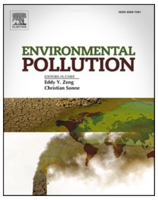
Environmental Pollution 307 (2022) 119575

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



# Exposure to the novel insecticide flupyradifurone impairs bumblebee feeding motivation, learning, and memory retention☆

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A R T I C L E  I N F O

*Keywords:*
Pollinator
Olfactory learning
Colour learning
Pesticides
Cognition
Sub-lethal

## A B S T R A C T

Bees are vital pollinators of crops and wildflowers and as such, wild bee declines threaten food security and functioning ecosystems. One driver of bee declines is the use of systemic insecticides, such as commonly used neonicotinoids. However, rising pest resistance to neonicotinoids, and restrictions on their use in the EU, has increased the demand for replacement insecticides to control crop pests. Flupyradifurone is a novel systemic insecticide that is thought to be relatively 'bee safe' although it can be present in the nectar and pollen of bee-attractive crops. Bumblebees rely on learning to forage efficiently, and thus detriments to learning performance may have downstream consequences on their ability to forage. While neonicotinoids negatively influence bumblebee learning and memory, whether this is also the case for their replacements is unclear. Here, we exposed bumblebees (*Bombus impatiens*) to an acute, field-realistic dose of flupyradifurone before training them to learn either an olfactory or colour association. We found that flupyradifurone impaired bumblebees' learning and memory performance in both olfactory and visual modalities. Flupyradifurone-treated bees were also less motivated to feed. Given the similarity between the detriments to cognition found here and those previously reported for neonicotinoids, this implies that these insecticides may have similar sub-lethal effects on bees. Restrictions on neonicotinoid use are therefore unlikely to benefit bees if novel insecticides like flupyradifurone are used as an alternative, highlighting that current agrochemical risk assessments are not protecting bees from the unwanted consequences of pesticide use. Sub-lethal assessments on non-*Apis* bees should be made mandatory in agrochemical regulation to ensure that novel insecticides are indeed 'bee safe'.

## 1. Introduction

Bee declines are driven by a multitude of anthropogenic stressors, including loss of habitat, intensive farming, parasite exposure, climate change, as well as the interactions between multiple stressors (Cameron et al. 2011; Samuelson et al. 2018; Soroye et al., 2020; Siviter et al. 2021a). One key driver of declines is exposure to agrochemicals, such as neonicotinoid insecticides (Rundlöf et al. 2015; Woodcock et al. 2016; Siviter et al., 2021c). As systemic insecticides, neonicotinoids are expressed throughout plant tissue including the nectar and pollen of target crops and wildflowers (Bonmatin et al. 2015; Rundlöf et al. 2015; Wood et al. 2019) where foraging bees may be exposed. Neonicotinoids have significant negative effects on bees and other pollinators (Pisa et al. 2017; Siviter et al. 2021c). Importantly, these negative impacts are often sub-lethal and environmental risk assessments, which are largely based on measures of toxicity, often fail to detect these more subtle, but important, effects (Sgolastra et al. 2020; Siviter et al. 2021c). Neonicotinoids (imidacloprid, thiamethoxam and clothianidin) are now banned from use in the European Union (EFSA, 2018). Outside of the EU, rising pest resistance has increased the demand for novel insecticides that are effective at controlling pest-species resistant to neonicotinoids (Simon-Delso et al. 2015; Brown et al. 2016). Understanding whether new-generation insecticides have similar sub-lethal impacts on bees is therefore of utmost importance (Brown et al. 2016; Siviter et al., 2018a; Siviter and Muth, 2020).

Flupyradifurone is a butanolide insecticide which has been registered for use globally, including in the EU and the United States (Nauen et al. 2015). It is effective at controlling pest species resistant to neonicotinoids (Jeschke et al. 2015; Nauen et al. 2015) and can be applied as either a seed or foliar treatment (Nauen et al. 2015). Like neonicotinoids, flupyradifurone is expressed throughout plant tissue, including in floral rewards. It also shares the same mode of action as

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☆ This paper has been recommended for acceptance by Jiayin Dai.

