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Bumblebees (*Bombus terrestris*) forage on plants treated with glyphosate-based herbicides despite potential behavioral consequences

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ABSTRACT

Pesticides are a recognized cause of global insect pollinator decline. Herbicides, which inhibit the growth of weeds, may pose ecotoxicological risks to pollinators. Foraging insect pollinators can encounter herbicides orally when visiting contaminated flowers between the time of application and the plant's death. However, the effects of pesticides on pollinator foraging behavior remain inconsistent. We studied whether buff-tailed bumblebees (*Bombus terrestris*) visited plants exposed to glyphosate-based herbicides (GBH) when non-contaminated plants were available. Additionally, we examined whether oral exposure to GBH and the presence of an invasive plant (*Lupinus polyphyllus*) influenced bumblebee foraging behavior. Our findings revealed that bumblebees visited recently GBH-treated plants in both field and flight cage experiments. Furthermore, bumblebees did not discriminate between GBH-treated and uncontaminated plants when the choice was based solely on the plant's emitted volatile compounds, which changed slightly after exposure. Oral GBH treatment reduced the foraging activity of bumblebees; a higher proportion of exposed bumblebees compared to control bees did not visit any plants. Nevertheless, in the presence of the plant invader, control bees visited fewer plants than GBH-exposed bees. Our results indicate that bumblebees can be exposed to GBH by foraging on recently treated plants, which may have consequences for their foraging behavior, necessitating careful consideration when using GBH products.

1. Introduction

Insect pollination is a crucial ecosystem service for agriculture and terrestrial biodiversity, with 35 % of crop plants and 78 %–94 % of flowering plants relying on insect pollinators (Klein et al., 2007; Ollerton et al., 2012). Pollinators use floral signals to identify the most rewarding plants for nectar and pollen (Goulson, 2010). They differentiate between flowers based on visible morphological characteristics such as floral display (Russo et al., 2020) and color (Gumbert, 2000; Kunze and Gumbert, 2001; Peitsch et al., 1992). Additionally, olfactory stimuli attract pollinators (Kunze and Gumbert, 2001). Some pollinators, particularly bees, possess olfactory systems specialized in processing complex odors and detecting subtle differences in floral scents (Laska et al., 1999). Flowers emit hundreds of volatile organic compounds (VOCs), creating unique scents that help pollinators find, recognize, and distinguish between flowers (Kunze and Gumbert, 2001). Certain pesticides, such as glyphosate, can impact VOC emission (D'Alessandro et al., 2006), inhibiting the synthesis of aromatic amino acids essential for protein and natural product biosynthesis (Fuchs et al., 2021, 2024; Maeda and Dudareva, 2012). Furthermore, invasive plant species, typically having large floral displays, can alter pollination dynamics by competing for pollinators with native species (Jakobsson and Padrón, 2014; Ramula and Pihlaja, 2012; Thijs et al., 2012; Valtonen et al., 2006), reducing pollinator visits to native plants and causing hetero-specific pollen transfer (Brown et al., 2002; Thijs et al., 2012).

Globally, insect pollinator populations have been declining (Goulson et al., 2008; Potts et al., 2010), with several potential drivers identified, including the increasing use of pesticides (Potts et al., 2010, 2016). Pesticides are agrochemicals designed to eliminate pests that harm crops, livestock, and the environment. Among them, herbicides are the most widely used, as they control weeds in agriculture, horticulture, silviculture, recreational areas, and home gardens.

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Commercial herbicides consist of active ingredients, co-formulants, inerts, adjuvants, and other components. Glyphosate is the most commonly used active ingredient in herbicides, with over 750 glyphosate-based herbicide (GBH) commercial products available (Guyton et al., 2015). This non-selective herbicide targets and inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimate pathway found in all green plants and many fungi and bacteria (Duke and Powles, 2008; Leino et al., 2021). Without EPSP, plants cannot produce chorismate, which is necessary for synthesizing the aromatic amino acids tryptophan, phenylalanine, and tyrosine (Fuchs et al., 2021; Helander et al., 2012). The absence of these amino acids ultimately leads to plant death (Duke and Powles, 2008). Glyphosate has been considered safe for non-target organisms due to the absence of a shikimate pathway in animals. However, recent studies have indicated its adverse effects on soil, rhizosphere, and endophytic microbes (Caggìa et al., 2023; Fuchs et al., 2023; Helander et al., 2018; Newman et al., 2016), water ecosystems (Brovini et al., 2021; Carles et al., 2019; Milan et al., 2018), and both above- and below-ground fauna (Berger et al., 2013; Carpenter et al., 2016; Evans et al., 2010; Gaupp-Berghausen et al., 2015; Jarrell et al., 2020; Maderthaner et al., 2020; Stellin et al., 2018; Takahashi, 2007; Zaller et al., 2014). Additionally, several studies have reported harmful effects of glyphosate and/or GBHs on pollinators, ranging from reduced learning performance (Helander et al., 2023a; Herbert et al., 2014; Kaakinen et al., 2024) to delayed larval development (Odemer et al., 2020; Vázquez et al., 2020; Weidenmüller et al., 2022) and changes in gut microbiota (Helander et al., 2023b; Motta and Moran, 2023; Motta et al., 2018, 2020).

Pollinators may encounter glyphosate when consuming nectar or pollen from GBH-exposed flowers, touching contaminated surfaces, or being directly sprayed during GBH application (Böhme et al., 2018; Gradish et al., 2019). Bumblebee foragers may be exposed to significant glyphosate residues while foraging on recently treated flowers (Thompson et al., 2014). However, the exposure window is brief, as bumblebees must visit sprayed flowers after GBH application but before the plant's death. Following GBH exposure, plants quickly lose their green coloration, and their tissues begin to die. Previous studies have shown that the application of certain herbicides can reduce flower visitation. For instance, spray drift from the herbicide dicamba (Bohnenblust et al., 2016) and sublethal levels of glyphosate (Russo et al., 2020) resulted in decreased pollinator visits to treated plants. Conversely, other research has suggested that bumblebees are poor at detecting nectar toxins in flowers (Tiedeken et al., 2014) and may even prefer flowers (Kessler et al., 2015) and sucrose feeders (Arce et al., 2018) containing commonly used neonicotinoid insecticides. Thompson et al. (2022) reported that bumblebees visited plants treated with pure glyphosate, and floral resources were still available several days post-treatment.

