nature sustainability

Article

https://doi.org/10.1038/s41893-024-01432-5

Ingestible hydrogel microparticles improve bee health after pesticide exposure

Received: 19 December 2023

Accepted: 15 August 2024

Published online: 05 September 2024

Check for updates

Julia S. Caserto[1], Lyndsey Wright[2], Corey Reese[3], Matthew Huang[4], Mary K. Salcedo[2], Stephanie Fuchs[2], Sunghwan Jung[2], Scott H. McArt[3] & Minglin Ma[2]

Bees provide crucial pollination services for crop cultivation, contributing billions of dollars to the global agricultural economy. However, exposure to pesticides such as neonicotinoids represents a major problem for bee health, necessitating strategies that can improve agricultural sustainability and pollinator health. Here we report a simple and scalable solution, through ingestible hydrogel microparticles (IHMs), which can capture neonicotinoids in vitro and in the bee gastrointestinal tract to mitigate the harmful effects of pesticides. Using the common eastern bumblebee (*Bombus impatiens*) as a model species and the neonicotinoid imidacloprid, we demonstrated by means of lethal and sublethal assays the substantial benefits of IHM treatments. Under lethal exposure of imidacloprid, bumblebees that received IHM treatment exhibited a 30% increase in survival relative to groups without IHM treatment. After a sublethal exposure of 5 ng, IHM treatment resulted in improved feeding motivation and a 44% increase in the number of bees that engaged in locomotor activity. Wingbeat frequency was significantly lower after a single 5 or 10 ng imidacloprid dose; however, IHM treatment improved wingbeat frequency. Overall, the IHMs improved bumblebee health, and with further optimization have the potential to benefit apiculture and reduce risk during crop pollination by managed bees.

Pollinators, especially bees, provide essential pollination services to 76% of crops[1] and contribute ~US$195–387 billion annually to the global agricultural economy[2]. However, pollinators are now experiencing unsustainable losses, range contractions, and population declines. Between 2017 and 2020, annual losses of managed US honeybee hives averaged 40–44% (ref. 3) and dozens of studies have now documented regional and global declines of wild bees[4,5]. There are several factors contributing to pollinator declines, including loss of habitat, pesticides, invasive species, climate change, and disease[6]. Of these, there is considerable interest in the effect that pesticides are having on bees. For example, honeybees and bumblebees are exposed to an average of 35 and 19 pesticides, respectively, during commercial blueberry pollination[7]. High-risk neonicotinoid and organophosphate insecticide exposures occur frequently during blueberry and apple pollination[8,9] and near seed-treated corn and soybean fields[10], with exposure levels that can influence susceptibility to parasites and pathogens[11], foraging behaviors[12], and growth and survival of bees[13]. Although much effort has gone into developing integrated pest management (IPM) tools meant to reduce reliance on pesticides, there remains limited adoption of IPM techniques by farmers and continued widespread use of pesticides[14]. A complementary approach could be to develop technology that minimizes the impact of pesticides on bees. To this end, we previously aimed

[1]Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA. [2]Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA. [3]Department of Entomology, Cornell University, Ithaca, NY, USA. [4]Department of Biological Sciences, Cornell University, Ithaca, NY, USA. e-mail: mm826@cornell.edu

to tackle the issue of organophosphate toxicity in bumblebees using a phosphotriesterase enzyme encapsulated in calcium carbonate microparticles to break down organophosphate insecticides through the hydrolysation of their triester linkages[15]. Besides this study, there have been limited solutions that aimed to detoxify or degrade other pesticides inside bees after exposure. Therefore, pesticide detoxification in bees remains an unaddressed challenge.

Neonicotinoids are nicotinic acetylcholine receptors (nAChR) agonists and are one of the most widely used classes of pesticides. They leach into groundwater and subsequently enter pollen and nectar that are foraged by bees. The first neonicotinoid produced was imidacloprid, which was patented in 1985, first sold in 1991, and approved by the US Environmental Protection Agency (EPA) in 1994[16]. The appeal of neonicotinoids originally stemmed from their lesser toxicity to vertebrates and preferential binding to insect nAChRs, which benefits human health but does not consider the perspective of non-target organisms such as bees and invertebrate aquatic wildlife[17,18]. These nAChRs are ionotropic membrane receptors, which play key roles in membrane depolarization and neuronal signal transmission[18]. Many studies have reported both sublethal and lethal effects of neonicotinoids on both bumblebees and honeybees[18–22]. Impairments of learning and behavioral activities, such as flight and memory, are examples of these sublethal effects[23]. Neonicotinoids have also been shown to induce oxidative stress which can result in consequences such as mitochondrial damage and shortened lifespan[24,25]. Imidacloprid has been widely used since its introduction to the pest management market. For example, from 2005 to 2015, 700,000 lb yr^{-1} of imidacloprid were applied to crops in the form of seed treatments[26]. In 2020, the US EPA released interim decisions on imidacloprid usage including mitigation measures such as minimizing application rates, invoking targeted and restrictive application protocols, and prohibiting some of its uses in specified cases[26]. In addition, the European Union has banned outdoor use of imidacloprid, clothianidin and thiamethoxam[27].

Nitro-substituted neonicotinoids, such as imidacloprid, clothianidin and thiamethoxam, have been shown to have a higher toxicity to bees compared to cyano-substituted neonicotinoids, such as thiacloprid and acetamiprid[28]. Owing to the increased toxicity of nitro-substituted neonicotinoids, they were chosen as the compounds of interest for this study. Taken together, the detrimental effects neonicotinoids can have on bees and the importance of bees to agriculture and crop pollination provide motivation for the development of strategies that could address these problems and offer solutions that are both economically and environmentally feasible. In this work, we aimed to develop a detoxification strategy in bees to combat neonicotinoid exposure, thereby lessening or eliminating their toxicity and sublethal effects (Fig. 1a). In this work, we developed ingestible hydrogel microparticles (IHMs) to capture imidacloprid in the bee gastrointestinal (GI) tract and mitigate the harmful effects of this pesticide to improve pollinator health. Using the common eastern bumblebee (*Bombus impatiens)* as a model species, we demonstrated that IHM treatment not only improved bee survival by 30% after a lethal imidacloprid exposure but also improved feeding motivation, locomotor activity and wingbeat frequency (WBF) at sublethal doses. Overall, this proof-of-concept study provides evidence that the IHMs may have the potential to benefit apiculture and consequently agriculture.

Results

In vitro adsorption capacity of IHMs

The first step in assessing the efficacy of these IHMs (Fig. 1b) was to determine if they could adsorb neonicotinoids in vitro at physiologically relevant pH values (Fig. 1c,d). R^2 values for imidacloprid were higher for pseudo-first-order model fits. Pseudo-first-order fits indicate that the limiting mechanism is physisorption[29]. Model parameters including pseudo-first- and pseudo-second-order rate constants, equilibrium constants and R^2 values are summarized in Supplementary Fig. 1. In addition, imidacloprid capture with varying IHM concentrations was also investigated. It was found that imidacloprid capture was concentration dependent and up to 78% of imidacloprid removal could be achieved with an IHM concentration of 5 mg ml^{-1} (Supplementary Fig. 3).

Bee survival after high imidacloprid exposure

First, bee survival without imidacloprid exposure was assessed to determine if IHMs alone have effects on mortality. As shown in Fig. 2a, the survival of bees provided with plain sucrose syrup was not statistically significantly different from the survival of bees provided with IHM syrup ($\beta = -0.005$, s.e. = 0.583, hazard ratio (HR) = 0.995, $P = 0.993$). The variance of the cage random effect was 0.01. Results of the integrated log-likelihood and penalized log-likelihood tests were ($\chi^2 = 0.01$, d.f. = 2.00, $P = 0.997$, Akaike information criterion (AIC) = −3.99, Bayesian information criterion (BIC) = −4.96) and ($\chi^2 = 0.21$, d.f. = 1.08, $P = 0.679$, AIC = −1.95, BIC = −2.47), respectively. To confirm that any improvements in survival would be the sole effect of IHMs reducing the free imidacloprid in the bee body and not caused by a lower pollen consumption and hence less pesticide intake, we examined the pollen consumption with and without IHM treatment. The differences in pollen consumption were not found to be statistically significant, with all P values being >0.05 (Fig. 2b). These data demonstrate that bees still consume pollen even with concurrent IHM consumption.

To test the effect of IHMs on survival, we first determined the concentrations for the survival assays by evaluating survival after exposure to different imidacloprid concentrations (Fig. 2c). The concentration chosen for further experiments was 4 ppm, which resulted in a more moderate rate of mortality relative to the other concentrations tested. Using the 4 ppm concentration, survival assays were then conducted to determine IHM efficacy in improving survival. Bumblebees exposed to 4 ppm imidacloprid via a pollen ball exhibited a significant decrease in survival compared to groups receiving clean pollen balls without imidacloprid (Fig. 2d). However, imidacloprid-exposed bumblebee groups that received IHM treatment exhibited an ~30% increase in survival compared with those that did not receive IHM treatment (Fig. 2d). The survival probabilities for groups with and without IHM treatment were 87% and 58%, respectively. Survival of the IHM group was statistically significantly higher than the pesticide only group ($\beta = -1.283$, s.e. = 0.501, HR = 0.277, $P = 0.01$). The variance of the cage random effect was 0.209. Results of the integrated log-likelihood and penalized log-likelihood tests were ($\chi^2 = 24.24$, d.f. = 2.00, $P < 0.001$, AIC = 20.24, BIC = 16.42) and ($\chi^2 = 31.58$, d.f. = 3.33, $P < 0.001$, AIC = 24.92, BIC = 18.56), respectively. A concern was that the IHMs would have an impact on bumblebee survival over a duration longer than the 8 d assay presented in Fig. 2a. To address this concern, an extra survival assessment was performed over a 34 d period (Supplementary Fig. 4) and differences in survival were not observed. The differences in mortality rate between the preliminary tests (Fig. 2c) and larger scale survival assay (Fig. 2d) may be attributed to different colonies and the smaller sample sizes used in the preliminary tests. When the sample sizes are smaller, a single death would have a substantial impact on the results of the group as a whole.

