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Cite this article: Siviter H, Folly AJ, Brown MJF, Leadbeater E. 2020 Individual and combined impacts of sulfoxaflor and *Nosema bombi* on bumblebee (*Bombus terrestris*) larval growth. *Proc. R. Soc. B* **287**: 20200935. http://dx.doi.org/10.1098/rspb.2020.0935

Received: 24 April 2020
Accepted: 13 July 2020

Subject Category:
Ecology

Subject Areas:
ecology, environmental science

Keywords:
sulfoxaflor, sulfoximine, insecticide, parasite, bumblebee, larval growth

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5077706.

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Individual and combined impacts of sulfoxaflor and *Nosema bombi* on bumblebee (*Bombus terrestris*) larval growth

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Sulfoxaflor is a globally important novel insecticide that can have negative impacts on the reproductive output of bumblebee (*Bombus terrestris*) colonies. However, it remains unclear as to which life-history stage is critically affected by exposure. One hypothesis is that sulfoxaflor exposure early in the colony's life cycle can impair larval development, reducing the number of workers produced and ultimately lowering colony reproductive output. Here we assess the influence of sulfoxaflor exposure on bumblebee larval mortality and growth both when tested in insolation and when in combination with the common fungal parasite *Nosema bombi*, following a pre-registered design. We found no significant impact of sulfoxaflor (5 ppb) or *N. bombi* exposure (50 000 spores) on larval mortality when tested in isolation but found an additive, negative effect when larvae received both stressors in combination. Individually, sulfoxaflor and *N. bombi* exposure each impaired larval growth, although the impact of combined exposure fell significantly short of the predicted sum of the individual effects (i.e. they interacted antagonistically). Ultimately, our results suggest that colony-level consequences of sulfoxaflor exposure for bumblebees may be mediated through direct effects on larvae. As sulfoxaflor is licensed for use globally, our findings highlight the need to understand how novel insecticides impact non-target insects at various stages of their development.

1. Introduction

Agrochemical use is believed to contribute towards widely documented global declines in wild bee populations [1,2]. While a plethora of research has demonstrated negative impacts of insecticide use on bee behaviour [3–10], physiology [11–14] and ultimately reproductive output [15–22], it remains unclear as to which life-stages are critically affected by insecticide exposure and thus drive colony failure. In particular, direct effects of insecticide exposure on pre-adult stages have been relatively neglected in wild bees (but see [23,24]) due to a focus on adult bees, which are known to exhibit impaired foraging behaviour [3,4] and brood care [25] when exposed to certain agrochemicals. However, impacts on larval development in particular have the potential to severely reduce colony size, a critical correlate of reproductive potential [26]. As novel insecticides become licensed for use globally [27], there is an urgent need to understand the specific mechanisms by which insecticides bring about colony failure.

Sulfoxaflor, the first branded sulfoximine-based insecticide, acts as an agonist of nicotinic acetylcholine receptors in invertebrate nervous systems [28] and has been registered for use in 81 countries around the world. We have recently shown that sulfoxaflor exposure can have a negative impact on bumblebee (*Bombus terrestris*) colony reproductive output and worker production [16]. These effects were comparable with those elicited by neonicotinoid insecticides [15,17–19], which also act as NAChR agonists and are known to

compromise learning and memory abilities in worker bees [7,10], but a follow-up experiment found no effect of sulfoxaflor exposure on these traits [29]. In contrast, we found that sulfoxaflor exposure did impair egg-laying by worker bees in queenless micro-colonies and was associated with reduced numbers of larvae [14]. However, reduced egg-laying cannot be the primary explanation for the reduced worker production in sulfoxaflor-exposed colonies described by Siviter *et al.* [16], because worker production was reduced at an early stage in the colony cycle that corresponds with the emergence of maximally exposed larvae. We thus hypothesized that sulfoxaflor exposure may have important direct negative impacts on bumblebee larval development [16].

After hatching from the egg stage, bumblebees (*B. terrestris)* spend approximately two weeks developing as larvae, during which time they are fed pollen and nectar by foraging workers [30]. Little is known about the sulfoxaflor residue levels that are retained in colony food stores, but residue levels found in the pollen collected by foraging honeybees can be up to tenfold those typical of nectar [31]. Given that pollen is collected to feed developing larvae and that bumblebee exposure levels are thought to be greater than those experienced by honeybees as a consequence of differences in their life history [32], it seems likely that bumblebee larvae will be fed pollen containing sulfoxaflor in agricultural environments. These larvae develop into either the future workers or the colony's sexual offspring, so negative impacts of insecticide use on bumblebee larval growth and/or mortality could have significant downstream consequences for colony productivity and reproductive success [16].

While agrochemicals may be an important stressor for bees, they do not act in isolation, and one of the major challenges in understanding bee declines is the role of interactions between different classes of stressors [1,33]. Poor nutrition due to loss of habitat, climate change and simultaneous exposure to multiple agrochemicals can all potentially interact to harm bumblebees [1,33]. Furthermore, bumblebee colonies host a wide variety of different pathogens [34] and so it is likely that bumblebee larvae will be exposed to multiple stressors simultaneously [35]. *Nosema bombi* is a fungal parasite that is found in bumblebee colonies globally, and is thought to be a major driver of bumblebee declines in North America [36]. Laboratory experiments have demonstrated that colony-level exposure to *N. bombi* is associated with increased worker and male mortality [37], and that exposed bumblebee queens produce smaller colonies that have a lower reproductive output and reduced individual bee longevity [38,39]. However, our understanding of this potentially important pathogen is still limited [40], and how it interacts with other stressors (if at all) remains unknown. When an organism is exposed to multiple stressors, the resulting interaction many be additive, synergistic or antagonistic, whereby the effect of the stressors in combination, respectively, equals, exceeds or falls short of the predicted sum of the individual impacts [41,42]. Previous studies investigating the general relationship between pesticides and pathogens have largely focused on *Apis* adults/larvae [43–48] and have produced varied results, [41,49–51], with additive [41], synergistic [44,49,50] and antagonistic [44,51] interactions all documented (for review, see [52]). Despite the critical role of bumblebees as a wild ecosystem service provider, to date no research has focused on *Bombus* larvae, partly due to an absence of protocols [53].