In this study, we examined whether bumblebees are exposed to GBH while foraging and how this oral exposure affects their foraging behavior. Specifically, we addressed the following two questions: 1. Do individual bumblebees discriminate between GBH-treated and untreated plants? 2. How does oral exposure to GBH and the presence of a common invasive alien plant with showy inflorescences influence bumblebee foraging behavior? We predicted that bumblebees would visit GBH-treated plants less frequently than untreated controls, as GBH exposure may lead to rapid degradation of floral traits and alterations in VOC emissions. To further explore the potential mechanisms underlying these behavioral changes, we analyzed VOC emissions from untreated and GBH-treated plants, hypothesizing that glyphosate exposure would alter VOC composition. Additionally, we predicted that, in the presence of an invasive plant, bumblebees would prefer it over a crop plant due to its known attractiveness to bumblebees (Ramula and Sorvari, 2017), and that bumblebees exposed orally to GBH would visit fewer flowers and plants compared to untreated control bumblebees.

2. Materials and methods

Our study comprised several sub-studies conducted during the summers of 2022–2023, including a field study, observations of the longevity of oilseed rapes (*Brassica napus* ssp. *oleifera*) after GBH exposure, two flight cage experiments, two Y-maze experiments, and volatile analysis of GBH-exposed plants. The study can be divided into two sections reflecting our research questions: the first investigates whether bumblebees can be exposed to glyphosate while foraging, and the second examines how oral exposure affects them. Since we used the same materials and followed partially similar procedures, especially during the first two days of both flight cage experiments, shared methods and materials are described here collectively before detailing the specific methodologies.

We used a total of 13 colonies of buff-tailed bumblebees (*Bombus terrestris* obtained from Koppert B.V. The colonies were transferred from their commercial boxes to wooden nest boxes (14.5 cm × 15 cm × 9 cm) with entrance halls of the same size. The entrance halls were connected to larger flight arenas (60 cm × 45 cm × 25 cm) via tunnels (32 cm × 5 cm × 5 cm). Bees were allowed to enter the flight arena with a gravity feeder containing 40 % sucrose water for two days, allowing bumblebee foragers to gain foraging experience and become familiar with the tunnel and exit while remaining naïve. Ten to 14 h prior to each experiment, we removed the gravity feeder from the flight arena, returned all bumblebees from the arena to the nest boxes, and closed the tunnel between the nest boxes and the arena.

We chose oilseed rape as a test plant due to its attractiveness to pollinators (Ion et al., 2012; Rašić et al., 2018). It is one of the most productive oilseed crops globally and is widely cultivated in northern climates (Vuorinen et al., 2014). It is known to produce both nectar and pollen (Ion et al., 2012) and benefits, at least partially, from insect pollination (Ouvrard and Jacquemart, 2019). The oilseed rapes used in our study were grown in a greenhouse from seed to the flowering stage and were isolated from any pollinators before the experiment.

2.1. *Can bumblebees be exposed to glyphosate while foraging*

2.1.1. *Exposed vs. control flowers: field study*

To observe whether pollinators visit recently GBH-treated plants, we set up an experimental field study with natural flowering plants, bumblebees, and other free-flying natural pollinators. We established 16 observation squares (1 m^2 each located at least 2 m apart) in a flowering field of about 1 ha, spraying half of them (randomly selected) with GBH and the other half with water. The GBH solution was prepared by mixing 0.6 L of Roundup Flex (commercial product Roundup Flex, Bayer Agriculture BVBA, Belgium, registration number 3072; glyphosate concentration 480 g/L as glyphosate potassium salt CAS: 6229-014-3) with 10 L of plain water. This 6 % GBH solution corresponds to the maximum concentration recommended for weed control as specified on the product label. We treated the plants using Bürkle handheld pressure sprayers, applying approximately 10.6 mL of solution per square over a 2-s spray duration. Separate sprayers were utilized for the GBH solution and plain water to prevent cross-contamination. The volume of GBH solution applied corresponds to the standard glyphosate treatment dosage used for weed management in Finland, as indicated on the product label. All plants across the sub-studies were treated following this protocol.

The following flowering plant species were present in the squares: *Achillea millefolium*, *Trifolium hybridum*, *Cirsium arvense*, and *Tripleurospermum inodorum*. One hour after spraying, we began pollinator observations, recording the numbers of bumblebees and other insect pollinators visiting the flowers within the squares. Each square was observed six times over the following three days, once in the morning and once in the afternoon per day, with each observation period lasting 10 min. A landing on any flower in the same or a different plant within a square was recorded as a visitation, except for *A. millefolium* (due to its small flowers and compact inflorescence), where a landing on an inflorescence, instead of a single flower, was counted as a visitation. Before and between the observation periods, we did not attempt to prevent any pollinators from visiting flowers in the observation squares.

2.1.2. *Exposed vs. control flowers: longevity of oilseed rape*

To determine the longevity of GBH-treated flowering plants and the exposure window for pollinators, we sprayed five flowering oilseed rapes with Roundup Flex GBH solution and five with water. We then observed the plants daily for seven days, recording damage as the percentage of yellow area and the number of remaining open flowers.

2.1.3. *Exposed vs. control flowers: flight cage experiment*

We conducted a choice test in an outdoor flight cage (110 cm × 195 cm × 190 cm), which allowed us to use real flowering plants in a semi-natural environment and observe the foraging behavior of individual bumblebees (n = 28 bees from three colonies). The tests were conducted under suitable weather conditions, avoiding rain and strong winds, with temperatures ranging from 14 °C to 30 °C. The choice tests were performed between 8:00 and 14:00, preferably on consecutive days when weather conditions permitted. The outdoor flight cage was positioned under trees to shield the colony from direct sunlight.

