3.5 The homing flight ring test: method for the assessment of sublethal doses of plant protection products on the honey bee in field conditions

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DOI 10.5073/jka.2018.462.032

Abstract

In the framework of the current revision of plant protection product risk assessment on the honeybee by European authority (EFSA, 2013), a European ring test is conducted with 11 voluntary laboratories to test a methodology assessing the effects of sublethal doses of a plant protection product administered in controlled conditions on the homing capacity of forager bees in the field.

Homing success is measured by monitoring free-ranging honey bees with radio-frequency identification (RFID) tagging technology. To do so, we capture at the hive entrance, foragers coming from a known site located at 1 km (+/- 100 m) away from the experimental colony, to ensure that the foragers have a prior knowledge of the pathway back to the colony. RFID-tagged bees are orally exposed to 3 sublethal dosing solutions (0.1, 0.3 and 1 ng/bee) of the reference item, thiamethoxam, or to a control in laboratory. The dosing solutions are collectively administered to the honeybees with 20 µl per bee of a 30% sucrose solution (w/v). Then foragers are released on the known site and the homing success is recorded at the hive entrance with RFID system for 24 hours after release. The test endpoint is defined as the determination of a No-Observed Effect Dose (NOED) on the homing success.

In the first year of the ring test (2015), 7 laboratories out of 10 could conduct the test and found a common NOED of 0.3 ng per bee. One important limiting point was the use of a Phacelia field planted at 1km from the colony in order to collect bees with specific bright blue pollen loads. Methodological improvements were also necessary to better maintain the foragers during the laboratory phase. In 2016, an alternative to the Phacelia field consisting in collecting bees previously powdered and released at 1km from the colony was tested. For the laboratory phase, a feeding ad libitum with candi or sucrose solution 30% (w/v) was also added to maintain the bees just before release. All the laboratories could conduct the test in 2016 and similar or better homing results in control bees were obtained, this validating the alternative method to the Phacelia field. The factors of variability due to the protocol and context have been discussed.

3.6 Non-uniform distribution of treated sucrose solution via trophallaxis by honeybees affects homing success variability and mortality

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DOI 10.5073/jka.2018.462.033

Abstract

Background: Food sharing in a group via trophallaxis might lead to a non-uniform distribution of pesticide spiked sucrose solution between caged honeybees. This can cause high variability in the homing success rate or mortality among group members and treatment replicates. In order to improve the oral food distribution of tested sucrose solution we compared two feeding schemes with two or ten bees per cage (20 µL/bee) and evaluated the impact on homing success rate and mortality.

Results: First results showed that food intake with the two-bees feeding regime is faster. Therefore, a more accurate dosing distribution among bees can be expected. We measured a less variable homing success rate and retuning time among runs and the corresponding treatments. Furthermore, mortality rate of the group-feeding scheme with ten bees per cage resulted in higher mortality values when compared to the two-bees feeding scheme. This might be an indication for a better and more uniform distribution of the treated sucrose solution among two caged bees.

Conclusion: Improving the uniform distribution of test items by orally treatment administration in smaller groups with honeybees should be discussed and considered, as toxicity endpoints of single-dosed wild bees

are compared with group-dosed honeybees. Furthermore, to minimize the trophallaxis dependency regarding food distribution in group dosed honeybees.

Introduction

The implementation of the EU Regulation 1107/2009, the publication of the EFSA Guidance Document, (EFSA 2013) and the requirements of US-EPA/PMRA require further efforts in method development and validation to evaluate the risk of bees exposed to pesticides for PPP registration in an appropriate and comparable way. As part of an international homing flight ring-test, we investigated and compared the impact of the feeding regime group dosing with 10 bees per cage versus group dosing with two bees per cage on the results of the homing success and mortality.

Based on our observations and a recently published article[1] food sharing in a group via trophallaxis (exchange of liquids between colony members) might lead to a non-uniform distribution of pesticide spiked sucrose solution between caged honeybees. This can cause high variability in the **homing success rate** or **mortality** among group members and treatment replicates. In order to improve the oral food distribution of tested sucrose solution we compared two feeding schemes with **two** or **ten** bees per cage (20 µL/bee) and evaluated the impact on homing success rate and mortality.

Method

RFID Homing flight ring-test: According to the homing flight ring-test protocol, bees were orally exposed to different sub-lethal concentrations of thiamethoxam (0, 0.11, 0.33 or 1 ng/bee). For each treatment scheme (two and ten bees/cage), three runs were conducted between June and July 2017 in Liebefeld, Switzerland (fig.1;2). In all treatment-groups, homing flight success was assessed after 24h.

Fig. 1 group feeding with 2 bees (tagged with RFID chip) per cage

Fig. 2 group feeding with 10 bees (tagged with RFID chip) per cage

Acute Toxicity Test: According to the TG OECD 213, bees were orally exposed to different concentrations of dimethoate (0, 0.033, 0.07, 0.1, 0.13, and 0.35 µg/bee). As above, oral treatment scheme was performed three times for both groups (two and ten bees/cage). Mortality was assessed after 24h (fig. 3;4).

