Occurrence of Guttation in Crops and its Relevance to Honeybee Colonies

- Results of Industry Studies in 2009 -

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Occurence of Guttation and Relevance to Honeybee Colonies

- Introduction
- Overview & design of studies performed by industry in 2009
- Study Results
  - Frequency and duration of guttation in investigated crops
  - Observation of honeybees in treated fields
  - Honeybee mortality rates
  - Colony condition and development during and after exposure
- Summary & Conclusions

Guttation: Introduction

What is guttation?

Guttation is a natural botanical phenomenon and describes the active excretion of liquid water (guttation fluid) by some vascular plants in form of droplets on the tips of leaves or on leaf edges.

Guttation must therefore not be mistaken with dew, which is a purely atmospheric phenomenon, describing the condensation of water vapor on surfaces (e.g. plants) in form of small droplets, or with transpiration, which is a passive process.

Nonetheless, those atmospheric conditions which favor dew formation, also favor guttation.

The guttation fluid is reported to be a 0.1 - 0.4% dilution of inorganic & organic substances; thus, the guttation fluid is unlikely to be of any energetic relevance for honey bees.

Guttation: Introduction

Guttation of seedlings and uptake of the guttation fluid by honey bees

A video published by [redacted] demonstrated in a laboratory study the intrinsic honey bee toxicity of guttation water exuded by young maize seedlings grown from neonicotinoid treated seeds.

However, at that point in time, the overall frequency of guttation occurrence and the relevance of the guttation fluid as a potential route of honey bee exposure to systemic insecticides under realistic use conditions was unknown.

To clarify the relevance of the guttation fluid as a water source for honey bee colonies under conditions of agronomic practice, various studies and monitorings were performed by industry in 2009.

Overview Industry Studies

In 2009, guttation studies were conducted in 6 crops and evaluates the development of > 120 honeybee hives in vicinity to treated fields

No.	Crop	Country	Com-pound class	Guttation assessm.	Bee exposure assessm.	Bee mortality	Bee hive develop-ment	No. treatment sites	No. control sites	No. treatment hives	No. control hives	Com-pletion
1	Maize	A	CNI	X	X	X	X	30	-	60	0	2009
2	Maize	F	CNI	X	X	X	X	4	4	24	24	2009
3	Maize	F	CNI	X	X	X	X	1	1	6	6	2009
4	Potato	D	Bait	X	(X)	-	-	1	2	-	-	2009
5	Sugar Beet	D	generic	X	-	-	-	(12)	-	-	-	2009
6	Winter OSR	D	CNI	X	X	X	X	3	3	15	15	2010
7	Winter Wheat	D	CNI	X	X	X	X	2	2	10	10	2010
8	Winter Barley	D	CNI	X	X	X	X	2		10		2010

Study design and key evaluation parameter are summarized in the next slides

Study 1 -Multiple site study <u>without control</u> plots in Maize

Study Design:

- Two typical maize growing regions in Austria, 15 field in each were monitored
- Exclusive CNI seed treatment due to high pest pressure
- Pedological and climatic conditions favouring guttation
- Two bee colonies each placed at the margin of surveyed fields or between fields
- Very low presence of bee attractive crops in surroundings, providing nectar
- ≥ 300m distance to permanent surface water bodies
- On 5 out of 15 fields per region, water trays were provided

=> Study design represents unrealistic worst-case conditions

Monitored parameter (3 – 6 weeks after emergence):

- Occurrence of guttation and residue analysis of droplets
- Observation of honeybees in corn fields
- Honeybee mortality and residue analysis
- Honeybee hive development
- Climatic conditions (11 out of 30 fields)

Upper / Lower Austria

Styria / Burgenland

Study 1 -Multiple site study <u>without control</u> plots in Maize

Study 2 -Multiple site study with control plots in Maize

Study Design:

- Four regions, representing different climatic and pedological conditions
- Per region, one treatment and one control plot with 6 commercial bee hives
- Treatment and control plots were well separated
- Size of the bee hives reflects typical commercial apicultural conditions
- Fields mostly not directly adjacent to permanent water bodies
- Rather low presence of bee attractive crops in surroundings

=> Study allows identification of effects and differentiation from other factors

Monitored parameter (3 – 9 weeks after emergence):

- Occurence of guttation
- Observation of honeybees in corn fields
- Honeybee mortality
- Honeybee hive development

Study 2 -Multiple site study <u>with control</u> plots in Maize

Study 3 - Study with control plot in Maize

Study Design:

- [redacted], France in spring 2009.
- Treated field plot (1.3 ha) matched with a similar sized field plot (2 ha) sown with untreated maize (control); plots separated by more than 2 km
- The experimental phase started with the arrival of the bee colonies at the field site in spring 2009 (prior to maize seeding) and ended at the last brood assessment on 3rd September 2009 at a remote monitoring location
- Six bee colonies were placed at the treated and six at the control field plots. At edge of field facing away from crop

Monitored parameter

- Mortality, foraging activity of the bees and the condition of the colonies were assessed during the period of seeding and subsequent guttation.
- After the maize had emerged, honeybee mortality and flight intensity were observed during guttation.
- Assessment of brood development was conducted at least once shortly before the set-up of the colonies at the fields and at several points during the exposure and monitoring phase.

