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Pesticide-exposed bees fail to thermoregulate leading to cold colonies with consequences for offspring development

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For many organisms, effective thermoregulation is needed to cope with changing environmental temperatures. But if exposure to pesticides in the environment were to impair this homoeostatic process, reproduction and population viability could be at risk. Focusing on an important insect pollinator, we conducted three complementary experiments exposing bumblebees to a pesticide under different temperature challenges and measuring the impacts on thermoregulatory ability and brood development. First, we reveal that pesticide-exposed individuals (food spiked with a neonicotinoid insecticide) cannot maintain a stable thorax temperature, especially at lower temperatures. Second, that reductions in body temperature are accompanied by behavioural changes with pesticide-exposed colonies failing to maintain the brood temperatures that control bees achieved. Third, that such collective impairment of brood thermoregulation (not the pesticide toxicity to offspring per se) leads to delayed pupal development and reduced adult population growth. Our study provides a plausible mechanistic pathway for why terrestrial insects requiring brood thermoregulation may be vulnerable to decline. With frequent extreme weather events forecasted, our findings have concerning implications for how populations will adequately persist and grow under current pesticide-use regimes with ramifications for pollination services.

Environmental pesticide exposure poses potential health risks to a range of insects that perform vital ecosystem functions[1–3]. Whilst mortality from acute pesticide exposure is rare in non-target insects[4], a culmination of sublethal effects on physiology and behaviour may translate to population-level impacts[5–7]. For the ecologically and economically important social insects (i.e., ants, termites, species of bees and wasps), theory predicts that subtle impairments to individual function could build up to result in colony collapse[5,8]. Direct empirical support for this, however, remains surprisingly limited, as pesticide exposure studies do not commonly test across multiple levels of biological organisation[9–11]. For instance, whilst pesticide exposure has been associated with lower colony productivity[12–17], it is unclear how this has emerged from pesticide induced changes to individual behaviour and physiology. Identifying this link is important for revealing causative pathways and determining how to engineer and target biological solutions[18]. Furthermore, when quantifying sublethal effects, we must consider the environmental context dependency of pesticide toxicity[19]. For instance, quantifying how pesticide impacts are modulated by different ambient temperatures is crucial if we are to forecast pesticide risks across climatic regions and under climate change[20,21], especially with social bee colonies increasingly experiencing extreme weather events and extended activity periods across the year[22]. Indeed, as has been shown in bumblebees and honeybees, the toxicity of pesticides can be temperature dependent[23–27] with the direction of effect (becoming less toxic or more toxic under warming) depending on the behavioural endpoint[27].

A key adaptive strategy of bees to buffer climatic challenges is for adult individuals to regulate their own metabolic activity to reach and maintain a body temperature needed for contributing to nest/brood thermoregulation. The translation of individual capability to collective performance is required

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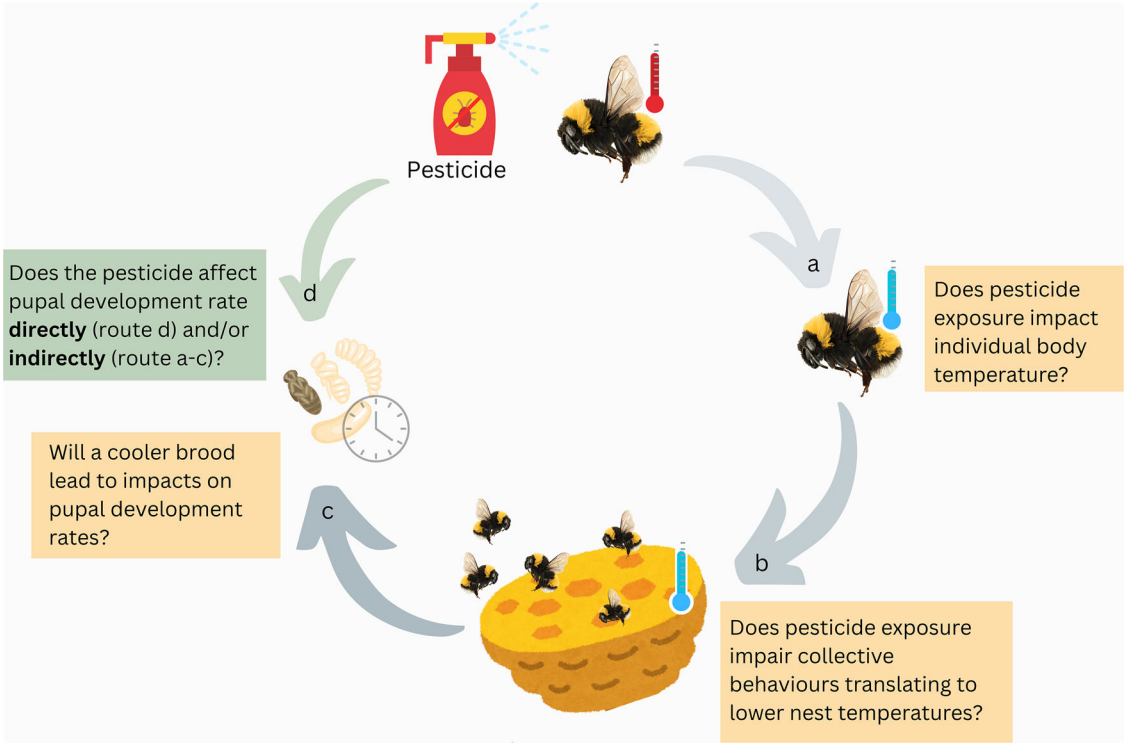


Fig. 1 | Conceptual diagram outlining our multi experimental approach and hypotheses to understand how exposure to a pesticide can affect bumblebee individual and colony thermoregulation and impacts on offspring development. We test how pesticide exposure affects individual body temperature (arrow a, experiment 1), how this translates to colony behaviours and nest thermal homoeostasis (arrow b, experiment 2), and if this indirectly culminates in impacting pupal development rates (arrow c, experiment 2 and 3). We further distinguish whether the impact on pupae is from the indirect route of impaired thermoregulation (yellow boxes and grey arrows a–c) or if direct pesticide toxicity can also explain our results (green box and arrow d, experiment 3). We test each hypothesis using a range of environmental temperatures. This figure was created by S. E.G. Hawthorne with some of the illustrative elements sourced from Canva's Elements library and used in accordance with Canva's Content License Agreement.

for successful rearing of colony offspring[28–30]. This is important for social insects with permanent nest sites, as they must deal with unfavourable environmental temperatures that frequently arise. It is thus concerning that a variety of pesticides have been reported to affect the molecular and cellular processes involved in energy metabolism and mitochondrial function placing thermoregulatory behaviours at potential risk[31–36]. Indeed, there is growing evidence that pesticide exposure can impact bee body temperature[37–39], but studies are few with each testing different pesticides under different environmental conditions and contexts, whilst using alternate study systems (i.e. different model bee species). To date, therefore, systematic data to link how individual thermoregulatory impairment translates to temperature-dependent colony-level performance are generally lacking (but see refs. 10,11).

