Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen
Federal Research Centre for Cultivated Plants

Testing methodology

Dr. Jens Pistorius

Institute for Bee Protection
Alemania

Programa de conferencias

- *Miércoles 6 de febrero* a las 17 hs
  “Pesticides and risks to bees” en Facultad de Química

- *Jueves 7 de febrero* a las 17 hs
  “Pesticide testing methodologies for bees” en IIBCE

- *Viernes 8 de febrero* a las 16 horas
  “Routes of exposure for bees and pesticide risk assessment” en Facultad de Química
- *Viernes 8 de febrero* a las 17.15 horas
  “Bee poisoning incidents and international activities to protect pollinators”

- *Lunes 11 de febrero* a las 10 hs
  - **“Bees and beekeeping ”** en INIA (a definir)

Bees gone. Shelf empty.

Fotos:
Utopia.de

www.julius-kuehn.de

pesticide exposure: routes, levels, effects?

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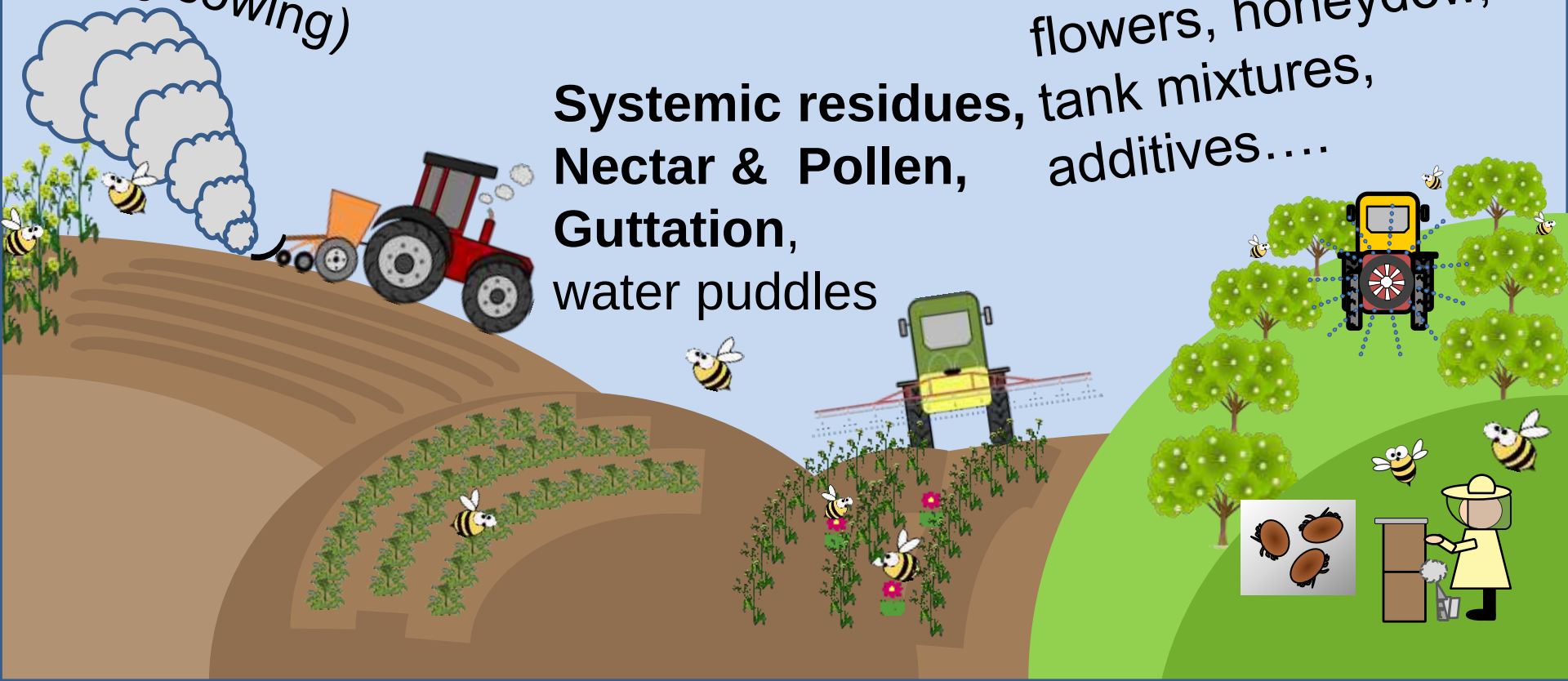
Seed treatments

Spray applications

Spray drift
dust drift
(during sowing)

Systemic residues, Nectar & Pollen, Guttation, water puddles

Spray applications:
Overspray, bees, flowers, honeydew, tank mixtures, additives….



JKI- Bundesforschungsinstitut für Kulturpflanzen

Julius Kühn-Institut
10 Jahre
1898 * 2008 * 2018
120 Jahre
Forschung für Kulturpflanzen von morgen

Federal Research Centre for Cultivated Plants

federal risk assessment authority and research institute

Mandated by legislation (Pflanzenschutzgesetz, Gentechnikgesetz, Chemikaliengesetz)

- 17 subinstitutes at 10 different locations
- 600 hectars research field area
- ca. 35.500 m^2 greenhouses
- ca. 20.000 m^2 laboratorys

Bundesministerium für Ernährung und Landwirtschaft

Institute for Bee Protection
Braunschweig & Berlin

Brunswick

Berlin

Research

Scientific assessment

Policy-Advice

JKIs major fields of competence

- plant genetics
- plant cultivation systems
- plant nutrition and soil science
- plant pests
- plant protection and plant health
- Bee Protection

Institute for Bee Protection

Tasks related to: Plant Protection Products (PPP)

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I. Risk assessment of plant protection products/bees

II. Examination center for bee poisoning incidents

III. Research on risks of pesticides to bees





www.jki.bund.de

Risk assessment vs. Risk management

- Risk assessment: What happens to the bees, are there adverse effects, of which nature, sublethal-lethal, damage to individuals / colonies, to which extent?

- Risk Management: are there specific actions that can be taken, to come from „unacceptable“ risk to „acceptable“ risks?

  → Example: avoid contact exposure to reduce risk: spray only at night time

Acute effects on honey bees and other bees

- Most insecticides: high acute toxicity to bees
- Active substance & Formulation, Application rate & timing of application, attractivity of the contaminated source to bees

→ **Importance of different routes of exposure?**

→ **Which applications are critical? Example: Neonicotinoids**

→ **Acute effects on mortality: magnitude of effects?**

→ **Importance of sublethal effects- relevance for the colony?**

→ **Consequences for individual, colony, population?**

Principle of the tiered approach Risk assessment Laboratory, Semi-field, Field

„Triggervalues“ – when to proceed to which test?

1. Laboratory, Toxicity ($LD_{50\ ,,,,}$): Adult oral, contact, chronic, Larvae



2. Triggers (ETR, PEC) exceeded → (Semi-) Field

Below Trigg.: no hazard

Semi-field / Tunnels with small bee colonies



…. And/Or….. Field- studies with normal bee colonies

Risk management: Final decision: (No) application on flowering crops during or after beeflight?

Which expsoure scenarios need to be considered- Spray vs. Seed treatment?

	Contact		Oral			
	Overspray	On leaves	Nectar	Honey dew	Pollen	Guttation
Treated crop						
Weeds						
Adjacent crops						
Off-field						
Following crops						
Dust drift						

What is the ***potential*** risk to bees? What is the ***real*** risk to bees?





Which scenarios need to be considered- Systemics?

	Contact		Oral			
	Overspray	On leaves	Nectar	Honey dew	Pollen	Guttation
Treated crop						
Weeds						
Adjacent crops						
Off-field						
Following crops						
Dust drift						

Different risk assessment schemes- weigh the importance of these routes differently, **use same exp. data but different calculation**

What is the potential risk to bees? What is the real risk to bees?





Which scenarios need to be considered- Non-Systemics?

	Contact		Oral			
	Overspray	On leaves	Nectar	Honey dew	Pollen	~~Guttation~~
Treated crop						
Weeds						
Adjacent crops						
Off-field						
~~Following crops~~						
Dust drift						

Different risk assessment schemes- weigh the importance of these routes differently, **use same exp. data but different calculation**

What is the ***potential*** risk to bees? What is the ***real*** risk to bees?





Fundamentals of risk assessment

- **Tier I** (screening level), **exposure estimates:** intended to be conservative "reasonably conservative", " relatively conservative", "highly conservative"?

- Relatively limited data set that measure ***potential* oral** and **contact** exposure
- complicated to measure- real **uptake/consumption** of nectar, pollen, water…
- **Tier II/Tier III**: exposure is based on measured values and more realistic scenarios with bee colonies- need to deal with uncertainties
- Proposed guidance on statistical evaluation of (semi-) field trials is complicated. No standard requirements on number of fields or colonies can currently be given. This makes it difficult to evaluate (and perform) a (semi-) field trial. Clear harmonised guidance on this is necessary.

