LETTER

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Sulfoxaflor exposure reduces bumblebee reproductive success

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Intensive agriculture currently relies on pesticides to maximize crop yield[1,2]. Neonicotinoids are the most widely used insecticides globally[3], but increasing evidence of negative impacts on important pollinators[4–9] and other non-target organisms[10] has led to legislative reassessment and created demand for the development of alternative products. Sulfoximine-based insecticides are the most likely successor[11], and are either licensed for use or under consideration for licensing in several worldwide markets[3], including within the European Union[12], where certain neonicotinoids (imidacloprid, clothianidin and thiamethoxam) are now banned from agricultural use outside of permanent greenhouse structures. There is an urgent need to pre-emptively evaluate the potential sub-lethal effects of sulfoximine-based pesticides on pollinators[11], because such effects are rarely detected by standard ecotoxicological assessments, but can have major impacts at larger ecological scales[13–15]. Here we show that chronic exposure to the sulfoximine-based insecticide sulfoxaflor, at dosages consistent with potential post-spray field exposure, has severe sub-lethal effects on bumblebee (*Bombus terrestris*) colonies. Field-based colonies that were exposed to sulfoxaflor during the early growth phase produced significantly fewer workers than unexposed controls, and ultimately produced fewer reproductive offspring. Differences between the life-history trajectories of treated and control colonies first became apparent when individuals exposed as larvae began to emerge, suggesting that direct or indirect effects on a small cohort may have cumulative long-term consequences for colony fitness. Our results caution against the use of sulfoximines as a direct replacement for neonicotinoids. To avoid continuing cycles of novel pesticide release and removal, with concomitant impacts on the environment, a broad evidence base needs to be assessed prior to the development of policy and regulation.

The widespread global use of highly effective neonicotinoid-based pesticides has led to the evolution of resistance among several insect crop pests[16] and has generated worldwide interest in emerging sulfoximine-based alternatives that have been shown to be effective in targeting some neonicotinoid-resistant species[17–19]. This potential lack of cross-resistance may reflect differences in the three-dimensional molecular structure that preclude the breakdown of sulfoximines by enzymes that are involved in neonicotinoid metabolism[20], supporting the claim that sulfoximines and neonicotinoids are chemically distinct[17]. However, as selective agonists of insect nicotinic acetylcholine receptors[17], the two pesticide groups share a common biological mode of action. This raises major concerns about potential effects on non-target species, and particularly on bees. Neonicotinoids, while not lethal to bees at field-realistic levels, have severe sub-lethal effects on both social and solitary bees, influencing cognition, foraging ability, homing ability, reproductive output, colony initiation[5,7,8,15,21–25], and, potentially, pollination services[26]. Mathematical modelling has shown that these sub-lethal stressors can have considerable negative consequences for colony fitness downstream in the colony cycle[14,15].

To assess whether sulfoxaflor, the first marketed sulfoximine-based pesticide, has similar negative effects on bees, we fed either untreated sucrose solution (1.8 M) or a sucrose solution containing 5 μg dm^{-3} (5 ppb) of sulfoxaflor to nascent *Bombus terrestris* colonies reared from wild-caught queens. We based this concentration on available estimates for sulfoxaflor residues in forager-collected nectar post-spray[27] (Extended Data Fig. 1a), because spray application is currently the most common application procedure (although products containing sulfoxaflor have also been developed for seed treatments and are already available for use on bee-pollinated crops in some markets[28]). After two weeks of laboratory-based exposure, size-matched colonies were placed in the field around a university parkland campus following a paired design and were no longer provided with additional resources. Staggered weekly nocturnal censuses revealed a clear difference in colony demographics between control and experimental colonies. The bumblebee colony cycle is characterized by an early growth phase in which worker numbers increase rapidly to create a large workforce, followed by a switch to production of reproductive brood later in the season. Between two and three weeks after exposure, detectable differences in worker numbers between treated and control colonies began to emerge, persisting until close to the end of the colony cycle (Fig. 1a and Supplementary Table 2d; analysis using a generalized linear mixed-effects model: treatment parameter estimate = −0.28, 95% confidence interval = −0.48 to −0.01; treatment:week interaction parameter estimate = −0.06, 95% confidence interval = −0.11 to −0.01; treatment:week^2 interaction parameter estimate = 0.11, 95% confidence interval = 0.05 to 0.16).

