

RESEARCH ARTICLE

Bees exposed to climate change are more sensitive to pesticides

Sergio Albacete^{1,2}  | Gonzalo Sancho^{1,2}  | Celeste Azpiazu^{1,3}  | Anselm Rodrigo^{1,2}  | Roberto Molowny-Horas^{1,2}  | Fabio Sgolastra⁴  | Jordi Bosch^{1,2} 

¹Universitat Autònoma de Barcelona, Bellaterra, Spain

²Centre for Ecological Research and Forestry Applications (CREAF), Bellaterra, Spain

³Institute of Evolutionary Biology (CSIC–Universitat Pompeu Fabra), Barcelona, Spain

⁴Dipartimento di Scienze e Tecnologie Agro-Alimentari, Alma Mater Studiorum Università di Bologna, Bologna, Italy

Correspondence

Sergio Albacete, Universitat Autònoma de Barcelona and CREAM (Centre for Ecological Research and Forestry Applications), 08193 Bellaterra, Spain. Email: s.albacete@creaf.uab.cat

Funding information

Spanish Ministry of Science and Innovation, Grant/Award Number: PRE2019-090375, PRE2019-088817, PID2021-128938OB-I00 and RTI2018-098399-B-I00; Spanish Ministry of Universities, Grant/Award Number: Margarita-Salas-scholarship

Abstract

Bee populations are exposed to multiple stressors, including land-use change, biological invasions, climate change, and pesticide exposure, that may interact synergistically. We analyze the combined effects of climate warming and sublethal insecticide exposure in the solitary bee *Osmia cornuta*. Previous *Osmia* studies show that warm wintering temperatures cause body weight loss, lipid consumption, and fat body depletion. Because the fat body plays a key role in xenobiotic detoxification, we expected that bees exposed to climate warming scenarios would be more sensitive to pesticides. We exposed *O. cornuta* females to three wintering treatments: current scenario (2007–2012 temperatures), near-future (2021–2050 projected temperatures), and distant-future (2051–2080). Upon emergence in spring, bees were orally exposed to three sublethal doses of an insecticide (Closer, a.i. sulfoxaflor; 0, 4.55 and 11.64 ng a.i./bee). We measured the combined effects of wintering and insecticide exposure on phototactic response, syrup consumption, and longevity. Wintering treatment by itself did not affect winter mortality, but body weight loss increased with increasing wintering temperatures. Similarly, wintering treatment by itself hardly influenced phototactic response or syrup consumption. However, bees wintered at the warmest temperatures had shorter longevity, a strong fecundity predictor in *Osmia*. Insecticide exposure, especially at the high dose, impaired the ability of bees to respond to light, and resulted in reduced syrup consumption and longevity. The combination of the warmest winter and the high insecticide dose resulted in a 70% longevity decrease. Smaller bees, resulting from smaller pollen–nectar provisions, had shorter longevity suggesting nutritional stress may further compromise fecundity in *O. cornuta*. Our results show a synergistic interaction between two major drivers of bee declines, and indicate that bees will become more sensitive to pesticides under the current global warming scenario. Our findings have important implications for pesticide regulation and underscore the need to consider multiple stressors to understand bee declines.

KEYWORDS

body size, climate change, global warming, longevity, nutritional stress, *Osmia cornuta*, pesticide exposure, sulfoxaflor, synergistic effects, toxicity

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. *Global Change Biology* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Bees play a key role in the functioning of terrestrial ecosystems and provide essential pollination services for agricultural crops (Klein et al., 2007; Ollerton et al., 2011). However, bees have experienced significant declines in abundance and diversity over the last century (Goulson et al., 2015; Potts et al., 2010; Sánchez-Bayo & Wyckhuys, 2019). The causes of these declines are at least partially known, and include land-use change, biological invasions, climate change, and the increasing use of pesticides associated with agricultural intensification (Goulson et al., 2015; IPBES, 2021). Importantly, these stressors do not act in isolation and sometimes interact synergistically (Castelli et al., 2020; Goulson et al., 2015; Siviter et al., 2021). Various studies show that the combined exposure of multiple stressors at sublethal levels may cause lethal effects (Doublet et al., 2015; Vanbergen, 2021). For example, disease-induced stress may result in increased sensitivity to pesticides, and, at the same time, sublethal exposure to toxicants may cause immunosuppression and increased vulnerability to pathogenic infections (Aufauvre et al., 2012; Doublet et al., 2015; Grassl et al., 2018; Pettis et al., 2012; Vidau et al., 2011). Insecticide exposure and nutritional stress may also interact synergistically, resulting in reduced thermoregulation capability, food consumption, flight ability, and survival (Castle et al., 2022; Linguadoca et al., 2021; Tong et al., 2019; Tosi et al., 2017). These studies underscore the need to address the combined effects of various stressors to understand the drivers of bee declines.

Like other ectotherms, insects are highly dependent on environmental temperatures for adequate growth, development, and reproduction, which makes them particularly vulnerable to global warming (Deutsch et al., 2008; Portner, 2002). In general, exposure to warm temperatures increases metabolic activity in insects, affecting development rates and overall physiological functioning (González-Tokman et al., 2020; Neven, 2000). These effects are accompanied by an increase in energy expenditure (Brown et al., 2004), resulting in the mobilization and consumption of stored metabolic resources (Storey & Storey, 2004), ultimately affecting body size, longevity, and fecundity (Blanckenhorn & Henseler, 2005; Fischer et al., 2014; Kierat et al., 2017; Vesterlund et al., 2014; Zhang et al., 2015). Several studies show that thermal stress enhances the depletion of fat body reserves in different insect groups (Jean et al., 1990; Klepsatel et al., 2016; Williams et al., 2012), including bees (Bosch et al., 2010; Fliszkiewicz et al., 2012; Sgolastra et al., 2011). Fat bodies play a crucial role not only in the storage and release of energy in response to metabolic demands (Arrese & Soulages, 2010), but also in the functioning of key physiological processes such as diapause development (Hahn & Denlinger, 2007), hormone regulation, and immune response (Keeley, 1985; Skowronek et al., 2021). Importantly, fat bodies are also the main tissue in which detoxification enzymes, such as cytochrome P450 monooxygenases, are produced and expressed (David et al., 2006; Petersen et al., 2001). Therefore, the alteration of fat bodies caused by heat stress may also impair detoxification

processes in insects (Costa et al., 2020; Linguadoca et al., 2021; Lycett et al., 2006).

In this study we explored the combined effects of increasingly warm wintering temperatures associated with the current scenario of global warming and exposure to sublethal levels of insecticide in the solitary bee *Osmia cornuta*. We expected exposure to warm temperatures to result in increased sensitivity to the insecticide. To the best of our knowledge, the interaction between pesticide exposure and climate change has not been previously addressed in solitary bees. *Osmia* spp. are appropriate organisms for a study of this sort because both their wintering ecophysiology and their sensitivity to insecticides have been studied in some detail. *Osmia* spp. overwinter on a fixed energetic budget (Bosch et al., 2010). Adult eclosion takes place in autumn, but adults remain inside their cocoons within their natal nest, without access to food until next spring. Therefore, wintering adults rely totally on the energy reserves derived from the food provisions ingested by the larval stage in early summer. This is in contrast to bumblebees and honey bees, which overwinter as emerged adults and therefore are able to feed before, and sometimes during, the winter. Previous *Osmia* studies have shown that warm pre-wintering and wintering temperatures enhance fat body depletion, lipid consumption, and body weight loss (Bosch et al., 2000, 2010; Bosch & Kemp, 2003, 2004; Fliszkiewicz et al., 2012; Sgolastra et al., 2011). *Osmia* spp. nest above ground, and therefore are more directly exposed to ambient temperatures than ground nesting bees (Dorian et al., 2022). *O. cornuta* has a strong affinity for fruit tree pollen (Jaumejoan et al., 2023) and, along with other *Osmia* species, is commercially managed for orchard pollination, resulting in frequent exposure to pesticides (Bosch & Kemp, 2002). In 2013, the European Food Safety Authority recommended the use of *O. cornuta* and *O. bicornis* as model species in pesticide risk assessment (EFSA, 2013), and information on the effects of pesticides on these species has notably increased since then (Sgolastra et al., 2019).

The aim of this study is to understand whether climate warming may exacerbate the sensitivity of solitary bees to pesticides. We exposed *O. cornuta* females to three simulated wintering treatments and then assessed their sensitivity to acute oral exposure to the insecticide CloserTM® (a formulation of sulfoxaflor) at two sublethal concentrations. We then measured the combined effects of these two stress factors on the propensity of the bees to respond to a light stimulus (phototactic response), feeding behavior, and longevity.

2 | MATERIALS AND METHODS

2.1 | Life history of *O. cornuta*

O. cornuta, known as the European orchard bee or the horned mason bee, occurs in most of central and southern Europe. Its populations fly in late winter and early spring. Females are active for approximately 20 days (Bosch & Vicens, 2006), during which time they build one or more nests in pre-existing cavities. Each nest consists of a linear series of cells, delimited by mud partitions, each containing a

pollen–nectar provision and an egg. After consuming the provision, the larva spins a cocoon and undergoes a summer diapause in the prepupal stage (Sgolastra et al., 2012). Pupation occurs in mid or late summer, and adult eclosion in late summer or early autumn. Eclosed adults remain inside their cocoons and lower their metabolic rates (Bosch et al., 2010; Sgolastra et al., 2010). Adults require exposure to cold temperatures (wintering) to complete diapause and emerge the following spring as temperatures increase (Bosch & Kemp, 2004; Sgolastra et al., 2010).

