Kaila *et al. Environmental Sciences Europe* (2023) 35:39
https://doi.org/10.1186/s12302-023-00744-1

Environmental Sciences Europe

RESEARCH

Open Access

Chronic oral exposure to Amistar fungicide does not significantly affect colour discrimination but may impact memory retention in bumblebees

Lotta Kaila[1,2*], Léo Despains[3], Danae Nyckees[4], Marjaana Toivonen[5], Marja Jalli[6] and Olli J. Loukola[7,8]

Abstract

Background Intensive agriculture, including pesticides, is one of the many reasons for pollinator decline. The EU legislation on plant protection products (hereon pesticides) demands that the risks of active substances and their use in pesticide products are assessed for bees. However, the risk assessment is not always sufficient as shown, for example, in the case of the fungicide Amistar. The fungicide has been shown to cause lethal and sublethal effects on bumblebees at levels that, according to the EU risk assessment, do not require risk mitigation measures to protect bees. In order to understand the effects of chronic Amistar exposure on bumblebees, we studied whether 5 days of oral exposure to 0.015 µl Amistar (3.75 µg azoxystrobin/day) impairs bumblebees' learning and memory performance in the 10-colour discrimination task.

Results Chronic Amistar treatment did not impair the learning of the bees, but a statistically non-significant negative trend was observed in memory retention between the final learning bout and the subsequent memory test.

Conclusions The results of our study suggest that chronic sublethal exposure to Amistar fungicide did not significantly impair the learning ability of bumblebees. However, there was a trend towards impaired memory retention, although this was not statistically significant. These findings provide further support for the hypothesis that Amistar may have a negative effect on bee cognitive performance. It is important to continue studying the effects of widely used pesticides on pollinators, as their decline is a complex issue with multiple contributing factors. Understanding the effects of different pesticide residue levels on bumblebees can inform policymakers in making more sustainable pesticide legislation and help protect pollinators.

Keywords Fungicide, Bumblebee, Behaviour, Pollinator, Residue

*Correspondence:
Lotta Kaila
lotta.kaila@helsinki.fi; ext.lotta.kaila@luke.fi
Full list of author information is available at the end of the article

Background

Pollinators, such as bees, butterflies, moths, birds, and bats, play a crucial role in the reproduction of plants by transferring pollen between flowers, enabling fertilization and the production of fruits and seeds. It has been estimated that about 90% of flowering plant species across the globe rely on animal pollinators [30]. Moreover, about 35% of agricultural crop species depend on animal pollination [25]. Yet, reduced land use heterogeneity and intensive agricultural practices, such as the use of pesticides, have detrimental effects on pollinators, including one of the most efficient pollinators, bumblebees (*Bombus* spp.) [12, 19].

Some insecticides have been banned in the European Union (EU) because of their potentially detrimental effects on bees [13–16]. Fungicides, however, are generally considered less toxic for bees. Yet, studies have shown that they can contribute to bee decline, for example by impairing learning performance [11], disrupting nest recognition [2] as well as impairing metabolic functions [10, 45]. Furthermore, fungicides, in combination with other pesticides, can produce synergistic effects, increasing the toxicity of the chemicals to pollinators [23, 40].

Amistar (Syngenta) is a systemic broad-spectrum fungicide commonly used in agriculture in Europe. The active substance in Amistar is azoxystrobin. Amistar was the representative product for azoxystrobin in the previous EU-evaluation [17]. EU regulators assessed the use of Amistar as having a low risk to honeybees based on the current agreed risk assessment which includes assessment of the ratio of the application rate (g active substance/ha) and median acute lethal dose (LD_{50}) (hazard quotient <50) [17]. Based on the assessed low risk, several Member States have authorized the product to be applied on bee-attractive flowering crops without any mitigation measures to protect bees. In the assessment, the acute oral and contact LD_{50} values of Amistar were >200 µg azoxystrobin/honeybee [17]. Straw and Brown [38] challenged the use of the LD_{50} value by studying the effects of 0.8 µl Amistar/bumblebee (corresponding 200 µg azoxystrobin/bumblebee). The study showed that the exposure level caused 30% mortality, reduction in appetite, weight loss and gut melanization in the treated bumblebees. The effects were caused by a co-formulant, alcohol ethoxylates, not the active substance azoxystrobin. The study stated that the EU risk assessment relies too much on mortality (LD_{50}) and underestimates sublethal effects.