As the colony cycle progressed, negative impacts on the reproductive output of the treated colonies became apparent. Treated and control colonies were equally likely to produce male reproductive offspring, but treated colonies produced significantly fewer males in total (zero-inflated count model, binomial section, treatment parameter estimate = 0.71, 95% confidence interval = −0.67 to 2.09; count section, treatment parameter estimate = −0.54, 95% confidence interval = −0.72 to −0.37; Fig. 2). This difference became apparent from approximately week 9 onwards (Fig. 1b). The dry mass of these males was no different from those produced by control colonies (w_i (null model) = 0.974), indicating that our results cannot be explained by differential investment in reproductive biomass. Neither treated nor control colonies produced an abundance of queens, but control colonies produced more gynes than treated colonies (in total, 36 new gynes from 3 out of 26 control colonies, no new gynes were produced by any of the 25 treated colonies); thus our findings hold when the total number of sexual offspring is analysed (zero-inflated count model, binomial section, treatment parameter estimate = 0.71, 95% confidence interval = −0.67 to 2.09; count section, treatment parameter estimate = −0.64, 95% confidence interval = −0.81 to −0.46). The timing of reproductive onset, queen longevity and colony survival did not differ between control and treated colonies (Extended Data Fig. 2; survival analyses, treatment parameter estimate for reproductive onset = −0.05, 95% confidence interval = −0.41 to 0.31; colony longevity = −0.03, 95% confidence interval = −0.43 to 0.38; queen survival = −0.07, 95% confidence interval = −0.47 to 0.33).

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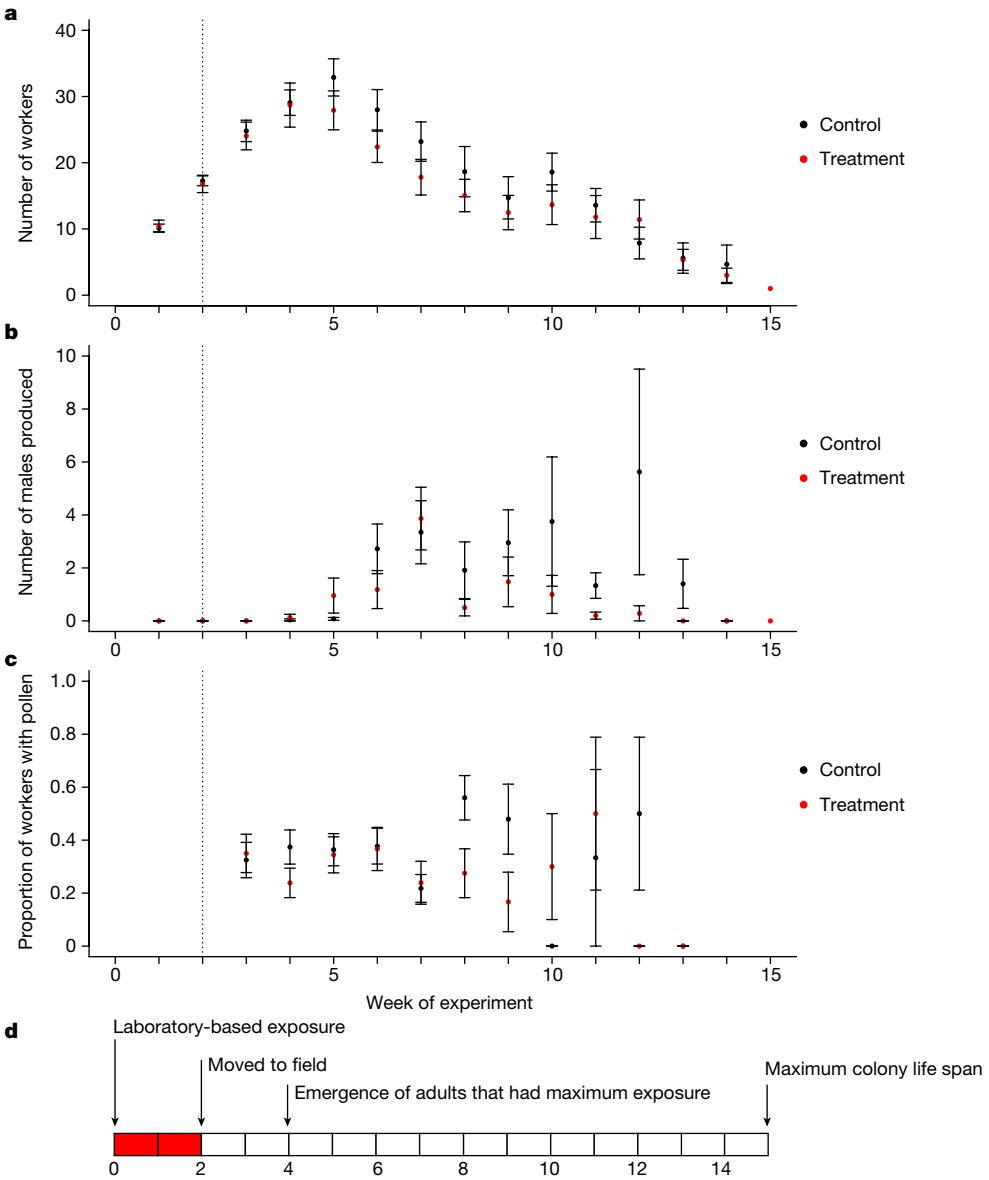

Fig. 1 | The impact of sulfoxaflor exposure on life-history trajectories of bumblebee colonies. a–c, Week-by-week colony field census data. **a**, Number of workers from treated ($n = 26$) and control colonies ($n = 26$). **b**, Number of sexual offspring. **c**, Proportion of workers returning to the colony with pollen for treated and control colonies ($n = 25$ and 26 respectively; reduced sample size for treated colonies reflects the death of one queen in week 2, see Methods). Data are mean ± s.e.m. **d**, Demographic timeline indicates the time points at which the laboratory-based exposure started (the exposure period is indicated in red); the colonies were moved into the field; adults that encountered maximum exposure as larvae should begin to emerge[29] and the maximum lifespan of the colony.

