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Pesticide and resource stressors additively impair wild bee reproduction

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Bees and other beneficial insects experience multiple stressors within agricultural landscapes that act together to impact their health and diminish their ability to deliver the ecosystem services on which human food supplies depend. Disentangling the effects of coupled stressors is a primary challenge for understanding how to promote their populations and ensure robust pollination and other ecosystem services. We used a crossed design to quantify the individual and combined effects of food resource limitation and pesticide exposure on the survival, nesting, and reproduction of the blue orchard bee *Osmia lignaria*. Nesting females in large flight cages accessed wildflowers at high or low densities, treated with or without the common insecticide, imidacloprid. Pesticides and resource limitation acted additively to dramatically reduce reproduction in free-flying bees. Our results emphasize the importance of considering multiple drivers to inform population persistence, management, and risk assessment for the long-term sustainability of food production and natural ecosystems.

1. Introduction

Agricultural intensification is a primary driver of global insect declines [1,2]. This intensification has led to a loss of flowering plants and widespread pesticide use that impact pollinators and other beneficial insect populations, diminishing their ability to deliver ecosystem services critical to human food supplies [2–7]. Disentangling the effects of simultaneous flowering resource scarcity and pesticide exposure is a primary challenge for understanding how to mitigate ongoing pollinator declines and develop strategies for the long-term sustainability of our food systems [8].

The effects of individual stressors on beneficial insects have been documented. For instance, limited floral resources, and resulting poor nutrition, reduces fecundity, longevity, and stress resistance [8–11]. Pesticide exposure can directly kill beneficial insects or cause sublethal effects that reduce reproduction and impair behaviour [12–15]. However, there remain significant knowledge gaps on the interactive effects of combined stressors. There is evidence that stressors have additive, synergistic, and/or antagonistic interactions through physiological mechanisms [16,17], behavioural responses [14,18], and demographic changes [12,19,20] (figure 1), yet these remain untested through controlled, field-realistic experiments.

Understanding the interplay of these drivers is particularly important for pollinator conservation in agroecosystems, where limited floral resources and widespread pesticide use commonly co-occur and are at odds with the demands for crop pollination services [21,22]. Under laboratory conditions, good nutrition can improve honey bee resistance to pesticides [16], and combined exposure to pesticides and nutritional stress synergistically reduced survival in honey bees in the laboratory over four days [17]. However, there has been no comparable research on wild bees in field or semi-field conditions, despite significant differences in resource acquisition and routes of exposure to toxins between species, as well as evidence for significant differences among species responses to pesticide exposure [23] (but see Ellis *et al*. [24]).

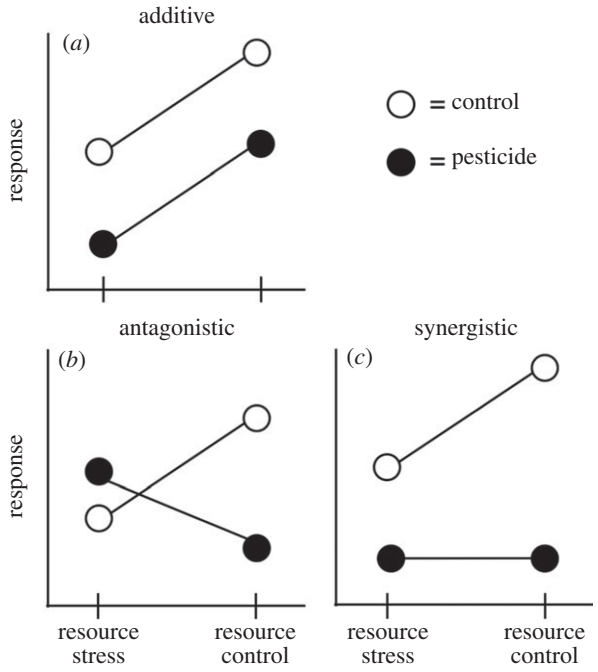

Figure 1. Interactions among multiple environmental stressors. Resource and pesticide stressors, for example, may have (*a*) additive, (*b*) antagonistic, and/or (*c*) synergistic interactions.

