



PoshBee

Manuscript on the nutritional mitigation of agrochemical effects in bees

Deliverable 5.2

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**Pan-european assessment, monitoring, and mitigation
of stressors on the health of bees**



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Summary

A reduction in floral resource abundance and diversity due to landscape simplification and habitat loss is generally observed in agro-ecosystems, along with widespread exposure to pesticides. Therefore, a better understanding of how the availability and quality of pollen diets can modulate bee sensitivity to pesticides is required. As bees can show interspecific variation in pesticide sensitivity and nutritional requirements, it is also important to test the pesticide/diet interaction on different bee species. To this end, we considered three species, *Apis mellifera*, *Bombus terrestris* and *Osmia bicornis*, the three most important managed pollinators in Europe. Protocols were adapted according to previous experiments evaluating specific constraints of the three species under laboratory conditions, resulting in 3 independent studies.

In the first experiment on *Apis mellifera*, we evaluated the toxicity of an acute single exposure and chronic exposure to field realistic and higher concentrations of the fungicide azoxystrobin and the insecticide sulfoxaflor in workers provided with pollen diets of differing qualities: *Salix* pollen (S pollen) as an average pollen diet, and Brassicaceae/*Quercus* (BQ pollen) as a high quality diet.

In the second study on *Bombus terrestris*, we assessed the impact of the interaction between nutritional and agrochemical stresses on development and resource collection. We used diets of different quality (one poor diet, *Cistus* pollen, C pollen, and one good diet, *Salix* pollen) and quantity (starvation treatment), and we exposed bumble bee micro-colonies chronically to different concentrations of insecticides (Sulfoxaflor, Cyantraniliprole), fungicide (Amistar® with azoxystrobin as active compound) or herbicide (Glyphosate).

In the third experiment, we investigated the development and survival of solitary Red mason bee *Osmia bicornis* larvae provisioned with four distinct pollen provision types and exposed to sulfoxaflor or azoxystrobin in a full-factorial design in the laboratory.

We found that pollen intake (vs no pollen) reduced the toxicity of the acute concentrations of pesticides for *Apis mellifera*. Contrary to azoxystrobin, chronic exposure to sulfoxaflor increased by 12-fold bee mortality, which was reduced by pollen intake. Most importantly, the risk of death upon exposure to a high concentration of sulfoxaflor was significantly lower for the S pollen diet as compared to the BQ pollen diet. This reduced pesticide toxicity was associated with higher expression of the vitellogenin gene, which produces a glycoprotein that promotes bee longevity and protects bees from oxidative stress, faster sulfoxaflor metabolism, and a lower concentration of the phytochemical p-coumaric acid, previously found to upregulate detoxification enzymes.

In *B. terrestris*, we found that Amistar® and glyphosate had no impact on development and resource collection. On the contrary, we observed effects of both sulfoxaflor (1000 and 2000ppb) and cyantraniliprole on every parameter. Interestingly, the impact of nutritional stress on these effects was different depending on the insecticide. When stressed from sulfoxaflor exposure, micro-colonies fed with a low-quality pollen diet (*Cistus*) were more sensitive to the agrochemical stress compared to those fed with high-quality pollen (*Salix*). On the other hand, micro-colonies exposed to Cyantraniliprole were affected similarly regardless of the pollen diet, indicating that the good diet cannot compensate for the impact of Cyantraniliprole on micro-colonies performances. Our results highlight that diet effects on bumble bees can be different depending on the stress level caused by agrochemicals.

We discovered pronounced sublethal negative effects of sulfoxaflor on *O. bicornis* development: sulfoxaflor reduced survival, cocoon weight, pollen efficacy and pollen consumption and elongated development time. We further found indications that azoxystrobin might negatively affect survival and development time. Our results on mason bees do not support the hypothesis that a more diverse pollen nutrition mitigates the observed negative effects of the pesticides.

Overall, our studies revealed that pollen quality can influence the ability of honey bees to metabolize pesticides and withstand their detrimental effects, providing another strong argument for the restoration of suitable foraging habitat. However, we did not find this effect in *Bombus terrestris*

colonies and we observed a mitigated effect only for the good pollen diet in the experiment with insecticide exposure on *Osmia bicornis* larvae. Our results highlight the importance of diverse floral resources for bee development as well as minimising pesticide exposure. Moreover, we showed the need for targeted studies of pesticide exposure alone, and in combination with variable nutrition, on all life history stages of bees.

1. Introduction

The availability of nutritive resources has long been acknowledged as a key ecological factor affecting the expression of several life-history traits (Simpson & Raubenheimer 2012). Notably, the quantity and balance of macro- and micronutrients, as well as secondary metabolites, in the diet of insects, can determine their longevity and ability to respond to environmental pressures, such as xenobiotics (Simpson & Raubenheimer 2012). For instance, variation in the protein:carbohydrate ratio can modulate their sensitivity to toxins (Deans et al. 2017), and secondary metabolites may increase their tolerance to various pesticides by stimulating the production of detoxification enzymes (Johnson et al. 2012; Terriere 1984).

In this context, the contribution of resource availability and quality to the overall health of bees, major pollinator of crops and wild plants (Hung et al. 2018), has received increased attention (Brodschneider & Crailsheim 2010). Indeed, like many organisms, their environment has rapidly changed under the influence of human activity. They are thus exposed to more frequent and diverse sources of stress, including pesticides, along with a reduction of floral resource abundance and diversity due to landscape simplification and habitat loss (Goulson et al. 2015).

Among the different stress factors threatening bees, pesticides have attracted most of the attention and debate (Durant 2020, Sgolastra et al. 2020, Storck 2017). The toxicity of a large range of pesticides has been documented (Aliouane et al. 2019; Alkassab & Kirchner 2017; Balbuena et al. 2015; Belzunces et al. 2012; Blacquière et al. 2012; Johnson 2015 ; Siviter et al. 2018). Research on the modulation of pesticide toxicity by nutritional factors, while still in its infancy, could lead to a better understanding of the impact of pesticides on bee populations and the design of more supportive habitats. The amount of nutrients in nectar and pollen can indeed differ between plant species (6.3–85% for sugar concentration in nectars (Pamminger et al. 2019), and 2.5- 61 % and 1-20% for protein and lipid contents in pollens, respectively (Roulston & Cane 2000; Vaudo et al. 2020). In addition, both pollen and nectar are nutritional sources of several amino acids, minerals, micronutrients and secondary metabolites (Wright et al. 2018; Palmer-Young et al. 2019). As a consequence, the quality of bee diets varies greatly over time and according to landscape features (Avni et al. 2014; Requier et al. 2015). Therefore, these variations in nutritional content may provide a basis for nutritional modulation of pesticide toxicity.

Confirmation of this hypothesis was tested for nectar by providing bees with limited access or access to low concentrations of sugar. The survival of bees was synergistically reduced by the combination of poor nutrition and field-realistic exposure to neonicotinoids (~50%) (Tosi et al. 2017). However, most data on the nutritional modulation of pesticide toxicity comes from studies that have tested pollen diets, likely because pollen is essential to the physiological development of bees (Brodschneider & Crailsheim 2010; Abdulaziz 2006; Haydak 1970; Pernal & Currie 2000). Wahl and Ulm (1983) were the first to report that feeding bees with pollen increased their tolerance to pesticides. They notably found that pollen intake as well as the quality of pollen (protein content) increased the median lethal concentration (LD50) of several pesticides. Later, Schmehl et al. (2014) demonstrated that pollen intake reduced bee sensitivity to chronic exposure to chlorpyrifos compared to bees fed without pollen. At the same time, Archer et al. (2014) showed that bees with access to an artificial protein-rich diet were more able to withstand nicotine exposure than bees provided with a protein-poor diet. More recently, Crone & Grozinger (2021) found that artificial and pollen diets characterized by different protein to lipid ratios can influence the survival time of bees chronically exposed to chlorpyrifos. Endpoints other than mortality rate have been used to assess the influence of pollen quality and availability on pesticide sensitivity in honey bees

(development of glands producing larval food ; Renzi et al. 2016), as well as in bumble bees (micro-colony performance, nest founding ; Barraud et al. 2020; Dance et al. 2017; Leza et al. 2018) and *Osmia* (reproduction ; Stuligross & Williams 2020), however, these studies generally reported a lack of interactions between these two factors.

Regarding the potential mechanisms underlying this nutritional modulation of pesticide sensitivity, pollen intake upregulates the expression of several xenobiotic-metabolizing cytochrome P450 genes (Schmehl et al. 2014), as well as the activity of glutathione S-transferases (Di Pasqual et al. 2013), which are involved in Phases I and II of the detoxification pathways, respectively (Berenbaum & Johnson 2015; Claudianos et al. 2006; Gong & Diao 2017). More specifically, upon ingestion, several constituents of pollen, like the phytochemicals p-coumaric acid and quercetin, can upregulate the expression of cytochrome P450 genes (Mao et al. 2011; 2013) and increase the survival rate of bees exposed to pesticides (Johnson et al. 2012; Liao et al. 2017, 2020; Wong et al. 2018). Such an effect on the detoxification capacity of bees was further confirmed by measuring pesticide metabolism. Ardalani (2021) found a reduction in the concentration of imidacloprid in honeybee bodies fed with quercetin, although no such effect was observed for either tebuconazole or tau-fluvalinate. Similarly, adding p-coumaric acid to a sucrose diet led to faster disappearance of coumaphos (Mao et al. 2013). Overall, these studies indicate that pollen may influence the ability of bees to metabolize pesticides, which was recently confirmed (Ardalani et al. 2021). Lastly, we cannot exclude an influence of pollen on the ability of bees to withstand the effects of pesticides given that the impact of pesticides depends not only on the fate of the molecule in the body (uptake, distribution, biotransformation, elimination), but also on its interaction with the biological target and effects at the physiological level. Due to its positive effect on bee longevity and on several molecular pathways and physiological functions (e.g., energy storage, immunocompetence, nutrient metabolism, protection against oxidative stress) (Alaux et al. 2011; Castelli et al. 2020; Jack et al. 2016; Rutter et al. 2019), pollen consumption might help bees to better tolerate the wide range of physiological impairments caused by pesticides, notably on nutrient metabolism, immunity, cell signalling and developmental processes (Schmehl et al. 2014; Aufauvre et al. 2014; Christen et al. 2018; Gao et al. 2020; Ye et al. 2020).

Overall, available studies showed that pollen nutrition can influence the survival rate of pesticide-exposed bees, particularly honey bees, and the metabolism of pesticides. However, the nutritional modulation of pesticide sensitivity in other bees, as well as the potential underlying mechanisms have rarely been studied.

To address these knowledge gaps, we evaluated the nutritional mitigation of agrochemical effects on key life-history traits regulated by pollen consumption in three European bee species (2 Apidae species: *Apis mellifera* (Apini) and *Bombus terrestris* (Bombini); 1 Megachilidae species: *Osmia bicornis*). We developed experiments in controlled conditions and monitored key life-history traits in bees fed with these pollen diets (e.g. survival for honey bees, brood production for bumble bees and larval development for mason bees) and exposed to pesticides.

2. Methodology

2.1 Overview of the study

According to the grant agreement, we planned to use protocols from Task 5.1 (described in Barraud et al. 2022) to test how our three model bee species are affected by three key aspects of the pollen diet and thus nutrition of bees: quantity, quality and diversity. We also planned to test how bee nutrition modulates the response of bees to three major classes of agrochemicals at chronic, sub-lethal concentrations. For honey bees and bumble bees, agrochemicals were planned to be administered via syrup, for mason bees via pollen provisions to larvae. The experimental design was organised to be fully crossed across agrochemicals and levels of nutrition. In the proposal we considered the same endpoints described in Task 5.1 (described in Barraud et al. 2022) for each model species. Additionally, we were

hoping to run the same protocol with an acute oral exposure of a single concentration (LD50) of the most important agrochemical to newly-emerged individuals. Samples from this task were used for proteomic analysis in WP9 to test for consistency of marker fingerprints of stressors (i.e., one stress or two or more stress combinations) across our three model bee species.

We adapted the ideas from the grant agreement according to the results from Task 5.1 (described in Barraud et al. 2022 and in the deliverable D5.1) and preliminary analyses. We did not study sperm quality as we used only females in the experiments on *Apis* and *Osmia*. The results of the sperm quality study of *Bombus* are not yet available and will be included in a separate manuscript. Evaluation of the fat body was not undertaken since this parameter seems to remain constant across diet measurements. Moreover, from other experiments with *O. bicornis* we showed that fat body depletion can be robustly approximated by measuring cocoon weight. Instead of measuring fat body, the expression of detoxification genes was analysed in bees exposed to different nutrition and specific pesticides at different concentrations in *Apis*.

Regarding honey bees, we provided bees with two pollen diets of different qualities (protein, lipid, p-coumaric acid contents) or no pollen (starvation treatment). The impact of diversity in pollen diet was not tested because it was not possible to obtain enough strictly monofloral pollens for the different experimental conditions and replicates. We exposed them to either sulfoxaflor, a new neurotoxic insecticide that shares its mode of action with neonicotinoids (Watson et al. 2011; Zhu Y et al. 2011), or azoxystrobin, an inhibitor of mitochondrial respiration in fungi and one of the most frequently detected fungicides in pollen collected by bees (34 to 87.5% of samples) (Long & Krupke 2016). We did not test a herbicide (glyphosate) because preliminary results showed a very low toxic effect on honey bee mortality of this chemical at environmental concentrations. We then determined whether the survival of bees exposed to a single concentration of pesticide (previously identified as the median lethal concentration) or chronically to field realistic and higher concentrations of pesticides is affected by pollen intake and/or the quality of pollens. Finally, we investigated the mechanisms underlying the modulation of pesticide sensitivity by testing whether pollen consumption induces a decrease in the concentration of pesticides in bees and/or help to tolerate the detrimental effect of pesticides on bee vitality. The latter was determined by measuring the gene expression level of vitellogenin, a well-established marker of bee health and longevity (Amdam et al. 2012; Seehuus et al. 2006).

To address knowledge gaps on *Bombus terrestris* we tested the effects of pesticides on colonies fed on two pollen diets showing different chemical qualities: a good pollen diet (*Salix* mix) as control, and a low-quality pollen diet (*Cistus* mix). Each diet was also tested by giving the bees only half the pollen normally consumed (starvation treatment). The impact of diversity in pollen diet was not tested because it was already explored previously. We then crossed these diet treatments with various concentrations of 4 pesticides: Glyphosate (herbicide), Amistar® (fungicide), Sulfoxaflor (insecticide) and Cyantraniliprole (insecticide). For each colony we monitored the rate of resource collection (the quantity of pollen and syrup collected) and the rate of brood development and pollen efficacy (larval mass developed per gram of consumed pollen).

In the study on *Osmia bicornis*, we used three nutrition types characterized by a low diversity of pollen (dominated by one or two plant species) and a mixture of these (higher diversity nutrition) and investigated two field-realistic concentrations of the potential neonicotinoid successor sulfoxaflor as well as the fungicide azoxystrobin. We did not test the herbicide glyphosate because preliminary results showed a very low effect of this chemical at environmental concentrations. With this experimental design we aimed to determine (1) whether a higher diversity nutrition would be beneficial for *O. bicornis* development and survival, (2) how the pesticides affect different fitness measures and (3) whether negative pesticide impacts are mitigated by a higher diversity pollen nutrition.

