BEENET: NATIONAL-WIDE MONITORING PROJECT IN ITALY

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BeeNet

- ✓ BeeNet is a network for studying bees-environment interactions and monitoring honeybee mortality and colony losses in Italy
- ✓ The BeeNet monitoring network was activated in September 2011, with an increase of the number of apiaries compared to the previous ApeNet network (2009-2010)
- ✓ In 2011 there were 97 apiaries for a total of approximately one thousand beehives
- ✓ In 2012 the monitoring network progressed up to 303 apiaries located in all the Italian regions with approximately 3000 beehives

Location of the **monitoring units** involved in the BeeNet project

▲ Coordination centre

● Apiary composed of 10 hives

Each **monitoring unit** is composed of five apiaries with ten beehives each managed by a referent person

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Bee Net

BeeNet monitoring network

The investigation aimed to:

- Colony development and environment data collection
- *Nosema apis/Nosema ceranae*
- Deformed Wing Virus (DWV)
- Acute Bee Paralysis Virus (ABPV)
- Chronic Bee Paralysis Virus (CBPV)
- Beebread protein content
- Beebread pesticide residues

BeeNet monitoring network

Colonies are visually inspected at 4 different time points:

-1st, end of Winter;
-2nd, Spring-Summer;
-3rd, end of Summer-beginning of Autumn
-4th, before wintering.

In each inspection several parameters of each colony are considered:

✓health and nutritional condition,
✓number of bees and brood,
✓queen’s age,
✓climate, land use

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Samples and analyses

- At visit 1 and 3, samples of beehive matrices are collected: **<u>beebread</u>** and live **<u>honey bees</u>**

- Analyses

Chemical (beebread pesticides), **pathology** (*Nosema*, virus and *Varroa*) and **nutritional** (beebread raw protein) analyses

BeeNet monitoring network

Analyses on adult bees

From Autumn 2011 to Autumn 2012

Nosema

620 samples of asymptomatic live adult honey bees

Viruses

636 samples of asymptomatic live adult honey bees

for DWV, ABPV and CBPV

Varroa destructor

The infestation level of *Varroa destructor* (% calculated on a sample of 250 honey bees) was estimated in the apiary using the "powder sugar roll method" during the 3rd visit, i.e. end of Summer-beginning of Autumn

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Bee Net

BeeNet monitoring network

Analyses on pollen (beebread)

From Autumn 2011 to Autumn 2012

Beebread protein content

- ✓ The protein content determined using the Kjeldhal method.
- ✓ The protein content calculated by multiplying the nitrogen content (obtained from NH_3 value) by 6.25

Pesticides

- ✓ The determination of pesticides performed by extraction and purification using the QuEChERS® technique
- ✓ The instrumental determination performed by GC-ECD and HPLC-MS using a quantification by external standards
- ✓ The number of tested pesticides greater than **150**

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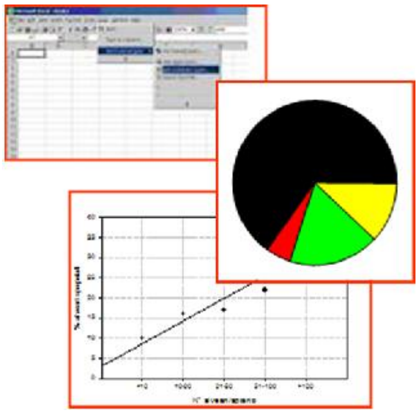
BeeNet monitoring network

- ✓ The data observed are transmitted by referents person of the monitoring unit using software facilities for data-entry
- ✓ the software facilities are activated via internet connections
- ✓ the data collected are stored in a georeferred database



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Results

***Varroa destructor* infestation**

- In Marche (6.3%) and Lazio (3.9%) (central Italy), Campania (3.3%) and Puglia (3.0%) (southern Italy) regions the highest infestation levels were recorded,
- the lowest ones in Toscana (0.3%) (central Italy) and Emilia-Romagna (0.5%) (northern Italy) regions at the 3rd visit (end of Summer-beginning of Autumn)

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Results

- *N. ceranae* was present in all Italian regions
- *N. apis* or *N. apis/N. ceranae* co-infection were not detected
- Of the 620 samples analyzed, 454 were positive for *N. ceranae* with an overall positivity rate of **73%**
- Only in **3.4%** of the samples more than 10 million *N. ceranae* spores per bee were detected

Nosema ceranae

a

Sampling 2011	*Nosema ceranae*
Total number of samples	122
POSITIVE	**46** (37.7%)
of which with more than 10^7 *N. ceranae* spores per bee	0
NEGATIVE	**76** (62.3%)

b

1^{st} sampling 2012	*Nosema ceranae*
Total number of samples	207
POSITIVE	**178** (86%)
of which with more than 10^7 *N. ceranae* spores per bee	6 (3.4%)
NEGATIVE	**29** (14%)

c

2^{st} sampling 2012	*Nosema ceranae*
Total number of samples	291
POSITIVE	**230** (79%)
of which with more than 10^7 *N. ceranae* spores per bee	15 (6.5%)
NEGATIVE	**61** (21%)

Results of the analyses directed to the determination of *Nosema* spp.

Viruses

- DWV, ABPV and CBPV were detected in Italian apiaries in different combinations

- **DWV** was present in almost all samples (96.7%)

  In 40% of cases exceeded 10 million viral copies per bee

- For **ABPV** and **CBPV** the percentages were lower, 53.6 and 57.7% respectively

  The samples that exceeded 10 million viral copies per bee were only 5.9 and 5.4%, respectively

Viruses

Results of the analyses directed to the determination of viruses

a

Sampling 2011	DWV	ABPV	CBPV
Total number of samples	126	126	126
POSITIVE	**118** (93.6%)	**65** (51.6%)	**54** (42.9%)
of wich with more than 10^7 viral copies per bee	88 (74.6%)	9 (13.8%)	5 (9.2%)
NEGATIVE	**8** (6.3%)	**61** (48.4%)	**72** (57.1%)

b

1st sampling 2012	DWV	ABPV	CBPV
Total number of samples	218	218	218
POSITIVE	**208** (95.4%)	**129** (59.2%)	**170** (78%)
of which with more than 10^7 viral copies per bee	43 (20.7%)	4 (3.1%)	11 (6.5%)
NEGATIVE	**10** (4.6%)	**89** (40.8%)	**48** (22%)

c

2st sampling 2012	DWV	ABPV	CBPV
Total number of samples	292	292	292
POSITIVE	**289** (99%)	**147** (50.3%)	**143** (49%)
of which with more than 10^7 viral copies per bee	116 (40.1%)	7 (4.8%)	4 (2.3%)
NEGATIVE	**3** (1%)	**145** (49.7%)	**149** (51%)

Beebread pesticides content

In Autumn 2011, **22** different active ingredients were found:

carbaryl (7.2% of the samples),

chlorpyriphos (4.0%, 8-47 ppb)

fluvalinate (3.2%, 17-150 ppb)

- Only one sample contained neonicotinoids (imidacloprid, 16 ppb)

Beebread pesticides content

In Spring 2012, **50** different active ingredients were found:

Neonicotinoid

- Imidacloprid (7),
- Acetamiprid (6)
- Thiametoxam, (2)

between 0,006 and 0,99 mg/Kg.

