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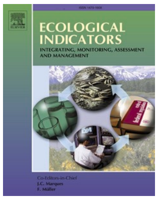

Original Articles

Honey bees and mason bees as biological indicators of landscape pesticide loads: Different results but similar conclusions

F. Sgolastra [a,*], X. Arnan [b], C. Azpiazu [c,d], A. Bordoni [a], J. Bosch [c], L. Lenzi [a], C. Martins [a], M. Parrilli [a], C. Porrini [a]

[a] *Dipartimento di Scienze e Tecnologie Agro-alimentari, Alma Mater Studiorum Università di Bologna, Italy*
[b] *Universidade de Pernambuco, Brazil*
[c] *CREAF, Centre de Recerca Ecològica i Aplicacions Forestals, Universitat Autònoma de Barcelona, Bellaterra, Spain*
[d] *Universidad Politécnica de Madrid, Madrid, Spain*

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ABSTRACT

Agricultural environments, including crop and non-crop areas, often provide important pollen and nectar resources for managed and wild bees. However, these resources may be contaminated with pesticides detrimental to bees and other non-target organisms, including humans. Differences in life-history traits among bee species influence food resource exploitation and pesticide exposure. This study assesses the potential of honey bees (*Apis mellifera*) and mason bees (*Osmia*), two bee species with highly contrasting functional traits, as biological indicators of pesticide exposure in 34 Italian agricultural farms. Pollen loads of both species were used to identify pollen species collected and to analyse multi-residue levels of pesticides. Pesticide risk indexes were calculated for honey bees, mason bees and humans. In mason bees, pesticide risk was not influenced by plant diversity. In agreement with their pollen preferences and short foraging ranges, mason bees collected a high proportion of pollen from flowers of the target crop. Conversely, pesticide risk decreased with increasing pollen diversity in honey bees. In agreement with their generalist foraging habits and long foraging ranges honey bees collected a greater diversity of pollen species and a lower proportion of target crop pollen. Although honey bees and mason bees showed similar toxic loads and pesticide composition, at a field scale pesticide risk of one species is not a good indicator of the risk to the other species. Our study confirms that bees in agricultural environments are pervasively exposed to multi-residue pesticide loads. Exposure is conditioned by specific bee traits but is also highly context-dependent.

1. Introduction

Agricultural intensification is considered one of the main drivers of insect biodiversity decline, with potential consequences on the delivery of ecosystem services (Sánchez-Bayo and Wyckhuys, 2019). Declines in pollinator populations, mainly bees, are of particular concern because crop production depends on pollination services provided by these animals (IPBES, 2016; Potts et al., 2010). More than 75 % of crops worldwide require animal pollination to produce fruits and seeds (Klein et al., 2007) and the economic value of this ecosystem service is estimated at US$ 235–577 billion per year (IPBES, 2016). One of the main components of agricultural intensification is the widespread use of pesticides, which may become important environmental contaminants in agricultural areas, posing a threat to the health of non-target organisms, including humans (Rani et al., 2021). Despite advances in alternative pest control methods and pesticide regulation, pesticide use is not declining worldwide (Sharma et al., 2019). Pesticides withdrawn from the market due to their hazard to non-target organisms are quickly being replaced by new products and there are currently more than 1,000 different active ingredients commercially available (Lewis et al., 2016).

Importantly, bees living in agricultural environments are simultaneously exposed to combinations of pesticides, some of which may have been applied weeks or even months before exposure occurs (Azpiazu et al., 2023; Zioga et al., 2023a). Also importantly, exposure does not only occur in crop fields, but also in off-crop adjacent areas that become contaminated through spray drift and/or leaching (Botías et al., 2015). The temporal and spatial dynamics of pesticides and their residues hinder our ability to quantify bee exposure. Screening for multiple

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* Corresponding author.
E-mail address: fabio.sgolastra2@unibo.it (F. Sgolastra).

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compounds in the wide array of flowers and other matrices contacted by bees in a given area is not feasible and, for this reason, the western honey bee, *Apis mellifera* L. has been used as a bioindicator of the environmental load of pesticides and other pollutants (Cabrera et al., 2016; Girotti et al., 2020; Porrini et al., 2003). During their foraging bouts, honey bees intercept many pollutants and, as central place foragers, concentrate these contaminants in the hive, where different bee matrices such as pollen, honey, and wax can be sampled (Porrini et al., 2003).

Yet, *A. mellifera* is only one of ca. 20,000 bee species described worldwide and its ecological traits, including its high level of sociality, long foraging range, and highly generalist diet, differ widely from those of other species. Most of bee species are solitary and show specific foraging and nesting habits that influence their exposure to pesticides (Sgolastra et al., 2019). Among solitary bee species, mason bees in the genus *Osmia* have received special attention due to their use as managed crop pollinators (Bosch et al., 2008) and their inclusion as model species in pesticide risk assessment (EFSA, 2013). Honey bees and mason bees show widely contrasting ecological traits. *A. mellifera* has a long foraging range (ca. 1.5 Km) and widely polylectic pollen collection habits. Mason bees, on the other hand, have much shorter foraging ranges (ca. 100–500 m) and, although polylectic, have marked pollen preferences (Sgolastra et al., 2019). Therefore, honey bees and mason bees may respond quite differently to the floral landscape context and, consequently, be exposed to different pesticides.

In this study we analysed pesticide residues in pollen-nectar loads from honey bees and two common European mason bees (*Osmia cornuta* and *Osmia bicornis*). We had three objectives: 1) To analyse the effect of landscape context on pollen choice and pesticide load in honey bees and mason bees; 2) To establish whether pesticide residues in bee-collected pollen in agricultural landscapes pose a threat to honey bees and mason bees. Because honey bee-collected pollen can also be consumed by humans, we also address whether these residues could pose a threat to human health; 3) Given their differences in biological traits, to establish whether honey bees and mason bees provide different assessments of landscape pesticide risks.

2. Materials and methods

2.1. Study sites and bee populations

The study was conducted in 2021 in 34 agricultural sites (farms) in Italy covering a range of entomophilous crops (Table A1). Most farms followed integrated pest management but some were organic (Table A1). At the beginning of the blooming period, honey bee colonies and/or mason bee (*O. bicornis* or *O. cornuta*) nesting stations were installed in each farm. These two mason bee species have very similar biological traits and occur across wide areas of Europe, including Italy.

Three healthy, queen-right honey bee colonies (*A. mellifera ligustica*) per site were installed in 31 of the farms. The main crops were apple and pear, but also cherry, kiwifruit, citrus and alfalfa. Following beekeeper practices, colonies were placed on the edge of the crop fields and remained on site for the duration of bloom. Mason bee nesting stations were installed in 27 sites, mostly fruit tree orchards. Each station contained 160–180 reed segments (*Arundo donax*) with front and back entrances. Nesting stations were located inside the orchards (at least 200 m from the edge). In each station, 550–600 *Osmia* cocoons (sex ratio mm:ff = 1.5:1) supplied by Pollinature Srl were released. The number of stations was adjusted to the size of the farm to optimize pollination services and ranged from 2 to 8. Overall, 24 sites had both honey bees and mason bees. However, due to extreme weather conditions during the study, pollen collection was not possible in some farms, reducing the number of sites with both pollen data for honey bees and mason bees to 17, while 13 sites had only honey bees and 4 had only mason bees (total sites: 34).

2.2. Landscape variables

We used the QGIS 3.22 version (https://qgis.osgeo.org) to digitize the landscape surrounding each the 34 farms. Land use categories were characterized based on a vector open geodata available for the region and the CORINE Land Cover nomenclature (Feranec et al., 2016). The intersection geoprocessing tool and the regional land use vector file were used to quantify the area occupied by each land use category within a radius of 500 m and 1500 m from the centre of the farm, which correspond to the typical foraging distances of *Osmia* sp. and *A. mellifera*, respectively (Gathmann and Tscharntke, 2002). We focused on the category “proportion of agricultural landscape” because most pesticide residues are expected to originate from this type of landscape. Since the proportion of agricultural landscape between both radii was highly correlated (Spearman rho $= 0.88$, $p < 0.0001$), analyses were only performed with the 500 m radius data.

