

Effects of a pesticide cocktail on food consumption, learning and food discrimination in honeybees

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Background

Animal pollination services are essential in agriculture and natural ecosystems, yet there is growing concern about worldwide decline of pollinator populations, especially honeybees. Various stressors, including pesticides, contribute to losses in honeybee colonies. Recently, the impairment of honeybee's learning and orientation behavior after ingestion of sublethal doses of pesticides has been demonstrated [1,2]. Here, the proboscis extension response (PER) was studied after the application of three insecticides (imidacloprid, cypermethrin, dimethoate). In a path-finding experiment the discrimination of pesticide spiked food sources was investigated. The pesticides were chosen to address major pesticides classes and applied at concentrations representing different fractions of published LD₅₀-values [3].

Materials and Methods

Hive bees at the age of 13-16 days were caged and fed with 50% sucrose solution for 48h, containing the mixture of the following amounts of insecticides (ng/bee): cypermethrin 4, dimethoate 24 and imidacloprid 0.8. To carry out PER conditioning, the beefix-bracket, a novel method of harnessing bees for the PER assay was used (Fig. 1). In a second experiment the feeding preferences of 12-16 day old hive bees were tested. Around 30 bees were caged in cages (n=3-5 per group) with two food sources for 48h (Fig. 2), containing the following amounts of insecticides (ng/bee): cypermethrin 20, dimethoate 60 or imidacloprid 4, as well as a mixture of the three.

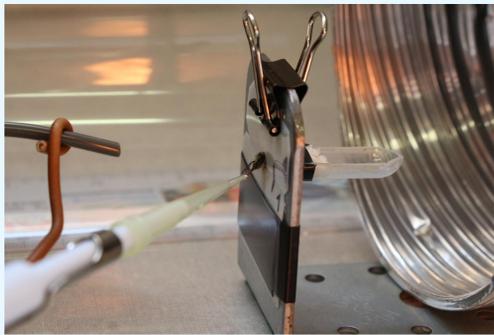


Fig.1. PER-conditioning in the beefix-bracket



Fig.2. Food choice experiment with caged honeybees

Results & Discussion

Here, for the first time honeybee treatment with a pesticide mixture of three insecticides was applied to approach a more field realistic level [4]. The consumption of pesticide spiked food was significantly reduced after 48h (Fig. 3), which might be due to repellent effects or impairments of neuronal activity.

In contrast, learning and memory as evaluated through the PER was not affected by the orally applied pesticide mixture (Fig. 4). The mortality rate in both groups was < 4%.

Choice behavior of honeybees plays an important role in effective foraging [5]. A choice experiment was used to test discrimination ability of pesticide spiked food vs. sucrose solution. A trend towards reduced consumption of single pesticide spiked food was found. Interestingly, such a trend did not occur for food sources containing a mixture of the three insecticides (Fig. 5). Mortality (1-34%) after 48h of treatment was only observed in cages in which dimethoate was applied.

Additive or synergistic effects of the ternary mixture could be responsible for reduced food discrimination as found for the food sources in which the insecticides were applied separately.

Acknowledgments

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www.zukunft-biene.at

Literature

- [1] Giurfa M. & Sandoz J.-C., (2012) Invertebrate learning and memory: Fifty years of olfactory conditioning of the proboscis extension response in honeybees. *Learning & memory* (Cold Spring Harbor, N.Y.), 19(2), pp.54–66
- [2] Fischer J, Müller T, Spatz A-K, Greggers U, Grünewald B. (2014) Neonicotinoids interfere with specific components of navigation in honeybees. *PLoS one* 9(3): e91364
- [3] http://bienen.jki.bund.de/dokumente/upload/bedbd_57_dpst_berlin_2010_poster_200_bu_bischoff.pdf (Julius-Kühn-Institut)
- [4] Sanchez-Bayo F. & Goka K., (2014) Pesticide residues and bees—a risk assessment. *PLoS one*, 9(4), p.e94482.
- [5] Menzel R. & Müller U., (1996) Learning and memory in honeybees : From Behavior to Neural Substrates. *Annu. Rev. Neurosci.*, 19, pp.379–404.