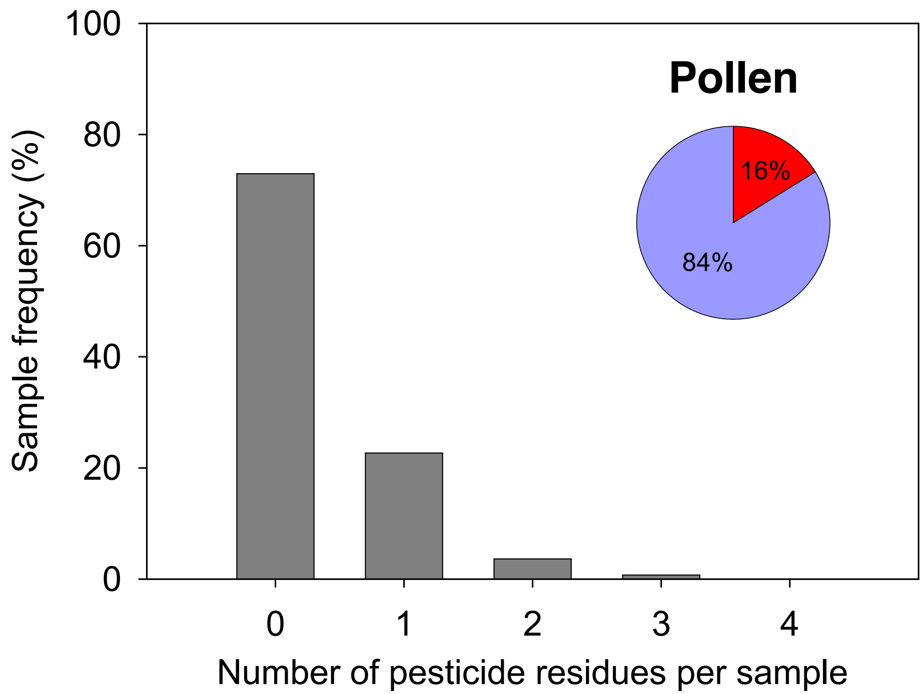
Acaricide/insecticide

- Fluvalinate, Chlorphenvinfos, Chlorpyrifos

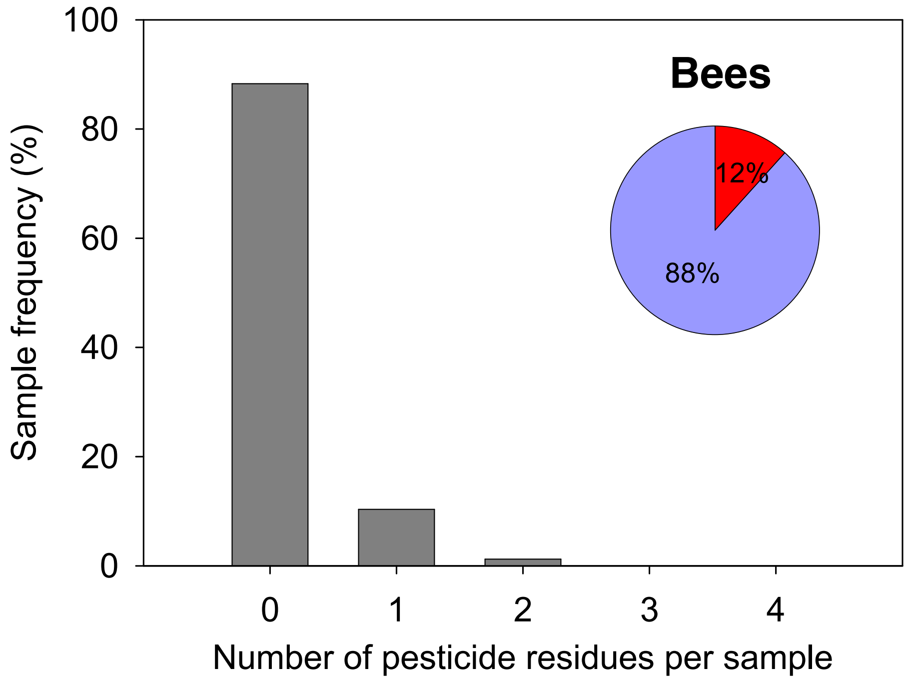
Fungicide

- Thiophanate Methyl e Fenamidone

On average, **34%** of the beebread samples results with residues.



Frequencies of numbers of different pesticides in bee bread (N=551). In the pie graph the percentage of the positive samples with more than one compound is in red.



Frequencies of numbers of different pesticides in honey bees (N=726). In the pie graph the percentage of the positive samples with more than one compound is in red.

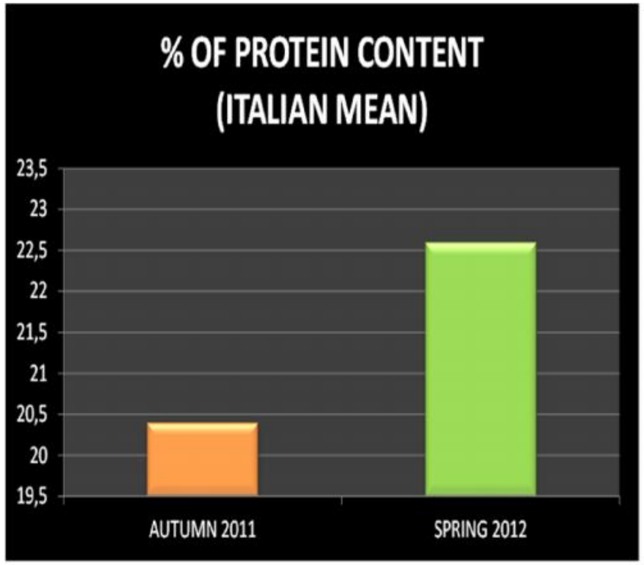
Cocktail of pesticides

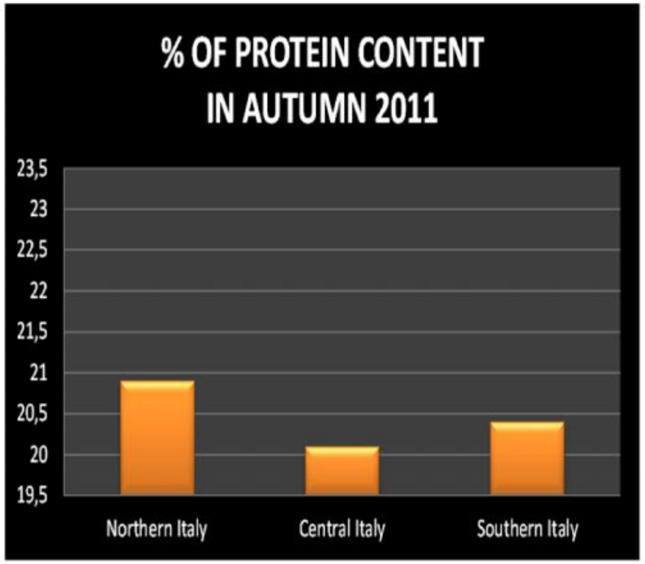
In most of the scientific experiments, only one pesticide at a time is used.

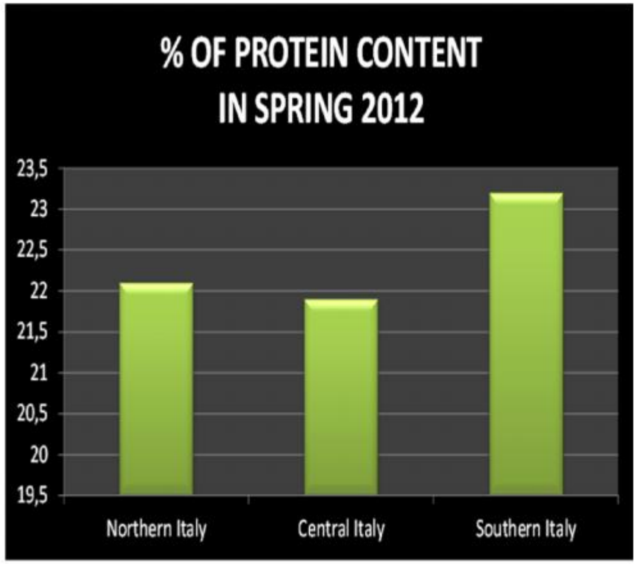
Multiple exposure to pesticides (including substances used in bee medication) is an established fact and potential additive and cumulative effects have to be considered in future studies;



Beebread protein content







Results of the analyses directed to the determination of protein content

- In Autumn 2011, the beebread contained a lower percentage of protein and pesticides, compared to Spring 2012
- In Spring 2012, the colonies located in the south of Italy contained beebread with the highest protein content and positivity to pesticides of the country

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Bee Net

B.E.S.T.
(Bee Emergency Service Team)

Objectives

- To Integrate the National Monitoring Network (BeeNet);
- To study the events of bee mortality and colony loss when the causes are difficult to identify;
- to analyse the event in real time when the <u>phenomenon is still in action</u>.

BEST Steps

Reporting via several information systems → **Filling out a simple form :**
Who:……………
Where:…………
When:…………
What:……………
Hypothesis:….