To address these knowledge gaps, we quantified the individual and combined effects of resource stress (limited floral resource availability) and sublethal, field-realistic insecticide exposure on the survival, nesting, and reproduction of the blue orchard bee *Osmia lignaria*. We focused on the systemic neonicotinoid insecticide imidacloprid, the most widely used neonicotinoid insecticide in the United States as of 2015 [25]. Neonicotinoids comprise nearly a quarter of the total insecticide market and pose a high risk to bees [26]. Bees can be exposed to neonicotinoids by consuming pollen and nectar which absorb pesticides during flower development [27]. We established nesting females in 16 flight cages using a crossed resource and pesticide design; cages contained spring wildflowers at high or low densities, treated with imidacloprid or without imidacloprid. Using cages allowed us to partition variation in pesticide exposure and resource abundance that may be correlated in real-world landscapes. Although field cages may limit additional risks bees face during long-distance foraging (e.g. predation, parasitism), they reduce variance from other environmental factors while still allowing bees to forage and nest freely. We hypothesize that resource and pesticide stressors will each directly reduce bee fitness. We expect that combined stressors will be additive or synergistic. For example, in a high-resource environment with high pesticides, the benefits conferred by increased resource availability may be negated by pesticide exposure. Alternatively, resource benefits may offset the deleterious effects of pesticide exposure.

2. Methods

(a) Study system and experimental design

The blue orchard bee *Osmia lignaria* is a solitary univoltine species native to North America. It and other *Osmia* species are widely used as alternative pollinators to honey bees and/or in combination with them in fruit orchards [12,21,22]. Females nest above the ground inside pre-existing tunnels (e.g. abandoned wood-boring beetle burrows in nature) but will readily nest in artificial tunnels (e.g. drilled holes, reed cane, cardboard paper tubes). Females collect pollen and nectar to mass-provision sequentially arranged brood chambers, which are separated by mud partitions. Within each chamber, a single egg is laid on or within the provision. Larvae hatch and consume the provision before spinning a cocoon and pupating. Offspring overwinter as adults and emerge the following spring. Bees for this experiment were collected in their overwintering state within nests from local sites in California (CA).

We conducted this experiment in 3 × 3 × 1.8 m flight cages at the UC Davis Bee Research Facility in North Central CA during the spring of 2018 (electronic supplementary material, figure S1). *Osmia* will readily nest in field cages. We established a factorial design with two levels of floral resource availability (high and low) and the presence or absence of pesticide. We allocated four cages per treatment for a total of 16 cages. In each cage, we placed a wooden nesting block with 12 pre-drilled holes, 7.8 mm in diameter and 13 cm in length. We lined each hole with a translucent paper straw, which we removed and replaced as they were filled with nests. When flowers approached full bloom (late April 2018), we released six newly emerged and individually marked female and 12 male *O. lignaria* per cage. We measured the body size of all females (intertegular span; ITS) prior to release, and body size did not differ between treatments ($\chi^2 = 2.69$, $p = 0.442$). We added new bees periodically as bees died to maintain an average of four actively nesting females in each cage. To control for possible effects of timing, we balanced bee additions across treatments, and we also included release date as a covariate in models. In total, we released 121 bees across all cages ($n = 34$ bees each for high-resource treatments; $n = 27$ and 26 bees, respectively, for low-resource pesticide and unexposed control treatments). We monitored nesting activity daily for a minimum of 20 min per cage by watching females take foraging trips in and out of their nests; this allowed us to associate each nest with a nesting female. We measured nesting progression daily by temporarily removing the nest straw and marking the nest progress on the outside of the straw.