The honey bee study was published in 2021 under the following reference:

Barascou, L., Sene, D., Barraud, A., Michez, D., Lefebvre, V., Medrzycki, P., di Prisco, G., Strobl, V., Yañez, O., Neumann, P., le Conte, Y., Alaux, C. (2021). Pollen nutrition fosters honeybee tolerance to pesticides. Royal Society Open Science, 8(9). <https://doi.org/10.1098/rsos.210818>

The publications of the results on *Bombus terrestris* and *Osmia bicornis* are under preparation:

Barraud A., Askri D., Barascou L., Schwartz J., Toktas Y., Nicodème L., Alaux C., Albrecht M., Vanderplanck M. and Michez D. Can a poor diet increase the impact of pesticides on bumblebees?

Schwarz J. M., Knauer A. C., Barraud A., Michez D., Barascou L., Dievart V., Alaux C., Ghazoul J., Albrecht M. A more diverse pollen nutrition matters for developing solitary bees but does not mitigate the negative impact of pesticides.

2.2 Bee model species

This study was conducted on three common pollen generalist bee species recorded in Europe which forage in the same habitat for part of the year (Michez et al., 2019). We selected the Western honey bee *Apis mellifera* (Hymenoptera, Apidae, Apini), a domesticated eusocial species; the buff tailed bumble bee *Bombus terrestris* (Hymenoptera, Apidae, Bombini), a wild social species (Rasmont et al., 2008); and the Red mason bee (*Osmia bicornis*; Hymenoptera, Megachilidae, Osmini), a wild solitary species. They are commonly used as model species because of their easy management in laboratory conditions. Bumble bee colonies were provided by Biobest NV (Westerlo, Belgium); honey bees were obtained from local apiaries at the “Institut National de la Recherche pour l’Agriculture, l’Alimentation et l’Environnement” (INRAE) in Avignon (France) and the mason bees were provided by Wildbiene + Partner (Switzerland).

2.3 Pollen diet

2.3.1 Diet of honey bees (*Apis mellifera*)

In order to assess the influence of pollen intake and pollen quality on bee sensitivity to pesticides we used two pollen blends that differed regarding their nutritional properties: one predominantly composed of Brassicaceae (36%) and *Quercus robur* (35%) (“BQ pollen”), and the other primarily composed of *Salix* (89%) (“S pollen”). They were purchased fresh from Abeille heureuse® (France). We analysed protein and lipid content and their ratio following protocols detailed in Vaudo et al. (2020).

The chemical composition of these two pollen types was analysed and confirmed by the University of Mons, Belgium [see Barraud et al. (2022) for details of the palynological and chemical analysis].

The BQ pollen had higher protein and lipid content (respectively 28.39 ± 0.72 % and 18.7 ± 1.6 %, $n = 9$) than the S pollen (21.49 ± 1.05 % and 14.07 ± 1.5 %, $n = 9$). The protein:lipid ratio was similar between pollen mixes (BQ pollen: 1.52 and S pollen: 1.53). We also determined the concentrations of two phytochemicals, p-coumaric acid and quercetin. The p-coumaric acid concentrations reached 244.7 mg/kg (1491.6 μ M) in the BQ pollen and 104.5 mg/kg (637 μ M) in the S pollen. The level of quercetin was under the quantification limit of the analysis method for both pollens, i.e. below 10 mg/kg. The presence of pesticide residues in one extract of each pollen blend was determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) with a limit of quantification of 0.01 mg/kg and a limit of detection of 0.005 mg/kg following the European Standard EN 15662:2018 procedure. Only residues of 2,4-dimethylformamide (DMF, degradation products of amitraz) and tau-fluvalinate were detected in both pollen blends, but both were below the limit of quantification. These compounds used as chemical treatments against the honey bee parasite *Varroa destructor* are consistently found in pollens (47.4% and 88.3% of trapped pollens for amitraz and tau-fluvalinate, respectively; Calatayud-Vernich et al. 2019; Mullin et al. 2010) and are considered as relatively safe for honey bees with an oral

LD₅₀ of 75 µg/bee for amitraz (contact exposure) and 45 µg/bee for tau-fluvalinate (oral exposure) (U.S. EPA). Both pollen blends were gamma irradiated to avoid parasite contamination and stored at -20°C.

2.3.2 Diet of bumble bees (*Bombus terrestris*)

Two different organic pollen blends of different quality were purchased from "Abeille heureuse" (France) and used in this study: *Salix* pollen blend as a good diet ("S pollen") and *Cistus* pollen blend ("C pollen") as a poor diet. Prior to the experiment, the pollen was treated with gamma-ray to eliminate potential pathogens and stored at -80°C.

As for the honey bee diet, the chemical composition of these two pollen types was analysed and confirmed by the University of Mons, Belgium. Diet composition has been fully analysed regarding palynology, protein content, lipid content, amino-acids, and sterol composition [see Barraud et al. (2022) for details of the palynological analysis].

2.3.3 Diet of mason bees (*Osmia bicornis*)

The same certified organic honey bee-collected pollen was purchased from *Abeille heureuse*, France, and subsequently gamma irradiated in order to prevent contamination by pathogens and parasites. Four different nutrition treatments were used. Three nutrition treatments consisted of provisions containing low pollen diversity, and one treatment consisted of a mixture of these three pollens, yielding a high-diversity pollen. The low diversity pollen types were dominated by (1) *Cistus ladanifer* (95%; "C"), (2) *Salix* spp. (89%; "S") and (3) Brassicaceae/*Quercus* (71% (36% + 35%, respectively); "BQ"). The higher diversity nutrition ("MIX") consisted of a mixture of the three low diversity pollen types.

These pollen batches were the same as those chosen for honey bees (S pollen and BQ pollen) and bumble bees (S pollen and C pollen). They were selected based on previously measured differences in protein, lipid and amino acid contents of the *Cistus*, *Salix* and Brassicaceae/*Quercus* pollen assessed at the University of Mons. The chemical composition of these two pollen types was analysed and confirmed by the University of Mons, Belgium [see Barraud et al. (2022) for details of the chemical and palynological analysis].

2.4 Experimental protocols

2.4.1 Honey bee (*Apis mellifera*)

Experiments were performed at the Institut National de la Recherche Agronomique (INRAE) in a semi-urban area (Avignon, France, 43°54'N-4°52'E) with honey bees (*Apis mellifera*) from our local apiary. To obtain one-day-old bees, brood frames containing late-stage pupae were removed from 8 to 10 colonies (depending on the experiments) and kept overnight in an incubator under controlled conditions (34°C, 50-70% relative humidity (RH)). The next day, newly-emerged bees (less than 1 day old) were collected, mixed and placed in cages (10.5 cm x 7.5 cm x 11.5 cm) [66]. To better simulate colony rearing conditions, cages were equipped with Beeboost® (Ickowicz, France), releasing one queen-equivalent of queen mandibular pheromone per day.

Caged bees were kept in an incubator (30°C and 50-70% RH) and provided with water and Candy (Apifonda® + powdered sugar) ad libitum. Except for the control groups, bees were also provided with one of the fresh pollen blends (BQ or S pollens) via an open tube feeder for 7 days. To prevent potential nutritive compensation of bees fed with one of the pollens, they were not provided with ad libitum pollen, but with a determined quantity of pollen each day, representing the minimum daily consumption of pollen: 4 mg/bee/day for the first two days, 5 mg/bee/day for the next two days, 3 mg/bee/day for the fifth day, and 2 mg/bee/day for the last two days, as described in Di Pasquale et al. (2013). If bees

died during the pollen-feeding period (7 days), pollen amount was adjusted to the number of surviving bees. Both pollen diets were fully consumed every day.

Acute single exposure

In the first experiment, we tested whether pollen intake and pollen quality could modify the sensitivity of bees to a single concentration of pesticide previously identified as a LD50 (data not published). Groups of 20 one-day old bees were placed in cages, which were randomly assigned to the different experimental groups: control (sucrose solution only), BQ pollen, S pollen, azoxystrobin, sulfoxaflor, BQ pollen/azoxystrobin, S pollen/azoxystrobin, BQ pollen/sulfoxaflor, and S pollen/sulfoxaflor (n = 10 cages per experimental group).

On day 5, bees were sugar-starved for two hours and then fed with a solution of 50 % (w/v) sucrose and azoxystrobin (4600 µg/ml, 1.14 % acetone) or sulfoxaflor (3.67 µg/ml, 0.37 % acetone) accordingly to the experimental group. Sugar solutions were provided via a feeding tube with a hole at its end. Each treated cage received 200 µl of the solution laced with pesticides. Solutions were provided for 4 hours and all of them were consumed within this time period. Assuming that the bees equally consumed the solutions, pesticide treatments resulted in a theoretical exposure to 46 µg/bee of azoxystrobin and 36.7 ng/bee of sulfoxaflor, corresponding to the LD50 levels previously determined. Control groups were fed with pesticide-free sugar solution (50% w/v sucrose, 1% acetone). After exposure to pesticides, bees were provided with water and Candy (Apifonda® + powdered sugar) ad libitum. Mortality was recorded 48 hours after exposure.

Stock solutions of sulfoxaflor (Techlab, France) and azoxystrobin (Sigma Aldrich, France) in acetone were previously aliquoted and kept at -20°C. The exact concentrations were confirmed with LC-MS/MS (see below) and resulted in 5746 µg/ml for azoxystrobin and 3.62 µg/ml for sulfoxaflor, which corresponds to a real exposure of 57.5 µg/bee and 36.2 ng/bee, respectively.

Chronic exposure

In the second experiment, bees were chronically exposed to two concentrations of pesticides: a concentration that was considered to be field realistic and a higher concentration representing a worst-case exposure scenario. Groups of 30 one-day old bees were placed in cages (n = 10 cages per experimental group) and treatment groups were provided with one of the pollen blends as described above. On day 5, caged bees were provided with a solution of 50 % (w/v) sucrose, 0.1% acetone and azoxystrobin or sulfoxaflor at either a low or high environmental concentration which corresponded to theoretical values of: 0.02 and 2 µg/ml for sulfoxaflor and 0.2 and 2 µg/ml for azoxystrobin according to the experimental group. Control groups were fed with pesticide-free sugar solution (50 % w/v, 0.1 % acetone). The concentrations were chosen based on pesticide residue data found in pollen and nectar. Different application rates of sulfoxaflor before or during flowering of cotton resulted in 6.6 to 13.8 ppb of sulfoxaflor in nectar and 7.7 to 39.2 ppb in pollen of cotton flowers (Jiang et al. 2020). However, other field residue studies with cotton, buckwheat and phacelia reported higher levels of sulfoxaflor ranging from 0.05 to 1 ppm in nectar and from 0.22 to 2.78 ppm in pollen collected by honeybees during the flowering period (U.S. EPA. 2010, 2019). Azoxystrobin was found at levels ranging from 0.03 to 0.11 ppm in pollen collected by honeybees in North America [64]. In France, these levels ranged from 0.01 to 1.9 ppm (Observatory of Pesticide Residue, ITSAP- Institut de l'Abeille, personal communication). The chronic pesticide treatments were performed over 10 days and the syrup feeders were replaced every day. For each cage, individual syrup consumption was assessed daily by weighing feeders and dividing the consumed food by the number bees remaining alive. The cumulative syrup consumption over the 10 days of exposure to pesticide was then determined. After exposure to pesticides, bees were provided with water and Candy (Apifonda® + powdered sugar) ad libitum. Dead bees were counted daily and removed over a 16-day period (i.e., when the high sulfoxaflor concentration group reached 100% mortality). Following the chemical analyses (LC-MS/MS) the real concentrations of tested diets resulted in 0.02 and 2.35 µg/ml for sulfoxaflor and 0.22 and 1.90 µg/ml for azoxystrobin, respectively, for the low and high exposure rates.

Potential mechanisms underlying the nutritional modulation of pesticide sensitivity

In order to investigate the mechanisms underlying the beneficial effect of pollen on pesticide sensitivity, we compared the gene expression level of vitellogenin and the amount of pesticide among groups. Groups of 80 one-day old bees were placed in cages and, as above, fed with one of the pollen diets ($n = 10$ cages per experimental group). On day 5, bees were sugar-starved for 2 hours and then fed with the highest concentration of azoxystrobin and sulfoxaflor ($2 \mu\text{g/ml}$), or sugar solution only. Each cage received $800 \mu\text{L}$ of sugar solution. Solutions were provided for 4 hours and all of them were consumed within this time period, giving a theoretical concentration of 20 ng of pesticide per bee (19 ng of azoxystrobin and 23.5 ng of sulfoxaflor based on the real concentration of the tested solution, see above). After exposure to pesticides, bees were provided with water and Candy (Apifonda® + powdered sugar). At 8 and 24 hr post-exposure (i.e., once all the solutions were consumed), 25 and 35 bees per cage were respectively sampled on dry ice and stored at -80°C for later analysis.

Influence of pollen nutrition and pesticides on vitellogenin expression level

For each cage, the abdomens of 6 bees sampled at 24 hr post-exposure were pooled in groups of 3. Abdomen pools were homogenised in $800 \mu\text{L}$ of Qiazol reagent (Qiagen) with a Tissue Lyser (Qiagen) ($4 \times 30 \text{ s}$ at 30 Hz). RNA extraction was then carried out as indicated in the RNeasyPlus Universal kit (Qiagen) with DNase treatment (Qiagen). RNA yields were measured with a Nanodrop (Thermo Scientific) and cDNA synthesis was carried out on $1 \mu\text{g}$ of RNA per sample using the High capacity RNA to cDNA Kit (Applied Biosystems®, Saint Aubin, France). cDNA samples were diluted ten-fold in molecular grade water. The expression level of vitellogenin was determined by quantitative PCR using a Step One-Plus Real-Time PCR System (Applied Biosystems) and the SYBR green detection method. Three μL of cDNA were mixed with $5 \mu\text{L}$ SYBR Green Master Mix, $1 \mu\text{L}$ of forward primer ($10 \mu\text{mol}$) and $1 \mu\text{L}$ of reverse primer ($10 \mu\text{mol}$) of the target gene. A dissociation stage for the subsequent melting curve analysis was included. All qPCR reactions were run in duplicate. The average cycle threshold values of vitellogenin were normalised to the geometric mean of the housekeeping genes actin and RPS18, which proved to have rather stable expression levels [70]. We used sequences of primers previously published [71,72]. The ΔCt value of each group was subtracted by the ΔCt value of the control group (sugar syrup only) to yield $\Delta\Delta\text{Ct}$.

Influence of pollen nutrition on pesticide detoxification

Pesticide concentrations were analysed from a pool of 25 bees per cage and time point in the following groups: sulfoxaflor, azoxystrobin, BQ pollen/sulfoxaflor, BQ pollen/azoxystrobin, S pollen/sulfoxaflor, S pollen/azoxystrobin.