To confirm IHM ingestibility and movement through the digestive tract, we studied their in vivo distribution using fluorescence microscopy (Fig. 3a–e). At 0 and 1 h timepoints, IHMs were observed in the crop and ventriculus. After 8 h, IHMs had traveled further into the ventriculus and into the hindgut (Fig. 3c and Supplementary Fig. 5), and at the 48 h timepoint IHMs were primarily in the hindgut with some remaining fluorescence signal in the ventriculus depending on the bee sample. In some cages, the excrement had a blue colour, indicating that IHMs were fully exiting the body. Lyophilized cyanine 5-amine (Cy5)-labeled IHMs had a blue colour able to be visually seen with the naked eye (Supplementary Fig. 5).

Imidacloprid consumption and exposure has been shown to decrease feeding in bumblebees[30,31]. We aimed to confirm that

https://doi.org/10.1038/s41893-024-01432-5

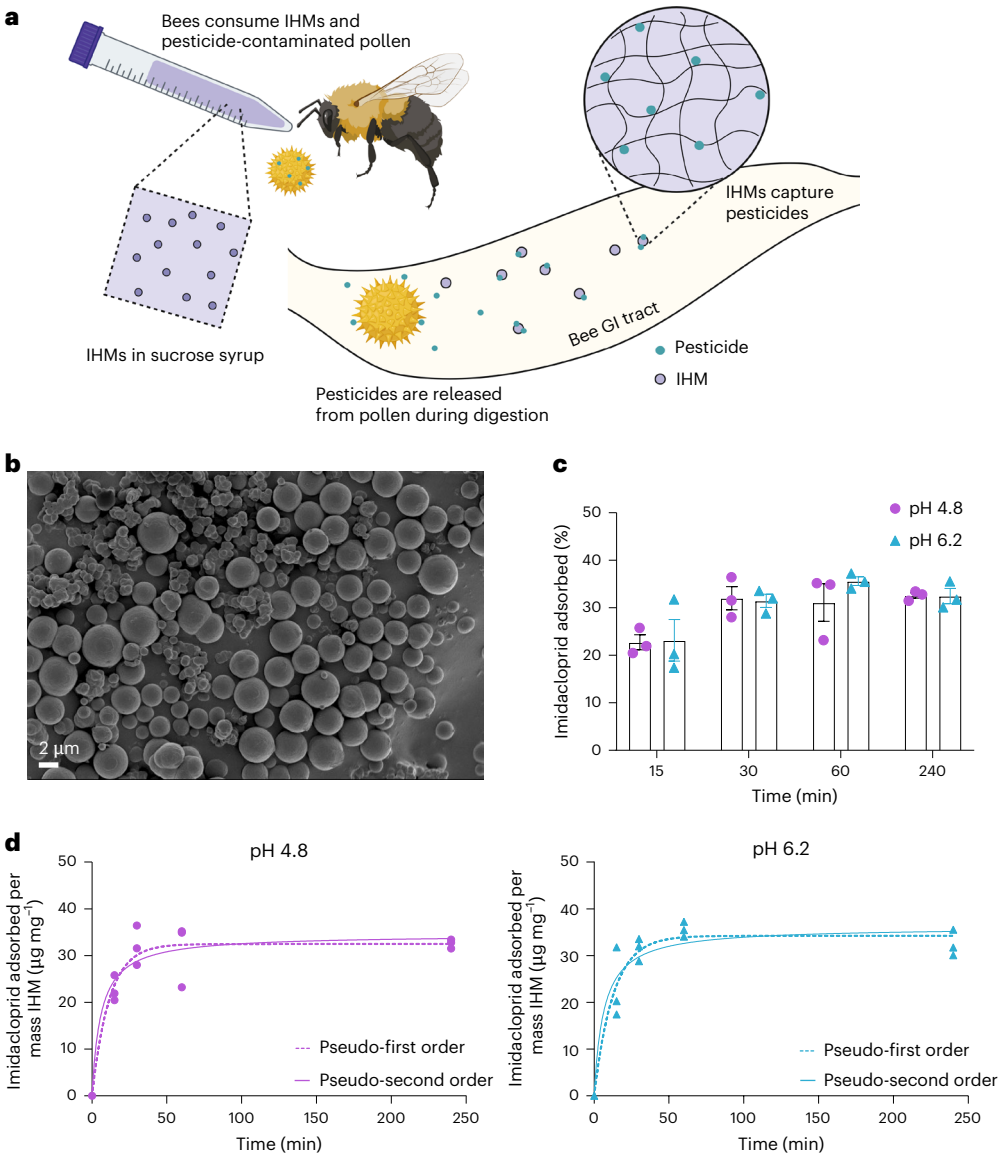

Fig. 1 | Schematic of pesticide detoxification strategy using IHMs and IHM characterization. a, Schematic of pesticide detoxification approach in the bee GI tract. **b**, Scanning electron microscopy image of lyophilized IHMs. **c**, Adsorption of imidacloprid by IHMs. **d**, Pseudo-first- and second-order model fits for pH 4.8 (left) and pH 6.2 (right). Data are represented as mean ± s.e.m. The $n = 3$ experimental replicates for each timepoint were exposed to identical environmental and experimental conditions. Specifically, there were three individual sample tubes for each timepoint. At each timepoint, an aliquot of each sample tube was taken for HPLC analysis. Schematic in **a** was created with BioRender.com.

bumblebees were consuming the IHM syrup throughout the survival assay. As seen in Fig. 3d, IHMs were observed inside bumblebee digestive tracts throughout the 8 d assay, confirming that the bees were still consuming the IHM syrup. Figure 3d provides representative examples of images taken on days 0, 3, 5 and 8 and more images can be found in Supplementary Fig. 6. IHM morphology was also maintained throughout digestion as seen in the insets of Fig. 3e.

Bee health after sublethal imidacloprid exposure

After observing improved survival with IHM administration, sublethal effects of imidacloprid were tested. Preliminary testing of 5, 10, 50 and 500 ng doses of imidacloprid were performed. Within 20 h, all bees that received a 500 ng dose were deceased and those that received 50 ng were very lethargic, non-responsive and would not fly so they were not deemed suitable for further testing. Therefore, 5 and 10 ng doses were used for all sublethal assessments. A timeline of experimental procedures is shown in Fig. 4a.

First, effects on syrup consumption were investigated. To confirm the health and feeding capabilities of each bee, syrup consumption was recorded before imidacloprid dosing. As shown in Supplementary Fig. 7, there were no significant differences in feeding behavior between groups before receiving an imidacloprid dose. After receiving an imidacloprid dose of either 5 or 10 ng, bees exhibited substantial decreases in syrup consumption both with and without IHM treatment (Fig. 4b). The decreases in consumption relative to the no-pesticide control group were 78%, 61%, 84% and 75% for 5, IHM-5, 10 and IHM-10 experimental groups, respectively. The effects of imidacloprid dose (sum of squares (SS) = 41.4, $F_{2,272} = 185.10$, $P < 0.001$) and IHM presence (SS = 1.236, $F_{1,274} = 11.05$, $P = 0.001$) were statistically significant. However, the interaction between imidacloprid dose and IHM presence was not found to be statistically significant (SS = 0.04, $F_{2,274} = 0.18$, $P = 0.83$). There was a statistically significant increase in syrup consumption with IHM treatment after a 5 ng imidacloprid dose (s.e. = 0.07, d.f. = 276, $P = 0.02$), with an average increase of 0.176 µl mg^{-1}. For 0 ng (s.e. = 0.07,