Here, we look to ascertain if temperature dependent impairments of bee colony thermal homoeostasis and subsequent reductions in brood development are a symptom of individual thermoregulation being affected. We use the neonicotinoid insecticide, imidacloprid, as our test pesticide stressor, designed to build from previous reports of imidacloprid affecting bee thermogenesis[37] and being associated with reduced nighttime colony temperatures and altered collective behaviours[10]. Our aim is to reveal a set of first principles for how a given pesticide can impair organisms across different levels of biological organisation (e.g. individual physiology to population-level dynamics) that can improve understanding of both past responses and future risks[40]. Despite certain restrictions, neonicotinoids remain as one of the most used insecticide classes globally[41,42] with bee exposure via contaminated nectar and pollen seeming to be a common occurrence along with residue accumulation inside colonies[9,43,44].

We conducted three complementary experiments involving sublethal imidacloprid oral exposure assays (via bees feeding on a nectar substitute spiked with a field-relevant concentration of 10 µg/L; see Methods). Each experiment was conducted under different controlled temperatures to place different demands on individuals to raise and maintain their body temperature. From this we tested the hypothesis that under cooler temperatures pesticide-exposed bees are less able to maintain nest temperature with impacts on brood development (Fig. 1). For experiment 1 we determined whether exposed individual bumblebee workers can maintain body temperature (Fig. 1 arrow a; Fig. S1). For experiment 2, we linked how exposure altered the collective behaviours of individuals, with a focus on brood attendance, and tested how this was associated with brood temperature and colony adult population growth (Figs. 1 arrows b, c and S1). For experiment 3, we directly manipulated brood temperature and pesticide exposure to test if any reduced colony growth is due either to direct imidacloprid toxicity on brood, or via the behavioural disruption of adult workers that we observe. We did this by tracking (in vitro) development of isolated early-stage pupae under a low and high incubation temperature (informed by brood temperatures at the end of experiment 2) which were removed from control and pesticide exposed source colonies (the natal queen-right colonies that larvae are reared in) (Fig. 1 arrows c, d).

Results

Experiment 1: Temperature dependent effects of pesticide exposure on individual thorax temperature profiles

Control bees held a stable relative thermal profile at all three ambient temperatures over the 6-h trial, maintaining a mean (±standard deviation (SD)) thorax surface temperature of 28.3 ± 2.44 °C when at 15 °C ambient, 30.7 ± 1.88 °C at 20 °C ambient, and 32.6 ± 1.57 °C at 25 °C ambient. For pesticide-exposed bees, however, we detected drops in thorax temperature and higher variation between individuals. We can first confirm that pesticide bees were exposed to a similar mean estimated dosage across temperatures, being 0.010 ± 0.0036 ng imidacloprid per mg of body mass at 15 °C ambient, 0.010 ± 0.0022 ng/mg at 20 °C ambient, and 0.0096 ± 0.0018 ng/mg at 25 °C ambient. But despite the similar level of oral

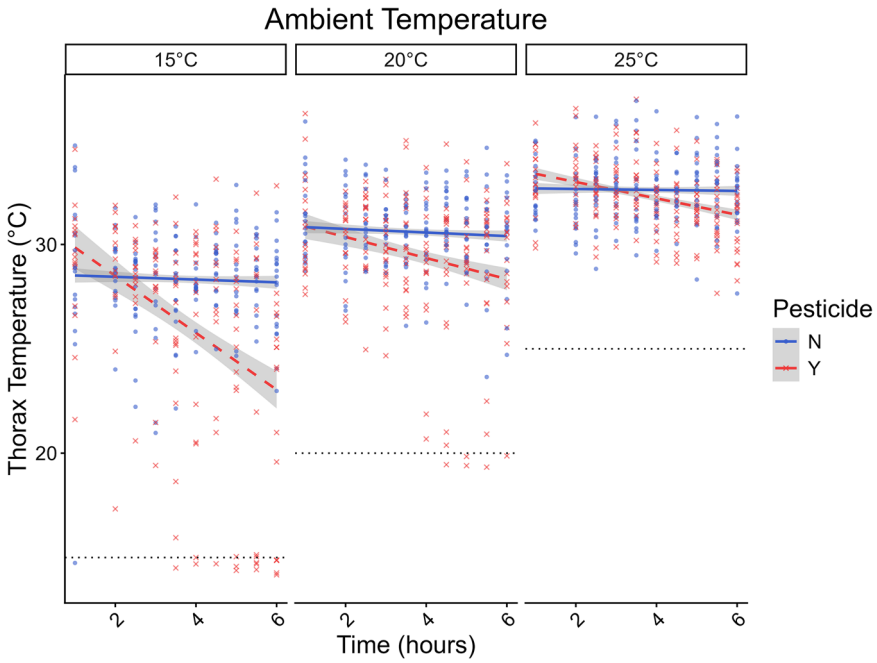


Fig. 2 | Pesticide-exposed bumblebees are unable to maintain a consistent thorax temperature (experiment 1). Bee thorax surface temperature for pesticide-exposed (red crosses and dashed line) and control (blue circles and solid line) bees over the 360-min trial at the three ambient temperatures (15, 20 and 25 °C, also shown as horizontal black dotted reference lines). Data lines represent mean estimates from the GLMM model with grey ribbons showing 95% CIs, with raw datapoints (all representing living bees) shown in the background ($n = 119$ individual bees across treatments).

pesticide exposure, we found temperature dependent effects on thorax temperature. Whilst we detected a lower (relative to the controls) estimated mean thorax temperature of 26.2 ± 5.13 °C at 15 °C ambient and 29.6 ± 3.10 °C at 20 °C ambient, thorax temperature was similar 32.6 ± 1.57 °C at 25 °C ambient. Furthermore, this temperature dependency was time dependent (*pesticide x temperature x time*: GLMM: $ß = 0.0020$, $p = 0.009$) showing that the reduction in thorax temperature was more severe at the lower temperature (Fig. 2; Table S8; Supplementary Data 1).