→ **Central or regional BEST referents**

In 2012, 43 intervention requests have been recorded

North: 58%

Centre: 7%

South: 35%

Reports distribution

Main crops surrounding the affected apiary

In the first 6months of 2013, **48** interventions have been done, expecially in north Italy (75%)

The case study of bee poisoning in citrus orchards during aphids control

Area: Sibari Valley;
Crop: Citrus;
Plant phenology: flowering (end);
Anamnesis: many dead bees, reduction colonies size; abnormal behaviors;
Period: May;
Agronomic operation: aphid control
Reports: 5 (2009)
Colonies involved: 150, (estimated: ~ 20,000) (2009)

Matrices/Sample	Residue analysis (2009)
Bees - 1	Imidacloprid: 25 ng/g Thiamethoxam: 10 ng/g Acetamiprid: 17 ng/g Metomil: 63 ng/g Nosema: negative
Citrus leaves - 1	Imidacloprid: 17 ng/g Thiamethoxam: 30 ng/g Acetamiprid: 13 ng/g Metomil: 580 ng/g
Citrus leaves - 2	Metomil: 1,000 ng/g
Bees - 2	Imidacloprid: 15 ng/g Clothianidin: 159 ng/g Acetamiprid: 36 ng/g
Citrus - 3	Thiamethoxam: 11 ng/g Acetamiprid: 21 ng/g
Bees - 3	Imidacloprid: 90 ng/g Clothianidin: 113 ng/g Acetamiprid: 203 ng/g Thiamethoxam: 107 ng/g Thiacloprid: 79 ng/g Nosema: negativo
Brood Comb - 1	AFB: negative EFB: negative

The case study of bee poisoning in maize during sowing operation

Area: Po Valley;
Crop: Maize;
Plant phenology: -;
Anamnesis: many dead bees, reduction colonies size; abnormal behaviors;
Period: March to May;
Agronomic operation: maize sowing
Reports: 185 (2008) – 2 (2009)*;
Colonies involved: 6,328 (2008) – ~50 (2009)*

Residues Analysis (2008)	TOTAL	
	Number	*%*
Analysed samples	**109**	
Dead bee samples	**105**	
Positive dead bee samples	**52**	**49.5**
Dead bee samples positive to imidacloprid	**27**	**25.7**
Dead bee samples positive to thiamethoxam	**3**	**2.8**
Dead bee samples positive to clothianidin	**27**	**25.7**
Dead bee samples positive to fipronil	**0**	**0**
Dead bee samples positive to both imidacloprid and clothianidin	**5**	**4.7**
Pollen samples	**4**	
Positive pollen samples	**3**	**75.0**
Pollen samples positive to imidacloprid	**3**	**75.0**
Pollen samples positive to thiamethoxam	**0**	**0**
Pollen samples positive to clothianidin	**1**	**25.0**
Pollen samples positive to both imidacloprid and clothianidin	**1**	**25.0**

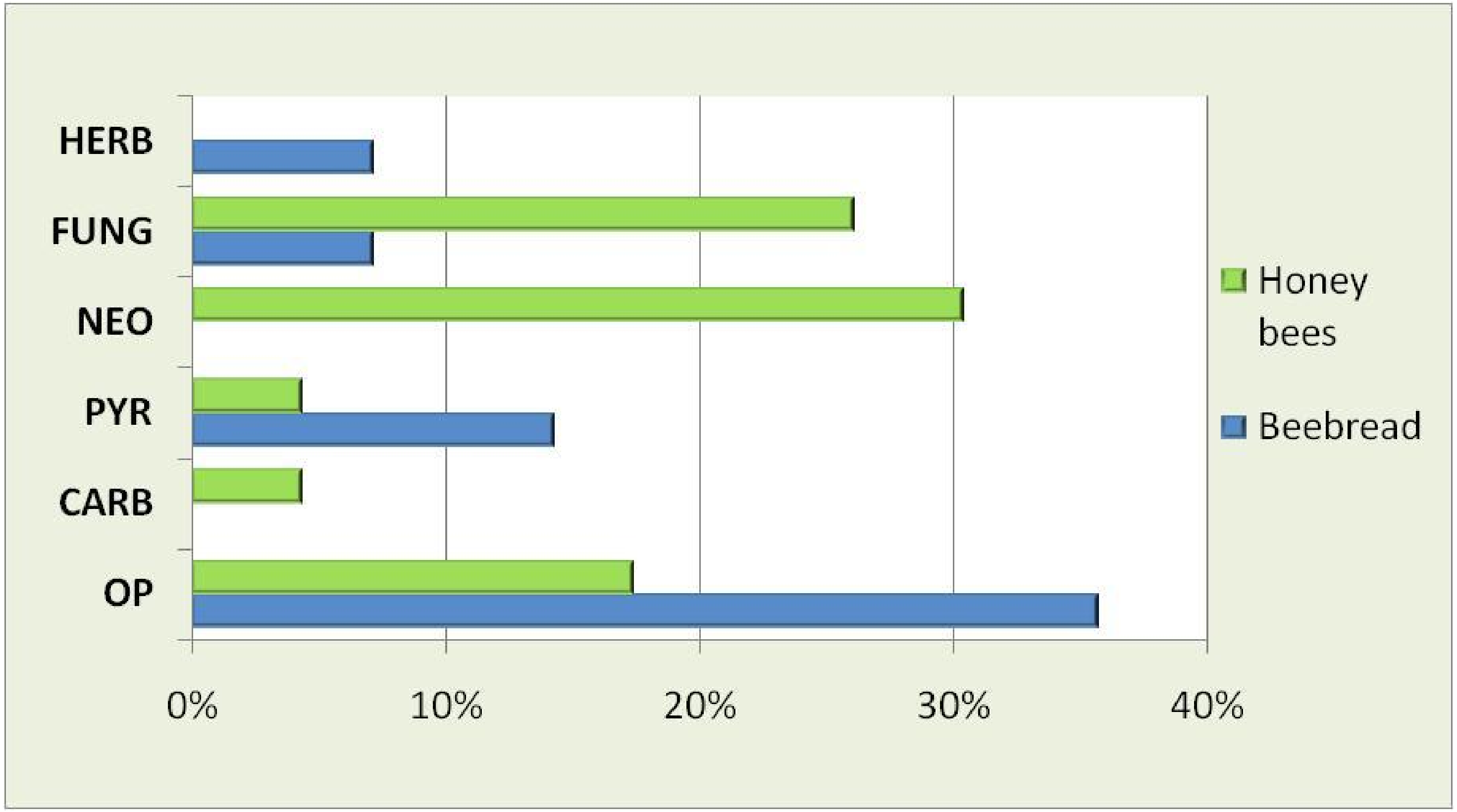
*after the suspension of imidacloprid, clothianidin, thiametoxam and fipronil used as maize seed dressing

The case study of bee poisoning in orange during scale insects (*Protopulvinaria piryformis)* control in presence of honeydew

Area: Eastern Sicily;
Crop: Orange;
Plant phenology: fruit-set;
Anamnesis: many dead bees, colonies collapse (30%);
Period: September (only in dry years);
Agronomic operation: scale insects control;
Reports: 3 (2011);
Colonies involved: 200 - (estimated: ~ 4,000) (2011)

Matrices/Sample	Residue analysis (2011)
Dead bees - 1	Clorpiriphos 0.28 ng/g; Deltamethrin 1.7 ng/g;
Dead bees - 2	Deltamethrin 0.38 ng/g

B.E.S.T.
(Bee Emergence Service Team)
2012



Percentage of different residues found in honey bees and beebread (data 2012

Pesticides not authorized

2012

Principio attivo	numero campioni	range (mg/kg)
Acrinathrin	1	0,014
Aldicarb *	1	0,016
Azinphos-Ethyl *	1	0,010
Azoxystrobin	4	0,02 - 0,075
Benalaxyl	1	0,019
Benzoximate *	1	0,010
Bitertanol	1	1,100
Boscalid	1	0,011
Captan	2	0,075 - 0,137
Carbaryl *	1	0,270
Chlorfenvinphos *	15	0,019 - 0,126
Chlorpyriphos-Ethyl	10	0,008 - 0,109
Coumaphos *	5	0,017 - 0,057
Cyproconazole	1	0,037
Deltamethrin	1	0,025
Dimethoate	3	0,018 - 0,338
Dimethomorph	5	0,005 - 0,069
Diniconazole *	1	0,101
Diuron	1	0,005
Dmst (Metabolite Of Tolyfluanid) *	1	0,018
Endosulfan Sulfate *	4	0,017 - 0,034
Etaconazole *	1	0,003
Fenamidone	8	0,01 - 0,471
Fenhexamid	3	0,018 - 0,392
Fipronil	1	0,020
Fludioxonil	1	0,019
Fluvalinate-Tau	17	0,015 - 0,134
Imidacloprid	3	0,012 - 0,062
Iprovalicarb	5	0,01 - 0,077
Lambda-Cyhalothrin	2	0,016 - 0,066
Metalaxyl-M	6	0,044 - 0,382
Metazachlor	1	0,025
Methoxyfenozide	1	0,047
Metrafenone	2	0,026 - 0,121
Myclobutanil	3	0,018 - 0,036
Penconazole	3	0,023 - 0,038
Pendimethalin	1	0,120
Piperonyl Butoxide	1	0,016
Profenophos *	1	0,005
Propamocarb	1	0,030
Propargite	3	0,026 - 0,073
Pyrimethanil	1	0,033
Tebuconazole	8	0,006 - 0,464
Tebufenozide	1	0,025
Tebufenpyrad	1	0,018
Terbuthylazine	4	0,015 - 0,034
Tetramethrin *	4	0,112 - 0,164
Thiamethoxam	1	0,018
Thiophanate-Methyl	2	0,01 - 0,058
Trifloxystrobin	2	0,033 - 0,042

