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Decreased efficiency of pollen collection due to Sulfoxaflor exposure leads to a reduction in the size of bumble bee workers in late European summer

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Bumble bees (*Bombus terrestris*) are important pollinators of wild and crop plants. Despite their importance in the process of fruit and seed production on crop sites, their activity may be impaired due to exposure to pesticides. This species has a yearly life cycle and colony success may rely on effective foraging of workers on ruderal plants late in summer when most crops are no longer flowering. In the current study, we investigated the effect of chronic exposure to Sulfoxaflor on aspects of the foraging behavior of bumble bees and whether Sulfoxaflor influences the body size of workers of *B. terrestris* in a crop landscape. We found that 2 weeks of continuous exposure to Sulfoxaflor influenced workers' foraging dynamics and collection of resources. However, there was no evidence that the 5 ppb dose of the pesticide impacted the ability of bees to handle flowers with different traits. Workers from colonies exposed to Sulfoxaflor were smaller. The effect on worker size may be explained as a consequence of the reduced pollen income per unit of worker foraging. Thus, if the effects of Sulfoxaflor applied directly to crops had the same effect as that observed on commercial bumble bees after our chronic exposure, it might negatively impact colony success due to the impact on pollen collection and the reduction in the size of workers.

KEYWORDS

Bumble terrestris, flight dynamics, flower handling abilities, pollen metabarcoding, ruderal plants, bee size

Introduction

The ecosystem services provided by bees are among the most important for the reproduction of wild plants (Ollerton et al., 2011) and crop production (Klein et al., 2007; Giannini et al., 2015; Sagwe et al., 2021). Nutritional resources that bees collect from flowers (e.g., nectar, pollen) during flower visits are usually consumed to supply their own metabolic demand or carried into the nest to feed the young and larvae. The nutrition of immatures is often based on a mixture of nectar and pollen (Donkersley et al., 2017). The latter is rich in protein, lipids, and carbohydrates (Kendel and Zimmermann, 2020), important components for the development of the brood (Watrous et al., 2019).

The ability of a given bee to collect pollen from a flower may depend on the evolutionary process between bees and plants. Specialized bees, for example, may be adapted to collect resources only from a few floral types (Konzmann et al., 2020), while generalist species explore resources in a wide variety of flowers (Wilson et al., 2021). Among the generalist social bee species, such as *Bombus terrestris*, diet composition may depend on the environment including the presence of competitors (Balfour et al., 2015; Lihoreau et al., 2016). Some flowers can also present a set of floral traits (e.g., shape, color, and resources) that influence generalists' foraging behavior (Spaethe et al., 2001; Wood et al., 2021). Social bees (e.g., honey bees) are organisms performing complex tasks (Nityananda and Chittka, 2021) that together with their social communication work synergistically to mitigate the negative effects of landscape (Nürnbergberger et al., 2019). However, it has been shown that unhealthy or stressed bees are less efficient at performing social tasks (Boff et al., 2018; Crall et al., 2018; Raine, 2018). Among environmental stressors, we highlight systemic insecticides. These agrochemicals (e.g., neonicotinoids, sulfoximines, butenolides) target nicotinic acetylcholine receptors (nAChRs) in insect nervous systems which, depending on concentration and class, can impair common bee activities, such as pollination (Stanley et al., 2015a; Whitehorn et al., 2017), social communication (Boff et al., 2018), antennal sensitivity (Straub et al., 2021), thermoregulation (Tong et al., 2019), and memory (Williamson and Wright, 2013; Stanley et al., 2015b; Tison et al., 2019) but see Siviter et al. (2019).

Workers of *B. terrestris audax* exposed to the neonicotinoid Thiamethoxan displayed a different choice for floral resource collection compared to control bees (Stanley and Raine, 2016). Moreover, the number of manipulated flowers increased significantly for pesticide exposed bumble bees, suggesting an impact on maximum foraging ability (Stanley and Raine, 2016). The ability of bumble bee workers to handle tomato flowers was also impacted when they were exposed to Thiamethoxan. According to Whitehorn et al. (2017), the bees were less able to remove pollen from flowers by sonication, averaging about 50% less pollen transportation than control bees. Similarly,

Switzer and Combes (2016) found that chronic exposure to a dose of Imidacloprid, below LD50, significantly reduced the number of individuals performing sonication on tomato flowers. Additionally, workers exposed to the pesticide were less likely to perform additional sonication events (Switzer and Combes, 2016). Therefore, since colony development and individual health rely on diversified pollen feeding (Kaluza et al., 2018; Vaudo et al., 2018), impaired individual ability to collect pollen (Stanley et al., 2016; Switzer and Combes, 2016; Whitehorn et al., 2017) can have a negative impact on colony development and in the nutritional aspects of foragers and other members of the colony.

