Environmental Toxicology and Chemistry, 2025, **44(1),** 232–239
https://doi.org/10.1093/etojnl/vgae007
Advance access publication: January 6, 2025
Original Article

Hazard/Risk Assessment

Bumblebees prefer sulfoxaflor-contaminated food and show caste-specific differences in sulfoxaflor sensitivity

Sarah E. Orr[1,*], Jixiang Xu[1], Wanvimol C. Juneau[1], and Michael A.D. Goodisman [1]

[1]Georgia Institute of Technology, School of Biological Sciences, Atlanta, GA, United States

*Corresponding author: Sarah E. Orr. sorr8@gatech.edu

Abstract

More than 30% of human food crop yield requires animal pollination. In addition, successful crop production depends on agrochemicals to control pests. However, agrochemicals can have negative consequences on beneficial insect pollinators, such as bees. We investigated the effects of an emerging class of pesticides, sulfoximines, on the common eastern bumblebee, *Bombus impatiens*. We performed a series of 96-hour toxicity tests on microcolonies of laboratory-reared *B. impatiens*. Our data showed that sulfoxaflor (SFX) is significantly less toxic to *B. impatiens* than historically used neonicotinoid pesticides, such as thiamethoxam. Further, for the first time, we found significant differences among castes in sensitivity to SFX; workers and drones were more sensitive than queens. These findings are notable because they reveal both caste and sex-specific differences in bumblebee sensitivity to pesticides. Interestingly, we found no evidence that bumblebees avoid SFX-contaminated sugar syrup. To the contrary, *B. impatiens* workers had an apparent preference for SFX-contaminated sugar syrup over sugar syrup alone. Overall, our investigation provides novel information on an important pesticide and may help inform regulatory decisions regarding pesticide use.

Keywords: pesticides, terrestrial invertebrate toxicology, contaminants of emerging concern, environmental toxicology, hazard/risk assessment

Introduction

Most agricultural practices depend on agrochemicals to control pest insects and support human food supply. However, many agrochemicals are also toxic to nontarget wildlife, such as bees, that are essential for pollination activity (Potts et al., 2016). In particular, neonicotinoid chemicals are a historically important class of pesticides used in agriculture. However, because of the strong evidence of toxicity to nontarget insects (Blacquière & van der Steen, 2017; Dirilgen et al., 2023), most neonicotinoids (e.g., clothianidin, imidacloprid, and thiamethoxam [THX]) have been banned from outdoor agricultural use in the European Union. Nevertheless, neonicotinoid chemicals, although strictly regulated, are still used commonly to cultivate many crops across the United States. In fact, the United States Department of Agriculture (USDA) Pesticide Data Program found that 15.5% and 10.8% of fruits and vegetables sampled contained the residues of the neonicotinoids imidacloprid and THX, respectively (USDA, 2020).

Recently, sulfoxaflor (SFX; market names: Isoclast, Transform, Closer), has been marketed as a replacement for neonicotinoids. Sulfoximines, like neonicotinoids, function as an agonist at insect nicotinic acetylcholine receptors (nAChRs; Sparks et al., 2013). They are considered as effective at controlling pest insect populations as popular pesticides like imidacloprid and THX (Babcock et al., 2011). Sulfoximines are particularly effective against the growing populations of neonicotinoid-resistant pests. Moreover, sulfoximines have been shown to be less toxic on nontarget insect pollinators than historically used pesticides, like THX (Kenna et al., 2023; Mundy-Heisz et al., 2022; Watson et al., 2021). However, preliminary data suggest that SFX can still have negative impacts on survival, fecundity, and pollination efficiency in honeybees (Capela et al., 2022; El-Din et al., 2022), stingless bees (Albacete et al., 2023), and bumblebees (Boff et al., 2022; Kenna et al., 2023; Linguadoca et al., 2021; Siviter et al., 2018, 2020). Sulfoxaflor is used globally with varying levels of restrictions but was notably banned for outdoor use in Europe in 2022 (Directorate-General for Health and Food Safety, 2022).

Ecological protection from harmful chemicals is especially important for native and unmanaged insect pollinators that play essential roles in agricultural production. Interestingly, native bumblebees have shown greater sensitivity to SFX than honeybees (Farnan et al., 2023; Gradish et al., 2019; Mundy-Heisz et al., 2022). This result emphasizes the need to better understand how native bee populations will fare in a polluted environment. Further, research has shown that some species of bees are incapable of avoiding nectar spiked with certain neonicotinoids. In fact, some bees actually prefer spiked nectar, despite the fact that they consume less spiked nectar than control (Arce et al., 2018; Kenna et al., 2023; Kessler et al., 2015; Muth et al., 2020). Notably, *Bombus terrestris* bumblebees were not able to detect pesticides, including SFX, and consumed lethal concentrations of the pesticide in controlled experiments (Parkinson et al., 2023). This suggests that some pollinators are unable to avoid contaminated plants in nature. However, it is unknown whether other

Received: March 07, 2024. **Revised:** August 01, 2024. **Accepted:** August 26, 2024

bee species would behave similarly towards SFX-contaminated foods.

The common eastern bumblebee, *Bombus impatiens*, is a native bee species in North America that is particularly important for plants that require buzz-pollination, which cannot be performed by honeybees (*Apis sp.*; Drummond, 2012; Goulson, 2003). Buzz-pollination is required by over 15,000 plants, including many important food crops, such as tomatoes, eggplant, and blueberries (Buchmann, 1983). Further, *B. impatiens* is a highly social bee species characterized by division of labor within colonies and a phenotypically distinct caste system (Goulson, 2003). Annual colonies are typically headed by a single queen bee and supported by hundreds of mostly sterile workers (Wilson, 1971). These characteristics make *B. impatiens* an excellent model organism to study the effects of agrochemicals on native social insect pollinators.

