



Effects of chronic exposure to the new insecticide sulfoxaflor in combination with a SDHI fungicide in a solitary bee



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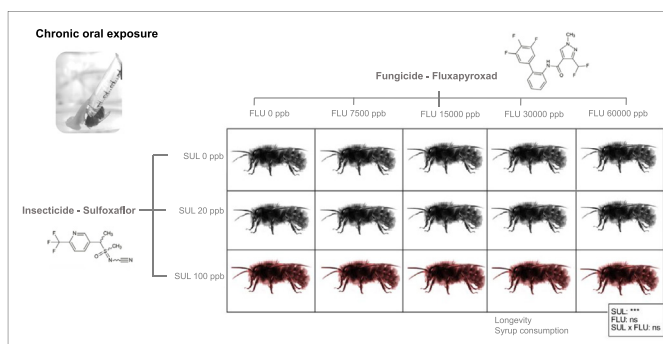
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HIGHLIGHTS

- Chronic oral exposure to 100 ppb of sulfoxaflor reduces the survival of *Osmia bicornis*.
- The chronic exposure up to 60,000 ppb of fluxapyroxad does not reduce bee survival.
- Syrup consumption increases at 20 ppb of sulfoxaflor and decreases at 100 ppb.
- We found no synergistic interaction between sulfoxaflor and fluxapyroxad.

GRAPHICAL ABSTRACT



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ABSTRACT

The recent EU ban of the three most widely used neonicotinoids (imidacloprid, thiamethoxam and clothianidin) to all outdoors applications has stimulated the introduction of new insecticides into the market. Sulfoxaflor is a new systemic insecticide that, like neonicotinoids, acts as a modulator of nicotinic acetylcholine receptors. In agro-environments, bees can be exposed to this compound via contaminated pollen and nectar for long periods of time. Therefore, it is important to assess the potential effects of chronic exposure to sulfoxaflor, alone and in combination with fungicides, on pollinators. In this study, we tested the effects of chronic exposure to two field concentrations of sulfoxaflor (20 and 100 ppb) alone and in combination with four concentrations of the fungicide fluxapyroxad (7500, 15,000, 30,000 and 60,000 ppb) on syrup consumption and longevity in females of the solitary bee *Osmia bicornis* L. Exposure to 20 ppb of sulfoxaflor, alone and in combination with the fungicide, stimulated syrup consumption, but did not affect longevity. In contrast, syrup consumption decreased in bees exposed to 100 ppb, all of which died after 2–6 days of exposure. We found no evidence of synergism between the two compounds at any of the two sulfoxaflor concentrations tested. Comparison of our findings with the literature, confirms that *O. bicornis* is more sensitive to sulfoxaflor than honey bees. Our results highlight the need to include different bee species in risk assessment schemes.

1. Introduction

The last decades have seen dramatic declines in wild bee diversity at local and regional scales (Bartomeus et al., 2013; Biesmeijer et al., 2006; Ollerton et al., 2014) together with unusual honeybee colony losses in various parts of the world (Chauzat et al., 2013; Lee et al., 2015). Although

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these declines are caused by a combination of factors, evidence suggests that the use of pesticides is one of the main drivers (Goulson et al., 2015). Among the various pesticide groups, neonicotinoid insecticides, which have been widely used to control insect pests since the 1990s, have raised particular concern (Godfray et al., 2015; Goulson, 2013; Maini et al., 2010; Sgolastra et al., 2020; Woodcock et al., 2017). As a result, the outdoors use of three neonicotinoids -clothianidin, imidacloprid, and thiamethoxam- has been banned in the EU and restricted in some areas of the US. Along with the appearance of pest resistance to neonicotinoids (Bass et al., 2015), these restrictions have prompted the search for new insecticides. One of the substances that is being considered as a potential alternative to neonicotinoids is sulfoxaflor, a newly registered sulfoximine insecticide. Like neonicotinoids, sulfoxaflor targets the insect nervous system, acting as a selective agonist of Nicotinic Acetyl Choline Receptors (NACHRs) (Sparks et al., 2013; Zhu et al., 2011). However, based on different NACHR binding sites and the absence of an sp³ nitrogen (Brown et al., 2016; Casida, 2018; Matsuda et al., 2020), sulfoxaflor and neonicotinoids belong to different IRAC (Insecticide Resistance Action Committee) categories (4C and 4A, respectively).

