Science of the Total Environment 857 (2023) 159298

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

Field-realistic acute exposure to glyphosate-based herbicide impairs fine-color discrimination in bumblebees

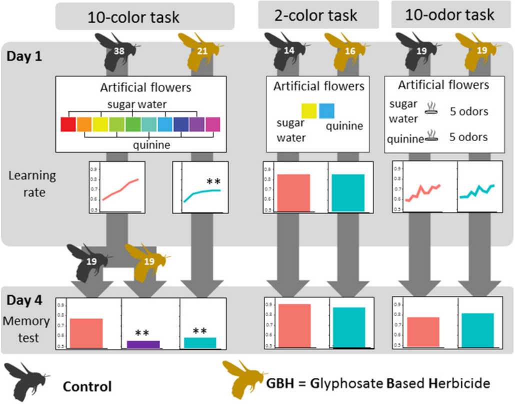
Marjo Helander[a,*], Topi K. Lehtonen[b,c], Kari Saikkonen[d], Léo Despains[e], Danae Nyckees[f], Anna Antinoja[b], Cwyn Solvi[b], Olli J. Loukola[b]

[a] Department of Biology, University of Turku, FI-20014 Turku, Finland
[b] Ecology and Genetics Research Unit, University of Oulu, FI-90570 Oulu, Finland
[c] Natural Resources Institute Finland, FI-90570 Oulu, Finland
[d] Biodiversity Unit, University of Turku, FI-20014 Turku, Finland
[e] Centre de Recherches sur la Cognition Animale, Centre de Biologie Intégrative, Université de Toulouse, CNRS, UPS, 118 route de Narbonne, F-31062 Toulouse cedex 9, France
[f] Laboratory of Entomology, Wageningen University, 6700 AA Wageningen, the Netherlands

HIGHLIGHTS

- Use of glyphosate-based herbicides (GBHs) is linked to pollinator crisis.
- Acute field-realistic exposure to GBH impairs bees' fine-color discrimination.
- Acute field-realistic exposure to GBH does not affect olfaction or general vision.
- GBHs may pose a greater threat to bumblebee colony survival than previously thought.
- Risk assessments should include tests for learning and memory.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Kevin V. Thomas

Keywords:
Glyphosate
Bumblebee
Pollinator decline
Color discrimination
Odor discrimination

ABSTRACT

Pollinator decline is a grave challenge worldwide. One of the main culprits for this decline is the widespread use of, and pollinators' chronic exposure to, agrochemicals. Here, we examined the effect of a field-realistic dose of the world's most commonly used pesticide, glyphosate-based herbicide (GBH), on bumblebee cognition. We experimentally tested bumblebee (*Bombus terrestris*) color and scent discrimination using acute GBH exposure, approximating a field-realistic dose from a day's foraging in a patch recently sprayed with GBH. In a 10-color discrimination experiment with five learning bouts, GBH treated bumblebees' learning rate fell to zero by third learning bout, whereas the control bees increased their performance in the last two bouts. In the memory test, the GBH treated bumblebees performed to near chance level, indicating that they had lost everything they had learned during the learning bouts, while the control bees were performing close to the level in their last learning bout. However, GBH did not affect bees' learning in a 2-color or 10-odor discrimination experiment, which suggests that the impact is limited to fine color learning and does not necessarily generalize to less specific tasks or other modalities. These results indicate that the widely used pesticide damages bumblebees' fine-color discrimination, which is essential to the pollinator's individual success and to colony fitness in complex foraging environments. Hence, our study suggests that acute sublethal exposure to GBH poses a

---

* Corresponding author.
E-mail address: helander@utu.fi (M. Helander).

http://dx.doi.org/10.1016/j.scitotenv.2022.159298
Received 12 June 2022; Received in revised form 2 October 2022; Accepted 4 October 2022

greater threat to pollination-based ecosystem services than previously thought, and that tests for learning and memory should be integrated into pesticide risk assessment.

1. Introduction

Pollinators are key players in global biodiversity and ecosystem functioning, providing essential services to both wild plant communities and agricultural productivity. More than 80 % of flowering plant species rely on pollinators to reproduce, and 35 % of the world's leading crops benefit from pollinating animals (Klein et al., 2007; Ollerton et al., 2011). Both the abundance and diversity of insect pollinators have alarmingly declined globally during the last decades (Potts et al., 2010; Hallmann et al., 2017; Reilly et al., 2020; Zattara and Aizen, 2021). Anthropogenic factors, such as changes in land cover, configuration and management, and pesticide use, are indisputably linked to the pollinator crisis (Dicks et al., 2021).

Commercial pesticides combine an active ingredient, for example glyphosate, and a complex mixture of co-formulants that are added to increase the efficiency of the active ingredient (Maldonado-Reina et al., 2022). These co-formulants can be more toxic to pollinators than the active ingredients (Straw and Brown, 2021). Glyphosate-based herbicides (GBHs) are globally the most widely used pesticides (Benbrook, 2016), and are commonly used in agriculture, horticulture, silviculture, landscaping and urban environments. In agriculture, the use of glyphosate-tolerant canola and soybean (which require or benefit from insect pollination) has promoted the popularity of GBHs (Benbrook, 2016). Glyphosate residues and their degradation products have accumulated across the natural environment, in soils, plants and waters, further raising concerns about the potential for non-target species being exposed to and harmfully impacted by this pesticide (Helander et al., 2012).