Study 4 - Guttation Monitoring in Potato

Study Design:

- Monitoring in typical potato growing area in Germany ([redacted])
- Bee hives set up in some distance to the fields (18-209 m)
- Regular assessment of guttation on 3 fields for 4 weeks (May 28th till June 24th, growth stages BBCH 15-64)
- Field visits every day in the morning and evening
- Evaluation of bee activity

=> Study allows assessment of guttation appearance in potatoes and bee activities

Monitored parameters (4 weeks):

- frequency and timing of guttation events in potatoes
- bee presence in potatoes and attractiveness of guttation

Study 5 – Guttation Monitoring in Sugar Beet

Study Design:

- Monitoring in typical sugar beet growing area in Germany (West of Cologne)
- Regular assessment of guttation on 12 fields from emergence until BBCH 19
- Field visits only performed on days with meteorological conditions favouring guttation

=> Study design allows assessment of overall relevance of guttation in sugar beet

Monitored parameter

- Occurence of guttation in sugar beet
- Occurence of guttation in adjacent crops and off-crop areas

Studies 6, 7, 8 - Guttation in Winter OSR & Winter Cereals

Study Design:

- Two regions in Germany (Baden-Württemberg, Lower Saxony)
- Guttation monitoring in winter barley or winter wheat and OSR in each of the regions
- Treatment (CNI seed treatment) vs. "untreated" (non-systemic seed treatment) plots
- Study started in September 2009 and will continue until spring 2010
- Five bee colonies each placed at the margin of surveyed fields
- Fields provide suitable conditions for hibernation of bee colonies

=> Study design allows integrative assessment over several months

Monitored parameter (autumn 2009 & spring 2010)

- Occurence of guttation
- Residues in guttation droplets
- Observation of honeybees in fields
- Honeybee mortality
- Honeybee hive development
- Climatic conditions (7 out of 12 fields)

Results of Industry Studies

Key **preliminary** * results and conclusions are summarized with regard to

- Frequency and duration of the occurrence of guttation in surveyed crops
- Presence and behavior of honeybees in treated fields
- Daily honeybee mortality rates (maize only)
- Honeybee hive development

* As bee hive assessments, residue analysis, overall evaluation, and reporting are ongoing for most studies, the data and conclusions should be considered preliminary

Frequency and Duration of Guttation (1 / 2)

1. Maize:

Guttation occurred regularly and spontaneously on all investigated fields and lasted for several weeks. Example study 1 (large-scale field study, Austria):

	[redacted]	[redacted]	Total
Total Number of Assessment Days [No. fields x No. assessment days per field]	123	208	331
Frequency of Guttation [No. of assessment days at which guttation of maize seedlings were observed	118	158	276

High soil moisture conditions, drizzly weather and a high air humidity favoured guttation. Under windy conditions, guttation droplets disappeared very quickly or even did not appear at all.

Guttation was regularly present during early morning hours; under favouring conditions, guttation droplets could persist into the early afternoon, occasionally guttation appeared again in the late afternoon or during evening hours.

Overall, the guttation fluid was often still present during daily bee activity, i.e. honey bees could potentially encounter and collect the guttation fluid exuded from maize seedlings.

CNI residue levels in guttation droplets are in the 2- to 3-digit ppm range directly after emergence but quickly decline with further plant growth.

Frequency and Duration of Guttation (2 / 2)

2. Sugar beet: Guttation occurred far less pronounced if compared to maize.

- Even if monitored only at days without previous rainfall, guttation in sugar beet could only be clearly observed and recorded during one out of 98 field visits (1 %).
- In 16 other cases, moisture on leafs originated most likely from dewfall.
- Guttation in adjacent cereal fields and grassland observed during 83 % of the field visits.

3. Potato: Guttation observed on 19 of 28 days (5x no guttation, 4x not determined)

- More frequent in the morning (50%) than in the evening (23%)
- More frequent at earlier (61%) than at later (28%) growth stages
- Influence of weather conditions (guttation increased at lower temperatures)

4. Winter cereals & Oilseed rape: Quantitative evaluation ongoing.

Summary – Frequency of guttation:

Frequency of guttation differs between different crops (pronounced in maize, less in potato, low in sugar beet)

Due to the concurrent presence of guttation fluid and honeybees in seed-treated maize fields, individual honeybees could be exposed to systemic insecticides via guttation fluid

Observation of Honeybees in Fields (1/4)