At the time this study was conducted (2019), regulation on the use of sulfoxaflor varied widely across European countries. The product was banned in France (ANSES, 2019) and could not be used outdoors in the UK (Corteva UK, 2021). However, in other countries, such as Ireland, Italy and Spain, its outdoors use was allowed up to 5–6 days before bloom (Corteva Ireland, 2021; Corteva Italy, 2021; Corteva Spain, 2021). Later, a report from the European Food Safety Authority report on sulfoxaflor risk assessment (EFSA, 2020a) identified this compound as highly hazardous to two social bee species, the western honey bee (*Apis mellifera* L.) and the buff-tailed bumblebee (*Bombus terrestris* L.). Then, in April 2022, the European Commission imposed further restrictions on the use of sulfoxaflor which is now only allowed in permanent greenhouses (OJEU, 2022). In the USA, sulfoxaflor applications during flowering are forbidden in some crops, such as pome fruits, citrus and berries, but allowed in others, such as cucurbits, tomato and strawberries (Corteva US, 2022; US EPA, 2019). In Australia and South Africa applications are also allowed during bloom (Corteva Australia, 2022; Corteva South Africa, 2022).

Some studies have shown that long term exposure to field-realistic doses of sulfoxaflor, can affect egg-laying rates, colony growth, foraging performance, and reproductive success in bumblebees (Siviter et al., 2019, 2018; Tamburini et al., 2021a). However, the impact of sulfoxaflor on solitary bees has received less attention. A recent study has shown that the solitary bee *Osmia bicornis* L. is much more sensitive to acute sulfoxaflor exposure than both honey bees and bumblebees (Azpiazu et al., 2021). Another study, using the same bee species in the laboratory, shows that long-term exposure to sulfoxaflor at field-realistic doses impairs survival, foraging performance and flower visitation rate (Boff et al., 2021).

In agricultural environments, bees are often exposed to pesticide combinations, rather than single compounds. Pollen and nectar from insect-pollinated crops (Heller et al., 2020; Kubik et al., 2000; Skerl et al., 2010), and adjacent wildflowers (Botías et al., 2015; Krupke et al., 2012) often contain residues of multiple pesticides. Yet, risk assessment protocols target single compounds, thus potentially underestimating the risks posed by pesticide mixtures, which may result in synergistic toxic effects (Gill et al., 2012; Sgolastra et al., 2017, 2018; Siviter et al., 2021). Bee exposure to pesticide mixtures is particularly likely to occur when insect-pollinated crops whose pollen and nectar contain residues of pre-bloom systemic insecticides (such as sulfoxaflor) are sprayed during bloom with fungicides. Fluxapyroxad is a succinate dehydrogenase inhibitor fungicide (SDHI) that is considered non-harmful to bees. For this reason, it is routinely sprayed during bloom on many insect-pollinated crops that are also treated with sulfoxaflor, including apple, pear, peach, melon, tomato or strawberry (EFSA, 2020b; US EPA, 2019). Therefore, co-exposure with sulfoxaflor is likely in those countries in which outdoor application is still allowed. Fluxapyroxad has experienced a rapid growth in the agricultural pesticide market (Chaulagain et al., 2019; Fernández-Ortuño et al., 2017; Sierotzki

and Scalliet, 2013) and has been found in several bee matrices (Simon-Delso et al., 2017).

In a previous study we showed that oral exposure of sulfoxaflor in combination with fluxapyroxad resulted in synergistic effects in *O. bicornis* and *A. mellifera*, but not in *B. terrestris* (Azpiazu et al., 2021). Bees in that study were acutely exposed. However, under field conditions bees may be exposed to pesticides for long periods of time, either due to persistence of the compounds in the environment or to multiple applications in the same or in different fields within the bee foraging range. In the current study, we test the combined toxicity effects of sulfoxaflor and fluxapyroxad through chronic oral exposure, by means of evaluation of two endpoints: longevity and syrup consumption. Longevity is ecologically relevant because it is strongly related to fecundity in *Osmia* spp. (Bosch and Vicens, 2006; Sgolastra et al., 2016). Syrup consumption, on the other hand, is a necessary measure to calculate the amounts of active ingredient ingested. In addition, pesticides may have a repellent effect (Barascou et al., 2021; Siviter et al., 2019; Thompson and Wilkins, 2003), but also an attractant effect (Baron et al., 2017; Kessler et al., 2015; Sgolastra et al., 2018), thus affecting syrup consumption. We have three objectives: 1) To measure the effects of chronic exposure to sulfoxaflor on syrup consumption and longevity in a solitary bee; 2) To determine whether the synergistic effects between sulfoxaflor and fluxapyroxad observed in previous studies are maintained when the two compounds are administered chronically *ad libitum*; 3) If so, to establish whether synergism is dose-dependent.