2.2 | Wintering and emergence

We used the progeny of an *O. cornuta* population that nested in February–March 2021 in an almond orchard near Lleida (Catalonia, Spain). In mid-September, when bees reached the adult stage, we dissected a subset of nests and collected 825 female cocoons. Cocoon sex can be reliably established based on size and position within the nest (Bosch, 1994). The collected cocoons were individually weighed and randomly assigned to one of three simulated wintering treatments ($n=275$ females per treatment): (a) Current scenario (average hourly temperatures recorded in the area of origin of the population during 2007–2012); (b) near-future scenario (projected temperatures for 2021–2050); and (c) distant-future scenario (projected temperatures for 2051–2080). Future wintering treatments were based on the temperature projections of the 8.5 Representative Concentration Pathways (RCPs) used in the IPCC AR5 assessment report on climate change (Moss et al., 2010). Our projections cover a 30-year period and reflect a mean temperature increase of 0.6 and 2.9°C for the near-future and distant-future scenarios, respectively, compared to the winter temperatures of 2007–2012 (Table 1). Data for the area of origin of the study population were extracted from the Escenarios-PNACC 2017 project (<http://escenarios.adaptecca.es>). We simulated daily temperature curves for each treatment and month (Figure S1; Supporting Information Methods). Cocoons were individually placed in multi-well plates and transferred to temperature-controlled chambers on 1 October until emergence the following year. Actual temperatures within the three chambers were monitored with data loggers throughout the wintering period.

TABLE 1 Mean weight loss and % winter mortality in *Osmia cornuta* females exposed to three wintering treatments representing increasingly warm climate change scenarios.

Wintering treatment	N	Temperatures (°C) ($\bar{X} \pm SE$) (range) ^{1,2}	Wintering duration (days) ¹	Pre-wintering weight (mg) ($\bar{X} \pm SE$) ³	% Winter mortality ($\bar{X} \pm SE$) ³	% Weight loss ($\bar{X} \pm SE$) ³
Current	275	9.4 ± 0.3 (2.6–20.1)	141	224.2 ± 1.6a	2.2 ± 0.8a	3.9 ± 0.04a
Near-future	275	10.0 ± 0.4 (3.2–22.5)	134	224.0 ± 1.6a	1.8 ± 0.6a	5.8 ± 0.05b
Distant-future	275	12.3 ± 0.4 (4.4–25.3)	128	227.0 ± 1.6a	4.0 ± 1.2a	8.4 ± 0.09c

¹From 1 October until 5% female emergence.

²Daily temperature curves provided in Figure S1.

³Values followed by different letters are significantly different (Tukey HSD test, $p > .05$).

Bees of the climate change treatments were expected to emerge before bees of the current treatment (Bosch & Blas, 1994a; Bosch & Kemp, 2003, 2004). Cocoons were checked daily, and when emergence of a given treatment reached 5%, cocoons of that treatment were transferred to an incubation chamber in which they were exposed to 15°C for 24 h and subsequently to 20°C until 100% emergence. Before being transferred to the incubation chamber cocoons were again individually weighed to obtain a measure of weight loss over the winter.

2.3 | Pesticide exposure

Upon emergence, bees were individually kept for 24 h in transparent plastic containers (diameter: 11 cm; height: 7 cm) capped with a pin-perforated lid to allow them to deposit the meconium. Bees were then orally and acutely exposed to one of three insecticide treatments using the “petal method” (Azpiazu et al., 2023). Each bee was offered 20 µL of test solution. Only bees that consumed 100% of the test solution within 3 h ($\geq 90\%$ in all treatments) were included in the analyses. Following this exposure phase, bees were left in the plastic cages and fed ad libitum with a 1-mL calibrated syringe filled with a feeding solution (henceforth syrup; 33% w/w sucrose–water). A petal of *Euryops* spp. (Asteraceae) was attached to the tip of the syringe to enhance prompt location by the bees (Azpiazu et al., 2023). During the experiment, test containers were maintained at $20 \pm 2^\circ\text{C}$ and 50%–70% relative humidity, and received indirect natural light.

We worked with the commercial formulation of sulfoxaflor CloserTM® (Sulfoxaflor 11.43% SC, Corteva Agriscience, Spain S.L.U.). We tested two doses of sulfoxaflor (SUL), 4.55 ng/bee (henceforth low dose) and 11.64 ng/bee (high dose), along with a control (0 ng/bee; 0 dose). The two SUL doses correspond to the LD₁₀ and LD₅₀ at 72 h after exposure of female *O. bicornis* (Azpiazu et al., 2021), adjusted to the greater body weight of *O. cornuta* ($0.149 \text{ g} \pm 0.0142$; Bosch & Vicens, 2002). To obtain the test solutions (20 µL/bee), we prepared a primary stock solution of 1.16 g/L SUL by dissolving Closer in distilled water. This solution was subsequently diluted and finally mixed with syrup at a ratio of 50 µL/mL to achieve the desired doses.

2.4 | Phototaxis test

Two hours after the exposure phase, approximately 25 individuals of each wintering-pesticide treatment were subjected to a phototactic test in which bees in a dark chamber were exposed to a light stimulus. The response of each bee was scored as either positive (the bee walked to the light source within 60s) or negative (the bee walked but did not reach the light source; see [Supporting Information Methods](#) for details).

2.5 | Syrup consumption and survival

Test cages were inspected daily to monitor syrup consumption (assessed by checking the level of syrup in the calibrated syringe; accuracy: 0.01mL) and bee mortality. Three additional containers without bees were used as controls to measure and account for changes in syrup levels due to evaporation. The syrup solution was renewed every 3–4 days until all the bees died. Sample sizes for syrup consumption and survival were approximately 60 individuals per wintering treatment and insecticide dose.

2.6 | Data analysis

To test the effect of wintering treatment on winter mortality we used a binomial generalized linear model (GLM). To determine the effect of wintering treatment on percent weight loss, we used a beta regression model, with individual pre-wintering weight as a covariate (betareg package; Cribari-Neto & Zeileis, 2010).

We used GLMs to analyze the effects of wintering treatment, insecticide exposure and their interaction on phototactic response (binomial error distribution and identity link function), daily syrup consumption (zero-inflated Gamma error distribution and log link function), and longevity (Poisson error distribution and log link function). Because body size may affect food consumption (Azpiazu et al., 2019; Sgolastra et al., 2018) and pesticide sensitivity (Thompson, 2016), pre-wintering weight was added as a covariate in the syrup consumption and longevity analyses. Pairwise comparisons were done with Tukey's p -value adjustment method (emmeans-package; Lenth et al., 2019).

We used Kaplan–Meier (K-M) survival curves to illustrate the combined effects of wintering and insecticide treatments on post-exposure survival. Then, we ran a log-rank omnibus test to explore overall differences among treatments (survdiff function of the survival R package with $\rho=0$; Therneau et al., 2020). Pairwise comparisons between survival curves were done with Holm multi-comparison corrections and $\rho=0$ (pairwise_survdiff function of the survminer package; Kassambara et al., 2020).

To explore potential synergistic effects between wintering and insecticide exposure on survival we used the Bliss drugs' independence criterion (Demidenko & Miller, 2019) to compare the observed survival curves of the near- and distant-future populations (at the

different insecticide doses) with the corresponding expected survival probability curves, built assuming no interaction between the two factors. The analyses (details provided in [Supporting Information Methods](#)) were conducted with the Fhtestrc function of the Fhtest package (Oller & Langohr, 2017).

All analyses were conducted in R (R Core Team, 2020). Primary data and associated R scripts are accessible on the repository Dryad (Albacete et al., 2023).

3 | RESULTS

3.1 | Winter mortality and weight loss

As expected, bees exposed to climate change wintering treatments emerged earlier than bees of the current climate scenario (Table 1). Winter mortality was very low and was not affected by wintering treatment (GLM, $\chi^2=2.8$; $df=2$; $p=.2516$). On the other hand, body weight loss increased with wintering temperature (GLM, $\chi^2=2311.8$; $df=2$; $p<.0001$). Bees of the distant-future scenario lost twice as much body weight as bees of the current scenario (Table 1). Regardless of the wintering treatment, bees with lower pre-wintering weight lost a greater proportion of body weight (GLM, $\chi^2=16.7$; $df=1$; $p<.0001$).

3.2 | Phototactic response

Phototactic response was affected by wintering treatment (GLM, $\chi^2=14.7$; $df=2$; $p=.001$), insecticide dose (GLM, $\chi^2=109.2$; $df=2$; $p<.0001$), and their interaction (GLM, $\chi^2=16.1$; $df=4$; $p=.003$). The vast majority of bees not exposed to the insecticide (0 dose) responded positively to light, irrespective of the wintering treatment (Figure 1). The percentage of bees that responded positively to light decreased with increasing insecticide dose, and this decrease was especially pronounced in bees of the distant-future scenario (Figure 1). In the current and near-future scenarios, the proportion of positively responding bees was hardly affected by the low insecticide dose, but dropped to ca. 50% when bees were exposed to the high dose. In bees of the distant-future scenario, the proportion of positively responding bees went from 95.8% (0 dose) to 73.1% (low dose) and 11.1% (high dose).