In this study, we investigated the acute toxicity of the emerging pesticide SFX on *B. impatiens* bumblebees (Figure 1). We had three primary objectives in our investigation. First, we sought to document the toxicity of SFX compared to historically used THX in *B. impatiens* workers. Sulfoxaflor has been shown to be significantly less toxic to nontarget insects than neonicotinoids (Azpiazu et al., 2021; Kenna et al., 2023; Mundy-Heisz et al., 2022). We were interested in confirming if this was the case for *B. impatiens* under controlled laboratory conditions.

We also hypothesized that drones (males) may be more susceptible to SFX exposure than workers or queens. Social insect drones are historically understudied compared with workers and queens (Belsky et al., 2020). Drones are typically more sensitive to environmental stressors than their female counterparts and have been observed to have a significantly shorter life span (Amin et al., 2012). Further, drones are haploid and therefore may be less able to handle a toxic chemical exposure due to their limited levels of genetic variation (Kraaijeveld, 2009). Previous research has shown that drones are more sensitive to SFX exposure than workers and queens in *B. terrestris* (Linguadoca et al., 2022). Therefore, we tested the acute toxicity of SFX in all three castes of *B. impatiens*.

Finally, we quantified sugar syrup consumption and feeding preferences of microcolonies of *B. impatiens* workers exposed to sublethal concentrations of SFX. We predicted that bees may not be able to taste or distinguish between spiked and clean sugar syrup when given a choice between the two. Previous studies have demonstrated a lack of avoidance for pesticide-contaminated food in bumblebees (Muth et al., 2020) and hoverflies (Clem et al., 2020). Interestingly, a preference for contaminated food has been previously demonstrated in other bee species with various pesticides (Kenna et al., 2023; Kessler et al., 2015; Parkinson et al., 2023). However, *B. impatiens* preference for SFX has not yet been studied. Overall, this study has far-reaching agricultural implications regarding the use of SFX and the potential toxicological consequences on native bee populations.

Methods

Bumblebee husbandry

We obtained commercially produced, queenright colonies of *B. impatiens* from Plant Products (Leamington, ON, Canada). On receipt, colonies were placed at 4°C for an hour to slow bee movement and assist in handling. Then, we transferred each colony into a clean, 25 × 25 × 12 cm transparent acrylic cage with holes for ventilation. The colonies were immediately provided unlimited 50% sugar syrup through a reservoir with a wick and fresh pollen balls. Bee colonies were left in a warm, dark room (26.3°C ± 0.4; 41.4% rh ± 1.4). Temperature and relative humidity were measured every 30 minutes with a LogTag recorder (Auckland, New Zealand). Sugar syrup and pollen were changed weekly and were closely monitored for any bacterial and fungal growth. Bee manipulation was conducted under red light to prevent bees from flying and behaving aggressively.

Pesticide chemistry

We created stock solutions of pure THX and SFX obtained from ThermoFisher (Waltham, MA, USA) in diH_20. To create spiked sugar syrup, we performed a serial dilution of the stock solution into 50% sugar syrup. Preliminary experiments were conducted to identify the appropriate range of concentrations to use in acute toxicity tests. We then used the nominal concentrations of 3.00, 1.80, 1.50, 1.08, 0.65, 0.39, and 0 g/L SFX and 0.2, 0.1, 0.05, 0.025, 0.0125, and 0 g/L THX. The nominal concentration of the stock solutions and control sugar syrup was verified by AGQ Labs (Oxnard, CA, USA; Table 1) and used in all subsequent analyses.

Acute toxicity tests

To assess the acute toxicity of each pesticide, we performed 96-hour toxicity tests. Bees for tests were sourced from four different queenright colonies. Small experimental cages (15 × 15 × 5 cm) were prepped with clean sugar syrup and pollen (Figure 2). Worker experiments for SFX were performed with 15–24 bees per microcolony and three to four microcolonies per pesticide concentration. Worker experiments for THX were performed with six to eight bees per microcolony and seven to eight microcolonies per pesticide concentration. Worker bees were housed in microcolonies with sisters from the same source queenright colony. In total, 436 worker bees were used to determine SFX toxicity and 312 worker bees were used to determine THX toxicity. During the worker experiments, we used nontoxic paint on the thorax to distinguish bees from different colonies.

Queenright colonies were monitored closely, and callow reproductive bees were removed on observation. Young, unmated gynes (prereproductive queens, hereafter, queens) and males

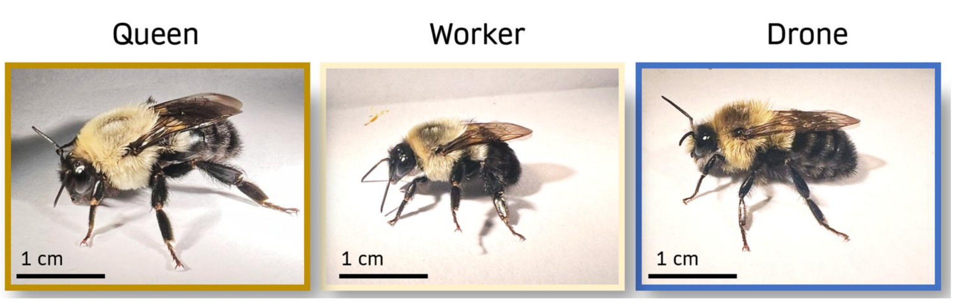

Figure 1. *Bombus impatiens* queen, worker, and drone castes.

Table 1. Nominal and measured concentrations of sulfoxaflor (SFX) and thiamethoxam (THX) used in toxicity experiments.

SFX nominal (mg/L)	SFX measured (mg/L)	THX nominal (mg/L)	THX measured (mg/L)
0	**0**	0	**0**
0.39	0.38	0.0125	0.01
0.65	0.63	0.025	0.02
1.08	1.04	0.05	0.04
1.5	1.45	0.1	0.08
1.8	1.74	0.2	**0.16**
3	**2.9**	-	-

Note: Bold values represent the stock solutions that were chemically verified.

