



Pesticide mixtures detected in crop and non-target wild plant pollen and nectar

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HIGHLIGHTS

- Pesticide residues in plant pollen and nectar may pose a hazard for pollinators.
- We evaluated the pesticide residues in a crop and a wild plant species in Ireland.
- Most detections were in fields with no recent application of the compounds detected.
- Azoxystrobin, boscalid and clothianidin was the most common compound mixture.
- Clothianidin was detected in both plant species several years after its application.

GRAPHICAL ABSTRACT



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ABSTRACT

Cultivation of mass flowering entomophilous crops benefits from the presence of managed and wild pollinators, who visit flowers to forage on pollen and nectar. However, management of these crops typically includes application of pesticides, the presence of which may pose a hazard for pollinators foraging in an agricultural environment. To determine the levels of potential exposure to pesticides, their presence and concentration in pollen and nectar need assessing, both within and beyond the target crop plants. We selected ten pesticide compounds and one metabolite and analysed their occurrence in a crop (*Brassica napus*) and a wild plant (*Rubus fruticosus* agg.), which was flowering in field edges. Nectar and pollen from both plants were collected from five spring and five winter sown *B. napus* fields in Ireland, and were tested for pesticide residues, using QuEChERS and Liquid Chromatography tandem mass spectrometry (LC-MS/MS). Pesticide residues were detected in plant pollen and nectar of both plants. Most detections were from fields with no recorded application of the respective compounds in that year, but higher concentrations were observed in recently treated fields. Overall, more residues were detected in *B. napus* pollen and nectar than in the wild plant, and *B. napus* pollen had the highest mean concentration of residues. All matrices were contaminated with at least three compounds, and the most frequently detected compounds were fungicides. The most common compound mixture was comprised of the fungicides azoxystrobin, boscalid, and the neonicotinoid insecticide clothianidin, which was not recently applied on the fields. Our results indicate that persistent compounds like the neonicotinoids, should be continuously monitored for their presence and fate in the field environment. The toxicological evaluation of the compound mixtures identified in the present study should be performed, to determine their impacts on foraging insects that may be exposed to them.

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1. Introduction

Insect pollination (i.e., the transfer of pollen from the male flower parts to the female, within or between flowers by foraging insects), is a key ecosystem service, providing a range of benefits to global food production and human welfare (Garibaldi et al., 2022; Fisher et al., 2009). Animal pollinators benefit the production of 75 % of the globally most common food crop species, are responsible for 35 % of world's crop production (Klein et al., 2007), and contribute \$235 billion - \$577 billion to world's agriculture (IPBES, 2016). Even in partially wind-pollinated crop species like oilseed rape (OSR, *Brassica napus* L.), bees' presence can increase the average seed yield, the oil content, and improve the seed pod characteristics (e.g., weight and set) (Bartomeus et al., 2014; Garratt et al., 2014; Jauker et al., 2012; Perrot et al., 2018). Such mass flowering crops can be very attractive to pollinators (Beyera et al., 2021; Silva et al., 2021), since they flower for several weeks (Stanley et al., 2013) and produce large amounts of pollen and nectar (Holzschuh et al., 2013; Westphal et al., 2003; Bommarco et al., 2012). OSR is cultivated in several countries all over the world (Stanley et al., 2013), and is visited by various insect species (Stanley and Stout, 2014), with honey bees being the most common visitors of OSR in UK (Garratt et al., 2014), but various wild bee species being more effective pollinators of OSR (Woodcock et al., 2013).

However, OSR crops tend to receive high inputs of pesticides (DAFM, 2016; Karlise et al., 2017; Raimets et al., 2020) which can be detected in plant nectar and pollen of both crops (Henry et al., 2015; Pohorecka et al., 2012; Botías et al., 2015; Xu et al., 2016) and wild plant species growing on the edges of the OSR crop fields (Botías et al., 2015; David et al., 2016). Plant pollen and nectar are considered the most relevant and an important pesticide exposure route for pollinating insects, particularly bees (Sgolastra et al., 2019; Zioga et al., 2020), as these are their main nutrition sources during adult and larval stages (Rortais et al., 2005; Sgolastra et al., 2019). Global declines in pollinating insects have been attributed to agricultural intensification, which involves increased pesticide application (Arena and Sgolastra, 2014; Goulson et al., 2015; Dudley and Alexander, 2017; Rundlöf et al., 2015; Sánchez-Bayo and Wyckhuys, 2019; Williams et al., 2015). In a recent review of the pesticide residues detected in plant pollen and nectar (Zioga et al., 2020), the importance of quantifying the concentrations and identifying the mixtures (Sgolastra et al., 2017, 2019), of pesticide active substances contaminating pollen and nectar of both crops and wildflowers growing nearby, was highlighted. There is a paucity of information on how various pesticide compounds behave in different climates and landscapes worldwide, or even on the same field over time following their application (Sgolastra et al., 2020), and an urgent need to expand the range of compounds evaluated in plant pollen and nectar pesticide residue studies, beyond that of neonicotinoids in particular, and insecticides in general (Kyriakopoulou et al., 2017; Zioga et al., 2020).

In the present study, we wanted to evaluate whether compounds from different pesticide categories (fungicides, herbicides, and insecticides) contaminate pollen and nectar of OSR crops and wild plants in Ireland, and whether their presence may pose a hazard for bees during the period that they are actively foraging on OSR crops (March until July). For this, we chose winter and spring sown OSR because a) OSR is proposed by the existing literature as a suitable crop for pesticide residue studies, b) it is the most abundant entomophilous crop in Ireland, c) it is very attractive to pollinators, d) it has a relatively long flowering period (~ 20 days), and e) due to different sowing times it flowers both in spring and summer in Ireland (DAFM, 2016; Pohorecka et al., 2012; Stanley and Stout, 2013; Stanley et al., 2013; Zioga et al., 2020). Blackberry (BAB, *Rubus fruticosus* agg.) was chosen as wild plant species because a) it has been evaluated in previous residue studies (Botías et al., 2015; David et al., 2015), b) it is an abundant wild flowering species on the margins of Irish crop fields, c) it is a valuable source of nectar and pollen for pollinators, and d) it has a long flowering period (~3 months) (Kavanagh, 2019; Russo et al., 2022; Wignall et al., 2020; Zioga et al., 2020). The potential risk that these pesticide residues

in plant pollen and nectar may pose to honey bees was then evaluated by calculating Risk Quotients.

2. Materials and methods

2.1. Field locations

In February 2019, ten conventional fields (i.e., farming that relies on chemical intervention to control pests and weeds and to provide plant nutrition) of winter sown (WOR, five fields) and spring planted (SOR, five fields) oilseed rape (OSR) were randomly selected in the East and Southeast Ireland (Fig. 1). For each field, all information related to pesticide applications during the crop season (e.g., products applied, date and rate of application etc), along with general information regarding the crop (e.g., crop variety, sowing date etc.) was compiled by interviewing the farmers (Tables S1 and S2).

The compounds selected for evaluation were three fungicides commonly applied on OSR crops in Ireland (azoxystrobin, boscalid, and prothioconazole), one herbicide applied on cereals used in rotation with OSR on the same fields (fluroxypyr), the herbicide glyphosate and its metabolite aminomethylphosphonic acid (AMPA) (DAFM, 2016), and five neonicotinoid insecticides (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam) that have not been recently applied on the fields but may have long half-lives in the environment (e.g., clothianidin) (PPDB, 2022) and some are toxic to bees (e.g., clothianidin, imidacloprid and thiamethoxam) (Wood and Goulson, 2017). Each compound chemical class, mode of action and status according to the EC regulation 1107/2009 (European Commission, 2009) is recorded based on the University of Hertfordshire database (PPDB, 2022) and shown in Table 1.