3.3 | Syrup consumption

Daily consumption of the feeding solution was not affected by wintering treatment (GLM, $\chi^2=2.5$; $df=2$; $p=.2827$) but varied with insecticide exposure (GLM, $\chi^2=65.6$; $df=2$; $p<.0001$). The interaction between wintering and pesticide exposure was significant (GLM, $\chi^2=17.7$; $df=4$; $p=.0014$) because differences in syrup consumption across insecticide doses were especially pronounced in bees of the distant-future treatment (Figure 2A). The effect of

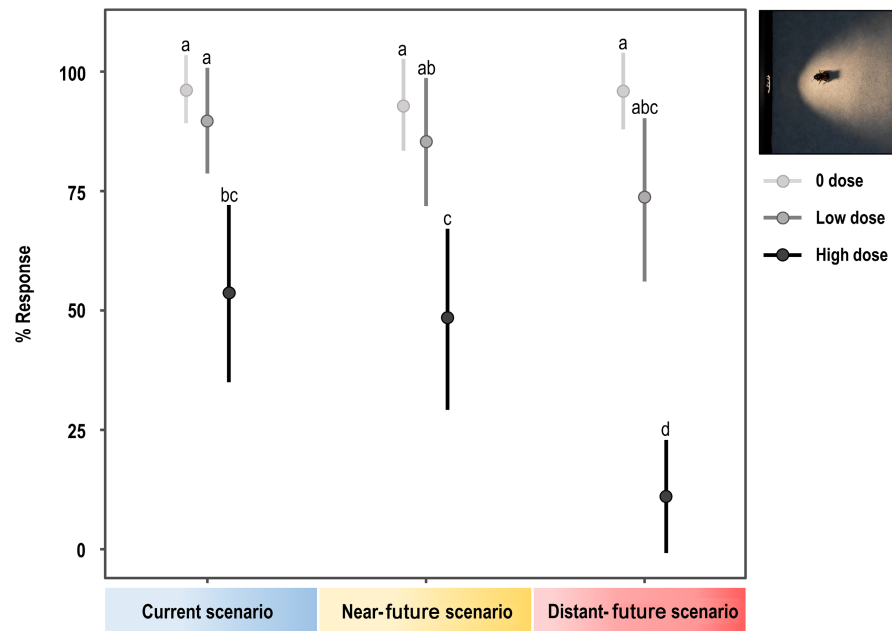


FIGURE 1 Model-estimated means and 95% confidence intervals of percent positive response to a phototaxis test in *Osmia cornuta* females exposed to three wintering treatments representing increasingly warm climate change scenarios, and three orally administered doses of the insecticide sulfoxafloer (0, 4.55 and 11.64 ng/bee). Different letters denote significant differences (Tukey HSD test, $p < .05$). $N = 24$ – 29 individuals per wintering scenario and dose.

the high insecticide dose on syrup consumption was evident from day 1 (Figure S2). We expected large bees to consume more syrup than small bees, but the relationship between pre-wintering body weight and daily syrup was not significant (GLM, $\chi^2 = 1.6$; $df = 1$; $p = .1987$).

3.4 | Survival probability and longevity

Survival curves differed significantly among treatments (log-rank test: $\chi^2 = 244.0$; $df = 8$; $p < .0001$; Figure 3). For a given insecticide dose, the current and near-future survival curves were similar ($p > .05$). Survival probability dropped faster in bees of the distant-future scenario than in bees of the current and near-future scenarios at all the insecticide doses tested, including 0 ng/bee. Consequently, longevity differed significantly across different treatments (Figure 2B). Longevity was affected by wintering treatment (GLM, $\chi^2 = 262.1$; $df = 2$; $p < .0001$), insecticide exposure (GLM, $\chi^2 = 613.3$; $df = 2$; $p < .0001$), and their interaction (GLM, $\chi^2 = 39.6$; $df = 4$; $p < .0001$). Pre-wintering body weight had a significant effect on longevity (GLM, $\chi^2 = 27.7$; $df = 1$; $p < .0001$), with large bees living longer than small ones.

To assess potential synergism between wintering treatment and insecticide exposure on survival probability, we compared the observed survival curves of the near and distant-future populations at the two insecticide doses with the expected survival curves based on the Bliss criterion for drugs independence. We found that the combination of the distant-future scenario and exposure to the high insecticide dose resulted in a synergistic decrease in survival

(Figure 4a). We also found a significant antagonistic effect in bees of the near-future scenario exposed to the low insecticide dose (Figure 4b).

4 | DISCUSSION

The aim of this study was to establish whether climate warming could affect the ability of adult bees to cope with pesticide exposure. We used climate change projections from IPCC RCP8.5 and realistic concentrations of a widely used insecticide to assess the combined effects of increasingly warm wintering temperatures and sublethal insecticide exposure on the behavior and longevity of a solitary bee. Our results demonstrate synergistic effects between realistic scenarios of two of the main drivers of bee declines. Importantly, these effects were obtained following acute oral exposure to a single insecticide. In field conditions bees may experience repeated and/or chronic exposure to a variety of chemicals via different exposure routes (Knapp et al., 2023; Sanchez-Bayo & Goka, 2014). At the same time, the temperatures of our most pessimistic climate scenario are already occurring in particularly warm winters (winter temperatures in Europe in 2019–2020 were 3.4°C higher than the mean of 1981–2010; AEMET, 2021; Copernicus, 2020). Therefore, both our pesticide and climate scenarios may be considered conservative. Our results underscore the need to study combinations of stressors to fully understand the effects of global change on bee populations (Goulson et al., 2015). Our findings also have important implications for pesticide regulation. Pesticide risk assessment is based on median lethal and effect doses (LD50 and ED50). Given

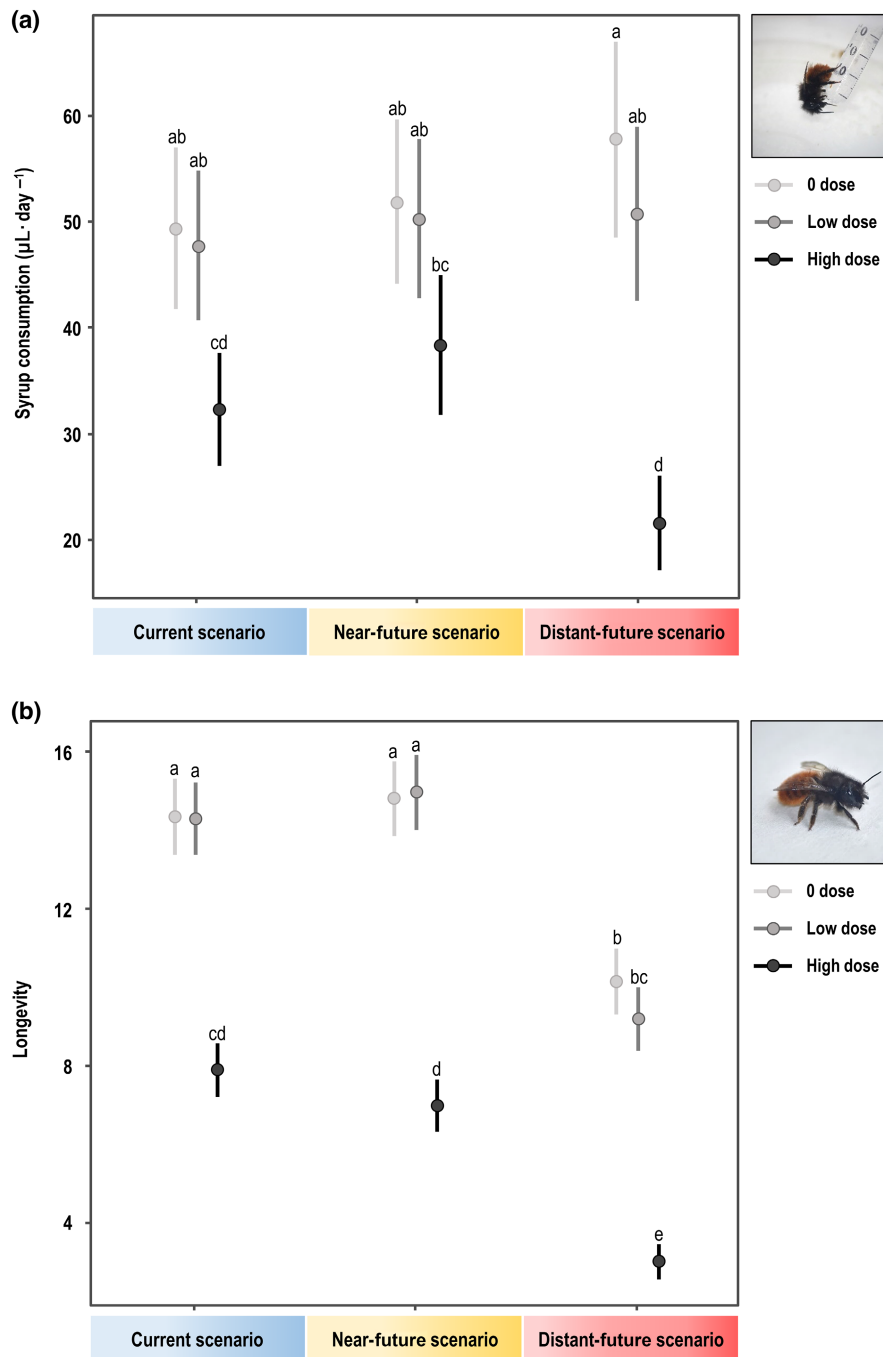


FIGURE 2 Model-estimated means and 95% confidence intervals of daily syrup consumption (a) and longevity (b) in *Osmia cornuta* females exposed to three wintering treatments representing increasingly warm climate change scenarios, and three orally administered doses of the insecticide sulfoxafloer (0, 4.55, and 11.64 ng/bee). Different letters denote significant differences (Tukey HSD test, $p < .05$). $N = 53$ –65 individuals per wintering scenario and dose.

our results, current LD50 and ED50 values may not represent sufficiently protective thresholds as bee populations are confronted with increasingly warm temperatures.