On the basis of the neonicotinoid literature, we considered whether this difference in the production of sexual offspring was mediated through poor provisioning of larvae by foraging workers[9,21], at the time when sexual offspring were developing. However, daytime foraging censuses revealed no significant differences in the relative number of bees returning to control and treated colonies (generalized linear mixed model, treatment parameter estimate = −0.07, 95% confidence interval = −0.32 to 0.19). Similarly, although visual inspection of the data suggested that a lower proportion of workers returned with pollen to pesticide-treated compared to control colonies from week eight onwards (Fig. 1c), this effect did not receive statistical support (generalized linear mixed model, week:treatment interaction parameter estimate = −0.14, 95% confidence interval = −0.29 to 0.001; treatment parameter estimate = 0.46, 95% confidence interval = −0.38 to 1.31) and furthermore occurred too late in the colony cycle to explain the differences in production of male offspring, which became apparent at approximately the same time. We also found no significant differences in the size of pollen loads collected between control and pesticide-treated colonies (Extended Data Fig. 3). Instead, consideration of the timing of differences between control and treated colonies suggests that the effects of sulfoxaflor exposure on reproductive output were mediated by the early drop in worker numbers that began at 2–3 weeks after exposure. Bumblebee worker pupae take approximately 14 days to develop[29], so the onset of deceleration of the growth of the colony workforce corresponds to the eclosion of individuals that had encountered maximum exposure as larvae (Fig. 1d). It remains unclear whether this failure to eclose was driven by direct effects on exposed larvae[30], or indirect effects, perhaps mediated by poor provisioning[9,21] by exposed workers (although note that colonies were provided with pollen and sucrose in the laboratory during this time). In either case, the resultant drop in worker numbers led to differences in the life-history trajectories of control and sulfoxaflor-treated colonies, with consequent effects on the reproductive output of treated colonies[14]. These knock-on effects of early exposure to a small cohort of colony

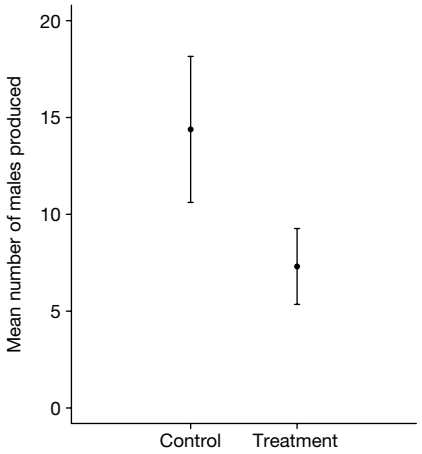

Fig. 2 | Male offspring production. The number of male sexual offspring produced in sulfoxaflor-treated ($n = 25$) and control ($n = 26$) colonies. Data are mean ± s.e.m.

members are entirely consistent with the results of mathematical explorations of stress impacts on bee colonies, which predict that chronic stress at an early stage can push bee colonies beyond a 'tipping point', increasing the likelihood of colony failure[14].

Sulfoxaflor is a systemic pesticide that is soluble in water and is thus transported around plant tissues following foliar or seed application. The likely exposure trajectory of pesticide treatments on crops differs between seed treatments, which deliver prolonged exposure, and spray applications, which deliver a short-term dose that is initially high but typically declines rapidly. Sulfoxaflor, like neonicotinoid-based pesticides, can be administered using both methods, and sulfoxaflor-based products that are used as a seed treatment have recently been developed for crops that attract bees (including oilseed crops)[31]. However, most currently marketed preparations are spray applications. The dosage used in this study is below US Environmental Protection Agency estimates for field-realistic immediate post-spray concentrations of sulfoxaflor in forager-collected nectar, and remains below residual concentrations estimated at 10 days after spray application (the maximum period for which data are available; concentration range over the whole period: 5.41–46.97μg active ingredient (a.i.) per kg, application rate: 0.045 pounds (0.020 kg) of active ingredient per acre applied twice[27]; Extended Data Fig. 1a, b). Note that our treatment protocol is particularly conservative in that our nascent colonies were fed untreated pollen in addition to the syrup provided, potentially producing underestimates of the effects on larvae. Post-spray sulfoxaflor residues in pollen have been documented to be more than tenfold higher than those in forager-collected nectar (Extended Data Fig. 1a, b), ranging from 510.95 to 50.12 μg a.i. per kg over the same post-spray period[27]. Mitigation measures can be used to reduce bee exposure to sulfoxaflor when used as spray treatments (for example, spray application to crops that attract bees during bloom is prohibited by law in the United States)[32]. Globally, however, under current usage, such measures are often either absent[33] or limited to product label recommendations to avoid spraying six days before bloom[34]. No such measures are possible for those products that have been developed as a seed treatment[31].

