Laboratory of Molecular Entomology
and Bee Pathology

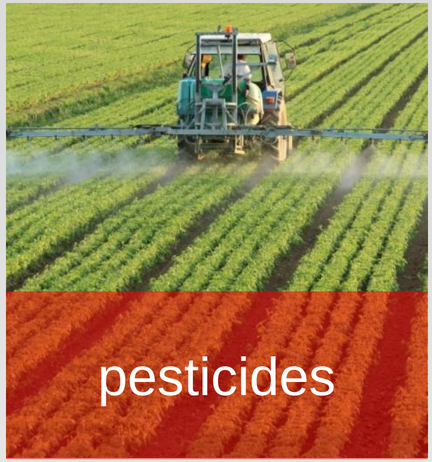
UNIVERSITEIT GENT

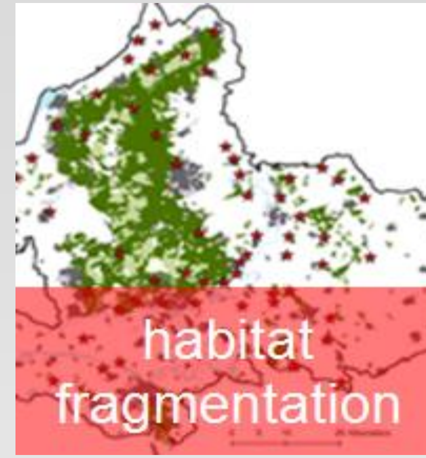
New insights into the honey bee diseases (*health*) as a lever to address the bee mortality

prof. Dirk de Graaf

Colloquium, RBINS, Brussels, 15/5/2017

multiple (environmental) stressors

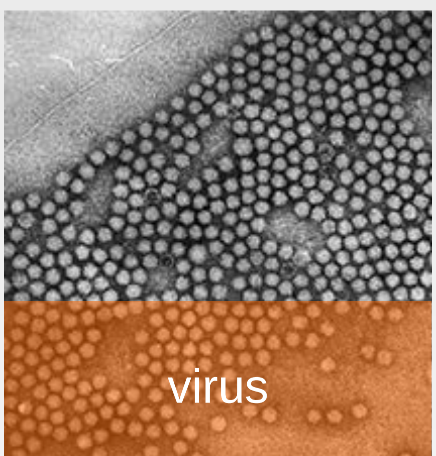


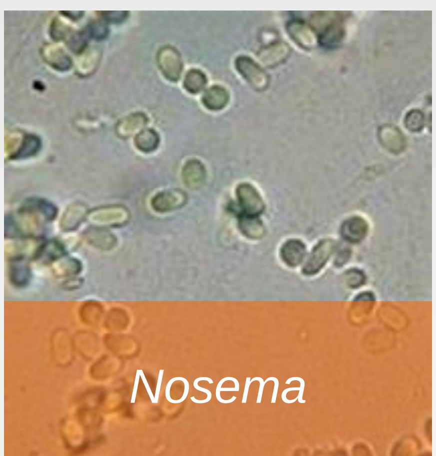


diseases: basic research questions

1. do we know all pathogens?
2. which pathogens (genotypes) do matter?
3. can bees protect themselves against pathogens?
4. what is the (molecular) mechanism behind this?

target pathogens:





Varroa destructor

3. can bees protect themselves against pathogens?

4. what is the (molecular) mechanism behind this?

funding:

VARRESIST

Vlaanderen
verbeelding werkt

EUROPEAN COMMISSION

Vlaams Bijenteelt-programma

Ricola Foundation

Ring-test

SIBELCO

BeeBond

VARRESIST

mechanisms of *Varroa*-tolerance (VT):
- hygienic behaviour
- grooming behaviour
- brood effect

no offspring

normal offspring

VARRESIST

comparison of different European VT-strains

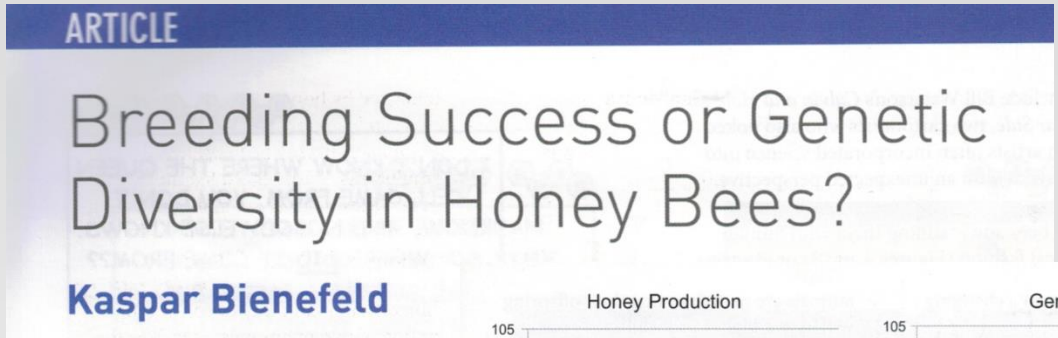
data not shown for reasons of confidentiality

ongoing: finding the responsible genes

>> establishment of VT by 'marker assisted selection'

Vlaams Bijenteeltprogramma

establishment of VT by 'breeding value estimation'


ARTICLE

Breeding Success or Genetic Diversity in Honey Bees?

Kaspar Bienefeld

genetic improvement by:
- motivated mating
(528 queens)
- breeding value estimation
(373 queens)
>> mechanisms: hygienic behaviour & brood effect
- breeding from selected queens

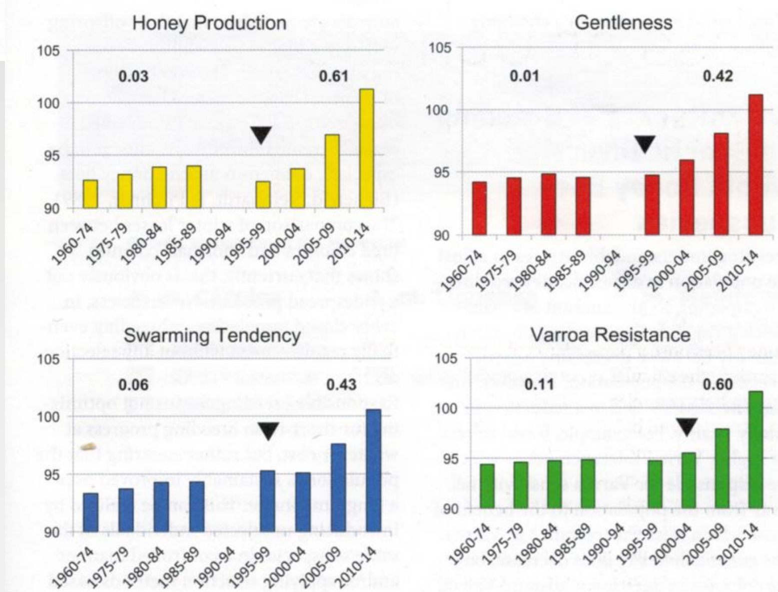


Figure 1. Genetic improvement in four traits, before and after BVE. The columns represent the average over five years. The numbers (regression coefficients) directly above the columns represent the average annual improvement in each trait for the time period before or after the introduction of BVE (black triangle). Due to insufficient data available between 1990 and 1994, the average values for these years are excluded.