Wintering treatment did not have a significant effect on winter mortality, which was very low and similar to mortality values found in studies in which *Osmia* populations were wintered under natural conditions (Bosch & Blas, 1994b; Bosch & Kemp, 2000; CaraDonna et al., 2018; Sgolastra et al., 2012). Similarly, wintering

treatment by itself did not impair the ability of females to respond to light. On the other hand, wintering treatment had an important impact on weight loss, which, in agreement with previous studies, increased with wintering temperatures. Because body weight loss during wintering is accompanied by the depletion of energy reserves in *Osmia* (Fliszkiewicz et al., 2012; Sgolastra et al., 2011), we expected that bees exposed to climate warming would consume more syrup to rebuild their metabolic reserves (Nestel et al., 2016).

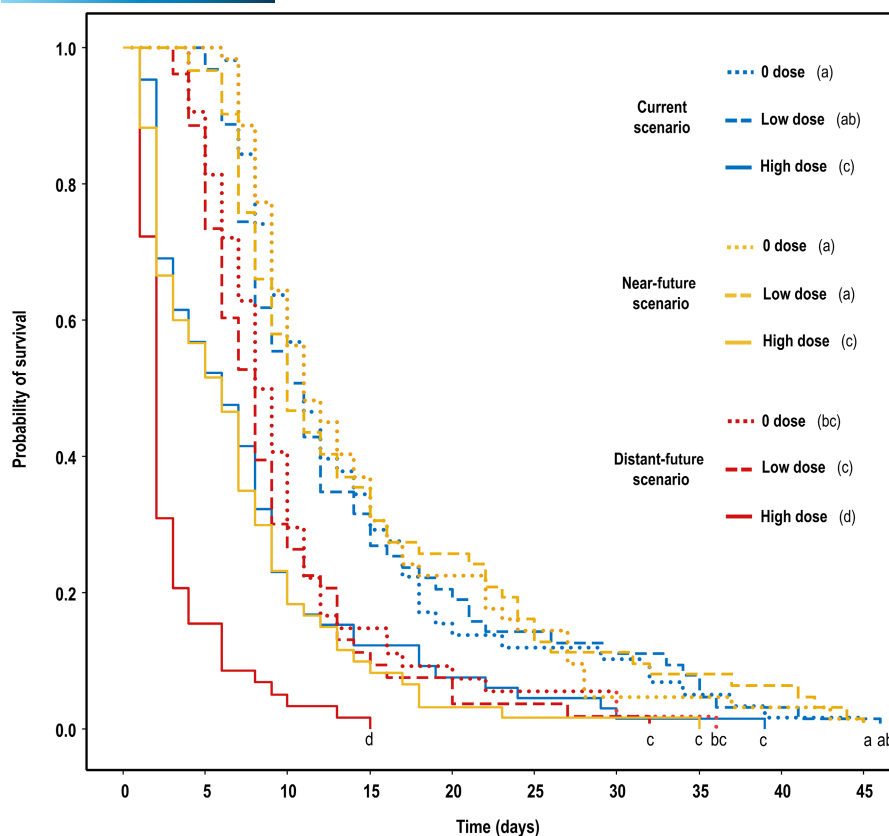


FIGURE 3 Cumulative survival probability of *Osmia cornuta* females exposed to three wintering treatments representing increasingly warm climate change scenarios, and three orally administered doses of the insecticide sulfoxaflor (0, 4.55, and 11.64 ng/bee). Curves with different letters are significantly different (pairwise comparisons, log-rank test, $p < .05$).

Contrary to this expectation, wintering treatment did not affect daily syrup consumption. So far, these findings suggest that the climate change scenarios tested would not compromise winter survival, feeding success and post-emergence performance in *O. cornuta*. However, even with similar feeding rates, bees wintered at the warmest temperature regime had significantly reduced mean longevity (10 days compared to 14–15 days in bees of the other two wintering scenarios), a strong predictor of realized fecundity in *Osmia* (Bosch & Vicens, 2006; Sgolastra et al., 2016). Longevity was also significantly affected by body size, with small individuals having shorter life spans. Adult body size in *Osmia* is directly dependent on the amount of food provision allocated to the larva by the nesting female (Bosch & Vicens, 2002). Therefore, historical declines in bee body size attributed to scarcity of floral resources in simplified landscapes may be further compromising bee longevity and realized fecundity (Chole et al., 2019; Grab et al., 2019; Herrera et al., 2023; Oliveira et al., 2016).

Like most solitary bees, *Osmia* spp. overwinter on a fixed budget, and experience a considerable depletion of lipid reserves during wintering (Fliszkiewicz et al., 2012; Sgolastra et al., 2011). Upon emergence, adults fly to nearby flowers and take a first, long, nectar meal that presumably allows them to rebuild their energetic resources (Bosch & Kemp, 2001; Nestel et al., 2016). In agricultural environments, however, the nectar ingested in this first meal may

contain sublethal levels of pesticides (David et al., 2016; Dively & Kamel, 2012; Heller et al., 2020; Hladik et al., 2016). To mimic this scenario, we exposed newly emerged bees to two sublethal doses of Closer, a widely used insecticide. The doses used correspond to concentrations of 0.58 and 0.23 ppm of active ingredient, which fall within the range of sulfoxaflor residues found in the nectar of various crop flowers 2–7 days after application (USEPA, 2019). Our results show that these realistic levels of exposure, especially the high dose, impaired the ability of bees to respond to light resulting in a drastic reduction of phototactic response in bees of the distant-future climate scenario (Figure 1). The inability to respond to light is indicative of an alteration of the sensitivity or the functioning of the visual system (Klein et al., 2017). Exposure to sublethal doses of imidacloprid, a neonicotinoid insecticide with a mode of action similar to sulfoxaflor (Sparks et al., 2013), increases the expression of genes related to biogenic amine receptors, such as serotonin, tyramine, and octopamine, associated with a lack of phototactic response in *Drosophila* (Martelli et al., 2020). High levels of octopamine have also been linked to reduced response to light in honey bees (Bloch & Meshi, 2007; Scheiner et al., 2014).

The interaction between wintering and pesticide exposure was also evident in the rate of daily syrup consumption. Exposure to the insecticide depressed feeding rates in bees of the three wintering treatments, but this decrease was especially pronounced in

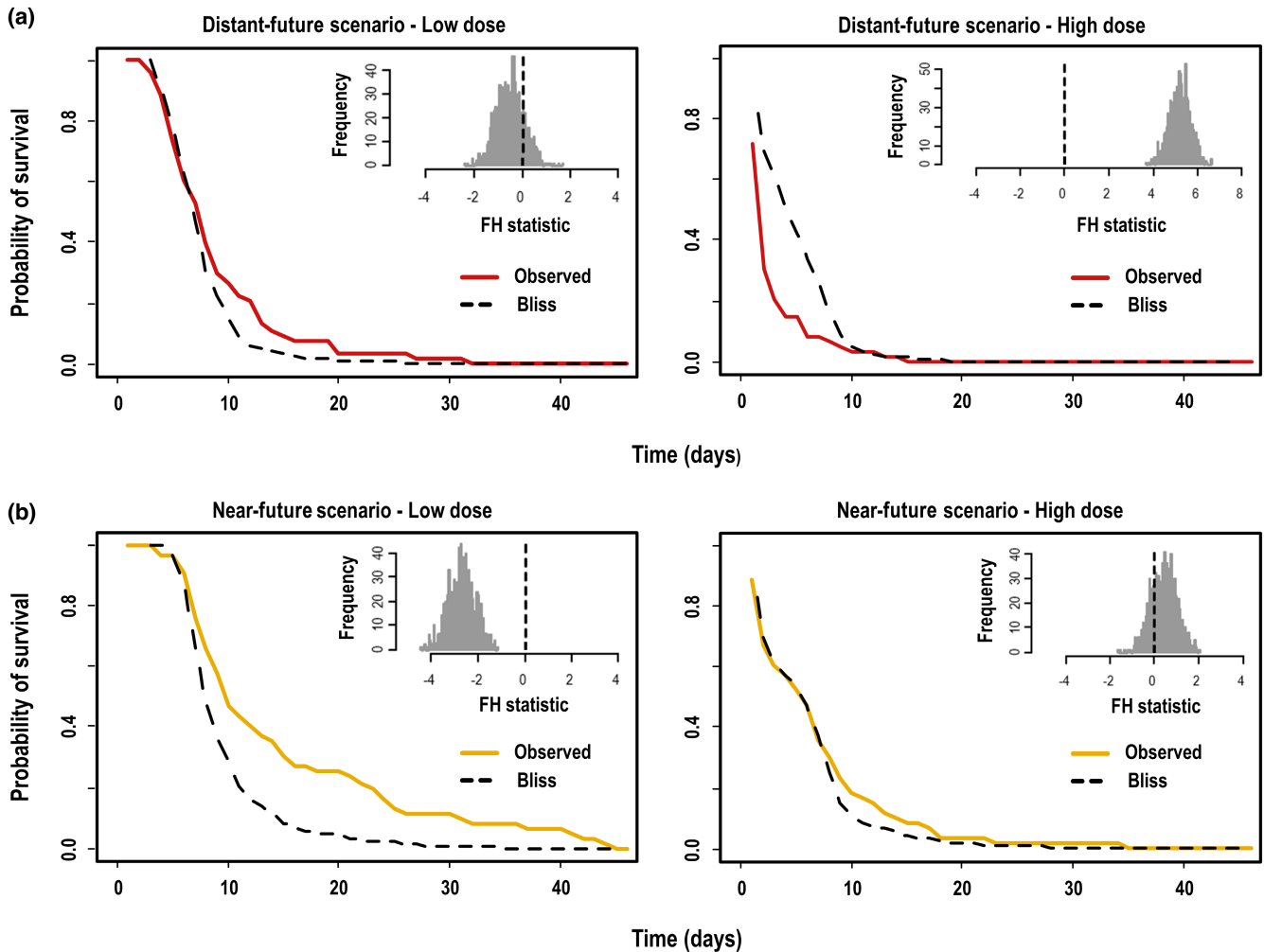


FIGURE 4 Observed (red/yellow lines) and expected (black dashed lines) survival probability curves of *Osmia cornuta* females exposed to distant- (a) and near-future (b) climate change scenarios and to two orally administered doses of the insecticide sulfoxaflor (4.55 and 11.64 ng/bee). Interaction significance is based on the Bliss criterion of drugs independence (FH statistic distribution >0 indicates synergism between the two stressors; FH statistic distribution <0 indicates antagonism).