A 10-colour learning paradigm has been used to study the sublethal effects of pesticides on bumblebees, which is designed and shown to be able to detect large variations in learning and memory performance between individuals [27]. Bumblebees foraging for nectar and/or pollen use sophisticated visual learning and discrimination strategies that are mainly based on colour information [9]. These cognitive traits have a strong effect on the foraging success of bees and therefore their fitness [34]. Pesticides, such as neonicotinoids (reviewed in [37]), and glyphosate-based herbicides [22] have been shown to impair bumblebees' learning and long-term memory. However, the effects of fungicides on bumblebee cognition have not been studied before. This research aimed to examine the cognitive effects of chronic exposure to Amistar fungicide on bumblebees. We studied whether 5 days of oral exposure to 0.015 µl Amistar (3.75 µg azoxystrobin/day) impairs buff-tailed bumblebees' (*B. terrestris*) learning and long-term memory. The cognitive abilities were studied by utilizing a 10-colour learning paradigm [27].

Materials and methods

Bumblebees

Seven bumblebee colonies were purchased from Koppert (Berkel en Rodenrijs, The Netherlands). The colonies were transferred into two-chamber wooden nesting boxes [$31\times13.5\times11.5$ (height) cm] the same day they arrived in the laboratory. The nesting boxes were connected to a flight arena [60c$\times45\times25$ (height) cm] by a transparent acrylic tunnel [$25\times3.5\times3.5$ (height) cm], where the movement of the bumblebees from the nest to the arena was controlled with white plastic sliding doors. The setup was kept indoors under standardized light (LED, 2700 K, 230 VAC) with temperatures ranging between 19 and 22 °C and a photoperiod of L12/D12. Foragers of each colony were marked with individual number tags (Opalithplättchen, Warnholz & Bienenvoigt, Ellerau, Germany). The tags were attached to their thorax by using Super Glue Gel (Loctite, OH, USA). A mass feeder containing 40% (*w*/*v*) sucrose solution was placed inside the nesting box, and the bees had free access to it. In addition, the bees were given approximately 7 g of commercial pollen into the nesting box every second day (Koppert B.V., Berkel en Rodenrijs, The Netherlands).

Pre-training of the foragers

The forager bees were pre-trained to forage sucrose solution from transparent chips. The bees were allowed to move freely to the flight arena where they encountered ten transparent chips, each attached to the top of a 4-cm-high transparent stand with 10 µl of 40% (*w*/*v*) sucrose solution on top. Only active forager bees from each colony were selected in the pre-training phase. Each bee was allowed to enter the flight arena when it exhibited signs of willingness to forage and waited in the tunnel. Individual bumblebees were considered ready for the learning phase after they had successfully visited the arena three times and foraged from the transparent chips each visit.

Pesticide exposure

The commercial product Amistar (Syngenta, Switzerland, Finnish Reg.no 1836) containing azoxystrobin 250 g/l was used in the study. A total of 50 pre-trained foragers from seven colonies were assigned to the following treatments: (0) no Amistar (control, 29 bees) and (1) 0.015 µl Amistar/bee (3.75 µg azoxystrobin/bee, 21 bees).

The pre-trained foragers of the treatment group were exposed to Amistar three times a day for five consecutive days (Fig. 1). During each exposure, they received 0.005 µl Amistar (1.25 µg azoxystrobin) diluted in 10 µl sucrose solution (1.25 µg × 3 exposures = 3.75 µg/day). To ensure a minimum one-hour interval between exposures, each bee was exposed to the exact level of Amistar inside the tunnel that connected the nest box and the flight arena. In total during the five days of exposure, the bees received 0.075 µl Amistar, corresponding to 18.75 µg azoxystrobin per bee. The pre-trained control bees were treated similarly, except that they were exposed only to 10 µl pure sucrose solution during each exposure. Each exposure was given to the bee inside the tunnel between