In this experiment we consider the potential impact of simultaneous sulfoxaflor exposure and *N. bombi* inoculation on bumblebee (*B. terrestris*) larval development. In Experiment 1, we assessed the impact of chronic sulfoxaflor exposure at various concentrations on bumblebee larval mortality and growth, and in Experiment 2, we further investigated the combined impacts of sulfoxaflor and *N. bombi* exposure. Our study followed an Open Science protocol whereby each step was pre-registered, including both methods and analysis, and Experiments 1 and 2 were large-scale follow-ups to two similar smaller initial studies (Experiments S2 and S3) that are reported in full in the electronic supplementary material (also pre-registered). This repetition reflects the contrasting results that we obtained in Experiments S2 and S3. In Experiments 1 and 2, which each involved a sample size of eight colonies and were analysed separately to the smaller experiments, we found consistent evidence for effects of sulfoxaflor on larval growth and, in some cases, mortality.

2. Experiment 1: does sulfoxaflor exposure influence bumblebee larval mortality and development?

(a) Methods

(i) Sulfoxaflor exposure

Data from the United States Environmental Protection Agency (EPA) have shown that the sulfoxaflor residue levels in the nectar of a cotton crop sprayed twice with 0.45 pounds of sulfoxaflor per acre over an 11-day period did not fall below 5 ppb, with pollen levels higher by a factor of approximately 10 [31]. It should be noted that spraying flowering crops is prohibited in Europe [54,55], but this is not the case globally [56–58], and recent legislative changes in the USA mean that sulfoxaflor can now be sprayed on numerous bee attractive crops during flowering (including, with restrictions, cucurbits, strawberries and ornamental plants) [59]. Based on the EPA data above, we chose to expose the larvae to sulfoxaflor at a concentration of 5 ppb, which is the same concentration used in previous work [14,16]. We also included a treatment group that were exposed to 0.28 ppb, based on data from the Pest Management Regulatory Agency Canada [60] that demonstrated that sulfoxaflor residue levels in the nectar of seed-treated crops may be significantly lower than in sprayed crops. A higher concentration of 500 ppb was also included. Fresh treatment solutions were made every 3–4 days and solutions were stored at 4°C in glass, tin-foil-covered containers to reduce the potential degradation of the active ingredient.

(ii) Experimental protocol

Eight commercially obtained bumblebee colonies (*B. terrestris audax;* Biobest, Belgium), with approximately 150 workers each, were housed in a room at 26°C (50–60% humidity) with ad libitum access to sucrose solution. Five workers per colony were arbitrarily removed from the comb of the colony with forceps and were faecally screened for common bumblebee parasites (*Apicystis bombi, Crithidia bombi, Nosema* spp.) [39,61]. None of the colonies were found to contain any of these parasites.

We removed all living early larvae ($n = 692$, instar stages 1 and 2; less than planned on pre-registration because fewer were present in the colonies) and placed each one in an individual well lined with filter paper (24 wells per plate; four rows, one row per treatment). Plates were then incubated (Sanyo MIR-554; 32°C; approx. 60% humidity [62]). Larvae were starved for an hour, and then fed untreated sucrose solution (50% w/w) before examination under a dissection microscope (Nikon SM2800) to confirm (through observation of movement) that the larva was still alive. 28 larvae died in transit. The larvae were then left overnight, during which time 14 more died, resulting in a final sample size of $n = 650$ (control $n = 166$, 0.28 ppb $n = 162$, 5 ppb $n = 157$, 500 ppb $n = 165$). Based on the results of a pilot experiment that aimed to establish a feeding regime that minimized mortality (electronic supplementary material, Experiment S1; figure S1), early larvae were fed pollen (Biobest, Belgium) suspended in sucrose solution (35.12 g pollen per litre of 50% w/w sucrose solution) and containing the relevant concentration of sulfoxaflor, for 10 days [63] with each larva receiving 4 feeds of 2 µl a day. Given that our commercially sourced pollen had been originally collected by free-flying honeybees, its nutritional composition was unknown but consistent across treatment groups; likewise, the likelihood that this pollen contained other agrochemicals was unknown, but consistent across treatment groups. After the last feed of each day we observed each larva under a dissection microscope (Nikon SM2800). If the larva did not respond with movement to (a) the feeding solution alone or (b) subsequent touch with forceps, it was categorized as dead. Otherwise, pictures (iPhone 7) were taken for ImageJ analysis to record growth (days 1, 5 and 10). After day 10, the larvae were frozen at −20°C.

(iii) Statistical analysis

We used an information theoretic approach based on AICc values. For each response variable tested, we created a full model containing all fixed and random measured factors, for comparison with all subsets of that full model (retaining all the random factors in each case) and a null model containing just the intercept and random factors (see electronic supplementary material, table S3). We selected a 95% confidence set of models based on Akaike weights derived from AICc values, and parameter estimates, and confidence intervals are based on model averaging of this set.

Larval mortality was analysed via survival analysis (mixed-effects Cox model) with treatment, size at the start of the experiment and the interaction between them included within the model, and with colony of origin and plate included as random factors. As larval size varied considerably between individuals, we analysed larval growth during the experiment (rather than absolute larval size, see pre-registration). Larval growth (day 5 growth = surface area on day 5—surface area on day 1; day 10 growth = surface area on day 10—surface area on day 5) was analysed with a linear mixed effects model (lmer) with treatment, day (day 5 or 10), size at the start of the experiment and two interactions (day and treatment; size and treatment) included within the model [64]. Colony, plate and Individual ID were also included as random factors.

We made two deviations from the original pre-registered analysis plan (see electronic supplementary material); (i) here, and with Experiment 2 (below), we pre-registered that we would consider larval growth at day 10 as (larval growth = larval surface area on day 10—larval surface area on day 1). However, we realized that this approach did not allow us to understand larval growth at different ages, and thus chose to analyse growth at day 10 as (larval growth = larval surface area on day 10—larval surface area on day 5). (ii) We did not specify in our pre-registered design that we would include the interaction between day (the day the measurement was taken) and treatment within the analysis. However, we realized that including this interaction could provide information about differences in growth trajectories across treatments, and therefore in all growth analyses we considered this interaction with treatment within the analysis. Note that excluding this interaction does not qualitatively change the main effects.

We used the R packages Hmisc, lme4, coxme & MuMIn [64–67].

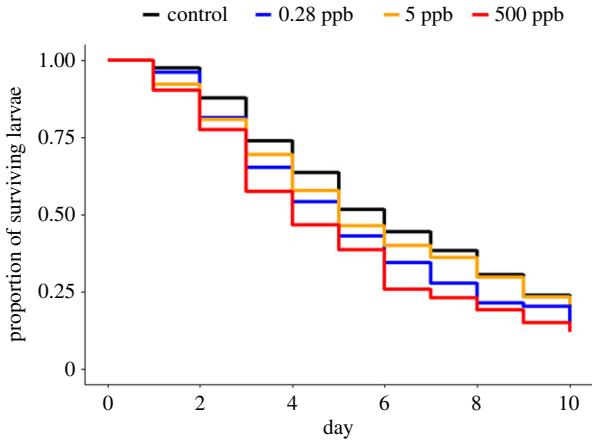

Figure 1. Experiment 1, larval mortality. Kaplan–Meier survival curves for early larvae chronically exposed to varying dosages of sulfoxaflor (coxme, all PE relative to negative control: 0.28 ppb PE = 0.26, 95% CI = −0.13 to 0.65; 5 ppb PE = 0.17, 95% CI = −0.34 to 0.68, 500 ppb PE = 0.42, 95% CI = 0.06 to 0.78). (Online version in colour.)