Glyphosate has had a reputation for being non-toxic to animals because, as an herbicide, its main effect (inhibiting 5-enolpyruvylshikimate-3-phosphate synthase enzyme in the shikimate pathway) targets plants (Helander et al., 2012), and may affect fungi and some bacteria (Leino et al., 2021). However, recent studies show that GBHs can negatively affect animals, including pollinators (Farina et al., 2019). These studies, typically on honeybees (*Apis mellifera*) (Tan et al., 2022), have often used chronic glyphosate or GBH doses, while examining survival, development, physiology, colony thermoregulation or gut microbiota of bees (Motta et al., 2020; Castelli et al., 2021; Almasri et al., 2022; Weidenmüller et al., 2022). In contrast, very few studies have tested field-realistic doses of glyphosate on bees' cognitive performance (Hernández et al., 2021), and no studies have examined this question in a non-honeybee pollinator.

Here, to investigate the potential impact of GBH on cognition, we tested free-flying bumblebees (*Bombus terrestris*) in a series of learning and memory experiments. We focused on the cognitive traits of the bees because these traits determine the successful foraging and social behavior of social insects and therefore their fitness (Raine and Chittka, 2008). These require bees in search of nectar and/or pollen to distinguish flowers based mainly on color information. Our first results on the effects of GBH on bees' fine-color discrimination inspired us to test whether their vision in general and/or odor discrimination are also affected.

2. Material and methods

2.1. Bumblebee exposure to GBH

Bumblebees do not avoid glyphosate treated plants and thus can be exposed to glyphosate when they are foraging in recently (one to three days) sprayed fields (Thompson et al., 2022). We expect wild bumblebees to encounter GBH exposure equivalent to 0.1 μL within a 24 hour period following GBH application on flowering plants within their foraging zone. The reference points for this calculation are the instructions for the application of the commercial formulation Roundup Gold and the known foraging behavior of *B. terrestris* foragers. According to the directions in the product label of Roundup Gold, the recommended dilution for GBH-spray in the field is 3–6 % in water. Once sprayed on flowering plants, GBH will ultimately mix with the nectar of the flowers (Thompson et al., 2014). Because the plants and their flowers start to wither within 2–3 days of GBH spraying, after which they no longer attract pollinators, we use the term 'recent spraying' to refer to the period after spraying when the pollinators are still attracted by the flowers. During a single nectar foraging trip, *B. terrestris* foragers often visit hundreds of flowers and can collect over 100 μL of nectar (Osborne et al., 2008). The foraging bumblebees can make dozens of trips per day, which provides ample opportunity for exposure to 0.1 μL GBH in a day. Moreover, the major pesticide exposure routes for bumblebees include nectar, pollen, plant surfaces, water, soil surfaces around nests, and air particles (Gradish et al., 2019). Therefore, we expect bumblebees to face GBH exposures equivalent to at least 0.1 μL within any one day shortly following GBH application on flowering plants.

To simulate approximated field-realistic exposures of GBH, we provided each focal bumblebee with 60 % sucrose solution with or without 0.1 μL GBH (commercial product Roundup Gold, Monsanto Europe S.A., Belgium, registration number 1934; glyphosate concentration 450 g/L, as glyphosate isopropylamine salt CAS: 3864194–0). We first diluted 1 mL of Roundup Gold with 99 mL of 60 % sucrose solution, creating a 1 % Roundup solution. We then offered each bee 10 μL of this solution (which amounted to 0.1 μL of Roundup Gold and 0.045 mg of active ingredient glyphosate). Thompson et al. (2014) estimated the total maximum daily intake of glyphosate residues in honeybee broods to be 66 mg, which equals 1500 glyphosate doses in our experiment. We found that bees would not initiate drinking 1 % GBH in water, however, they readily began to drink a mixture of 1 % GBH in 60 % sugar water. Some of the bees did not immediately finish the initially provided 20 μL mixture, and for those bees, we added 60 % sugar water (up to 100 μL total volume) until the entire droplet was consumed. Control group bees drank 20 μL of 60 % sucrose solution. After GBH- or control-treatment, bees were isolated until they finished the droplet. Once the droplet was consumed, and 5 min had passed, the bee was left to return to the nest and empty its crop load. Exposure (control and GBH) was done only once before the initiation of training. Because bees deposited their crop load into the nest before training, and in between each training bout, motivation during their next foraging bout is not affected. This was verified by the control and GBH bees behaving equally well in the first three learning bouts (Fig. 1A) regardless of the consumed amount of sugar solution during the exposure.

2.2. Testing individual bee learning and memory

2.2.1. Animals and pretraining

All the experiments were conducted in the spring of 2021 at the University of Oulu, Finland. Buff-tailed bumblebees (*B. terrestris*; from Koppert, The Netherlands) were housed in a two-chamber wooden nesting box connected to a flight arena (l = 60 cm, w = 45 cm, h = 25 cm, with a transparent top) by a transparent acrylic tunnel (l = 25 cm, 3.5 cm × 3.5 cm). The movement of individual bees from the nesting box to the arena was controlled with sliding doors in the tunnel. Individual forager bees were marked by super-gluing a small plastic number tag (Bienen-Voigt & Warnholz, Germany) on their thorax. Forager bees used in the experiments were randomly selected from 18 colonies (six colonies for each experiment). The nesting boxes and arenas were placed indoors under standardized light (LED, 2700 K, 230 VAC) with temperature between 19 and 22 °C and light-dark cycle of 12 h/12 h.

