3.10 Bumble bee semi-field studies: choice and management of colonies to reduce variability in assessment endpoints

O. Klein[1], L. Franke[1], J. Fricke[1], J. Sorlí[2], S. Knaebe[1]

[1]Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany, [2]Eurofins-Trialcamp SLL, Valencia, Spain

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Abstract

The publication of the proposed EFSA risk assessment for pollinators resulted in an increasing demand for experiments with non-Apis pollinators (EFSA 2013). However, no official guideline for the standardized semi-field trials exists so far. To overcome this lack of guidance, the development of semi-field study designs are under way. The methodology is concurrently be developed by an ICPPR working group (non-Apis working group).

A major challenge in higher tier studies is the variability of the different endpoints. Hive development and particularly the production of young queens are very variable (Cabrera et al. 2016). With the current knowledge it seemed crucial to select appropriate colonies for the tests to reduce variability. The aim was to evaluate different strategies for the selection of bumble bee colonies and to improve the data quality with regard to the most important endpoints in bumble bee semi-field studies.

Methods

Semi-field tests according to the ICPPR non-Apis working group protocol were performed in Germany (test 1) and Spain (test 2). Bumble bee colonies were selected which were as similar as possible with regard to:

- Number of workers and brood stages
- Brood (larvae)/worker ratio
- Increase (development speed)

For most of the parameters it is sufficient to count the number of different life stages, whereas for the evaluation of the development speed it is necessary to perform an initial brood assessment at a very early stage of colony development followed by an approx. 2 week period where the colonies are kept in the lab. After this period, the brood assessment is repeated. The increase was calculated as given in the formula:

Increase = (count SB – count IB)/ count IB

(with IB = initial brood assessment, SB = second brood assessment, counts = total number of workers + brood)

All parameters were compared between the different bumble bee colonies for final selection.

Results

For the selection of bumble bee colonies priority was given to the two endpoints: number of workers and development speed (increase). Other endpoints (brood, total (workers + brood) as well as the larvae/worker ratio) were also considered. Some of the colonies were excluded from the selection process due to visible deficiencies (marked with greyish bars; i.e. weak foundress queens, deformed wings, significantly smaller size of workers). For the remaining colonies the upper and lower limits for the two main endpoints were set. The aim was to keep the variation between hives as small as possible (rectangular frames). In the end 12 colonies were selected based on the range chosen for the endpoints. The colonies outside the range were taken out. Selected colonies (white background) were distributed over the two treatment groups (control = green and test item treatment = red bars).

The variability of the the final colony weight was low, confirming that an additional brood assessment improves the test design. For the important endpoints mortality and young queen production MDDs also improved markedly if compared to ringtest data (Knäbe et al. 2017). There MDDs ranging from 50 to 285 were prepared for 8 separate studies while in the two tests described here MDDs for queen productions were 52 and 67.