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https://doi.org/10.1016/j.envpol.2022.119575
Received 22 January 2022; Received in revised form 19 May 2022; Accepted 1 June 2022
Available online 9 June 2022

neonicotinoids, acting as an agonist of insect nicotinic acetylcholine receptors (nAChRs), which play an important role in learning and memory (Tomizawa and Casida, 2009; Nauen et al. 2015). Despite this similarity, flupyradifurone is classified in a distinct chemical group by the Insecticide Resistance Action Committee (IRAC) (neonicotinoids = group 4 A, flupyradifurone = group 4D) (Nauen et al. 2015). Furthermore, because flupyradifurone is thought to be relatively 'bee safe' it can be used on bee-attractive crops when they are actively flowering (EPA, 2014; Nauen et al. 2015). This means that bees can be acutely exposed to high levels of flupyradifurone both through being directly sprayed by the insecticide and when feeding on the nectar or pollen of treated crops (EPA, 2014). Exposure to flupyradifurone at field realistic levels can impair honeybee (Apis spp.) olfactory learning, flight, foraging, and sucrose consumption (Tosi and Nieh, 2019; Tong et al., 2019; Hesselbach et al. 2020; Tosi et al. 2021), but beyond honeybees, little is known about its sub-lethal impacts (reviewed in Siviter and Muth (2020)).

A worker bumblebee can visit hundreds to thousands of flowers a day to collect nectar and pollen for her colony. When foraging, bumblebees use multiple sensory modalities, such as vision and olfaction, to detect and learn about rewarding flowers in an ever-changing floral marketplace (Leonard & Masek, 2014). Bumblebee learning speed is positively correlated with foraging performance at a colony level (Raine & Chittka, 2008,but see (Evans, Smith & Raine, 2017)). This suggests that if pesticide exposure impairs learning it could have downstream consequences for foraging efficiency and colony growth (Klein et al. 2017). A plethora of research has shown that pesticides and other agrochemicals, including neonicotinoids, can impair bees' learning and memory performance (Samuelson et al. 2016; Siviter et al. 2018b; Siviter, Johnson & Muth, 2021b; Muth, Francis & Leonard, 2019; DesJardins et al. 2021). However, the majority of these studies have been conducted with honeybees (*Apis mellifera*) in an olfactory learning task where the bee is restrained (reviewed in Siviter et al. 2018b; Muth & Leonard, 2019). Honeybees are an important model species for pesticide regulation, but their unique life history means that they are not necessarily representative of wild bees more broadly (Franklin & Raine, 2019). Furthermore, restraining bees may interfere with their motivation to participate in the experiment (Siviter et al. 2019; Muth & Leonard, 2019). More broadly, the impact of novel insecticides on non-*Apis* bee cognition and behaviour is poorly understood (Siviter & Muth, 2020).

Here we assessed the impact of acute exposure to flupyradifurone on bumblebee (*B. impatiens)* olfactory and visual leaning. We chose bumblebees as they are (i) essential pollinators of crops and wildflowers and (ii) a model species for assessing the impact of pesticide on bee cognition (Samuelson et al. 2016; Siviter et al. 2018b). Previous work with neonicotinoid pesticides has found that impairments to learning may be modality-specific, with olfactory, but not visual, learning being affected by exposure (Lämsä et al. 2018; Muth et al. 2019; Muth & Leonard, 2019). Considering the similar mode of action between neonicotinoids and flupyradifurone, we expected a larger impact on olfactory learning relative to visual learning. We also expected that pesticide-exposed bees would have a lower motivation to feed, based on well-established findings from neonicotinoids (Laycock et al. 2012, 2014; Cresswell et al. 2014; Lämsä et al. 2018; Muth & Leonard, 2019).

## 2. Methods

### *2.1. Subjects and general protocol*

We used four commercial bumblebee (*B. impatiens)* colonies (Koppert Biological Systems, USA) (n = 2 for each experiment) that were connected via plastic tubes to flight arenas (l × w × h: 122 × 61 × 61 cm). These colonies were maintained on an *ab libitum* supply of 50% (w/w) sucrose solution that foragers collected from feeders placed within the flight arenas. We also supplemented colonies with honeybee-collected pollen (Koppert Biological Systems, USA), placed directly into colonies (1 tbsp every 2–3 days).