In addition to sonication on buzz pollination flowers, the sub-lethal effects of pesticides on bees include impairment of navigation (Fischer et al., 2014; Balbuena et al., 2015), flying, and homing (abilities to return to nest) (e.g., Stanley et al., 2016). For instance, acute (one time exposure) and chronic exposure to Thiamethoxam changed flight dynamics in honey bees (Tosi et al., 2017). While the acute dose increased flight distance, chronic exposure decreased flight duration, distance, and velocity. Similar impacts were recorded for bumble bee species exposed to a different Neonicotinoid. Bumble bee workers exposed to Imidacloprid were faster but achieved a shorter distance and reduced flight time in the flight cage experiment (Kenna et al., 2019). Recently, studies with a new acetylcholinesterase inhibitor pesticide (Sulfoxaflor) have shown that harmful effects on foraging are likely to vary with dosage and bee species (Siviter et al., 2018; Boff et al., 2021; Tamburini et al., 2021a,b). For example, in a previous study, flight dynamics of solitary mason bees were adversely affected when exposed to field-realistic doses of Sulfoxaflor (Boff et al., 2021). In this study, Sulfoxaflor had the effect of increasing the time necessary for a bee to land in a flower (Boff et al., 2021). Further studies, with honey bees (Tamburini et al., 2021b) and bumble bees (Tamburini et al., 2021a), showed that Sulfoxaflor may have species-specific effects, supporting previous reports of different bee species having different responses when exposed to the same pesticide (Cresswell et al., 2012; Arena and Sgolastra, 2014; Mayack and Boff, 2019; Azpiazu et al., 2021; Schmolke et al., 2021). A negative effect of Sulfoxaflor on non-commercial bumble bee colony development was suggested by the impact on reproductive output by lowering larvae (Linguadoca et al., 2021) and male production (Siviter et al., 2018), but did not appear to affect foraging and weight of pollen loads of workers (Siviter et al., 2018).

In the current study, we investigated the effect of a field-realistic dose of Sulfoxaflor on workers of commercial standard colonies of *B. terrestris* by recording (1) worker foraging activities, (2) the weight of their pollen loads, (3) and the plant species from which pollen grains were brought to the colonies. Foraging activities also included whether (4) workers exposed to Sulfoxaflor interacted with flowers with similar traits to those visited by unexposed workers; and

finally, (5) whether Sulfoxaflor influenced the body size of workers. Given the results of previous studies, we expected impairment of reproduction to explain the amount of collected resources and changes in collected plant species composition. Since impairment at different levels on foragers across bee species (Boff et al., 2021; Tamburini et al., 2021a,b) has been associated with this insecticide, studying the effect of Sulfoxaflor in commercial bumblebees is an important approach to the safety of pollinators and pollination outcomes in conventional agricultural environments late in the summer.

Materials and methods

Study area

The study site where bumblebee nests were located corresponds to the orchard farm of the University of Milan, at Arcagna (45° 20' 31"N, 9° 27' 5.18" E), Lodi province, at about 25 km away from the city center of Milan, Italy. The area is characterized by the presence of cultivars of different fruit species such as apple trees (1.5 ha), pear trees (1.0 ha), peach trees (5.0 ha), apricot trees (0.2 ha), plum trees (1.5 ha), cherry trees (0.15 ha) and other small fruits (0.15 ha). The study was performed in late summer (August 2019) when local fruit crop species were no longer blooming.

Pesticide preparation

In this study, we exposed commercial colonies of bumble bees *B. terrestris* to a field-realistic concentration of Sulfoxaflor according to the amount of the pesticide found in the nectar of cotton flowers, the United States Environmental Protection Agency (2016) and used in previous studies (e.g., Siviter et al., 2018; Boff et al., 2021). To reach a sucrose solution with a final dose of 5 ppb, 1.8 M sucrose solution (616.19 gr of sugar) was diluted in 1 L of distilled water. The stock solution of 1 mg of Sulfoxaflor was mixed with 100 ml of pure acetone (Medrzyck et al., 2021). The Sulfoxaflor solution of use (5 ppb, 5×10^{-6} gr Sulfoxaflor L^{-1}) was prepared by diluting 500 μ l of the stock solution in 1 L of the sucrose solution 1.8 M.

Colony treatment

Standard commercial colonies of *B. terrestris* ($n = 8$) type Natupol (Koppert) were delivered in a plastic cage set in a cardboard box. Two of the colonies were delivered approximately 2 weeks earlier than the rest. Bees from these two colonies were let free to forage at our experimental site. These two colonies (herein called old colonies) were brought back to the laboratory at night when the other six colonies were

delivered. In the laboratory, on the following day, one of the old colonies was added to the pool of Sulfoxaflor exposed colonies, and the other four (one old and three new) were assigned to the control. *Ad libitum* supply of 1.8 M sucrose solution with 5 ppb of Sulfoxaflor was made available to the treated colonies ($n = 4$) attaching a bag with 1.5 L of the mixed solution to the feeder at the bottom of the nest box. Control colonies were allowed access to *ad libitum* 1.8 M sucrose solution without Sulfoxaflor, plus an equivalent concentration of the acetone added in the treatment group. Colonies were maintained closed in a laboratory shielded from direct light exposure with a temperature set at 25°C for the period of 12 days (considered week 1 and week 2 of the experiment). Additionally, 16 g of commercial dry honeybee collected pollen were provided daily to the colonies. At the end of the 12th day, the colonies were transferred and positioned at the study site and the feeder containing sucrose solution with or without Sulfoxaflor was removed. The colonies were set in a covered area near the crops site (~10 m) circa 20 cm from the ground. Colonies exposed to Sulfoxaflor were set on one side of the covered area attached to a lateral wall with a 2 m distance between each colony. Control colonies were set on the other side of the covered area at a distance of circa 6 m from the closest Sulfoxaflor exposed colony (Supplementary Figure 1). The day after installing the colonies, in the morning, entrances were opened one by one in an interval of 30 min allowing bumblebees to forage. Once a week, the entrances of the colonies were closed at night. The following morning, before opening the colony entrances, the colonies were weighed. Weight of colonies did not differ across treatments throughout the experiment (LM, $F = 2.78$, $df = 1$, $p = 0.10$).

