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Toxic temperatures: Bee behaviours exhibit divergent pesticide toxicity relationships with warming

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Abstract

Climate change and agricultural intensification are exposing insect pollinators to temperature extremes and increasing pesticide usage. Yet, we lack good quantification of how temperature modulates the sublethal effects of pesticides on behaviours vital for fitness and pollination performance. Consequently, we are uncertain if warming decreases or increases the severity of different pesticide impacts, and whether separate behaviours vary in the direction of response. Quantifying these interactive effects is vital in forecasting pesticide risk across climate regions and informing pesticide application strategies and pollinator conservation. This multi-stressor study investigated the responses of six functional behaviours of bumblebees when exposed to either a neonicotinoid (imidacloprid) or a sulfoximine (sulfoxaflor) across a standardised low, mid, and high temperature. We found the neonicotinoid had a significant effect on five of the six behaviours, with a greater effect at the lower temperature(s) when measuring responsiveness, the likelihood of movement, walking rate, and food consumption rate. In contrast, the neonicotinoid had a greater impact on flight distance at the higher temperature. Our findings show that different organismal functions can exhibit divergent thermal responses, with some pesticide-affected behaviours showing greater impact as temperatures dropped, and others as temperatures rose. We must therefore account for environmental context when determining pesticide risk. Moreover, we found evidence of synergistic effects, with just a 3°C increase causing a sudden drop in flight performance, despite seeing no effect of pesticide at the two lower temperatures. Our findings highlight the importance of multi-stressor studies to quantify threats to insects, which will help to improve dynamic evaluations of population tipping points and spatiotemporal risks to biodiversity across different climate regions.

KEYWORDS
bumblebee, climate change, colony, flight, foraging, neonicotinoid, pollination, pollinator, responsiveness, sulfoximine, walking

1 | INTRODUCTION

Declining populations of beneficial insects is a worldwide problem, making identifying the drivers a research priority and crucial for developing mitigative strategies (Gill et al., 2016; Potts et al., 2016; Wagner et al., 2021; Zattara & Aizen, 2021). Widespread pesticide usage is one proposed driver (Bryden et al., 2013; Goulson et al., 2015; Woodcock et al., 2017), with studies showing that beneficial insects exposed to field-realistic concentrations can exhibit impeded development and impaired behaviour (Crall et al., 2018; Kenna et al., 2019; Siefert et al., 2020; Siviter, Brown, et al., 2018; Siviter, Koricheva, et al., 2018; Smith et al., 2020), which can translate to reduced fitness (Arce et al., 2017; Bryden et al., 2013; Gill et al., 2012; Rundlöf et al., 2015; Whitehorn et al., 2012; Willis Chan & Raine, 2021). Another increasingly scrutinised driver is climate change, with recent decades of environmental warming associated with insect population range shifts (e.g. Halsch et al., 2021; Kerr et al., 2015; Rasmont et al., 2015; Raven & Wagner, 2021) and localised extinctions (Janzen & Hallwachs, 2021; Soroye et al., 2020). However, despite the ubiquity with which these factors are simultaneously experienced (Dicks et al., 2021), our understanding of how they interact is surprisingly poor. Specifically, we have limited quantification of how temperature modulates the toxic effects of pesticide exposure in terrestrial beneficial insects. This is concerning, because to reveal the true risk that field realistic concentrations of pesticides pose, we must consider how the environmental context at the time of exposure influences an organism's response (Camp & Buchwalter, 2016; Holmstrup et al., 2010). Indeed, with future landscapes projected to experience increased pesticide application and changing temperature regimes under climate change (Deutsch et al., 2018; Tang et al., 2021; Zhang, 2018), there is an urgent need to fill this evidence gap. Considering the universally governing role of temperature in determining metabolic and physiological rates (Abram et al., 2017; Archer et al., 2019), quantifying temperature-dependent responses has fundamental implications for current pesticide regulatory guidelines, and will improve mapping and forecasting of spatiotemporal risks of pesticide exposure across the world's different climate regions.

For insect pollinators, exposure to pesticides can lead to reduced mitochondrial activity (Moffat et al., 2015; Powner et al., 2016) and change the expression of genes involved in mitochondrial function (Colgan et al., 2019). Similarly, variation in ambient temperature can affect insect metabolic and physiological rates (Gillooly et al., 2001; Huey & Kingsolver, 2019; Huey & Stevenson, 1979), such as chemical detoxification and excretion (Harwood et al., 2009; Khan & Akram, 2014; Lydy et al., 1999; Weston et al., 2009), rates of consumption (Camp & Buchwalter, 2016; Holmstrup et al., 2010; Noyes et al., 2009), as well as altering pesticide binding efficiency at target-receptors (Boina et al., 2009). These temperature-dependent processes provide a rationale to why we might expect temperature to modulate pesticide sublethal impacts on insect behaviour. For instance, the effect of neonicotinoid exposure on honeybee homing success and on bumblebee foraging behaviour has been reported to be more pronounced under colder conditions (Henry et al., 2014; Kolano et al., 2021; Monchanin et al., 2019). Additionally, neonicotinoid impacts on bumblebee colony worker activity and nursing behaviour were reported to be more pronounced at night relative to daytime (Crall et al., 2018). However, potential confounding factors make interpreting such findings difficult, as controlled temperature effects were not explicitly tested, and it is difficult to understand which functional behaviours are being affected. Hence, conducting tightly controlled pesticide exposure studies under different temperatures can help to reveal causal links, quantify the direction and scaling of this interactive relationship, and reveal evidence for additive effects or antagonisms/synergisms.

Insect pollinators are exposed to pesticides under wide thermal ranges due to seasonal patterns of usage, location of application, and the long periods that pesticide active ingredients (AIs) can reside in the environment (Holmstrup et al., 2010; Woodcock et al., 2018). However, pesticide risk assessments often recommend carrying out single end-point response studies, typically at single temperatures, and primarily focusing on lethal [not sublethal] behaviours. This limits our capacity to build a thermal response framework. By testing across different temperatures we can quantify the negative or positive temperature response relationships with pesticides (the degree to which toxicity to exposed bees decreases or increases as temperature rises; Glunt et al., 2013; Mansoor et al., 2015). This can provide the baseline data necessary to determine how daily, seasonal, or annual temperature variation should be considered when assessing pesticide risk. Furthermore, by studying multiple functional behaviours under a single study framework we can gain a more holistic understanding of organismal responses, as well as reveal what functional roles are likely to be at risk and whether separate behavioural endpoints respond differently.

Given the above evidence gaps, we here designed a multi-stressor study to quantify temperature-dependent effects of two insecticides on a model insect pollinator—the bumblebee *Bombus terrestris*—exposed to either imidacloprid (a neonicotinoid) or sulfoxaflor (a sulfoximine). A neonicotinoid was chosen as they represent the most widely used insecticide class on the global market and are frequently encountered by insect pollinators (David et al., 2016; Simon-Delso et al., 2015; Woodcock et al., 2018). Furthermore, with growing pest resistance to pesticides and growing restrictions to field applications based on unacceptable risks, new pesticides are being licensed and approved but we are unsure of their sublethal effects. Therefore, we also chose a sulfoximine as it represents one of the most promising replacements of neonicotinoids (Sparks et al., 2013) (see Section 2 for further justification of chosen AIs).

Being also a multi-response study, we undertook three complementary experiments to investigate a total of six different behavioural responses representing important bee life-history and ecological functions (Figure 1), and reveal the direction of the temperature and insecticide relationship (Boina et al., 2009; Khan & Akram, 2014; Muturi et al., 2011). Specifically, we investigated how temperature modulated the effect of exposure to: (1) a 150 μg/L (128 parts per billion [ppb]) concentration of each

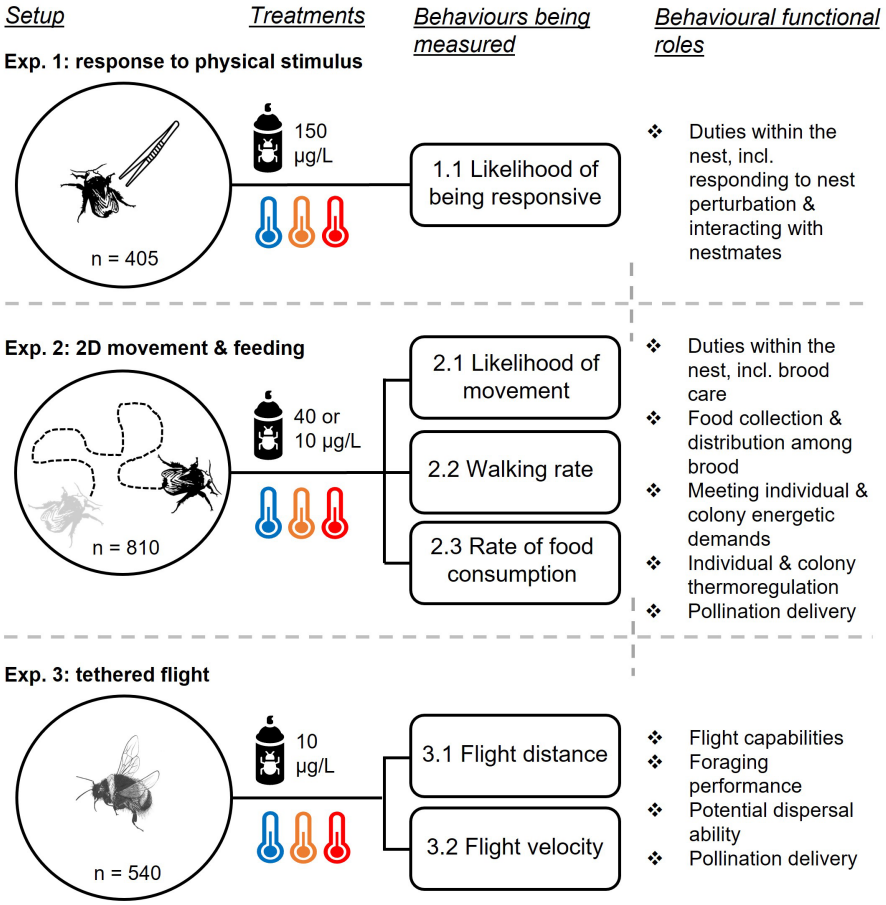

FIGURE 1 Overview of the three separate experiments (*n* = number of individual workers tested), investigating six functional behavioural responses (rectangular boxes). All three experiments were tested over the same temperature gradient (left to right: low°C = blue thermometer; mid°C = orange; high°C = red). The pesticide concentrations, however, differed in accordance with the types of behaviour being measured, and oral exposure was achieved through the provision of spiked sucrose solution (nectar substitute). Experiment 1, which chronically exposed individuals over a 144 h period, repeatedly tested an individual's response to a physical stimulus every 12 h in cages consisting of three workers (15 cages per treatment). Experiment 2, which chronically exposed individuals over a 72 h period, repeatedly recorded individual movement rates every 24 h also in cages of three workers, while recording per capita food consumption (18 cages per treatment). Experiment 3, which involved an acute single dosage exposure per individual, measured tethered flight performance of individuals over a 90 min period after being removed from the nest and acutely exposing bees to the pesticide. To justify the importance of each behaviour being investigated, we list some key functional roles they contribute towards.

insecticide on the likelihood of an individual being responsive to a physical stimulus (behaviour important for within nest activity); (2) a 40 µg/L (34 ppb) or 10 µg/L (8.5 ppb) concentration on the likelihood of movement, walking rate, and consumption rate (behaviours important for nesting duties, colony thermoregulation, meeting energy demands, and food collection); (3) a 10 µg/L concentration on flight endurance and velocity (behaviours crucial for foraging performance and dispersal). Using a fully factorial design with appropriate control groups, each experiment for each insecticide was conducted at a relative low[21]°C, mid[27]°C, and high[30]°C temperature, with these values informed by a recently established bumblebee flight thermal performance curve (Kenna et al., 2021). We tested if insecticide toxicity via oral exposure showed a negative or positive relationship with temperature, how the relationship varied between insecticides, and how it differs between the six behavioural response endpoints.

