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Original article

Flupyradifurone-based insecticide influences foraging behaviour but does not alter chemosensory orientation of *Bombus terrestris*

Brigitta JUHÁSZ[1,2], Zsolt BERTÓK[3], Magdolna O. SZELÉNYI[4,5], Zsolt KÁRPÁTI[4], and Zoltán TÓTH[1]

[1] Department of Zoology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary
[2] Doctoral School of Biology, ELTE Eötvös Loránd University, Budapest, Hungary
[3] University of Veterinary Medicine Budapest, Budapest, Hungary
[4] Department of Chemical Ecology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary
[5] GLORIA coordination, Institute for Interdisciplinary Mountain Research, Austrian Academy of Sciences, Vienna, Austria

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Abstract – In recent decades, pollinating insect populations have drastically declined, partly due to the extensive use of various agrochemicals. Flupyradifurone is a systemic butanolide insecticide that has been considered to pose low risk to bees; however, recent studies have shown that it can cause various sublethal effects (e.g. reduced learning ability and memory) in various pollinators. In this study, we examined how exposure to a flupyradifurone-based insecticide formulation for 1, 2, or 3 weeks affected peripheral olfactory detection of a synthetic floral blend and the foraging behaviour of buff-tailed bumblebee (*Bombus terrestris*) workers. We found that pesticide-treated bumblebees approached the two visually cued target locations (scentless or scented with the floral blend) significantly less frequently, but they did not choose the floral blend at a lower rate than the control animals did. The latency to first approach a target location also differed between treatment groups as pesticide-treated bees reached the first location with a 37% lower probability than control animals at any time during the behavioural trial. The antennal response of bumblebees was dose-dependent, but time also modulated its magnitude at higher doses, and this effect was observed in both control and treated individuals. Overall, exposure to a low concentration of flupyradifurone altered some aspects of bumblebees' foraging behaviour regardless of exposure length, but did not influence their chemosensory perception or orientation. Future investigations should examine whether these sublethal effects identified at the individual level may scale up to impair colony growth or reproductive output in this important pollinator species.

Foraging behaviour / Floral volatiles / Pesticide exposure / Wind-tunnel test / Sublethal effects

1. INTRODUCTION

Bee populations have experienced a dramatic decline in recent decades, driven by several environmental factors (Soroye et al. 2020). Among these, the use of pesticides poses a significant threat to bee species, though they have an indispensable role in agricultural practices (Potts et al. 2010; Goulson et al. 2015; Siviter and Muth 2020). The active ingredients in insecticides are not only harmful to the targeted pest species, but they often have detrimental effects on a wide range of non-target species as well (Carriger et al. 2006), including pollinating insects

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Corresponding author: Z. Tóth, toth.zoltan@atk.hun-ren.hu
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that are essential for crop production (Tscharntke et al. 2012; Arena and Sgolastra 2014; Goulson et al. 2015). Currently, pesticide development is challenged by the increasing production demands and the emergence of pesticide resistance in pest populations (Nauen et al. 2015). The ban of most neonicotinoids in 2018 (EC 2018) has driven the urgent need for alternative plant protection products (Siviter et al. 2018), and in response to this demand, flupyradifurone was successfully registered as an active ingredient in 2013 (Jeschke et al. 2015).

Flupyradifurone is a butenolide insecticide and a synthetic analogue of the alkaloid stemofoline naturally occurring in plants of the *Stemona* genus (Nauen et al. 2015). Flupyradifurone is systemically absorbed into plant tissues following its application and is translocated throughout all parts of the plant, including the nectar and pollen (EPA 2014). Having high selectivity for target pests such as aphids, hoppers, and whiteflies, this active ingredient can be applied in numerous crops and vegetable and fruit production systems, and is also recommended in Integrated Pest Management programmes and resistance management strategies (Jeschke et al. 2015; Nauen et al. 2015).

Pre-authorisation testing indicated that this active ingredient poses a low risk to bees, and in formulations it is often applied to flowering crops during blooming (Nauen et al. 2015; Siviter et al. 2024). Similar to neonicotinoids, this substance targets insect nicotinic acetylcholine receptors (nAChRs), but acts as a partial agonist and binds reversibly to these receptors (Jeschke et al. 2015). However, an increasing number of studies have shown that flupyradifurone induces a range of sublethal and lethal effects in a wide variety of pollinator species. First, flupyradifurone can exert a detrimental effect on learning, memory, and other processes linked to the central nervous system. For instance, exposure to a field-realistic dose of flupyradifurone significantly impaired the olfactory learning and memory in the eastern honey bee, *Apis cerana* (Tan et al. 2017) and in *Bombus impatiens* (Siviter and Muth 2022). In western honey bees, *Apis mellifera*, exposure to flupyradifurone caused thermoregulatory disturbances (Tong et al. 2019) and led to increased occurrences of poor coordination, apathy, and hyperactivity (Tosi and Nieh 2019; Tosi et al. 2021). In *B. impatiens*, flupyradifurone-exposed individuals exhibited impaired cognitive abilities (Siviter and Muth 2022; Gray et al. 2024) and disruptions in thermoregulation (Fischer et al. 2023). In *Osmia lignaria*, exposure to this substance substantially increased mortality (Siviter et al. 2024). Second, flupyradifurone can influence foraging behaviour and activity. For example, exposure to this substance reduces the amount of sugar solution consumed in *A. mellifera* (Tosi et al. 2021; Wu et al. 2021), impairs foraging efficiency in *O. lignaria* (Siviter et al. 2024), and decreases feeding motivation in *B. impatiens* (Siviter and Muth 2022). Third, flupyradifurone can also affect reproductive processes in pollinators, causing disrupted sexual communication (Boff and Ayasse 2024), decreased nesting success (Siviter et al. 2024), reduced reproductive output (Richardson et al. 2024; Knauer et al. 2022), and impairment in offspring caretake (Richardson et al. 2025). Overall, the growing body of evidence raises concerns about the potential risks of applying flupyradifurone without restriction to the health and survival of pollinating insects (Siviter and Muth 2020).