With individuals having no significant difference in mean body mass between control and pesticide treatments per ambient temperature (Wilcoxon test: $p \geq 0.08$; see Supplementary Results for details), body mass is unlikely to be driving the comparative treatment difference. When incorporated as a covariate, however, a significant interactive term of body mass x pesticide ($ß = 0.0050$, $p = 0.011$) indicates that whilst unstressed (control) smaller bees can perform as well as larger bees in maintaining thorax temperature, this was not the case for smaller bees that were pesticide exposed. Furthermore, in accordance with the hypothesis that energy input will influence thermogenesis, our estimates of sugar consumption (mg) relative to bee mass (per mg) showed a positive association with respective thorax temperature across treatments (GLMM: $ß = 0.37$, $p < 0.001$). Therefore, we additionally compared consumption rates between treatments at each ambient temperature. At 15 °C, we found that pesticide exposed bees compared to control bees did consume less sugar (mean (± SD) = 0.41 ± 0.14 vs 0.57 ± 0.17 mg/mg; Wilcoxon test: $W = 281$, $p < 0.001$) suggesting that the impact of pesticide exposure on thorax temperature may be an indirect consequence of lower carbohydrate input. However, at 20 °C, with no detected difference in sugar consumption found between pesticide-exposed and control bees (0.40 ± 0.088 vs 0.44 ± 0.10 mg/mg; $W = 286$, $p = 0.19$) the significant drop in thorax temperature does also suggest a direct physiological disruption of thermogenic mechanisms. We also detected no significant difference in sugar consumption between control and pesticide-exposed bees at 25 °C (0.39 ± 0.073 vs 0.42 ± 0.11 mg/mg; $W = 221$, $p = 0.40$).

Experiment 2: Temperature dependent pesticide effects on worker activity, brood care behaviour and cohort brood temperature

Comparative worker behaviour at the start and end of the trial. The behaviour of each bee in the microcolonies per segment of video was categorised as one of three classifications: (i) active, showing clear signs of defined movement whilst in physical contact with the nest structure and brood; (ii) active, but not in physical contact with the nest structure and brood; or (iii) inactive (Table S5). On the morning of day-1 of the trial, 80.0% of control group bees at 19 °C ambient were active and in contact with the brood and 72.0% at 25 °C ambient. In the pesticide-exposed groups 81.7% of bees at 19 °C were in contact with the brood and 57.0% at 25 °C (Table S9; Supplementary Data 2). By the end of day 5, the behaviour between pesticide and control groups developed to be distinctively different, with 93.3% and 72% showing a behaviour classified as inactive in the pesticide group when at 19 °C and 25 °C, respectively. In contrast, bees in the control group tended to either maintain brood care or transition to other active behaviours (i.e., being active on the brood to exhibiting activity off the brood), with only 1.4% transitioning to inactivity at 19 °C and 0.5% at 25 °C on day 5.

Workers attending brood over time. The raw number of bees observed on the brood per snapshot image of the microcolonies, increased over time for the control groups at both ambient temperatures. This seems to be due to an increased adult population in the microcolonies (see section 2.2.5) because the proportion of adult bees on the brood remained the same (Fig. 3A, B, Tables S10-11; Supplementary Data 3). In contrast, pesticide-exposed groups showed a decline over time in the raw number of bees on the brood (GLMM: *pesticide x time*: $t = -5.52$, $p < 0.001$). With limited adult population growth over time (see section 2.2.5), this also equated to a drop in the relative proportion of bees observed on the brood (*pesticide x time*: $Z = -3.17$, $p = 0.0015$; Fig. 3A, B, Tables S10 and 11). This divergence in the proportion of bees on the brood, with the control remaining stable whilst the pesticide groups declined, became apparent from day 2 of the experiment when at 25 °C (Wilcoxon test: $W = 2$, $p = 0.006$), and day 4 at 19 °C ($W = 14.5$, $p = 0.008$).

Brood temperature. We found a chronic effect of pesticide exposure that was greater at the lower temperature (19 °C) as evidenced by the three-way *pesticide* x *temperature* x *time* interaction (GLMM: $Z = 2.08$, $p = 0.037$; Fig. 3C, Table S12; Supplementary Data 3). Brood thermal profiles of the control group remained consistent over the 5 days, with a mean brood hotspot temperature in the control colonies of 33.2 ± 1.47 °C at 19 °C ambient and 36.3 ± 0.84 °C at 25 °C ambient. In contrast, pesticide-exposed colonies experienced significantly lower temperatures compared to the control at both the 19 °C and 25 °C ambient temperature

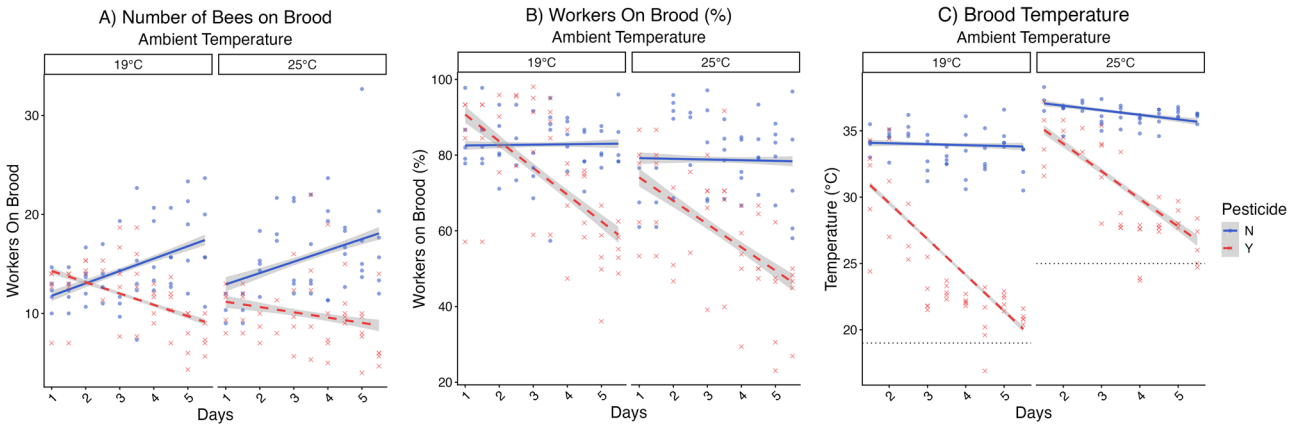


Fig. 3 | Chronic effects of pesticide exposure on bumblebees leads to decreases in brood attendance behaviour with associated reductions in brood temperature (experiment 2). A Mean across microcolonies ($n = 20$) of the (**A**) total number and (**B**) percentage of adult workers observed on the brood (based off three photos per microcolony per time point, with 185 complete observations), in pesticide-exposed (red crosses, dashed line) and control (blue circles, solid line) cohorts at the two ambient temperatures. **C** Average warmest surface temperature (mean of three thermal images per microcolony per time point) of the brood in pesticide-exposed and control microcolonies ($n = 5$ per ambient temperature-pesticide exposure combination, with 83 complete observations). Lines represent model estimates with grey ribbons showing 95% CIs, with raw data points in the background. The horizontal black dotted reference line shows the set ambient temperature of the room.