2 | MATERIALS AND METHODS

2.1 | Colonies, pesticides and temperatures tested

2.1.1 | Bumblebee colonies

Natal colonies from which workers were tested were supplied by Biobest (distributed by Agralan Ltd). On arrival, colonies were kept in a controlled environment (CE) room at 21°C and 60% relative humidity (RH) under constant red light. Colonies were also censused, ensuring a healthy queen and removal of any dead individuals, with supplied sugar feeders and pollen patties removed. Colonies were then provisioned with freshly made *ad-libitum* 40% sucrose solution and irradiated honeybee collected pollen (Agralan Ltd) on Mondays (6 g), Wednesdays (6 g) and Fridays (9 g). No single colony was used for multiple experiments and testing of bees was conducted

in separate CE rooms at the respective testing temperatures under 60% RH (Table S1).

2.1.2 | Justification for pesticide concentrations tested

For each experiment, we decided to mirror the concentrations between imidacloprid (I) and sulfoxaflor (S) to facilitate direct comparisons and to expose bees via a spiked nectar substitute (40% w/w sucrose/water solution). The choice of concentrations across the experiments were primarily based off knowledge on neonicotinoid field levels at the start of our study, as sulfoximines had been a commercial option for a comparatively shorter period with less known about the concentrations present in the environment. For experiment 1 (responsiveness), we chose 150 μg/L as whilst it can be within the field range for both compounds in different parts of the globe (Azpiazu et al., 2019; Cheng et al., 2018; Johnson et al., 2010; Linguadoca et al., 2021; US EPA, 2016; Zhou et al., 2022; Zhu et al., 2017), it presents a worst-case exposure scenario for imidacloprid. Consequently, we would expect to see impacts on behaviour and functioning at this concentration enabling us to first confirm whether temperature can have a modulatory effect on sublethal toxicity. This is important not only when considering unacceptable risks but would further warrant our investigations of impacts at lower concentrations on finer behavioural activity rates. Indeed, for experiment 2 (motivation to move, walking and consumption rates), we used much lower concentrations of 40 and 10 μg/L (for both imidacloprid and sulfoxaflor) which can be found in pollen and nectar of treated plants and within bee material (Blacquière et al., 2012; Goulson, 2013; Jiang et al., 2018, 2020; Johnson et al., 2010; Krischik et al., 2007; Stoner & Eitzer, 2012; Tong et al., 2018; Zhou et al., 2022). The 10 μg/L concentration is also applicable to experiment 3 (flight performance).

2.1.3 | Temperatures tested under

With biological rates responding to temperature change in a non-linear fashion (Huey & Stevenson, 1979), we avoided testing across evenly spaced temperatures. Instead, we informed our relative low, mid, and high temperatures based-off a previous thermal performance curve of *B. terrestris* workers when measuring tethered flight (Kenna et al., 2021). The mid°C was 27°C, which represented peak performance (when considering motivation to fly and flight endurance), with low°C and high°C representing a ca. 25% performance reduction either side (21°C & 30°C, respectively). This allowed us to test between temperatures that behaviours are known to be thermally sensitive to, and a temperature range experienced by temperate bee species during the summer.

2.1.4 | Experiment 1: Likelihood of being responsive (1.1)

On arrival and from each of the five natal colonies, we removed 81 bees (workers) under red light, and cooled them to 4°C. We then distributed bees in groups of three into 340 mL circular plastic cages (base dimensions = 110 mm diameter, 60 mm depth) ensuring each cage contained three bees from the same natal colony (equating to 27 cages per colony; totalling 135 cages). Using groups of three workers is consistent with previous chronic exposure (multi-day) tests using feeding cage designs (Heard et al., 2017) and given the social nature of *Bombus terrestris* allows social contact even if one worker died. There was no difference between treatments in mean intertegular span (ITS) (ANOVA: $p > .1$ for all pairwise comparisons) or variance (Bartlett's $K^2 = 5.49$, df = 8, $p = .70$). Cages had a ventilated lid, circular piece of filter paper (diameter = 100 mm) on the floor, and two holes on opposing sides in which perforated Eppendorf 'feeding' tubes were inserted to provision set volumes of 40% sucrose solution.

We evenly assigned cages to a chronic pesticide exposure (control, Imidacloprid 150 μg/L (I150) or Sulfoxaflor 150 μg/L (S150)) and temperature (low°C, mid°C or high°C) combination ($n = 9$ treatments), with 15 cages examined (total = 45 bees) per treatment. For each colony, a further nine bees were removed and distributed evenly across three reserve cages, with one assigned per temperature. All cages were initially provisioned with two feeding tubes each containing 1.5 mL of untreated sucrose solution and were moved to their respective CE room before the end of the first day to allow acclimation. The following morning (06:00–07:00) cages were inspected, and any bees that had died were replaced by live bees taken from reserve cages of the corresponding natal colony and temperature. Directly following this (07:00–08:00), each cage had its feeding tubes replaced with ones containing 1.5 mL of assigned treatment sucrose solution. Along with the filter paper, feeding tubes were replaced every 48 h allowing bees to feed ad libitum.

Bees were exposed to their respective treatments for 144 h (6 days) under red light throughout. Chronic toxicity assays better reflect field exposure scenarios and are now seen as a priority by pesticide regulatory organisations (EFSA, 2013). Cages were monitored every 12 h (07:00–08:00 and 19:00–20:00) using the following protocol: (1) cage was tapped three times, and individuals observed walking (movement of both thorax and legs) within the following 10 s were recorded as 'mobile'; (2) individuals that did not walk were turned onto their back using metal forceps via the hind leg and monitored for 30 s. If the individual successfully self-righted within this timeframe it was recorded as 'mobile', but if unable was classed as 'immobile'; (3) any dead bees were recorded, transferred to an Eppendorf tube, and frozen (−20°C); (4) individuals classed as immobile were righted using forceps and left in the cage for future monitoring (N.B. no bee was actually observed to revert from being immobile to mobile). We examined the probability of survival and immobility (collectively termed 'responsiveness'), and this dataset

used for our analysis outlined the time to event for every individual bee when classifying the event as when a bee is 'unresponsive'. On days where sucrose solution was replenished, monitoring was conducted beforehand. After the last monitoring event each bee was placed in a separate Eppendorf tube and frozen (−20°C). Considering the presence of bees in an immobile or moribund state aligns with behavioural abnormality classifications in OECD (OECD, 2017) guidelines of standard bee chronic toxicity tests. Our approach also assesses individual sensitivity to vibration and physical contact both of which are adaptive behaviours in preparing to defend the nest to intruders and interacting with nestmates and brood (Goulson, 2010).

2.1.5 | Experiment 2: Likelihood of movement, and rates of walking and consumption (2.1–2.3)

On arrival and for each of nine colonies, 90 bees (workers) were removed, placed on ice to immobilise, and a small circular uniquely numbered plastic tag (Abelo Ltd; diameter = 2 mm) attached to the thorax using superglue. Tagged bees were then placed in separate plastic pots for 60 min to rest before being returned to their natal colony. Once all tagged, bees were placed in cages assigned to a chronic pesticide exposure (control, Imidacloprid 40 or 10 μg/L (I40 or I10), Sulfoxaflor 40 or 10 μg/L (S40 or S10)) and temperature (low°C, mid°C or high°C) combination (n = 15 treatments), with 18 cages examined per treatment (n = 54 bees, giving a total of 810 bees in experiment 2). Cage setups were the same as in experiment 1, each having three bees from the same natal colony. Bees per cage spanned a range of body sizes, with no significant difference between treatments in dry mass mean (ANOVA: $p > .1$ for all pairwise comparisons) or variance (Bartlett's K^2 = 23.4, df = 14, p = .054). Cages for this experiment were provisioned with a single perforated Eppendorf feeding tube containing 2 mL of untreated sucrose solution. Cages were moved to their respective CE room before the end of the first day. The following morning, cages were inspected for dead bees which [if present] were replaced by healthy individuals from reserve cages of the corresponding natal colony and temperature following the same protocol as outlined for experiment 1.

To record individual bee movement, particularly walking behaviour, we employed a similar approach to that used in previous studies (Crall et al., 2018; Cresswell et al., 2012; Williamson et al., 2014). After letting bees settle for 4 h, we took 'pre-exposure' recordings by filming cages in pairs to establish baseline behaviour, conducted under red light for 30 s using a Panasonic HC-V160 video camera (Figure S1), with a ruler to allow distance calibration. At the start of each recording, the position of each bee ID was mapped, and cross-referenced with the video file when analysing behaviour. On finishing the 'pre-exposure' video recordings, the feeding tube for each cage was replaced with a tube containing 2 mL of assigned sucrose solution pesticide (or control) treatment, which was weighed prior to being provisioned. After 20 h post onset of exposure, cages were surveyed and any dead individuals removed. Feeders were then replaced, with the mass of both the new and used feeders being recorded. After 4 h (24 h since onset of exposure), filming of cages began again as described for the pre-exposure recordings. This protocol of removing any dead bees, replenishing feeders, and video recording cages, was repeated every 24 h, with the last analysed recording made 72 h post onset of pesticide exposure. The following day, feeders were removed and weighed, and cages were frozen (−20°C). Later, bees were placed in an oven at 80°C for 48 h and the dry mass per bee weighed.

All video recordings were initially examined within the video tracking programme Kinovea (version 0.8.15), whereby the demonstration (yes/no) of a key set of behaviours (walking, flight, feeding, and general movement) was recorded over a specified timeframe (20 s) per individual (detailed in Supplementary Methods). We used the 'tracking' functionality within the Kinovea software, which allowed automatic motion tracking with manual supervision, to record the distance walked per bee over the same 20 s timeframe that the initial round of monitoring was conducted on. From this information, we produced a walking rate (cm/s) for each bee that walked; calculated as total walking distance (cm) divided by the time (s) the individual was visible and not flying or feeding (bees were sometimes not visible due to crawling under the filter paper).

2.1.6 | Experiment 3: Flight distance and velocity (3.1 and 3.2)

During the 3 days following colony arrival, 120–125 bees (all workers) per natal colony (total = 6 colonies) were randomly removed, placed on ice, and a small circular galvanized iron tag (diameter = 2 mm) attached using super glue to the centre of each individual's thorax following a previously described protocol (Kenna et al., 2019, 2021). Each tagged bee was then allowed to rest in a separate plastic pot for 60 min before being returned to their natal colony. Bees remained in their natal colonies until being removed for flight tests. Flight testing was run in bouts, with three or four bouts run daily. Each bout consisted of six flight mills tested in parallel (one bee per mill) using the apparatus described in Kenna et al. (2021). A magnet hanging from one end of the mill arm allowed attachment to the bee's metal tag. The suspended bee could fly without carrying the load of the tag, and the number and duration of subsequent full rotations of the mill arm (from here-on termed 'circuits') were automatically recorded (Raspberry Pi 3 computer, Model B).

When removing bees from their natal colony for testing, each was assigned to a pesticide treatment (control, Imidacloprid 10 μg/L (I10), Sulfoxaflor 10 μg/L (S10)) and temperature (low°C, mid°C or high °C) combination, resulting in nine treatments with 60 bees tested per treatment (10 bees per colony; total number of bees = 540). To ensure an equal distribution of body sizes across treatments, bees were assigned based on their wet mass directly following removal from the natal colony, resulting in no significant difference in mean dry mass (ANOVA: $p > .1$ for all pairwise comparisons) or variance (Bartlett's K^2 = 6.31, df = 8, p = .61) between treatments. Prior to flight testing, each bee was placed inside a

self-contained transparent Perspex feeding tube with one end sealed using a mesh. Each bee was left to acclimatise for 2 min, immediately after which a droplet (min. 100 mg [with no bee consuming the entire amount]) of the untreated or treated (I10 or S10) sucrose solution was presented on a cover slip on the outer side of the mesh allowing the bee to reach the droplet with its antennae and proboscis. The cover slip and droplet were presented for 5 min to provide enough time for individuals to feed to satiation (Kenna et al., 2021). Direct feeding was identified as the proboscis extending into the sucrose droplet, and each bee was allowed to feed multiple times within this period. The slip was weighed immediately before and after this presentation period to calculate the mass of sucrose solution consumed. With bees varying in the mass consumed over the 5 min period, we produced a 'mass-specific consumption' value per bee, calculated as the total mass of sucrose consumed (mg) per unit of body mass (mg of dry mass). Feeding trials were run in the same 21°C room that natal colonies were housed in. Any bees observed not feeding within the 5 min ($n = 22$), were visibly unhealthy or possessed damaged wings ($n = 3$), were removed from further testing. A further six bees were excluded due to mill technical issues (Table S2).