In this study, we investigated the potential effects of 1, 2, or 3 weeks of exposure to a flupyradifurone-containing insecticide formulation on the olfactory detection responses and foraging decisions of buff-tailed bumblebee, *Bombus terrestris*, workers. Bumblebees are vital pollinators of both agricultural crops and wildflowers in the temperate regions (Hutchinson et al. 2021). The species *B. terrestris* is widespread in Europe and also reared commercially for crop pollination. We conducted choice tests on control and pesticide-treated bumblebee workers in a wind tunnel and subsequently measured the electrophysiological recordings of their antennal response. We used the synthetic floral blend derived from white mustard (*Sinapis alba*), an annual plant of the Brassicaceae family with a Mediterranean origin, as the floral scent source. This crop species, being partially pollinated by bumblebees, is cultivated globally and has considerable agronomic importance owing to its diverse applications (Mitrović et al. 2020). The composition of the floral blend and the relative proportions of its components were determined following Saunier et al. (2023). We predicted that the applied pesticide treatment may have the following adverse sublethal effects on exposed individuals compared to controls: (1) impaired ability to detect the volatile blend due to reduced antennal sensitivity; (2) lower probability or increased latency to locate the floral scent source; (3) delayed initiation of foraging bouts, resulting in lower probability of successful foraging. We also expected that these potential effects will be most pronounced after 3 weeks of exposure.

2. MATERIALS AND METHODS

2.1. Animal husbandry

We purchased eight Natupol Excel hives of *B. terrestris* from the local distributor of Koppert® on 20 March, 2025 (Szentes-Bio Ltd., Hungary). We kept the colonies in their original brood box, with the top of the cardboard outer box left partly open for better ventilation. Bumblebees were fed through an adjacent plastic pouch, which contained a sugar solution provided by the supplier. In addition, we provided freezer-stored (at −20 °C) organic flower pollen (ApiLand SRL, Baia Mare, Romania) to the animals ad libitum. We randomly assigned the colonies to two treatment groups: control and pesticide-treated. Colonies were arranged randomly on the laboratory shelves according to their treatment. The ambient temperature was 21.5 ± 1.67 °C, and the relative humidity ranged between 35 and 60%. Red light (Philips LED red bulbs; Philips International BV, Amsterdam, The Netherlands) has been used to illuminate the laboratory during the daytime (in a 13.5:10.5 h L:D).

2.2. Preparation of floral volatiles

We prepared a synthetic floral volatile blend consisting of 10 synthetic compounds based on Saunier et al. (2023). We combined the individual, synthetic, neat compounds in the following proportions: 0.2% of phenylacetaldehyde (CAS: 122–78-1; Sigma-Aldrich, 95%); 1.2% of β-caryophyllene (CAS: 87–44-5; Sigma-Aldrich, ≥ 80%); 1.4% of acetophenone (CAS: 98–86-2; Sigma-Aldrich, 99%); 1.4% of benzyl alcohol (CAS: 100–51-6; Sigma-Aldrich, ≥ 99%); 2% of (Z)−3-hexen-1-ol (CAS: 928–96-1; Sigma-Aldrich, > 98%); 2.8% of methyl salicylate (CAS: 119–36-8; Sigma-Aldrich, ≥ 99%); 3% of β-ocimene (CAS: 13877–91–3; Sigma-Aldrich, ≥ 90%); 8% of (Z)−3-hexenyl acetate (CAS: 3681–71-8; Sigma-Aldrich, ≥ 98%); 30% of anisaldehyde (CAS:123–11-5; Sigma-Aldrich, 98%); and 50% of benzaldehyde (CAS: 100–52-7; Sigma-Aldrich, ≥ 99%). For the electroantennogram (EAG) measurements, the blend was dissolved in mineral oil (CAS: 8042–47-5) and 10 µl of each dilution (0.125, 1.25, 12.5, and 125 µg/µl) was applied to a filter paper disk (cotton liner, diameter: 12.7 mm; Carl Roth GmbH, Karlsruhe, Germany). Then, the treated disk was inserted into a Pasteur pipette, which served as a stimulus cartridge. For the behavioural observations, one brown vial-wick dispenser (Molnár et al. 2017) was filled with 1 ml of the neat blend (Saunier et al. 2023), while another dispenser was filled with 1 ml of mineral oil. The first served as a rewarding target location and the second as a control target location in the behavioural trials.

