Project "MELISSA"

μέλισσα

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

? ? ? ……..?

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)

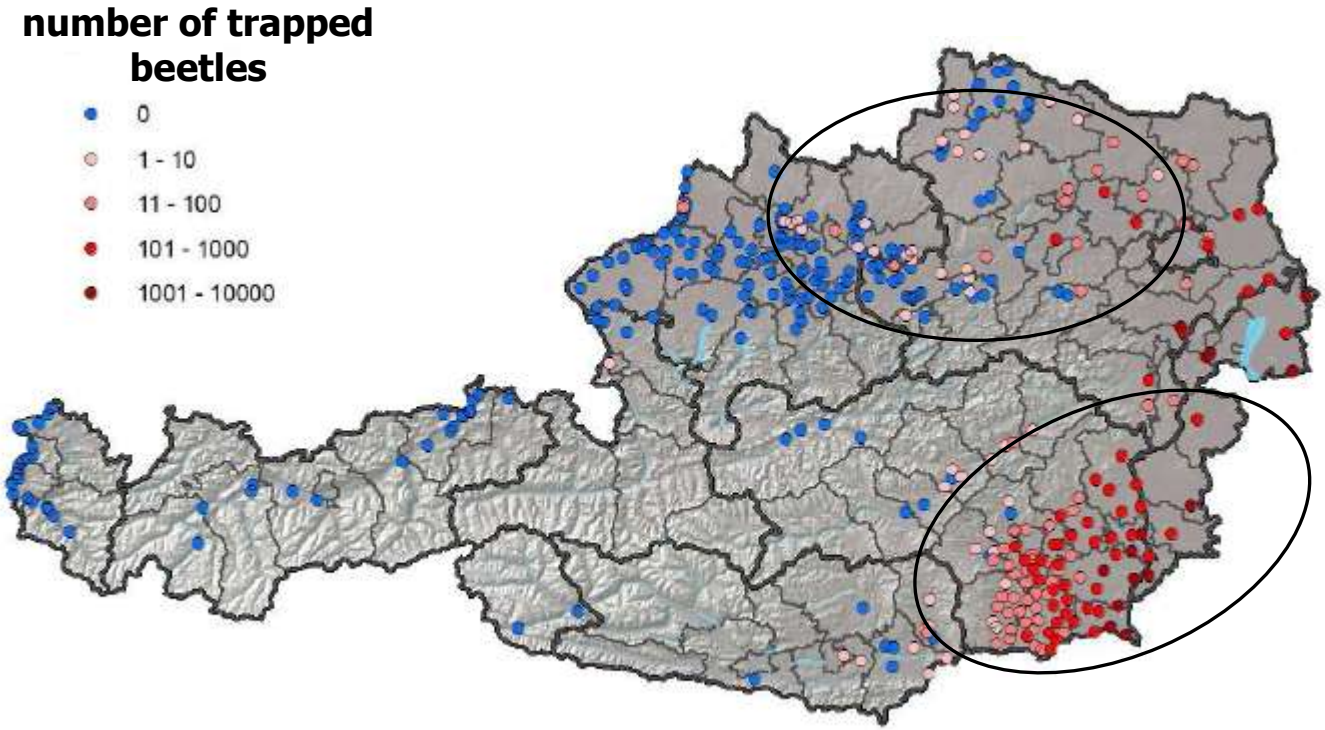
major part of the total feed cereals production

