



Pesticide hazard, floral resource availability and natural enemies interactively drive the fitness of bee species depending on their crop fidelity

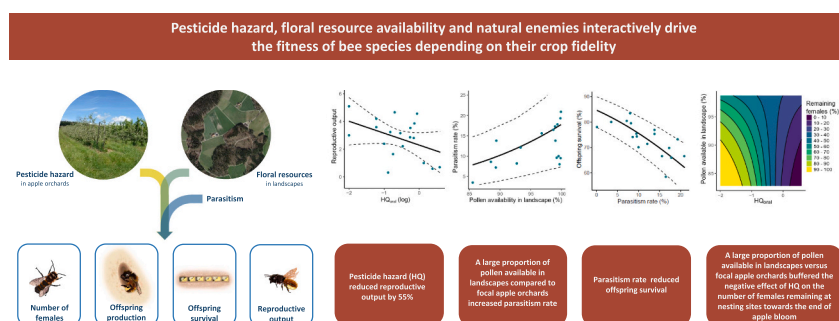
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HIGHLIGHTS

- How drivers of bee decline interact is poorly understood under field conditions.
- We tracked the fitness of two solitary bee species released in apple orchards.
- Pesticide hazard reduced the fitness of the bee with higher crop fidelity.
- Floral resource distribution in landscapes modulated larval parasitism rates.
- The findings show that drivers of bee decline can act interdependently.

GRAPHICAL ABSTRACT



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ABSTRACT

Pesticides, loss of floral resources and parasitism are considered drivers of wild bee decline in agroecosystems, but their interactions are poorly understood under field conditions. Negative impacts are predicted to vary across species of distinct crop fidelity because of the associated consequences for pesticide exposure and dependence on wild flowers. We experimentally established populations of two solitary bee species in apple orchards with distinct pesticide hazard and flower availability in surrounding landscapes. The number of nesting females, their reproduction and offspring survival and parasitism rate were tracked throughout apple bloom. We show that in the solitary bee *Osmia cornuta*, which showed a strong preference for forage in apple orchards, the number of females remaining at nesting sites towards the end of apple bloom and the overall reproductive success of the released bees declined with pesticide hazard. In contrast, the closely related *Osmia bicornis*, which showed a lower preference for apple, was not affected by pesticide use in orchards. Floral resource distribution furthermore modulated parasitism rates in both species: while large amounts of locally available resources reduced offspring parasitism, it increased with relatively higher food availability in the landscapes surrounding focal orchards, probably due to the bees foraging on a larger scale and thus longer periods during which nests remained unattended. Our findings provide novel insights into the complex interplay of pesticide exposure, flower availability and parasitism risk driving population dynamics of bees in agroecosystems. They show that in particular the most reliable crop pollinator species are especially harmed by high pesticide use in crops. Our study underpins the key role of reducing pesticide risks for pollinators through effective regulation of potentially hazardous

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agrochemicals and the promotion of alternative pest regulation approaches along with conserving and restoring flower-rich habitats to sustain pollinator populations in agroecosystems.

1. Introduction

The yield of the majority of crops depends at least partly on pollination services provided by insect pollinators (Klein et al., 2007; Garibaldi et al., 2013). Wild bees play a key role for crop pollination, but their decline in many regions of the world has raised concerns about an impaired pollination service (Garibaldi et al., 2011; Reilly et al., 2020). Besides the loss of suitable habitat, a main driver of population declines in wild bees is the high use of agrochemicals accompanying agricultural intensification (Potts et al., 2016; Woodcock et al., 2016; Douglas et al., 2020; Nicholson et al., 2023). In bees, the major exposure to agrochemicals occurs via residues in nectar and pollen of crop flowers and through direct contact exposure during spray application (Zioga et al., 2020; Thompson, 2021). Consequently, agrochemicals may especially jeopardize bee species that provide important pollination services to crops.

It has been hypothesized that pesticide exposure is particularly severe in simplified agricultural landscapes dominated by crops with low amounts of alternative food resources, while a high proportion of complementary floral resources provided by wild plant species may reduce pesticide exposure (Pettis et al., 2013; Park et al., 2015; Colwell et al., 2017; Bednarska et al., 2022; Rundlöf et al., 2022). The extent to which bee species are affected by pesticide use in crops and consequently can benefit from such a potential buffering by floral resources in surrounding landscapes can be expected to depend on the fidelity of the bee species to forage on crop flowers: species regularly foraging on crops, but with preferences also for non-crop flowers, should benefit more from buffering through reduced exposure than species with a high preference for crops. In contrast, species with a very low crop preference, are expected to experience low pesticide exposure and therefore have limited possibilities to benefit from buffering. However, despite the relevance of this hypothesis for the prediction of pesticide exposure and risk assessment, it has rarely been tested (but see e.g. Graham et al., 2021).