2.3. Pesticide treatment

During the total exposure period (21 days), behavioural and physiological tests were performed after 1 week, 2 weeks, and 3 weeks. The exact duration was ensured by randomly selecting one control and one pesticide-treated colony for 1 of 4 exposure-starting days; bumblebees from these pairs of colonies participated in the tests on the same day on each week (i.e.

the colonies were tested after all three exposure lengths). At the start of the experiment, we added 18.4 µl of Sanium® System (25 g/l flupyradifurone content; Bayer AG, Germany) to 460 ml syrup of the pesticide-treated colony on its assigned exposure-starting day. The added volume of insecticide formulation resulted in a nominal concentration of 1 ppm flupyradifurone in the syrup of the pesticide-treated colonies, which can be regarded as a field-relevant dose (EFSA PPR Panel et al. 2022). This process was repeated each week to ensure that the concentration of flupyradifurone in the sugar syrup remained relatively stable. In the control colonies, we pipetted the same amount of filtered water (instead of the formulation) to the syrup. We also pipetted 10 µl of the neat synthetic floral blend into the sugar syrup, so bumblebees could associate the floral scent with a nutritional reward.

2.4. Behavioural observations

Behavioural trials were conducted on 4 consecutive days following each exposure period (between 9:30 and 15:10) in a wind tunnel, measuring 110 cm long, 30 cm wide, and 30 cm high (for a schematic figure, see Kárpáti et al. 2024). Charcoal-filtered air was introduced into the tunnel at a temperature of 21.6–24.2 °C and relative humidity of 31–46%. The rate of the airflow was 0.1 m/s, directed from the target locations toward the test animals through a fine-mesh screen. An exhaust expelled the wind tunnel air outside the building at the tunnel's far end. The scent sources were two vial-wick dispensers, which were embedded into the bottom of the wind tunnel (made of fibreboard). These dispensers were positioned 8.5 cm from the sidewalls and 15 cm apart from each other (Fig. S1). To provide visual cues for orientation towards the dispensers, blue coloured and round plastic disks (2.4 cm in diameter) were attached below their black caps. We termed these visually cued dispensers as 'target locations'. One dispenser contained mineral oil (i.e. scentless, unrewarding location) and the other contained the neat floral blend (i.e. rewarding location; see the rationale at the end of the 'Pesticide treatment' section). The appointed pair of colonies (one control and one pesticide-treated) was moved into the preparation room next to the wind tunnel 1 h before the experiment. During a trial, we first positioned the scent sources into their respective assigned locations within the wind tunnel, following a random determination. Next, we selected an individual at random from one of the two colonies (the order of colony testing was also determined randomly) and placed it inside an upside-down wire mesh cylinder, referred to as a 'releasing cage' (length, 5.5 cm; internal diameter, 3 cm). The individual remained in the cage for 5 min, with the distance between the target locations and the releasing cage was 65 cm. The room was lit only by a red LED light source during this period. After the 5-min acclimatisation period, the observer turned on the light (similar to daylight [5500–6500 K], turned the releasing cage in a lateral position so the focal bee could leave the cage, and then left the room. During the following 10 min, we recorded the behaviour of the focal bee at the releasing cage and near the target locations with two Panasonic HC-V380 video cameras from above. After 10 min, we switched off the main light and illuminated the area using only red light. We removed the focal individual from the wind tunnel in a sampling tube and put it aside for the EAG measurement. After each trial, the observer cleaned the releasing cage, the disks of the dispensers, and the wind tunnel using 70% ethanol and wiped them with paper towels. The continuous airflow between trials ensured that no traces of the synthetic blend or alcohol remained in the tunnel.

The video files were analysed using the event recorder BORIS 8.25 (Friard and Gamba 2016). The video files were named based on their date and time of creation and did not refer to the treatment of the observed animal, thus ensuring that observer bias was avoided. Recordings were also analysed in a random order. The following behavioural measures were determined from the video recordings: (1) latency to leave the releasing cage after the observer turned the releasing cage in a lateral position, (2) type of the first- and second-approached target locations, and (3) latency to approach the first and second target location. 'Approach' was defined as an event when the focal individual got so close to a target location that its head crossed the edge of the blue disk attached to the dispenser (as in Kárpáti et al. 2024).

2.5. Antennal detection—EAG

To assess how pesticide treatment affects bumblebees' ability to detect floral volatiles, we performed electroantennography (EAG) recordings using the synthetic floral blend. All bumblebees were tested within 10 min after their behavioural trials in the wind tunnel. EAG recordings quantify the depolarisation amplitude of all responsive olfactory sensory neurons when exposed to a stimulus (Roelofs 1984). For the EAG recordings, the antennal flagellum of a *B. terrestris* worker was excised, then the half of the last (distal) segment was cut and both sides of the antenna were inserted into two glass electrodes (ID: 1.17 mm, Syntech, Kirchzarten, Germany), which were filled with Ringer solution (Ephrussi and Beadle 1936). The antennal signal underwent a pre-amplification of tenfold, was then converted into a digital signal using a DC amplifier interface (IDAC-2, Syntech), and subsequently recorded utilising GC-EAD software (GC-EAD 2024, version 1.3.1, Syntech). Antennae were stimulated for 0.5 s using a Stimulus Controller (CS-55, Syntech), while being continuously exposed to a humidified, charcoal-filtered airstream (1 l/min) both during and between stimulus sessions. Mineral oil, the solvent of the floral volatiles, served as the control stimulus. Stimuli were prepared at the beginning of the 3-week experimental period. Odour cartridges were stored individually at −20 °C, wrapped in four layers of aluminium foil, between experimental days to minimise the loss of volatile compounds. The same cartridges were reused throughout the experiment and were applied repeatedly across recording sessions. Before and after each stimulus session, we measured the antennal responses of the focal individual to the mineral oil; within sessions, four doses of the synthetic blend (1.25, 12.5, 125, and 1250 µg; see the dilution protocol in 'Preparation of flower volatiles' paragraph) were tested in ascending order. We also counted the antennal segments of each animal to confirm that the tested individual was a worker. After the EAD measurements, each individual was euthanized by freezing.