On the third day of the experiment, nest boxes containing bumblebee colonies were connected to the cage via the tunnel, allowing one bumblebee at a time to enter the cage to forage (Fig. 1A). The flight cage contained four oilseed rape plants, two of which were sprayed with Roundup Flex GBH solution (GBH treatment) and two with water (control treatment). Plants were sprayed outside 16–24 h (<24 h treatment) or 40–48 h (>40 h treatment) before the experiment. After spraying, we brought the plants inside to prevent pollinators from visiting them before the experiment. We kept the plants separate to avoid contact between GBH-treated and water-sprayed plants. Before the experiment, we counted the number of open flowers on each plant.

In the choice test, we arranged the plants in a square formation inside the cage with the same treatments crosswise. We then introduced a naïve bumblebee (starved for 10–14 h before the experiment) into the experimental cage and observed it for 10 min from its first foraging interaction, recording the number of plant and flower visits. A plant visit (**No. of plants visited**) was defined as the bumblebee landing on a specific plant and visiting one or multiple flowers; the visit ended when the bumblebee clearly departed from the plant. A flower visit (**No. of flowers visited**) was defined as the bumblebee landing on a single flower and actively gathering either pollen or nectar. Additionally, we documented the temperature and time of day for each observation. After each bout, we removed the bumblebee from the flight cage and euthanized it to prevent any interaction with its colony. Bumblebees that did not interact with any plant within 10 min of entering the cage were excluded from the experiment. Furthermore, individual plants visited by bumblebees were removed from the cage and disposed of. If bees did not visit the plants, we did not remove the plants from the cage, but changed their order after every bout.

The flight cage experiment was conducted over two days for each colony, ideally planned to be consecutive. On the first day, bees foraged on oilseed rape exposed to glyphosate about 24 h prior to the experiment, and on the second day, they foraged on oilseed rape exposed about 48 h earlier. However, we had to incorporate off-days due to unpredictable weather conditions, resulting in non-consecutive experimental days. On off-days, bees were kept inside and fed with sucrose solution and pollen to prevent starvation.

2.1.4. *Exposed vs. control flowers: Y-maze experiment*

We conducted a Y-maze olfactometer test in a greenhouse where bumblebees (n = 40 bees from two colonies) could choose between GBH-treated and untreated control plants based solely on the emitted volatile compounds. The Y-maze is a transparent, hollow structure with a central stem branching into two arms, commonly used to evaluate insect responses to different scents (Simonnet et al., 2014). Prior to testing, we enclosed two oilseed rape plants, one treated with GBH and the other with water (control), in plastic oven bags (polyethylene terephthalate; 30 cm × 55 cm; Pirkka® Paistopussi Kesko Oyj). As in the

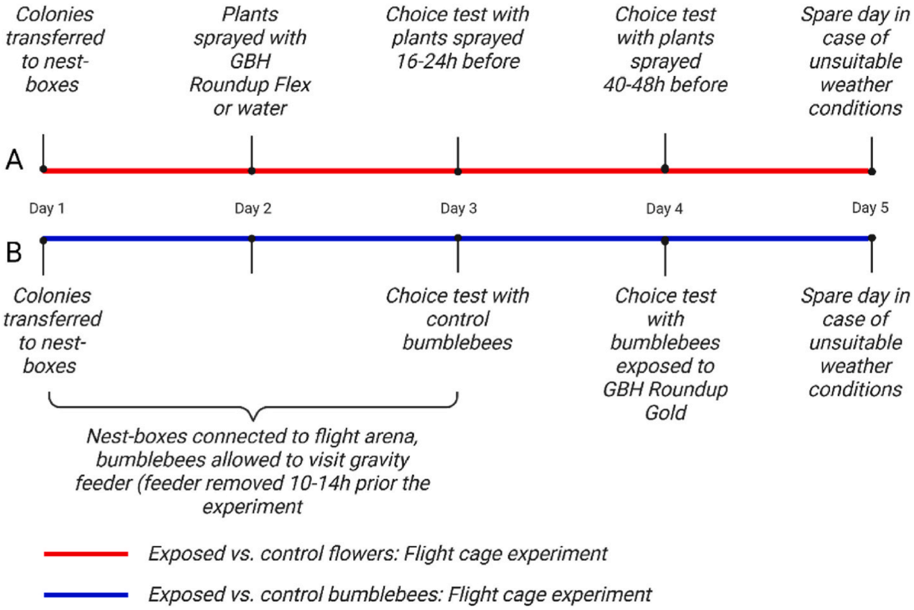

Fig. 1. Timeline of both flight cage experiments. The experiments began with naïve bumblebees (*Bombus terrestris*) feeding on a gravity feeder containing 40 % sugar water during the first two days. To motivate bees to visit flowers during the choice tests, they were deprived of sugar water 10–14 h prior to testing. (A) Exposed vs. control flowers: On the third day, we observed whether bumblebees preferred plants sprayed with water or GBH (Roundup Flex) 16–24 h earlier. On the following day, the experiment was repeated with new bees from the same colonies, but the plants had been sprayed either with water or GBH 40–48 h earlier. (B) Exposed vs. control bumblebees: On the third day, we recorded the preferences of control bumblebees between lupine (an invasive plant) and oilseed rape. On the following day, the experiment was repeated with GBH-treated bumblebees. Experimental days were planned to occur consecutively; however, in cases of unsuitable weather, choice tests were conducted on spare days while ensuring that the plants were sprayed as originally scheduled.

cage experiment, the plants were sprayed 16–24 h or 40–48 h prior to the experiment. Flexible hoses connected a compression pump to each oven bag, creating an airflow of 225 mL/min, with additional hoses directing this airflow from the bags to the corresponding arms of the Y-maze. We introduced individual bumblebees one at a time into the main stem of the Y-maze and allowed them to move freely to choose between the two arms. Each bee was given 2 min to explore and make a decision i.e. to choose one arm. After completing the test, the bumblebee was removed from the Y-maze and euthanized. To prevent scent contamination from bees, we cleaned the Y-maze with alcohol after each test, and changed the orientation of the arms to ensure that the water- and GBH-sprayed oilseed rapes were positioned on opposite sides.

