Biological control agents (BCAs) and the pollinator *Bombus terrestris*

Veerle Mommaerts, Jana Boulet, Guido Sterk* & Guy Smagghe

Bee Research Group, Free University of Brussels, Brussels, Belgium

* Biobest NV, Westerlo, Belgium

Vrije Universiteit Brussel

biobest BIOLOGICAL SYSTEMS

Overview

- **Introduction**
- **Materials and Methods**
- **Results**
- **Conclusion**
- **Future perspectives**

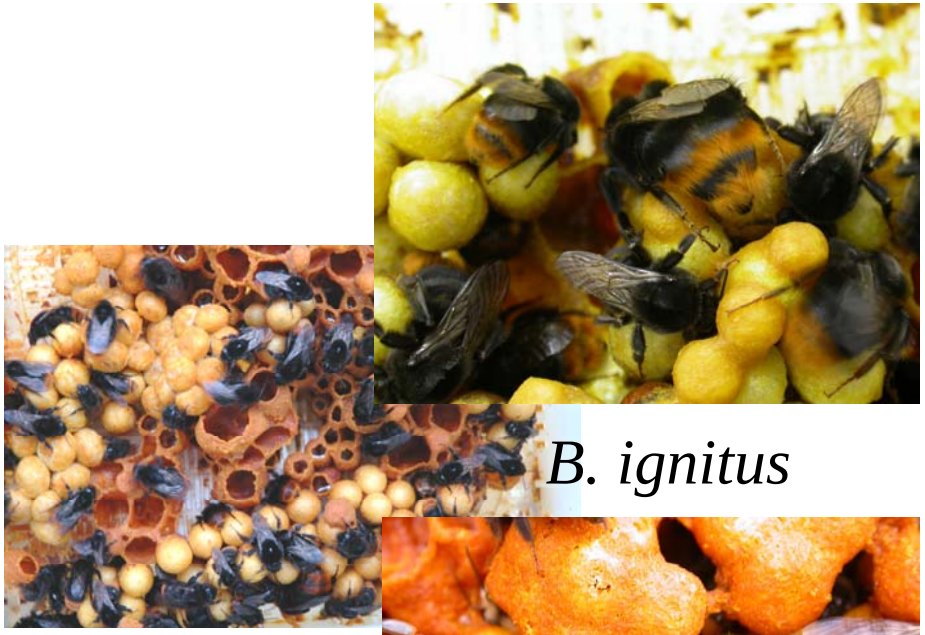
Introduction

1. Bumblebees

Order Hymenoptera

B. terrestris dalmatinus

B. impatiens



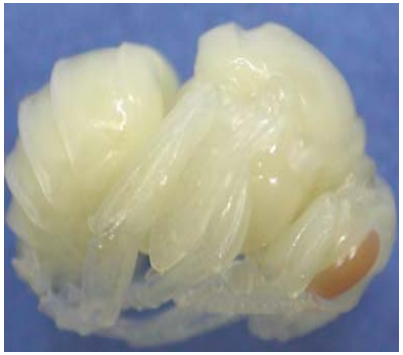
B. ignitus

B. terrestris canariensis

B. occidentalis

2. Biology of bumblebees

Life cycle

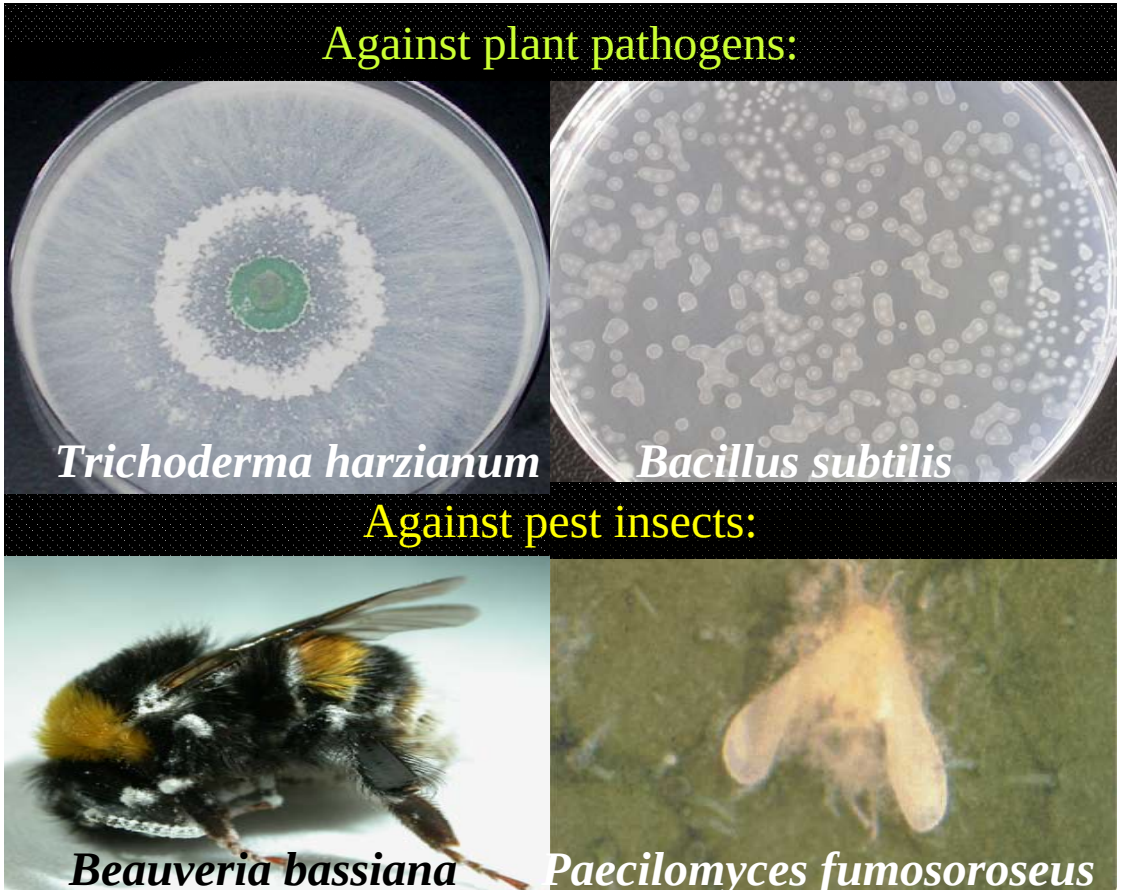


The hive

3. Bumblebees for pollination

4. BCAs

- Microbiologicals:
  - Fungi
  - Bacteria
  - Yeast
  - Viruses



What is their potential risk against pollinators?

↓

Limited data

micro**BIOLOGICAL** ≠ **SAFE**

Product name	Antagonist	Mode of action
BINAB products	*Trichoderma harzianum* *Trichoderma polysporum*	Competition for nutrients and space + production of antibiotics, induced resistance, inhibition of degrading enzymes + mycoparasitism
Trianum	*Trichoderma harzianum* strain T-22	Competition for nutrients and space + production of antibiotics
Prestop Mix	*Gliocladium catenulatum J1446*	(Indirect) mycoparasitism + competition for nutrients and space
AQ10	*Ampelomyces quisqualis*	hyperparasitism
Serenade Rhapsody	*Bacillus subtilis QST 713*	Competition for nutrients and space + production of antibiotics