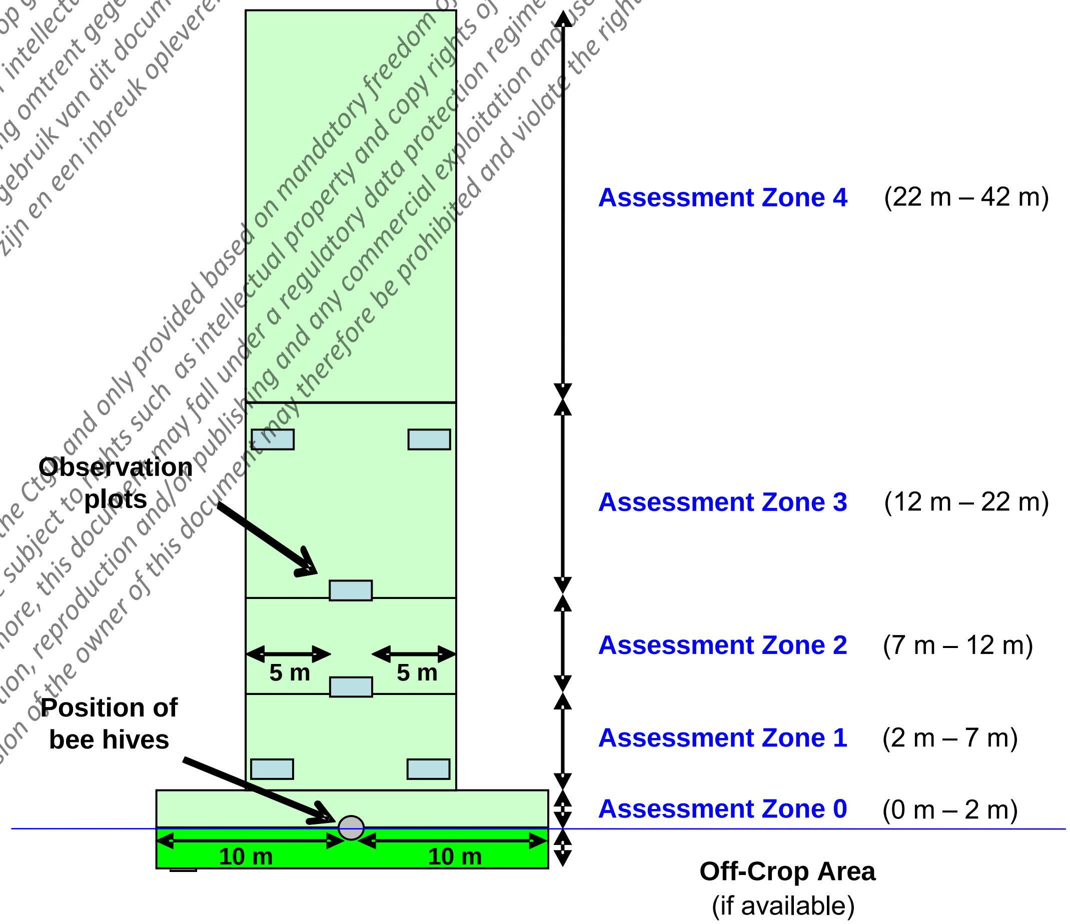
During the maize studies, regular and intensive assessments were made to clarify if honeybees visit the fields and take up guttation droplets.

Example assessment scheme study 1 & 2:

The total assessment area per individual field covered ≈500 m².

The in-field area was divided into five observation zones.

Within the observation zones, guttation and bee monitoring followed a pre-defined assessment scheme; in addition, six 2 m² plots were set up for detailed behavioral observations.



Observation of Honeybees in Fields (2 / 4)

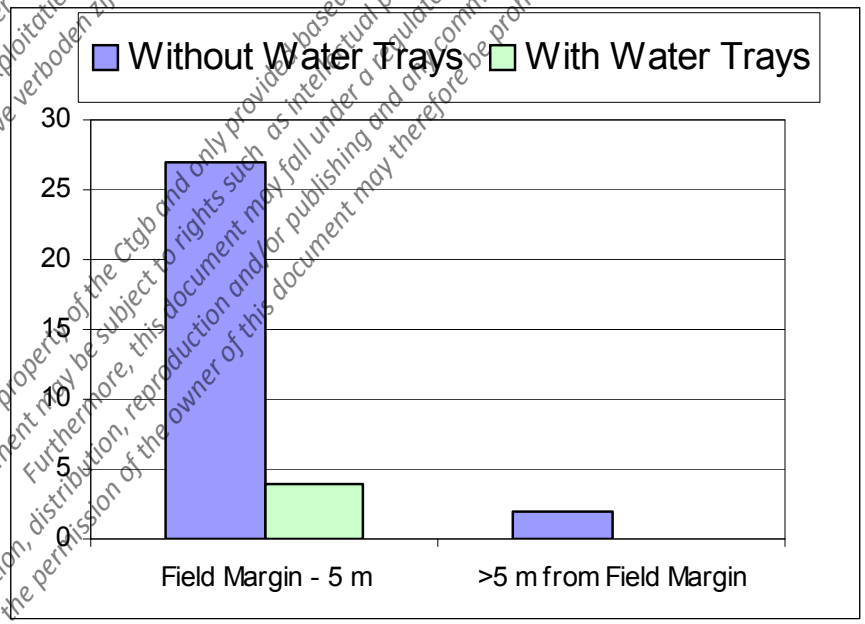
Honeybees visited maize seedlings rather infrequently. Visits were recorded during a few days only and not on all surveyed study plots. Occasionally, individual honey bees could be observed on maize seedlings when beehives were placed directly at the field margin (Study 1).

Access to an alternative water source reduced the frequency visitation even further.

When honey bees were observed on maize seedlings, they were encountered almost exclusively in close proximity to the beehive, i.e. within a range of 7 m from the field margin.

Example from study 1:

No. of assessment days at which bees were present on guttating maize seedlings



Observation of Honeybees in Fields (3 / 4)

Where beehives were placed in some distance to the field margins, visits of bees in the field were hardly observed.

Example **study 3** (maize, few meter distance from field margins):

- During the 40 day assessment period, two honeybees were seen flying over the maize crop at 10 assessment areas.
- Bees sitting on the ground were seen in the treated field on two occasions.
- No honeybees were seen interacting with guttation droplets despite intensive observation.