Sulfoxaflor and azoxystrobin content were analysed via LC MS/MS. The QuEChERS method was used for the extraction of the active ingredients from samples, following the European Standard EN 15662. Briefly, samples were ground in liquid nitrogen and 2 g of the crushed sample were mixed with 15 mL of a 1:2 water and acetonitrile mixture and a bag containing 4 g of magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate tribasic dihydrate, and 0.5 g of sodium citrate dibasic sesquihydrate. An aliquot of the supernatant was mixed with 900 mg of magnesium sulfate, 150 mg of PSA and 150 mg of C18-E. After centrifugation, $2 \mu\text{L}$ of extract were injected into an Accela 1250 ultra-high performance liquid chromatography (UHPLC) system for sulfoxaflor or azoxystrobin detection. The UHPLC system was coupled to a TSQ Quantum Access MAX Triple-Stage Quadrupole Mass Spectrometer, equipped with a heated-electrospray ionisation (H-ESI) source working in positive polarity. The mobile phase used for the analysis consisted of 4 mM ammonium formate in water and 4 mM ammonium formate in MeOH, both containing 0.1% formic acid. The fragments analysed were at m/z 372.1; 329.1; 344.1 (products) generated by the ion at m/z 404.12 (parent, azoxystrobin), and the fragments at m/z 154.1 and 104.2 (products) generated by the ion at m/z 278.1 (parent, sulfoxaflor). Quantification was performed using acetamiprid as an internal standard. The limit of quantification (LOQ) for azoxystrobin and sulfoxaflor was 0.001 and 0.01 mg/kg , respectively.

2.4.2 Bumble bee (*Bombus terrestris*)

A total of 25 queen-right colonies of 100 *Bombus terrestris* workers were used to build-up 480 queen-less micro-colonies of five workers in plastic boxes (8*16*16 cm). This number of individuals per micro-colony has been optimized during previous bioassays (Moerman et al., 2016; Roger et al., 2017b; Vanderplanck et al., 2018) and has been shown to be the most favourable for male offspring production (Gradish et al., 2013). Moreover, using more workers can dilute the brood tending responsibilities across more individuals, inducing a microclimate temperature elevation (Klinger et al., 2019). The large number of conditions required the experiment to be spread over several months, using different queen-right colonies for each month. For each experimental condition, a total of 10 micro-colonies were used with two micro-colonies coming from each of five queen-right colonies to avoid any colony-related bias. All micro-colonies were maintained in the same room in constant darkness with a relative humidity of 60–65%. They were manipulated under red light to minimize disturbance (Sadd, 2011) for a period of 28 days. Micro-colonies were then fed *ad libitum* with either *Salix* or *Cistus* pollen mixed with sugar syrup to obtain pollen candies (Fig. 1). A starvation condition was also tested by giving to the bees only half the quantity of the pollen they usually eat. New pollen candies were provided every three days, while the previous ones were removed before drying or decaying and weighed to assess pollen collection. Pollen pesticides residues were also analysed to avoid unwanted pesticide interaction during the experiment.

Pesticide exposure

Four different pesticides at different concentrations were used in this study: Sulfoxaflor (insecticide, 20, 300, 1000, 2000ppb), Cyantraniliprole (insecticide, 6200 ppb), Azoxystrobin (Amistar®, fungicide, 200, 1900ppb) and Glyphosate (herbicide, 3800, 7600ppb). A mixture of Sulfoxaflor (1000ppb) and Amistar® (1900ppb) was also tested. Pesticides were administered chronically for 28 days in syrup feeders while syrup used to prepare pollen candies remained pesticide free. The concentrations were chosen based on pesticide residue data in pollen and nectar found in literature and databases. A study showed that sulfoxaflor residues can range from 6.6–13.8 ppb in nectar and 7.7–39.2 ppb in pollen of cotton flowers (Jiang et al., 2020) while another one reported higher level of sulfoxaflor, ranging from 0.05 to 1 ppm in nectar and from 220 to 2780 ppb in pollen collected by honeybees during the flowering period (EPA, 2010, 2019). The highest concentration of residues that have been detected were quantified at 3000ppb shortly after sulfoxaflor application in flowering apple (EFSA, 2019, 2020). Azoxystrobin was found at levels ranging from 30 to 110 ppb in pollen collected by honey bees in North America (Mullin et al., 2010) which is similar to another study in the US also in honey bee-collected pollen (4.6 to 1870ppb) (Rennich et al., 2013). Concentrations of glyphosate were chosen based on studies that detected sublethal effects on honey bees when exposing bees to concentrations ranging from 500 to 10000ppb (Herbert and Farina, 2014; Balbuena, 2015), which is in the range of what is measured in natural environments (from 1400 to 7600ppb) (Goldsborough and Brown, 1988; Feng and Thompson, 1990; Giesy et al., 2000). Finally, due to the difficulty in finding much information about cyantraniliprole residues, we chose a concentration based on the few analyses (Dinter and Samel, 2015; Kyriakopoulou et al., 2017; Lee et al., 2019) and sub-lethal observations on food intake (Barraud et al. unpublished data).

To reach these concentrations, pesticides were first diluted in acetone, then in water and in sugar syrup. Solutions were given *ad libitum* to the bees in a 100 mL container with a capillary. Control and contaminated syrup were replaced every four days to avoid degradation and weighed to assess sugar and pesticide consumption. To avoid any bias, acetone was added to control syrup.

Experimental set up/assessed parameters

Each nutritional condition previously described was crossed with all the pesticides treatments and the control, which led to a total of 29 experimental conditions. To estimate performance and development of bumble bee microcolonies, several parameters were evaluated: (i) total pollen and syrup collection, which can impact brood production and development (e.g. Plowright et al., 2008; Sutcliffe and

Plowright, 2008); (ii) colony growth after 28 days of development [i.e. mass of individuals from all brood stages (dead larvae, eggs, larvae, pupae, non-emerged and emerged males)] (Vanderplanck et al., 2014, 2018); (iii) worker mortality. For each micro-colony, all the measured parameters were divided by the total mass of the five workers to standardize the results and avoid potential effects of worker activities related to their size (i.e., consumption and brood care) (Cnaani and Hefetz, 1994). Additionally, we calculated the pollen efficacy as the mass of total offspring divided by the total pollen collection to estimate the colony performance. As the experiments were carried out over several different months, all the parameters measured were represented in terms of difference from the control of the corresponding month.

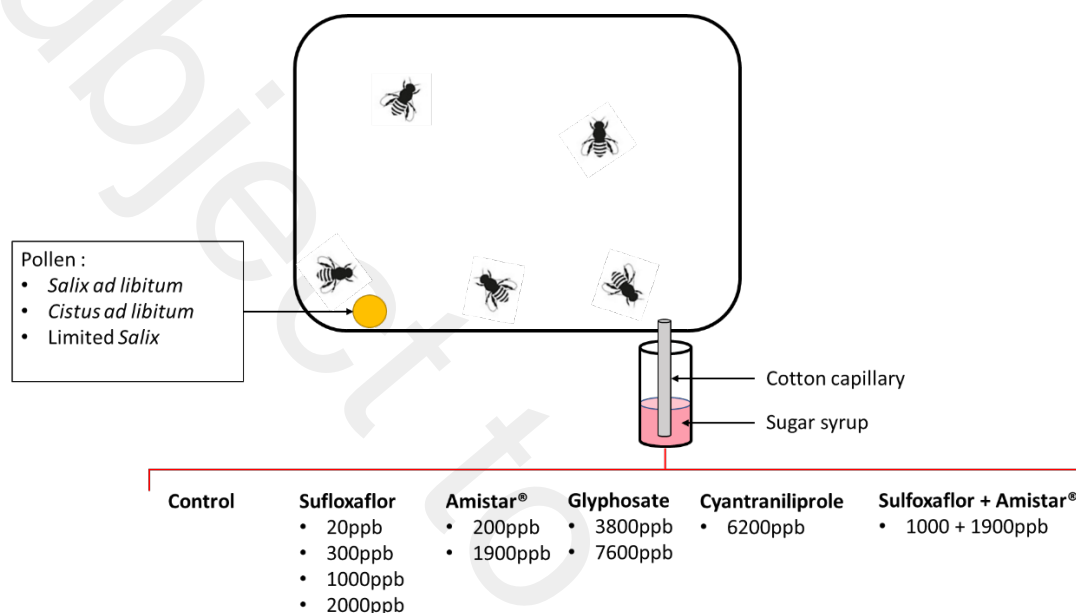


Figure 1. Experimental set-up. Bumble bees were fed with sugar syrup (treated or not) and pollen (*Salix/Cistus ad libitum* or limited amount) for 28 days and maintained in constant darkness at 26°C and 60% relative humidity.

2.4.3 Mason bee (*Osmia bicornis*)

The interactive effects of nutrition and pesticide exposure on solitary bee (*O. bicornis*) development were tested at Agroscope, Zürich, from April 2020 to April 2021. We reared freshly laid *O. bicornis* eggs on artificial honey bee collected pollen provisions in the laboratory (Fig. 2) and spiked the pollen with the insecticide sulfoxaflor (sulfoximine) or with the fungicide azoxystrobin (strobilurin). Two concentrations (low, high) were tested for each pesticide and as they were dissolved in acetone, separate water-control and acetone-control treatments were tested for each pesticide-nutrition combination. The larvae were reared on four different nutrition types containing either a high or a low pollen diversity (see above). In each treatment combination, 30 female *O. bicornis* larvae were reared. The developing bees were kept in an incubator in the dark under controlled conditions (23 °C and 60% RH) and checked daily for their developmental progress. After completion of metamorphosis in mid-September (Bosch et al. 2008, Splitt et al. 2021), the cocoons were cleaned and weighed. Overwintering took place at a sheltered place outdoors. In the following spring, the emergence rates of the bees as adults were assessed and bees were released into the wild.

Pesticide exposure

We tested the impacts of two field-realistic concentrations (high, low) of the insecticides sulfoxaflor and the fungicide azoxystrobin. We used residue values from published literature to determine the

concentrations used in the study. The high concentration approximately corresponds to the highest residue values found in pollen and can be considered a worst-case exposure scenario. The low concentration was determined as a 10-fold lower value. We used 3 ppm as the high sulfoxaflor concentration (EFSA 2019) and 0.3 ppm as the low concentration. For azoxystrobin, we used 1.9 ppm as the high concentration (Observatory of Pesticide Residue, ITSAP – Institute de l'Abeille 2014, personal communication, Rennich et al. 2013) and 0.19 ppm as the low concentration.

The pesticides sulfoxaflor and azoxystrobin were dissolved in acetone and then diluted to the desired concentrations with distilled water. Both pesticide solutions contained the same amount of acetone. A separate water-acetone control as well as a water-only control were also prepared. To prepare artificial pollen provisions, honey bee collected pollen was finely ground and mixed with pesticide solution and sugar solution in a mortar. Then, provisions of 400 mg were formed by hand and stored at -20°C until use in the experiment.

***Osmia bicornis* egg collection and rearing of larvae**

To obtain freshly laid *O. bicornis* eggs, adult female bees were released next to suitable nesting aids close to Agroscope, Zürich. Two weeks after the release, the first eggs for use in the experiment could be collected.



Figure 2. Plastic boxes containing cocooned *Osmia bicornis* adults ready to emerge. Boxes were placed on custom-made wooden nesting aids at an outdoor sheltered place on the experimental field site of Agroscope near Zürich (Switzerland) to establish a large population of nesting *O. bicornis*. Laid eggs were collected and transferred onto different pollen provision treatments in the laboratory.

On every collection day, custom-made wooden plates containing different artificial pollen provisions (randomly distributed) were prepared. Then, the nesting aids were opened and freshly laid eggs were collected with a wet brush and carefully placed on the pollen provisions. We distinguished male and female eggs based on their position in the nest and the size of the pollen provision they were laid upon (Ivanov 2006, Seidelmann et al. 2010). After collection, the plates were covered with a transparent acetate sheet and placed in an incubator in the dark.

The development of the larvae was monitored daily, except on Sundays, during the following weeks. We assessed the following developmental stages: egg, larva, feeding larva, feeding and defecating larvae, spinning larva, cocoon. If a larva died, the date of death was noted. Larvae that did not hatch from the egg at the start of the experiment were excluded from analysis, as they were likely damaged during the egg collection process. After completion of the cocoons, the plates were left inside the incubator until mid-September 2020 to let the bees complete their metamorphosis. After metamorphosis, the cocoons were cleaned and weighed. They were then packed individually into labelled Eppendorf tubes (with a

hole to guarantee air circulation) and placed into cardboard boxes for overwintering. The boxes were stored outdoors at a sheltered space. In the following spring, the cocoons were placed at room temperature and it was assessed whether bees hatched or not. We also determined the sex of the bees, in order to be able to exclude male bees from the analysis. Approximately 10% of bees were identified as males.

Pollen consumption

For each developing bee, we measured how much pollen was eaten by weighing the leftover pollen. On some of the plates, however, pollen mites were detected (after cocoon completion). Since the mites consumed parts of the pollen, we excluded the affected pollen from the analysis. Only pollen with negligible levels of mites were included.

2.5 Statistical analyses

2.5.1 Honey bee (*Apis mellifera*)

Data were analysed using the statistical software R v3.3.3 (R Core Team. 2020). Since data from the acute toxicity tests were not normally distributed, the effect of pesticide and pollen treatments on bee mortality was analysed using Kruskal–Wallis, followed by Dunn’s multiple comparison tests with Benjamini-Hochberg correction. Then, the epsilon-squared (ϵ^2) was computed to obtain a measure of effect size between experimental groups (*epsilonSquared* function of the *rcompanion* package [74]). The interpretation values were as follows: $\epsilon^2 < 0.01$: very small effect, $0.01 < \epsilon^2 < 0.08$: small effect, $0.08 < \epsilon^2 < 0.26$: medium effect, and $\epsilon^2 \geq 0.26$: large effect [75]. Survival data from the chronic toxicity tests were analysed with a Cox proportional hazards regression model (*coxph* function of the *survival* package in R (Cox 1970)). Data were transformed in a survival table and the remaining bees were considered alive at day 16. The Cox model was used to calculate the Hazard Ratio (HR). The HR is defined as the ratio between the instantaneous risk in the treatment group (H_1) and the risk in the control group (H_0), occurring at a given time interval (Hoffman 2019). The influence of experimental treatments on the cumulative syrup consumption, vitellogenin expression level and pesticide detoxification were analysed using Kruskal-Wallis, followed by Dunn’s multiple comparison tests with Benjamini-Hochberg correction.

2.5.2 Bumble bee (*Bombus terrestris*)

We performed comparative analyses of micro-colony performance and feeding behaviour using R version 3.6.0 (R Core Team, 2017). Statistical analyses using two-way crossed analyses of variance (Two-Way crossed ANOVA) were conducted to evaluate the effect of diet and pesticide stress as well as their interaction. Since it is a parametric test, homoscedasticity (Bartlett test) and normality of the residuals (Shapiro test) were checked prior to the analyses. When violation occurred, data were log- or rank-transformed to normality of residuals (“*rnttransform*” function, R-package “GenABEL”) prior to the test. Multiple pairwise comparisons were conducted using Tukey HSD tests when ANOVA detected significant difference among pollen treatments ($P < 0.05$).

2.5.3 Mason bee (*Osmia bicornis*)

We analysed the obtained data in two steps. First, only the effects of the different nutrition types on survival, cocoon weight, development time and pollen efficacy (rate at which ingested pollen was transformed into body weight) were assessed by including only bees not exposed to pesticides. We fitted (generalised) linear ((G)LM) or generalised least squares fitted linear models (GLS) for testing the effect of the explanatory variables. The models included nutrition type (4 levels) and a factor accounting for the type of control (acetone-control or water-only control). We also included the egg collection day as covariate. The control treatment factor and the egg collection day covariate were dropped from the

model, if they did not improve model fit. Pairwise comparisons between nutrition types were analysed using Tukey's HSD post-hoc tests in the package emmeans (Lenth 2021). Additionally, we tested whether bees reared on the MIX nutrition performed significantly better compared to the average of bees reared on the low diversity nutrition (mean of C, S, BQ) using the multcomp package (Hothorn et al., 2008).