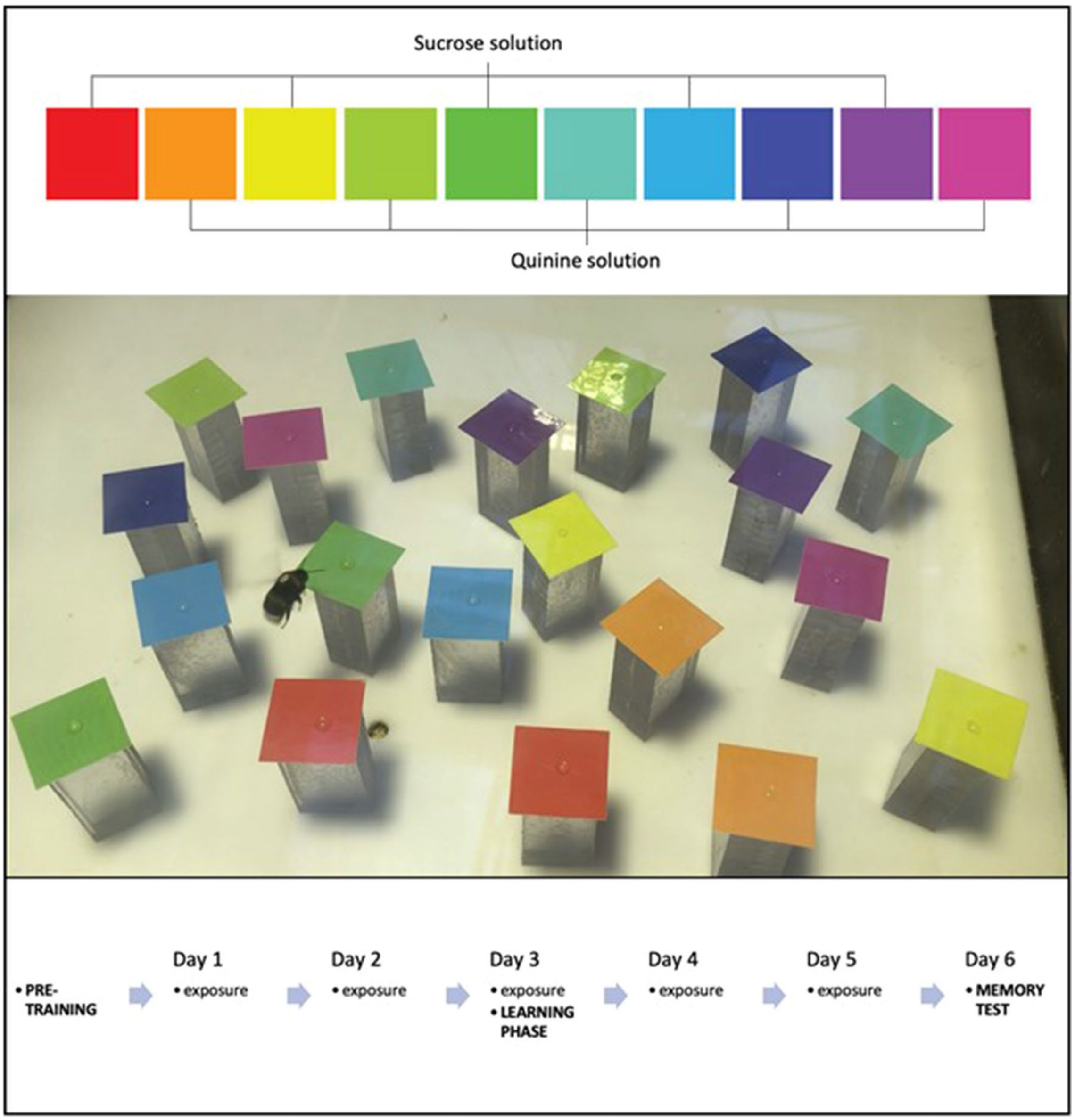

Fig. 1 Experimental setup and timeline of the experiment

the nest box and the flight arena with a minimum of 1-h intervals. The interval was based on the mean duration of foraging bouts in resource-abundant environments (66 ± 4.6 min) as determined by Westphal et al. [44]. During the exposure, each bee was kept in the tunnel for 10 min to ensure that the bee had fully consumed the solution before returning to the colony.

Colour discrimination task

The ten-colour discrimination task developed by Li et al. [27] was employed to test the learning and memory abilities of bumblebees in a way that is ecologically relevant. This task has been previously established, designed, and proven to result in significant variations in individual learning and memory performance [22, 27]. The learning phase was performed two days after the bee received its first Amistar or control exposure. In this phase, each bee was subjected to five learning bouts in the arena. In each learning bout, a single bumblebee was released in the arena, where it encountered 20 colour chips (flowers) of ten different colours (two flowers per colour) disposed of in random order. Five colours were considered as rewarding with 7 µl of sucrose solution (40% *w*/*v*), whereas five colours were aversive with 7 µl of a water solution saturated with quinine. Each learning bout lasted a maximum of ten minutes during which the bumblebee was expected to land on the flowers and to learn to dissociate between rewarding and aversive flower colours. The bout was stopped earlier if the bumblebee stopped foraging and attempted to return to the nest. After each learning bout, the bees returned to the nest for a minimum of ten minutes to empty their honey crops and started the next learning bout when they were ready and waited in the tunnel. This approach allowed us to ensure that the bees were in a foraging state and motivated to perform the task. A landing was defined as a bumblebee landing on top of a flower and touching the sucrose/quinine with its antennae or proboscis. The flowers were cleaned with 70% ethanol in water between each bout to ensure no scent marks were used to solve the task.

After the learning phase (learning bouts 1–5), the bees did not have an access to the flight arena for two days, but the Amistar and control exposures in the tunnel were continued (Fig. 1). The memory test was performed on the third day after the end of the learning phase. The experimental setup was the same as in the learning phase except that each flower contained 7 µl of water.