Agrochemicals and the availability and distribution of floral resources in arable landscapes may further influence the dynamics between wild bees and their parasites and parasitoids. Attack by natural enemies can be promoted by long time periods that nesting females have to leave their nest unattended due to long flight distances to suitable food resources (Gathmann and Tschamtko, 2002; Goodell, 2003) or because of the interference of agrochemicals with the bees' flight ability, memory and orientation (Artz and Pitts-Singer, 2015; Siviter et al., 2018; Anderson and Harmon-Threatt, 2021). On the other hand, agrochemicals may also reduce parasitism rates by increasing the mortality in parasites or parasitoids, if they visit crop flowers for food or get in contact with the agrochemical through spray drift in the vicinity of crops (Rodrigues et al., 2002). However, the interplay between agrochemicals, food availability and parasitism in driving the survival and reproductive success of wild bees in agricultural landscapes remains largely unexplored.

In this study, we assessed the survival and reproductive success of *Osmia cornuta* and *Osmia bicornis*, two closely related wild bee species, in 20 apple orchards with distinct pesticide use. We hypothesized that pesticide hazard mainly affects *O. cornuta*, an important pollinator of fruit trees in Europe (Bosch and Kemp, 2002), that also visits a range of wild plant species if available and shows pronounced pollen mixing (Haider et al., 2014). *Osmia bicornis*, the species mainly foraging on wild plants, was expected to be less affected by high pesticide use in the apple orchards, but rather by alternative floral resource availability in landscapes. Such alternative floral resources in surrounding landscapes were further expected to buffer negative pesticide effects in *O. cornuta* and to

modulate parasitism rates of nests by increasing flight durations.

2. Methods

2.1. Study organisms

The genus *Osmia*, mason bees, belongs to the family Megachilidae and includes important crop pollinators (Bosch and Kemp, 2002). *Osmia bicornis* and *O. cornuta* are univoltine solitary bees common in Central Europe. The natural flight period of *O. cornuta* is from mid-March until the beginning of May, the one of *O. bicornis* from beginning of April until mid-June (Westrich, 2019). Building their nests in existing cavities, they also readily colonize artificial nesting aids offering cavities of adequate diameter. Females provide each offspring with a pollen-nectar mixture in brood cells arranged linearly in the nesting cavity. The eggs hatch into larvae after a few days followed by the larval development, which takes about one month and is completed with the spinning of the cocoon (Bosch, 1994). Following a dormant period, the offspring finalizes metamorphosis in late summer or autumn. The fully developed adults hibernate inside the cocoons until emergence in the next spring (Sedivy and Dorn, 2014).

2.2. Experimental design

To obtain distinct pesticide exposure, we selected 10 organically and 10 conventionally managed apple orchards that strongly varied in pesticide use intensity (Fig. 1). Orchards ranging from 0.5 to 15 ha were selected to obtain a gradient of pollen availability provided by the crop relative to other land-use types in the surrounding landscape. All orchards were located in agricultural landscapes of the North-Eastern Swiss lowlands, with a minimal distance of 1 km between sites (Fig. 1). The organic orchards were managed according to the Swiss organic farming guidelines avoiding the use of synthetic agrochemicals. At the margin of each orchard, we placed three nesting aids adjacent to each other (Fig. S1). Each unit provided 100 cavities (300 cavities in total per site) of 6, 8 and 10 mm diameter in equal numbers, which cover the preferred cavity diameters of the two focal *Osmia* species (Vicens et al., 1993). Nesting units were fixed about 1.2 m above ground facing southeast and were covered with a wooden roof to protect them against sun and rain (Holzschuh et al., 2013). The nest cavities were drilled into the MDF (medium-density fibreboard) boards. They were half-round and open on top to allow observation of nesting progress. To ensure that bees and nests were not disturbed during observations, each layer was covered with a transparent plastic foil.

Ten days before apple bloom (beginning on 9 April 2020), approximately 100 female and 150 male cocoons per species (Bosch, 1994) were released in cardboard boxes (with a hole for hatched bees) in each orchard (Fig. S1). Prior to the release, *O. bicornis* cocoons were incubated at about 20 °C for two days to achieve a better synchronization of the hatching with *O. cornuta* (Bosch et al., 2000). The bees hatched within few days after the release and started nesting well synchronized with the beginning of apple bloom. The number of hatched females per site was measured by counting emerged cocoons with cocoon sizes typical for females with >12 mm length and >7 mm width for *O. cornuta* and >12 mm and >6 mm for *O. bicornis* (Fig. S2).