In a second step, we analysed the interactive effects of pesticide exposure and nutrition type on survival, development time, cocoon weight, pollen efficacy and pollen consumption. Each model was fitted including pesticide concentration and its interaction with nutrition type. Egg collection day (numeric) was included as additional covariate, but was dropped from the model if it did not significantly improve model fit ($P \leq 0.05$, based on a likelihood ratio test). Only the acetone-control bees served as controls in these models. Main pesticide effects were calculated for each model using Tukey's HSD pairwise comparisons in the package emmeans. Pairwise comparisons of pesticide concentrations within each nutrition type were also calculated in emmeans. Additionally, the effect of the high pesticide concentration in the MIX nutrition type (higher diversity pollen) was compared to the average high pesticide concentration effect in the lower diversity nutrition types (C, S, BQ) using the package multcomp.

Statistical analyses were performed in R version 4.2.1. (R Development Core Team 2022). Model assumptions of normality and homoscedasticity of residuals, as well as homogeneity of variances in different treatment groups were visually assessed (Zuur et al. 2009). Statistical inferences were calculated via type II ANOVA using the car package (Fox and Weisberg 2019) for linear models (LMs) and via likelihood ratio tests for generalised linear models (GLMs). Type II ANOVAs were computed manually for generalised least-squares models (GLS). Several larvae fell from the pollen provisions during development. These larvae were excluded from all analyses.

3. Results

3.1 Honey bee (*Apis mellifera*)

3.1.1. Influence of pollen nutrition on honeybee sensitivity to pesticides

Acute single exposure

The concentration of azoxystrobin significantly increased the mortality of bees that did not have access to pollen (Kruskal-Wallis test: $P < 0.05$, and Dunn post-hoc tests: $P < 0.05$; Fig.1A). Although, only 10% of bees were found dead 48 hours post-exposure (vs 0% in control groups), the size of the negative effect of azoxystrobin could be considered as medium ($\epsilon^2 = 0.163$; Table 1). Azoxystrobin did not increase bee mortality in bees fed with either the BQ or S pollen diets (Dunn post-hoc tests: $P = 0.41$ and $P = 0.79$ for BQ and S pollen, respectively; Fig.3A). However, bee mortality did not differ between the different pollen diets (no pollen, BQ and S pollen) irrespective of exposure to azoxystrobin (Fig.3A).

Table 1. Effect size (ϵ^2) of pesticide acute toxicity. The interpretation values are as follows: $\epsilon^2 < 0.01$: very small effect, $0.01 < \epsilon^2 < 0.08$: small effect, $0.08 < \epsilon^2 < 0.26$: medium effect, and $\epsilon^2 \geq 0.26$: large effect. 95% CI: 95 % confidence interval, p-values are derived from post-hoc Dunn tests.

comparison	ϵ^2	95% CI inf	95% CI sup	p-value
control versus azoxystrobin	0.163	0.059	0.301	<0.05
BQ pollen versus BQ pollen + azoxystrobin	0.011	4.01×10^{-5}	0.086	0.12
S pollen versus S pollen + azoxystrobin	0.001	3.73×10^{-5}	0.117	0.70
control versus sulfoxaflor	0.277	0.149	0.395	<0.001
BQ pollen versus BQ pollen + sulfoxaflor	0.183	0.065	0.313	<0.001
S pollen versus S pollen + sulfoxaflor	0.143	0.031	0.273	<0.001

Sulfoxaflor increased the mortality of bees over 48 hours (Kruskal-Wallis test, $P < 0.01$; Fig. 1B). For instance, the concentration of sulfoxaflor killed around 50% of the bees that did not ingest pollen (Fig. 3B). However, sulfoxaflor toxicity was also reduced by pollen consumption. First, the sulfoxaflor-induced mortality was significantly lower in bees fed with BQ or S pollen diets than in bees fed without pollen (BQ pollen: $P < 0.05$ and S pollen: $P < 0.05$). Second, the sulfoxaflor toxicity was reduced by half in bees provided with pollen (BQ pollen: $\epsilon^2 = 0.183$, S pollen: $\epsilon^2 = 0.143$ – medium effect) as compared to bees fed without pollen ($\epsilon^2 = 0.277$ – large effect; Table 1).

Ultimately, the two types of pollen diet did not differentially affect the acute toxicities of azoxystrobin and sulfoxaflor (BQ pollen vs S pollen: $P = 1.0$ for azoxystrobin and sulfoxaflor; Fig. 3A and B).

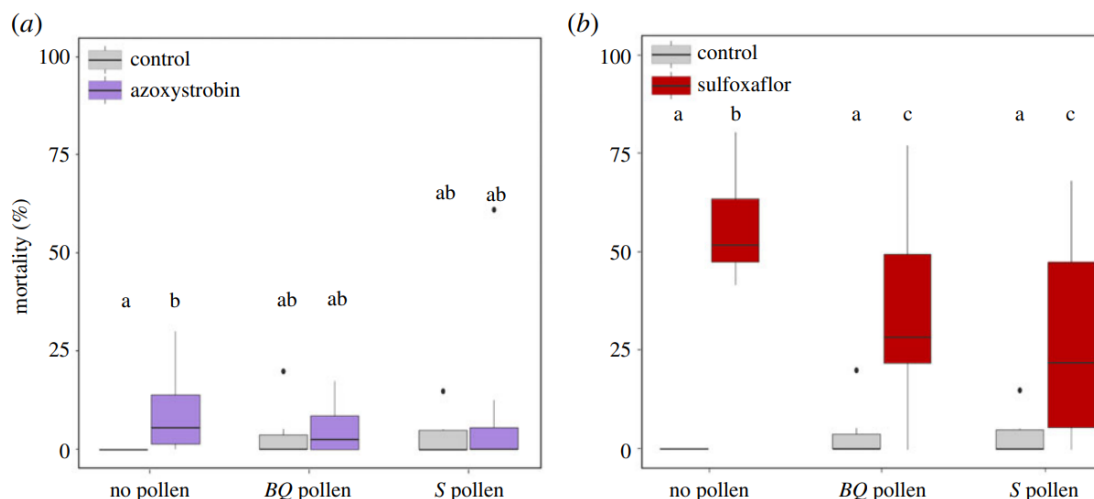


Figure 3. Acute toxicity of azoxystrobin (57.5 µg/bee) (A) and sulfoxaflor (36.2 ng/bee) (B) on bees fed with different pollen regimes. Data represent the 48 hr post-exposure mortality of bees ($n = 20$ bees per cage and 10 cages per modality). Boxes indicate the 1st and 3rd interquartile range with a line denoting the median. Whiskers include 90% of the individuals, beyond which circles represent outliers. Different letters indicate significant differences (Kruskal-Wallis tests followed by Dunn's multiple comparison test).

Chronic exposure

In the azoxystrobin experiment, we found that, regardless of exposure to pesticides, bees provided with pollen consumed more syrup than bees who did not receive pollen (Kruskal-Wallis test: $P < 0.001$; Fig. 4A). However, apart from the non-intoxicated bees who consumed less syrup than intoxicated bees in the BQ pollen groups, there was no difference in syrup consumption between pesticide treatments for a given pollen diet. In the sulfoxaflor experiment, the pollen effect on syrup consumption was only found in non-intoxicated bees: bees without pollen consumed less syrup than bees with pollen (Kruskal-Wallis test: $P < 0.001$; Fig. 4B). At the low and high concentration of sulfoxaflor, pollen diets did not affect syrup consumption. The main variation in syrup consumption was due to the high concentration of sulfoxaflor. Bees exposed to 2 ppm of sulfoxaflor consumed less syrup than bees exposed to 0 or 0.02 ppm of sulfoxaflor (although it was not significant for the BQ pollen groups).

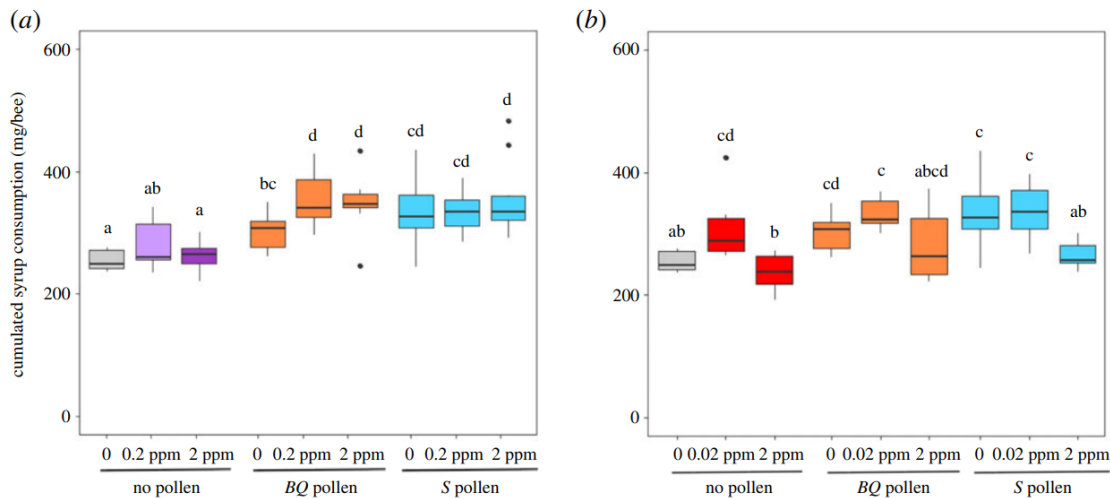


Figure 4. Individual syrup consumption according to pesticides and pollen feeding treatments. Cumulative individual consumption (mg/bee) are shown for bees exposed to azoxystrobin (A) and sulfoxaflor (B) ($n = 30$ bees per cage and 10 cages per experimental conditions). Boxes indicate the 1st and 3rd interquartile range with a line denoting the median. Whiskers include 90% of the individuals, beyond which circles represent outliers. Different letters indicate significant differences (Kruskal-Wallis tests followed by Dunn's multiple comparison test).

Chronic exposure to azoxystrobin (0–2 - 2ppm) did not affect bee survival whether bees consumed pollen or not (Cox model, $P = 0.17$; Fig. 5A). However, both sulfoxaflor concentrations decreased bee survival (Cox model, $P < 0.001$, Fig. 3B). While, in bees fed without pollen, the highest concentration of sulfoxaflor (2 ppm) caused 100% bee mortality within 16 days, the lowest concentration (0.02 ppm) reduced the survival probability by around 25%.

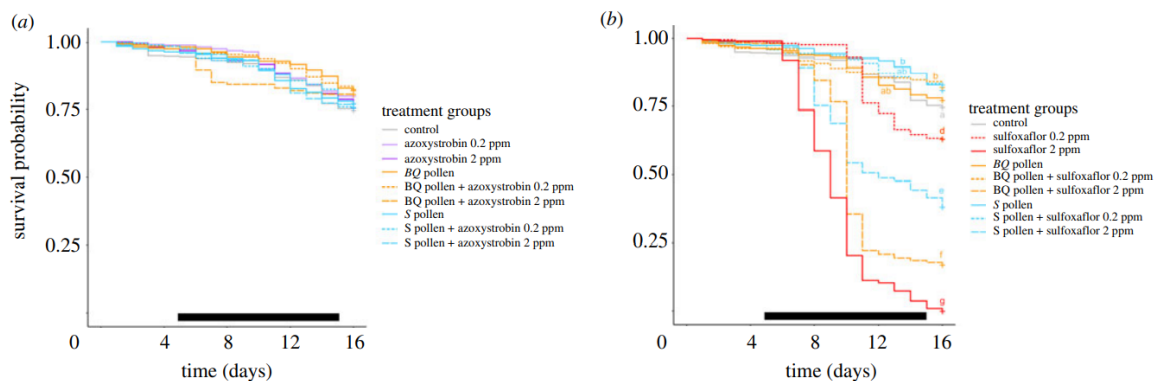


Figure 5. Chronic toxicity of environmentally-relevant concentrations of azoxystrobin (A) and sulfoxaflor (B) on bees fed with different pollen regimes. Data represent the survival probabilities of bees ($n = 30$ bees per cage and 10 cages per modality). Different letters indicate significant differences (Cox model) and the black bar represents the period of exposure to pesticides.

The survival probability of bees over 16 days was enhanced by the S pollen (no pollen, $P < 0.05$), but not by the BQ pollen ($P = 0.58$), although no difference in survival was found between the two pollen diets ($P = 0.14$; Fig. 3B). The survival probability of bees intoxicated with the low concentration of sulfoxaflor improved with pollen feeding (sulfoxaflor vs sulfoxaflor + BQ pollen: $P < 0.001$, sulfoxaflor vs sulfoxaflor + S pollen: $P < 0.001$), but no difference was found between the two pollen diets (sulfoxaflor + S pollen

vs sulfoxaflor + BQ pollen: $P = 0.68$). As a consequence, if sulfoxaflor (0.02 ppm) increased the risk of death in bees fed without pollen (HR = 1.53), feeding bees BQ pollen or S pollen reversed this risk (BQ pollen: HR = 0.76; S pollen: HR = 1.07; Fig. 6).

Similarly, the consumption of pollen lowered the negative effect of the highest concentration of sulfoxaflor (sulfoxaflor vs sulfoxaflor + BQ pollen: $P < 0.001$ and sulfoxaflor vs sulfoxaflor + S pollen: $P < 0.001$). However, the improvement of bee survival was significantly higher when bees consumed the S pollen as compared to the BQ pollen (sulfoxaflor + BQ pollen vs sulfoxaflor + S pollen: $P < 0.001$; Fig. 3B). Overall, the consumption of BQ pollen and S pollen decreased the mortality risk by 2 and 2.5-fold, respectively (BQ pollen: HR = 5.72, S pollen: HR = 4.79) compared to bees fed without pollen (HR = 12.01; Fig. 6).

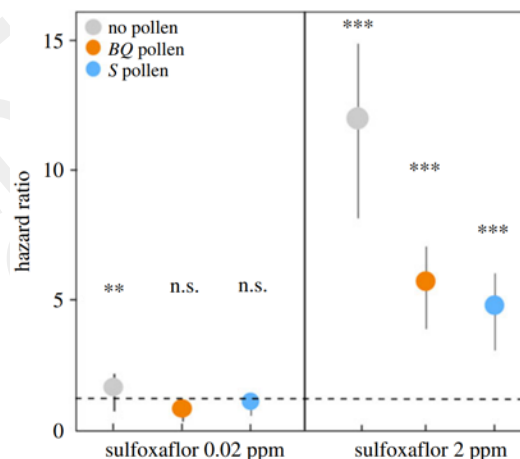


Figure 6. Hazard ratio for bees exposed to sulfoxaflor under different pollen feeding regimes. Bars indicate the 95 % confidence interval. Stars indicate statistically significant risks of death caused by the pesticide (* = $P < 0.05$; ** = $P < 0.01$ and *** = $P < 0.001$) and the dotted line represents HR=1.

3.1.2. Potential mechanisms underlying the nutritional modulation of pesticide sensitivity

Influence of pollen nutrition and pesticides on vitellogenin expression level

The expression level of vitellogenin was significantly affected by the different treatments (Kruskal-Wallis test, $P < 0.001$; Fig. 7A and B). In bees not exposed to pesticide, pollen feeding increased vitellogenin expression but the effect was significantly stronger with the S pollen (~ 30-fold) than with the BQ pollen (~ 8-fold, $P < 0.001$).