2.3. Chemical and palynological analysis

Bee-collected pollen samples were obtained during peak bloom of the main crop. For honey bees, pollen loads from the corbiculae of foragers were collected in three hives per site using pollen traps placed at the hive entrance. For mason bees, we collected pollen samples from the provisions stored in three recently completed nests per site. Honey bees add nectar to their pollen loads while foraging, and mason bees add nectar to their pollen provisions inside the nest. Therefore, the pollen samples of the two species also contained nectar. However, for convenience and because pesticide levels are usually higher in pollen than in nectar (Zioga et al., 2020)), we use the term “pollen samples” throughout the text. Pollen samples were maintained at −20 °C for two months until analysis. Pollen samples from different honey bee colonies and mason bee nesting stations of the same site were mixed to obtain a single sample of at least 5 g per species and site. Successively, two aliquots from each sample were used for chemical and palynological analysis, respectively.

Chemical analyses were conducted by Agriparadigma (Ravenna, Italy) on 2 ± 0.03 g of pollen per sample. Analysis was performed using UNI EN 15662:2018 (with GC/MS/MS and LC/MS/MS) for multi-residue analysis and with the CVUA EU RL-SRM QuPPe Vers 12 met 1.3 2021 (with LC/MS/MS) method for glyphosate. A total of 584 active ingredients were screened. The name of the active ingredients, their quantification limits (<LQ), and the analytical method used are provided in Appendix S1.

Palynological analysis was performed at CREA-AA (Bologna, Italy). For each sample, 2-g-subsamples of pollen were suspended in 50 mL of distilled water. Then, pollen grains in one 0.01-mL-aliquot of the suspension were identified under the microscope at 100 x. At least 1,000 grains were identified per sample. This analysis provided a plant diversity estimate for each site, expressed as Effective Number of Species (ENS) (Loujost, 2023). In the statistical analyses, pollens from crop plants were grouped under the category “crop pollen”.

2.4. Risk index calculation

With the pesticide residue data, we calculated a risk index for honey bees and mason bees, separately. Risk indexes were calculated as the sum of the “toxic units” of all the compounds found in a pollen sample consumed by an individual bee in one day. The toxic unit of a compound is the ratio between its level of exposure, estimated from the amount of residue detected in the sample, and its reference toxicity value (LD_{50} – Median Lethal Dose) (EFSA, 2019). Risk indexes provide a relative measure of the hazard of a daily ingestion of pollen in a given site.

The risk index for honey bees (RI_{hb}) was calculated as:

$$RIhb = \sum_{i=1}^{n} \frac{Ri*PC/1000}{LD50i}*A \quad (1)$$

Where: Ri is the residue of compound *i* expressed in mg/Kg found in the honey bee pollen sample; PC is the daily pollen consumption of nurse bees (0.012 g/day), the worker honey bee category that consumes the largest amounts of pollen (Sgolastra et al., 2019); $LD_{50}i$ is the acute oral lethal dose of compound *i* expressed in μg/bee (obtained from the PPBD Pesticide Properties Database (Lewis et al., 2016)); AF is an "Assessment Factor" that converts acute toxicity into chronic toxicity, that is, it converts LD_{50} values into LDD_{50} values (median lethal daily dose). We used AF=10, which is considered a sufficient safety factor when chronic toxicity values are estimated from acute toxicity tests in adult bees (Alix and Lewis, 2010).

The risk index for mason bees (RI_{mb}) was based on the amount residues of each compound found in mason bee food provisions (Centrella et al., 2020). As a worst-case scenario we used the larval daily pollen consumption (0.0152 mg/day) (Sgolastra et al., 2019). Because for most compounds toxicity values of mason bees are not available, we used toxicity values (acute oral LD50) of honey bees (Lewis et al., 2016).

Honey bee pollen is also consumed by humans. Therefore, we calculated a risk index for humans (RI_{hu}):

$$RIhu = \sum_{i=1}^{n} \frac{Ri^{*}PC/1000}{ADIi} \tag{2}$$

Where Ri is the residue of compound *i* in the pollen sample (collected by honey bees) and expressed in mg/Kg; PC is the daily pollen consumption in human diet (0.07 g of pollen per Kg of body weight) corresponding to the maximum value in the EU database for daily pollen consumption by different categories of consumers (EFSA, 2018); ADI*i*, the Acceptable Daily Intake of the compound *i,* was obtained from the PPBD Pesticide Properties Database (Lewis et al., 2016). We did not calculate RI_{hu} based on residues found in mason bee provisions because these are not used for human consumption. For each compound, we also indicate the maximum residue level (MRL) expressed in mg/Kg allowed in human food, considering the food category "*Honey and other apicultural products*" from the EU Pesticide database.

Risk index values higher than 1 indicate that the residues in the pollen samples pose a potential lethal risk for bees or represent a non-acceptable daily intake for humans. For an easier interpretation of the risk index values, we transformed them to percentage of ingested doses referred to LDD_{50} (median lethal daily dose) and to ADI, for bees and humans, respectively. For example, a Risk Index for bees of 0.10 is equivalent to a bee consuming 10 % of its daily LDD_{50}.

Sites were ranked according to their pesticide risk for honey bees, mason bees and humans, and the compounds that contributed most to each risk index were identified. The most frequent pesticide combinations were also identified. When available, we used information from the Phytosanitary Notebook (register of pesticides applied in each farm) to detect the potential presence of compounds that had not been applied in a given site.

2.5. Statistical analyses

All analyses were conducted using the R software v. 4.2.0 (R Core Team, 2022).

We tested for landscape effects on the proportion of crop pollen and pollen ENS for honey and mason bees using GLMMs. In these models, the response variables were the proportion of crop pollen and pollen ENS, and the explanatory variable was the proportion of agricultural land. Crop was again added as a random factor. To test for the effect of landscape on the composition of pollens collected, we run PERMANOVAs with the function adonis2 in vegan package (Oksanen et al., 2022). The response variables were the Euclidean distance matrices of pollens collected among sites and the proportion of agricultural landscape was the explanatory variable. We controlled for similar crops by grouping them using the *strata* function. We run separate PERMANOVAs for each bee species.

To detect significant (either positive or negative) associations among pesticides across farms, we applied the probabilistic model of species co-occurrence (Veech, 2013) to the presence-absence matrix of pesticides by site. To this aim, we used the function *cooccur* in the package cooccur (Veech, 2013) in R. The model computed the observed and expected frequencies of co-occurrence between each pair of pesticides. The expected frequency was based on the distribution of each pesticide being random and independent of the other pesticides. The model returns the probabilities that a more extreme (either low or high) value of co-occurrence may have been obtained by chance, and classifies pesticide pairs as having positive, negative, and random associations.

To test the relationships between pollen ENS and the number of compounds, and between the number of compounds and risk index we performed two general linear mixed models (GLMMs) using the function *lme* in package nlme (Pinheiro et al., 2022) in R. We performed separate analyses for each bee type. In the first model, the response variable was the number of compounds and the explanatory variable was pollen ENS. In the second model, the response variable was the risk index and the explanatory variable was the number of compounds. Crop was added as a random factor in both models. The replicates were the number of farms with the presence of each bee species, respectively (i.e., n = 30 for honey bees, and n = 21 for mason bees).

To test differences between bee species in the number of compounds, risk index, pollen diversity (ENS) and proportion of crop pollen, we performed four GLMMs. The response variables were number of compounds, risk index, pollen diversity (log-transformed) and proportion of crop pollen, and the explanatory variable was bee species. Crop and farm ID were added as random factors. The replicates were the farms in which both honey bee and mason bee pollen samples were available (n = 17). We also used GLMMs to analyze whether there was a relationship between honey and mason bees in risk index values and number of compounds. In the first model, the response variable was the risk index of one species (sqrt-transformed) and the explanatory variable the risk index of the other species (sqrt-transformed). In the second model, the response variable was the number of compounds of one species and the explanatory variable the number of compounds of the other species. Crop was added as a random factor. The replicates were the farms in which both honey bee and mason bee pollen samples were available.

To test similarity between bee species in pollen and pesticide residue composition we performed two PERMANOVA tests on the Euclidean distances from the pollen and pesticide residue composition matrices of each farm. In both tests the explanatory variable was bee species, and the farm was added as a strata factor to pair the samples for each bee species within each plot. We used the function *adonis2* in package *vegan* (Dixon, 2003).

Finally, we assessed, separately for honey and mason bees, whether chemical residue composition was correlated with pollen composition using Mantel tests (based on the Euclidean distance matrices of residue composition and pollen composition among sites) with the function mantel.rtest in ade4 package (Thioulouse et al., 2018).

In all analyses we checked for the normality and homoscedasticity assumptions for model residuals. When necessary, response variables were transformed.