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

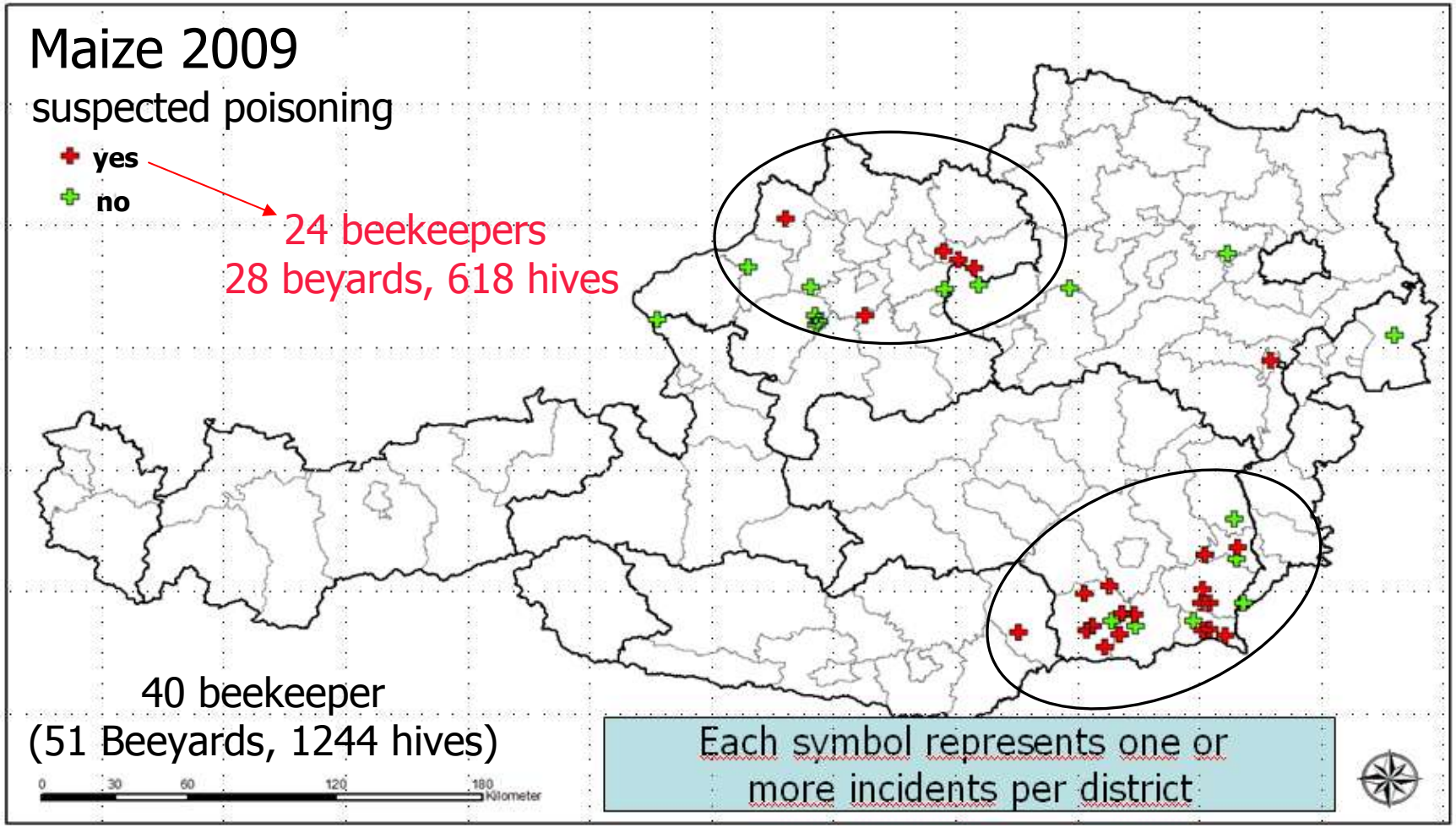
Distribution of western corn rootworm in Austria, 10/2009