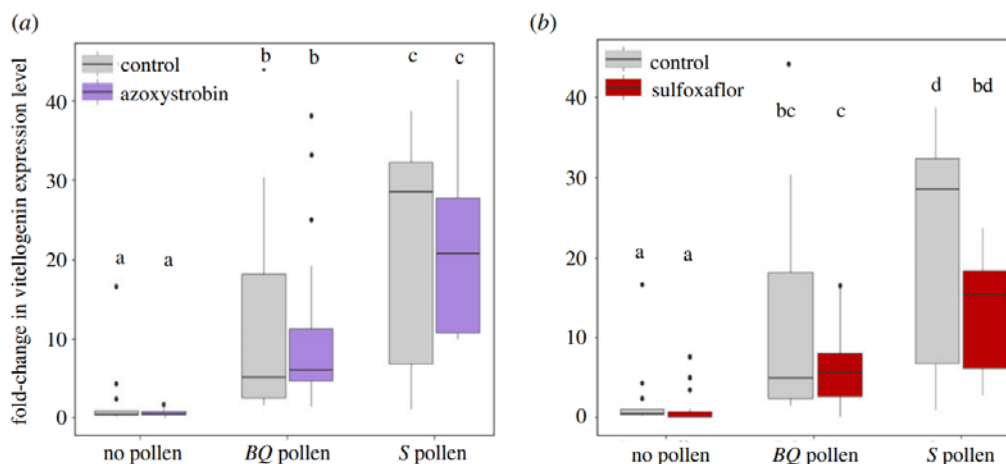


Figure 7. Gene expression levels of vitellogenin in response to pesticides and pollen feeding regimes. Vitellogenin expression levels are shown for bees exposed to azoxystrobin (2ppm) (A) and sulfoxaflor (2ppm) (B) and according to the pollen diets (n = 18-20 pools of 3 bees per experimental condition). Boxes indicate the 1st and 3rd interquartile range with a line denoting the median. Whiskers include 90% of the individuals, beyond which circles represent outliers. Different letters indicate significant differences (Kruskal-Wallis tests followed by Dunn's multiple comparison tests).

In all pollen treatments, we did not find any effect of azoxystrobin and sulfoxaflor exposure on vitellogenin expression levels (no pollen, BQ pollen and S pollen: $P = 1.0$). However, after both pesticide exposures, the level of vitellogenin remained significantly higher in bees fed with the S pollen as compared to bees who consumed the BQ pollen ($P < 0.05$).

Influence of pollen nutrition on pesticide detoxification

Residues of azoxystrobin were detected at very low concentrations at 8 hr post-exposure in all treatment groups (Fig. 8A). No significant difference was observed between experimental groups (Kruskal-Wallis test, $P = 0.71$). Since azoxystrobin concentrations were close to the LOQ at 8 hr post-exposure, samples at 24 hr post-exposure were not analysed.

Regarding sulfoxaflor, the concentrations of residues found in bees 8 hr post-exposure was significantly different between experimental groups (Kruskal-Wallis test, $P < 0.01$; Fig. 8B). The maximum concentrations were found in bees fed with sugar syrup alone (0.19 ± 0.02 mg/kg). Consumption of S pollen significantly decreased sulfoxaflor concentrations (0.13 ± 0.03 mg/kg) compared to bees who did not ingest pollen (1.5 times less, $P < 0.01$). The concentrations of sulfoxaflor in BQ pollen-fed bees were intermediate (0.17 ± 0.04 mg/kg) and did not differ from control ($P = 0.11$) and S pollen-fed bees ($P = 0.11$). For each treatment group, the concentration of sulfoxaflor significantly decreased between 8 and 24 hr post-exposure (Mann-Whitney test, $P < 0.001$ for each treatment group). It also differed between treatment groups at 24 hr post-exposure (Kruskal-Wallis test, $P < 0.001$). Sulfoxaflor concentration was more than two times lower in bees fed with the S or BQ pollen diets than in bees fed without pollen (S pollen: 0.04 ± 0.02 mg/kg, BQ pollen: 0.03 ± 0.02 mg/kg, and control: 0.10 ± 0.03 mg/kg; $P < 0.001$ for both diets). Finally, no difference in sulfoxaflor concentration was found between the two pollen diets at 24 hr post-exposure ($P = 0.63$).

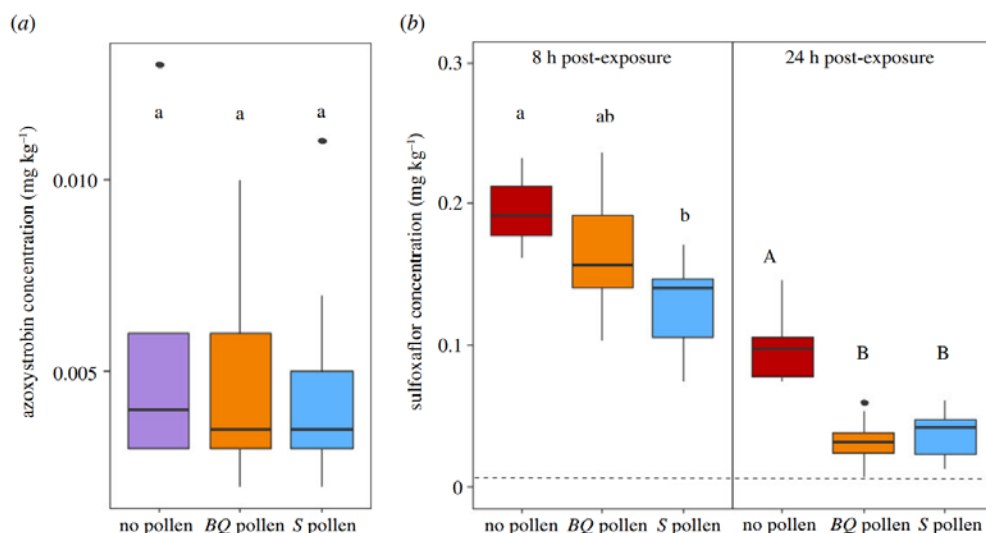


Figure 8. Concentration of (A) azoxystrobin and (B) sulfoxaflor in bees fed with different pollen regimes. Data represent the pesticide concentrations in 10 pools of 25 bees per experimental conditions. Boxes indicate the 1st and 3rd interquartile range with a line denoting the median. Whiskers include 90% of the individuals, beyond which circles represent outliers. Different letters indicate significant differences (Kruskal-Wallis tests followed by Dunn's multiple comparison tests) and the dotted line represents the limit of quantification.

3.2 Bumble bee (*Bombus terrestris*)

As the data were collected over several months, using different colonies in each month, variability was observable between the different controls, probably because they come from different colonies. To overcome this, the results of all measured parameters were subtracted by the mean of the corresponding control and divided by the standard deviation. Thus, the observed results reflect the difference (in grams for the weighed parameters) to the control.

Our results show that exposure to certain pesticides can have effects on both development and resource consumption of bumble bee micro-colonies, but not on mortality. Depending on the diet, which impacted the different parameters measured, these effects can be modulated.

Regardless of pollen diet, insecticide exposure significantly impacted brood development when micro-colonies were exposed to either cyantraniliprole (CY, $p < 0.001$), or the highest concentrations of sulfoxaflor (S3 and S4, $p = 0.013$ and $p < 0.0001$, respectively) (Fig. 11). No significant effects were observed for bumble bees exposed to glyphosate or azoxystrobin, or to the mixture (AS) (Figs 9-10, $p = 0.798$, $p = 0.747$ and $p = 0.190$, respectively). The total brood mass of micro-colonies fed with either low quality pollen (*Cistus*) or low quantity pollen (Starvation) was negatively impacted by these diets compared to bumble bees fed with *ad libitum* *Salix* pollen (Fig. 9-10, all $p < 0.001$). Under nutritional stress (i.e. quantitative or qualitative), total brood mass was not more affected when bees were exposed to sulfoxaflor, Amistar® or glyphosate, as the differences between *Salix*, *Cistus* and starvation conditions were not significantly different (all $p < 0.3$). However, the impact of nutritional stress (i.e. quantitative and qualitative) was no longer significant when the micro-colonies were exposed to Cyantraniliprole (Fig. 11, $p = 0.946$).

Regarding resource consumption, decreases were observed for pollen consumption in micro-colonies exposed to S4 ($p = 0.008$) or cyantraniliprole (CY, $p < 0.001$) (Fig. 11), and for syrup consumption in micro-colonies exposed to certain concentrations of sulfoxaflor (S3 and S4, $p = 0.011$ and $p < 0.001$, respectively), the azoxystrobin/sulfoxaflor mixture (AS, $p = 0.018$) or to cyantraniliprole (CY, $p = 0.008$) (Fig. 11). No significant effects were observed for bumble bees exposed to glyphosate or azoxystrobin

alone (Figs 9-10 all $p > 0.1$). While pollen consumption was impacted when bumble bees were put into quantitative stress conditions (STAR, $p < 0.001$), this was not observed during qualitative stress as no significant differences were observed between the pollen collection of those fed with *Salix* and those fed with *Cistus*, with the exception of bumble bees exposed to cyantraniliprolle, who consumed more pollen when fed with *Cistus* compared to those fed with *Salix* (Fig. 9, $p = 0.043$). Diet conditions also affected syrup consumption, with a higher consumption when micro-colonies were fed with *Salix* compared to those under a qualitative (C, $p = 0.011$) or quantitative (STAR, $p = 0.009$) stress but not for AS and CY (Figs 9-10).

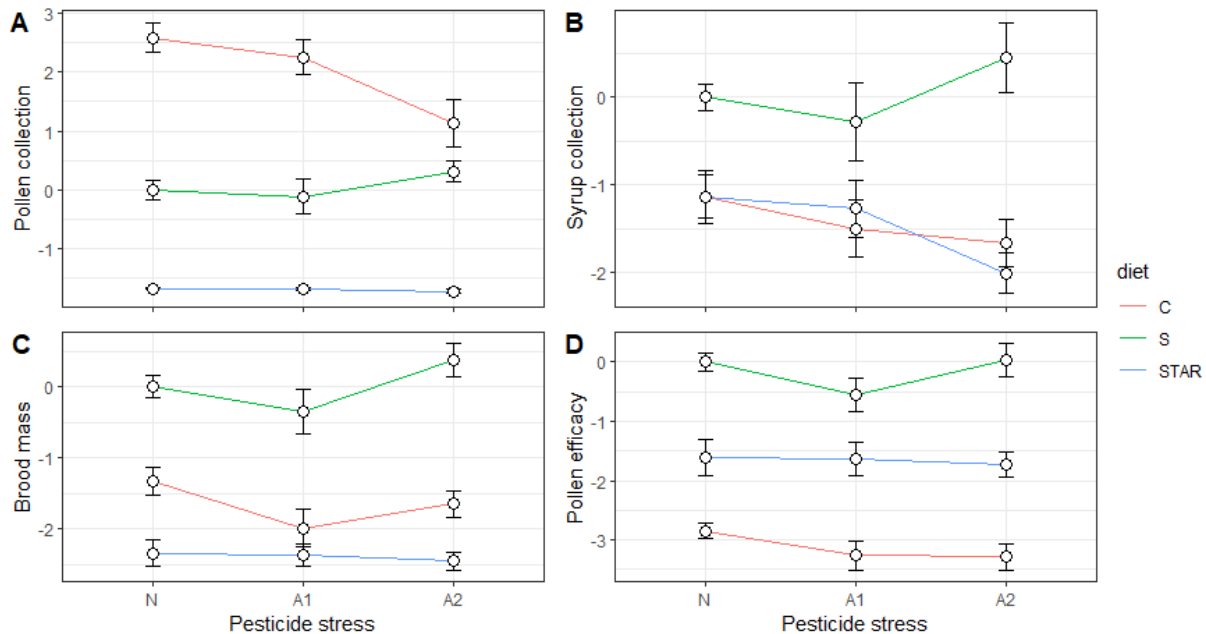


Figure 9. Resource collection and micro-colony development of bumble bees exposed to Amistar®. Pollen collection (a), syrup collection (b), brood mass (c) and pollen efficacy (d) of micro-colonies exposed to different levels of stresses (mean $\pm$ SE). C = *Cistus* diet, S = *Salix* diet, STAR = Starvation. Each treatment has 10 replicates.

Micro-colony efficacy was only impacted when bumble bees were exposed to high concentrations of sulfoxaflor (S3, $p = 0.014$ and S4, $p = 0.019$) or to cyantraniliprolle ($p < 0.001$) (Fig. 11). No significant differences were observed for bumble bees exposed to glyphosate or azoxystrobin (all $p > 0.4$) (Fig. 9-10). Micro-colonies fed with ad libitum *Salix* pollen were more efficient compared to those fed with limited amount of *Salix* pollen ($p = 0.038$) or with *Cistus* pollen ($p < 0.001$). Despite the fact that brood development was lower, bumble bees under quantitative stress (STAR) were still more efficient than those under qualitative stress (C) ($p = 0.028$). Diet effects were not mitigated by the pesticide's treatments, except with microcolonies exposed to cyantraniliprolle, for which the efficacy was the same, regardless of the diet ($p = 0.79$) (Fig. 11).

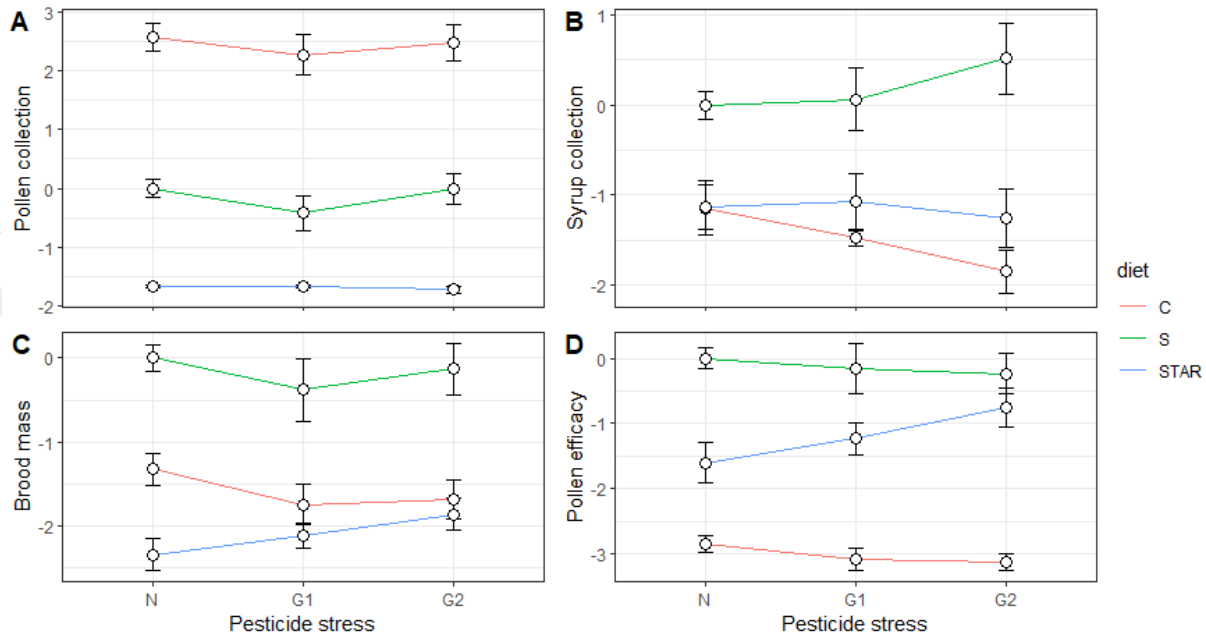


Figure 10. Resource collection and micro-colony development of bumble bees exposed to glyphosate. Pollen collection (a), syrup collection (b), brood mass (c) and pollen efficacy (d) of micro-colonies exposed to different levels of stresses (mean $\pm$ SE). C = *Cistus* diet, S = *Salix* diet, STAR = Starvation, N= no glyphosate; G1= lower concentration; G2= higher concentration. Each treatment has 10 replicates.