Xentari Dipel	*Bacillus thuringiensis aizaiwa* *Bacillus thuringiensis kurstaki*	Destruction/lysis of insect midgut epithelium
Botanigard	*Beauveria bassiana GHA*	Entomopathogen, hyphae are able to grow on the insect and will penetrate its cuticle

Product name	Antagonist	Mode of action
Naturalis L	*Beauveria bassiana* ATCC 74040	Entomopathogen against spider mites, …
PreFeRal	*Paecilomyces fumosoroseus strain Apopka 97*	Entomopathogen against whiteflies
Mycotal	*Verticillium lecanii*	Entomopathogen against whiteflies
Contans	*Coniothurium minitans*	Biological fungicide against Sclerotinia
BioAct	*Paecilomyces lilacinus*	Nematicide
Bionext	*Candida sake* and *Pichia anomala*	Competition for space
Granupom	*Cydia pomonella* granulosis virus	Able to multiply in the intestinal tract

Goal of the study

Bombus terrestris workers

- laboratory trials
- climate room
- not fertilised (haploid)

Effects of BCAs

- **Toxicity**
- **Reproduction and development**
- **Behaviour**

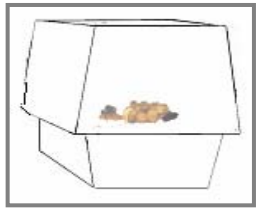
Materials and Methods

1. Tested BCA's

Class	Product	Species/ strain	Formulation	MFRC
Biological fungicides	Binab T-vector	*Trichoderma harzianum* ATCC 20476	WP (10^6 CFU/g)	1.25g/l
	Binab TF WP	AND	WP (10^5 CFU/g)	1.25g/l
	Binab TF WP Konc	*Trichoderma polysporum* ATCC 20475	WP (10^6 CFU/g)	1.25g/l
	Trianum	*Trichoderma harzianum* T- 22	WP (10^9 CFU/g)	0.6g/l
Biological insecticides	Botanigard	*Beauveria bassiana* GHA	ES (2×10^{10} CFU/ml)	1.25ml/l
	Naturalis	*Beauveria bassiana* ATCC 74040	ES (2.3×10^7 CFU/ml)	1.5ml/l
	PreFeRal	*Paecilomyces fumosoroseus* APOPKA 97	WG (10^6 CFU/g)	1g/l