Once the feeding trial had ended, bees were removed from their respective feeding tubes, placed into separate plastic holding pots (120 mL), and transferred to the testing CE room set at the respective treatment temperature. Still under red light, each bee was left to rest inside the pot for 5 min and then immediately removed and magnetically tethered to its respective flight mill. Once tethered, the room was switched to white light and a support stand held the bee in place for 10 min to allow metabolism of the ingested pesticide (Kenna et al., 2019; Suchail, De Sousa, et al., 2004; Suchail, Debrauwer, et al., 2004), after which the support stand was removed to stimulate flight. Flight trials were capped at a 90 min time limit, at which point the trial was terminated, as a previous thermal performance study that included the three tested temperatures used in this study showed that >90% of bees did not fly past this duration (Kenna et al., 2021). However, each time a worker stopped flying we classed it as a 'strike', with a flight trial being terminated prior to the end of the 90 min period if they reached three strikes (Kenna et al., 2021) (see Supplementary Methods for extended details). On flight trial termination, each bee was untethered, placed in a labelled Eppendorf tube and frozen at −20°C. This was followed by a measure of dry body mass by weighing the bee after oven warming at 80°C for 48 h. Of the 509 bees that were tested on the flight mills, 496 had a filtered flight distance >0 m (termed 'successful fliers') and were used in the data analysis.

2.2 | Data analysis

All statistical analyses were conducted in R v3.6.2 (R Core Team, 2019), and raw data with associated R scripts have been uploaded to the repository Dryad and accessible to the public (Kenna et al., 2023).

2.2.1 | Experiment 1: Likelihood of being responsive (1.1)

The Cox proportional hazards model (Therneau, 2020) was used to conduct an event analysis on the effect of pesticide, temperature, and interaction on the probability of responsiveness over time. Firth's penalised maximum likelihood bias reduction method was implemented using the 'coxphf' package (Heinze et al., 2020), due to some treatment combinations having zero events throughout the 6 day trial. Natal colony was included as an additional covariate to account for inherent differences between colonies. Hazard ratios (probability of the event occurring in the exposed versus control group) are reported for each pesticide-temperature combination (HR > 1 indicates a pesticide is positively associated with the event probability). Kaplan–Meier curves were produced using the 'survminer' package (Kassambara et al., 2020) to show event probability over the 6 days.

2.2.2 | Experiment 2: Likelihood of movement, and rates of walking and consumption (2.1–2.3)

Statistical analyses were conducted using the "lme4" package (Bates et al., 2015), with results reported using the package "lmerTest" (Kuznetsova et al., 2017) and pairwise contrasts using the Tukey method to account for multiple testing performed with the package "lsmeans" (Lenth, 2016). Diagnostic residual plots were examined using the "DHARMa" package (Hartig, 2021) to ensure models met all assumptions (see Supplementary Methods for model selection process).

The likelihood of movement was analysed by fitting generalised linear mixed models (GLMM) under a binomial family distribution (moved = 1, did not move = 0). Data were analysed for all live bees, with any bees not visible during the recording period being excluded. Models were fit with bee ID as a random intercept, to account for individual repeated measures over time. Colony ID was not retained as a random effect to prevent model overfitting and the variance was close to zero. Models were fit for each pesticide examining the effects of temperature, time and the interaction between these two categorical variables, with dry body mass removed as it did not significantly improve model explanatory power. Time was modelled as a categorical predictor allowing comparison of the likelihood of movement after each of 24, 48 and 72 h against the pre-exposure likelihood.

For walking rate (cm/s), we fitted linear mixed effects models (LMER) under a Gaussian distribution, with bee ID and cage ID included as random intercepts. Models were fit for each pesticide examining the effects of temperature, time and the interaction, with dry mass included as a continuous covariate. The response variable (walking rate) was transformed according to Box–Cox estimates calculated through the 'EnvStats' package (Millard, 2013), resulting in a square root transform in all instances except when examining treatment I40 for which the response variable was raised to the power 0.8.

We calculated per capita consumption rate of sucrose solution per 24 h period by taking the total mass of sucrose consumed divided by the number of bees alive in the respective cage at the end. To examine the effects of treatments, we fitted a GLMM under a Gaussian distribution, with the response variable square-root transformed, so that residual distributions met model assumptions. Consumption was examined as a function of pesticide, temperature, and time, as well as pesticide × temperature and pesticide × time interactions. We included cage ID as a random intercept, with the slope of the relationship between time and sucrose consumption allowed to vary between cages to account for repeated measures over time. Colony ID was included as a random intercept to account for inherent colony level differences.

2.2.3 | Experiment 3: Flight distance and velocity (3.1 and 3.2)

For successful fliers ($n = 496$), we calculated total distance (m) and duration (s) flown over the whole flight test. Mean and maximum flight velocity (m/s) were determined for each bee over four periods of flight: (i) 0–100 m; (ii) >100–500 m; (iii) >500–1000 m; and (iv) >1000–2000 m (see Supplementary Methods for justification). Statistical packages in R used in experiment 2 were also used here. For analyses of flight distance (m), mean velocity (m/s) and maximum velocity (m/s), we fitted GLMMs under a gamma family with log link function due to skewed error distributions. We ran analyses examining the likelihood of flying past two 'milestone' distances, 100 and 1000 m (Table S2), by fitting GLMMs under a binomial family distribution (flight surpassing milestone distance = 1, flight not reaching milestone distance = 0). All models were initially constructed considering the main effects of pesticide, temperature, dry body mass, mass-specific consumption and the interactions pesticide × temperature, pesticide × dry mass, temperature × dry mass, and pesticide × mass-specific consumption, with pesticide and temperature modelled as categorical variables and dry mass and mass-specific consumption modelled as continuous variables. Additionally, colony ID was included as a covariate to account for inherent colony-level differences. Models were then simplified through stepwise removal of non-significant terms, checking after each removal that the simplified model had not significantly decreased in explanatory power.

3 | RESULTS

3.1 | Experiment 1

3.1.1 | Likelihood of being responsive

While low°C and mid°C imidacloprid exposed bees were 113 and 16 times more likely, respectively, to be unresponsive relative to controls ($p \leq .005$; Figure 2; Figures S2 and S3), we detected no significant effect under high°C. Indeed, imidacloprid exposed bees were significantly more likely to be responsive when under the low°C ($p < .001$) and mid°C ($p = .033$) relative to high°C. Contrastingly, we detected no effect of sulfoxaflor on the likelihood of becoming unresponsive under any temperature.

3.2 | Experiment 2

Mortality was expectedly low over the trial with never more than a single death per treatment from 54 bees per treatment cohort.

3.2.1 | Likelihood of movement

Significant effects were detected in imidacloprid exposed bees but only under low°C and mid°C. Specifically, and relative to the pre-exposure response for that respective treatment, bees exposed to Imidacloprid 40 μg/L (I40) demonstrated a significantly reduced likelihood of movement when assessed at 24, 48 and 72 h under low°C, and at 24 and 72 h under mid°C (GLMM: $z \geq 2.84$, $p \leq .023$; Figure 3a; Tables S3a–S3e). Exposure to Imidacloprid 10 μg/L (I10) showed a reduced likelihood only at 72 h when under low°C and mid°C ($z \geq 2.60$, $p \leq .046$). Together the findings indicate that the degree to which likelihood of movement was reduced was greater as the temperature decreased. For instance, I40 exposed bees assessed under low°C showed a significantly greater reduction relative to high°C at 24 and 48 h, and relative to mid°C at 48 h ($z \geq 2.14$, $p \leq .033$). In contrast, we detected no effect of sulfoxaflor at either concentration under any temperature relative to the pre-exposure response.

3.2.2 | Walking rate

Significant effects were detected in imidacloprid exposed bees but again only under low°C and mid°C. Relative to pre-exposure responses, I40 exposed bees demonstrated a significantly increased walking rate when assessed at 24 and 48 h under low°C, and at 24 h under mid°C (LMER: $t \geq 2.68$, $p \leq .039$; Figure 3b; Tables S4a–S4e). Exposure to I10 showed an increased walking rate only at 24 h when under low°C ($t = 2.83$, $p = .026$). Intriguingly, the pattern of walking by imidacloprid exposed bees suggests an initial hyperactive effect over the first 24 or 48 h, with this lessening in magnitude by 72 h supporting an acute followed by chronic effect. But overall, our findings indicate that the degree to which walking rate increased was greater as the temperature decreased. For instance, I40 exposed bees assessed under low°C showed a significant increase in walking rate relative to high°C at 24 and 48 h ($t = -2.06$, $p = .040$). For sulfoxaflor exposed bees, while S40 exposed bees significantly increased walking rate under mid°C at 24 h after the onset of exposure ($t = 3.38$, $p = .005$), we detected no effect under any other temperature and at any other timepoint for either concentration.

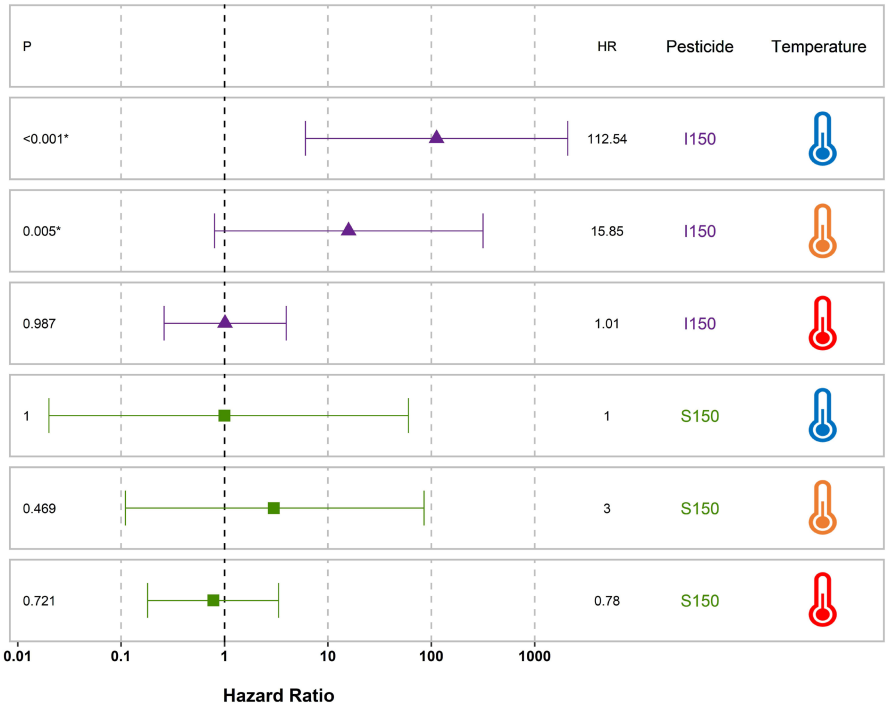

FIGURE 2 Experiment 1. Forest plot showing the hazard ratios (HR) of becoming unresponsive over a 144 h period of exposure to each pesticide (I150 = Imidacloprid 150 μg/L, purple triangle; S150 = Sulfoxaflor 150 μg/L, green square) at each of the three tested temperatures (top to bottom: low°C = blue thermometer; mid°C = orange; high°C = red). Vertical black dashed line shows the standardized control response for relative comparison per treatment, the error bars representing 95% confidence interval of hazard ratio, and *p*-value column denoting the statistical probability of a worker becoming unresponsive. Number of bees tested per pesticide-temperature combination treatment was standardized at 45 individuals.

3.2.3 | Sucrose consumption rate

When pooling bees across all pesticide treatments (including control), we found per capita sucrose consumption decreased as temperatures got warmer ($p < .001$ for all contrasts; Table S5). Bees exposed to I10 or I40 consumed significantly less relative to control bees under all temperatures (LMER: $z \geq 6.40$; $p < .001$; Figure 4). Our findings indicate that the degree to which consumption was reduced was greater as the temperature decreased. I40 exposed bees consumed significantly less under low°C than they did under both mid°C and high°C ($t \geq 2.57$; $p \leq .011$). Moreover, we found evidence of a chronic effect of imidacloprid exposure on consumption, as the slope over time significantly differed from control bees as evidenced by a significant interaction term (I10: $t = -4.89$, $p < .001$ & I40: $t = -7.02$, $p < .001$). In contrast, while we did observe S40 exposed bees to consume significantly less sucrose solution under mid°C relative to control bees ($t = -3.06$, $p = .021$), there was no detectable effect of sulfoxaflor on consumption under any other temperature or concentration.

