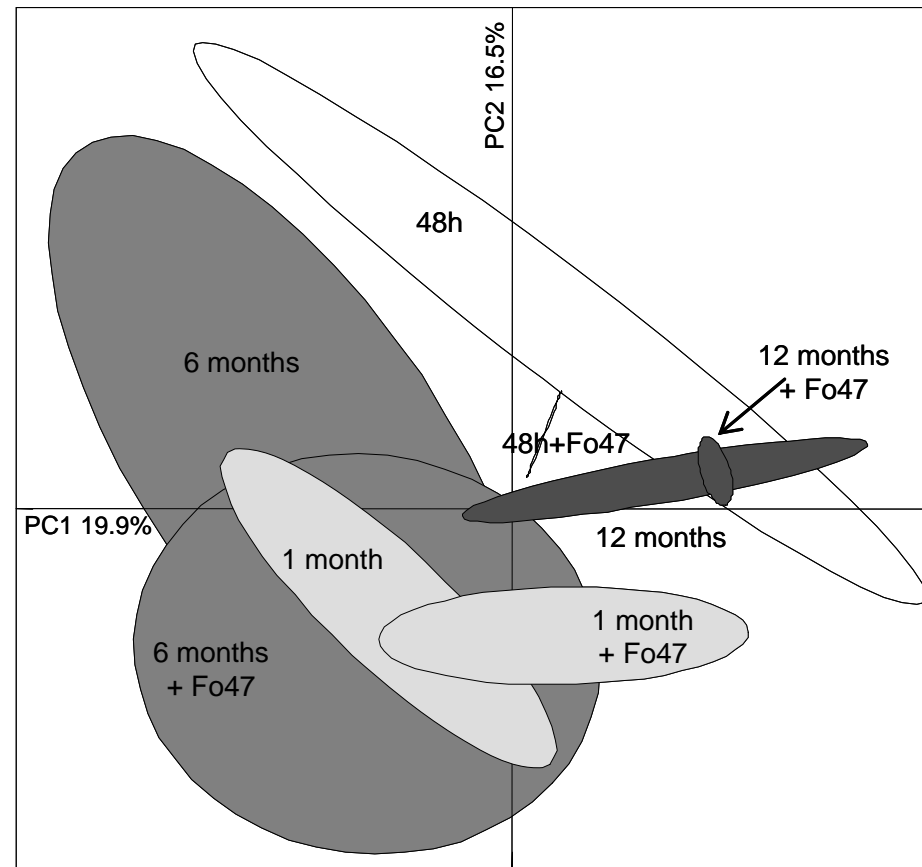
Soil of Epoisses

Fungi

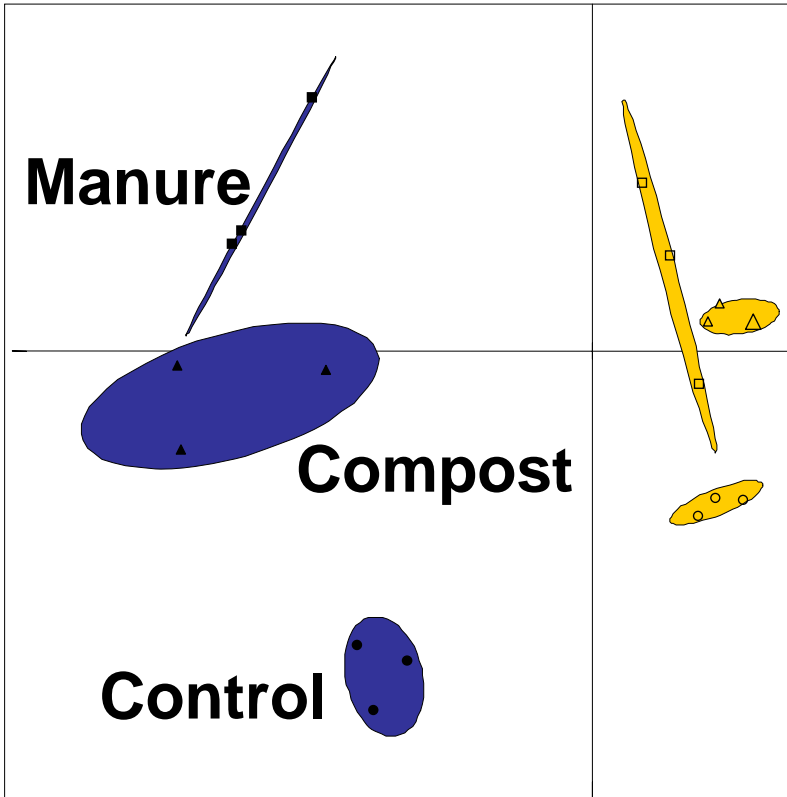


Soil of Epoisses

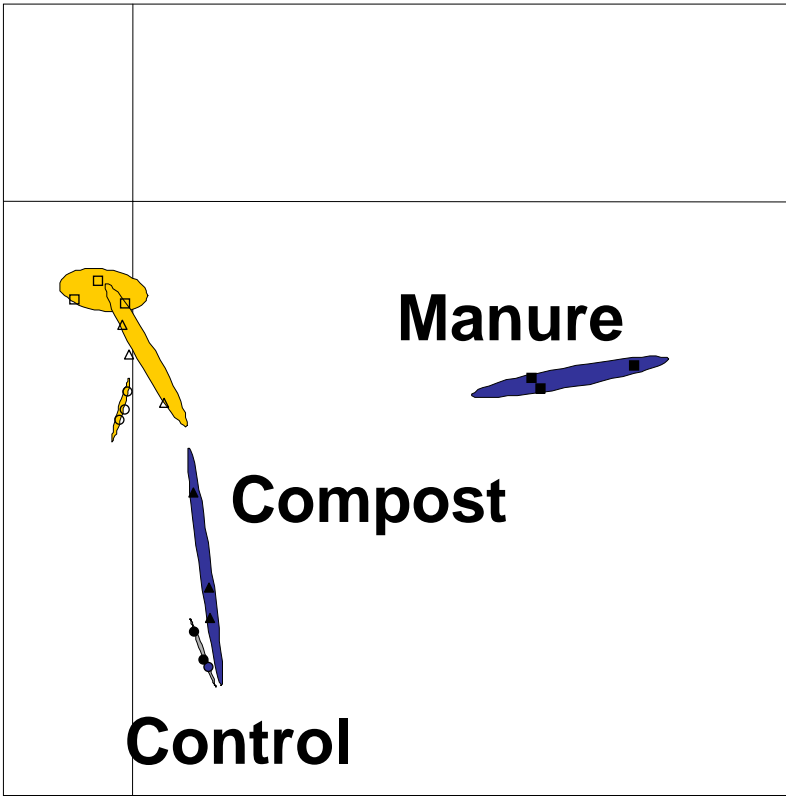
Bacteria



8/ Effects on non target organisms of cultural practices



Bacterial communities



Fungal communities



8/ Effects on non target organisms

- Regarding the microbial balance, some natural phenomena are more important than introduction of BCAs. For example the seasonal effect is very important, and often neglected. If we take it in consideration, we will get a more realistic view of the effects on non target organisms .



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Conclusion

- A large diversity of organisms, and natural substances can be used as alternatives to chemical control
- Their use needs to be regulated but with specific requirements adapted to each type of organisms /natural products
- A cost/benefit approach should be used to assess the risks



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Conclusion

- We should remember that a lot of cultural practices, which are not regulated, present risks (manure amendments, soil tillage...)
- We should admit that we do not have the tools to address some questions
- It is a loss of time and money to ask for studies when the methods are not adapted



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Based on this analysis of regulation versus risk assessment, REBECA is making several proposals to simplify registration without decreasing the level of safety

But whatever the regulation, experts should be open minded and accept waivers when the benefit of a study does not balance the cost of the study

Thank you