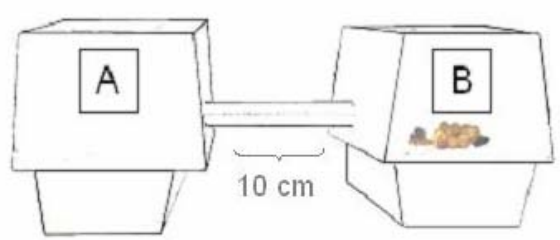
2. Toxicity test

2.1. Single design



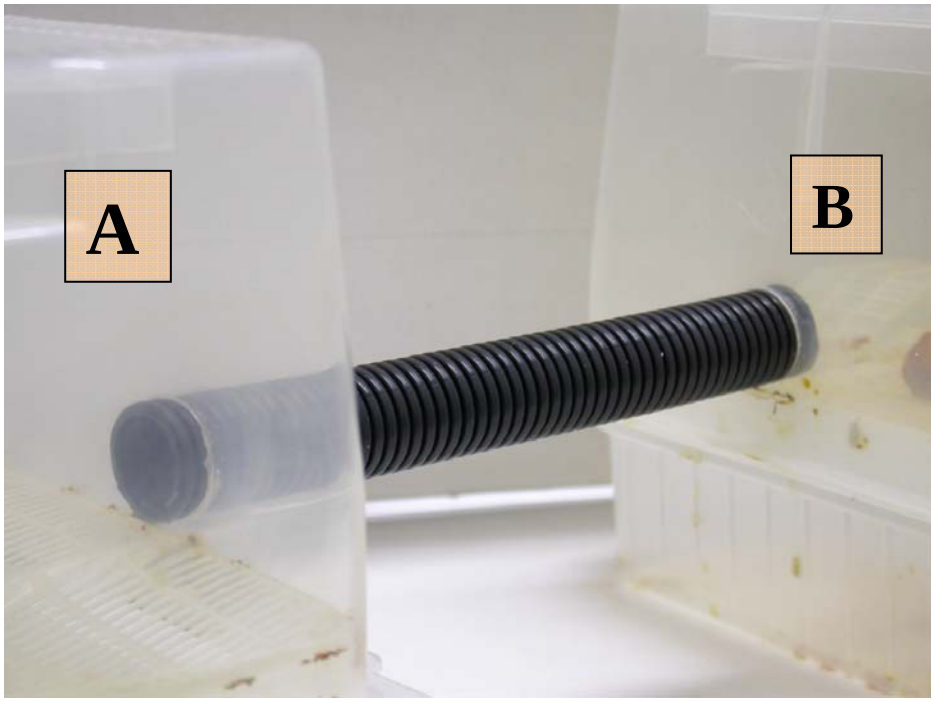
3. Behaviour tests

3.1. Double design



A

food compartment

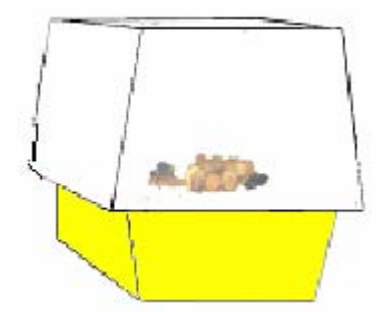


B

nest compartment

Artificial nests in the lab

Day 1



28°C and 60% RV

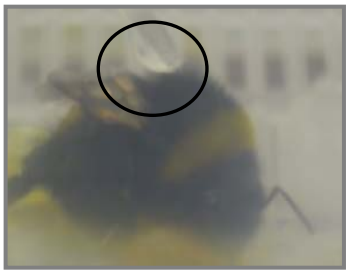
4 nests/product

5 workers of the same age per nest

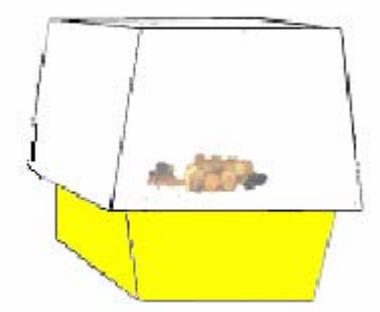
(Mommaerts et al., 2006)

Artificial nests in the lab

Day 1



✓ 50 µl test solution
✓ micropipet



After 1 week

✓ Treatment of pollen

5 workers

28°C and 60% RH

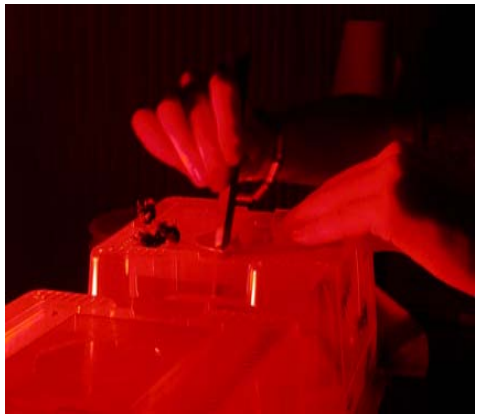
4 nests/product

✓ Sugar water treatment

(Mommaerts et al., 2006)