Flight activities

The number of individual foragers leaving each colony was counted twice a day (between 8 a.m. and 1 p.m.) for 5 min each time. The census was performed independently at each colony and repeated once a week for a total of 5 weeks, totaling 400 min of monitoring. Additionally, except for the first monitoring day (the third week of the experiment), the number of individuals returning to the nest and individuals returning with pollen were also recorded, totaling 320 min of observations.

Collection of pollen

After the census, between 2 and 4 p.m., workers returning to the hive with pollen loads were collected at the hive entrance, each week, during the 3rd, 4th, 5th, and 6th week, with the aid of tweezers. Captured workers were placed individually in a Petri dish (55 mm diameter) and maintained inside a styrofoam box with ice packs. Cooled immobilized workers ($n = 76$ for control and $n = 76$ for exposed colonies, $n_{\text{total}} = 152$, see Table 1

TABLE 1 Total and mean (\pm SD) number of individuals per colony from which pollen loads were analyzed.

Pollen weight	Number of individuals				Total
	Week 3	Week 4	Week 5	Week 6	
Control	16 (4 \pm 2)	20 (5 \pm 0.81)	20 (5 \pm 0.81)	20 (5 \pm 0.81)	76
Sulfoxaflor	17 (4.25 \pm 2.2)	21 (5.25 \pm 0.5)	19 (4.75 \pm 0.5)	19 (4.75 \pm 0.5)	76

for details of sample size) had their pollen loads removed with tweezers. The tweezers were washed with pure ethanol following heat sterilization after each pollen load removal. Pollen loads presented in the corbicula were placed in a sterile plastic tube, labeled, and transferred to a -80°C freezer in the laboratory. After the removal of pollen loads, workers were measured (see below), put back on the Petri dish, and released near their hive entrance. In the laboratory, pollen loads were lyophilized at -60°C and each pollen load from a given individual was individually weighed with a balance (precision 10^{-3}g).

Metabarcoding for identification of pollen grains

Metabarcoding followed strictly the strategy of Sickel et al. (2015) using the ITS2 region as suggested by Keller et al. (2015). Beside the biological samples ($n = 152$), negative PCR controls were added. Forward ITS-S2F (Chen et al., 2010) and reverse primers ITS4R (White et al., 1990) contained multiplex identifiers so each had a unique combination. Triplicate polymerase chain reactions were performed for each sample and marker using the following conditions: heat activation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min; followed by a final extension at 72°C for 10 min, holding at 10°C until storage. Amplification success was checked on a 1% agarose gel, using $2\ \mu\text{l}$ product and $4\ \mu\text{l}$ dye per sample, and electrophoresed at 100 V for 45 min. Amplicons were pooled by the sample in 96-well plates and concentrations were normalized using the SequalPrep Normalization Plate Kit (Invitrogen Corporation, CA). Samples were further pooled by plate (hereafter, “libraries”) and quantified with a Qubit (Invitrogen Corporation, CA) fluorometer. The final pooled library was spiked with 5% PhiX control. The library was sequenced on an Illumina MiSeq v2, 2×250 bp spiked with custom index, Read1 and Read2 sequencing primers as detailed in the protocol.

Bioinformatics followed mostly the protocol as described in Campos et al. (2021): subsequent filtering and data processing steps were carried out with VSEARCH (Rognes et al., 2016). First, forward and reverse reads were joined. We removed reads of low quality ($-\text{fastq_maxee } 1$) and subsequently removed reads with < 200 bp or > 500 bp. Second, filtered reads of all samples were dereplicated, denoised, and assigned to amplicon sequence

variants (ASVs). Sample-specific reads were then mapped to ASVs to generate count data for each sample and plant species. Subsequently, we classified the ASVs using global alignments and best hit selection (at least 97% identity), against pre-defined reference databases. For this, we used the BCdatabaser (Keller et al., 2020) to subset the NCBI database for the ITS2 marker and contain only reference sequences known to occur in the Milan wider region (see Bartolucci et al., 2018)¹ ASVs that remained unclassified were mapped against a global reference database (Ankenbrand et al., 2015). Lastly, SYNTAX was used to obtain a taxonomy of remaining unclassified reads as deep as possible to a maximum of genus level. Using the R package phyloseq (McMurdie and Holmes, 2013), we imported the final read abundance and taxonomic tables, normalized the read abundance by sample such that each sample totaled 100 (relative read abundances), and filtered taxa that represented less than 1% per sample.