To address flupyradifurone effects on learning, we used a Free-Moving Proboscis Extension Response (FMPER) protocol which allowed bumblebees to actively choose between two stimuli (Fig. 1) (Muth et al. 2018; Muth, 2021). We collected foraging bumblebees from feeders with forceps and placed them into individual transparent plastic rectangular tubes (l × w × h: 15 × 2.5 × 2.5 cm) (Muth et al. 2018). The ends of the tubes contained two holes through which we could present stimuli to bees for the learning trials (Fig. 1). We food-restricted bumblebees for 2h in the tubes to ensure that they were sufficiently motivated to participate in the experiment.

### *2.2. Pesticide treatment and dosing*

Flupyradifurone has been licenced for use on a broad range of crops and can be applied as both a seed and spray application. We based our exposure regime on Environmental Protection Agency (EPA) residue data from winter sown oil-seed rape treated with both seed and spray applications of flupyradifurone (10 g ai/kg seed; 0.28 lbs/A foliar spray prior to sowing and 2 × 0.18lbs ai/A during early and full flowering) (EPA, 2014). Residues in flowers ranged from 0.08 to 36 mg/kg (0.8 ppm–36 ppm) and foraging honeybees (*Apis mellifera*) had up to 4.3 mg/kg (4.3 ppm) and 21 mg/kg (21 ppm) of flupyradifurone in their nectar and pollen respectively (EPA, 2014).

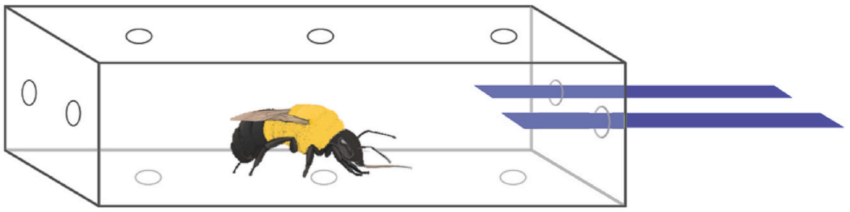
We combined flupyradifurone (powder form, analytical standard from Chem service, USA) with water to make a stock solution which was added to 50% (w/w) sucrose solution to create a 4 ppm solution. The pesticide solution was frozen, and a subset was defrosted for use as necessary to reduce the risk of degradation. To replicate a scenario where a foraging bumblebee is acutely exposed to flupyradifurone, we fed bees in the treatment group 18.85 μl of sucrose solution. This equated to 75.4 ng of flupyradifurone per bee. This concentration (4 ppm) is significantly lower than that found in the pollen of foraging honeybees (EPA, 2014). Control bees were fed 18.85 μl of untreated sucrose (50% w/w). Wild-foraging bumblebees consume approximately 37.7 μl when foraging for an hour (Samuelson et al. 2016). By feeding bees 18.85 μl we aimed to replicate the scenario of a forager being acutely exposed while foraging for approximately 30 min (Samuelson et al. 2016). We pipetted the treated or control sucrose into each bee's individual testing container for her to consume. Bumblebees that did not consume all the sucrose were removed from the experiment at this stage (olfactory learning experiment: control n = 3, treatment n = 3; colour learning experiment: control n = 9, treatment n = 6). In line with previous studies with neonicotinoids (Samuelson et al. 2016), we then waited 45 min before beginning either olfactory (experiment 1) or colour (experiment 2) conditioning trials to allow for maximal absorption of the pesticide (Samuelson et al. 2016) (See Fig. 1).

#### *2.2.1. Experiment 1) olfactory learning*

We trained 70 bumblebees (control n = 35, treatment n = 35) over a series of two 'pre-training' presentations, followed by three 'choice' training trials and two test trials. We used two scents in the experiment: linalool and geraniol (Sigma-Aldrich, USA). Both scents were diluted in hexane (Wright & Schiestl, 2009; Claudianos et al. 2014) using a dilution (1:100,000) used in previous work (Muth & Leonard, 2019). For each bumblebee, one scent (conditioned stimulus, CS+) was paired with a sucrose reward (50% w/w) and the other (CS-) with water which is unrewarding to bees. We pipetted 3 μl of each scent onto strips of dark blue coloured card (see Fig. 1).