Forager bees used in the experiments were individually pretrained to find sucrose solution from our artificial flowers constructed by attaching

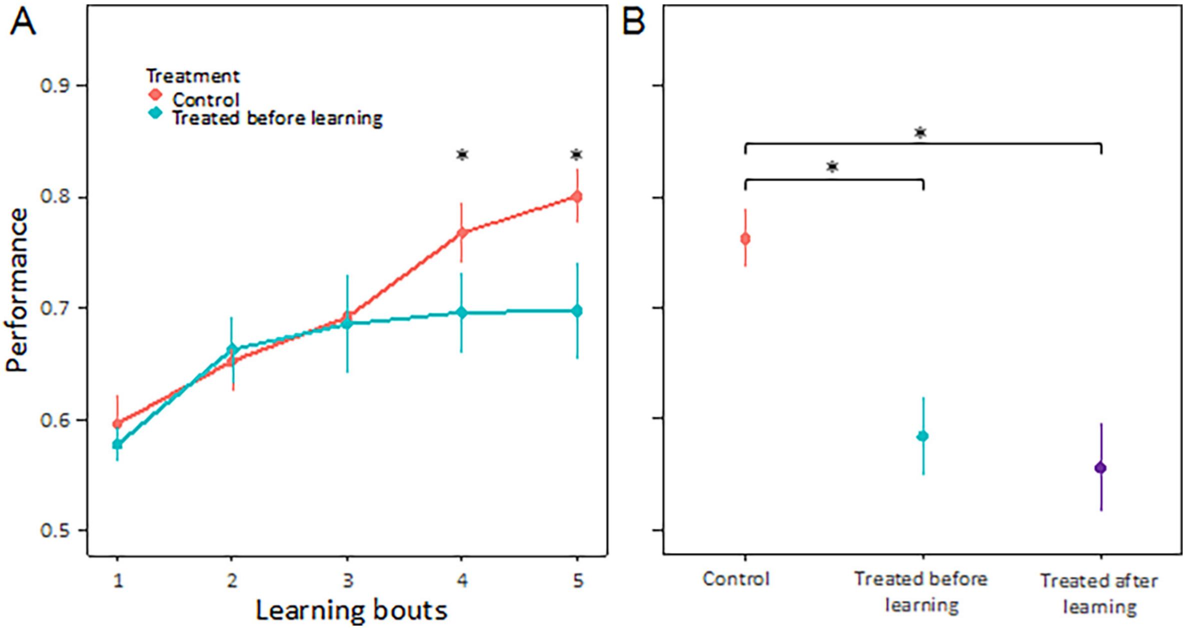


Fig. 1. Performance (proportion of correct landings) of bumblebees (*Bombus terrestris*) after acute glyphosate-based herbicide exposure (0.1 μL of Roundup Gold in 60 % sucrose solution) or controls (only 60 % sucrose solution) in the 10-color discrimination experiment (control n = 19; treated before learning n = 21; treated after learning n = 19). A) Learning phase, where the bees were allowed to choose between rewarding (40 % sucrose solution) and aversive (saturated quinine solution) flowers of 10 different colors. Here, the bees treated after the learning are pooled with the controls. B) Two full days after the learning phase the bees underwent a memory test with the same set-up as in the learning phase except that each flower contained unrewarding water. Vertical lines represent standard error of the mean of the raw data. Stars represent significant differences between the treatment groups.

a transparent chip to the top of a 40 mm high transparent stand. In all experiments, ten flowers were placed in the arena for pretraining, each with a 7 μL drop of sucrose (40 % w/v) on the top. Individual bees were considered ready for color/odor training after they had successfully foraged 5–6 times from the transparent flowers and returned to the colony.

Time-wise, experiments in a colony were carried out as follows. Bees were pretrained in a day. In the following day, we treated control bees with sugar water and carried out all learning trials. Usually, bees returned to the arena for the first learning bout within 30 min from the treatment. Two full days after the learning trials, the control bees underwent the memory test. After the control bees had accomplished the memory test, we started the GBH exposure treatments on GBH bees of the colony. Similarly to the control bees, GBH treated bees returned to the arena for the first learning bout within 30 min. The learning phase of the bees was completed within the same day in most of the cases and always within 24 h. Here again, we tested the bees' memory two full days later. For each colony, the experiment for all control bees took four or five days, at which point the experiments for all GBH exposed bees took four or five days. There is no indication from the literature on bee learning, memory and cognition that older bees or bees from slightly (days) older colonies have any deterioration in cognition (Raine et al., 2006; Riveros and Gronenberg, 2009).

2.2.2. Ten-color discrimination experiment

We used a 10-color discrimination task established by Li et al. (2017) that provides a sufficient and ecologically relevant challenge for bumblebees' learning and memory. For each colony, we assessed the control treatment group first to ensure that control bees were not exposed to GBH in the nest. We subsequently exposed pretrained bees from the same colony to GBH, as described above.

In the *learning phase*, each pretrained and tagged forager bumblebee was randomly assigned into one of three groups: control (19 bees), GBH exposed before the learning phase (21 bees), or GBH exposed after the learning phase (19 bees). Each bee underwent five bouts in which they were allowed to choose between flowers of 10 different colors. Each bout lasted a maximum of 10 min, or until the bee returned to the colony on her own. Flowers of five colors contained 7 μL of 40 % sucrose solution (reward) and the other five contained 7 μL of aversive saturated quinine solution. Two flowers of each color, for a total of 20 flowers, were randomly placed in the arena. All landings on the flowers were recorded both manually and by video. Flowers were cleaned with 70 % ethanol in water between trials to ensure no scent marks could be used to solve the task. A landing was defined as anytime a bee was positioned on the top of a flower and stopped flying and touched the sucrose/quinine with its antennae or proboscis. Once a bee completed the training phase, she was not allowed to enter the arena for two days to prevent any further foraging experience. Two full days after the learning phase, the same bee underwent a single-bout memory retention test, with the setup being the same as in training except that each flower contained 7 μL of unrewarding water. Again, all landings within 10 min of entering the arena were recorded.