3.3 | Experiment 3

3.3.1 | Flight distance

Under low°C and mid°C, control bees flew a similar mean (± s.e.m.) distance of 1368 ± 190 and 1323 ± 203 m, respectively. For Imidacloprid 10 μg/L (I10) exposed bees, we observed a 14.9% and 5.6% shorter distance reached relative to the controls for each respective temperature, and for Sulfoxaflor 10 μg/L (S10) exposed bees an 8.6% and 0.7% shorter distance, all of which we detected as non-significant reductions (GLMM: $z \leq 0.58$, $p \geq .83$; Figure 5; Table S6). Under high°C, control bees reached a longer distance of 1504 ± 192 m, but this time we saw a more dramatic and significant effect in I10 bees which showed a 53.2% reduction in distance reached ($z = -3.07$, $p = .006$). This indicates that as temperature increases the toxicity effect of imidacloprid on distance also increases, and in support we detected a significantly greater reduction in distance under high°C relative to mid°C ($t = -2.52$, $p = .012$; Figures S4 and S5). Whilst S10 bees under high°C showed a greater reduction in distance of 24.2%, relative to the other temperatures, we detected it as non-significant ($z = -1.42$, $p = .33$).

We further investigated the likelihood of bees reaching a 100 m milestone and a 1000 m milestone to see how flight trial termination (stopped flying three times) could explain the above observed flight distance patterns. For I10 bees we detected no significant difference relative to control bees in reaching either milestone under any temperature, except when under high°C in which I10 bees were significantly less likely to reach 1000 m (GLMM: $z = -2.93$, $p = .009$; Figures S5 and S6; Tables S7a and S7b). Accordingly, the likelihood of I10 bees reaching 1000 m was significantly reduced when under high°C relative to mid°C ($z = -2.02$, $p = .043$). For S10 bees, we detected no significant difference relative to control bees in reaching either milestone under any temperature.

3.3.2 | Flight velocity

We detected no significant effect of I10 or S10 on either mean or maximum velocity relative to control bees under any temperature, and this lack of effect remained relatively consistent across the four phases of flight we investigated (Figures S6 and S7; Tables S8a–S8h).

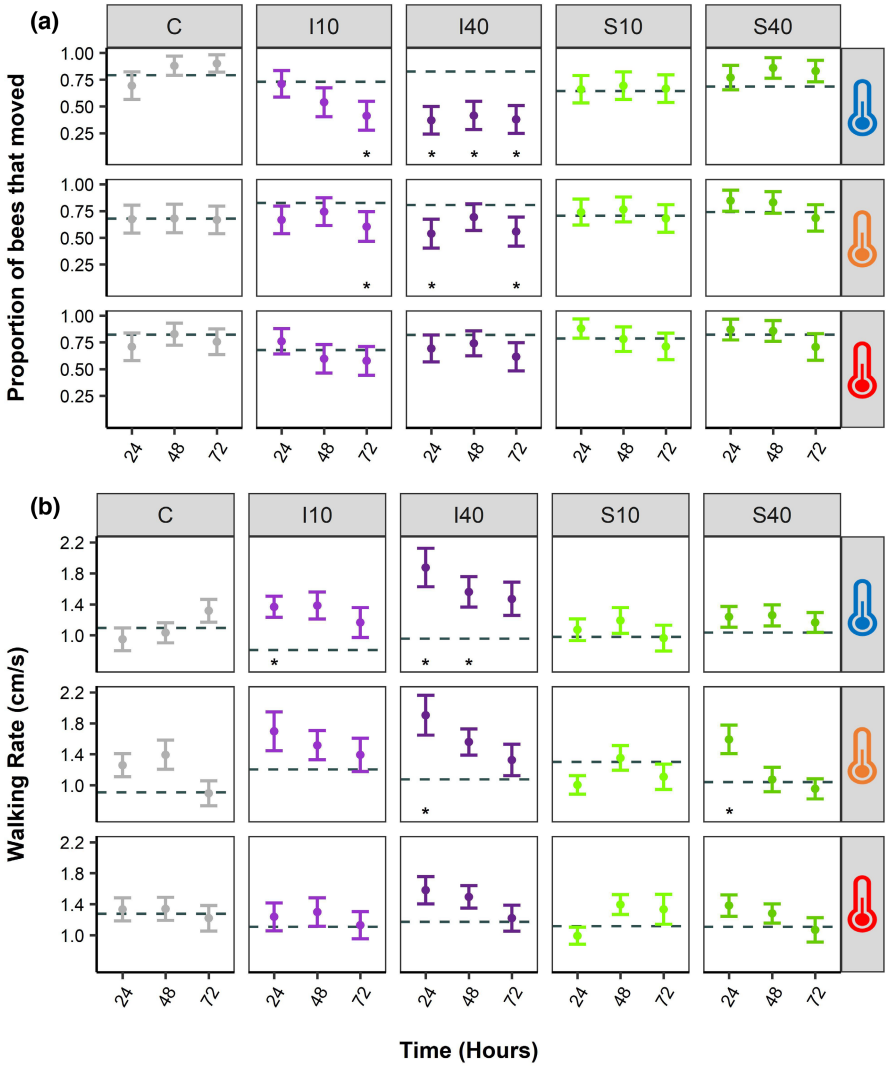

FIGURE 3 Experiment 2. Multi-panel plots representing two different aspects of movement and showing variation in responses under the different pesticide (C = control bees; I10 or S10 = Imidacloprid or Sulfoxaflor 10 μg/L; I40 or S40 = Imidacloprid or Sulfoxaflor 40 μg/L) and temperature (top to bottom: low°C = blue thermometer; mid°C = orange; high°C = red) combinations. Data points represent the raw data, the black horizontal dashed line shows the pre-exposure response (baseline) measured prior to the spiked sucrose treatments being provisioned, and asterisks denote a statistically significant change relative to the pre-exposure consumption for the respective treatment. (a) Proportion of bees that moved during the snap-shot video footage at each 24 h monitoring period, with error bars representing 95% confidence limits calculated by the 'Wald' method on the raw data. (b) For the bees that did move, the figure shows the walking rate (cm/s) per bee, with the error bars representing standard error. Number of bees per pesticide-temperature combination treatment that started the assay was standardized at 54 individuals.

4 | DISCUSSION

We quantify how environmental temperature can modulate the sublethal effects from pesticide exposure in an important insect pollinator and show that just a small temperature change (±3°C) can dramatically alter the behavioural impact of pesticide exposure. Toxicity of the neonicotinoid—imidacloprid—was strongly temperature dependent, but critically the direction of effect depended on the type of response being measured (Table 1). The energetically demanding behaviour of flight exhibited a positive relationship with pesticide toxicity increasing under higher temperatures, whereas the presumed less energetically demanding behaviours (responsiveness, walking, consumption) were negative with pesticide toxicity increasing under cooler temperatures. Our findings show we cannot assume warming and extreme temperature events to influence pesticide impacts on functional traits uniformly, highlighting the complexity of predicting how ecosystems will function under future stress. These findings show the need to consider the environmental context when assessing pesticide toxicity to beneficial organisms, otherwise we limit our accuracy in modelling pesticide risks across daily temperature cycles, seasons, and climatic regions, particularly when projecting responses under agricultural expansion and future climate change. Intriguingly, we found little consistent effect of the sulfoximine—sulfoxaflor—on *B. terrestris* behaviour, finding no impact at 150 μg/L (when assessing responsiveness), and any effect on walking and consumption rates were under mid°C, but not low°C and high°C.

4.1 | Temperature dependency of neonicotinoid sublethal effects varied across behaviours

The increasing pesticide impacts as temperature dropped found for responsiveness, walking rate and consumption rate (experiments 1 and 2 when exposed to imidacloprid) is interesting given neonicotinoids have typically been designated as positive temperature response coefficient insecticides (Arthur et al., 2004; Mansoor et al., 2015; Tsaganou et al., 2021), although a study by Boina et al. (2009) in a hemipteran pest (*Diaphorina citri*) did find imidacloprid to be more toxic at 17°C compared to 27°C. A possible reason for such contrasting results is that most previous studies have predominantly centred around lethality tests on target insect pests,

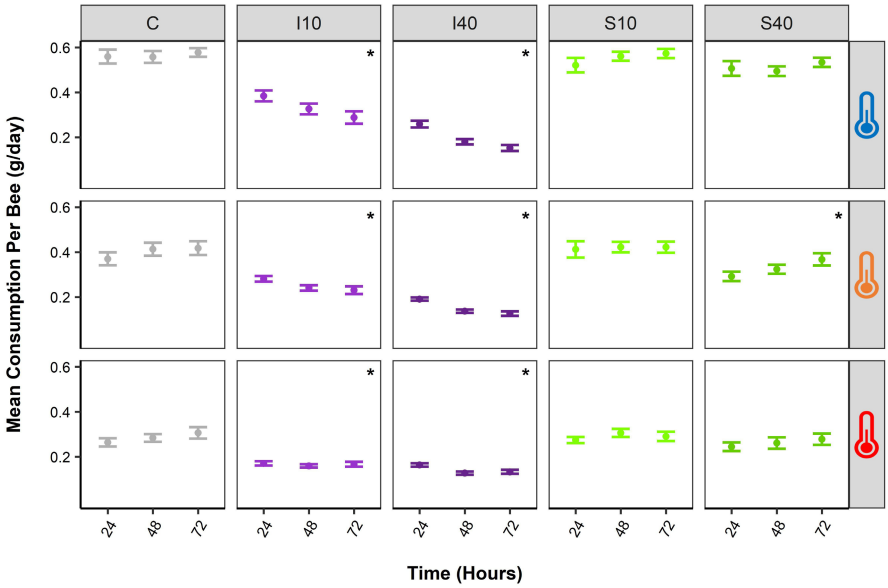

FIGURE 4 Experiment 2. Multi-panel plots showing per capita consumption rate of sucrose solution per bee for each 24 h period for each pesticide (C = control bees; I10 or S10 = Imidacloprid or Sulfoxaflor 10 μg/L; I40 or S40 = Imidacloprid or Sulfoxaflor 40 μg/L) and temperature (top to bottom: low°C = blue thermometer; mid°C = orange; high°C = red) combination. Data points represent the mean value of the raw data with standard error. Any asterisk placed in the top right corner of a panel denotes a statistically significant lower consumption for that treatment relative to the control at that respective temperature across all 72 h. Number of bees per pesticide-temperature combination treatment that started the assay was standardized at 54 individuals evenly distributed across 18 cages.

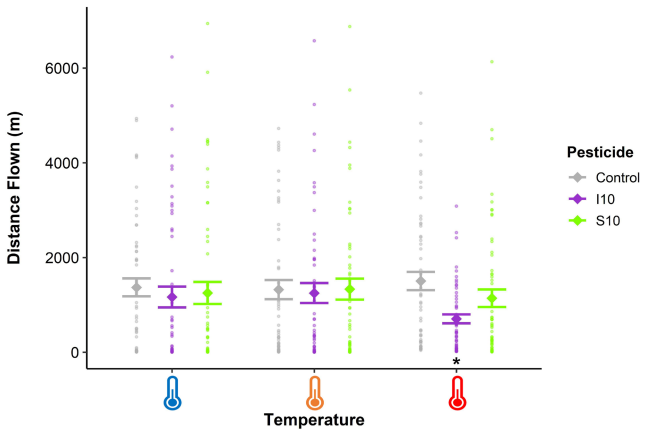

FIGURE 5 Experiment 3. Distance flown by tethered bees over the 90 min trial on a flight mill for each pesticide treatment cohort (Control; I10 or S10 = Imidacloprid or Sulfoxaflor 10 μg/L) under each temperature (low°C = blue thermometer; mid°C = orange; high°C = red). Raw data values are provided in the background (points), with the mean (diamond) and associated standard errors (whiskers) overlaid. The asterisk placed under the I10 treatment under high°C denotes a statistically significant lower distance relative to the control at that respective temperature. The figure represents all workers that were classed as successful fliers (i.e., had filtered flight distance >0 m) giving sample sizes of >50 individuals per pesticide-temperature combination treatment (control-low°C = 55; I10-low°C = 56; S10-low°C = 56; control-mid°C = 54; I10-mid°C = 53; S10- mid°C = 53; control-high°C = 57; I10-high°C = 56; S10-high°C = 56).

and not sublethal effects on non-target beneficial insects. Sublethal testing on a wider range of insect species would, therefore, improve our understanding of how conserved responses may be across the insect phylogeny. However, as shown by our study, the direction of the effect is also likely to depend on what responses are being studied, suggesting that accounting for taxonomic differences in physiological/morphological traits alongside which behaviours are being studied may be important. For instance, how species differ in body mass can determine responses to environmental temperature (Jenkins et al., 2007) and influence thermoregulatory ability (Bishop & Armbruster, 1999).

