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Honeybee's biological and behavioral processes can affect the outcome of the RFID homing flight test

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Introduction

The development of new regulatory ecotoxicological test methods with honeybees requires the inclusion of fundamental bee biology and corresponding behavioral processes, to improve the explanatory power of a new method as much as possible. In the RFID homing flight ring-test protocol (Fourrier et al. 2018), the test-substance is administered orally with sugar solutions to groups of ten foragers. Due to methodological reasons of the present protocol, the age and task composition of collected foragers may not be homogenous. As a result, the corresponding food uptake could vary considerably among individual bees and/ or groups. Similarly, group-based food distribution via trophallaxis might not homogenously distribute the feeding solution among individual bees. We tested those critical parameters as follow.

Methods

1. Basic set up:

Three colonies were kept in specifically configured hives equipped with a RFID device. For each colony, ~700 foragers (comprising both pollen- and nectar foragers) were collected at the hive entrance and coloured with a pink powder. Coloured bees were then released 1 km away from their hive and collected again at the entrance. These bees were then individually equipped with a RFID tag- coupled with a 90 min starvation phase. This procedure was followed by acute oral exposure to different test item solutions under controlled lab conditions. After a 40 min starvation phase, bees were finally released at the same place as before and their homing rate was automatically recorded.

1.1. Crop content:

To prevent interferences with natural honey stomach content, honey stomachs should be empty at two time points during the test:

a) Before exposure with the test substance;
b) Before release of the chipped bees.

To check the honey stomach level of the bees during the test at the two critical time points, extra bees were collected, handled as the test bees and then analysed. For this, individual honey stomachs were dissected with tweezers and their filling level was visually categorized as "empty" or "full".

1.2. Single versus group feeding:

Here, we tested the effect of trophallaxis (food-distribution performance) on the outcome of the RFID homing test, by administering the 1 ng thiamethoxam spiked sugar solution to;

groups of ten bees (200µl), or to individual bees (20µl) in single cages.

For this, we mainly collected pollen foragers. Otherwise, the same conditions as mentioned above were used (see **point 1 above**). In the **control** we fed 30% sucrose solutions only. Return rates were analysed statistically for groupwise differences with *R*.

Results

2.1. Crop content:

At time point **a)** after the 90 min starvation phase, 40 % of all analyzed honey stomachs were empty, whereas 60 % were full. At time point **b)** after the 40 min starvation phase, 3% of analyzed honey stomachs were empty and 97 % were still full, respectively (see fig. 1).

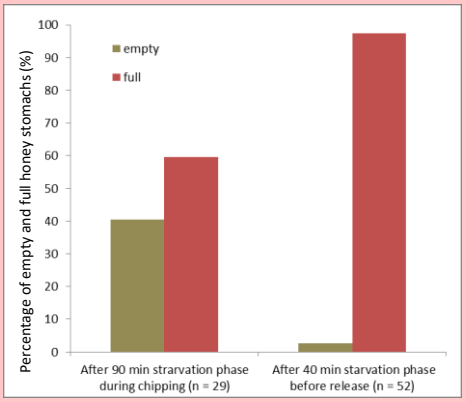

Fig. 1: Bars represent empty and full honey stomachs at two different time points during the homing test, Run 3.

2.2. Single versus group feeding:

We found that the pooled homing ability of thiamethoxam exposed foragers was statistically different when administered individually (38.9 %), compared to group-feeding (71.2 %); χ^2 test: **$p < 0.001$**; see fig. 2).

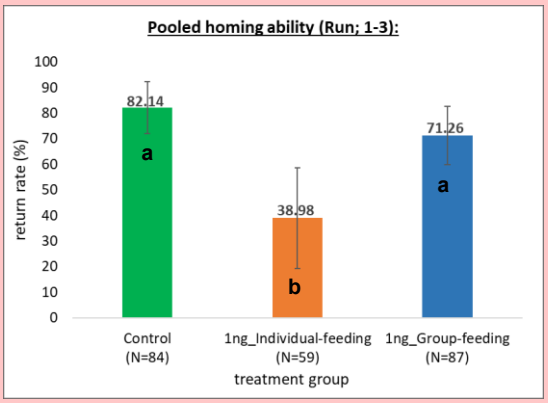

Fig. 2: Bars represent mean homing flight success (return rate) per treatment and thin lines indicate their corresponding standard deviation. Literals differentiate statistically significant groups (chi²-testing using R).

Discussion and Conclusions

More than half of the analyzed honey stomachs were still full before administering the test solution. Accordingly, bees with a full stomach might consume less (or even nothing) and thus could be exposed to lower levels than expected. However, other bees (with an empty stomach) in the feeding-group may be overdosed. The finding that before the bees' release, 97 % of honey stomachs were still full, suggests that feeding solutions might not be fully digested, which could influence the output of the homing ability. Similarly, our results from the group size experiments show that single-dosed bees produces different results compared to group-dosed bees, suggesting an inhomogeneous dose distribution via trophallaxis and thus represents another important parameter of this new method.

Our results bring new insights, how the RFID homing flight ring-test protocol could be improved, using individual feeding and / or choosing exclusively returning pollen foragers (assuming empty stomachs and similar contents) to create equivalent test units.

REFERENCE Fourrier et al. (2018) The homing flight ring test: method for the assessment of sublethal doses of plant protection products on the honey bee in field conditions. https://doi.org/10.5073/jka.2018.462.032

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Niedersachsen

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