BeeNet Network (Residues in beebread)

Data 2011: *3 not authorized a.s. (not included in the Annex 1 of EC 91/414) – 13.6%

Data 2012: *10 not authorized a.s. - 20%

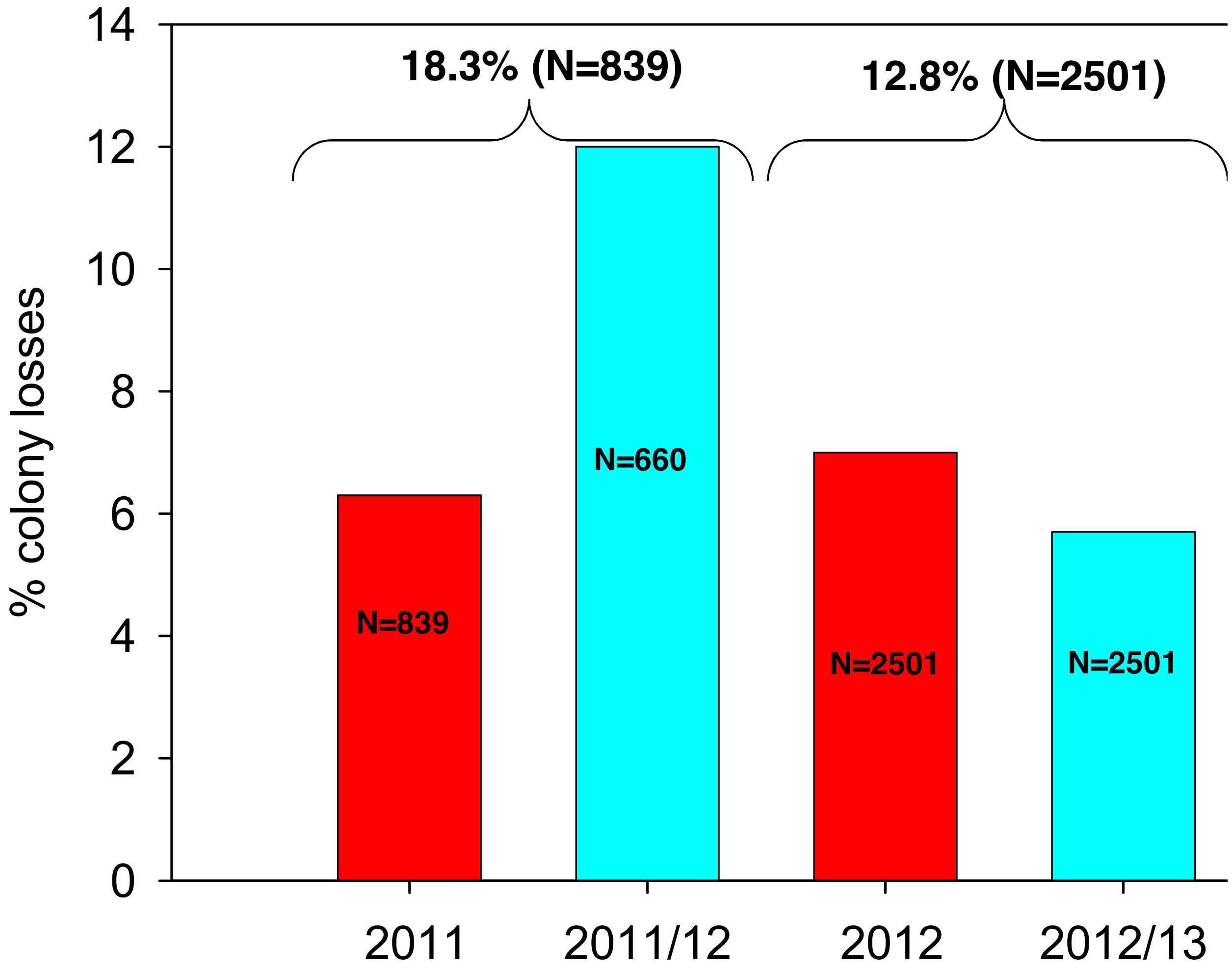
2011

Principio attivo	N° campioni positivi	Media residui (mg/Kg)	Range (mg/Kg)
Aldicarb *	1	0,079	
Azoxystrobin	1	0,018	
Boscalid	1	0,011	
Carbaryl *	9	0,031	0,011-0,082
Chlorfenvinphos *	2	0,008	0,004-0,011
Chlorpyriphos	5	0,022	0,008-0,047
Coumaphos *	1	0,330	
Dimethomorph	1	0,142	
fenamiphos sulfoxido	2	0,015	0,010-0,020
Fenazaquin	4	0,040	0,012-0,069
Fludioxonil	2	0,196	0,010-0,381
Flumethrin *	2	0,055	0,015-0,095
Fluvalinate	4	0,072	0,017-0,150
Heptenophos	1	0,010	
Imazalil	3	0,046	0,011-0,092
Imidacloprid	1	0,016	
Metalaxyl	2	0,017	0,016-0,018
Metazachlor	1	0,006	
Metribuzin	2	0,015	0,010-0,020
Propamocarb	2	0,348	0,042-0,653
Tebufenozide	2	0,026	0,013-0,038
Thiophanate methyl	2	0,106	0,040-0,173

*Used in veterinary medicine but not authorized in Italy

mutinelli

Honey bee colony losses



Honey bee colony losses

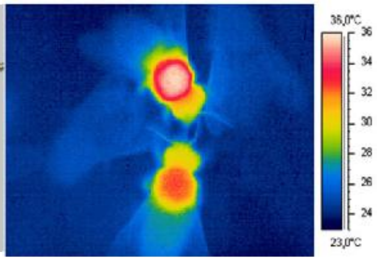
- In northern Italy colony mortality amounted to 4% (1227 monitored colonies) and Winter mortality to 8.5%;
- in central Italy colony mortality amounted to 12.9% (584 monitored colonies) and Winter mortality to 4.9%;
- in southern Italy colony mortality amounted to 7.6% (920 monitored colonies) and Winter mortality to 3%

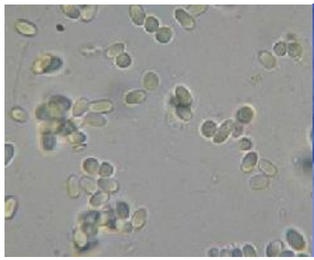
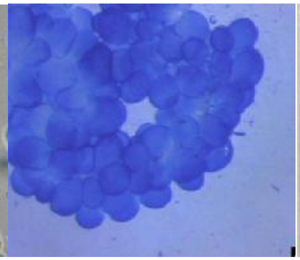
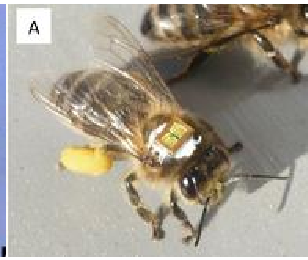
Question

- Do we need even more evidence and scientific validation concerning the toxicity of most of pesticides for the honeybee (and other pollinators)?

