species and have a smaller foraging radius than honeybees. Moreover, due to their social lifestyle and the large number of workers inside the hive, honeybees can often compensate for PPP effects. This is not possible for solitary wild bees. In addition, nurse bees can filter larval food before feeding honeybee larvae, so fewer PPPs are ingested by them. Particularly at the individual level, honeybees and wild bees also exhibit different sensitivities to PPPs (Drossart and Gérard, 2020; Thompson, 1999; Wood et al., 2020). This was already shown by investigating the effect of a neonicotinoid to three different bee species. The solitary wild bee O. bicornis was most sensitive to the insecticide clothianidin, followed by the social wild bee B. terrestris and finally the honeybee A. mellifera. Synergistic effects were also most evident in O. bicornis (Sgolastra et al., 2017). Wild bees, such as the bumblebee, also have different activity patterns compared to honeybees. They show higher activity in the morning and in the evening while honeybees display their activity peak in the midday. In addition, wild bees often fly in unfavorable weather conditions. These different flight times can also lead to higher exposure to PPPs for wild bees, as regulations for PPP application are usually based on honeybee activity (Thompson, 1999).

Wild bees are indispensable pollinators of crops but especially of wild plants. At the same time, they suffer from a significant decline. Thus, it is important to focus research on stressors and their effects on pollinators not only on the honeybee but also on wild bees (Drossart and Gérard, 2020).

5. Conclusion

A correct evaluation of food sources is crucial for the foraging success of a honeybee, as the nectar concentration determines whether a food source should be exploited (Seeley, 1995; von Frisch, 1965). Furthermore, individual responsiveness to sucrose has a direct impact on the learning behavior (Scheiner et al., 2005, 2001). A good learning performance also maximizes the foraging success, as honeybees have to learn important features of the food source like the shape, the color or the odor and have to be able to orient themselves to the sun compass and to landmarks. Sharing this information with other foragers increases the foraging efficiency (Menzel, 1993). Accordingly, adverse effects of PPPs on learning behavior or sucrose responsiveness would have far-reaching consequences for the honeybee colony.

Our behavioral experiments revealed no negative effects of a field-realistic treatment with the fungicide Cantus® Gold, the insecticide Mospilan® or the mixture of both PPPs on sucrose responsiveness or learning performance of honeybees. Nevertheless, the mortality rate was synergistically affected.

Although our finding suggests that the PPPs tested do not have a sublethal effect on the honeybees in the field realistic concentrations, this does not mean that higher concentrations would not have negative side effects. But our experiments were based on a field-realistic situation in Europe and should be interpreted in this background.

Additionally, the interaction of PPPs needs to be addressed further, because we could only test one combination of fungicides and a neonicotinoid, but other combinations of different PPPs are also frequent. In fact, future studies should try to investigate a matrix of different PPPs and their interaction using more concentrations to estimate the real threat of PPP mixtures on honeybee behavioral performance and cognition.

Since honeybees differ from wild bees in many aspects, no direct comparisons can be made with wild bees. However, as wild bees are suffering from a significant decline, further studies with different wild bee species are inevitable. Also other non-target organisms should be examined in more detail since negative effects of PPPs can occur (Drossart and Gérard, 2020; Thompson, 1999; Willow et al., 2019; Wood et al., 2020).

Ethical approval

Our protocols comply with standard welfare practice in our field. The experiment involved bees from an apiary dedicated to research.

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CRediT authorship contribution statement

Antonia Schuhmann: Data acquisition, Visualization, Writing – original draft, Writing – review & editing. **Ricarda Scheiner:** Conceptualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw data for the mortality studies, the PER tests and the learning experiments is available on Mendeley Data (doi: 10.17632/4r839t38sb.1).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.114850.

References

Agência Nacional de Vigilância Sanitária, 2022. Monografias de agrotóxicos - Monografias autorizadas. https://www.gov.br/anvisa/pt-br/setorregulado/regularizacao/agrotoxicos/monografias/monografias-autorizadas-por-letra. (Accessed 11 February 2023).

Aliouane, Y., El Hassani, A.K., Gary, V., Armengaud, C., Lambin, M., Gauthier, M., 2009. Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. Environ. Toxicol. Chem. 28, 113–122. https://doi.org/10.1897/08-110.1.

Almasri, H., Tavares, D.A., Pioz, M., Sené, D., Tchamitchian, S., Cousin, M., Brunet, J.L., Belzunces, L.P., 2020. Mixtures of an insecticide, a fungicide and a herbicide induce high toxicities and systemic physiological disturbances in winter *Apis mellifera* honey bees. Ecotoxicol. Environ. Saf. 203. https://doi.org/10.1016/j.ecoenv.2020.111013.