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

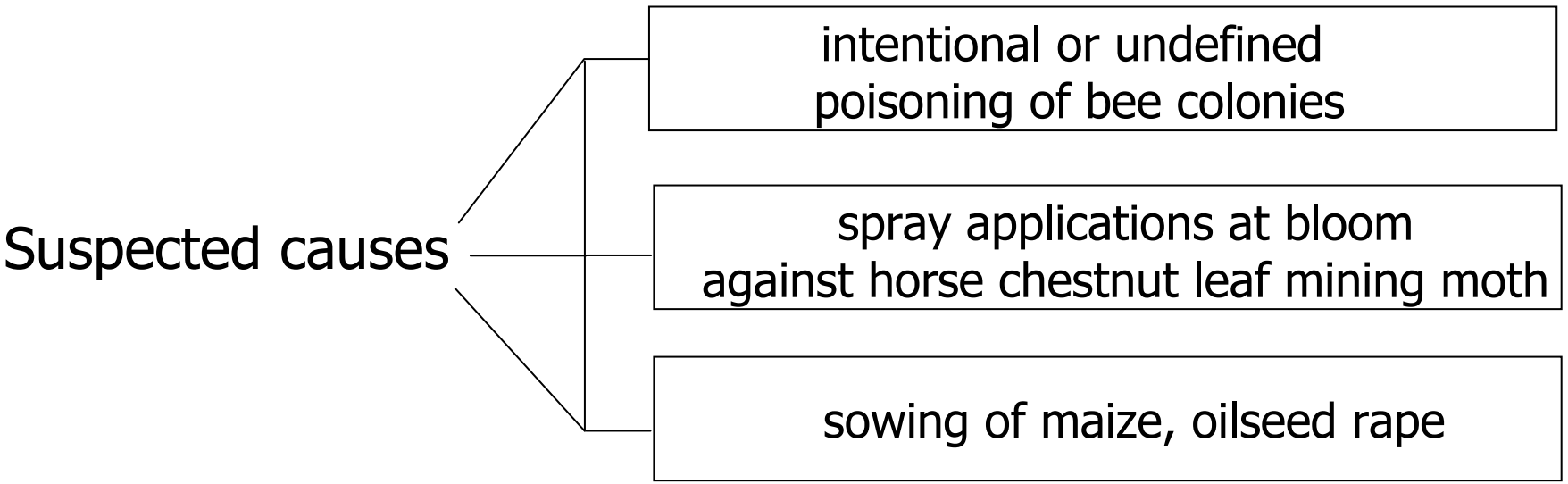
Bees and maize growing 2009

Distribution of records of beeyards with or without suspected bee poisoning



Starting Point for Melissa

2009 Incidents of suspected bee poisoning in Austria



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

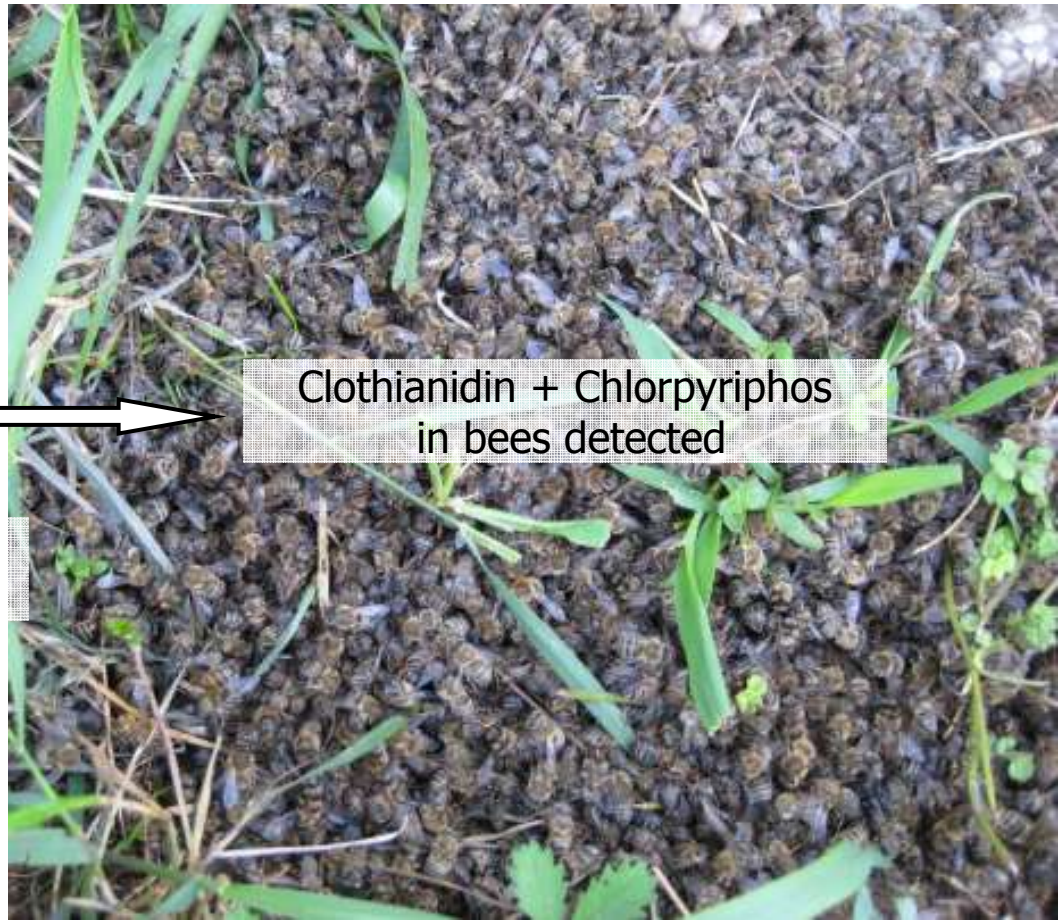
What are the objectives?