2.2.3. Two-color discrimination experiment

To test whether GBH exposure is affecting the general deterioration in color vision, we trained and tested bumblebees on a simple two-color discrimination task, in which the colors (blue and yellow) can normally be proficiently learned in a single bout. Bees were assigned into either the control (14 bees) or GBH exposed (16 bees) group. Ten flowers of each color were randomly placed in the arena, for a total of 20 flowers. Here, we followed the same procedure for both the learning phase and memory test as the 10-color discrimination experiment, except for the following three differences. First, all the bees in the treatment group were exposed to GBH *before* the learning phase. Second, only yellow and blue flowers were used. Third, training was stopped when bees reached a criterion performance of 80 % correct in the last 20 landings. For each bee, the rewarding color (blue or yellow) was assigned randomly.

2.2.4. Ten-odor discrimination experiment

To determine whether the GBH-effect was modality specific, we trained and tested 38 bumblebees, each randomly assigned into either the GBH exposed (19 bees) or control (19 bees) group. The odor experiment followed the same general procedure as in the 10-color discrimination experiment.

However, because odor discrimination is more challenging than color discrimination for the bees, they were given ten training bouts. All ten flowers were visually identical and differed only in odor. Flowers consisted of a transparent cylinder (petri dish with a lid, Ø: 31 mm, height: 13 mm) secured to a 40 mm transparent stand. 0.5 μL of scented liquid (100 % essential oils, VSADEY) was aliquoted onto a small piece of filter paper placed inside the cylinder. The top of the cylinder (i.e. petri dish lid) had eight 1-mm holes around the edge to allow the odors to exit around the reinforcement solution (sucrose/quinine) that was placed on the center top of the cylinder. Ten different odors were used, of which cinnamon, eucalyptus, frankincense, lemongrass and tea tree were always rewarded (had sucrose on top of the cylinder) and bergamot, ginger, peppermint, rosemary and sweet orange always had aversive (bad-tasting) quinine solution on top. Only one flower with each odor was available at a time. Flowers were placed in the arena immediately before each bout to help reduce the overaccumulation of odors in the arena. In addition, a fume hood ventilation tube was connected to the arena while replacement air flowed to the arena through numerous small holes in the arena walls. Two full days after having completed the learning phase, 18 treated and 17 control bumblebees were tested in a *memory* test. Here, the set-up was similar to the learning phase, except that instead of rewarding and aversive options, all 10 flowers with the different scents had 20 μL of water on the top. The landings were again recorded both by video and manually.

2.3. Statistical analysis

Statistical analyses were conducted to compare the performance between the treatments in the learning phase of each experiment as well as each of the memory tests. The analyses were conducted using R 4.1.1 software (R Core Team, 2021). Generalized linear mixed-effects models and generalized linear models (GLMM and GLM; 'glmer' and 'glm' functions in package lme4) (Bates, 2005) were used. In total, we fitted six models. The relative influence of each observation was adjusted in the models by using the 'weights' function. Post hoc tests were conducted with the Tukey HSD (R function: TukeyHSD) for performing multiple pairwise-comparison between the means of performances in learning bouts and memory tests.

Model 1 tested whether the GBH exposure before learning affected the bees' performance (proportion of correct landings) in the learning phase of the 10-color discrimination experiment. The following formula was used: glmer(performance ~ learning bout ∗ treatment + (1|colony/bee identity), family = 'binomial', weights = total number of landings). Data of control bees and bees treated after learning were pooled together as there was no difference in learning performance over bouts between these groups (GLMM; estimate = 0.06 ± 0.06, z = 1.00, p = 0.32).

Model 2 tested whether the GBH exposure before and after learning affected the bees' performance in the memory test of a 10-color discrimination experiment. The following formula was used: glm(performance ~ treatment, family = 'binomial', weights = total number of landings).

Model 3 tested whether the GBH exposure before learning affected the bees' performance in the learning phase of the 2-color discrimination experiment. The following formula was used: glmer(performance ~ treatment + flower color + (1|bee identity), family = 'binomial', weights = total number of landings).

Model 4 tested whether the GBH exposure before and after learning affected the bees' performance in the memory test of the 2-color discrimination experiment. The following formula was used: glmer(performance ~ treatment + flower color + (1|colony), family = 'binomial', weights = total number of landings).

Model 5 tested whether the GBH exposure before learning affected the bees' performance in the learning phase of the 10-odor discrimination experiment. The following formula was used: glmer(performance ~ learning bout ∗ treatment + (1|colony/bee identity), family = 'binomial', weights = total number of landings).

Model 6 tested whether the GBH exposure before learning affected the bees' performance in the memory test of the 10-odor discrimination experiment. The following formula was used: glmer(performance ~ learning bout ∗ treatment + (1|colony/bee identity), family = 'binomial', weights = total number of landings).