Artificial nests in the lab

ENDPOINTS



During 11 weeks →

-Survival of workers

-Production of drones

-Dead larvae

Evaluation of the worker mortality at MFRC

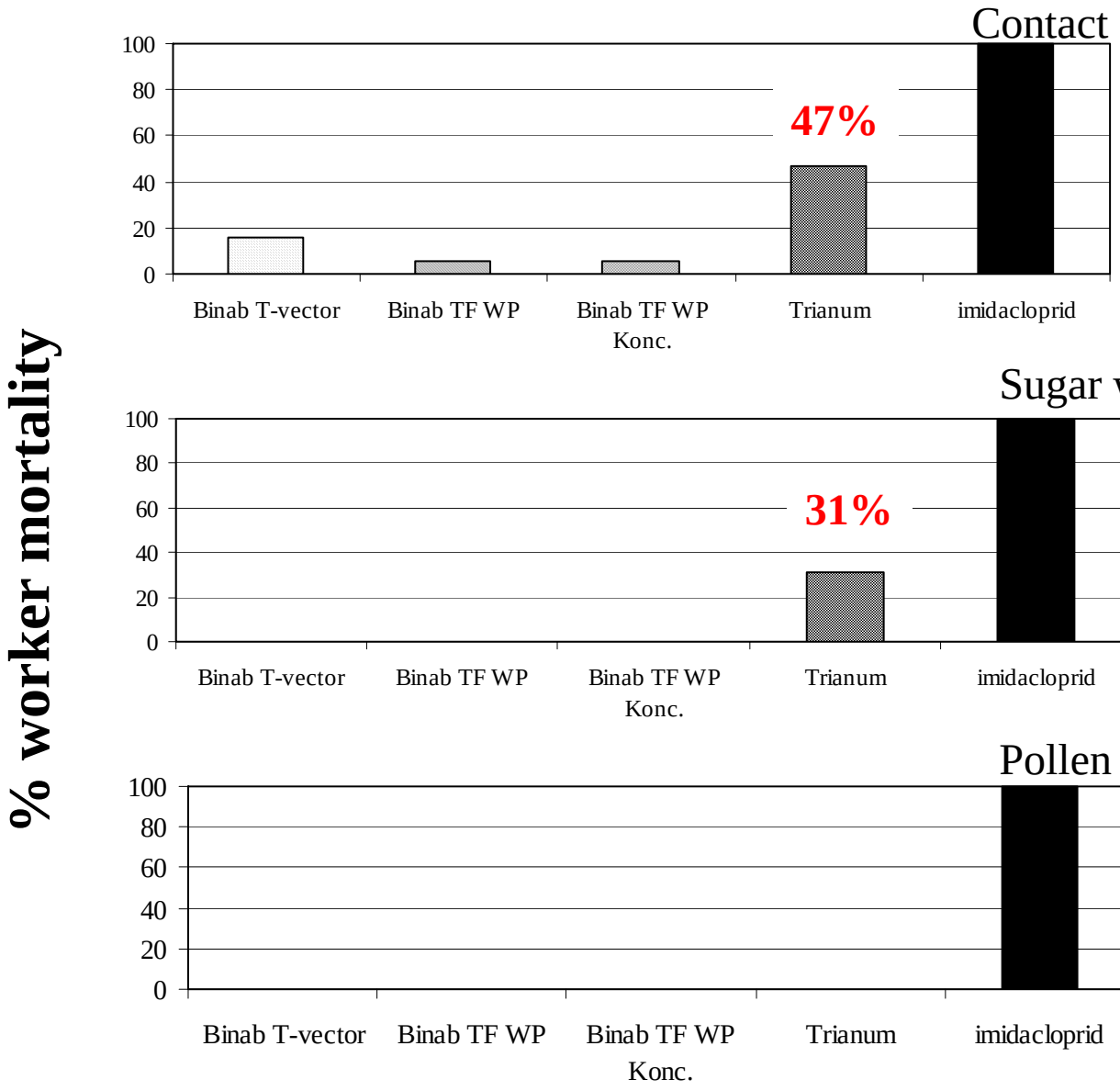
→ IOBC-classes

Toxicity	% mortality
Not toxic	< 25%
Weakly toxic	25 - 50%
Moderately toxic	51 – 75%
Highly toxic	>75%

(Mommaerts et al., 2006)

Results

1. Biofungicides MFRC: mortality



1. Binab products

ACUTE TOXICITY

2. Trianum

Contact

Sugar water

ACUTE TOXICITY

= WEAKLY

1.1. Biofungicides MFRC: reproduction

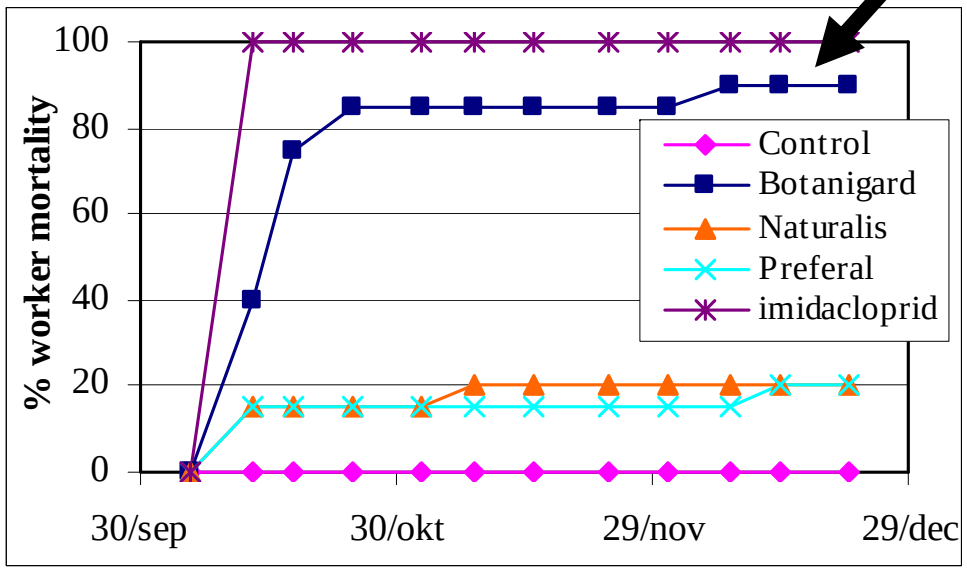
Detrimental effects on reproduction

Analysis via χ^2 gives significance compared to the control (*0,01<p<0,05; **0,001<p<0,01; ***p<0,001).