2.2. Collection of flowers from *Brassica napus* and *Rubus fruticosus* agg. plants

Crop flowers were collected during the blooming periods of the two different OSR crop types: from 19th March to 13th April (WOR) and from 17th to 27th June (SOR). During the sample collection, OSR crops were between GS63 to GS67 of the BBCH growth stages (Meier, 2001). Flowers were also collected from wild blackberries (BAB) growing in the margins and hedges of the OSR fields (~1.5 m average distance from the crop edge) during the period from 4th to 23rd July. BAB flowers were collected from eight of the ten fields, as in the remaining two sites the crop had already started to form seeds when BAB started flowering, creating an impenetrable stand of plant stems, which made the BAB plants growing in the edges of the crop inaccessible. Evaluating the temporal distribution of pesticides applied was feasible only in three fields (2, 3 and 5) (Tables S1 and S2). In field 3, OSR samples were collected twice after the prothioconazole application (seven and 16 days after); in field 5, OSR sampling took place both before and within a period of 20 days after the recorded azoxystrobin application; and BAB sampling was performed twice after glyphosate application on the crops (three and five days in field 2 and two and seven days in field 5). To obtain enough pollen and nectar for chemical analysis (100 mg and 100 µL respectively), a minimum of 1000 flowers from each plant species in each field and each sampling event (pre/post pesticide application) were collected. Flowers were stored in coolers during sampling and when transferred in the lab, they were weighed and subsequently stored at -25 °C prior to pollen and nectar extraction.

2.3. Nectar and pollen extraction from flowers

The flowers were defrosted at room temperature. The flower petals were carefully removed from each flower for easier access to the nectar droplets located at the base of the filaments. The nectar was collected with a precision-bore glass microcapillary tube (1 µL - 5 µL, Hirschmann®). Using a micropipette, nectar was extracted from the microcapillary tubes and transferred in a labelled and pre-weighed plastic 2 mL centrifuge vial.

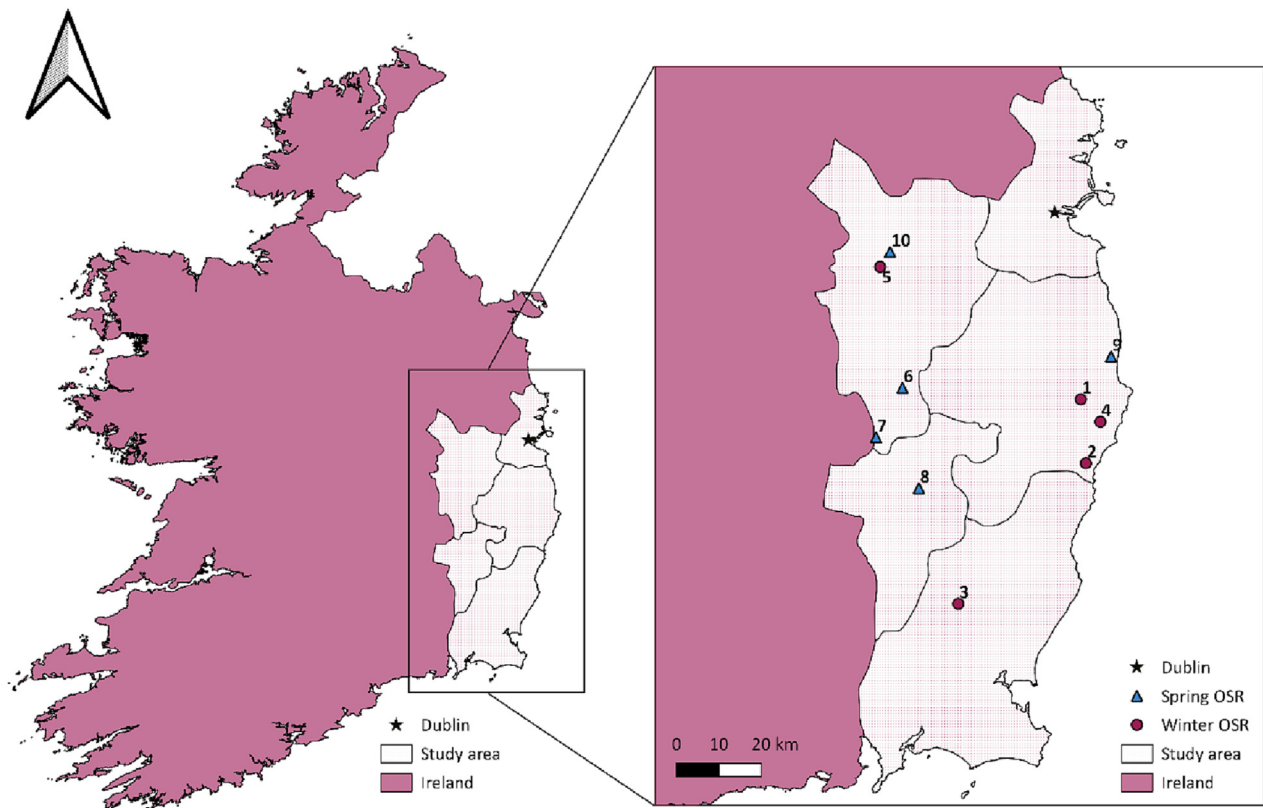


Fig. 1. Map of the Republic of Ireland, the study area and location of the ten oilseed rape (OSR) fields, divided in five fields of spring and five fields of winter oilseed rape varieties.

For each sample, the final number of flowers processed was recorded and the respective amount of nectar was weighed. Sugar content (as sucrose equivalent) in OSR and BAB floral nectar was measured using a handheld refractometer and was found to be $39 \pm 1.8 \%$ and $28 \pm 2.3 \%$ respectively. Nectar samples were stored in the freezer at $-25 \text{ }^{\circ}\text{C}$ prior to chemical analysis.

Our pollen extraction process follows the protocol of Botías et al. (2015). Specifically, after the nectar extraction, the stamens of the flowers in both plant species were cut from the rest of the flower parts and they were incubated at $37 \text{ }^{\circ}\text{C}$ for 24 h, to facilitate pollen release from the anthers. Then, the stamens were brushed over sieves to separate pollen from the anthers and sifted through multiple sieves of decreasing pore size (250 to $45 \text{ }\mu\text{m}$). The final amount of pollen collected was weighed following

the same process with nectar, and pollen samples were evaluated under the microscope for impurities. The pollen samples were stored in the freezer at $-25 \text{ }^{\circ}\text{C}$ prior to chemical analysis.

2.4. Pesticide residue analysis

2.4.1. Chemicals and reagents

Certified analyte standards of thiamethoxam, thiacloprid, acetamiprid, clothianidin, imidacloprid, prothioconazole, fluroxypyr, boscalid, azoxystrobin, internal standards (IS) (clothianidin-d3, imidacloprid-d4, thiamethoxam-d3, thiacloprid-d4, acetamiprid-d3, malathion-(diethyl-d10), MCPA-(methyl-d3)), formic acid, ammonium formate, Supel™ QuE, PSA/C18/ENVI-Carb, Millipore Millex syringe

Table 1
The ten pesticide compounds evaluated, their chemical class, their mode of action, their current status and expiry date according to the European regulation 1107/2009.

Compound	Chemical class	Mode of action	EC regulation 1107/2009	
			Status	Expiry date
Neonicotinoids				
Acetamiprid	Pyridylmethylamine	Nicotinic acetylcholine receptor (nAChR) competitive modulator	Approved	28/02/2033
Clothianidin	Nitroguanidine, Thiazole,		Not approved	Expired
Imidacloprid	Chloronicotine, Guanidine		Not approved	Expired
Thiacloprid	Pyridylmethylamine, Thiazolidine		Not approved	Expired
Thiamethoxam	Nitroguanidine, Thiazole		Not approved	Expired
Herbicides				
Fluroxypyr	Pyridinecarboxylic acid	Synthetic auxin	Approved	31/12/2024
Glyphosate	Organophosphate, Phosphonoglycine	Inhibition of EPSP synthase	Approved	15/12/2023
Fungicides				
Azoxystrobin	Methoxyacrylate, Strobilurin	Respiration inhibitor	Approved	31/12/2024
Boscalid	Pyridine-carboxamide, Anilide	Succinate dehydrogenase Inhibitor	Approved	31/07/2023
Prothioconazole	Conazole	Sterol biosynthesis inhibitor	Approved	31/07/2023

filters with hydrophobic PTFE membrane (pore size 0.22 μm and 20 mm diameter) and low-adsorption LC-MS certified vials were obtained from Sigma Aldrich, Ireland. The XBridge UPLC BEH column 4.6 $\times$ 100 mm i. d., 3.5 μm particle size using XBridge BEH C18 VanGuard Cartridge (3.5 μL 3.9 $\times$ 5 mm) and VanGuard cartridge holder were all purchased from Waters Chromatography, Ireland. All pesticide standards were > 98 % compound purity and deuterated standards >98 % isotopic purity. Ultrapure water was generated using ELGA Purelab Ultra SC MK2 (ELGA, UK), and LC-MS grade acetonitrile (ACN) was obtained from Sigma-Aldrich, Ireland. Individual standard pesticide (native and deuterated) stock solutions (1 mg/mL) were prepared in ACN. An additional IS mixture of the deuterated pesticides at 1000 ng/mL was also prepared. Calibration points in $\text{H}_2\text{O}/\text{ACN}$ (70:30) were freshly prepared from the stock solutions. All stock solutions were preserved at -25°C in dark conditions. The glyphosate and AMPA chemical analysis protocols are described in full detail in Zioga et al. (2022).