2. Materials and methods

2.1. Bee population and test conditions

We worked with a local *O. bicornis* population reared in a semi-natural area of La Garrotxa (Girona, NE Spain). Palynological analysis of the provisions showed that females collected mostly oak pollen. Adult-containing cocoons were wintered at 3–4 °C until May when large cocoons (presumed to contain females) were incubated in the laboratory at 22–23 °C until emergence. Because emergence time influences sensitivity to pesticides (females taking longer to emerge are more sensitive; Sgolastra et al., 2018), we only used bees that emerged over three consecutive days during the peak of the emergence period (days 5–7 of incubation). Upon emergence (<24 h), bees were transferred to a Plexiglas holding cage (50 × 50 × 50 cm) for ca. 4 h so they could deposit their meconium. Then, bees were individually placed in plastic ice cream cups (Maximum diameter: 8 cm; height: 7 cm) capped with transparent pin-perforated lids. Each cup contained a 1-ml calibrated syringe (Tuberculin Beroject® III, Beromed; accuracy: 0.01 ml) filled with syrup and inserted through the lid. The syrup was prepared by diluting sucrose in distilled water (33 % w/w). A petal of *Euryops* (Asteraceae) was attached to the tip of the syringe to enhance the prompt location of the feeder by the bee. Cups were maintained at 22 ± 2 °C and 50–70 % relative humidity and received indirect natural light throughout the experiment.

Females emerging on any given day were evenly distributed among 16 treatments (compounds added to the syrup): water control (water), solvent control (acetone 1.3 %), two sulfoxaflor concentrations (20 and 100 ppb), four fluxapyroxad concentrations (7500, 15,000, 30,000 and 60,000 ppb; geometric series factor of 2), and the combinations of the two sulfoxaflor and the four fluxapyroxad concentrations (eight combinations). The test concentrations of sulfoxaflor correspond to residue levels found in honeybee collected nectar 1–5 days after spray application in cotton (20 ppb) (US EPA, 2016), and to residue levels found in cucumber flowers 6 days after application (100 ppb) (Cheng et al., 2018). The two concentrations are also within the range of concentrations found in nectar in other field studies (US EPA, 2010, 2019). The fluxapyroxad concentrations tested range from mean levels of SDHI fungicides found in different bee matrices (7500 ppb; Stejskalová et al., 2018; Wallner, 2010) to the application rate of fluxapyroxad commercial formulate Sercadis® (60,000 ppb). Bees were exposed *ad libitum* to their respective treatments until they died.

The fluxapyroxad stock solution was prepared by diluting 100 µl of Sercadis® (300 g of a.i./L, BASF S.A.) in 50 ml of purified distilled water. For sulfoxaflor (purity 98 %; A2S Analytical Standard Solution) a stock solution with a concentration of 0.88 mg sulfoxaflor/mL acetone was used. These solutions were diluted in the syrup to reach the desired concentrations. The final concentration of acetone in the feeding solution was adjusted to 1.3 % (v:v) in all treatments (except for the water control) by adding pure acetone.

Cups were inspected daily to monitor syrup consumption (assessed by checking the level of syrup in the calibrated syringe) and bee mortality. Three additional containers without bees were used as controls to measure and account for changes in syrup levels due to evaporation. Bees that consumed <10 µL during the first 2 days of the exposure were considered non-feeders and were excluded from the analyses. The initial sample size was 15 females per treatment. However, as many as 26.6 % of the bees exposed did not reach the established feeding threshold, which reduced sample sizes to an average of 11 bees per treatment (range: 7–15; Table 1). Future studies should increase initial sample sizes to account for non-feeding individuals. The syrup was renewed every 3–4 days. Once a bee died, it was frozen (–20 °C) for later measurement of the head width under a stereomicroscope at 240×. Head width is a good proxy of body size in *Osmia* (Bosch and Vicens, 2002).

2.2. Statistical analysis

We used a generalized linear model (GLM) to analyse the effect of treatment on longevity. We accounted for overdispersion by fitting a negative binomial error distribution and using a log link function. We used sulfoxaflor concentration (0, 20 and 100 ppb) and fluxapyroxad concentration (0, 7500, 15,000, 30,000 and 60,000 ppb) and their interaction as continuous fixed effect factors, and body size as a covariate. We tested the significance of the main effects using the likelihood ratio test ($p < 0.05$). To determine the main effects of sulfoxaflor concentration (continuous fixed factor, three levels), fluxapyroxad concentration (continuous fixed factor, five levels) and their interaction on syrup consumption, log-transformed daily syrup consumption was analysed using a linear mixed-effect model (LMM) with the sampling dates as the repeated measures factor, the bee as the random factor and body size as a covariate. To avoid the effects of declining syrup consumption due to bee ageing, we only used data up to the day on which 50 % mortality of the control was reached. Because body size can affect pesticide sensitivity (Thompson, 2016) and food consumption (Azpiazu et al., 2019; Sgolastra et al., 2018) it was added as a covariate in our analysis. Linearly independent pairwise comparisons of estimated marginal means were performed using the Fisher least significant difference (LSD) test ($p < 0.05$). To analyse differences in the total and daily amount of sulfoxaflor ingested among treatments

containing this compound, we used the non-parametric Kruskal–Wallis test followed by Dunn's test with Bonferroni correction for specific pairwise comparisons. Statistical analyses were conducted with IBM SPSS Statistics 26.0 software (Chicago, IL, USA).