Floral diet diversity

The final list of plant species identified using metabarcoding was investigated with regard to their species richness (just number of species) and composition in control and treated colonies.

Floral traits and resource exploitation

The most common plant species ($n = 40$) identified in the pollen diet of broods from control and treated colonies were investigated regarding their floral traits. The description of the floral shape was performed based on Faegri and Van Der Pijl (1979) and Machado and Lopes (2004). Floral shape types were the followings: tube, gullet, dish, brush, flag, bell-funnel, chamber, and inconspicuous (< 4 mm) (Faegri and Van Der Pijl, 1979; Machado and Lopes, 2004). The resource types were classified as predominantly nectar flower, pollen flower, or flower offering both resources simultaneously (Faegri and Van Der Pijl, 1979). Flower colors were classified according to human perception and classified as greenish, purple, red, pink, white, and yellow.

¹ <http://ossnat.biodiversita.lombardia.it>

Body size of workers

Workers returning from foraging with pollen loads were sized with a digital pachymeter by measuring the thorax size at the insertion point of wings. In the same manner, at the end of the experiment, workers were sized and remained frozen at -20°C .

Statistical analyses

Model fit to all General Linear Mixed Models (GLMM) was assessed with the package DHARMA (Hartig, 2020). All analyses were performed in R 1.4.1106 (R Core Team, 2018).

Flight activities

We used GLMM to test the effect of Sulfoxaflor exposure and its interaction with time (temporal effect, “weeks”) on the number of individuals leaving the nest, the number of individuals returning to the nest, and the proportion of individuals returning to the nest carrying pollen loads. In our models, colony identity was used as a random factor to account for multiple measurements from individuals from the same colony. A Poisson distribution of errors was used in the model for workers leaving and returning to the colonies. The proportion of individuals carrying pollen was arcsine square root transformed to avoid variance dependence (Gotelli and Ellison, 2013). For the proportion of individuals with pollen loads, we used a model with a negative binomial distribution of errors. Model selections were based on AIC comparisons. Models with delta AIC smaller than 2 were considered similar and selection was based on the lowest p -value.

Pollen collection

To test the effect of Sulfoxaflor exposure, temporal effects (weeks) and nest age on the weight of pollen loads sampled per worker, we used a GLMM with colony identity as a random factor to account for multiple measurements of multiple individuals from the same colony and a negative binomial distribution of errors.

Floral diet diversity

The number of plant species visited was compared among treatments and weeks of observation. The size of the workers and the dry weight of pollen grains were additionally considered dependent variables with nest identity and nest

age as random factors in a model with a negative binomial distribution of errors.

Distance-based redundancy analysis (dbRDA), a constrained ordination method, was used to quantify the proportion of variation in community composition in a site by species matrix. The matrix can be explained by variables represented in a site by an environmental data matrix and a matrix of spatial variables (Legendre and Legendre, 2012). Bray-Curtis distances (capscale function from vegan R package, Oksanen et al., 2022) were used to ordinate workers by floral species composition (identified from worker’s pollen load) considering pesticide treatment, age of the nest, and period (weeks) from the 3rd to 6th week. Permutational ANOVA was used to test the proportion of variance explained by each of these variables and the variance in the floral species composition.

Floral traits and resource exploitation

We used the procedure of dbRDA with Bray-Curtis distances and permutational ANOVA to test the proportion of variance explained by flower shape, color, and resources, as well as the variance in flowers, with respect to the composition of specific floral traits.

Body size of workers

We used a GLMM to test whether exposure to Sulfoxaflor during the immature phase (workers at larvae-feeding phase during pesticide exposure) affected the size of workers. In this model, the average body size of workers was compared between treatments taking into consideration the temporal effect (week) and the age of nests with colony identity used as a random factor with Gaussian and a log link function. Workers measured in week 3 and week 4 were removed from the model to mitigate a potential inclusion of individuals that were already adults at the beginning of the experiment. Model selections were performed based on AIC comparison with the ANOVA function.

Results

Flight activities

Both week and treatment affected the number of individuals leaving the colonies. More individuals were observed leaving treated colonies (mean \pm SD) 2.95 ± 2.41 5 min^{-1} relative to the control group 2.2 ± 1.98 5 min^{-1} (GLMM, $\chi^2 = 3.89$, $df = 1$, $p = 0.04$, Figure 1A). Overall, after week four, there was a reduction in the number of individuals leaving the colonies as the weeks progressed (GLMM, $\chi^2 = 10.42$, $df = 4$, $p = 0.005$, Figure 1A for Tukey HSD *post hoc* test). The number of

returning workers to control colonies $2.47 \pm 1.94 \text{ 5 min}^{-1}$ and to treated colonies $3.31 \pm 3.04 \text{ 5 min}^{-1}$ were not statistically different (GLMM, $\chi^2 = 2.0$, $df = 1$, $p = 0.157$, **Figure 1B**). Yet, the number of individuals returning to the colonies significantly decreased as the weeks progressed (GLMM, $\chi^2 = 48.73$, $df = 3$, $p < 0.001$, **Figure 1B** for Tukey HSD *post hoc* test). No significant difference between the number of individuals returning with pollen (average for control colonies $1.18 \pm 1.30 \text{ 5 min}^{-1}$, average for treated colonies $1.5 \pm 2.2 \text{ 5 min}^{-1}$) was found between treatments (GLMM, $\chi^2 = 0.52$, $df = 1$, $p = 0.468$, **Figure 1C**); the number of pollen loads collected by workers decreased as the week progressed (GLMM, $\chi^2 = 48.73$, $df = 3$, $p < 0.001$, **Figure 1C** for Tukey HSD *post hoc* test).