Statistical analyses

The effects of Amistar treatment on bumblebee performance in the learning phase and in the memory tests were analysed using generalized linear mixed models (GLMM). The analyses were conducted using R 4.1.1 software [31], and the models were fitted using the glmmTMB function of the *glmmTMB* package [6]. The models were fitted with a binomial distribution, and the regression lines were extracted using the *effects* package [18]. Individual bees were considered as the unit of replication and the colony was used as a random effect in the models to control for potential pseudoreplication. The relative influence of each observation was adjusted in the models by using the 'weights' function. We performed residual diagnostics and checked the dispersion of our models using the DHARMa package in R [21].

Model 1 tested whether Amistar exposure affected the bumblebees' performance (proportion of correct landings) in the learning phase. The following formula was used: glmmTMB (Performance ~ learning bout (bouts 1–5) * treatment + (1|colony/bee identity), family = "binomial", weights = a total number of landings).

Model 2 tested whether Amistar exposure affected the bumblebees' memory retention between the final learning bout and the subsequent memory test. The following formula was used: glmmTMB(Performance ~ bout (5th learning bout and memory test) * treatment + (1|colony/bee identity), family = "binomial", weights = a total number of landings).

Results

Based on Model 1, the bumblebees' overall performance (i.e. the proportion of correct landings) significantly increased over the learning bouts (glmmTMB; estimate = 0.27, SE = 0.04, $z = 7.15$, $p \leq 0.01$). Control bees and the Amistar-treated bees did not significantly differ from each other in overall performance (glmmTMB; estimate = − 0.34, SE = 0.26, $z = -1.30$, $p = 0.193$, Fig. 2) or in the change of performance over the learning bouts (bouts 1–5) (glmmTMB; estimate = 0.03, SE = 0.07, $z = 0.45$, $p = 0.652$, Fig. 2, left side).

Model 2 did not reveal a statistically significant difference between the Amistar-treated and control bees in memory retention between the final learning bout and the subsequent memory test. However, we did observe a non-significant trend that suggests a potential effect (glmmTMB; estimate = − 0.57, SE = 0.33, $z = -1.70$, $p = 0.088$, Fig. 2, right side).

Discussion

In this research, we studied whether chronic Amistar exposure impairs bumblebees' learning and long-term memory in the 10-colour discrimination task. Our analysis did not reveal a statistically significant difference in performance between the Amistar-treated and control bees in the learning phase. However, we observed a non-significant trend in memory retention between the final learning bout and the subsequent memory test of the