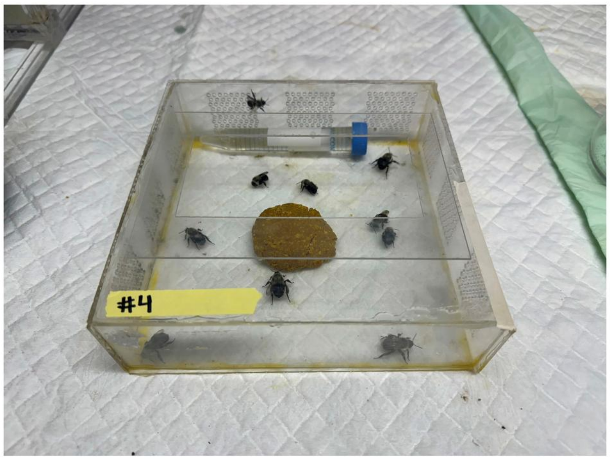

Figure 2. Example of experimental cage used for acute toxicity test of *B. impatiens* bees. A 15 mL tube of 60% sugar syrup and fresh pollen ball was provided for all microcolonies. The mass in the middle of the cage is the pollen ball that served as the base for the developing bumblebee nest.

(hereafter, drones) were housed separately. Sample size for queen experiments consisted of six to eight bees per microcolony, three to four microcolonies per pesticide concentration, and a total of 148 queen bees. Total sample size for drone experiments consisted of five to eight bees per microcolony, three to four microcolonies per pesticide concentration, and a total of 175 unmated drone bees.

Bees were allowed to acclimate to their new microcolonies for 72 ± 1 hours. The experiments began when tubes containing spiked sugar syrup were given to the appropriate cages. Bees were allowed to feed ad libitum on the spiked sugar syrup and clean pollen for 96 hours. Thus, bees were dosed based on the amount of sugar syrup they consumed. Every 24 hours, we monitored the microcolonies for mortalities, which were removed, and refilled sugar syrup and pollen as needed. After 96 hours, any survivors were euthanized via rapid cooling. Finally, we obtained the final wet mass for every bee in the experiment.

Feeding choice trials

To test the prediction that bees imbibe spiked and clean sugar syrup differentially, we created 18 microcolonies of eight to nine *B. impatiens* worker bees from four distinct queenright colonies. Thus, this experiment used a total of 150 worker bees. We provided each microcolony with fresh pollen and two feeding tubes: one with clean sugar syrup and one spiked with a low concentration of SFX (0.4 mg/L). We chose a sublethal concentration of SFX (0.4 mg/L) to prevent excessive mortality during these specific experiments. This concentration exceeded field concentrations (~0.05 mg/L; Siviter et al., 2018), because the goal of this experiment was to assess the feeding preferences of bumblebees at a physiological level without a field component. Bees were allowed to feed freely for 96 hours and tubes were weighed before and after the experiment to determine the amount of sugar syrup consumed from each tube. We also normalized data for number of bees per microcolony to quantify the average sugar syrup consumption per bee.

Data analysis

Statistical analyses were performed using JMP software. We also used the Environmental Protection Agency's (USEPA's, 2019) TRAP software to calculate 96-hour median lethal concentration (LC50) for each experiment (Erickson, 2020). We performed a student's t-test to test for differences between sensitivity between THX and SFX. Caste-specific sensitivity differences were tested by fitting a mixed effect model for time of death with SFX concentration, cage, and caste as fixed effects, and mass as a random effect. We also ran a one-way analysis of variance (ANOVA) between the caste-specific LC50 estimates to determine whether castes differed significantly in sensitivity to SFX. Finally, we used a matched pair's *t*-test to determine whether bees had a feeding preference between control and SFX-contaminated sugar syrup.

Results

THX and SFX acute toxicity in *B. impatiens* workers

Increased concentrations of both SFX and THX led to increased bee mortality (Figure 3). We found that the LC50s for worker *B. impatiens* bumblebees exposed to THX and SFX for 96 hours were 0.044 ± 0.0085 and 1.22 ± 0.038 mg/L, respectively (Table 2). A Student's *t*-test verified that these LC50s were significantly different ($t = 33.12$, $p < .0001$). Thus, SFX is more than 40-fold less toxic to *B. impatiens* bumblebee workers than THX.

Caste sensitivity differences in acute toxicity of SFX

Next, we determined the acute toxicity of SFX in queens, drones, and workers with 96-hour toxicity tests (Figure 3). Our model found significant differences in sensitivity among castes by comparing least square means among males, queens, and workers (LSMean = 51.1, 39.4, and 32.4, respectively, $p < .05$). Notably, queens were substantially larger than workers and drones; the mass ± SEM of queens, drones, and workers was 419.2 mg ± 10.4, 141.5 mg ± 3.8, and 142.8 mg ± 3.1, respectively. However, our mixed effects model revealed that wet mass did not explain caste-specific differences in SFX sensitivity ($p = .6937$).