BeeBond

establishment of VT by 'natural selection'


Apidologie (2011) 42:533–542
© INRA, DIB-AGIB and Springer Science+Business Media B.V., 2011
DOI: 10.1007/s13592-011-0029-5

Original article

Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation

Barbara Locke, Ingemar Fries

Department of Ecology, Swedish University of Agricultural Sciences, P.O. Box 7044, 750 07 Uppsala, Sweden

Received 7 June 2010 – Revised 7 October 2010 – Accepted 13 October 2010

Abstract – A population of European honey bees (*Apis mellifera*) surviving *Varroa destructor* mite infestation in Sweden for over 10 years without treatment, demonstrate that a balanced host–parasite relationship may evolve over time. Colony-level adaptive traits linked to *Varroa* tolerance were investigated in this population to identify possible characteristics that may be responsible for colony survival in spite of mite infestations. Brood removal rate, adult grooming rate, and the mite distribution between brood and adults were not significantly different in the untreated population compared with treated control colonies. However, colony size and the reproductive success of the mite were significantly reduced in surviving colonies compared with control colonies. Our data suggest that colony-level adaptive traits may limit mite population growth by reducing mite reproduction opportunities and also by suppressing the mite reproductive success.

***Varroa destructor* / *Apis mellifera* / natural selection / tolerance / host–parasite interaction**

1. INTRODUCTION

Host–parasite interactions in social insects are intricate with two different levels in which social insects can defend themselves against parasites: (1) by innate individual-level immune responses and (2) by adaptive colony-level defence mechanisms. At the individual level, the immune system of the European honey bee, *Apis mellifera*, is not well-developed compared with other insects (Evans et al. 2006), and rather, they rely heavily on colony-level adaptive mechanisms for defence. The parasitic *Varroa destructor* mite has become a major threat to apiculture with European races of *A. mellifera* throughout most of the world in contrast to the African honey bee race *A. mellifera scutellata* and the Africanized bees in South America (Rosenkranz et al. 2010).

By feeding on the hemolymph of adult bees (during their phoretic phase) and developing bees (during their reproductive phase), the mite vectors naturally occurring otherwise latent viruses which can develop into severe overt infections and potentially lead to colony mortality (Allen and Ball 1996; Nordström et al. 1999; Martin 2001; Sumpter and Martin 2004).

Mite control methods, which are used in apiculture to limit the mite population and avoid colony losses, can be problematic for several reasons. Chemical residues can build up in hive products (Bogdanov et al. 1998; Wallner 1999); mites can develop resistance to effective acaricides (Sammataro et al. 2005); some methods cause damage to bees (Imdorf et al. 1990, 1999; Charrièr and Imdorf 2002), but most importantly, they remove the selective pressures on the mites and the host that may otherwise produce a stable host–parasite relationship through co-adaptive evolution (Fries and Camazine 2001).

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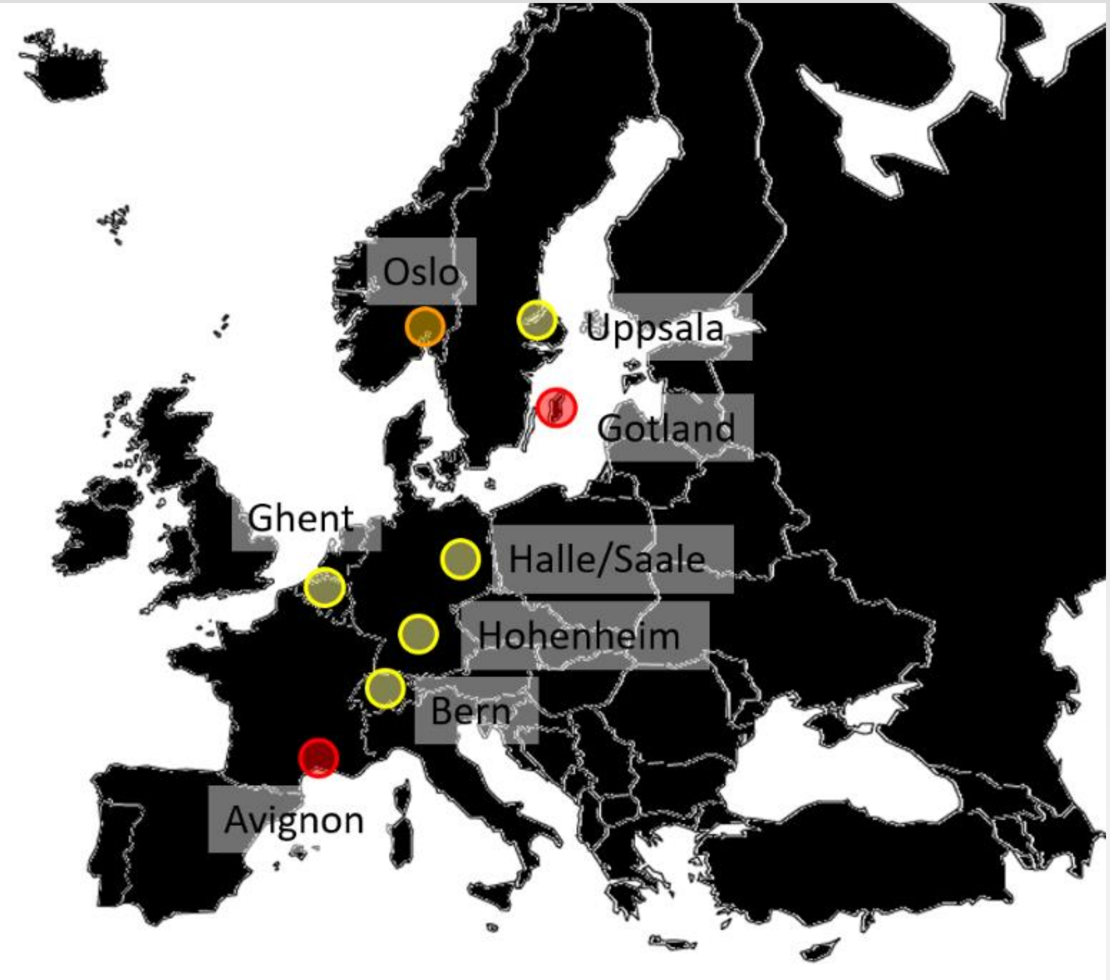
barbara.locke@ekol.slu.se
Manuscript editor: David Tarpy

INRA DIB Springer

treetrunk hive

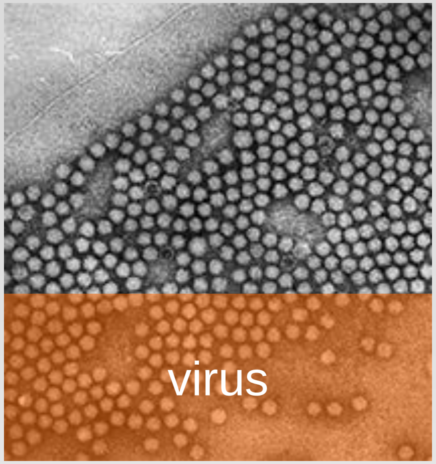
Ring-test

what is the relative impartance of genetics versus environment?



setup at the apiary

virus



1. do we know all pathogens?