Bumblebees are considered heterothermic, as they vary between unregulated and self-regulated control of body temperature, and are capable of generating heat endogenously in a process known as non-flight thermogenesis (Potts et al., 2018). For bumblebees under our low and possibly mid temperatures (21 and 27°C) to have performed the behavioural assays optimally, it would have required individuals to maintain a body temperature above the ambient (Heinrich, 1975). However, neonicotinoids have been shown to affect non-flight thermogenesis in individual honey bees (Tosi et al., 2016) and bumblebees (Potts et al., 2018), which could explain why we found pesticide impacts on responsiveness and likelihood of movement to increase as temperature cooled. In addition, elevation and regulation of body temperature above ambient requires an increased energy intake to meet these metabolic demands, yet in our experiment 2 we found imidacloprid caused significantly lower sucrose consumption. From

TABLE 1 Results summary across the three experiments for all measured functional behavioural responses. Thermometers (low°C = blue; mid°C = orange; high°C = red) denote the temperature under which a statistically significant effect of pesticide exposure was detected for the respective behaviour, with 'n.s.' denoting a non-significant effect. A downward blue arrow depicts if we found evidence for pesticide toxicity decreasing as it got warmer for that respective behaviour, whereas an upward red arrow if pesticide toxicity increased as the temperature got warmer.

		Temperatures under which a pesticide effect was detected		Direction of pesticide toxicity with increasing temperature	
Exp.	Response measured	*Imidacloprid*	*Sulfoxaflor*	*Imidacloprid*	*Sulfoxaflor*
1	Likelihood of being responsive	🌡🌡	n.s.	↓	–
2	Likelihood of movement	🌡🌡	n.s.	↓	–
2	Walking rate	🌡🌡	🌡	↓	None
2	Rate of food consumption	🌡🌡	🌡	↓	None
3	Flight distance	🌡	n.s.	↑	–
3	Flight velocity	n.s.	n.s.	–	–

our study, it is difficult to distinguish between this being a direct effect on appetite (Laycock et al., 2012; Muth et al., 2020; Zhu et al., 2017), an adaptive response to reducing active ingredient intake (although unlikely given work by Arce et al., 2018), or an indirect effect of lowering activity (Azpiazu et al., 2019). Furthermore, xenobiotic chemicals should be metabolized at a faster rate under warmer conditions making them less toxic (Harwood et al., 2009; Khan & Akram, 2014; Noyes et al., 2009; Weston et al., 2009), and it has been suggested that imidacloprid can have a greater binding efficiency to aphid neuronal membranes at lower temperatures (Wellmann et al., 2004), which all provide potential rationale for findings in experiments 1 and 2.

The potential explanation that imidacloprid exposed bees were unable to generate appropriate body temperatures to perform the measured responses (experiments 1 and 2), however, do not concur with our findings of pesticide impacts on flight performance increasing as temperatures got warmer (experiment 3). This is surprising, as bumblebees need to attain and maintain a minimum thoracic temperature typically around 35°C to support take-off and continuous flight (Heinrich, 1975; Mapalad et al., 2008; Nieh et al., 2006), which would encourage the assumption that impacts of neonicotinoid exposure on flight would be greatest at lower ambient temperatures. A possible reason for this divergence in relationships between temperature and pesticide toxicity (going from negative in experiments 1–2 to positive in experiment 3) could be down to rates of biotransformation increasing with temperature. Biotransformation, the process whereby pesticides are enzymatically modified to facilitate excretion from the body, can sometimes yield toxic metabolites in a process known as bioactivation, and a primary metabolite in the case of imidacloprid is an olefin compound that is actually more toxic to insects than the parent compound (Suchail, De Sousa, et al., 2004; Suchail, Debrauwer, et al., 2004). Under a one-shot acute dose of imidacloprid in our flight experiment, where there was no time for imidacloprid to accumulate within individuals like there was in the cage trials, conversion of imidacloprid to olefin at a faster rate provides a rationale for impairment to flight performance at higher temperatures. Indeed, a similar process has been proposed to explain the increased toxicity of organophosphates to insects at warmer post-exposure temperatures (Lydy et al., 1999). Additionally, bumblebees need to utilise complex thermoregulatory processes to prevent overheating during flight (Dudley, 2000; Heinrich, 1975), and recent research suggests that some bumblebee populations may be moving towards their flight thermal limits around 30°C (Kenna et al., 2021). As neonicotinoids are known to have detrimental impacts on other thermoregulatory processes in individuals (Potts et al., 2018; Tosi et al., 2016), it is possible imidacloprid exposed workers edged too close to their thermal limits leading to earlier termination of flight. This impairment seemed to stem from larger workers prematurely terminating flight, which are known to be less able to dissipate heat to the environment (Harrison & Roberts, 2000; Rubalcaba & Olalla-Tárraga, 2020). Our findings may therefore help to further elucidate how bee species of different sizes will respond to pesticide-temperature interactions in the future.

4.2 | Imidacloprid exposure showed temperature dependent hyperactivity

Whilst chronic imidacloprid exposure led to fewer bees initiating movement in experiment 2, those bees that did move walked at a faster rate, which again was temperature dependent. As agonists of insect nicotinic acetylcholine receptors, neonicotinoids stimulate

neuronal activity (Tomizawa & Casida, 2005), potentially leading initially to hyperactivity (Tosi & Nieh, 2017). Indeed, a previous study did show faster bumblebee flight shortly after being acutely exposed to imidacloprid (Kenna et al., 2019), but this was followed by hypoactivity, likely reflecting a cumulative dose-dependent effect. Interestingly, Crall et al. (2018) found that whilst chronic exposure to imidacloprid increased movement velocity in nest bumblebee workers, it decreased the amount of time workers were active, suggesting our findings translate to colony level impacts. Indeed, continued imidacloprid exposure may eventually result in reduced muscular activity (Almeida et al., 2021; Williamson et al., 2014) as result of neuronal dysfunction and oxidative stress (Martelli et al., 2020; Palmer et al., 2013), mitochondrial damage (Lu et al., 2020; Moffat et al., 2015), and impacts on the regulation of genes involved in muscle function (Colgan et al., 2019; Wu et al., 2017). Further to this, a recent study by Manzi et al. (2020) demonstrated that imidacloprid can lead to changes in the expression of genes in honeybees relating to both stress response and ionic channels in the brain that are key in neuronal excitability. Expression of these genes was increased following imidacloprid exposure, and this overexpression was further intensified at 20°C but not at warmer temperatures of 28 or 36°C.

4.3 | Comparative lower sublethal impacts of sulfoxaflor on behaviour

At the concentrations tested in this study, sulfoxaflor had a comparatively lesser effect on behaviour relative to controls than did imidacloprid. We observed no effect of sulfoxaflor on responsiveness (experiment 1), and whilst in experiment 2 we did see an effect on walking rate at 24 h since the onset of exposure, and on consumption measured across the course of the trial, this was only at the mid temperature. Whilst imidacloprid bees exhibited a >50% reduction in flight distance (experiment 3) relative to controls under the high°C, sulfoxaflor exposed bees exhibited a 24% reduction under the same temperature. We did not find this drop to be statistically different from controls, but this may have been due to the distribution of the data showing a long right-tail, with a few individuals skewing the mean. A 24% reduction, however, still has a potentially large implication on foraging range and further investigation on how sulfoxaflor can affect different components of flight would be recommended. The slow detoxification of neonicotinoids has been proposed as a reason for their high toxicity compared to other insecticides (Zhang et al., 2021), and relative to some neonicotinoids, sulfoxaflor is thought to have a higher degradation rate in nectar and pollen (Linguadoca et al., 2021), which may also help to explain our results.

For each separate experiment, we used the same concentrations between imidacloprid and sulfoxaflor to enable true comparative tests, but it is important to question how our chosen concentrations reflect exposure across landscapes. For neonicotinoids, which global regions are being considered (as national regulations and farming practices differ), what treated or contaminated flowering plants are being foraged upon (Bonmatin et al., 2015; Kyriakopoulou et al., 2017), alongside whether nectar or pollen is being analysed, can change exposure concentrations dramatically. Indeed, studies across the world have found imidacloprid concentrations in certain plants and bees to range greatly (Azpiazu et al., 2019; Blacquière et al., 2012; Botias et al., 2015; David et al., 2016; Goulson, 2013; Jiang et al., 2018; Johnson et al., 2010; Mitchell et al., 2017; Mullin et al., 2010; Simon-Delso et al., 2015; Stewart et al., 2014; Stoner & Eitzer, 2012), from lower than our 10 μg/L to higher than our 150 μg/L. In a commercial melon field in Spain (Azpiazu et al., 2019), whilst a mean (± s.e.m.) of 15 ± 8 ppb imidacloprid was detected in nectar - which is a higher concentration than used in our experiments 2 and 3—a mean of 370 ± 186 ppb was detected in pollen—which is higher than what we used even in our experiment 1. For sulfoxaflor, application concentrations are typically higher in the field (relative to imidacloprid), and recent studies have found sulfoxaflor residues in nectar and pollen of a variety of treated crops >10 μg/L (Jiang et al., 2020) and >150 μg/L (Cheng et al., 2018; US EPA, 2016; Zhou et al., 2022) when applied close to the blooming period. Whilst bees in experiment 2 provisioned with sulfoxaflor sucrose solution consumed more on average than imidacloprid bees (and thus received a higher sulfoxaflor dosage) we still found little consistent effect of sulfoxaflor, despite other studies exposing bees to concentrations similar or under that used in our experiments 2 and 3 finding impacts on different behaviours (El-Din et al., 2022; Linguadoca et al., 2021; Siviter et al., 2020; Siviter, Brown, et al., 2018; Siviter, Koricheva, et al., 2018; Taning et al., 2019). Interestingly, Boff et al. (2021) found sulfoxaflor exposure at 50 ppb, but not 10 ppb, led to impaired flight and foraging in a solitary bee. Whilst our results support sulfoxaflor concentrations ≤150 μg/L across our tested temperature range being a lower risk to bumblebees relative to nitrimine-based neonicotinoids, the small effect we observed on walking and consumption rates together with other study findings still question how 'bee safe' sulfoxaflor is—especially given it is often sprayed directly on crops potentially increasing contact exposure.

4.4 | Implications of temperature modulating pesticide effects

Our work provides evidence of a synergistic impact between temperature and neonicotinoid exposure on bee health, and highlights the need to account for this in future studies and risk assessments. Using temperature groupings derived from non-linear thermal flight performance data (Kenna et al., 2021), we found little behavioural change in pesticide response between two sequential temperatures followed by an effect under the lowest or highest temperature suggesting a thermal tipping-point had been crossed.

The potential impact of this should not be under-appreciated, as pesticides can accumulate and temperatures fluctuate even within nests (Crall et al., 2018). Our results suggest that during cold-snaps some within-nest behaviours may be more substantially impaired by pesticides than expected. This highlights the importance of understanding interactive effects, and supports recent work in freshwater mayflies, where high temperatures were suggested to have a synergistic interaction with imidacloprid exposure (Camp & Buchwalter, 2016; Macaulay et al., 2021). Using controlled lab studies like ours should remain a crucial approach to understanding inadvertent pesticide effects under future climate scenarios, as it is difficult for contemporary field studies to capture interactions between projected conditions (Kolano et al., 2021). Field experiments typically can only account for short-term high or low temperatures in the range experienced at that time, which may not simulate future climatic conditions and not always detect synergies (Burgess et al., 2021). Furthermore, future risk assessment guidelines for active substance authorization could incorporate our approach as current requirements for supporting evidence are primarily for single response studies at standardized temperatures that assess lethal [not sublethal] endpoints. Whilst we acknowledge the additional burden associated, a potential policy recommendation is that risk assessment requires ecotoxicology studies on bees [and other insects] to be conducted across a standardized temperature gradient to enable toxicity to be linked better with the climate region(s) where the substance will be used. Our study thus raises the need for discussion around how different climatic temperatures should determine the choice of insecticide and the timing of insecticide application in sustainable agricultural management practices.