3. Results

3.1. Longevity

Longevity was affected by sulfoxaflor concentration (GLM: $\chi^2 = 287.0$, $df = 2$; $p < 0.001$), but not by fluxapyroxad concentration (GLM: $\chi^2 = 1.0$, $df = 4$; $p = 0.907$) or the sulfoxaflor-fluxapyroxad interaction (GLM: $\chi^2 = 7.1$, $df = 8$; $p = 0.527$). Body size had no effect on longevity (GLM: $\chi^2 = 0.456$, $df = 1$; $p = 0.499$). Mean longevity in treatments with sulfoxaflor at 100 ppb was 3.5–4 days, compared to 13–29 days in treatments without sulfoxaflor (Fig. 1).

3.2. Syrup consumption

Daily syrup consumption was affected by sulfoxaflor concentration (LMM: $F = 11.2$; $df = 2$, 231.5; $p < 0.001$; Fig. 2), but not by fluxapyroxad concentration (LMM: $F = 0.3$, $df = 4$, 279.4; $p = 0.884$; Fig. 2), or their interaction (LMM: $F = 1.5$, $df = 8$, 244.4; $p = 0.172$; Fig. 2). Compared to the control, bees consumed more syrup at sulfoxaflor 20 ppb and less at 100 ppb (Fig. 2). Body size had no effect on daily syrup consumption (LMM: $F = 0.5$, $df = 1$, 239.8; $p = 0.501$; Fig. 2).

3.3. Amount of sulfoxaflor ingested

The mean total amount of sulfoxaflor ingested by bees throughout the entire exposure period did not differ among treatments (Kruskal–Wallis: $\chi^2 = 4.84$, $df = 9$, $p = 0.848$; Table 1). However, we found differences in the mean daily sulfoxaflor dose ingested (Kruskal–Wallis: $\chi^2 = 77.86$, $df = 9$, $p < 0.001$; Table 1), which was lower in bees of the 20 ppb sulfoxaflor treatments compared to bees of the 100 ppb sulfoxaflor treatments (Table 1). Individual patterns of daily sulfoxaflor intake fluctuated widely, ranging from 0 to 22 ng in bees exposed to the 100 ppb sulfoxaflor concentration, and from 0 to 5 ng in bees exposed the 20 ppb concentration (Fig. 3).

4. Discussion

We evaluated the chronic toxicity of sulfoxaflor at two concentrations likely to be encountered in flowers in agricultural environments alone and in combination with a SDHI fungicide in the solitary bee *O. bicornis*. We found reduced longevity at the higher sulfoxaflor concentration (100

Table 1

Mean \pm SE and maximum total and daily amount of sulfoxaflor (SUL) and fluxapyroxad (FLU) ingested via syrup in *O. bicornis* females chronically exposed to two SUL concentrations (20 and 100 ppb) alone and in combination with four FLU concentrations (7500, 15,000, 30,000 and 60,000 ppb).

Treatment	N	SUL ingested		FLU ingested	
		Total (mean ng·bee ⁻¹)	Daily (mean ng·bee ⁻¹ ·day ⁻¹)	Total (mean µg·bee ⁻¹)	Daily (mean µg·bee ⁻¹ ·day ⁻¹)
SUL 20	12	16.52 \pm 6.24 a	0.91 \pm 0.16 ab		
SUL 20 + FLU 7500	13	17.73 \pm 6.19 a	0.74 \pm 0.13 a	6.62 \pm 2.31	0.28 \pm 0.05
SUL 20 + FLU 15000	14	17.68 \pm 3.99 a	0.88 \pm 0.13 a	13.26 \pm 2.99	0.66 \pm 0.10
SUL 20 + FLU 30000	13	15.46 \pm 3.99 a	0.95 \pm 0.12 ab	23.15 \pm 5.97	1.42 \pm 0.18
SUL 20 + FLU 60000	15	20.87 \pm 3.44 a	0.83 \pm 0.09 a	62.64 \pm 10.32	2.48 \pm 0.27
SUL 100	10	20.25 \pm 5.98 a	5.35 \pm 1.09 c		
SUL 100 + FLU 7500	12	13.37 \pm 3.38 a	4.58 \pm 0.72 c	1.01 \pm 0.25	0.26 \pm 0.05
SUL 100 + FLU 15000	9	11.78 \pm 2.97 a	3.09 \pm 0.64 bc	1.77 \pm 0.45	0.41 \pm 0.07
SUL 100 + FLU 30000	10	11.60 \pm 2.89 a	2.71 \pm 0.44 bc	3.49 \pm 0.87	0.93 \pm 0.19
SUL 100 + FLU 60000	12	16.08 \pm 2.95 a	3.43 \pm 0.72 c	9.67 \pm 1.77	2.75 \pm 0.43
FLU 7500	10			8.84 \pm 2.18	0.28 \pm 0.02
FLU 15000	9			11.27 \pm 4.23	0.45 \pm 0.09
FLU 30000	7			31.20 \pm 11.90	1.03 \pm 0.26
FLU 60000	10			38.18 \pm 13.98	1.87 \pm 0.27