The impact of sulfoxaflor identified here can be compared with previous experiments that focused on exposure to neonicotinoids. For example, bumblebee colonies placed next to oilseed rape fields that were treated with neonicotinoids showed a 71% reduction in the mean number of queen cocoons found within the nest[6] and a 32–36% reduction in the mean number of males and/or workers produced[7]. Similarly, colonies foraging next to thiacloprid-treated raspberry crops had a 46% reduction in reproductive output[35] and commercial bumblebee colonies exposed to imidacloprid for a period of two weeks had an 85% reduction in the number of new queens produced[8]. Here, we found that sulfoxaflor-exposed colonies had a 54% reduction in the total number of sexual offspring produced compared with control colonies, suggesting that from the perspective of wild pollinators, sulfoxaflor exposure could lead to similar environmental impacts as neonicotinoids if used on crops that attract bees in the absence of evidence-based legislation.

Sulfoximine-based pesticides are a newly emerging class of product, but are already licensed in many countries worldwide, including China[3], Canada[28] and Australia[36]. Within the European Union, where the use of certain neonicotinoids is now banned for open-field crops, substances containing sulfoxaflor as an active ingredient have been assessed by the European Food Safety Authority[37] and approval has been granted for use in five member states, and applications from seven more member states are currently in progress[38]. Our results provide pre-emptive evidence that, if exposure at equivalent dosages to those used in our study occurs via bee-attractive crops before or during bloom, either through spray or seed treatment applications, these products could pose a substantial risk to pollinators. The effects that we identified were the longer-term outcome of initial short-term exposure, and were only detected by monitoring the full colony cycle. Bans and restrictions on neonicotinoid-based pesticides have largely been implemented to protect important pollinators such as bees, following years of widespread use with potential long-term population-level consequences. To avoid a situation in which pesticides such as neonicotinoids are replaced by products that are similarly contentious, regulatory bodies should move towards an evidence-based approach that assesses both the lethal and sub-lethal consequences of novel insecticides such as sulfoxaflor on non-target organisms, and incentivises integrated pest-management approaches before products are licensed for use[39].

Online content

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0430-6

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Competing interests The authors declare no competing interests.

Additional information
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METHODS

Exposure regime. Sulfoxaflor-based preparations have been developed for use on a wide range of bee-attractive crops that flower at varying times of the year. The regime used in our study most closely mimics spring-flowering crops in temperate environments, allowing comparison with similar neonicotinoid-based studies[6,7,15] that also exposed colonies for a short period during the early growth phase.

Preparations containing sulfoxaflor as an active ingredient are currently most commonly applied as a foliar spray. We thus based our pesticide concentrations on the best available information from a realistic and bee-relevant spray experiment reported by the US Environmental Protection Agency (EPA), in which sulfoxaflor was applied to a cotton crop at an application rate of 2 × 0.045 pounds of active ingredient per acre. Under this application regime, mean sulfoxaflor residue levels in honeybee-collected nectar did not drop below 5 μg a.i. per kg over an 11-day period[27] (the maximum period for which data are available; Extended Data Fig. 1a). We are confident that our exposure is conservative, because (a) in the same experiment, pollen residue levels did not drop below 50 μg a.i. per kg[3,27] (Extended Data Fig. 1b), while we provided all colonies with untreated pollen ad libitum; and (b) this application rate is similar to label recommendations for at least some sulfoxaflor-based products[33]. A second study has also measured residues (in cucumber), but application rates were 1.5 times above recommended usage, and the relevance of this experiment for bees is unclear as the cucumber tissue that was sprayed and sampled was not described[40].

In terms of current usage, our data are most relevant to sulfoxaflor preparations when sprayed on crops immediately before or during bloom (note that this practice has recently been reviewed and prohibited in the United States[27]). Although some product labels recommend avoidance of spraying six days before bloom[34], this ignores experimental data showing that residues could remain present in pollen at levels that we show to have sub-lethal impacts after this six-day period[27] (Extended Data Fig. 1d). Other labels allow spraying during bloom at night[33]. To the best of our knowledge, no data are currently available on field-realistic residues for seed treatment preparations that have been developed for use on oilseed crops and are already available in some markets[28].