3. Results

3.1. Ten-color discrimination experiment

When learning to discriminate 10 different colors, overall bumblebees' performance (proportion of correct landings) increased over learning bouts (Generalised Linear Mixed Model (GLMM); estimate [±SE] = 0.26 ± 0.03, z = 8.12, p ≤ 0.001; see Fig. 1A for the mean values and standard error of means). The performance is not expected to be below 0.5. even if the bumblebee does not understand the task and chooses the flowers at random (starting from the chance of a 50/50 outcome). In our experimental set-up we had two replicates of each color, and thus the bumblebees were able to learn in the first learning bout to avoid or prefer the second artificial flower of the same color. This increases the chance to be >0.5 in the first learning bout.

Bees exposed to GBH before learning performed equally well with control bees in the first three learning bouts. However, some hours later the performance of GBH exposed bees leveled off and their learning did not advance in the last two learning bouts (Tukey HSD post-test; difference = 0.01, p = 0.99, 95 % CI [−0.13, 0.16]), which resulted in significantly lower learning performance of GBH bees compared to control bees (GLMM; estimate = −0.18 ± 0.05, z = −3.71, p ≤ 0.001; see Fig. 1A for the mean values and standard error of means).

In the memory test bees treated with GBH before or after the learning bouts performed at the same level with bees in the first learning bout (Fig. 1) (Tukey HSD post-test: GBH treatment before learning, difference = 0.006, p = 1.00, 95 % CI [−0.15, 0.16]; GBH treatment after learning, difference = −0.04, p = 0.97, 95 % CI [−0.17, 0.10]). This indicates total loss of memory in fine color discrimination due to GBH treatment. GBH exposure both before (GLM; estimate = −0.86 ± 0.17, z = −5.2, p ≤ 0.001, 95 % CI [0.51, 0.61]) and after (GLM; estimate = −0.88 ± 0.18, z = −4.94, p ≤ 0.001, 95 % CI [0.49, 0.61], see Fig. 1B for the mean values and standard error of means) learning diminished bumblebees' performance in the memory test compared to the controls (95 % CI [0.70, 0.79]).

3.2. Two-color discrimination experiment

Acute exposure to the same field-realistic amount of GBH used in the 10-color discrimination task did not affect bees' ability to discriminate (GLMM; estimate = 0.07 ± 0.25, z = 0.27, p = 0.79; treated before learning: 95 % CI [0.81, 0.92], control: 95 % CI [0.80, 0.91], see Fig. 2A for the mean values and standard error of means), or recall (GLMM; estimate ≤ 0.01 ± 0.39, z = 0.01, p = 0.99; treated before learning: 95 % CI [0.80, 0.96], control: 95 % CI [0.81, 0.96], see Fig. 2B for the mean values and standard error of means) two disparately colored flowers (yellow and blue). The bees did not prefer either color over the other (GLMM; estimate = 0.23 ± 0.27, z = 0.86, p = 0.39).

3.3. Ten-odor discrimination experiment

When learning to discriminate 10 different odors, the overall performance of the bees increased over learning bouts (GLMM; estimate = 0.07 ± 0.02, z = 3.47, p ≤ 0.001, see Fig. 3A for the mean values and standard error of means). GBH exposure before the training phase did not affect learning performance (GLMM; estimate = −0.02 ± 0.03, z = −0.51, p = 0.61; see Fig. 3A for the mean values and standard error of means) or memory performance two full days later (GLMM; estimate = 0.10 ± 0.29, z = 0.33, p = 0.74; treated before learning: 95 % CI [0.61, 0.69], control: 95 % CI [0.61, 0.69], see Fig. 3B for the mean values and standard error of means), which suggests that the observed GBH-induced effects were specific to vision.