Different letters within a column indicate significant differences ($p < 0.05$) according to Dunn's test with Bonferroni correction.

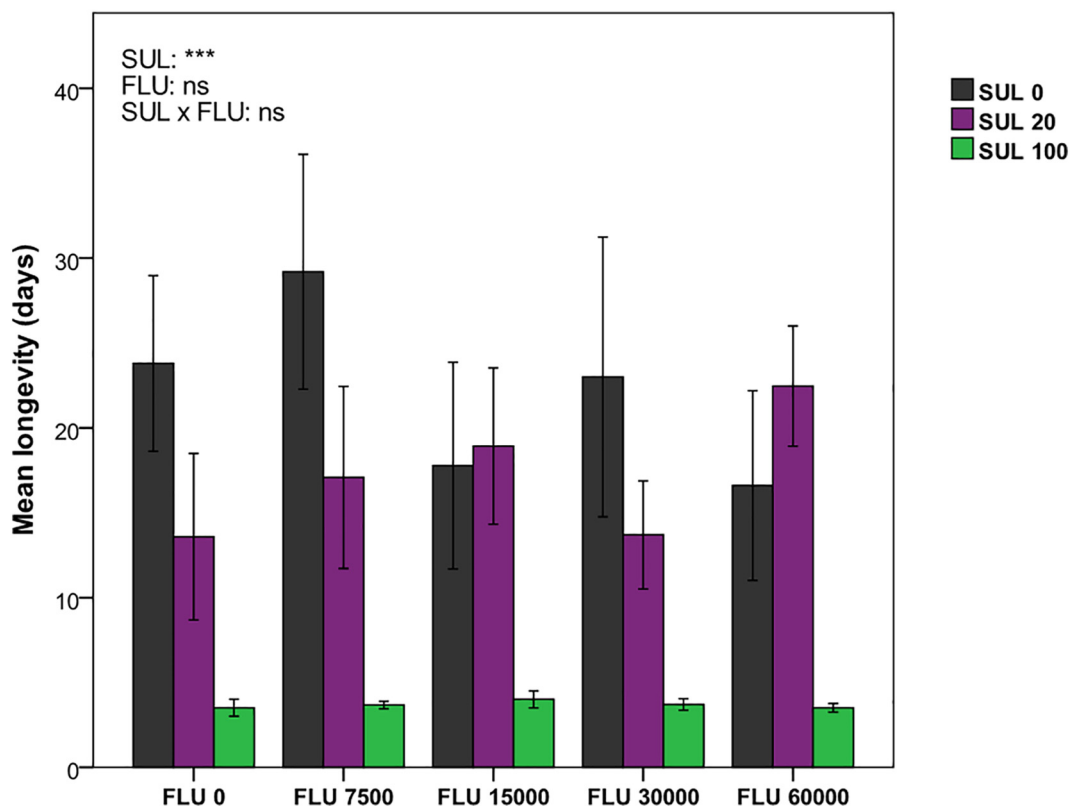


Fig. 1. Mean \pm SE longevity of *O. bicornis* females orally and chronically exposed to three field-realistic concentrations of sulfoxaflor (SUL: 0, 20 and 100 ppb) alone and in combination with 5 concentrations of fluxapyroxad (FLU: 0, 7500, 15,000, 30,000 and 60,000 ppb). *p*-Values are from generalized lineal model (** $p < 0.001$; ns, non-significant). Body size was included as a covariate.

ppb), but no interaction with fluxapyroxad at any tested concentration. Mean longevity of bees chronically exposed to sulfoxaflor at 20 ppb (13–23 days) was not significantly different from that of the controls (20–26 days), and similar to mean longevity of *Osmia* females nesting in greenhouses (16.2 days; Sgolastra et al., 2016) and in the field (17.3–30.5

days; Bosch and Vicens, 2006). By contrast, the longevity of *Osmia* females exposed to sulfoxaflor at 100 ppb was drastically reduced (100 % mortality after 2–6 days). Bees exposed to the 100 ppb treatments ingested total amounts of sulfoxaflor (13.31–23.22 ng) that were similar to those ingested by bees exposed to the 20 ppb treatments (17.67–23.91 ng), but over a