3. Results

3.1. Pollen composition

In total we found 103 pollen types of different botanical origin. The number of plant species identified ranged from 5 to 26 in honey bees (mean = 12.78; median = 12; n = 30 sites) and from 2 to 21 in mason bees (mean = 8.68; median = 9; n = 19 sites) (Appendix S2). Pollen of the target crops represented 14.5 % (range = 0–86.3 %) and 35.2 % (range = 0–99.7 %) of the pollen collected by honey bees and mason bees, respectively. However, when considering the pollen collected from all crop plants, the differences between the two species were smaller

(57.1 % and 59.2 %, respectively). In farms in which both bee species were present, pollen diversity was significantly higher in honey bees (GLMM; $X^2_1 = 48.4$, $p < 0.0001$), and the proportion of crop pollen was higher in mason bees (GLMM; $X^2_1 = 10.6$, $p = 0.001$). Overall, pollen composition differed significantly between the two bee species ($F_{1,\,31} = 1.93$, $p < 0.009$).

Neither the proportion of pollen from crop plants (honey bees: GLMM, $t_{19} = -1.3$, $p = 0.224$; mason bees: GLMM, $t_{11} = -0.9$, $p = 0.387$) nor pollen diversity (ENS) (honey bees: GLMM, $t_{18} = 1.23$, $p = 0.234$; mason bees: GLMM, $t_{11} = 0.42$, $p = 0.682$) were affected by the proportion of agricultural landscape. Similarly, the proportion of agricultural landscape had no effect on pollen composition (PERMANOVA; honey bees: $F_{1,\,28} = 1.25$, $p = 0.38$; mason bees: $F_{1,\,17} = 1.11$; $p = 0.16$).

3.2. *Pesticide residues*

We found 65 different active ingredients. Of these, 55 were found in honey bee and 50 in mason bee samples (Tab. A2). The number of active ingredients per sample ranged from 0 to 23 (mean = 10.7; median = 12) in honey bees, and from 3 to 22 (mean = 12.4; median = 13) in mason bees. All samples except two had more than one active ingredient. In sites with honey bees and mason bees, the number of compounds per pollen sample was correlated (GLMM, $t_{10} = 2.28$, $p = 0.046$) and did not differ between the two bee species (GLMM, $X^2_1 = 0.0$, $p = 0.991$). In addition, pesticide residue composition did not differ between the two species ($F_{1,\,33} = 0.93$, $p = 0.25$).

In honey bees, the number of chemical compounds marginally and negatively decreased with pollen diversity (GLMM, $t_{18} = -1.97$, $p = 0.065$, Fig. 1) and was positively related to the proportion of crop pollen (GLMM, $t_{19} = 2.5$, $p = 0.021$). By contrast, in mason bees, the number of compounds was neither related to pollen diversity (GLMM, $t_{11} = -0.63$, $p = 0.541$, Fig. 1) nor to the proportion of crop pollen (GLMM, $t_{11} = 0.5$, $p = 0.660$). Compound composition was not related to pollen composition in either bee group (honey bees: Mantel test, $r = 0.11$, $p = 0.21$; mason bees: Mantel test, $r = 0.12$, $p = 0.25$).

In honey bees, the most frequently detected pesticide was the herbicide glyphosate (70 % of the samples), followed by the fungicides fluazinam (60 %), tebuconazole (50 %), pyrimethanil and cyprodinil (46.7 %). The most frequently detected insecticides in honey bee samples were flonicamid and tau-fluvalinate (both 40 %). In mason bees, the most frequently detected pesticides were the fungicides fluazinam (66.7 %) and captan (+its metabolite tetrahydrophthalimide (THPI); 66.7 %), followed by dithianon (61.9 %) and tebuconazole (61.9 %). As with honey bees, the most frequently detected insecticides in mason bee samples were tau-fluvalinate (33.3 %) and flonicamid (28.6 %). Only about a third of the compounds found in honey bee (mean: 30.5 %; range: 0–88.9 %) and mason bee pollen (mean: 34.5 %; range: 0–88.4 %) had been applied to the target crop (Fig. 2).

Pesticide co-occurrence networks show that the compounds with a central role (represented by highly connected nodes in Fig. 3) were glyphosate and fluazinam in honey bees and fluazinam, captan, and dithianon in mason bees. The most likely pesticide binary combinations (i.e., pesticide pairs likely to co-occur) are reported in Fig. 4.

Pesticide concentrations ranged from 0.01 to 1217 mg/Kg, with a median of 0.06 mg/Kg. Captan, difenoconazole, and THPI were the compounds with the highest concentrations (Table A2). As many as 34 out of 55 compounds detected in honey bee pollen showed residue levels exceeding the MRL in humans (Table A3).

3.3. *Pesticide risk*

The potential risk of pesticides for bee and human health varied widely among farms (Fig. 5). The risk index ranged from 0 to 217.8 % (mean: 8.85 %, median: 0.76 %) in honey bees, from 0.01 to 67.4 % (mean: 5.36 %, median: 1.35 %) in mason bees, and from 0 to 163.2 % (mean: 6.89 %, median: 0.36 %) in humans. Compounds with particularly high incidence in some farms were captan for honey bees (risk index = 119.2 %) and humans (85.2 %), and imidacloprid for mason bees (65.7 %). All other compounds showed risk levels lower than 10 %. In general, the compounds with the highest risk levels were insecticides for honey bees and mason bees, and fungicides for humans (Fig. 6). The risk index was positively related to the number of compounds in both honey bees (Fig. 6; GLMM, $t_{18} = 3.02$, $p = 0.0074$, Fig. 1) and mason bees (GLMM, $t_{13} = 2.76$, $p = 0.016$, Fig. 1).

On a site by site basis, the risk indices of honey bees and mason bees were not significantly correlated (GLMM, $t_8 = 0.17$, $p = 0.869$; two outliers removed from the analysis). However, overall, the risk index did not differ between honey bees and mason bees (GLMM, $X^2_1 = 0.499$, $p = 0.480$).

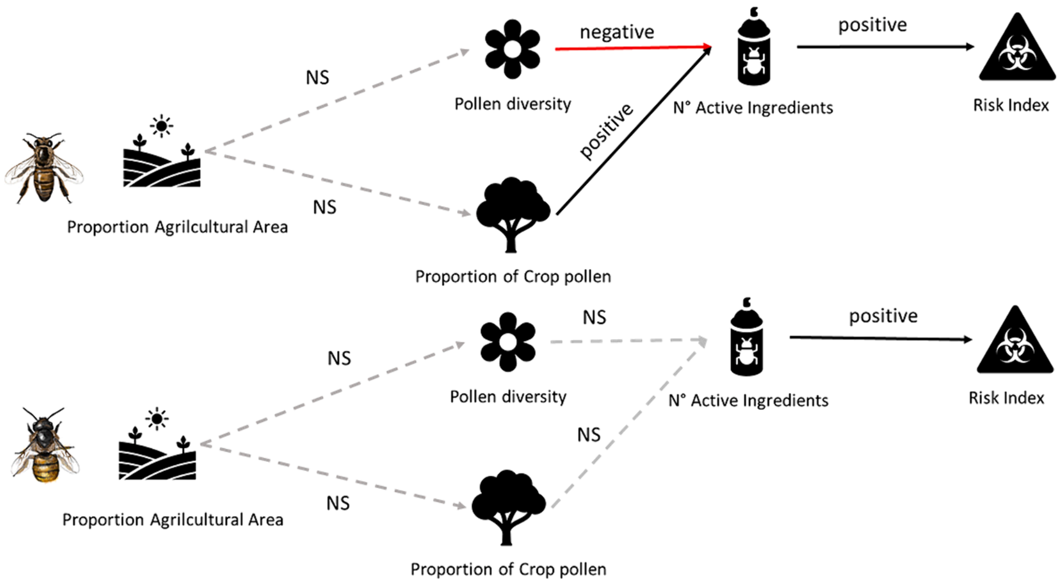

Fig. 1. Impact of different variables that affect the pesticide risk index for honey bees and mason bees. Solid lines indicate a significant relationship between variables and their colour indicates direction (black: positive; red: negative). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