2.3. Proxies of solitary bee fitness

Both *Osmia* species started their nesting activity roughly at the beginning of the apple bloom (with a value of 60 on the BBCH scale for the phenological stages) (Meier, 1997), whereby the phenological

timing (i.e., start of flowering) between the sites varied up to three days. Successful establishment of the bees was checked at the beginning of apple bloom, while after peak bloom (BBCH 65), the number of nesting females of both *Osmia* species were counted at night while bees were roosting inside the nests. At the end of the apple bloom (BBCH 67, on 1 May 2020), we recorded the nesting progress on the transparent foil covering the nest cavities. Finally, after the cocoons had been spun the following data was collected for each species: the total number of cells built and of these the number of developed cocoons and dead eggs or larvae. For dead offspring, we recorded additionally if the brood cells were parasitized by the fruit fly *Cacoxenus indagator* or by pollen mites (*Chaetodactylus* spp.), in which case the developing larvae died most likely due to attack by these natural enemies. In March 2021, a sample of up to 100 cocoons were hatched to measure hatching rates of *Osmia* offspring as well as to quantify rates of parasitism by the bombyliid fly *Anthrax anthrax*. These three taxa are typically among the most common natural enemies attacking *O. cornuta* and *O. bicornis* in the study region (Albrecht et al., 2007; Schüepp et al., 2011; Coudrain et al., 2014) and their parasitism rates were therefore used as a proxy of parasitism in this study.

2.4. Quantification of pesticide use in orchards

Application rates correlate well with pesticide residues found in crop flowers (MacLachlan and Hamilton, 2011). Pesticide hazard for bees and natural enemies within orchards was therefore calculated based on the farmers spray records of the focal apple orchards where bees were placed (Table S1), considering fungicide and insecticide applications from the hatching of bees until the end of apple bloom as:

$$HQ_{\text{oral}} = \sum_{n=1}^N \frac{\text{Application rate (kg active ingredient per ha)}}{LD_{50}}$$

The HQ_{oral} summed up all N applications of a site considering the application rate of the active ingredient and the toxicity of the pesticide based on oral LD_{50} from honeybees (*Apis mellifera*). Although LD_{50} from *Osmia* are available for certain active ingredients, we used values obtained from honeybees to avoid biasing the relative toxicity of certain pesticides compared to others as LD_{50} from *Osmia* typically reach higher values than the ones from honeybees (Arena and Sgolastra, 2014). Oral LD_{50} from honeybees were obtained from the Pesticide Properties Database (PPDB) and Bio Pesticides Database (BPDB) (Lewis et al.,

2016), while the information for the concentration of active ingredients in the applied products were compiled from the Swiss plant protection products database (BLW, 2021). The HQ_{oral} showed low, non-significant correlation with pollen availability in landscapes ($r = -0.10$) and with orchard size ($r = 0.27$).

Given the different application dates and dissipation rates of the used pesticides in this study, a quantification on the basis of residues would require a continuous sampling of provisions throughout the nesting period. Such continuous sampling was not possible here however, because of the associated confounding effect on the measurements of nesting progress, offspring survival and reproductive output. For this reason, pesticide hazard was calculated from application rates (e.g. Park et al., 2015) rather than pesticide residues in pollen-nectar provisions (e.g. Rundlöf et al., 2022) in our study. Our approach has the advantage that it also takes into account the risk of pesticides with lethal effects on foraging females (or sublethal effects leading to e.g. severe disorientation without return to the nest), while such effects are not considered by residues measured in females captured at the nest or in the pollen stores they build. To estimate pesticide hazard for larvae, however, HQs based on residue levels are more appropriate as they directly measure their exposure. Another limitation of the HQ_{oral} used here is that pesticides potentially applied to other orchards in the landscape surrounding focal apple orchards are not considered, as no information on the management of those fields was available. However, as bees are central place foragers with an optimal foraging strategy (Jha and Kremen, 2013), it is likely that apple pollen was mainly collected from the focal orchards. This assumption is also supported by our finding that *O. cornuta*, which showed a preference to forage on apple, collected more apple pollen with increasing pollen availability in focal orchards (Fig. S3).

2.5. Composition of pollen provisions

At each site, we collected five pollen-nectar provisions per *Osmia* species from cells built during apple bloom. Species could be identified based on the clay fastener and the pollen provision, which has a higher nectar content in *O. cornuta* (Tasei and Picart, 1973; Westrich, 2019). The samples were stored at -20°C (Kratschmer et al., 2020) and analysed by acetolysis with subsequent staining by Safranin O according to Jones (2012). Before acetolysis, the pollen samples were rinsed in 95% ethanol, followed by a glacial acetic acid rinse. Acetolysis was performed by adding a 9:1 mixture of acetic anhydride and sulphuric acid