Queen rearing. In total, 332 bumblebee (*Bombus terrestris audax*) queens were caught between the 28 February and the 23 March 2017 in Windsor Great Park, Surrey, UK. Chilled queens were transported to the laboratory, where their faeces were microscopically examined for parasites (*Nosema* spp., *Apicystis bombi*, *Sphaerularia bombi* and *Crithidia bombi*; 400× magnification). Parasitized individuals ($n = 54$) were removed from the experiment. A second parasite screening was repeated after one week (29 further queens were removed, $n = 249$ queens remained).

Queens were placed in rearing boxes (67 mm (width) by 127 mm (length) by 50 mm (depth); Allied Plastics) and were provided with a gravity feeder containing an ad libitum supply of 1.8 M sucrose solution (changed weekly; Thorne) and a pollen ball (changed twice weekly, unless the queen was laying eggs in which case more pollen was added; Biobest). Each queen was housed in a dark/red-lit room maintained at 26 °C and 50–60% relative humidity. Queens that did not produce eggs after eight weeks were removed from the experiment ($n = 107$). Once a queen had produced at least six workers, the colony was moved into a wooden nest box (280 mm (width) by 320 mm (length) by 160 mm (depth)) and randomly assigned to a treatment group (see 'Pesticide exposure'). The time taken to reach this stage varied but was on average 7.2 weeks (±s.d. of 1.5 weeks). On transfer, the queens underwent a final parasite screening (2 queens removed). Two queens died before transfer, therefore, 52 colonies reached this stage. The use of colonies from wild-caught queens is a key feature of our experimental design that enabled us to (a) have a complete overview of the lifecycle of these colonies (both in the laboratory and the field, see below), and (b) use colonies with a life history that was adapted to the local environment.

Pesticide exposure. Prior to pesticide exposure, colonies were allocated randomly to control and treatment groups and paired for size according to the number of workers present (mean ± s.d.= 8.43 ± 1.87). Each colony was then provided with an ad libitum supply of either 1.8 M sucrose solution containing 5 μg dm^{-3} (5 ppb) sulfoxaflor (derived from a stock solution of 1 g dm^{-3} in acetone; Greyhound Chromatography and Allied Chemicals) or 1.8 M sucrose containing an equivalent concentration of acetone but no sulfoxaflor for a two-week period. Sucrose solution was weighed on placement in and removal from the colony; no differences in consumption were found between treatment groups (w_i (null model) = 0.985). During the exposure period, we recorded the number of workers produced, colony mass and the number of dead workers on a weekly basis. One queen died during the exposure period, thus 51 colonies were present at the start of the field experiment ($n = 26$ control colonies and $n = 25$ pesticide-treated colonies).

Field placement. After two weeks of exposure in the laboratory, colonies were moved into the field. Nest boxes were placed within plastic field boxes (440 mm (width) by 710 mm (length) by 310 mm (depth); Really Useful Box) containing insulation wrap (Thermawrap) and aluminium foil, and placed at locations around the Royal Holloway University of London campus, Egham, UK (45 ha; Extended Data Fig. 4). Paired colonies were matched for location within the campus, and were positioned at least 20 m from one another to reduce drifting. Each colony entrance was demarcated by a distinctive visual pattern. Colonies were placed in discreet, shaded and southeast-facing locations, and secured with a ratchet strap to avoid badger damage. To prevent usurpation attempts from other queens and social parasite species *(Bombus vestalis)*, queen excluders were placed on each colony. Upon initial placement in the field, the colonies were supplied with a gravity feeder containing 46 g 1.8 M sucrose solution, after which they received no further food supplements. The process of field placement was staggered over six weeks (10 April to 21 May 2017) owing to variation in the date at which queens were initially caught. The week of placement was included as a predictor in each statistical analysis (see 'Statistical analysis').

Data collection. We combined methodological approaches from previous studies on the effects of neonicotinoids on bumblebees[8,21], as well as studies on bumblebee life history[41] to maximize our measurement of both impacts and potential mechanisms. We conducted censuses every night such that each colony was visited once per week, between the hours of 21:30 and 04:00. Using a red-light torch, we recorded the number of live workers (average of three counts), dead workers, males and new queens. We also recorded the state of the original queen (dead or alive), the presence of gyne larvae and/or pupae, the presence of worker larvae and/or pupae, the number of pollen and nectar pots containing stores, and the mass of the colony (average of three recordings; EM-30KAM balance, A&D Instruments). In cases in which the wax covering prevented observation, we peeled it back in order to conduct the count. Weekly censuses continued until moribundity, defined as either a live queen and three or fewer workers, or no queen and 10 workers or fewer[42]. After the experiment, all sexual offspring that had been found in the colonies ($n = 600$) were dried for 72 h and weighed (accuracy of ±0.001 g).

All 51 colonies were also visited during daylight hours twice per week. Colony traffic (number of bees entering and leaving the nest) was recorded during 10-min counts, once between 9:00 and 13:00 and once between 14:00 and 18:00. We also recorded whether returning workers had large (pollen basket was over-flowing) or small (pollen enclosed within pollen basket) pollen loads relative to their body size. Control and pesticide pairs were always observed directly after one another, in a random order. The average daily temperature, humidity and total rainfall were obtained from a local weather station (https://wunderground.com).