2.6. Body size

After the experiment, we measured the length of the marginal cell of bumblebee wings and used it as a proxy for body size (Medler 1962; Nooten and Rehan 2020). First, we cut off the left forewing of each frozen individual from the mesothorax and placed it between two microscope glass slides so the wing was completely flattened. Then, we photographed the wings using a microscope camera (Oplenic Pro-MicroScan, HangZhou, China) attached to a stereo microscope. To measure the length of the marginal cell, we first made the photographs compatible with the TPS series using the program tpsUtil v1.83 (Rohlf 2015). Then, we identified two landmarks at the proximal and distal ends of the marginal cell (Owen 1988) on the wing and measured the distance between these landmarks using the program tpsDig2 v2.32.

2.7. Statistical analysis

All tests were conducted in R 4.5.0 (R Core Team 2025). A total of eight colonies, four control and four pesticide-treated, were included in the analyses, each tested at every exposure length (resulting in a sample size of four colonies at each treatment × exposure length combination). We used a mixed-effect Cox proportional hazards model ('coxme' R package; Therneau 2022a) to examine how starting time of trial (decimal time divided into three equal-length periods; as a factor), body size (as a covariate), pesticide treatment (as a factor), exposure length (as a factor), and the interaction of the latter two affected workers' latency to leave the releasing cage. We fitted a generalised linear mixed model (GLMM) with binomial error distribution using Template Model Builder ('glmmTMB' R Package; Brooks et al. 2017) to investigate how these predictors influenced whether workers approached any target locations (with the value of 0 for not visiting any and 1 for approaching at least one). We applied the same model type to examine the effect of these explanatory variables on the type of the first-approached scent source (with the value of 0 for mineral oil and 1 for the floral blend), and a GLMM with betabinomial error distribution on the proportion of visited target locations (i.e. zero, one, or two, out of the two locations). We used a mixed-effect Cox proportional hazards model to examine how starting time of trial (as a factor), body size (as a covariate), pesticide treatment (as a factor), length of exposure (as a factor), and the interaction of the latter two affected the latency to approach the first target location. Compliance with the proportional hazard assumption was confirmed for both fitted Cox models. To check compliance with the assumptions of the GLMMs, we applied residual plot diagnoses using the 'DHARMa' R package (Hartig 2022). We included 'Colony' nested in 'Day' as a random term in these mixed-effect models to account for non-independence between repeated measurements (Pinheiro and Bates 2000). We analysed the behavioural data of 144 bumblebee workers (i.e. six individuals per colony at each week), out of which 73 were control and 71 were pesticide-treated.

We fitted a GLMM with Gaussian error distribution ('glmmTMB' R package) to investigate the effects of pesticide treatment and exposure length on the bumblebees' antennal response to the floral blend at five different doses (0, 1.25, 12.5, 125, and 1250 µg). For that, we averaged first the EAG amplitudes of the mineral oil control stimuli measured before and after each session (as in Kárpáti et al. 2024). We also log-transformed the response data ($\log(x+0.1)$) to improve its fit to normal distribution. In this model, we included body size (as a covariate), pesticide treatment (as a factor), exposure length (as a factor), stimulus dose (as a factor), and the three-way interaction between pesticide treatment, exposure length and stimulus dose as potential predictors. The random structure included 'Individual identity' nested in 'Colony' nested in 'Day'. We excluded one measurement of a single test subject due to its indistinguishable response to the smallest dose from the noisy baseline, and all measurements on the first and eleventh trial days because of issues with the instrument (unusually large measurements throughout the first day) or unintentional mistake in the applied protocol (eleventh day). These exclusions resulted in a total sample size of 584 antennal responses of 117 workers (58 control and 59 pesticide-treated, respectively) for this analysis.

Finally, we investigated potential differences in body size between bumblebees ($N=144$) from the eight colonies using a linear model with Gaussian error distribution ('glmmTMB' R package). In this model, we included radial cell length as the response variable, and colony identity (as a factor), experimental period (1, 2, or 3 weeks; as a factor), and their two-way interaction as explanatory variables. Experimental period was considered here as a proxy for colony age in the experiment.

To estimate the significance of potential predictors in all fitted models, we applied type III Wald χ^2-tests using the Anova function of the 'car' R package (Fox and Weisberg 2019). Random effects were retained in all models regardless of the proportion of variance they explained (Meteyard and Davies 2020); their inclusion did not lead to convergence or fitting issues. Estimated marginal means (EMMs) with 95% confidence intervals were calculated and post hoc comparisons were performed with FDR adjustment using the 'emmeans' R package (Lenth 2023). All tests were two-tailed with α set to 0.05.