Next, we found that the LC50s were 1.54 ± 0.041, 1.26 ± 0.071, and 1.22 ± 0.038 mg/L for queens, drones, and workers, respectively (Figure 4). We performed a one-way ANOVA among calculated LC50 values that revealed that queens were 20.0% more

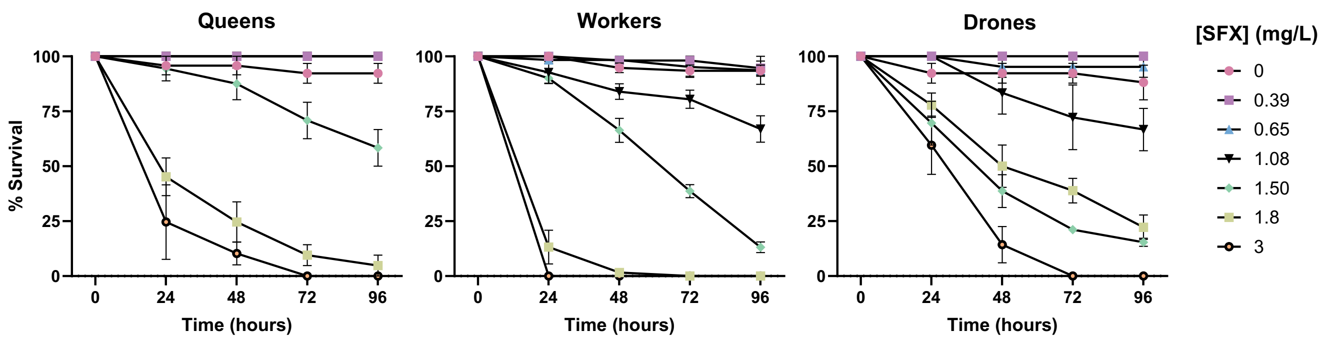

Figure 3. Percent survival of queen, male, and worker *B. impatiens* bees in microcolonies exposed to various concentrations of sulfoxaflor (SFX) over a 96-hour exposure period. Error bars represent standard error of the mean.

Table 2. Median lethal dose (LC50) for thiamethoxam and sulfoxaflor for bumblebee workers with lower (LCL) and upper (UCL) 95% confidence limits.

Pesticide	LC50 (mg/L)	LCL (mg/L)	UCL (mg/L)
Thiamethoxam	0.044	0.026	0.063
Sulfoxaflor	1.222	1.144	1.3

Note: Sulfoxaflor is significantly less toxic than thiamethoxam ($p < .0001$).

tolerant to SFX than workers and 23.2% more tolerant to SFX than drones ($F = 11.63$, $p = .006$, Figure 4).

Feeding choice trials

We investigated whether bees fed from pesticide-spiked and non-spiked sugar syrup with equal frequency in choice trials. Our goal was to understand whether bees showed evidence of being able to detect or avoid pesticide exposure. We found that microcolonies of worker bees did not avoid SFX-spiked sugar syrup. In fact, they preferred it. On average, bees drank 61.5% more SFX-spiked sugar syrup than control ($t = 1.973$, $p = .0325$, $n = 18$, Figure 5).

Discussion

The aim of this study was to investigate the caste-specific sensitivity and feeding preferences of the common eastern bumblebee, *B. impatiens*, to SFX. We found that SFX was significantly less toxic than THX to *B. impatiens* workers. Toxicity tests revealed that queens were significantly more tolerant to SFX than workers or drones. Finally, we showed that *B. impatiens* workers had an apparent preference for SFX-contaminated food, which has important implications for nontarget animal safety in agricultural practices.

SFX is less toxic than THX

Neonicotinoid pesticides have been used widely in agriculture since the 1990s. In particular, THX was used heavily as a promising pesticide to prevent pest infestations in important crops (Maienfisch et al., 2001). However, THX has also gained a reputation for killing nontarget insects, such as important pollinating bees (Gajger et al., 2017; Kozii et al., 2021; Laycock et al., 2014; Potts et al., 2010, 2016). The widespread use of THX has also led to resistant strains of pest insects (Feng et al., 2010; Khan et al., 2015). For these reasons, there has been a substantial push to formulate new pesticide chemicals with less nontarget insect toxicity.

SFX, approved for use by the USEPA in 2013, has been marketed as a replacement for harmful neonicotinoids in agriculture (Babcock et al., 2011). Interestingly, SFX shares a common mode of action to neonicotinoids (Linguadoca et al., 2021; Sparks et al., 2013; Watson et al., 2021). However, SFX has a similar level of efficacy on target pest insects to THX and is much less toxic to nontarget wildlife (Babcock et al., 2011; Mundy-Heisz et al., 2022). The difference in effects between SFX and neonicotinoids lies in the binding affinity to nAChRs; research has shown that SFX binds less strongly to nAChRs than THX (Sparks et al., 2013). Therefore, we aimed to quantify the acute toxicity of both SFX and THX in the native pollinator, *B. impatiens*.

We found that *B. impatiens* workers were much less sensitive to SFX than THX. This result is consistent with prior investigations. For example, a previous study found that *B. impatiens* had an 11-fold greater acute toxicity to THX than SFX (Mundy-Heisz et al., 2022). Our results demonstrate an even larger difference in LC50 (~40-fold). Notably, toxicity test methods varied between studies; our investigation allowed bees to feed ad libitum whereas Mundy-Heisz et al. fed bees prescribed dosed droplets. Regardless, taken together, SFX appears to be much less acutely toxic to *B. impatiens* than THX.

The risk that SFX may pose on bumblebees in the environment depends on how it is used and regulated. Sulfoxaflor is used globally with varying levels of environmental restrictions. For example, in Europe, SFX was banned from outdoor agricultural use by the European Commission in 2022 (Directorate-General for Health and Food Safety, 2022). Moreover, agencies attempt to prevent unintentional pollinator deaths by providing recommendations for applying pesticides depending on the time of day, temperature, timing of peak bloom, and more (European Food Safety Authority et al., 2022). In the United States, regulations require a maximum seasonal dose of 0.133 lbs/ac (~149 g/ha) along with many specifications for particular crops (USEPA, 2016). Therefore, bumblebees may be unintentionally exposed to toxic levels of SFX depending on how SFX is used in the environment.