(b) Floral resource treatments

In each cage, we sowed a mix of three common wildflowers: *Phacelia tanacetifolia*, *Phacelia ciliata*, and *Collinsia heterophylla* (electronic supplementary material, table S1). These flowers are known to be used by *O. lignaria* and bloom during their foraging period [28–30]. We planted all cages with a high density of flowers in November 2017. Our goal was to create two resource levels: high, essentially not limiting to the bees, and low, which would limit resource availability during the foraging day. We based the floral availability for each resource treatment on published data on the amount of pollen per *O. lignaria* provision and per *P. tanacetifolia* flower to calculate how many flowers would be needed for each female to provision a single offspring [30–32]. Cages receiving a high-resource treatment had (mean ± s.e.) 2034 ± 77 flowers open at a time. We created low-resource cages by removing and covering plants to limit cages to (mean ± s.e.) 498 ± 27 open flowers at a time (electronic supplementary material, figure S2). We conducted weekly flower counts to ensure that treatments were consistent across cages and made adjustments to add or remove flowers as necessary. High-resource cages contained sufficient flowers such that pollen was leftover in many of them at the end of the day; low-resource cages were stripped of pollen by the afternoon each day (electronic supplementary material, table S2), indicating the treatments achieved the desired goal. All cages contained high resources when we released the first cohort of bees; we established the low-resource treatment when females commenced nesting, 4–8 days after release. We released subsequent bees first

into high-resource cages to facilitate nest initiation [29,33]. Upon nest initiation, we immediately moved them within their nests after sundown to the same location in low-resource cages of the same pesticide treatment. The move was only a few metres, and all females re-commenced foraging at the beginning of the next day; thus, we are confident that this moving had minimal impact on nesting females [29]. We provided each cage with a consistent mud source for nesting using moistened soil from each cage.

(c) Neonicotinoid treatments

We applied a soil drench of the neonicotinoid insecticide imidacloprid (AdmirePro®, Bayer Crop Science) six weeks prior to releasing bees in cages at the maximum label rate (10.5 oz/acre; 767 ml/ha) for herbs and orchard fruit crops. Imidacloprid is the most frequently and heavily applied insecticide in California [34] and the United States [26]. AdmirePro® is the most common commercial imidacloprid product in California [34]. Imidacloprid has also been found in *O. lignaria* nests in agricultural landscapes (CS and NMW 2017, unpublished data). To prevent lateral movement of the pesticide through the soil, we buried eight layers of 4 mm clear plastic sheeting 40 cm into the ground between treated and untreated cages. We measured pesticide exposure based on neonicotinoid residues from the pollen provisions within nests, a single male larval provision per cage, which were sent for analysis at the Metabolomics Research Laboratory at Purdue University. Individual samples were prepared using the QuEChERS method [35] and analysed using liquid chromatography triple quadrupole mass spectrometry (LC/QQQ; see electronic supplementary material, table S3 and methods).

(d) Offspring outcomes

Completed nests were stored in darkness at 22°C for six months, followed by four months at 6°C to overwinter. The following spring, we X-rayed all nests with brood inside before opening them. This allowed us to determine the number, sex, and condition of all offspring matched to each mother. We weighed each bee within its cocoon, visually determined the offspring sex, and measured the ITS of each female offspring.

(e) Statistical analysis

We conducted all statistical analyses in R (version 3.4.1). To test for differences in body size of parent females between treatments, we used a Kruskal–Wallis test based on differences of ITS among bees assigned to the different experimental treatments. To test the effects of pesticide exposure and resource availability on offspring production in *O. lignaria*, as well as total nesting duration, we used a generalized linear mixed model (GLMM) with negative binomial error distribution and log link. We included pesticides (treated, not treated), resources (high, low), and date deployed in cage as fixed effects and cage as a random effect. We used a GLMM with binomial error distribution and logit link to test the difference in nesting probability, overwinter mortality, and offspring sex ratio between treatments. *p*-values from GLMMs were calculated using likelihood ratio tests. We tested differences in offspring body size and nest construction rate using a linear mixed model with normal error distribution. In our analysis of offspring body size, we also included parent female ITS as a fixed effect.

3. Results

Resource limitation and pesticide exposure acted individually, and combined additively, to reduce bee reproductive fitness (figure 2). The total impact on reproduction is a function of two processes: first, the probability of nesting, and second, the total number of offspring produced.

The probability of nesting was affected only by chronic exposure to field-realistic concentrations of imidacloprid. Female *O. lignaria* exposed to imidacloprid were 10% less likely to produce offspring, although these borderline statistical results should be interpreted with caution ($\chi^2 = 3.2$, d.f. = 1, $p = 0.074$; figure 2*a*). Resource limitation did not influence nesting probability ($\chi^2 = 0.03$, d.f. = 1, $p = 0.86$).