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4. what is the (molecular) mechanism behind this?

funding:

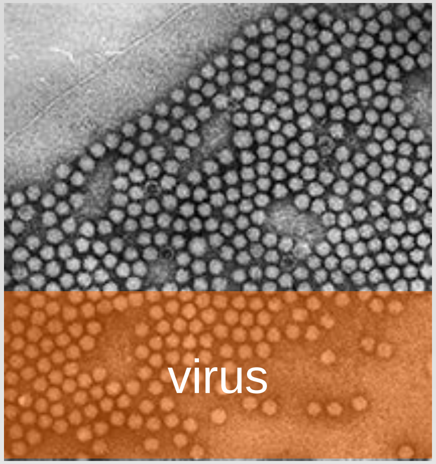
Deformed Wing Virus-project & Honeybee viruses in *Bombus*

ViroBee

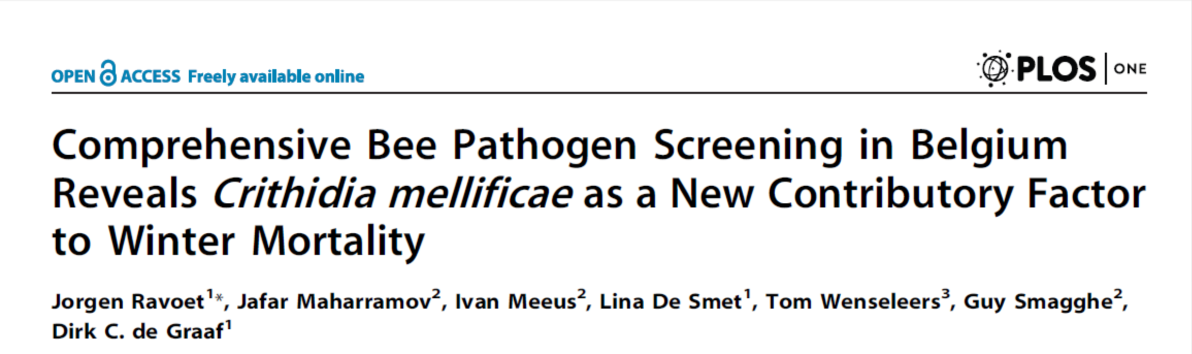
BELBEES

Vlaams Bijenteelt-programma

Deformed Wing Virus-project



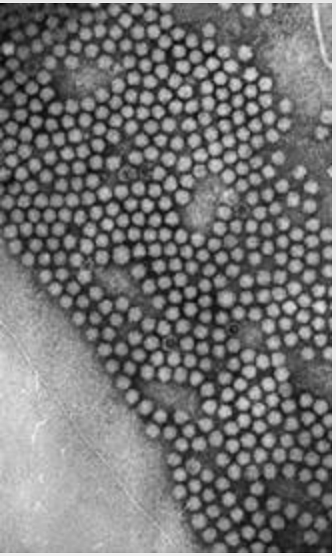
expanding the known honeybee viruses (and more)



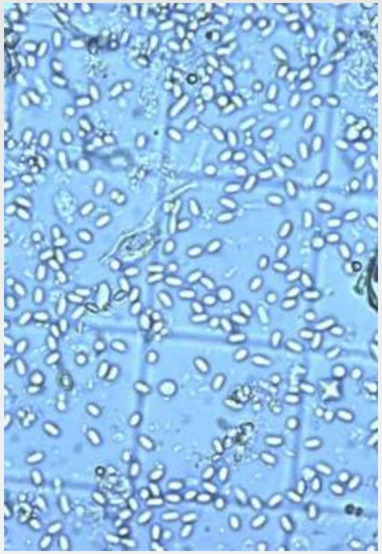
OPEN ACCESS Freely available online PLOS | ONE

Comprehensive Bee Pathogen Screening in Belgium Reveals *Crithidia mellificae* as a New Contributory Factor to Winter Mortality

Jorgen Ravoet[1]*, Jafar Maharramov[2], Ivan Meeus[2], Lina De Smet[1], Tom Wenseleers[3], Guy Smagghe[2], Dirk C. de Graaf[1]

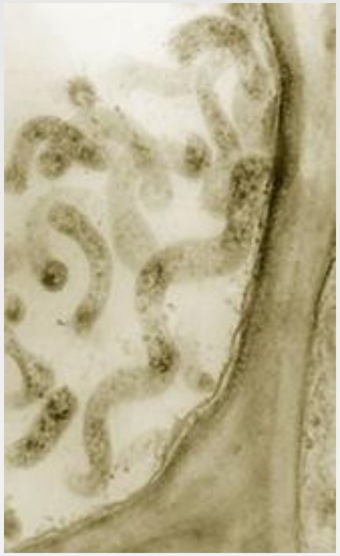


ALPV, VdMLV, LSV



Nosema ceranae

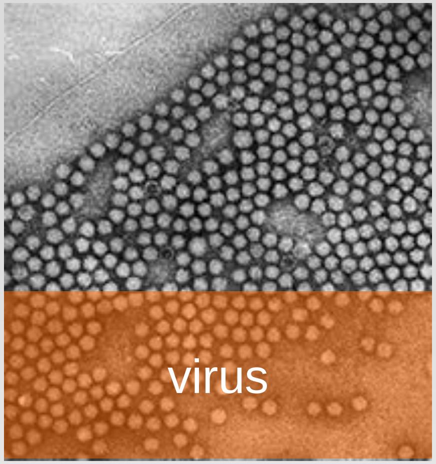
Crithidia mellificae



Spiroplasma melliferum and *S. apis*

Apocephalus borealis

Deformed Wing Virus-project



virus sequence heterogeneity in a single bee

Virus Research 201 (2015) 67–72

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

ELSEVIER

Genome sequence heterogeneity of Lake Sinai Virus found in honey bees and Orf1/RdRP-based polymorphisms in a single host

Jorgen Ravoet[a,*], Lina De Smet[a], Tom Wenseleers[b], Dirk C. de Graaf[a]

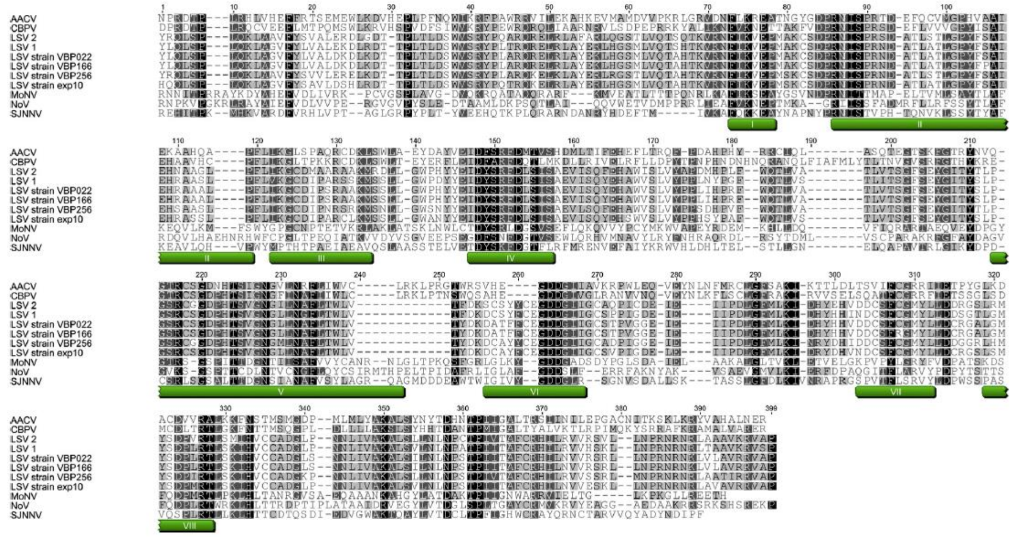
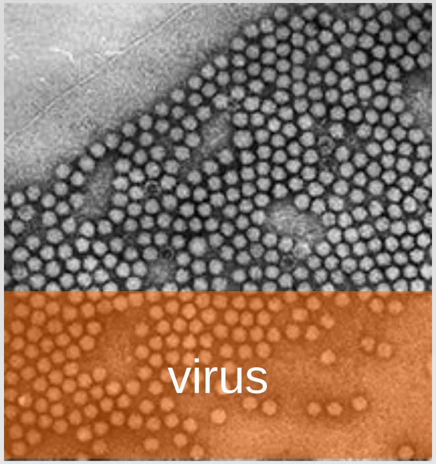