Notably, other studies have documented that at sublethal concentrations, SFX may still impair the fecundity and foraging ability of nontarget insects (Siviter & Muth, 2020). For example, several studies reported a decrease in food consumption by bees when offered SFX-contaminated sugar syrup (Albacete et al., 2023; Kenna et al., 2023). Other investigations documented that SFX caused a reduction in bee foraging (Boff et al., 2022; El-Din et al., 2022) and homing ability (Capela et al., 2022). These findings suggest that pesticides may be detrimental to pollination activity in agriculture. Moreover, SFX also appears to impair egg laying and overall fecundity (Linguadoca et al., 2021; Siviter et al., 2020), which may be explained by a reduction in food consumption or an impairment in ovary development. Other groups,

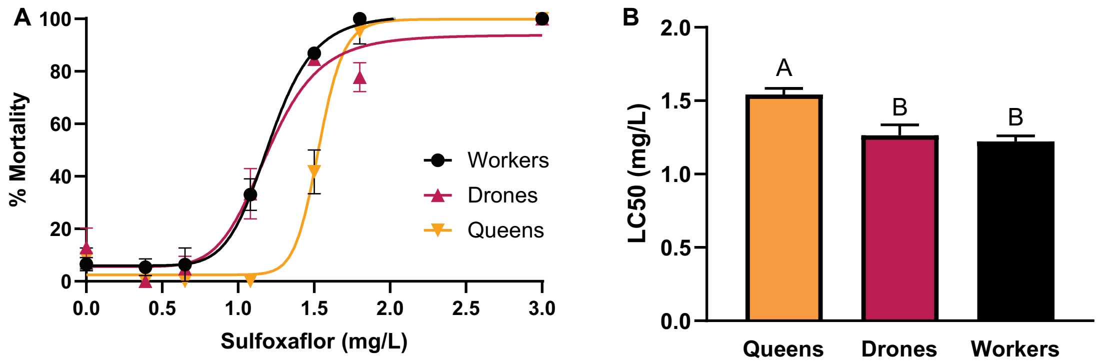

Figure 4. (A) Percent mortality and (B) lethal concentration 50% values (LC50s) of queen (orange), drone (black), and worker (maroon) bumblebees to sulfoxaflor (SFX). Queens are significantly more tolerant of SFX than drones or workers ($p = .006$). Error bars represent standard error of the mean.

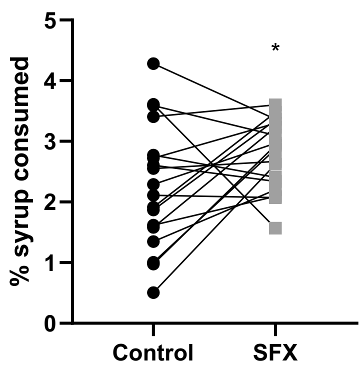

Figure 5. Percent sugar syrup consumed per bee in microcolonies of *B. impatiens* bees given a choice between clean sugar syrup (black) and sugar syrup spiked with 0.4 mg/L sulfoxaflor (SFX, gray). Lines connect values from the same microcolony. On average, bees fed from pesticide-spiked sugar syrup 61.5% more often when given a choice between spiked and clean sugar syrup ($p < .05$, $n = 18$).

however, have reported minimal or no effects of SFX on bee foraging or pollination efficiency in field studies (Straw et al., 2023; Tamburini et al., 2021). And SFX does not appear to influence olfactory conditioning, memory, or learning in bumblebees (Siviter et al., 2019; Vaughan et al., 2022). Clearly, more research is needed to fully understand the potential risks and sublethal impacts of SFX on beneficial pollinators.

Queens are more tolerant of SFX than workers or drones

Most pesticide toxicology research is performed using the most abundant caste of social insect colonies, workers. However, queens and drones are essential for future generational success, despite being less abundant (Belsky et al., 2020). Therefore, we prioritized assessing SFX sensitivity within all castes of *B. impatiens*.

We found that queens were significantly more tolerant to SFX than workers or drones when controlling for differences in mass. Our work is the first to report caste sensitivity differences to SFX in *B. impatiens*. We were somewhat surprised to find that drones were not more sensitive to SFX than workers. Previous work has shown that honeybee drones exposed to neonicotinoid pesticides during development were more susceptible than workers (Friedli et al., 2020). Our contrasting results are important because they show that castes respond differently to pesticides in a way that body mass alone cannot explain. The differences in caste sensitivity to SFX may result from differences in physiology between castes. Linguadoca et al. (2022) posited that the expression of cytochrome P450 enzymes in the fat stores of queens may improve the efficacy of detoxification for the queen caste (Alford, 1969; Costa et al., 2020). This metabolic variation may explain both the caste- and sex-specific differences found in our study. Future studies should explore the intraspecific physiological variation in bees to improve our understanding of caste and sex differences in pesticide sensitivity.

Other studies have also found caste differences in pesticide sensitivity. For example, *B. terrestris* queens were found to be more tolerant to SFX than drones or workers (Linguadoca et al., 2022). Moreover, honeybee queens were more tolerant to several acaricides than workers (Dahlgren et al., 2012). Other social insects have been used to demonstrate caste-specific differences to pesticide sensitivity. For example, thiamethoxam was found to lead to decreased body size in *Lasius niger* ant workers but not queens (Schläppi et al., 2021). Interestingly, SFX sensitivity also differed between honeybee worker type; foragers were more sensitive to SFX than nurses (Barascou et al., 2022). Overall, exposure to chemicals can affect different castes of the same species differently, which could have important effects on colony viability and reproductive capacity.

Interestingly, different castes may experience different dosing of pesticides because of their behavioral and developmental differences. New queens forage at the onset of a colony initiation for several weeks until workers are produced and able to take over foraging tasks. Queens may also be exposed through contact with contaminated soil during overwintering (Gradish et al., 2019). Drones may be exposed to chemicals later in the season when they leave to go on mating flights. Foraging workers are likely to be constantly exposed to chemicals in the environment throughout their life. In addition, all individuals within colonies are likely indirectly exposed by the sharing of food (trophallaxis) within colonies, which is a characteristic of virtually all social insects. Indirect exposure through trophallaxis is likely to be important to understanding toxicity in colonial organisms. Therefore, the higher sensitivity of workers and drones could indicate lethal consequences for both nonreproductive and reproductive castes of *B. impatiens*.