2.1.5. *Exposed vs. control flowers: volatile organic compound analysis*

VOCs were collected through dynamic headspace sampling from seven oilseed rape plants at 6 h and 24 h after GBH treatment, as well as from nine corresponding control plants. The treated flowers were placed in an oven bag (polyethylene terephthalate; 30 cm × 55 cm; Pirkka® Paistopussi Kesko Oyj), which was connected to a flow of filtered air via a Teflon tube attached to an air pump (225 mL/min) and a suction pump (200 mL/min) attached to a Tenax TA adsorbent filter. Volatile collection was conducted in pairs, with one randomly selected plant from each treatment. The VOC samples were analyzed using gas chromatography–mass spectrometry (GC–MS), and ChemStation software was used for peak identification and quantification. We identified 31 volatile compounds through mass comparison with external standards or by comparing fragmentation patterns to published fragmentations in the NIST compound library (Version 2.11, Supplementary Fig. 1). Most detected compounds fell into one of the following three classes: green leaf volatiles, monoterpenes, or sesquiterpenes.

2.2. *Effect of GBH exposure on bumblebees foraging behavior*

2.2.1. *GBH-exposed vs. control bumblebees: flight cage experiment*

We conducted a flight cage experiment to investigate the effect of oral GBH exposure on the foraging behavior of bumblebees in the presence and absence of an invasive plant. For the first two days, the procedure mirrored that of the initial cage experiment with GBH-exposed and control flowers (Fig. 1B). However, on the second day, we selected the most active bumblebees—those that entered the arena—and marked them with tiny numbered tags (Bienen-Voigt & Warnholz, Ellerau, Germany). We affixed the numbered tags to the thorax of approximately 30 bumblebees per colony using super glue, i.e., we gently pressed the tag to the thorax of each bumblebee while holding it in place. At the beginning of the third day, we connected the nest box and entrance halls with marked and unmarked bees to the large outdoor flight cage. We used two test plants: the invasive garden lupine (*Lupinus polyphyllus*, Fabaceae) and the crop plant oilseed rape. The garden lupine was chosen for its attractiveness to bumblebees (Ramula and Sorvari, 2017), despite not producing nectar (Haynes and Mesler, 1984). Lupine can grow up to 1.5 m high and features large blue, pink, or white inflorescences. Bumblebees have an innate preference for the blue range of the spectrum (Chittka et al., 2004; Müller, 1881; Willmer, 2011), so we used only lupines with a blue inflorescence. The two test plants were arranged in the flight cage in different combinations (lupine-rapeseed and rapeseed-rapeseed), and an individually marked (untreated) forager bumblebee was allowed to enter the flight cage through a tube to forage (n = 116 bees from five colonies). Each bumblebee was observed for 10 min, during which all landings and the number of plant and flower visits were recorded as described above. After the observation, the bumblebee was captured and euthanized to prevent it from interacting with its colony. All test plants visited by the bumblebees were also removed and disposed of.

After the third day, the remaining marked bumblebees from each colony were exposed to 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, 11 mL Roundup Gold/L sugar water). Each bumblebee received 7 μL of a 5 g/L Roundup/sugar water solution (equivalent to 35 μg of glyphosate and being a field realistic dose). On the fourth day, the colony was reconnected to the outdoor flight cage, and the same procedure from the previous day was repeated with the exposed bumblebees.

2.2.2. *GBH-exposed vs. control bumblebees: Y-maze experiment*

To investigate bumblebees' foraging behavior based solely on olfactory cues, we repeated the Y-maze olfactometer test for both GBH-exposed and unexposed bumblebees. The olfactory test was similar to the previous one (1.3. Exposed vs. Control Flowers: Y-Maze Experiment); however, this time, we enclosed lupine in one plastic oven bag and oilseed rape in another. Following repetitions with unexposed bees (n = 30 bees from three colonies), the colonies were brought into the laboratory and exposed to GBH (Roundup Gold) by feeding each bee a droplet of sugar water containing 35 μg of glyphosate. The treated bees were marked with a white dot on their thorax for identification, and the experiment was repeated with them (n = 29 bees from three colonies).

2.3. *Statistical analysis*

All statistical analyses were conducted using R version 4.3.2 (R Core Team, 2023).

In the field study (2.1.1), we used linear mixed-effects models (LMMs) (lme4lmer; Bates et al., 2015) to examine whether GBH treatment of squares with flowering plants, time from treatment (in days), their interactions, and/or temperature affected flower visitation. The number of flower visits was square root-transformed to improve the fit of the model residuals. Flowering squares were treated as a random factor to control for repeated observations from them. The significance of the fixed explanatory variables was assessed using F-tests with the type III Kenward-Roger method for adjusting the denominator degrees of freedom (lmerTestanova; Kuznetsova et al., 2017), and model assumptions were visually verified through residual plots. This analysis was conducted separately for all insect pollinators and for the observed bumblebees.

In the flight cage experiment based on GBH-exposed flowers (2.1.3), we first examined bumblebees' foraging behavior on oilseed rape using a generalized linear mixed model (GLMM) with a binomial distribution and a logit-link function (glmmTMBglmmTMB; Magnusson et al., 2017). In particular, we explored whether the treatment (GBH-exposed vs. unexposed plants), time from plant treatment (<24 h or >40 h), their interaction, temperature, and the number of open flowers per plant influenced plant preference (whether a bumblebee visited a plant). Plant tetrad and bumblebee colony were included as random factors. The significance of the fixed explanatory variables was determined using type II Wald's $\chi 2$ test (carAnova; Fox and Weisberg, 2019), and the binomial model was validated by visually examining residual plots and checking model dispersion, which was close to 1 (0.688) (DHARMatestDispersion; Hartig, 2018). For a subset of plants that received a pollinator visit, we then examined whether the treatment, time from treatment, their interaction, temperature, and the number of flowers influenced the number of plant and flower visits using LMMs, as described above. The number of flower visits was log-transformed to improve the fit of the model residuals with plant tetrad and colony as a random factor.