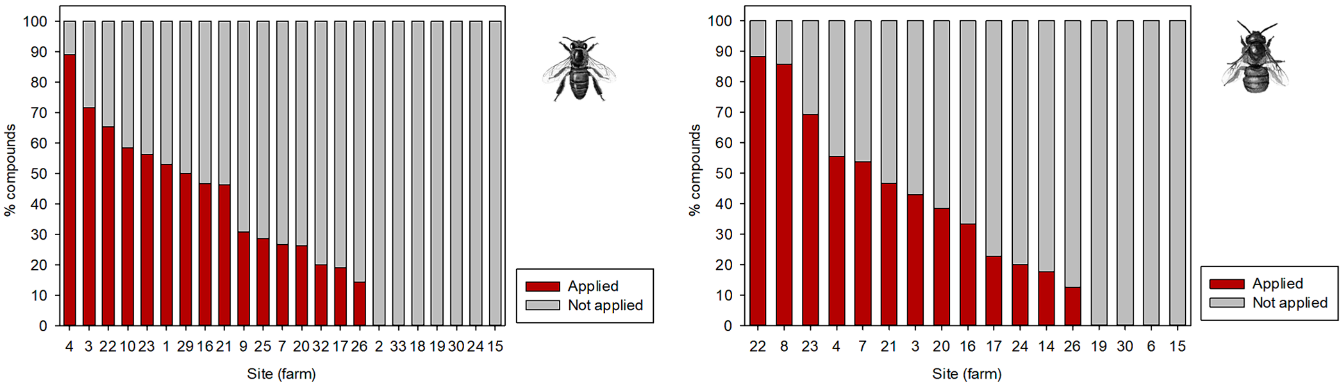

Fig. 2. For each site, percentage of compounds detected in honey bee and mason bee pollen samples that were either applied or not applied by the producer in the year of the study.

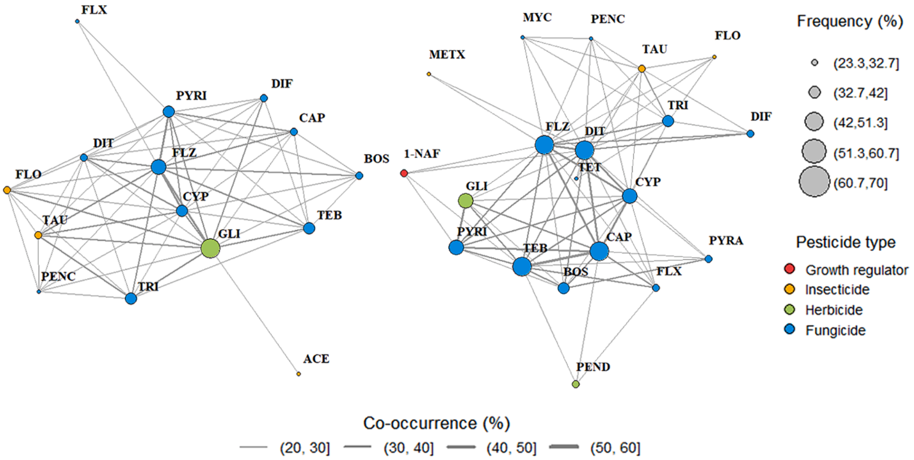

Fig. 3. Co-occurrence network of pesticide residues detected in honey bee and mason bee pollen samples. Node sizes indicate pesticide occurrence (proportion of samples with residues of the compound). Link width represents the probability of co-occurrence. Only combinations with more than 20 % co-occurrence are shown. Probabilistic models for pair-wise patterns and the *co-occur* package in R were used. 1-NAF=1- Naftil acemide; ACE=Acetamiprid; BOS=Boscalid; CAP=Captan; CYP=Cyprodinil; DIF=Difenoconazole; DIT=Dithianon; FLO=Flonicamid; FLZ=Fluazinam; FLX=Fluxapyroxad; GLY=Glyphosate; METX=Methoxyfenozide; MYC=Myclobutanil; PENC=Penconazole; PEND=Pendimethalin; PYRA=Pyraclostrobin; PYRI=Pyrimethanil; TAU=Tau-fluvalinate; TEB=Tebuconazole; TET=Tetraconazole; TRI=Trifloxystrobin.

4. Discussion

Agricultural landscapes provide important floral resources for insect pollinators (Roulston and Goodell, 2011). However, these food resources may be contaminated with pesticides, affecting bees (Tosi et al., 2018) and other non-target organisms, including humans (Kim et al., 2017). We assessed pesticide toxic loads in 34 Italian agricultural farms using social bees, *A. mellifera*, and solitary bees, *O. bicornis* and *O. cornuta*, as bioindicators. Given the differences between honey bees and mason bees in functional traits, including pollen preferences and foraging range, we expected to find differences in the way they interacted with landscape pesticide levels. In partial agreement with this expectation, we found significant differences between honey bees and mason bees in the composition of pollens collected but, surprisingly, pesticide composition was not related to pollen composition and, as a result, pesticide composition did not vary between the two groups of bees. On a site by site basis, pesticide risk indexes of honey bees and mason bees were not correlated, but overall risk index values did not differ between the groups of bees.

4.1. *Landscape pesticide exposure*

In our study, all but two pollen samples were contaminated with at least two pesticides, with an average of 11–12 compounds per sample. The maximum number of active ingredients detected in a single sample (farm) was 23 and 22 in honey bees and mason bees, respectively. These findings are in line with other studies showing that bees are commonly exposed to a wide variety pesticides in agricultural environments (Azpiazu et al., 2023; Knapp et al., 2023; Mullin et al., 2010; Nicholson et al., 2024b; Tosi et al., 2018).

Of the 584 compounds screened for, 65, including 31 fungicides, 18 insecticides, 10 herbicides and 6 other compounds (plant growth regulators, metabolites, synergists) were detected in the pollen samples. Glyphosate, the most widely used herbicide world-wide, was the compound most frequently found (70 % and 52.4 % in honey bees and mason bee samples, respectively). Despite its wide use and potential detrimental effect on bees (Helander et al., 2023; Motta et al., 2018; Straw et al., 2021), glyphosate is rarely screened in bee monitoring studies because it requires a separate analytical procedure, representing an extra cost (Toselli and Sgolastra, 2020). As far as we know, our study is the first that shows the presence of glyphosate in *Osmia* provisions, confirming its widespread occurrence in bee collected-pollen in agricultural areas.

The two most frequent insecticides in our study were flonicamid and tau-fluvalinate. However, compared to other insecticides, these two compounds are less toxic to bees (Frost et al., 2013; Meikle and Weiss, 2022), and contributed only marginally to risk indices. Although fungicides usually have low toxicity to bees (Ladurner et al., 2005), and for this reason are commonly applied during bloom, several studies show that their risk is often overlooked (Rondeau and Raine, 2022). Captan was the most frequent fungicide in our samples. This fungicide (and its metabolite) reached levels as high as 1,217 mg/Kg, potentially able to cause lethal effects on honey bees ($\mathrm{RI_{hb}} = 119$ %). A laboratory study found that *Osmia lignaria* was much more sensitive to captan than *A. mellifera* (Ladurner et al., 2005). Other fungicides commonly detected in our samples (difenoconazole, penconazole, tebuconazole) belong to the SBI (sterol-biosynthesis-inhibiting) group. These compounds alter the metabolic detoxification pathways of bees (Berenbaum and Johnson, 2015), synergistically enhancing the effects of some insecticides such as acetamiprid, imidacloprid, flupyradifurone and tau-fluvalinate, that were also found in our pollen samples (Carnesecchi et al. 2019; Sgolastra et al., 2017; Tosi and Nieh, 2017). Tau-fluvalinate and acetamiprid in particular were strongly associated to SBI fungicides based on our co-occurrence analysis. A recent study shows that the reproductive success of *O. cornuta* females is not affected by exposure to field-realistic levels of acetamiprid or tebuconazole alone, but females exposed simultaneously to both compounds have decreased nesting success and reduced longevity, resulting in negative population growth (Albacete et al., n.d.). Importantly, risk indexes are based on risk additivity and therefore do not account for synergistic interactions, thus underestimating the hazard of “pesticide cocktails”.

Only about a third (30–35 %) of the compounds detected in the

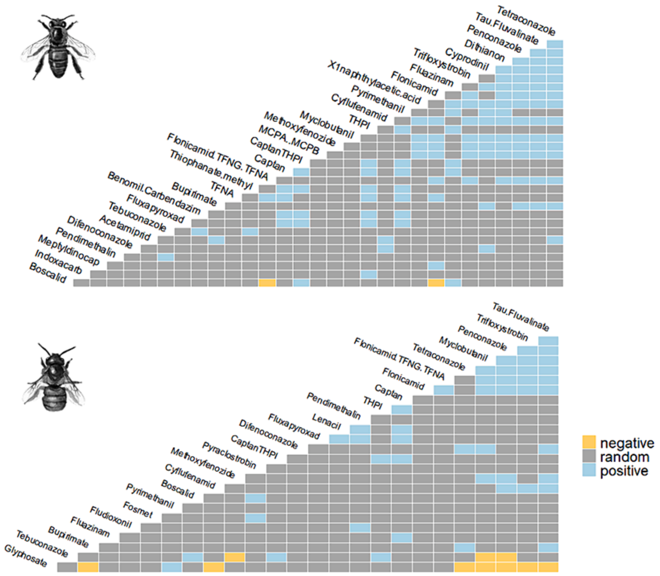

Fig. 4. Binary pesticide co-occurrence matrices obtained from honey bee and mason bee pollen samples, indicating negative, positive and random correlations.