Example **study 4** (potato; beehives placed 18-209 meters away from the field margins):

Low frequency of honeybee visits in the fields

- Some visits during the occurrence of guttation
- Observed visits more frequent and longer in morning than evening
- No bees observed interacting with guttation droplets

Observation of Honeybees in Fields (4 / 4)

Although observations of honeybees in fields were rare, analytical findings in dead bees (Study 1) give evidence that under aggravated exposure conditions (exposure for up to six weeks directly at field margin) individual bees can ingest residues of systemic insecticides, most likely by collecting guttation fluid (see next slides)

Summary - Observation of honeybees in fields:

- Visits of bees in guttating fields were rarely observed
- Where observed, visits took only place in direct proximity to the bee hives
- Residue detects in dead honeybees provide evidence that – under aggravated exposure conditions – individual bees apparently may collect guttation fluid
- Number of visits is strongly reduced if alternative water sources are available or bee colonies are placed in off-crop structures rather than directly at the field margin

Daily Honeybee Mortality Rate

Mortality data are currently only available for maize (Studies 1, 2, and 3)

Even under aggravated exposure conditions (example study 1: honeybee hives directly placed at maize field margin for 3 – 6 weeks), the overall daily mortality was low.

Residues analysis has been performed in study 1:

A total of 1518 individual daily mortality assessments was conducted on 30 treatment sides. Whenever 10 or more dead bees were found, a residue analysis was conducted

Out of these 1518 samples, no or only few dead bees (0 – 9 bees per hive) were recorded in 1292 assessments (=> no reside analysis)

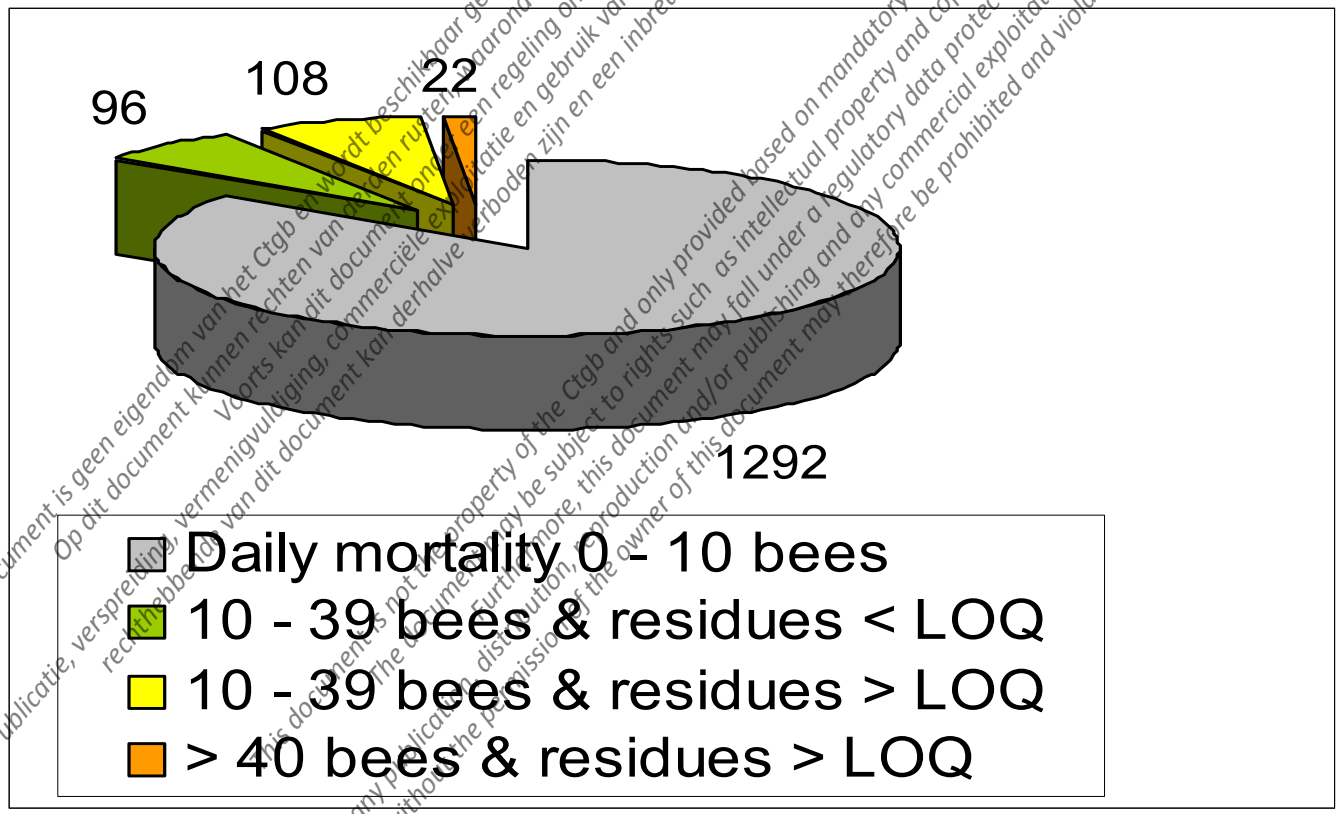
All remaining 226 samples (10 or more dead bees per hive) were subject to CNI residue analysis (LOQ: 0.001 mg/kg).