bees exposed to the warmest winter (Figure 2A). The inhibitory effect of sulfoxaflor on syrup consumption has been documented in both solitary and social bees (Azpiazu et al., 2022; Li et al., 2021; Siviter et al., 2020). Longevity was reduced by ca. 50% in bees of the current and near-future scenarios exposed to the high insecticide dose. In bees of the distant-future scenario, exposure to the low dose led to an estimated 11% reduction in longevity and exposure to the high dose resulted in a synergistic 70% reduction (Figure 3). As mentioned, the cognitive abilities of the bees of the latter treatment were significantly impaired, further reducing the effective time frame available for reproduction (Bosch & Vicens, 2006; Sgolastra et al., 2016). We also found that bees exposed to the near-future scenario and the low insecticide dose lived longer than expected. However, this antagonistic effect did not result in an increase in longevity compared to bees of the near-future scenario exposed to the 0 insecticide dose. Antagonistic interactions are not uncommon in studies addressing interactions between stressors (Bird et al., 2021; Bruckner et al., 2023; Siviter et al., 2021; Zaragoza-Trello et al., 2021).

The increased sensitivity to Closer in bees exposed to climate warming scenarios may be mediated by the effects of warm winter temperatures on the consumption of energy reserves and fat body depletion in *Osmia* (Bosch et al., 2000, 2010; Bosch & Kemp, 2003, 2004; Fliszkiewicz et al., 2012; Sgolastra et al., 2011). The fat body is a dynamic, “liver-like”, tissue that acts as an organ of energy storage and other metabolic functions in insects (Arrese & Soulages, 2010). Studies on butterflies and mosquitoes have found that exposure to xenobiotics increases the expression of cytochrome P450 monooxygenases genes, involved in detoxification, in the fat body (David et al., 2006; Petersen et al., 2001). In addition, studies on bumblebees have found that the expression of these genes in the fat body is enhanced by a sucrose-rich diet (Costa et al., 2020), and that queens, which have significantly higher fat body reserves than males and workers, are more resilient to pesticides (Linguadoca et al., 2022). In honey bees, attack by the mite *Varroa destructor*, that feeds on the fat body of adults (Ramsey et al., 2019) disrupts detoxification ability and increases sensitivity

to pesticides (Blanken et al., 2015; Schwartz et al., 2021; Zhu et al., 2022). Based on this line of evidence, we suggest that the ability to produce detoxification enzymes to confront sublethal levels of insecticide is depressed in solitary bees emerging with depleted fat bodies as a result of warm winter temperatures.

Our findings indicate that ongoing global warming may exacerbate the impact of pesticides on bee health and compromise bee reproductive success, with potentially important consequences on population dynamics. The magnitude of these impacts will depend on our ability to reduce the dependence of agriculture on pesticides (Siviter et al., 2023; Sponsler et al., 2019) and on the extent to which bee populations are able to adjust to the new climate scenario through adaptation and/or phenotypic plasticity. The distribution of *O. cornuta* ranges from northern Africa to southern Sweden (Müller, 2022), suggesting that the species has the capacity to adapt to local climates. Temperature increases such as those simulated in our study are likely to have a stronger impact in populations from already warm environments, living closer to the thermal safety limits of the species, than in northern populations (CaraDonna et al., 2018). Bees may also mitigate the effects of climate warming by actively selecting cooler nesting sites, as shown in *O. bicornis* (Ostap-Chec et al., 2021), or through latitudinal or altitudinal migration to cooler areas (Kerr et al., 2015). At any rate, the fact that temperature increases similar to those of our most pessimistic scenario have been recorded in recent years (AEMET, 2021; Copernicus, 2020) suggests that the combined effects of climate warming and pesticide exposure are already affecting bee population dynamics.

ACKNOWLEDGMENTS

The authors are grateful to three anonymous reviewers for their constructive comments. The authors are also grateful to M. Barnadas, E. Serratosa and P. Soler for their technical assistance throughout the experiment. The study was funded by the Spanish Ministry of Science and Innovation, through projects RTI2018-098399-B-I00 and PID2021-128938OB-I00, and PhD scholarships to SA and GS (PRE2019-088817 and PRE2019-090375). CA was supported by a Margarita Salas postdoctoral fellowship from the Spanish Ministry of Universities through the EU NextGeneration program.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support findings of this study are available on Dryad at <https://doi.org/10.5061/dryad.dz08kps3f>.

ORCID

Sergio Albacete  <https://orcid.org/0000-0001-7696-623X>
 Gonzalo Sancho  <https://orcid.org/0000-0001-7711-846X>
 Celeste Azpiazu  <https://orcid.org/0000-0001-8217-9347>
 Anselm Rodrigo  <https://orcid.org/0000-0001-6341-0363>
 Roberto Molowny-Horas  <https://orcid.org/0000-0003-2626-6379>
 Fabio Sgolastra  <https://orcid.org/0000-0002-2845-8297>
 Jordi Bosch  <https://orcid.org/0000-0002-8088-9457>

REFERENCES

- Agencia Estatal de Meteorología (AEMET). (2021). 2020, the warmest year in Spain, in Europe and in the world. https://www.aemet.es/es/noticias/2022/10/septiembre_2022_resumen_clima
- Albacete, S., Sancho, G., Azpiazu, C., Rodrigo, A., Molowny-Horas, R., Sgolastra, F., & Bosch, J. (2023). Bees exposed to climate change are more sensitive to pesticides. *Dryad Dataset*. <https://doi.org/10.5061/dryad.dz08kps3f>
- Arrese, E. L., & Soulaiges, J. L. (2010). Insect fat body: Energy, metabolism, and regulation. *Annual Review of Entomology*, 55, 207–225. <https://doi.org/10.1146/annurev-ento-112408-085356>
- Aufauvre, J., Biron, D. G., Vidau, C., Fontbonne, R., Roudel, M., Diogon, M., Viguès, B., Belzunces, L. P., Delbac, F., & Blot, N. (2012). Parasite-insecticide interactions: A case study of *Nosema ceranae* and fipronil synergy on honeybee. *Scientific Reports*, 2(1), 326. <https://doi.org/10.1038/srep00326>
- Azpiazu, C., Bosch, J., Bortolotti, L., Medrzycki, P., Teper, D., Molowny-Horas, R., & Sgolastra, F. (2021). Toxicity of the insecticide sulfoxafloir alone and in combination with the fungicide fluxapyroxad in three bee species. *Scientific Reports*, 11(1), 6821. <https://doi.org/10.1038/s41598-021-86036-1>
- Azpiazu, C., Bosch, J., Martins, C., & Sgolastra, F. (2022). Effects of chronic exposure to the new insecticide sulfoxafloir in combination with a SDHI fungicide in a solitary bee. *Science of the Total Environment*, 850, 157822. <https://doi.org/10.1016/j.scitotenv.2022.157822>
- Azpiazu, C., Bosch, J., Viñuela, E., Medrzycki, P., Teper, D., & Sgolastra, F. (2019). Chronic oral exposure to field-realistic pesticide combinations via pollen and nectar: Effects on feeding and thermal performance in a solitary bee. *Scientific Reports*, 9(1), 13770. <https://doi.org/10.1038/s41598-019-50255-4>
- Azpiazu, C., Hinarejos, S., Sancho, G., Albacete, S., Sgolastra, F., Martins, C. A. H., Domene, X., Benrezkallah, J., Rodrigo, A., Anran, X., & Bosch, J. (2023). Description and validation of an improved method to feed solitary bees (*Osmia* spp.) known amounts of pesticides. *Ecotoxicology and Environmental Safety*, 264, 115398.
- Bird, G., Wilson, A. E., Williams, G. R., & Hardy, N. B. (2021). Parasites and pesticides act antagonistically on honey bee health. *Journal of Applied Ecology*, 58(5), 997–1005. <https://doi.org/10.1111/1365-2664.13811>
- Blanckenhorn, W. U., & Henseler, C. (2005). Temperature-dependent ovariole and testis maturation in the yellow dung fly. *Entomologia Experimentalis et Applicata*, 116(3), 159–165. <https://doi.org/10.1111/j.1570-7458.2005.00316.x>
- Blanken, L. J., van Langevelde, F., & van Dooremalen, C. (2015). Interaction between *Varroa destructor* and imidacloprid reduces flight capacity of honeybees. *Proceedings of the Royal Society B: Biological Sciences*, 282(1820), 20151738. <https://doi.org/10.1098/rspb.2015.1738>
- Bloch, G., & Meshi, A. (2007). Influences of octopamine and juvenile hormone on locomotor behavior and period gene expression in the honeybee, *Apis mellifera*. *Journal of Comparative Physiology A*, 193, 181–199. <https://doi.org/10.1007/s00359-006-0179-5/tables/6>
- Bosch, J. (1994). The nesting behaviour of the mason bee *Osmia cornuta* (Latr) with special reference to its pollinating potential (Hymenoptera, Megachilidae). *Apidologie*, 25(1), 84–93. <https://doi.org/10.1051/apido:19940109>
- Bosch, J., & Blas, M. (1994a). Foraging behaviour and pollinating efficiency of *Osmia cornuta* and *Apis mellifera* on almond (Hymenoptera, Megachilidae and Apidae). *Applied Entomology and Zoology*, 29(1), 1–9. <https://doi.org/10.1303/aez.29.1>
- Bosch, J., & Blas, M. (1994b). Effect of over-wintering and incubation temperatures on adult emergence in *Osmia cornuta* Latr. (Hymenoptera, Megachilidae). *Apidologie*, 25(3), 265–277. <https://doi.org/10.1051/apido:19940301>