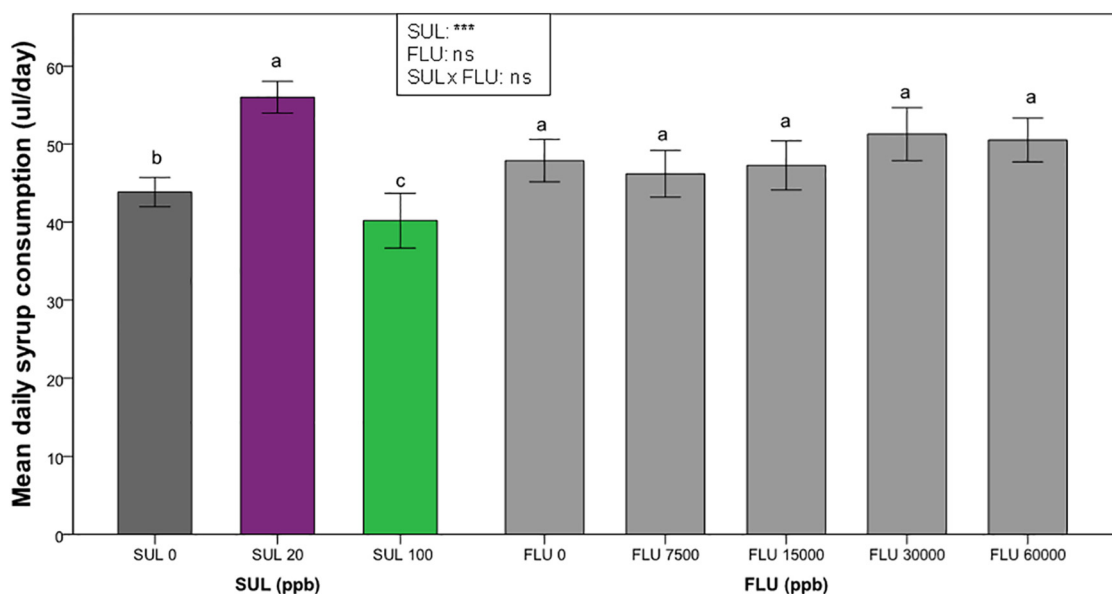


Fig. 2. Mean \pm SE daily syrup consumption (up to the day on which 50 % mortality of control bees was reached) in *O. bicornis* females orally and chronically exposed to three field-realistic concentrations of sulfoxaflor (SUL: 0, 20 and 100 ppb) alone and in combination with the 5 concentrations of fluxapyroxad (FLU: 0, 7500, 15,000, 30,000 and 60,000 ppb). *p*-Values are from linear mixed-effect model (** $p < 0.001$; ns: non-significant). Body size was included as a covariate and sampling dates as the repeated measures factor. Means with the same letter are not significantly different (Fisher's LSD post hoc; $p < 0.05$).

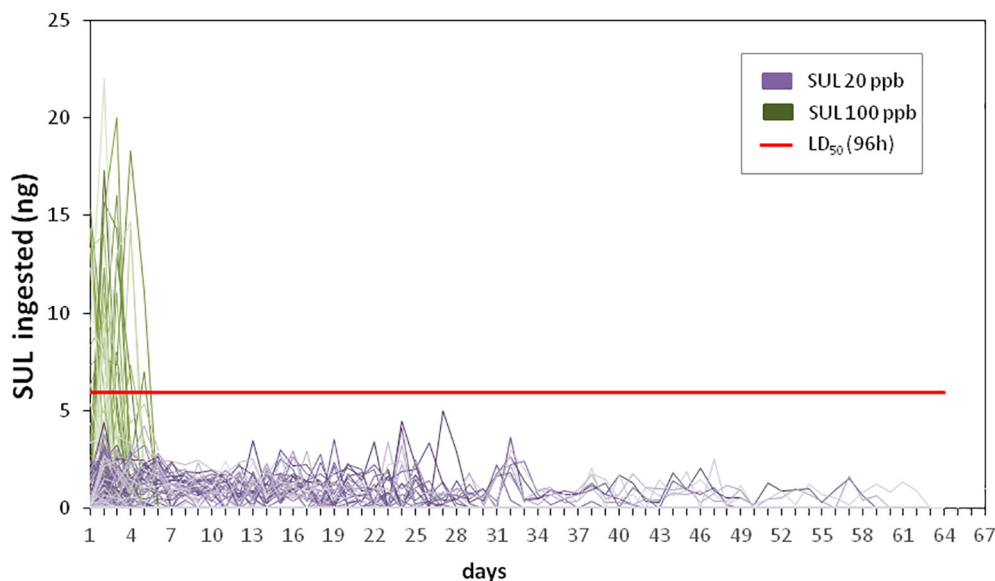


Fig. 3. Individual daily amounts of sulfoxaflor ingested by *O. bicornis* females chronically exposed via syrup to two sulfoxaflor concentrations (20 and 100 ppb). The red line indicates the LD₅₀ of sulfoxaflor at 96 h (Azpiazu et al., 2021).