➢ **All the different tiers have some weaknesses but also strengths!**

➢ **Goal: Protection of bee colonies** **(and all their services, pollination, honey,…)**

Exposure profile

- Crop dependend- every crop has a specific profile
- Crop attractivity – Nectar (Flower/extrafloral nectaries) and/or Pollen
- Plant protection Product (PPP) and active substance-properties, systemicity, persistence, mode of action
- Application type:
  - Spray – before, during or after flower
  - Seed treatment - time from seed to flower, crop type
  - Trunk/Stem injection
- Mode of application:
  - Type of sprayer, upward/downward application,
  - Nozzles and drift reduction
  - Sowing machinery, deflectors

→ Residue levels in bee relevant matrices

Risk assessment: tiered Approach

1st Tier / Labor

Trigger-Values indicate further higher tier studies necessary

2nd Tier / Semi-field

3rd Tier / field

Landscape level Monitoring

Standnardisatio

Ecological relevance, complexity

Guidelines for Risk assessment purpose

Methodology

- **ICPPR (**Internat. Commission Plant Pollinatorship) Bee Protection Group
  - Develops, discusses, ring tests, validates Tests in preparation for OECD

- **OECD** (Org. for Economic Co-operation and Development)
  - **Ring tested & validated, international agreed Guidelines**
  - Honey Bees: oral acute (213), adult chronic (245) contact acute (214), larval tests (237& 239), bumble bee acute contact (246) and oral (247), Brood semi-field test (75) , Osmia – ring test (**ICPPR**) completed, Homing Flight (RFID)- Ringtest ongoing

- **EPPO 170** (European Plant Protection Org.):
  - Outlines testing methodology for Lab, Semi-field and Field tests

Risk assessment schemes: use OECD (and EPPO) methods

- EPPO 170
- Newer Risk assessment schemes:
  - EFSA (European Food Safety Agency) (contains some method suggestions)
  - US-EPA/CalDPR/PMRA
  - IBAMA (Brasil)

Adult- Oral, chronic oral, contact

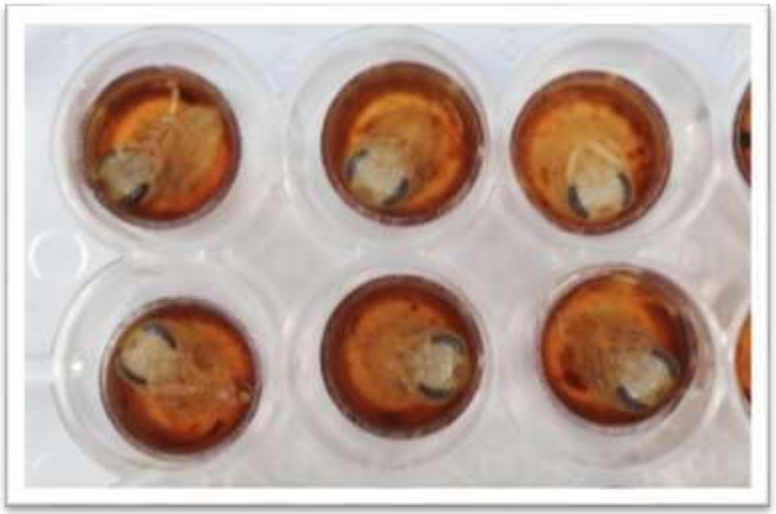
Endpoints: LD/C_5, LD/C_{10}, LD/C_{50}, LDD_{50}

Larvae

LC_{50} , LDD_{50}

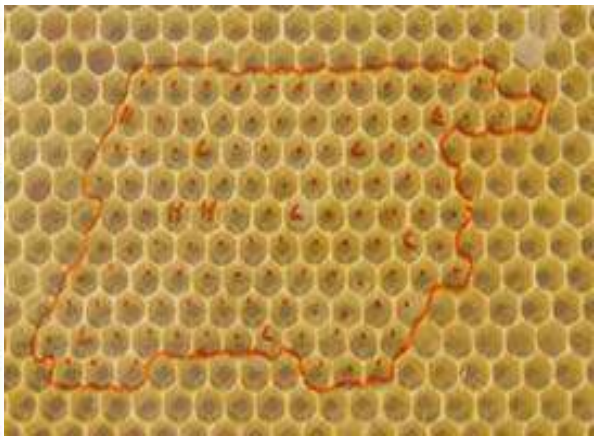
Sublethal effects to larve following pesticide exposure

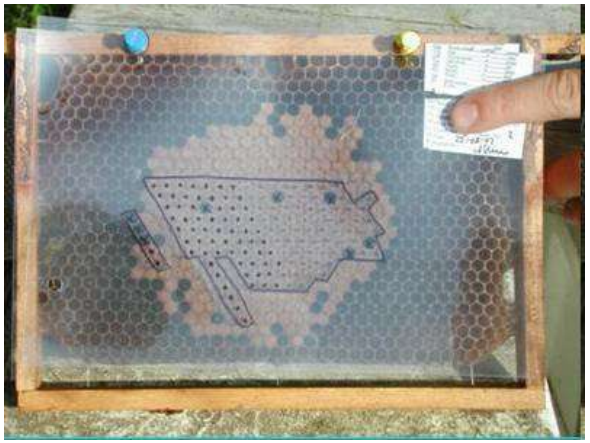
- molecular level
- gene expression? detoxification, Immune-system, development and energetic status?
- Measurements on basis of
- transkribierte RNA
- Proteine-expression

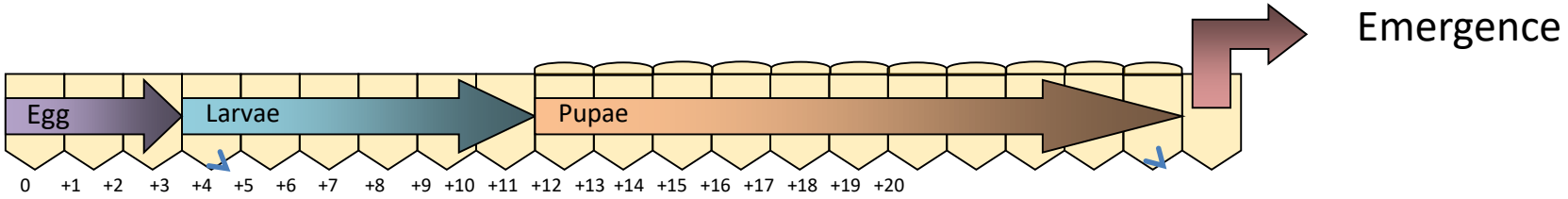


Testing of effects on bee brood

- Certain Insecticides (Insect growth regulators) may be untoxic to adults but may affect larvae
- Classical method- marking/tracking of individual cells, several assessments during development from egg to bee (21 days)
- Nowadays photographic assessment/evaluation







Semi-field

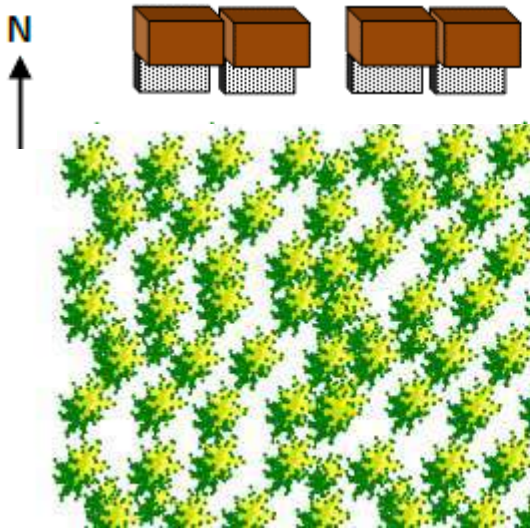
- Enclosed environment
- Worst-case exposure
- Either as „OECD 75 N Brood Test“
- Or „Mortality“ Test

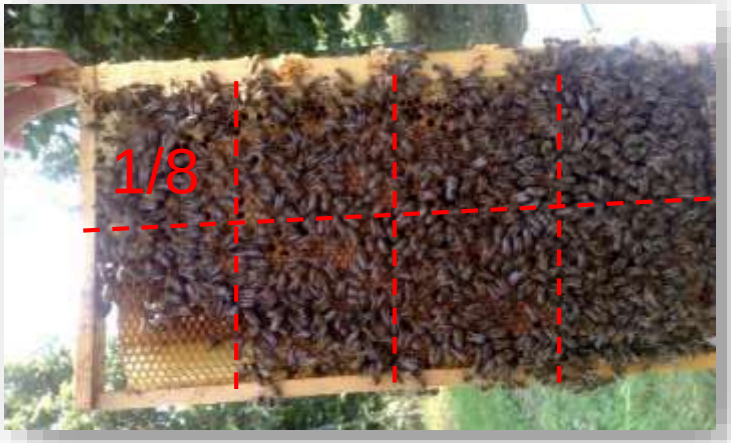
- However, the confinement often leads to brood reduction

Setup: Honey bees field 2014 & 2015

4 x Honey bee colonies

(10000 ± 2000 Bees).