For the first pre-training presentation, we dipped the card with the rewarding scent (CS+) into sucrose solution (50% w/w) and presented it to the bee by stimulating its antennae with the sucrose-laden strip of card. Once the bee started feeding, it was given 3 s of contact between the sucrose and its proboscis. 10 min later, we presented the bee with the unrewarding scent paired with water. Again, the bee was given 3 s to feed on the water, but seldom did and would either not extend its proboscis after antennating the unrewarding strip or would extend but then

**Experiment 1) Olfactory learning**



**Experiment 2) Colour learning**

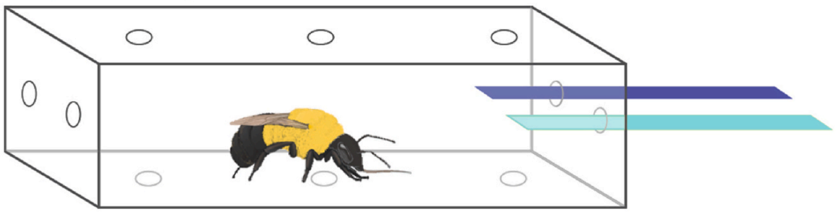


**Fig. 1.** Experimental set-up for experiments 1 and 2. In experiment 1, we trained bees to discriminate between two scents (linalool and geraniol), where one was rewarded and the other was unrewarded. Both stimuli were the same colour. In experiment 2, we trained bees to discriminate between two coloured stimuli (dark blue and light blue) that were unscented, one of which was rewarded and the other unrewarded. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

retract it immediately. These pre-training presentations served to motivate the bees to partake in the experiment and were also the first opportunity for them to learn that a specific scent predicted reward.

Following the pre-training presentations, we then trained bees over a series of three choice trials, each separated by an inter-trail interval of 10 min. On each of these trials, we simultaneously presented two strips of dark blue card, each containing one of the two scents (Fig. 1A). We recorded which scent the bee chose first, after which it was given the chance to choose the other strip, meaning that all bees encountered both the CS+ and CS- on each trial. A choice was defined as the bee making contact with a given strip of card, using either its antennae or proboscis. On a given trial, if the bee chose the CS + first, this was coded as a 'correct' choice; when it chose the CS- first, this was coded as an 'incorrect' choice. As with the pre-training trials, we allowed each bee to feed on both stimuli for 3 s. Bees that did not extended their proboscis in response to the sucrose reward in the training phase of the experiment were excluded from the experiment (control n = 1; treatment n = 8; Fig. 2A).

Following the three training trials, we gave bees two test (probe) trials. These trials were carried out in the same manner as the training trials, with the exception that both stimuli were unrewarding, containing water. The first test trial was conducted 10 min after the final training trial and the second was conducted 1 h later. At the end of the experiment, all bees were euthanized via freezing. We later measured the body size of bees using intertegular distance as a proxy (Cane, 1987).