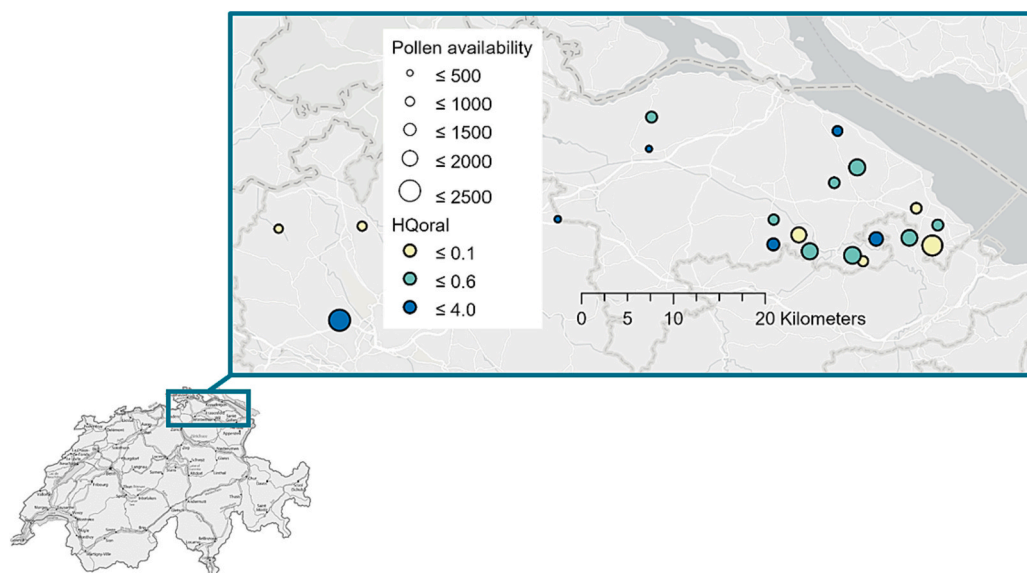


Fig. 1. Distribution of orchards in Northern Switzerland. The pesticide hazard (HQ_{oral}) in focal orchards and the pollen availability (volume in liter) in the landscapes surrounding these orchards (radius 500 m) are indicated.

and heating it for 3 min at 100 °C. The chemical process was terminated with another rinse in glacial acetic acid. Between each step, the samples were vortexed for 15 s, centrifuged at 1060 × g for 3 min and the supernatant was decanted (adapted from Jones, 2012). After this process, 300 pollen grains of each stained sample were inspected under a light microscope (DM 1000, Leica, magnification of 400) using at least three random transects across coverslips. Orchard pollen (apple and occasionally pear), was counted to calculate their average percentage for each *Osmia* species for each site. Pollen identification was done based on a reference library with pollen from apple and other fruit trees as well as the most abundantly flowering wild plant species and bee-attractive crops. Pollen of flowers collected for the reference library were processed with the same protocol as described above. Pollen grains from the family Rosaceae are difficult to determine to species level, but based on the reference library it was possible to distinguish between orchard pollen and pollen from co-flowering wild Rosaceae species. Besides apple pollen, pollen types that exceeded 10% of pollen in individual pollen provisions were identified and classified as important pollen food sources considered in the quantification of landscape-level pollen availability (see below). For *O. cornuta* this included *Prunus* spp., *Taraxacum* spp., *Brassica* spp., *Salix* spp. and *Acer* spp. For *O. bicornis* the identified dominant pollen types were *Ranunculus* spp., *Quercus* spp., *Salix* spp. and *Acer* spp. These pollen types represent wild plants by the vast majority; only *Brassica* spp. is likely dominated by oil seed rape, *Brassica napus*, which however made up only a very small proportion of the pollen volumes collected by the two *Osmia* species in the studied landscapes (Fig. S4). Moreover, pesticide use in oil seed rape is much lower on average compared to fruit tree orchards in Switzerland (Serra et al., 2021). Potential pesticide exposure through other forage plants than apple and pear are therefore negligible compared to exposure through orchards.

2.6. Estimation of pollen availability in the landscapes surrounding apple orchards

During peak bloom of apple, flower abundance of important food plants (as defined above) was recorded in the landscapes surrounding the apple orchards within a radius of 500 m, which is considered to well cover the foraging range of the two *Osmia* species (Gathmann and Tscharrntke, 2002; Greenleaf et al., 2007). Within these landscape sectors, we estimated for each land-use patch offering floral resources, the percentage cover of all present food plants. For open land-use types dominated by herbaceous vegetation (mainly grasslands, but also herbaceous and grassy field margins and arable crops) this was done on the horizontal view, while on a vertical view for land-use types covered by woody vegetation (i.e., hedgerows, forest edges). Flower abundance inside forests could not be assessed. All patches offering floral resources were mapped and digitalized in ArcGis Pro 2.6 (ESRI, 2020), and volumes of their vegetation containing flowers were subsequently calculated with heights measured for at least five patches of each land-use type (Table S2). The estimated flower cover was converted to number of flowers for each plant species and patch based on reference values. Reference values were obtained from counts of number of flowers in at least five sampled 1 m³ cubes for each plant species. Finally, pollen amount for each plant species and landscape sector was calculated using values of flower pollen volume (typically reported in µL) compiled from the literature (Table S3). Using the same method, pollen availability provided by focal apple orchards was quantified. None of the focal orchards reached the limit of the 500 m buffer; instead, they were all completely surrounded by agricultural landscapes with fragments of semi-natural habitats. As according to optimal foraging theory, resource distribution and diversity should modulate the bees' foraging routes and therefore pesticide exposure levels (Jha and Kremen, 2013; Pyke and Starr, 2021), we calculated the relative pollen availability in landscapes (excluding focal orchards) relative to focal apple orchards as *pollen amount in landscape / (pollen amount in orchards + pollen amount in*