- **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

Clothianidin + Chlorpyriphos + Fipronil
in bees detected



Clothianidin + Chlorpyriphos
in bees detected

Increased bee mortality in front of the hives

What were the observed symptoms in bees and bee colonies?

Dead, trembling, flightless bees, crawlers, disturbed coordination

(A)

(B)

(C)

(A), (C) detectable residues of Clothianidin (0,003 mg/kg)
(B) no residues found

clustered groups of bees in the grass in front of the hives

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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

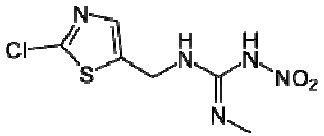
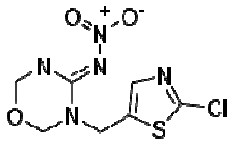
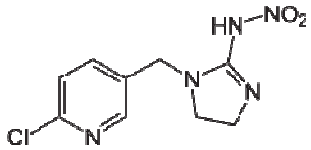
detectable (in some samples):

- *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

From the neighbourhood of maize fields

Plants

Honey

Processed pollen – made by bees

Bee bread

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Development and validation of methods

QuEChERS

2,5 g sample
Extraction with acetonitrile

addition of $MgSO_4$, 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)

formic acid / water

LC-MS/MS

method validation with fortified samples at

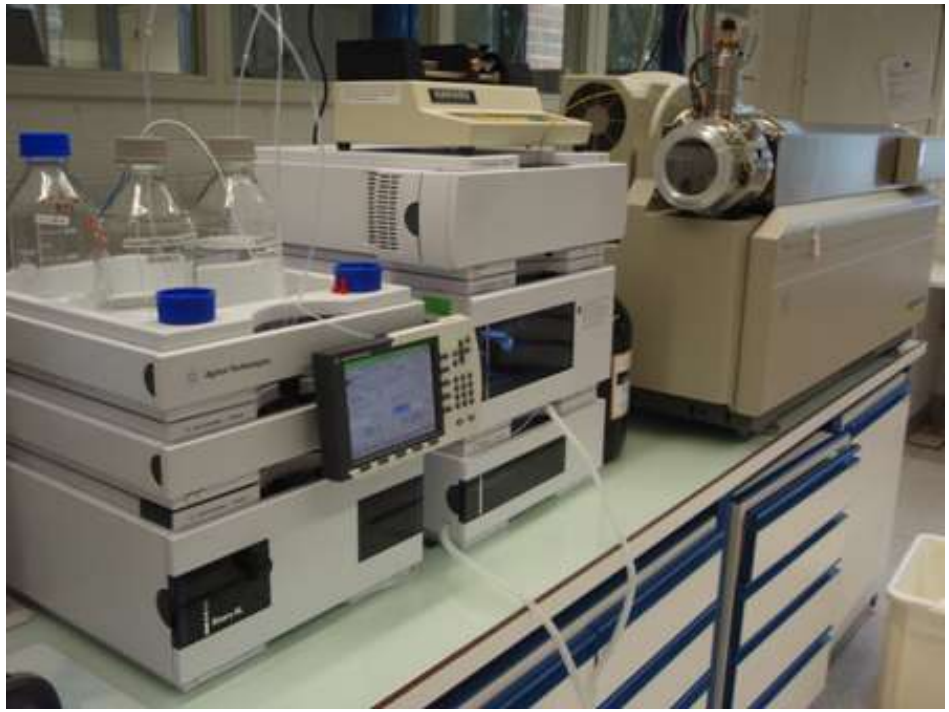
- LOQ (0.001 mg/kg)
- 10 x LOQ (0.01 mg/kg)
- 100 x LOQ (0.10 mg/kg)

mean recovery 70 to 110 %,

RSD ≤ 20 %.