2. Biological insecticides: contact

Worker mortality

90% acute toxicity



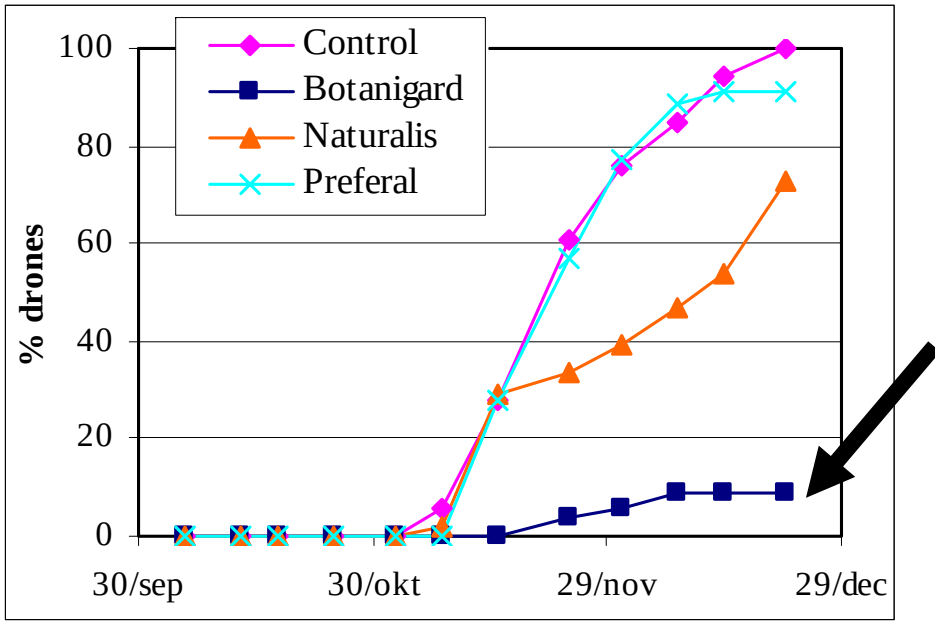
Botanigard ↔ Naturalis

≠ Formulation and CFU/ml

≠ Strain

⇨ Further research is necessary

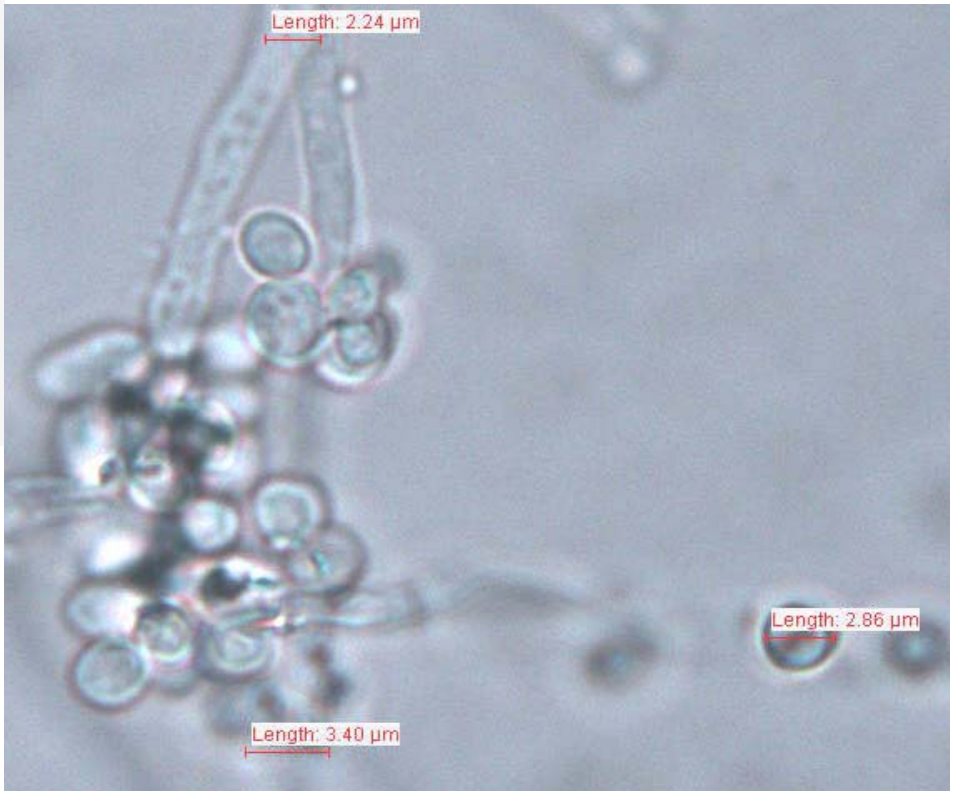
Reproduction



Botanigard

B. bassiana (Botanigard) grew on the adult body of a worker (topical exposure @ MFRC)

B. bassiana (under the light microscope)



Toxicity tests: summary

		Worker mortality (%)			Effect on reproduction (%)		
Class	**Product**	**Contact**	**Sugar water**	**Pollen**	**Contact**	**Sugar water**	**Pollen**
Biological fungicide	Binab T-vector	16	0	0	0	0	0
	Bianb TF WP	5,3	0	0	22	0	0
	Binab TF WP Konc	5,3	0	0	0	0	0
	Trianum	47	31	0	72	44	45
Biological insecticide	Botanigard	90	29	0	91	23	10
	Naturalis	20	12	6,7	24	0	29
	Preferal	20	31	0	5	41	6

Conclusion BCA's

- **Biological control agent does not automatically mean safe! -> Tests are necessary**
- **We performed risk assessment tests under laboratory conditions = worst case test.**
  - Binab T- vector, Binab TF WP and Binab TF WP Konc are safe for *B. terrestris*
  - Our current tests with Trianum suggest that it can be used with *B. terrestris* but with caution

  => differences between batches
  - Naturalis and Preferal are compatible with *B. terrestris*
  - Botanigard, highly toxic via contact exposure (MFRC)