- Bosch, J., & Kemp, W. P. (2000). Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology*, 29(1), 8–13. <https://doi.org/10.1603/0046-225x-29.1.8>
- Bosch, J., & Kemp, W. P. (2001). *How to manage the blue orchard bee as an orchard pollinator. Handbook series book 5. Sustainable Agriculture Network.*
- Bosch, J., & Kemp, W. P. (2002). Developing and establishing bee, species as crop pollinators: The example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. *Bulletin of Entomological Research*, 92(1), 3–16. <https://doi.org/10.1079/ber2001139>
- Bosch, J., & Kemp, W. P. (2003). Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology*, 32(4), 711–716. <https://doi.org/10.1603/0046-225x-32.4.711>
- Bosch, J., & Kemp, W. P. (2004). Effect of pre-wintering and wintering temperature regimes on weight loss, survival, and emergence time in the mason bee *Osmia cornuta* (Hymenoptera: Megachilidae). *Apidologie*, 35(5), 469–479. <https://doi.org/10.1051/apido:2004035>
- Bosch, J., Kemp, W. P., & Peterson, S. S. (2000). Management of *Osmia lignaria* (Hymenoptera: Megachilidae) populations for almond pollination: Methods to advance bee emergence. *Environmental Entomology*, 29(5), 874–883. <https://doi.org/10.1603/0046-225x-29.5.874>
- Bosch, J., Sgolastra, F., & Kemp, W. P. (2010). Timing of eclosion affects diapause development, fat body consumption and longevity in *Osmia lignaria*, a univoltine, adult-wintering solitary bee. *Journal of Insect Physiology*, 56(12), 1949–1957. <https://doi.org/10.1016/j.jinsphys.2010.08.017>
- Bosch, J., & Vicens, N. (2002). Body size as an estimator of production costs in a solitary bee. *Ecological Entomology*, 27(2), 129–137. <https://doi.org/10.1046/j.1365-2311.2002.00406.x>
- Bosch, J., & Vicens, N. (2006). Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behavioral Ecology and Sociobiology*, 60, 26–33. <https://doi.org/10.1007/s00265-005-0134-4>
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85(7), 1771–1789. <https://doi.org/10.1890/03-9000>
- Bruckner, S., Straub, L., Neumann, P., & Williams, G. R. (2023). Negative but antagonistic effects of neonicotinoid insecticides and ectoparasitic mites *Varroa destructor* on *Apis mellifera* honey bee food glands. *Chemosphere*, 313, 137535. <https://doi.org/10.1016/j.chemosphere.2022.137535>
- CaraDonna, P. J., Cunningham, J. L., & Iler, A. M. (2018). Experimental warming in the field delays phenology and reduces body mass, fat content and survival: Implications for the persistence of a pollinator under climate change. *Functional Ecology*, 32(10), 2345–2356. <https://doi.org/10.1111/1365-2435.13151>
- Castelli, L., Branchiccela, B., Garrido, M., Invernizzi, C., Porrini, M., Romero, H., Santos, E., Zunino, P., & Antúnez, K. (2020). Impact of nutritional stress on honeybee gut microbiota, immunity, and *Nosema ceranae* infection. *Microbial Ecology*, 80, 908–919. <https://doi.org/10.1007/s00248-020-01538-1>
- Castle, D., Alkassab, A. T., Bischoff, G., Steffan-Dewenter, I., & Pistorius, J. (2022). High nutritional status promotes vitality of honey bees and mitigates negative effects of pesticides. *Science of the Total Environment*, 806, 151280. <https://doi.org/10.1016/j.scitotenv.2021.151280>
- Chole, H., Woodard, S. H., & Bloch, G. (2019). Body size variation in bees: Regulation, mechanisms, and relationship to social organization. *Current Opinion in Insect Science*, 35, 77–87. <https://doi.org/10.1016/j.cois.2019.07.006>
- Copernicus Climate Change Service (Copernicus). (2020). *The boreal winter season 19/20 was by far the warmest winter season ever recorded in Europe.* <https://climate.copernicus.eu/boreal-winter-season-1920-was-far-warmest-winter-season-ever-recorded-europe-0>
- Costa, C. P., Duennes, M. A., Fisher, K., Der, J. P., Watrous, K. M., Okamoto, N., Yamanaka, N., & Woodard, S. H. (2020). Transcriptome analysis reveals nutrition- and age-related patterns of gene expression in the fat body of pre-overwintering bumble bee queens. *Molecular Ecology*, 29(4), 720–737. <https://doi.org/10.1111/mec.15361>
- Cribari-Neto, F., & Zeileis, A. (2010). Beta regression in R. *Journal of Statistical Software*, 34, 1–24. <https://doi.org/10.18637/jss.v034.i02>
- David, A., Botías, C., Abdul-Sada, A., Nicholls, E., Rotheray, E. L., Hill, E. M., & Goulson, D. (2016). Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environment International*, 88, 169–178. <https://doi.org/10.1016/j.envint.2015.12.011>
- David, J. P., Boyer, S., Mesneau, A., Ball, A., Ranson, H., & Dauphin-Villemant, C. (2006). Involvement of cytochrome P450 monooxygenases in the response of mosquito larvae to dietary plant xenobiotics. *Insect Biochemistry and Molecular Biology*, 36(5), 410–420. <https://doi.org/10.1016/j.ibmb.2006.02.004>
- Demidenko, E., & Miller, T. W. (2019). Statistical determination of synergy based on bliss definition of drugs independence. *PLoS One*, 14(11), e0224137. <https://doi.org/10.1371/journal.pone.0224137>
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 105(18), 6668–6672. <https://doi.org/10.1073/pnas.070947210>
- Dively, G. P., & Kamel, A. (2012). Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. *Journal of Agricultural and Food Chemistry*, 60(18), 4449–4456. <https://doi.org/10.1021/jf205393x>
- Dorian, N. N., McCarthy, M. W., & Crone, E. E. (2022). Ecological traits explain long-term phenological trends in solitary bees. *Journal of Animal Ecology*, 92(2), 285–296. <https://doi.org/10.1111/1365-2656.13778>
- Doublet, V., Labarussias, M., de Miranda, J. R., Moritz, R. F. A., & Paxton, R. J. (2015). Bees under stress: Sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environmental Microbiology*, 17(4), 969–983. <https://doi.org/10.1111/1462-2920.12426>
- European Food Safety Authority (EFSA). (2013). Guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). *EFSA Journal*, 11(7), 3295. <https://doi.org/10.2903/j.efsa.2013.3295>
- Fischer, K., Klockmann, M., & Reim, E. (2014). Strong negative effects of simulated heat waves in a tropical butterfly. *Journal of Experimental Biology*, 217(16), 2892–2898. <https://doi.org/10.1242/jeb.106245>
- Fliszkiewicz, M., Giejdasz, K., Wasielewski, O., & Krishnan, N. (2012). Influence of winter temperature and simulated climate change on body mass and fat body depletion during diapause in adults of the solitary bee, *Osmia rufa* (Hymenoptera: Megachilidae). *Environmental Entomology*, 41(6), 1621–1630. <https://doi.org/10.1603/EN12004>
- González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R. A., & Villalobos, F. (2020). Insect responses to heat: Physiological mechanisms, evolution and ecological implications in a warming world. *Biological Reviews*, 95(3), 802–821. <https://doi.org/10.1111/BRV.12588>
- Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347(6229), 1255957. <https://doi.org/10.1126/science.1255957>
- Grab, H., Brokaw, J., Anderson, E., Gedlinske, L., Gibbs, J., Wilson, J., Loeb, G., Isaacs, R., & Poveda, K. (2019). Habitat enhancements rescue bee body size from the negative effects of landscape simplification. *Journal of Applied Ecology*, 56(9), 2144–2154. <https://doi.org/10.1111/1365-2664.13456>