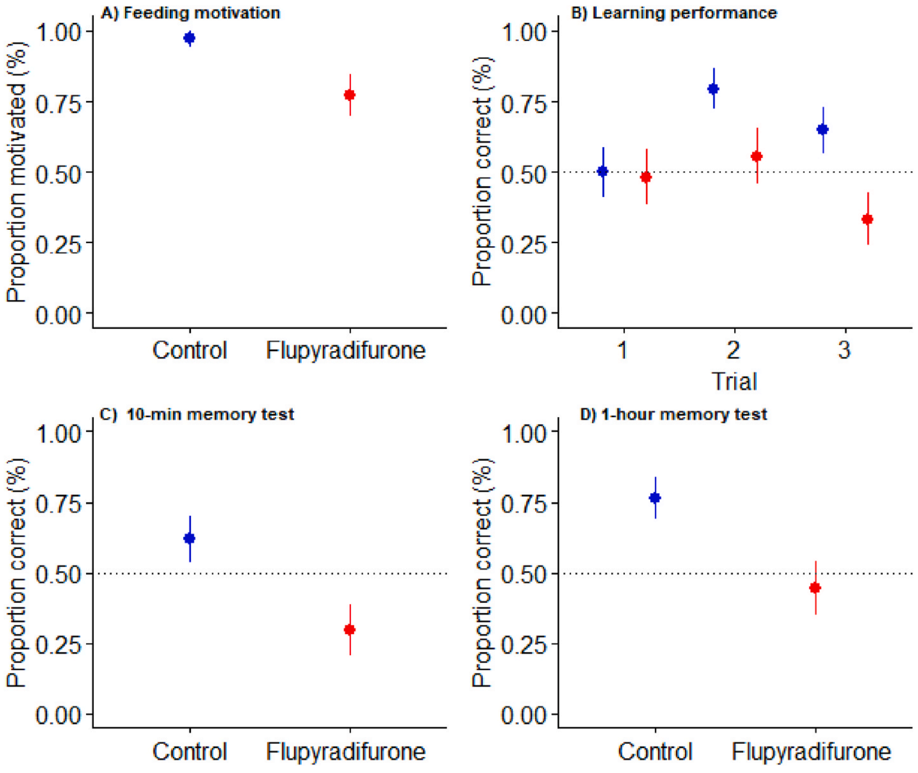


**Fig. 2.** (A) The mean proportion (± SE) of bumblebees that participated in the olfactory learning training, consuming sucrose across all three trials. The mean proportion (± SE) of bumblebees that chose the correct scent in the (B) training phase, (C) test phase and (D) 1-h memory test. Blue = control bumblebees and red = treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 2.2.2. *Experiment 2) colour learning*

The colour learning protocol was conducted in the same way as the olfactory learning protocol, with the exception that instead of training bees using olfactory cues, we used dark and light blue strips of card (Fig. 1), as in Muth (2021).

We tested 98 bees that consumed the experimental or control solutions (control n = 41, treatment n = 57, taken from 2 colonies). 27 bees, most of which were exposed to the pesticide, did not complete training and were excluded from the experiment (control n = 5; treatment n = 22; Fig. 3A).

### 2.2.3. *Statistical analysis*

We based our analysis on an information theoretic, model selection approach. For each response variable analysed we created a full model containing all the measured factors/covariates, subsets of the full model, and a null model containing just the intercept. We selected models based on AICc values, and in cases when ΔAICc >2 we used model averaging to determine the parameter estimates and 95% confidence intervals. We used R (version April 1, 1717) (R core Team, 2021) and the packages *Hmisc, ggplot2, lme4, MuMIn* (Wickham, 2009; Bates et al. 2015; Barton, 2016; Harrell & Dupont, 2018).

To determine if feeding motivation differed between treatment groups, we compared the number of bees that exhibited PER in response to the sucrose reward in all trials of each experiment to the number of bees that did not exhibit PER in response to sucrose on at least one trial. To do this, we used generalized linear models (GLMs) with a binomial error structure. For the analysis for the olfactory learning experiment, we included 'treatment' (control vs pesticide-treated) and colony as a fixed factors and bee size as a covariate. For the colour learning data, we also included the interaction between bee size and treatment (note, this interaction failed to run when included in the olfactory data analysis, likely because there were fewer 'unmotivated' bees in this experiment (9/70 unmotivated)).

To determine if learning performance differed between treatment groups, we analysed the training data using generalized linear mixed-effect models (GLMMs) and test trials using GLMs. A binomial error structure was used for each dependent variable. For the training data, treatment, bee size, trial, rewarding scent/colour, colony, and the interactions between (i) treatment and bee size and (ii) treatment and trial were included in the full model. Bee was also included as a random factor. For the test trials, treatment, bee size, rewarding scent/colour, colony, and the interactions between (i) treatment and bee size and (ii) treatment and rewarding scent/colour were included in the full model. For a full list of candidate models selected, and all outputs see Table S1 and S2.

## 3. Results

### 3.1. *Feeding motivation*

In both experiments, bumblebees fed flupyradifurone were less motivated to consume sucrose compared to control bees (Fig. 2A: Olfactory learning: treatment, parameter estimate (PE) = −2.61, 95% confidence intervals (CI) = −5.62 to −0.74; Fig. 3A, treatment (colour learning), PE = −1.52, CI = −2.63 to −0.41).