Arıcan, E.Y., Gökçeoğlu Kayalı, D., Ulus Karaca, B., Boran, T., Öztürk, N., Okyar, A., Ercan, F., Özhan, G., 2020. Reproductive effects of subchronic exposure to acetamiprid in male rats. Sci. Rep. 10, 8985. https://doi.org/10.1038/s41598-020-05037

Artz, D.R., Pitts-Singer, T.L., 2015. Effects of fungicide and adjuvant sprays on nesting behavior in two managed solitary bees, Osmia lignaria and Megachile rotundata. PLoS One 10. https://doi.org/10.1371/journal.pone.0135688.

- BASF SE, 2021. Gebrauchsanleitung Cantus. https://www.agrar.basf.de/Dokumente/Produkte/Cantus-Gold/ga-cantus-gold.pdf. (Accessed 12 February 2023).
- Berenbaum, M.R., Johnson, R.M., 2015. Xenobiotic detoxification pathways in honey bees. Curr. Opin. Insect Sci. 10, 51–58. https://doi.org/10.1016/j.cois.2015.03.005.
- Bokšová, A., Kazda, J., Stejskalová, M., Šubrt, T., Uttl, L., Mráz, P., Bartoška, J., 2021. Findings of herbicide and fungicide residues in bee bread. Plant Soil Environ. 67, 343–352. https://doi.org/10.17221/135/2021-PSE.
- Brattsten, L.B., Berger, D.A., Dungan, L.B., 1994. *In vitro* inhibition of midgut microsomal p450s from *Spodoptera eridania* caterpillars by demethylation inhibitor fungicides and plant growth regulators. Pestic. Biochem. Physiol. 49, 234–243. https://doi.org/10.1006/pest.1994.1025.
- Camp, A.A., Batres, M.A., Williams, W.C., Koethe, R.W., Stoner, K.A., Lehmann, D.M., 2020. Effects of the neonicotinoid acetamiprid in pollen on *Bombus impatiens* microcolony development. Environ. Toxicol. Chem. 39, 2560–2569. https://doi.org/ 10.1002/etc.4886.
- Casida, J.E., 2018. Neonicotinoids and other insect nicotinic receptor competitive modulators: progress and prospects. Annu. Rev. Entomol. 63, 125–144. https://doi. org/10.1146/annurev-ento-020117-043042.
- Cedergreen, N., 2014. Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS One 9. https://doi.org/10.1371/ journal.pone.0096580.
- Chauzat, M.P., Faucon, J.P., 2007. Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera L.*) in France. Pest Manag. Sci. 1100–1106. https://doi.org/10.1002/ps.1451.
- Cox, C., Surgan, M., 2006. Unidentified inert ingredients in pesticides: implications for human and environmental health. Environ. Health Perspect. 114, 1803–1806. https://doi.org/10.1289/ehp.9374.
- Daniele, G., Giroud, B., Jabot, C., Vulliet, E., 2018. Exposure assessment of honeybees through study of hive matrices: analysis of selected pesticide residues in honeybees, beebread, and beeswax from French beehives by LC-MS/MS. Environ. Sci. Pollut. Res. 25, 6145–6153. https://doi.org/10.1007/s11356-017-9227-7.
- DeGrandi-Hoffman, G., Chen, Y., Watkins Dejong, E., Chambers, M.L., Hidalgo, G., 2015. Effects of oral exposure to fungicides on honey bee nutrition and virus levels. J. Econ. Entomol. 108, 2518–2528. https://doi.org/10.1093/jee/tov251.
- DesJardins, N.S., Fisher, A., Ozturk, C., Fewell, J.H., DeGrandi-Hoffman, G., Harrison, J. F., Smith, B.H., 2021. A common fungicide, Pristine, impairs olfactory associative learning performance in honey bees (*Apis mellifera*). Environ. Pollut. 288. https://doi.org/10.1016/j.envpol.2021.117720.