To examine the effect of GBH treatment 6 h and 24 h following application on the composition of floral VOC emissions (2.1.5), we performed a nonmetric multidimensional scaling (NMDS) analysis of all 31 compounds (veganmetaMDS; Oksanen et al., 2025). We used a Bray–Curtis dissimilarity matrix (veganvegdist) with Wisconsin double standardization and two dimensions (stress = 0.167) for ordination. We tested whether the time after GBH application (6 h and 24 h) affected VOC emissions using permutational analysis of variance (PERMANOVA;

9999 permutations; veganadonis2). Significant PERMANOVA results were obtained, and then a post hoc test (multcomp glht; Hothorn et al., 2002) was conducted for each volatile compound separately testing for differences in concentrations between the treatments.

In the flight cage experiment with GBH-exposed bumblebees (2.2.1), we examined whether oral exposure and the presence of an invasive plant (lupine) affected bumblebee foraging probability (using a GLMM with a binomial distribution and a logit-link function) and the number of plant and flower visits (using LMMs). Oral exposure, presence of an invasive plant, their interaction, the number of open flowers, and temperature were treated as fixed explanatory variables, with colony included as a random factor. The number of flower visits was log-transformed to improve the fit of the model residuals. Model assumptions and the significance of the fixed explanatory variables were examined as previously described, with the dispersion factor in the GLMM being close to 1 (0.784).

Differences in bumblebees' choices in both Y-maze experiments were analyzed using a Chi-square test. In the first experiment with GBH-exposed and unexposed control oilseed rapes (2.1.4), we tested whether treatment and time from exposure (<24 h or >40 h) influenced bumblebees' first-choice decisions between the control and exposed oilseed rapes. In the second experiment, where half of the bumblebees were orally exposed to GBH (2.2.2), we examined whether the exposure affected their preference for lupine or oilseed rape.

3. Results

3.1. Exposed vs. control flowers: field study

Neither GBH treatment, time from treatment, nor their interaction affected the visitation rates of all observed pollinator insects in the field study (Table 1; Supplementary Table S1.). Temperature during observations had a significant effect on visitation rate (Table 1), with higher temperatures being associated with increased pollinator visits (intercept = −0.37, slope = 0.23). When analyzing only bumblebee visits, neither GBH treatment, time from treatment, their interaction, nor temperature had a significant effect on visitation rates (Table 1; Supplementary Table S1.).

3.2. Exposed vs. control flowers: longevity of oilseed rape

In the longevity observation, oilseed rapes remained visually alive at least three days after the application of GBH. After this period, the green parts of the GBH-treated plants started to turn yellow (particularly after 5 days), but they lost their flowers about the same rate as the control plants (Fig. 2).

Table 1

Summary of test statistics from the field study with glyphosate-based herbicide (GBH)-treated and control flowers based on linear mixed-effects models (LMMs), using flowering squares as random factors. df and ddf denote the degrees of freedom in the numerator and denominator in LMMs, respectively.

	No. of flowers visited (all observed pollinators)		No. of flowers visited (bumblebees)	
	$F_{\mathrm{df,ddf}}$	p	$F_{\mathrm{df,ddf}}$	p
Treatment (GBH-exposed, control observation squares)	$0.19_{1,90}$	0.66	$0.86_{1,82}$	0.36
Time from treatment	$0.44_{1,77}$	0.51	$2.49_{1,77}$	0.12
Treatment × Time from treatment	$0.02_{1,77}$	0.88	$0.01_{1,77}$	0.93
Temperature	$4.55_{1,77}$	<0.05	$0.04_{1,77}$	0.84

3.3. Exposed vs. control flowers: flight cage experiment

Neither treatment (GBH-exposed vs. control flowers), time from plant treatment (<24 h or > 40 h), nor their interaction affected bumblebees' plant preference in the flight cage experiment (Table 2, Fig. 3A). However, the number of open flowers significantly affected bumblebee preference (Table 2), with bumblebees favoring oilseed rape plants that had more open flowers (intercept = −6.93, slope = 0.12). Treatment, time from treatment, or their interaction did not affect the number of flowers visited (Table 2, Fig. 3B) or the number of plants visited (Table 2). Only the number of open flowers positively influenced flower visitations (Table 2; intercept = −1.16, slope = 0.039).

3.4. Exposed vs. control flowers: Y-maze experiment

In the volatile choice test with exposed flowers in the Y-maze, neither the plant treatment (GBH, control) nor the time from treatment (<24 h, >40 h) affected bumblebees' choices ($\chi^2 = 0$, df = 1, $p = 1$ in both cases). Fifty-two percent of the bumblebees chose the control oilseed rape first, while 48 % chose the GBH-treated oilseed rape first.

3.5. Exposed vs. control flowers: volatile measurements

NMDS analyses based on 31 volatile compounds revealed a significant shift in the emitted volatile composition between control plants and GBH-treated plants (df = 2, $F = 3.89$, $R^2 = 0.28$, $p = 0.024$), most notably 24 h after GBH treatment (as indicated by only partially overlapping ellipse with that of the control treatment in Fig. 4). At the individual compound level, the concentrations of three compounds, alpha-farnesene, beta-ocimene, and myrcene, were significantly lower 24 h after GBH treatment compared to control and 6 h after GBH treatment (Supplementary Fig. S1).

3.6. GBH-exposed vs. control bumblebees: flight cage experiment

Oral exposure to GBH had a significant negative effect on bumblebees' foraging behavior, with control bumblebees having about double the probability of foraging on a plant compared to GBH-exposed bumblebees (Table 3, Fig. 5A). The presence of an invasive plant (lupine) or the interaction between oral exposure and the presence of an invasive plant did not affect foraging probability (Table 3). The interaction between oral exposure and the presence of an invasive plant did not significantly affect the number of flowers visited, but it did affect the number of plants visited (Table 3). In the presence of an invasive plant, control bumblebees tended to visit fewer plants than GBH-exposed bumblebees, while there was no difference in the number of plant visits when only oilseed rapes were available (Fig. 5B).