Fig. 2. Trimmed alignment of the RdRP proteins from AACV (Genbank: YP_009011225), CBPV (Genbank: YP_001911137A), LSV strains from the USA (LSV 1 and 2; Genbank: AEH26187, AEH26192) and Belgium (LSV strains VBP022, VBP166, VBP256, exp10; Genbank: KM886902, KM886905), MoNV (Genbank: AIO11151) and the Nodaviridae types Nodamura virus (Genbank: NP_077730) and Striped Jack nervous necrosis virus (Genbank: NP_599247). The eight conserved viral RdRP domains (Koonin and Dolja, 1993) are shown below the alignment in green boxes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Deformed Wing Virus-project



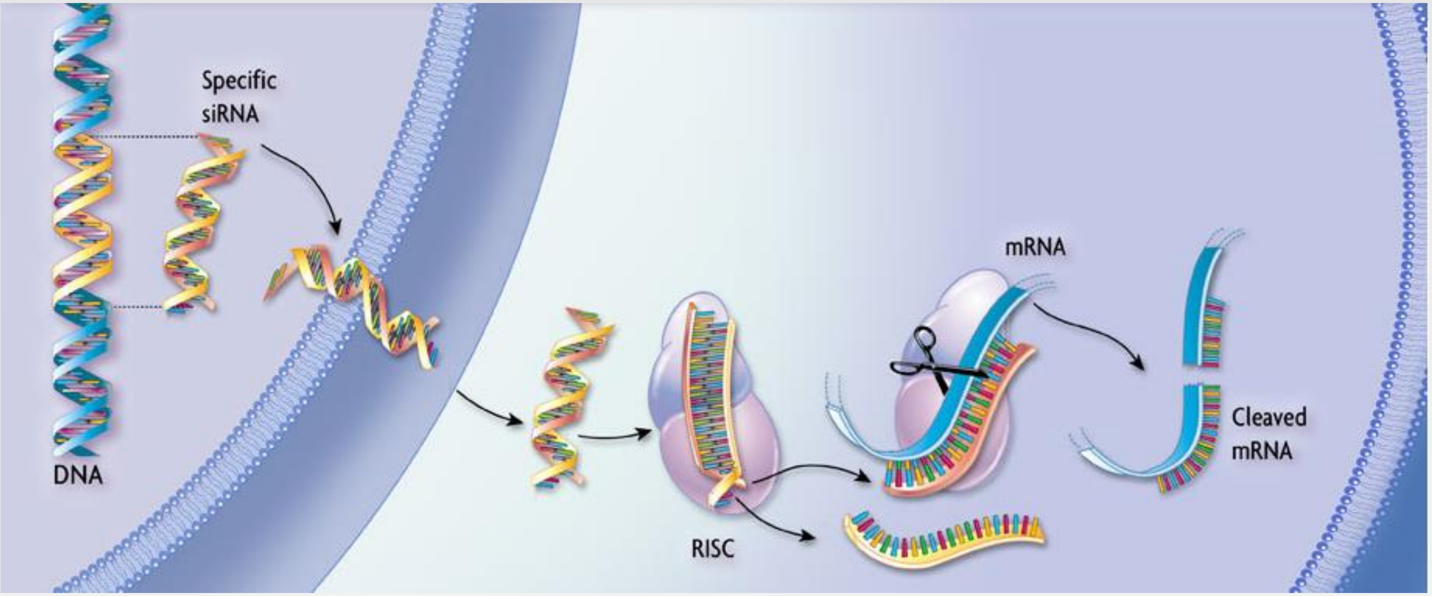
relationship virus load - antiviral immunity (RNAi)

Entomological Science (2017) **20**, 76–85 doi: 10.1111/ens.12227

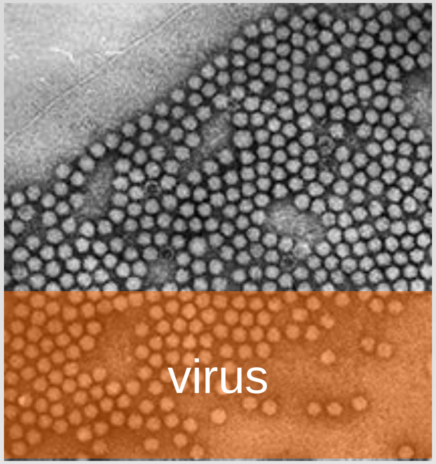
ORIGINAL ARTICLE

Expression of key components of the RNAi machinery are suppressed in *Apis mellifera* that suffer a high virus infection

Lina DE SMET[1]*, Jorgen RAVOET[1]*, Tom WENSELEERS[2] and Dirk C. DE GRAAF[1]



Deformed Wing Virus-project



long-term deleterious effects of type B-DWV

PROCEEDINGS B

rspb.royalsocietypublishing.org

Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival

Kristof Benaets[1], Anneleen Van Geystelen[1], Dries Cardoen[1], Lina De Smet[2], Dirk C. de Graaf[2], Liliane Schoofs[3], Maarten H. D. Larmuseau[1,4,5], Laura E. Brettell[6], Stephen J. Martin[6] and Tom Wenseleers[1]

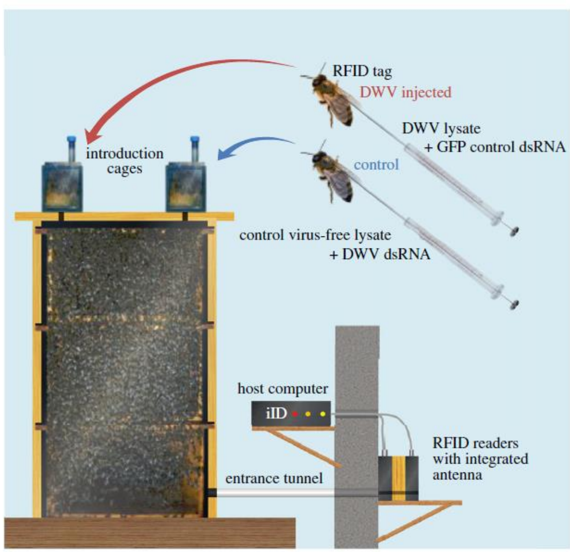
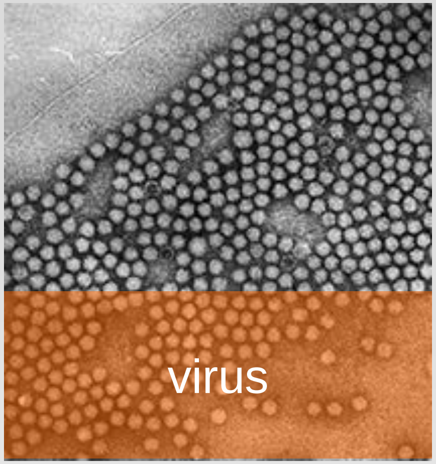


Figure 1. Experimental set-up. Observation hives were installed indoors with two RFID readers at the hive entrance to detect and log RFID-tagged bees entering or leaving the hive. The two RFID readers modules, connected to the host computer, were placed in series to determine the walking direction of detected bees. Tagged bees which were or were not experimentally infected with deformed wing virus were introduced into the host colony via separate introduction cages shown at the top ($n = 400$ bees per treatment and host colony). (Online version in colour.)