NEVENEFFECTENGIDS

SIDE EFFECTS MANUAL

MANUEL DES EFFETS SECONDAIRES

biobest N.V.
BIOLOGICAL SYSTEMS

Side-effects list Biobest

ACTIVE INGREDIENT ACTIEVE STOF MATIERE ACTIVE		BUMBLEBEES HOMMELS BOURDONS		Predatory mites Roofmijten Acariens predateurs										Predatory insects Roofinsecten Insectes predateurs								
				Amblyseius californicus		Amblyseius cucumeris		Amblyseius degenerans		Phytoseiulus persimilis		Hypoaspis Hypoaspis miles aculeifer		Aphidoletes Therodiplosis		aphidimyza persicae	Chrysopa carnea			Coccinellidae (1)		
INSECTICIDES / ACARACIDES	Application/Toepassing	kolonie/colony	persist. (5)	nymph(e)/adult(e)	persist.	nymph(e)/adult(e)	persist.	nymph(e)/adult(e)	persist.	nymph(e)/adult(e)	persist.	nymph(e)/adult(e)	persist.	larva/larve	adult(e)	persist.	larva/larve	adult(e)	persist.	larva/larve	adult(e)	persist.
abamectin	s	😐	24 h	4	5 d	2	5 d	4	1 w	4	1 w	2	5 d	4	4	1 w	1	4	1 w	1	3	1w
acephate	s	☹	-	4	>2 w	4	>8 w	4	>8 w	4	1 w	-	-	2	4	>8 w	4	4	>6 w	-	4	>6w
acetamiprid	s	😐	48 h	3	5 d	-	-	-	-	3	1 w	-	-	4	-	-	-	-	-	3	3	-
acetamiprid	i	🙂	-	1	-	1	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-
acrinathrin	s	😐	72 h	4	-	4	-	4	-	4	-	-	-	4	-	-	-	1	-	4	4	-
Adoxophyes orana Granulose Virus	s	🙂	-	1	-	1	-	1	-	1	-	1	-	1	-	-	1	1	-	1	1	-
aldicarb	s	☹	-	4	-	4	>8 w	4	>8 w	4	>8 w	4	-	-	4	>8 w	-	-	-	-	4	-
alphacypermethrin	s	☹	-	-	-	4	>8 w	4	>8 w	4	>8 w	3	-	4	4	>8 w	4	4	-	4	4	-
amitraz	s	🙂	-	4	2 w	4	2 w	4	2 w	4	3 w	2	-	3	-	-	1	-	-	-	2	-
amitraz	st	-	-	-	-	4	>2 w	4	>2 w	4	>2 w	2	-	-	-	-	-	1	-	-	1	-
azadirachtin	s	🙂	-	1	-	1	-	1	-	2	-	1	-	-	1	-	-	1	-	-	-	-
azinfos-methyl	s	☹	-	2	2 w	4	>8 w	4	>8 w	3	2 w	2	-	3	4	>8 w	3	4	>6 w	-	4	>6w
azocyclotin	s	😐	36 h	2	-	4	-	4	-	3	3 d	2	-	-	-	-	4	2	-	3	4	-
Bacillus thuringiensis var. aizawai	s	🙂	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus thuringiensis var. israelensis	s	🙂	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus thuringiensis var. kurstaki	s	🙂	-	1	-	1	-	1	-	1	-	-	-	1	1	-	1	1	-	-	1	-
Bacillus thuringiensis var. kurstaki	d	🙂	-	-	-	2	-	2	-	2	-	-	-	1	-	-	1	-	-	-	1	-
Bacillus thuringiensis var. tenebrionis	s	🙂	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	1	-
bendiocarb	s	☹	-	-	-	4	3 w	4	3 w	4	3 w	4	6 w	-	4	3 w	-	-	-	-	-	-
benzoximate	s	🙂	-	1	2 w	1	-	1	-	2	1 w	2	-	-	-	-	1	1	-	-	-	-
bioresmethrin	s	😐	48 h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
biphentrin	s	☹	-	4	-	4	>8 w	4	>8 w	4	>8 w	4	>8 w	4	4	>8 w	4	4	>8 w	4	4	>8w
bromophos	s	☹	-	-	-	-	-	-	-	4	>3 w	2	-	2	4	-	3	4	-	3	1	-