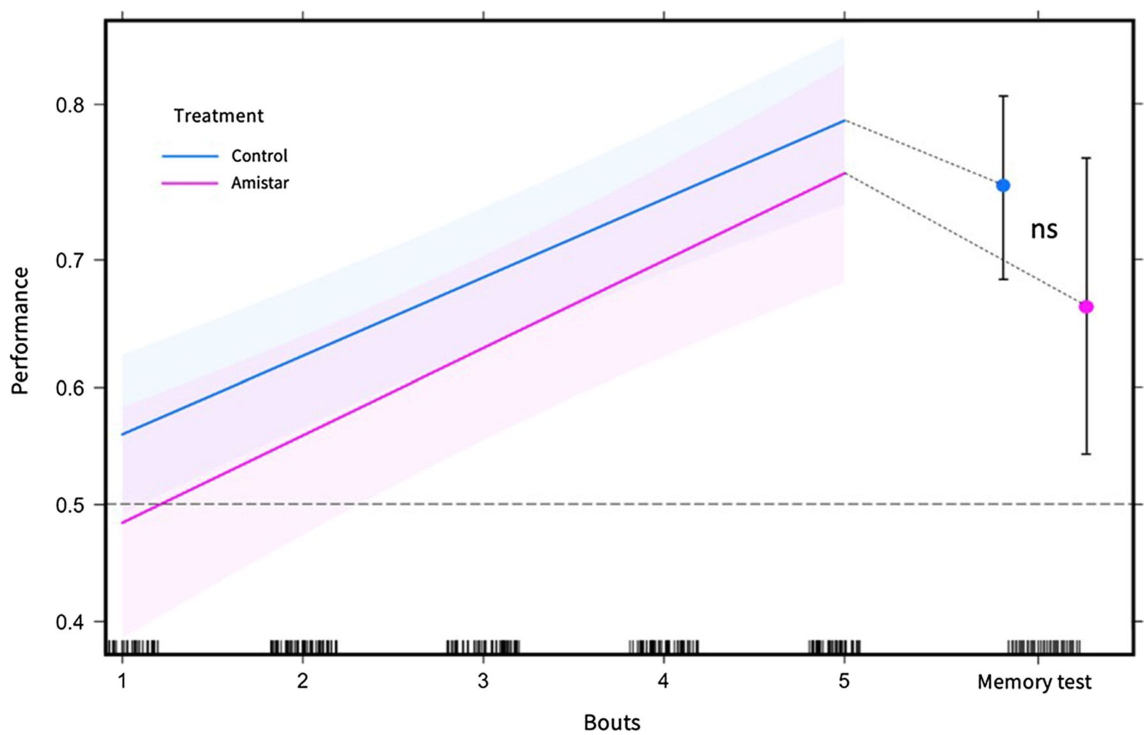

Fig. 2 The left side of the figure shows the predicted levels of performance (proportion of correct landings) and its 95% confidence band for the sample values of performance of control and treated bumblebees (three days of chronic exposure to Amistar (0.015 µl/day) in the learning phase (bouts 1–5) of the 10-colour discrimination experiment (control $n = 29$; treated with Amistar $n = 21$). Circles on the right side of the figure represent model estimates of performance and error bars represent confidence levels at 95% for the sample values of performance in the memory test (control $n = 29$; treated with Amistar $n = 17$). The dashed lines between the predicted levels of performance in the learning phase and the memory test represent memory retention between the final learning bout and the subsequent memory test. The label 'ns' indicates a non-significant memory retention trend between the treatments. The horizontal dashed line indicates the chance level (50%). Black bars above each learning bout represent the number of sample values per bout

Amistar-treated bees, which was negative when compared to the control bees. These new findings provide further support for our hypothesis that Amistar may have a negative effect on bee cognitive performance. Further studies with larger sample sizes and longer exposure periods are needed to confirm these findings. Nonetheless, our study highlights the importance of evaluating the sublethal effects of pesticides on pollinators, as even non-lethal doses can potentially affect their cognitive abilities and fitness.

Environmental conditions and agricultural practices affect pesticide residue levels in the environment and thus, the residue levels vary across time and geographical location [5, 29, 46]. Azoxystrobin is one of the most widely detected pesticides in bee-relevant matrixes like pollen and nectar [35], though worldwide, pesticide residues in these matrixes remain little studied [3, 46]. Based on the studies reporting azoxystrobin residues in pollen, the residue levels vary from a few micrograms to more than 500 µg/kg [4, 5, 24, 26, 28, 29, 32, 35, 39, 43]. The only information about azoxystrobin levels in nectar we found, were unpublished data by the Finnish Food Authority, where the residue levels were maximum of 38.6 µg/kg and a study by Krupke et al. [26], where the residue levels were maximum of 0.6 µg/kg. Overall, we found it challenging to estimate the field-realistic residue levels in both pollen and nectar, due to the high variability in the reported azoxystrobin levels in pollen, and the lack of studies on the residue levels in nectar.

There is a debate in the science community about whether the pesticide doses studied correspond to the actual exposure of bees in the field [7, 8, 37]. In this study, based on the residues found in nectar (Finnish Food Authority, unpublished data) and pollen [24], the daily exposure level was a hundred times higher than the daily oral exposure of bumblebees in Finnish agriculture (Additional file 1:). However, it is irrelevant whether the tested dose is field-realistic or not, as the sublethal effects of Amistar are not well known. Therefore, it is crucial to provide information about the potential risks of the product on bees, especially since there are gaps in our knowledge about pesticides. Testing higher doses can help establish a dose–response relationship, which can help predict the effects of lower doses in the field. Additionally, testing a range of doses can help identify potential risks and provide a basis for further research, even in cases where the field-realistic dose is unknown.

Amistar has been shown to decrease bumblebees' foraging performance and pollen deposition [41], though in honeybees (*Apis mellifera*) no effects on colony development and foraging activity were detected [42]. In the field, pollinators are rarely exposed to individual stressors, but they encounter various stress factors simultaneously and these may act synergistically [20]. Fungicides have been shown to synergize the effects of insecticides in several studies (e.g. [23, 33]). The synergistic effects of azoxystrobin together with other pesticides are studied less and the existing studies concentrate mainly on the synergistic effects of Amistar and Closer (pesticide product containing sulfoxaflor). In semi-field experiments, no synergistic effects of Closer and Amistar were found on bumblebees [41] or on solitary bees [36]. In contrast, Naggar et al. [1] showed that azoxystrobin together with insecticides flupyradifurone and sulfoxaflor reduced honeybee health and caused dysbiosis. Due to the limited number of studies concentrating mainly on the synergistic effects of Amistar and sulfoxaflor, the harmful effects of the studied exposure levels of Amistar on bumblebee cognition cannot be ruled out when bumblebees are exposed to other pesticides.

Better knowledge of pesticide residue levels in the environment is essential in order to further the sustainable use of pesticides. In our opinion, residue monitoring should be regulated in the EU and not rely on separate studies made by academia. The lack of a comprehensive database on the residue levels in the environment leaves too much uncertainty on the field-realistic exposure levels of pollinators and other environments, and thus hinders the critical evaluation of the protectiveness of the EU pesticide legislation. Likewise, the dataset would reveal which pesticides and pesticide combinations are the most relevant ones in different geographical regions. With help of this knowledge, policymakers and academia could prioritize specific pesticides in their actions.

