Science For A Better Life

Bee Safety of Neonicotinoids - Evidence from Studies Conducted Under Realistic Field Conditions

Bayer CropScience, Environmental Safety - Ecotoxicology

7 Nov 2016 / Janine Doering

Introduction

- Neonicotinoid insecticides are used as seed treatment in various crops. Among these there are bee-attractive crops like oilseed rape, sunflower, and maize
- After neonicotinoid seed treatment, traces of systemic residues can be found in nectar and pollen of treated crops
- This has led to concerns about their safety to bees and other pollinators
- Although monitoring data do not suggest a systematic correlation between the exposure of bees to neonicotinoids and bee health issues, the use of neonicotinoids in bee-attractive crops has been restricted in the EU in 2013
- In order to complement existing field data, a large scale field study with neonicotinoid-treated oilseed rape (treatment with Elado® - 10 g clothianidin & 2 g β-cyfluthrin/kg seed) was conducted in Northern Germany

Setup and Purpose of the Study

Conceptual approach of the monitoring study in Mecklenburg Vorpommern (Germany)

Monitoring potential adverse effects at landscape level

- Comparable landscapes for „control“ und „treatment“
- Spatial proximity of both landscapes: close enough to ensure comparable climatic and topographical conditions distant enough to avoid honeybees to cross-forage
- Agricultural landscapes with significant oilseed rape cultivation and low abundance of other flowering crops or wild plants distracting bees from the target crop
- Common oilseed rape cultivation practice with pre-agreed and defined crop protection measures
- Large-scale farm operations required to ensure regulated crop protection regimen

Study design

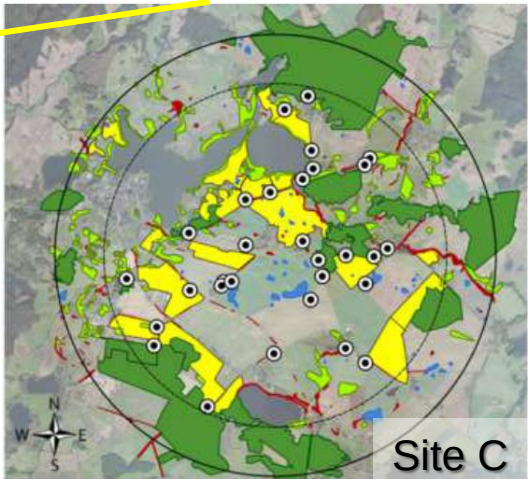
- Monitoring of the clothianidin residue levels in nectar and pollen during bloom
- Monitoring potential adverse effects on pollinators which differ in life history traits

Similarity of Control and Treatment Sites

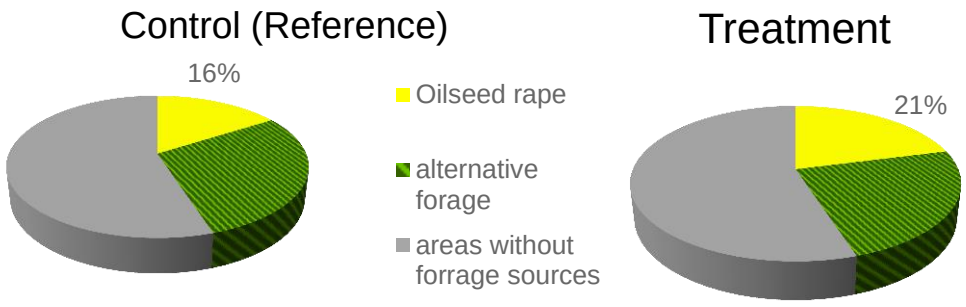
Attractiveness for Bee Pollinators

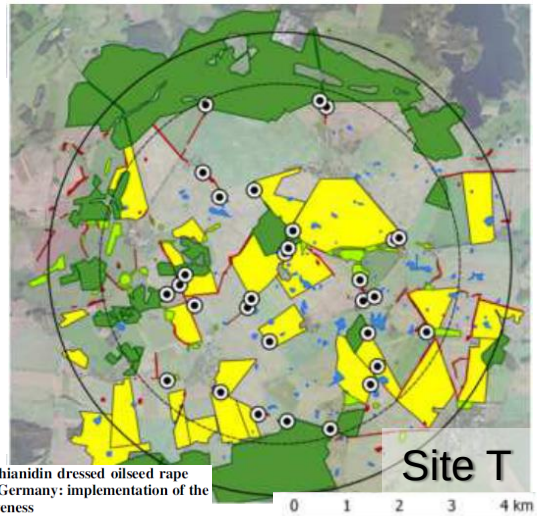
Location of surveyed habitats | Hedge, shrubs | Forest | Grove of trees | 0 1 2 3 4 km
Oilseed rape field | Kettle | Edge of the forest | Edge of grove of trees

Study area



Two sites of approx. 65 km² each were selected according to field cropping area, statistic and land cover data





F. Heimbach et al.

Table 2 Summary of parameter comparison between the study sites

Dependent variable	Test	Test statistic	*P*	Reference site	Test site
Land cover					
Total OSR at Core area				614.6 ha	791.7 ha
Median OSR field size	Wilcoxon rank sum test	W = 170	0.999	33.5 ha	35.3 ha
Percentage OSR of arable land				32.3 %	28.5 %
Percentage arable land				49.5 %	72.2 %

Large-scale monitoring of effects of clothianidin dressed oilseed rape seeds on pollinating insects in Northern Germany: implementation of the monitoring project and its representativeness

Fred Heimbach[1] · Anja Russ[1] · Maren Schimmer[1] · Katrin Born[2]