LOQ of 0.001 mg/kg

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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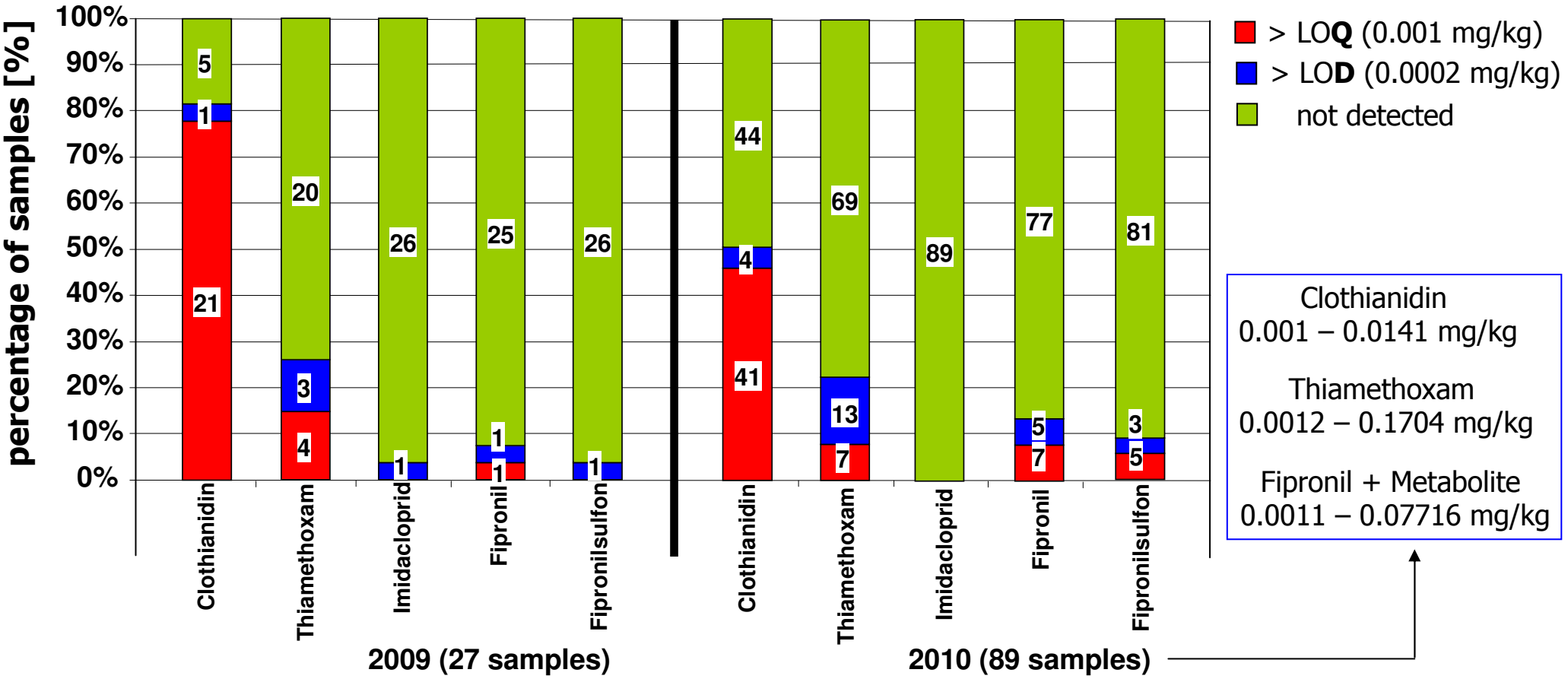
Results: Residue analysis of **bees**