landscape). As measure of pollen diversity we calculated the Shannon index as

$$\text{Pollen diversity} = \sum_{i=1}^n p_i \times \ln(p_i)$$

where *p* is the proportion of pollen provided by one plant species in a set of *n* species in the landscape including orchards.

2.7. Statistical analysis

We modeled the effect of pesticide exposure (HQ_{oral}) and pollen availability and diversity on the fitness of the two *Osmia* species with either generalized linear mixed-effects models (GLMMs) or linear models (LMs) using model selection and model averaging based on AICc (Burnham and Anderson, 2002; Cade, 2015). Model averaging was done with the MuMIn package (Barton, 2020). For each proxy of bee fitness as well as parasitism rate, a global model was generated and a set of top models was identified as those that were within two AICc compared to the best model. This set of models was used for model averaging weighted by AICc.

We used the proportion of released bees that were still nesting inside the provided nesting units after peak bloom as a proxy for survival, which may also include some migration of females to (or from) surroundings, however. Offspring survival was calculated as the proportion of produced offspring that reached the adult stage and successfully hatched. The proportion of nesting females and offspring survival were analysed using GLMs with a binomial error distribution. An observation level random term was included to control for overdispersion (Harrison, 2014). The number of produced offspring (fertility) was calculated as the average number of produced brood cells per nesting female at a site, which was analysed with an LM. Similarly, the reproductive output was calculated as the number of hatched daughters (calculated as *hatching rate of cocoons × number of female cocoons*) per nesting female of a site, which was also analysed with an LM. These response variables were analysed for each of the two *Osmia* species in separate models. For both *Osmia* species we had to exclude one site, in one case because *O. cornuta* did not establish in the nesting unit at the beginning of the experiment, and in the other case because the apple producer released a high number of additional *O. bicornis* in an orchard site to improve apple pollination.

For all proxies of fitness (number of nesting females as proxy for survival, number of produced offspring, offspring survival, and reproductive output) the global models included the HQ_{oral}, the percentual pollen availability provided by landscapes surrounding orchards (excluding the pollen offered by focal orchards, see above) and the Shannon index of pollen diversity in the entire landscapes (including orchards). To test for potential buffering effects (antagonistic interaction) of pesticide exposure by the landscape context, the interaction terms between the HQ_{oral} and pollen availability and diversity were included in the global models. In the global model testing effects on offspring survival and reproductive output, we additionally included parasitism rate as explanatory variable.

Parasitism rate was analysed separately for each bee species using a GLM with binomial error distribution and the proportion of parasitized brood cells as response variable. The global model included pollen availability in landscapes and pesticide use as explanatory variables to test for potential indirect effects of these variables on females' foraging times and consequential parasitism rates of their nests. Additionally, the proportion of semi-natural habitat in the landscapes (permanent grasslands, hedgerows and forest lots) was included as covariate to account for parasitism pressure from surrounding landscapes (Maalouly et al., 2013; Bihaly et al., 2021) and an observation level random term was included to control for overdispersion.

To investigate how the relative pollen availability in focal orchards and pollen diversity influences the proportion of apple pollen collected, these variables were included in a GLMM with the average proportion of apple pollen in the pollen-nectar provisions per site as response and an

observation level random term to control for overdispersion.

As flower abundance is very high in Swiss landscapes in spring, bees were highly unlikely to be limited by total pollen availability in landscapes. This was confirmed by fitting models considering the pollen amount of entire landscapes including orchards. No effect of overall pollen availability was found and the variable got dropped from all models except one. Therefore, and to avoid overfitting, we excluded the variable from the final analysis. Since the relative pollen availability in landscapes and orchards was fully correlated due to the calculation (see above), we always selected the variable that was directly related to the research question. Furthermore, models with the HQ_{oral} calculated as described above were compared with models including an HQ based on contact toxicity (HQ_{contact}), but as the HQ_{oral} showed the better predictions (according to AICc and model fit) in all models, it was chosen as explanatory variable. The HQ_{oral} was log-transformed to achieve normality and homoscedasticity of the model residuals, which were validated graphically (Zuur et al., 2009). All analyses were done in R 4.1.2 (R Core Team 2021).

