Project "MELISSA"

μέλισσα

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3rd Latin American Pesticide Residue Workshop Food and Environment
Montevideo, URUGUAY, May 8 - 11, 2011

www.ages.at Austrian Agency for Health and Food Safety (AGES)
What have maize, insecticides and the western corn rootworm to do with bees?
The “Billion Dollar Bug”

Importance of maize cultivation in Austria

approx. 300,000 ha cultivated (22 % arable land)

major part of the total feed cereals production

Maize roots and plants damaged by corn rootworm
Western corn rootworm in Austria

Imported from North America to Eastern Europe - beginning of the 1990s
Infestations in Austria since 2001

Distribution of western corn rootworm in Austria, 10/2009

number of trapped beetles
- 0
- 1 - 10
- 11 - 100
- 101 - 1000
- 1001 - 10000

G. Grabenweger und M. Schwarz, AGES; Monitoring-Daten: Amtliche Pflanzenschutzdienste der Bundesländer

www.ages.at Austrian Agency for Health and Food Safety (AGES)
Bees and maize growing 2009

Distribution of records of beeyards with or without suspected bee poisoning

Maize 2009
suspected poisoning

- yes
- no

24 beekeepers
28 beeyards, 618 hives

40 beekeeper
(51 beeyards, 1244 hives)

Each symbol represents one or more incidents per district.
2009 Incidents of suspected bee poisoning in Austria

Suspected causes

- intentional or undefined poisoning of bee colonies
- spray applications at bloom against horse chestnut leaf mining moth
- sowing of maize, oilseed rape
Are insecticidal seed dressings putting our honeybees at risk?

Yes!

Massive bee damage related to sowing of insecticide treated maize in 2008 in DE, I, SLO through dust emission on flowering plants

Insufficient quality of seed dressings and subsequent emission of abraded dust particles into environment with pneumatic (vacuum) sowing machines
Records of suspected poisoning incidents in maize areas

Correlation between poisoning incidence and maize sowing period

Overlapping of the maize sowing period and the spring bloom period of fruit trees, dandelion, hedges and meadows
Project “MELISSA”

“Investigations in the incidence of bee losses in corn and oilseed rape growing areas of Austria and possible correlations with bee diseases and the use of insecticidal plant protection products”

Project initiator: BMLFUW Ministry for agriculture, environment and water management
duration: 2009 – 2010

Extension until 31.01.2012
What are the objectives?

- **Documentation** of bee damage incidents
- **Investigation** / analysis of causes
- **Development** and **validation** of **methods**
  for the analysis of residues in bee products for the most frequently used insecticides
- **Evaluation of results**
- **Implementation of measures** to avoid bee losses
Bee losses 2010 in Austria

Increased bee mortality in front of the hives

Clothianidin + Chlorpyriphos + Fipronil in bees detected

Clothianidin + Chlorpyriphos in bees detected
What were the observed symptoms in bees and bee colonies?

Dead, trembling, flightless bees, crawlers, disturbed coordination

(A) clustered groups of bees in the grass in front of the hives

(B) detectable residues of Clothianidin (0.003 mg/kg)

(C) no residues found
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Examination of bee samples for pathogens / parasites

not detectable:
- Israel acute paralysis virus (IAPV)
- Chronic bee paralysis virus (CBPV)
- Kashmir bee virus (KBV)
- Tracheal mite
- Malpighamoeba

detectable (in some samples):
- Nosema ceranae
- Black Queen Cell Virus (BQCV)
- Acute bee paralysis virus (ABPV)
- Deformed Wing Virus (DWV)
- Sacbrood virus (SBV)
- Varroa destructor mites

observed symptoms - not linked to pathogens or parasites
Analysis of insecticides

Determination of residues

- Clothianidin
- Thiamethoxam
- Imidacloprid
- Fipronil and Fipronil sulfon

Bees

Plants

Honey

Processed pollen – made by bees

From the neighbourhood of maize fields

Bee bread
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Development and validation of methods

**QuEChERS**

- 2.5 g sample
- Extraction with acetonitrile

- Addition of MgSO₄, NaCl
- Buffering citrate salts (pH 5-5.5)

- Centrifugation

- Clean-up / organic phase (aliquot)
  - by freezing out any oil/wax/fat
  - dispersive SPE (PSA, MgSO₄ and C18-material)

Method validation with fortified samples at:

- LOQ (0.001 mg/kg)
- 10 x LOQ (0.01 mg/kg)
- 100 x LOQ (0.10 mg/kg)

Mean recovery 70 to 110 %,
RSD ≤ 20 %.