3.7. GBH-exposed vs. control bumblebees: Y-maze experiment

In the volatile choice test involving lupine and oilseed rape in the Y-maze, the treatment of the bumblebees (GBH, control) had no effect on bees' plant choices (lupine or oilseed rape; $\chi^2 = 0.89$, df = 1, $p = 0.35$).

4. Discussion

We found that bumblebees did not avoid GBH-treated crop plants that still offered floral resources, even when untreated plants were available, leading to exposure to glyphosate during foraging. This pattern was consistent across all experiments: in the field experiment, pollinators selected between treated and untreated areas of flowering plants; in the flight cage experiment, bumblebees chose between GBH-treated and untreated oilseed rape plants; and in the Y-maze experiment, their choices relied solely on the volatiles emitted by treated or untreated oilseed rape plants. In our volatile measurements, we observed a shift in the VOC composition between the control and GBH-

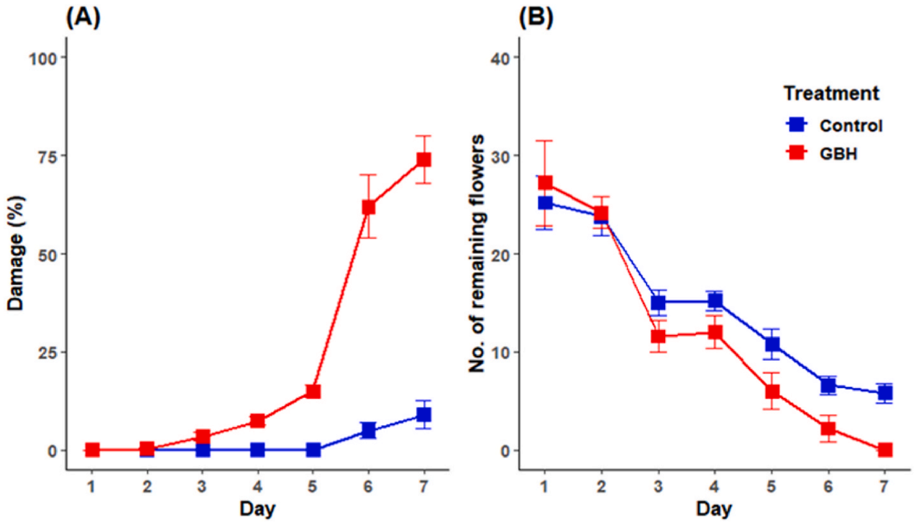

Fig. 2. Summary of the longevity observation for five oilseed rapes sprayed with water (control) and glyphosate-based herbicide (GBH). Damage (%) represents the mean percentage of yellow parts, and No. of remaining flowers indicates the mean number of open flowers on oilseed rapes (mean ± SE). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Summary of test statistics from the flight cage experiment with glyphosate-based herbicide (GBH)-treated and control flowers based on linearized (GLMM) and linear (LMM) mixed models, using plant tetrad and bee colony as random factors. df and ddf denote the degrees of freedom in the numerator and denominator in LMMs, respectively (for GLMMs, ddf is not applicable).

	Plant preference			No. of plants visited		No. of flowers visited	
	χ^2	df	*p*	$F_{df,ddf}$	*p*	$F_{df,ddf}$	*p*
Treatment (GBH-exposed, control flowers)	0.15	1	0.698	$1.20_{1,\ 46}$	0.278	$0.46_{1,\ 39}$	0.501
Time from treatment (<24 h, >40 h)	0.69	1	0.405	$0.00_{1,\ 23}$	0.995	$0.17_{1,\ 26}$	0.687
Treatment × Time from treatment	1.42	1	0.233	$1.47_{1,\ 45}$	0.232	$1.98_{1,\ 39}$	0.168
Temperature	1.58	1	0.208	$0.19_{1,\ 1}$	0.760	$0.96_{1,\ 2}$	0.461
No. of open flowers	11.04	1	<0.001	$0.00_{1,\ 41}$	0.991	$14.47_{1,\ 45}$	<0.001

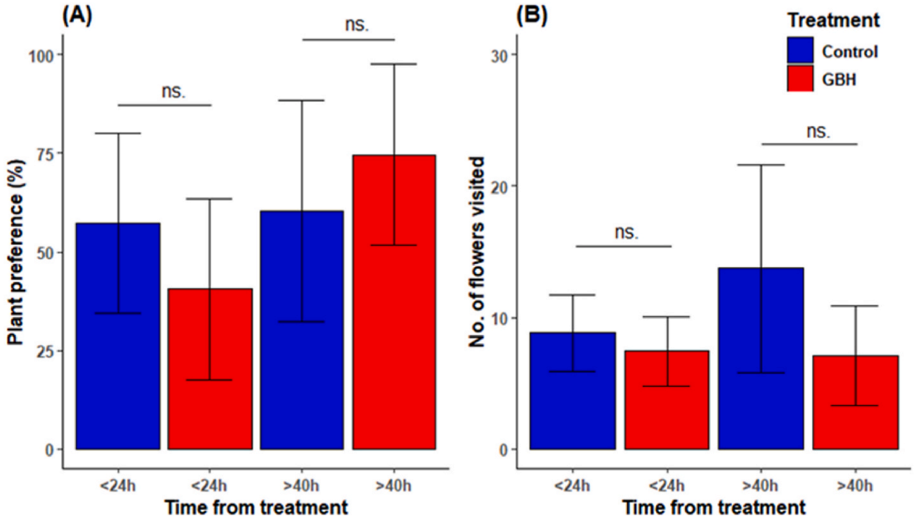

Fig. 3. Back-transformed least square mean (±SE) for the effects of glyphosate-based herbicide (GBH) on bumblebee foraging behavior in two time points: (A) probability of bumblebees landing on control oilseed rape plants and those exposed to GBH, (B) number of oilseed rape flowers visited by bumblebees.