Combined resource and pesticide stressors reduced female fecundity. Of the female *O. lignaria* that initiated nesting, those exposed to imidacloprid produced 42% fewer surviving offspring than unexposed controls (mean ± s.e. 19.1 ± 1.9 versus 32.7 ± 2.9, respectively; $\chi^2 = 17.59$, d.f. = 1 , $p < 0.001$; figure 2*b*). Bees with low resources produced 26% fewer surviving offspring than bees with abundant resources (mean ± s.e. 22.1 ± 2.2 versus 29.8 ± 2.7, respectively; $\chi^2 = 7.17$, d.f. = 1, $p = 0.007$; figure 2*b*). Together, unstressed females produced approximately 21 more offspring on average than resource and pesticide-stressed females. Pesticide exposure and resource limitation acted additively to reduce reproduction (no significant interaction; $\chi^2 = 0.61$, d.f. = 1, $p = 0.44$; figure 2*b*). Nearly all provisioned cells successfully developed into adults, and overwinter offspring mortality did not differ among treatments ($\chi^2 = 3.23$, d.f. = 1, $p = 0.20$; electronic supplementary material, figure S3).

In addition to direct effects on reproduction, resource and pesticide stressors led to male-biased sex ratios, further limiting reproductive output. Pesticide exposure caused a 33% reduction in the proportion of daughters produced ($\chi^2 = 8.32$, d.f. = 1, $p < 0.004$; figure 2*c*). Resource limitation caused a 48% reduction in the proportion of daughters produced ($\chi^2 = 15.29$, d.f. = 1, $p < 0.001$; figure 2*c*). The two stressors combined additively to reduce the female : male offspring sex ratio (no significant interaction; $\chi^2 = 1.04$, d.f. = 1, $p = 0.31$; figure 2*c*), similar to effects on total offspring reproduction.

Surviving offspring differed by an average of 0.13 mm in body size (intertegular span; ITS) between treatments; female offspring were 5% larger in high-resource treatments ($\chi^2 = 19.92$, d.f. = 1, $p < 0.001$) and 3% larger in pesticide treatments ($\chi^2 = 12.46$, d.f. = 1, $p < 0.001$; figure 2*d*). This pattern is similar for males (electronic supplementary material, figure S4).

Bees produced fewer offspring via multiple mechanisms: changing nesting rate, onset, and duration. Stressed bees constructed nests slower and nested for fewer days than unstressed bees. Resource limitation slowed nest construction by 32% (approx. 0.5 cells/day; $\chi^2 = 23.73$, d.f. = 1, $p < 0.001$), and pesticide exposure slowed nesting by 20% (approx. 0.3 cells/day; $\chi^2 = 11.62$, d.f. = 1, $p < 0.001$; figure 2*e*). Again, the effects were additive (no significant interaction; $\chi^2 = 0.02$, d.f. = 1, $p = 0.89$; figure 2*e*). Females exposed to pesticides also spent 6.33 (28%) fewer days nesting than bees that were not exposed ($\chi^2 = 9.54$, d.f. = 1, $p = 0.002$; figure 3). Pesticide-exposed females started nesting an average of 49% later than unexposed bees, about 3.6 days ($\chi^2 = 16.54$, d.f. = 1, $p < 0.001$; figure 3). Pesticide and resource stressors acted additively on the total nesting duration and delayed start (no significant interaction on nesting duration, $\chi^2 = 2.40$, d.f. = 1, $p = 0.12$; delay $\chi^2 = 0.09$, d.f. = 1, $p = 0.76$).

Despite significant responses of behaviour and reproduction of bees exposed to pesticides, only two of eight pesticide-treated cages had detectable levels of imidacloprid in pollen provisions (electronic supplementary material, table S3). None of the pollen provisions from untreated control cages contained detectable imidacloprid levels (electronic supplementary material, table S3).