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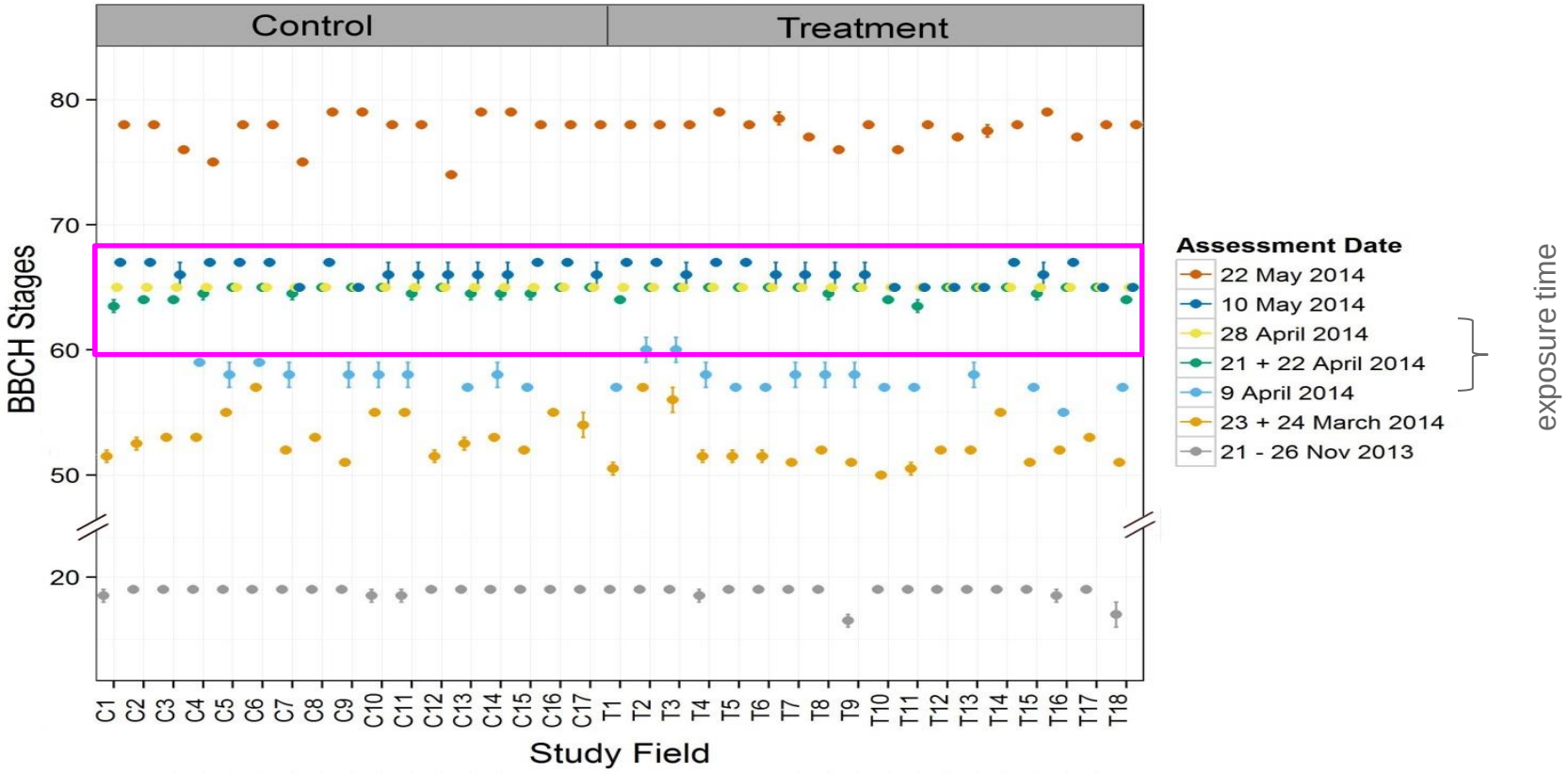
Similarity of Control & Treatment Sites

Crop phenology



Control fields received 1 – 2 additional pyrethroid applications to ensure comparable oilseed rape emergence

Homogenous flowering period across control (reference) and treatment and over time

Similarity of Control & Treatment Sites

Crop phenology

Table 2 Summary of parameter comparison between the study sites

Dependent variable		Test	Test statistic	*P*	Reference site	Test site
OSR development						
Date of OSR drilling	🟢	ANOVA	$F_{1, 33} = 1.09$	0.305	18 August 2013 ± 2.5 days	19 August 2013 ± 4.4 days
OSR emergence rate	🟢	Wilcoxon rank sum test	$W = 669.5$	0.800	68 ± 21 %	68 ± 28 %
OSR plant density	🟡	Wilcoxon rank sum test	$W = 391.5$	0.004	26.0 ± 7.3 plants/ m^2	32.6 ± 11.4 plants/ m^2
OSR development (BBCH stages)	🟢	ANOVA	$F_{1, 232} = 0.00$	0.972		
OSR yield	🟡	General linear model	$F_{1, 33} = 8.08$	0.008	33.9 ± 7.1 dt/ha	38.6 ± 6.3 dt/ha

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Characterization of Exposure

Residue levels of Clothianidin in OSR nectar & pollen at bloom

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in northern Germany: residues of clothianidin in pollen, nectar and honey

Daniel Rolke[1] · Markus Persigehl[2] · Britta Peters[2] · Guido Sterk[3] · Wolfgang Blenau[1,2]

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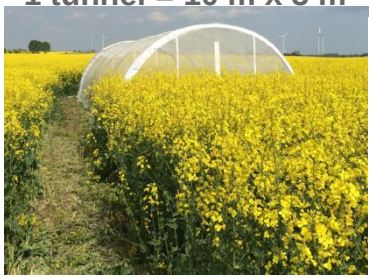
1 tunnel = 10 m x 5 m



39 x 50 m² = 0.2 ha [18 fields, Total oilseed rape area 792 ha]

Experimental conditions	Bee species	Pollen		Nectar		Honey	
		Reference site[a]	Test site[b]	Reference site[a]	Test site[b]	Reference site[a]	Test site[b]
Semi-field (tunnel)	*Apis mellifera*	< LOD (n = 34)	1.67 (n = 39)	<LOD (n = 34)	1.31 (n = 39)		
Field (free flying)	*Apis mellifera*	1st sampling: < LOD (n = 48) 2nd sampling: < LOD (n = 48)	1st sampling: 0.50 (n = 48) 2nd sampling: 0.97 (n = 48)	1st sampling: < LOD (n = 46) 2nd sampling: < LOD (n = 48)	1st sampling: 0.68 (n = 48) 2nd sampling: 0.77 (n = 48)	< LOQ (n = 48)	1.35 (n = 48)
	Bombus terrestris	< LOD (n = 6)	0.88 (n = 6)				
	Osmia bicornis	< LOD (n = 6)	0.88 (n = 6)				

[a] Minimum 90 % of samples with residues < LOD (= 0.3 µg/kg) or < LOQ (= 1.0 µg/kg), respectively

[b] Calculation: residues < LOD = 0.0 µg/kg; residues < LOQ but >LOD = 0.65 µg/kg; residues > LOQ as quantified. See material and methods for details on calculation and text for standard deviations

- No residue detects in majority of oilseed rape pollen or nectar in “control” landscape (n = 34)
- Trace residue levels in few of control pollen samples (tunnel) & nectar samples (field)
- Residue levels in treatment landscape (n = 46/48 + 48) comparable to tunnel samples (n = 39)

Investigation of Potential Adverse Effects

Three bee pollinator species with different life history traits

Honeybee (*Apis mellifera*)

Life history traits:

- complex social community with task share
- Comb cells of wax
- Hives with 50.000+ workers
- Thermoregulation
- Full colony overwintering
- Winter food stores

Bumblebee (*Bombus terrestris*)

Life history traits:

- Less complex social structure and task share
- Comb cells of wax
- Hives with 500+ workers
- Thermoregulation
- Only queens overwinter
- Limited food storage

Red Mason bee (*Osmia bicornis)*

Life history traits:

- Solitary bee
- Nests in tube-shaped structures e.g. in deadwood
- Overwinter as pupa (cocoons)

Monitoring Results

Honey Bees (*Apis mellifera*)

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (*Apis mellifera*)

Daniel Rolke[1] · Stefan Fuchs[1] · Bernd Grünewald[1] · Zhenglei Gao[2] · Wolfgang Blenau[1]

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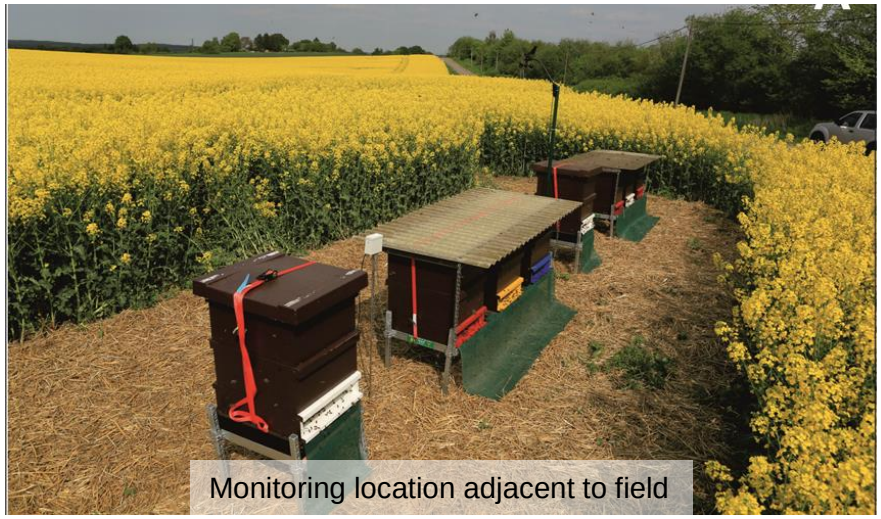
tier3 solutions

- Eight bee colonies per monitoring location (=1 replicate)
- Six replicates per site (treatment and control)
- Distribution of replicates per site: 3 replicates adjacent to oilseed rape fields, 3 replicates in 400 m distance.



Monitoring location adjacent to field

Post-exposure phase: west-central Germany

Monitoring Results

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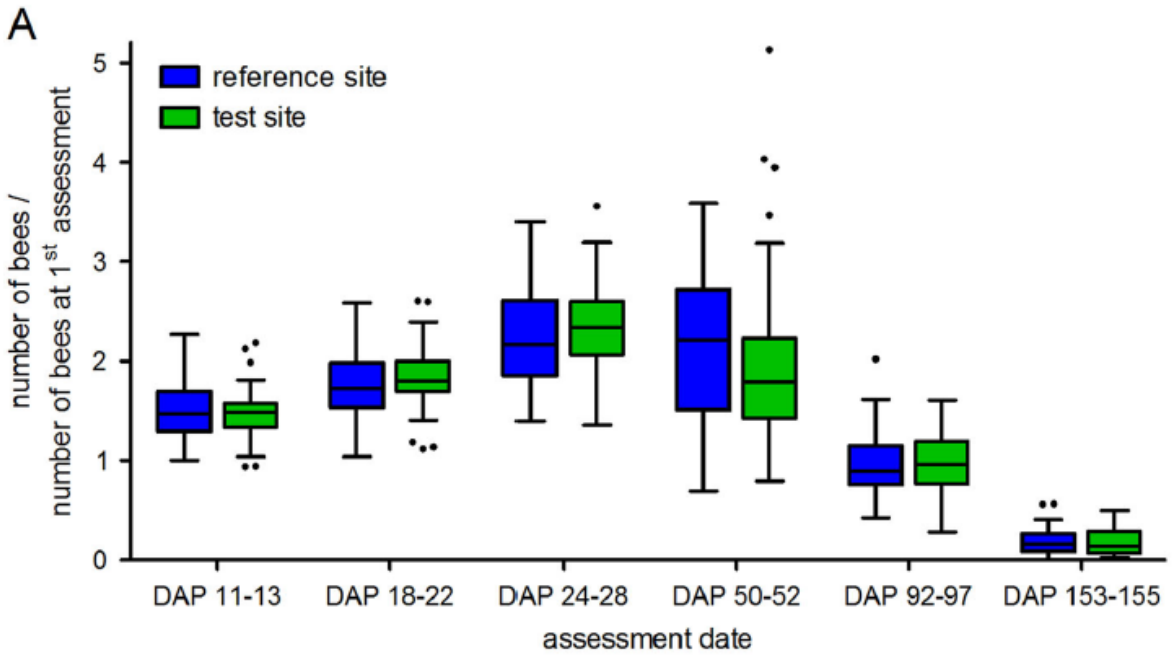
(1) Adult honey bees

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating…

Fig. 2 Development of the numbers of adult honey bees (**a**), capped brood cells (**b**), and open brood cells (**c**) in colonies located at study locations in the reference site (no clothianidin seed-dressing) and the test site (clothianidin seed-dressing). The box plots contain the 1st and 3rd quartiles, split by the median; traditional Tukey whiskers go 1.5 times the interquartile distance or to the highest or lowest point, whichever is shorter. Any data beyond these whiskers are shown as points. *DAP* day after placement



- Typical seasonal colony development pattern
- No statistically significant effect of treatment or distance to fields