Bumblebees prefer SFX-contaminated food

We tested whether *B. impatiens* bumblebee workers preferred SFX-contaminated sugar syrup over uncontaminated control sugar syrup. We found that *B. impatiens* workers consumed more SFX-contaminated sugar syrup, indicating an apparent preference for contaminated food (Figure 5). Sulfoxaflor preference has been found in *B. terrestris* workers previously (Parkinson et al., 2023). Further, bees have frequently been found to prefer sugar syrup contaminated with pesticides (Arce et al., 2018, but see Muth et al., 2020; Kessler et al., 2015). These studies have also documented a decrease in overall consumption of pesticide-contaminated foods, which suggests that negative physiological consequences do not deter bees from consuming contaminated foods. Overall, our results support the growing body of literature suggesting that there may not be a safe level of pesticide usage if bees preferentially feed on contaminated food. Future studies could further pinpoint the range of SFX concentrations that this preference occurs including field realistic doses.

Electrophysiological experiments show that honeybees are not able to taste neonicotinoids with their gustatory neurons in their mouthparts (Kessler et al., 2015; Parkinson et al., 2023). Instead, SFX alters neurological pathways in the brain based on its target of nACHRs (Sparks et al., 2013; Watson et al., 2021). Indeed, SFX and other pesticides may act on cholinergic circuits and encode a reward learning behavior that occurs post ingestion (Arce et al., 2018; Barnstedt et al., 2016; Palmer et al., 2013; Parkinson et al., 2023). Taken together, it appears that bees may become addicted to pesticide-contaminated foods and preferentially feed on them. However, the exact physiological mechanism behind this preference remains unclear and should be a focus of future research.

Conclusion

In this study, we aimed to better understand the toxicological consequences of the pesticide SFX on the native social bumblebee, *B. impatiens*. We showed a dramatic 40-fold decrease in toxicity of bumblebee workers for SFX compared with the historically used neonicotinoid THX. We also demonstrated that queens were more tolerant of SFX compared to workers or drones, which had similar LC50s. This result suggests strong intraspecific variability to chemical sensitivity. Finally, we demonstrated that *B. impatiens* workers have a significant preference for SFX-contaminated food, which has important implications for agricultural production and risk management.

Data availability

Data is available at https://datadryad.org/stash/share/DD4YuW_NXbTgGjMa-mIdw4hEKM0ue8hGKa3OAUoRZKI.

Author contributions

Sarah E. Orr (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—Original draft, Writing—review and editing), Jixiang Xu (Data curation, Methodology), Wanvimol C. Juneau (Data curation, Methodology), Michael A.D. Goodisman (Funding acquisition, Project administrations, Resources, Supervision, Writing—review and editing)

Funding

Support for this research was provided by the United States Department of Agriculture National Institute of Food and Agriculture: 2023-67012-39886 to S.E. Orr; and National Science Foundation Divison of Environmental Biology: 2105033 and National Science Foundation Division of Integrative Organismal Systems: 2019799 to M.A.D. Goodisman.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors would like to acknowledge N. Hedrick, E. Bossard, and K. Murray for assistance with bee husbandry.

References

Albacete, S., Sancho, G., Azpiazu, C., Rodrigo, A., Molowny-Horas, R., Sgolastra, F., & Bosch, J. (2023). Bees exposed to climate change are more sensitive to pesticides. *Global Change Biology*, *29*, 6248–6260.

Alford, D. V. (1969). Studies on the fat-body of adult bumble bees. *Journal of Apicultural Research*, *8*, 37–48.

Amin, M. R., Bussière, L. F., & Goulson, D. (2012). Effects of male age and size on mating success in the bumblebee *Bombus terrestris*. *Journal of Insect Behavior*, *25*, 362–374.

Arce, A. N., Ramos Rodrigues, A., Yu, J., Colgan, T. J., Wurm, Y., & Gill, R. J. (2018). Foraging bumblebees acquire a preference for neonicotinoid-treated food with prolonged exposure. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20180655.

Azpiazu, C., Bosch, J., Bortolotti, L., Medrzycki, P., Teper, D., Molowny-Horas, R., & Sgolastra, F. (2021). Toxicity of the insecticide sulfoxaflor alone and in combination with the fungicide fluxapyroxad in three bee species. *Scientific Reports*, *11*, 6821.

Babcock, J. M., Gerwick, C. B., Huang, J. X., Loso, M. R., Nakamura, G., Nolting, S. P., Rogers, R. B., Sparks, T. C., Thomas, J., Watson, G. B., & Zhu, Y. (2011). Biological characterization of sulfoxaflor, a novel insecticide. *Pest Management Science*, *67*, 328–334.

Barascou, L., Sene, D., Le Conte, Y., & Alaux, C. (2022). Pesticide risk assessment: Honeybee workers are not all equal regarding the risk posed by exposure to pesticides. *Environmental Science and Pollution Research International*, *29*, 90328–90337.

Barnstedt, O., Owald, D., Felsenberg, J., Brain, R., Moszynski, J.-P., Talbot, C. B., Perrat, P. N., & Waddell, S. (2016). Memory-relevant mushroom body output synapses are cholinergic. *Neuron*, *89*, 1237–1247.

Belsky, J. E., Camp, A. A., & Lehmann, D. M. (2020). The importance of males to bumble bee (*Bombus* species) nest development and colony viability. *Insects*, *11*, 506.

Blacquière, T., & van der Steen, J. J. (2017). Three years of banning neonicotinoid insecticides based on sub-lethal effects: Can we expect to see effects on bees? *Pest Management Science*, *73*, 1299–1304.

