Do pollen foragers represent a more homogenous test unit for the RFID homing test, when using group-feeding?

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Introduction

The radio-frequency identification (RFID) homing flight ring test aims at developing a method, which can assess sublethal effects of xenobiotic substances on the navigation of foraging bees (RFID ring-test protocol; Fourrier et al. 2018). Thereby, honeybee's *(Apis mellifera*) biological parameters and corresponding behavioral processes might strongly influence the output of this test-method. Accordingly, previous experiments demonstrated that the homing ability of nectar foragers differed between group- and single-bee-feeding based on uneven crop content of returning bees and/or due to uneven food distribution via trophallaxis. Therefore, we here evaluated if pollen foragers represent a more homogenous test unit, when test item solutions are administered to groups of bees and thus are distributed between each other via trophallaxis, assuming that pollen foragers have more similar (empty) crop contents when returning from foraging flights.

Methods

To do so, approx. 600 pollen foragers were collected at the hive entrance (N = 5 colonies tested consecutively) and were coloured with a pink powder. Coloured bees were then released 1 km away from their hive and were again collected at the entrance. These bees were then individually equipped with a RFID tag. This procedure was followed by acute oral exposure to different test item solutions under controlled lab conditions. Here, we tested the effect of thiamethoxam and thiacloprid (both neonicotinoid insecticides) at field realistic doses by orally exposing pollen foragers either in: groups of ten bees, or in individual cages to evaluate the effect of trophallaxis (food-distribution performance) on the outcome of the RFID homing test. As control groups, bees were fed with 30% sucrose solutions only. Bees were finally released at the same place as before and their homing rate was automatically recorded with the RFID-device. Gained return rates were analysed statistically with nominal pairwise matrices using conservative Bonferroni correction in *R*.

Results

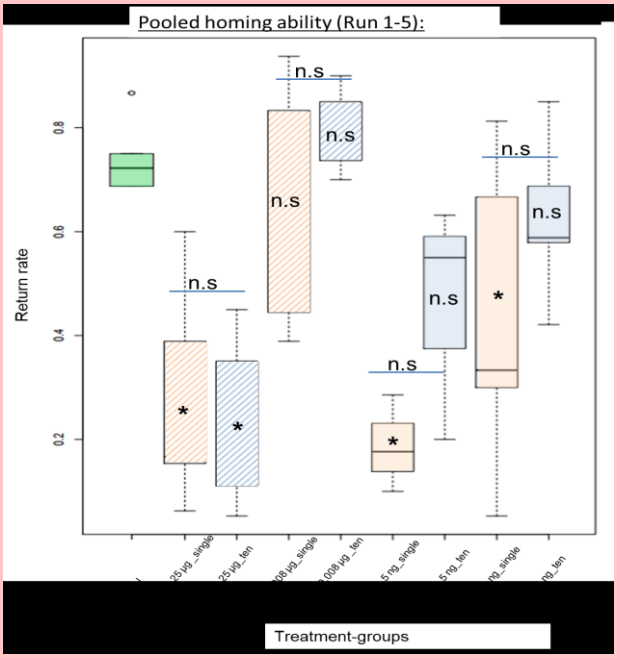

Fig. 1: Boxplots represent median, first and third quartile of homing flight success (return rate) per treatment (thiaclopid = dashed boxes; thiamethoxam = solid boxes). Stars represent statistically significant different groups from the control (for statistical details see in methods).

We found that the homing ability of thiamethoxam (1ng /bee and 1.5 ng/bee) exposed pollen foragers was significantly different from the untreated control in the single-bee-feeding approach, but not in the group-feeding approach. Similar tests with thiacloprid (0.008 µg/bee 0.25 µg/bee), revealed not such clear differences between the two feeding approaches. However, the higher dose had a generally strong impact on bee's return rate.

Discussion and Conclusions

Our results demonstrate that single-bee- and group-feeding, indeed, differentially affect the return rate under the conditions tested here. Although, it seems that the effect of group-size on the homing ability of pollen foragers seems to be compound/dose specific. Nevertheless, our results suggest that single-bee-feeding reveal biologically more robust results in context of homing ability compared to group feeding, which should be considered in the development of this new test guideline by ideally performing such tests using single-bee-feeding.
Moreover, pollen instead of nectar foragers should be preferentially chosen, since they consumed the feeding solution quicker and more reliably compared to previous trials with nectar foragers. We also strongly suggest to perform further experiments with different compounds, in order to evaluate the robustness of the method under scrutiny.

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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