3. RESULTS

3.1. Foraging behaviour

We found that significantly less pesticide-treated bees approached a target location

Table I Test statistics and significance of the explanatory variables from the fitted mixed-effect models on the behavioural data of *Bombus* workers. Random terms are given as SD ± 95% confidence intervals (please note that upper confidence intervals could not be reliably estimated for very small random effects). Test statistics and *P*-values for the non-significant predictors were computed by including them one by one in the final models

Response variable	Model type	Random term	Predictors	χ^2	df	*P*
Latency to leave the releasing cage	Mixed-effect Cox model	Day/colony: 0.12 [0; 0.55] Day: 0.24 [0; 0.95]	Starting time of trial	3.66	1	0.16
			Body size	**4.67**	**1**	**0.031**
			Pesticide treatment	2.14	1	0.144
			Exposure length	0.63	2	0.729
			Pesticide treatment × Exposure length	0.65	2	0.723
Approaching any target locations	GLMM with binomial error distribution	Day/colony: < 0.01 [0; -] Day: 0.34 [0.08; 1.51]	Starting time of trial	1.63	2	0.443
			Body size	0.06	1	0.807
			Pesticide treatment	**4.06**	**1**	**0.044**
			Exposure length	4.97	3	0.084
			Pesticide treatment × Exposure length	1.23	2	0.542
Proportion of visited target locations	GLMM with betabinomial error distribution	Day/colony: < 0.01 [0; -] Day: 0.27 [0.06; 1.16]	Starting time of trial	2.81	2	0.245
			Body size	0.79	1	0.374
			Pesticide treatment	**5.35**	**1**	**0.021**
			Exposure length	**10.59**	**2**	**0.005**
			Pesticide treatment × Exposure length	4.36	2	0.113
Type of the first-approached target location	GLMM with binomial error distribution	Day/colony: < 0.01 [0; -] Day: 0.42 [0.1; 1.78]	Starting time of trial	3.2	2	0.202
			Body size[†]	0.03	1	0.869
			Pesticide treatment	1.31	1	0.253
			Exposure length	1.5	2	0.473
			Pesticide treatment × Exposure length	0.14	2	0.932
Latency to approach the first target location	Mixed-effect Cox model	Day/colony: 0.03 [0; 0.57] Day: 0.24 [0; 0.93]	Starting time of trial	2.39	2	0.302
			Body size	< 0.01	1	0.974
			Pesticide treatment	**4.59**	**1**	**0.032**
			Exposure length	**6.68**	**2**	**0.036**
			Pesticide treatment × Exposure length	1.04	2	0.596

[†] when the model was fitted with body size as a single predictor, the quantile deviation plot and the combined adjusted quantile test indicated a difference between the observed and expected data (although the 'diagnose' function of the DHARMa package did not report any issues); therefore, the estimated effect of this predictor should be treated with caution

Significant predictors in the final models and their values are shown in bold

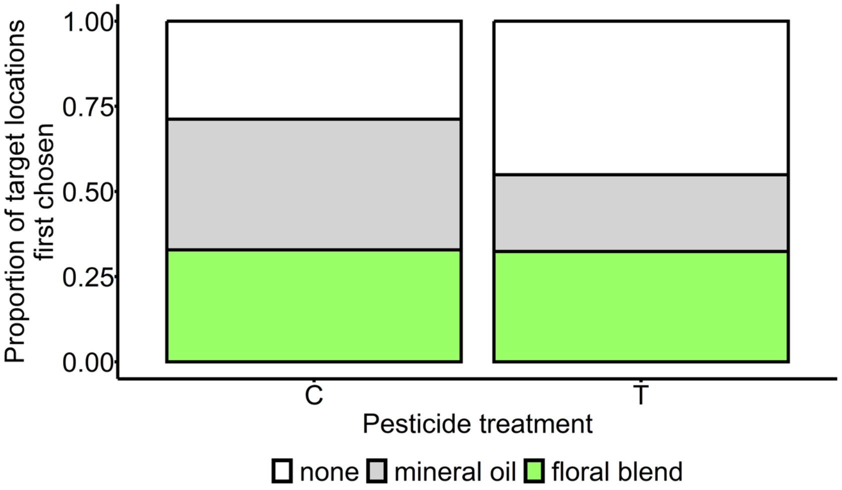

Figure 1. The proportion of tested *B. terrestris* workers that approached the blend-containing (green) and the mineral oil-containing (grey) target locations, or did not approach any locations (white) in the two treatment groups.

compared to controls (EMMs with 95% CI: 0.55 [0.41; 0.69] versus 0.72 [0.58; 0.82]; T-C contrast ± SE = −0.17 ± 0.08, *z*-ratio = −2.05, $P = 0.041$; Table I; Figure 1), and the proportion of approached locations during the behavioural trial was also significantly lower in the pesticide-treated than in the control individuals (0.39 [0.28; 0.5] versus 0.55 [0.43; 0.66]; −0.16 ± 0.07, *z*-ratio = −2.36, $P = 0.019$; Figure 2). Furthermore, the latency to approach the first target location was affected by the pesticide treatment as pesticide-treated bees reached the first scent source with 37% lower probability (hazard ratio [95% CI]: 0.63 [0.41; 0.96]) than control animals at any time of the trial (0.79 [0.64; 0.98] versus 1.26 [1.02; 1.55]; −0.46 ± 0.22, *z*-ratio = −2.112, $P = 0.035$; Figure 3). The latter two measures were also significantly affected by