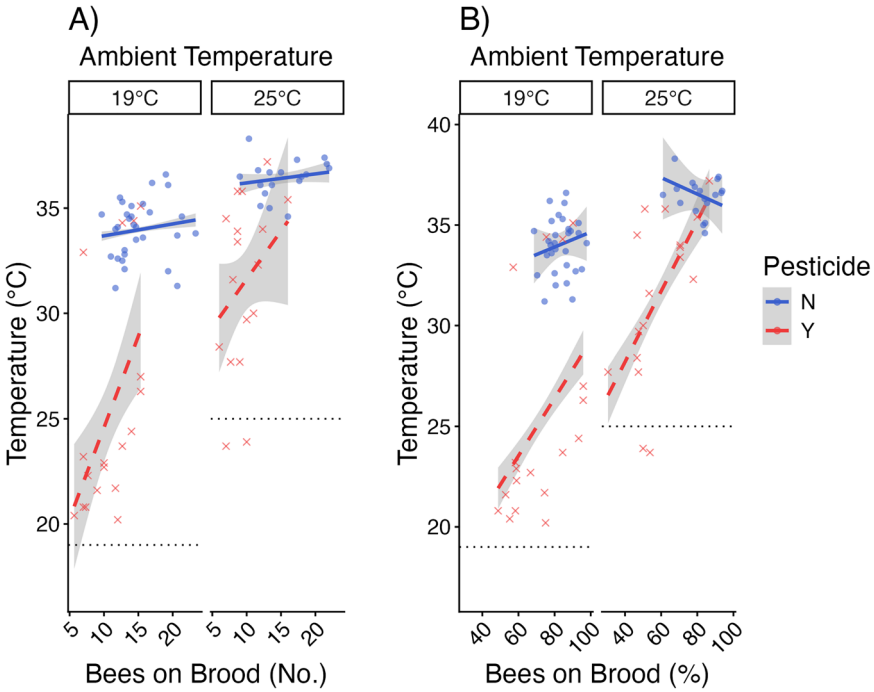


Fig. 4 | Relationship between brood attendance (*x* axis) and nest temperature (*y* axis) supports pesticide exposure leading to ineffective brood thermoregulation (experiment 2). Even when the (**A**) raw number and (**B**) proportion of adult bees on the brood in pesticide-exposed cohorts (red crosses, dashed line) overlaps with control (blue circles, solid line) the surface brood temperature in pesticide-exposed cohorts was significantly cooler on average. Data lines represent estimates from the GLMM model with grey ribbons showing 95% CIs, and the horizontal black dotted reference lines show the set ambient temperatures. The raw data in the background represents the mean value of three thermal images of the brood sourced from five microcolonies per ambient temperature-pesticide exposure combination (due to three missing observations, there were 165 complete observations in total).

(Wilcoxon test: $W = 120$, $p < 0.001$ and $W = 70.5$, $p < 0.001$, respectively). The thermal profiles of the pesticide-exposed groups varied more, with a mean brood hotspot temperature of 25.1 ± 5.08 °C at 19 °C ambient, and 30.7 ± 3.85 °C at 25 °C ambient. By the end of the experiment, brood temperatures of pesticide-exposed cohorts were only ~1–3 °C above ambient in both ambient temperature treatments, whilst control colonies were able to maintain brood at around 30–37 °C.

Testing the relationship between worker brood attendance and brood temperature. The temporally repeated video recordings and brood thermal imaging were in tandem, enabling us to examine how the number of bees on the brood was associated with brood temperatures. We found that the decreased number of bees on the brood associated with pesticide exposure had a negative impact on brood temperature (*Pesticide x Average bees on brood*: $t = -1.93$, $p = 0.054$; Table S12; see Table S13 for analysis on % bees on brood). Control colonies maintained high brood temperatures regardless of the number of bees on the brood, whereas pesticide-exposed bees required higher attendance to increase brood temperature but rarely reached that of the controls (Fig. 4).

Adult population growth. The total number of bees present in microcolonies at the end of the five-day experiment (sum of surviving original adults and emerged bees whilst accounting for any mortalities; Table S14) was significantly lower in pesticide-exposed microcolonies by a mean of 35.4% at 19 °C and 21.4% at 25 °C relative to controls (Wilcoxon test: $W = 19.5$, $p = 0.022$; Fig. 5; Table S15; Supplementary Data 4). Considering only surviving adults, pesticide-exposed cohorts had lower final

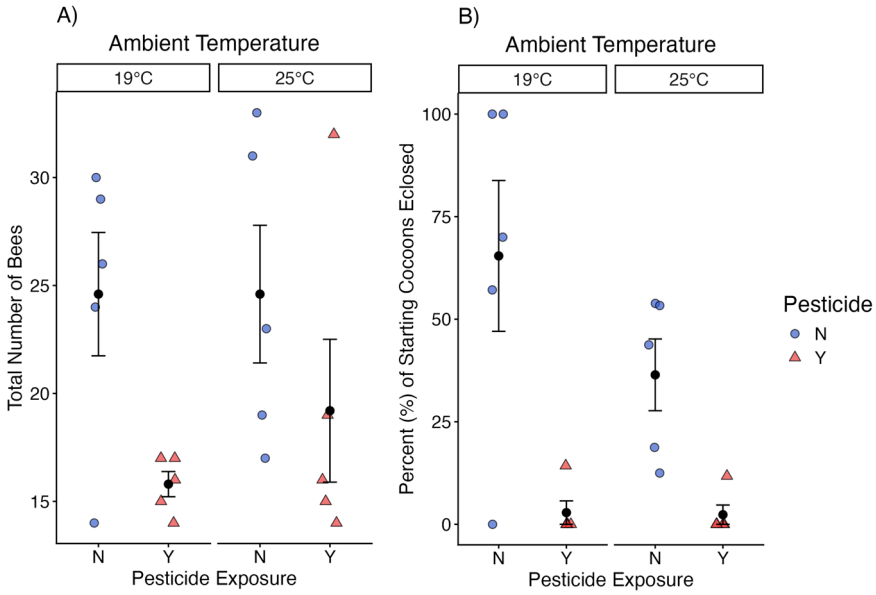


Fig. 5 | Pesticide-exposed microcolonies having a significantly lower adult bumblebee population at the end of the assay (experiment 2). A Total number of adult bees present at the end of the five-day trial, reflecting a dramatically lower net increase of the population in pesticide-exposed cohorts (red triangles) relative to control (blue circles; Wilcoxon test (two-sided) = $p < 0.01$). **B** Percentage of starting cocoons that had visibly hatched from video images by the end of the five-day trial reflects a significant reduced rate in pesticide-exposed cohorts (Wilcoxon test (two-sided) = $p < 0.01$). For both (**A**, **B**), black points represent the mean values across five microcolonies per pesticide-temperature combination (shown as jittered raw census data in the background totalling 20 microcolonies) with bars representing the standard error.