Pollen collection

Treatment and week affected the weight of pollen loads per forager brought back to the colonies. Bees brought heavier pollen loads back to the hive in the treated group $20.03 \pm 13 \text{ mg}$, relative to the untreated group $16.3 \pm 13 \text{ mg}$ (GLMM, $z = 1.991$, $p = 0.04$). The age of the nest had no effect on the weight of pollen load carried by the workers (GLMM, $\chi^2 = 2.83$, $df = 1$, $p = 0.09$); the period (weeks) had a significant effect on decreasing pollen weight (see **Supplementary Table 1**, Tukey HSD *post hoc* test).

Floral diet diversity

Only treatment influenced the number of plant species found in pollen loads. The number of plant species found in the pollen loads removed from the corbiculae ranged from one to 20 species. Workers from treated colonies visited (mean \pm SD) 5.95 ± 3.4 species per foraging event; while control bees visited (mean \pm SD) 5.0 ± 3.8 species; thus, Sulfoxaflor influenced the average number of plant species (alpha diversity; GLMM, $\chi^2 = 4.13$, $df = 1$, $p = 0.04$) identified in the pollen loads of workers from treated in comparison to control colonies. However, there were no effects of weeks, bee size, or pollen load weight on alpha diversity (**Table 2**). Only week affected the composition of plant species (dbRDA, $R^2_{\text{adj}} = 0.14$, $F = 8.03$, $df = 3$, $p = 0.001$, **Supplementary Figure 2**). There was no effect of treatment (dbRDA, $R^2_{\text{adj}} = 0$, $F = 0.53$, $df = 1$, $p = 0.76$) or nest age ($R^2_{\text{adj}} = 0.01$, $F = 1.99$, $df = 1$, $p = 0.07$) on the composition of plant species.

Floral traits and resources exploitation

Only week and nest age (but not treatment) affected the type of flowers bees interacted with. The combined floral traits of plant species making up the diet of controls and treated

colonies did not differ (dbRDA, $R^2_{\text{adj}} = 0$, $F = 0.52$, $df = 1$, $p = 0.76$). Floral traits differed between nests of different ages (dbRDA, $R^2_{\text{adj}} = 0.01$, $F = 2.47$, $df = 1$, $p = 0.04$) and over weeks (dbRDA, $R^2_{\text{adj}} = 0.14$, $F = 8.06$, $df = 3$, $p = 0.001$, **Supplementary Figure 3**).

The body size of workers

Treatment, age of the colony, and week affected the body size of workers. Sulfoxaflor exposure had an impact on the size of workers (GLMM, $\chi^2 = 4.82$, $df = 1$, $p = 0.02$). Workers exposed to Sulfoxaflor were significantly smaller (mean \pm SD) $5.76 \pm 1.27 \text{ mm}$ than workers from control colonies (mean \pm SD) $6.05 \pm 1.26 \text{ mm}$ ($t = 2.185$, $p = 0.02$, **Figure 2**). A reduction in size was observed in workers from older colonies (GLMM, $t = 2.482$, $p = 0.013$). We also found a temporal effect on the size of workers, with an increase in the size of the bees at the end of the experiment (GLMM, Tukey HSD *post hoc* test; 5th–6th weeks, $z = 1.691$, $p = 0.202$; 5th–7th weeks, $z = 8.112$, $p < 0.001$; 6th–7th weeks, $z = 9.604$, $p < 0.001$).

Discussion

We tested whether a realistic dose of 5 ppb of Sulfoxaflor influenced flight dynamics, foraging strategies, or affected body sizes of commercial bumble bees, *B. terrestris* during late European summer. We found that a field-realistic dose of Sulfoxaflor affected foraging activities, leading to a reduction in the amount of pollen per forager brought back to the colony. The insecticide did not affect the plant composition bees foraged on. The sub-lethal dose studied here did not impair the ability of workers to handle flowers with different traits. We found a reduction in the size of workers exposed to Sulfoxaflor during the larval period.

Sub-lethal doses of insecticide neonicotinoids often affect the flight behavior of bumble bees (Gill and Raine, 2014; Kenna et al., 2019), the duration of foraging trips for bumble and honey bees (Stanley et al., 2016; Prado et al., 2019, respectively), and the amount of pollen collected by the bumble bees (Gill et al., 2012; Feltham et al., 2014). Sublethal effects of these pesticides impact flight motivation (Lämsä et al., 2018), foraging ability (Gill et al., 2012; Feltham et al., 2014), and foraging performance (Colin et al., 2019), leading to reduced pollination efficiency (Stanley et al., 2015a; Whitehorn et al., 2017). In addition to neonicotinoids, the sub-lethal doses of sulfoxamine-based pesticides were recently found to impair the survival of wild bees (Azpiazu et al., 2021; Boff et al., 2021). Moreover, mason bees exposed to Sulfoxaflor fly longer distances and for a longer time in relation to the control bees during foraging (Boff et al., 2021).