DWV-infected bees:
- started to forage at an earlier age
- showed reduced lifespans
- showed reduced total activity spans

Honeybee viruses in *Bombus*



honeybee viruses affect *Bombus* reproductivity

Journal of Invertebrate Pathology 121 (2014) 64–69

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

ELSEVIER

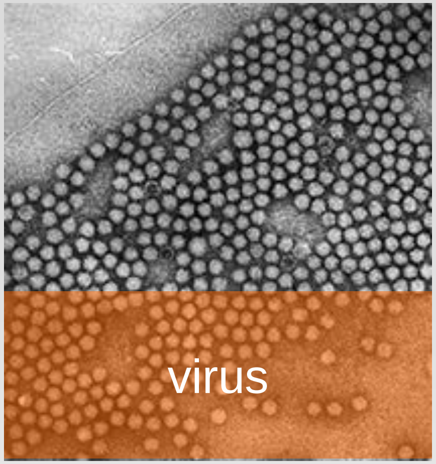
Effect of oral infection with Kashmir bee virus and Israeli acute paralysis virus on bumblebee (*Bombus terrestris*) reproductive success

Ivan Meeus [a,*], Joachim R. de Miranda [b], Dirk C. de Graaf [c], Felix Wäckers [d], Guy Smagghe [a]

Table 1
The number of micro-colonies with a regular and delayed time until oviposition (a), and with a without drone production (b).

(a)	The number of micro-colonies (mean oviposition day)			
	Regular oviposition	Delayed oviposition		χ^2
Control	8 (10.5)	2 (16.5)	Expected	
IAPV	6 (10.5)	4 (14)	Observed	$\chi^2 = 2.5$, df = 1, $P = 0.11$
KBV	4 (10.5)	6 (16.3)	Observed	$\chi^2 = 10$, df = 1, $P = 0.002$
(b)	The number of micro-colonies			
	With drone production	Without drone production		χ^2
Control	9	1	Expected	
IAPV	9	1	Observed	$\chi^2 = 0$, df = 1, $P = 1$
KBV	5	5	Observed	$\chi^2 = 17.778$, df = 1, $P < 0.001$

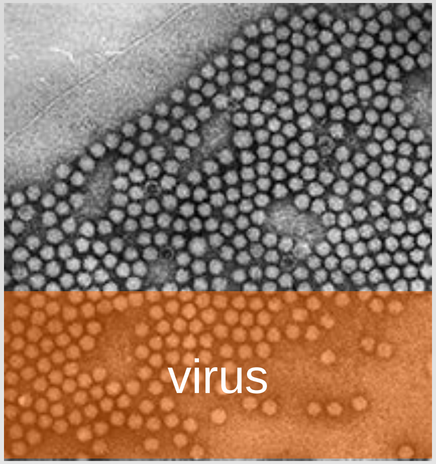
ViroBee



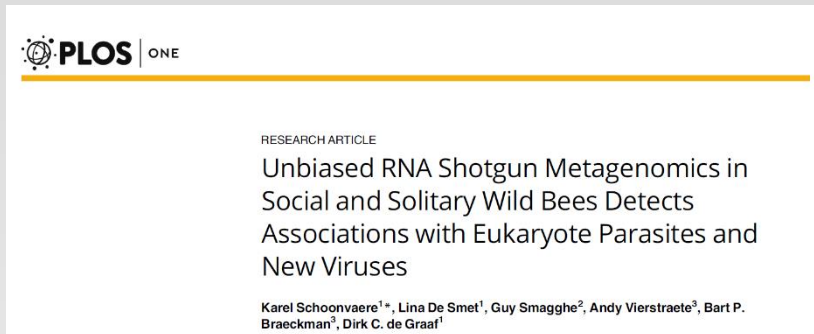
expanding the known honeybee viruses

data not shown for reasons of confidentiality

BELBEES



expanding the known wild bee viruses (and more)

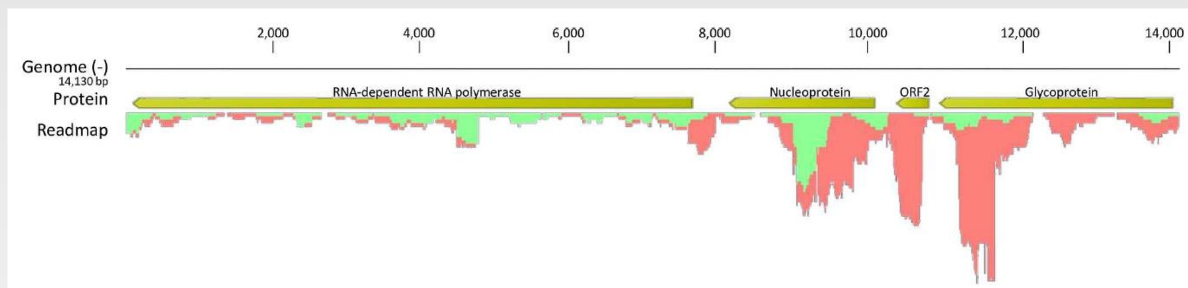

PLOS | ONE

RESEARCH ARTICLE

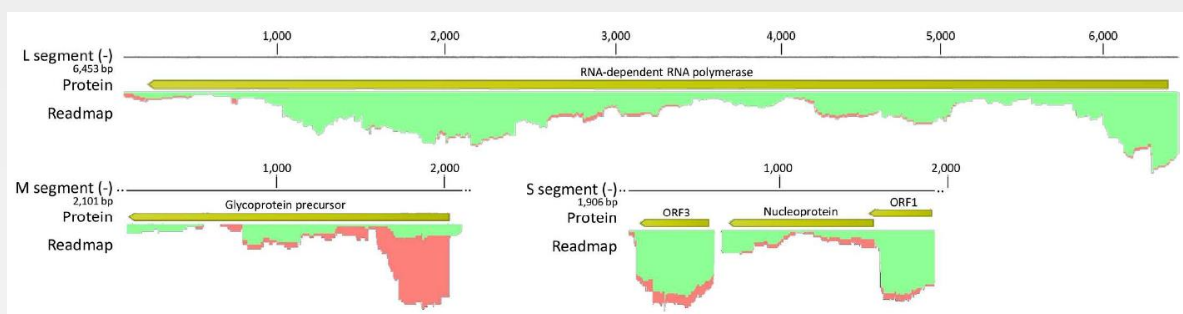
Unbiased RNA Shotgun Metagenomics in Social and Solitary Wild Bees Detects Associations with Eukaryote Parasites and New Viruses

Karel Schoonvaere[1]*, Lina De Smet[1], Guy Smagghe[2], Andy Vierstraete[3], Bart P. Braeckman[3], Dirk C. de Graaf[1]

