“Honey bee colony disorder in crop areas: the role of pesticides and viruses”

Noa SIMON-DELSO

Noa Simon-Delso¹,², Gilles San Martin³, Etienne Bruneau¹, Laure-Anne Minsart¹, Coralie Mouret¹, Louis Hautier³

¹ Centre Apicole de Recherche et d’Information
² Utrecht University
³ Centre wallon de Recherches Agronomiques
First objective of the study

Honey bee Colony disorders

Virus

Pesticides
(acaricides, insecticides, fungicides, herbicides)
Second objective of the study

Honey bee Colony Disorders

Agricultural Land Use
Honey bee disorders?

Include several unspecific clinical symptoms for which no causal agent can be identified:

1. dead and disappeared colonies:
2. weak colonies
3. queen loss
4. problems linked to brood and not related to any known disease

Discard:

1. pesticides (acute intoxication)
2. Varroa destructor
3. starvation
(1) Link between disorders and virus – pesticide residues
**Strategy**

The condition of honey bee colonies was follow-up 3 times: 2 before and 1 after the winter with the help of questionnaires and clinical visits done by beekeeping technicians.

At each visit, samples were collected for residues and viral analysis: wax, honey, beebread, honeybees.

On basis of field observations, set up of two groups ("with disorders" and "healthy" group), to constitute the group of colonies whose samples will be analyzed.

Compare the content of virus and residues between the two groups.
Selection of beekeepers and colonies

• Need more than 300 colonies (prevalence rate of disorder ~ 10-30%\(^1\) and statistical analysis with model requires \(n \sim 30\))

• Request of voluntary beekeepers to participate in the study, fulfilling 3 criteria:
  1) have a minimum of 5 production colonies per apiary in June 2011;
  2) regularly follow up the health status and development of their colonies;
  3) apply good beekeeping practices: monitor the varroa infestation level and carry out a varroa control

• Five well-developed colonies selected from each apiary

\(^1\)CARI 2003-2004, Gembloux Agro-Bio Tech 2008-2009, 2009-2010
Selection of beekeepers and colonies

July 2011 (n=330 colonies & 66 apiaries)

- Ruchers suivis (n= 66 ruchers & 330 colonies)
Data collection

• Apiaries were visited by beekeeping technicians and paper questionnaires were filled at each visit: (1) mid-July to mid-August 2011, (2) mid-September to mid-October 2011, (3) March to April 2012

• Specific questionnaires for beekeeper, apiary and colony

• Clinical inspection of the colony
  • Information on the queen
  • Varroa treatment
  • Colony status (possible clinical symptoms): mortality, weakness, brood problems, queen loss, diarrhoea, deformed wings, …
  • Level of reserves
Matrix sampling

- **3 Sample periods:** (1) mid-July to mid-August 2011, (2) mid-September to mid-October 2011, (3) March to April 2012

- **Honey bees**
  100 alive bees from brood frames

- **Beebread, honey, wax**
  cutting of frames: 10x10cm or 5x20cm
  (100 cm²)
Apiaries with matrices and fully completed forms

April 2012 (n=173 colonies & 35 apiaries)
173 colonies

Selection according the availability
Honey bees / honey / beebread Period 1 & 2 (n=107)

Clustering
Symptoms (n=75)
1) mortality
2) weakening
3) brood problem
4) queen loss

Clustering
Colony condition (n=75)
1) Colony treated against varroa
2) Food reserves before and after the winter
3) drone-laying queens
1) Food reserve before the winter : B1/B2/B3
2) Year of creation
3) Queen color

Selection for analyses
25 colonies with disorders
29 healthy colonies

Virus & pesticides residues analyses
Virus analyses

Quantitative RT-PCR by the National Bee Unit laboratory, Food and Environment Research Agency (Sand Hutton, York, United-Kingdom).

1. Black Queen Cell Virus (BQCV)
2. Chronic Bee Paralysis Virus (CBPV)
3. Acute Bee Paralysis Virus (ABPV)
4. Deformed Wings Virus (DWV)
5. Sac Brood Virus (SBV)
Pesticide analyses

Eurofins Chemiphar NV, Brugge, Belgium and analysed by SOFIA GmbH Chemisches Labor für Softwareentwicklung und Intelligente Analytik, Berlin, Germany.