Sulfoxaflor belongs to a class of pesticides that has been shown to impact the reproduction of bumble bees

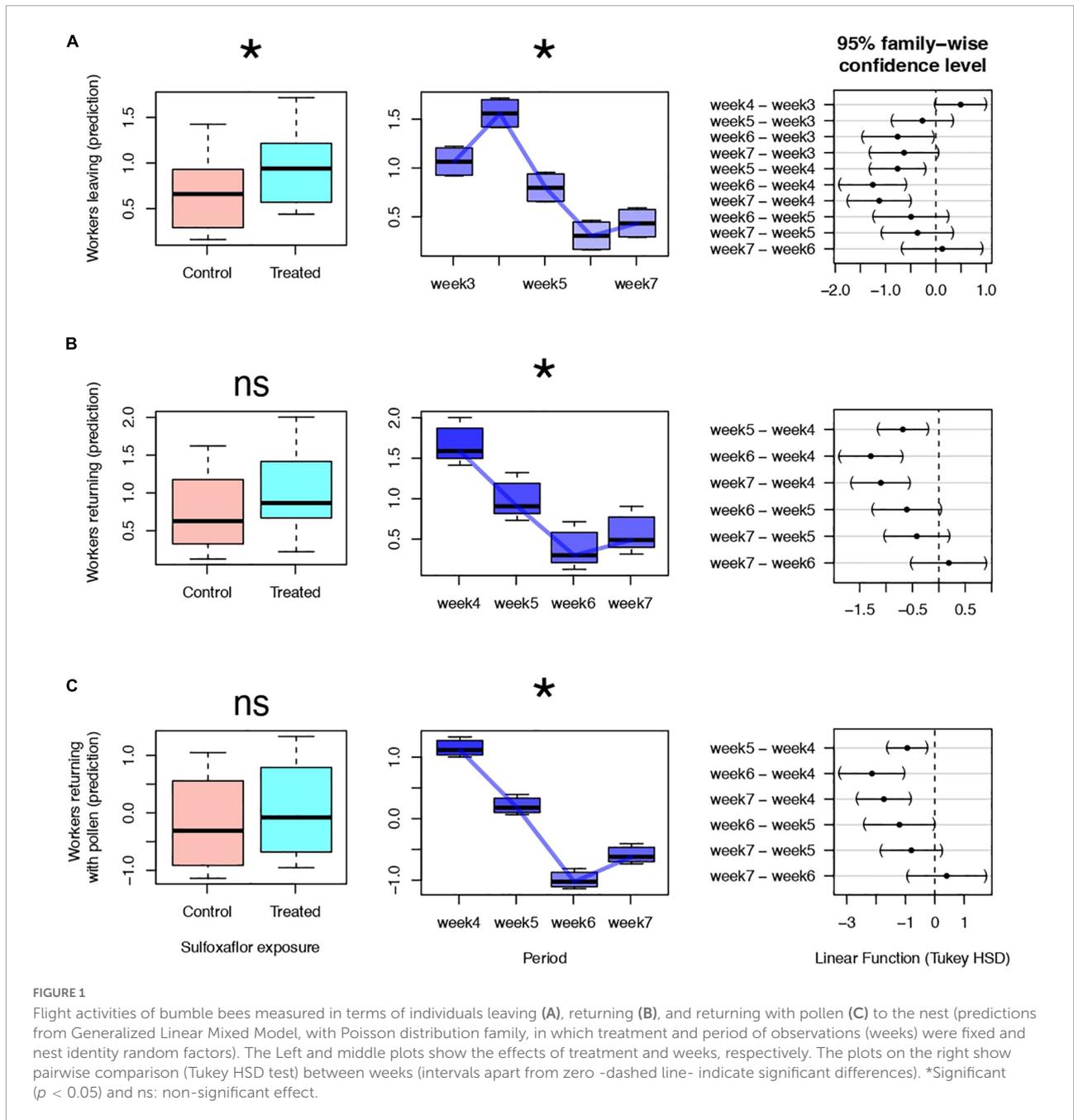


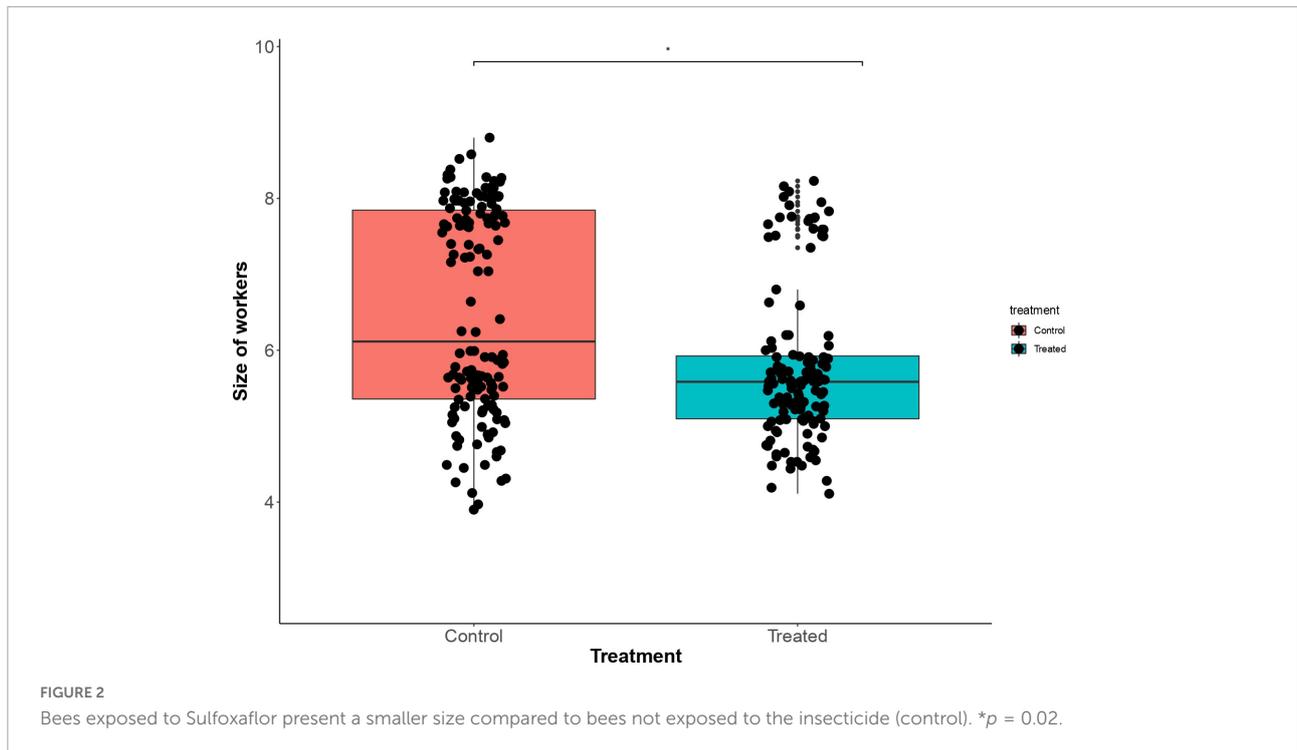
TABLE 2 Variables affecting alpha diversity of ruderal plant species visited by *Bombus terrestris* in late summer.

Variable	Number of plant species (Alpha diversity)			
	Sulfoxaflor	Week	Bee size	PL* weight
	$\chi^2 = 4.13, df = 1, p = 0.04$	$\chi^2 = 1.86, df = 2, p = 0.39$	$\chi^2 = 0.01, df = 1, p = 0.89$	$\chi^2 = 0.002, df = 2, p = 0.96$

*Pollen loads.

(Siviter et al., 2018; Linguadoca et al., 2021). For instance, in a previous study, colonies of non-commercial bumble bees exposed to 5 ppb of Sulfoxaflor (the same dose as tested

here) produced a smaller amount of males than control colonies (Siviter et al., 2018). In this study, Siviter et al. (2018) performed in an urbanized environment in England, exposure



to Sulfoxaflor did not affect the foraging behavior of workers. In the conventional farming area where the current study was performed, we did find an effect of Sulfoxaflor on the foraging of bumble bees. Indeed, agricultural areas may impose an extra impairment on bumble bees due to potential reduced floral diversity compared to rich flower areas in urban sites (Theodorou et al., 2016). Here, the colonies were similar in weight, independent of treatment, suggesting a similar number of workers, yet the number of foragers leaving the colonies exposed to Sulfoxaflor differed from the control colonies. Interestingly, the frequency of workers returning with pollen did not differ between treatments, indicating that bees exposed to Sulfoxaflor may have experienced an impact on foraging. A similar finding was reported earlier when bumble bee workers were exposed to Imidacloprid (Gill et al., 2012). These results suggest that individuals exposed to Imidacloprid and Sulfoxaflor need more time to return from foraging events due to impairment over navigation, as observed in bumble bees exposed to Imidacloprid (Gill and Raine, 2014), and bumble bees (current study), mason bees (Boff et al., 2021), and honey bees (Barascou et al., 2022) exposed to Sulfoxaflor. Moreover, pollen loads collected by workers from Sulfoxaflor exposed colonies were on average heavier, indicating the successful foragers in a colony are probably working harder, collecting more pollen to offset the poorer foraging performance of their nest mates. Additionally, a higher proportion of foragers carrying pollen from control compared to Sulfoxaflor treated colonies, suggests that pesticide-exposed colonies had to send out more foragers to bring in the same amount of pollen in

comparison with control colonies (see, Gill et al., 2012; Feltham et al., 2014).