treated plants, driven by a few specific compounds. In the second part of the study, where bumblebees could choose between invasive lupine and oilseed rape, the presence of lupine on its own did not significantly influence their foraging behavior. However, oral exposure to GBHs prior to the experiment reduced bumblebees' activity, which was seen as a reduced visitation rate on plants. Only 20 % of GBH-exposed bumblebees visited at least one plant, compared to 41 % of control bees that made at least one landing on plants. We also found a significant interaction between oral exposure to GBH and the presence of an invasive plant, with control bumblebees tending to visit fewer plants when lupine was present. Since oral exposure was administered a day before the choice test and the bees were kept without sugar water for the

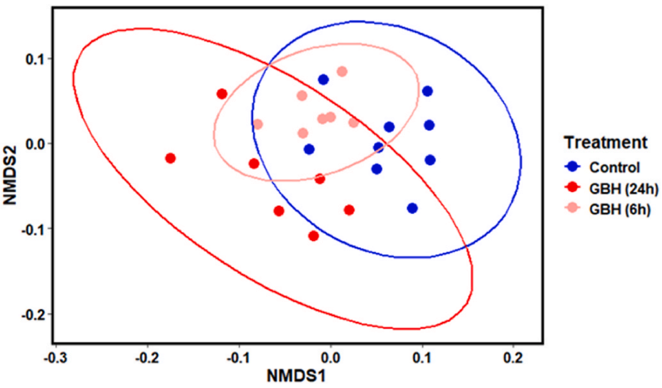

Fig. 4. Non-metric multidimensional scaling ordination based on 31 floral VOC emission rates (ng g−1 min−1) collected from the flowerheads of individual oilseed rapes (n = 16). GBH (6 h) and GBH (24 h) refer to volatiles collected 6 h and 24 h after glyphosate-based herbicide (GBH) application. Ellipses with 95 % confidence intervals are drawn by treatment.

Table 3
Summary of test statistics from the flight cage experiment comparing GBH-exposed and control bumblebees, based on linearized (GLMM) and linear (LMM) mixed models, with bee colony as a random factor. df and ddf denote the degrees of freedom in the numerator and denominator in LMMs, respectively (for GLMMs, ddf is not applicable).

	Foraging probability			No. of plants visited		No. of flowers visited	
	χ^2	df	*p*	$F_{\mathrm{df,ddf}}$	*p*	$F_{\mathrm{df,ddf}}$	*p*
Presence of an invasive plant (lupine)	0.12	1	0.733	$0.06_{1,27}$	0.813	$1.51_{1,27}$	0.230
Oral exposure (GBH-exposed, control bees)	5.56	1	0.0184	$0.67_{1,28}$	0.420	$0.42_{1,28}$	0.521
Presence of an invasive plant × oral exposure	0.63	1	0.436	$7.78_{1,29}$	0.009	$3.31_{1,30}$	0.079
Temperature	1.91	1	0.167	$0.51_{1,4.51}$	0.511	$0.03_{1,4.40}$	0.870
No. of open flowers	0.01	1	0.920	$0.72_{1,27.52}$	0.404	$0.02_{1,27.54}$	0.898

same duration as the control group, their reduced foraging activity was not due to satiety.

Our results support those of Thompson et al. (2022), who found that bumblebees foraged on glyphosate-exposed *Phacelia tanacetifolia* flowers even when unexposed flowers were available. A key difference between the two studies is that we used a field-recommended dose of the commercial GBH Roundup rather than pure glyphosate. Since GBHs include co-formulants and other added ingredients, we considered it essential to investigate their effects using the commercial formulation commonly applied in agriculture. The composition of co-formulants is often classified as confidential business information (Weinhold, 2010). Some co-formulants in GBHs are suspected to be more toxic to non-target organisms than glyphosate itself. For example, certain surfactants, which help glyphosate in penetrating plant cuticles, have been shown to be harmful. Especially when applied topically, they can obstruct the tracheal systems of insect pollinators, posing significant risks (Straw et al., 2021). However, our findings suggest that the co-formulants in GBHs do not influence bumblebees' foraging decisions, a possibility previously considered by Thompson et al. (2022).

Our results were also consistent with those of Thompson et al. (2022), who reported that *P. tanacetifolia* exhibited only minimal changes in physical floral characteristics within the first three days following glyphosate treatment, after which substantial alterations became apparent. Similarly, in our longevity observations, oilseed rape plants showed little physical change during the initial days. However, between day 5 and day 6, the percentage of the yellow area increased dramatically from 15 % to 64 %, with nearly all flowers lost. These results highlight a delay in floral withering following glyphosate spraying, during which foraging pollinators can encounter herbicide.

In the European Union, crop plants such as oilseed rape are not exposed to GBH during flowering. The practice of forced ripening, where crops are sprayed with glyphosate before harvest, is not considered good agricultural practice according to The Commission Implementing Regulation (EU) 2016/1313. Additionally, glyphosate-resistant genetically modified crops, which allow multiple glyphosate applications during the growing season, are not cultivated in the European Union (Finger et al., 2023; Schütte et al., 2017). However, outside the European Union practices with herbicides vary, and oilseed rape is one of the most commonly grown glyphosate-resistant genetically modified crop (Bansal and Kour, 2022). Also, glyphosate is applied by aerial or terrestrial spraying mainly during tillage, and herbicide exposure can occur through spray drift, where tiny droplets of herbicide are transported by the wind beyond the target area (Wang and Rautmann, 2008). This drift can affect field margins containing flowering plants that pollinators may visit. The extent of spray drift depends on factors such as the application method, weather conditions, and droplet formation, and under favorable conditions, it can affect a wide area (Linhart et al., 2019). Additionally, when herbicides are applied either before sowing seeds or after harvest, early- or late-flowering weeds in the targeted field may attract pollinators, potentially exposing them to glyphosate. GBHs are also used outside of agriculture, exposing pollinators to glyphosate when sprayed on flowering plants in public spaces, private gardens, roadsides, and other non-agricultural areas. Fortunately, the use of herbicides is relatively limited in this context (Benbrook, 2016).