Two multi-residues methods (SF146 and SF150) were used searching pesticides residues:

- 99 residues in wax (54 samples),
- 93 residues in bee bread (108 samples)
- 96 residues in honey (107 samples – one sample did not contain enough matrix)
# Pesticide analyses

| Active ingredient or metabolite* | Class | LOQ (mg/kg) |
|---------------------------------|-------|-------------|
|                                 |       | Wax | Beebread | Honey |
| 2,4-D                           | H     | 0.1 | -       | 0.01  |
| Abamectin                       | I     | 0.1 | 0.1     | 0.005 |
| Acetamiprid                     | I     | 0.1 | 0.1     | 0.005 |
| Aldicarb                        | I     | 0.1 | 0.1     | 0.005 |
| Alpha-cypermethrin              | I     | 0.1 | 0.1     | 0.005 |
| Amitraz                         | A     | 0.1 | 0.1     | 0.01  |
| Azoxystrobin                    | F     | 0.1 | 0.1     | 0.005 |
| Bentazon                        | H     | 0.2 | -       | 0.02  |
| Benthiavalicarb                 | F     | 0.1 | 0.1     | 0.005 |
| Beta-cyfluthrin                 | I     | 0.1 | 0.1     | 0.01  |
| Bifenthrin                      | I     | 0.1 | 0.1     | 0.005 |
| Boscalid                        | F     | 0.1 | 0.1     | 0.005 |
| Captan                          | F     | 0.1 | 0.1     | 0.01  |
| Carbaryl                        | I     | 0.1 | 0.1     | 0.005 |
| Chlorpyriphos                   | I     | 0.1 | 0.1     | 0.005 |
| Chlorpyriphos-methyl            | I     | 0.1 | 0.1     | 0.005 |
| Chlorothalonil                  | F     | 0.1 | 0.1     | 0.005 |
| Clothianidin                    | I     | 0.1 | 0.1     | 0.01  |
| Coumaphos                       | A     | 0.1 | 0.1     | 0.05  |
| Coumaphos oxon *                | A     | 0.1 | 0.1     | -     |
| Coumaphos phenolic*             | A     | 0.1 | 0.1     | -     |
| Cyazofamid                      | F     | 0.2 | 0.2     | 0.02  |
| Cyfluthrin                      | I     | 0.1 | 0.1     | 0.01  |
| Cymoxanil                       | F     | 0.1 | 0.1     | 0.01  |
| Cypermethrin                    | I     | 0.1 | 0.1     | 0.005 |
| Cyproconazole                   | F     | 0.1 | 0.1     | 0.005 |
| Cyprodinil                      | F     | 0.1 | 0.1     | 0.01  |
| DDT                             | I     | 0.1 | 0.1     | 0.005 |
| Deltamethrine                   | I     | 0.1 | 0.1     | 0.005 |
| Dichlorprop-P                   | H     | 0.1 | -       | 0.01  |
| Difenoconazole                  | F     | 0.1 | 0.1     | 0.005 |
| Diflubenzuron                   | I     | 0.2 | 0.2     | 0.02  |
| Dimethenamid-P                  | H     | 0.1 | 0.1     | 0.005 |
| Dimethoate                      | I     | 0.1 | 0.1     | 0.005 |

**43 insecticides**

**Acaricides & metabolites**

**41 fungicides**

**14 herbicides**

**1 synergist**
Statistics

DESCRIPTIVE ANALYSES - Linear model (two way ANOVA)

> dependent variable: Ct or number of residues

> explanatory variables: visit (first or second), group (with disorder or healthy) or type (fungicide or insecticide/acaricide) and their interaction

RELATIONSHIP BEE COLONY DISORDERS-POTENTIAL STRESSORS – GLM models (with binomial distribution and logit link function)

> dependent binary variable: group (with disorder or healthy)

> explanatory variables: total number of fungicides, insecticides-acaricides, viruses (visits 1 & 2)

> random effect: apiary

Likelihood Ratio (LR) - Tests to evaluate the significance of the explanatory variables
(2) Link between disorders and land use
Spatial analysis

- Land Parcel Identification System (CAP)
- Geographical Coordinates of Apiaries
- Delimitation of « buffer » around apiary: 1.5 km
- Calculation of crop and grassland surfaces
- Statistical analysis
  
  Disorder ~ surf. crops * surf. grassland
Spatial analysis around 3 apiaries

|     | Grasslands (ha) | Crops (ha) |
|-----|-----------------|------------|
| 40A | 8.4             | 20.9       |
| 40B | 5.8             | 18.4       |
| 40C | 6.0             | 23.8       |
RELATIONSHIP BEE COLONY DISORDER-APIARY ENVIRONMENT - GLM models (with binomial distribution and logit link function)

> dependent binary variable: group (with disorder or healthy)

> explanatory variables: surface of grasslands and crops *sensu lato* (cereals, potatoes, beet, oilseed rape, maize, flax, ...) and fruit, vegetable, fodder, horticulture productions

> random effect: apiary

Likelihood Ratio (LR) - Tests to evaluate the significance of the explanatory variables
Main results
Observed disorders

- Rucher sans aucun dépérissement (n=7)
- Rucher avec des dépérissements (n=14)

n=29 healthy
n=24 with disorders (+ 5A4 mortality)
Detected virus (National Bee Unit, UK)

- Molecular analysis (RT-PCR) on 53 samples
- 3 dominant virus: DWV, BQCV, SBV
- No significant difference between with disorders and healthy:
  - DWV: $p = 0.731$
  - BQCV: $p = 0.373$
  - SBV: $p = 0.54$
Content of residues

With disorders vs. healthy

- Significantly more fungicides residues \( (p = 0.008) \), 2 times more!