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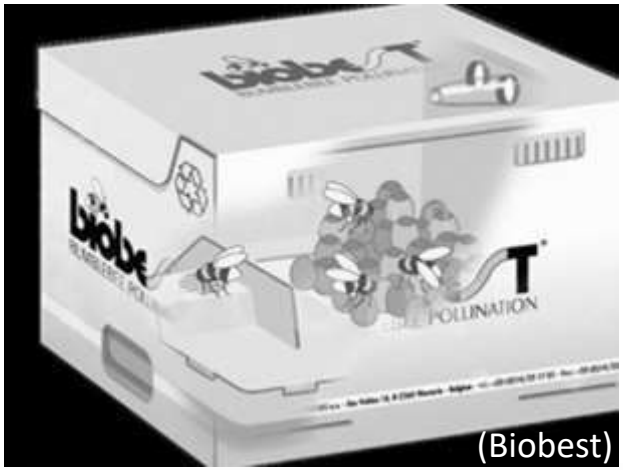
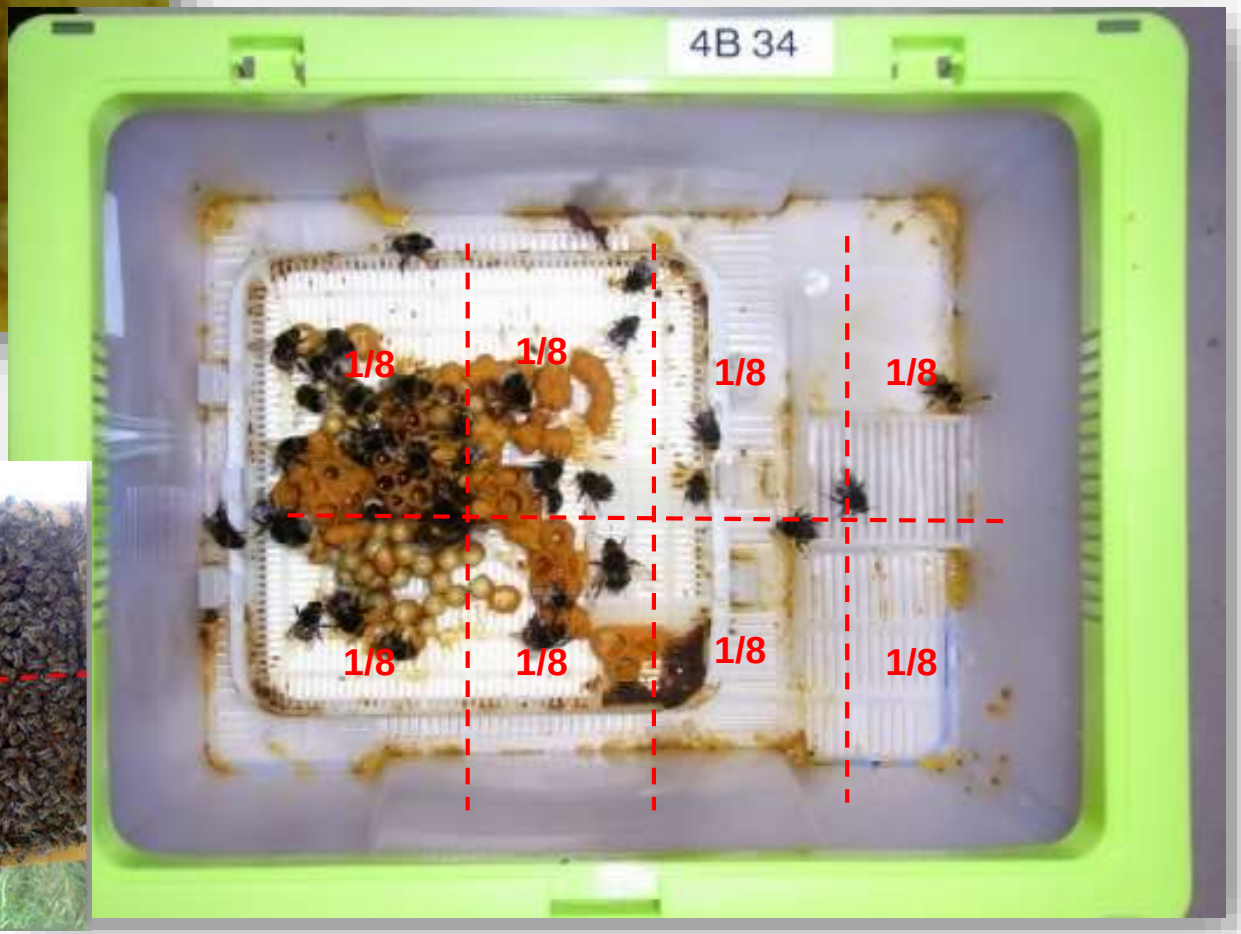
Bumble Bees- commercially available (Bombus terrestris)