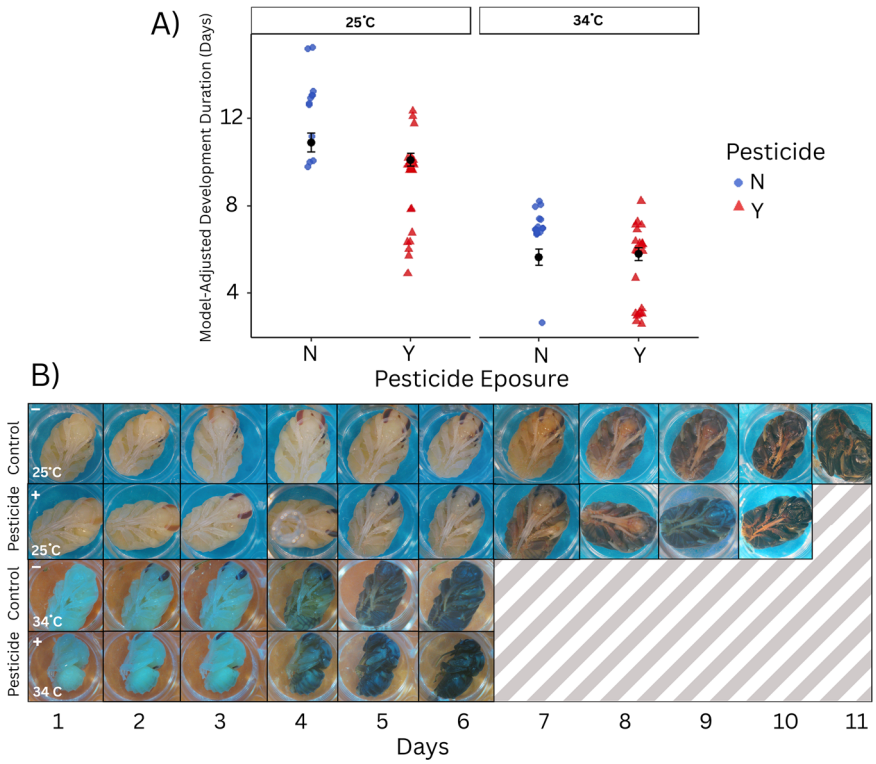


Fig. 6 | Reduced pupal development rate is driven by lowered brood temperature (an indirect effect of pesticide exposure on nest thermoregulation) and not the direct impact of pesticide toxicity on the individual pupae (experiment 3). A Duration in days each pupae took to reach the developmental milestone in which an individual had a fully melanized cuticle and showed spontaneous leg twitching. The black points representing linear model (LM) marginal mean estimates (accounting for initial developmental stage) with the bars representing the standard error. The jittered data points (total = 77 pupae) in the background represent the raw number of developmental days in which initial developmental stage is not considered. **B** Time series images of representative individuals taken daily throughout the pupation period, showing the typical morphological changes from initial eye pigmentation to fully melanised cuticle before emergence.

populations compared with control cohorts ($W = 11.5$, $p = 0.004$), with the population actually decreasing at 19 °C (mean = 14.5 final adults) and only slightly increasing at 25 °C (mean = 17.5). In contrast, control cohorts grew by a mean of 50.2% at 19 °C (mean final bees = 21.9) and 65.1% at 25 °C (mean final bees = 24.4). Data collected on visible cocoon hatching suggests that the lower population growth in pesticide-exposed cohorts was due to limited adult emergence. This is because we visibly observed less than one eclosed (empty) pupal cocoons on average in the pesticide-exposed cohorts at either temperature (19 °C mean eclosed cocoons = 0.2; 25 °C mean eclosed cocoons = 0.4), compared to a mean of 3.6 and 5.2 visibly eclosed cocoons in the control at 19 °C and 25 °C, respectively ($W = 7$, $p < 0.001$).

Experiment 3: Temperature dependent developmental rates of control and pesticide-exposed pupae

Over the 15 days of in vitro monitoring, the proportion of pupae that reached our defined final stage of development did not significantly differ between treatment cohorts (control 25 °C = 0.61; pesticide 25 °C = 0.81; control 34 °C = 0.75; pesticide 34 °C = 0.65; Pearson's $\chi^2 = 4.95$, df = 4, $p = 0.29$; Fig. 6; Table S16). However, the rate at which they reached this stage did. The lower temperature imposed a strong limiting factor on development rate (LM: $F(4, 72) = 80.44$, $p < 0.001$; Table S16), with pupae reared at 25 °C taking an estimated 5.24 days longer relative to 34 °C (when accounting for pupal age at the start of the assay; $\beta = -0.84$, $p < 0.001$). We therefore found no evidence that direct pesticide toxicity during larval

development was causing the delayed pupal development rates (no independent effect of pesticide or interactive effect with temperature: $\beta \leq 0.93$, $p \geq 0.149$).

Discussion

We show that oral exposure to a pesticide (specifically feeding on sugar solution spiked with the neonicotinoid imidacloprid) prevents adult bumblebees from maintaining a suitable [above ambient] body temperature whilst decreasing behavioural activity, including brood attendance. This dual impairment occurred quickly after the onset of exposure which led to a gradual but distinct decline in brood temperature. This effect was exacerbated at the experimental lower temperatures (15-20°C), reinforcing the notion that neonicotinoids impair non-target insect thermogenesis (in this case the ability of a bee to warm up[37,38]. Furthermore, our analyses suggest that pesticide exposure was associated with reduced efficiency in how adults collectively heated the brood. Despite a similar number of workers attending the brood for some control and pesticide exposed microcolonies in the first couple of days, the brood temperature of pesticide exposed microcolonies never reached that of the controls. Concerningly this was associated with little to no growth of the colony adult population and our in vitro rearing assay supports that this is due to the indirect effects of exposure on brood thermoregulation rather than due to direct physiological effects from the pesticide.

Looking at the control bees in experiment 1 shows that bumblebee workers (at least *B. terrestris*) are hardwired to heating and maintaining their body temperature above the ambient temperature (even when not in flight[45]. Exposure to the neonicotinoid imidacloprid, however, places this adaptation at risk. Generally, we found control bumblebee adults consistently held their body temperature over 6 h between an average of 29 °C (at 15 °C ambient) and 32 °C (at 25 °C ambient), with <3% variation from trial start to end. This range of non-flight body temperatures aligns with reported optimal brood rearing temperatures[46], supporting individual thermoregulation to be an evolved response for enabling brood thermoregulation. But we also show that pesticide exposure can leave adults unable to maintain suitable body temperatures, with a 21.3%, 6.6%, and 7.3% average reduction in maximal thorax surface temperature over the 6 h when at 15 °C, 20 °C and 25 °C, respectively.

