Xenobiotics sublethal effects on chemical communication and associative learning and peripheral olfaction of honeybees

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Honeybees rely on a variety of chemical signals to coordinate their activities within the colony and locate food sources. These signals include pheromones like cuticular hydrocarbons, which play a role in recognizing colony members, and ethyl oleate, which regulates temporal polyethism. When it comes to foraging, honeybees utilize signals such as flower volatiles, which they can learn to associate with floral rewards after detecting them with their antennae. Additionally, honeybees exhibit an innate Proboscis Extension Reflex (PER), wherein they extend their proboscis upon perceiving a suitable reward, such as sucrose. PER can be linked to external conditioning stimuli, such as floral volatiles.

To evaluate the sublethal effects of xenobiotics on *Apis mellifera* (Hymenoptera: Apidae) honeybees, we conducted studies to investigate their impact on cuticular hydrocarbons (CHC), ethyl oleate, and peripheral olfaction. We conducted controlled laboratory and field experiments using various varroacide substances as potential stressors to assess their sublethal effects on honeybees of different ages. We analyzed the mentioned pheromones using gas chromatography-mass spectrometry and evaluated olfaction through electroantennography (EAG) and olfactory learning, as indicated by the conditioning of the PER response.

Our findings revealed that different stressors had varying effects on pheromones, with some displaying dose-dependent effects. However, we observed no discernible impact of the tested varroacides on EAG responses in any of the experiments. Furthermore, exposure to the tested products did not significantly affect associative learning and memory.

In summary, our results suggest that the tested products may influence chemical communication within the colony but do not yield short- or medium-term effects on peripheral olfaction and learning processes. Consequently, their use as potential varroacides is unlikely to hinder the pollination process, which relies on bees' search for and collection of food from flowers.

Key words: honeybee pheromones, learning, memory, sublethal effect

Porrini, M.; Garrido, P.; Umpiérrez, M.; Porrini, L.; Cuniolo, A.; Davyt, B.; González, A.; Eguaras, M.; Rossini, C. *Veterinary Sciences* **2020,** *7* (4), 199.

Porrini, M. P.; Garrido, P. M.; Gende, L. B.; Rossini, C.; Hermida, L.; Marcángeli, J. A.; Eguaras, M. J. *Journal of Apicultural Research* **2017,** *56* (5), 616-624.

Rossini, C.; Rodrigo, F.; Davyt, B.; Umpierrez, M. L.; Gonzalez, A.; Garrido, P. M.; Cuniolo, A.; Porrini, L. P.; Eguaras, M. J.; Porrini, M. P. *PLoS One* **2020,** *15* (11), e0241666.

Pellegrini, C.; Alonso Salces, R.; Rossini, C.; Fuselli, S. *Chemistry & Biodiversity* **2017,** *14* (4), e1600382.

Umpiérrez, M.; Santos, E.; Mendoza, Y.; Altesor, P.; Rossini, C. *Parasitology Research* **2013,** *112* (10), 3389-400.

Umpiérrez, M. L.; Santos, E.; González, A.; Rossini, C. *Phytochemistry Reviews* **2011,** *10* (2), 227-244.

Bragunde, G.; González, G.; Rossini, C., The use of Arduino controller to deliver volatiles in a system to evaluate learning and memory in honeybees. *submitted* **2023**.