(Biobest)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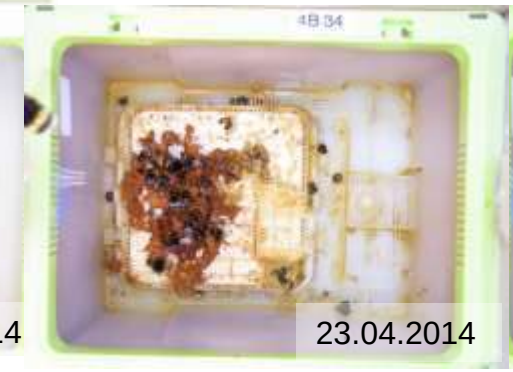
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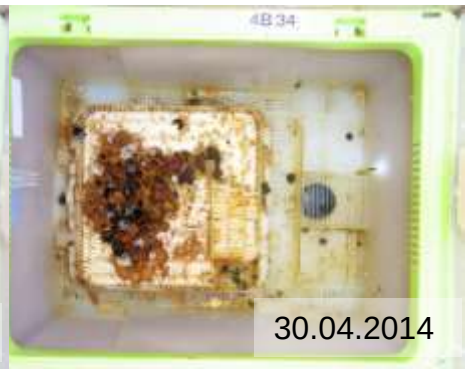
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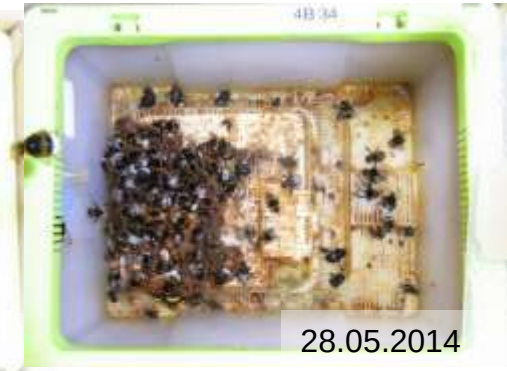
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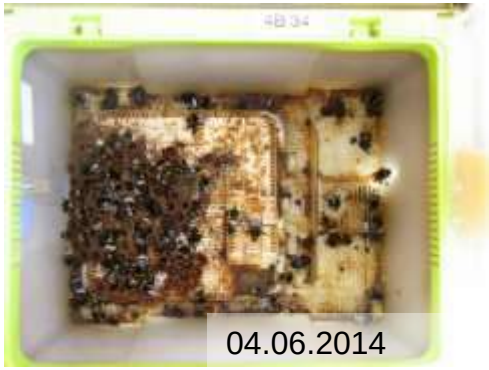
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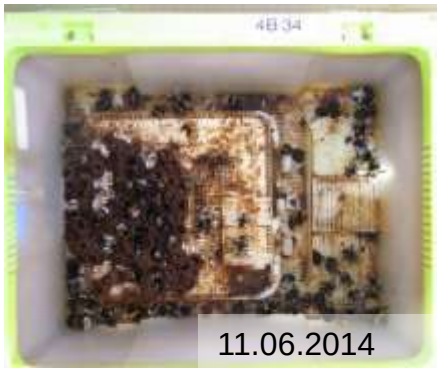
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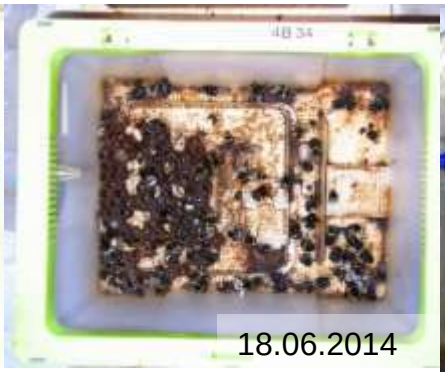
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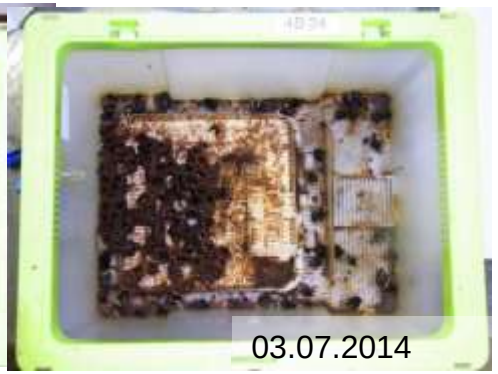
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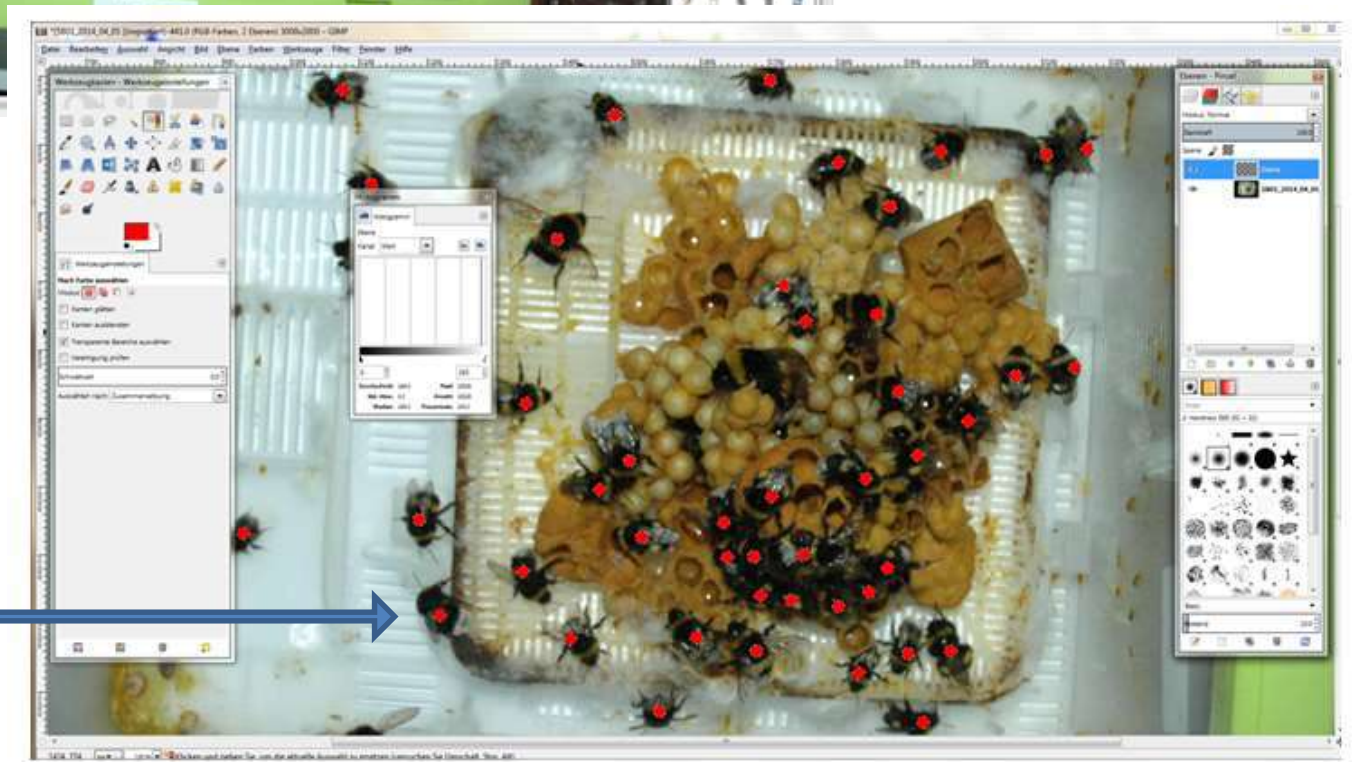


Red spots: counting workers…

Dunkle Erdhummel (*Bombus terrestris*)

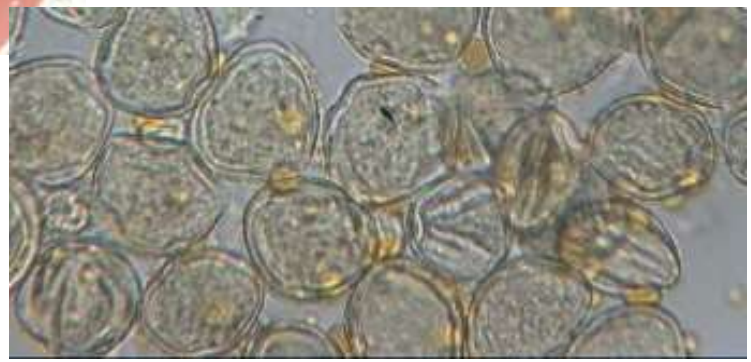
Brood (Eggs, young and old larvae, Pupae), Pollen, Nectar

Red Mason Bee (*Osmia bicornis*)

Sammelaktivität

Nistaktivität & Entwicklung

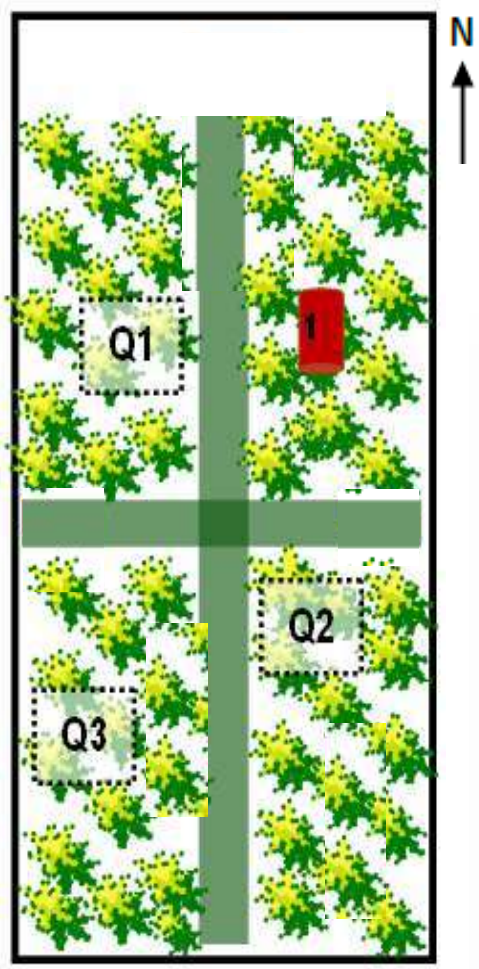
Pollenanalyse



Rückstände

Additional route of exposure: contaminated soil?

General setup: Semi-field (SF, 40 m²)



1 x *Apis mellifera* colony

2 x *Bombus terrestris* colonies

1 x *Osmia bicornis* trap nest

3 x Quadrats (flight activity)

This setup was done in 2013 only, due to competition it is better to always separate the 3 species…2014-2018 only 1 species/tent

Data collected

	Amount of active substance in	Assessment parameters	Assessment endpoints
A. mellifera	Pollen, nectar, dead bees	Mortality, activity, colony development	Overwintering success
B. terrestris	Pollen, nectar	Mortality, activity, colony development	Overwintering queens
O. bicornis	Pollen, mud	Activity, number of cells, male/female ratio, cocoon weight	Hatching success of offspring
Other parameters	Soil, plants		