Decrease 2009-2010
Clothianidin 81 % to 51 %

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

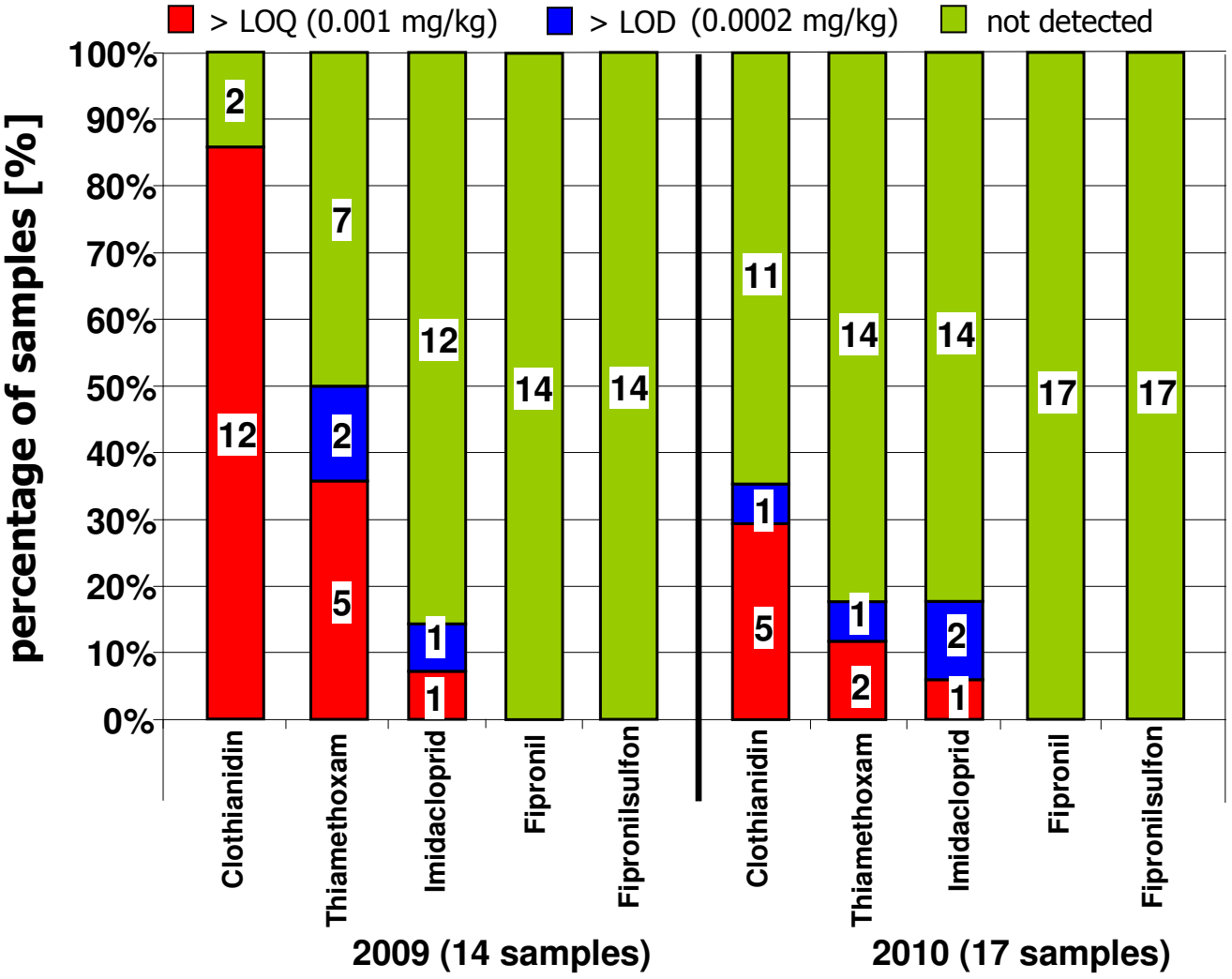


Clothianidin
0.001 – 0.0141 mg/kg

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 %

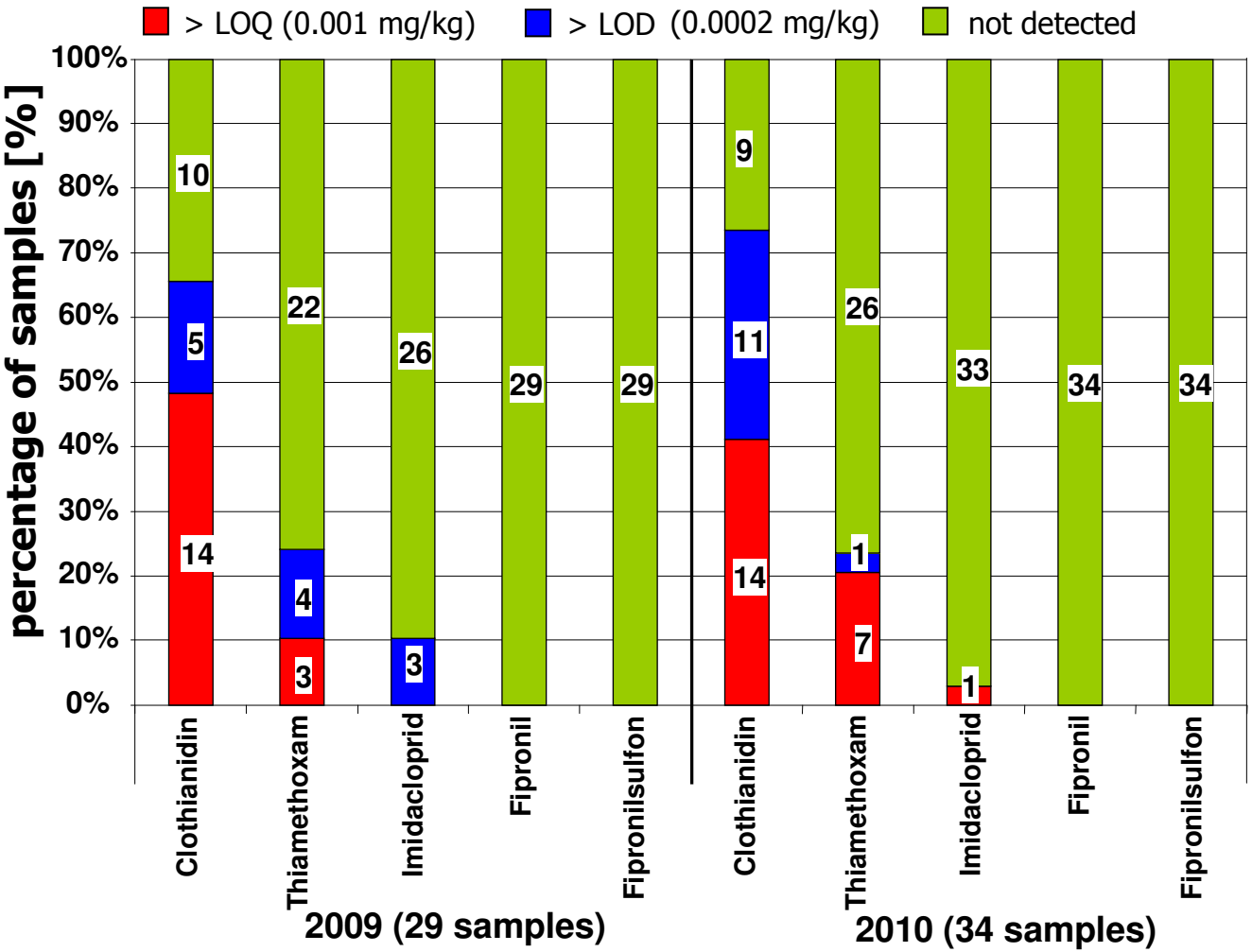
Indication for dustdrift

Clothianidin
0.0016 – 2.648 mg/kg

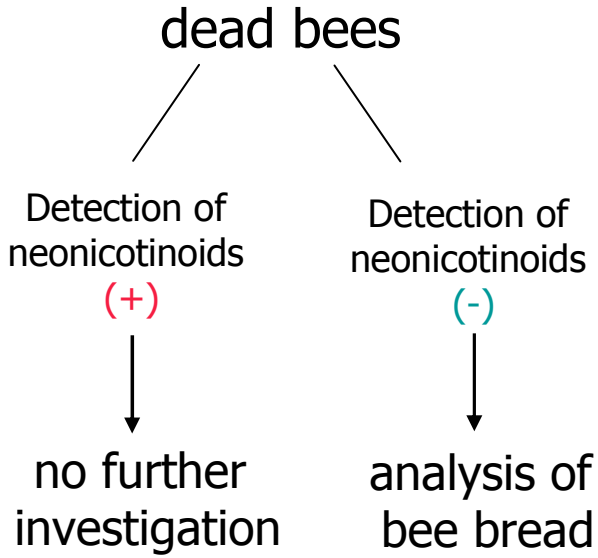
Thiamethoxam
0.0023 – 0.0122 mg/kg

Imidacloprid
0.001 – 0.035 mg/kg

Results: Residue analysis of **bee bread**



after the cultivation of maize, from apiaries with suspected poisoning



Clothianidin
0.0011 – 0.0060 mg/kg

Thiamethoxam
0.001 – 0.0052 mg/kg

Imidacloprid
0.0011 mg/kg

Risk assessment for bees

Clothianidin[1)]

Acute oral toxicity: LD50 = 0.00379 µg Clothianidin/bee
Acute contact toxicity: LD50 = 0.04426 µg Clothianidin/bee

LD50 = median lethal dose (24h)