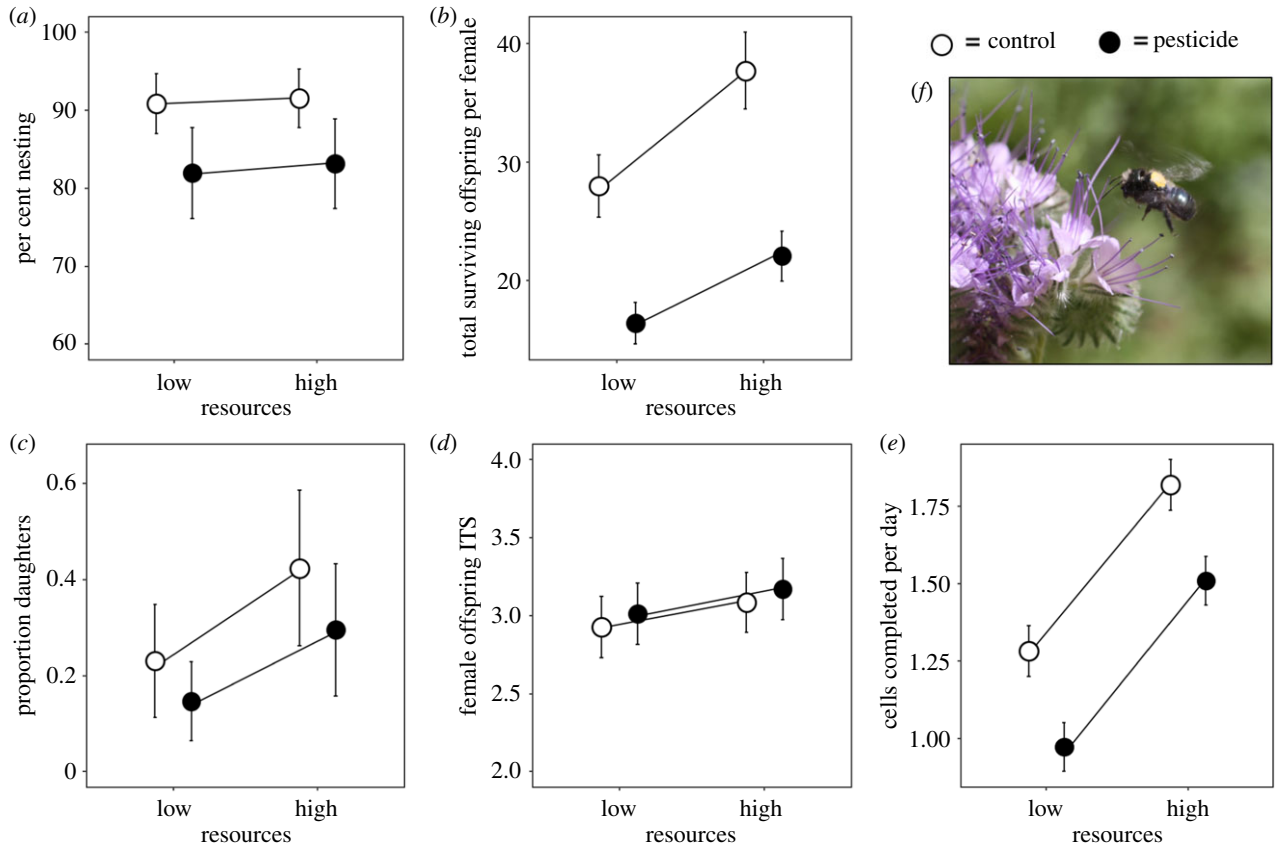

Figure 2. Effects of resource limitation and pesticide exposure on bee performance. (*a*) Per cent of female *Osmia lignaria* that produced at least one offspring; (*b*) mean number of surviving offspring per nesting female; (*c*) proportion of daughters produced per nesting female; (*d*) ITS of female offspring; (*e*) mean number of cells completed per day per nesting female *O. lignaria* in 16 field cages with pesticides (black) or without pesticides (white) in high- and low-floral resource environments. Error bars show s.e.; $N = 121$. (*f*) Photo of a paint-marked *O. lignaria* female approaching a *Phacelia tanacetifolia* flower. (Online version in colour.)