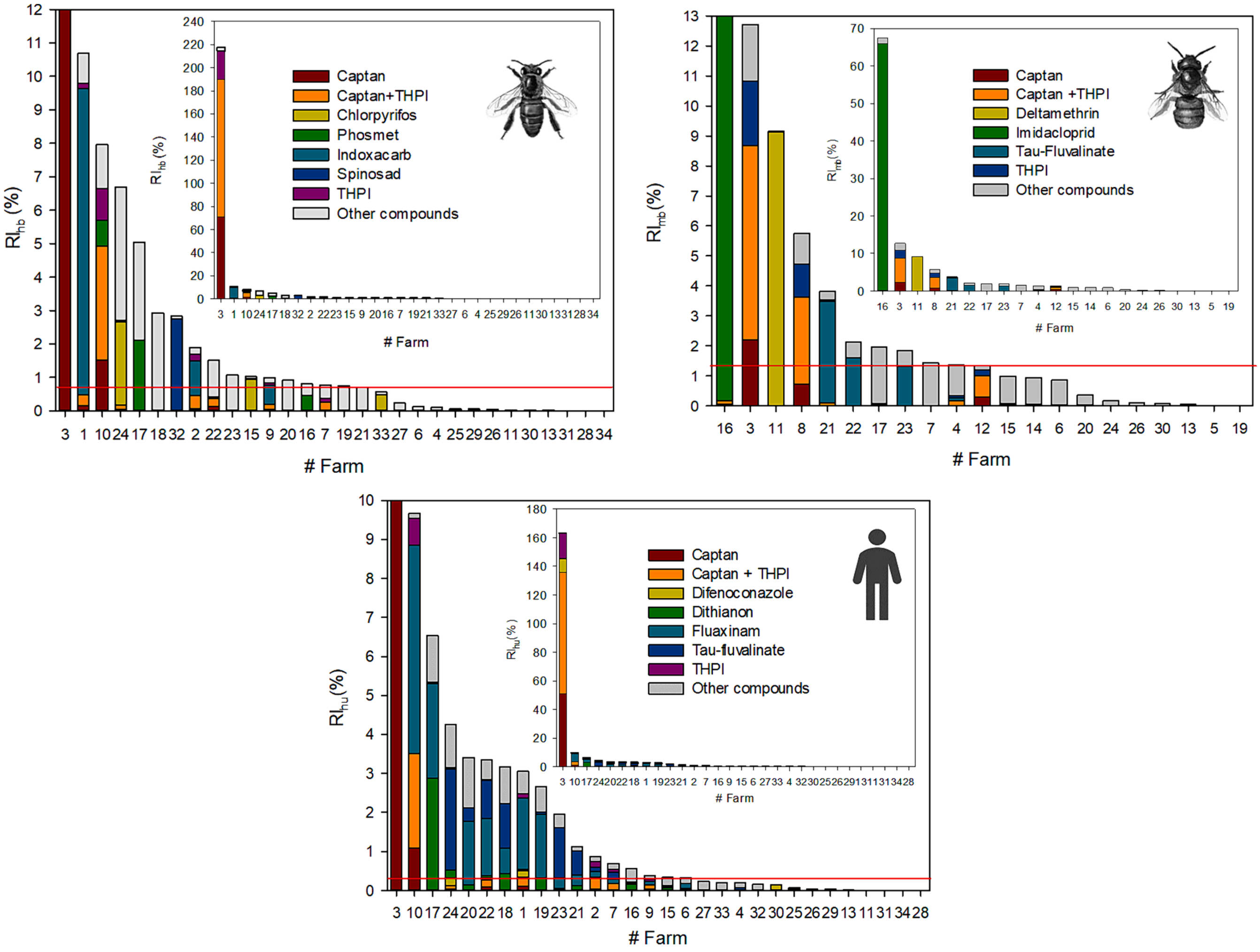

Fig. 5. Risk index and main compounds found in each farm for honey bees, mason bees and humans. The red line indicates the median value. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

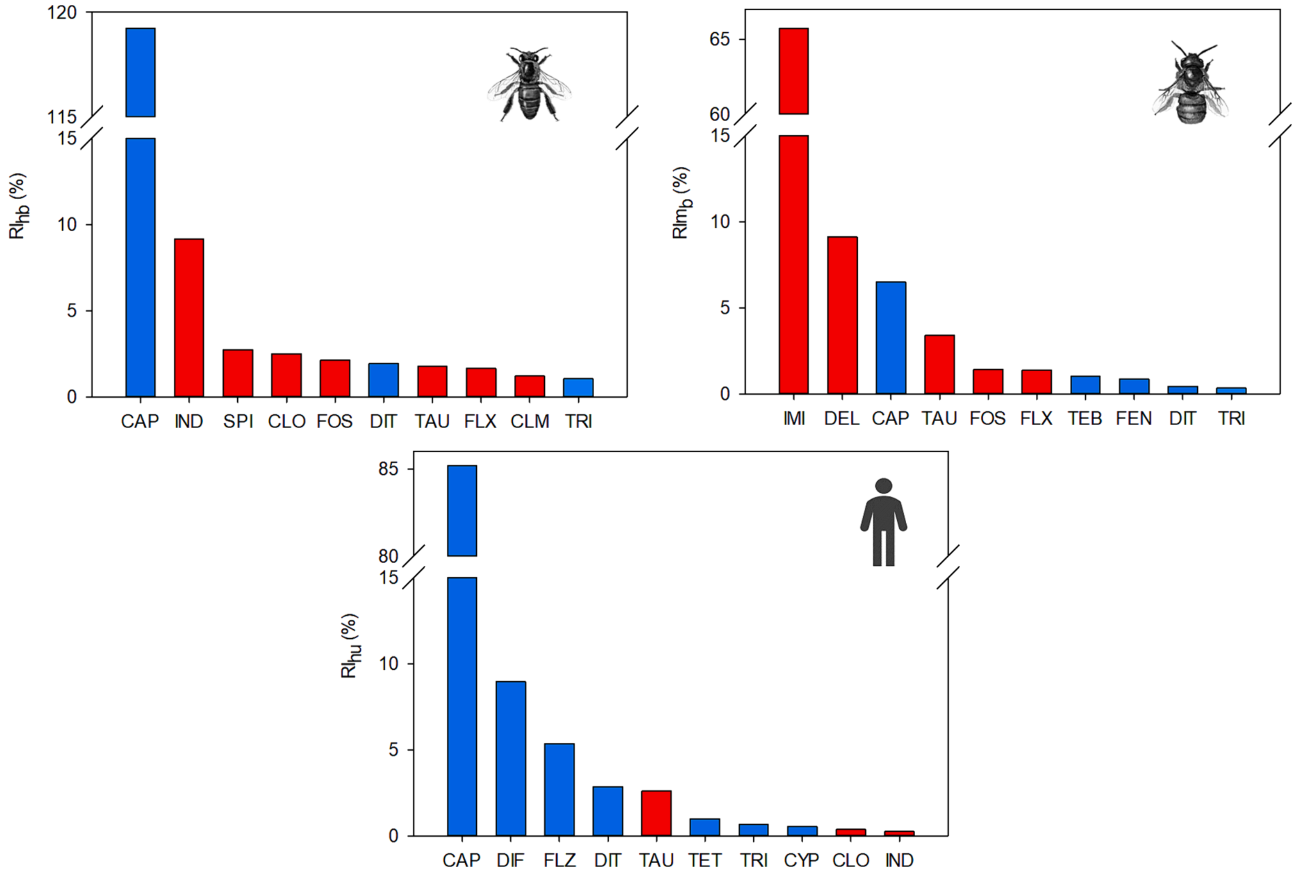

Fig. 6. Risk index of the main compounds, calculated using the maximum residue level, for honey bees, mason bees and humans. CAP=Captan; CLO=Chlorpyrifos; CYP=Cyprodinil; DEL=Deltamethrin; DIF=Difenoconazole; DIT=Dithianon; FEN=Fenbuconazole; FLZ=Fluazinam; FLX=Fluxapyroxad; FOS=Fosmet; IMI=Imidacloprid; IND=Indoxacarb; SPI=spynosad; TAU=Tau-fluvalinate; TEB=Tebuconazole; TET=Tetraconazole; TRI=Trifloxystrobin. Blue bars indicate fungicides while red bars insecticides. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pollen samples were actually applied by farmers on the target fields in the study year. This result is in line with reports from other agricultural systems, such as blueberry farms in Michigan, USA, and in oilseed rape and broad bean fields in Ireland, in which pesticide risk assessed from pollen collected by honey bees and bumblebees was primarily driven by pesticides not applied in the study fields (Graham et al., 2022; Zioga et al., 2023b). These results can be explained by cross-contamination from adjacent fields via pesticide drift (Cech et al., 2023) or by bee visitation to other fields within their foraging range (Botías et al., 2015; Favaro et al., 2019; McArt et al., 2017). In conclusion, bee exposure in agricultural areas is determined as much by landscape-level (surrounding fields) as by local-level (target field) pesticide use (Bloom et al., 2021).

4.2. *Pesticide risk levels*

Pesticide risk levels were low in most farms (median risk index = 0.76 % for honey bees and 1.35 % for mason bees). However, two farms showed risk index values higher than 100 % for both honey bees (farm #3) and mason bees (farm #16), indicating a pesticide toxic load higher than the cumulative LDD_{50}. The fungicide captan (plus its metabolite, THPI) was the main compound contributing to the high-risk index of *A. mellifera* in farm #3. In farm #16, the neonicotinoid insecticide imidacloprid, at a concentration of 0.016 mg/Kg, strongly contributed to high risk level of *Osmia*. Therefore, three years after its ban in the EU (Goulson, 2013; Wintermantel et al., 2020), bees are still being exposed to this neonicotinoid in some areas.

RI_{hu} values in our study sites were very low (0–9.7 %), except in farm #3 (163.20 %), indicating that, for the most part, the ingestion of pollen collected by honey bees does not represent a potential risk for human health. However, as many as 34 compounds exceeded pollen MRLs for humans at certain sites. In addition, some detected compounds (e.g., boscalid, tebuconazole) are considered endocrine disruptors that can interfere with endogenous hormones, resulting in negative effects on human health (Vandenberg et al., 2012).

4.3. *Comparison between honey bees and mason bees*

Landscape pesticide exposure is the result of the organism's interaction with landscape patterns, including a variety of flower resources, and the toxic load (i.e. the pesticides that have been used in the landscape) (Nicholson et al., 2024b). Considering their specific ecological traits, we expected differences between honey bees and mason bees in pollen choices and therefore pesticide exposure. As expected, pollen diversity was higher and crop pollen was lower in honey bees than mason bees. However, pollen diversity and the proportion of crop pollen were not influenced by the proportion of agricultural area surrounding the nesting sites, not even for honey bees, with a longer foraging range and lower affinity for pollen of the target crop. As a result, the number and composition of active ingredients and pesticide risk were similar in the two species. However, there was a marginal negative relationship between the number of compounds and pollen diversity in honey bees, indicating that in landscapes with higher flower diversity honey bees were able to visit a higher proportion of uncontaminated flowers. This "diluting effect" was not observed in mason bees. In honey bees, but not in mason bees, the proportion of crop pollen collected was positively related to pesticide risk. These results are congruent with differences in foraging behaviour between the two species. With a much shorter foraging range and a stronger affinity for the target crop, mason bees are exposed to pesticides close to their nesting site. In contrast, pesticide exposure in honey bees, with a much longer foraging range and a wider diet breadth, is mainly linked to pesticide contamination at wider spatial scales and to the probability to visit areas with uncontaminated flowers.

The various studies that have analysed the relationship between landscape and/or pollen composition and pesticide risk in different groups of bees provide highly contrasting results. Knapp et al. (2023) found no relationship between agricultural landscape and pesticide risk in honey bees. However, we found a positive relationship between the proportion of crop pollen collected and pesticide diversity (positively related to pesticide risk). For mason bees, the study of Knapp et al. (2023) found a positive relationship between agricultural landscape and pesticide risk, but this relationship was not significant in the study of Misiewicz et al. (2023). Also in mason bees, the relationship between pesticide risk and pollen diversity has been found to be either positive (Bednarska et al., 2022) or negative (Misiewicz et al., 2023), whereas in our study pesticide diversity was not related to either pollen diversity or proportion of crop pollen. Overall, these highly contrasting results across studies suggest that the ways in which bees get exposed to pesticides is not only determined by species foraging ranges and pollen choices (Jaumejoan et al., 2023), but is also highly context-dependent (Nicholson et al., 2024b). Cross-contamination across fields, including contamination of wild flowers in field margins (Botías et al., 2015) may explain this context dependency and the lack of correspondence between pollen composition and pesticide composition in our study. There are two limitations inherent to this kind of study. First, for most pesticides, *Osmia* LD50s are lacking. Because different bee species show differences in sensitivity to given pesticides, the use of honey bee LD50s to estimate the risk in *Osmia* may hinder the ability to detect differences between the two species. Second, the risk index does not account for potential synergism between compounds, thus possibly underestimating actual risk. At any rate, even with overall similar pesticide composition between honey bees and mason bees, local pesticide risk in our study was not correlated between honey bees and mason bees, raising concerns about the use of honey bees in post-approval pesticide monitoring (Ward et al., 2022; but see Knapp et al., 2023).

5. Conclusions

Two main conclusions can be drawn from our study. First, agricultural landscapes are pervasively contaminated with background levels of multiple pesticide combinations. Based on the risk indexes the hazard associated to these background levels appear to be low, but information on potential interactions is lacking for most compound combinations. A recent pan-European study shows that landscape pesticide exposure reduces bumble bee colony performance and this effect is especially strong in simplified landscapes with intensive agricultural practices (Nicholson et al., 2024a). Even at sublethal levels, pesticide exposure may interact with other biotic and abiotic stressors further compromising bee health (Albacete et al., 2023; Goulson et al., 2015).

Second, honey bees and mason bees interact differently with floral resources, but overall provide a similar assessment of landscape pesticide exposure. Our study shows that the way in which honey bees and mason bees intercept pesticides in the environment depends on specific traits but is also highly context dependent. These findings agree with a recent study concluding that simple metrics of landscape pattern, such as proportion of agricultural land, are not sufficient to predict pesticide risk (Nicholson et al., 2024b). In our study, overall pesticide composition and risk index were similar for honey bees and mason bees but, at a local scale, the combined use of honey bees and mason bees as bioindicators of pesticide contamination provided a complementary assessment of the risk of pesticides for bee health, as indicated by the lack of correlation between the two (honey bee and mason bee) risk indexes.

Our results have important consequences for risk assessment. The positive relationship between pesticide risk and number of active ingredients for both honey and mason bees underscores the importance of assessing multiple-pesticide exposure scenarios, a currently important gap in bee environmental risk assessment schemes (Sgolastra et al., 2020). In contrast to bees, whose pesticide risk was mostly driven by insecticides, fungicides were the main contributor to human pesticide risk. Therefore, measures to mitigate pesticide risk for bees may not necessarily be useful for human health, and vice-versa. Our findings emphasize the need to change our approach to environmental toxicology assessment, moving from a traditional farm-centred view of pesticide applications to a landscape-scale view considering both pesticide translocation and persistence, as well as the foraging ranges of non-target-organisms (Focks, 2014; Sgolastra et al., 2020; Topping et al., 2020). To reach this aim, access to pesticide-use data at field scale is an essential policy measure (Mesnage et al., 2021).

CRediT authorship contribution statement

F. Sgolastra: Writing – original draft, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **X. Arnan:** Writing – review & editing, Methodology, Formal analysis, Data curation. **C. Azpiazu:** Writing – review & editing, Methodology, Formal analysis. **A. Bordoni:** Writing – review & editing, Formal analysis, Data curation. **J. Bosch:** Writing – review & editing, Validation, Methodology, Formal analysis. **L. Lenzi:** Writing – review & editing, Methodology, Data curation. **C. Martins:** Writing – review & editing, Methodology, Data curation. **M. Parrilli:** Writing – review & editing, Methodology, Formal analysis, Data curation. **C. Porrini:** Writing – review & editing, Methodology, Conceptualization.

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 will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.112425.