- Drossart, M., Gérard, M., 2020. Beyond the decline of wild bees: optimizing conservation measures and bringing together the actors. Insects 11. https://doi.org/10.3390/insects11090649
- Dupuis, J., Louis, T., Gauthier, M., Raymond, V., 2012. Insights from honeybee (*Apis mellifera*) and fly (*Drosophila melanogaster*) nicotinic acetylcholine receptors: from genes to behavioral functions. Neurosci. Biobehav. Rev. 36, 1553–1564. https://doi.org/10.1016/j.neubiorev.2012.04.003.
- El Hassani, A.K., Dacher, M., Gary, V., Lambin, M., Gauthier, M., Armengaud, C., 2008. Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). Arch. Environ. Contam. Toxicol. 54, 653–661. https://doi.org/10.1007/s00244-007-9071-8.
- El-Nahhal, Y., 2020. Pesticide residues in honey and their potential reproductive toxicity. Sci. Total Environ. 741, 139953 https://doi.org/10.1016/j.scitotenv.2020.139953.
- European Commission, 2022. EU Pesticides Database Active substances, safeners and synergists. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/ start/screen/active-substances. (Accessed 13 December 2022).
- Fantke, P., Juraske, R., 2013. Variability of pesticide dissipation half-lives in plants. Environ. Sci. Technol. 47, 3548–3562. https://doi.org/10.1021/es303525x.
- Fisher, A., DeGrandi-Hoffman, G., Smith, B.H., Johnson, M., Kaftanoglu, O., Cogley, T., Fewell, J.H., Harrison, J.F., 2021. Colony field test reveals dramatically higher toxicity of a widely-used mito-toxic fungicide on honey bees (*Apis mellifera*). Environ. Pollut. 269, 115964 https://doi.org/10.1016/j.envpol.2020.115964.
- FMC Agricultural Solutions, 2021. Mospilan SG in Raps Insektizid. https://www.fmcagro.de/de/produkte/a-z/mospilan-sg-im-raps.htm. (Accessed 12 February 2023).
- Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr. 44, 864–877. https://doi.org/10.4319/ lo.1999.44.3 part 2.0864.
- Fungicide Resistance Action Commitee, 2021. FRAC Code List 2022: Fungal control agents sorted by cross resistance pattern and mode of action (including coding for FRAC Groupson product labels). https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2022-final.pdf?sfvrsn=b6024e9a_2. (Accessed 10 February 2023).
- Gong, Y., Diao, Q., 2017. Current knowledge of detoxification mechanisms of xenobiotic in honey bees. Ecotoxicology 26, 1–12. https://doi.org/10.1007/s10646-016-1742-
- Hadar, R., Menzel, R., 2010. Memory formation in reversal learning of the honeybee. Front. Behav. Neurosci. 4. https://doi.org/10.3389/fnbeh.2010.00186.
- Han, W., Yang, Y., Gao, J., Zhao, D., Ren, C., Wang, S., Zhao, S., Zhong, Y., 2019. Chronic toxicity and biochemical response of *Apis cerana cerana* (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined. Ecotoxicology 28, 399–411. https://doi.org/10.1007/s10646-019-02030-4.
- Hesselbach, H., Scheiner, R., 2018. Effects of the novel pesticide flupyradifurone (Sivanto) on honeybee taste and cognition. Sci. Rep. 8. https://doi.org/10.1038/ s41598-018-23200-0.
- Hesselbach, H., Scheiner, R., 2019. The novel pesticide flupyradifurone (Sivanto) affects honeybee motor abilities. Ecotoxicology 28, 354–366. https://doi.org/10.1007/ s10646-019-02028-y.