https://doi.org/10.1038/s41893-024-01432-5

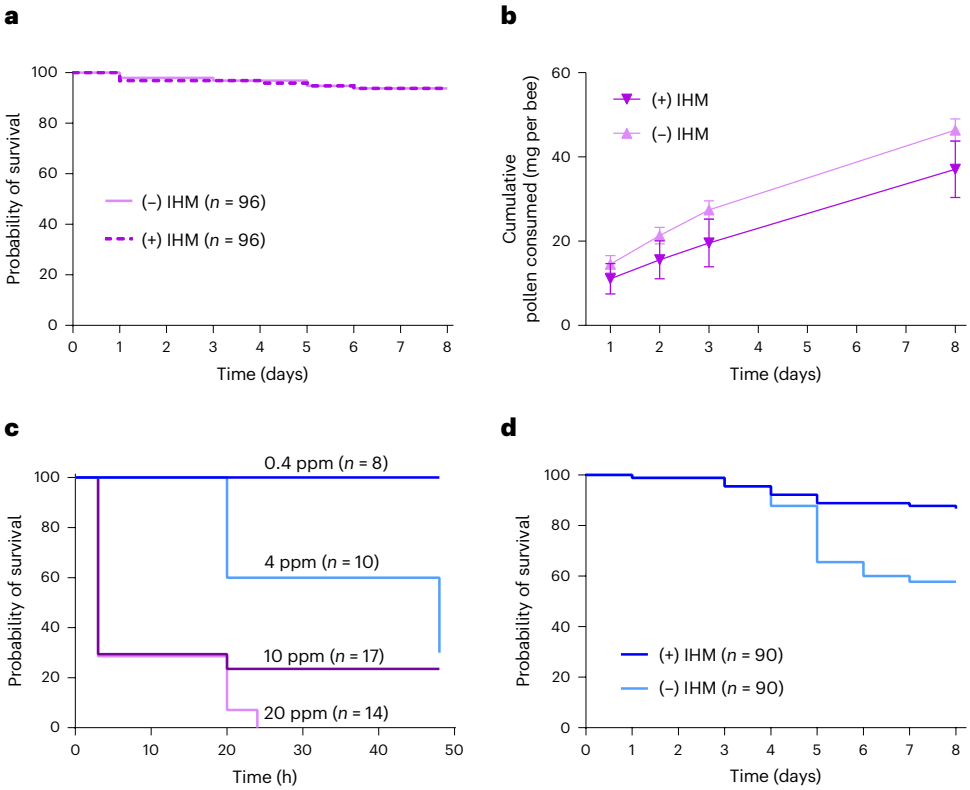

Fig. 2 | Survival assays and pollen consumption. a, Control groups that were not exposed to imidacloprid. The survival of bees provided with plain sucrose syrup was not statistically significantly different from the survival of bees provided with IHM syrup ($\beta = -0.005$, s.e. = 0.583, HR = 0.995, $P = 0.993$). **b**, Cumulative mass of pollen consumed over time (mg per bee) corresponding to the survival data presented in **a**. There were no statistically significant differences in pollen consumption between bees provided with IHMs and bees provided with plain sucrose feeding syrup ($n = 3$). P values were 0.4658, 0.3359, 0.2987 and 0.3016 for days 1, 2, 3 and 8, respectively. We performed unpaired t-tests with Welch's correction (two-tailed, 95% confidence). Data are presented as mean ± s.e.m. **c**, Testing different imidacloprid concentrations for survival assays. **d**, Exposure to imidacloprid in pollen (4 ppm). Survival of the IHM group was statistically significantly higher than the imidacloprid-only group ($\beta = -1.283$, s.e. = 0.501, HR = 0.277, $P = 0.01$). All survival data are presented as Kaplan–Meier survival curves. A Cox model with a fixed effect of syrup composition (with or without IHMs) and a random effect of cage was used to compare survival between the treatment groups (two-tailed, 95% confidence). Each treatment was done in triplicate with $n = 30$–32 bees in each group. (+) indicates IHM syrup and (−) indicates plain sucrose syrup.

d.f. = 273, $P = 0.07$) and 10 ng (s.e. = 0.07, d.f. = 273, $P = 0.11$) doses, there were no statistically significant differences in syrup consumption observed with IHM treatment. This indicates that the IHMs themselves do not have an impact on feeding behavior but further studies must be conducted to identify potential long-term effects. The random intercept variance associated with trial and colony contributed to 1.6% and 3.9% of the total variance, respectively (trial variance = 0.002 and colony variance = 0.005). The residual variance contributed to 94.5% of the total variance (residual variance = 0.111).

After observing improvements in feeding behavior, locomotor behavior was assessed. The percentage of bees that crossed the channel during the 1 h recording period was 42%, 86%, 38% and 52% for 5, IHM-5, 10 and IHM-10 groups, respectively (Fig. 4c). Therefore, the number of bees that did engage in locomotor activity and crossed the channel was improved by IHM treatment, as demonstrated by the 44% and 14% increases in the percentages of bees in 5 and 10 ng dose groups, respectively. The differences in the number of crossings after a 0 ng dose were not statistically significant (s.e. = 0.61, $P = 0.75$). The differences in the number of crossings after a 5 ng (s.e. = 0.09, $P < 0.001$) or 10 ng (s.e. = 0.14, $P = 0.01$) imidacloprid dose were statistically significant. The average number of crossings increased fivefold when bees were provided IHM syrup and given a 5 ng imidacloprid dose (Fig. 4d). Also, the increase for 10 ng dose groups was over twofold (Fig. 4d). However, all groups given an imidacloprid dose showed substantial decreases in locomotion relative to no-pesticide control groups, even with IHM syrup. Therefore, IHMs improve locomotion but do not return it back to normal levels at the timeframe studied in this experiment. The interaction between imidacloprid dose and IHM presence was statistically significant ($\chi^2 = 7.26$, d.f. = 2, $P = 0.03$). The effect of imidacloprid dose was also statistically significant ($\chi^2 = 80.05$, d.f. = 2, $P < 0.001$). However, the effect of IHM presence was not statistically significant ($\chi^2 = 0.099$, $P = 0.75$).

The final sublethal assessment was WBF. Images showing the progression of a bumblebee downstroke (highest to lowest point of vertical wing movement) are shown in Fig. 5a. There was an 11% and 15% decrease in WBF for the 5 and 10 ng groups without IHM treatment, respectively (Fig. 5b). The effects of imidacloprid dose (SS = 2,710.3, $F_{2,78} = 3.97$, $P = 0.02$) and IHM presence (SS = 2,184.4, $F_{1,77} = 6.40$, $P = 0.01$) were statistically significant. However, the interaction between imidacloprid dose and IHM presence was not found to be statistically significant (SS = 1,426.7, $F_{2,78} = 2.09$, $P = 0.13$). There was a statistically significant difference in WBF with IHM treatment after a 5 ng imidacloprid dose (s.e. = 8.03, d.f. = 73.0, $P = 0.02$). For 0 ng (s.e. = 7.81, d.f. = 77.7, $P = 0.89$) and 10 ng (s.e. = 7.36, d.f. = 64.9, $P = 0.06$) doses, there were no statistically significant differences in WBF observed with IHM treatment. The random intercept variance associated with trial and colony contributed to 0% and 7% of the total variance, respectively (trial variance = 0, colony variance = 26.55). The residual variance contributed to 93% of the total variance (residual variance = 344.88). Factors such as wing length and environmental conditions have been shown to affect

https://doi.org/10.1038/s41893-024-01432-5

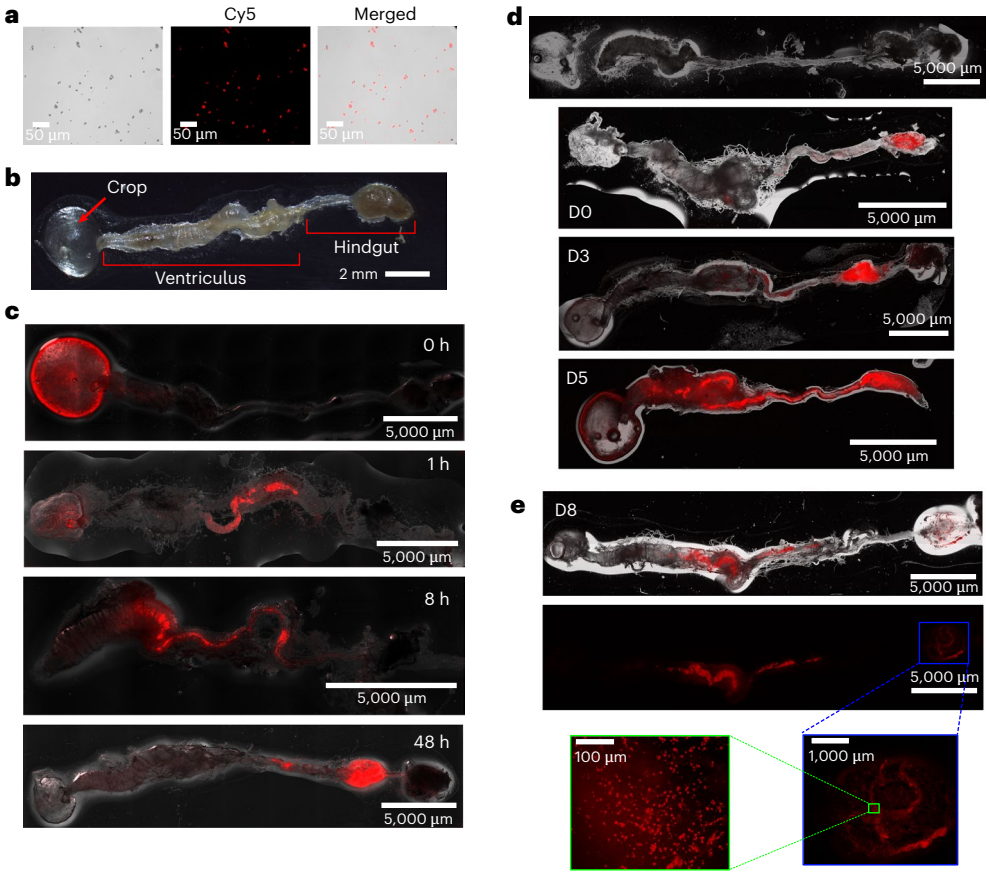

Fig. 3 | In vivo IHM tracking. a, Image of fluorescently labeled IHMs. From left to right: phase contrast, fluorescence (Cy5), merged phase contrast and fluorescence. **b**, Brightfield image of excised bumblebee GI tract. **c**, Bees were individually dosed at the same time with Cy5-labeled IHMs. **d**,**e**, IHMs were observed in bee GI tracts throughout an 8 day survival assay with exposure to 4 ppm of imidacloprid in pollen. **d**, The topmost image is from a control bee fed only 50% sucrose without IHMs. Days 0, 3 and 5 are presented. **e**, Day 8 fluorescence images at 4× (right) and 40× (left) magnifications for visualization of IHM morphology. For all images, tissues are presented in the same orientation from left (crop) to right (rectum).