Our current knowledge on the impact of Sulfoxaflor on bees is diverse, indicating these impairments may differ among species, the season, and the route of exposure; for example, Sulfoxaflor did not have an effect on the flight intensity of honey bees after administration of two different doses on cucumber flowers (Cheng et al., 2018). Similarly, no effect was found on honey bees foraging on *Phacelia* flowers exposed to Sulfoxaflor (Tamburini et al., 2021b). In a different study, the authors, using the same high recommended maximum rates of Sulfoxaflor (Tamburini et al., 2021b) found an impact on bumble bee flight activities (Tamburini et al., 2021a). The contrasting results between honey bees and bumble bees in sensitivity to the same pesticide, reinforce that sub-lethal effects of pesticides can be species-specific, as mentioned in previous studies on a variety of pesticides (Mayack and Boff, 2019; Azpiazu et al., 2021; Mundy-Heisz et al., 2022).

Bombus terrestris is a generalist flower visitor. Here, when set free to forage, the workers interacted with a wide diversity of ruderal flower types. According to previous studies, pesticides, such as Thiamethoxam and Imidacloprid, impact the foraging ability of bumble bees, likely reducing pollination of tomato flowers and collection of pollen (Switzer and Combes, 2016; Whitehorn et al., 2017). Sulfoxaflor at 5 ppb impaired the amount of pollen (in mg) brought to the colony in comparison to the control group; however, their ability to handle flowers of several traits might not have been impaired enough to be detected, as observed with bumble bees handling flowers

with poricidal anthers (Whitehorn et al., 2017). We found that bees visited a similar set of plant species, but workers from Sulfoxaflor exposed colonies explored on average a greater pollen diversity. It would be interesting to test in the future whether these differences resulted from a lower capacity of competition for the best source of resources compared to control bees and whether workers from treated colonies are able to identify potential income from new food sources to balance the lack of primary sources due to reduction in competitiveness. Here, since workers became smaller, it might be that they added additional sources of food, probably becoming relatively more corpulent (Couvillon et al., 2011; Kelemen et al., 2020), independent of floral architecture.

The observed reduction in the size of workers from treated colonies may be explained by two non-exclusive hypotheses. First, despite workers from Sulfoxaflor exposed colonies collecting heavier pollen loads, the extra amount collected per returning worker might not have been sufficient to compensate for the loss of pollen income per unit of a worker leaving the colony. Yet, a substantial loss of adult foragers in pesticide exposed colonies may be neglected since colony weight remained stable over time. For instance, of 10 workers leaving control colonies to forage, 60% were returning with pollen loads. These percentages decreased to 49% in workers from Sulfoxaflor exposed colonies. This finding is also supported by the significant interaction factor between treatment and the period after exposure (weeks). For instance, while the number of individuals leaving the colony reduced over time, it was always higher for Sulfoxaflor exposed colonies. Moreover, there was an interesting trend showing that while the number of workers returning with pollen remained mostly constant over time in the control colonies, pollen income decreased sharply in the first 3 weeks of monitoring for Sulfoxaflor exposed colonies. Second, exposing queens to Sulfoxaflor might have led to a reduction in egg size leading to a reduction in workers' size. Although the current study did not directly investigate effects on queen reproductive cells, egg plasticity in regard to size is known to occur in honey bees in response to environmental stressors (Amiri et al., 2020), therefore, it may possibly also be the case in bumble bees. For example, *B. terrestris* exposed chronically to Pyrethroid resulted in the production of smaller workers (Baron et al., 2014). Thus, in addition to impacting the reproductive output by reducing the production of males over time (Siviter et al., 2018), our study provides evidence of the impairment by Sulfoxaflor of reproductive potential by decreasing the size of workers but does not let us solve completely the mechanism driving the reduction in size. Nonetheless, bee size is correlated with flight distance ability, especially in the summer period when low local resources require significantly greater distances in foraging (Pope and Jha, 2018), reduced worker size may play an important role in colony success, especially in environments with low food quality (Osborne et al., 2008). Bees of smaller sizes patrol at smaller distances when compared to bigger bees and

therefore they could be more susceptible to higher costs and competition due to local resource constraints (Osborne et al., 2008). Moreover, since workers from the 5th and 6th weeks were in general smaller than workers from the 7th week, it is possible that the pollen diet offered as dry pellets in the laboratory phase had a negative effect on growth. A full four factorial experiment with pesticide and variable food quality (Straub et al., 2022) may help us, in the future, to understand the mechanism through which food quality can offset the effects of pesticides on bees (Klaus et al., 2021).

Conclusion

Sulfoxaflor at the field-realistic dose of 5 ppb disrupted the foraging output of workers of *B. terrestris* in an area with ruderal plants when forced feeding was imposed continuously for a period of 12 days. A reduction in the proportion of returning workers with pollen per unit of a worker leaving the colony suggests a deficit in pollen income. Workers from Sulfoxaflor exposed colonies carried heavier pollen loads, possibly to compensate for the reduced pollen income in treated colonies. Workers from all colonies foraged on plant populations of similar composition and handled flowers of similar architecture and resources. Thus, 5 ppb of Sulfoxaflor seems to not impose a barrier for bee-plant interaction in the study environment. Our study is limited to comprehending whether the impact on non-reproductive workers occurs only due to reduced pollen income or might also be due to an impact on queen reproductive cells.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

Author contributions

SB and DL designed the experiment and performed the data collection. SB, JR, and AK performed the data analysis. SB drafted the manuscript. SB, AK, JR, and DL contributed to the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.842563/full#supplementary-material>

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