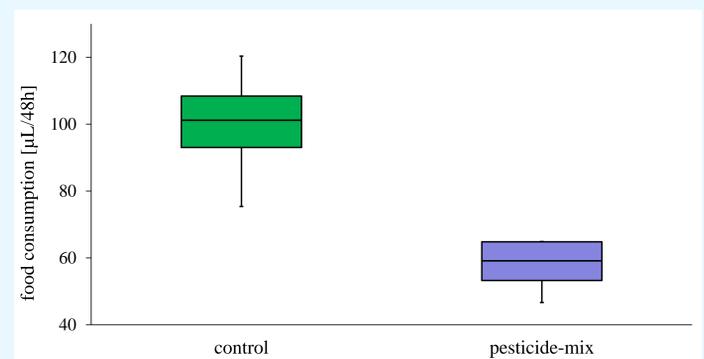


Fig.3. Significant difference in mean food consumption between bees fed with sucrose solution (n=191) and bees fed with pesticide-mix solution (n=164); (t-test, 9 df, $P < 0.001$).

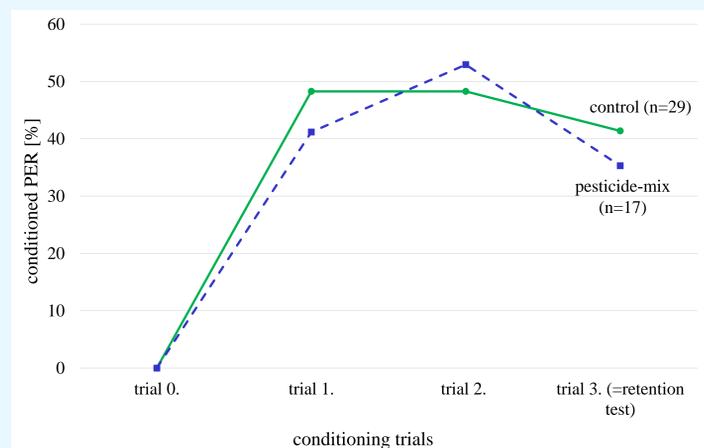


Fig.4. Conditioning rates of bees fed either with sucrose solution or pesticide-mix solution. In the retention test only the conditioned stimulus (i.e. odor) was presented. Differences in the conditioning rates at each trial were non-significant (χ^2 -test, 1df, $P > 0.05$).

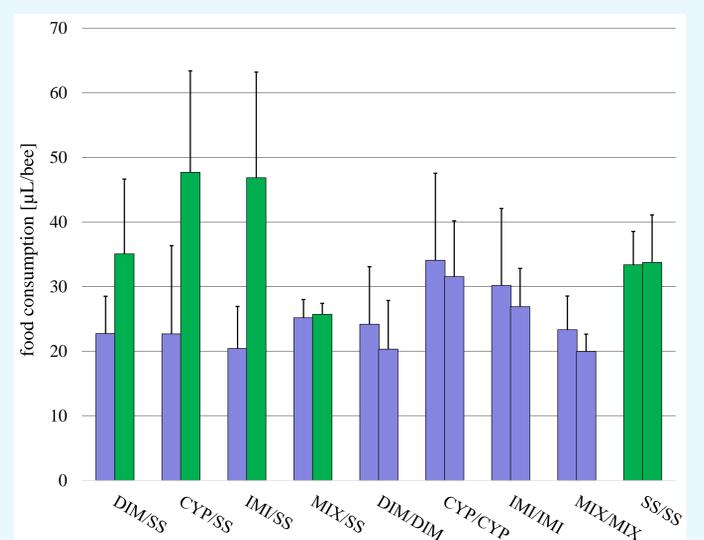


Fig.5. Mean food consumption per bee within 48h of treatment. Purple bars indicating food sources containing pesticide solution and green bars indicating food sources containing control solution (SS = sucrose solution, DIM = dimethoate, CYP = cypermethrin, IMI = imidacloprid, MIX = mix of all three pesticides). Error bars represent the standard deviation of the replicates.