- Iverson, A., Hale, C., Richardson, L., Miller, O., McArt, S., 2019. Synergistic effects of three sterol biosynthesis inhibiting fungicides on the toxicity of a pyrethroid and neonicotinoid insecticide to bumble bees. Apidologie 50, 733–744. https://doi.org/ 10.1007/s13592-019-00681-0.
- Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, R.M., 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Prot. 23, 371–378. https://doi.org/10.1016/j.cropro.2003.08.018.
- Jiang, X., Wang, Z., He, Q., Liu, Q., Li, X., Yu, L., Cao, H., 2018. The effect of neonicotinoid insecticide and fungicide on sugar responsiveness and orientation behavior of honey bee (*Apis mellifera*) in semi-field conditions. Insects 9. https://doi. org/10.3390/insects9040130
- Jin, N., Klein, S., Leimig, F., Bischoff, G., Menzel, R., 2015. The neonicotinoid clothianidin interferes with navigation of the solitary bee *Osmia cornuta* in a laboratory test. J. Exp. Biol. 218, 2821–2825. https://doi.org/10.1242/jeb.123612.
- Johnson, R.M., Wen, Z., Schuler, M.A., Berenbaum, M.R., 2006. Mediation of pyrethroid insecticide toxicity to honey bees (Hymenoptera: Apidae) by cytochrome P450 monooxygenases. J. Econ. Entomol. 99, 1046–1050. https://doi.org/10.1093/jee/ 99.4.1046.
- Kinnberg, K., Holbech, H., Petersen, G.I., Bjerregaard, P., 2007. Effects of the fungicide prochloraz on the sexual development of zebrafish (*Danio rerio*). Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 145, 165–170. https://doi.org/10.1016/j. cbpc.2006.11.002.
- Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274, 303–313. https://doi.org/10.1098/ rspb.2006.3721.
- Köhler, H.R., Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond. Science 341, 759–765. https://doi.org/ 10.1126/science.1237591
- Komischke, B., Giurfa, M., Lachnit, H., Malun, D., 2002. Successive olfactory reversal learning in honeybees. Learn. Mem. 9, 122–129. https://doi.org/10.1101/lm.44602.
- Kubo, S., Hirano, T., Miyata, Y., Ohno, S., Onaru, K., Ikenaka, Y., Nakayama, S.M.M., Ishizuka, M., Mantani, Y., Yokoyama, T., Hoshi, N., 2022. Sex-specific behavioral effects of acute exposure to the neonicotinoid clothianidin in mice. Toxicol. Appl. Pharmacol. 456, 116283 https://doi.org/10.1016/j.taap.2022.116283.
- La Ville de Montréal, 2015. La Ville de Montréal annonce l'adoption prochaine du Règlement modifiant le règlement sur l'utilisation des pesticides. http://ville.montreal.qc.ca/portal/page?_pageid=5798,42657625&_dad=portal&schema=PORTAL&id=26124&ret=http://ville.montreal.qc.ca/pls/portal/url/page/prt_vdm_fr/rep_annonces_ville/rep_communiques/communiques. (Accessed 14 February 2023).
- Ladurner, E., Bosch, J., Kemp, W.P., Maini, S., 2005. Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria Say* and *Apis mellifera*. Apidologie 36, 449–460. https://doi.org/10.1051/apido:2005032.
- Luken, D.J., von der Ohe, W., 2018. A research about different residues in pollen and honey samples. Hazards Pestic. Bees 198–202. https://doi.org/10.5073/ ika.2018.462.064.
- Manning, P., Ramanaidu, K., Cutler, G.C., 2017. Honey bee survival is affected by interactions between field-relevant rates of fungicides and insecticides used in apple and blueberry production. Facets 2, 910–918. https://doi.org/10.1139/facets-2017-0025
- Medrzycki, P., Giffard, H., Aupinel, P., Belzunces, L.P., Chauzat, M.-P., Claßen, C., Colin, M.E., Dupont, T., Girolami, V., Johnson, R., Le Conte, Y., Lückmann, J., Marzaro, M., Pistorius, J., Porrini, C., Schur, A., Sgolastra, F., Delso, N.S., van der Steen, J.J.M., Wallner, K., Alaux, C., Biron, D.G., Blot, N., Bogo, G., Brunet, J.-L., Delbac, F., Diogon, M., El Alaoui, H., Provost, B., Tosi, S., Vidau, C., 2013. Standard methods for toxicology research in Apis mellifera. J. Apic. Res. 52, 1–60 https://doi.org/10.3896/IBRA.1.52.4.14.
- Meng, Z., Liu, L., Jia, M., Li, R., Yan, S., Tian, S., Sun, W., Zhou, Z., Zhu, W., 2019. Impacts of penconazole and its enantiomers exposure on gut microbiota and metabolic profiles in mice. J. Agric. Food Chem. 67, 8308–8311. https://doi.org/ 10.1021/acs.iafc.9b02856.
- Mengoni Goñalons, C., Farina, W.M., 2018. Impaired associative learning after chronic exposure to pesticides in young adult honey bees. J. Exp. Biol. 221. https://doi.org/ 10.1242/jeb.176644.
- Menzel, R., 1993. Associative learning in honey bees. Apidologie 24, 157–168. https://doi.org/10.1051/apido:19930301.
- Mokkapati, J.S., Bednarska, A.J., Laskowski, R., 2021a. Physiological and biochemical response of the solitary bee *Osmia bicornis* exposed to three insecticide-based agrochemicals. Ecotoxicol. Environ. Saf. 230, 113095 https://doi.org/10.1016/j. ecoenv.2021.113095.
- Mokkapati, J.S., Bednarska, A.J., Laskowski, R., 2021b. The development of the solitary bee Osmia bicornis is affected by some insecticide agrochemicals at environmentally relevant concentrations. Sci. Total Environ. 775, 145588 https://doi.org/10.1016/j. scitotenv.2021.145588.
- Mondal, S., Ghosh, R.C., Mate, M.S., Karmakar, D.B., 2009. Effects of acetamiprid on immune system in female wistar rats. Proc. Zool. Soc. 62, 109–117. https://doi.org/ 10.1007/s12595-009-0012-6.
- Mussen, E.C., Lopez, J.E., Peng, C.Y., 2004. Effects of selected fungicides on growth and development of larval honey bees, *Apis mellifera L.* (Hymenoptera: Apidae). Environ. Entomol. 33, 1151–1154. https://doi.org/10.1603/0046-225X-33.5.1151.
- New York State, 2023. Pesticides: Reclassification of Certain Neonicotinoid Pesticide Products to Restricted Use Pesticides. https://www.dec.ny.gov/chemical/298.html. (Accessed 14 February 2023).
- Papaefthimiou, C., Theophilidis, G., 2001. The cardiotoxic action of the pyrethroid insecticide deltamethrin, the azole fungicide prochloraz, and their synergy on the