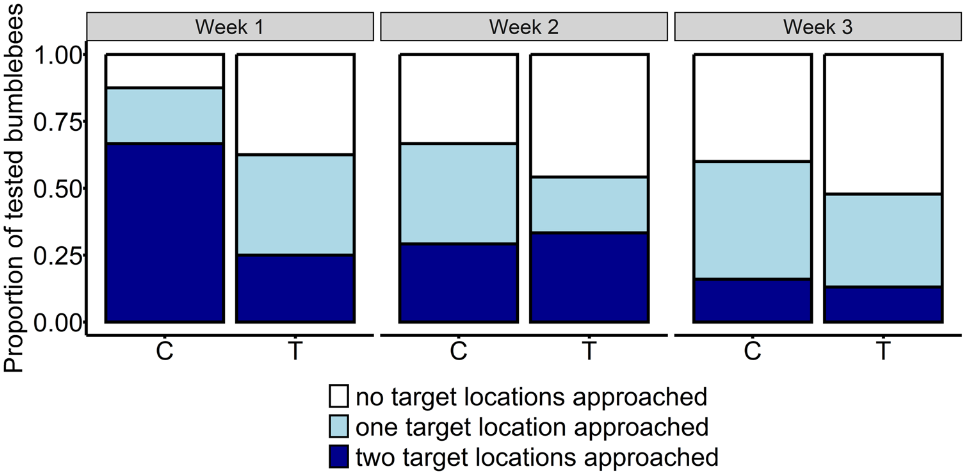

Figure 2. The proportion of individuals that approached no (white), one (light blue), or two (dark blue) target locations during the behavioural trial in the two treatment groups and in the three exposure periods

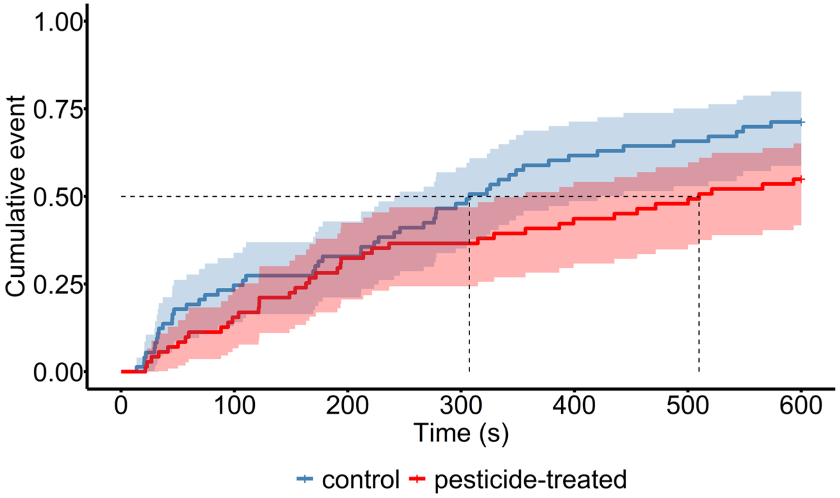

Figure 3. Kaplan–Meier curves for the cumulative incidences of approaching the first target location in the two treatment groups (blue: control; red: pesticide-treated). Curves are shown with 95% confidence intervals. Dashed lines indicate the median time from the start of the trial at which half of the tested individuals approached the first location (control: 307 s; pesticide-treated: 510 s). For graphical presentation only, we used the survfit function of the 'survival' R package (Therneau and Grambsch 2000; Therneau 2022b) without including the random term.

exposure length in itself (number of approached locations: $\chi^2_2 = 10.59$, $P = 0.005$; latency to approach the first location: $\chi^2_2 = 6.68$, $P = 0.036$), indicating that the probability of approaching the target locations decreased over the weeks both in control and pesticide-treated bees. On the other hand, neither pesticide treatment nor exposure length influenced which type of target locations animals approached first (all $P \geq 0.202$; Table I; Figure 1). Pesticide treatment, either in itself or in interaction with exposure length, did not affect workers' latency to leave the releasing cage at the start of the trials either (Table I). However, this measure was negatively related to worker body size ($\chi^2_1 = 4.67$, $P = 0.031$).

3.2. Antennal detection

Antennal responses of workers to the synthetic floral blend were dose-dependent and were significantly affected by the interaction of concentration and exposure length ($\chi^2_8 = 197.89$, $P < 0.001$; Table II). EAG amplitudes increased with concentration in all experimental periods and were the highest at the highest dose level, with all doses significantly differing from the control (i.e. mineral oil) and from each other (including consecutive doses; Table S1). However, amplitudes were significantly lower after 3 weeks compared to the shorter experimental periods at the 12.5 and 125 µg doses, and differed between all periods at the 1250 µg dose level (Table S2; Figure 4). Pesticide treatment did not affect this measure either by itself or in interaction with concentration, exposure length, or both (all $P \geq 0.156$; Table II).

3.3. Differences in workers' body size between colonies

Bumblebee body size, measured as marginal cell length, ranged from 2.56 to 3.33 mm. We found significant difference in this measure between the eight colonies ($\chi^2_7 = 32.96$, $P < 0.001$; Fig. S2), but experimental period did not affect body size either alone ($\chi^2_2 = 0.08$, $P = 0.961$) or in interaction with colony ($\chi^2_{14} = 11.92$, $P = 0.612$).

Table II Test statistics and significance of the examined predictors from the fitted mixed-effect model on workers' antennal response. Random terms are given as SD ± 95% profile confidence intervals (please note that upper confidence intervals could not be reliably estimated for very small random effects). Test statistics and *P*-values for non-significant predictors were obtained by individually incorporating each variable into the final models

Response variable	Model type	Random term	Predictors	χ^2	df	*P*
Antennal response (log-transformed)	GLMM with Gaussian error distribution	Day/colony/individual identity 0.15 [0.13; 0.17] Day/colony: < 0.01 [0; -] Colony: 0.06 [0.03; 0.11]	Body size	1.26	1	0.262
			Pesticide treatment	0.42	1	0.519
			Exposure length	**2.91**	**2**	**0.233**
			Concentration	**12,984.43**	**4**	**< 0.001**
			Exposure length × concentration	**197.89**	**8**	**< 0.001**
			Pesticide treatment × concentration	6.65	4	0.156
			Pesticide treatment × exposure length	0.68	2	0.712
			Pesticide treatment × exposure length × concentration	3.89	8	0.867