Seasonal patterns of pesticide application to agricultural landscapes makes exposure to insects under wide thermal ranges a realistic scenario. Additionally, future landscapes are predicted to experience increased rates of pesticide application along with warmer and more extreme climates, increasing the likelihood that insect pollinators will experience these stressors simultaneously (Noyes & Lema, 2015). Our study highlights the need for further quantification on how temperature variations during the day/season alter the risks of exposure, impacts foraging activity and consequently pollination services (Greenop et al., 2020; Stanley et al., 2015). Furthermore, understanding how application rates translate to actual exposure across landscapes, and importantly how degradation and risk of exposure change under different temperatures is an integral future avenue of work. Findings can better inform pesticide users on recommended application timing based on predicted thermal conditions alongside known pollinator susceptibility windows (e.g., when colonies are being founded, when nutritional landscapes are limiting). It is possible that insecticides could be used on a rotational basis, using knowledge of temperature response coefficients to limit harm to beneficial insect pollinators, and this targeted approach to pesticide use could be incorporated into integrated pest management (IPM) strategies currently being developed and promoted (Creissen et al., 2021). Furthermore, our findings on neonicotinoid toxicity are also especially pertinent given current climate predictions suggest increased weather extremes, and average temperatures to rise. Of concern has been lower latitude and southern hemisphere regions where neonicotinoids are still routinely applied and are vulnerable areas to climate impacts (Dicks et al., 2021; Potts et al., 2016). Understanding temperature dependencies can enable interpolation and extrapolation of pesticide exposure responses across large and natural geographic ranges to inform model forecasts of risk under changing climates.

AUTHOR CONTRIBUTIONS

Daniel Kenna and Richard J. Gill conceived the project; Daniel Kenna, Peter Graystock and Richard J. Gill designed the experiments; Daniel Kenna undertook the experiments; Daniel Kenna and Richard J. Gill analysed the data and wrote the manuscript; Peter Graystock provided feedback on the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no known conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings are available on Dryad at https://doi.org/10.5061/dryad.9ghx3ffnf.

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REFERENCES

Abram, P. K., Boivin, G., Moiroux, J., & Brodeur, J. (2017). Behavioural effects of temperature on ectothermic animals: Unifying thermal physiology and behavioural plasticity. *Biological Reviews*, *92*(4), 1859–1876. https://doi.org/10.1111/brv.12312

Almeida, C. H. S., Haddi, K., Toledo, P. F. S., Rezende, S. M., Santana, W. C., Guedes, R. N. C., Newland, P. L., & Oliveira, E. E. (2021). Sublethal agrochemical exposures can alter honey bees' and Neotropical stingless bees' color preferences, respiration rates, and locomotory responses. *Science of the Total Environment*, *779*, 146432. https://doi.org/10.1016/J.SCITOTENV.2021.146432

Arce, A. N., David, T. I., Randall, E. L., Ramos Rodrigues, A., Colgan, T. J., Wurm, Y., & Gill, R. J. (2017). Impact of controlled neonicotinoid exposure on bumblebees in a realistic field

setting. *Journal of Applied Ecology*, *54*(4), 1199–1208. https://doi.org/10.1111/1365-2664.12792

Arce, A. N., Rodrigues, A. R., 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. https://doi.org/10.1098/rspb.2018.0655

Archer, L. C., Sohlström, E. H., Gallo, B., Jochum, M., Woodward, G., Kordas, R. L., Rall, B. C., & O'Gorman, E. J. (2019). Consistent temperature dependence of functional response parameters and their use in predicting population abundance. *Journal of Animal Ecology*, *88*(11), 1670–1683. https://doi.org/10.1111/1365-2656.13060

Arthur, F. H., Yue, B., & Wilde, G. E. (2004). Susceptibility of stored-product beetles on wheat and maize treated with thiamethoxam: Effects of concentration, exposure interval, and temperature. *Journal of Stored Products Research*, *40*(5), 527–546. https://doi.org/10.1016/J.JSPR.2003.08.001

Azpiazu, C., Bosch, J., Viñuela, E., Medrzycki, P., Teper, D., & Sgolastra, F. (2019). Chronic oral exposure to field-realistic pesticide combinations via pollen and nectar: Effects on feeding and thermal performance in a solitary bee. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-50255-4

Bates, D., Machler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Bishop, J. A., & Armbruster, W. S. (1999). Thermoregulatory abilities of Alaskan bees: Effects of size, phylogeny and ecology. *Functional Ecology*, *13*(5), 711–724. https://doi.org/10.1046/J.1365-2435.1999.00351.X

Blacquière, T., Smagghe, G., Van Gestel, C. A. M., & Mommaerts, V. (2012). Neonicotinoids in bees: A review on concentrations, side-effects and risk assessment. *Ecotoxicology*, *21*, 973–992. https://doi.org/10.1007/s10646-012-0863-x

Boff, S., Scheiner, R., Raizer, J., & Lupi, D. (2021). Survival rate and changes in foraging performances of solitary bees exposed to a novel insecticide. *Ecotoxicology and Environmental Safety*, *211*, 111869. https://doi.org/10.1016/J.ECOENV.2020.111869

Boina, D. R., Onagbola, E. O., Salyani, M., & Stelinski, L. L. (2009). Influence of Posttreatment temperature on the toxicity of insecticides against *Diaphorina citri* (Hemiptera: Psyllidae). *Journal of Economic Entomology*, *102*(2), 685–691. https://doi.org/10.1603/029.102.0229

Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A., Noome, D. A., Simon-Delso, N., & Tapparo, A. (2015). Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research*, *22*(1), 35–67. https://doi.org/10.1007/S11356-014-3332-7/TABLES/5

Botias, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., & Goulson, D. (2015). Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. *Environmental Science and Technology*, *49*(21), 12731–12740. https://doi.org/10.1021/acs.est.5b03459

Bryden, J., Gill, R. J., Mitton, R. A. A., Raine, N. E., & Jansen, V. A. A. (2013). Chronic sublethal stress causes bee colony failure. *Ecology Letters*, *16*(12), 1463–1469. https://doi.org/10.1111/ele.12188

Burgess, B. J., Purves, D., Mace, G., & Murrell, D. J. (2021). Classifying ecosystem stressor interactions: Theory highlights the data limitations of the additive null model and the difficulty in revealing ecological surprises. *Global Change Biology*, *27*(13), 3052–3065. https://doi.org/10.1111/GCB.15630

Camp, A. A., & Buchwalter, D. B. (2016). Can't take the heat: Temperature-enhanced toxicity in the mayfly Isonychia bicolor exposed to the neonicotinoid insecticide imidacloprid. *Aquatic Toxicology*, *178*, 49–57. https://doi.org/10.1016/J.AQUATOX.2016.07.011

Cheng, Y., Bu, Y., Tan, L., Wu, W., Li, J., Zhou, J., Zhai, A., & Shan, Z. (2018). A semi-field study to evaluate effects of sulfoxaflor on honey bee (*Apis mellifera*). *Bulletin of Insectology*, *71*(2), 225–233.

Colgan, T. J., Fletcher, I. K., Arce, A. N., Gill, R. J., Ramos Rodrigues, A., Stolle, E., Chittka, L., & Wurm, Y. (2019). Caste- and pesticide-specific effects of neonicotinoid pesticide exposure on gene expression in bumblebees. *Molecular Ecology*, *28*, 1964–1974. https://doi.org/10.1111/mec.15047

Crall, J. D., Switzer, C. M., Oppenheimer, R. L., Ford Versypt, A. N., Dey, B., Brown, A., Eyster, M., Guérin, C., Pierce, N. E., Combes, S. A., & de Bivort, B. L. (2018). Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. *Science*, *362*(6415), 683–686. https://doi.org/10.1126/science.aat1598

Creissen, H. E., Jones, P. J., Tranter, R. B., Girling, R. D., Jess, S., Burnett, F. J., Gaffney, M., Thorne, F. S., & Kildea, S. (2021). Identifying the drivers and constraints to adoption of IPM among arable farmers in the UK and Ireland. *Pest Management Science*, *77*(9), 4148–4158. https://doi.org/10.1002/PS.6452

Cresswell, J. E., Page, C. J., Uygun, M. B., Holmbergh, M., Li, Y., Wheeler, J. G., Laycock, I., Pook, C. J., de Ibarra, N. H., Smirnoff, N., & Tyler, C. R. (2012). Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). *Zoology*, *115*(6), 365–371. https://doi.org/10.1016/j.zool.2012.05.003

David, A., Botías, C., Abdul-Sada, A., Nicholls, E., Rotheray, E. L., Hill, E. M., & Goulson, D. (2016). Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environment International*, *88*, 169–178. https://doi.org/10.1016/j.envint.2015.12.011

Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B., & Naylor, R. L. (2018). Increase in crop losses to insect pests in a warming climate. *Science*, *361*(6405), 916–919. https://doi.org/10.1126/SCIENCE.AAT3466/SUPPL_FILE/AAT3466-DEUTSCH-SM.PDF

Dicks, L. V., Breeze, T. D., Ngo, H. T., Senapathi, D., An, J., Aizen, M. A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L. A., Gemmill-Herren, B., Howlett, B. G., Imperatriz-Fonseca, V. L., Johnson, S. D., Kovács-Hostyánszki, A., Kwon, Y. J., Lattorff, H. M. G., Lungharwo, T., Seymour, C. L., … Potts, S. G. (2021). A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nature Ecology & Evolution*, *5*(10), 1453–1461. https://doi.org/10.1038/s41559-021-01534-9

Dudley, R. (2000). *The biomechanics of insect flight: Form, function, evolution*. Princeton University Press. https://doi.org/10.2307/j.ctv301g2x

EFSA. (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin, thiamethoxam and imidacloprid. *EFSA Journal*, *11*(1), 3066–3068.

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*(2), 1–12. https://doi.org/10.1007/S13592-022-00937-2/FIGURES/3

Gill, R. J., Baldock, K. C. R., Brown, M. J. F., Cresswell, J. E., Dicks, L. V., Fountain, M. T., Garratt, M. P. D., Gough, L. A., Heard, M. S., Holland, J. M., Ollerton, J., Stone, G. N., Tang, C. Q., Vanbergen, A. J., Vogler, A. P., Woodward, G., Arce, A. N., Boatman, N. D., Brand-Hardy, R., … Potts, S. G. (2016). Protecting an ecosystem service: Approaches to understanding and mitigating threats to wild insect pollinators. *Advances in Ecological Research*, *54*, 135–206. https://doi.org/10.1016/bs.aecr.2015.10.007

Gill, R. J., Ramos-Rodriguez, O., & Raine, N. E. (2012). Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature*, *491*(7422), 105–108. https://doi.org/10.1038/nature11585

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*(5538), 2248–2251. https://doi.org/10.1126/SCIENCE.1061967

Glunt, K. D., Blanford, J. I., & Paaijmans, K. P. (2013). Chemicals, climate, and control: Increasing the effectiveness of malaria vector control tools by considering relevant temperatures. *PLoS Pathogens*, *9*, e1003602. https://doi.org/10.1371/journal.ppat.1003602

Goulson, D. (2010). Bumblebees. In *Bumblebees: Behaviour, ecology and conservation* (2nd ed.). Oxford University Press.

Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*, *50*, 977–998. https://doi.org/10.1111/1365-2664.12111

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, *347*, 1–16. https://doi.org/10.1126/science.1255957

Greenop, A., Mica-Hawkyard, N., Walkington, S., Wilby, A., Cook, S. M., Pywell, R. F., & Woodcock, B. A. (2020). Equivocal evidence for Colony level stress effects on bumble bee pollination services. *Insects*, *11*(3). https://doi.org/10.3390/INSECTS11030191

Halsch, C. A., Shapiro, A. M., Fordyce, J. A., Nice, C. C., Thorne, J. H., Waetjen, D. P., & Forister, M. L. (2021). Insects and recent climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(2), 2002543117. https://doi.org/10.1073/PNAS.2002543117

Harrison, J. F., & Roberts, S. P. (2000). Flight respiration and energetics. *Annual Review of Physiology*, *62*, 179–205. https://doi.org/10.1146/ANNUREV.PHYSIOL.62.1.179

Hartig, F. (2021). *DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models* (R package version 0.4.1). https://cran.r-project.org/package=DHARMa

Harwood, A. D., You, J., & Lydy, M. J. (2009). Temperature as a toxicity identification evaluation tool for pyrethroid insecticides: Toxicokinetic confirmation. *Environmental Toxicology and Chemistry*, *28*(5), 1051–1058. https://doi.org/10.1897/08-291.1

Heard, M. S., Baas, J., Dorne, J. L., Lahive, E., Robinson, A. G., Rortais, A., Spurgeon, D. J., Svendsen, C., & Hesketh, H. (2017). Comparative toxicity of pesticides and environmental contaminants in bees: Are honey bees a useful proxy for wild bee species? *Science of the Total Environment*, *578*, 357–365. https://doi.org/10.1016/j.scitotenv.2016.10.180

Heinrich, B. (1975). Thermoregulation in bumblebees. *Journal of Comparative Physiology*, *96*(2), 155–166. https://doi.org/10.1007/BF00706595

Heinze, G., Ploner, M., & Jiricka, L. (2020). *coxphf: Cox regression with Firth's penalized likelihood* (R package version 1.13.1). https://cran.r-project.org/package=coxphf

Henry, M., Bertrand, C., Le Féon, V., Requier, F., Odoux, J. F., Aupinel, P., Bretagnolle, V., & Decourtye, A. (2014). Pesticide risk assessment in free-ranging bees is weather and landscape dependent. *Nature Communications*, *5*, 4359. https://doi.org/10.1038/ncomms5359

Holmstrup, M., Bindesbøl, A. M., Oostingh, G. J., Duschl, A., Scheil, V., Köhler, H. R., Loureiro, S., Soares, A. M. V. M., Ferreira, A. L. G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P. E., Bayley, M., Svendsen, C., & Spurgeon, D. J. (2010). Interactions between effects of environmental chemicals and natural stressors: A review. *Science of the Total Environment*, *408*, 3746–3762. https://doi.org/10.1016/j.scitotenv.2009.10.067

Huey, R. B., & Kingsolver, J. G. (2019). Climate warming, resource availability, and the metabolic meltdown of ectotherms. *The American Naturalist*, *194*, E140–E150. https://doi.org/10.1086/705679

Huey, R. B., & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *Integrative and Comparative Biology*, *19*(1), 357–366. https://doi.org/10.1093/ICB/19.1.357

Janzen, D. H., & Hallwachs, W. (2021). To us insectometers, it is clear that insect decline in our costa Rican tropics is real, so let's be kind to the survivors. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(2). https://doi.org/10.1073/PNAS.2002546117

Jenkins, D. G., Brescacin, C. R., Duxbury, C. V., Elliott, J. A., Evans, J. A., Grablow, K. R., Hillegass, M., Lyon, B. N., Metzger, G. A., Olandese, M. L., Pepe, D., Silvers, G. A., Suresch, H. N., Thompson, T. N., Trexler, C. M., Williams, G. E., Williams, N. C., & Williams, S. E. (2007). Does size matter for dispersal distance? *Global Ecology and Biogeography*, *16*(4), 415–425. https://doi.org/10.1111/J.1466-8238.2007.00312.X

Jiang, H., Chen, J., Zhao, C., Tian, Y., Zhang, Z., & Xu, H. (2020). Sulfoxaflor residues in pollen and nectar of cotton applied through drip irrigation and their potential exposure to *Apis mellifera* L. *Insects*, *11*(2), 114. https://doi.org/10.3390/INSECTS11020114

Jiang, J., Ma, D., Zou, N., Yu, X., Zhang, Z., Liu, F., & Mu, W. (2018). Concentrations of imidacloprid and thiamethoxam in pollen, nectar and leaves from seed-dressed cotton crops and their potential risk to honeybees (*Apis mellifera* L.). *Chemosphere*, *201*, 159–167. https://doi.org/10.1016/J.CHEMOSPHERE.2018.02.168

Johnson, R. M., Ellis, M. D., Mullin, C. A., & Frazier, M. (2010). Pesticides and honey bee toxicity—USA. *Apidologie*, *41*, 312–331. https://doi.org/10.1051/apido/2010018

Kassambara, A., Kosinski, M., & Biecek, P. (2020). *Survminer: Drawing survival curves using "ggplot2"* (R package version 0.4.8).

Kenna, D., Cooley, H., Pretelli, I., Ramos Rodrigues, A., Gill, S. D., & Gill, R. J. (2019). Pesticide exposure affects flight dynamics and reduces flight endurance in bumblebees. *Ecology and Evolution*, *9*, 5637–5650. https://doi.org/10.1002/ece3.5143

Kenna, D., Graystock, P., & Gill, R. J. (2023). Toxic temperatures: Bee behaviours exhibit divergent pesticide toxicity relationships with warming. *Dryad Dataset*. https://doi.org/10.5061/dryad.9ghx3ffnf

Kenna, D., Pawar, S., & Gill, R. J. (2021). Thermal flight performance reveals impact of warming on bumblebee foraging potential. *Functional Ecology*, *35*(11), 2508–2522. https://doi.org/10.1111/1365-2435.13887

Kerr, J. T., Pindar, A., Galpern, P., Potts, S. G., Roberts, S. M., Rasmont, P., Schweiger, O., Colla, S. R., Richardson, L. L., Wagner, D. L., Gall, L. F., Sikes, D. S., & Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science*, *349*(6244), 177–180. https://doi.org/10.1126/science.aaa7031

Khan, H. A. A., & Akram, W. (2014). The effect of temperature on the toxicity of insecticides against *Musca domestica* L.: Implications for the effective management of diarrhea. *PLoS ONE*, *9*(4). https://doi.org/10.1371/JOURNAL.PONE.0095636

Kolano, P. J., Borgå, K., & Nielsen, A. (2021). Temperature sensitive effects of the neonicotinoid clothianidin on bumblebee (*Bombus terrestris*) foraging behaviour. *Journal of Pollination Ecology*, *28*, 138–152. https://doi.org/10.26786/1920-7603(2021)633

Krischik, V. A., Landmark, A. L., & Heimpel, G. E. (2007). Soil-applied Imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (hymenoptera: Encyrtidae). *Environmental Entomology*, *36*(5), 1238–1245.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Kyriakopoulou, K., Kandris, I., Pachiti, I., Kasiotis, K. M., Spyropoulou, A., Santourian, A., Kitromilidou, S., Pappa, G., & Glossioti, M. (2017). *Collection and analysis of pesticide residue data for pollen and nectar*. EFSA Supporting Publication. https://doi.org/10.2903/sp.efsa

Laycock, I., Lenthall, K. M., Barratt, A. T., & Cresswell, J. E. (2012). Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology*, *21*, 1937–1945. https://doi.org/10.1007/s10646-012-0927-y

Lenth, R. V. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software*, *69*(1), 1–33.

Linguadoca, A., Rizzi, C., Villa, S., & Brown, M. J. F. (2021). Sulfoxaflor and nutritional deficiency synergistically reduce survival and fecundity in bumblebees. *Science of the Total Environment*, *795*, 148680. https://doi.org/10.1016/J.SCITOTENV.2021.148680

Lu, C., Chang, C. H., Lemos, B., Zhang, Q., & MacIntosh, D. (2020). Mitochondrial dysfunction: A plausible pathway for honeybee Colony collapse disorder (CCD). *Environmental Science & Technology Letters*, *7*(4), 254–258. https://doi.org/10.1021/ACS.ESTLETT.0C00070/SUPPL_FILE/EZ0C00070_SI_001.PDF

Lydy, M. J., Belden, J. B., & Ternes, M. A. (1999). Effects of temperature on the toxicity of M-parathion, chlorpyrifos, and pentachlorobenzene to Chironomus tentans. *Archives of Environmental Contamination and Toxicology*, *37*, 542–547. https://doi.org/10.1007/s002449900550

Macaulay, S. J., Hageman, K. J., Piggott, J. J., & Matthaei, C. D. (2021). Time-cumulative effects of neonicotinoid exposure, heatwaves and food limitation on stream mayfly nymphs: A multiple-stressor experiment. *Science of the Total Environment*, *754*, 141941. https://doi.org/10.1016/J.SCITOTENV.2020.141941

Mansoor, M. M., Afzal, M., Raza, A. B. M., Akram, Z., Waqar, A., & Afzal, M. B. S. (2015). Post-exposure temperature influence on the toxicity of conventional and new chemistry insecticides to green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *Saudi Journal of Biological Sciences*, *22*(3), 317–321. https://doi.org/10.1016/J.SJBS.2014.10.008

Manzi, C., Vergara-Amado, J., Franco, L. M., & Silva, A. X. (2020). The effect of temperature on candidate gene expression in the brain of honey bee *Apis mellifera* (hymenoptera: Apidae) workers exposed to neonicotinoid imidacloprid. *Journal of Thermal Biology*, *93*, 102696. https://doi.org/10.1016/J.JTHERBIO.2020.102696

Mapalad, K. S., Leu, D., & Nieh, J. C. (2008). Bumble bees heat up for high quality pollen. *Journal of Experimental Biology*, *211*(14), 2239–2242. https://doi.org/10.1242/jeb.016642

Martelli, F., Zhongyuan, Z., Wang, J., Wong, C. O., Karagas, N. E., Roessner, U., Rupasinghe, T., Venkatachalam, K., Perry, T., Bellen, H. J., & Batterham, P. (2020). Low doses of the neonicotinoid insecticide imidacloprid induce ROS triggering neurological and metabolic impairments in drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(41), 25840–25850. https://doi.org/10.1073/PNAS.2011828117

Millard, S. P. (2013). *EnvStats: An R package for environmental statistics*.

Mitchell, E. A. D., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., & Aebi, A. (2017). A worldwide survey of neonicotinoids in honey. *Science*, *358*(6359), 109–111. https://doi.org/10.1126/science.aan3684

Moffat, C., Pacheco, J. G., Sharp, S., Samson, A. J., Bollan, K. A., Huang, J., Buckland, S. T., & Connolly, C. N. (2015). Chronic exposure to neonicotinoids increases neuronal vulnerability to mitochondrial dysfunction in the bumblebee (*Bombus terrestris*). *FASEB Journal*, *29*, 2112–2119. https://doi.org/10.1096/fj.14-267179

Monchanin, C., Henry, M., Decourtye, A., Dalmon, A., Fortini, D., Bœuf, E., Dubuisson, L., Aupinel, P., Chevallereau, C., Petit, J., & Fourrier, J. (2019). Hazard of a neonicotinoid insecticide on the homing flight of the honeybee depends on climatic conditions and Varroa infestation. *Chemosphere*, *224*, 360–368. https://doi.org/10.1016/J.CHEMOSPHERE.2019.02.129

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., VanEngelsdorp, D., & Pettis, J. S. (2010). High levels of miticides and agrochemicals in north American apiaries: Implications for honeybee health. *PLoS ONE*, *5*(3), e9754. https://doi.org/10.1371/journal.pone.0009754

Muth, F., Gaxiola, R. L., & Leonard, A. S. (2020). No evidence for neonicotinoid preferences in the bumblebee *Bombus impatiens*. *Royal Society Open Science*, *7*(5). https://doi.org/10.1098/RSOS.191883

Muturi, E. J., Lampman, R., Costanzo, K., & Alto, B. W. (2011). Effect of temperature and insecticide stress on life-history traits of *Culex restuans* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, *48*(2), 243–250. https://doi.org/10.1603/ME10017

Nieh, J. C., León, A., Cameron, S., & Vandame, R. (2006). Hot bumble bees at good food: Thoracic temperature of feeding Bombus wilmattae foragers is tuned to sugar concentration. *Journal of Experimental Biology*, *209*(21), 4185–4192. https://doi.org/10.1242/JEB.02528

Noyes, P. D., & Lema, S. C. (2015). Forecasting the impacts of chemical pollution and climate change interactions on the health of wildlife. *Current Zoology*, *61*(4), 669–689. https://doi.org/10.1093/CZOOLO/61.4.669