### 3.2. *Learning and memory*

Flupyradifurone impaired both olfactory and colour learning and memory (Fig. 2C and D; Fig. 3B and D). In the olfactory learning experiment, bumblebees acutely exposed to flupyradifurone showed a strong trend towards performing worse in the training phase (Fig. 2B, treatment, PE = −0.59, CI = −1.87 to 0.68; treatment × trial, PE = −0.11, CI = −0.67 to 0.45). In the unrewarded test trials, flupyradifurone impaired olfactory memory at both 10 min and 1 h after training (Fig. 2C, olfactory probe test, treatment, PE = −1.36, CI =

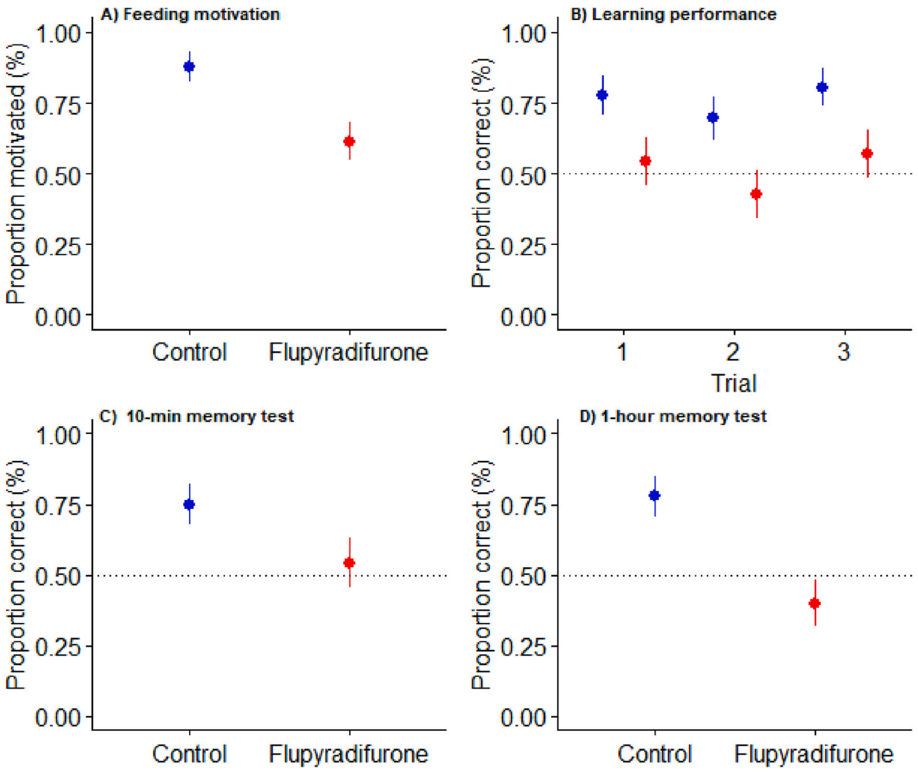


**Fig. 3.** (A) The mean proportion (±SE) of bumblebees that participated in the colour learning training, consuming sucrose across all three trials. The mean proportion (±SE) of bumblebees that chose the correct colour in the (B) training phase, (C) test phase and (D) 1-h memory test. Blue = control bumblebees and red = treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

−2.46 to −0.25; Fig. 2D, olfactory memory test, treatment, PE = −1.42, CI = −2.54 to −0.29).

In the colour learning experiment, flupyradifurone impaired performance in the training phase, and this impairment was equal across learning trials (Fig. 3B, treatment, PE = −1.14, CI = −1.75 to −0.54; treatment × trial, AICc >2). There was a strong trend towards flupyradifurone impairing bees' memory after 10 min (Fig. 3C, colour probe test, treatment, PE = −0.70, CI = −1.88 to 0.47), a difference that was significant 1 h later (Fig. 3D, colour memory test, treatment, PE = −1.65, CI = −2.71 to −0.60).

We found no effect of the specific scent or colour a bee was trained to, colony, body size or trial on performance in either experiment, in both the training and test phases (Table S1 & S2; Figure S2, Figure S3).