Significant explanatory variables in the final models, along with their corresponding values, are presented in bold

4. DISCUSSION

Bumblebees play a vital role as pollinators in sustaining ecosystem functions (Gallai et al. 2009; Klein et al. 2007), but their frequent exposure to agrochemicals and other anthropogenic stressors are threatening their natural populations and contribute to their widespread decline in recent decades (Goulson et al. 2008, 2015). In this study, we investigated the sublethal effects of chronic exposure to the novel butenolide insecticide flupyradifurone on the chemosensory detection and foraging behaviour of buff-tailed bumblebee workers. We found that fewer pesticide-exposed individuals approached a target location at all, explored both locations, and approached their first chosen target location more slowly, but these pesticide-related effects were not influenced by exposure length. In addition, treated and control bees approached the reward-associated floral blend with similar probability and did not differ in their latency to initiate foraging. Antennal responses were dose-dependent, with amplitudes increasing with floral scent concentration as expected, but were also unaffected by the pesticide treatment. These findings indicate that exposure to flupyradifurone can alter some important aspects of foraging behaviour in *B. terrestris*. While a previous study reported more pronounced negative effects of a contemporary systemic insecticide on chemosensory orientation in this species (Kárpáti et al. 2024), direct comparison should be treated cautiously due to differing experimental conditions.

Pesticide-exposed bees were less likely to approach targets and exhibited longer latencies

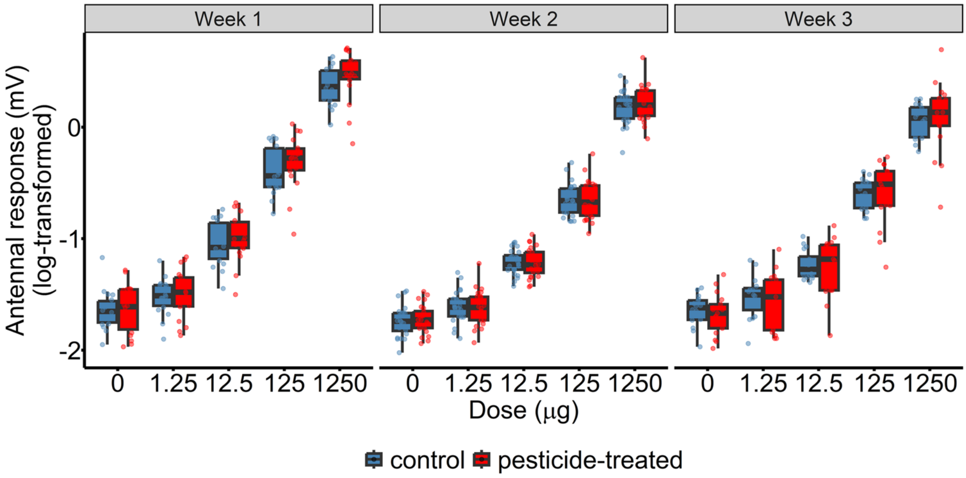

Figure 4. Electroantennographic dose responses of *B. terrestris* workers to the synthetic blend. Boxplots show the median and interquartile range, whiskers indicate values within 1.5-fold of the interquartile range, and dots represent individual data points. The categories on the *x*-axis represent the quantity of volatile blend loaded into the stimulus cartridge. The zero dose indicates the mineral oil control; we measured the EAG responses to this stimulus before and after the blend dose series and averaged the two measurements for each individual.

to approach the first target, whereas the probability of choosing the scented target first did not differ from controls. This pattern is consistent with reduced foraging engagement and may be attributable to several, non-mutually exclusive mechanisms. It may suggest an adverse effect of flupyradifurone on the motivational aspect of foraging behaviour. Previous studies have reported similar effects of this substance, where exposed bumblebees exhibited reduced sucrose responsiveness and impaired learning of olfactory and visual cues (Siviter and Muth 2022; Gray et al. 2024). This effect may stem from flupyradifurone's agonistic action on the nicotinic acetylcholine receptors (nAChRs) in the central nervous system, modulating cognitive functions including spatial orientation and reward processing (Paoli and Giurfa 2024). Alternatively, pesticide-exposure could induce reduced activity or diminished responsiveness in the testing environment. In bumblebees, experimental evidence suggests that agonistic effects on nAChRs can also lead to hypoactivity, disorientation, or reduced exploratory behaviour (Gill et al. 2012; Feltham et al. 2014; Gill and Raine 2014). However, we found no effect of flupyradifurone exposure on the latency to leave the releasing cage at the beginning of the behavioural trials, implying that a deficit was more likely to occur during active decision-making phases. Finally, even though control and exposed colonies experienced the same laboratory conditions throughout the experiment, variation in colony development could still influence the measured foraging behavioural parameters.