Monitoring Results

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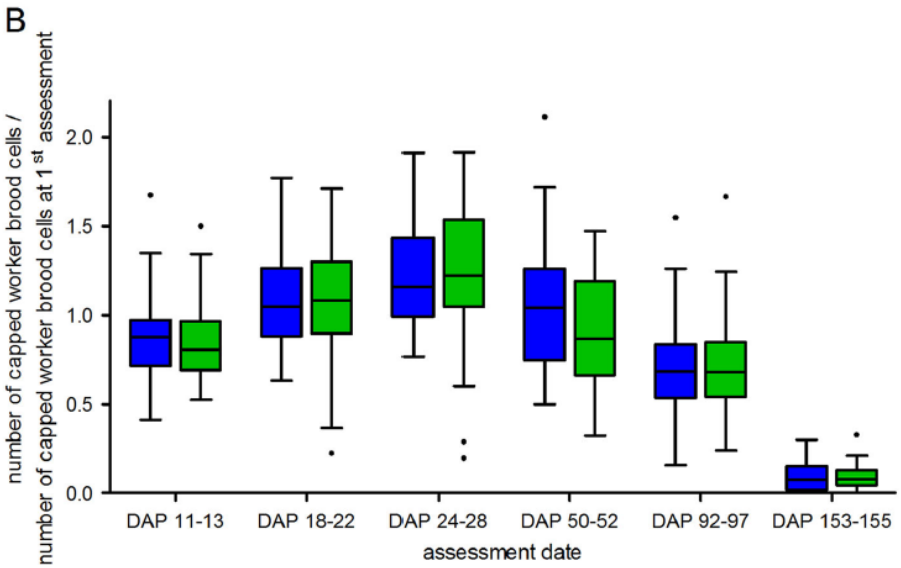
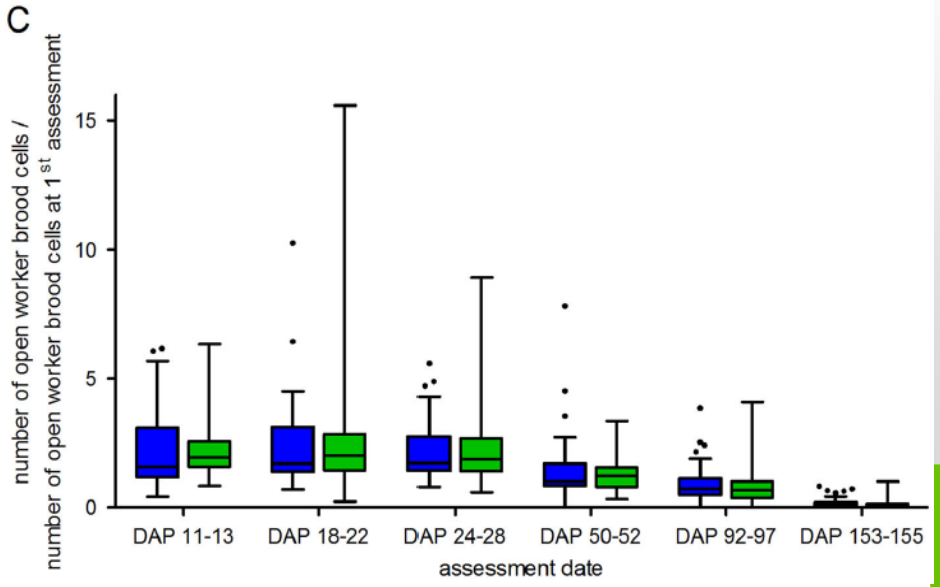
(2) Bee brood





- Typical seasonal growth pattern
- No statistically significant effect of treatment or distance to fields

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Monitoring Results

Honey Bees (*Apis mellifera*)

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (*Apis mellifera*)

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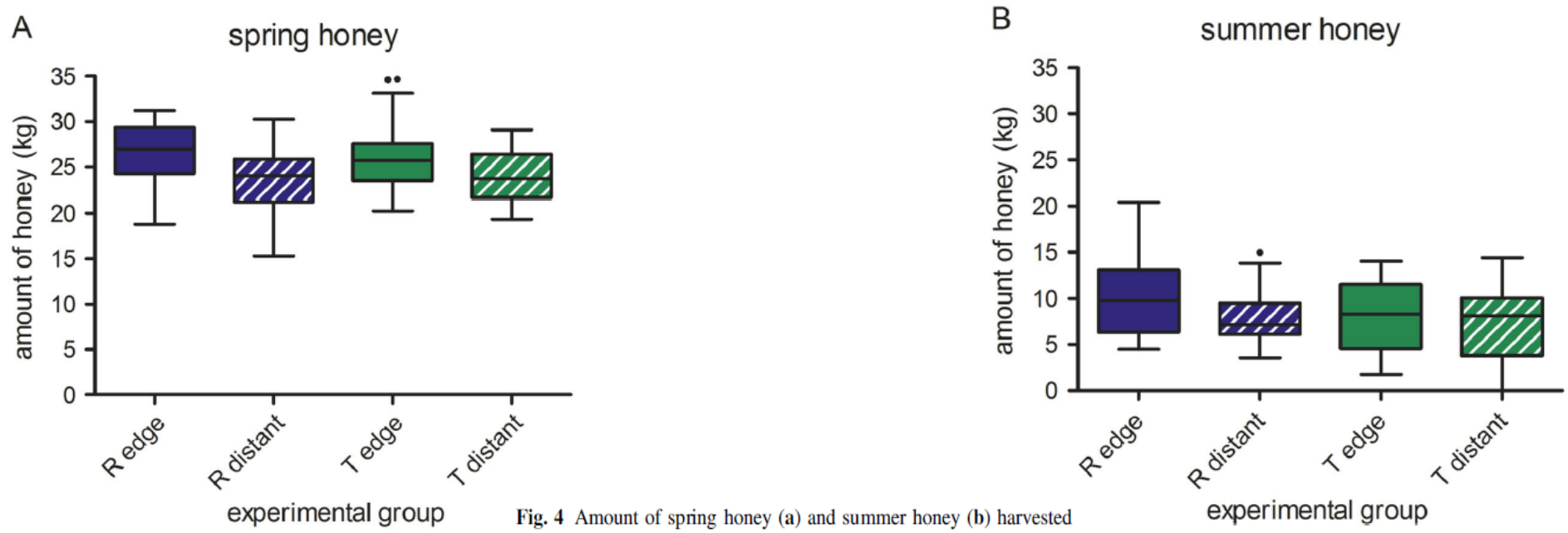

Fig. 4 Amount of spring honey (**a**) and summer honey (**b**) harvested from colonies from different experimental groups. The box plots contain the 1st and 3rd quartiles, split by the median; traditional Tukey whiskers go 1.5 times the interquartile distance or to the highest or lowest point, whichever is shorter. Any data beyond these whiskers are shown as points. *R* reference site, *T* test site

- No statistically significant effect of treatment
- Statistically significant effect of distance to oilseed rape fields

Characterization of Exposure

Sampling for residues

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in northern Germany: residues of clothianidin in pollen, nectar and honey

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Honey Bees *(Apis mellifera)*

- **Tunnels**:

  One Mini-Plus hive (30 x 30 x 30 cm, 6 combs, approx. 2500 bees) per tunnel, tunnels set-up inside study fields, sampling period 4 weeks,

  min. one pollen sample per tunnel (pollen traps, 100 mg / tunnel collected from morning to noon),

  nectar from 200 forager bees (content of honey stomachs), sample size 200 mg

- **Free-flying**:

  Eight Zander hives (50 x 43.5 x 23 cm, 10 combs, brood chamber plus 1-2 honey supers) at 12 locations = from 96 colonies,

  each colony sampled twice for pollen (pollen traps, 300 mg over one day),

  nectar from 200 forager bees (content of honey stomachs), sample size 200 mg

Conclusions

Honey Bees (*Apis mellifera*)