Conclusions

Our experiment provides evidence that chronic exposure to the fungicide Amistar does not seem to impair the learning ability of bumblebees, although a negative trend was observed in memory retention between the final learning bout and the subsequent memory test. The consistent trend from the last of the five learning bouts to the memory test suggests that exposure to Amistar may have a sublethal effect on memory retention in bumblebees. However, further research is needed to confirm this effect and its magnitude. To fully understand the potential sublethal effects of commonly used pesticides, including fungicides, and potential synergies with other pesticides, more information on pesticide residues in different environments is needed. This knowledge is essential to tackle the drivers of pollinator decline and to provide regulatory bodies with information to design more sustainable directives for pesticide use in agroecosystems.

Limitations of the study

This study has limitations that should be considered. Firstly, the laboratory dose used may not reflect the actual exposure of bumblebees in the field. Secondly, exposure levels may vary in the field, affecting actual exposure. Thirdly, the sample size was small, limiting statistical power and generalizability. Finally, the study focused on a single species of bees and cognitive task, while bumblebees are often exposed to multiple pesticides simultaneously in the field. Future research should investigate these questions in greater depth and explore the potential long-term effects of Amistar on bee health and behaviour. Despite these limitations, this study emphasizes the need for sustainable agricultural practices that protect both crop yields and pollinator health.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-023-00744-1.

Additional file 1. Additional Table S1

Acknowledgements
We thank Némo Fontanié for helping in the lab.

Author contributions
LK, OJL, DN and LD conceived the ideas and designed the methodology; DN and LD collected the data; OJL analysed the data; LK led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. All authors read and approved the final manuscript.

Funding
Open Access funding provided by University of Helsinki including Helsinki University Central Hospital. LK was supported by the Maj and Tor Nessling Foundation. OJL was supported by the Kone Foundation (Grant number 202010852).

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Agricultural Sciences, University of Helsinki, 27, 00014 Helsinki, Finland. [2]Natural Resources Institute Finland (Luke), Latokartanonkaari 9, 00790 Helsinki, Finland. [3]Université de Toulouse, CRCA, UPS, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France. [4]Wagenigen University, 6700 Wagenigen, The Netherlands. [5]Biodiversity Centre, Finnish Environment Institute (SYKE), Latokartanonkaari 11, 00790 Helsinki, Finland. [6]Natural Resources Institute Finland (Luke), Tietotie 4, 31600 Jokioinen, Finland. [7]Ecology and Genetics Research Unit, University of Oulu, 3000, 90014 Oulu, Finland. [8]Biodiversity Unit, University of Oulu, 3000, 90014 Oulu, Finland.