Seed treatments

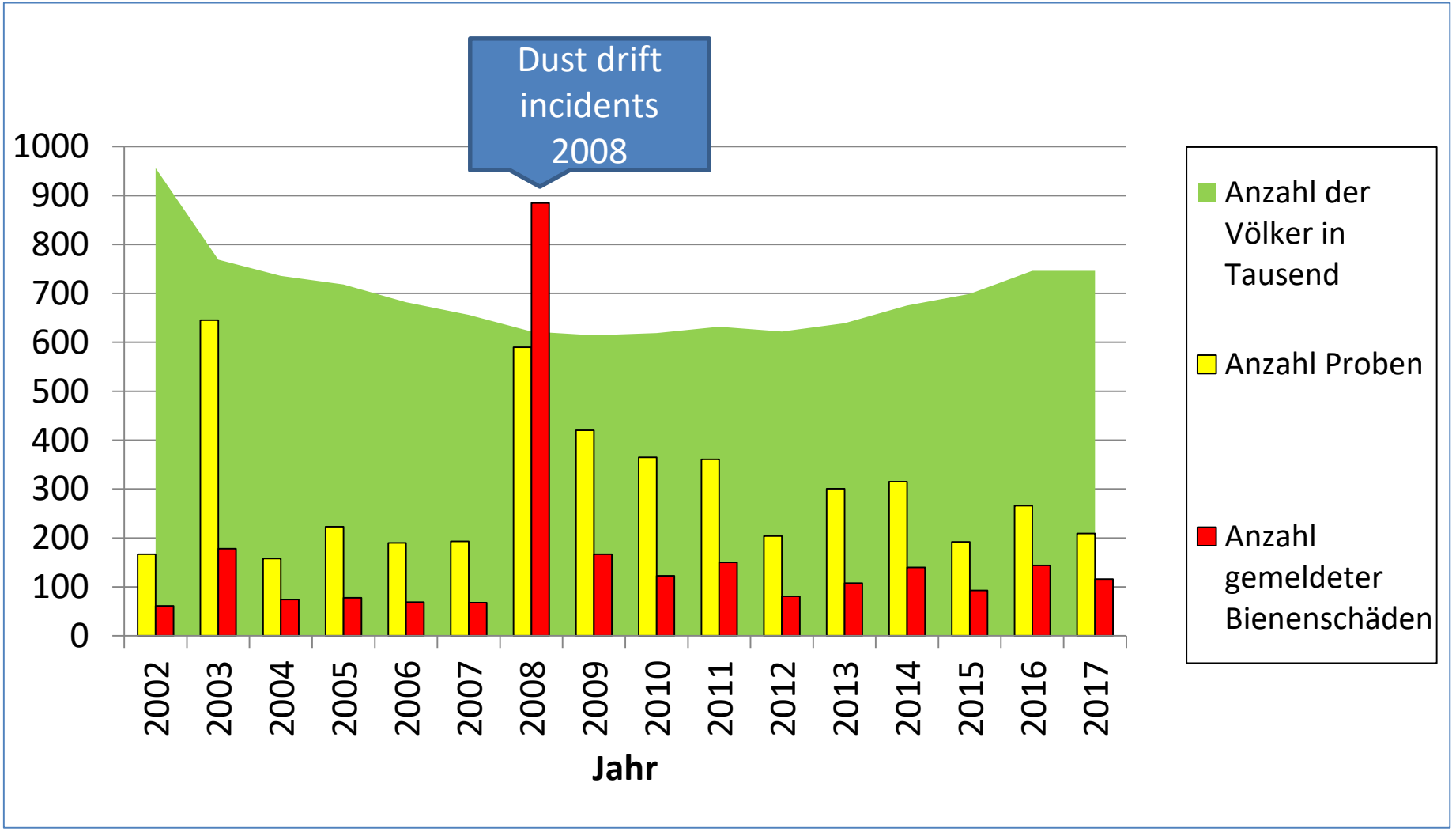
- Important for crop protection, principally environmentally friendly
- Different types of seeds and type of seed treatment

→ **different amounts of abradable dusts and dust in seed bag (depending on crop, facility, treatment)**

- Different machinery used for sowing

→ **Different measures possible (and necessary) to mitigate risk to acceptable levels**

Bee colonies in Germany, Reported incidents, number of samples 2002 - 2017



Exposure and risk under realistic conditions- Dusts

Dust Emission depends on e.g. seed quality, sowing technique, wind conditions,…

Exposure depends on dust emission and

- distance of flowering plants/crops or honey dew to sowing
- Structure and stickiness of neigboring crops

Risk for bees depends on exposure and

- amount of alternative, uncontaminated forage in flight range
- Attractivity of crop
- Weather conditions

Testing dust abrasion (fine dust only!) from seeds
Heubach Dustmeter (s.a. www.jki.bund.de/heubach.html)

Dusts of different maize batches from market in 2008

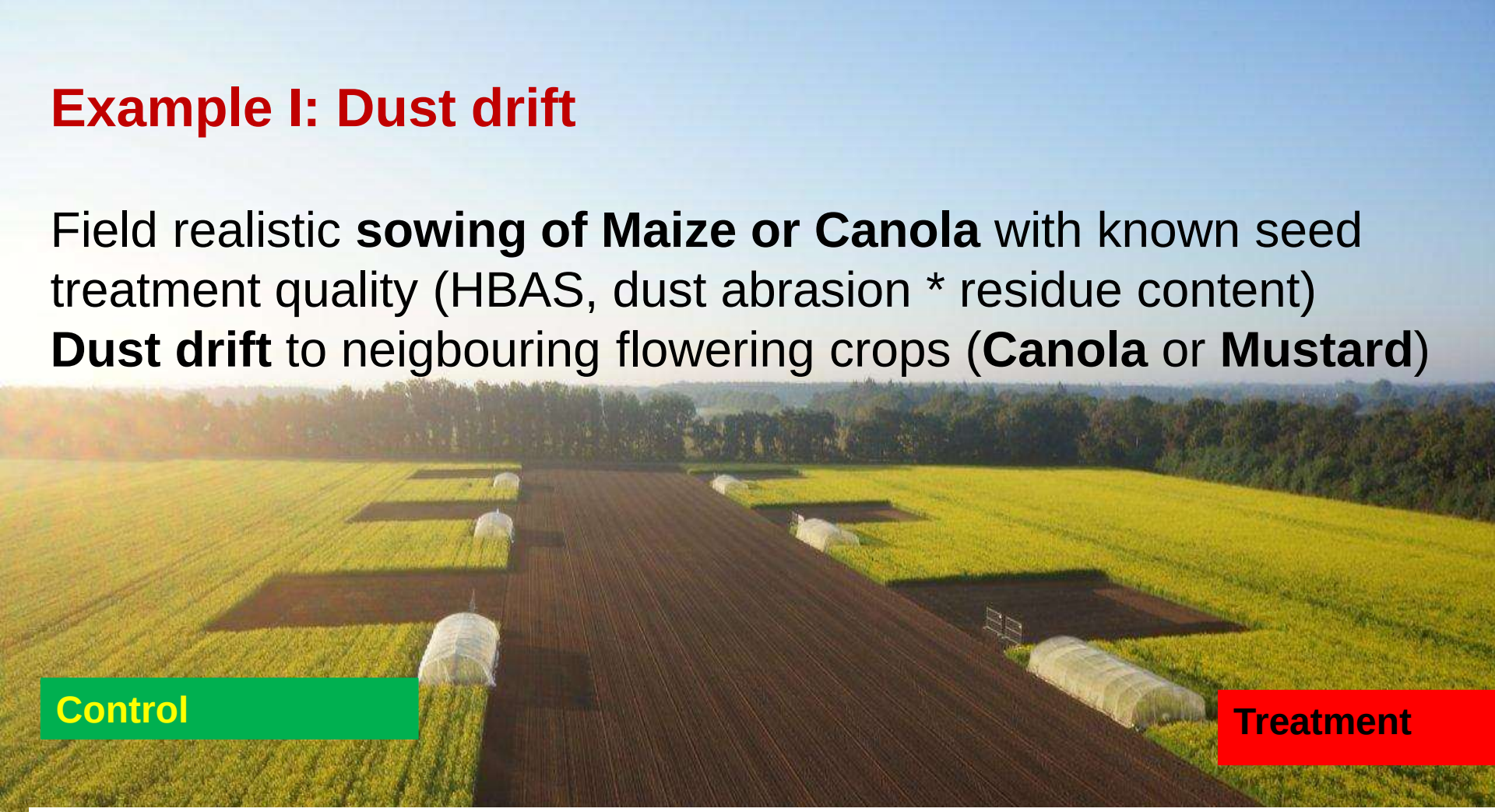
mean fine dust (left, blue): 3,6 g with 24,3 % Clothianidin

mean corse staub (right, purple): 4,9 g with 13,4 % Clothianidin

Graph: u. Heimbach, JKI, Institut für Pflanzenschutz in Ackerbau und Grünland

Example I: Dust drift

Field realistic **sowing of Maize or Canola** with known seed treatment quality (HBAS, dust abrasion * residue content)
Dust drift to neigbouring flowering crops (**Canola** or **Mustard**)



→Correlation: **Seed treatment quality/ exposure / effects**

Exposure Non – target areas & Bees: Petridishes, Flowers, Crop area; nectar, pollen, bee bread,
Effects on Bees: Mortality, Brood & Colony development, residues bees