WBF[32,33]. The wing lengths corresponding to bees whose WBFs were measured are presented in Fig. 5c. Wing length between groups were found to not be statistically significant (SS = 23.11, d.f. = 5, $F_{5,78} = 1.65$, $P = 0.16$). In addition, the difference in AIC was <1 when including wing length in the model. For these reasons, wing length was not included in the model. More details about WBF measurements can be found in Supplementary Fig. 8.

Discussion

Bees contribute substantially to crop pollination and are important to agricultural and apicultural success. Worldwide, bee health is being compromised and bees are facing notable declines due to a variety of different factors such as habitat loss, agrochemicals and climate change[34]. Our group has previously developed pollen-inspired enzymatic microparticles that aimed to address the issue of organophosphate exposure by encapsulating and delivering an enzyme that can degrade organophosphates[15]. This approach was specific to organophosphates, so we sought to find a solution that could be more broadly applied. The first step was to investigate a different class of insecticide and develop a strategy that could help with its toxicity. Neonicotinoids are a class of insecticides that has been shown to have many harmful effects on bee health and survival. They have been found in nectar, pollen and other agricultural samples that bees may come in contact with in the environment, thus putting bees at risk[35]. In this work, we aimed to develop an inexpensive, scalable solution to alleviate the harmful effects of these pesticides on bees. The objective was to capture neonicotinoids in vivo, thus lowering their free concentrations in the bee body. In terms of size, it has been reported that the size range for pollen to be handled by bee mouthparts, travel through the oesophagus and be subsequently filtered by the proventriculus is 0.5–100 µm in diameter[36]. Therefore, it was crucial for our IHMs to be within this range. We synthesized IHMs ~5 µm in diameter using a facile precipitation polymerization approach as a result of this method not requiring time-consuming or expensive steps or reagents. Another benefit of this polymerization is that it does not require the use of any stabilizers or surfactants[37]. Hydrogel microparticles have been shown to adsorb neonicotinoids for use in in vitro applications such as pesticide removal and detection in water, fruit or other agricultural samples[38–40]. These studies implemented a molecular imprinting approach to improve microparticle selectivity; however, we did not use molecular imprinting and our IHMs are not inherently selective to a specific type of pesticide. To establish a proof-of-concept for our approach, we chose imidacloprid to be the neonicotinoid of interest for our experiments. Adsorption relied on interactions between methacrylic acid and imidacloprid, including hydrogen bonding and ionic interactions as well as hydrophobic effects. In vitro studies confirmed that imidacloprid can be captured by IHMs through a physisorption mechanism. After IHM characterization and in vitro testing, exposure to a lethal concentration of imidacloprid was used to assess survival of bees with and without IHM treatment. Bees exhibited a 30% increase in survival with IHM treatment. After seeing improvements in survival, we used fluorescence microscopy to confirm that bees were consuming IHMs continuously throughout the survival assay since imidacloprid has been widely demonstrated to negatively impact feeding behavior.

https://doi.org/10.1038/s41893-024-01432-5

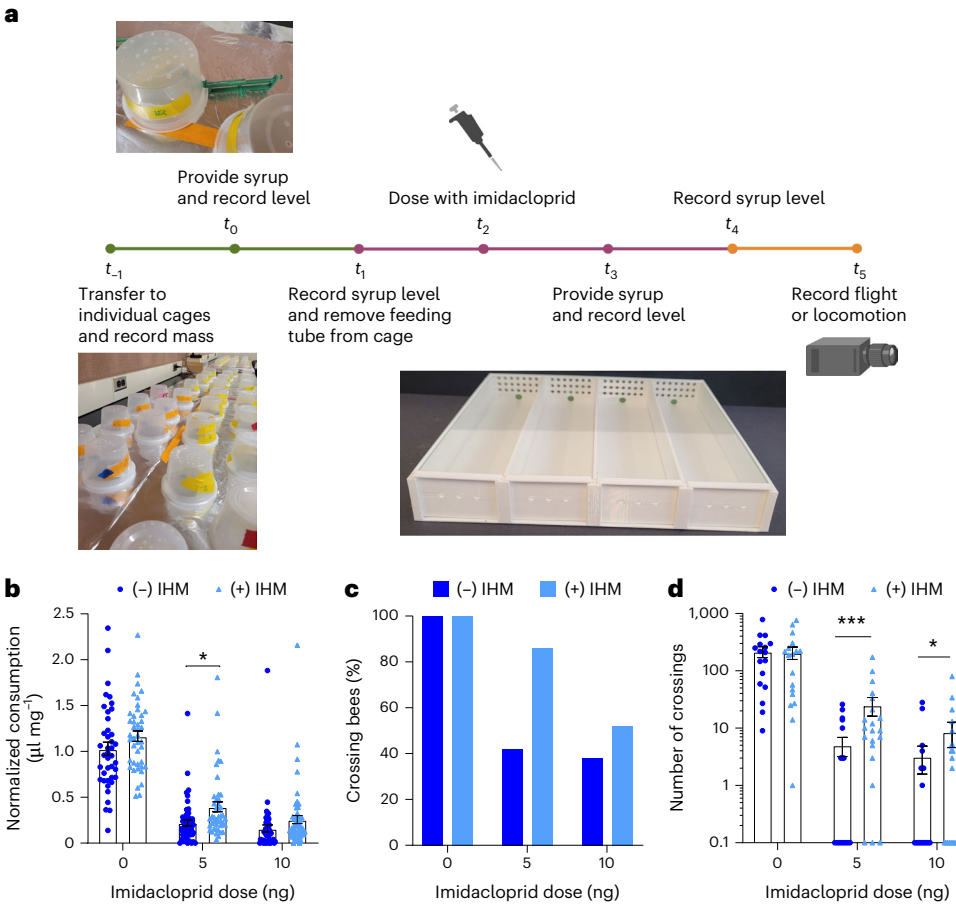

Fig. 4 | Syrup consumption and locomotor activity. a, Timeline of experimental procedures for sublethal assessments. **b**, There was a statistically significant difference in syrup consumption with IHM treatment after a 5 ng imidacloprid dose (s.e. = 0.07, d.f. = 276, $P = 0.02$). As presented on x axis from left to right: $n = 41, 42, 51, 44, 49, 57$. Data were fitted using a linear mixed effects model (lme4 package) with fixed effects of imidacloprid dose and IHM presence using R statistical software (two-tailed, 95% confidence). The model included an interaction term between imidacloprid dose and IHM presence. Trial and colony source were also included as random effects. Fixed effects were tested using F tests with Satterthwaite's method approximation for the degrees of freedom. **c**, Percentage of bees that crossed the channel. **d**, Number of times a bee crossed the midline of the channel over 1 h. Data are presented on a logarithmic scale and include bees that did not cross the channel. Bees that did not cross are shown at the 0.1 mark on the y axis for visualization purposes. $n = 17, 18, 19, 21, 21, 21$. There were statistically significant improvements in the number of crossings with IHM treatment after a 5 ng (s.e. = 0.09, $P = 0.0005$) and 10 ng (s.e. = 0.14, $P = 0.013$) imidacloprid dose. Data were analyzed using a negative binomial mixed model with fixed effects of imidacloprid dose and IHM presence using R statistical software (two-tailed, 95% confidence). This model was used because of the number of crossings being a count response. The model included an interaction term between imidacloprid dose and IHM presence and a random effect of trial. Fixed effects were tested using a Wald chi-square test. $^*P < 0.05$, $^{***}P < 0.001$. Data are presented as mean ± s.e.m. (+) indicates IHM syrup and (−) indicates plain sucrose syrup. Schematic in **a** created with BioRender.com.

The IHMs were also able to be filtered through the proventriculus and travel through the GI tract, without getting stuck in the crop or other tissues, while maintaining their particle morphology.

The concentration of imidacloprid used in this study exceeds field-realistic concentrations[7] but was used to simulate a high concentration scenario that would induce mortality. It was expected that a higher concentration would be required to achieve mortality after exposure via pollen compared to exposure via sucrose solution because bees typically consume more nectar than pollen[41]. This deviance from a field-realistic scenario motivated experiments assessing sublethal effects of imidacloprid. The 24 h LD_{50} value for imidacloprid in *Bombus terrestris* has been reported to be 40 ng per bee[42]. However, the lethal dose or concentration of imidacloprid varies between bee species and season[43]. Many field-realistic doses have also been reported and often vary depending on factors such as geographical location, crop of interest and application method[44]. For example, 5, 20 and 100 ppb have been reported as field-realistic concentrations in nectar[45,46] and, assuming that an adult bumblebee worker consumes up to 400 mg of nectar per day[47], this can result in an exposure of 2.5–40 ng per day. For this work, doses within this range were desired. Imidacloprid has been proven to have negative impacts on the feeding motivation of bees[30,31,48]. Both 5 and 10 ng imidacloprid doses considerably decreased feeding motivation of bumblebees; however, IHMs improved feeding in the case of a 5 ng dose. In these experiments, bees only received a single dose, which demonstrates the toxicity of imidacloprid and how detrimental it can be to bee health. In reality, bees are more likely to experience repeated exposures, so further testing with alternative exposure schemes and timeframes is necessary to gain a better understanding of real-life scenarios. Locomotor activity was also improved, with increases in the number of crossings and the percentages of bees that crossed the channel. It has been shown that the effects of imidacloprid on locomotor activity are both dose and time dependent[49]; therefore, it is probable that observations at longer timeframes, for example 2 or 10 d after exposure, may provide different results. For example, others evaluated the locomotion of *B. impatiens* after receiving imidacloprid doses ranging from 0.2 to 2.2 ng and found that bees were less active <1 h after exposure but were more active 1–2 h after exposure[31]. Further work should be done in investigating the locomotion of bumblebees over longer timeframes to provide further validation of IHM efficacy in helping lessen the effects of imidacloprid on locomotor activity.