Whilst we suspect pesticide effects on body temperature to be an issue for other insect taxa, impacts on generating and maintaining body temperature in bumblebees are problematic given they are heterotherms and as cold adapted specialists are frequently required to heat their body for important tasks[45,47]. Our findings can provide a mechanistic basis for explaining why previous results have shown temperature dependent effects of neonicotinoid toxicity on bumblebee locomotive behaviours[48] and survival[25]. It also expands on previous work showing that imidacloprid exposed bumble bees[37], honey bees[25], and solitary bees[49] struggle to survive and recover from cold temperatures. The worsening effects of lower ambient temperatures for pesticide-exposed bees over time suggests that with projected increases in the frequency and intensity of extreme low temperature events[50], alongside the ubiquity to which bees are exposed to pesticides[43], individuals could be at risk of becoming less active at key phases in the season.

Past studies have shown neonicotinoid exposure to be associated with reduced appetite in bees[48,51], potentially leaving bees with less energy for thermogenesis/endothermy[32]. Our results showed that appetite responses across a temperature gradient are non-uniform. Neonicotinoid exposed bees consumed a similar amount of food relative to control bees at the higher temperatures (20 and 25 °C) but consumed less at the low (15 °C). Whilst the lower body temperature of pesticide exposed bees at 15 °C could be due to reduced energy intake, this does not explain why a similar impact was found at 20 °C when food intake was similar to that of control bees. This points towards a direct effect of the neonicotinoid on molecular and neuronal pathways preventing bumblebees from being physically capable of controlling their own body temperature. Previous neonicotinoid exposure experiments in bumblebees have shown the expression of genes involved in energetic metabolic pathways to be affected[26,31,52], and that mitochondrial dysfunction may be symptomatic of exposure[33,34]. It thus seems that internal pathways involved in thermogenesis and homoeostasis are being affected, as suggested in honeybees[36,38]. However, future experiments to distinguish and quantify the contributions of direct toxicity versus indirect impacts of lowered food consumption through varying dosage assays would be a valuable contribution to our understanding.

Whilst control cohorts maintained an ~34–36 °C brood temperature over the trial (aligning with that expected;[46], neonicotinoid cohorts at 19 °C and 25 °C exhibited a gradual but distinct 42% and 20% average reduction in brood temperature, by the end of the trial, respectively. For control cohorts, there was no relationship between the number of workers attending brood and brood temperature, indicating that just a relatively small number of workers are needed to maintain thermal homoeostasis. Yet we found a positive relationship for pesticide-exposed cohorts indicating the need to compensate for neonicotinoid induced reductions in individual body temperature. However, even where a high proportion of workers were observed on the brood, brood temperatures still rarely reached that of controls. This suggests that pesticide exposure reduced not only the number of workers attending the brood, but also the functional efficiency of each attending worker as a heat source. Therefore, collective thermoregulation failed through both behavioural and physiological routes[53]. To compound this issue, once around day 3 had passed, worker collective behaviours in cohorts showed a distinct change, with a significant drop in active workers and brood attendance. Such impacts on collective behaviours echoes the conclusions by Crall and colleagues[10] who reported neonicotinoid impacts on social interaction networks within bumblebee colonies associated with a drop in nighttime colony temperatures. For our studied bumblebees, the consequences of an inability to rear a large enough adult population are that brood care and foraging activities will not be able to keep up with the energetic needs of overlapping adult generations[54]. For instance, in *Bombus huntii*, experimental removal of incubating bees can increase incubation times of remaining brood carers, rather than task switching of other bees to incubation (Gardner et al.[56]). Though some thermal buffering can be achieved by having underground nests, trade-offs between trying to counter brood getting cold with other important colony functions (e.g. foraging to meet metabolically energetic demands) could follow Allee type patterns, reinforcing negative feedback loops and leading to colony collapse[5,55].

Using a combined colony level exposure followed by in vitro assay (experiment 3), we looked to test whether delays in developing pupae was indeed down to the indirect effects of pesticide affecting nest temperatures, or because the direct toxic effects of the pesticide itself, or both. By rearing the control and exposed pupae in vitro at temperatures measured in experiment 2 for end of trial control nests (34 degrees) and neonicotinoid nests (25 degrees), we found evidence for the indirect hypothesis. Indeed, brood temperature should be important for successful larval and pupal development of many holometabolous insects, particularly in bumblebees[28–30,56]. Our findings, therefore, contribute a potential mechanism explaining why lower productivity in bumblebee colonies has been found near pesticide treated agricultural fields[14,57] and lower offspring hatching in solitary bee nests in which parents have been foraging on neonicotinoid treated plants[58]. It can further explain why controlled neonicotinoid exposure of bumblebee colonies in the field by Arce and colleagues[12] found lower numbers of reared gynes and males despite foraging patterns appearing little affected (also see[17]). It should be noted, however, that in the study presented here, we used cohorts of 15 workers which is smaller compared to typical field colony sizes nearer the end of the season (>100 workers). Larger bumblebee colonies have been suggested to better buffer impacts of imidacloprid on thermoregulation and population growth[59], yet with pesticides heavily applied at the start of the season, colony sizes are naturally small at that time.

In conclusion

Colonial living in social insect is considered an adaptation for buffering environmental pressures, such as temperature extremes[60]. Our result,

however, suggests pesticide exposure undermines this strategy. We show explicitly that pesticide effects on thermogenesis and nest thermal homoeostasis translates to impacts on brood development, and yet this is unlikely to be exclusive to bumblebees as other insects require suitable body temperatures for foraging and suitable nest temperatures for rearing offspring[61,62]. The multi stressor nature of modern landscapes suggests that care should be given when assessing the context dependency of pesticide risks[48,63]. Furthermore, in environments where food resources are heterogeneous and there is competition for resources, the additional impairments that pesticides have on locomotion[48,51,64] and cognition[51,65] could exacerbate the situation. Further research is needed to understand multi stressor effects on beneficial insect fitness, particularly temperature and pesticide combinations, to inform targeted mitigative action and to inform risk assessments and environmental policy. Overall, our findings reveal how sublethal pesticide exposure can impair a core adaptive trait providing a mechanistic link between environmental stress, failures in colony function, and the decline of beneficial insect populations.