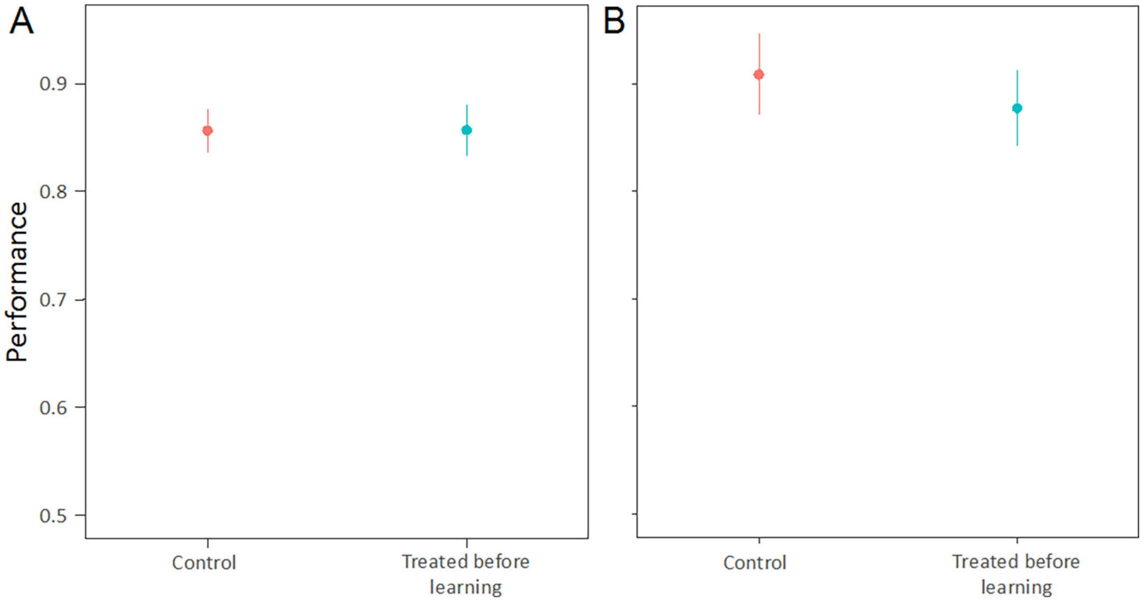


Fig. 2. Performance of bumblebees (*Bombus terrestris*) after acute glyphosate-based herbicide exposure (0.1 μL of Roundup Gold in 60 % sucrose solution) or controls (only 60 % sucrose solution) in the 2-color discrimination experiment (control n = 14; treated before learning n = 16). A) Learning phase, where the bees were allowed to choose between rewarding (40 % sucrose solution) and aversive (saturated quinine solution) flowers of two different colors (blue or yellow). B) Two full days after the learning phase the bumblebees underwent a memory test with the same set-up as in the learning phase except that each flower contained unrewarding water. Vertical lines represent standard error of the mean of the raw data.

Across experiments, during training and tests, we did not notice any appreciable differences in foraging behavior, i.e. flying and landing between treatment groups and controls. Further, treated bees performed just as well in the 2-color and the 10-odor discrimination experiments.

4. Discussion

Our results demonstrate that acute exposure to GBH, equivalent to one to several foraging bouts within a recently sprayed area, significantly impairs bumblebees' fine-color discrimination and long-term memory, which may decrease individual and colony fitness. Pollinators rely heavily on their visual system to be successful in their complex foraging environment, which includes unrewarding flowers that closely resemble, or even mimic, rewarding ones (Dafni, 1984). Moreover, the survival and success of bumblebee colonies are highly dependent on the foraging success of their first brood workers (Goulson, 2003). Other important pollinators may suffer similar (or additional) negative effects. For instance, studies with honeybees showed that glyphosate traces in food delayed molting

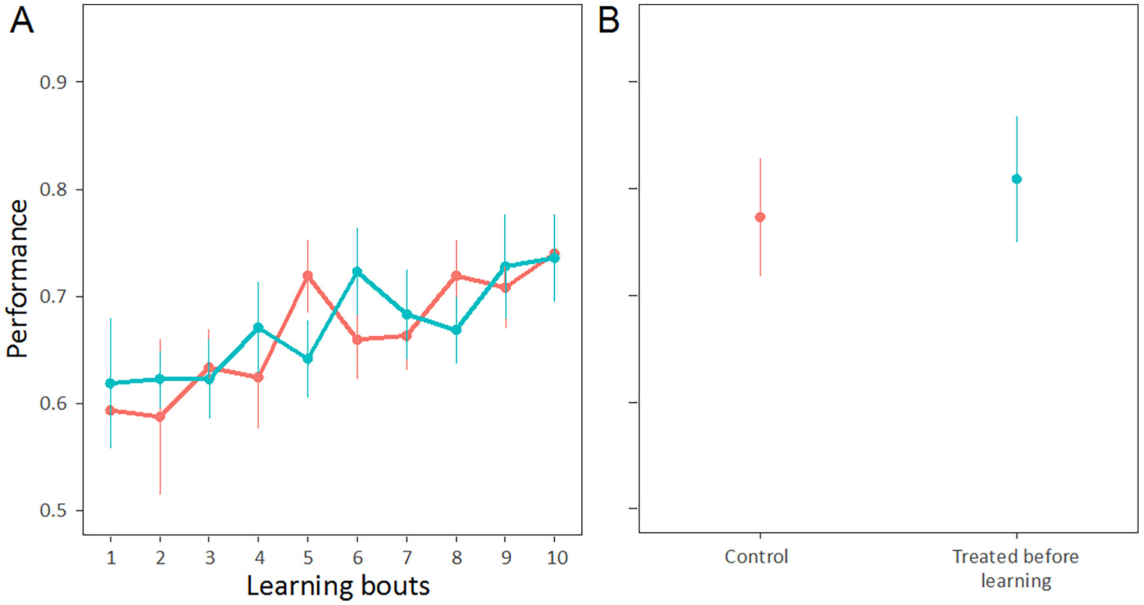


Fig. 3. Performance of bumblebees (*Bombus terrestris*) after acute glyphosate-based herbicide exposure (0.1 μL of Roundup Gold in 60 % sucrose solution) or controls (only 60 % sucrose solution) in the 10-odor discrimination experiment (control n = 19; treated before learning n = 19); A) Learning phase, where the bees were allowed to choose between rewarding (40 % sucrose solution) and aversive (saturated quinine solution) flowers presenting different odors. B) Two full days after the learning phase the bumblebees underwent a memory test with the same set-up as in the learning phase except that each flower contained unrewarding water. Vertical lines represent standard error of the mean of the raw data.

(Vázquez et al., 2018), decreased weight of larvae (Vázquez et al., 2018), increased mortality (Motta et al., 2020) and decreased parasite resistance (Faita et al., 2020). Furthermore, glyphosate was found to affect honey bees' proboscis extension reflexes (Herbert et al., 2014; Hernández et al., 2021) and, in field conditions, to cause honeybees to perform more indirect homing flights (Balbuena et al., 2015), indicating a decrease in the bees' memory capacity.

Our results suggest that, in contrast, GBH exposure does not negatively affect the cognitive ability of bumblebees either at simple 2-color or odor tests. In the wild, even when discriminating between very different colored flowers, bees must distinguish flowers that are much closer in color than dark blue and light yellow. Thus, we suppose that under most environmental settings the 2-color task is much less ecologically relevant than the 10-color task. However, the relative biological importance of bees' contrasting color and fine-color discrimination performance as well as their ability to discriminate between different odors remains to be revealed in future studies.