References

Albacete, S., Sancho, G., Azpiazu, C., Rodrigo, A., Molowny-Horas, R., Sgolastra, F., Bosch, J., 2023. Bees exposed to climate change are more sensitive to pesticides. Glob. Chang. Biol. 00, 1–13. https://doi.org/10.1111/gcb.16928.

Albacete, S., Sancho, G., Azpiazu, C., Sgolastra, F., Rodrigo, A., Bosch, J., n.d. Exposure to realistic levels of insecticide-fungicide mixtures affect reproductive success and population growth rates in a solitary bee (submitted).

Alix, A., Lewis, G., 2010. Guidance for the assessment of risks to bees from the use of plant protection products under the framework of Council Directive 91/414 and Regulation 1107/2009. EPPO Bull. 40, 196–203. https://doi.org/10.1111/j.1365-2338.2010.02376.x.

Azpiazu, C., Medina, P., Sgolastra, F., Moreno-Delafuente, A., Viñuela, E., 2023. Pesticide residues in nectar and pollen of melon crops: Risk to pollinators and effects of a specific pesticide mixture on Bombus terrestris (Hymenoptera: Apidae) microcolonies. Environ. Pollut. 326, 121451 https://doi.org/10.1016/j.envpol.2023.121451.

Bednarska, A.J., Mikołajczyk, Ł., Ziółkowska, E., Kocjan, K., Wnęk, A., Mokkapati, J.S., Teper, D., Kaczyński, P., Łozowicka, B., Śliwińska, R., Laskowski, R., 2022. Effects of agricultural landscape structure, insecticide residues, and pollen diversity on the life-history traits of the red mason bee Osmia bicornis. Sci. Total Environ. 809, 151142 https://doi.org/10.1016/j.scitotenv.2021.151142.

Berenbaum, M.R., Johnson, R.M., 2015. Xenobiotic detoxification pathways in honey bees. Curr. Opin. Insect Sci. 10, 51–58. https://doi.org/10.1016/j.cois.2015.03.005.

Bloom, E.H., Wood, T.J., James, L., John, H., Ingwell, L.L., Goodell, K., Kaplan, I., Szendrei, Z., 2021. Synergism between local- - and landscape-level pesticides reduces wild bee floral visitation in pollinator-dependent crops. J. Appl. Ecol. 58, 1187–1198. https://doi.org/10.1111/1365-2664.13871.

Bosch, J., Sgolastra, F., Kemp, W.P., 2008. Life cycle ecophysiology of Osmia mason bees used as crop pollinators. In: Bee Pollination in Agricultural Ecosystems. Oxford University Press, Oxford, UK, pp. 83–104.

Botías, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., Goulson, D., 2015. Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for Bees. Environ. Sci. Technol. 49, 12731–12740. https://doi.org/10.1021/acs.est.5b03459.

Cabrera, R., Oliveira, D., Claudia, S., Fernandes, C., Silveira, R., Rath, S., 2016. Bee pollen as a bioindicator of environmental pesticide contamination. Chemosphere 163, 525–534. https://doi.org/10.1016/j.chemosphere.2016.08.022.

Cech, R., Zaller, J.G., Lyssimachou, A., Clausing, P., Hertoge, K., Linhart, C., 2023. Pesticide drift mitigation measures appear to reduce contamination of non-agricultural areas, but hazards to humans and the environment remain. Sci. Total Environ. 854, 158814 https://doi.org/10.1016/j.scitotenv.2022.158814.

Centrella, M., Russo, L., Moreno Ramírez, N., Eitzer, B., Dyke, M., Danforth, B., Poveda, K., 2020. Diet diversity and pesticide risk mediate the negative effects of land use change on solitary bee offspring production. J. Appl. Ecol. 57, 1031–1042. https://doi.org/10.1111/1365-2664.13600.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x.

Efsa, 2013. Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA J. 11, 3295. https://doi.org/10.2903/j.efsa.2013.3295.

Efsa, 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA J. 17, 1–77. https://doi.org/10.2903/j.efsa.2019.5634.

EFSA, 2018. European Food Safety Authority, 'The EFSA Comprehensive European Food Consumption Database', version 2 [WWW Document]. URL http://data.europa.eu/88u/dataset/the-efsa-comprehensive-european-food-consumption-database.

Favaro, R., Bauer, L.M., Rossi, M., D'Ambrosio, L., Bucher, E., Angeli, S., 2019. Botanical Origin of Pesticide Residues in Pollen Loads Collected by Honeybees During and After Apple Bloom. Front. Physiol. 10, 1069. https://doi.org/10.3389/fphys.2019.01069.

Feranec, J., Hazeu, G., Kosztra, B., Arnold, S., 2016. CORINE Land Cover Nomenclature. European Landscape Dynamics. CRC Press 47–56.

Focks, A., 2014. The challenge: Landscape ecotoxicology and spatially explicit risk assessment. Enviromental Toxycology Chem. 33, 1193.

Frost, E.H., Shutler, D., Hillier, N.K., 2013. Effects of fluvalinate on honey bee learning, memory, responsiveness to sucrose, and survival. J. Exp. Biol. https://doi.org/10.1242/jeb.086538.

Gathmann, A., Tscharntke, T., 2002. Foraging ranges of solitary bees. J. Anim. Ecol. 71, 757–764. https://doi.org/10.1046/j.1365-2656.2002.00641.x.

Girotti, S., Ghini, S., Ferri, E., Bolelli, L., Colombo, R., Serra, G., Porrini, C., Sangiorgi, S., 2020. Bioindicators and biomonitoring: honeybees and hive products as pollution impact assessment tools for the Mediterranean area. Euro-Mediterranean J. Environ. Integr. 5, 62. https://doi.org/10.1007/s41207-020-00204-9.

Goulson, D., 2013. An overview of the environmental risks posed by neonicotinoid insecticides. J. Appl. Ecol. 50, 977–987. https://doi.org/10.1111/1365-2664.12111.

Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Sci. 347, 1255957. https://doi.org/10.1126/science.1255957.

Graham, K.K., Milbrath, M.O., Zhang, Y., Baert, N., McArt, S., Isaacs, R., 2022. Pesticide risk to managed bees during blueberry pollination is primarily driven by off-farm exposures. Sci. Rep. 12, 7189. https://doi.org/10.1038/s41598-022-11156-1.

Helander, M., Lehtonen, T.K., Saikkonen, K., Despains, L., Nyckees, D., Antinoja, A., Solvi, C., Loukola, O.J., 2023. Field-realistic acute exposure to glyphosate-based herbicide impairs fine-color discrimination in bumblebees. Sci. Total Environ. 857, 159298 https://doi.org/10.1016/j.scitotenv.2022.159298.

Ipbes, 2016. The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production, Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Secretariat of the Intergovernmental Science-Policy Platform. on Biodiversity and Ecosystem Services, Bonn, Germany.

Jaumejoan, X., Arnan, X., Hagenbucher, S., Rodrigo, A., Sédivy, C., Bosch, J., 2023. Different effects of local and landscape context on pollen foraging decisions by two managed orchard pollinators, Osmia cornuta and Bombus terrestris. Agric. Ecosyst. Environ. 353, 108528 https://doi.org/10.1016/j.agee.2023.108528.

Kim, K., Kabir, E., Jahan, S.A., 2017. Exposure to pesticides and the associated human health effects. Sci. Total Environ. 575, 525–535. https://doi.org/10.1016/j.scitotenv.2016.09.009.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. r. Soc. B Biol. Sci. 274, 303–313. https://doi.org/10.1098/rspb.2006.3721.

Knapp, J.L., Nicholson, C.C., Jonsson, O., de Miranda, J.R., Rundlöf, M., 2023. Ecological traits interact with landscape context to determine bees' pesticide risk. Nat. Ecol. Evol. 7, 547–556. https://doi.org/10.1038/s41559-023-01990-5.

Ladurner, E., Bosch, J., Kemp, W.P., Maini, S., 2005. Assessing delayed and acute toxicity of five formulated fungicides to Osmia lignaria Say and Apis mellifera. Apidologie 36, 449–460. https://doi.org/10.1051/apido:2005032.

Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess. an Int. J. 22, 1050–1064. https://doi.org/10.1080/10807039.2015.1133242.

Loujost,, 2023. The new synthesis of diversity indices and similarity measures. and Physics/Diversity and Similarity/EffectiveNumberOfSpecies.htm [WWW Document].

McArt, S.H., Fersch, A.A., Milano, N.J., Truitt, L.L., Böröczky, K., 2017. High pesticide risk to honey bees despite low focal crop pollen collection during pollination of a mass blooming crop. Sci. Rep. 7, 46554. https://doi.org/10.1038/srep46554.