LOQ of 0.001 mg/kg

- Formic acid / water
- LC-MS/MS
Development and validation of methods

Agilent 1200 Series HPLC system

Waters Atlantis T3 C18 column
(length: 150 mm, i.d.: 3.0 mm, particle size: 3 µm)

Applied Biosystems MDS Sciex API 4000 triple quadrupole LC-MS/MS system
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Results: Residue analysis of bees

Decrease 2009-2010
Clothianidin 81% to 51%

Increase 2009-2010
Fipronil (+Met) 11% to 22%

Clothianidin
0.001 – 0.0141 mg/kg

Thiamethoxam
0.0012 – 0.1704 mg/kg

Fipronil + Metabolite
0.0011 – 0.07716 mg/kg
Results: Residue analysis of plants

Decrease 2009-2010
Clothianidin 86 to 35 %
Thiamethoxam 50 to 18 %

Indication for dustdrift

- Clothianidin
  0.0016 – 2.648 mg/kg
- Thiamethoxam
  0.0023 – 0.0122 mg/kg
- Imidacloprid
  0.001 – 0.035 mg/kg
Results: Residue analysis of bee bread

Detection of neonicotinoids

- Clothianidin: 0.0011 – 0.0060 mg/kg
- Thiamethoxam: 0.001 – 0.0052 mg/kg
- Imidacloprid: 0.0011 mg/kg

No further investigation

- Fipronil: 0.0011 mg/kg
- Fipronilsulfon: 0.0011 mg/kg

after the cultivation of maize, from apiaries with suspected poisoning
Risk assessment for bees

Clothianidin\(^1\)

Acute oral toxicity: \(LD50 = 0.00379 \, \mu g \text{ Clothianidin/bee}\)
Acute contact toxicity: \(LD50 = 0.04426 \, \mu g \text{ Clothianidin/bee}\)

\(LD50 = \text{median lethal dose (24h)}\)

1 kg bees \(\sim\) 10 000 bees
0,0141 mg/kg in bee sample \(\sim\) 0.00141 \(\mu g \text{ Clothianidin/bee}\)

\[\rightarrow\] 37 % of LD50 for acute oral toxicity

0,006 mg/kg in beebread \[\rightarrow\] acute and chronic risk

\(^1\) Clothianidin SANCO/10533/05 – Final 18 January 2005
Results: Residue analysis of honey

2009: 8 samples
2010: 13 samples

Apiaries with suspected poisoning

+ 49 samples from food safety authorities

Results:

No residues found
Clothianidin, Thiamethoxam, Imidacloprid

LOQ of 0.002 mg/kg
Results: Residue analysis with multimethod

- **bee samples** (62 samples)
  - Chlorpyrifos, Phosalone (toxic to bees) 0,01 - 0,018 mg/kg
  - Thiacloprid, Azoxystrobin, Bitertanol, Fludioxonil, Mepanipyrim, Metamitron, Metolachlor, Propiconazole, Prosulfocarb, Spiroxamn, Terbutylazin

- **plant samples** (10 samples blooming plants; apple blossom)
  - Dimethoat, Cypermethrin, Chlorpyrifos, Chlorpyrifos-methyl, Deltamethrin (toxic to bees) 0,01 - 1,1 mg/kg
  - Thiacloprid, Boscalid, Dimethomorph, Fludioxonil, Metconazole, Pendimethalin, Pyraclostrobin, Tebuconazol

- **Bee bread** (10 samples)
  - Chlorpyrifos, Chlorpyrifos-methyl, Chlorfenvinfos (toxic to bees) 0,03 - 0,086 mg/kg
  - Acetamiprid, Kresoxim-methyl, Cyprodinil, t-Fluvalinat, Tebuconazol, Thiaclorid, Trifloxystrobin, Benalaxyl, Metolachlor, Prosulfocarb
Guttation drops and bees

Guttation droplets were observed regularly:

- on young maize plants
- on other plants in maize fields
Guttation drops and bees

Fluid drop in the leaf axil of a maize plant, sucked up by a bee

Analytical results from a collective sample of corn stalk residues of Thiamethoxam (systemic action)

Analytical results from dead bees and bee bread no residues detected

Day with observed high bee mortality

Pictures: beekeeper 07/2010
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Risk-Minimising Strategies to protect honey bees

1. For insecticidal seed dressings

Improving the abrasion resistance by the use of stickers (criteria for quality - Heubach-test)

< 0,75 g abrasion/100 000 seeds

COMMISSION DIRECTIVE 2010/21/EU
Risk-Minimising Strategies to protect honey bees

2. Use of vacuum systems for insecticides treated seeds only when the exhaust air pipes were modified with proved air deflectors (wind speed < 5 m/s)

User protection  protection of the environment
Risk-Minimising Strategies to protect honey bees

3. Regulatory measures for seed dressing labelling, sowing etc. were extended
   - Cautious handling of the seed bags to avoid dust formation
   - Strict avoidance of dust drift into adjacent areas (wind speed)
   - Use of adequate seed drilling equipment
   - Ensure a high degree of incorporation in soil (minimisation of spillage)
   - No use of treated seeds after crop rotation / without infestation
Summary

Bees and agriculture interact closely
Protection of bees
   precondition for the success of beekeepers and farmers
Plant pests
   threat for cultivation of different crops
Safety requirements
   insecticide treated seeds - effective plant protection measure
Results of 2009/2010
   need for further improvement
   information of farmer / application of pesticides

Create awareness for bee protection
   avoid beelosses due to pesticide residues
Acknowledgments

Project management

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