Received: 21 December 2022 Accepted: 19 May 2023
Published online: 30 May 2023

References

1. Al Naggar Y, Singavarapu B, Paxton RJ, Wubet T (2022) Bees under stressors: the novel insecticides flupyradifuorne and sulfoxaflor along with the fungicide azoxystrobin disturb the gut microbiota of honey bees and increase opportunistic bacterial pathogens. Sci Total Environ 849:157941. https://doi.org/10.1016/j.scitotenv.2022.157941
2. Artz DR, Pitts-Singer TL (2015) Effects of fungicide and adjuvant sprays on nesting behavior in two managed solitary bees, Osmia lignaria and Megachile rotundata. PLoS ONE 10(8):e0135688. https://doi.org/10.1371/journal.pone.0135688
3. Benuszak J, Laurent M, Chauzat MP (2017) The exposure of honey bees (*Apis mellifera*; Hymenoptera: Apidae) to pesticides: room for improvement in research. Sci Total Environ 587–588:423–438. https://doi.org/10.1016/j.scitotenv.2017.02.062
4. Beyer M, Lenouvel A, Guignard C, Eickermann M, Clermont A, Kraus F, Hoffmann L (2018) Pesticide residue profiles in bee bread and pollen samples and the survival of honeybee colonies—a case study from Luxembourg. Environ Sci Pollut Res 25(32):32163–32177. https://doi.org/10.1007/s11356-018-3187-4
5. Böhme F, Bischoff G, Zebitz CPW, Rosenkranz P, Wallner K (2018) Pesticide residue survey of pollen loads collected by honeybees (*Apis mellifera*) in daily intervals at three agricultural sites in South Germany. PLoS ONE. https://doi.org/10.1371/journal.pone.0199995
6. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9(2):378–400. https://doi.org/10.32614/RJ-2017-066
7. Campbell PJ (2013) Declining European bee health: banning the neonicotinoids is not the answer. Outlooks Pest Manag 24:52–57. https://doi.org/10.1564/v24_apr_02
8. Carreck L, Ratnieks FLW (2014) The dose makes the poison: have "field realistic" rates of exposure of bees to neonicotinoid insecticides been overestimated in laboratory studies? J Apic Res 53(5):607–614. https://doi.org/10.3896/IBRA.1.53.5.0
9. Chittka L, Thomsson J (2001) Cognitive ecology of pollination : animal behaviour and floral evolution. Cambridge University Press, Cambridge
10. Chmiel JA, Daisley BA, Pitek AP, Thompson GJ, Reid G (2020) Understanding the effects of sublethal pesticide exposure on honey bees: a role for probiotics as mediators of environmental stress. Front Ecol Evol. https://doi.org/10.3389/fevo.2020.00022
11. DesJardins NS, Fisher A, Ozturk C, Fewell JH, DeGrandi-Hoffman G, Harrison JF, Smith BH (2021) A common fungicide, Pristine®, impairs olfactory associative learning performance in honey bees (*Apis mellifera*). Environ Pollut 288:17720–117720. https://doi.org/10.1016/j.envpol.2021.117720
12. Dicks LV, Breeze TD, Ngo HT, Senapathi D, An J, Aizen MA, Basu P, Buchori D, Galetto L, Garibaldi LA, Gemill-Herren B, Howlett BG, Imperatriz-Fonseca VL, Johnson SD, Kovacs-Hostyanszki A, Kwon YJ, Lattorff HMG, Lungarwo T, Seymour CL, Vanbergen AJ, Potts G (2021) A global-scale expert assessment of drivers and risks associated with pollinator decline. Nat Ecol Evol 5(2021):1453–1461
13. European Food Safety Authority (2016) Peer review of the pesticide risk assessment for the active substance clothianidin in light of confirmatory data submitted. EFSA J 14(11):4606. https://doi.org/10.2903/j.efsa.2016.4606
14. European Food Safety Authority (2016) Peer review of the pesticide risk assessment for the active substance imidacloprid in light of confirmatory data submitted. EFSA J 14(11):4607. https://doi.org/10.2903/j.efsa.2016.4607
15. European Food Safety Authority (2016) Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for thiamethoxam in light of confirmatory data. EFSA supporting publication 2016:EN-1020. 27 pp. https://doi.org/10.2903/j.efsa.2018.5177
16. European Food Safety Authority (2013) Conclusions on the peer review of the pesticide risk assessment for bees for the active substance fipronil. EFSA J 11(5):3158. https://doi.org/10.2903/j.efsa.2013.3158
17. European Food Safety Authority (2010) Conclusions on the peer review of the pesticide risk assessment for bees for the active substance azoxystrobin. EFSA J 8(4):1542. https://doi.org/10.2903/j.efsa.2010.1542
18. Fox J (2003) Effect displays in R for generalised linear models. J Stat Softw 8(15):1–27
19. Goulson D, Lye GC, Darvill B (2008) Decline and conservation of bumble bees. Annu Rev Entomol 53:191–208
20. Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347(6229):1255957
21. Hartig F (2022) DHARMa: residual diagnostics for hierarchical (Multi-Level/Mixed) Regression Models. R package version 0.4.6. Accessed 31 Aug 2022. http://florianhartig.github.io/DHARMa/
22. Helander M, Lehtonen T, Saikkonen K, Despains L, Nyckees D, Antinoja A, Solvi C, Loukola O (2023) Field-realistic acute exposure to glyphosate-based herbicide impairs fine-color discrimination in bumblebees. Sci Total Environ 857:159298–159298. https://doi.org/10.1016/j.scitotenv.2022.159298
23. 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 neonicotionoid insecticide to bumble bee. Apidologie 50:733–744. https://doi.org/10.1007/s13592-019-00681-0
24. Kaila L, Ketola J, Toivonen M, Loukola O, Hakala K, Raiskio S, Hurme T, Jalli M (2022) Pesticide residues in honeybee-collected pollen: does the EU regulation protect honeybees from pesticides? Environ Sci Pollut Res 29:18225–18244. https://doi.org/10.1007/s11356-021-16947-z
25. Klein AM, Vaissiere BE, Cane JH, Steffan-Dewneter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for word crops. Proc R Soc B Biol Sci 274(1608):303–313
26. Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K, Smagghe G (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS ONE 7(1):29268. https://doi.org/10.1371/journal.pone.0029268
27. Li L, MaBouDi H, Egertová M, Elphick MR, Chittka L, Perry CJ (2017) A possible structural correlate of learning performance on a color discrimination task in the brain of bumblebee. Proc R Soc B 284:20171323. https://doi.org/10.1098/rspb.2017.1323
28. Long EY, Krupke CH (2016) Non-cultivated plants present a season-long route of pesticide exposure for honey bees. Nat Commun 7(1):11629–11629. https://doi.org/10.1038/ncomms11629
29. Niell S, Jesus F, Perez C, Mendoza Y, Diaz R, Franco J, Cesio V, Heinzen H (2015) QuEChERS adaptability for the analysis of pesticide residues in beehive products seeking the development of an agroecosystem sustainability monitor. J Agric-Tural Food Chem 63(18):4484–4492. https://doi.org/10.1021/acs.jafc.5b00795
30. Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals ? Oikos 120(3):321–326. https://doi.org/10.1111/j.1600-0706.2010.18644
31. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Accessed 5 Feb 2023. https://www.R-project.org/.
32. Raimets R, Bontšutšnaja A, Bartkevics V, Pugajeva I, Kaart T, Puusepp L, Pihlik P, Keres I, Viinalass H, Mänd M, Karise R (2020) Pesticide residues in beehive matrices are dependent on collection time and matrix type but independent of proportion of foraged oilseed rape and agricultural land in foraging territory. Chemosphere 238:124555. https://doi.org/10.1016/j.chemosphere.2019.124555