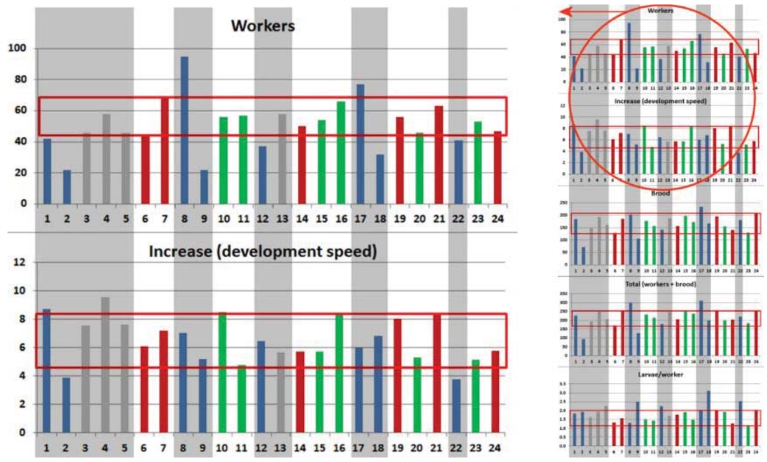

Figure 1 Initial brood assessment exemplarily for test 1

Table 1 Results of colony selection based on initial brood assessment

Treatment group	**Control**		**Treatment**	
Endpoint	**Number**	*SD*	**Number**	*SD*
		Test 1		
Workers	55	*7*	55	*9*
Brood	165	*23*	169	*32*
Total (workers + brood)	220	*26*	223	*34*
Larvae/worker	1.6	*0.3*	1.7	*0.5*
Increase (development speed)	2.3	*0.5*	2.2	*0.3*
		Test 2		
Workers	25	*6*	24	*6*
Brood	77	*9*	81	*14*
Total (workers + brood)	102	*10*	105	*16*
Larvae/worker	2.6	*0.7*	2.6	*0.9*
Increase (development speed)	1.8	*0.5*	1.9	*0.3*

Table 2 Results of colony selection: endpoints of final brood assessment

Treatment group	**Control**		**Treatment**		
Endpoint	**Number**	*SD*	**Number**	*SD*	MDD
Weight test 1	543	*19*	480	*21*	***4***
Weight test 2	481	28	421	20	***5***
Mortality test 1	46	*31*	81	*8*	***45***
Mortality test 2	7.7	6.4	64.5	22.3	***49***
Queen production test 1	11.8	*8.3*	0.0	*0.0*	***52***
Queen production test 2	30.7	*24.4*	5.5	*13.5*	***67***

Discussion and conclusions

To reduce the variability in relevant endpoints (mortality, hive development and young queen production), the selection of colonies should consider the development speed of the colonies besides the number of workers, brood and the larvae/worker ratio. Improved selection of bumble bee colonies, can reduce variability of developmental endpoints.

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3.11 Bumble bee queen production in semi-field studies: assessment of endpoints and challenges

L. Franke[1], O. Klein[1], J. Fricke[1], J. Sorlí[2], S. Knaebe[1]

[1]Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany, [2]Eurofins-Trialcamp SLL, Valencia, Spain

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Abstract

Bumble bees (*Bombus terrestris* L; Hymenoptera, Apidae) provide important pollination services and are commercially used, e.g. in greenhouse cultures. Consequently, the impacts of pesticides on bumble bees were already tested in the past. In the light of the newest EFSA guidance document on the risk assessment of plant protection products for pollinators standardized higher tier studies for pollinators are needed (EFSA 2013). For that reason a ringtest protocol for a bumble bee semi-field study design was developed in the ICPPR Non-Apis working group starting in 2015 to date.

The central endpoint in a higher tier bumble bee study is the colony reproduction success (production of young queens, Cabrera et al. 2016). The endpoint is chosen because at the end of the annual life cycle of a bumble bee colony all workers die and only young queens overwinter. Queens that survive establish a new colony in the following year. However, assessing queen reproduction is challenging. Many variables can influence the number of produced queens, such as the right timing for the termination of the study or the condition of the colony at study start. Furthermore, young queen weights are measured. Weight is used as indicator of diapause survival. Literature values of average weight needed for survival before overwintering state 0.8 g for a young queen for successful overwintering (Beekman et al. 1998).

Based on data from ring tests of 2016 and 2017 we tried to answer several open questions concerning queen reproduction, i.e. how can the experimental set-up influence queen weights and how high is the natural variation in queen numbers and queen weight/size?

Methods

The test design of the ring-tests conducted in Germany (test 1) and Spain (test 2) followed the ICPPR working group semi-field test protocol 2016 and 2017, respectively, with *Phacelia tanacetifolia* as a crop. One bumble bee colony was placed in each of the 6 replicate tunnels per treatment group. Dimethoate was tested as reference substance and was compared to an untreated control. At the end of flowering of *Phacelia* plants in the semi-field tunnels the colonies were moved to a monitoring site with flowers in the surroundings to provide enough food for their further development. Queen production was closely monitored. To prevent young queens from leaving the hives queen excluder were installed at the hive entrances. Hatched young

462

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Foto

Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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