(b) Results

We found no significant effect of sulfoxaflor exposure on larval mortality at either 0.28 or 5 ppb, although larvae exposed to 500 ppb died earlier than control larvae (figure 1; electronic supplementary material, table S4A; coxme, 0.28 ppb PE = 0.26, 95% CI = −0.13 to 0.65; 5 ppb PE = 0.17, 95% CI = −0.34 to 0.68, 500 ppb PE = 0.42, 95% CI = 0.06 to 0.78).

In contrast, there was a significant negative effect of sulfoxaflor exposure on larval growth at both 5 and 500 ppb, although there was no detectable effect at 0.28 ppb (figure 2; electronic supplementary material, table S4B; lmer, 0.28 ppb PE = −1.08, 95% CI = −2.18 to 0.02; 5 ppb PE = −1.03, 95% CI = −2.05 to −0.01, 500 ppb PE = −1.45, 95% CI = −2.62 to −0.28). There was also no interaction effect between day and treatment, suggesting that the growth trajectories did not differ significantly from the negative control in any treatment groups (figure 2, electronic supplementary material, table S4B; lmer, day × 0.28 ppb PE = 1.41, 95% CI = −0.86 to 3.69; day × 5 ppb PE = −0.68, 95% CI = −1.05 to 2.40, day × 500 ppb PE = 1.07, 95% CI = −1.02 to 3.16; all comparisons are relative to day × 0 ppb).

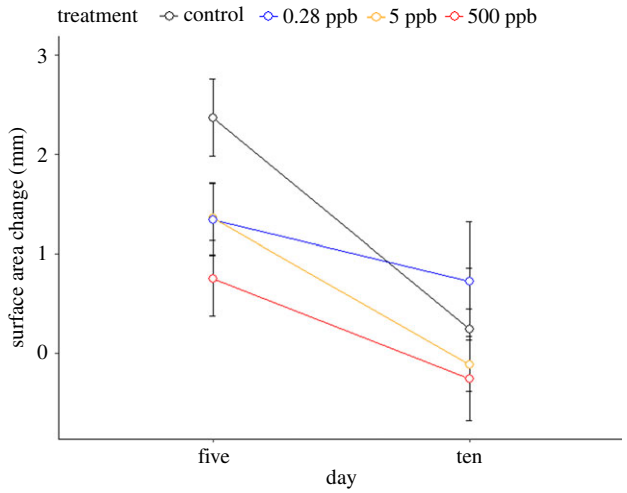

Figure 2. Experiment 1, larval growth. Surface area change ($mm^2 \pm SE$) of larvae at day 5 and 10. Day 5 surface area change = individual larval surface area day 5—surface area at the start of the experiment. Day 10 surface area change = larval surface area day 10—surface area at day 5 (lmer, 0.28 ppb PE = −1.08, 95% CI = −2.18 to 0.02; 5 ppb PE = −1.03, 95% CI = −2.05 to −0.01, 500 ppb PE = −1.45, 95% CI = −2.62 to −0.28). (Online version in colour.)

3. Experiment 2: do sulfoxaflor and *N. bombi* influence bumblebee larval mortality and growth when in combination?

(a) Methods

(i) Parasite preparation

A wild bumblebee queen (*B. terrestris)* infected with *N. bombi* (determined through faecal examination, as described above) was collected from Windsor Great Park in 2016. The infected queen was dissected, and the fat body and gut were homogenized in 0.01 M NH_4Cl. Then, as described in Rutrecht & Brown [68], the spore solution was placed in a centrifuge set to 4°C and 5000 rpm for 10 min to isolate and purify the spore pellet. The spore solution was then resuspended in 0.01 M NH_4Cl and the concentration of *N. bombi* spores was calculated using a Neubauer improved haemocytometer. This inoculum was used to infect three bumblebee colonies (*B. terrestris audax)* from which we sampled bees to create the inoculum used in the present experiment.

(ii) Experimental protocol

The same basic experimental protocol was used as in experiment 1. We used a fully crossed design that included four treatment groups, (control (no sulfoxaflor or *N. bombi*), *N. bombi* alone, sulfoxaflor alone, *N. bombi* and sulfoxaflor). Larvae that were allocated to receive sulfoxaflor exposure were fed a 5 ppb sulfoxaflor in sucrose/pollen solution (see Experiment 1) throughout, and the control and *N. bombi* larvae were fed a sucrose/pollen solution containing just acetone.

Following Folly *et al.* [69], we combined our *N. bombi* stock solution with 1000 µl of a sucrose/pollen mixture to make a stock solution of 50 000 spores per µl for larval inoculation. In the first feed of the experiment, each of the larvae in the parasite treatment groups were fed 2 µl of the *N. bombi* solution (paired with either control or sulfoxaflor laced sucrose/pollen solution respectability), and from this the bee received approximately 50 000 spores, a quantity that is known to infect 45% of larvae [69]. 50 000 spores is well within the range of exposure that would be expected within an infected colony, when exposure occurs through faecal contamination [69]. After the experiment (10 days after inoculation) all surviving larvae were frozen (−80°C) and we later counted *N. bombi* spores in each surviving larva. We found no extracellular spores, in line with previous work which demonstrates it takes bumblebees between two and three weeks to develop extracellular spores [70,71]—due to the process required to count extracellular spores, we were not able to assess the presence of intracellular infections in our larval material.

The rest of the experiment used identical methodology to Experiment 1.

We were able to graft 768 larvae from eight colonies. Seven larvae died during the plating process and 15 died over night and were thus not included in the experiment. Eight larvae were removed due to experimental error, so our final sample size was 738 (control $n = 186$, *N. bombi* $n = 187$, sulfoxaflor $n = 182$, *N. bombi* × sulfoxaflor $n = 183$).