GBHs have become the most widely used pesticides globally, which has been associated with an increase in the use of genetically engineered glyphosate-tolerant crops, such as soy and canola (Benbrook, 2016). Their use allows GBH application throughout the growing season. This dramatically increased GBH exposure can have diverse consequences for ecosystem functions and services. Moreover, the risks of GBHs for pollinators are not limited to the effects of glyphosate because other ingredients in commercialized products may be substantially toxic (Straw et al., 2021). GBHs contain various co-formulants, in particular surfactants that enhance the uptake and translocation of glyphosate in plants (Li et al., 2005) and possibly other organisms. Some of the co-formulants may be more toxic than glyphosate (Defarge et al., 2018; Helander et al., 2019; Straw et al., 2021).

Our findings stress the urgent need to assess the potential impacts of GBH on bees and their ecosystem services. We have shown that acute exposure to field-realistic amounts of GBH can have both immediate and lasting (for several days) detrimental effects on bumblebee visual cognition. Hence, our results emphasize the imperative need to direct our collective research focus on the substantial, complicated and ecologically relevant risk scenarios rather than lethal doses alone. These risks are not limited to agro-ecosystems because glyphosate residues are near-ubiquitous in wild environments as well (van Bruggen et al., 2018), and a vast majority of plant species are animal pollinated. Thus, sublethal consequences of GBHs should be considered not only in future research but also in public discussion, decision making and development of environmentally friendly pesticides.

CRediT authorship contribution statement

Marjo Helander: Conceptualization, Investigation, Writing – original draft, Project administration, Funding acquisition. **Topi K. Lehtonen:** Conceptualization, Investigation, Writing – review & editing. **Kari Saikkonen:** Conceptualization, Investigation, Writing – review & editing. **Léo Despains:** Investigation, Writing – review & editing. **Danae Nyckees:** Investigation, Writing – review & editing. **Anna Antinoja:** Investigation, Writing – review & editing. **Cwyn Solvi:** Conceptualization, Visualization, Writing – review & editing. **Olli J. Loukola:** Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

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.

Acknowledgments

We are grateful for the support of the Biodiversity Unit, University of Oulu, Finland. We thank Enni Manninen for helping in the lab. This work was supported by the Academy of Finland (grant number 309995 to OJL, grant number 311077 to MH) and the Kone Foundation (grant number 202010852 to OJL).

References

Almasri, H., Liberti, J., Brunet, J.-L., Engel, P., Belzunces, L.P., 2022. Mild chronic exposure to pesticides alters physiological markers of honey bee health without perturbing the core gut microbiota. Sci. Rep. 12, 4281.

Balbuena, M.S., Tison, L., Hahn, M.-L., Greggers, U., Menzel, R., Farina, W.M., 2015. Effects of sublethal doses of glyphosate on honeybee navigation. J. Exp. Biol. 218, 2799–2805.

Bates, D., 2005. Fitting linear mixed models in R. R News 5, 27–30.

Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28, 3.

van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., Morris Jr., J.G., 2018. Environmental and health effects of the herbicide glyphosate. Sci. Tot. Environ. 616–617, 255–268.

Castelli, L., Balbuena, S., Branchiella, B., Zunino, P., Liberti, J., Engel, P., Antúnez, K., 2021. Impact of chronic exposure to sublethal doses of glyphosate on honey bee immunity, gut microbiota and infection by pathogens. Microorganisms 9, 845.

Dafni, A., 1984. Mimicry and deception in pollination. Annu. Rev. Ecol. Syst. 15, 259–278.

Defarge, N., Spiroux de Vendômois, J., Séralini, G.E., 2018. Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides. Toxicol. Rep. 5, 156–163.

Dicks, L.V., Breeze, T.D., Ngo, H.T., Senapathi, D., An, J., Aizen, M.A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L.A., Gemmill-Herren, B., Howlett, B.G., Imperatriz-Fonseca, V.L., Johnson, S.D., Kovács-Hostyánszki, A., Kwon, Y.J., Lattorff, H.M.G., Lungharwo, T., Seymour, C.L., Vanbergen, A.J., Potts, S.G., 2021. A global-scale expert assessment of drivers and risks associated with pollinator decline. Nat. Ecol. Evol. 5, 1453–1461.

Faita, M.R., Cardozoa, M.M., Amandio, D.T.T., Orth, A.I., Nodar, R.O., 2020. Glyphosate-based herbicides and Nosema sp. microsporidia reduce honey bee (Apis mellifera L.) survivability under laboratory conditions. J. Apic. Res. 59, 332–342.

Farina, W.M., Sol Balbuena, M., Herbert, L.T., Goñalons, C.M., Vázquez, D.E., 2019. Effects of the herbicide glyphosate on honey bee sensory and cognitive abilities: individual impairments with implications for the hive. Insects 10, 354.

Goulson, D., 2003. Bumblebees: Their Behaviour and Ecology. Oxford University Press, USA.

Gradish, A.E., Van der Steen, J., Scott-Dupree, C.D., Cabrera, A.R., Cutler, G.C., Goulson, D., Klein, O., Lehmann, D.M., Lückmann, J., O'Neill, B., Raine, N.E., Sharma, B., Thompson, H., 2019. Comparison of pesticide exposure in honey bees (Hymenoptera: Apidae) and bumble bees (Hymenoptera: Apidae): implications for risk assessments. Environ. Entomol. 48, 12–21.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., de Kroon, H., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE 12, e0185809.