3. Results

Osmia cornuta showed a strong preference for apple pollen, which made up 63% ($\pm 6\%$) of the overall collected pollen by this species. *Osmia bicornis*, in contrast, preferably collected pollen from wild plants and only 4% ($\pm 2\%$) of the pollen provisions were constituted by apple pollen on average. In some pollen provisions, however, apple pollen exceeded 30%, which shows that apple can be a suitable pollen host although it is not the preferred one. Considering all food plants of the two *Osmia* species, overall pollen availability in landscapes including orchards was on average 5.2 times higher for *O. bicornis* than for *O. cornuta*. The main difference was caused by oak (*Quercus* spp.) pollen, which made up 82% of the pollen volume per landscape considering the pollen host plants of *O. bicornis* (Fig. S4). For *O. cornuta*, maple (*Acer* spp.) offered the largest pollen volumes covering 67% of the pollen volume on average, followed by Rosaceae pollen with 29% (Fig. S4). In *O. cornuta*, the average proportion of apple pollen that female bees collected at a site increased with relative pollen availability in orchards ($Z = 2.07$, $p = 0.038$) (Fig. S3), while in *O. bicornis* this was not the case ($p = 0.151$).

The pesticide hazard quotient (HQ_{oral}) did not differ between conventionally and organically managed apple orchards ($p = 0.892$), rather the HQ_{oral} distributed along a gradient in both production systems (Fig. S5). Consistent with our hypothesis that pesticide use in apple orchards mainly impairs fitness of crop pollinators with a high fidelity to

forage on crop flowers, in *O. cornuta* the number of females remaining at nesting sites towards the end of apple bloom and the total reproductive output (the number of female offspring per released female) declined with the HQ_{oral} (Fig. 2a), while *O. bicornis* was not affected (Table S4). Total reproductive output declined by 55% along the full gradient of HQ_{oral} (Fig. 2b). Moreover, the negative effect of pesticide hazard on the number of nesting females was antagonistically mitigated by a high proportion of pollen available in landscapes (excluding pollen offer from focal orchards where bees were placed) (Fig. 2a). In both *Osmia* species, female fertility (number of brood cells produced per female) and offspring survival were neither affected by pesticide hazard nor by pollen availability or diversity, whereas offspring survival was reduced by parasitism rate (Fig. 3, Table S4). Parasitism rate itself, however, was positively associated with the relative pollen availability in landscapes (Fig. 3, Table S4).

4. Discussion

How pesticide exposure, the loss of floral resources and attack by natural enemies interact with each other in driving bee decline is still poorly understood under field conditions. Consistent with the hypothesis that intensive pesticide use has a greater impact on pollinators that exhibit higher foraging fidelity for crop flowers, pesticide hazard substantially reduced fitness in the species *O. cornuta*, that collected a large proportion of its pollen from apple, while *O. bicornis*, which preferentially visited wild plants, was not impacted by pesticide hazard in apple orchards. Our findings further support the hypothesis that high pollen availability in landscapes surrounding focal orchards can buffer against negative impacts of pesticides in pollinators with a relatively high crop fidelity. However, also large amounts of pollen offered by focal apple orchards benefited both species by reducing their offspring parasitism rate. These findings provide insights into the interplay of pesticide exposure, availability of floral resources offered by crops and wild plants as well as top-down control by natural enemies in shaping fitness parameters of two closely related bee species with similar life-history traits but distinct preferences for crop flowers.

Osmia cornuta is known as important wild and managed pollinator species of European fruit trees such as apple and cherry (Márquez et al., 1994). Its large thermal niche breadth and ability to pollinate fruit trees also under adverse weather conditions (Vicens and Bosch, 2000) combined with the high preference for plants in the Rosaceae family contribute to the high effectiveness as a pollinator of these crops (Bosch, 1994; Kratschmer et al., 2020; Jaumejoan et al., 2023). Consistent with these previous findings, *O. cornuta* collected the majority of pollen from