(iii) Statistical analysis

Our statistical analysis followed the same approach as described above (also pre-registered; see electronic supplementary material), whereby each treatment group was compared to the negative control (for both larval mortality and growth). However, since this approach simply treats the combined stressor group as an extra level in the factor 'treatment', it provides no information as to whether any interaction is antagonistic, additive or synergistic. We therefore also conducted an additional, post hoc analysis (not pre-registered), to confirm whether our results provided support for antagonistic, additive or synergistic effects of the two stressors (see electronic supplementary material, table S3D and S3F). For the mortality data we used a survival analysis (mixed-effects Cox model) with sulfoxaflor, *N. bombi*, *N. bombi*:sulfoxaflor and larva initial size included within the model, and colony and plate included as random factors (see electronic supplementary material, table S3D for full model). For the growth data we used a linear model with sulfoxaflor, *N. bombi*, *N. bombi* × sulfoxaflor, day, initial size, *N. bombi* × day, sulfoxaflor × day and *N. bombi* × sulfoxaflor × day included within the model, and colony, larva and plate included as random factors (see electronic supplementary material, table S3F for full model).

(b) Results

We found no effect of sulfoxaflor or *N. bombi* exposure on bumblebee larval mortality when tested in isolation, but when used in combination there was a significant negative impact on larval mortality (figure 3; electronic supplementary material, table S4C; coxme, *N. bombi* PE = 0.00, 95% CI = −0.33 to 0.33; sulfoxaflor 5 ppb PE = 0.14, 95% CI = −0.16 to 0.44, *N. bombi* × sulfoxaflor PE = 0.40, 95% CI = 0.09 to 0.70). Our follow-up analysis confirmed that this was due to an additive (not synergistic) impact of sulfoxaflor and *N. bombi* exposure on larval mortality (figure 3; electronic supplementary material, table S4D; coxme, *N. bombi* × sulfoxaflor PE = 0.10, 95% CI = −0.21 to 0.41), suggesting that smaller individual impacts of each stressor summed to produce a detectable negative impact of multiple stressors on larval mortality (figure 3).

Both sulfoxaflor and *N. bombi* exposure, in isolation, reduced bumblebee larval growth (figure 4; electronic

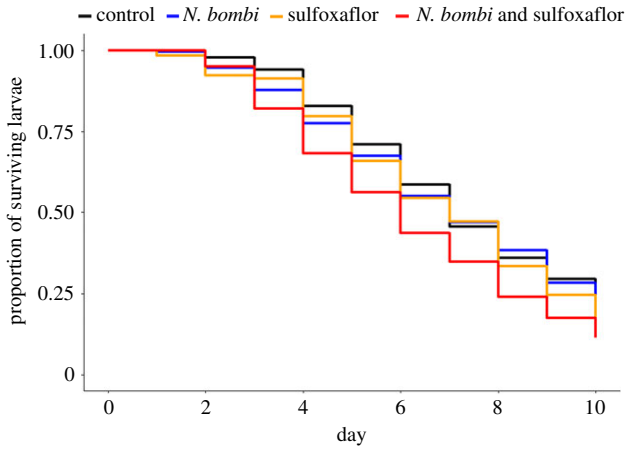

Figure 3. Experiment 2, larval mortality. Kaplan–Meier survival curves for early larvae exposed to sulfoxaflor and *N. bombi* (coxme, all parameter estimates relative to negative control, *N. bombi* PE = 0.00, 95% CI = −0.33 to 0.33; sulfoxaflor 5 ppb PE = 0.14, 95% CI = −0.16 to 0.44, *N. bombi* and sulfoxaflor PE = 0.40, 95% CI = 0.09 to 0.70). (Online version in colour.)

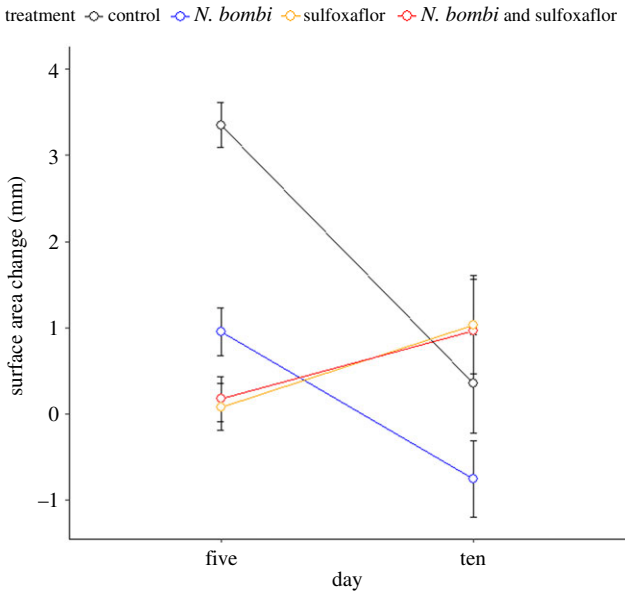

Figure 4. Experiment 2, larval growth. Surface area change ($mm^2 \pm SE$) of larvae at day 5 and 10 from the start of the experiment for larvae. Day 5 surface area change = individual larval surface area day 5—surface area at the start of the experiment. Day 10 surface area change = larval surface area day 10—surface area at day 5 (lmer, *N. bombi* PE = −2.45, 95% CI = −3.14 to −1.76; sulfoxaflor 5 ppb PE = −3.35, 95% CI = −4.04 to −2.64; combined PE = −3.29, 95% CI = −4.02 to −2.56). (Online version in colour.)

supplementary material, table S4E; lmer, *N. bombi* PE = −2.45, 95% CI = −3.14 to −1.76; sulfoxaflor 5 ppb PE = −3.35, 95% CI = −4.04 to −2.64), and further, the combined treatment also had a significant negative impact (combined PE = −3.29, 95% CI = −4.02 to −2.56). A follow-up analysis, designed to assess whether the combined effects were synergistic, additive or antagonistic, revealed that sulfoxaflor and *N. bombi* in combination interacted antagonistically in terms of their effect on bumblebee larval growth, such that their combined impact fell short of the predicted sum of each effect when in isolation (figure 4; electronic supplementary material, table S4F lmer: *N. bombi* × sulfoxaflor PE = 2.41, 95% CI: 1.38 to 3.44). Although visual inspection of the results (figure 4) suggested potential differences in the temporal trajectory of these effects across treatments, we found no statistical support for this (figure 4; electronic supplementary material, table S4F, lmer: *N. bombi* × sulfoxaflor × day PE = −1.20, 95% CI: −3.11 to 1.34).

