On the way to a new guideline: Results of three years of bumble bee semi-field testing

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Introduction

According to the EFSA Guidance Document on bees (EFSA, 2013), not only honey bees but also bumble bees should be considered in the risk assessment of plant protection products. Up to now, no official guideline for standardised semi-field trials is available to assess effects on bumble bees. The existing methods for honey bees cannot be easily transferred as bumble bees, e.g. have an annual life cycle and build smaller nests with irregular structures.

Open questions are: What are the relevant endpoints for bumble bee semi-field testing? How can these endpoints be assessed in a reliable, reproducible way?

To answer these questions, tests have been performed from 2014 to 2016 based on the recommendations of the ICPPR non-*Apis* workgroup. Experiences were gained regarding different crops, season and size of colonies as well as different endpoint assessments.

General test design

Single colonies were placed in tunnels with an attractive crop (winter oilseed rape, *Phacelia tanacetifolia)*. After acclimatisation, spray application was performed. Colonies remained in the tunnels until the crop had faded (exposure phase) and were transferred to a monitoring site until colonies started to produce reproductive offspring (males and queens). Colonies were then frozen and stored for final colony assessments.

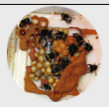
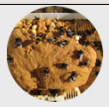
View inside a tunnel

Colony set up in the tunnel

Monitoring site

Season and size of colonies

For a more realistic scenario, colonies were used in a size which is comparable to the developmental stage found in nature at the time of year the test was performed.

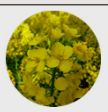
Colony size	 Small	 Large
Start of test	Spring (end of April)	Summer (end of June)
Advantages and problems	• Colonies easier to handle • Small tunnel size sufficient • Colonies likely more sensitive	• Assessments difficult due to more irregular nest structure • Susceptible to overheating in summer on open fields

Quality of colonies

Colonies were ordered at a commercial supplier, but were always larger than ordered and varied strongly in number of workers and condition of brood nests. As large colonies tend to be at the end of their annual development cycle, there is the risk of colonies having already started to produce queens before exposure.

To obtain comparable and meaningful results, a reliable quality of colonies has to be ensured in future.

Year	Colony size [no. of workers] ordered	received
2014	50 – 70	71 – 137
2015	10 – 20	17 – 34
2016	50 – 60	230 – 340

Crop	 Winter oilseed rape	 *Phacelia tanacetifolia*
Crop area	30 m²	90 m²
Colony size	17 – 34 workers	230 – 340 workers
Time colonies lived in tunnels	18 days	13 days
Additional feeding	None	None

Test crop

Bumble bees foraged well on both crops (winter oilseed rape and *Phacelia tanacetifolia).* Colonies gained weight during the exposure phase. Therefore, it can be concluded that the crop and crop area were sufficient to provide enough nectar and pollen for an undisturbed brood development.

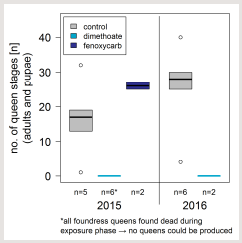
Toxic reference

Dimethoate showed acute effects at maximum field application rate on bumble bee colonies during the exposure phase, similar to the effects observed on honey bees. No effects on queen production could be noted.

Active substance	Application rate	Observed effects
Dimethoate	400 g a.s./ha	• Increased worker mortality • Inhibited foraging activity • Weight loss of colonies
Fenoxycarb	300 g a.s./ha	None

For Fenoxycarb, no effects were observed at all. One would expect effects on the brood caused by the application of this insect growth regulator, regularly used in honey bee brood studies. But due to the irregular nest structure, brood development can only be determined destructively and was therefore only assessed once at the end of the test.

Endpoints



Production of young queens

As only queens survive the winter and will build up new colonies, the number and fitness of newly hatched queens is crucial. But even untreated colonies do not all produce queens and if they do, the number varies greatly, also at colonies of a similar initial size.

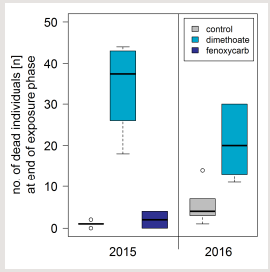
With this variation in mind, it remains unclear, if effects of substances on the number of queens produced per colony can be detected reliably.

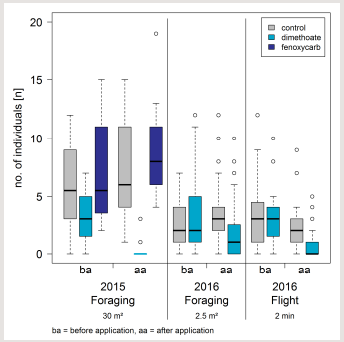
Mortality

Dead workers and also foundress queens can easily be counted inside the colonies and removed without strong disturbance of the colonies. As long as colonies are small, this assessment is reliable.

Brood development

Up to now, there is no method available to measure effects on brood during the test. Dead pupae and larvae can only be counted by destroying the nest and were therefore assessed only once at the end of the test. The only option is to stop the test after the exposure phase inside the tunnels but thereby loose the possibility to detect effects on reproduction.





Foraging and flight activity

Foraging: Number of bumble bees observed foraging on a defined crop area.

Flight: Number of bumble bees observed entering the colony during a defined time period.

Absolute numbers were low and varied strongly for these two assessments. Especially when small colonies are used, the area or time period should be as large or long as possible to gain reproducible results.

Colony weight

Weight was measured weekly and was a good indicator of colony development without strong disturbance of the colonies. It was basically measured to determine the time point at which colonies had switched from colony growth and worker production to reproduction (queens and males), which results in strongly declining colony weight.

Test duration

To measure reproductive success, colonies have to be observed until they switch from colony growth to reproduction. From then on, colonies were observed two more weeks and than frozen for final colony assessments.

Test end was determined individually for each colony, as the time point of switching can in itself be meaningful.

Conclusions

- Small colonies at an early developmental stage should be preferred.
- A better colony quality has to be ensured.
- For small colonies, a crop area of 30 m² is sufficient.
- Dimethoate (400 g a.s./ha) is a suitable toxic reference to show acute effects.
- Queen production is a highly variable endpoint.
- As bumble bee colonies vary strongly in size and development, a minimum of six replicates per treatment group is recommended.

References

EFSA, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal, 11(7): 3295, 268 pp., doi:10.2903/j.efsa.2013.3295