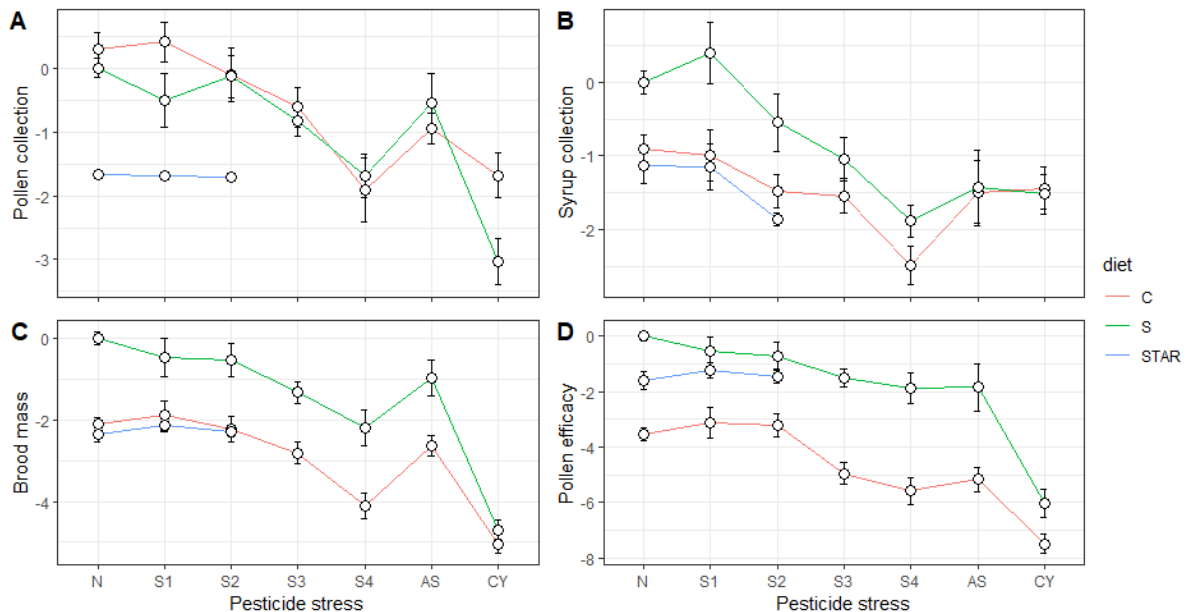


Figure 11. Resource collection and micro-colony development of bumble bees exposed to sulfoxaflor, Cyantraniliprole, or Sulfoxaflor + Amistar®. Pollen collection (a), syrup collection (b), brood mass (c) and pollen efficacy (d) of micro-colonies exposed to different levels of stresses (mean $\pm$ SE). C = *Cistus* diet, S = *Salix* diet, STAR = Starvation, N= no pesticide, S1= lowest concentration of sulfoxaflor, S2= second lowest concentration of sulfoxaflor, S3= third lowest concentration of sulfoxaflor, S4= highest concentration of sulfoxaflor, AS= Amistar® + sulfoxaflor, CY= Cyantraniliprole. Each treatment has 10 replicates.

3.3 Mason bee (*Osmia bicornis*)

3.3.1. Effects of nutrition

On average, over 90% of developing *O. bicornis* survived until adult emergence. The survival probability was not significantly affected by the type of nutrition. The nutrition type affected development time from hatching from the egg until initiation of cocoon spinning (Fig. 12a), cocoon weight before overwintering (Fig. 12b), pollen efficacy and the bees' probability to consume the whole pollen provision. The MIX pollen was beneficial for bees. Development time on MIX pollen was 2.8% faster compared to the average in the lower diversity pollen types. Additionally, cocoons were 9.6% heavier when bees fed on MIX compared to the average low diversity pollen types. Pollen efficacy was also higher (+5.6%) in MIX compared to the average low diversity nutrition. The probability of bees consuming the whole pollen provision was highest when they were feeding on C pollen (probability = 98.1%), followed by MIX pollen (probability = 78.3%), S pollen (probability = 29.5%) and BQ pollen (probability = 21.3%). Bees feeding on MIX pollen were more likely to consume the whole pollen provision than the average of bees feeding on the individual lower diversity nutrition types.

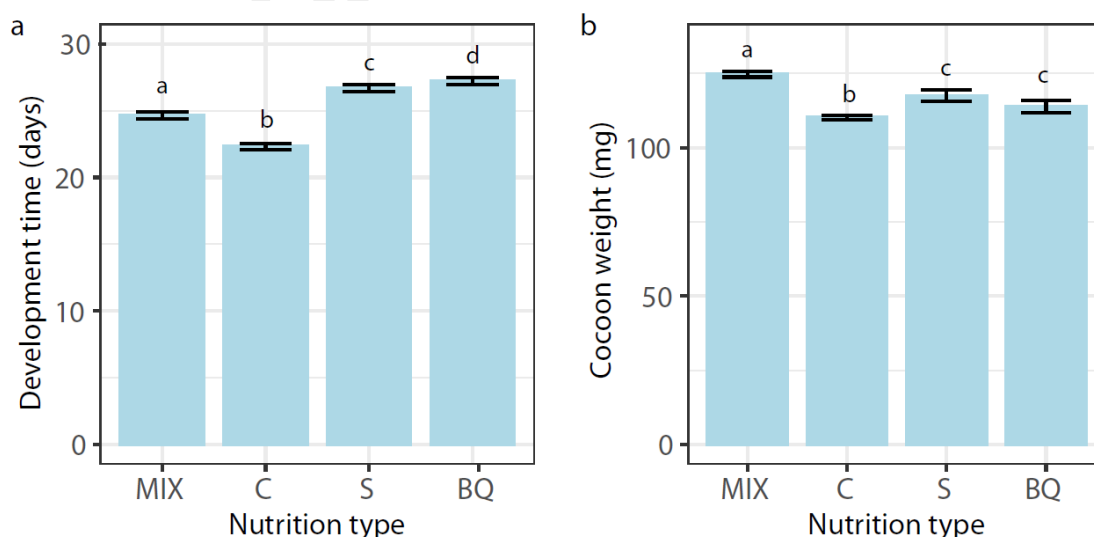


Figure 12. Effects of nutrition type on *O. bicornis* (a) development time needed from hatching until cocoon spinning (days) and (b) cocoon weight at pre-wintering (mg). Nutrition (pollen provision) types: MIX: Mixture of *Cistus*, *Salix* and Brassicaceae/*Quercus* pollen, C: *Cistus* pollen, S: *Salix* pollen, BQ: Brassicaceae/*Quercus* pollen. Estimated means and 95% confidence intervals are shown. Significant differences ($P \leq 0.05$) between nutrition types are indicated with different letters. Only *O. bicornis* not exposed to pesticides (water control, water-acetone control) were included in these analyses.

3.3.2. Effects of pesticides and their interaction with nutrition

The survival probability of *O. bicornis* exposed to 3 ppm sulfoxaflor was reduced by 32.4% compared to the control and by 30.3% compared to bees exposed to 0.3 ppm sulfoxaflor. High exposure to sulfoxaflor further elongated development time by 16.7% (ca. 4 days) compared to the control and by 15.8% compared to the low concentration. Additionally, high exposure to sulfoxaflor reduced cocoon weight before overwintering (-24.7% and -26.3% compared to control and low concentration, respectively) and pollen efficacy. (-11.8% and -13.7% compared to control and low concentration, respectively). Bees feeding on pollen contaminated with the high sulfoxaflor concentrations were less likely to consume the whole pollen provision (probability = 5.8%) compared to bees feeding on control pollen (probability = 41%) and bees feeding on pollen containing the low concentration of sulfoxaflor (probability = 60.4%).

We found an interaction of sulfoxaflor exposure and nutrition type in case of cocoon weight and pollen efficacy: the high concentration of sulfoxaflor negatively affected these measures in all nutrition types, however, the magnitudes of the effects were larger in MIX and S compared to C and BQ pollen. We did not find that the MIX nutrition mitigated the negative effects of sulfoxaflor in any of the analysed endpoints.

Azoxystrobin exposure affected survival until adult emergence, cocoon weight, and pollen efficacy. For survival, we found, however, no significant differences between the two azoxystrobin concentrations (low: 0.19 ppm, high: 1.9 ppm) and the control group. There was only a marginally significant difference between the low and the high azoxystrobin concentration. The high concentration of azoxystrobin tended to reduce the survival probability by 10.8% compared to the low concentration. We did not find significant differences between the different azoxystrobin concentrations for cocoon weight. Pollen efficacy was 3.4% higher in the bees treated with the low azoxystrobin concentration compared to those of the control group, but low and high concentrations did not significantly differ. The probability of larvae consuming the whole pollen provision was unaffected by exposure to azoxystrobin. Azoxystrobin exposure and nutrition type did not interact, and the effect of the high azoxystrobin concentration in the MIX nutrition did not differ from its average effect in the low diversity nutrition.

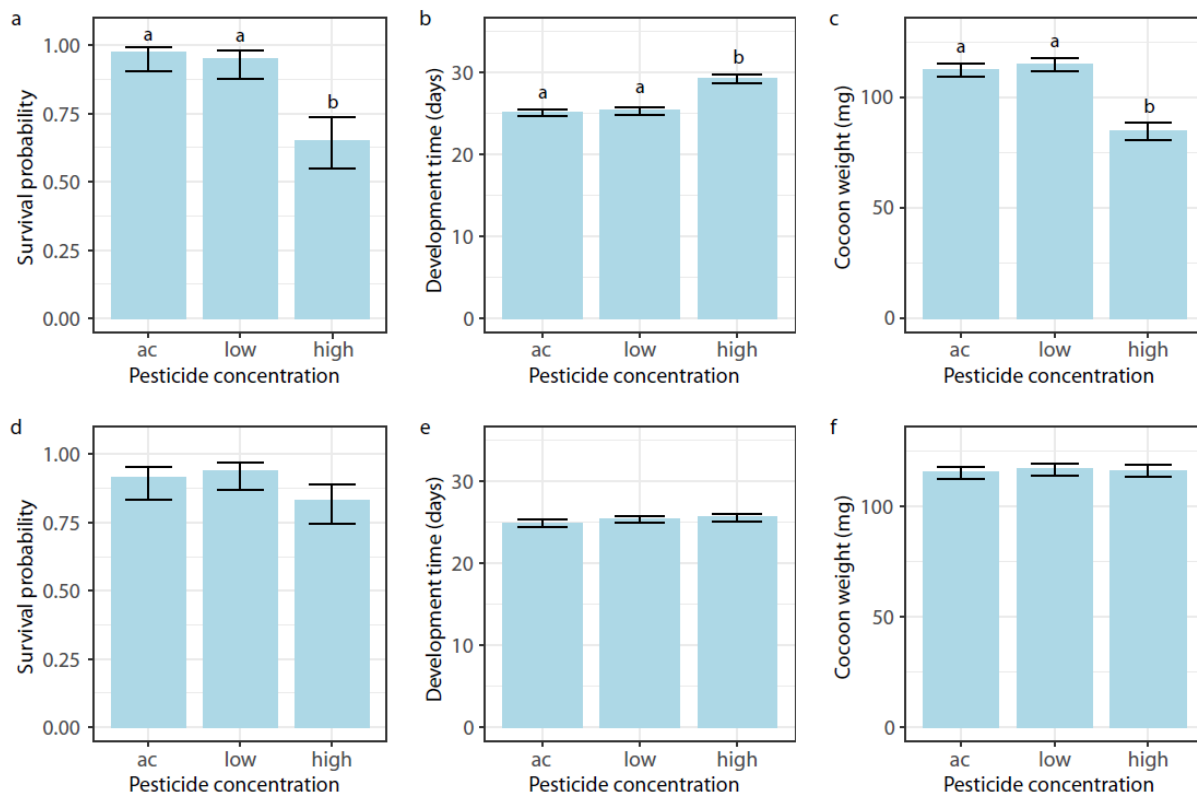


Figure 13. Pesticide main effects on *O. bicornis*: Main effects of sulfoxaflor 0.3 / 3 ppm (SUL, a-c) and azoxystrobin 0.19 / 1.9 ppm (AZO, d-f) on *O. bicornis* survival, development time from hatching to cocoon spinning (days) and cocoon weight at pre-wintering (mg). Estimated means (average across the four nutrition types) and 95% confidence intervals are shown. Significant differences ($P \leq 0.05$) between pesticide concentrations are indicated with different letters.

4 Discussion

Honey bees (Apis mellifera)

In the present study, we showed that pollen consumption, besides its well-known positive effect on honeybee longevity (Stuligross & Williams, 2020; Schmidt et al. 1987), can reduce the mortality risk caused by pesticides across different conditions of exposure. In addition, we found that the quality of pollen diets can substantially affect the toxicity of pesticides. These results may help to explain the variability of responses often observed at a given concentration or concentration of pesticide (Poquet et al. 2016).

Similar to Wahl and Ulm (1983), the negative effect of an acute concentration of pesticide was reduced by pollen consumption. The tested concentration of azoxystrobin was found to be non-lethal (over 48 hr) to bees fed with pollen, while it slightly increased the mortality level of bees fed without pollen. Interestingly, the LD50 of azoxystrobin determined during preliminary assays appeared to be less toxic here, providing another example of response variability to pesticides. Experiments were performed in different years and with different colonies (bee genetics), which likely explain the different responses across LD50 experiments (Rinkevich et al. 2015). Contrary to Wahl and Ulm (1983), pollen quality did not influence the sensitivity of bees to the tested concentrations of pesticides. This lack of effect here might be due to the concentrations or the pollen diets we used. Consequently, measurements should be repeated over a range of dosages to derive more general conclusions about a potential influence of pollen quality. It is also possible that the differences in our pollen diets were not strong enough to influence bee sensitivity to pesticides in the short-term (i.e. over 48 hours). However, similar differences in the nutritional quality of pollens were previously found to affect the chronic susceptibility of honey bees to a parasitic infection (Di Pasquale et al. 2013), suggesting that effects might rather be observed over the long-term as indicated by our chronic exposure experiment.

In the chronic exposure experiment, pollen diets increased the consumption of sugar syrup. Such results are consistent with previous studies, which showed that in response to pollen nutrients, genes involved in carbohydrate metabolism are upregulated (Alaux et al. 2011; Annoscia et al. 2017). This may reflect a higher energy demand since pollen consumption stimulates tissue growth (e.g., hypopharyngeal glands and fat body) (Brodschneider & Crailsheim 2010), which is an energetically costly process. However, this phenomenon was not observed in bees exposed to sulfoxaflor. Syrup consumption did not differ between pollen groups and thus bees provided with different pollen diets were exposed to similar amounts of pesticides. Only bees exposed to the high concentration of sulfoxaflor (2 ppm) tended to consume less syrup. There is now strong evidence for preference or avoidance of sugar syrup laced with pesticides, and this food choice was found to be dependent on pesticide concentration (Kessler et al. 2015; Liao et al. 2017). Even though bees are capable of taste perception (de Brito Sanchez 2011), the underlying mechanisms of food choice are not clearly known (Kessler et al. 2015). In our experiments, bees were not provided with a food choice but it is possible that sulfoxaflor at high concentration gives syrup a bitter taste as previously found with high concentration of nicotine (Köhler et al. 2012), which target nicotinic receptors like sulfoxaflor.