2.4.2. Nectar extraction

100 μL of nectar was placed into a 15 mL PTFE centrifuge tube. A solution of $\text{H}_2\text{O}/\text{ACN}$ (70:30) was added so that the volume of the final extract was 1 mL. The nectar sample was then homogenized using a vortex and centrifuged at 2500 rpm for 5 min. The solution was filtered through 20 mm PTFE hydrophilic syringe filters (Sigma-Aldrich) into an LC-MS vial.

2.4.3. Pollen extraction

Samples were extracted using a modified QuEChERS method as described by Anastassiades et al. (2003). Briefly, 100 mg were weighed in a 50 mL PTFE centrifuge tube to which 1 mL of ultrapure H_2O was added. The tube was vortexed for 1 min and then sonicated for 30 min. Then 4 mL of acidified ACN (5 % formic acid) were added and the tube was vortexed for 1 min. For the partitioning, 0.5 g ammonium formate salt was added, and the sample was vortexed for 1 min. The tube was centrifuged at 2500 rpm for 2 min. The supernatant was transferred into a 15 mL PTFE centrifuge tube and mixed with the SupelTM QuE, PSA/C18/ENVI-Carb. The 15 mL tube was vortexed for 1 min and then centrifuged at 4500 rpm for 10 min. A 400 μL aliquot of the supernatant was mixed with 100 μL ACN and diluted with 500 μL acidified ultrapure water (0.2 % formic acid). The solution was filtered through 20 mm PTFE hydrophilic syringe filters (Sigma-Aldrich) into an LC-MS vial.

2.4.4. LC-MS/MS analysis

The analysis of the nine compounds in both nectar and pollen, was performed on an Agilent 1290 Infinity II Multisampler LC System coupled to an Agilent 6470 Triple Quadrupole mass spectrometer (LC-MS/MS). Compound separation was achieved on an XBridge UPLC BEH column 4.6 $\times$ 100 mm i.d., 3.5 μm particle size, Waters) using a column oven at 30°C and a flow rate of 0.5 mL/min. Mobile phase A consisted of 5 mM ammonium formate with 0.1 % formic acid and mobile phase B comprised ACN with 0.1 % formic acid. The following gradient elution programme was used: linear increase from 30 to 50 % B in 5.0 min, 50 to 80 % B in 3 min, 80 to 94 in 2 min, 94 to 98 in 1 min, 98 to 100 in 1 min, holding at 100 % B for 2 min and returning to initial conditions at 30 % B in 1 min. Total time of the LC-MS/MS analysis was 15 min. The injection volume was 10 μL . The ion spray voltage was set at 3500 V and - 3500 V for positive and negative ionization respectively. Source temperature was set at 325°C . Nitrogen was used as a curtain gas (8 L/min), nebulizer gas (25 psi), sheath gas flow (11 L/min), and sheath gas temperature was 375°C . Agilent Mass Hunter Workstation Data Acquisition Version 10.0 software was used to control LC-MS/MS system and for data acquisition. Quantitative and qualitative analysis was done with Agilent Mass Hunter Workstation Qualitative Analysis software version 10.0, based on two most abundant precursor ion and product ion MRM transitions, and their characteristic retention time. The compound-specific LC-MS/MS retention times (R_t), quantifying transition ions (Q) and qualifier transition ions (q) for the nine compounds analysed are shown in Table S3.

2.4.5. Method validation

Validation experiment was carried out according to requirements of Document No. SANTE/12682/2019 (European Commission, 2020) guidance to evaluate linearity, matrix effect, limit of detection and quantification, sensitivity, accuracy, and precision (Table S4) (European Commission, 2020). To prepare the pollen matrix-matched calibration, pollen from organic beekeeping was used as blank. For the nectar matrix-matched calibration, a nectar surrogate was created by mixing 6 g of glucose and 3 g of fructose in 25 mL of ultrapure water (Martel et al., 2013). Linearity was evaluated both in pure solvent and matrices, using nine-point matrix-matched calibration curves ($R^2 > 0.99$). The matrix effect of nectar and pollen extracts was evaluated by comparing the slopes of the calibration curves in pure solvent ($\text{H}_2\text{O}/\text{ACN}$; 70:30) with those in the respective matrix. Method sensitivity was examined by calculating the limit of quantification (LOQ) as the minimum amount of analyte detected with a signal-to-noise ratio of ten, and the limit of detection (LOD) as the minimum amount of analyte detected with a signal-to-noise ratio of 3 (David et al., 2015). Recovery (%) and precision (RSD%) were performed by fortifying blank pollen and nectar matrix samples at three concentration levels of the test analytes, for three replicates. Recoveries between 70 and 120 % with RSD <20 % were considered satisfactory (European Commission, 2020). Concentrations were determined using a least-square linear regression analysis of the peak area ratio versus the concentration ratio (native analyte to deuterated IS) (David et al., 2015).

2.4.6. Quality control

The instrument was calibrated between sample batches to monitor sensitivity changes after a batch analysis. A workup sample (i.e., standard solution) per batch of ten samples was included in the worklist of the LC-MS/MS run to ensure that no contamination occurred during the sample preparation and that there was no carryover during the injections (David et al., 2015). Three replicates per sample were analysed, and analyses were performed twice for the samples where pesticides were detected.

2.5. Risk evaluation

For evaluating the potential risk that the active substances detected in plant pollen and nectar pose to honey bee adult foragers and nurse bees, we estimated the acute and chronic Risk Quotients (RQs) using the BeeREX risk assessment tool suggested by US EPA as tier 1 risk assessment approach (US EPA, 2015). The respective RQ values were calculated by using the value of acute dosage that kills 50 % of honey bees within a given period after first exposure (LD_{50}) and were retrieved from the University of Hertfordshire online database (PPDB, 2022). The chronic 10-day oral NOED value (no observed effect dose) for clothianidin was retrieved from the US EPA OPP Pesticide Ecotoxicity Database (US EPA OPP, 2017) and for thiacloprid was retrieved from the respective conclusion on the peer review of the pesticide risk assessment document of the European Food Safety Authority (EFSA, 2019). In cases where the 10-day oral NOED data from chronic oral toxicity tests on adult honey bees were not available, we used 1/10th of the oral LD_{50} value as a worst-case scenario (e.g., boscalid, glyphosate and prothioconazole), while information for the compound azoxystrobin was not available. For nectar, the daily consumption rates are suggested to be 292 mg/bee for foragers and 140 mg/bee for in-hive workers, and for pollen the daily consumption is 9.6 mg per in-hive worker and zero for foragers (US EPA, 2014). Acute and chronic RQ values above the level of concern (LOC, 0.4 and 1 respectively), would imply that further assessment is needed (Thompson, 2021).

2.6. Statistical analysis

The concentrations of residues and the RQ values were calculated in Excel (version 2108, Microsoft®, Redmond, WA, USA). The software used for map creation was QGIS Geographic Information System (QGIS, version 3.12.1), and part of graph creation was GraphPad Prism Software (version 9.3.1). Non-parametric Mann-Whitney (M-W) U tests were used

to compare the concentrations of compounds present in pollen and nectar collected from OSR flowers, to compare pollen and nectar collected from OSR flowers versus pollen and nectar from BAB flowers growing in the OSR field margins, and for pollen and nectar collected from WOR versus those collected from SOR. To perform the statistical analyses, all concentrations that were $\geq$ LOD but $<$ LOQ were assigned half of the LOQ value for each compound (Dively and Kamel, 2012; Zioga et al., 2020) (Table S4). Concentrations below the LOD were assigned a zero value. The coefficient of variation in the concentrations of compounds found in OSR pollen and nectar, and in BAB pollen and nectar, was used to analyse the consistency in the residue levels found in these sets of samples, using non-parametric tests to compare between the variability found in OSR pollen and OSR nectar, and in OSR pollen and BAB pollen. The relationship between the concentrations of residues in nectar and pollen was assessed using a paired Generalized Linear Mixed Model approach following the method described in detail in Zioga et al. (2020). The concentration of residues in pollen ($\mu\text{g}/\text{kg}$) was fitted as the response variable. The natural log of residue concentrations in pollen ($\mu\text{g}/\text{kg}$), and the compound type, were fitted as predictor variables (fixed factors). The model accounted for non-independence of data within 'Field', and 'Species' by fitting these as random non-nested factors. The model was fitted using a Gamma error distribution, and a log link function. Analyses were conducted using R version 4.2.1 (R Core Team, 2022).

3. Results

3.1. Pesticide active substances applied on the OSR fields

All ten OSR crop fields sampled in 2019 were treated with pesticides. In total, 19 different active substances were applied, the majority of which were herbicides ($n = 11$), followed by fungicides ($n = 7$), and one molluscicide (Fig. 2). No insecticides were applied during the year of sampling on any of the OSR fields. According to the information on pesticide applications provided by the farmers, the fungicide prothioconazole was the most applied compound both in winter and spring fields. Comparing the two different crop types (spring and winter OSR) the WOR crops received more active substances and had a higher chemical input of both fungicides and herbicides. Of the other compounds that were the focus of the present study, boscalid and azoxystrobin were applied on one field each, while the herbicide fluroxypyr and the five neonicotinoid compounds were not applied on any of the OSR crops before or during the cropping period.

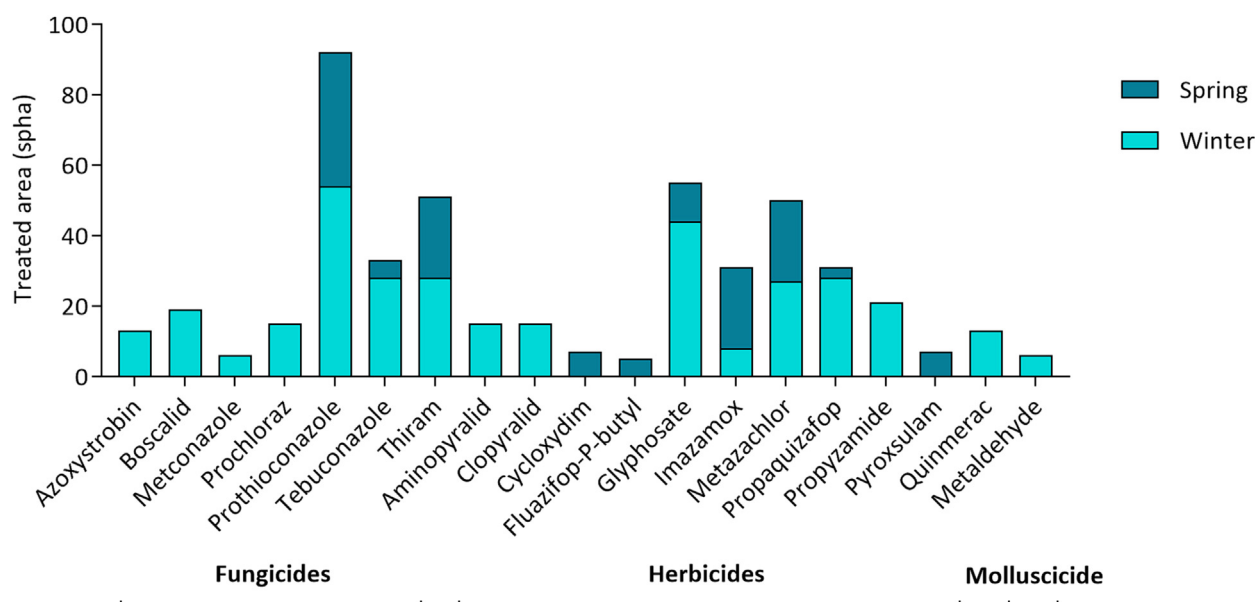


Fig. 2. Active substances applied on the spring and winter oilseed rape fields sampled, their respective pesticide category (fungicides, herbicides, or molluscicides), and their treated area (the total area treated with a pesticide, which includes all repeated pesticide applications, and it is measured in spray hectares).

3.2. Pesticide active substances occurrence on a field scale

Six out of the 11 evaluated compounds were found to be present in several of the samples analysed, even in cases where there were no reports of them having been applied on the fields (Fig. 3). Specifically, the majority of azoxystrobin and boscalid detections and all the clothianidin and thiacloprid detections came from fields in which there were no reports of their application during the year of sampling. Conversely, the compound prothioconazole was only detected in some of the fields that it was applied to. Azoxystrobin and boscalid were detected in all ten fields, clothianidin in four fields, prothioconazole and glyphosate in three, and thiacloprid only in one field. The detected combinations of compounds that co-occurred were azoxystrobin with boscalid ($n = 10$), azoxystrobin, boscalid and clothianidin ($n = 4$), azoxystrobin with boscalid and prothioconazole ($n = 3$), azoxystrobin, boscalid and glyphosate ($n = 2$), azoxystrobin with boscalid, clothianidin and prothioconazole ($n = 1$) and azoxystrobin, boscalid, glyphosate and thiacloprid ($n = 1$) (Fig. S1). In field 5 where OSR sampling was performed before and after azoxystrobin and boscalid application, both residues were detected in both sampling events, but the concentrations were higher in the post application event (Fig. 3). No temporal variation of the detected compound mixtures was observed between the SOR and WOR crops, except for thiacloprid and glyphosate that were only detected in BAB from WOR fields. The compounds acetamiprid, imidacloprid, thiamethoxam, fluroxypyr, and the main metabolite of glyphosate (AMPA), were not detected in any of the matrices evaluated in the present study. None of these four compounds were reported to have been applied in any of the ten OSR fields.

3.3. Pesticide active substances detections at individual sample scale

3.3.1. Compound and matrix specific detections

On a per sample basis, we recorded 67 pesticide detections ($29 > \text{LOQ}$). The overall most frequently detected compound was boscalid ($n = 27$ detections/4 applications), followed by azoxystrobin ($n = 23$ detections/4 applications), glyphosate ($n = 6$ detections/15 applications), prothioconazole ($n = 5$ detections/26 applications), clothianidin ($n = 5$ detections/0 applications) and thiacloprid ($n = 1$ detection/0 applications) (Fig. 3). Of the compounds applied, glyphosate was never applied during OSR flowering, but was applied as WOR desiccant when BAB plants were flowering, azoxystrobin and boscalid were applied during OSR flowering, but no BAB plants were flowering

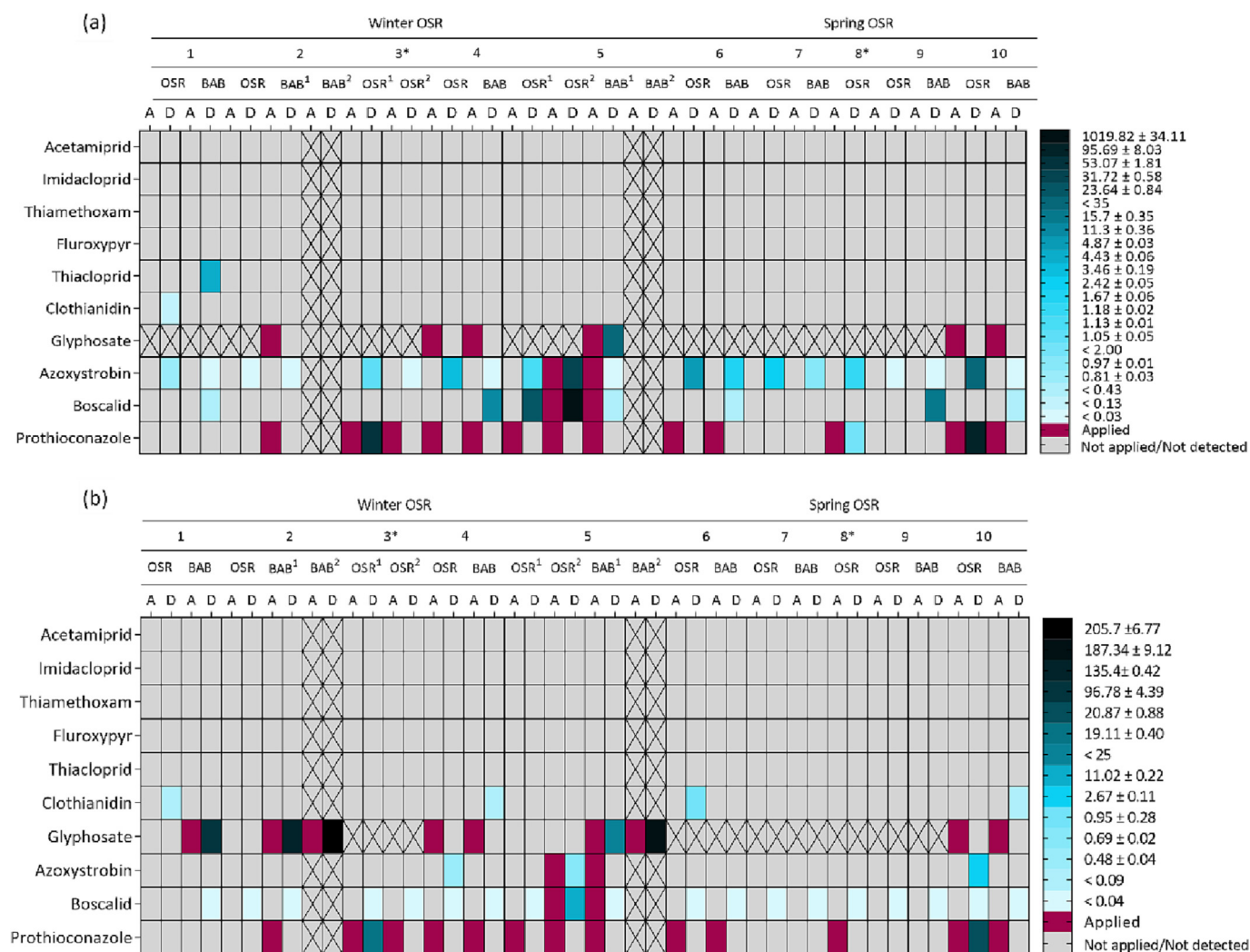


Fig. 3. Occurrence of the ten evaluated active substances in (a) pollen and (b) nectar samples. The first column under each plant species *Brassica napus* (OSR) or *Rubus fruticosus* agg. (BAB) provides information on the application of the active substance (A). If it is coloured in magenta, it means that the active substance was applied on the field where the sample was collected, while a grey cell implies no application. The second column indicates whether that active substance was detected or not in the respective sample (D). The blue-black colour scale shows each compound detection in the samples, while each grey cell indicates there was no detection. The concentrations are measured in $\mu\text{g}/\text{kg}$. The cells marked with an X refer to samples that were not evaluated for the respective compound.

¹ Refers to the first sampling event of OSR or BAB flowers on a specific field.

² Refers to the second sampling event of OSR or BAB flowers on a specific field.

*No BAB flowers were collected from fields 3 and 8. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

at the time, and prothioconazole was mainly applied when OSR was not flowering (60 % of applications). Individual compounds were almost equally detected in pollen and nectar ($n = 34 > n = 33$). OSR pollen and nectar had the same number of detections ($n = 18$), followed by BAB pollen ($n = 16$) and BAB nectar ($n = 15$) (Fig. S2a). The most common compounds detected in pollen were azoxystrobin ($n = 20$) and boscalid ($n = 8$), and for nectar, boscalid ($n = 19$) and glyphosate ($n = 5$), though prothioconazole was most frequently applied. Fungicides were the most common compounds detected in all matrices. Azoxystrobin was more frequently detected in pollen samples ($n = 20$), and it was not detected in any of the BAB nectar samples. Conversely, boscalid was more frequently detected in nectar ($n = 19$) than in pollen samples ($n = 8$). Both compounds were equally detected in BAB flowers from OSR and WOR fields, but there were more detections in WOR flowers. Both compounds were detected more frequently than they were applied. Glyphosate was mainly detected in BAB nectar (only one detection in BAB pollen), while it was not detected in OSR samples. Prothioconazole was detected equally in both WOR and OSR nectar and pollen but was not found in any of the BAB samples, and in

all matrices was not detected in most sites where it was applied. Although neonicotinoids were not applied, clothianidin was detected, mainly in nectar of both crop and wild species and was also found in WOR pollen. Thiacloprid was only detected once, in BAB pollen collected from a WOR field.

3.3.2. Concentration specific contaminations

The number of detections and median concentrations of residues were higher in WOR than OSR flower pollen and nectar (Fig. S2a, b), which corresponded with the number of applications of pesticides being higher in WOR than OSR. However, no statistically significant differences were observed between the concentrations of residues in OSR and WOR (M-W test, $U = 468$, $p = 0.345$). The overall concentrations of residues were significantly higher in OSR pollen than OSR nectar (M-W test, $U = 94$, $p = 0.29$) and BAB pollen residues (M-W test, $U = 86$, $p = 0.04$) (Fig. 4). Boscalid had the highest residue recorded and the highest variability in concentrations, ranging from 0.02 to 1020 $\mu\text{g}/\text{kg}$, followed by azoxystrobin with a range of residues from 0.02 to 31.7 $\mu\text{g}/\text{kg}$ (Table 2). The concentrations of azoxystrobin were similarly highly variable in the

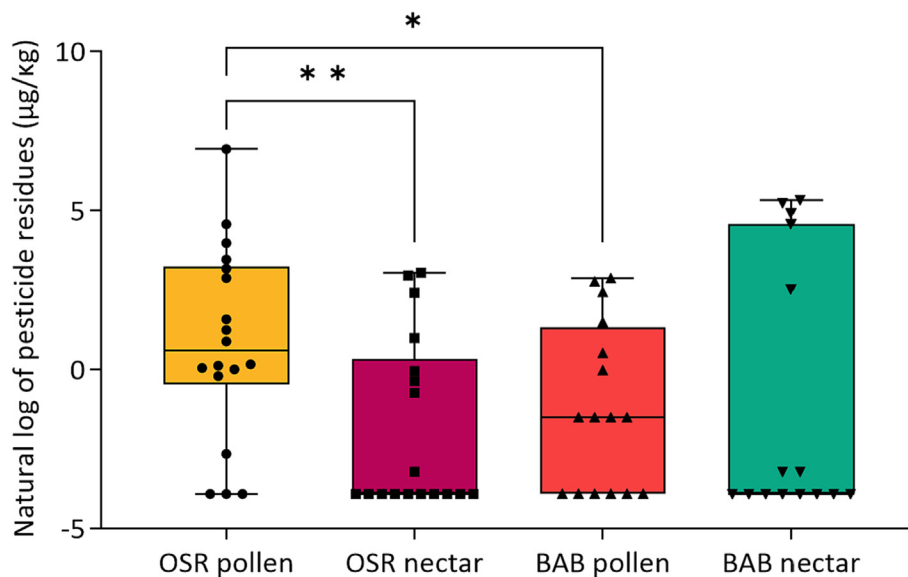


Fig. 4. Concentration levels of pesticide residues detected in oilseed rape (OSR) and *Rubus fruticosus* (BAB) in the four different matrices. The line inside the box indicates the median value, the whiskers represent the maximum and minimum values, and each point on the graph represents a pesticide detection. The simple asterisk indicates a p value of 0.043 and the double asterisks imply a more significant p value of 0.007.

matrices they were detected in, and the residue levels in OSR pollen were significantly higher than in OSR nectar and BAB nectar (M-W test, $U = 8$, $p = 0.009$; $U = 0$, $p < 0.001$ respectively) and the concentrations in BAB pollen were significantly higher to those in BAB nectar (M-W test, $U = 0$, $p < 0.001$). Boscalid residues were highly variable in both BAB and OSR pollen, and significant differences were observed between OSR nectar and OSR pollen, OSR pollen and BAB pollen, OSR pollen and BAB nectar and OSR nectar and BAB pollen residues (M-W test, $U = 11.5$, $p = 0.01$; $U = 15$, $p = 0.04$; $U = 8$, $p = 0.001$; $U = 15$, $p = 0.04$). No concentration differences between the samples were observed in the residues of clothianidin and prothioconazole in any of the matrices (Table S5). There was a strong positive correlation between the overall azoxystrobin residues detected in pollen and those detected in nectar ($\beta = 3.332 \pm 0.965$, $p = 0.00055$) and negative correlations were observed for the overall clothianidin residues ($\beta = -4.945 \pm$

1.184 , $p = 0.00003$) and glyphosate residues in BAB flowers ($\beta = -4.07611 \pm 1.936$, $p = 0.03529$) (Fig. 5).

3.3.3. Temporal distribution of glyphosate, prothioconazole, azoxystrobin and boscalid

In fields 2 and 5, glyphosate was detected in BAB nectar samples when sampling was performed three and five, and two and seven days after its application on the crop respectively. In the second sampling event of both fields, the concentrations were higher than the first sampling (Fig. 3b). In field 3, where WOR sampling was performed seven and 16 days after prothioconazole application, no residues of the compound were detected in the second sampling (Fig. 3). In field 5, when sampling of WOR was performed prior to azoxystrobin application, $1.13 \mu\text{g/kg}$ were detected in pollen and no detections was observed in nectar, but when sampling was performed 20 days after the application, the concentrations were

Table 2

Frequency of detection, range, mean and median values of the pesticide residues detected in *Brassica napus* (OSR) and *Rubus fruticosus* agg. (BAB) pollen and nectar.

Matrix	Number of samples evaluated		Insecticides		Fungicides		Herbicides	
	Insecticides and Fungicides	Glyphosate	Clothianidin	Thiacloprid	Azoxystrobin	Boscalid	Prothioconazole	Glyphosate
OSR pollen	12	2	Frequency of detection (%)	8.3	100.0	16.7	25	
			Frequency of detection >LOQ (%)	0	66.7	100	66.7	
			Range (ng/g)	< 0.13	< 0.03–31.72	23.64–1019.82	< 2.00–95.69	ND
			Mean $\pm$ SD (ng/g)	–	4.94 ± 9.32	521.73 ± 704.41	49.92 ± 47.42	
			Median (ng/g)	–	1.13	521.73	53.07	
OSR nectar	12	6	Frequency of detection (%)	16.7	25	91.7	16.7	
			Frequency of detection >LOQ (%)	50	100	9.1	100	
			Range (ng/g)	< 0.09–0.95	ND	0.48–2.67	< 0.04–11.02	ND
			Mean $\pm$ SD (ng/g)	0.5 ± 0.64	1.28 ± 1.21	0.94 ± 3.17	19.99 ± 1.24	
			Median (ng/g)	0.5	0.69	0.02	19.99	
BAB pollen	8	4	Frequency of detection (%)		12.5	100.0	75.0	25
			Frequency of detection >LOQ (%)		100	25.0	33.3	0
			Range (ng/g)	ND	4.43	< 0.03–1.67	< 0.43–15.7	ND
			Mean $\pm$ SD (ng/g)		4.43 ± 0.06	0.34 ± 0.63	4.65 ± 7.00	–
			Median (ng/g)		4.43	0.02	0.22	–
BAB nectar	8	7	Frequency of detection (%)	25.0		100		71.4
			Frequency of detection >LOQ (%)	0		0		80
			Range (ng/g)	< 0.09	ND	ND	< 0.04	ND
			Mean $\pm$ SD (ng/g)	–		–		127.54 ± 77.34
			Median (ng/g)	–		–		135.41

^a The ND value implies that the certain pesticide compound was not detected (< LOD) in the respective matrix, in any of the samples evaluated.

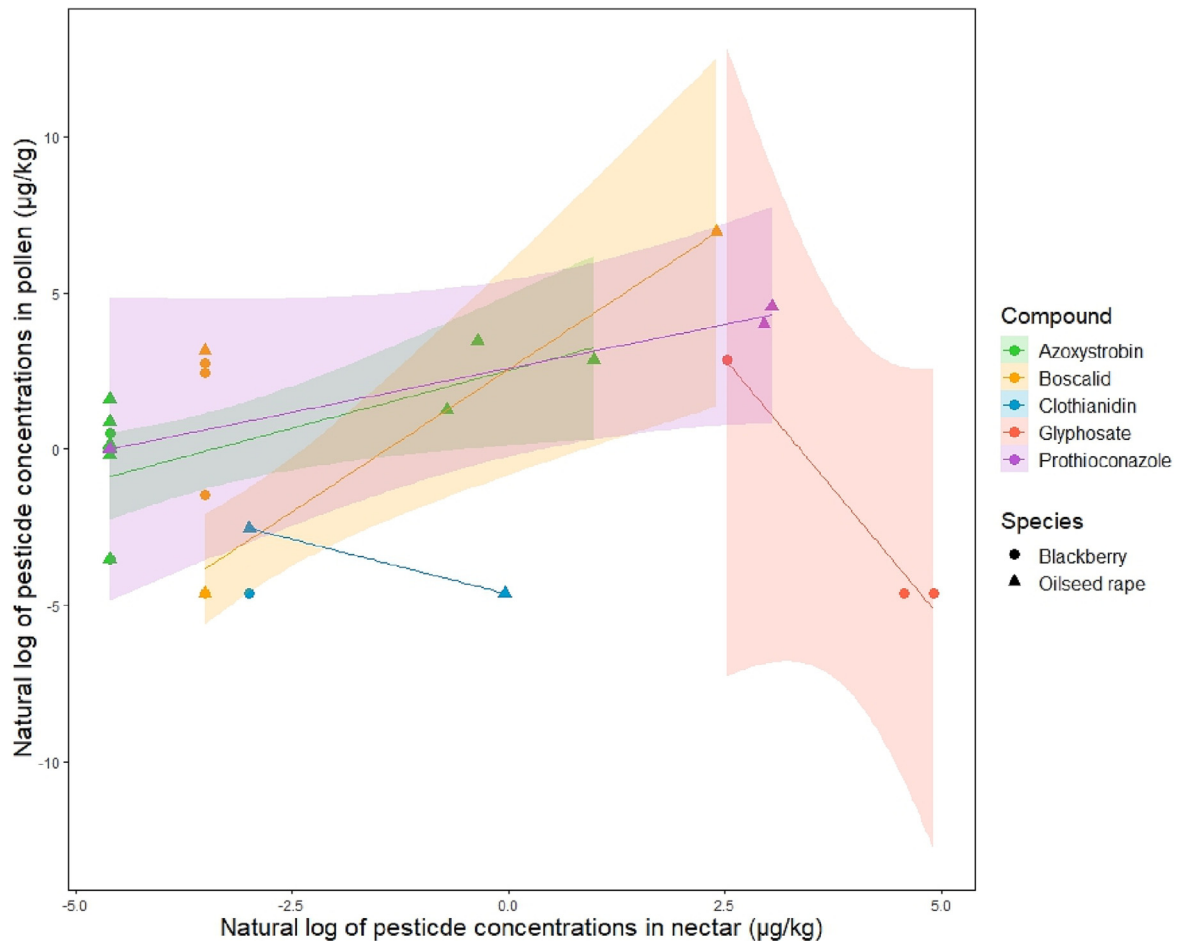


Fig. 5. Relationship between the pesticide residues detected in nectar and pollen. Points show data from fields where both nectar and pollen were measured concurrently. Pesticide detections in the two plant species are represented by a circle for *Rubus fruticosus* agg. (blackberry) and by a triangle for *Brassica napus* (oilseed rape). Colours indicate the pesticide compounds evaluated. Fitted lines show the relationship between pollen and nectar residues from the Generalized Linear Mixed Model. Shaded areas indicate the 95 % confidence intervals on fitted lines.

31.72 µg/kg in pollen and 0.69 µg/kg in nectar. In the same field, boscalid was detected in WOR nectar (<LOQ) and pollen (23.64 µg/kg) prior to the product application, and 11.02 µg/kg and 1019.82 µg/kg seven days after the pesticide application (Fig. 3).

3.4. Risk quotients

We used the maximum residues detected in plant pollen and nectar to calculate the acute and the mean residues for the chronic Risk Quotient calculation for honey bees (*Apis mellifera*) - we were limited to this species due to lack of data for LD₅₀s for other bee species (Table S6). Based on our results no pesticide compound was detected above the level of concern for causing acute or chronic toxicity to honey bees. By plotting the acute and chronic RQs for each plant species (Fig. S3), higher mean RQ values were observed for residues detected in OSR.

4. Discussion

Several detections of selected pesticide compounds were recorded in plant pollen and nectar of both crops and non-target wild plants in agricultural fields in Ireland. Evaluating the application timing, behavior, and chemical characteristics of each compound separately we identified different detection patterns. The most common compound combination was between the pesticide categories of fungicides and insecticides.

4.1. Pesticide applications and detections in an Irish context

When considering the treated area (i.e., the total area treated with a pesticide, which includes all repeated pesticide applications, and it is measured in spray hectares, see López-Ballesteros et al., 2021), the number of applications in each field, and the number of all individual pesticide compounds applied in OSR fields in the present study, herbicides were more widely applied than fungicides, which is in accordance with the results of the Irish Arable Crops Survey Report (DAFM, 2016). However, no insecticides were used on the OSR crops, and the fungicide prothioconazole was more applied than the herbicide glyphosate. It was also observed that some compounds applied in WOR were not applied in OSR and vice versa. During spring time (for Northern hemisphere, from March to May), pesticide applications are typically highest, so bees are expected to be more exposed to these contaminants in this season (Murcia-Morales et al., 2021; Tong et al., 2018). Indeed, our findings show greater number and concentrations of pesticides residues in WOR (flowering in early spring during middle spring months) rather than OSR (flowering in early to middle summer months), which can be justified by the higher number of pesticide input in WOR due to its longer cropping period and is in accordance with the findings of similar studies (Raimets et al., 2020). It is likely that fluroxypyr applied on crops used in rotation with OSR on the same fields, will not be translocated in OSR or BAB plants, since it was not detected in any of our samples.

4.2. Pesticide residue detections - a global perspective

All the compounds evaluated in the present study have been previously detected in bee related matrices like honey (El Agrebi et al., 2020a; Gawel et al., 2019; Karise et al., 2017; Muli et al., 2018; Raimets et al., 2020; Thompson et al., 2019), bee bread (Bergero et al., 2021; El Agrebi et al., 2020a; Lozano et al., 2019; Simon-Delso et al., 2014) and corbicular pollen (David et al., 2016; Favaro et al., 2019; Graham et al., 2021; Raimets et al., 2020; Tosi et al., 2018). Considering that different matrices may accumulate different pesticide compounds for various reasons (Gierer et al., 2019) and since there is a need for elucidating the primary contamination source of these compounds in space and time (Graham et al., 2021), we focused our comparison on residues detected in pollen and nectar sampled directly from plants. To put the pesticide residue results of the 11 compounds evaluated in the present study into a global perspective, we collated information on previously reported detections of these compounds in plant pollen and nectar from 21 studies (Fig. S4). These are studies included in Zioga et al. (2020) review, with additional studies that were published after 2019. Studies took place from 1999 to 2018 (published from 2001 to 2021) and they originate from the Northern hemisphere, representing three continents (Asia, Europe, and North America). The compounds glyphosate, AMPA, prothioconazole, and fluroxypyr have not been previously detected in plant pollen and nectar, presumably due to their chemical properties that constitute them hard to fit in multiresidue analysis. Analytical methodologies used in various studies to date may have underestimated the pesticide exposure and presence since the detections in the various matrices are highly dependent on the number and types of compounds analysed and the sensitivity and type of the analytical method (Toselli and Sgolastra, 2020). This makes our detections of glyphosate in BAB pollen and nectar, and prothioconazole in OSR pollen and nectar, unique. On the other hand, the fungicides azoxystrobin and boscalid were previously detected only in plant pollen, whereas we detected both in pollen and nectar.

4.2.1. Fungicides

Prothioconazole residues were only detected in OSR crop pollen and nectar when the compound was applied within a seven-day period from sampling. This is in accordance with the results of Wallner (2009), where prothioconazole was detected in honey bee collected nectar in decreasing concentrations until it was not detected seven days after its application to the OSR crop. No prothioconazole residues were detected in BAB pollen or nectar, in OSR crops when the compound was not applied, or when it was applied >16 days prior to sampling (80 % of the sprayed fields). Based on our results, and due to its low solubility in water, prothioconazole is not likely to translocate from soil to pollen and nectar (Long and Krupke, 2016).

Widespread detections of azoxystrobin and boscalid were observed not only in pollen, but also in nectar from both crop and non-target plants. Azoxystrobin concentrations in our study were higher in OSR than in BAB plants, and much lower than the respective concentrations detected in the United States (Bloom et al., 2021). The latter could be attributed to the different crops evaluated (Brassicaceae versus Cucurbitaceae) and/or different application characteristics (e.g., application rate, timing in relation to flowering etc.) (Gierer et al., 2019), which are frequently missing from pesticide residue studies for bee related matrices (Zioga et al., 2020). Most observations of azoxystrobin were recorded in pollen matrices, whereas the opposite scenario applies for boscalid. It is suggested that hydrophobic interactions between the pesticide and lipophilic sites of pollen are responsible for the sorption process (Campos et al., 2008). The octanol-water partition coefficients for these compounds are similar (2.50 and 2.96 respectively), implying similar lipophilicity for both compounds (PPDB, 2022). To predict pesticide mobility in plants, we need to also consider the dissociation constant of the compounds (pKa), which is higher in boscalid (14.3) than azoxystrobin (1.9) (PPDB, 2022). Decreasing pKa is associated with increase in plant systemicity. Hence, according to the Bromilow and Chamberlain (1991) model for the prediction of pesticide mobility in plants, azoxystrobin appears to be more mobile than boscalid.

However, more research is required to investigate potential relationships between the physicochemical characteristics of an active substance to the translocation to pollen or nectar and to the fate and dissipation after the application (Crenna et al., 2020; Gierer et al., 2019). It can also be hypothesised that boscalid dissipates faster in pollen than in nectar (Choudhary and Sharma, 2008), and/or the fact that with our analytical method, boscalid detection limit was lower for nectar than for pollen. Pollen residue concentrations of boscalid in our study were much higher than those reported from Canada and United Kingdom (David et al., 2015, 2016; Willis Chan et al., 2019). This could be attributed to the fact that our sampling took place relatively close to the application time of the compound.

Both azoxystrobin and boscalid were also detected in OSR and BAB pollen and nectar of fields where they were not intentionally applied, most likely due to translocation from soil residues from past pesticide applications (Riedo et al., 2022). The persistence in soil of these compounds (DT₅₀ 8.6 and 10.3 months respectively) (PPDB, 2022) could explain the low concentrations detected ($\leq$ LOQ). However, higher concentrations were observed especially after another fungicide was applied on the same fields. Upon discussion with the farmers, it was noticed that it was a common practice to not wash the tank in between applications of products belonging to the same pesticide category (e.g., fungicides). This could partially explain their detections in fields where they were not intentionally applied.

A positive relationship was observed between the fungicide residues detected in pollen and nectar, especially for azoxystrobin residues and this in line with previous findings (Botfás et al., 2015; Kyriakopoulou et al., 2017; Zioga et al., 2020).

4.2.2. Glyphosate and AMPA

In our study, glyphosate was applied as desiccant on the OSR crops and detected in the non-target BAB pollen and nectar, but not in OSR crops. The off-target contamination of flower tissues with high concentrations of glyphosate (up to 1.25 mg/kg) has been previously reported (Cebotari et al., 2018), and should not be overlooked. We detected glyphosate in higher concentrations in plant nectar than in pollen as long as seven days after its application, which is contradictory to the results of Thompson et al. (2014) where pollen and nectar collected by honey bees foraging on directly sprayed plants was evaluated, and concentrations in pollen were 10 times higher than in nectar. The difference in the pattern of the detected concentrations could be a result of the different matrix evaluated, the different plant structure (Gierer et al., 2019), or the different contamination pathway (direct spray versus spray drift or soil translocation) (Zioga et al., 2022). Future work should include a temporal investigation of the pesticide's behavior over multiple days to elucidate the number of days it is detected in plant pollen and nectar and how the concentrations behave over time (Zioga et al., 2022). The metabolite AMPA was not detected in any of the evaluated samples in our study.

4.2.3. Neonicotinoids

Various neonicotinoid residues have been recorded in the literature over the years. Neonicotinoid insecticides are still legally applied globally, but the European Union has taken measures that altered the application status of these compounds over the last decade: in 2013 the use of clothianidin, imidacloprid and thiamethoxam as seed treatments was restricted (European Commission, 2013), in 2018 their outdoor use was banned (EFSA, 2018a,b,c), and their approvals expired on 31 January 2019, 30 April 2019 and 1 December 2020. Thiacloprid's approval was withdrawn on 3 February 2020, while acetamiprid is still approved for use (European Commission, 2022). Recently, the European Court of Justice confirmed that Member States will no longer be allowed to grant neonicotinoid derogations (ECJ, 2023).

There are several reported neonicotinoid detections in bee related matrices (El Agrebi et al., 2020b; Erban et al., 2019; Favaro et al., 2019; Friedle et al., 2021; Gawel et al., 2019; Kaila et al., 2022; Šlachta et al., 2020; Végh et al., 2022). However, there is scarce information on studies

showing the neonicotinoid contamination of bee related matrices after the European Commission regulation amendments for those compounds (e.g., see Odemer et al., 2023; Thompson et al., 2022), and no study has evaluated pollen and nectar from both crop and non-target plants under the same agricultural conditions, several years after neonicotinoid application. In our study, only the compounds clothianidin and thiacloprid were detected, even though there were no reports for neonicotinoid application on the respective fields for at least three years prior to 2018 for most of the fields. Clothianidin, a metabolite of thiamethoxam, is a very persistent compound with a half-life that can last up to ~3 years and that could explain its presence in our samples (PPDB, 2022). Clothianidin was mainly detected in OSR rather than BAB plants, a pattern that has been observed before in a similar study (Botías et al., 2016). The residues of clothianidin detected in the present study were lower than those reported for oilseed rape in similar studies (e.g., Botías et al., 2015; David et al., 2016) and within the range of those detected in the study of Thompson et al. (2022), where residues were detected in pollen and nectar of succeeding crops, a year after planting seed-treated sugar beet. Clothianidin was mainly detected in nectar, and we observed a strong negative correlation between nectar and pollen residues. This is in contrast with the results of Zioga et al. (2020), who found a positive relationship. This could be attributed to the fact that during the studies included in the 2020 review there was a more recent application of clothianidin on the fields, as opposed to our study, where clothianidin has not been applied for years. More research could elucidate whether clothianidin residues tend to appear more in nectar than pollen. The concentrations of clothianidin detected in both pollen and nectar in our study were much lower than those reported for Europe in previous studies (Botías et al., 2015; David et al., 2015, 2016; Thompson et al., 2016; Pohorecka et al., 2012), and both our maximum and mean concentrations of clothianidin detected did not pose a hazard for bees based on the RQ estimation. Thiacloprid was detected in BAB pollen in only one field at a concentration similar to the average European detections. Thiacloprid was still legally applied during the sampling period, hence the recorded detection could be a result of spray drift or run-off from adjacent fields.

4.3. Compound mixtures

In our study, the detection of several compound mixtures in the same matrix and the same field was more the rule rather than the exception. This is not surprising as multiple pesticide detections in floral pollen and nectar have been previously reported (Bloom et al., 2021; Botías et al., 2015; David et al., 2015, 2016; Pohorecka et al., 2012). However, an evaluation of the number and combinations of the compounds that were co-detected highlighted some interesting observations. A minimum of three and a maximum of four compounds contaminated the evaluated matrices, while azoxystrobin and boscalid - both fungicides, seem to be the common denominators in the field detections. Fungicides were co-detected with other fungicides (azoxystrobin, boscalid and prothioconazole), insecticides (azoxystrobin, boscalid, prothioconazole, and clothianidin), herbicides (azoxystrobin, boscalid, and glyphosate), and all three categories together (azoxystrobin, boscalid, glyphosate, and thiacloprid).

Mixtures of compounds in pollen and nectar are a potential hazard for sublethal effects (Toselli and Sgolastra, 2020; Tosi et al., 2022), and contrary to what was previously thought, recent evidence shows lethal and sublethal impacts as a result of exposure to fungicides and herbicides alone or in combinations with insecticides for various bee species (Belsky and Joshi, 2020; Cullen et al., 2019; Tosi et al., 2022). However, it is important to account for the field relevant potential exposure concentration and concentration ratio when trying to calculate the synergistic relationship between pesticide compounds belonging to various categories (Belden, 2022; Belden and Brain, 2018). In our study, most pesticide detections occurred in sublethal concentrations, and only one pesticide detection reached the mg/kg level (boscalid).

More specifically, of the compounds detected in the present study, glyphosate was found to frequently cause sublethal effects at

concentrations on a mg/kg level (Cullen et al., 2023; Tosi et al., 2022), while chronic exposure to glyphosate concentrations alone (below the spectrum of those we detected) or in combination with neonicotinoid and fungicide compounds may induce high toxicity in winter honey bees (Almasri et al., 2020). Field realistic levels of boscalid have been shown to interact with neonicotinoid insecticides such as thiamethoxam and clothianidin, increasing their toxicity to honey bees (Peghaire et al., 2020; Tsvetkov et al., 2017). Its presence was also linked with weak honey bee colonies (Simon-Delso et al., 2014) and consumption of its commercial formulation can lead to chronic and cumulative toxicity in adult honey bees (Simon-Delso et al., 2018). Thus, the co-occurrence of boscalid with several other active substances requires further investigation. Moreover, since the presence of azole fungicides is known to have synergistic activity with neonicotinoid insecticides (Belden, 2022), we would recommend evaluating whether the relative ratio of prothioconazole and clothianidin residues and the absolute residue of clothianidin are likely to be of concern. Azoxystrobin can act as synergist with other pesticide compounds (Van Dyke et al., 2018), and alone has shown weak toxicity to honey bees (Barascou et al., 2022), induced gene transcriptional alterations (Christen et al., 2019) and histopathological and cytotoxic changes in the midgut of honey bees (Serra et al., 2023), at concentrations higher to those detected in the present study.

Even without potential synergistic effects with other pesticides, many studies report lethal and sublethal effects of clothianidin to various bee species and life stages (Charvet et al., 2004; Colgan et al., 2019; López et al., 2017; Williams et al., 2015; Wood et al., 2018), in some cases at concentrations similar to those detected in our study (e.g., 0.96 µg/kg). In our study we found several clothianidin detections, the application of which dates at least three years back in time. Since various bee species can react differently to exposure to clothianidin (Rundlöf et al., 2015), clothianidin and its co-occurred compounds should be monitored and assessed for both lethal and sublethal effects on various bee species and not just honey bees. A similar spectrum of impacts has also been reported for thiacloprid. By itself, it induces behavioral sublethal effects in bees affecting foraging, homing success and navigation (Fent et al., 2020; Tison et al., 2016), causes adverse effects to their immune system (Brandt et al., 2016) and impacts colony development of bumble bees under field conditions (Ellis et al., 2017), at much higher concentrations than those we detected. It also shows synergistic activity when co-detected with azole fungicides (Johnson, 2015; Iwasa et al., 2004; Schmuck et al., 2003; Wernecke et al., 2019), and exhibits sublethal effects (Vanderhaegen, 2017). Even though it is not applied anymore in a European context, it is still in use in other parts of the world, hence its co-occurrences with other pesticides in those areas should be further evaluated.

5. Conclusions

In our study we found several pesticide compound detections in the main source of pollinators' food: plant pollen and nectar, though these detections did not correspond with the pesticides recently applied in most cases. Residues were detected not only in matrices of the OSR crops, but also in nearby growing non-target wild plants. Higher concentrations were observed in the OSR crops for all compounds except for glyphosate and thiacloprid that were only detected in BAB pollen and nectar. We show that during springtime - a crucial time of the year for bees, the latter can be exposed to high concentrations and a variety of chemical compounds through their food. The RQ thresholds for honey bees were not exceeded for any of the compounds evaluated in the present study, however there are currently insufficient data on toxicological endpoints for wild bees and for some compounds (e.g., azoxystrobin). The compounds azoxystrobin, boscalid and clothianidin co-occurred in several fields, illustrating that in field conditions bees can be simultaneously exposed to combinations of pesticides from different chemical groups that are known to act synergistically, with negative impact on bee' health. However, fluroxypyr applied in rotation crops was not detected and prothioconazole seems to

not present a chronic toxicity concern. The persistence of the neonicotinoid insecticide clothianidin in plant pollen and nectar several years after its outdoor ban in Europe, and since its actual application on the fields, is of concern, and suggests that continuous monitoring of neonicotinoid presence and fate in the field environment is warranted.

CRedit authorship contribution statement

Elena Zioga: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - Original draft preparation. **Blánaid White:** Conceptualization, Methodology, Supervision, Writing - Reviewing and Editing, Validation, Resources, Funding acquisition. **Jane Stout:** Conceptualization, Methodology, Supervision, Writing - Reviewing and Editing, Validation, Resources, Funding acquisition.

Data availability

All necessary data is provided in Appendix A.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.162971>.

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