Up to ca. 40 dead bees per hive were observed in 204 of these 226 samples. From these, the number with CNI residues > LOQ did not substantially differ from the number without CNI residues (108 samples > LOQ; 96 samples < LOQ)

In only 22 out of the 1518 daily mortality assessments, and thus only in single hives and on single days, a higher number of dead bees (> 40 bees) was found.
In most of the samples collected during these assessments, potentially detrimental CNI residue levels were found.

Daily Honeybee Mortality Rate

When beehives were exposed directly at field margins and under worst-case conditions over several weeks, increased mortality which can be related to exposure to CNI residues was observed in only 1.7% of the assessments (i.e. only on single days and for individual hives).



Daily Honeybee Mortality Rate

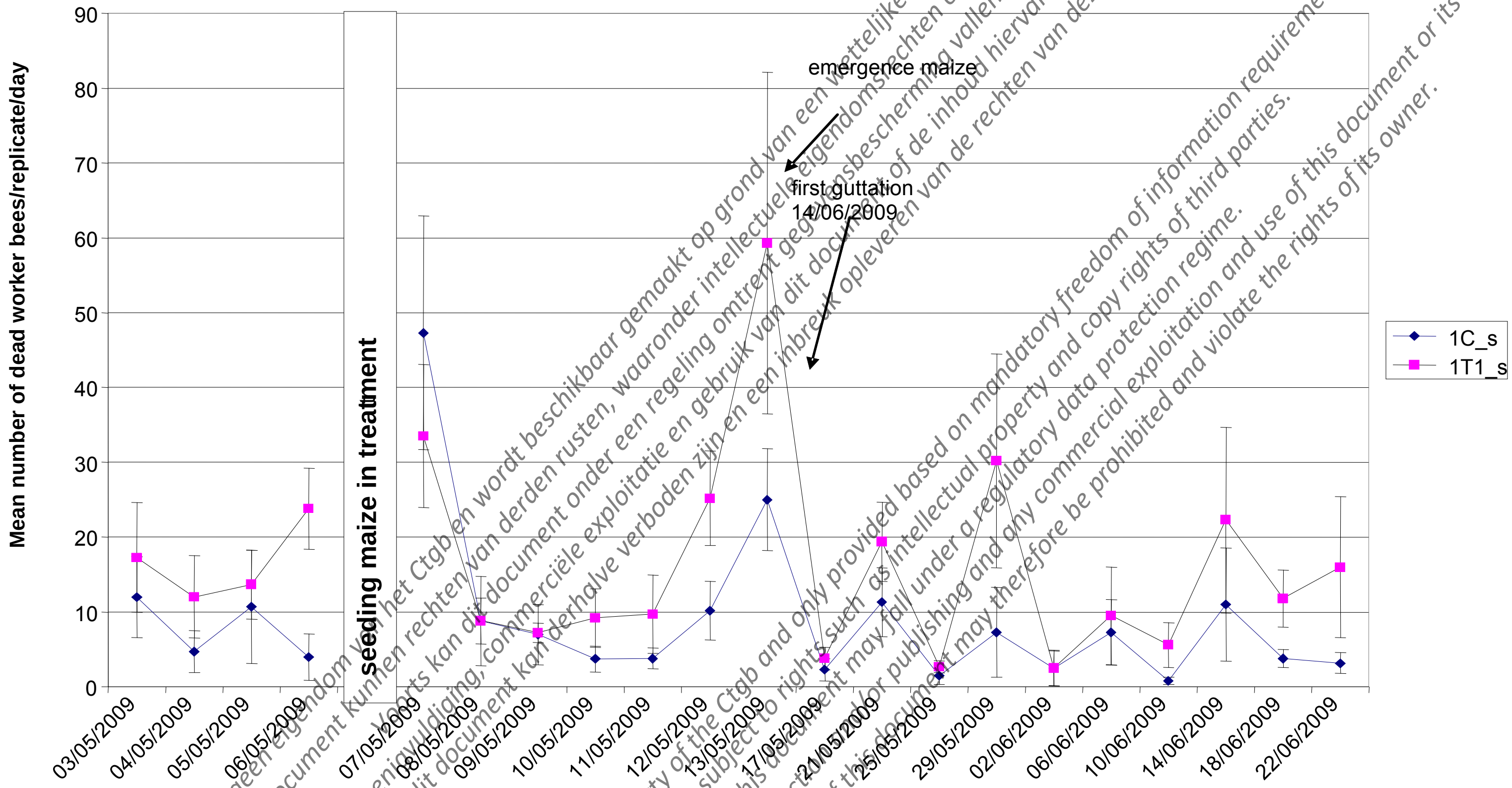
Due to possibility to compare treated sites with untreated control sites, studies 2 & 3 allow a more conclusive evaluation whether systemic bee-toxic seed treatment products could cause an increased honeybee mortality. Moreover, these studies were conducted under experimental conditions which are more representative of typical apicultural practices, i.e. larger colony size and hives placed on sheltered ground with surrounding non-crop vegetation.

In study 2 occasionally peak mortalities were seen in both, treatment and control groups, frequently synchronous or slightly phase-shifted, suggesting non-treatment related factors. Frequently, such peak mortalities were correlated with in-hive assessments (see example of Gard site) indicating hive disturbance as a relevant detrimental factor.

Summary – Daily Honeybee Mortality Rate:
Overall, mortality rates did not differ between treatment and control plots.