Survival data from the chronic exposure experiment further confirmed the beneficial effect of pollen on tolerance to pesticides: the risk of death upon exposure to the low and high sulfoxaflor concentrations disappeared or was significantly reduced by pollen feeding, respectively. This is in accordance with a previous study, which showed that bees fed over several days with a pollen-based diet exhibit reduced sensitivity to a daily exposure to chlorpyrifos (Schmehl et al. 2014). Both pollen diets contained traces of DMF and tau-fluvalinate (below the LOQ), introducing possible interactive effects with the experimental pesticides (Johnson 2015). However, this was not the case for azoxystrobin since no toxic effect were found on bee survival. Regarding sulfoxaflor, it may have increased or lowered its toxicity but to a small extent since survival upon exposure to sulfoxaflor remained lower in bees fed without pollen than in bees provided with pollen.

Interestingly, bees fed with the S pollen were less sensitive to the high sulfoxaflor concentration as compared to the BQ pollen diet. This suggests that the quality of pollen diets can also affect their capacity to tolerate chronic exposure to pesticides. The higher protective effect of S pollen might result from an improved physiological state. For instance, regardless of exposure to pesticides, the expression level of vitellogenin was much higher in bees fed with the S pollen as compared to bees provided with the BQ pollen. As a glycoprotein with antioxidant functions that protects bees from oxidative stress (Havukainen et al. 2013; Park et al. 2018), vitellogenin promotes bee longevity but may also have reduced the effects of sulfoxaflor. Indeed, a recent study found that sulfoxaflor increases the level of reactive oxygen/nitrogen species and thus significantly elevates oxidative stress in bees (Chakrabarti et al. 2020). The higher vitellogenin expression level induced by the S pollen might have thus contributed to better protect bees from exposure to sulfoxaflor, assuming that changes in transcript levels translated into different protein levels. This latter point is supported by the significant decrease in haemolymph vitellogenin concentration upon inhibition of vitellogenin gene activity via RNA interference (Nelson et al. 2007), and the concomitant change in the gene and protein expression of vitellogenin between nurses and foragers (Fluri et al. 1982; Ament et al. 2011).

We did not find any pesticide-induced differences in vitellogenin levels between bees, either in the presence or absence of a pollen diet. This suggests that the negative impact of pesticides on bee survival does not involve a decline in vitellogenin level, although we cannot eliminate long-term exposure effects. Reported effects of pesticides on vitellogenin in the literature have been contradictory across studies, ranging from no effects (acute exposure to fipronil and deltamethrin; Bordier et al. 2017), to increasing (chronic exposure to neonicotinoids; Christen et al. 2016) and also inhibitory effects (chronic exposure to imidacloprid (Abbo et al. 2017). This indicates that effects on vitellogenin may be quite variable and possibly depend upon multiple factors, e.g. age of the bees, season, pesticide type and mode of exposure (acute, chronic).

In response to exposure to dietary toxins, organisms have developed elimination mechanisms (e.g., detoxification) that prevent their accumulation in organs and tissues. How the body is able to handle pesticides can therefore affect its pesticide sensitivity. The analysis of pesticide residues showed that azoxystrobin was eliminated much faster than sulfoxaflor (~100-fold difference between the 2 pesticide concentrations at 8 hr post-exposure), even though the same concentrations were given to bees. The mechanisms underlying this faster metabolization of azoxystrobin are not known, but enzymes from the detoxification pathways, like the cytochrome P450 monooxygenases, often exhibit substrate-specificity. For instance, cytochrome P450 members of the CYPQ9 family were found to be responsible for tau-fluvalinate metabolism (Mao et al. 2011). Honey bees might thus possess cytochrome P450s that can dock and metabolize azoxystrobin better than sulfoxaflor. This rapid azoxystrobin metabolization might also contribute to its lower toxicity as compared to sulfoxaflor. However, since azoxystrobin is a fungicide we obviously cannot exclude that it is less efficient in reaching its biological target and/or has a different mode of action in insects.

Finally, sulfoxaflor concentration decreased more quickly in bees fed with the S pollen as compared to bees provided with the BQ pollen. This faster metabolization may play a causal role in the reduced sulfoxaflor toxicity upon ingestion of the S pollen diet. Such results also confirm a recent study, which reported that some pollens are better than others in promoting pesticide metabolization (Ardalani et al. 2021). Different pollens have different nutritional values, which generally translate into differences in bee physiology and longevity (Omar et al. 2017; Standifer 1967). Among the pollen nutrients that have positive effects on bee health, the amount of protein plays a substantial role (Frias et al. 2017), although it does not result systematically in healthier bees, especially regarding pathogen resistance (Di Pasquale et al. 2013). Our study further indicates that the quality of pollen should not just be estimated based on protein content since the S pollen had a lower concentration of protein than the BQ pollen. For instance, the amount of other nutrients, such as amino acids, sterols, vitamins, minerals, nutrient ratio can also influence bee physiology and longevity (Corby-Harris et al. 2018; de Groot 1953; Moerman et al. 2017; Stabler et al. 2021; Vanderplanck et al. 2014, 2018). More specifically, the macronutrient ratio in pollen

may also influence the sensitivity of bees to pesticides. This was demonstrated by testing diets with modified protein to lipid ratios (P:Ls) and several pollen diets with different P:Ls (Crone & Grozinger 2021). The pollen-induced differences in pesticide sensitivity reported in our study could not be explained by this nutritional factor since both pollen diets had similar P:Ls. However, several studies have shown that the pollen phytochemicals quercetin and p-coumaric acid, upon ingestion, can significantly enhance bee longevity (Bernklau et al. 2019) and also tolerance to several pesticides (Liao et al. 2020). However, the effects of these phytochemicals are concentration-dependent; lower concentrations tend to have a positive effect on honey bee longevity (p-coumaric acid at 5, 50 and 500 μM and quercetin at 12.5, 25 and 250 μM), while higher natural concentrations (1000 μM) have no effects (Liao et al. 2020). Similarly, the reduced mortality risk upon exposure to pesticide was observed over a range of relatively low natural concentrations (5, 50 and 500 μM) for p-coumaric acid. Higher concentrations (1000 μM) increased or did not change the toxicity of pesticides (Liao et al. 2020). Our results are therefore consistent with these data given that the S pollen, containing 637 μM of p-coumaric acid, was better in improving bee longevity and tolerance to sulfoxaflor when compared to the BQ pollen (1491.6 μM of p-coumaric acid). This former concentration might be more optimal for stimulating detoxification enzymes [43] and thereby more quickly eliminating sulfoxaflor, as indicated by our results. It was not possible to quantify quercetin, but its concentration below 33.09 μM likely falls in the range of beneficial concentrations for both pollens, and therefore does not explain the differences in pollen quality.

Overall, our study demonstrated the modulation of pesticide toxicity by the nutritional state of worker honeybees. Pollen availability and quality, by modifying the physiological background of bees, can improve their ability to eliminate pesticides and withstand their detrimental effects (e.g., protective effect of vitellogenin against oxidative stress), as observed with the high concentration of sulfoxaflor. This nutritional modulation may cause a large range of pesticide responses in the field, given that the abundance and composition of honeybee pollen diets can be highly variable across landscapes and seasons (Galimberti et al. 2014; Richardson et al. 2015; Danner et al. 2017; de Vere et al. 2017; Kamo et al. 2018; Elliott 2021). A decline in resource availability and biodiversity in agro-ecosystems (Lichtenber et al. 2017) might therefore impair the bee's ability to deal with pesticides (Klaus et al. 2021), giving another strong argument for the restoration of floral resource abundance and diversity in such habitats (introduction of extensive grasslands and flower strips, protection of semi-natural habitats) (Decourtye et al. 2019; Scheper et al. 2013). Further research is therefore needed to evaluate the influence of a larger range of pollens of different qualities on pesticide toxicity to better mitigate the impact of exposure to pesticides.

Bumble bees (*Bombus terrestris*)

Our results show that the micro-colonies were affected differently depending on the diet they were given. Bumble bees fed with *Cistus*, or with a limited amount of *Salix* were negatively impacted compared to those fed with ad libitum *Salix* pollen, indicating that both qualitative and quantitative stress had an impact on micro-colony performances.

Qualitative stress (*Cistus* diet) had no impact on pollen consumption. There is therefore no alteration of nutritional intake to compensate the bad diet, underlining the importance of balancing the availability of nutrients in order to have an optimal diet (Crone and Grozinger, 2021). If this is in line with other results of previous studies using this pollen diet (Génissel et al., 2002; Tasei and Aupinel, 2008; Vanderplanck et al., 2014; Roger et al., 2017a; Barraud et al., 2022), it is important to notice that this is not necessarily the case with every other unfavourable diet. Pollen resources coming from plants of the Asteraceae genera for example, like *Taraxacum* sp. or *Cirsium* sp., are considered to be bad diets and are less consumed by micro-colonies compared to other more beneficial pollen diets (Roger et al., 2017b; Vanderplanck et al., 2018, 2019). This can be explained by the fact that these pollen diets are unbeneficial for different reasons: while pollen from Asteraceae contains toxic compounds (i.e.

alkaloids) and few sterol compounds, *Cistus* pollen does not contain any toxic compounds but only low levels of amino acids and sterols (Barraud et al., 2022).

As expected, reproduction parameters of bees fed with *Cistus* pollen were negatively impacted with a lower brood mass and pollen efficacy compared to the bees fed with *Salix* pollen. This has already been observed in various other studies showing that an imbalanced supply of nutrients can lead, e.g., to delayed development, reduced body weight, or even mortality (Sutcliffe and Plowright, 1990; Moerman et al., 2016, 2017; Archer et al., 2021). If there is a common assumption that proteins or protein/ lipid ratio are positively related to performance (Regali and Rasmont, 1995; Génissel et al., 2002; Roulston and Cane, 2002; Smeets and Duchateau, 2003; Alaux et al., 2010; Nicolson, 2011; Stabler et al., 2015), other results showed that amino-acid and sterol composition play a crucial role in diet quality. In this regard, studies concluded that amino acid intake was positively correlated with bumble bee body mass, and underlined that the effects of total amino acids intake may depend on the blend of individual amino acids (Archer et al., 2021; Barraud et al., 2022). Furthermore, some sterolic compounds have already been reported to be positively correlated with larval growth or brood development (Vanderplanck et al., 2014; Barraud et al., 2022). For example, 24-methylenecholesterol is known to influence moulting and ovary development (Svoboda et al., 1978, 1983; Regali, 1996; Human et al., 2007), while B-sitosterol and D5-avenasterol are supposedly involved in metabolic pathways of *B. terrestris* (Regali, 1996). Here, the *Cistus* pollen had a lower total amino acid content and sterol content (125.04mg/g and 4.54mg/g, respectively) compared to *Salix* pollen (181.35mg/g and 8.22mg/g, respectively, see Barraud et al. (2022), with a low quantity of 24-methylenecholesterol (1.43mg/g for *Cistus*, 2.48mg/g for *Salix*) and B-Sitosterol (0.73mg/g for *Cistus*, 2.42mg/g for *Salix*). However, it is important to keep in mind that those nutritional requirements are not necessarily the same among the bee species (Scwhartz et al, unpublished, Barascou et al., 2021; Barraud et al., 2022).

Quantitative stress (STAR diet) impacted pollen consumption, and also the total brood mass compared to *Salix*, and the efficacy compared to both other diets, with more efficacy compared to *Cistus* and less compared to *Salix*. While it was expected to observe a reduction in brood mass in this condition, it is interesting to see an efficacy difference between the ad libitum *Salix* diet and the limited *Salix* diet. Since it is the same pollen, we could have expected a similar efficacy. While we cannot conclude the reasons behind this difference, we can hypothesise that, as workers still need to consume pollen for their own health, they use relatively less pollen to feed the larvae compared to the bees fed ad libitum.

Depending on the agrochemical and the concentration to which the bumble bees were exposed, impacts on the micro-colonies on both resource consumption and reproduction were different.

Glyphosate had no impact neither on feeding or reproduction parameters. Studies exploring the impact of glyphosate on bees, especially on bumble bees, are not numerous but a recent meta-analysis (Battisti et al., 2021) gathered the different results to draw a picture of the current state of knowledge. When foraging, bees can be highly exposed during spraying (up to 2 g/L) or collect pollen and nectar from freshly sprayed plants (up to 629 and 31.3 mg/kg, respectively) (Thompson et al., 2014; Zhu et al., 2015; Abraham et al., 2018; Motta and Moran, 2020). With the concentrations used in this experiment (3.6 and 7.8mg/L), previously observed effects are contradictory. Vázquez et al. (2018) showed that *A. mellifera* larvae fed with 1.25 mg/L and 5 mg/L of glyphosate presented delayed ecdysis, and reduction in weight and survival. Conversely, another study showed that the mortality of adult workers of *A. mellifera* exposed orally, for 15 days, to two concentrations of GLY, 2.5 mg/L and 5 mg/L, was not impacted (Herbert et al., 2014). To our knowledge, the only effect of glyphosate on bumble bees is an impact on collective thermoregulation after chronic exposure (Weidenmüller et al., 2022). Interestingly, various studies have shown that newly emerged larvae and adults of *A. mellifera* exposed to glyphosate showed reduced expression of several detoxification-related genes (Gregorc et al., 2012; Tomé et al., 2020; Vázquez et al., 2020), suggesting that although glyphosate is not directly harmful, it can make bees more sensitive to other agrochemicals. Finally, glyphosate being an herbicide, it is important to consider that it can reduce food resources by reducing the diversity of plants around the crop and, therefore, reducing pollen and nectar accessibility (Battisti et al., 2021).

Fungicides, although lacking acute toxicity against insects, may impact bees directly by altering metabolism, reproduction and food consumption (Bernauer et al., 2015; Liao et al., 2017; Mao et al., 2017). Here, Amistar® alone did not impact any of the measured parameters. If our results are in line with recent studies that used *A. mellifera* (Barascou et al., 2021) and *O. bicornis* as models (Schwartz et al., unpublished) under laboratory conditions, other studies have found effects of this agrochemical at realistic levels in semi-field conditions, using *B. terrestris* as a model species. In these studies, various parameters including foraging performance, body mass, colony growth or pollen deposition have been negatively impacted by Amistar® when the maximum application rate was applied to crops (Tamburini et al., 2021a; Wintermantel et al., 2022). The mechanisms underlying the observed patterns are not evident, as only a few studies explored the effects of azoxystrobin on bees. However, it has been shown that azoxystrobin can influence the hormone system regulation of honey bees, which could result in disturbance of bee development into foragers (Christen et al., 2019). These differences between laboratory and semi-field experiments are interesting. Although the reasons are difficult to explain, it can be hypothesised that the semi-field conditions are more energetically demanding (e.g. foraging) and that other stresses may be involved, making the bees more sensitive to agrochemical stress. Another reason for this resistance could be that bees are good at detoxifying azoxystrobin compared to other pesticides. In a recent study, Barascou et al. (2021) showed that azoxystrobin was eliminated 100-fold faster than sulfoxaflor. If the mechanisms underlying this faster metabolism of azoxystrobin are unknown, the authors suggested a potential substrate specificity of the cytochrome P450 monooxygenases, as is already known to be the case for the metabolism of tau-fluvalinate that is driven by the CYPQ9 family of cytochrome P450s (Mao et al., 2011).