Control individuals had a relatively poor performance in approaching the scent sources and choosing the floral blend first. These observations were unexpected as in our previous experiments, where we used the same experimental protocol and wind tunnel (Kárpáti et al. 2024; Tóth Z, Juhász B, Kárpáti Zs, Schultheiss P, Nooten SS, unpubl. data), all control bumblebees approached at least one scent source and approx. 80% of them oriented to the floral blend first. In the present study, control individuals also showed reductions in some aspects of foraging behaviour similarly to pesticide-treated bumblebees as the study progressed, although the observed differences between treatment groups persisted over time. We do not have a definitive explanation for these discrepancies; however, certain details may help to clarify this pattern. First, we used colonies from a different commercial source than before, and bumblebees' learning ability to associate a novel floral scent with a nutritional reward could be lower in the present than in past colonies. Second, this study was conducted in the spring when the ambient temperature and relative humidity was lower and more variable throughout the study period than during earlier experiments, which could negatively affect both control and pesticide-treated workers' foraging performance. Third, we found significant differences in wing size among colonies in this study, indicating substantial inter-colonial variation. This variation may also extend to differences in foraging activity or motivation during the behavioural trial, potentially leading to a lower average behavioural output. We acknowledge that these explanations remain tentative in the absence of additional analyses and that the moderate number of colonies used in the study limits the generalisability of our results. Nevertheless, differences between treatment groups in the probability of approaching a target location and the latency to do so reflect a consistent effect of flupyradifurone exposure in the tested individuals.

Chronic exposure to a pesticide regularly leads not only to a single, but multiple sublethal effects (Desneux et al. 2007). While we found only moderate reductions in some aspects of foraging behaviour, other studies reported more substantial adverse effects of flupyradifurone in bumblebees. For instance, Richardson et al. (2024), Richardson et al. (2025)) showed impaired alloparental care and reproductive output in *B. impatiens* microcolonies, with larval mass reductions driven by the exposure of nursing adults rather than direct larval toxicity. Fischer et al. (2023) found that flupyradifurone disrupts collective thermoregulation in bumblebee microcolonies, halving reproductive output. However, the applied exposure regimes were different in these studies compared to ours. Exposure levels experienced by pollinators can vary depending on crop species, application regime, and environmental context, and information on how residues of this active ingredient may accumulate and persist in agricultural and semi-natural habitats is very limited (e.g. EPA 2014). The field-realistic concentration of this substance in nectar and pollen was also shown to cover a wide range (0.3–4.3 ppm; Campbell et al. 2016; Wu et al. 2021). The concentration used in our study (approx. 1 ppm) falls within this range and represents a conservative estimate of flupyradifurone bumblebees can be exposed to (EFSA PPR Panel et al. 2022). Thus, our findings indicate that even a short-term exposure to an intermediate concentration of flupyradifurone could compromise bumblebees' ability to exploit floral resources efficiently, potentially reducing the pollination service they provide.

Despite the induced behavioural bias, pesticide treatment had no effect on antennal responses in our study. Previous studies on other insecticides have reported mixed effects on antennal responses. For instance, larval exposure to thiacloprid similarly altered antennal selectivity to floral volatiles (Ke et al. 2023). Clothianidin exposure reduced antennal sensitivity in *Osmia bicornis* and *B. terrestris*, with corresponding declines in foraging performance, such as fewer flowers visited per flight and longer search times in *O. bicornis* (Straub et al. 2021). On the other hand, buff-tailed bumblebees exposed to acetamiprid exhibited disrupted activity and orientation, even though their olfactory detection of a floral blend remained unaffected (Kárpáti et al. 2024). Our findings suggest that antennal sensitivity and thus peripheral detection abilities remain intact in bumblebees even after 3 weeks of exposure to flupyradifurone. As odour cartridges were prepared at the beginning of the experiment and reused throughout the recording sessions, a gradual decrease in stimulus intensity cannot be excluded. Therefore, the temporal decrease in antennal responses may reflect stimulus depletion rather than solely physiological changes in the bees. However, this did not affect the comparison between treatment groups as control and pesticide-treated individuals were tested under identical conditions, i.e. using the same cartridges.

In conclusion, our study demonstrates that chronic exposure to flupyradifurone at a conservative, field-realistic dose can reduce foraging engagement in *B. terrestris* without affecting peripheral olfactory detection. The observed sublethal behavioural changes may have the potential to contribute to reduced resource acquisition, compromising colony growth and reproductive success found in previous studies (Fischer et al. 2023; Richardson et al. 2024). Landscape-level investigations, which integrate multiple stressors (e.g. pesticide exposure, nutrition deficiency, elevated temperature regimes) and assess how individual impairments scale up to detrimental alterations in colony dynamics and population viability (Whitehorn et al. 2012; Gill and Raine 2014; Lonsdorf et al. 2024), could yield critical insights for regulatory frameworks, ensuring that novel pesticides do not perpetuate further declines in pollinator populations. The development of insecticides that selectively suppress pest populations while minimizing adverse effects on non-target insects is of critical importance for both agricultural productivity and conservation management. In order to achieve that goal, more comprehensive risk assessments are needed that incorporate sublethal behavioural endpoints (Fisher et al. 2023), allowing to assess how emerging pesticides would influence the population dynamics of pollinators in nature.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

BJ, ZsB, MOSz, ZsK, and ZT contributed to the investigation and commented on previous versions of the manuscript. BJ also participated in formal analysis and writing of the original draft. ZT performed data curation, methodology, software, formal analysis, validation, and participated in the writing of the original draft.

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DATA AVAILABILITY

Data supporting the results and R scripts for the statistical analyses are archived and available at Figshare (https://doi.org/10.6084/m9.figshare.30675419).

DECLARATIONS

Ethics approval No permits or approvals were necessary for the experiment involving commercially available buff-tailed bumblebees in Hungary. After completing the experiment, we euthanized the animals by freezing them to ensure they did not mix with the natural population.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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