4. Discussion

In the previous work [16], we observed that sulfoxaflor exposure early in the bumblebee colony cycle was associated with a subsequent reduction in worker numbers, and a later reduction in reproductive offspring. We hypothesized that sulfoxaflor exposure might increase larval mortality, driving the observed downstream consequences on reproductive output. Here, we find no evidence that sulfoxaflor exposure in isolation increased larval mortality in bumblebees, except at extremely high doses. We did find, however, that sulfoxaflor exposure (5 ppb) interacted additively with the common bumblebee parasite *N. bombi*, resulting in increased larval mortality. We also found that both sulfoxaflor exposure and *N. bombi* inoculation individually negatively influenced larval growth, but additionally found evidence of a possible antagonistic interaction between the two stressors on larval growth, such that the observed impact of the combined treatment was negative, but smaller than predicted (figure 4). To our knowledge, this is the first assessment of how environmental stressors impact a key developmental stage in this important genus of wild pollinators.

Colonies in our previous work [16] were parasite-free during the exposure period of the experiment and so the effects that we observed there cannot be explained by direct impacts of sulfoxaflor exposure on larval mortality. However, in the present experiment, we found that sulfoxaflor exposure had a significant negative impact on bumblebee larval growth, at similar dosages to those observed in previous research (5 ppb) [14,16]. The time it takes for a bumblebee larva to develop varies [63] and impaired growth could result in (i) larvae taking longer to start pupating or (ii) larvae starting to pupate at a smaller size so emerging bees are smaller. In our previous work [16], it was not possible to measure whether there were differences in the size of workers between control and sulfoxaflor treatment groups, but the results from the present experiment suggest that colonies exposed to sulfoxaflor may produce smaller bees, a hypothesis that requires future research. Similarly, if during sexual production larvae are exposed to sulfoxaflor, then it is possible that emerging males and gynes might be less healthy than unexposed bees [13]. Given that gyne larvae take longer to develop into adults than workers [63] and males, it might be the case that gyne larvae are particularly vulnerable to sulfoxaflor exposure. Such potential knock-on consequences for emerging adult bees require urgent attention.

We found that larval mortality was greater when larvae had been exposed to both sulfoxaflor and *N. bombi*. Whether increased larval mortality benefits or hinders the parasite is unclear, as we do not know whether increases in larval mortality result in increased or reduced intra-nest transmission of *N. bombi*. Our results also showed that field-realistic inoculation of *N. bombi* probably impairs larval growth, which could in turn have downstream consequences on emerging adults and contribute to bumblebee declines [36]. A prolonged developmental period, however, is arguably advantageous to the

parasite, as it could potentially increase parasite intensity within emerging bees, possibly leading to higher rates of faecal transmission both in and outside the nest [72,73]. In Experiment 2, we found no evidence for additive or synergistic interactions between *N. bombi* and sulfoxaflor on larval growth, but rather, that exposure to sulfoxaflor and *N. bombi* in combination has a less severe effect on bumblebee larvae growth than when larvae are exposed to *N. bombi* in isolation (figure 4; electronic supplementary material, table S4F). Whether sulfoxaflor overwhelms the impact of this co-evolved parasite is unclear, but previous studies have shown that certain pesticides can reduce parasite intensity (for review, see [52]). If sulfoxaflor has a similar effect on either *N. bombi* prevalence and/or intensity, this could explain our results, but future experiments would be required to confirm or refute this hypothesis, across a range of different *N. bombi* and sulfoxaflor dosages. Furthermore, although adult bumblebees do not remove larvae infected with *N. bombi* [74], if sulfoxaflor exposure reduces growth, workers could be more likely to remove larvae, exacerbating effects on larval mortality. More broadly, an understanding of why certain pesticides have a synergistic interaction with parasites, and others do not [52] could be invaluable in the future development of insecticides that are less harmful to beneficial insects, such as bees.

Regulators and governing bodies are under increasing pressure to consider the potential impact of agrochemicals on non-*Apis* bees so there is a need to develop new methodologies and frameworks that can be used as a standardized methodology [75–77]. While rearing honeybee larvae *in vitro* has been established over decades [78], our results here are some of the first to demonstrate how to rear and monitor bumblebee larvae *in vitro* [62]. Despite this, there are large gaps in our understanding of how to rear bumblebee larvae, and efforts need to be made to increase baseline survival in larvae reared *in vitro* (see control group figures 1 and 3). A standardized methodology that can be used to assess the impact of plant protection products on bumblebee larvae has yet to be developed [53], but our research here provides the first step in this direction. Now, specific experiments are required aimed at understanding how variation in environmental factors (nutrition, humidity, etc.) impact bumblebee larvae development, akin to those that have been performed for honeybees [78].

In its current form, the insecticide licensing process focuses on how agrochemicals in isolation impact bees. However, bees encounter a plethora of different anthropogenic, and co-evolved environmental stressors [1,33]. Previous research has shown that the interactions between pesticides and pathogens can impact honeybee mortality [41], pathogen load [45], behaviour [79] and immune response [47]. Bees are also likely to come into contact with multiple agrochemicals, increasing the likelihood of both lethal [20,80] and sub-lethal consequences [4]. Within our experiment, larvae were fed honeybee-collected pollen, which (like pollen collected by wild colonies) may potentially also contain degraded residues of other agrochemicals. Potential additional interactions are thus not taken into consideration in our results, and invite further study [80,81]. Given that bees, and other pollinators, are likely to be exposed to a multitude of different anthropogenic, and co-evolved environmental stressors, we suggest that regulatory bodies and policy-makers should increasingly consider how novel insecticides interact with other environmental and anthropogenic factors such parasites/pathogens, at various stages of their life history. While considering every potential interaction between stressors is likely to be impractical in the pre-approval period, improvements to the post-licensing assessment process (which is currently minimal [82]) would achieve this aim by monitoring safety in real-world landscape-scale applications.

While significant research has been conducted on the impact of environmental stressors on adult bumblebees, impacts on larvae remain under-researched [53]. We show here that both sulfoxaflor exposure and *N. bombi* inoculation can negatively impact bumblebee larval growth and that larval mortality is significantly higher when larvae are exposed to both sulfoxaflor and *N. bombi* simultaneously. Given the growing global importance of sulfoxaflor, and the increasing prevalence and intensity of *N. bombi* in bumblebee populations [36], such effects may provide a potential mechanism through which exposure to these stressors can reduce bumblebee colony fitness. Our results highlight the need to understand how novel insecticides influence beneficial insects, such as bumblebees, at various stages of their life cycle.

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.zcrjdfn7d [83].

Authors' contributions. H.S., M.J.F.B. and E.L. conceived and designed the experiments. H.S. collected the data, conducted the statistical analyses and wrote the first version of the manuscript. A.J.F. designed the parasite inoculation protocol. All authors contributed to subsequent drafts.