In laboratory

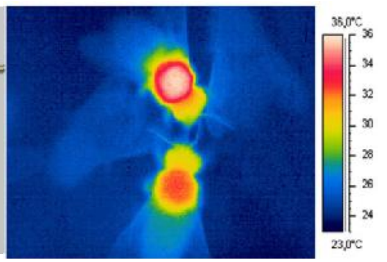
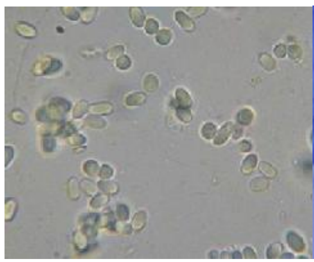
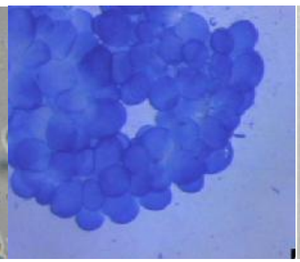
Effects on	Compound	Dose/ exposure	Other stressors	References
Homing ability	Thiametoxam	1.34 ng/bee [acute oral]	-	Henry et al. 2012 Science
Homing ability	Imidacloprid	100, 500 ppb [acute oral]	-	Bortolotti et al. 2003 B. Insect.
Foraging duration	Imidacloprid	>50 ppb [acute oral]	-	Yang et al. 2008 J. Econ. Ent.
Comunication	Parathion	0.03 µg/bee [acute oral]	-	Schricker and Stephen, 1970 J. Apic. Res.
Comunication	Imidacloprid	100, 500 ppb [acute oral]	-	Medrzycki et al. 2003 B. Insect.
Thermoregulation	Delthametrin	2.5-4.5 ng/bees [acute contact]	-	Vandame and Belzunces, 1998 Neurosc. Lett.
Learning ability	Imidacloprid	0.1-10 ng/ape [chronic oral]	-	Guez et al. 2001. Neurobiol. Learn. & Mem.

In laboratory

Effects on	Compound	Dose/ exposure	Other stressors	References
Queen rearing	Coumaphos	From 1 to 1000 ppm in wax queen cup	-	Pettis et al. 2004 Apidologie
Hypopharingeal gland development and respiratory rhythm	Imidacloprid	2 ppb in nectar + 3 ppb in pollen [chronic ingestion]	-	Hatjina et al. 2013 Apidologie
Thermoregulation	Delthametrin	1.5-2.5 ng/bees [acute contact]	850 ng of difenoconazole	Vandame and Belzunces, 1998 Neurosc. Lett.
Longevity	Dimethoate	0.15 µg/bee [acute oral]	Brood rearing temperature	Medrzycki et al. 2010 J. Apic. Res.
Survival	Imidacloprid	0.7 ppb [chronic ingestion]	Nosema	Alaux et al. 2009. Env. Microbiol.
Mortality	Fipronil	1ppb [chronic oral]	Nosema	Vidau et al. 2011 PlosOne


In semi-field and field test

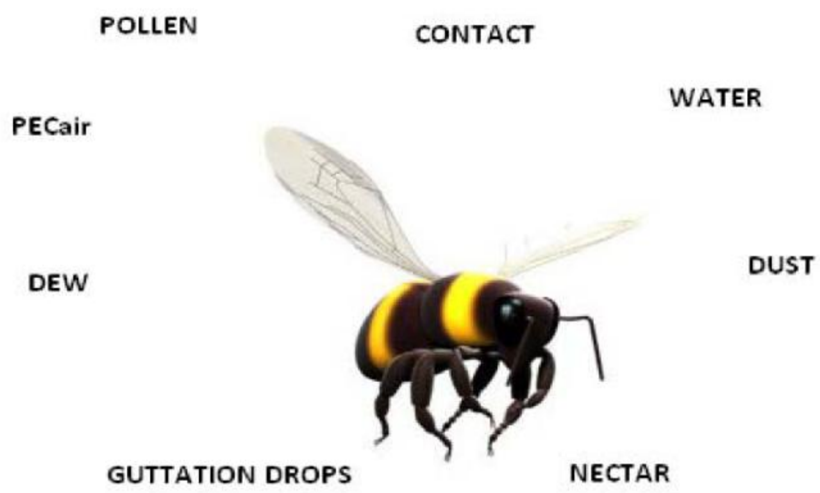
Effects on	Compound	Use	Conditions	References
Mortality rate	Clothianidin	Seed-dressing	Cages in a oilseed rape field	Sgolastra et al. 2012 B. Insect.
Mortality and foraging rate	Thiametoxam	Seed-dressing	Corn field during sowing	Tremolada et al. 2010 B. Env. Cont. Tox.
Foraging bees mortality	Imidacloprid	Seed-dressing	Corn field during sowing	Girolami et al. 2012 J. Appl. Ent.; Marzaro et al. 2012 B. Insect.
Mortality rate	Quinalphos	Spray application	Alfalfa	Porrini et al. 1991 Congress Proceeding, Bari
Mortality rate	Acephate	Spray application	Oilseed rape field	Sabatini et al. 1992 Congress Proceeding, Firenze
Colony size	Imidacloprid	Fertigation	Melon field	ApeNet report 2010 in publishing
Colony survival	Imidacloprid	High-fructose corn syrup	*In situ* study	Lu et al. 2012 B. Insect.






Question

- Are honey bee and other beehive matrices appropriate sentinels for monitoring anthropogenic contamination in the environment?

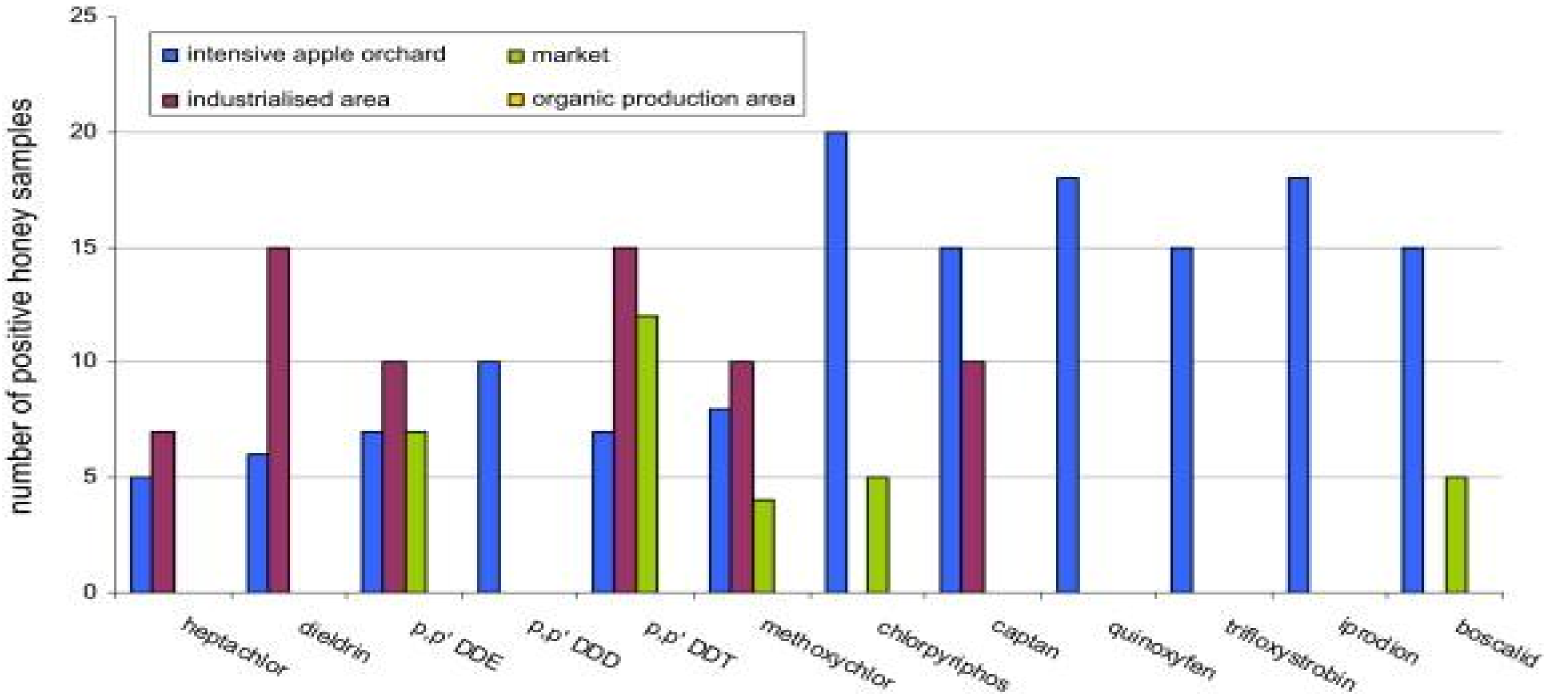


S. Panseri , A. Catalano , A. Giorgi , F. Arioli , A. Procopio , D. Britti , L.M. Chiesa

Occurrence of pesticide residues in ITALIAN honey from different areas in relation to its potential contamination sources

Food Control null 2013 Milan University

http://dx.doi.org/10.1016/j.foodcont.2013.10.024



The analysis of 72 honey samples produced in different geographic areas in order to screen and then relate the presence of pesticide residues to their potential contamination source confirming honey as a suitable indicator of environmental pollution as well as an indicator of the presence of pesticides utilised in crop protection management.