Semi-field ~5000 Bees/Colony/Tent

Petridishes w. Filters, Leaves, Honey

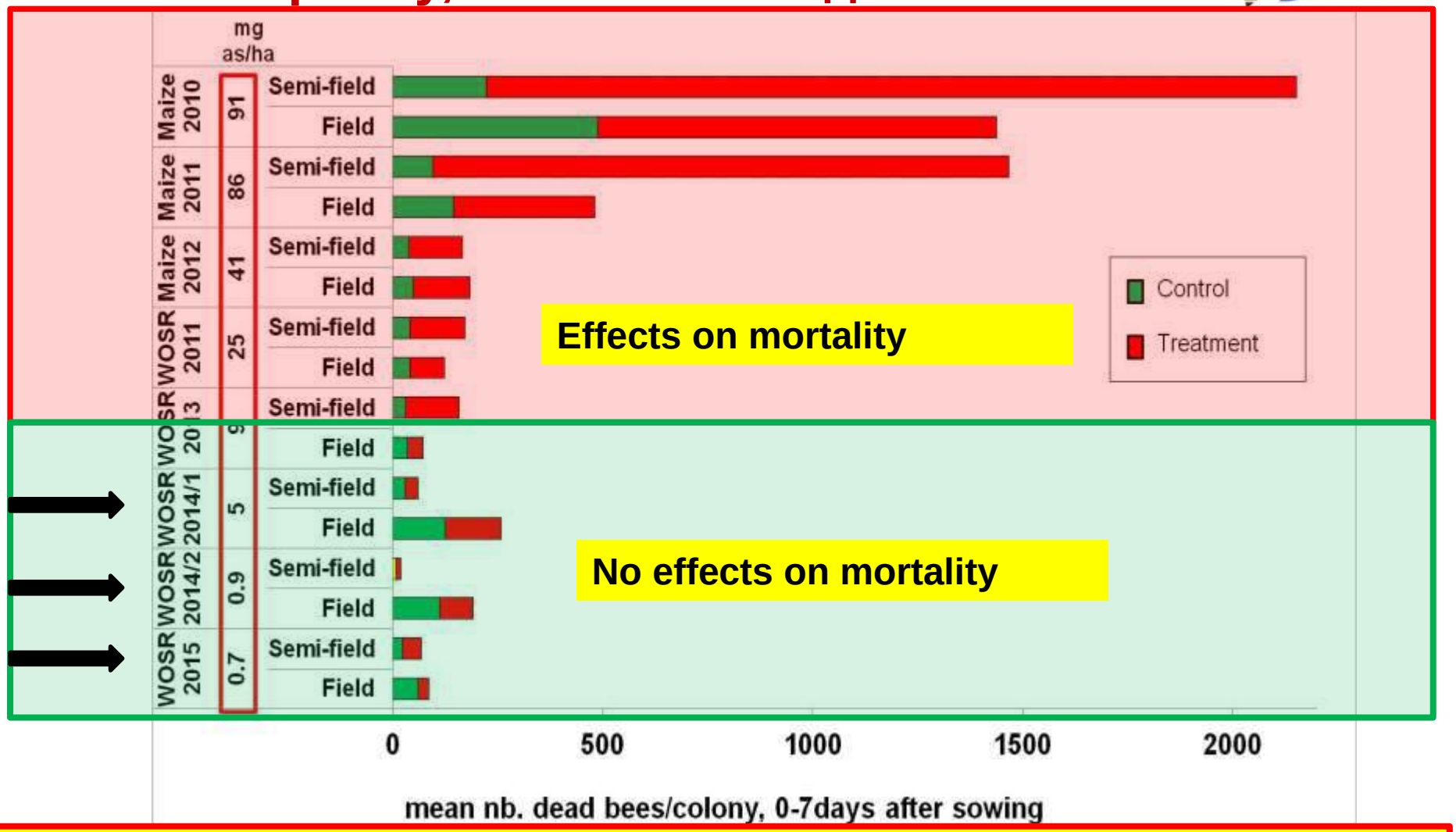
Flower samples

Bees in wire cages, direct dust exposure

Field – colonies ~25000 Bees, free flying

„sowing“ (most dust is soil dust)

Insecticidal dust drift: risk depends on abrasion quality, not/a lot less on application rate!!!



Improving seed treatment quality & testing machinery: risk mitigation to achieve acceptable levels of environmental risk (for honeybees and other organisms)

Exposure and risk under realistic conditions- Guttation

Occurence of Guttation **depends on** e.g. plant type, environmental conditions,…

Exposure depends on Occurence of Guttation and

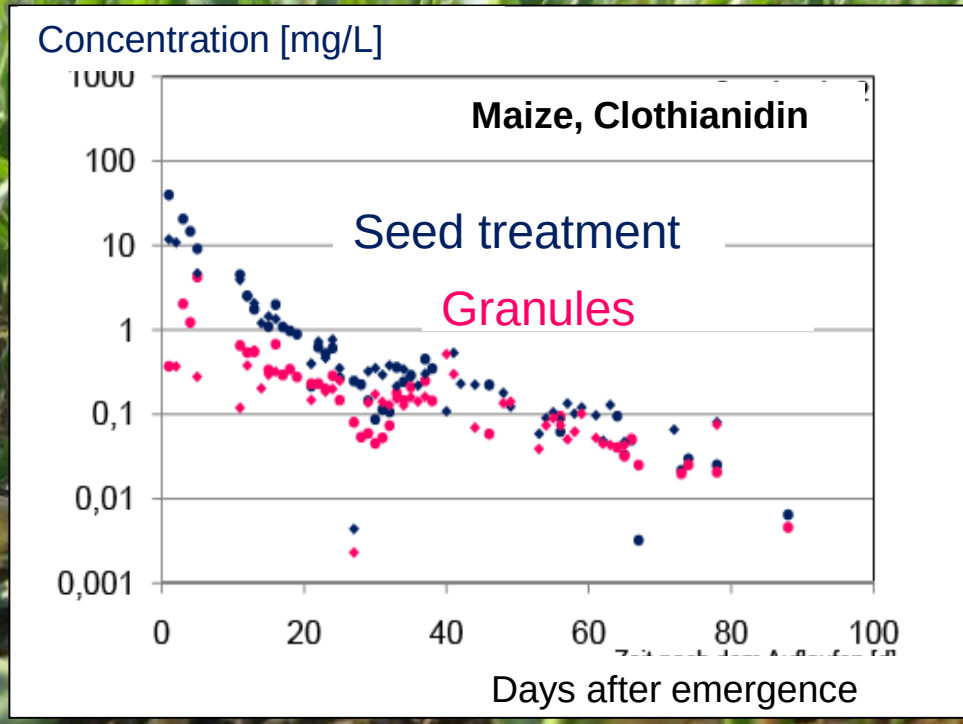
- active substance, seed loading
- distance of bee colonies to treated crop

Risk for bees depends on exposure and

- amount of alternative, uncontaminated water sources in flight range
- Use of guttation droplets from treated plants
- Weather conditions

Guttation – hazard yes, but which risk?