Competing interests. The authors declare they have no competing interests.

Funding. H.S. was supported by a Royal Holloway University of London Reid PhD Scholarship and by contributions from High Wycombe Beekeeper's Association. This project has received funding from the European Horizon 2020 research and innovation programme under grant agreement no. 773921 and ERC Starting Grant BeeDanceGap 638873, and from the Biotechnology and Biological Sciences Research Council, Grant/Award no.: BB/N000668/1.

Acknowledgements. We would like to thank Ash Samuelson, Callum Martin and Romain Willemet for technical assistance, and Rosaline Hulse and Joanne Carnell for offering advice on how to rear bumblebee larvae. We are grateful to the editor and two anonymous reviewers for their helpful suggestions, which significantly improved the manuscript.

References

1. Goulson D, Nicholls E, Botias C, Rotheray EL. 2015 Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **347**, 1255957. (doi:10.1126/science.1255957)
2. Woodcock BA, Isaac NJB, Bullock JM, Roy DB, Garthwaite DG, Crowe A, Pywell RF. 2016 Impacts of neonicotinoid use on longterm population changes in wild bees in England. *Nat. Commun.* **7**, 12459. (doi:10.1038/ncomms12459)
3. Feltham H, Park K, Goulson D. 2014 Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* **23**, 317–323. (doi:10.1007/s10646-014-1189-7)
4. Gill RJ, Ramos-Rodriguez O, Raine NE. 2012 Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* **491**, 105–108. (doi:10.1038/nature11585)

5. Muth F, Leonard AS. 2019 A neonicotinoid pesticide impairs foraging, but not learning, in free-flying bumblebees. *Sci. Rep.* **9**, 4764. (doi:10.1038/s41598-019-39701-5)
6. Muth F, Francis JS, Leonard AS. 2019 Modality-specific impairment of learning by a neonicotinoid pesticide. *Biol. Lett.* **15**, 20190359. (doi:10.1098/rsbl.2019.0359)
7. Siviter H, Koricheva J, Brown MJF, Leadbeater E. 2018 Quantifying the impact of pesticides on learning and memory in bees. *J. Appl. Ecol.* **55**, 2812–2821. (doi:10.1111/1365-2664.13193)
8. Arce AN, Ramos Rodrigues A, Yu J, Colgan TJ, Wurm Y, Gill RJ. 2018 Foraging bumblebees acquire a preference for neonicotinoid-treated food with prolonged exposure. *Proc. R. Soc. B* **285**, 20180655. (doi:10.1098/rspb.2018.0655)
9. Tong L, Nieh JC, Tosi S. 2019 Combined nutritional stress and a new systemic pesticide (flupyradifurone, Sivanto®) reduce bee survival, food consumption, flight success, and thermoregulation. *Chemosphere* **237**, 124408. (doi:10.1016/j.chemosphere.2019.124408)
10. Samuelson EEW, Chen-Wishart ZP, Gill RJ, Leadbeater E. 2016 Effect of acute pesticide exposure on bee spatial working memory using an analogue of the radial-arm maze. *Sci. Rep.* **6**, 38957. (doi:10.1038/srep38957)
11. Baron GL, Jansen VAA, Brown MJF, Raine NE. 2017 Pesticide reduces bumblebee colony initiation and increases probability of population extinction. *Nat. Ecol. Evol.* **1**, 1308–1316. (doi:10.1038/s41559-017-0260-1)
12. Baron GL, Raine NE, Brown MJF. 2017 General and species-specific impacts of a neonicotinoid insecticide on the ovary development and feeding of wild bumblebee queens. *Proc. R. Soc. B* **284**, 20170123. (doi:10.1098/rspb.2017.0123)
13. Straub L *et al*. 2016 Neonicotinoid insecticides can serve as inadvertent insect contraceptives. *Proc. R. Soc. B* **283**, 20160506. (doi:10.1098/rspb.2016.0506)
14. Siviter H, Horner J, Brown MJF, Leadbeater E. 2020 Sulfoxaflor exposure reduces egg laying in bumblebees *Bombus terrestris*. *J. Appl. Ecol.* **57**, 160–169. (doi:10.1111/1365-2664.13519)
15. Whitehorn PR, O'Connor S, Wackers FL, Goulson D. 2012 Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* **336**, 351–352. (doi:10.1126/science.1215025)
16. Siviter H, Brown MJF, Leadbeater E. 2018 Sulfoxaflor exposure reduces bumblebee reproductive success. *Nature* **561**, 109–112. (doi:10.1038/s41586-018-0430-6)
17. Ellis C, Park KJ, Whitehorn P, David A, Goulson D. 2017 The neonicotinoid insecticide thiacloprid impacts upon bumblebee colony development under field conditions. *Environ. Sci. Technol.* **51**, 1727–1732. (doi:10.1021/acs.est.6b04791)
18. Rundlöf M *et al*. 2015 Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* **521**, 77–80. (doi:10.1038/nature14420)
19. Woodcock BA *et al*. 2017 Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science* **356**, 1393–1395. (doi:10.1126/science.aaa1190)
20. Tsvetkov N, Samson-Robert O, Sood K, Patel HS, Malena DA, Gajiwala PH, Maciukiewicz P, Fournier V, Zayed A. 2017 Chronic exposure to neonicotinoids reduces honey bee health near corn crops. *Science* **356**, 1395–1397. (doi:10.1126/science.aam7470)