https://doi.org/10.1038/s41893-024-01432-5

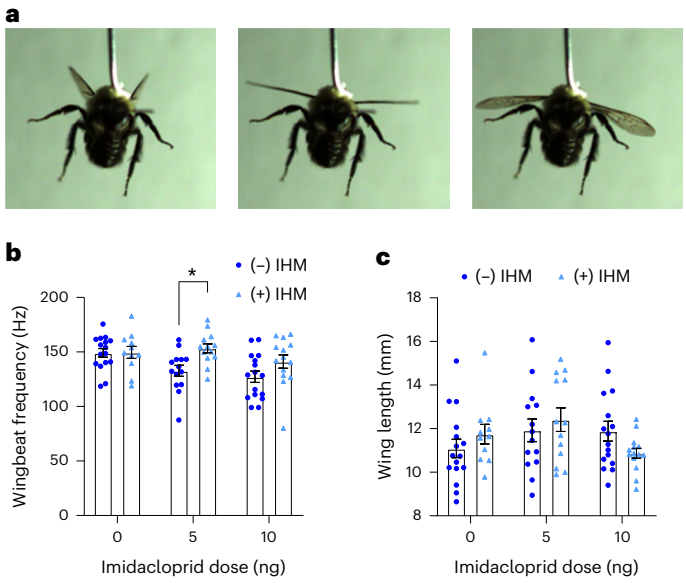

Fig. 5 | Wingbeat frequency. a, Examples of frames showing the progression of a single downstroke. **b**, WBF of bees. There was a statistically significant difference in WBF with IHM treatment after a 5 ng imidacloprid dose (s.e. = 8.03, d.f. = 73.0, $P = 0.02$). Data were fitted using a linear mixed effects model (lme4 package[68]) with fixed effects of imidacloprid dose and IHM presence using R statistical software (two-tailed, 95% confidence). The model included an interaction term between imidacloprid dose and IHM presence. Trial and colony source were also included as random effects. Fixed effects were tested using *F* tests with Satterthwaite's method of approximation for the degrees of freedom. **c**, Wing lengths of bees whose WBF measurements were obtained. Mean wing lengths between groups were found to not be statistically significant using one-way ANOVA (two-tailed, 95% confidence) in GraphPad Prism (SS = 23.11, $F_{5,78} = 1.65$, $P = 0.16$). All data are represented as mean ± s.e.m. In order presented from left to right on *x* axis, $n = 16, 11, 14, 13, 16, 14$. *$P < 0.05$. (+) indicates IHM syrup and (−) indicates plain sucrose syrup.

There have been several reports of imidacloprid having negative effects on bee flight behavior[50,51]. For example, ref. 50 found that WBF and glucose levels were appreciably lower in honeybees dosed with 28 ng of imidacloprid. They observed a 19 Hz decrease in WBF by 24 h after exposure relative to bees not exposed to imidacloprid and bees also exhibited shorter moving distances. In our study, IHMs improved WBFs of imidacloprid-exposed bees after a 5 ng dose of imidacloprid and decreases in WBF exceeded 10% for bees exposed to imidacloprid but not supplied with IHM syrup. To quantify the impact of reduction in flight performance, an 11% decrease in WBF leads to a 21% reduction in lift force, assuming the same stroke trajectory and amplitude[52]. Consequently, the affected bee may struggle to carry even a reduced load, including its own weight[52].

A limitation of this study is that a single imidacloprid dose does not reflect a true exposure; however, this was done to ensure bees received the same dose for comparative purposes. It is also important to note that bees will typically be exposed to a combination of different pesticides, rather than just one[53]. So, it would be critical to test combinations of different pesticides as well in the future. For example, it would be interesting to test neonicotinoid and fungicide combinations because they have been shown to have harmful synergistic effects[54]. In addition, thorough testing of these IHMs in managed pollinators, such as honeybees, would be valuable since they are the target species for this technology. In this study, WBF was the only flight characteristic investigated; however, other flight characteristics, such as flight distance, duration and velocity[55], have also been shown to be affected by imidacloprid exposure.

Future studies that aim to more directly measure imidacloprid capture by IHMs in vivo and determine whether or not IHMs prevent imidacloprid metabolization must be conducted before large-scale application can be considered. For example, such studies may include performing residue analysis at various timepoints after exposure to quantify levels of imidacloprid and its metabolites. In addition, microparticles with superior biodegradability should be considered. For implementation, IHMs could be added to products already commercially available, such as pollen patties or syrup supplements. These IHMs were synthesized using commonly used monomers and solvents which makes them desirable for usage in apiculture. In addition, the current formulation does not include biologically derived molecules or compounds that would raise the cost of production and market price. Future work will also aim to design IHMs that can promote a more selective binding mechanism and/or accommodate for adsorption of other pesticides. Alternative methods that may be used to detoxify neonicotinoids involve the use of membrane receptor subunits conjugated to the microparticle surfaces or the encapsulation and delivery of bacteria or enzymes that can degrade these pesticides[56].

Imidacloprid served as the model neonicotinoid in this work; however, this IHM strategy may be applicable to other neonicotinoids or even other classes of pesticides after appropriate optimizations are performed, such as improvements in selectivity or the addition of bioactive substances such as enzymes. The methodologies presented in this work provide useful insights and areas of focus for future experimental design and testing of IHM-based treatments for pesticide detoxification in bees. As previously described, bees play an essential role in the pollination of a variety of crops and in the maintenance of agricultural sustainability. These roles ultimately promote food security and prompt the necessity for technologies, such as the IHMs presented in our study, that have the potential to provide a defense for managed bees against harmful pesticides that they encounter in the field.

Methods

Materials

Methacrylic acid (≥99.0%, MEHQ stabilized), ethylene glycol dimethacrylate (>97.0%, HQ stabilized) and *N*-hydroxysuccinimide were purchased from TCI America. The 2,2′-azobis(2-methylpropionitrile (98%), citric acid, acetone and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich. Cy5 was purchased from Lumiprobe. Phosphate buffer saline (PBS) pH 7.4 was purchased from Corning Life Sciences. The 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDC) was purchased from Chem Impex International. Bee pollen granules collected by honeybees were purchased from All Star Health and the manufacturer was CC Pollen. Pollen was ground into a fine powder before use. Sodium citrate was purchased from VWR. Sugar was purchased from Domino Foods. Sucrose solutions were prepared by dissolving appropriate amounts of white sugar in deionized (DI) water to obtain desired concentrations. Analytical grade imidacloprid was purchased from Agilent Technologies. Stock solutions (50 mg ml^{-1}) for adsorption experiments were prepared by dissolving imidacloprid in HPLC grade acetonitrile. Stock solutions (10 μg ml^{-1}) for bee inoculation and in vivo experiments were prepared by dissolving imidacloprid in a 50% sugar solution. Stock solutions were stored at 4 °C away from light to prevent photolysis.

Fabrication of IHMs

The hydrogel microparticles were synthesized using precipitation polymerization[37,40,57]. Briefly, methacrylic acid (0.8 mmol), ethylene glycol dimethacrylate (3.84 mmol) and 2,2′-azobis(2-methylpropionitrile (0.25 mmol) were fully dissolved in 25 ml of acetonitrile in a round-bottom flask using gentle shaking at room temperature. The flask was sealed and degassed with nitrogen for 20 min at room temperature. Next, the reaction flask was placed in an oil bath on a shaker (140 rpm) and heated to 60 °C to initiate free-radical precipitation polymerization. After 24 h, the reaction flask was opened to air to stop any remaining polymerization. Acetonitrile was then

removed using a Rotovap. The remaining white precipitate was washed with 70% ethanol and DI water three and two times, respectively, to remove excess initiator and unreacted monomers. Each wash step included adding fresh washing solution (either ethanol or DI water), vortexing and centrifuging at 17,000*g* for 20 min. After the final wash, IHMs were filtered through a 40 µm cell strainer and then lyophilized to form a white powder.

Adsorption of imidacloprid onto IHMs

A total of 1 mg of imidacloprid was mixed with 10 mg of IHMs in citric acid buffer solutions with a total sample volume of 10 ml. Two different physiologically relevant pH buffer solutions were tested, 4.8 and 6.2, which are representative of the crop and ventriculus of bees, respectively[58]. Samples were incubated at room temperature in a rotator shaker. At designated timepoints, samples were centrifuged and the supernatant collected for high-performance liquid chromatography (HPLC) measurements to quantify remaining free imidacloprid concentrations. The pH of the crop and ventriculus have been reported as 4.8 and 6.5, respectively[58]. We wanted to ensure that the IHMs could perform comparably in both pH conditions. Data for each experimental condition were fit using nonlinear regression according to both Lagergrens's first-order rate equation[59] for pseudo-first-order kinetics and a pseudo-second-order kinetics model[60–62] (Supplementary Fig. 1) in GraphPad Prism 9. To test how adsorption changes with increasing IHM concentration, samples consisted of 1, 2, 3 or 5 mg ml^{-1} of IHMs and 1 mg of imidacloprid in citric acid buffer (pH 6.2) for 4 h (Supplementary Fig. 3).