Helander, M., Saloniemi, I., Saikkonen, K., 2012. Glyphosate in northern ecosystems. Trends Plant Sci. 17, 569–574.

Helander, M., Pauna, A., Saikkonen, K., Saloniemi, I., 2019. Glyphosate residues in soil affect crop plant germination and growth. Sci. Rep. 9, 19653.

Herbert, L.T., Vázquez, D.E., Arenas, A., Farina, W.M., 2014. Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour. J. Exp. Biol. 217, 3457–3464.

Hernández, J., Riveros, A.J., Amaya- Márquez, M., 2021. Sublethal doses of glyphosate impair olfactory memory retention, but not learning in the honeybee (Apis mellifera). J. Insect Conserv. 25, 683–694.

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 274, 303–313.

Leino, L., Tall, T., Helander, M., Saloniemi, I., Saikkonen, K., Ruuskanen, S., Puigbò, P., 2021. Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase) for assessing sensitivity of organisms to the herbicide. J. Hazard. Mater. 408, 124556.

Li, J., Smeda, R.J., Sellers, B.A., Johnson, W.G., 2005. Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. Weed Sci. 53, 153–159.

Li, L., MaBouDi, H.D., Egertova, M., Elphick, M.R., Chittka, L., Perry, C.J., 2017. A possible structural correlate of learning performance on a colour discrimination task in the brain of the bumblebee. Proc. R. Soc. B 284, 20171323.

Maldonado-Reina, A.J., López-Ruiz, R., Romero-González, R., Martínez Vidal, J.L., Garrido-Frenich, A., 2022. Assessment of co-formulants in marketed plant protection products by LC-Q-Orbitrap-MS: application of a hybrid data treatment strategy combining suspect screening and unknown analysis. J. Agric. Food Chem. 70, 7302–7313.

Motta, E.V.S., Mak, M., De Jong, T.K., Powell, J.E., O'Donell, A., Suhr, K.J., Riddington, I.M., Moran, N.A., 2020. Oral or topical exposure to glyphosate in herbicide formulation impacts the gut microbiota and survival rates of honey bees. Appl. Environ. Microbiol. 86, e01150-20.

Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120, 321–326.

Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J., Sanderson, R.A., 2008. Bumblebee flight distances in relation to the forage landscape. J. Anim. Ecol. 77, 406–415.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollination declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353.

R Core Team, 2021. R: a language and environment for statistical computing. URLR Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Raine, N.E., Ings, T.C., Ramos-Rodriguez, O., Chittka, L., 2006. Intercolony variation in learning performance of a wild British bumblebee population (Hymenoptera: Apidae: Bombus terrestris audax). Entomol. Gener. 28, 241–256.

Raine, N.E., Chittka, L., 2008. The correlation of learning speed and natural foraging success in bumble-bees. Proc. R. Soc. B 275, 803–808.

Reilly, J.R., Artz, D.R., Biddinger, D., Bobiwash, K., Boyle, N.K., Brittain, C., Brokaw, J., Campbell, J.W., Daniels, J., Elle, E., Ellis, J.D., Fleischer, S.J., Gibbs, J., Gillespie, R.L., Gundersen, K.B., Gut, L., Hoffman, G., Joshi, N., Lundin, O., Mason, K., McGrady, C.M., Peterson, S.S., Pitts-Singer, T.L., Rao, S., Rothwell, N., Rowe, L., Ward, K.L., Williams, N.M., Wilson, J.K., Isaacs, R., Winfreet, R., 2020. Crop production in the USA is frequently limited by a lack of pollinators. Proc. R. Soc. B 287, 20200922.

Riveros, A.J., Gronenberg, W., 2009. Olfactory learning and memory in the bumblebee Bombus occidentalis. Naturwissenschaften 96, 851–856.

Straw, E.A., Brown, M.J.F., 2021. Co-formulant in a commercial fungicide product causes lethal and sub-lethal effects in bumble bees. Sci. Rep. 11, 21653.

Straw, E.A., Carpentier, E.N., Brown, M.J.F., 2021. Roundup causes high levels of mortality following contact exposure in bumble bees. J. Appl. Ecol. 58, 1167–1176.

Tan, S., Li, G., Liu, Z., Wang, H., Guo, X., Xu, B., 2022. Effects of glyphosate exposure on honeybees. Environ. Toxicol. Pharmacol. 90, 103792.

Thompson, L.J., Smith, S., Stout, J.C., White, B., Zioga, E., Stanley, D.A., 2022. Bumblebees can be exposed to the herbicide glyphosate when foraging. Environ. Toxic. Chem. https://doi.org/10.1002/etc.5442.

Thompson, H.M., Levine, S.L., Doering, J., Norman, S., Manson, P., Sutton, P., von Mérey, G., 2014. Evaluating exposure and potential effects on honeybee brood (Apis mellifera) development using glyphosate as an example. Integr. Environ. Assess. Manag. 10, 463–470.

Vázquez, D.E., Ilina, N., Pagano, E.A., Zavala, J.A., Farina, W.M., 2018. Glyphosate affects the larval development of honey bees depending on the susceptibility of colonies. PLoS ONE 13, e0205074.

Weidenmüller, A., Meltzer, A., Neupert, S., Schwarz, A., Kleineidam, C., 2022. Glyphosate impairs collective thermoregulation in bumblebees. Science 376, 1122–1126.

Zattara, E.E., Aizen, M.A., 2021. Worldwide occurrence records reflect a global decline in bee species richness. One Earth 4, 114–123.