Photo: Wallner, 2009

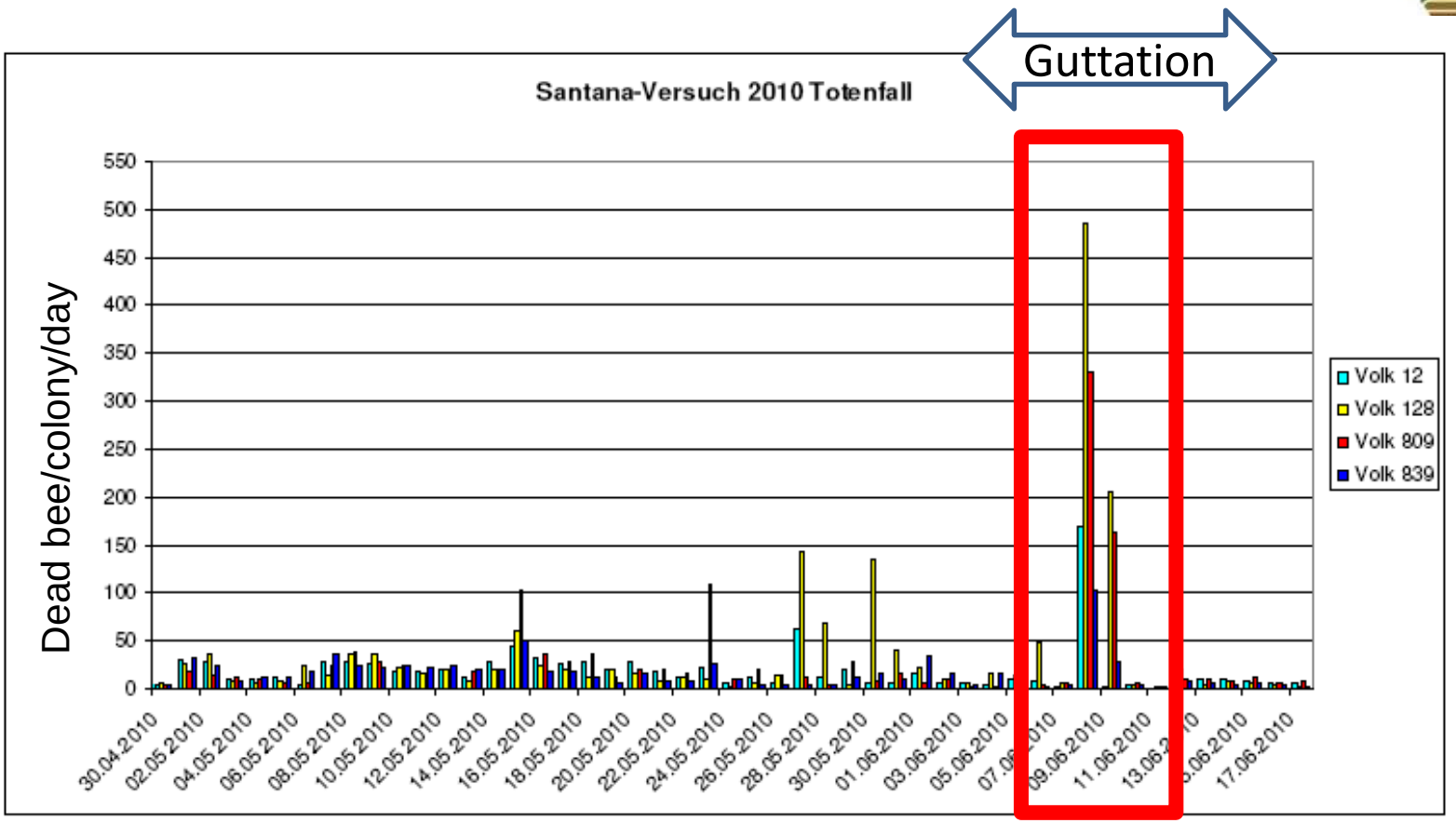


Maize up to 100 mg/L, Cereals up to 5, Canola (WOSR) up to 1 mg/L.
For Clothianidin ~ 4 µL water contain = LD50

Summary of JKI field and semi-field trials

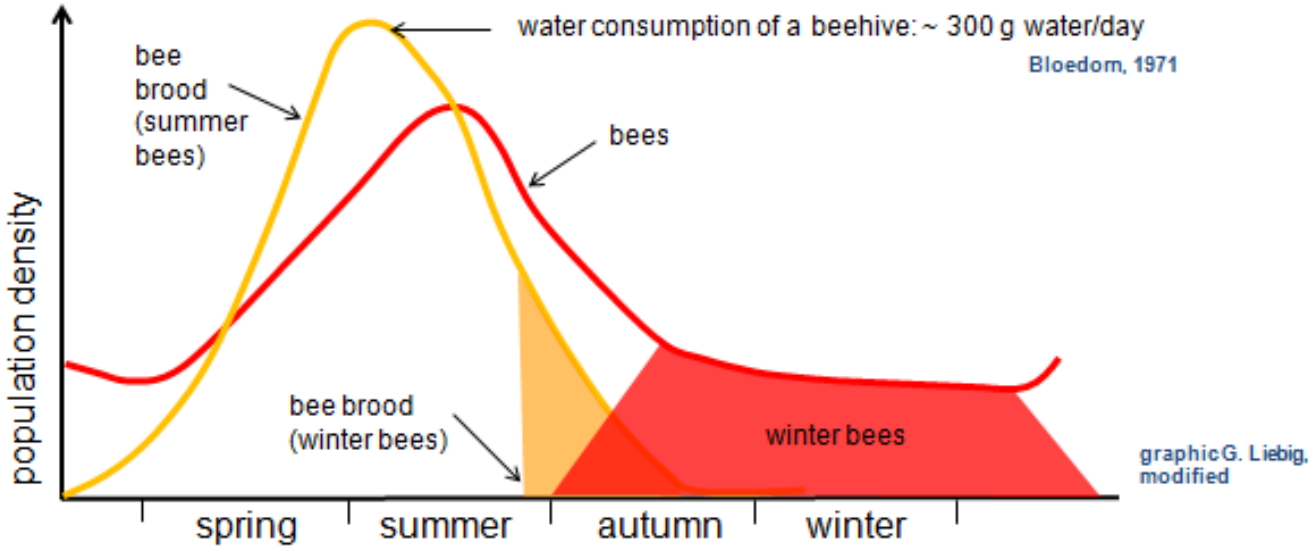
Crop	Type	Honeybee mortality	Colony and brood development
Maize	Field trials (3 trials, 2009-2012)	Mortality not increased	No effects on colony and brood development
	Semi-field trials (trials , 2009-2012)	**With** water supplied: Mortality not increased	No effects on colony and brood development
		Without water supplied: Mortality increased	Effects on colony and brood development **(„worst case"- artificial situation!)**
WOSR + Cereals	Field trials [3] (3 + 3 trials, 2010-2012) (incl. overwintering)	Mortality not increased	No effects on colony and brood development
	Semi-field trial (1 trial, 2009-2012)	Mortality not increased	No effects on colony and brood development

Monitoring, granules, maize [a.i.clothianidin]

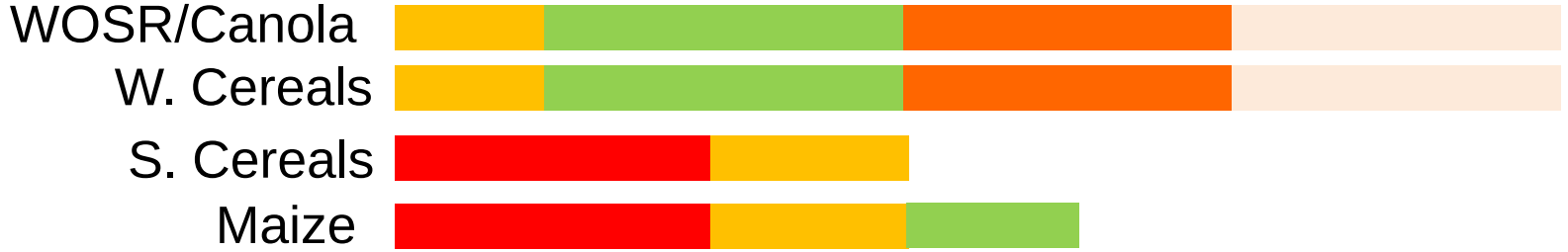


Monitoring (2010, Bee Institute Celle, Dr. von der Ohe)
worst case placement of colonies directly at field border. Several days with Guttation and no effects, but some days with clear acute high mortality. No long term impact

Bee biology, water demand and potential guttation exposure?

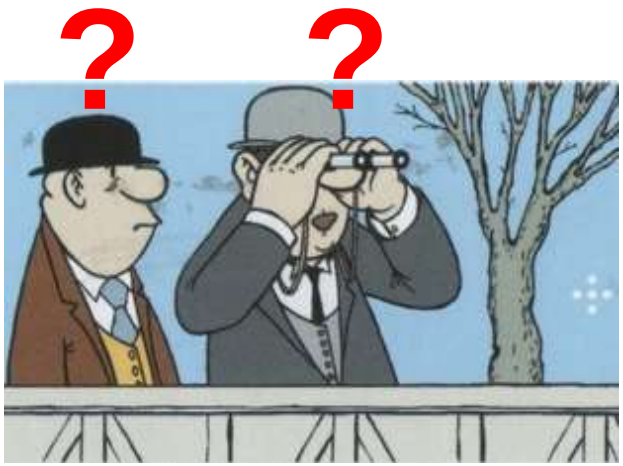


Potential risks for water foraging bees during the season



→ increased mortality may occur but only in very rare situations and **usually** affects only single foragers!