Side-effects list Biobest

									Parasitoïds			Sluipwespen						Insectes parasites											ACTIVE INGREDIENT AKTIEVE STOF MATIERE ACTIVE
Macrolophus caliginosus			Orius insidiosus Orius laevigatus			Anthocoris nemoralis			Aphidius spp. (2)			Dacnusa sibirica Diglyphus isaea			Encarsia formosa			Eretmocerus spp.			Trichogramma spp. persicae			Nematodes (3)		Paecilomyces fumosoroseus			
nymph(e)	adult(e)	persist.	nymph(e)	adult(e)	persist.	nymph(e)	adult(e)	persist.	larva/larve	adult(e)	persist.	larva/larve	adult(e)	persist.	larva/larve	adult(e)	persist.	larva/larve	adult(e)	persist.	larva/larve	adult(e)	persist.	nematode	persist.	spore	persist.	Application/Toepassing	**INSECTICIDES / ACARICIDES**
2	4	1w	3	3	1w	4	4	1w	-	4	1w	-	4	1w	1	3	5d	1	4	5 d	2	4	1 w	1	1 w	1	-	s	abamectin
4	4	>6w	4	4	-	-	-	-	-	4	-	-	4	-	4	4	>8w	4	4	>6 w	2	4	>4 w	2	3 d	3	-	s	acephate
4	-	>2w	4	-	2w	3	-	1w	1	-	-	-	-	-	3	4	2w	-	-	-	-	-	-	-	-	-	-	s	acetamiprid
4	-	-	2	-	1w	3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	i	acetamiprid
4	4	-	4	4	-		-	-	2	4	-	-	4	-	4	4	-	4	4	-	4	4	-	-	-	-	-	s	acrinathrin
1	1	-	1	1	-	1	1	-	1	1	-	1	1	-	1	1	-	1	1	-	1	1	-	1	-	-	-	s	Adoxophyes orana Granulose Virus
-	-	-	-	-	-	-	-	-	-	4	-	-	4	-	4	4	>8w	-	-	-	-	-	-	4	-	-	-	s	aldicarb
4	4	-	4	4	-	4	4	-	-	4	-	-	4	-	4	4	>8w	-	4	-	-	4	-	-	-	-	-	s	alphacypermethrin
-	-	-	3	2	1w	4	1	1w	-	1	-	1	1	-	4	4	3w	2	2	2 w	2	4	>4 w	1	-	3	-	s	amitraz
3	3	-	-	-	-	-	-	-	-	-	-	-	1	-	4	4	>2w	-	-	-	-	-	-	-	-	1	-	st	amitraz
2	2	-	2	1	-	-	-	-	1	1	-	1	1	-	1	2	-	-	-	-	-	-	-	1	-	-	-	s	azadirachtin
-	-	-	3	3	-	-	-	-	-	4	-	-	4	-	4	4	>8w	-	3	-	2	4	>4 w	1	-	4	-	s	azinfos-methyl
-	-	-	-	2	-	-	-	-	-	-	-	-	4	-	-	3	-	-	-	-	1	4	>2 w	-	-	-	-	s	azocyclotin
-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s	Bacillus thuringiensis var. aizawai
-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s	Bacillus thuringiensis var. israelensis
1	1	-	1	1	-	1	1	-	1	1		1	1	-	1	1	-	1	1	-	1	1	-	1	-	-	-	s	Bacillus thuringiensis var. kurstaki
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	d	Bacillus thuringiensis var. kurstaki
1	1	-	1	1	-	1	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	-	1	-	-	-	s	Bacillus thuringiensis var. tenebrionis
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	3w	-	-	-	-	-	-	4	1 w	-	-	s	bendiocarb
-	-	-	-	1	-	-	-	-	-	-	-	-	3	-	1	3	1w	-	-	-	-	2	-	-	-	-	-	s	benzoximate
2	2	1w	-	-	-	-	-	-	-	-	-	-	-	-	1	4	1w	1	4	1 w	-	-	-	-	-	-	-	s	bioresmethrin
4	4	>8w	4	4	>8w	4	4	-	2	4	>8w	4	4	>8w	4	4	>8w	4	4	>8 w	4	4	>8 w	1	-	-	-	s	biphentrin
-	-	-	4	4	-	-	-	-	4	4	-	-	-	-	-	4	-	-	-	-	4	4	>4 w	2	-	3	-	s	bromophos

CD-ROM

Future perspectives (1)

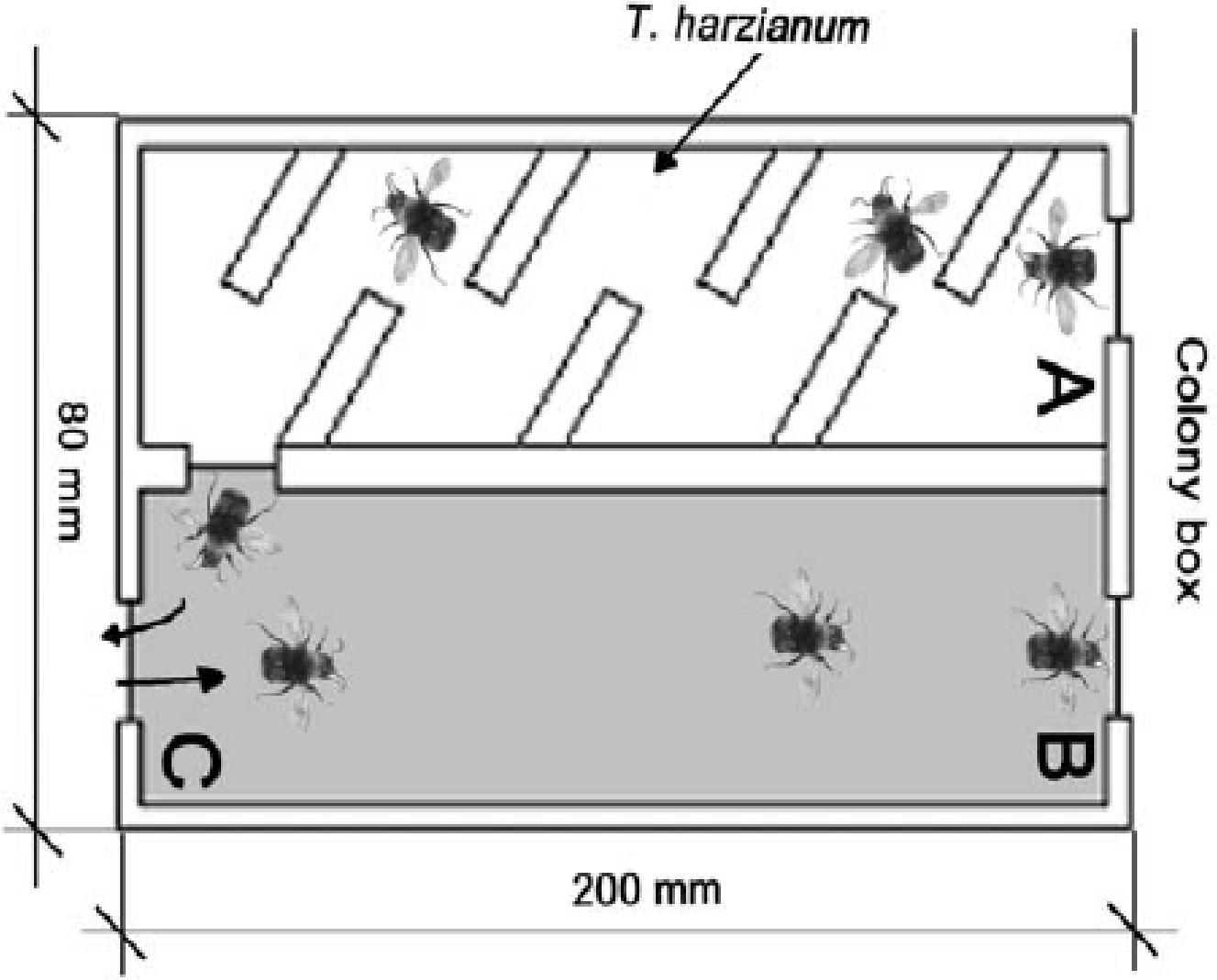
- **Further tests are necessary to test the BCAs under more practical, field-related conditions.**
- **Sublethal effects against fouraging/behaviour → guarantee of pollination**
- **We also envisage to test other strains and species of bumblebees for the compatibility with BCAs**