Pesticides detection frequency in honey samples in relation to their sampling area.

S. Panseri , A. Catalano , A. Giorgi , F. Arioli , A. Procopio , D. Britti , L.M. Chiesa

Occurrence of pesticide residues in ITALIAN honey from different areas in relation to its potential contamination sources

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http://dx.doi.org/10.1016/j.foodcont.2013.10.024

"The majority of honey samples (94%) contained at least one of the pesticides even if their concentrations were found to be lower than its MRL. DDT, DDD and DDE were the compounds isolated with higher frequency in honey samples produced in the industrialised area. Chlorpyrifos and quinoxyfen were the residues frequently detected in samples coming from the apple orchard area.
No residues were isolated in honey coming from the mountain area dedicated to organic production."

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Widespread Occurrence of Chemical Residues in Beehive Matrices from Apiaries Located in Different Landscapes of Western France

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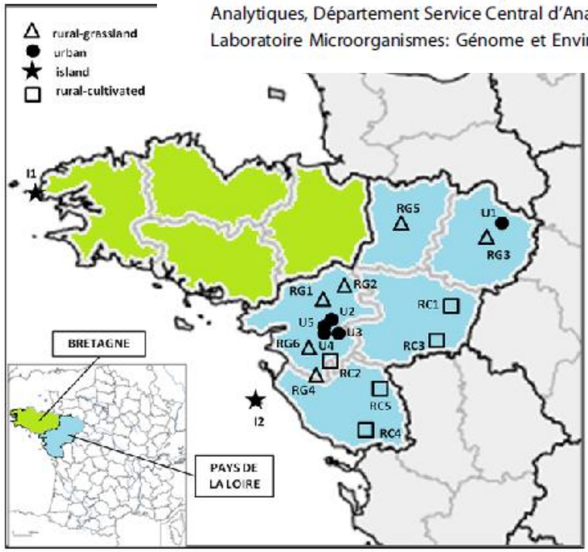

Location of the 18 surveyed apiaries.

Lambert O, Piroux M, Puyo S, Thorin C, et al. (2013) Widespread Occurrence of Chemical Residues in Beehive Matrices from Apiaries Located in Different Landscapes of Western France. PLoS ONE 8(6): e67007. doi:10.1371/journal.pone.0067007
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0067007

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Figure 3. Number of compounds in beehive matrices (honey bees, honey and pollen) detected according to the period.

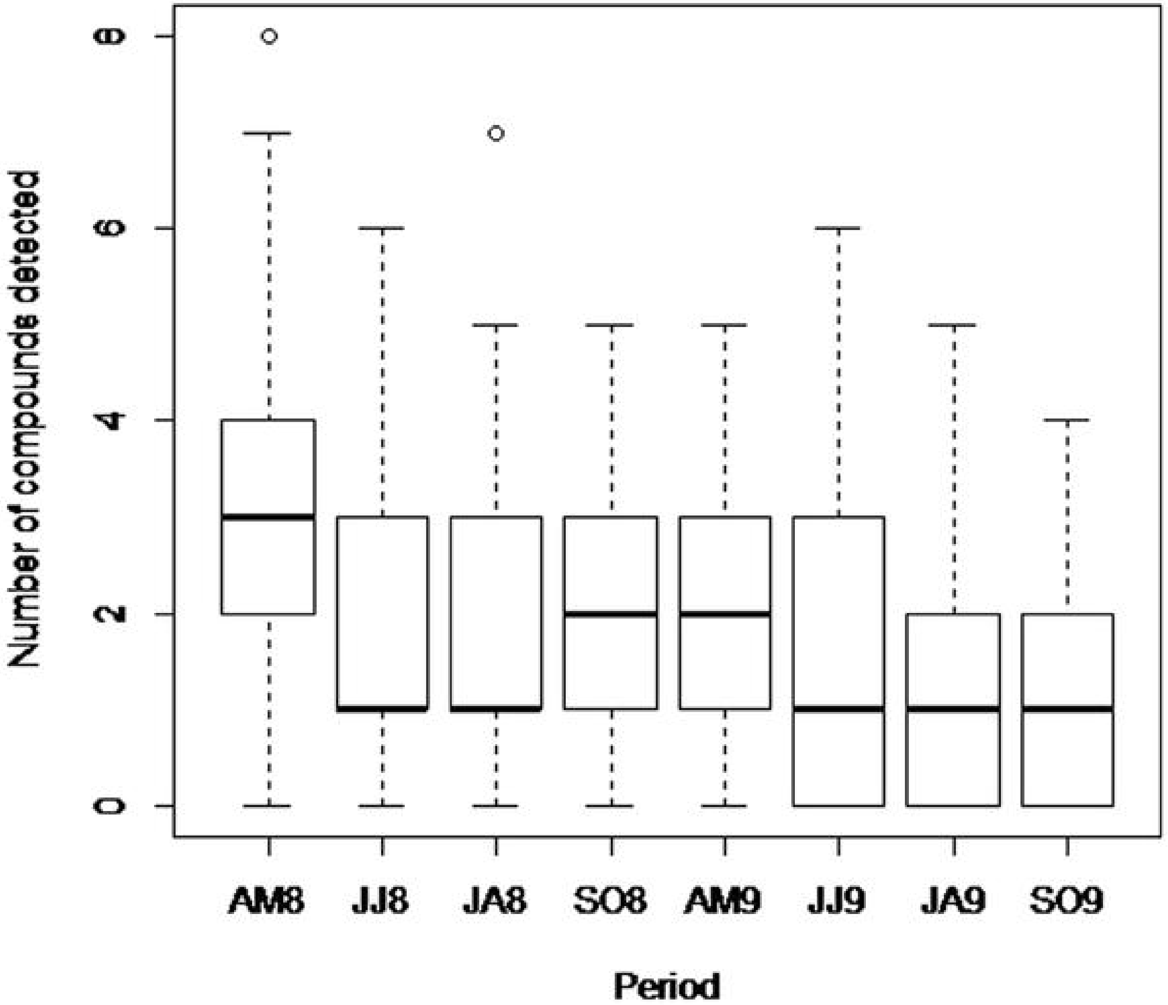

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Table 4. Summary of contaminant residues detections in pollen samples from western France honey bee colonies.

Compound	Class[1]	Effect[2]	%[3]	Detections				
				Min[4]	Max[4]	Mean[5]	Median[5]	SD[5]
Amitraz I	FORM	A	1.6	> LOD and < LOQ	115.20	24.14	23.15	8.67
Amitraz II	FORM	A	14.8	> LOD and < LOQ	129.40	7.39	4.10	13.82
Bupirimate	PYRI	F	0.8	> LOD	< LOQ	1.48	1.40	0.95
Carbaryl	CARB	I	7.8	> LOD and < LOQ	14.67	0.70	0.35	1.68
Carbendazim	CARB	F	34.4	> LOD and < LOQ	2595.00	24.31	0.05	229.47
Carbofuran	CARB	I	1.6	> LOD and < LOQ	2.30	0.22	0.20	0.19
Chlorpyrifos	OP	I	3.9	> LOD and < LOQ	139.50	5.61	4.00	12.53
Coumaphos	OP	A	3.9	> LOD and < LOQ	40.40	1.95	0.90	5.10
Cyprocanozole	TRIA	F	0.8	22.30	22.30	1.66	1.50	1.84
Dieldrin	OH	I	0.8	> LOD	< LOQ	5.0	4.90	1.09
Diethofencarb	CARB	F	0.8	2.60	2.60	0.32	0.30	0.32
Dimethoate	OP	I	0.8	> LOD	< LOQ	4.73	4.55	2.01
Flusilazole	TRIA	F	2.3	19.90	51.60	2.60	1.80	5.53
Imidacloprid	NEO	I	0.8	> LOD	< LOQ	1.35	1.30	0.53
Iprodione	DICA	F	0.8	> LOD	< LOQ	7.99	7.80	2.15
Phosmet	OP	I	7.4	> LOD and < LOQ	78.10	9.38	7.40	8.80
Piperonyl Butoxide	BENZ	I	0.8	> LOD	< LOQ	3.49	3.40	1.00
Pyriproxyfen	PHENP	I	4.7	> LOD	< LOQ	5.85	5.35	2.28
Tau-fluvalinate	PYRE	I	3.1	> LOD and < LOQ	85.42	3.52	2.30	8.31
Thiophanate-methyl	CARB	F	1.6	1395.00	3674.00	47.72	8.25	345.52
Triadimenol	TRIA	F	2.3	34.30	35.70	8.64	8.00	4.12
Triphenylphosphate	OP	I	9.4	> LOD	< LOQ	0.69	0.25	1.36
Vinclozolin	DICA	F	0.8	70.31	70.31	1.29	0.75	6.15