shorter period of time. Mean daily dose of sulfoxaflor ingested by bees of the 100 ppb treatment (3.11–5.25 ng; Table 1) did not exceed acute LD₅₀ values found in *O. bicornis* for sulfoxaflor (5.9 ng/bee at 96 h; Azpiazu et al., 2021). However, daily syrup consumption fluctuated widely (see also Azpiazu et al., 2019), and as a result most bees of the 100 ppb treatment ingested levels of sulfoxaflor well above this dose (Fig. 3). On the other hand, bees of the 20 ppb treatment never reached ingestion levels over the LD₅₀ value (Fig. 3).

We detected no effects on longevity at any of the five fluxapyroxad tested concentrations (7500, 15,000, 30,000 and 60,000 ppb), alone or in combination with sulfoxaflor at 20 ppb. Depending on the treatment, the total amount of fluxapyroxad ingested was 6.62–62.64 µg/bee and the mean daily ingested dose was 0.28–2.48 µg/bee (Table 1). These doses are much lower than the acute oral LD₅₀ of fluxapyroxad reported for honey bees (110.9 µg/bee; EFSA, 2012).

Longevity of bees exposed to sulfoxaflor, either at 20 or 100 ppb, did not decrease with the addition of fluxapyroxad at any of the concentrations tested. Therefore, our results provide no evidence of interaction between these two compounds. This is in contrast to a previous study in which we found synergism between these two pesticides in *O. bicornis* after acute oral exposure (Azpiazu et al., 2021). In that study, synergism was tested with sulfoxaflor doses ranging from 2.75 to 88 ng/bee combined with a single fluxapyroxad dose (1200 ng/bee) and synergism was observed only at intermediate insecticide doses (11 ng/bee). Our ability to detect potential synergism under chronic exposure in the current study may have been hindered by the choice of test concentrations, with 20 ppb being too low to cause mortality, and 100 ppb quickly yielding high levels of mortality irrespective of the addition of fluxapyroxad. The various fluxapyroxad concentrations tested, on the other hand, did not influence mortality at any of the two sulfoxaflor concentrations. Therefore, future studies should address intermediate insecticide concentrations at the expense of fungicide concentrations.

Our study shows that sulfoxaflor affects feeding behaviour in *O. bicornis* by increasing (at 20 ppb) and decreasing (at 100 ppb) syrup consumption compared to control bees. This result is in partial agreement with previous studies which found inhibitory effects of sulfoxaflor in bumblebees (Siviter et al., 2019) and honey bees (Barascou et al., 2021; Li et al., 2021). Similar results have been found in *A. mellifera* (Zhu et al., 2017), *B. terrestris* (Laycock et al., 2012; Thompson et al., 2015) and *O. bicornis* (Azpiazu et al., 2019) exposed to neonicotinoids. For this group of insecticides, some studies have demonstrated that bees prefer solutions containing low

concentrations of neonicotinoids indicating that feeding behaviour is dose-dependent (Kessler et al., 2015; Sgolastra et al., 2018).

Bees in our study were chronically exposed to pre-determined concentrations of sulfoxaflor and fluxapyroxad, an approach that does not account for pesticide degradation, which effectively decreases pesticide concentration over time (Cheng et al., 2018; Kyriakopoulou et al., 2017; US EPA, 2019). However, levels of sulfoxaflor higher than 20 ppb (the lowest concentration we tested) have been found in the nectar of several crop flowers up to 14 days after bloom application (US EPA, 2019). Bees of the 100 ppb treatment in our study lived for 2–6 days and, again, levels of sulfoxaflor above this concentration have been found in the nectar of some crop flowers up to one week after application (US EPA, 2019). A recent study (Schwarz et al., 2022) in which *Phacelia* plants were treated with sulfoxaflor before bloom showed levels of 74–129 ppb in pollen 5–8 days after the application. Sulfoxaflor levels in nectar were not measured in that study, but they are usually lower than levels found in pollen (US EPA, 2019). The use of sulfoxaflor during bloom is still allowed in many countries (Corteva Australia, 2022; Corteva South Africa, 2022; Corteva US, 2022) and wild flowers growing near field crops may also become contaminated from sprays conducted outside of the blooming period of the target crop (Botías et al., 2015).