- Grassl, J., Holt, S., Cremen, N., Peso, M., Hahne, D., & Baer, B. (2018). Synergistic effects of pathogen and pesticide exposure on honey bee (*Apis mellifera*) survival and immunity. *Journal of Invertebrate Pathology*, 159, 78–86. <https://doi.org/10.1016/j.jip.2018.10.005>
- Hahn, D. A., & Denlinger, D. L. (2007). Meeting the energetic demands of insect diapause: Nutrient storage and utilization. *Journal of Insect Physiology*, 53(8), 760–773. <https://doi.org/10.1016/j.jinsphys.2007.03.018>
- Heller, S., Joshi, N. K., Chen, J., Rajotte, E. G., Mullin, C., & Biddinger, D. J. (2020). Pollinator exposure to systemic insecticides and fungicides applied in the previous fall and pre-bloom period in apple orchards. *Environmental Pollution*, 265, 114589. <https://doi.org/10.1016/j.envpol.2020.114589>
- Herrera, C. M., Núñez, A., Valverde, J., & Alonso, C. (2023). Body mass decline in a Mediterranean community of solitary bees supports the size shrinking effect of climatic warming. *Ecology*, e4128. <https://doi.org/10.1002/ecy.4128>
- Hladik, M. L., Vandever, M., & Smalling, K. L. (2016). Exposure of native bees foraging in an agricultural landscape to current-use pesticides. *Science of the Total Environment*, 542, 469–477. <https://doi.org/10.1016/j.scitotenv.2015.10.077>
- IPBES. (2021). *Scoping report for a thematic assessment of the underlying causes of biodiversity loss and the determinants of transformative change and options for achieving the 2050 vision for biodiversity (transformative change assessment). Annex II to decision IPBES/8/1. IPBES Secretariat.*
- Jaumejoan, X., Arnan, X., Hagenbucher, S., Rodrigo, A., Sédivy, C., & Bosch, J. (2023). Different effects of local and landscape context on pollen foraging decisions by two managed orchard pollinators, *Osmia cornuta* and *Bombus terrestris*. *Agriculture, Ecosystems & Environment*, 353, 108528. <https://doi.org/10.1016/j.agee.2023.108528>
- Jean, C., Coderre, D., & Tourneur, J. C. (1990). Effects of temperature and substrate on survival and lipid consumption of hibernating *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae). *Environmental Entomology*, 19(6), 1657–1662. <https://doi.org/10.1093/EE/19.6.1657>
- Kassambara, A., Kosinski, M., Biecek, P., & Fabian, S. (2020). *Survminer: Drawing Survival Curves using "ggplot2"*. R package version 0.4, 8, 556. <https://CRAN.R-project.org/package=survminer>
- Keeley, L. L. (1985). Physiology and biochemistry of the fat body. In G. A. Kerkut & L. I. Gilbert (Eds.), *Comprehensive insect physiology, biochemistry and pharmacology* (Vol. 3, pp. 211–248). Pergamon Press.
- Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., Rasmont, P., Schweiger, O., Colla, S. R., Richardson, L. L., Wagner, D. L., Gall, L. F., Sikes, D., & Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science*, 349(6244), 177–180. <https://doi.org/10.1126/science.aaa7031>
- Kierat, J., Szentgyörgyi, H., Czarnoleski, M., & Woyciechowski, M. (2017). The thermal environment of the nest affects body and cell size in the solitary red mason bee (*Osmia bicornis* L.). *Journal of Thermal Biology*, 68, 39–44. <https://doi.org/10.1016/j.jtherbio.2016.11.008>
- Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274(1608), 303–313. <https://doi.org/10.1098/rspb.2006.3721>
- Klein, S., Cabirol, A., Devaud, J. M., Barron, A. B., & Lihoreau, M. (2017). Why bees are so vulnerable to environmental stressors. *Trends in Ecology & Evolution*, 32(4), 268–278. <https://doi.org/10.1016/j.tree.2016.12.009>
- Klepsatel, P., Gálíková, M., Xu, Y., & Kühnlein, R. P. (2016). Thermal stress depletes energy reserves in drosophila. *Scientific Reports*, 6(1), 1–12. <https://doi.org/10.1038/srep33667>
- Knapp, J. L., Nicholson, C. C., Jonsson, O., de Miranda, J. R., & Rundlöf, M. (2023). Ecological traits interact with landscape context to determine bees' pesticide risk. *Nature Ecology & Evolution*, 7(4), 547–556. <https://doi.org/10.1038/s41559-023-01990-5>
- Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2019). *Emmeans: Estimated marginal means, aka least-squares means*. R package version, 1(3.4). <https://CRAN.R-project.org/package=emmeans>
- Li, J., Zhao, L., Qi, S., Zhao, W., Xue, X., Wu, L., & Huang, S. (2021). Sublethal effects of Isoclast™ active (50% sulfoxaflor water dispersible granules) on larval and adult worker honey bees (*Apis mellifera* L.). *Ecotoxicology and Environmental Safety*, 220, 112379. <https://doi.org/10.1016/j.ecoenv.2021.112379>
- Linguadoca, A., Jürison, M., Hellström, S., Straw, E. A., Šima, P., Karise, R., Costa, C., Serra, G., Colombo, R., Paxton, R. J., Mänd, M., & Brown, M. J. F. (2022). Intra-specific variation in sensitivity of *Bombus terrestris* and *Osmia bicornis* to three pesticides. *Scientific Reports*, 12(1), 17311. <https://doi.org/10.1038/s41598-022-22239-4>
- Linguadoca, A., Rizzi, C., Villa, S., & Brown, M. J. F. (2021). Sulfoxaflor and nutritional deficiency synergistically reduce survival and fecundity in bumblebees. *Science of the Total Environment*, 795, 148680. <https://doi.org/10.1016/j.scitotenv.2021.148680>
- Lycett, G. J., McLaughlin, L. A., Ranson, H., Hemingway, J., Kafatos, F. C., Loukeris, T. G., & Paine, M. J. I. (2006). *Anopheles gambiae* P450 reductase is highly expressed in oenocytes and in vivo knockdown increases permethrin susceptibility. *Insect Molecular Biology*, 15(3), 321–327. <https://doi.org/10.1111/j.1365-2583.2006.00647.x>
- Martelli, F., Zhongyuan, Z., Wang, J., Wong, C. O., Karagas, N. E., Roessner, U., Rupasinghe, T., Venkatachalam, K., Perry, T., Bellen, H. J., & Batterham, P. (2020). Low doses of the neonicotinoid insecticide imidacloprid induce ROS triggering neurological and metabolic impairments in drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, 117(41), 25840–25850. https://doi.org/10.1073/pnas.2011828117/suppl_file/pnas.2011828117.sapp.pdf
- Moss, R. H., Edmonds, J. A., Hibbard, K. A., Manning, M. R., Rose, S. K., Van Vuuren, D. P., Carter, T. R., Emori, S., Kainuma, M., Kram, T., Meehl, G. A., Mitchell, J. F. B., Nakicenovic, N., Riahi, K., Smith, S. J., Stouffer, R. J., Thomson, A. M., Weyant, J. P., & Wilbanks, T. J. (2010). The next generation of scenarios for climate change research and assessment. *Nature*, 463(7282), 747–756. <https://doi.org/10.1038/nature08823>
- Müller, A. (2022). *Palaeartic osmiine bees*. ETH Zürich. <http://blogs.ethz.ch/osmiini>
- Nestel, D., Papadopoulos, N. T., Pascacio-Villafán, C., Righini, N., Altuzar-Molina, A. R., & Aluja, M. (2016). Resource allocation and compensation during development in holometabolous insects. *Journal of Insect Physiology*, 95, 78–88. <https://doi.org/10.1016/J.JINSPHYS.2016.09.010>
- Neven, L. G. (2000). Physiological responses of insects to heat. *Postharvest Biology and Technology*, 21(1), 103–111. [https://doi.org/10.1016/S0925-5214\(00\)00169-1](https://doi.org/10.1016/S0925-5214(00)00169-1)
- Oliveira, M. O., Freitas, B. M., Scheper, J., & Kleijn, D. (2016). Size and sex-dependent shrinkage of dutch bees during one and a half centuries of land-use change. *PLoS One*, 11(2), e0148983. <https://doi.org/10.1371/journal.pone.0148983>
- Oller, R., & Langohr, K. (2017). FHtest: An R package for the comparison of survival curves with censored data. *Journal of Statistical Software*, 81, 1–25. <https://doi.org/10.18637/jss.v081.i15>
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321–326. <https://doi.org/10.1111/j.1600-0706.2010.18644.x>
- Ostap-Chec, M., Kierat, J., Kuszewska, K., & Woyciechowski, M. (2021). Red mason bee (*Osmia bicornis*) thermal preferences for nest sites and their effects on offspring survival. *Apidologie*, 52(3), 707–719. <https://doi.org/10.1007/s13592-021-00858-6>
- Petersen, R. A., Zangerl, A. R., Berenbaum, M. R., & Schuler, M. A. (2001). Expression of CYP6B1 and CYP6B3 cytochrome P450 monooxygenases and furanocoumarin metabolism in different tissues of *Papilio polyxenes* (Lepidoptera: Papilionidae). *Insect Biochemistry*