Boff, S., Keller, A., Raizer, J., & Lupi, D. (2022). Decreased efficiency of pollen collection due to Sulfoxaflor exposure leads to a reduction in the size of bumble bee workers in late European summer. *Frontiers in Ecology and Evolution*, *10*

Buchmann, S. L. (1983). Buzz pollination in angiosperms. In C. E. Jones & R. J. Little (Eds.), *Handbook of Experimental Pollination Biology* (pp. 63–113). Van Nostrand Reinhold Company, New York.

Capela, N., Sarmento, A., Simões, S., Azevedo-Pereira, H. M. V. S., & Sousa, J. P. (2022). Sub-lethal doses of sulfoxaflor impair honey bee homing ability. *The Science of the Total Environment*, *837*, 155710.

Clem, C. S., Sparbanie, T. M., Luro, A. B., & Harmon-Threatt, A. N. (2020). Can anthophilous hover flies (Diptera: Syrphidae) discriminate neonicotinoid insecticides in sucrose solution? *PLoS One*, *15*, e0234820.

Costa, C. P., Duennes, M. A., Fisher, K., Der, J. P., Watrous, K. M., Okamoto, N., Yamanaka, N., & Woodard, S. H.,. (2020).

Transcriptome analysis reveals nutrition- and age-related patterns of gene expression in the fat body of pre-overwintering bumble bee queens. *Molecular Ecology*, *29*, 720–737.

Dahlgren, L., Johnson, R. M., Siegfried, B. D., & Ellis, M. D. (2012). Comparative toxicity of acaricides to honey bee (Hymenoptera: Apidae) workers and queens. *Journal of Economic Entomology*, *105*, 1895–1902.

Directorate-General for Health and Food Safety (2022). Sulfoxaflor: Commission restricts the use of harmful pesticide for pollinators. https://food.ec.europa.eu/news/sulfoxaflor-commission-restricts-use-harmful-pesticide-pollinators-2022-04-07_en

Dirilgen, T., Herbertsson, L., O'Reilly, A. D., Mahon, N., & Stanley, D. A. (2023). Moving past neonicotinoids and honeybees: A systematic review of existing research on other insecticides and bees. *Environmental Research*, *235*, 116612.

Drummond, F. (2012). Commercial bumble bee pollination of lowbush blueberry. *International Journal of Fruit Science*, *12*, 54–64.

El-Din, H. S., Helmy, W. S., Al Naggar, Y., & Ahmed, F. S. (2022). Chronic exposure to a field-realistic concentration of Closer® SC (24% sulfoxaflor) insecticide impacted the growth and foraging activity of honey bee colonies. *Apidologie*, *53*, 22.

Erickson, R. J. (2020). *Toxicity Relationship Analysis Program (TRAP)*. United States Environmental Protection Agency.

European Food Safety Authority, Bellisai, G., Bernasconi, G., Brancato, A., Carrasco Cabrera, L., Castellan, I., Ferreira, L., Giner, G., Greco, L., Jarrah, S., Leuschner, R., Oriol Magrans, J., Miron, I., Nave, S., Pedersen, R., Reich, H., Robinson, T., Ruocoo, S., Santos, M., Pia Scarlato, A., Theobal, A., & Verani, A. (2022). Modification of the existing maximum residue levels for sulfoxaflor in various crops. *EFSA Journal European Food Safety Authority*, *20*, e07283.

Farnan, H., Yeeles, P., & Lach, L. (2023). Sublethal doses of insecticide reduce thermal tolerance of a stingless bee and are not avoided in a resource choice test. *Royal Society Open Science*, *10*, 230949.

Feng, Y., Wu, Q., Wang, S., Chang, X., Xie, W., Xu, B., & Zhang, Y. (2010). Cross-resistance study and biochemical mechanisms of thiamethoxam resistance in B-biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science*, *66*, 313–318.

Friedli, A., Williams, G. R., Bruckner, S., Neumann, P., & Straub, L. (2020). The weakest link: Haploid honey bees are more susceptible to neonicotinoid insecticides. *Chemosphere*, *242*, 125145.

Gajger, I. T., Sakač, M., & Gregorc, A. (2017). Impact of thiamethoxam on honey bee queen (*Apis mellifera carnica*) reproductive morphology and physiology. *Bulletin of Environmental Contamination and Toxicology*, *99*, 297–302.

Goulson, D. (2003). *Bumblebees: Their behaviour and ecology*. Oxford University Press.

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

Kenna, D., Graystock, P., & Gill, R. J. (2023). Toxic temperatures: Bee behaviours exhibit divergent pesticide toxicity relationships with warming. *Global Change Biology*, *29*, 2981–2998.

Kessler, S., Tiedeken, E. J., Simcock, K. L., Derveau, S., Mitchell, J., Softley, S., Stout, J. C., & Wright, G. A. (2015). Bees prefer foods containing neonicotinoid pesticides. *Nature*, *521*, 74–76.

Khan, H. A. A., Akram, W., Iqbal, J., & Naeem-Ullah, U. (2015). Thiamethoxam resistance in the house fly, *Musca domestica* L.: Current status, resistance selection, cross-resistance potential and possible biochemical mechanisms. *PloS One*, *10*, e0125850.

Kozii, I. V., Barnsley, S., Silva, M. C. B. D., Wood, S. C., Klein, C. D., de Mattos, I. M., Zabrodski, M. W., Silva, R. d. C. M., Fabela, C. I. O., Guillemin, L., Dvylyuk, I., Ferrari, M. C. O., & Simko, E. (2021). Reproductive fitness of honey bee queens exposed to thiamethoxam during development. *Veterinary Pathology*, *58*, 1107–1118.

Kraaijeveld, K. (2009). Male genes with nowhere to hide; sexual conflict in haplodiploids. *Animal Biology*, *59*, 403–415.