Methods

Bumblebee colonies, controlling pesticide and temperature exposure

We obtained five, eight, and five *Bombus terrestris audax* colonies for experiments 1, 2 and 3, respectively, to act as source colonies for our assays from the commercial supplier Biobest (distributor Agralan Ltd). Source colonies (all experiments), individual pots (experiment 1) and microcolonies (experiment 2) were held in controlled environment (CE) rooms at 60% relative humidity. For all experiments, the *ad libitum* nectar substitute that bees were provisioned was a 40% w/w sucrose/water solution, with the pesticide treatment group being spiked with 10 µg/L imidacloprid representing a concentration found in nectar and pollen of flowers in the field[66]. For experiment 1, focusing on individual relative surface body temperatures, we tested bee responses at ambient temperatures of 15, 20 and 25 °C. For experiment 2 on whole cohort responses, we tested under 19 and 25 °C to mimic mean Spring-Summer temperatures recorded at Silwood Park (Ascot, UK) from 2010-2019[67] and within the range tested in experiment 1. For experiment 3, we reared pupae in vitro at 25 °C and 34 °C to simulate approximate surface brood temperatures that neonicotinoid exposed and control microcolonies (respectively) showed near the end of experiment 2 (see Results 3.2.3).

Thermal imaging (experiments 1 and 2)

A FLIR ONE Pro camera (FLIR Systems) captured thermal images (emissivity set at 0.95) of test subjects (Fig. 1). Pilot work testing the thermal imaging showed that use of black electrical tape (3 M Scotch super 33 + PVC tape with known emissivity of $\varepsilon = 0.95 \pm .\ 0.05$) enabled us to calibrate camera readings with high accuracy[68] (see Supplementary Methods). FLIR images were processed using a hotspot tool (FLIR Tools v.5.13.18031.2002) to return relative thermal readings for each subject. When imaging subjects, we found that 93% of images taken by the camera read the tape temperature within 0.2 °C of the known and tightly controlled room temperature[48], and we further excluded any images above this margin of error. For this comparative treatment experiment, this provided a suitable standardised process to thermally profile between adults and between brood to gain relative comparisons of thermoregulation at the individual and colony-level. For experiment 1, hotspots always represented a thoracic pixel of a bee. For experiment 2, if hotspots were not automatically assigned to the brood by the FLIR tools package, polygons were manually assigned to represent the brood area with the hotspot then extracted from this portion of the image. We validated that the thermal hotspot could be used as a proxy of wider brood relative thermal profiles by showing that the hotspot has a strong correlation with the mean temperature of three randomly selected points across the brood ($R^2 = 0.85$; Fig. S2; Table S1; Supplementary Data 5).

Experiment 1: Temperature dependent effects of pesticide exposure on individual thorax temperature profiles. On arrival, source colonies were allowed to acclimate to lab conditions for five days before experimental assays started. These were kept in a red-lit CE room set at 25 °C and provided with *ad libitum* plain nectar substitute and four grams of honeybee collected pollen daily (BioBest). For the trial we took individual workers from their respective source colony (Table S2) and placed each in a separate container (120 mL) for the duration of the experiment. In total, we randomly selected 119 workers (22–25 per source colony, see Supplementary methods for protocol).

For each of the three temperature treatments ($n = 37$–43 workers per temperature), half the workers were fed either the untreated or pesticide-spiked nectar substitute. This was done via an Eppendorf tube in which a small hole was made at the bottom (using tip of a soldering iron) and then the tube being inserted in the container side wall just above the floor. Beginning when the food was provisioned, thermal images (taken once the pot cover was removed) were captured at 60 min, 120 min, and then at 30-min intervals until 6 h had elapsed, resulting in a total of ten images. Six hours was selected to ensure metabolism of imidacloprid[69] whilst maintaining a feasible recording period. For each thermal image, six pots were configured in a way that allowed thermal profiling of six workers under a single field of view. At the end of the 6-h trial, workers were frozen at −20 °C, with individual dry mass calculated post-trial by drying inside 80 °C ovens for 72 h and weighed.

Experiment 2: Temperature dependent pesticide effects on worker activity, brood care behaviour and cohort brood temperature. Within two days of arrival, from each source colony we created 2–3 cohorts with each cohort comprising fifteen randomly selected worker bees (twenty cohorts in total; Table S3). Each cohort was provisioned 10–16 g of the source colony's brood with a balanced composition of comb type (same approximate ratio of eggs, larvae, pupae, and nectar pots), with no significant difference in brood mass between treatments (Table S4). The resultant 'microbroods' were placed on a 45 mm diameter plastic petri dish in the middle of a ventilated high-density polypropylene microcolony box with the selected workers. The cohorts (microcolonies) made from each source colony were evenly split between our two experimental temperatures (19 or 25 °C) and allowed to acclimate for two days with *ad libitum* provision of nectar substitute via gravity feeders and 2.5 g of honeybee collected pollen inside a 45 mm petri dish provisioned on days 0, 2 and 4. Any workers found dead prior to the start of the assay were removed and replaced with a randomly chosen worker from the respective source colony to maintain the worker population at fifteen at the start of the trial.

After acclimation, half of the cohorts in each CE room were assigned to control and the other half to pesticide treatment. Both groups were supplied with 2.5 g of pollen on days 1, 3, and 5 of the five-day experiment. Nectar consumption was measured by weighing the tubes daily, and pollen consumption by weighing the pollen dish at the end of the trial. Mortality was visually recorded each day with dead workers removed and frozen at −20 °C. At the end of the experiment, all cohorts were frozen (−20 °C) and later censused with final mass and composition of the brood recorded. Given that the duration to develop from a first day pupa to an adult bumblebee is more than five days, the emergence of any bees during our five-day experiment will be indicative of pupal development. Crucially pupae do not require any further food provision from adult workers, but their development rate and likelihood of eclosion should be dependent on temperature[46].

Three repeat (in-triplicate) thermal images were taken within a 60 s period, and this was done twice a day for each cohort (i.e. total of six images per day per microcolony). Top-down video recordings of within cohort behaviour was conducted for three minutes in the morning and evening prior to thermal imaging using either a Canon (LEGRIA HFR606) or Panasonic (HC_V160) camera. Behavioural data was obtained by manually scoring behaviours for each cohort individual at the 60th, 120th and 180th second in the footage for (i) the ratio of bees in contact with brood and (ii) the type of behaviour each bee was exhibiting, categorised as either active on the brood, active off the brood, or inactive based off an ethogram (Table S5).

Experiment 3: Temperature dependent developmental rates of control and pesticide-exposed pupae. On arrival source colonies were maintained at 25 °C and allowed to acclimate for 1 week with pollen provided *ad libitum* and untreated sucrose solution *ad libitum* via gravity feeders. Once the week was up, three colonies were randomly assigned as the pesticide exposure treatment and two colonies as control. We continued this exposure for seven days because this is the average time taken to develop from an egg to a 1-day old pupa, and thus the early-stage pupae in the colonies at this end point must have developed during the source colony exposure treatment period. After this week of exposure, brood cells (pupal cases) were collected from each colony ($n = 20–30$ per treatment). Early-stage pupae could be distinguished based on the texture and colour of the pupal case (lighter colour indicating younger). Then, once we had removed the pupae from the case, we could more accurately determine age (development stage). Pupae were aseptically removed from the pupal case using sterilised forceps. Individuals were selected at either the final larval stage or early pupal stage, the latter defined by the presence of white eyes, indicating the onset of pupation[70]. Pupae were placed individually into sterile 48-well plates (1 ml) wells, with lids modified by drilling 2 mm holes to allow for airflow. These plates were then placed into sealed plastic containers (Tupperware, 10 × 5 × 5 in.), containing saturated NaCl solution to maintain a stable relative humidity of 75%, following standard bee rearing protocols[71,72].