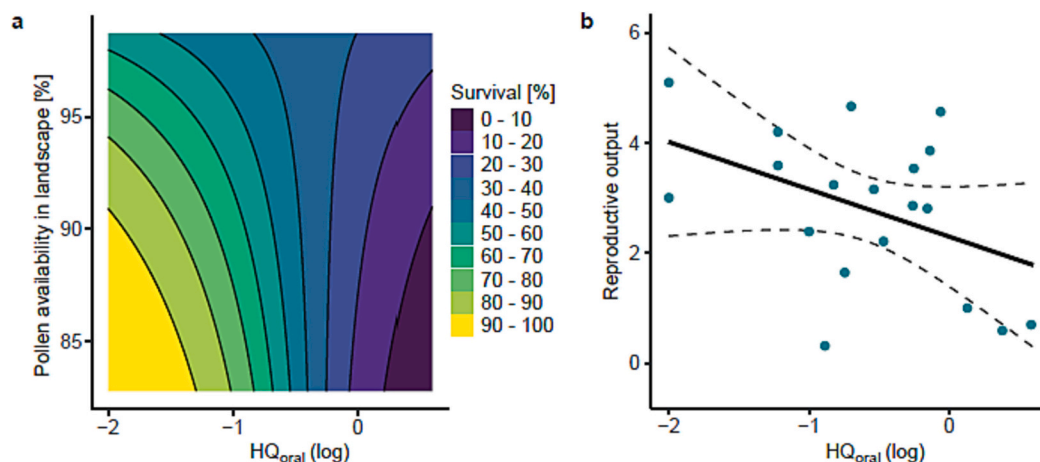


Fig. 2. Effect of pesticide hazard (HQ_{oral}) on the performance of *Osmia cornuta*. (a) Interactive effect of HQ_{oral} and the pollen availability in agricultural landscapes surrounding focal apple orchards on the number of females remaining at nest sites towards the end of apple bloom. (b) Relationship between HQ_{oral} and the reproductive output (number of female offspring per released female). Dashed lines reflect 95% confidence intervals.

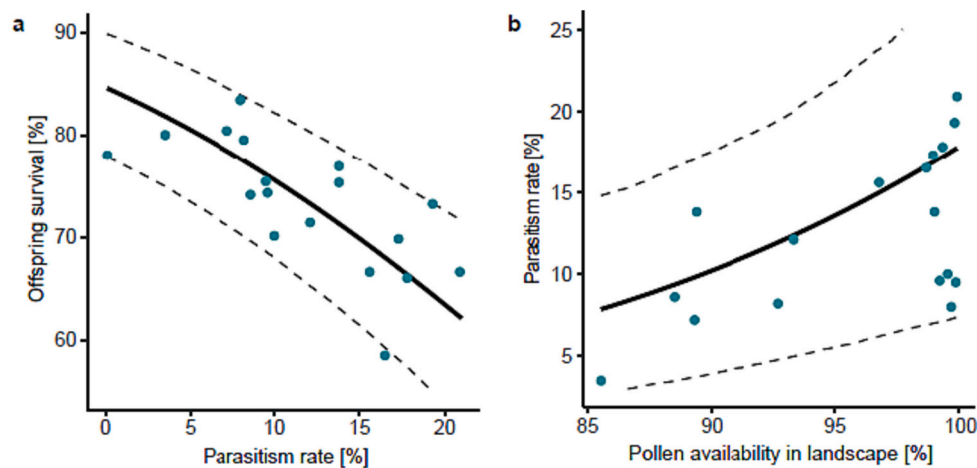


Fig. 3. Parasitism in *Osmia cornuta*. (a) Relationship between parasitism rate and offspring survival. (b) Relationship between pollen availability in agricultural landscapes surrounding focal apple orchards and parasitization of *Osmia cornuta* nests. Dashed lines reflect 95% confidence intervals.

apple trees in our study, while in the pollen provisions of *O. bicornis*, the proportion of apple pollen was much lower, and included the previously documented wild pollen hosts, such as *Quercus*, *Acer*, *Salix* and *Ranunculus* (Coudrain et al., 2016; Bertrand et al., 2019). These findings indicate that *O. cornuta* has substantially higher preference and fidelity for fruit trees of the Rosaceae family, and thus should be the more suitable pollinator of these crops. In fact, the proportion of pollen that *O. cornuta* collected from apple increased with the proportion of pollen provided by focal orchards where bees were placed. Also, as indicated by the interaction between pesticide hazard and the proportional pollen availability in landscapes (Fig. 2a), adult bees benefit from a high availability of floral resources from apple trees in the immediate vicinity of their nest, if these flowers do not expose them to pesticides; possibly due to short foraging distances and more efficient resource allocation (Pyke and Starr, 2021).

Floral resource distribution may further shape parasitism rates of the studied solitary bees, as foraging beyond the focal apple orchards on more distant complementary floral resources should be associated with longer time periods that nesting females leave their nest unattended, which can facilitate attack by parasites and parasitoids (Goodell, 2003; Ganser et al., 2021). In fact, in both bee species studied, parasitism rates increased with pollen availability in surrounding landscapes compared to focal orchards. Similarly, parasitism of nests could also be facilitated by pesticide exposure of foraging females, if sublethal effects cause prolonged foraging (Stanley et al., 2016). Alternatively, pesticide use may have direct harmful effects on parasites and parasitoids through spray drift in the proximity of bee nests or through oral exposure in species as the parasitoid fruit fly *Anthrax anthrax*, in which adults feed on flowers (El-Moursy et al., 1999). However, in this study we did not find support for any of these hypotheses as parasitism rate was not affected by pesticide hazard.