Laycock, I., Cotterell, K. C., O'Shea-Wheller, T. A., & Cresswell, J. E. (2014). Effects of the neonicotinoid pesticide thiamethoxam at field-realistic levels on microcolonies of *Bombus terrestris* worker bumble bees. *Ecotoxicology and Environmental Safety*, *100*, 153–158.

Linguadoca, A., Jürison, M., Hellström, S., Straw, E. A., Šima, P., Karise, R., Costa, C., Serra, G., Colombo, R., Paxton, R. J., Mänd, M., & Brown, M. J. F. (2022). Intra-specific variation in sensitivity of *Bombus terrestris* and *Osmia bicornis* to three pesticides. *Scientific Reports*, *12*, 17311.

Linguadoca, A., Rizzi, C., Villa, S., & Brown, M. J. F. (2021). Sulfoxaflor and nutritional deficiency synergistically reduce survival and fecundity in bumblebees. *The Science of the Total Environment*, *795*, 148680.

Maienfisch, P., Angst, M., Brandl, F., Fischer, W., Hofer, D., Kayser, H., Kobel, W., Rindlisbacher, A., Senn, R., Steinemann, A., & Widmer, H. (2001). Chemistry and biology of thiamethoxam: A second generation neonicotinoid. *Pest Management Science*, *57*, 906–913.

Mundy-Heisz, K. A., Prosser, R. S., & Raine, N. E. (2022). Acute oral toxicity and risks of four classes of systemic insecticide to the common eastern bumblebee (*Bombus impatiens*). *Chemosphere*, *295*, 133771.

Muth, F., Gaxiola, R. L., & Leonard, A. S. (2020). No evidence for neonicotinoid preferences in the bumblebee *Bombus impatiens*. *Royal Society Open Science*, *7*, 191883.

Palmer, M. J., Moffat, C., Saranzewa, N., Harvey, J., Wright, G. A., & Connolly, C. N. (2013). Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees. *Nature Communications*, *4*, 1634.

Parkinson, R. H., Jennifer, S., Anna, D., Hannah, J., Martha, H., R Alex, M., & Wright, G. (2023). Bumblebee mouthparts exhibit poor acuity for the detection of pesticides in nectar. *eLife*, *18*, RP89129.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology & Evolution*, *25*, 345–353.

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to human well-being. *Nature*, *540*, 220–229.

Schläppi, D., Kettler, N., Glauser, G., Straub, L., Yañez, O., & Neumann, P. (2021). Varying impact of neonicotinoid insecticide and acute bee paralysis virus across castes and colonies of black garden ants, *Lasius niger* (Hymenoptera: Formicidae). *Scientific Reports*, *11*, 20500.

Siviter, H., Brown, M. J. F., & Leadbeater, E. (2018). Sulfoxaflor exposure reduces bumblebee reproductive success. *Nature*, *561*, 109–112.

Siviter, H., Scott, A., Pasquier, G., Pull, C. D., Brown, M. J. F., & Leadbeater, E. (2019). No evidence for negative impacts of acute sulfoxaflor exposure on bee olfactory conditioning or working memory. *PeerJ*, *7*, e7208.

Siviter, H., Horner, J., Brown, M. J. F., & Leadbeater, E. (2020). Sulfoxaflor exposure reduces egg laying in bumblebees *Bombus terrestris*. *The Journal of Applied Ecology*, *57*, 160–169.

Siviter, H., & Muth, F. (2020). Do novel insecticides pose a threat to beneficial insects? *Proceedings Biological Sciences*, *287*, 20201265.

Sparks, T. C., Watson, G. B., Loso, M. R., Geng, C., Babcock, J. M., & Thomas, J. D. (2013). Sulfoxaflor and the sulfoximine insecticides:

Chemistry, mode of action and basis for efficacy on resistant insects. *Pesticide Biochemistry and Physiology*, *107*, 1–7.

Straw, E. A., Cini, E., Gold, H., Linguadoca, A., Mayne, C., Rockx, J., Brown, M. J. F., Garratt, M. P. D., Potts, S. G., & Senapathi, D. (2023). Neither sulfoxaflor, *Crithidia bombi*, nor their combination impact bumble bee colony development or field bean pollination. *Scientific Reports*, *13*, 16462.

Tamburini, G., Wintermantel, D., Allan, M. J., Dean, R. R., Knauer, A., Albrecht, M., & Klein, A.-M. (2021). Sulfoxaflor insecticide and azoxystrobin fungicide have no major impact on honeybees in a realistic-exposure semi-field experiment. *The Science of the Total Environment*, *778*, 146084.

United States Environmental Protection Agency (USEPA). (2019). Sulfoxaflor. Overviews and Factsheets. https://www.regulations.gov/document/EPA-HQ-OPP-2010-0889-0570. Date accessed March 1, 2024.

United States Department of Agriculture (USDA). (2020). Pesticide Data Program. Agricultural Marketing Service. https://www.ams.usda.gov/sites/default/files/media/2020PDPAnnualSummary.pdf. Date accessed March 1, 2024.

Vaughan, O. P., Straw, E. A., Linguadoca, A., & Brown, M. J. F. (2022). No effect of dual exposure to sulfoxaflor and a trypanosome parasite on bumblebee olfactory learning. *Scientific Reports*, *12*, 8611.

Watson, G. B., Siebert, M. W., Wang, N. X., Loso, M. R., & Sparks, T. C. (2021). Sulfoxaflor—a sulfoximine insecticide: Review and analysis of mode of action, resistance and cross-resistance. *Pesticide Biochemistry and Physiology*, *178*, 104924.

Wilson, E. O. (1971). *The insect societies*. Harvard University Press.

Environmental Toxicology and Chemistry, 2025, 44, 232–239
https://doi.org/10.1093/etojnl/vgae007
Original Article