High-performance liquid chromatography

Samples were analyzed using reverse-phase HPLC (Agilent Technologies) with a flow rate of 1.0 ml min^{-1}, mobile phase of acetonitrile/water (v/v, 90/10) and an injection volume of 20 µl. The column temperature was 22–23 °C for all measurements. Imidacloprid concentrations were quantified at an absorption wavelength of 270 nm. Limits of detection and quantification were determined according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The limit of detection and limit of quantification of imidacloprid were 11.1 and 33.6 µg ml^{-1}, respectively. Imidacloprid standard curve data are presented in Supplementary Fig. 2. Samples were filtered through a 0.22 µm syringe filter before analysis. Column specifications are Ultra C18 5 µm, 250 × 4.6 mm^2.

Bumblebee colony maintenance

B. impatiens research colonies were purchased from Biobest (now Plant Products). Before experiments, bees were housed together and provided a 30% by weight sugar solution (sucrose) in DI water and pollen balls. For example, sucrose was made by dissolving 600 g of sugar in 1,400 g of DI water. Bee colonies and experimental bees were always kept indoors at room temperature. Whenever bees were transferred from the colony into tubes or smaller cages for experiments, they were transferred under red light to limit their flight and vision[63].

Individual bumblebee dosing

Bees were taken from the colony and placed individually in 5 ml tubes. Each bee was then anaesthetized with carbon dioxide (CO_2). Once anaesthetized, the bee was removed from the tube and a 5 µl drop of a pesticide solution, sucrose or IHM syrup was placed on the proboscis depending on the experiment. IHM syrup contained 1 mg of IHMs per ml of sucrose (50%, w/w). Bees were observed carefully to ensure that each bee received the full dose. After the drop of solution was consumed, bees were either placed back in individual tubes or into experimental group housing, depending on the experiment.

Survival testing in bumblebees

Bumblebees were transported under red light from colonies to cages with a mesh bottom. After being transferred to experimental cages, bees were given sucrose (50%, w/w) or IHM solutions (1 mg ml^{-1}) immediately. After a 24 h cage acclimation period, any dead bees were counted, recorded and eliminated from the total group number. During the acclimation period, bees were fed either IHM or plain syrup to ensure that bees were able to access the IHM treatment and have it in their systems before pesticide exposure. Cages were provided with either a clean or imidacloprid-containing pollen ball; this day was considered day 0. Each succeeding day, the number of deceased bees was recorded. Experimental bee cages were kept in the dark and at room temperature throughout the experiments. The 50% sucrose or IHM solutions were provided to bees ad libitum throughout survival assays by means of a 15 ml tube inserted into the cage. IHM syrup concentration was 1 mg ml^{-1} for all tests.

Pollen consumption without neonicotinoid exposure

Bumblebees were transferred to experimental cages with 32 bees in each cage and three cages per treatment. After 24 h of cage acclimation, each cage was given a clean pollen ball without pesticide. On days 0, 1, 3 and 8, the pollen ball was weighed and recorded. Pollen balls stored under the same conditions but without bees were used as controls to correct for mass loss due to evaporation ($n = 3$).

IHM labeling for in vivo distribution imaging

IHMs were labeled using a method adapted from a previously reported protocol[64]. A total 100 mg of IHMs, 100 mg of EDC and 100 mg of *N*-hydroxysuccinimide were dissolved in 10 ml of MilliQ water in a round-bottom flask with a magnetic stir bar. The mixture was stirred at room temperature for 1.5 h. After EDC/*N*-hydroxysuccinimide activation, the IHM suspension was centrifuged at 14,000*g* for 20 min and resuspended in 10 ml of PBS (pH 7.4). The suspension was then added to a glass vial with 0.5 mg of Cy5 and incubated at room temperature overnight, protected from light. After dye conjugation, IHMs were washed three times with MilliQ water and redispersed in PBS.

In vivo IHM visualization and tracking

For biodistribution studies to assess how long IHMs travel through the digestive tract, individual bees were dosed once with 10 µl of a solution containing Cy5-labeled IHMs. Bees were housed individually and fed sucrose (50%, w/w) for the duration of the experiment after inoculation. For studies to determine if bees consume IHMs throughout the 8 day assay, bees were continuously provided with Cy5-labeled IHMs (1 mg ml^{-1} in 50% sucrose) ad libitum and housed together. To prepare samples for IHM visualization using fluorescence microscopy, bees were first anaesthetized with CO_2. Then the head and legs were removed in preparation for dissection. Each dorsal tergite was removed individually to expose the inside of the abdomen and the digestive tract was then carefully removed. Excised digestive tracts were placed on glass cover slips and imaged using a Nikon ECLIPSE Ti inverted microscope. All images are presented in the same orientation. From left to right: crop, ventriculus, ileum and rectum. At 30 min after IHM administration was considered time zero.

Feeding behavior

Bees were weighed and transferred into individual cages. Then either plain sucrose (50%, w/w) or IHM syrup (1 mg ml^{-1} in 50% sucrose) was provided to each bee via a borosilicate glass tube (5 mm optical density, 3 mm inner diameter) and the syrup level was recorded. After 20 h, the syrup level was recorded again and the amount consumed before imidacloprid dosing was calculated. The feeding tubes were then removed and the bees were starved for 2 h in preparation for imidacloprid dosing. After the 2 h, bees were removed from their cages, anaesthetized with CO_2 and a 5 µl drop of imidacloprid solution was administered onto the proboscis as they woke up from anaesthesia. Bees were anaesthetized to dose them individually and in a controlled manner via the proboscis. Bees were observed carefully to ensure they

consumed the entire drop before transferal back into their respective cages. Bees received either 5 or 10 ng of imidacloprid. Control bees (not in pesticide groups) received plain sucrose instead of the imidacloprid solution. Feeding tubes were then re-introduced into the cages and the syrup level replenished and recorded. After 18 h, the syrup level was measured to calculate the amount of syrup consumed after imidacloprid dosing and then normalized by the body mass of the bee. Then bees were either tethered for flight recordings or underwent locomotion studies. During data analysis, loss of syrup due to evaporation was accounted for. There were five evaporation controls for both plain syrup and IHM syrup included in every trial and syrup consumption values were adjusted accordingly. There were eight trials conducted.

Locomotion

Locomotion chambers consisted of a three-dimensional printed base with a glass panel on the top which allowed for bee visualization. The base was printed using white polylactic acid filament (1.75 mm) and featured air holes and vertically sliding entry doors for transferring bees into and out of the chambers. Each chamber contained four separate channels (8×2 in^2, L × W) and only a single bee was permitted in a given channel. For experiments, each bee was removed from its individual cage and transferred into one of the channels. Bees were recorded from above for 1 h using a AKASO EK7000 HD Action Camera using a frame rate of 60 fps. The number of times each bee crossed the midpoint line of the channel was counted. Three locomotion trials were conducted and a single source colony was used.

Wingbeat frequency

Bees were removed from their respective cages and transferred to 5 ml tubes. They were anaesthetized on ice and subsequently tethered to eye pins (0.2 g) using Loctite gel control super glue. Bees were mounted to a ring stand and filmed using a colour Photron high-speed camera (NOVA; Supplementary Fig. 6) with 1,024 × 1,024 pixel resolution interfaced with Photron Fastcam Viewer v.4.0 software. Flight recordings were taken at a frame rate of 9,000 fps. WBFs were calculated by counting the number of complete wing strokes using Fiji (ImageJ)[65] and multiplying that value by the frame rate and dividing by the number of frames elapsed. Bees that would not fly right away after being put in position for filming were agitated slightly using a piece of paper or small tube by placing the object underneath them and dropping it suddenly, which prompted them to start flying. Throughout all of the four trials conducted, researchers who recorded the videos and calculated the WBFs were blind to which experimental group each bee belonged to.

Statistics

To compare results between groups for pollen consumption tests, unpaired *t*-tests were performed with Welch's correction (two-tailed, 95% confidence). Kaplan–Meier survival curves were made using GraphPad Prism 9. A Cox model with a fixed effect of IHM presence and a random effect of cage was used to compare survival between the treatment groups using R statistical software (two-tailed, 95% confidence)[66,67]. IHM presence refers to a binary representation of whether or not IHMs were provided in the feeding syrup with '0' indicating that no IHMs were provided and '1' indicating IHMs were provided. For WBF and syrup consumption experiments, data were fitted using a linear mixed effects model (lme4 package[68]) with fixed effects of imidacloprid dose and IHM presence using R statistical software (two-tailed, 95% confidence). The model included an interaction term between imidacloprid dose and IHM presence. Trial and colony source were also included as random effects. Fixed effects were tested using *F* tests with Satterthwaite's method approximation for the degrees of freedom. Assumptions of normality and homogeneous variance were assessed visually using residual plots. Differences in wing lengths between groups were determined using a one-way analysis of variance (ANOVA) in GraphPad Prism (two-tailed, 95% confidence). Locomotion data were analyzed using a negative binomial mixed model with fixed effects of imidacloprid dose and IHM presence using R statistical software (two-tailed, 95% confidence). This model was used because of the number of crossings being a count response. The model included an interaction term between imidacloprid dose and IHM presence and a random effect of trial. Fixed effects were tested using a Wald chi-square test. All data are presented as mean ± s.e.m. unless otherwise stated.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Wingbeat frequency videos were captured using a high-speed camera, which resulted in the raw data files being exceedingly large; therefore, they are available from the corresponding author upon reasonable request. Videos acquired during locomotion studies can be found at https://github.com/julia-caserto/Bee-Locomotion-Analysis. Source data are provided with this paper.