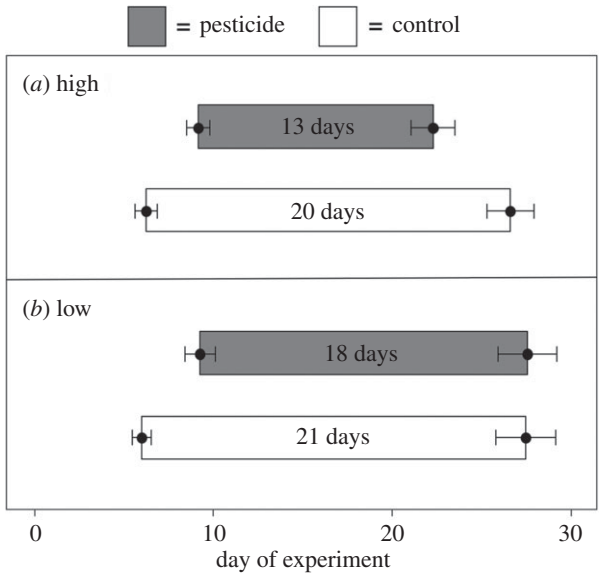

Figure 3. Nesting onset and duration is moderated by pesticide exposure and resource limitation. Mean (± s.e.) number of days between first and last offspring provisioned by each nesting female in (*a*) high- and (*b*) low-floral resource environments exposed to pesticides (grey) or unexposed controls (white). $N = 121$ bees.

4. Discussion

We show in free-foraging trials that field-realistic pesticide exposure and floral resource scarcity combine additively to dramatically reduce multiple vital rates of a solitary bee. Unlike recent predictions of worst-case scenarios of negative synergies [8,17,36], effects—although substantial—were additive. The additive effects of exposure to pesticides and food limitation reduced reproduction by 57% compared to unexposed control populations. These combined stressors could dramatically impede population growth and jeopardize population persistence. Pesticide exposure had the greatest impact on offspring production and nesting activity, reducing overall reproduction 1.75 times more than food limitation. Negative effects of systemic pesticides on bee survival and reproduction are well documented but based largely on correlative (rather than experimental) field and laboratory studies [8,12,37].

In *Osmia*, reduced offspring production by pesticide-exposed bees resulted from a dramatically delayed onset of nesting, earlier cessation, and a substantially lower rate of offspring provisioning. *Osmia lignaria* females exposed to imidacloprid began nesting 3.6 days later and spent 5.2 fewer days nesting than unexposed control bees—eliminating nearly a week of potential nesting days. This could be due to a delay in ovary maturation, as well as decreased longevity, that together curtailed nesting at both ends [38–40].

Resource limitation also reduced the rate of offspring provisioning among nesting females, likely due to the lack of pollen and nectar available for nest provisioning [11,41]. This reduced the rate at which bees could complete nest provisions, as well as potentially reducing overall exposure to pesticide-treated flowers.

Interestingly, pesticide exposure affected total nesting duration differently between resource treatments. Pesticides

had a particularly large influence on nesting duration for bees with abundant resources, which stopped nesting two days earlier than bees in all other treatments. It is possible that resource-stressed bees nested longer to make up for a slower overall nesting rate. This seems unlikely because slower nesting was not observed in pesticide-free resource treatments. Instead, we suspect that a faster provisioning rate and associated greater number of flowers visited in the high-resource treatment increased pesticide exposure. Increased chronic exposure to pesticides reduced bee longevity despite their access to sufficient forage resources [39], suggesting that bees are not rescued by more forage resources when it also exposes them to more toxins [17,42].

Both pesticide exposure and resource scarcity biased offspring sex ratio toward more males. Females of *O. lignaria* and most other solitary bee species are larger and are provisioned with more food than males, thus they cost more to produce [43,44]. Pesticide exposure dramatically reduced the probability that a bee produced even a single daughter. Indeed, of all nesting females, only 62% of pesticide-exposed individuals produced at least one daughter compared to 92% of unexposed individuals. This suggests sublethal effects on foraging ability whereby females shifted to produce less costly males [11,38,45].

