

## 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.

## References

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

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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

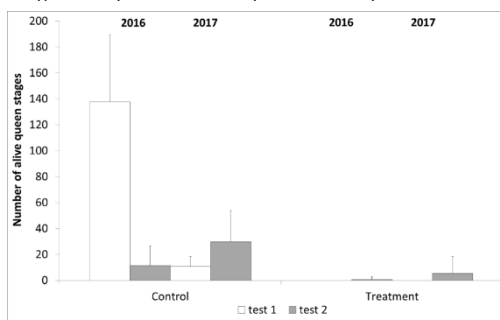
The test design of the ring-tests conducted in Germany (test 1) and Spain (test 2) followed the ICPPR working group semi-field test protocol 2016 and 2017, respectively, with *Phacelia tanacetifolia* as a crop. One bumble bee colony was placed in each of the 6 replicate tunnels per treatment group. Dimethoate 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

queens were regularly collected from the hives to avert overcrowding and associated food shortage in the hives. At the end of each study, bumble bee colonies were deep-frozen and the queen production (number of queen larvae, pupae and remaining hatched young queens) was assessed. Also, hatched young queens were weighed individually (wet weight) to determine their health and nutritional status.

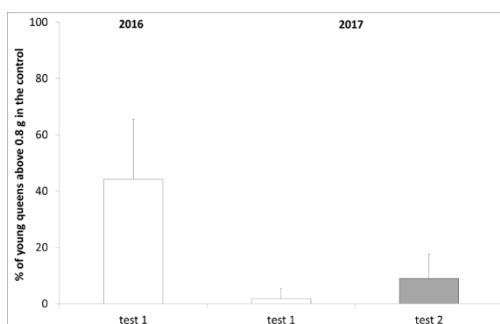
## Results

In all four studies the majority of control colonies entered the reproduction phase and produced young queens (67 to 100% of colonies). However, queen production of the control colonies was quite variable between studies. A general trend could be observed with higher numbers of young queens produced in all colonies when food availability was high, i.e. good crop conditions in the tunnel throughout the exposure phase and high quality of the monitoring site. In tests, where food supply was not plentiful throughout the study period (e.g. due to unfavorable weather or seasonal low supply of flowering vegetation or crops on monitoring site), 1 or 2 out of 6 control colonies did not enter the reproductive phase. In all four tests queen production was low in the dimethoate treated colonies (0 to 17% of colonies producing queen brood).

Concerning queen weights a high natural variation was observed with weights ranging from 0.4 g to more than 1.2 g. The majority of young queens weighed between 0.6 and 0.8 g in three tests (queen weights were not measured in test 2 in 2016). Queen weights were high, when food supply was plentiful (mean weight of 0.81 g in test 1 in 2016). In comparison tests with less food available either through less flowers at monitoring sites or weather conditions queen mean weight was 0.63 g (test 1) and 0.69 g (test 2) in 2017. The percentage of young queens with a wet weight above 0.8 g was 44.2 % (test 1 in 2016), 1.8 % (test 1 in 2017) and 8.9 % (test 2 in 2017).



**Figure 1** Mean number of alive queen stages (= queen larvae, pupae and hatched young queens) in the control and the dimethoate treatment in the four tests in 2016 and 2017



**Figure 2** Mean percentage of young queens with a weight above 0.8 g in the colonies of the controls in three tests (test 1 in 2016, test 1 and 2 in 2017)

**Table 1** Mean values of queen production (queen larvae, pupae and hatched young queens) and weight of young queens in the four studies in 2016 and 2017

Treatment group		Control		Treatment	
Endpoint		Mean	SD	Mean	SD
<b>2016</b>					
Test 1	Queen production	137.8	51.4	0.0	0.0
	Queen weight (g)	0.81	0.15	-	-
Test 2	Queen production	11.5	15.2	0.8	2.0
	Queen weight (g)	n.a.	-	n.a.	-
<b>2017</b>					
Test 1	Queen production	10.8	7.6	0.0	0.0
	Queen weight (g)	0.63	0.11	-	-
Test 2	Queen production	29.8	23.9	5.3	13.1
	Queen weight (g)	0.69	0.10	0.75	0.14

### Discussion and conclusions

Selection of the monitoring site is very important as availability of flowering resources influences queen production.

Queen production in control colonies naturally varies due to food supply, temperatures and genetic factors and only under optimal conditions 100% of colonies can be expected to produce young queens emphasizing the need to use 6 replicates.

Young queen weight in this test system was mainly between 0.6 g and 0.8 g, as young queens are collected and weighed before they start foraging and fatten up for hibernation.

Weight of queens needs to be compared between treatments to find out if the test item might affect survival since the value given in the literature is not based on semi field conditions.

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