Code availability

Code used to analyze locomotion videos can be found at https://github.com/julia-caserto/Bee-Locomotion-Analysis.

References

1. Klein, A. M. et al. Importance of pollinators in changing landscapes for world crops. *Proc. Biol. Sci.* **274**, 303–313 (2007).
2. Porto, R. G. et al. Pollination ecosystem services: a comprehensive review of economic values, research funding and policy actions. *Food Secur.* **12**, 1425–1442 (2020).
3. Bruckner, S. et al. A national survey of managed honey bee colony losses in the USA: results from the Bee Informed Partnership for 2017–18, 2018–19 and 2019–20. *J. Apic. Res.* **62**, 429–443 (2023).
4. Bartomeus, I. et al. Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proc. Natl Acad. Sci. USA* **110**, 4656–4660 (2013).
5. LeCroy, K. A., Savoy-Burke, G., Carr, D. E., Delaney, D. A. & Roulston, T. H. Decline of six native mason bee species following the arrival of an exotic congener. *Sci. Rep.* **10**, 18745 (2020).
6. Goulson, D., Nicholls, E., Botías, C. & Rotheray, E. L. Bee declines driven by combined stress from parasites, pesticides and lack of flowers. *Science* **347**, 1255957 (2015).
7. Graham, K. K. et al. Identities, concentrations and sources of pesticide exposure in pollen collected by managed bees during blueberry pollination. *Sci. Rep.* **11**, 16857 (2021).
8. Graham, K. K. et al. Pesticide risk to managed bees during blueberry pollination is primarily driven by off-farm exposures. *Sci. Rep.* **12**, 7189 (2022).
9. McArt, S. H., Fersch, A. A., Milano, N. J., Truitt, L. L. & Böröczky, K. High pesticide risk to honey bees despite low focal crop pollen collection during pollination of a mass blooming crop. *Sci. Rep.* **7**, 46554 (2017).
10. Krupke, C. H., Hunt, G. J., Eitzer, B. D.,Andino, G. & Given, K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS ONE* **7**, e29268 (2012).
11. Pettis, J. S. et al. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLoS ONE* **8**, e70182 (2013).
12. Stanley, D. A. et al. Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. *Nature* **528**, 548–550 (2015).
13. Whitehorn, P. R., O'Connor, S., Wackers, F. L. & Goulson, D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* **336**, 351–352 (2012).

14. Deguine, J.-P. et al. Integrated pest management: good intentions, hard realities. A review. *Agron. Sustain. Dev.* **41**, 38 (2021).
15. Chen, J. et al. Pollen-inspired enzymatic microparticles to reduce organophosphate toxicity in managed pollinators. *Nat. Food* **2**, 339–347 (2021).
16. Camp, A. A. & Lehmann, D. M. Impacts of neonicotinoids on the bumble bees *Bombus terrestris* and *Bombus impatiens* examined through the lens of an adverse outcome pathway framework. *Environ. Toxicol. Chem.* **40**, 309–322 (2021).
17. Liu, G. Y., Ju, X. L. & Cheng, J. Selectivity of Imidacloprid for fruit fly versus rat nicotinic acetylcholine receptors by molecular modeling. *J. Mol. Model.* **16**, 993–1002 (2010).
18. Grünewald, B. & Siefert, P. Acetylcholine and its receptors in honeybees: involvement in development and impairments by neonicotinoids. *Insects* **10**, 420 (2019).
19. Alkassab, A. T. & Kirchner, W. H. Sublethal exposure to neonicotinoids and related side effects on insect pollinators: honeybees, bumblebees and solitary bees. *J. Plant Dis. Prot.* **124**, 1–30 (2017).
20. Gregorc, A. et al. Effects of coumaphos and imidacloprid on honey bee (Hymenoptera: Apidae) lifespan and antioxidant gene regulations in laboratory experiments. *Sci. Rep.* **8**, 15003 (2018).
21. Wu, Y.-Y. et al. Sublethal effects of imidacloprid on targeting muscle and ribosomal protein related genes in the honey bee *Apis mellifera* L. *Sci. Rep.* **7**, 15943 (2017).
22. Yao, J., Zhu, Y. C. & Adamczyk, J. Responses of honey bees to lethal and sublethal doses of formulated clothianidin alone and mixtures. *J. Econ. Entomol.* **111**, 1517–1525 (2018).
23. Tasman, K., Hidalgo, S., Zhu, B., Rands, S. A. & Hodge, J. J. L. Neonicotinoids disrupt memory, circadian behaviour and sleep. *Sci. Rep.* **11**, 2061 (2021).
24. He, B. et al. Imidacloprid activates ROS and causes mortality in honey bees (*Apis mellifera*) by inducing iron overload. *Ecotoxicol. Environ. Saf.* **3**, 112709 (2021).
25. Xu, X. et al. Neonicotinoids: mechanisms of systemic toxicity based on oxidative stress-mitochondrial damage. *Arch. Toxicol.* **96**, 1493–1520 (2022).
26. *Imidacloprid: Proposed Interim Registration Review Decision. Case Number 7605* (EPA, 2020); www.epa.gov/sites/default/files/2020-01/documents/imidacloprid_pid_signed_1.22.2020.pdf
27. Klingelhöfer, D., Braun, M., Brüggmann, D. & Groneberg, D. A. Neonicotinoids: a critical assessment of the global research landscape of the most extensively used insecticide. *Environ. Res.* **213**, 113727 (2022).
28. Hirata, K., Jouraku, A., Kuwazaki, S., Kanazawa, J. & Iwasa, T. The R81T mutation in the nicotinic acetylcholine receptor of *Aphis gossypii* is associated with neonicotinoid insecticide resistance with differential effects for cyano- and nitro-substituted neonicotinoids. *Pestic. Biochem. Physiol.* **143**, 57–65 (2017).
29. Sahoo, T. R. & Prelot, B. in *Nanomaterials for the Detection and Removal of Wastewater Pollutants* (eds Bonelli, B. et al.) 161–222 (Elsevier, 2020).
30. Azpiazu, C. et al. Chronic oral exposure to field-realistic pesticide combinations via pollen and nectar: effects on feeding and thermal performance in a solitary bee. *Sci. Rep.* **9**, 13770 (2019).
31. Muth, F., Gaxiola, R. L. & Leonard, A. S. No evidence for neonicotinoid preferences in the bumblebee *Bombus impatiens*. *R. Soc. Open Sci.* **7**, 191883 (2020).
32. Parmezan, A. R. S., Souza, V. M. A., Žliobaitė, I. & Batista, G. E. A. P. A. Changes in the wing-beat frequency of bees and wasps depending on environmental conditions: a study with optical sensors. *Apidologie* **52**, 731–748 (2021).
33. Santoyo, J., Azarcoya, W., Valencia, M., Torres, A. & Salas, J. J. P. A. Frequency analysis of a bumblebee (*Bombus impatiens*) wingbeat. *Pattern Anal. Applic.* **19**, 487–493 (2016).
34. Panziera, D., Requier, F., Chantawannakul, P., Pirk, C. W. W. & Blacquière, T. The diversity decline in wild and managed honey bee populations urges for an integrated conservation approach. *Front. Ecol. Evol.* **10**, 767950 (2022).
35. Ward, L. T. et al. Pesticide exposure of wild bees and honey bees foraging from field border flowers in intensively managed agriculture areas. *Sci. Total Environ.* **831**, 154697 (2022).
36. Peng, Y.-S. & Marston, J. M. Filtering mechanism of the honey bee proventriculus. *Physiol. Entomol.* **11**, 433–439 (1986).
37. Sambe, H., Hoshina, K., Moaddel, R., Wainer, I. W. & Haginaka, J. Uniformly-sized, molecularly imprinted polymers for nicotine by precipitation polymerization. *J. Chromatogr. A* **1134**, 88–94 (2006).
38. Chen, J. et al. Dummy template surface molecularly imprinted polymers based on silica gel for removing imidacloprid and acetamiprid in tea polyphenols. *J. Sep. Sci.* **43**, 2467–2476 (2020).
39. Kumar, N., Narayanan, N. & Gupta, S. Application of magnetic molecularly imprinted polymers for extraction of imidacloprid from eggplant and honey. *Food Chem.* **255**, 81–88 (2018).
40. Wang, X., Mu, Z., Liu, R., Pu, Y. & Yin, L. Molecular imprinted photonic crystal hydrogels for the rapid and label-free detection of imidacloprid. *Food Chem.* **141**, 3947–3953 (2013).
41. Russell, A. L., Morrison, S. J., Moschonas, E. H. & Papaj, D. R. Patterns of pollen and nectar foraging specialization by bumblebees over multiple timescales using RFID. *Sci. Rep.* **7**, 42448 (2017).
42. Marletto, F., Patetta, A. & Manino, A. Laboratory assessment of pesticide toxicity to bumblebees. *Bull. Insectol.* **56**, 159–164 (2003).
43. Chen, Y. R., Tzeng, D. T. W. & Yang, E. C. Chronic effects of imidacloprid on honey bee worker development—molecular pathway perspectives. *Int. J. Mol. Sci.* **22**, 11835 (2021).
44. Démares, F. J. et al. Honey bee (*Apis mellifera*) exposure to pesticide residues in nectar and pollen in urban and suburban environments from four regions of the United States. *Environ. Toxicol. Chem.* **41**, 991–1003 (2022).
45. Kim, S. et al. Chronic exposure to field-realistic doses of imidacloprid resulted in biphasic negative effects on honey bee physiology. *Insect Biochem. Mol. Biol.* **144**, 103759 (2022).
46. Sampson, B. et al. Sensitivity to imidacloprid insecticide varies among some social and solitary bee species of agricultural value. *PLoS ONE* **18**, e0285167 (2023).
47. Gradish, A. E. et al. Comparison of pesticide exposure in honey bees (Hymenoptera: Apidae) and bumble bees (Hymenoptera: Apidae): implications for risk assessments. *Environ. Entomol.* **48**, 12–21 (2018).
48. Paus-Knudsen, J. S., Sveinsson, H. A., Grung, M., Borgå, K. & Nielsen, A. The neonicotinoid imidacloprid impairs learning, locomotor activity levels and sucrose solution consumption in bumblebees (*Bombus terrestris*). *Environ. Toxicol. Chem.* **42**, 1337–1345 (2023).
49. Lambin, M., Armengaud, C., Raymond, S. & Gauthier, M. Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. *Arch. Insect Biochem. Physiol.* **48**, 129–134 (2001).
50. Lin, Y. C., Lu, Y. H., Tang, C. K., Yang, E. C. & Wu, Y. L. Honey bee foraging ability suppressed by imidacloprid can be ameliorated by adding adenosine. *Environ. Pollut.* **332**, 121920 (2023).
51. Switzer, C. M. & Combes, S. A. The neonicotinoid pesticide, imidacloprid, affects *Bombus impatiens* (bumblebee) sonication behavior when consumed at doses below the LD_{50}. *Ecotoxicology* **25**, 1150–1159 (2016).
52. Combes, S. A., Gagliardi, S. F., Switzer, C. M. & Dillon, M. E. Kinematic flexibility allows bumblebees to increase energetic efficiency when carrying heavy loads. *Sci. Adv.* **6**, eaay3115 (2020).