- No significant difference for acaricides/insecticides \( (p = 0.402) \)
Fungicide residues
Insecticide/acaricide residues
Disorders ~ virus*pesticides

- Significant positive correlation between the number of fungicides residues and the disorder
- No significant correlation between viral charge or residues of insecticides-acaricicides
- No interactions
  > fungicides*virus
  > insecticides-acaricicides*virus
  > fungicides*insecticides-acaricicides

|                  | LR   | df | p(>Chisq) |
|------------------|------|----|-----------|
| totfungicides    | 7.128| 1  | 0.008     |
| totinsaca        | 0.005| 1  | 0.943     |
| totvirus         | 0.136| 1  | 0.712     |
| totfungicides:totvirus | 2.222| 1  | 0.136     |
| totinsaca:totvirus | 0.975| 1  | 0.323     |
| totfungicides:totinsaca | 0.901| 1  | 0.342     |
Disorder $\sim$ agricultural land use

Disorder probability:

- with the surface of crop ($LR = 8.052$, $df = 1$, $p = 0.0045$)
- with the surface of grassland ($LR = 14.527$, $df = 1$, $p = 0.0001$)

=> Apiaries surrounded by a crop environment have more probability to have a disorder!
Conclusions

There is a significant positive correlation between the appraisal of bee disorders and

1) the fungicides residues

2) the surface of crops around apiaries

These results questioned the:

• General acknowledgement of the non-toxicity of fungicides on honey bee colonies

• Compatibility between the intensive agricultural model and pollinators well-being
Thanks to

Beekeeping technicians & beekeepers

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| Active ingredient | Group | LOQ (mg/kg) | Number of samples (n=54) | Residues amount (mg/kg) | LOQ (mg/kg) | Number of samples (n=108) | Residues amount (mg/kg) | LOQ (mg/kg) | Number of samples (n=107) | Residues amount (mg/kg) |
|-------------------|-------|-------------|--------------------------|-------------------------|-------------|--------------------------|-------------------------|-------------|--------------------------|-------------------------|
| Amtraz            | D     | 0.1         | < LD Detected Quantified | Range Mean s.d.         | 0.1         | < LD Detected Quantified | Range Mean s.d.         | 0.01        | 49 - 1                   | 0.022 - 0.02           |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Boscalid          | D     | 0.1         | 16 8 1 0.29 0.29         | 39 7 4 0.4-1.3 0.68    | 0.005       | 46 - 4                   | 0.005-0.026 0.01       | 0.01        |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Captan            | D     | 0.1         | 24 - 1 3.1              | 48 - 1 1.9 1.9         | 0.01        |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Carbaryl          | D     | 0.1         | - - - -                  | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Chlorpyriphos     | D     | 0.1         | 25 -                  | 54 -                   | 0.005       | 56 - 1                   | 0.058       | 0.06 -                   |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Chlorothalonil    | D     | 0.1         | 24 - 1                | 58 -                   | 0.005       |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Coumaphos         | D     | 0.1         | 14 9 2 0.32-0.34 0.33   | 48 - 1                  | 0.05        | 49 - 1                   | 0.012       | 0.01 -                   |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Cyprodimil        | D     | 0.1         | 22 3                   | 0.1                     | 0.01        |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Fenpropimorph     | D     | 0.1         | 24 1                   | 0.1                     |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Indoxacarb        | D     | 0.1         | 22 1                   | 0.1                     |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Iprodione         | D     | 0.1         | 22 1                   | 0.24-1.5 0.87 0.89     | 0.1         | 45 - 4                   | 0.34-1.5 0.90 0.48      | 0.005       | 48 - 2                   | 0.017-0.022 0.02       |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Piperonyl butoxide| D     | 0.1         | 21 4                   | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Propamocarbe      | D     | 0.1         | - - -                  | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Pyraclostrobin    | D     | 0.1         | - - -                  | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Pyrimethanil      | D     | 0.1         | 21 4                   | 0.1                     |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Tau-fluvalinate   | D     | 0.1         | 12 9 4 0.29-0.46 0.4   | 0.1                     |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Tebuconazole      | D     | 0.1         | 24 1                   | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Tebufenozide      | D     | 0.1         | 23 2                   | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Terbutylazine     | D     | 0.1         | 24 1                   | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Thiadiazofos      | D     | 0.1         | - - -                  | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Thiophanate-methyl| D     | 0.1         | - - -                  | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Trifloxystrobin   | D     | 0.1         | 23 2                   | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
| Zoxamide          | D     | 0.1         | - - -                  | -                       |             |                         |                         |             |                         |                         |
|                   | H     |             |                          |                         |             |                          |                         |             |                         |                         |
Hierarchical clustering?

**Why**: in order to build groups with the lowest variation within the group and the highest variation between group (see Zuur *et al.* 2007)

**How**: Ward aggregation method