[1]Class: BENZ = benzodioxole; CARB = carbamate; DICA = dicarboximide; FORM = formamidin; NEO = neonicotinoid; OH = organohalogenus; OP = organophosphorus; PHENP = phenylpyrazole; PYRE = pyrethroid; PYRI = pyrimidin; TRIA = triazole.
[2]Effect: A = acaricide; F = fungicide; I = insecticide.
[3]n = 128 honey bee samples.
[4]Min = minimum in ng/g; LOD = limit of detection; LOQ = limit of quantification.
[5]Mean, Median and SD (standard deviation) were calculated taking into account all the analyzed samples.
doi:10.1371/journal.pone.0067007.t004

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Answer

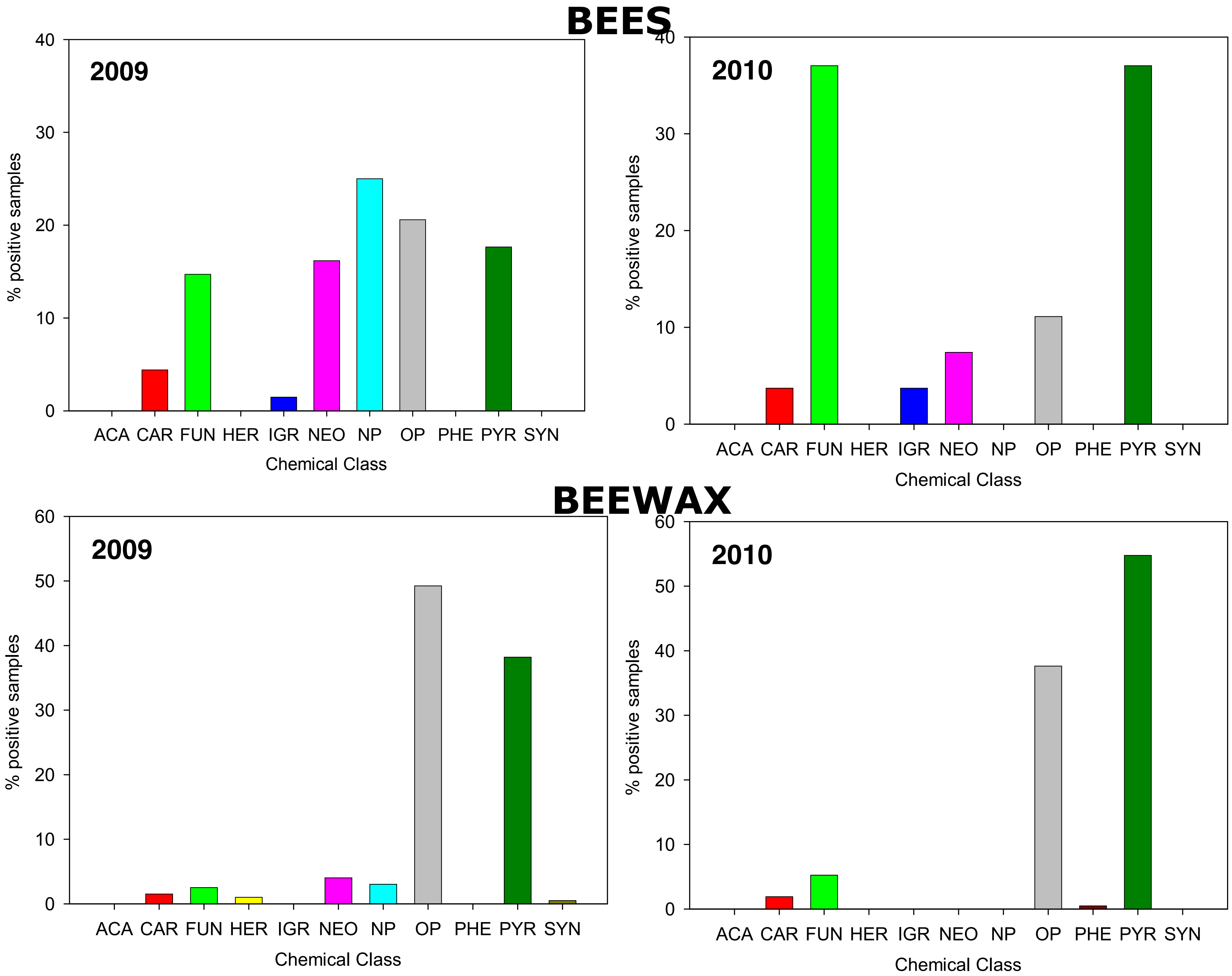
"Contamination of honey is strictly related to the contamination source and could reflect the specific pollution of a given environment, confirming honey bee and beehive matrices as appropriate sentinels for monitoring contamination in the environment"

Question

Fungicides are typically seen as fairly safe for honey bees. Is it thrue?

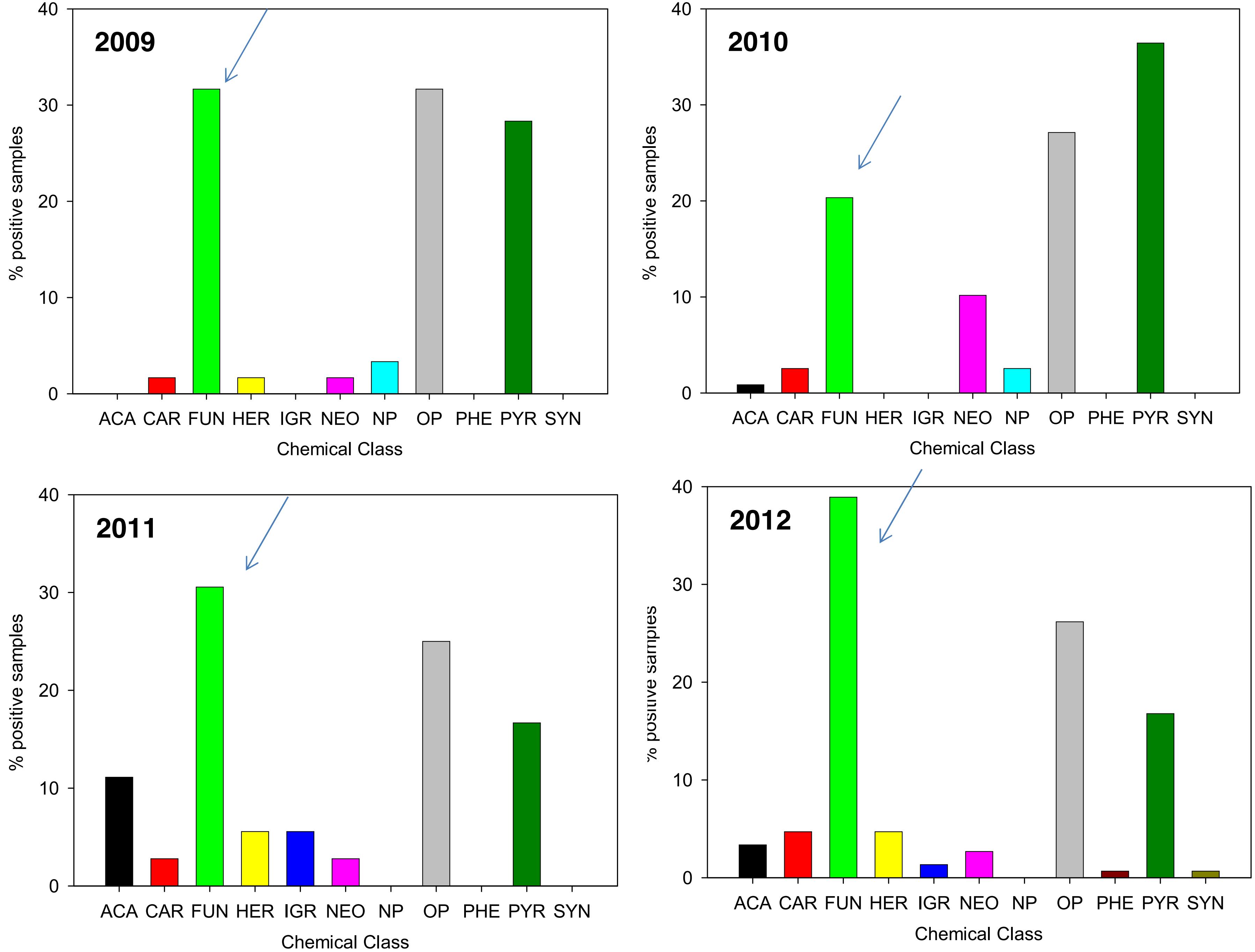
Chemical groups of a.i. found in BeeNet samples