33. Raimets R, Karise R, Mänd M, Kaart T, Ponting S, Song J, Cresswell JE (2018) Synergistic interactions between a variety of insecticides and an ergosterol biosynthesis inhibitor fungicide in dietary exposures of bumble bees (*Bombus terrestris* L.). Pest Manag Sci 74:541–546. https://doi.org/10.1002/ps.4756
34. Raine NE, Chittka L (2008) The correlation of learning speed and natural foraging success in bumble-bees. Proc R Soc B Biol Sci 275(1636):803–808
35. Rondeau S, Raine N (2022) Fungicides and bees: a review of exposure and risk. Environ Int 165:107311. https://doi.org/10.1016/j.envint.2022.107311
36. Schwarz JM, Knauer AC, Allan MJ, Dean RR, Ghazoul J, Tamburini G, Wintermantel D, Klein A-M, Albrecht M (2022) No evidence for impaired solitary bee fitness following pre-flowering sulfoxaflor application alone or in combination with a common fungicide in a semi-field experiment. Environ Int 164:107252–107252. https://doi.org/10.1016/j.envint.2022.107252
37. Siviter H, Koricheva J, Brown MJF, Leadbeater E (2018) Quantifying the impact of pesticides on learning and memory in bees. J Appl Ecol 55:2812–2821. https://doi.org/10.1111/1365-2664.13193
38. Straw EA, Brown MJF (2022) Co-formulant in a commercial fungicide product causes lethal and sub-lethal effects in bumble bees. Sci Rep 11:21653. https://doi.org/10.1038/s41598-021-00919-x
39. Stoner KA, Eitzer BD (2013) Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in connecticut. PLoS ONE 8(10):e77550. https://doi.org/10.1371/journal.pone.0077550
40. Sgolastra F, Medrzycki P, Bortolotti L, Renzi MT, Tosi S, Bogo G, Teper D, Porrini C, Molowny-Horas R, Bosch J (2017) Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. Pest Manag Sci 73(6):1236–1243
41. Tamburini G, Pereira-Peixoto M-H, Borth J, Lotz S, Wintermantel D, Allan MJ, Dean R, Schwarz JM, Knauer A, Albrecht M, Klein A-M (2021) Fungicide and insecticide exposure adversely impacts bumblebees and pollination services under semi-field conditions. Environ Int 157:106813–106813. https://doi.org/10.1016/j.envint.2021.106813
42. Tamburini G, Wintermantel D, Allan MJ, Dean RR, Knauer A, Albrecht M, Klein A-M (2021) Sulfoxaflor insecticide and azoxystrobin fungicide have no major impact on honeybees in a realistic-exposure semi-field experiment. Sci Total Environ 778:146084–146084. https://doi.org/10.1016/j.scitotenv.2021.146084
43. Tosi S, Costa C, Vesco U, Quaglia G, Guido G (2018) A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides. Sci Total Environ 615:208–218. https://doi.org/10.1016/j.scitotenv.2017.09.226
44. Westphal C, Stefan-Wewenter I, Tscharntke T (2006) Foraging trip duration of bumblebees in relation to landscape-wide resource availability. Ecol Entomol 31(4):389–394
45. Zaluski R, Bittarello AC, Vieira JCS, Braga CP, Pedro de Magalhes P, da Silva FM, de Souza BT, de Oliveira OR (2020) Modification of the head proteome of nurse honeybees (*Apis mellifera*) exposed to field-relevant doses of pesticides. Sci Rep 10(1):2190. https://doi.org/10.1038/s41598-020-59070-8
46. Zioga E, Kelly R, White B, Stout JC (2020) Plant protection product residues in plant pollen and nectar: a review of current knowledge. Environ Res 189:109873. https://doi.org/10.1016/j.envres.2020.109873

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.