1 kg bees ~ 10 000 bees

0,0141 mg/kg in bee sample ~ 0.00141 µg Clothianidin/bee

⇨ 37 % of LD50 for acute oral toxicity

0,006 mg/kg in beebread ⇨ 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, Spiroxamin, 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, Thiacloprid, 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

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

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

Pictures: beekeeper 07/2010

Day with observed high bee mortality

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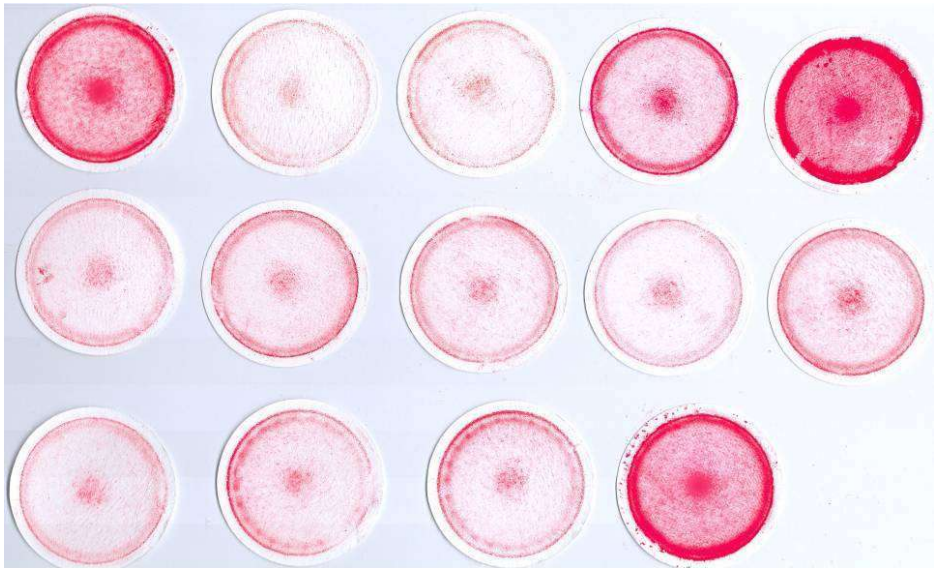
Risk-Minimising Strategies to protect honey bees

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



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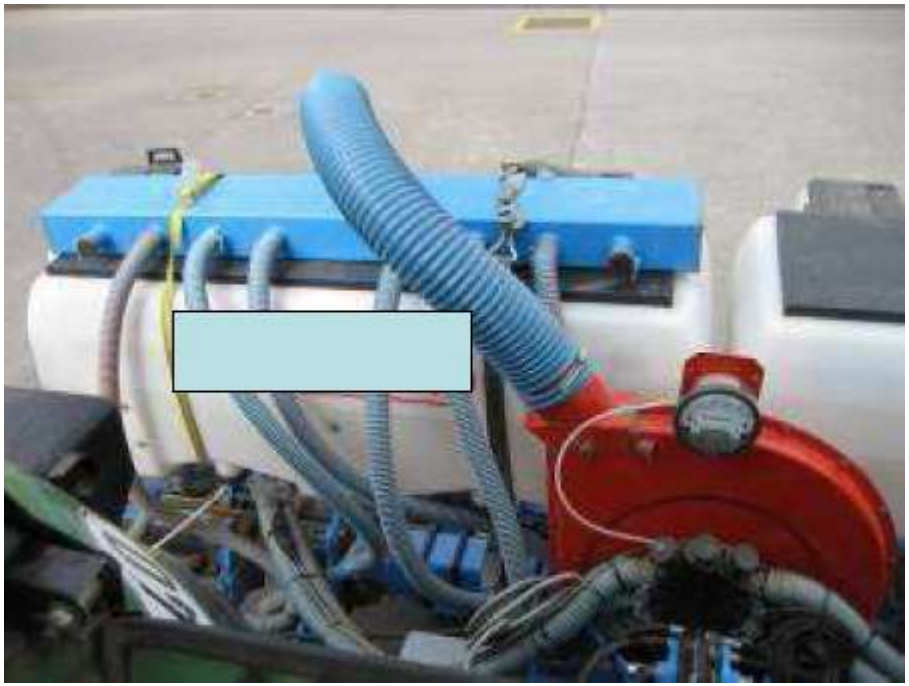
Risk-Minimising Strategies to protect honey bees

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 dustdrift 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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