## 4. Discussion

Flupyradifurone is a novel insecticide that is replacing neonicotinoids over a large geographical range (Nauen et al. 2015), yet few studies have addressed its sub-lethal effects on non-*Apis* pollinators (Siviter & Muth, 2020). We found that bumblebees exposed to an acute dose of flupyradifurone were less motivated to forage and had impaired olfactory learning; these results are similar to those observed with neonicotinoids (Stanley, Smith & Raine, 2015; Siviter et al. 2018b; Muth et al. 2019). In addition, we also found that flupyradifurone exposure impaired colour learning, in contrast to what has generally been found with neonicotinoids (Lämsä et al. 2018; Muth et al. 2019; Muth & Leonard, 2019). Taken as a whole, our results indicate that new-generation 'replacement' insecticides may have similar sub-lethal effects to neonicotinoids on bee behaviour (Siviter & Muth, 2020).

We found that 32% of bumblebees exposed to flupyradifurone did not consume sucrose rewards across training, compared to 8% of bees in the control groups. When bumblebees were motivated to feed, both olfactory and colour memory retention were impaired. Only 44% and 40% of bumblebees exposed to flupyradifurone were able to recall the rewarding odour and colour respectively, compared to 76% and 77% in the control treatments. The results are similar to those observed with honeybees (*Apis* spp.) exposed to flupyradifurone (reviewed in Siviter & Muth, 2020). For example, exposure to flupyradifurone reduces honeybee (*A. mellifera*) feeding rates (Tong et al. 2019; Wu et al. 2021) and consuming low concentrations of flupyradifurone impairs *A. cerana* worker olfactory learning when individuals are exposed at either the adult or larval stage (Tan et al. 2017). High concentrations of flupyradifurone can also impair *A. mellifera* olfactory learning (Hesselbach & Scheiner, 2018), but Bell et al. (2020) found no evidence to suggest that exposure to low, acute concentrations of flupyradifurone impaired *A. mellifera* olfactory learning. Considering these results alongside each other, this suggests that *A. cerana* and *B. impatiens* are more vulnerable to flupyradifurone than *A. mellifera*, highlighting the importance of testing the impact of agrochemical exposure on a range of species when conducting environmental risk assessments (Franklin & Raine, 2019; Sgolastra et al. 2020; Siviter & Muth, 2020).

Flupyradifurone and neonicotinoids share the same mode of action, and as such, the sub-lethal effects these chemicals have on bees may be underpinned by the same or similar physiological mechanisms (Nauen et al. 2015). Both pesticides act as agonists of nAChRs which are present throughout the insect nervous system (Nauen et al. 2015), including in the mushroom bodies, a neural region associated with sensory integration, learning, and memory (Hourcade et al. 2010; Devaud et al. 2015). As agonists of nAChRs, neonicotinoids disrupt cholinergic transmission which can result in neural cells in the mushroom bodies being inactivated or failing to develop (Palmer et al. 2013; Peng & Yang, 2016). However, while less is known about the effects of flupyradifurone at a neural level, Hesselbach et al. (2020) found no evidence that flupyradifurone increased apoptosis of Kenyon cells in the mushroom bodies of honeybees as is the case for neonicotinoids (Wu et al. 2015). Aside from its effects within the brain, honeybees exposed to Sivanto (a commercial formula with flupyradifurone as an active ingredient) had increased levels of apoptosis compared with unexposed bees when whole bees were sampled (Chakrabarti et al. 2020). Flupyradifurone can also alter the expression of genes associated with olfactory learning (Wu et al. 2021) and detoxification (Al Naggar & Baer, 2019) in honeybees, all of which could offer a potential mechanisms for observed sub-lethal effects on bees. While our results cannot speak to the mechanism driving the sub-lethal impacts of flupyradifurone on bees, the similarities between neonicotinoids and flupyradifurone are clear (Siviter & Muth, 2020).