Despite the shift in the VOC emission of GBH-treated oilseed rape plants after 24 h in the present study, there was no difference in bumblebees' flower preferences. This indicates that GBH-elicited changes in the olfactory cues of oilseed rape flowers do not determine the behavior of bees during foraging. We hypothesized that differences in VOC profiles and Y-maze experiments would occur since glyphosate exposure prevents the production of phenylalanine, an essential amino acid and vital precursor for several VOCs (Vogt, 2010). Alpha-farnesene, myrcene and beta-ocimene, compounds with a significantly lower concentration in the 24 h treatment than in the control and 6 h treatment observed here, are known to be highly attractive to *Apis mellifera* (Blight et al., 1997; Knudsen et al., 2006; Dötterl and Vereecken, 2010); however, their effects on bumblebees are not well understood.

Bumblebees' low interest in invasive lupine challenges our initial hypothesis that the presence of lupine would reduce visitations to oilseed rape, as we expected bumblebees to prefer lupine flowers. Sites occupied by lupines are known to host an abundance of bumblebee visitors (Ramula and Sorvari, 2017), and the color and shape of lupine flowers are particularly suitable for large bumblebees (Chittka et al., 2004; Müller, 1881; Willmer, 2011). Our flight cage experiment indicated that lupine did not compete with crop plants, as previously suspected (Brown et al., 2002; Thijs et al., 2012), nor did it benefit other plants as a magnet species (Jakobsson et al., 2015; Lopezaraiza–Mikel et al., 2007). However, our experiment involved only two plants at a time in the cage. It is possible that oilseed rape, known for producing nectar and pollen (Ion et al., 2012), was simply more appealing than lupine for naïve bumblebees that had been starved for 10–14 h before the experiment. Nevertheless, the volatile choice test with lupine and oilseed rape in the Y-maze revealed no difference in bees' plant preference. Although our study primarily focused on potential changes in preference following GBH exposure rather than on the preference between lupine and crop plants, our methods could effectively investigate this topic further in a controlled environment with other plant species.

The apathy toward foraging observed after oral exposure to GBHs in

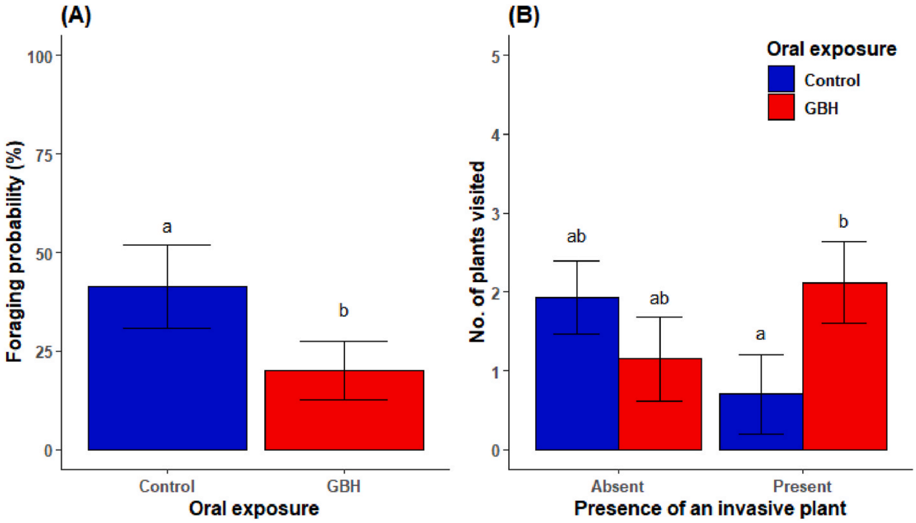

Fig. 5. Back-transformed least square mean (±SE) for the effects of glyphosate-based herbicide (GBH) and the presence of an invasive plant on bumblebee foraging behavior: (A) effect of oral GBH exposure on overall foraging probability and (B) effects of oral GBH exposure and presence of an invasive plant on the number of plants visited. Differences between treatments are indicated by different letters ($p < 0.05$ for foraging probability and $p = 0.07$ for the number of plants visited).

our experiment has not been previously studied with real flowers, although glyphosate has been shown to reduce sensitivity to nectar and food uptake in proboscis extension response experiments (Herbert et al., 2014; Mengoni Goñalons and Farina, 2018). Glyphosate is also known to impair the learning and memory of bees (Helander et al., 2023a; Herbert et al., 2014; Kaakinen et al., 2024). However, since our study used naïve bumblebees with no prior foraging experience or learned preferences, the experimental design minimized the potential confounding effects of glyphosate on cognitive processes related to memory. Our study did not determine the duration of this apathy; however, since the bees were treated orally with GBH 10–14 h before the experiment, the apathy appeared to last at least a day. If a significant proportion of foraging bees in a colony is exposed to GBH and subsequently loses interest in foraging, it may weaken the entire colony. Even a short-term food and energy shortage can increase a colony's vulnerability to predators and parasites, prolong the development time of larvae, lower brood temperature, and affect colony growth (Cartar and Dill, 1991; Requier et al., 2020). Thus, further research is needed to determine whether the reduced foraging activity of GBH-exposed bumblebees on real flowers is merely a short-term effect or if it persists over a more extended period.

The effects of glyphosate and GBH on the foraging behavior of bumblebees and other bees have been relatively understudied (Bokšová et al., 2023), with existing studies focusing primarily on Western honey bees. Our study confirms that bumblebees visit recently GBH-treated flowers and can thus be exposed to glyphosate residues while foraging. We observed a shift in VOC composition between control and GBH-treated flowers, but the treatment did not affect bumblebees' choices. Although flowering crop plants are not sprayed with GBH in the EU due to legislation, our results highlight the risks posed by spray drift affecting the margin zones of agricultural fields. Additionally, the use of GBH in silviculture and recreational areas may significantly threaten pollinator populations, warranting further attention and mitigation efforts.

CRediT authorship contribution statement

Kimmo Kaakinen: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Satu Ramula:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Benjamin Fuchs:** Writing – review & editing, Methodology, Formal analysis. **James D. Blande:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Eva-Maria Vaajamo:** Writing – review & editing, Methodology, Data curation. **Marjo Helander:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Grammarly and ChatGTP in order to rephrase individual sentences for improved clarity. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.123017.

Data availability

Data will be made available on request.

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