Statistical analyses. We used an information theoretical model selection approach. For each response variable, the initial candidate set included a full model and all subsets, including a null model. Reported parameter estimates and confidence intervals are based on full-set averaging of the 95% confidence set (that is, the set of models with cumulative Akaike weight ≥0.95). Model types, error structuring, a list of parameters included within each model and parameter estimates are provided in Supplementary Tables 1, 2. In brief, to analyse the number of workers produced per week, we used a generalized linear model (glmer; Poisson error structure) with colony nested within the pair as a random factor, and the week of initial field placement (week started), treatment, week of experiment and a two-way interaction between treatment and week of experiment as fixed factors. Because the number of workers increased to a maximum and then decreased for each colony, 'week of experiment' was modelled as a quadratic factor (ΔAIC between full linear and full quadratic model: 1206.40). Many colonies did not produce sexual offspring, so we used zero-inflated generalized linear models (zeroinfl) to analyse the differences in both the overall number of sexual offspring and the number of males produced by colonies, with the week of initial field placement, treatment and their interaction as predictors. The number of workers returning to the nest was analysed using a zero-inflated generalized linear model (glmmadmb; negative binomial error structure) in which treatment, week started, colony week and temperature were included as fixed factors and colony as a random factor. The proportion of workers returning with pollen was also analysed using a generalized linear model (glmmadmb; binomial error structure) with treatment, colony week and their interaction, week started, temperature and time of day included as fixed factors and colony/pair included as a random factor. Week of reproductive onset and queen survival were analysed using a Cox proportional hazards survival analysis that contained treatment and week started as fixed factors. All analyses were conducted in R studio (version 1.0.136) using the R packages pscl[43], lme4[44], glmm[45], MuMin[46] survival[47] and glmmadmb[48].

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. The full dataset is available as an open science framework project (https://osf.io/acrsy/).