Homing behaviour/disorientation



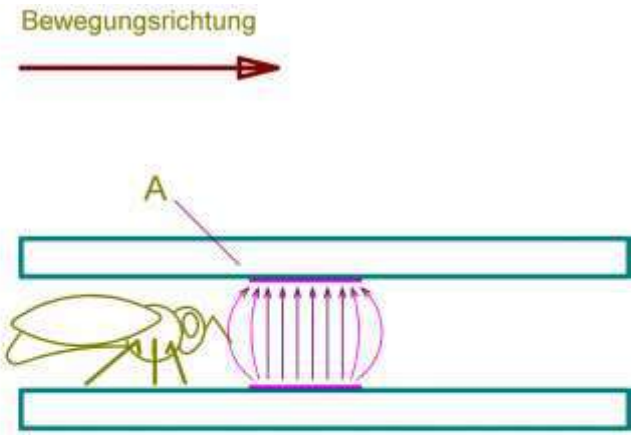
RFID- tracking individuals…

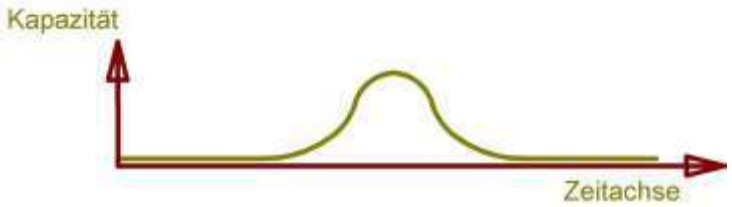
Individual marking of bees

Needs a reader – when the marked bee passes, it is registered

Labor intensive, not too many individuals possible

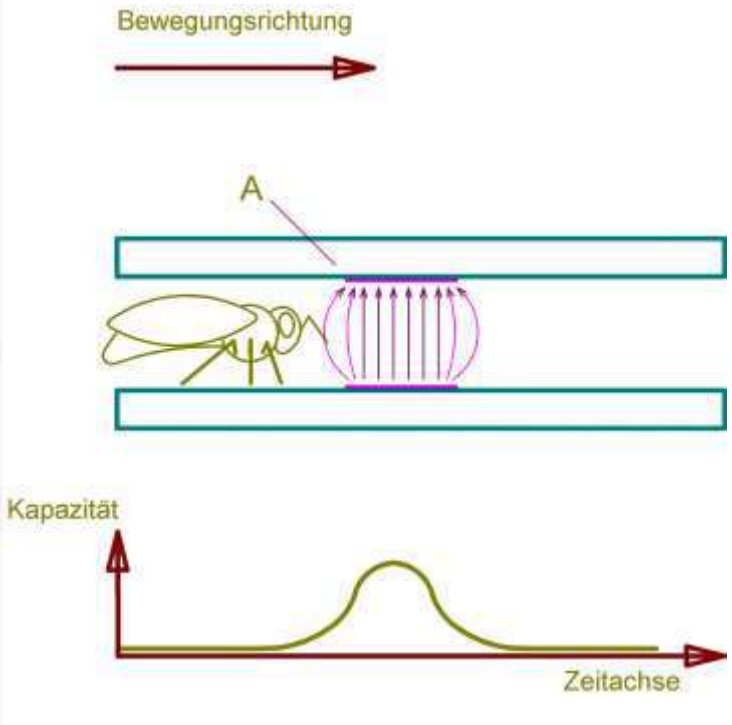
Beecheck I vs. Beecheck II

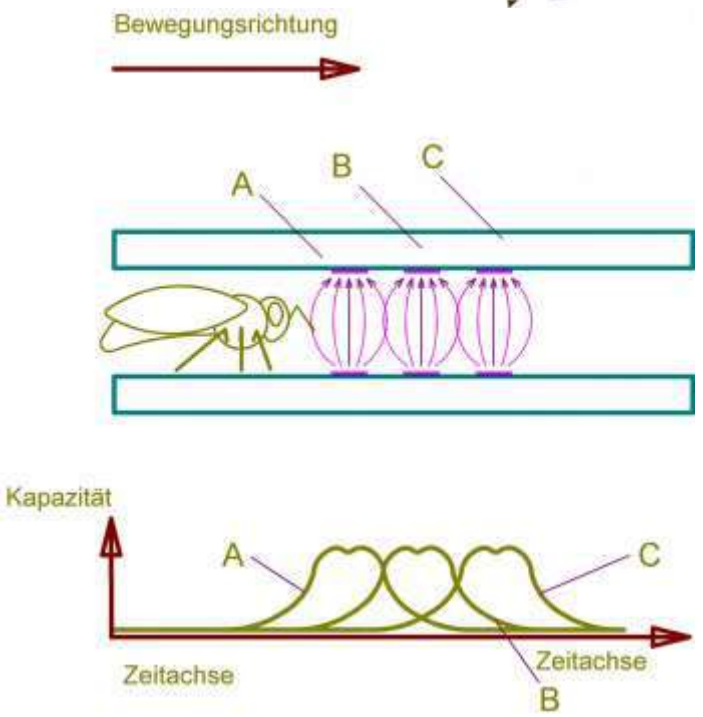




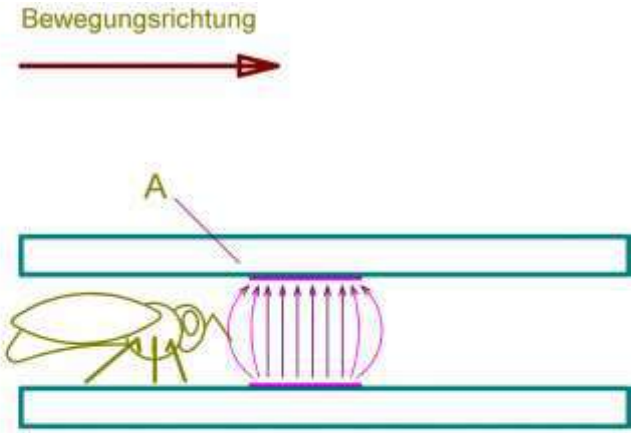
Counting all in- out movements

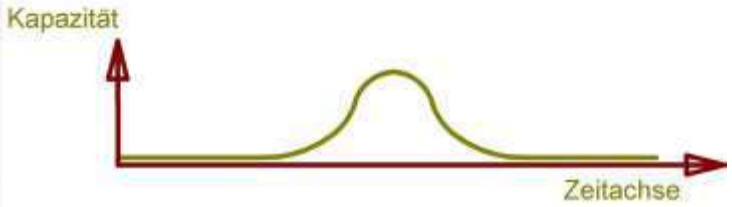
Beecheck I vs. Beecheck II

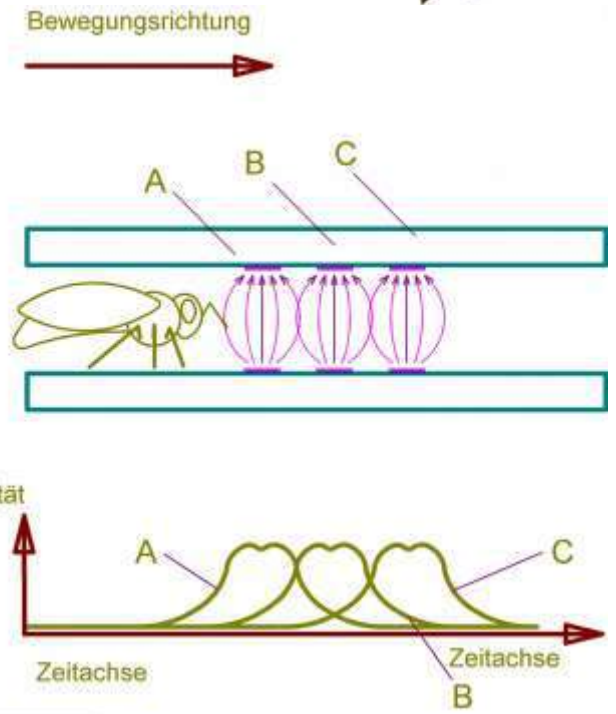


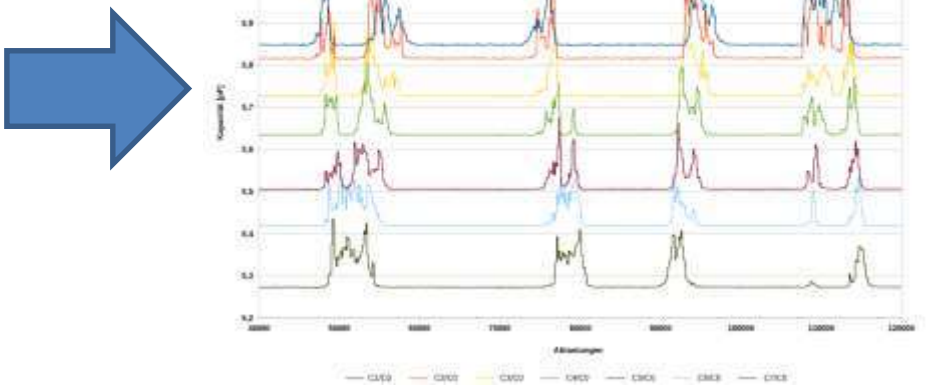


Beecheck I vs. Beecheck II









Big Data!

10000 Din A4 Pages
Data/Hour/Machine

Methodolgy- Risk assessment and Research

- For research a high number of methodological approaches to test specific reactions of indivudual bees, bee larvae and the colony- mostly with specific questions

- For risk assessment, specific tests generating „endpoints“ are required

- In Risk assessment, also tests beyond the „standard lab-semi-field field“ may be requested for risk assessment

What do Risk assessors need from research?

→ **Better and more refined methodologies.** For different exposure routes and bee species specific testing strategies are required with different need to emphasize acute versus chronic or sublethal effects

→ **More research with investigation of the risks in realistic conditions and realistic doses**, not just the investigation of substances properties in laboratories.

→ **More research on suitable risk mitigation measures (e.g. toxicity of mixtures, additives,…)**

Thank you for your attention!

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