High pesticide exposure, in particular to insecticides, may exert negative lethal and a series of sublethal impacts on bees (Siviter et al., 2021) with implications for fitness, reproductive success and population development of wild bees (Whitehorn et al., 2012; Rundlöf et al., 2015; Woodcock et al., 2016; Willis Chan and Raine, 2021; Bednarska et al., 2022). Here, we show that for the important crop-pollinating solitary bee species *O. cornuta*, the number of females remaining at nesting sites towards the end of apple bloom and the total reproductive output of the released bees decrease with pesticide-use intensity in the focal apple orchards. As pesticide use was not correlated with food availability in orchards or surrounding landscapes in our study, the lower number of nesting females in orchards with high pesticide hazard is unlikely connected to migration to more suitable nesting sites, but likely caused by an increased mortality through agrochemicals. Also, several dead

females were found inside nests at the three sites with the highest pesticide exposure during apple bloom, while nothing similar was observed at the other sites. This is further underpinned by the finding that the closely related solitary bee species *O. bicornis*, which visited apple flowers much less frequently, was not affected by pesticide hazard, despite nesting within apple orchards. This result, together with the fact that consideration of hazard through contact exposure did not improve prediction of bee fitness proxies, suggests that the main exposure pathway was oral exposure through contamination of nectar and pollen in crop flowers, which mainly puts crop pollinators at risk. Hence, our findings imply that while aiming at maximizing crop yield, such high pesticide use may jeopardize pollination services in the long term. Although our results also imply that the negative effect of high pesticide exposure in apple orchards can be partially buffered by high availability of complementary floral resources in agricultural landscapes surrounding focal apple orchards, this mitigation effect was not transferred to reproductive performance. The benefit of such buffering for the population development of *O. cornuta* therefore remains uncertain.

It is good practice and well established in Switzerland to apply insecticides only when pests have reached certain thresholds. For this reason, most farmers (both conventional and organic producers) try to avoid the use of insecticides as long as they can still meet the quality requirements for selling fruits. Nevertheless, the highest hazards through oral exposure were reached by applications of relatively bee-toxic insecticides such as chlorpyrifos-methyl or spinetoram shortly before or during the bloom of apple. Although the Swiss organic farming guidelines exclude the use of synthetic pesticides, insecticides with pyrethrins as natural active ingredients also reached comparably high hazard values per application (Table S4). Moreover, in their sum, the very high number of fungicide applications also contributed substantially to pesticide hazards, which was the case for both production systems. In general, many labels of relatively bee-toxic insecticides state that they should not be applied when bees are foraging. However, this criterion is difficult to fulfil as different bee species forage at different times of the day and entire bee communities cover almost the entire light period (Karbassioon and Stanley, 2023). At the same time, pesticides must be applied to dry surfaces, which limits the application window. Therefore, to reduce oral exposure of crop pollinators to bee-toxic products, applications one week before and during the bloom of bee-attractive crops should be avoided.

5. Conclusions

Our study shows that pesticide hazard, floral food availability in landscapes surrounding crops and parasitism risk act interdependently

on crop pollinating bee species. Moreover, findings indicate that this interplay is shaped by the foraging traits of pollinator species: while reproductive output of *Osmia cornuta*, which showed a high fidelity to visit crop flowers, strongly decreased with pesticide hazard, no such negative impacts of pesticide use were found for the closely related *O. bicornis*, characterized by a relatively low preference to visit crop flowers. Hence, our study highlights a potentially important trade-off between short-term crop protection through high use of pesticides to optimize yields and adverse effects by pesticides on crop pollinators, potentially jeopardizing sustainable pollination services and yields in the long-term. However, these negative effects were partly mitigated by a high complementary floral resource availability in the surrounding landscape showing the importance of enhancing complementary floral resources in agricultural landscapes through habitat conservation or agri-environment schemes. In addition to negative impacts from pesticide applications, bees also benefited from the high pollen availability in crop fields, which reduced offspring parasitism rates and consequently increased offspring survival. Therefore, to protect bees and maintain pollination services for crops, reducing pesticide risks is all the more urgent. In addition to stricter regulations of potentially hazardous pesticides and avoidance of applications during bee flight, alternative pest regulation approaches such as ecological intensification through agro-ecological practices appear to be promising ways to mitigate these trade-offs for a sustainable crop production, while at the same time contributing to a better protection of pollinators from pesticides.

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CRediT authorship contribution statement

Anina Knauer: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft. **Carmen Naef:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Matthias Albrecht:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available via the Dryad Digital Repository under <https://doi.org/10.5061/dryad.j6q573nn2>

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Appendix A. Supplementary data

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