Meikle, W.G., Weiss, M., 2022. Field and Cage Studies Show No Effects of Exposure to Flonicamid on Honey Bees at Field-Relevant Concentrations. Insects 13, 845. https://doi.org/10.3390/insects13090845.

Mesnage, R., Straw, E.A., Antoniou, M.N., Benbrook, C., Brown, M.J.F., Chauzat, M., Finger, R., Goulson, D., Leadbeater, E., López-Ballesteros, A., Möhring, N., Neumann, P., Stanley, D., Stout, J.C., Thompson, L.J., Topping, C.J., White, B., Zaller, J.G., Zioga, E., 2021. Improving pesticide-use data for the EU. Nat. Ecol. Evol. 5, 1560. https://doi.org/10.1038/s41559-021-01574-1.

Misiewicz, A., Mikołajczyk, Ł., Bednarska, A.J., 2023. Floral resources, energetic value and pesticide residues in provisions collected by Osmia bicornis along a gradient of oilseed rape coverage. Sci. Rep. 13, 13372. https://doi.org/10.1038/s41598-023-39950-5.

Motta, E.V.S., Raymann, K., Moran, N.A., 2018. Glyphosate perturbs the gut microbiota of honey bees. Proc. Natl. Acad. Sci. 115, 10305–10310. https://doi.org/10.1073/pnas.1803880115.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., VanEngelsdorp, D., Pettis, J.S., 2010. High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. PLoS One 5, e9754.

Nicholson, C.C., Knapp, J., Kiljanek, T., Albrecht, M., Chauzat, M.-P., Costa, C., De la Rúa, P., Klein, A.-M., Mänd, M., Potts, S.G., Schweiger, O., Bottero, I., Cini, E., de Miranda, J.R., Di Prisco, G., Dominik, C., Hodge, S., Kaunath, V., Knauer, A., Laurent, M., Martínez-López, V., Medrzycki, P., Pereira-Peixoto, M.H., Raimets, R., Schwarz, J.M., Senapathi, D., Tamburini, G., Brown, M.J.F., Stout, J.C., Rundlöf, M., 2024a. Pesticide use negatively affects bumble bees across European landscapes. Nature 628, 355–358. https://doi.org/10.1038/s41586-023-06773-3.

Nicholson, C.C., Lonsdorf, E.V., Andersson, G.K.S., Knapp, J.L., Svensson, G.P., Gönczi, M., Jonsson, O., de Miranda, J.R., Williams, N.M., Rundlöf, M., 2024b. Landscapes of risk: A comparative analysis of landscape metrics for the ecotoxicological assessment of pesticide risk to bees. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.14622.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. De, Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., O.Hill, M., Lahti, L., McGlinn, D., Ouellette, M.-H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.F., Weedon, J., 2022. Vegan: Community Ecology Package version 2.6-4. https://cran.r-project.org/web/packages/vegan/index.html.

Pinheiro, J., Bates, D., Team, R.C., 2022. nlme: Linear and Nonlinear Mixed Effects Models. R Packag. version 3.1-160. https//CRAN.R-project.org/package=nlme.

Porrini, C., Sabatini, A.G., Girotti, S., Fini, F., Monaco, L., Celli, G., Bortolotti, L., Ghini, S., 2003. The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators. Bull. Insectology 56, 147–152.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.

Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A.S., Srivastav, A.L., Kaushal, J., 2021. An extensive review on the consequences of chemical pesticides on human health and environment. J. Clean. Prod. 283, 124657 https://doi.org/10.1016/j.jclepro.2020.124657.

Rondeau, S., Raine, N.E., 2022. Fungicides and bees: a review of exposure and risk. Environ. Int. 165, 107311 https://doi.org/10.1016/j.envint.2022.107311.

Roulston, T.H., Goodell, K., 2011. The Role of Resources and Risks in Regulating Wild Bee Populations. Annu. Rev. Entomol. 56, 293–312. https://doi.org/10.1146/annurev-ento-120709-144802.

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2019. Worldwide decline of the entomofauna: A review of its drivers. Biol. Conserv. 232, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020.

Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M.T., Tosi, S., Bogo, G., Teper, D., Porrini, C., Molowny-Horas, R., Bosch, J., 2017. Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. Pest Manag. Sci. 73, 1236–1243. https://doi.org/10.1002/ps.4449.

Sgolastra, F., Hinarejos, S., Pitts-Singer, T.L., Boyle, N.K., Joseph, T., Lückmann, J., Raine, N.E., Singh, R., Williams, N.M., Bosch, J., 2019. Pesticide Exposure Assessment Paradigm for Solitary Bees. Environ. Entomol. 48, 22–35. https://doi.org/10.1093/ee/nvy105.

Sgolastra, F., Medrzycki, P., Bortolotti, L., Maini, S., Porrini, C., Simon-Delso, N., Bosch, J., 2020. Bees and pesticide regulation: Lessons from the neonicotinoid experience. Biol. Conserv. 241, 108356 https://doi.org/10.1016/j.biocon.2019.108356.

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G.P.S., Handa, N., Kohli, S.K., Yadav, P., Bali, A.S., Parihar, R.D., Dar, O.I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., Thukral, A.K., 2019. Worldwide pesticide usage and its impacts on ecosystem. SN Appl. Sci. 1, 1446. https://doi.org/10.1007/s42452-019-1485-1.

Straw, E.A., Carpentier, E.N., Brown, M.J.F., 2021. Roundup causes high levels of mortality following contact exposure in bumble bees. J. Appl. Ecol. 58, 1167–1176. https://doi.org/10.1111/1365-2664.13867.

Thioulouse, J., Dray, S., Dufour, A.-B., Siberchicot, A., Jombart, T., Pavoine, S., 2018. Multivariate Analysis of Ecological Data with ade4. Springer, New York, New York, NY. https://doi.org/10.1007/978-1-4939-8850-1.

Topping, C.J., Aldrich, A., Berny, P., 2020. Overhaul environmental risk assessment for pesticides. Science 367, 360–363. https://doi.org/10.1126/science.aay1144.

Toselli, G., Sgolastra, F., 2020. Seek and you shall find: An assessment of the influence of the analytical methodologies on pesticide occurrences in honey bee-collected pollen with a systematic review. Chemosphere 258, 127358. https://doi.org/10.1016/j.chemosphere.2020.127358.

Tosi, S., Nieh, J.C., 2017. A common neonicotinoid pesticide, thiamethoxam, alters honey bee activity, motor functions, and movement to light. Sci. Rep. 7, 15132. https://doi.org/10.1038/s41598-017-15308-6.

Tosi, S., Costa, C., Vesco, U., Quaglia, G., Guido, G., 2018. A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides. Sci. Total Environ. 615, 208–218. https://doi.org/10.1016/j.scitotenv.2017.09.226.

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050.

Veech, J.A., 2013. A probabilistic model for analysing species co-occurrence. Glob. Ecol. Biogeogr. 22, 252–260. https://doi.org/10.1111/j.1466-8238.2012.00789.x.

Ward, L.T., Hladik, M.L., Guzman, A., Winsemius, S., Bautista, A., Kremen, C., Mills, N.J., 2022. Pesticide exposure of wild bees and honey bees foraging from field border flowers in intensively managed agriculture areas. Sci. Total Environ. 831, 154697 https://doi.org/10.1016/j.scitotenv.2022.154697.

Wintermantel, D., Odoux, J., Decourtye, A., Henry, M., Allier, F., Bretagnolle, V., 2020. Neonicotinoid-induced mortality risk for bees foraging on oilseed rape nectar persists despite EU moratorium. Sci. Total Environ. 704, 135400 https://doi.org/10.1016/j.scitotenv.2019.135400.

Zioga, E., Kelly, R., White, B., Stout, J.C., 2020. Plant protection product residues in plant pollen and nectar: A review of current knowledge. Environ. Res. 189 https://doi.org/10.1016/J.ENVRES.2020.109873.

Zioga, E., White, B., Stout, J.C., 2023a. Pesticide mixtures detected in crop and non-target wild plant pollen and nectar. Sci. Total Environ. 879, 162971 https://doi.org/10.1016/j.scitotenv.2023.162971.

Zioga, E., White, B., Stout, J.C., 2023b. Honey bees and bumble bees may be exposed to pesticides differently when foraging on agricultural areas. Sci. Total Environ. 896, 166214 https://doi.org/10.1016/j.scitotenv.2023.166214.