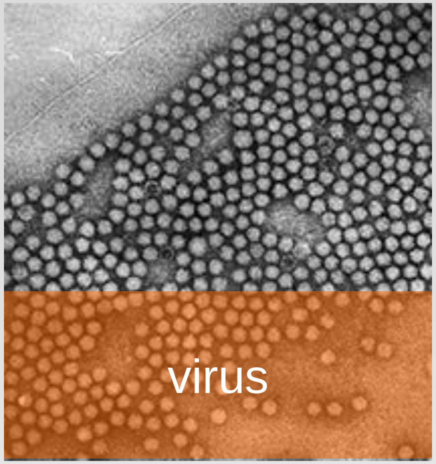
Scaldis River bee virus



Ganda bee virus



Vlaams Bijenteeltprogramma

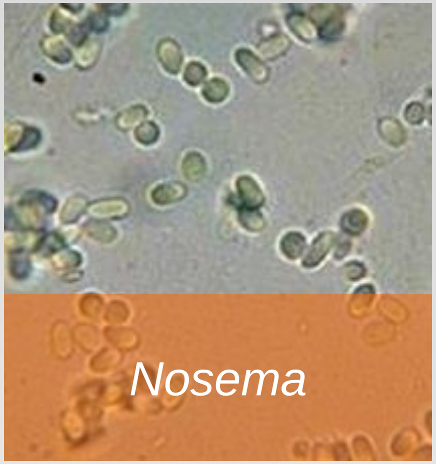


establishment of virus resistance by 'breeding value estimation'
+ inheritance of the phenotype

data not shown for reasons of confidentiality

data not shown for reasons of confidentiality

Nosema



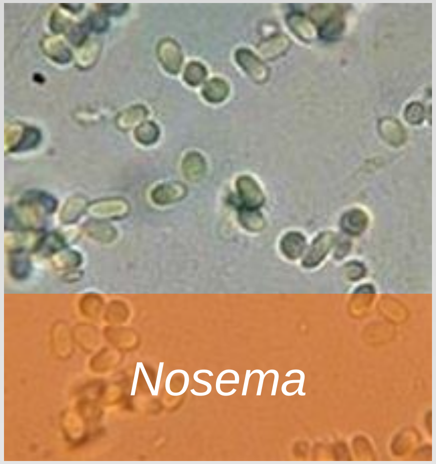
1. do we know all pathogens?

funding:

CARIBU
www.caribu.be

Ugandan-project

Ugandan-project



discovery of a new *Nosema* spp. in Ugandan honeybees

data not shown for reasons of confidentiality

Paenibacillus

2. which pathogens (genotypes) do matter?

funding:

fwo
Opening new horizons

AFB-project

AFB-project

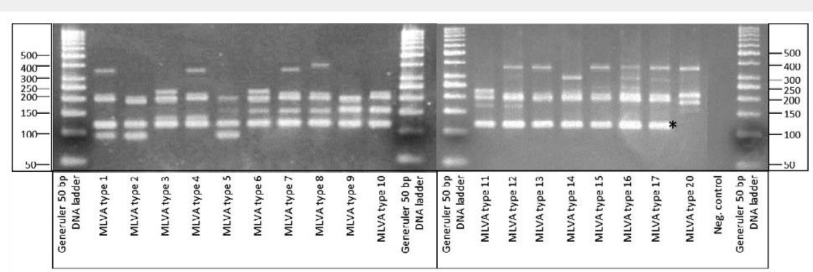
development of a new genotyping tool

microbial biotechnology
Open Access

Multiple Locus Variable number of tandem repeat Analysis: A molecular genotyping tool for *Paenibacillus larvae*

Tine Descamps,[1,*] Lina De Smet,[1] Pieter Stragier,[2] Paul De Vos[2] and Dirk C. de Graaf[1]

MLVA patterns of different P. larvae *strains*



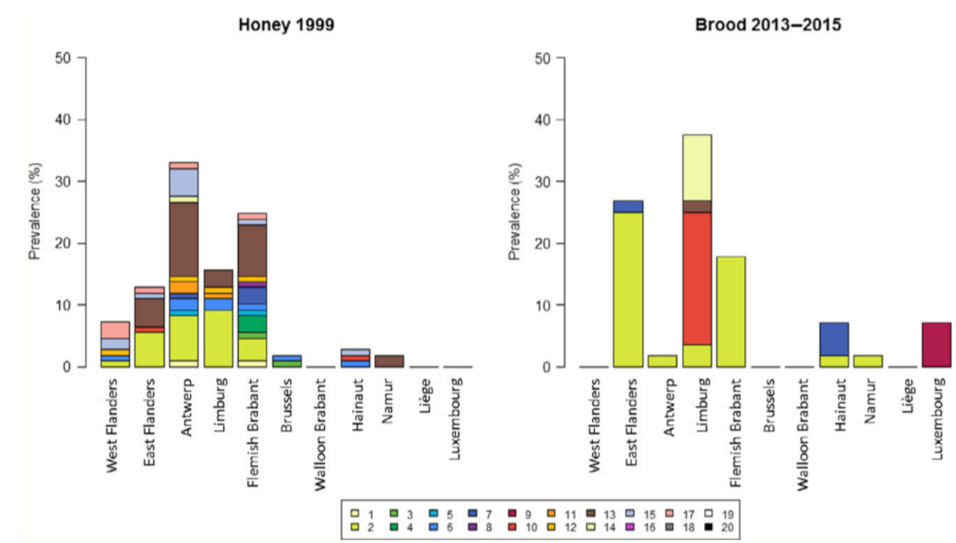


Fig. 4. Distribution of MLVA-types. The geographical location of each isolate from honey in 1999 and diseased brood in 2013–2015 was recorded. A significant difference in distribution over the provinces was observed. A much higher diversity in MLVA-types was found in the honey samples than in the brood samples.

AFB-project

identification of virulence genes by transposon mutagenesis

data not shown for reasons of confidentiality

in vitro *rearing of larvae*

management: basic research questions

1. which techniques are used by beekeepers?

2. do they influence mortality rates?

funding:

BeeBestCheck

Vlaanderen
verbeelding werkt

Vlaams Bijenteelt-programma

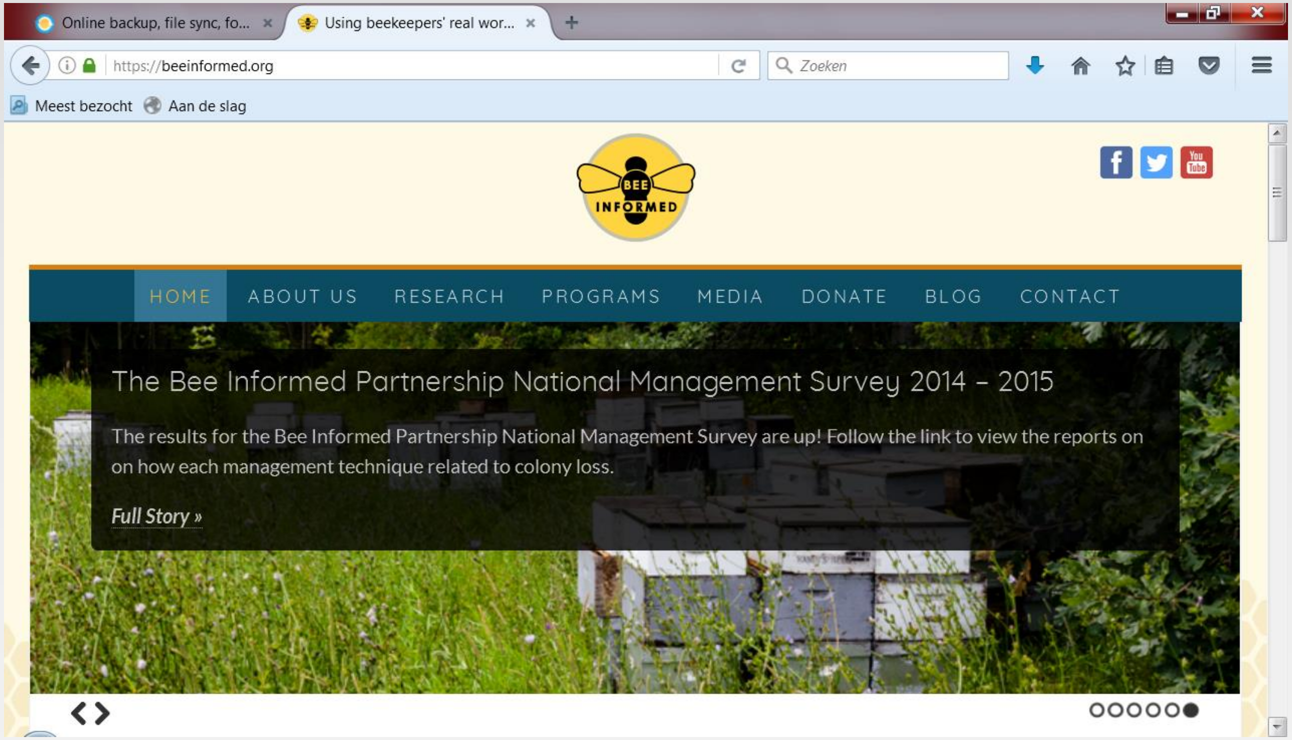
EUROPEAN COMMISSION

Ricola Foundation

COLOSS-monitoring

BeeBestCheck

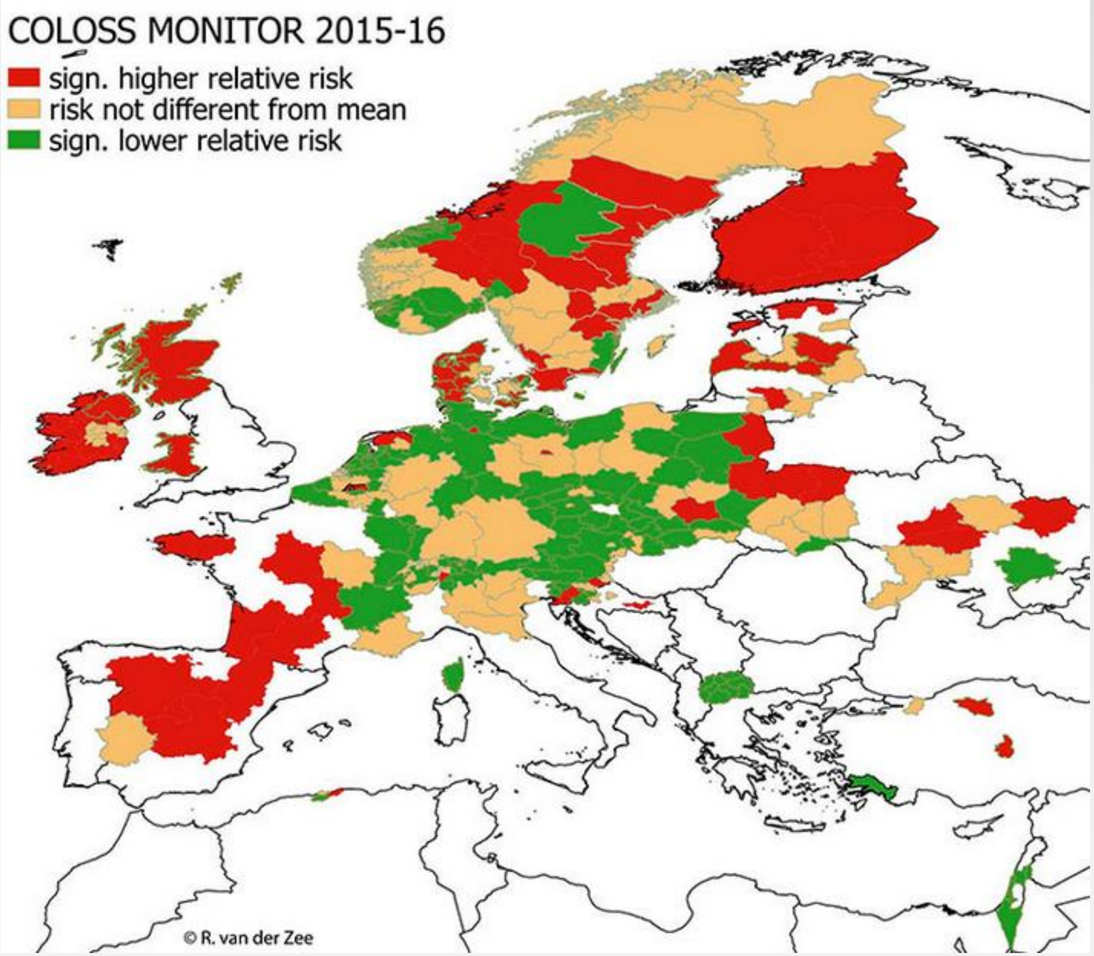
survey on beekeeping management
cfr. USA vanEngelsdorp-group



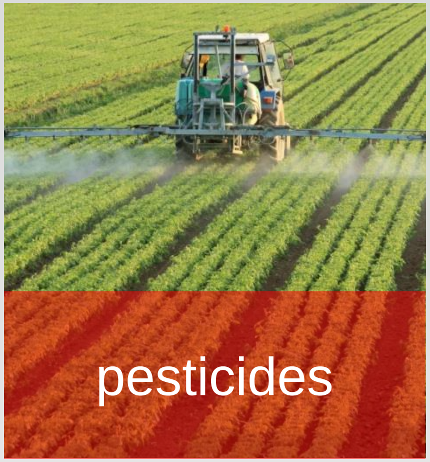
to report and to inform about beekeeping techniques and management

COLOSS-monitoring

uniform questionnaire about bee mortality



pesticides: basic research questions



1. where are bees exposed to?
2. which compounds/mixtures are dangerous?
3. can bees protect themselves against pesticides?
4. what is the (molecular) mechanism behind this?

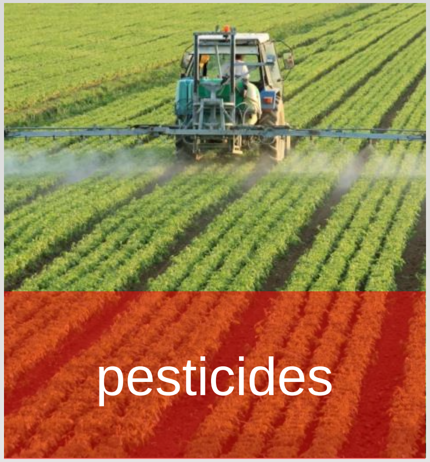
funding:

Vlaams Bijenteelt-programma

BELBEES

BeeHappy

Vlaams Bijenteeltprogramma



pesticide residues in beeswax

Bull Environ Contam Toxicol (2015) 94:543–548
DOI 10.1007/s00128-015-1511-y

Pesticides for Apicultural and/or Agricultural Application Found in Belgian Honey Bee Wax Combs

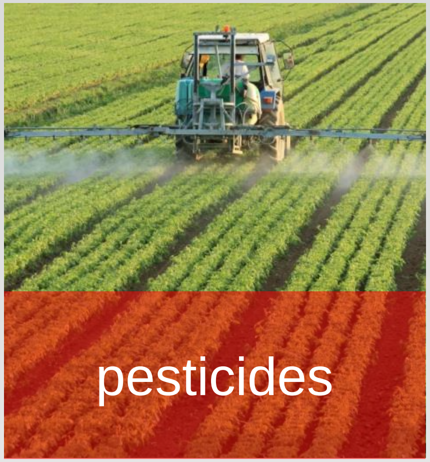
Jorgen Ravoet · Wim Reybroeck · Dirk C. de Graaf

Table 1. Pesticides found in Belgian beeswax samples (n= 10).