Noyes, P. D., McElwee, M. K., Miller, H. D., Clark, B. W., Van Tiem, L. A., Walcott, K. C., Erwin, K. N., & Levin, E. D. (2009). The toxicology of climate change: Environmental contaminants in a warming world. *Environment International*, *35*(6), 971–986. https://doi.org/10.1016/J.ENVINT.2009.02.006

OECD. (2017). *Test No. 245: Honey bee (Apis mellifera L.), chronic Oral toxicity test (10-day feeding). OECD guidelines for the testing of chemicals, section 2*. OECD Publishing. https://doi.org/10.1787/9789264284081-en

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. https://doi.org/10.1038/ncomms2648

Potts, R., Clarke, R. M., Oldfield, S. E., Wood, L. K., Hempel de Ibarra, N., & Cresswell, J. E. (2018). The effect of dietary neonicotinoid pesticides on non-flight thermogenesis in worker bumble bees (*Bombus terrestris*). *Journal of Insect Physiology*, *104*, 33–39. https://doi.org/10.1016/j.jinsphys.2017.11.006

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. https://doi.org/10.1038/nature20588

Powner, M. B., Salt, T. E., Hogg, C., & Jeffery, G. (2016). Improving mitochondrial function protects bumblebees from neonicotinoid pesticides. *PLoS ONE*, *11*(11), e0166531. https://doi.org/10.1371/JOURNAL.PONE.0166531

Rasmont, P., Franzén, M., Lecocq, T., Harpke, A., Roberts Stuart, P. M., Biesmeijer, J., Castro, L., Cederberg, B., Dvorák, L., Fitzpatrick, Ú., Gonseth, Y., Haubruge, E., Mahé, G., Manino, A., Michez, D., Neumayer, J., Ødegaard, F., Paukkunen, J., Pawlikowski, T., … Schweiger, O. (2015). Climatic risk and distribution atlas of European bumblebees. *BioRisk*, *10*, 1–236. https://doi.org/10.3897/biorisk.10.4749

Raven, P. H., & Wagner, D. L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(2). https://doi.org/10.1073/PNAS.2002548117

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rubalcaba, J. G., & Olalla-Tárraga, M. (2020). The biogeography of thermal risk for terrestrial ectotherms: Scaling of thermal tolerance with body size and latitude. *Journal of Animal Ecology*, *89*(5), 1277–1285. https://doi.org/10.1111/1365-2656.13181

Rundlöf, M., Andersson, G. K. S., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B. K. B. K., Pedersen, T. R., Yourstone, J., Smith, H. G., Rundlof, M., Andersson, G. K. S., Bommarco, R., Fries, I., Hederstrom, V., Herbertsson, L., Jonsson, O., Klatt, B. K. B. K., … Smith, H. G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature*, *521*(7550), 77–U162. https://doi.org/10.1038/nature14420

Siefert, P., Hota, R., Ramesh, V., & Grünewald, B. (2020). Chronic within-hive video recordings detect altered nursing behaviour and retarded larval development of neonicotinoid treated honey bees. *Scientific Reports*, *10*(1), 1–15. https://doi.org/10.1038/s41598-020-65425-y

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D. W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C. H., Liess, M., Long, E., Mcfield, M., Mineau, P., Mitchell, E. A., Morrissey, C. A., … Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research*, *22*(1), 5–34. https://doi.org/10.1007/s11356-014-3470-y

Siviter, H., Brown, M. J. F., & Leadbeater, E. (2018). Sulfoxaflor exposure reduces bumblebee reproductive success. *Nature*, *561*, 109–112. https://doi.org/10.1038/s41586-018-0430-6

Siviter, H., Horner, J., Brown, M. J. F., & Leadbeater, E. (2020). Sulfoxaflor exposure reduces egg laying in bumblebees *Bombus terrestris*. *Journal of Applied Ecology*, *57*(1), 160–169. https://doi.org/10.1111/1365-2664.13519

Siviter, H., Koricheva, J., Brown, M. J. F., & Leadbeater, E. (2018). Quantifying the impact of pesticides on learning and memory in bees. *Journal of Applied Ecology*, *55*(6), 2812–2821. https://doi.org/10.1111/1365-2664.13193

Smith, D. B., Arce, A. N., Ramos Rodrigues, A., Bischoff, P. H., Burris, D., Ahmed, F., & Gill, R. J. (2020). Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bumblebees. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1922), 20192442. https://doi.org/10.1098/rspb.2019.2442

Soroye, P., Newbold, T., & Kerr, J. (2020). Climate change contributes to widespread declines among bumble bees across continents. *Science*, *367*, 685–688. https://doi.org/10.1126/science.aax8591

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. https://doi.org/10.1016/j.pestbp.2013.05.014

Stanley, D. A., Garratt, M. P. D., Wickens, J. B., Wickens, V. J., Potts, S. G., & Raine, N. E. (2015). Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. *Nature*, *528*, 548–550. https://doi.org/10.1038/nature16167

Stewart, S. D., Lorenz, G. M., Catchot, A. L., Gore, J., Cook, D., Skinner, J., Mueller, T. C., Johnson, D. R., Zawislak, J., & Barber, J. (2014). Potential exposure of pollinators to neonicotinoid insecticides from the use of insecticide seed treatments in the mid-southern United States. *Environmental Science and Technology*, *48*(16), 9762–9769. https://doi.org/10.1021/ES501657W/SUPPL_FILE/ES501657W_SI_001.PDF

Stoner, K. A., & Eitzer, B. D. (2012). Movement of soil-applied Imidacloprid and Thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*). *PLoS ONE*, *7*(6), e39114. https://doi.org/10.1371/JOURNAL.PONE.0039114

Suchail, S., De Sousa, G., Rahmani, R., & Belzunces, L. P. (2004). In vivo distribution and metabolisation of ^{14}C-imidacloprid in different compartments of *Apis mellifera* L. *Pest Management Science*, *60*(11), 1056–1062. https://doi.org/10.1002/ps.895

Suchail, S., Debrauwer, L., & Belzunces, L. P. (2004). Metabolism of imidacloprid in *Apis mellifera*. *Pest Management Science*, *60*(3), 291–296. https://doi.org/10.1002/ps.772

Tang, F. H. M., Lenzen, M., McBratney, A., & Maggi, F. (2021). Risk of pesticide pollution at the global scale. *Nature Geoscience*, *14*(4), 206–210. https://doi.org/10.1038/s41561-021-00712-5

Taning, C. N. T., Vanommeslaeghe, A., & Smagghe, G. (2019). With or without foraging for food, field-realistic concentrations of sulfoxaflor are equally toxic to bumblebees (*Bombus terrestris*). *Entomologia Generalis*, *39*(2), 151–155. https://doi.org/10.1127/ENTOMOLOGIA/2019/0784

Therneau, T. (2020). *A package for survival analysis in R* (R package version 3.2-7). https://cran.r-project.org/package=survival

Tomizawa, M., & Casida, J. E. (2005). Neonicotinoid insecticide toxicology: Mechanisms of selective action. *Annual Review of Pharmacology and Toxicology*, *45*, 247–268. https://doi.org/10.1146/ANNUREV.PHARMTOX.45.120403.095930

Tong, Z., Duan, J., Wu, Y., Liu, Q., He, Q., Shi, Y., Yu, L., & Cao, H. (2018). A survey of multiple pesticide residues in pollen and beebread collected in China. *Science of the Total Environment*, *640–641*, 1578–1586. https://doi.org/10.1016/J.SCITOTENV.2018.04.424

Tosi, S., Démares, F. J., Nicolson, S. W., Medrzycki, P., Pirk, C. W. W., & Human, H. (2016). Effects of a neonicotinoid pesticide on thermoregulation of African honey bees (*Apis mellifera scutellata*). *Journal of Insect Physiology*, *93*, 56–63. https://doi.org/10.1016/j.jinsphys.2016.08.010

Tosi, S., & Nieh, J. C. (2017). A common neonicotinoid pesticide, thiamethoxam, alters honey bee activity, motor functions, and movement to light. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/s41598-017-15308-6

Tsaganou, F. K., Vassilakos, T. N., & Athanassiou, C. G. (2021). Influence of temperature and relative humidity on the efficacy of thiamethoxam for the control of three stored product beetle species. *Journal of Stored Products Research*, *92*, 101784. https://doi.org/10.1016/J.JSPR.2021.101784

United States Environmental Protection Agency. (2016). *2016 addendum to the environmental fate and ecological risk assessment for sulfoxaflor registration*.

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(2), e2023989118. https://doi.org/10.1073/PNAS.2023989118

Wellmann, H., Gomes, M., Lee, C., & Kayser, H. (2004). Comparative analysis of neonicotinoid binding to insect membranes: II. An unusual high affinity site for [3H]thiamethoxam in Myzus persicae and Aphis craccivora. *Pest Management Science*, *60*(10), 959–970. https://doi.org/10.1002/ps.920

Weston, D. P., You, J., Harwood, A. D., & Lydy, M. J. (2009). Whole sediment toxicity identification evaluation tools for pyrethroid insecticides: III. Temperature manipulation. *Environmental Toxicology and Chemistry*, *28*(1), 173–180. https://doi.org/10.1897/08-143.1

Whitehorn, P. R., O'Connor, S., Wackers, F. L., & Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, *336*(6079), 351–352. https://doi.org/10.1126/science.1215025

Williamson, S. M., Willis, S. J., & Wright, G. A. (2014). Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology*, *23*, 1409–1418. https://doi.org/10.1007/s10646-014-1283-x

Willis Chan, D. S., & Raine, N. E. (2021). Population decline in a ground-nesting solitary squash bee (*Eucera pruinosa*) following exposure to a neonicotinoid insecticide treated crop (*Cucurbita pepo*). *Scientific Reports*, *11*(1), 4241. https://doi.org/10.1038/S41598-021-83341-7

Woodcock, B. A., Bullock, J. M., Shore, R. F., Heard, M. S., Pereira, M. G., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S., & Pywell, R. F. (2017). Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science*, *356*(6345), 1393–1395. https://doi.org/10.1126/science.aaa1190

Woodcock, B. A., Ridding, L., Freeman, S. N., Gloria Pereira, M., Sleep, D., Redhead, J., Aston, D., Carreck, N. L., Shore, R. F., Bullock, J. M., Heard, M. S., & Pywell, R. F. (2018). Neonicotinoid residues in UK honey despite European Union moratorium. *PLoS ONE*, *13*(1), e0189681. https://doi.org/10.1371/JOURNAL.PONE.0189681

Wu, Y. Y., Luo, Q. H., Hou, C. S., Wang, Q., Dai, P. L., Gao, J., Liu, Y. J., & Diao, Q. Y. (2017). Sublethal effects of imidacloprid on targeting muscle and ribosomal protein related genes in the honey bee *Apis mellifera* L. *Scientific Reports*, *7*(1), 15943. https://doi.org/10.1038/S41598-017-16245-0

Zattara, E. E., & Aizen, M. A. (2021). Worldwide occurrence records suggest a global decline in bee species richness. *One Earth*, *4*(1), 114–123. https://doi.org/10.1016/J.ONEEAR.2020.12.005

Zhang, W. (2018). Global pesticide use: Profile, trend, cost/benefit and more. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *8*(1), 1–27.

Zhang, Y., Chen, D., Du, M., Ma, L., Li, P., Qin, R., Yang, J., Yin, Z., Wu, X., & Xu, H. (2021). Insights into the degradation and toxicity difference mechanism of neonicotinoid pesticides in honeybees by mass spectrometry imaging. *Science of the Total Environment*, *774*, 145170. https://doi.org/10.1016/J.SCITOTENV.2021.145170

Zhou, H. X., Chen, M. H., Gu, W. J., Hu, M. F., Liu, X. Y., Zhou, J., Song, Y. Q., & Zha, H. G. (2022). Identification and quantitation of the novel insecticide sulfoxaflor and its metabolites in floral nectar from *Salvia splendens* Ker Gawl. (Lamiaceae). *Ecotoxicology*, *31*(8), 1310–1320. https://doi.org/10.1007/S10646-022-02590-Y/TABLES/1

Zhu, Y. C., Yao, J., Adamczyk, J., & Luttrell, R. (2017). Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*). *PLoS ONE*, *12*(6), e0178421. https://doi.org/10.1371/JOURNAL.PONE.0178421

SUPPORTING INFORMATION

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