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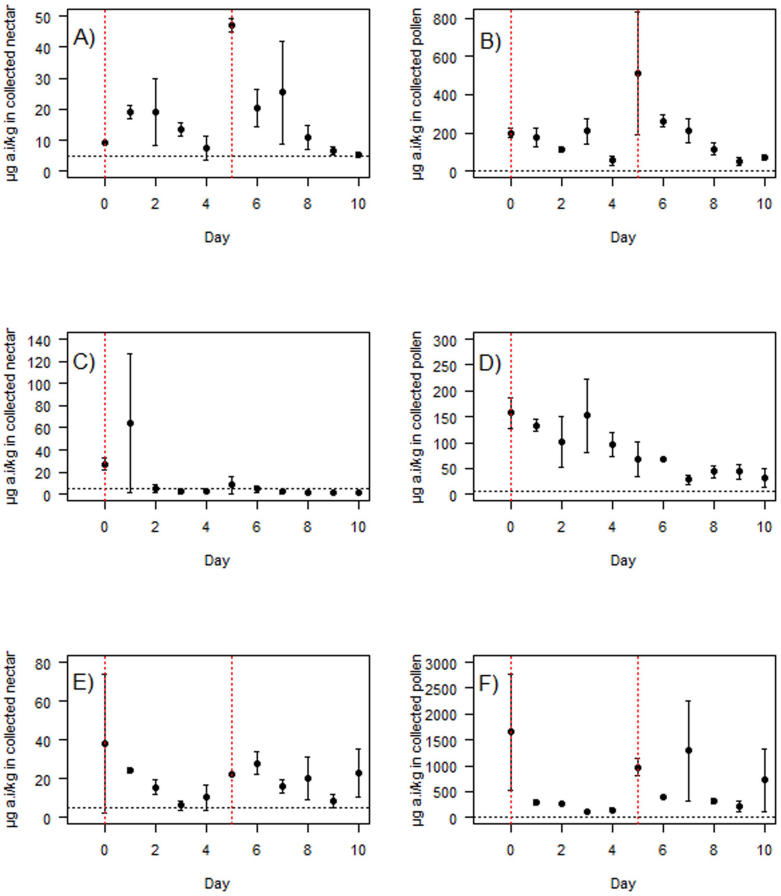
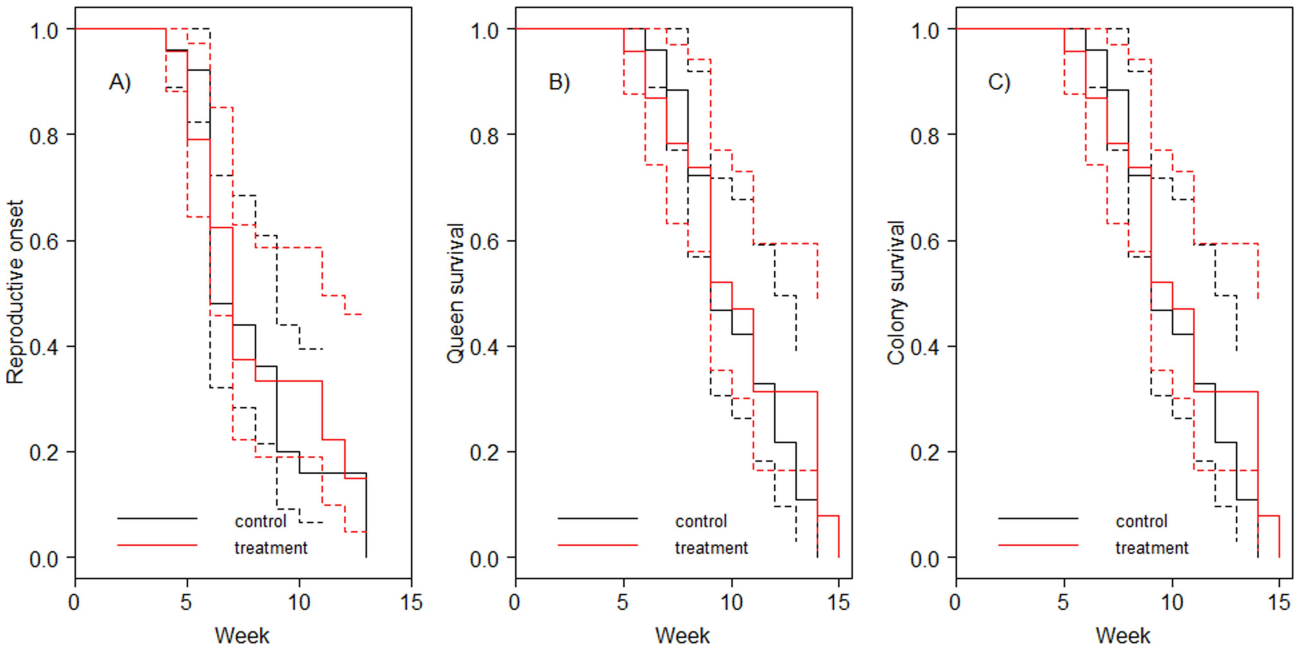
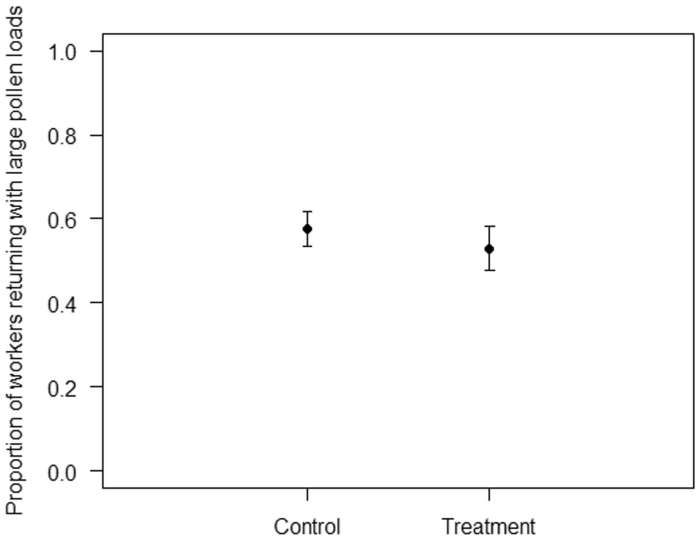