53. David, A. et al. Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environ. Int.* **88**, 169–178 (2016).
54. Schuhmann, A. & Scheiner, R. A combination of the frequent fungicides boscalid and dimoxystrobin with the neonicotinoid acetamiprid in field-realistic concentrations does not affect sucrose responsiveness and learning behavior of honeybees. *Ecotoxicol. Environ. Saf.* **256**, 114850 (2023).
55. Kenna, D. et al. Pesticide exposure affects flight dynamics and reduces flight endurance in bumblebees. *Ecol. Evol.* **9**, 5637–5650 (2019).
56. El Khoury, S., Giovenazzo, P. & Derome, N. Endogenous honeybee gut microbiota metabolize the pesticide clothianidin. *Microorganisms* **10**, 493 (2022).
57. Zhou, T., Jørgensen, L., Mattebjerg, M. A., Chronakis, I. S. & Ye, L. Molecularly imprinted polymer beads for nicotine recognition prepared by RAFT precipitation polymerization: a step forward towards multi-functionalities. *RSC Adv.* **4**, 30292–30299 (2014).
58. Rademacher, E., Harz, M. & Schneider, S. Effects of oxalic acid on *Apis mellifera* (Hymenoptera: Apidae). *Insects* **8**, 84 (2017).
59. Lagergren, S. About the theory of so-called adsorption of sobule substances. *Kungl. Svenska Vetenskapsakad. Handl.* **24**, 1–39 (1898).
60. Ho, Y. S. & McKay, G. A comparison of chemisorption kinetic models applied to pollutant removal on various sorbents. *Process Saf. Environ. Prot.* **76**, 332–340 (1998).
61. Wang, M., Yang, C., Cao, J., Yan, H. & Qiao, F. Dual-template hydrophilic imprinted resin as an adsorbent for highly selective simultaneous extraction and determination of multiple trace plant growth regulators in red wine samples. *Food Chem.* **411**, 135471 (2023).
62. Blanchard, G., Maunaye, M. & Martin, G. Removal of heavy metals from waters by means of natural zeolites. *Water Res.* **18**, 1501–1507 (1984).
63. Skorupski, P. & Chittka, L. Photoreceptor spectral sensitivity in the bumblebee, *Bombus impatiens* (Hymenoptera: Apidae). *PLoS ONE* **5**, e12049 (2010).
64. Kántor, I. et al. Biocatalytic synthesis of poly[ε-caprolactone-co-(12-hydroxystearate)] copolymer for sorafenib nanoformulation useful in drug delivery. *Catal. Today* **366**, 195–201 (2021).
65. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682 (2012).
66. Therneau, T. M. coxme: Mixed effects Cox models. R package version 2.2-18.1 (2022).
67. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2023).
68. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).

Acknowledgements

Cornell University Department of Human Ecology for the use of HPLC. Fluorescence microscopy was carried out at the Cornell Institute of Biotechnology's BRC Imaging Facility. We acknowledge the use of field emission scanning electron microscopy supported by NSF through the Cornell University Materials Research Science and Engineering Center DMR-1719875. This work was supported by the New York State Environmental Protection Fund. We acknowledge the following USDA NIFA grants: 2021-22-127 (M.M.) and 2021-08373 (M.K.S.). We thank A. Rios Tascon for helping design locomotion chambers and providing code to analyze locomotion videos. Schematics in Fig. 1a and 4a were created with BioRender.com.

Author contributions

J.S.C. and M.M. conceived the study. J.S.C., M.M. and S.H.M. designed experiments. J.S.C. conducted and supervised all experiments and data collection. L.W. prepared materials, performed colony maintenance and assisted with bee transfers and experiments. S.F. assisted with bee transfers and experiments. C.R., M.H., S.J. and M.K.S. collected WBF data and calculated WBF. J.S.C. analyzed all data collected. J.S.C., S.H.M. and M.M. reviewed and interpreted the results. J.S.C. wrote the paper; M.M. provided substantial edits. All authors reviewed and commented on the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41893-024-01432-5.

Correspondence and requests for materials should be addressed to Minglin Ma.

Peer review information *Nature Sustainability* thanks Rachel Parkinson and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

nature portfolio

Corresponding author(s): Minglin Ma

Last updated by author(s): Jul 30, 2024

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒ A statement on 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
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Fluorescent images were acquired using a Nikon ECLIPSE Ti inverted microscope.
Data analysis	GraphPad Prism version 9, R statistical software version 4.4.0, ImageJ version 2.14.0 (64-bit Java 1.8.0_172), code used to analyze locomotion videos can be found at https://github.com/julia-caserto/Bee-Locomotion-Analysis

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Wingbeat frequency videos were captured using a high-speed camera which resulted in the raw data files being exceedingly large; therefore, they are available from the corresponding author upon reasonable request. Videos acquired during locomotion studies can be found at https://github.com/julia-caserto/Bee-Locomotion-Analysis. Source data are provided with this paper.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Sample sizes for in vivo studies were chosen according to sizes used in related published studies. Studies investigating bee survival after imidacloprid exposure used group sizes consisting of three biological replicates each containing 35-40 bees [DOI: 10.1111/1744-7917.12335; DOI: 10.1038/s41598-017-16245-0]. Studies quantifying effects of neonicotinoids on wingbeat frequency used sample sizes ranging from 10-34 bees per experimental group [DOI: 10.1007/s10646-016-1669-z ; DOI: 10.1016/j.envadv.2021.100051; DOI: 10.1016/j.envpol.2023.121920]. For feeding and locomotion studies, samples sizes of 30-50 bees and 15-24 bees per experimental group, respectively, have been used [DOI: 10.1098/rsos.191883]. For our experiments we chose sample sizes within these ranges. Final sample sizes varied due to bees dying or escaping cage enclosures. For wingbeat frequency experiments there were bees that did not fly as described in further detail in supplementary figure 8. Exact sample sizes can be found in the figure captions. The design of the IHMs was inspired by previous studies that have demonstrated that microparticles can successfully adsorb neonicotinoids. Further details are provided in the discussion section. Due to the synthesis and design of the IHMs themselves not being the focus of our study, we chose a sample size of three replicates for each experimental condition for in vitro adsorption studies in order to calculate means and standard errors.
Data exclusions	None
Replication	Survival studies presented within the main text were performed with three biological replicates. Replicate experiments containing 1-3 biological replicates were successful and yielded results showing improvement in survival with IHM treatment. For feeding studies, eight experimental trials were conducted and within each trial there were 3-6 bees (biological replicates) that underwent procedures for each experimental group. For locomotion and wingbeat frequency studies, three and four experimental trials, respectively, were conducted and within each trial there were 3-6 bees (biological replicates) that underwent procedures for each experimental group. In vitro adsorption experiments contained three replicates for each experimental condition, as described in the figure captions and all attempts at replication were successful.
Randomization	Bee subjects were randomly selected from the source colony and randomly assigned to experimental groups.
Blinding	Researchers that filmed bee flight and calculated wingbeat frequencies were blind to which group bees belonged to. Otherwise, no blinding was performed due to requirements for treatment group labeling and the predetermined nature of the measurements.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Bumblebees (bombus impatiens) were purchased from Plant Products and Koppert. Colonies from Koppert were only used to obtain data shown in supplementary figure 7. All other data were collected using colonies from Plant Products.
Wild animals	No wild animals were used in this study.
Reporting on sex	Female worker bumblebees were used.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	No ethical approval or guidance was required to use invertebrate research subjects.

Note that full information on the approval of the study protocol must also be provided in the manuscript.