Pesticide	Concentration (µg/kg)	# Pos.	Acute 48h LD50 (µg/bee)	Type	MRL in honey (µg/kg)	Registration in Belgium
Pesticides used in beekeeping						
coumaphos	39; 6; 7; 16; 15; 31; 8; 66; 35	9	no data	aca	100	no[c] (until 2009)
bromopropylate	7; 46; 11; 18; 46; 78; 89	7	183	aca	10	no[c] (until 2007)
Pesticides used in beekeeping and applied to crops						
fluvalinate	27; 40; 12; 28; 17; 83; 17; 11; 30; 23	10	--	ins, aca	N.A.	yes[d]
amitraz	11; 19	2	50	ins, aca, vet	200	no[c] (until 2006)
Pesticides applied to crops						
delta-HCH	5; 29; 8; 9; 9; 8; 13	7	0.011[a]	ins, aca	10	no
alpha-HCH	9	1	0.011[a]	ins, aca	10	no
gamma-HCH	7	1	0.011	ins, aca	10	no
DEET	41; 38; 35; 13; 36	5	--	ins, rep	10*	no[e]
propargite	17; 27; 12; 43; 65	5	47.9	aca	10*	no
chlorfenvinphos	11; 9; 15; 8; 13	5	0.55	ins, aca, vet	10	no
p,p'-DDT	44; 6; 8	3	5[b]	ins	50	no
p,p'-DDE	10	1	5[b]	ins	50	no
o,p'-DDT	11	1	5[b]	ins	50	no
4,4'-dibromobenzophenone	5; 7; 8	3	no data		10*	
boscalid	13; 12	2	100	fun	500	yes
parathion-methyl	16	1	19.5	ins	10	no
piperonyl butoxide	10	1	294	opc, vet	10*	yes
bromophos	6	1	0.44	ins	10*	no

18 pesticides found
3-13/samples
most abundant: apicultural application
also: lindane
DDT
> historic contamination by re-use

BELBEES



comparison between cage and field exposure
+ molecular responses of the bees

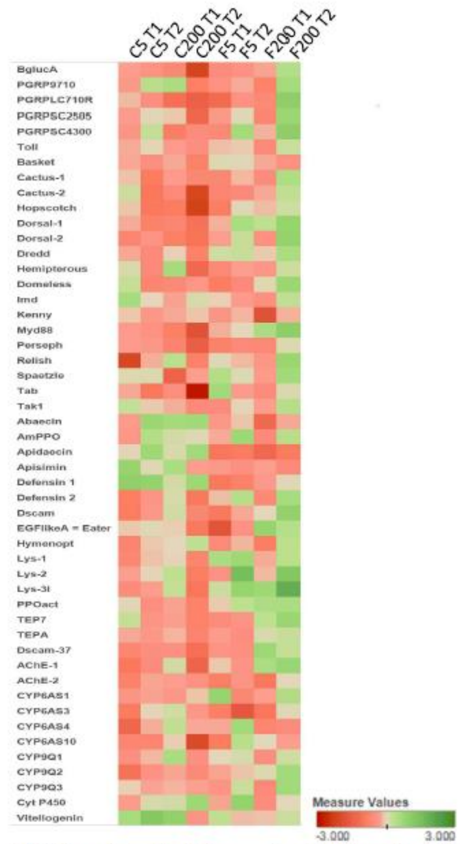


Fig 4. Heat map showing the relative expression profiles of immunity related and detoxification genes. C5 T1 and C5 T2: cage experiment treated with 5 ppb for 10 and 20 days, respectively, compared to the cage control at 10 and 20 days; C200 T1 and C200 T2: cage experiment treated with 200 ppb for 10 and 20 days, respectively, compared to the cage control at 10 and 20 days; F5 T1 and F5 T2 a: field experiment treated with 5 ppb for 10 and 20 days, respectively, compared to the control at 10 and 20 days; F200 T1 and F200 T2: field experiment treated with 200 ppb for 10 and 20 days, respectively, compared to the field control at 10 and 20 days.

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RESEARCH ARTICLE

Stress indicator gene expression profiles, colony dynamics and tissue development of honey bees exposed to sub-lethal doses of imidacloprid in laboratory and field experiments

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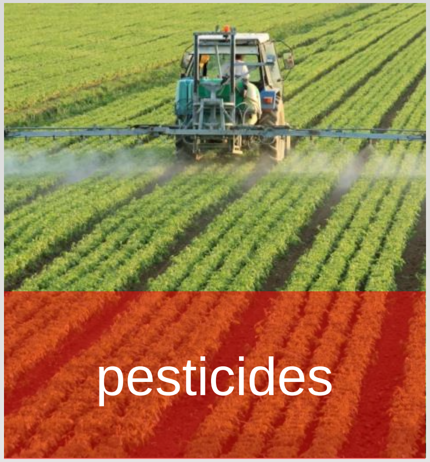
BeeHappy



spatially-explicit study of the relationship between environmental variables and bee mortality

data not shown for reasons of confidentiality

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diseases: answer to the questions

1. do we know all pathogens?

never-ending list of new pathogens & genotypes

2. which pathogens (genotypes) do matter?

gap in our knowledge of their importance

3. can bees protect themselves against pathogens?

bees seems to be much more resilient than we thought, and we should exploit this fully

4. what is the (molecular) mechanism behind this?

gene (expression) profiles may serve for understanding and predicting

management: answers to the questions

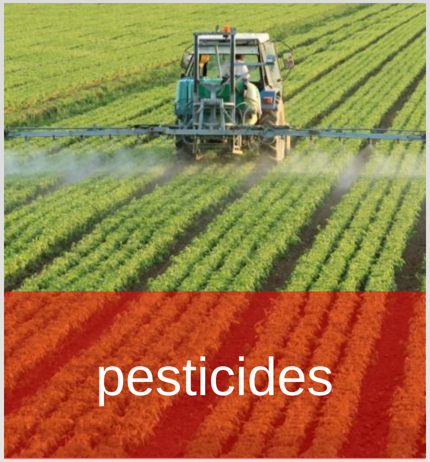
1. which techniques are used by beekeepers?

everybody seems to have his/her own methods

2. do they influence mortality rates?

it seems reasonable to believe they do, so this is also a issue for improvement

pesticides: answers to the questions



1. where are bees exposed to?

only data from beeswax

2. which compounds/mixtures are dangerous?

to be determined; can be mixtures of chemical or any other environmental variable

3. can bees protect themselves against pesticides?

there is a discrepancy between cage and field trials; colonies are much more resilient than cages

4. what is the (molecular) mechanism behind this?

gene (expression) profiles may serve for understanding and predicting

tips and tricks

- avoid import of bees >> source of new pathogens
- choose locally adapted bees
- invest fully in selection for resilient bees
- beekeepers' management should be in harmony with the bees' biology and environment
- see medical treatment only as a solution on the short term
- respect the carrying capacity of the landscape
- invest fully in a bee friendly vegetation >> food from April to September
- avoid dangerous mixtures of chemicals and environmental variables
- ornamental plants should be disease resistent >> no pesticides required
- let apiculture and agriculture exist in harmony

OR IN SHORT: more food
less poison
less mobility
breed wisely
good beekeeping practices

thank you