Section 4 – Testing methodologies for non-Apis bees

4.1 Progress of working group Non-Apis testing

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See summary of progress of the Non-*Apis* group on page 8 Thomas Steeger: Working Groups of the ICP-PR Bee Protection Group – Developments and Progress

4.2 Summary of an ICPPR Non-Apis workshop – Subgroup higher tier (bumble bees and solitary bees) with recommendations for a semi-field experimental design

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DOI 10.5073/jka.2018.462.041

Introduction

The publication of the proposed EFSA risk assessment guidance document of plant protection products for pollinators [1] highlighted that there are no study designs for non-Apis pollinators available. Since no official guidelines exist for semi-field testing at present, a protocol was proposed and a ringtest was conducted in 2016 to develop a general test set-up. The ringtest design was based on the draft EFSA guidance document [1], OEPP/EPPO Guideline No. 170 [2] and results of discussions regarding testing solitary bees during the meetings of the ICPPR non-Apis workgroup in 2015, 2016 and 2017 [3, 4, 5] and an hand on workshop in May 2017 [6].

Materials and Methods

Ring-tests were conducted with two different test organisms, one representative of a social bumble bee species (*Bombus terrestris* L; Hymenoptera, Apidae) and one representative of a solitary bee species (*Osmia bicornis* L; Hymenoptera, Megachilidae). Both are polylectic and foraging on a diverse spectrum of flowering crops. In addition, they are common species in Europe, commercially available and widely used for pollination services.

Several laboratories participated in the higher-tier ring test. Seven semi-field tests were conducted with *B. terrestris* and 8 semi-field tests were done with *O. bicornis* in 2016. In 2017 8 semi-field tests with bumble bees and 8 semi-field tests with solitary bees were run.

Two treatment groups were always included in the ringtest: an untreated control (water treated) and dimethoate as a toxic reference item (optional other i.e. brood affecting substances (fenoxycarb, diflubenzuron)). The toxic reference items were chosen based on their mode of action and long term experience in honey bee testing.

In the solitary bee study design adult bees (both sexes) were caged in tunnels containing a bee attractive flowering crop and exposed during their reproductive period. After the application of the respective reference items, the adult female bees collected the relevant food items from the treated crop, providing their offspring with exposed pollen and nectar as the only food source during brood development. The final result on developing and hatching success of the progeny was assessed in the following year.

In the bumble bee study design only the early part of the colony development took place during the exposure phase in the tunnels. At the end of flowering, the bumble bee colonies were transferred to a monitoring site until they produced queens and drones ("switch-point").

Results and discussion

Test design

A general test design was developed for a solitary bee and a bumble bee semi-field study based on results of the first year of testing 2016.

For the solitary bees the aim of the second run of the ring-test was to define a more standardized test design to reduce the variability between study results and to guarantee reproducible test conditions (e.g. nesting material, latest study start, assessments, overwintering procedure). The replication was under discussion since MDDs (Minimum Detectabel Differences) calculated from ring-test results of 2016 were high and there was no information what are expected variations for this kind of tests. Furthermore, immature mortality of the bee brood needed to be lowered by reducing parasitation and improving handling of the sensitive eggs and larvae. Also, the optimal timing of spray applications was under discussion.

For the bumbe bees the aim of the second ringtest run was also to define a more standardized test design to reduce the variability between study results and to guarantee reproducible test conditions (e.g. worker number per m^2 crop, colony composition at study start, assessment of endpoints, determination of switch point, timing of deep-freezing). The replication was under discussion since one of the most important endpoints, i.e. the queen reproduction, showed high variability [7]. To reduce variability between replicates a special focus was upon the origin of the hives and the selection of colonies for the test. One further challenge was and is the best timing for the termination of a study allowing the assessment of the most important endpoint, queen reproduction, which was discussed in detail.

The basis requirements for studies after the first ring test of 2016 and discussions are given in the following table.

	Buff-tailed bumble bee (*Bombus terrestris* L.)	**Red mason bee** (*Osmia bicornis* L.)
Replicates	6	4
Size of tunnels	≥ 30 m^2	
Number of test organisms	Initial colony size 10 bumble bees, approx. colony size 20 bumble bees after 14 days in laboratory	1 ♀ /m^2 / 1.5 ♂ /m^2
Nests	Commercial bumble bee hives with queen excluder	Chipboard units MDF (100 cavities)
Test item[a]	Dimethoate (600 g a.i./ha)	Dimethoate (75 g a.i./ha)
Exposure	Flowering period of crop	Flowering period of crop after first cells are produced
Test duration	6 - 15 weeks	10 - 12 months
Time of testing	April - August	April – May (- July)
Crop	Oil seed rape, *Phacelia*	Oil seed rape, *Phacelia*

[a]optional additional test of other substances

Table 1 Test design of semi-field studies with solitary bees and bumble bees for 2017

All ring test participants agreed on the design for studies run in 2017. For the ring test in 2017 bumble bee colonies from one distributor were used to reduce the variability.