Future perspectives (2)

- **More new BCAs are under investigation**
- **Compatible BCAs open opportunities to be used with bumblebees as vector = “flying doctors” concept**

Side by side passageway (SSP)

- SSP

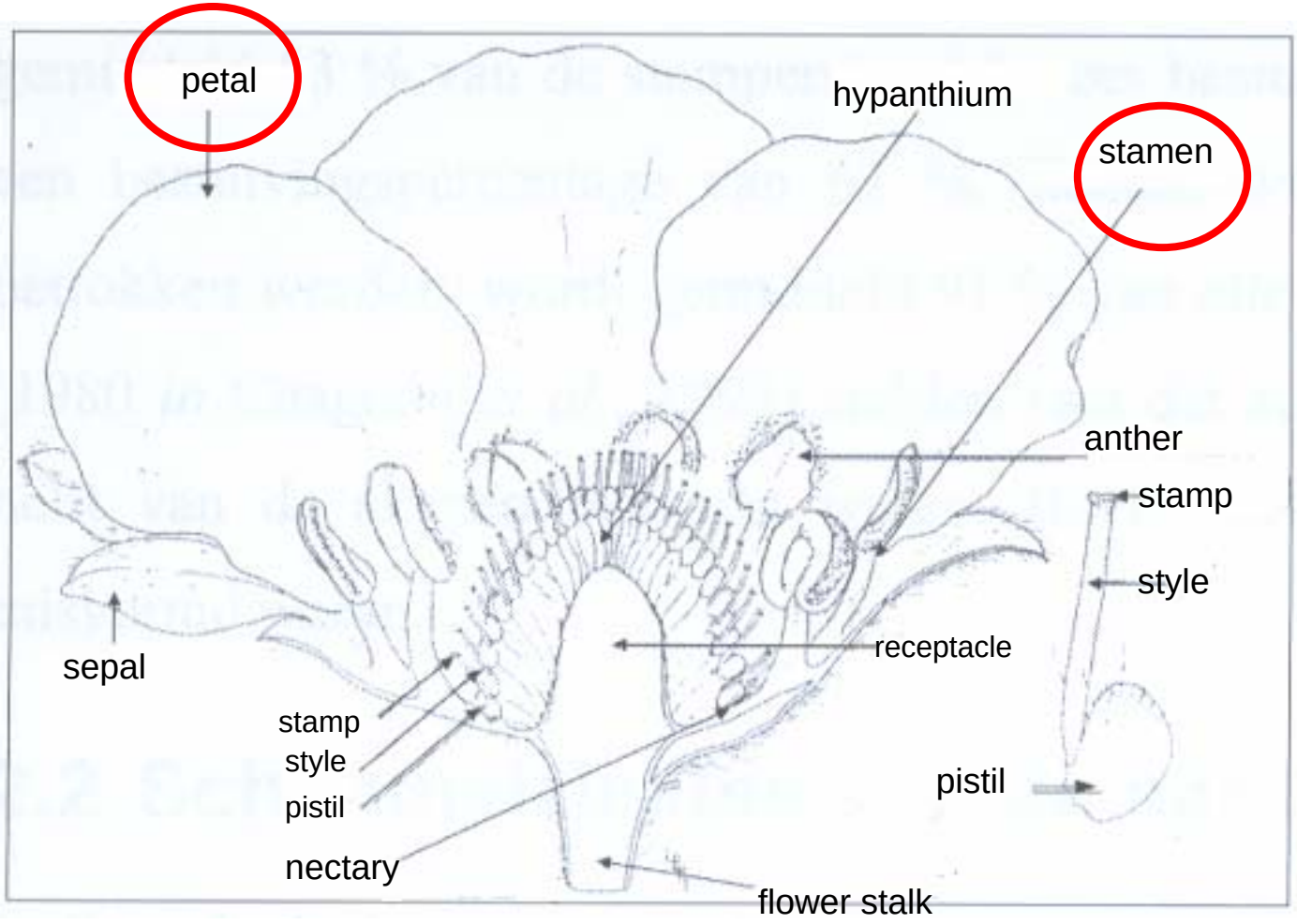


(Yu and Suton, 1997)

- only contact with legs (?)
- entrance = exit

Bumblebees versus spraying

- Target sites are treated



Bumblebees versus spraying

- Less product is necessary

  10^2 spores/flower to control plant pathogens

- Less labour intensive: bumblebees do the work

- Low degree of infection: able to control

  High degree of infection: combination of fungicides and BCAs

⇒ Due to the **lower amount of applications** of fungicides, problems of **resistance** ↓

Thank you for the attention!

Questions?