Insecticide exposure impacted every measured parameter. Both resource consumption and brood development were negatively affected when the bees were exposed to the highest concentrations of sulfoxaflor (S3 and S4). If studies focusing on sulfoximine insecticides are currently limited (Siviter and Muth, 2020), this class of insecticide is similar to neonicotinoids and shares the same mode of action: by interfering with nerve impulse transmission of the central nervous system via modulation of the nicotinic acetylcholine receptors, these molecules cause tremors followed by paralysis and death of the exposed targets (Matsuda et al., 2001; Tomizawa and Casida, 2003; Cutler et al., 2013). Even if bees are not the target of these insecticides, neonicotinoids are known to interfere with many different parameters like locomotion, reproduction, cognition, olfaction or nutritional intake (van Tomé et al., 2012; Thompson et al., 2015; Kessler et al., 2016; Tosi et al., 2017a, 2017b; Hesselbach and Scheiner, 2018; Barraud et al., 2020).

Reduction of resource consumption following sulfoxaflor exposure have already been reported in other laboratory experiments with different species (Barascou et al., 2021; Linguadoca et al., 2021; Schwartz et al., unpublished). The reduction of both syrup and pollen collection observed in this study may imply that we are not observing a simple repellent effect, given that pollen did not contain any pesticide in our experiment, and that sulfoxaflor exposure leads directly to a reduction in nutritional intake, as has already been suggested in other studies using neonicotinoids (Thompson et al., 2015; Kessler et al., 2016; Tosi et al., 2017b). Observed effects on brood development and pollen efficacy are in line with other studies (Schwartz et al., unpublished; Linguadoca et al., 2021). However, the low concentrations used did not show effects on bumble bees while Linguadoca et al. (2021) found lethal effects at 1.37mg/kg and sublethal effects at 0.16mg/kg. Here, with realistic concentrations (S1 and S2), we did not witness any effect on our measured parameters, while witnessed effects at the highest concentration were not realistic, or at best the worst-case scenario possible. Nonetheless, Tamburini et al. (2021) observed reductions in foraging performance and colony growth in a semi-field experiment, using regulatory application rates of sulfoxaflor. While this reinforces the idea that bees are less sensitive in the laboratory, it also suggests that further studies are needed to better assess the risks associated with this pesticide.

Given that bumble bees are impacted by sulfoxaflor, the interaction of these effects with the pollen diet were investigated in this study. It has been hypothesised that adequate nutrition could mitigate the

negative effects of pesticides on bees (Wahl and Ulm, 1983; Wong et al., 2018; Ardalani et al., 2021b, 2021a; Crone and Grozinger, 2021), for example through increased expression of detoxification genes (Johnson et al., 2012; Schmehl et al., 2014). Proteins, lipids and carbohydrates serve as important energy sources for the detoxification of toxic compounds (Even et al., 2012) and adequate intake of these nutrients could therefore compensate for the increased energy demand during the detoxification process (du Rand et al., 2015). However, the present work only found additive effects of these two stresses following a sulfoxaflor exposure. Bees fed with *Cistus*, with or without sulfoxaflor, were less efficient compared to exposed bees fed with *Salix*. If some studies have demonstrated synergistic interactions between pesticide exposure and nutritional stress in honey bees, bumble bees and *Osmia* (Tosi et al., 2017b; Linguadoca et al., 2021), a recent meta-analysis reviewing interactive effects of pesticides with further stressors such as food stress concluded that food stress and pesticide exposure generally act additively (Siviter et al., 2021), which is in line with our results. Nonetheless, other nutritional stresses could affect the bees in another way that could lead to variability in the sensitivity to pesticides. In our study, the bad quality of the pollen diet was mostly linked to a lack of total amino-acids and specific sterols compounds (Barraud et al., 2022). Given the importance of proteins in detoxification mechanisms, it could be interesting to test a pollen diet with a low protein content, as it could be also interesting to use pollen from some specific plants (like Asteraceae) that contain toxic compounds which could, coupled with a pesticide exposure, overwhelm the detoxification system already weakened by this pollen diet. In addition, several studies have shown that the pollen phytochemicals quercetin and p-coumaric acid can significantly enhance tolerance to several pesticides (Liao et al., 2017, 2020; Wong et al., 2018). It would therefore be interesting to test pollen diet with various quantities of these phytochemicals. Finally, we have to keep in mind that nutritional stresses can also be caused by the quality/quantity of the consumed nectar. Linguadoca et al. (2021) already explored this question and showed that sulfoxaflor coupled with a nutritional deficiency using different sugar syrups synergistically reduced the survival and fecundity of bumble bee microcolonies.

Bumble bees exposed to cyantraniliprole were highly impacted, with a reduction of resource consumption and a higher reduction of both brood development and pollen efficacy compared to the other treatments. As, to our knowledge, no other studies have looked at sub-lethal parameters using this pesticide, we cannot put in perspective what was observed in this work. Interestingly, with this pesticide, no additive effects were observed. Instead, there were no difference between the measured parameters of exposed bees fed with the beneficial and unbeneficial diet. This kind of phenomenon has already been observed in a previous study, in which bumble bees were exposed to imidacloprid and brood development was more impacted compared to the colonies exposed to sulfoxaflor in this present study (Barraud et al., 2020). Therefore, we hypothesise that bumble bees that are highly affected by a pesticide cannot benefit from a good diet anymore.

Finally, the effects of the interaction between sulfoxaflor and Amistar® have been explored in this work. Fungicides, although lacking acute toxicity against insects, may impact bees directly by altering metabolism, reproduction and food consumption (Bernauer et al., 2015; Liao et al., 2017; Mao et al., 2017), and indirectly by increasing insecticide toxicity (Tosi et al., 2017b; Tsvetkov et al., 2017; Sgolastra et al., 2018; Tosi and Nieh, 2019). Here, no significant differences were observed between the mixture (AS) and the same concentration of sulfoxaflor alone (S3). So far, while various studies have been conducted using different species in the laboratory or under semi-field conditions, the results are mixed. While some studies have established an interaction between sulfoxaflor and a fungicide (Linguadoca et al., 2021; Tamburini et al., 2021a), others have not (Azpiazu et al., 2021; Tamburini et al., 2021b; Wintermantel et al., 2022). More studies need to be conducted on this topic in order to better understand in which situation an interaction can take place.

Mason bees (*Osmia bicornis*)

Our study demonstrates a clear positive effect of a more diverse pollen diet for developing *O. bicornis* bees. On MIX pollen, larvae developed faster and transformed the ingested pollen more efficiently into body weight, resulting in heavier cocoons. The high concentration of sulfoxaflor (3 ppm) showed negative effects on *O. bicornis*: we observed increased mortality, elongated development time, reduced pollen efficacy and cocoon weight as well as lowered pollen consumption. We also found that azoxystrobin might have a negative influence on *O. bicornis* development time and survival. Our findings suggest that the impact of sulfoxaflor might be modulated by the type of pollen nutrition, but we did not find that negative effects were less strong in a more diverse pollen nutrition.

Pollen amino acid and protein content influences bee development (Roulston and Cane 2002, Vanderplanck et al. 2014, Moerman et al. 2016b). For an optimal diet, nutrients need to be balanced (Crone and Grozinger 2021). In our study, *Cistus* pollen had the lowest protein content (20.96%) and total and essential amino acids (12.50% and 5.26%, respectively), whereas *Salix* and *Brassicaceae/Quercus* diets contained more amino acids and/or proteins (*Salix*: protein content: 21.49%, total and essential amino acid content: 18.14%, 8.65%; *Brassicaceae/Quercus*: protein content: 28.39, total and essential amino acid content: 17.45%, 8.56%; Barraud et al. 2022). On average, *O. bicornis* females provision their offspring with pollen containing around 19% protein (Budde and Lunau 2007). Since none of our tested pollen diets had a protein content lower than 19%, this might explain why survival was not affected by the different nutrition types.

If bees are subjected to a suboptimal supply of nutrients, negative effects such as delayed development or reduced body weight can occur (Sutcliffe and Plowright 1990, Moerman et al. 2016b, Moerman et al. 2017, Archer et al. 2021). Even though *Cistus* had the lowest protein and amino acid content in our experiment, bees developed fastest on this pollen. This suggests that additional factors might have influenced development speed. For instance, it has been shown that the depletion of stored food is a critical cue for the initiation of metamorphosis in *O. lignaria* (Helm et al. 2017). Since most bees in our study consumed the whole *Cistus* pollen, this might have triggered the initiation of cocoon spinning earlier than in bees feeding on the other pollen types, which were less well consumed. Cocoon spinning might also be initiated after the bee has consumed a specific amount of carbohydrates (Austin and Gilbert 2021). However, since we used equal amounts of sugar solution in all treatments, this should not have affected development times. Even though most bees consumed the whole *Cistus* pollen, they showed the lowest cocoon weight before overwintering. This is in line with other studies reporting a reduced pollen efficacy and body weight when bees were feeding on *Cistus* pollen (Tasei and Aupinel 2008, Vanderplanck et al. 2014, Baloglu and Gurel 2015, Moerman et al. 2016a, Moerman et al. 2017, Leza et al. 2018).

Bees feeding on MIX pollen benefitted in terms of development time, cocoon weight, pollen consumption and pollen efficacy. Other studies also reported that bee larvae reached a higher body weight on mixed pollen, even when it was not the pollen with the highest protein content (Tasei and Aupinel 2008). Also pollen efficacy of a pollen mixture has been found to be higher than theoretically expected (Moerman et al. 2017). The fact that the pollen efficacy of the *Salix* pollen was similarly high compared to the MIX, however, suggests that it is not necessarily better to have a higher pollen diversity, as long as the right nutrients are available in adequate amounts (Radmacher and Strohm 2010, Moerman et al. 2017).

The high concentration of sulfoxaflor negatively affected development time, cocoon weight, pollen efficacy and pollen consumption. An elongated development time can be disadvantageous, as the larval stage of *O. bicornis* is especially vulnerable to parasitism, infection and environmental stresses (Eraerts et al. 2020). A reduced body size of females can further lead to fitness disadvantages, as smaller bees live shorter, have a lower foraging range and can only transport less pollen per flight compared to larger bees, which makes them less efficient foragers and leads them to produce fewer and smaller offspring (Bosch and Kemp 2004, Seidelmann et al. 2010).

Neurotoxic insecticides like sulfoxaflor interfere with the transmission of nerve impulses in insects (Matsuda et al. 2001, Tomizawa and Casida 2003, Cutler et al. 2013). Neonicotinoid insecticides can impair motor function, cognition and locomotion in bees (Tomé et al. 2012, Tosi and Nieh 2017, Jacob et al. 2019) and affect odor or taste perception (Hesselbach and Scheiner 2018). It is therefore possible that sulfoxaflor, with its similar mode of action, might impair a bee's ability to consume pollen provisions (Cresswell et al. 2012), ultimately leading to an elongated development time and reduced body weight. Similar findings have previously been reported (Abbott et al. 2008). The reduced pollen consumption under sulfoxaflor exposure has likely played a major role in reducing the survival probability of bees in our experiment. Exposure to sulfoxaflor might also have caused an increased energy demand due to detoxification (du Rand et al. 2017, Tosi et al. 2017). Further, a previous study demonstrated a negative correlation between development time and pollen efficacy for *O. bicornis* (Konrad et al. 2008).

For the fungicide azoxystrobin, we found indications that high exposure might negatively affect survival and development time (Fig. 13a, b). Azoxystrobin can influence the hormone system regulation of honey bees, potentially disturbing the development of foragers (Christen et al. 2019). Additionally, the azoxystrobin-containing product Amistar has been shown to increase adult mortality, reduce colony growth and worker body size (Wintermantel et al. 2022). Our study calls for further work on the impacts of azoxystrobin and other fungicides on solitary bees, especially given that these products are frequently sprayed into flowering crops, leading to high exposures of bees to fungicides (Cullen et al. 2019).

High quality nutrition might lower the negative effects of pesticides on bees (Wahl and Ulm 1983, Wong et al. 2018, Ardalani et al. 2021a, Ardalani et al. 2021b, Crone and Grozinger 2021), for instance by enhancing detoxification gene expression (Johnson et al. 2012, Schmehl et al. 2014). Proteins, lipids and carbohydrates are valuable energy sources and a good supply of these nutrients is likely essential to compensate for the high energy demand during detoxification (du Rand et al. 2017). Even though we found beneficial effects of a higher diversity nutrition, we did not find that the MIX pollen mitigated the effects of sulfoxaflor on development. Similar results were obtained for bumble bees exposed to imidacloprid fed with different quality pollen diets (Barraud et al. 2020).

While several studies on interactions of pesticides and nutritional stresses have been conducted on honey bees (Wahl and Ulm 1983, Di Pasquale et al. 2013, Schmehl et al. 2014, Renzi et al. 2016, Tosi et al. 2017, Tong et al. 2019, Barascou et al. 2021, Crone and Grozinger 2021, Linguadoca et al. 2021, Vodovnik et al. 2021) and bumble bees (Dance et al. 2017, Leza et al. 2018, Barraud et al. 2020, Wintermantel et al. 2022), only a few studies are available for solitary bees (Cecala et al. 2020, Stuligross and Williams 2020, Klaus et al. 2021, Kopit et al. 2022, Knauer et al. under review). Several studies have demonstrated synergistic negative interactions of food stress and pesticides (Tosi et al. 2017, Linguadoca et al. 2021, Knauer et al. under review), a recent meta-analysis concluded that the two stressors generally act additively (Siviter et al. 2021). The relatively high quality of the pollen used in our experiments might partly explain why we did not find a mitigation of the sulfoxaflor impacts by a more diverse nutrition. Additionally, a higher diversity pollen alone might not be better for bees, rather it is important that the pollen offers all essential nutrients (Bukovinszky et al. 2017, Filipiak et al. 2022).

In conclusion, our experiment demonstrated a beneficial effect of higher diversity nutrition for *O. bicornis* development. It is therefore crucial to offer abundant and suitable floral resources in agricultural landscapes to promote healthy populations of wild bees. Additionally, we found profound negative impacts of field-realistic worst-case sulfoxaflor exposure on *O. bicornis* larvae. This points to the necessity of including tests on bee larvae chronically exposed to pesticides in risk assessment. Moreover, potential carry-over effects to the next generation should be considered while testing the risks of pesticides (Stuligross and Williams 2021). More studies on interactions of pesticide exposure and nutritional stress are needed for a better understanding of the interplay of these stressors and to mitigate their consequences on the fitness and population dynamics of wild bees in agroecosystems.

General conclusions

Our studies revealed that pollen quality can influence the ability of honey bees to metabolize pesticides and withstand their detrimental effects, providing another strong argument for the restoration of suitable foraging habitat. However, we did not find this effect in *Bombus terrestris* colonies and *Osmia bicornis* larvae. Maybe the positive effects of pollen are only on lethal levels (for honey bees) and not at the sublethal level (for bumble bee and mason bee). Our results highlight the importance of diverse floral resources for bee development as well as low pesticide exposure to keep a low level of stress for bee species. Moreover, we show the need for targeted studies of pesticide exposure alone, and in combination with variable nutrition qualities, on all life history stages of bees.

5 References

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