The isolated pupae from each source colony were then divided equally and reared at either 25 °C or 34 °C. These two in vitro rearing temperatures were picked to represent a conservative differential temperature between our treatment combinations. The lower temperature of 25 °Cis close to the nest temperature of imidacloprid microcolonies at the end of the 5-day assay when kept at 25 °C ambient. The 34 °C was the nest temperature of control colonies at the end of the assay when kept under 19 °C. This means we tested between the narrowest margin of nest temperature difference found between our treatments. This experiment will enable us to distinguish if imidacloprid is affecting pupal development through direct toxicity or via the indirect effect of nest temperature being kept too low by adult nestmates. Of the 111 pupae that started the in vitro rearing assay, five were removed within 24 h due to signs of damage during the extraction and transfer process, with the probability of exclusion not significantly differing between the four treatments ($p = 0.89, \chi^2 = 0.58$, df = 3). This resulted in the following treatments and sample sizes: 25 °C control $n = 21$; 25 °C pesticide-exposed $n = 31$; 34 °C control $n = 33$; 34 °C pesticide-exposed $n = 21$ (Table S16). Pupal development was monitored daily using a digital stereomicroscope fitted with a high-resolution camera (Andonstar). Individual images were taken at the same time each day to ensure temporal consistency with key developmental stages timestamped using photographic records, with reference to described morphological criteria[70]. For this study, we classified the end of pupation as the point at which individuals displayed a fully melanized cuticle and spontaneous leg twitching, immediately after which each respective worker offspring was removed and frozen to avoid bees developing into mobile adults and escaping.

Statistical analyses

Analyses were run using R statistical software via RStudio using either Version 2023.06.2 + 561 or 2025.05.0 Build 496 'Mariposa Orchid'[73] with graphical plots made using ggplot2[74]. For constructing the mixed effects models (see Supplementary Data 6 for our analytical code), we first considered maximal models with all explanatory variables (using appropriate model functions according to data distributions) before dropping components and using AIC to select the best fit model[75]. Best fit models were then used to produce relationship estimates (with 95% CIs), with *p*-values computed using a Wald t-distribution approximation, producing a Nagelkerke's R value[76].

Experiment 1. Bee mass was scaled using an exponent of 2/3 to account for the surface area to volume ratio of bees and to consider the relationship between thermogenesis and body mass likely follows a power law[77]. The best fit model for predicting thorax temperature was a generalised linear mixed model (GLMM) fit with a log link function. The model contained a three-way interaction of *pesticide x temperature x time*, and a two-way interaction of *body mass x pesticide exposure*. The model included *colony of origin* as a random effect to account for any non-independence of workers originating from the same source (natal) colony. Additionally, time and bee identity were considered as random effects and structured to allow each bee to have its own thermal trajectory over time (slope) by comparing the impact of treatments relative to its own baseline (intercept) (see Tables S6-7). This was fitted to account for any underlying variation between individuals such as metabolic rate, energetic state and body condition[78,79]. This selected model had a total explanatory power of 68% of the thorax temperature variation. For direct comparisons of worker body mass and the mass of sugar consumption by workers between treatments, we used a Wilcoxon test (two-sided).

Experiment 2. Brood temperature data were averaged across the three thermal images captured per time step. The best fit model was a fixed effects GLMM using a log link function and structured as a three-way interaction between *pesticide* x *temperature* x *time*, interactive effects of the number of bees on the brood and pesticide exposure, and considering additive effects of *pre-trial brood mass* and random effects of *cohort ID*. The model's total explanatory power was 88% of brood temperature variation.

The behavioural data of the cohorts were first analysed as worker attendance to the brood (proportion of bees on the brood). The best fit model for brood attendance was a fixed effects linear model (LM) with interactions of *pesticide* x *temperature* and *pesticide x time*, with a random effect of *cohort ID*. The model's total explanatory power was 56% of the data variation. Behavioural data was then further analysed by calculating the transitions between behaviour types between day 1 and 5 of the experiment (active on the brood, active off the brood, or inactive) to assess trends in behaviour transitions between trial groups. We explored how behavioural data could predict thermal profile data by using a GLM that included a three-way interaction between *pesticide* x *temperature* x *time*, a two-way interaction between the *number of bees on brood* and *pesticide exposure*, and considered *starting brood mass* as an additive effect, with *cohort ID* included as a random effect. The model's total explanatory power was 88% of brood temperature variation. Data on brood mass and number of bees at the end of the trial were analysed using Wilcoxon tests (two-sided) to assess significant differences in final cohort population (starting adult bees and emerged adult bees minus mortality) and percentage of pupal emergence (visible eclosed pupal cells) between the trial groups.

Experiment 3. To test the interactive effect of *temperature x pesticide* on pupal development rate, we fitted a linear model (LM) while controlling for variation in initial developmental stage (*start age*). Because individuals entered the experiment at different developmental stages, an estimated *start age* was assigned based on visible eye and cuticle pigmentation and included as a covariate in the model. This correction ensured that comparisons reflected true differences in developmental rate rather than differences in starting stage. Post hoc pairwise comparisons of estimated marginal means were performed using the emmeans package[80,81].

Data availability

All data used to generate the results reported in this article are made available as Supplementary Files in CSV format. Source data for experiment 1 and Fig. 2 is in the file 'Supplementary_Data_1_Bee_-body_temp'. Source data for experiment 2 and Figs. 3–5 in the files 'Supplementary_Data_2_BB_behaviour' and 'Supplementary_Data_3_Brood_temp'. Source data for experiment 3 and Fig. 6 in the file 'Supplementary_Data_4_Bee_dev.'.

Code availability

All code used to produce the results and figures in this article is available as a single Supplementary File in RMD format for use in R.

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Author contributions

K.E.S. and R.J.G. contributed to conceptualisation and writing the original draft. K.E.S., S.H., C.L., F.M., D.K., T.Z. and R.J.G. planned, setups and conducted the experiments. K.E.S. and S.H. carried out the analyses and produced figures with advice from R.J.G. All authors contributed to editing of the later drafts. D.K., P.G. and R.J.G. provided supervision, funding and resources.

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Competing interests
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Additional information
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