Considering all findings, there is no evidence that guttation fluid of maize crops seed-treated with bee-toxic systemic compounds has a detrimental impact to exposed commercial-sized colonies (see next slides)

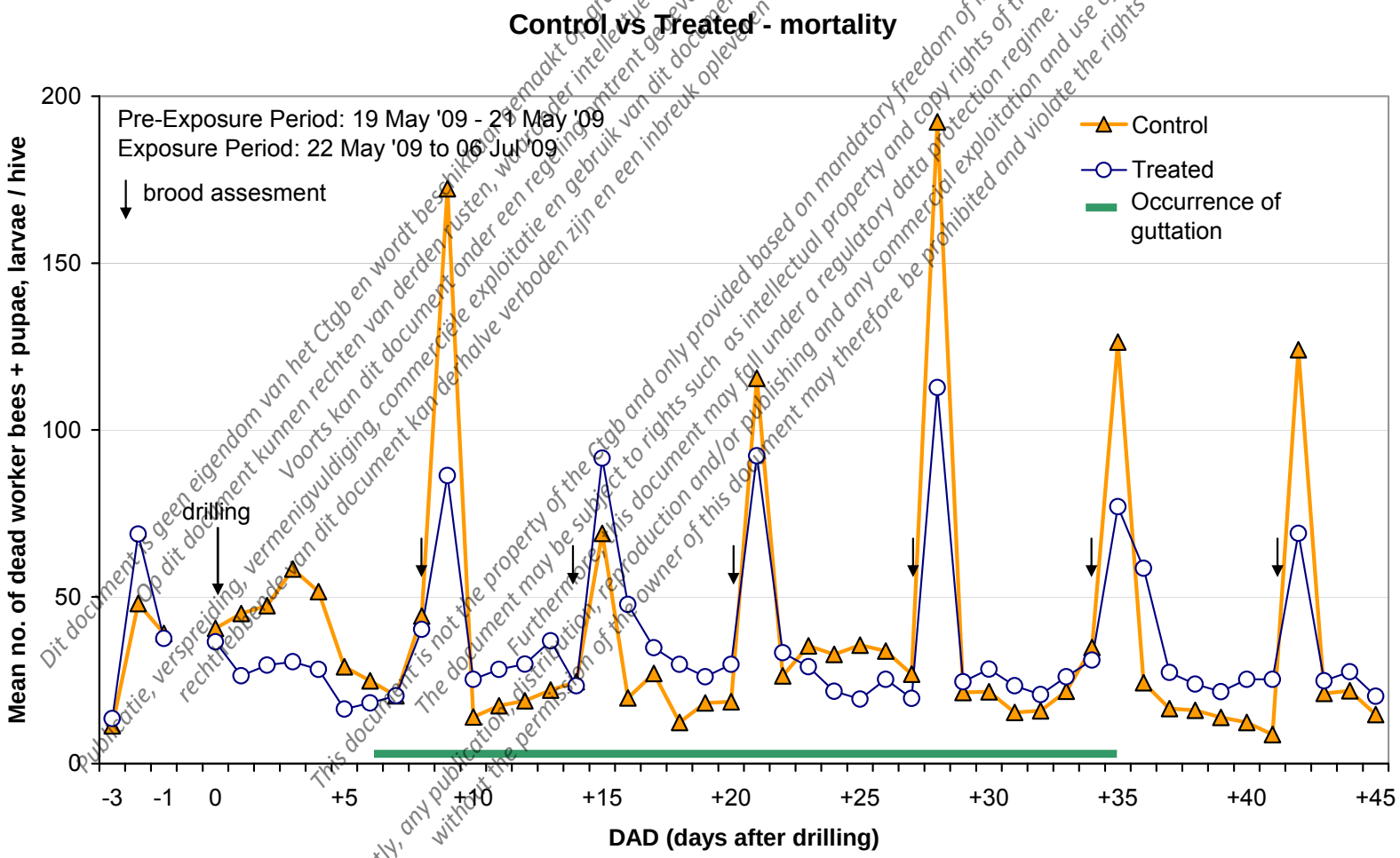
Daily Honey Bee Mortality Rate: Study 3



- Mean honeybee mortality in the test item was higher on some assessments days compared to the control treatment.
- However mortality remained at a low level during the exposure period (<50 dead bees/hive/day) except on one occasion prior to the start of guttation, when mean mortality in the test item group was approximately 60 bees/hive/day.

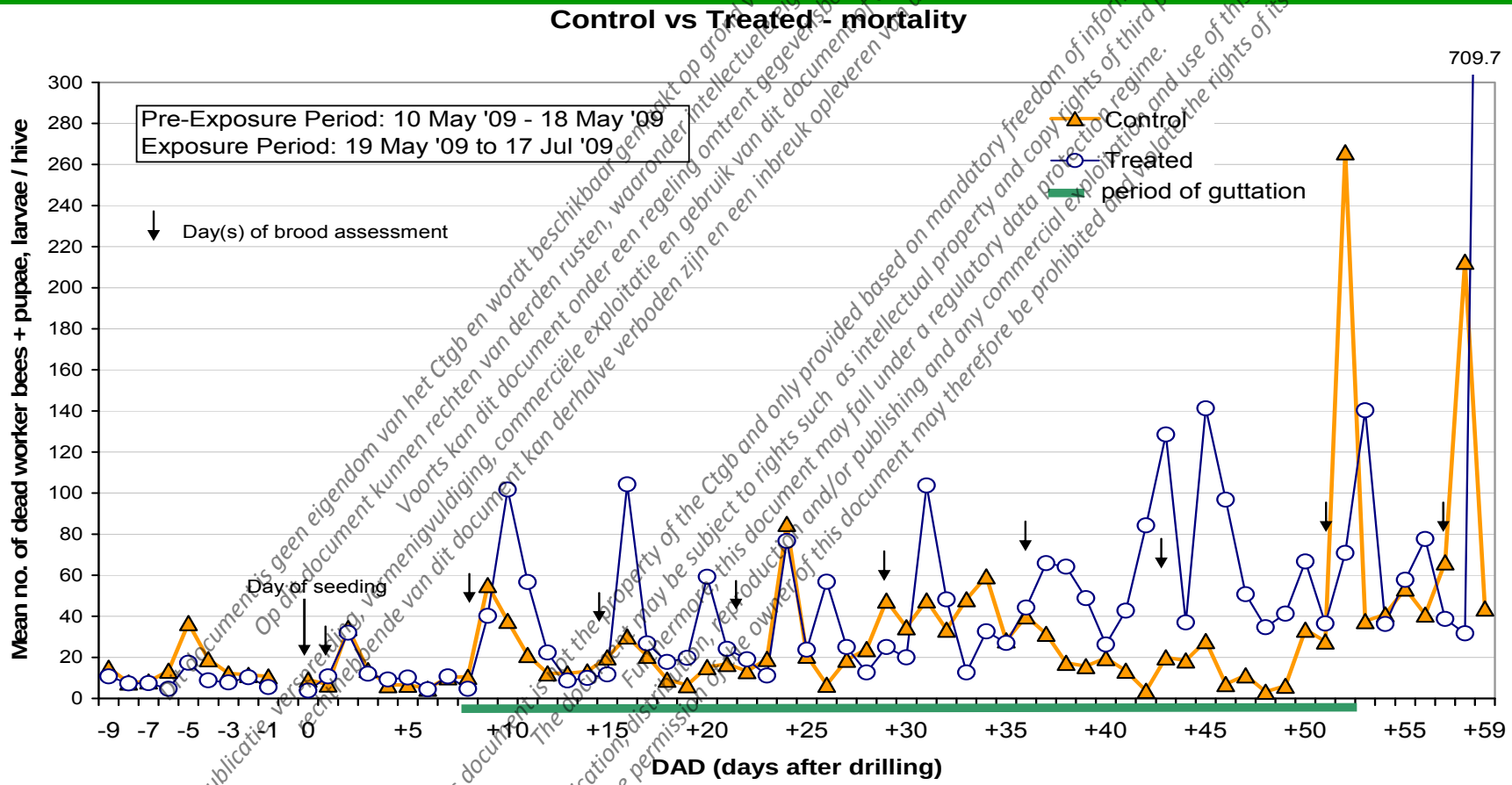
Daily Honey Bee Mortality Rate : Study 2

Total number of dead bees per day from 6 colonies per field (dead bee traps + linen sheets, combined), France: Gard site



Daily Honey Bee Mortality Rate : Study 2

Total number of dead bees per day from 6 colonies per field (dead bee traps + linen sheets, combined), France: Alsace site



Peak mortality on day 59 outside guttation period and caused by „robbery“

Daily Honey Bee Mortality Rate : Study 2

Daily average mortality at the different assessment sites during the guttation phase does not differ between control and treatment sites

C = Control

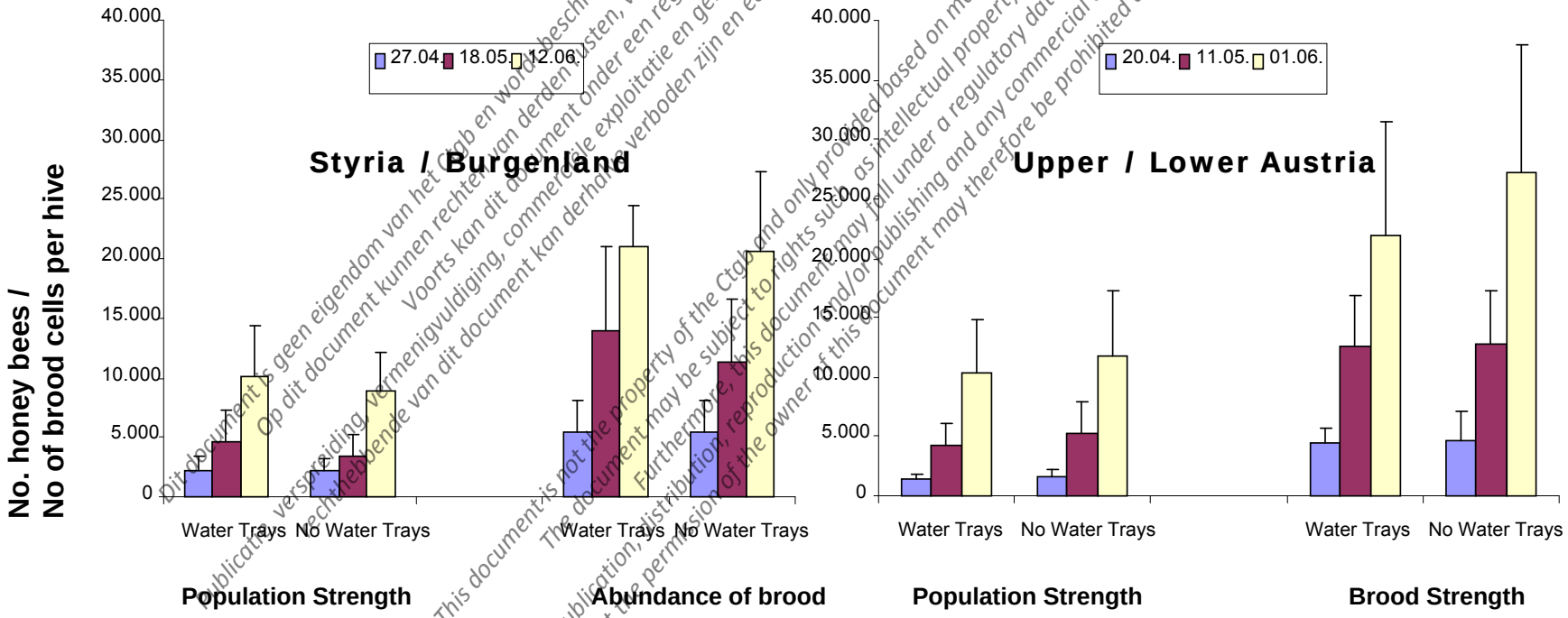
T = Treatment

	Alsace		Champagne		Aquitaine		Gard (Nîmes)	
	C	T	C	T	C	T	C	T
Average Number of Dead Bees per Hive and Day	29.8	46.3	11.4	9.5	9.0	12.7	42.6	38.4

Honeybee Hive Development – Study 1

Even the nearest exposure of bee hives to guttating maize seedlings had no influence on hive development.

This also applies to beehives which showed an increased bee mortality on 1 - 3 days during the 3-week observation period.



Honeybee Hive Development – Studies 2 & 3

Conclusions from the findings of the trials

Study 2:

- Colony condition, strength, and brood development were generally very variable between the different locations and different hives, and developed in a very dynamic way.
- Nevertheless, development of colony strength and brood-related endpoints largely followed the same trends in the colonies of the control and treatment groups.

Study 3:

- Colony strength in the control was slightly higher than the test item treatment before trial commencement and during the study period.
- During the assessment period, the number of bees per colony in both treatments showed the same tendency to increase and decrease. Although colony strength was higher in the control colonies compared to the treated colonies, by the last assessment in September the mean colony strength in both treatments was at the same level.
- The levels of brood and food during the assessment period were very similar throughout the trial.

Field Study of the Swiss Authorities

Results of CP industry studies in maize are in line with a field monitoring project of the Swiss BLW which investigated potential effects of dust-off at drilling and guttation fluid of maize seedlings to bee colony development

Two field trials were conducted with a setup comparable to the CP industry trials (without detailed assessments of bee behavior in the crop)

Conclusions of Swiss authorities about the results (September 2009):

- **1.** ***In both trials unnatural bee mortalities did not arise immediately after sowing and no clothianidin residues were detected in the bees. The results confirm that under practical conditions with moderately flowering plants in the neighbourhood of the sowing operation, the current regulations on the application of corn seeds dressed with clothianidin are adequate. However, adherence to the regulations should be strictly monitored in order to ensure protection of bees.***
- **2.** ***Increased bee mortality did not occur in either trial during the guttation period of the young corn plants. Clothianidin residues could not be detected in the bees or the honey. Harm to the health of bee colonies can be excluded on the conditions chosen for the trials.***

Guttation: Summary and Conclusions (1/3)

- Guttation is a botanical phenomenon which regularly occurs in maize, less frequently in potatoes and hardly in sugar beet crops. Other crops, e.g. OSR and cereals are currently under investigation.
- In spring, guttation fluid with high residue levels of bee-toxic systemic insecticides was present on maize seedlings during bee flight; investigations in crops sown in fall are ongoing
- Concerning soil applied baits limited results showed no detectable residue levels in guttation droplets
- When beehives are placed in nearest distance to maize fields without alternative water sources for several weeks during early summer, individual honey bees may collect guttation fluid from maize seedlings. In these case, guttation fluid can impact honeybees at the level of individuals. In contrast, no impact was recorded at the colony level even under such aggravated exposure conditions.
- Access to alternative water sources, e.g. guttating non-crop vegetation, and placing beehives in sheltered locations as practiced in good apicultural maintenance reduces exposure to negligible levels.

Guttation: Summary and Conclusions (2 / 3)

- As shown by 2 further field studies, guttation fluid of maize seedlings had no observed impact on either the daily honeybee mortality nor the colony development under more realistic apicultural conditions and commercially sized colonies.
- First evaluations from initiated studies in winter OSR and winter cereals also give no indication for treatment-related mortality or effects on hive development
- The presented data and conclusions are in line with an evaluation which has recently been published by the Swiss authorities

Guttation: Summary and Conclusions (3 / 3)

Also from a historical perspective, there is no indication that guttation fluid impacts bee colony viability under practical field conditions:

- During the last decade, bee samples submitted to the German Bee Incident Investigation Institute were all subjected to a CNI residue analysis. No residues of CNI were detected in these samples except for those of the 2008 bee incident in the Upper Rhine valley which was caused by enhanced seed abrasion
- In a recent survey in Belgium, no correlation was found between the intensity of exposure to CNI seed-treated maize fields and mortality rates of 16 apiaries which were regularly inspected between March 2004 and March 2005 (Imidacloprid; [redacted] 2009)