Endpoints

It was agreed on the most important, obligatory endpoints to be recorded for the tests.

In the test with solitary bees hatching success (1st generation) will be established since it will be the basis for later calcualtions of reproductive success and gives an information of the quality of the coccons. The next endpoints are the establishment at the nesting units (nest occupation), flight activity, reproduction and hatching success (2nd generation). The latter is the most important information that needs to be observed.

In the bumble bee trials endpoints are brood development, colony weight and colony reproduction (production of sexuals). It was agreed that the trial should only stopp when first queens have been hatched.

Test performace

For the solitary bee test will consist of two reatment groups, the untreated control C and the test item treatment group T (applied with Dimethoate). Bee cocoons, e.g. *O. cornuta* or *O. bicornis*, need to be placed in the tunnel when the first flowers are open (approx. BBCH 60). Nesting units are placed in each tunnel where the bees will establish their brood nests. The adult bees and their larvae will be exposed to the nectar and pollen of the crop throughout the flowering period. After the end of exposure the development of their progeny will be followed through to the following spring and the reproduction success will be determined by the number and vitality of hatched individuals.

For bumble bees the test will consist of two treatment groups, the untreated control C and the test item treatment group T (applied with Dimethoate). Additionally, brood-affecting substances can be added as further treatment groups, if required (i.e. Diflubenzuron). The application will take place as spray application during bee flight at least 3-6 days after set-up of the bumble bee colonies in the tunnels. Exposure will last until the end of flowering. After the exposure phase in the tunnels, the bumble bee colonies will be transferred to a remote site (natural area with foraging resources and minimal pesticide exposure) location in order to assess the development of the colonies and the reproduction of young queens and drones.

Outlook

Based on the results of the ringtest main open questions will be adressed and the aim will be to propose a guidance for the performance of semi-field studies. The open points at the moment are:

…for bumble bees:

- how many replicates are needed to see possible effects?
- how can minimal variation of endpoints be achieved and specifically what are realistic variations in queen number and size/weight?
- how can the “switch-point” be defined reliably for a test protocol?
- how can the assessment of hatched queens be handled?

…for solitary bees:

- how can cocoon incubation and hatching of bees be synchronised with the onset of flowering?
- how fit are solitary bees out of season (tests in summer)?
- which substance can be used as reference item for brood studies?

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4.3 An international workshop on pesticide exposure assessment for non-Apis bees

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DOI 10.5073/jka.2018.462.042

Abstract

The honey bee (*Apis mellifera*) is typically used as a surrogate to evaluate the risk of pesticides to all bee species. However, there is uncertainty regarding the extent to which honey bees can serve as surrogates for solitary bees, bumble bees and stingless bees given differences in their life history traits (*e.g.*, body size, feeding, sociality, flight/activity season, nesting materials, behavior, overwintering strategy, *etc.*). Lack of basic knowledge of non-*Apis* bee exposure scenarios has been among the biggest challenges in determining whether honey bees are sufficient surrogates for non-*Apis* bees. As a result of a tripartite effort between regulatory agencies, academia and agrochemical industry, an international workshop was organized in Washington D.C. on 10th-12th January 2017. Forty bee researchers and risk assessors from ten different countries gathered to discuss the current state of science on pesticides exposure to non-Apis bees, and to determine how well honey bee exposure estimates used by different regulatory agencies may be protective for non-*Apis* bee species. There was a general consensus that the current honey bee exposure assessment paradigm is highly conservative. However, several data gaps were identified that hindered a complete analysis of various routes of exposure between *Apis* and non-*Apis* bees, especially when non-Apis bees may be exposed via nesting materials such as soil (*e.g.*, blue orchard bees; *Osmia* spp., alkali bees; *Nomia* spp.), leaves (*e.g.*, alfalfa leafcutting bees, *Megachile rotundata*), or a combination of soil and leaves (*e.g.*, stingless bees; tribe Meliponini). Basic conceptual models and preliminary exposure equations were discussed that could help to quantify these exposure routes, allowing for future comparisons with honey bee exposure estimates. The workshop proceedings, along with a list of critical research needs identified to quantify non-*Apis* bee exposure routes, will be published as a series of peer-reviewed journal articles.