The decrease in female offspring has important consequences for populations; because males rarely limit population growth, fewer female progeny will reduce the reproductive potential of subsequent generations [19,46]. Combined with lower overall offspring production, as we found, it could create an extinction vortex, driving populations to decline or go extinct [19,47,48]. Consider, the average female in an optimal environment with abundant, pesticide-free forage resources can produce 37 offspring in her lifetime, of which approximately 10 are female (figure 2*b*,*c*). Pesticide and food-stressed females produce about 16 offspring each—a difference of 57%—of which a mere 1–2 are females (figure 2*b*,*c*). This difference is striking considering that even minor changes in offspring production can substantially influence population growth given solitary bees' relatively low reproductive rate [43,49].

Unsurprisingly, abundant resources led to relatively larger offspring [38,50]. We were surprised, however, that pesticide-exposed bees also produced larger offspring than unexposed bees, albeit by a small margin (3%; 0.09 mm ITS). Pesticide-exposed bees may have allocated larger food provisions (leading to larger offspring) to compensate for fewer overall offspring, although it is unclear what fitness benefit this confers. Body size positively correlates with nesting success in some studies [44,45], but not others [51,52] depending on environmental conditions, and it may be mediated by other differences in addition to body size. In our study, parent female body size did not influence realized fecundity.

We applied pesticide according to label instructions; thus, it is likely that bees were exposed to field-realistic pesticide levels throughout the experiment. We were therefore surprised that only two of the eight pesticide-treated cages contained detectable levels of imidacloprid. We discuss three possibilities for this ambiguous detection: (i) bees were not exposed to pesticides in the pollen. We think this is unlikely because we found strong differences in measured outcomes between pesticide-treated and untreated control cages. (ii) The residues in the pollen degraded. We applied the pesticide six weeks prior to releasing bees in cages; degradation occurs over time, but we nonetheless found residue in multiple samples. Additionally, the half-life of imidacloprid is relatively long (in soil: 28–1250 days [27]; in water: 30 days [53]). (iii) Given the small amount of pollen we sent for analysis, it is possible that low levels of pesticide residue could not be detected. Such variability in pesticide levels found in larval provisions may be a previously undocumented pattern, since samples are generally pooled for analysis, and is an important consideration for future studies.

The sublethal impacts of pesticides and resource limitation may be especially problematic in agricultural systems, which rely on robust pollinator populations. Establishing flower plantings to provide additional forage resources is a frequently implemented approach for mitigating pollinator decline [54–56]. We demonstrated that abundant floral resources yielded a 35% increase in bee reproduction. However, flower plantings could act as ecological traps if they also exposed bees to pesticides. This occurs via pesticide drift from agricultural crops onto nearby field margins and flower plantings [57,58]. In our study, bees with unlimited pesticide-treated forage produced 21% fewer offspring than those with critically limited but pesticide-free forage, indicating that additional resources do more harm than good if they become contaminated with pesticides [17,57–60]. Although we focused on pollinators, similar impacts likely apply to other beneficial insects in agriculture; parasitoids rely on nectar sources and generalist predators feed on prey found in planted field margins [57,61].

A critical challenge facing ecologists today is predicting and understanding the effects of multiple stressors [36,62]. Thus, we are encouraged that pesticide exposure and resource limitation combined additively, rather than synergistically, to affect bee health. The additive nature of the effects could enable us to make preliminary predictions about the effects of such environmental change from univariate experiments, although this must be approached with caution as chemicals are known to interact in different ways [15,63]. It is clear that insects encounter multiple stressors throughout their life cycles, each exacerbating the effects of the others. We show that pesticide exposure and resource limitation combined to additively limit bee reproduction through reduced offspring production, male-biased sex ratio, and shorter nesting duration. In addition to novel findings for understanding combined environmental stressors, our results inform practical decision-making for conservation and management of ecosystem services in agriculture. For example, they reinforce the need for caution in the placement of flower plantings intended to provide forage resources for bees to avoid them becoming traps that expose bees to potential additive negative effects of pesticides in agroecosystems.

Data accessibility. The datasets generated and analysed during this study are available from the Dryad Digital Repository: https://doi.org/10.25338/B83P6K [64].

Authors' contributions. C.S. and N.M.W. conceived and designed the experiments and analysed and interpreted data. C.S. collected the data and wrote the initial manuscript draft with revisions from N.M.W. Both authors read and approved the final manuscript.

Competing interests. We declare we have no competing interests.

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