Neonicotinoid exposure impairs olfactory learning in bees (Stanley et al. 2015; Piiroinen & Goulson, 2016; Siviter et al. 2018b; Muth et al. 2019), and here we found that flupyradifurone has comparable effects on bumblebee olfactory learning and memory. Flupyradifurone also impaired bumblebee colour learning, in contrast to previous studies finding no impact of the neonicotinoid imidacloprid on bumblebee colour learning (Lämsä et al. 2018; Muth et al. 2019; Muth & Leonard, 2019). We can only speculate as to why this is, but different neonicotinoids differentially activate different receptor subtypes throughout the bee brain (Moffat et al. 2016). As such, a broad variety of behaviours and cognitive abilities are impaired aside from associative learning, e.g. spatial learning and navigation (Tison et al. 2016; Samuelson et al. 2016), and exactly which aspects of behaviour are impaired may be determined by the specific activation of receptor subtypes by a given pesticide. One surprising result was that bumblebees exposed to flupyradifurone performed at below chance (30% correct) in the olfactory test trial following training. Bumblebees rarely consumed the water after tasting it (via their antennae or proboscis) and so it is unlikely that they were actively choosing the scent paired with the water stimulus. One possibility for a group-level preference against the CS + could be an untrained preference for the CS-. However, we did not see evidence for strong scent preferences that might bias our results: in the first training trials, bees in the pesticide treatment group showed a preference for geraniol, but by the third training trial both scents were chosen a similar amount (Figure S3). Moreover, since this bias away from the CS+ was not evident in the 1-h memory test, the most likely explanation for the below-chance performance in the first test is random chance.

While the effects of pesticides are often assessed individually, bees are more often exposed to multiple anthropogenic and environmental stressors simultaneously which can synergistically interact with each other (Siviter et al. 2021a). For example, synergistic interactions between flupyradifurone and the fungicide propiconazole increased honeybee mortality and hyperactivity (Tosi & Nieh, 2019). Similarly, the impact of flupyradifurone on honeybee flight success was worse when bees were nutritionally stressed (Tong et al. 2019) and exposure to flupyradifurone made honeybees more susceptible to the microsporidian *Nosema ceranae* (Al Naggar & Baer, 2019). Likewise, exposure to sulfoxaflor, another novel insecticide, and poor nutrition, synergistically increased bumblebee (*B. terrestris)* mortality, and co-exposure to both sulfoxaflor and the common bumblebee pathogen *Nosema bombi* additively increased bumblebee larval mortality (Siviter et al. 2020a; Linguadoca et al. 2021). Here, we show that by itself, flupyradifurone impairs bumblebee feeding motivation and cognition. However, as bees are likely to be exposed to a plethora of environmental stressors (Vanbergen & Insect Pollinators Initiative, 2013; Goulson et al. 2015; Siviter et al. 2021a), including surfactants and other co-formulants (Fine, Cox-Foster & Mullin, 2017; Straw, Carpentier & Brown, 2021), it is likely that our findings underestimate the potential sub-lethal impact of flupyradifurone on bumblebee behaviour. A clear next step to gain a full understanding of the effects of novel insecticides on bees would be to test commercial formulas and multiple agrochemicals simultaneously.

## 5. Conclusions

Our results contribute to the growing body of evidence that shows that flupyradifurone can have significant negative impacts on beneficial insects. To our knowledge, our results are the first to assess the sub-lethal effects of flupyradifurone on bees outside of the domesticated honeybee (Siviter & Muth, 2020). The colony-level consequences of these sub-lethal effects are not yet established (Campbell et al. 2016) but our results, and those previously reported (Tosi & Nieh, 2019; Hesselbach et al. 2020; Siviter & Muth, 2020), suggest that flupyradifurone, at label recommendations, is not 'bee safe'. In the short term, policy makers should advise against treating bee-attractive crops with flupyradifurone during flowering (Siviter & Muth, 2020; Tosi et al. 2021). More broadly, given the increasing evidence that licenced novel insecticides harm bees, this suggests that agrochemical risk assessments processes globally are failing to protect pollinators from the unwanted consequences of pesticide use (Siviter et al. 2018a, 2020b; Tosi et al. 2021). To better protect bees, regulators should consider (i) the sub-lethal impacts of pesticide use on a range of bee species before an insecticide is licenced for use and (ii) conduct post-monitoring observations akin to those conducted in pharmaceutical regulation once an agrochemical is licenced for use (Milner & Boyd, 2017; Siviter & Muth, 2020).

## Author contributions

HS & FM designed the experiment. HS conducted the experiment, the statistical analysis and wrote the first version of the MS. FM contributed to subsequent drafts.

## Data availability

All data is available online (https://osf.io/6cern/).

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We would like to thank Leeah Richardson for supplying the pesticide solutions used in this experiment, and Claire Hemingway for providing feedback on this manuscript. HS was funded by the Stengl-Wyer Scholars Program. F.M. received funding from NSF, award number IOS-2028613.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119575.

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