Comparisons of pesticide sensitivity across species need to be interpreted with caution because, due to differences in biological traits, experimental laboratory conditions cannot be exactly the same for different species (Sgolastra et al., 2019). For example, honey bee toxicity tests are usually conducted under group feeding conditions at 25 ± 2 °C in darkness (OECD, 2017). In contrast, *Osmia* spp. need to be individually fed and tests are usually conducted at lower temperatures (22 ± 2 °C) under light (Ladurner et al., 2003; Sgolastra et al., 2018; this study). Accepting this caveat, and assuming that experimental conditions have been tailored to mimic appropriate environmental conditions for each species, *Osmia* spp. appear to be more sensitive to sulfoxaflor than honey bees and bumblebees. Chronic exposure to 100 ppb strongly reduced *O. bicornis* survival in our study. By contrast, no increased mortality was observed in *A. mellifera* workers chronically exposed to 500 ppb for 10 days (EFSA, 2020a). This conclusion holds when the daily doses of sulfoxaflor ingested by the two species are compared (*O. bicornis*: 2.71–5.35 ng-*bee*⁻¹·*day*⁻¹; *A. mellifera*: 11.5 ng-*bee*⁻¹·*day*⁻¹, EFSA, 2020a), and after correcting for body weight (*O. bicornis*: 29.46–58.15 ng-*g*⁻¹·*day*⁻¹; *A. mellifera*: 125 ng-*g*⁻¹·*day*⁻¹). Other studies on honey bees also found no effects on survival after 6 days of exposure to either 20 ppb or 2000 ppb (Barascou et al., 2021), and

exposure to 47 ppb for 30 days (Al Naggar and Paxton, 2021). A previous study calculated the LD₅₀ of *O. bicornis* acutely exposed to sulfoxaflor and also concluded that this species was more sensitive than *A. mellifera* and *B. terrestris* (Azpiazu et al., 2021). Similarly, sulfoxaflor applications 6 days before bloom reduced colony growth, colony size and foraging performance in bumblebees (Tamburini et al., 2021a), but not in honey bees (Tamburini et al., 2021b). Studies on neonicotinoid insecticides have also found sensitivity differences between bee species, indicating that *Osmia* are more sensitive than honey bees to compounds that target NACHRs receptors (Arena and Sgolastra, 2014; Biddinger et al., 2013; Sgolastra et al., 2017; Uhl et al., 2019).

Sulfoxaflor has been proposed as an alternative to the use of neonicotinoids and its use has been increasing worldwide since its approval in 2013 and 2015 in US and EU, respectively. A previous study showed that, although less toxic to bees than nitro-substituted neonicotinoids, sulfoxaflor was much more toxic than cyano-substituted neonicotinoids (Azpiazu et al., 2021). Neonicotinoid toxicity is greatly enhanced by the presence of EBI fungicides (Biddinger et al., 2013; Gill et al., 2012; Sgolastra et al., 2018, 2017; but see Haas and Nauen, 2021). By contrast, our results and those of previous studies (Azpiazu et al., 2021; Tamburini et al., 2021a) indicate that synergism between sulfoxaflor and SDHI is weak and highly dose-dependent (although this could not be confirmed in the current study). Because both neonicotinoids and sulfoxaflor act on Nicotinic Acetyl Choline Receptors (NACHRs) (Sparks et al., 2013; Zhu et al., 2011), future studies should address potential synergism between sulfoxaflor and EBI fungicides. Ideally, these studies should be conducted on *Osmia* spp. given that these species are more sensitive to sulfoxaflor than honey bee and bumblebees. Notwithstanding the recent restrictions on outdoors use of sulfoxaflor in the EU, this active ingredient is still allowed in many other countries, even in bloom applications of entomophilous crops (Corteva Australia, 2022; Corteva South Africa, 2022; Corteva US, 2022).

In conclusion, our data showed that the highest tested concentration of sulfoxaflor causes a decline in syrup consumption and a marked decrease in longevity. By contrast, the low concentration stimulated syrup consumption, but did not affect longevity. In addition, we found no interaction between sulfoxaflor and fluxapyroxad administered chronically *ad libitum* at any of the concentrations tested and, therefore, no evidence of dose-dependent synergism.

CRediT authorship contribution statement

Celeste Azpiazu: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft; **Jordi Bosch:** Conceptualization, Funding acquisition, Writing - review & editing; **Catia Martins:** Methodology, Data Curation, Writing - review & editing; **Fabio Sgolastra:** Conceptualization, Methodology, Investigation, Data Curation, Funding acquisition, Supervision, Writing - review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare they have no conflict of interest.

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