Fig. 3 group feeding with 2 bees per cage (OECD 213)

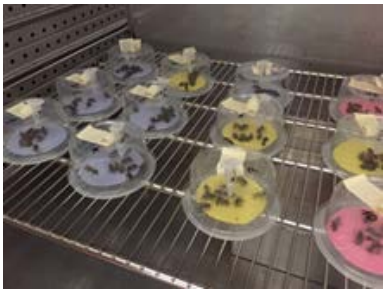

Fig. 4 group feeding with 10 bees per cage (OECD 213)

Results

First results showed that food intake with the two-bees feeding regime is faster. Therefore, a more accurate dosing distribution among bees can be expected. We measured a less variable homing success rate and retuning time among runs and the corresponding treatments. This might be an indication for a better and more uniform distribution of the treated sucrose solution among two-caged bees. **Homing flight** success rate, at 1 ng thiamethoxam per bee, was significantly lower in the group of ten bees compared to the two bees approach, as well as the control (fig. 5). Obviously, a large variability was found in the ten-bees feeding group. For the other doses, similar trends were obtained. **Acute toxicity data** with dimethoate showed that group feeding scheme with ten bees per cage resulted in higher mortality values when compared to the two bees feeding scheme (at same dosing levels). Consequently, the LD_{50} value is higher for the latter (fig. 6).

RFID: Homing success per treatment and feeding scheme

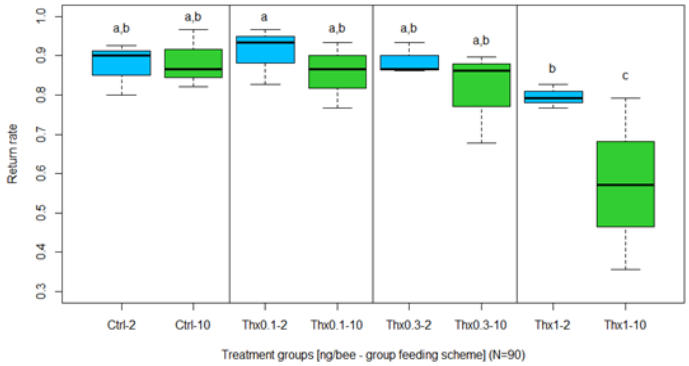

Fig. 5 Boxplot: Homing flight success per treatment and feeding scheme. Literals differentiate statistically significant ($p<0.05$) groups, validated by Chi-Square-Tests.

OECD 213: 24h mortality per group feeding scheme

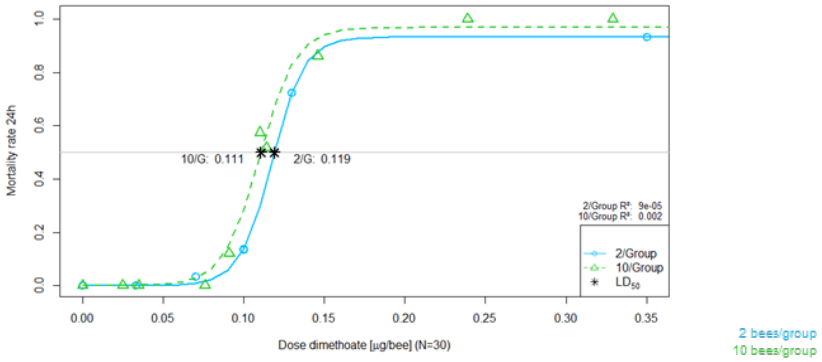

Fig. 6 LD_{50} dose-response model for dimethoate with two, resp. 10 group feeding schemes. 2 group feeding showed a more accurate and closer LD_{50} value compared to the reported LD_{50} value of 0.1257µg/bee by Baskar et al.[2]

Conclusion

High variability of homing success or mortality rate observed with the ten-bee group feeding scheme is most likely caused by inhomogeneous dose distribution among bees, or either by over- or underdosing of single bees within replicates. In contrast, food intake with the two bees feeding scheme is generally faster and more homogenous as the chance to feed directly on the offered sugar solution is increased. Hence, a more accurate and uniform dosing distribution can be expected resulting in less variable data between runs, replicates and treatments. We highlight that feeding (treatment of interest) in smaller groups of honeybees should be discussed and considered to **minimize the trophallaxis dependency** regarding food distribution in group dosed honeybees. Moreover, to compare endpoints of toxicological studies with single dosed wild bees for regulatory purposes.

Reference

[1]Brodschneider R, Libor A, Kupelwieser V, Crailsheim K, PloS ONE 12(3) (2017),
[2]Baskar K, Sudha V, Tamilselvan C ScholeReps 1(1) (2016)