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (*Apis mellifera*)

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tier3 solutions

- Honey bees intensively foraged in treated fields

  (73-83% oilseed rape pollen found in honey)
- Residues of clothianidin in tunnels:
  average: 1.7 µg/kg in pollen (max. 3.5 µg/kg),
  average: 1.3 µg/kg in nectar (max 3.6 µg/kg),
  metabolites TZMU & TZNG < LOD
- Residue of clothianidin from field sites:
  average: 0.5 & 0.97 µg/kg in pollen (max. 2.7 µg/kg),
  average: 0.68 & 0.77 µg/kg in nectar (max. 1.6 µg/kg),
  average: 1.35 µg/kg in honey (max. 2.1 µg/kg in honey),
  metabolites TZMU & TZNG < LOD or LOQ

Conclusions

Honey Bees (*Apis mellifera*)

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (*Apis mellifera*)

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tier3 solutions

- There were no treatment-related adverse effects observed on:
  - colony strength and development (adults, brood)
  - honey yield
  - pollen composition
  - infestation with parasites and diseases

Monitoring Results

Bumble bees (*Bombus terrestris*)

Large-scale monitoring of effects of clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (*Bombus terrestris*)

Guido Sterk[1] · Britta Peters[2] · Zhenglei Gao[2] · Ulrich Zumkier[2]

Accepted: 14 September 2016

IPM IMPACT

tier3 solutions

- Three Tripols (= 3 bumblebee colonies) per monitoring location (= 1 replicate)
- Six replicates per site (treatment and control)
- Distribution of replicates per site: 3 replicates adjacent to oilseed rape fields, 3 replicates in 400 m distance.

Monitoring location adjacent to field

Collection of bumblebee workers for pollen sampling

Monitoring Results

Bumble bees (*Bombus terrestris*)

Large-scale monitoring of effects of clothianidin-dressed seeds on pollinating insects in Northern Germany: effects large earth bumble bees (*Bombus terrestris*)

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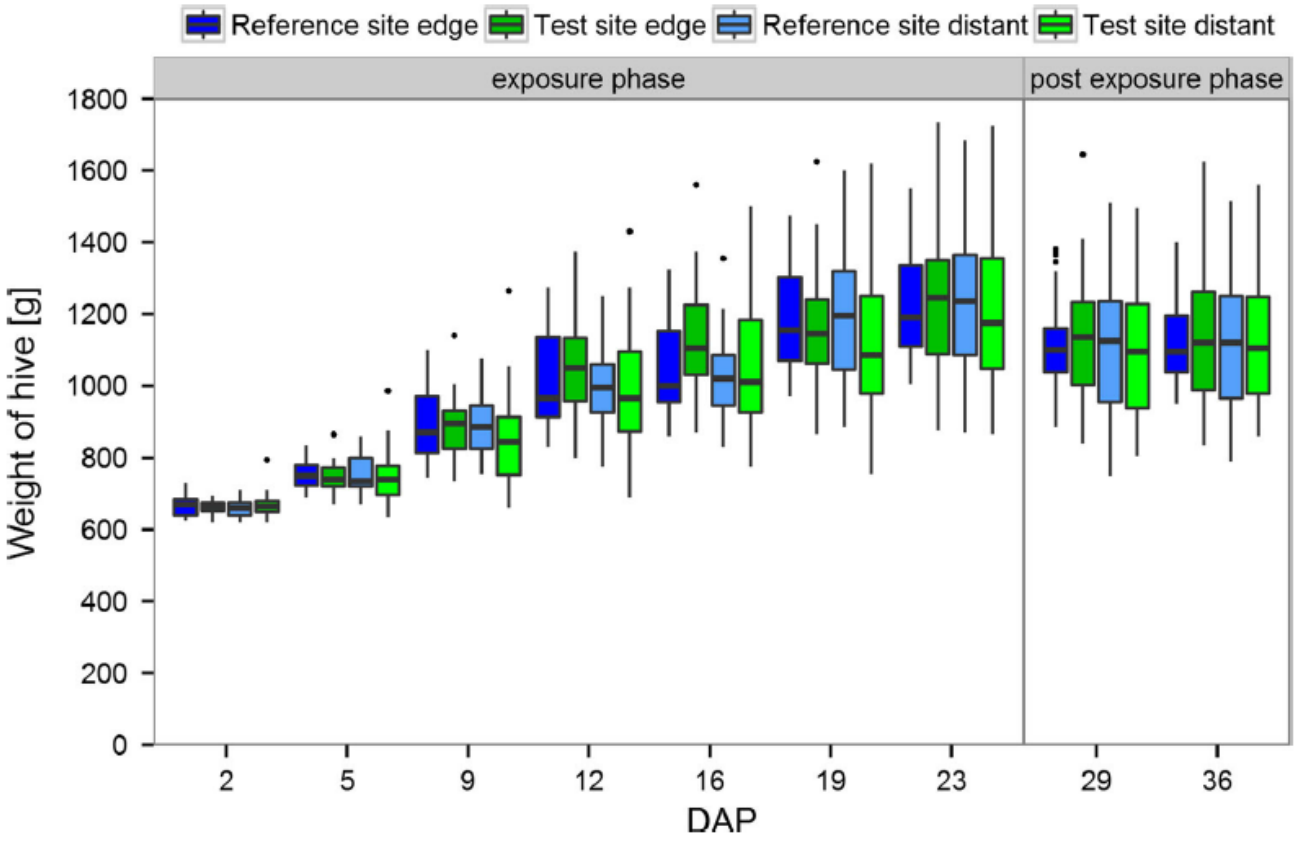
(1) Hive weight development

Fig. 5 Development of bumble bee hive weights. DAP: Days after Placement. After the exposure period colonies were relocated to a nature reserve area for further monitoring. The upper and lower hinges of the boxplot correspond to the first and third quartiles (the 25th and 75th percentiles). Whiskers extend from the highest value to the lowest value within 1.5 times the interquartile range. Data beyond the end of the whiskers are plotted as points



No statistically significant effect of treatment or distance to oilseed rape fields

Monitoring Results

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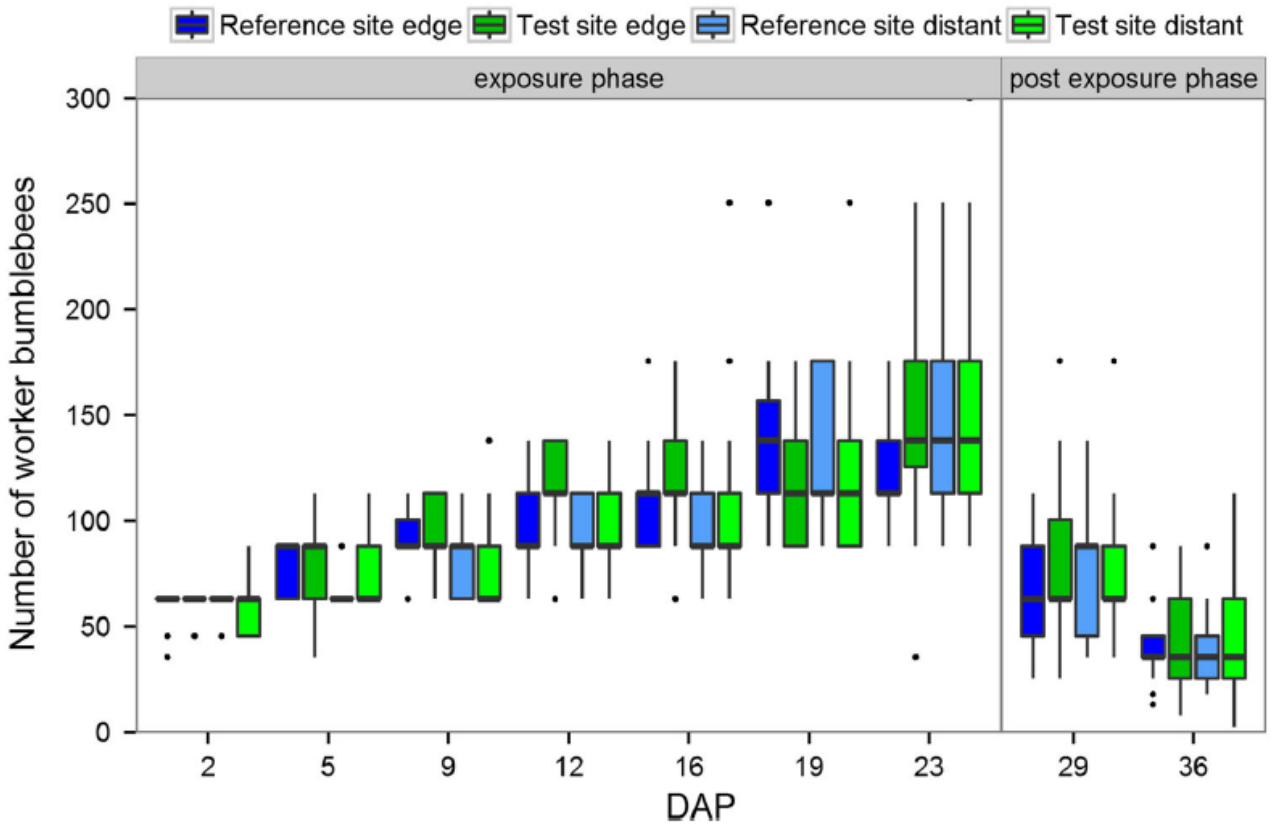
(2) Number of worker bees per colony

Fig. 6 Number of bumble bee workers as estimated by category. Bumble bee hives were either placed in a landscape with OSR fields treated with clothianidin seed dressing (test site) or untreated fields (reference site). Hives were either placed at the edge of the fields (edge) or ca. 400 m distant from the fields (distant). DAP: Days after Placement. After the exposure period colonies were relocated to a nature reserve area for further monitoring. The upper and lower hinges of the boxplot correspond to the first and third quartiles (the 25th and 75th percentiles). Whiskers extend from the highest value to the lowest value within 1.5 times the interquartile range. Data beyond the end of the whiskers are plotted as points



No statistically significant impact of treatment or distance to oilseed rape fields

Monitoring Results

Bumble bees (*Bombus terrestris*)

Large-scale monitoring of effects of clothianidin-dressed OSI seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (*Bombus terrestris*)

Guido Sterk[1] · Britta Peters[2] · Zhenglei Gao[2] · Ulrich Zumkier[2]

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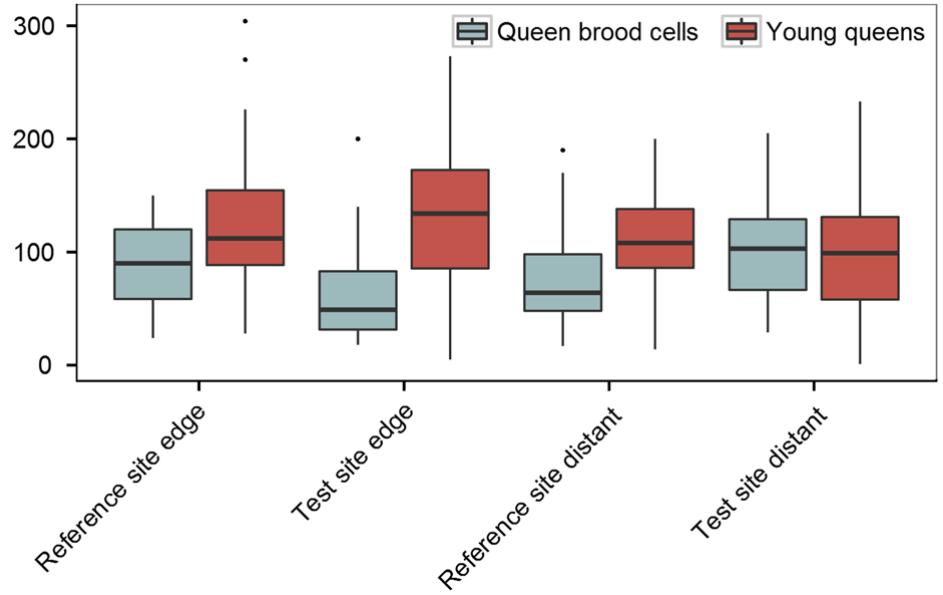
(3) Number of queens (including queen brood cells)

Fig. 7 Numbers of young queens and queen cells at the end of the experiment. Bumble bee colonies were frozen, and the number of young queens and queen cells were determined. Bumble bee hives were either placed in a landscape with OSR fields treated with clothianidin seed dressing (test site) or untreated fields (reference site). Hives were either placed at the edge of the fields (edge) or ca. 400 m distant from the fields (distant). During the study, all hives were fitted with a queen lock to prevent young queens from escaping, so that a reliable determination of their number was possible at the end of the experiment. The upper and lower hinges of the boxplot correspond to the first and third quartiles (the 25th and 75th percentiles). Whiskers extend from the highest value to the lowest value within 1.5 times the interquartile range. Data beyond the end of the whiskers are plotted as points



No statistically significant effect of treatment or distance to oilseed rape fields

Monitoring Results

Bumble bees (*Bombus terrestris*)

Large-scale monitoring of effects of clothianidin-dressed OSI seeds on pollinating insects in Northern Germany: effects or large earth bumble bees (*Bombus terrestris*)

Guido Sterk[1] · Britta Peters[2] · Zhenglei Gao[2] · Ulrich Zumkier[2]

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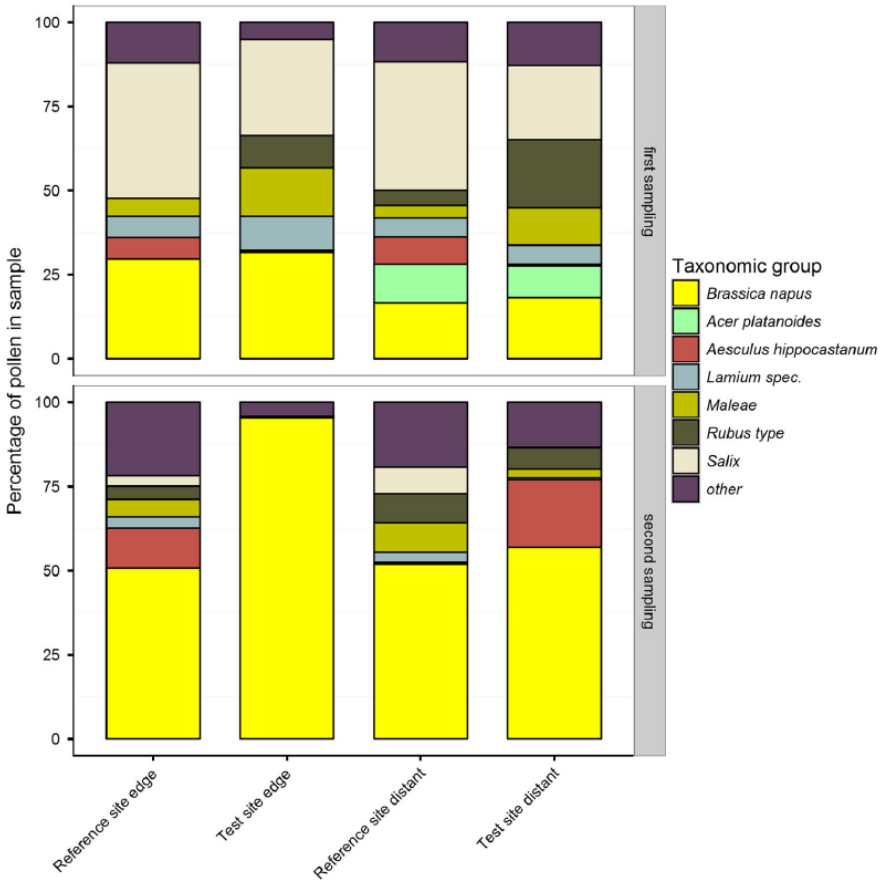
(4) Residue detects

Fig. 4 Composition of pollen samples. Bumble bee hives were either placed in a landscape with OSR fields treated with clothianidin seed dressing (test site) or untreated fields (reference site). Hives were either placed at the edge of the fields (edge) or ca. 400 m distant from the fields (distant). At each site one hive was designated to the collection of returning foragers carrying pollen. Two sampling events were conducted. The mean percentage of the different pollen species for each experimental group is shown. Pollen species representing less than 5 % of the total amount at the reference site were grouped as 'other'



- No residues in control pollen samples
- Residue levels in treatment landscape (bumble bee) comparable to tunnels (honeybees)

Characterization of Exposure

Sampling for residues

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in northern Germany: residues of clothianidin in pollen, nectar and honey

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Bumble bees *(Bombus terrestris)*

- One hive per study location used to sample 11-21 returning foragers,
- stored on dry ice,
- pollen loads were detached,
- sample size min 230 mg

Conclusions

Bumble bees (*Bombus terrestris*)

Large-scale monitoring of effects of clothianidin-dressed OS seeds on pollinating insects in Northern Germany: effects or large earth bumble bees (*Bombus terrestris*)

Guido Sterk[1] · Britta Peters[2] · Zhenglei Gao[2] · Ulrich Zumkier[2]

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- Bumble bees intensively foraged in treated fields
- Besides oilseed rape pollen, pollen from willow, chestnuts and wild berries were identified
- Clothianidin residues in pollen:
  average: 0.88 µg/kg (max. 1.3 µg/kg)
  metabolites TZMU & TZNG < LOD

- There were no treatment-related adverse effects observed on:
  - Colony development (workers, drones, queens)
  - Seasonal growth pattern (turning point to queen production)

Monitoring Results

Red Mason Bee (*Osmia bicornis*)

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (*Osmia bicornis*)

Britta Peters[1] · Zhenglei Gao[1] · Ulrich Zumkier[1]

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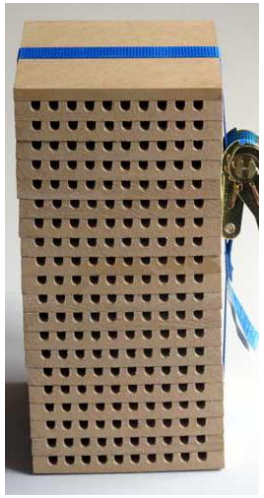
tier3 solutions

- Six Replicates per Control and Treatment with 3 nesting units adjacent to the oilseed rape field and 3 nesting units in 100 m distance
- 750 cocoons per nesting unit = **1500 cocoons per replicate released** (sex ratio 6:5 m:f)



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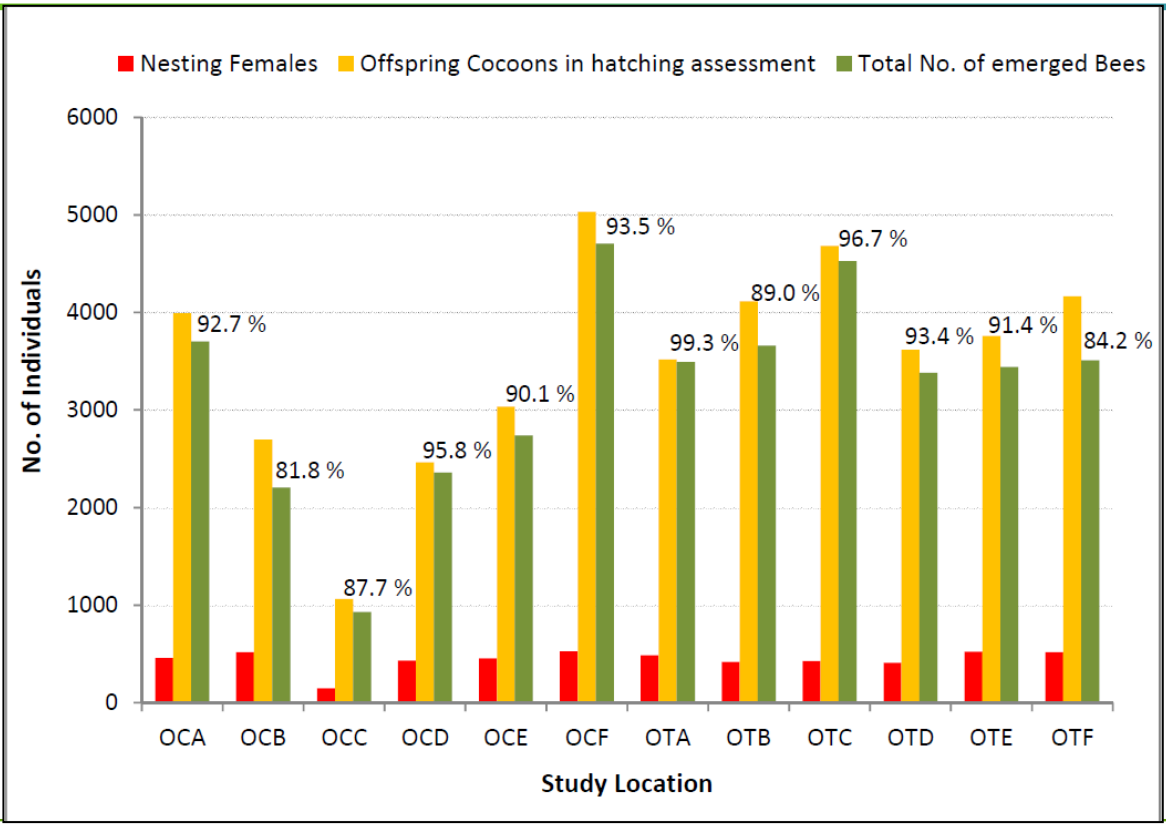

Reproduction rate =
Number of cocoons per nesting female (2014):

Control: 7.47
Treatment: 8.61

[1]Remark:
Control replicate OCC not taken into account for reproduction analysis. Breeding activity was low due to incorrect positioning of nesting units (shadow location) that caused site-related poor performance

Emergence rate spring 2015

Control : 91.0%
Treatment: 92.2%

- No statistically significant difference to the control on reproductive performance nor on cocoons emergence of offspring

Characterization of Exposure

Sampling for residues

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Red Mason Bees *(Osmia bicornis)*

- One sampling per study location,
- Nest blocks were opened to collect 10 subsamples from rear end of active provisioned nesting cell,
- Pollen retrieved with micro spoon,
- Combined sample amounted to min 200 mg

Conclusions

Red mason bees (*Osmia bicornis*)

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (*Osmia bicornis*)

Britta Peters[1] · Zhenglei Gao[1] · Ulrich Zumkier[1]

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- Red mason bees collected up to 32% of their pollen from oilseed rape fields (average 14%)
- Besides rape pollen, they collected pollen of roses and buttercups
- Clothianidin residues in pollen:
  average 0.88 µg/kg (max. 1.7 µg/kg)
  metabolites TZMU & TZNG < LOD

- There were no treatment-related adverse effects observed on:
  - Reproductive performance (nesting activity, offspring production)
  - Hatching success

Summary of Key Results

- Bee pollinators in the treatment site were exposed to low and representative levels of systemic Clothianidin residues
- Bee pollinators used oilseed rape pollen to different extent (honeybees > bumblebees > solitary bees)

Honeybee (*Apis mellifera*)

- No effects on colony development (adults & brood)
- No effects on honey yield and pollen composition
- No effects on infestation by parasites and diseases

Bumblebee (*Bombus terrestris*)

- No effects on colony development (no. of workers, drones, queens)
- No effects on seasonal growth pattern (switch point to queen production)

Red Mason bee (*Osmia bicornis*)

- No effect on reproductive performance (nesting activity, offspring production, hatching success)

Overall Conclusions

- The chosen landscapes “control” and “treatment” were sufficiently similar in terms of topography, climate, soil types and crop phenology to deliver a robust conclusion on whether or not Clothianidin poses an unacceptable risk to populations of insect pollinators
- Residue levels in pollen and nectar of oilseed rape in the treatment landscape were in the range of residue levels found in previous studies, i.e. highly representative exposure situation
- There were no treatment-related short- or long-term adverse effects recorded for the Clothianidin seed treatment to three bee species with different life history traits

Six publications available (open access)

Ecotoxicology
DOI 10.1007/s10646-016-1723-x

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in northern Germany: residues of clothianidin in pollen, nectar and honey

Daniel Rolke[1] · Markus Persigehl[2] · Britta Peters[2] · Guido Sterk[3] · Wolfgang Blenau[1,2]

Accepted: 2 September 2016

Ecotoxicology
DOI 10.1007/s10646-016-1730-y

Large-scale monitoring of effects of clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (*Bombus terrestris*)

Guido Sterk[1] · Britta Peters[2] · Zhenglei Gao[2] · Ulrich Zumkier[2]

Ecotoxicology
DOI 10.1007/s10646-016-1729-4

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (*Osmia bicornis*)

Britta Peters[1] · Zhenglei Gao[1] · Ulrich Zumkier[1]

Accepted: 14 September 2016

Ecotoxicology
DOI 10.1007/s10646-016-1734-7

Review of field and monitoring studies investigating the role of nitro-substituted neonicotinoid insecticides in the reported losses of honey bee colonies (*Apis mellifera*)

Richard Schmuck[1] · Gavin Lewis[2]

Accepted: 22 May 2016

Ecotoxicology
DOI 10.1007/s10646-016-1725-8

Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (*Apis mellifera*)

Daniel Rolke[1] · Stefan Fuchs[1] · Bernd Grünewald[1] · Zhenglei Gao[2] · Wolfgang Blenau[1]

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Ecotoxicology
DOI 10.1007/s10646-016-1724-9

Large-scale monitoring of effects of clothianidin dressed oilseed rape seeds on pollinating insects in Northern Germany: implementation of the monitoring project and its representativeness

Fred Heimbach[1] · Anja Russ[1] · Maren Schimmer[1] · Katrin Born[2]

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Science For A Better Life

Thank you!

Further Large-Scale Field Studies

Examples for other large-scale field studies under realistic field conditions:

- Studies in canola in Canada (2005 and 2012). Evaluation of colony health over one season and after overwintering
- Multi-site field study in maize in France. Locations in four different regions, study conduction over three consecutive years (2008-2010)
- Field study in Southwestern Germany (2008). Evaluation of colony health over one season and after overwintering.

In none of these studies, adverse effects of neonicotinoid seed treatment products to honey bee colonies have been observed.

Since field studies provide a direct insight into the interaction of pesticides with bee colonies, they provide highest-tier evidence for the ecotoxicological risk assessment