- and Molecular Biology, 31(6–7), 679–690. [https://doi.org/10.1016/S0965-1748\(00\)00174-0](https://doi.org/10.1016/S0965-1748(00)00174-0)
- Pettis, J. S., Vanengelsdorp, D., Johnson, J., & Dively, G. (2012). Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften*, 99, 153–158. <https://doi.org/10.1007/S00114-011-0881-1/tables/1>
- Portner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 132(4), 739–761. [https://doi.org/10.1016/S1095-6433\(02\)00045-4](https://doi.org/10.1016/S1095-6433(02)00045-4)
- Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345–353. <https://doi.org/10.1016/j.tree.2010.01.007>
- R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.r-project.org/>
- Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbranson, C., Mowery, J. D., Cohen, A., Lim, D., Joklik, J., Cicero, J. M., Ellis, J. D., Hawthorne, D., & Van Engelsdorp, D. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences of the United States of America*, 116(5), 1792–1801. <https://doi.org/10.1073/pnas.1818371116>
- Sanchez-Bayo, F., & Goka, K. (2014). Pesticide residues and bees—A risk assessment. *PLoS One*, 9(4), e94482. <https://doi.org/10.1371/journal.pone.0094482>
- Sánchez-Bayo, F., & Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, 232, 8–27. <https://doi.org/10.1016/j.biocon.2019.01.020>
- Scheiner, R., Toteva, A., Reim, T., Søvik, E., & Barron, A. B. (2014). Differences in the phototaxis of pollen and nectar foraging honey bees are related to their octopamine brain titers. *Frontiers in Physiology*, 5, 116. <https://doi.org/10.3389/fphys.2014.00116/bibtex>
- Schwartz, K. R., Minor, H., Magro, C., McConnell, J., Capani, J., Griffin, J., & Doebel, H. (2021). The neonicotinoid imidacloprid alone alters the cognitive behavior in *Apis mellifera* L. and the combined exposure of imidacloprid and *Varroa destructor* mites synergistically contributes to trial attrition. *Journal of Apicultural Research*, 60(3), 431–438. <https://doi.org/10.1080/00218839.2020.1866233>
- Sgolastra, F., Arnan, X., Cabbri, R., Isani, G., Medrzycki, P., Teper, D., & Bosch, J. (2018). Combined exposure to sublethal concentrations of an insecticide and a fungicide affect feeding, ovary development and longevity in a solitary bee. *Proceedings of the Royal Society B*, 285(1885), 20180887. <https://doi.org/10.1098/rspb.2018.0887>
- Sgolastra, F., Arnan, X., Pitts-Singer, T. L., Maini, S., Kemp, W. P., & Bosch, J. (2016). Pre-wintering conditions and post-winter performance in a solitary bee: Does diapause impose an energetic cost on reproductive success? *Ecological Entomology*, 41(2), 201–210. <https://doi.org/10.1111/een.12292>
- Sgolastra, F., Bosch, J., Molowny-Horas, R., Maini, S., & Kemp, W. P. (2010). Effect of temperature regime on diapause intensity in an adult-wintering hymenopteran with obligate diapause. *Journal of Insect Physiology*, 56(2), 185–194. <https://doi.org/10.1016/j.jinsphys.2009.10.001>
- Sgolastra, F., Hinarejos, S., Pitts-Singer, T. L., Boyle, N. K., Joseph, T., Lückmann, J., Raine, N. E., Singh, R., Williams, N. M., & Bosch, J. (2019). Pesticide exposure assessment paradigm for solitary bees. *Environmental Entomology*, 48(1), 22–35. <https://doi.org/10.1093/ee/nvy105>
- Sgolastra, F., Kemp, W. P., Buckner, J. S., Pitts-Singer, T. L., Maini, S., & Bosch, J. (2011). The long summer: Pre-wintering temperatures affect metabolic expenditure and winter survival in a solitary bee. *Journal of Insect Physiology*, 57(12), 1651–1659. <https://doi.org/10.1016/j.jinsphys.2011.08.017>
- Sgolastra, F., Kemp, W. P., Maini, S., & Bosch, J. (2012). Duration of prepupal summer dormancy regulates synchronization of adult diapause with winter temperatures in bees of the genus *Osmia*. *Journal of Insect Physiology*, 58(7), 924–933. <https://doi.org/10.1016/j.jinsphys.2012.04.008>
- Siviter, H., Bailes, E. J., Martin, C. D., Oliver, T. R., Koricheva, J., Leadbeater, E., & Brown, M. J. F. (2021). Agrochemicals interact synergistically to increase bee mortality. *Nature*, 596(7872), 389–392. <https://doi.org/10.1038/s41586-021-03787-7>
- Siviter, H., Horner, J., Brown, M. J. F., & Leadbeater, E. (2020). Sulfoxaflor exposure reduces egg laying in bumblebees *Bombus terrestris*. *Journal of Applied Ecology*, 57(1), 160–169. <https://doi.org/10.1111/1365-2664.13519>
- Siviter, H., Linguadoca, A., Ippolito, A., & Muth, F. (2023). Pesticide licensing in the EU and protecting pollinators. *Current Biology*, 33(2), R44–R48. <https://doi.org/10.1016/j.cub.2022.12.002>
- Skowronek, P., Wójcik, Ł., Strachecka, A., & Brivio, F. (2021). Fat body-multifunctional insect tissue. *Insects*, 12(6), 547. <https://doi.org/10.3390/insects12060547>
- Sparks, T. C., Watson, G. B., Loso, M. R., Geng, C., Babcock, J. M., & Thomas, J. D. (2013). Sulfoxaflor and the sulfoximine insecticides: Chemistry, mode of action and basis for efficacy on resistant insects. *Pesticide Biochemistry and Physiology*, 107(1), 1–7. <https://doi.org/10.1016/j.pestbp.2013.05.014>
- Sponsler, D. B., Grozinger, C. M., Hitaj, C., Rundlöf, M., Botías, C., Code, A., Lonsdorf, E. V., Melathopoulos, A. P., Smith, D. J., Suryanarayanan, S., Thogmartin, W. G., Williams, N. M., Zhang, M., & Douglas, M. R. (2019). Pesticides and pollinators: A socioecological synthesis. *Science of the Total Environment*, 662, 1012–1027. <https://doi.org/10.1016/j.scitotenv.2019.01.016>
- Storey, K. B., & Storey, J. M. (2004). Metabolic rate depression in animals: Transcriptional and translational controls. *Biological Reviews*, 79(1), 207–233. <https://doi.org/10.1017/S1464793103006195>
- Therneau, T., Crowson, C., & Atkinson, E. (2020). *Multi-state models and competing risks*. <https://cran.r-project.org/web/packages/survival/vignettes/compete.pdf>
- Thompson, H. (2016). Extrapolation of acute toxicity across bee species. *Integrated Environmental Assessment and Management*, 12(4), 622–626. <https://doi.org/10.1002/ieam.1737>
- Tong, L., Nieh, J. C., & Tosi, S. (2019). Combined nutritional stress and a new systemic pesticide (flupyradifurone, Sivanto®) reduce bee survival, food consumption, flight success, and thermoregulation. *Chemosphere*, 237, 124408. <https://doi.org/10.1016/j.chemosphere.2019.124408>
- Tosi, S., Nieh, J. C., Sgolastra, F., Cabbri, R., & Medrzycki, P. (2017). Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proceedings of the Royal Society B: Biological Sciences*, 284(1869), 20171711. <https://doi.org/10.1098/RSPB.2017.1711>
- United States Environmental Protection Agency (USEPA). (2019). *Ecological risk assessment for the registration review of sulfoxaflor*. United States Environmental Protection Agency.
- Vanbergen, A. J. (2021). A cocktail of pesticides, parasites and hunger leaves bees down and out. *Nature*, 596, 351–352. <https://doi.org/10.1038/d41586-021-02079-4>
- Vesterlund, S. R., Lilley, T. M., van Ooik, T., & Sorvari, J. (2014). The effect of overwintering temperature on the body energy reserves and phenoloxidase activity of bumblebee *Bombus lucorum* queens. *Insectes Sociaux*, 61, 265–272. <https://doi.org/10.1007/S00040-014-0351-9/figures/6>
- Vidau, C., Diogon, M., Aufauvre, J., Gis Fontbonne, R., Viguè, B., Brunet, J.-L., Texier, C., Biron, D. G., Blot, N., El Alaoui, H., Belzunces, L. P., & Delbac, F. (2011). Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLoS One*, 6, e21550. <https://doi.org/10.1371/journal.pone.0021550>
- Williams, C. M., Marshall, K. E., MacMillan, H. A., Dzurisin, J. D. K., Hellmann, J. J., & Sinclair, B. J. (2012). Thermal variability increases the impact of autumnal warming and drives metabolic depression

- in an overwintering butterfly. *PLoS One*, 7(3), e34470. <https://doi.org/10.1371/journal.pone.0034470>
- Zaragoza-Trello, C., Vilà, M., Botías, C., & Bartomeus, I. (2021). Interactions among global change pressures act in a non-additive way on bumblebee individuals and colonies. *Functional Ecology*, 35(2), 420–434. <https://doi.org/10.1111/1365-2435.13703>
- Zhang, W., Chang, X. Q., Hoffmann, A. A., Zhang, S., & Ma, C. S. (2015). Impact of hot events at different developmental stages of a moth: The closer to adult stage, the less reproductive output. *Scientific Reports*, 5(1), 1–9. <https://doi.org/10.1038/srep10436>
- Zhu, Y. C., Yao, J., & Wang, Y. (2022). Varroa mite and deformed wing virus infestations interactively make honey bees (*Apis mellifera*) more susceptible to insecticides. *Environmental Pollution*, 292, 118212. <https://doi.org/10.1016/j.envpol.2021.118212>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Albacete, S., Sancho, G., Azpiazu, C., Rodrigo, A., Molowny-Horas, R., Sgolastra, F., & Bosch, J. (2023). Bees exposed to climate change are more sensitive to pesticides. *Global Change Biology*, 29, 6248–6260. <https://doi.org/10.1111/gcb.16928>