21. Arce AN, David TI, Randall EL, Ramos Rodrigues A, Colgan TJ, Wurm Y, Gill RJ. 2017 Impact of controlled neonicotinoid exposure on bumblebees in a realistic field setting. *J. Appl. Ecol.* **54**, 1199–1208. (doi:10.1111/1365-2664.12792)
22. Tosi S, Nieh JC, Sgolastra F, Cabbri R, Medrzycki P. 2017 Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proc. R. Soc. B* **284**, 20171711. (doi:10.1098/rspb.2017.1711)
23. Sgolastra F, Arnan X, Cabbri R, Isani G, Medrzycki P, Teper D, Bosch J. 2018 Combined exposure to sublethal concentrations of an insecticide and a fungicide affect feeding, ovary development and longevity in a solitary bee. *Proc. R. Soc. B* **285**, 20180887. (doi:10.1098/rspb.2018.0887)
24. Smith DB, Arce AN, Ramos Rodrigues A, Bischoff PH, Burris D, Ahmed F, Gill RJ. 2020 Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bumblebees. *Proc. R. Soc. B* **287**, 20192442. (doi:10.1098/rspb.2019.2442)
25. Crall JD *et al*. 2018 Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. *Science* **362**, 683–686. (doi:10.1126/science.aat1598)
26. Muller CB, Schmid-Hempel P. 1992 Correlates of reproductive success among field colonies of *Bombus lucorum*: the importance of growth and parasites. *Ecol. Entomol.* **17**, 343–353. (doi:10.1111/j.1365-2311.1992.tb01068.x)
27. Brown MJF *et al*. 2016 A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ* **4**, e2249. (doi:10.7717/peerj.2249)
28. Sparks TC, Watson GB, Loso MR, Geng C, Babcock JM, Thomas JD. 2013 Sulfoxaflor and the sulfoximine insecticides: chemistry, mode of action and basis for efficacy on resistant insects. *Pestic. Biochem. Physiol.* **107**, 1–7. (doi:10.1016/j.pestbp.2013.05.014)
29. Siviter H, Scott A, Pasquier G, Pull CD, Brown MJF, Leadbeater E. 2019 No evidence for negative impacts of acute sulfoxaflor exposure on bee olfactory conditioning or working memory. *PeerJ* **7**, e7208. (doi:10.7717/peerj.7208)
30. Alford DV. 1975 *Bumblebees*. London: Davis-Poynter.
31. EPA. 2016 *Addendum to the environmental fate and ecological risk assessment for sulfoxaflor registration*. Washington, DC: EPA.
32. Gradish AE *et al*. 2019 Comparison of pesticide exposure in honey bees (Hymenoptera: Apidae) and bumble bees (Hymenoptera: Apidae): implications for risk assessments. *Environ. Entomol.* **48**, 12–21. (doi:10.1093/ee/nvy168)
33. Vanbergen AJ, Insect Pollinators Initiative. 2013 Threats to an ecosystem service: pressures on pollinators. *Front. Ecol. Environ.* **11**, 251–259. (doi:10.1890/120126)
34. Schmid-Hempel P. 1998 *Parasites in social insects*. Princeton, NJ: Princeton University Press.
35. Nicholls E *et al*. 2018 Monitoring neonicotinoid exposure for bees in rural and peri-urban areas of the U.K. during the transition from pre- to post-moratorium. *Environ. Sci. Technol.* **52**, 9391–9402. (doi:10.1021/acs.est.7b06573)
36. Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL. 2011 Patterns of widespread decline in North American bumble bees. *Proc. Natl. Acad. Sci.* **108**, 662–667. (doi:10.1073/pnas.1014743108)
37. Otti O, Schmid-Hempel P. 2007 *Nosema bombi*: a pollinator parasite with detrimental fitness effects. *J. Invertebr. Pathol.* **96**, 118–124. (doi:10.1016/j.jip.2007.03.016)
38. Otti O, Schmid-Hempel P. 2008 A field experiment on the effect of *Nosema bombi* in colonies of the bumblebee *Bombus terrestris*. *Ecol. Entomol.* **33**, 577–582. (doi:10.1111/j.1365-2311.2008.00998.x)
39. Rutrecht ST, Brown MJF. 2009 Differential virulence in a multiple-host parasite of bumble bees: resolving the paradox of parasite survival? *Oikos* **118**, 941–949. (doi:10.1111/j.1600-0706.2009.17392.x)
40. Brown MJF. 2017 Microsporidia: an emerging threat to bumblebees? *Trends Parasitol.* **33**, 754–762. (doi:10.1016/j.pt.2017.06.001)
41. Doublet V, Labarussias M, de Miranda JR, Moritz RFA, Paxton RJ. 2015 Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environ. Microbiol.* **17**, 969–983. (doi:10.1111/1462-2920.12426)
42. Folt CL, Chen CY, Moore MV, Burnaford J. 1999 Synergism and antagonism among multiple stressors. *Limnol. Oceanogr.* **44**, 864–877. (doi:10.4319/lo.1999.44.3_part_2.0864)
43. Alaux C *et al*. 2010 Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ. Microbiol.* **12**, 774–782. (doi:10.1111/j.1462-2920.2009.02123.x)
44. Vidau C *et al*. 2011 Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLoS ONE* **6**, e21550. (doi:10.1371/journal.pone.0021550)
45. Pettis JS, Vanengelsdorp D, Johnson J, Dively G. 2012 Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften* **99**, 153–158. (doi:10.1007/s00114-011-0881-1)
46. Locke B, Forsgren E, Fries I, de Miranda JR. 2012 Acaricide treatment affects viral dynamics in Varroa destructor-infested honey bee colonies via both host physiology and mite control. *Appl. Environ. Microbiol.* **78**, 227–235. (doi:10.1128/AEM.06094-11)

47. Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F. 2013 Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proc. Natl. Acad. Sci.* **110**, 18 466–18 471. (doi:10.1073/pnas.1314923110)
48. Fauser A, Sandrock C, Neumann P, Sadd BM. 2017 Neonicotinoids override a parasite exposure impact on hibernation success of a key bumblebee pollinator. *Ecol. Entomol.* **42**, 306–314. (doi:10.1111/een.12385)
49. Grassl J, Holt S, Cremen N, Peso M, Hahne D, Baer B. 2018 Synergistic effects of pathogen and pesticide exposure on honey bee (*Apis mellifera*) survival and immunity. *J. Invertebr. Pathol.* **159**, 78–86. (doi:10.1016/j.jip.2018.10.005)
50. López JH, Krainer S, Engert A, Schuehly W, Riessberger-Gallé U, Crailsheim K. 2017 Sublethal pesticide doses negatively affect survival and the cellular responses in American foulbrood-infected honeybee larvae. *Sci. Rep.* **7**, 40853. (doi:10.1038/srep40853)
51. Papach A, Fortini D, Grateau S, Aupinel P, Richard F-J. 2017 Larval exposure to thiamethoxam and American foulbrood: effects on mortality and cognition in the honey bee *Apis mellifera*. *J. Apic. Res.* **56**, 475–486. (doi:10.1080/00218839.2017.1332541)
52. Collison E, Hird H, Cresswell J, Tyler C. 2016 Interactive effects of pesticide exposure and pathogen infection on bee health—a critical analysis. *Biol. Rev.* **91**, 1006–1019. (doi:10.1111/brv.12206)
53. EFSA. 2013 EFSA guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, Bombus spp. and solitary bees). *EFSA J.* **11**, 3295.
54. Dow AgroSciences Ireland. 2018 Closer. See http://www.dowagro.com/en-ie/ireland/product-finder/insecticide/closer (accessed on 15 May 2018).
55. Dow AgroSciences USA. 2018 Closer. See https://s3-us-west-1.amazonaws.com/www.agrian.com/pdfs/Closer_SC1d_Label.pdf (accessed on 31 January 2019).
56. Dow AgroSciences South Africa. 2018 Closer 240 SC. See http://www.dowagro.com/en-za/southafrica/products/insecticide/closer-240-sc (accessed on 15 May 2018).
57. Dow AgroSciences New Zealand. 2018 TransformTM. See https://www.corteva.co.nz/content/dam/dpagco/corteva/au/nz/en/products/files/Label_029_Transform.pdf (accessed on 31 January 2019).
58. Dow AgroSciences Australia Limited. 2018 TransformTM. See https://www.corteva.com.au/content/dam/dpagco/corteva/au/au/en/products/files/LINK_LABEL_Transform.pdf (accessed on 31 January 2019).
59. EPA. 2019 *Decision memorandum supporting the registration decision for new uses of the active ingredient sulfoxaflor on alfalfa, cacao, citrus, corn, cotton, cucurbits, grains, pineapple, sorghum, soybeans, strawberries and tree plantations*. Washington, DC: EPA.
60. Pest Management Regulatory Agency (Canada). 2016 *Registration decision RD2016-12, Sulfoxaflor*. Saskatoon, Canada: Pest Management Regulatory Agency.
61. Folly AJ, Koch H, Stevenson PC, Brown MJF. 2017 Larvae act as a transient transmission hub for the prevalent bumblebee parasite *Crithidia bombi*. *J. Invertebr. Pathol.* **148**, 81–85. (doi:10.1016/j.jip.2017.06.001)
62. Pereboom JJM, Velthuis HHW, Duchateau MJ. 2003 The organisation of larval feeding in bumblebees (Hymenoptera, Apidae) and its significance to caste differentiation. *Insectes Soc.* **50**, 127–133. (doi:10.1007/s00040-003-0639-7)
63. Cnaani J, Borst DW, Huang ZY, Robinson GE, Abraham H. 1997 Caste determination in *Bombus terrestris*: differences in development and rates of JH biosynthesis between queen and worker larvae. *J. Insect Physiol.* **43**, 373–381. (doi:10.1016/S0022-1910(96)00106-0)
64. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 51. (doi:10.18637/jss.v067.i01)
65. Harrell FE, Dupont C. 2018 Hmisc: Harrell miscellaneous. R package version 4.1-1. See https://cran.r-project.org/package=Hmisc.
66. Therneau TM. 2018 coxme: Mixed effects Cox models. R package version 2.2-7. See https://cran.r-project.org/package=coxme.
67. Barton K. 2016 MuMIn: Multi-Model Inference. R package version 1.15.6. See https://cran.r-project.org/package=MuMIn.
68. Rutrecht ST, Brown MJF. 2008 Within colony dynamics of *Nosema bombi* infections: disease establishment, epidemiology and potential vertical transmission. *Apidologie* **39**, 504–514. (doi:10.1051/apido:2008031)
69. Folly AJ, Stevenson PC, Brown MJF. 2020 Age-related pharmacodynamics in a bumblebee-microsporidian system mirror similar patterns in vertebrates. *J. Exp. Biol.* **223**, jeb.217828. (doi:10.1242/jeb.217828)
70. McIvor CA, Malone LA. 1995 *Nosema bombi* a microsporidian pathogen of the bumble bee *Bombus terrestris* (L.). *New Zeal. J. Zool.* **22**, 25–31. (doi:10.1080/03014223.1995.9518020)
71. Imhoof B, Schmid-Hempel P. 1999 Colony success of the bumble bee, *Bombus terrestris*, in relation to infections by two protozoan parasites, *Crithidia bombi* and *Nosema bombi*. *Insectes Soc.* **46**, 233–238. (doi:10.1007/s000400050139)
72. Rutrecht ST, Klee J, Brown MJF. 2007 Horizontal transmission success of *Nosema bombi* to its adult bumble bee hosts: effects of dosage, spore source and host age. *Parasitology* **134**, 1719–1726. (doi:10.1017/S0031182007003162)
73. Folly AJ. 2019 The impact of plant chemicals on bee health: Interactions with parasites and immunity.
74. Munday Z, Brown MJF. 2018 Bring out your dead: quantifying corpse removal in *Bombus terrestris*, an annual eusocial insect. *Anim. Behav.* **138**, 51–57. (doi:10.1016/j.anbehav.2018.02.003)
75. Sanchez-Bayo F, Tennekes HA. 2017 Assessment of ecological risks of agrochemicals requires a new framework. *Environ. Risk Assess. Remediat.* **1**, 1–9.
76. Sgolastra F *et al*. 2019 Pesticide exposure assessment paradigm for solitary bees. *Environ. Entomol.* **48**, 22–35. (doi:10.1093/ee/nvy105)
77. Dorigo AS, de Souza Rosa-Fontana A, Soares-Lima HM, Galaschi-Teixeira JS, Nocelli RCF, Malaspina O. 2019 *In vitro* larval rearing protocol for the stingless bee species *Melipona scutellaris* for toxicological studies. *PLoS ONE* **14**, e0213109. (doi:10.1371/journal.pone.0213109)
78. Crailsheim K, Brodschneider R, Aupinel P, Behrens D, Genersch E, Vollmann J, Riessberger-Gallé U. 2013 Standard methods for artificial rearing of *Apis mellifera* larvae. *J. Apic. Res.* **52**, 1–16. (doi:10.3896/IBRA.1.52.1.05)
79. Blanken LJ, van Langevelde F, van Dooremalen C. 2015 Interaction between Varroa destructor and imidacloprid reduces flight capacity of honeybees. *Proc. R. Soc. B* **282**, 20151738. (doi:10.1098/rspb.2015.1738)
80. Tosi S, Nieh JC. 2019 Lethal and sublethal synergistic effects of a new systemic pesticide, flupyradifurone (Sivanto®), on honeybees. *Proc. R. Soc. B* **286**, 20190433. (doi:10.1098/rspb.2019.0433)
81. Carnesecchi E *et al*. 2019 Investigating combined toxicity of binary mixtures in bees: Meta-analysis of laboratory tests, modelling, mechanistic basis and implications for risk assessment. *Environ. Int.* **133**, 105256. (doi:10.1016/j.envint.2019.105256)
82. Milner AM, Boyd IL. 2017 Toward pesticidovigilance. *Science* **357**, 1232–1234. (doi:10.1126/science.aan2683)
83. Siviter H, Folly AJ, Brown MJF, Leadbeater E. 2020 Data from: Individual and combined impacts of sulfoxaflor and *Nosema bombi* on bumblebee (*Bombus terrestris*) larval growth. Dryad Digital Repository. (doi:10.5061/dryad.zcrjdfn7d)