ACA	Acaricide (not included in other classes) (e.g. Tebufenpyrad o Benzoximate)
CAR	Carbamate
FUN	Fungicide
HER	Herbicide
IGR	Insect Growth Regulator
NEO	Neonicotinoid
NP	Natural product (rotenone)
OP	Organophosphate
PHE	Phenylpyrazole
PYR	Pyrethroid
SYN	Synergist (piperonyl butoxide)



BEES
2009
2010
% positive samples
Chemical Class
ACA CAR FUN HER IGR NEO NP OP PHE PYR SYN
BEEWAX
2009
2010
% positive samples
Chemical Class
ACA CAR FUN HER IGR NEO NP OP PHE PYR SYN

BEEBREAD


2009
2010
2011
2012
% positive samples
0
10
20
30
40
ACA CAR FUN HER IGR NEO NP OP PHE PYR SYN
Chemical Class

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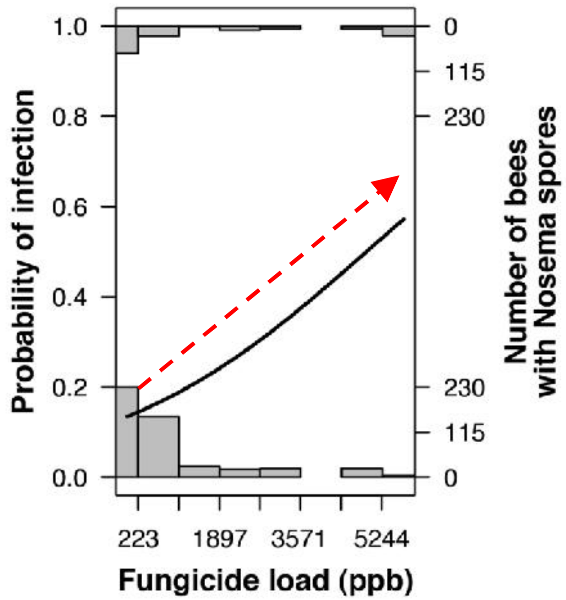
Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen *Nosema ceranae*

Jeffery S. Pettis[1], Elinor M. Lichtenberg[2], Michael Andree[3], Jennie Stitzinger[2], Robyn Rose[4], Dennis vanEngelsdorp[2]*

1 Bee Research Laboratory, USDA-ARS, Beltsville, Maryland, United States of America, **2** Department of Entomology, University of Maryland, College Park, College Park, Maryland, United States of America, **3** Cooperative Extension Butte County, University of California, Oroville, California, United States of America, **4** USDA-APHIS, Riverdale, Maryland, United States of America

"…we found an increased probability of Nosema infection in bees that consumed pollen with a higher fungicide load.
Our results highlight a need for research on sub-lethal effects of fungicides and other chemicals that bees placed in an agricultural setting are exposed to.»



Probability of Nosema infection increased with fungicide load in consumed pollen. The curve shows the predicted probability of Nosema infection.

Answer

- "Our results combined with several recent studies of specific pesticides' effects on Nosema infection dynamics indicate that a **detrimental interaction** occurs when honey bees are **exposed to both pesticides (fungicide) and Nosema**" *(Pettis et al., July 2013, Plosone)*

Question

- What happens if pesticide and pathogens interact for a negative synergy?
- How do field-relevant combinations and loads of pesticides affect **bee health?**

Synergic Effects

E.G: between stress agents e colony colaps

Interactions between diseases and susceptibility of bees to pesticides.

Proceedings of the National Academy of Sciences of the United States of America

PNAS

Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees

Gennaro Di Prisco[a], Valeria Cavaliere[b], Desiderato Annoscia[c], Paola Varricchio[a], Emilio Caprio[a], Francesco Nazzi[c], Giuseppe Gargiulo[b], and Francesco Pennacchio[a,1]

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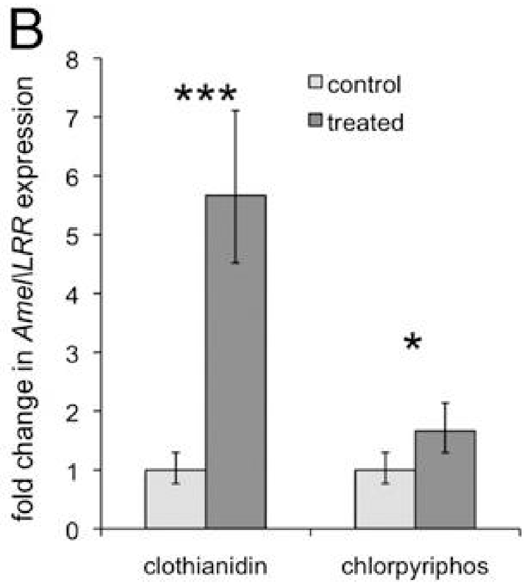
PNAS October 21, 2013



The immune-challenged larvae exposed to clothianidin showed an increased transcription of the gene Amel\LRR,

Clothianidin treatment significantly **reduced** the transcription level of apidaecin gene.

Synergic Effects

Impact of Clothianidin on the DWV virus replication

Edited by Gene E. Robinson, University of Illinois at Urbana-Champaign, Urb

Large-scale losses of honey bee colonies represent a poorly understood problem of global importance. Both biotic and abiotic factors are involved in this phenomenon that is often associated with high loads of parasites and pathogens. A stronger impact of pathogens in honey bees exposed to neonicotinoid insecticides has been reported, but the causal link between insecticide exposure and the possible immune alteration of honey bees remains elusive. Here, we demonstrate that the neonicotinoid insecticide clothianidin negatively modulates NF-κB immune signaling in insects and adversely affects honey bee antiviral defenses controlled by this transcription factor. We have identified in insects a negative modulator of NF-κB activation, which is a leucine-rich repeat protein. Exposure to clothianidin, by enhancing the transcription of the gene encoding this inhibitor, reduces immune defenses and promotes the replication of the deformed wing virus in honey bees bearing covert infections. This honey bee immunosuppression is similarly induced by a different neonicotinoid, imidacloprid, but not by the organophosphate chlorpyriphos, which does not affect NF-κB signaling. The occurrence at sublethal doses of this insecticide-induced viral proliferation suggests that the studied neonicotinoids might have a negative effect at the field level. Our experiments uncover a further level of regulation of the

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Answer

- *Di Prisco et al, October 2013 PNAS)* Clothianidin is able to depress (get down) the immune defence system of the honeybee, facilitating the DWV replication

Bee health

Spring-summer mortality
= poisoning

Winter mortality
= varroa/virus

Conclusions

- ✓ The monitoring units characterized by **different landscape** i.e. agricultural, urban, forest and humid areas according to the peculiarities of the regional territory

- ✓ Confirmed the enzootic condition of ***N. ceranae*** in Italy, with low spore concentration (in 2012, only in 3.4% of the samples with more than 10 million *N. ceranae* spores per bee)

- ✓ Systematically investigated the presence of **viruses** (DWV, ABPV, CBPV), their quantification and geographic distribution as well as **varroa** infestation

- ✓ Contributed to the knowledge of **beebread** nutritional value and the contamination level of residues (pesticides, acaricides and neonicotinoids).

Conclusions

- ✓ Contributed to quantify annual and Winter **colony losses** (Mean annual mortality and Winter mortality were remarkably lower in 2012 than those recorded in Italy during the previous years through Coloss and Apenet monitoring (19-37%).

- ✓ Contributed to create a **database** on honey bee colonies development and health condition in Italy

- ✓ The **B**ee **E**mergency **S**ervice **T**eam established with the Apenet project (2009-2010) has been reinforced and is active at national level for field intervention, samples and data collection and epidemiological investigation in case of mortality report.

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- Italian Beekeepers and their Associations
- Ministry of Agriculture Food and Forestry

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Thank you for your attention

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