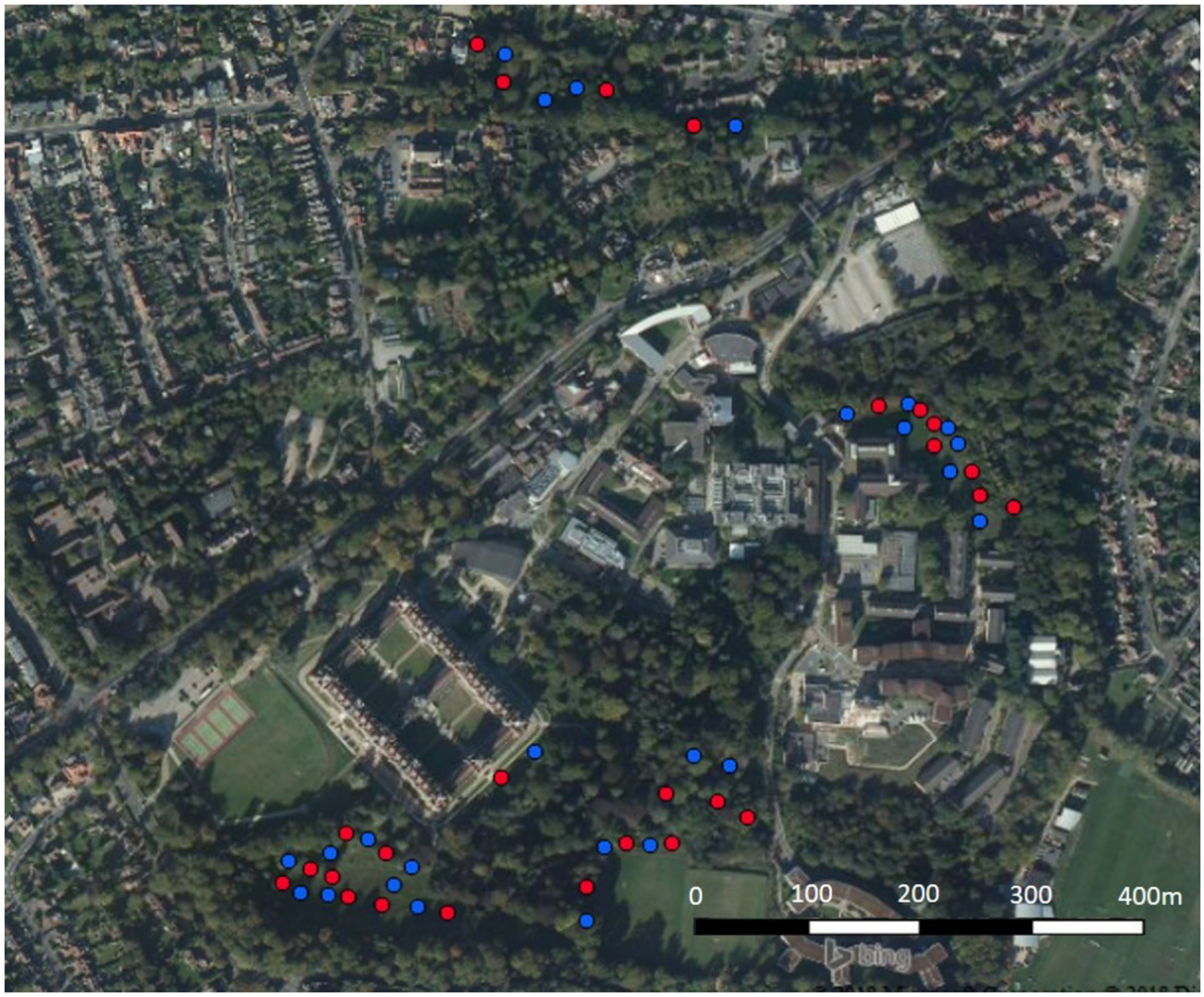
Extended Data Fig. 1 | Concentrations of sulfoxaflor in forager-collected resources from a USA EPA cotton study. Mean µg of active ingredient (a.i.) per kg (mean ± s.e.m.) found in the nectar (**a**, **c**, **e**) and pollen (**b**, **d**, **f**) of honeybees foraging on cotton crops sprayed with sulfoxaflor. Note the differences in *y*-axis scale between graphs, owing to considerably higher concentrations in pollen. Red lines indicate spray application. Dosage: twice over ten days at 0.045 pounds a.i. per acre (**a**, **b**); once over ten days at 0.045 pounds a.i. per acre (**c**, **d**); twice over ten days at 0.089 pounds a.i. per acre (**e**, **f**). The black dotted horizontal line indicates the equivalent amount of sulfoxaflor (5 ppb) that was fed to sulfoxaflor-treated colonies in sucrose in our experiment. Data are means from two hives; number of individual bees sampled is not published[27].



Extended Data Fig. 2 | Timing of colony life-history events. a–c, The probability of reproductive onset (**a**), queen survival (**b**) and colony survival (**c**) for control ($n = 26$) and sulfoxaflor-treated ($n = 25$) colonies (± confidence intervals).



Extended Data Fig. 3 | Pollen foraging. The proportion (mean ± s.e.m.) of foragers returning to the nest with large pollen loads, for control ($n = 25$) and pesticide-treated ($n = 22$) colonies (note that not all of the colonies in the experiment had pollen foragers).



Extended Data Fig. 4 | Distribution of colonies across the Royal Holloway Campus. Blue dots indicate control colonies; red dots indicate treated colonies. Grid reference: TQ000706; Imagery © Google, Map Data © 2018 Google.

nature research

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Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
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☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect data
Data analysis	R studio was used (version 1.0.136) and we used the packages nlme, pscl, survival, lme4, glmmadmb, glmm,Mumin

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets generated during and/or analysed during the current study are available in the an on line repository (likely to be Dryad)

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☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	52 bumblebee colonies at the start of the experiment (26 control & 26 pesticide). This sample size was based on wild queen production.
Data exclusions	No data was excluded
Replication	We have not attempted to replicate the results
Randomization	Within pairs that were matched for number of workers, colonies were allocated to the treatment or control groups at random
Blinding	Due to the large area that is experiment was conducted over and a limited number of researchers working on the project the experimenter was not blind the treatments

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	Wild caught bumblebee queens (Bombus terrestris) were caught from Windsor Great park in the spring of 2017.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging