Report on exposure of bees to agrochemicals

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PoshBee

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

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Summary

Honey bees and their products can be used to monitor the quality of the environment, because their foraging activity brings them in contact with various environmental compartments. Residues of active ingredients used for plant protection can be detected by analysing bee bodies and can therefore provide insights into the extent that these products can affect pollinators and living organisms in general. Until now, honey bees have been the most investigated insect, but recently attention has started to focus also on other pollinators.

In the POSHBEE project honey bees, bumble bees and mason bees, the latter as representatives of solitary bees, were placed in apple orchards and oilseed rape fields characterised by different levels of agricultural intensity, and samples of adult bees were collected during the blossoming of the crops, to understand whether and to what extent bees could become contaminated during their foraging activities in such conditions. A site network based in apple orchards and oilseed rape fields was set up in 8 European countries (Germany, Great Britain, Estonia, Ireland, Italy, Spain, Sweden, Switzerland). In each site, 3 honey bee hives, 3 bumble bee colonies and mason bee nests were deployed in 16 sites per country (8 in apple orchards and 8 in oilseed rape fields). Samples were collected during the flowering of the selected crops, and analysed by multiresidue analyses in a laboratory specialised in analyses of honey bee products.

Of the 128 samples of honey bees 51% were positive for at least one active ingredient (a.i.), and a total number of 27 a.i. were detected across all samples. A higher number of positive samples was found in apple orchards in all countries apart from Spain and Estonia. Apple orchards in Great Britain had the highest number of positive honey bee samples per a.i., followed by Italy, Germany and Switzerland. In Estonia, no positive samples were detected in any of the apple sites.

Of the 128 received samples of bumblebees 47% were positive for at least one a.i., and a total number of 24 a.i. were detected across all samples. Of these, the fungicides Chlorothalonil and Fenhexamid and the insecticide Phosmet were not detected in the honey bee samples. A higher number of positive samples was found in apple orchards in all countries apart from Estonia, similar to the pattern observed in honey bees. Bumble bees confirmed findings in honey bees that apple orchards in Great Britain had the highest number of positive honey bee samples per a.i., but differently from honey bees, bumble bees highlighted a high number of positive samples per a.i. in Ireland. Bumble bees mirrored honey bees with relatively high numbers of positive samples in Germany, Italy and Switzerland. In Spain, no positive samples were detected in any of the oil rape seed sites.

Mason bees were unexpectedly successful outside of the deployed nests, thus it was not possible to collect a sufficient number of them for analyses of chemical residues.

In apple orchard sites, the a.i. present in the most samples and at the highest residue level in both honey bees and bumble bees was 1,2,3,6-Tetrahydrophthalimide, a metabolite of the fungicide Captan, one of the most widely used fungicides in fruit orchards.

In the oilseed rape sites the a.i. present in the highest number of honey bee samples was Tau-Fluvalinate, while the one with the highest residue level was Boscalid, a synthetic carboxamide fungicide. Boscalid was the a.i. that was present in the highest number of bumble bee samples in the oil rape seed sites, while the one with the highest level of residues was the Captan metabolite 1,2,3,6-Tetrahydrophthalimide.

1. Introduction

Pollinators are responsible for pollinating more than 1,200 crops globally and about 75% of the leading world food crops depend on pollinators (Klein et al., 2007). Pollinators provide pollination services to over 180,000 different plant species, contributing to environmental and societal benefits, which include producing an important part of our food supply, providing food and cover for wildlife, preventing soil erosion, producing the oxygen we breathe and absorbing C02, thus counteracting global climate change (Potts et al. 2016). Bees (superfamily Apoidea) are the most effective group of pollinators, with approximately 20,000 species worldwide and 2,000 species in Europe (IPBES, 2016). The European honey bee (*Apis mellifera* L.) is the most widely managed pollinator species contributing to crop pollination (Aizen and Harder, 2009), but both managed and wild bee species contribute significantly to crop pollination globally (Garibaldi et al. 2013).

In recent years, honey bee populations have been subject to decline and beekeepers have suffered unprecedented losses (Brodschneider et al, 2016). This decline has been linked to several factors, including parasites and viral infections, monoculture farming which prevents bees from having a varied diet, and the use of pesticides which can have toxic effects on bees. There have been many studies and publications on effects of pesticides on bees (mainly on *Apis mellifera*), and results showing adverse effects have led to some pesticides being banned. Many studies were laboratory based experiments, and questions remain as to the real exposure of bees in field conditions.

Honey bees forage nectar, pollen, water, and resin from a wide area surrounding their hive. In this way, either orally, or by contact, they can absorb active ingredients present in the environment. The substances may be absorbed at sublethal levels and be brought back to the hive, where they can accumulate and cause chronic and sublethal effects (Johnson, 2015; Sanchez-Bayo & Goka, 2016).

Honey bees and their products can therefore be used to monitor the quality of the environment, but also, analyses of their bodies to detect the presence of residues of active ingredients used for plant protection can provide insights as to the extent that pollinators are exposed to, and thus may suffer effects from these products. Until now, honey bees have been the most investigated insect, but recently attention has started to focus also on other pollinators.

In the POSHBEE project honey bees, bumble bees and mason bees (as representatives of solitary bees), were placed in apple orchards and oilseed rape fields characterised by different levels of agricultural intensity. Samples of adult bees were collected during the blossoming of the crops, to understand whether the surrounding landscape could contaminate the bees during their foraging activities on these crops.

2. Methods

2.1. Field collection of samples from the site network

A site network based in apple orchards and oilseed rape fields was set up in 8 European countries (Germany, Great Britain, Estonia, Ireland, Italy, Spain, Sweden, Switzerland). In each site, 3 honey bee hives, 3 bumble bee colonies, and 3 mason bee nests (each with 100 cocoons) were deployed in 16 sites per country (8 in apple orchards and 8 in oilseed rape fields.

Samples were collected during the flowering of the selected crops, from the apiaries of the site network, as described in D1.1 Protocols for methods of field sampling (Stout & Hodge, 2020). After collection, samples were immediately frozen and kept at -20°C until analyses. The analysis which was

performed at CREA laboratory specialised in analyses of honey bees and honey bee products, accredited according to UNI CEI EN ISO/IEC 17025 norm. Samples were posted in dry ice.

2.1.1. Collection of honey bee samples

For sampling, hive entrances were closed and returning foragers collected. According to the sampling protocol, 20 bees per hive were collected and then pooled, yielding a sample per site of 60 bees.

2.1.2. Bumble bees

Entrances to colonies were closed and returning forager workers were captured using a net, tube or jar. About 10 returning foraging workers were collected per colony, yielding a site sample of 30 bumble bees.

2.1.3. Mason bees

Due to the behaviour and success of solitary bees in the wild (and thus not in the deployed nests), it was not possible to obtain sufficient numbers of mason bees to perform analyses for chemical residues.

2.2. Chemical analyses

The total number of molecules that was screened for, inclusive of isomers and metabolites, was 374. A simplified QuEChERS method was used for sample preparation, which consists of an extraction and a purification stage: in the extraction stage, $MgSO_4$ salts together with a solution of water and acetonitrile were added to the samples. The sample was then centrifuged and the supernatant was collected and purified with a PSA resin. The sample was again centrifuged, concentrated and a specific solvent added for the GC-MS/ MS (Fig. 1) or LC-MS/MS analysis (Fig. 2).

Quantification was performed by means of a calibration curve with the standards in solvent, which was conducted at each analytical cycle.

Figure 1: Gas chromatograph coupled to tandem mass spectrometer (GC-MS/MS)

Figure 2: Liquid chromatography mass spectrometer (LC-MS/MS)

3. Results

3.1. Honey bees

3.1.1. Residue type, level and rate in honey bees

All of the 128 received samples of honey bees were analysed; 51% of these were positive for at least one active ingredient (a.i.), and a total of 27 a.i. were detected across all samples. Figure 3 shows the distribution of the a.i. across the site network countries and in the two different crops. A higher number of positive samples was found in apple orchards in all countries apart from Spain and Estonia. Apple orchards in Great Britain had the highest number of positive honey bee samples per a.i., followed by Italy, Germany and Switzerland. In Estonia, no positive samples were detected in any of the apple sites.

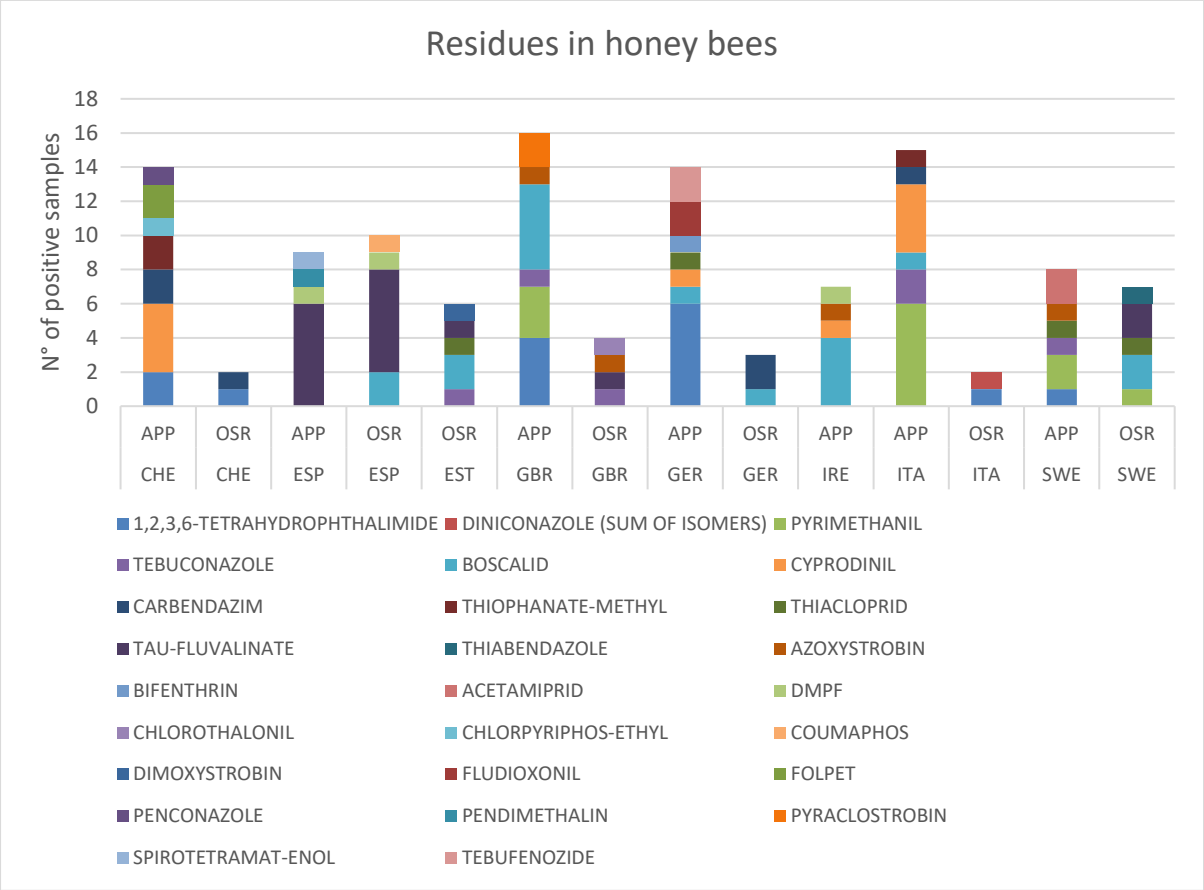

Figure 3: Active ingredients detected across the site network and number of positive samples in honey bee samples in different countries and crop types. APP = apple sites; OSR = oilseed rape sites; CHE = Switzerland; ESP = Spain; EST = Estonia; GBR = Great Britain; GER = Germany; IRE = Ireland; ITA = Italy; SWE = Sweden.

3.1.2. Residues in honey bees in apple sites

In honey bees collected in apple sites the a.i. that was present in the highest number of samples and which also had the highest level of residues was 1,2,3,6-Tetrahydrophthalimide, a metabolite of the

fungicide Captan, one of the most widely used fungicides in fruit orchards. Other a.i. detected in >10 samples were the fungicides Pyrimethanil and Boscalid.

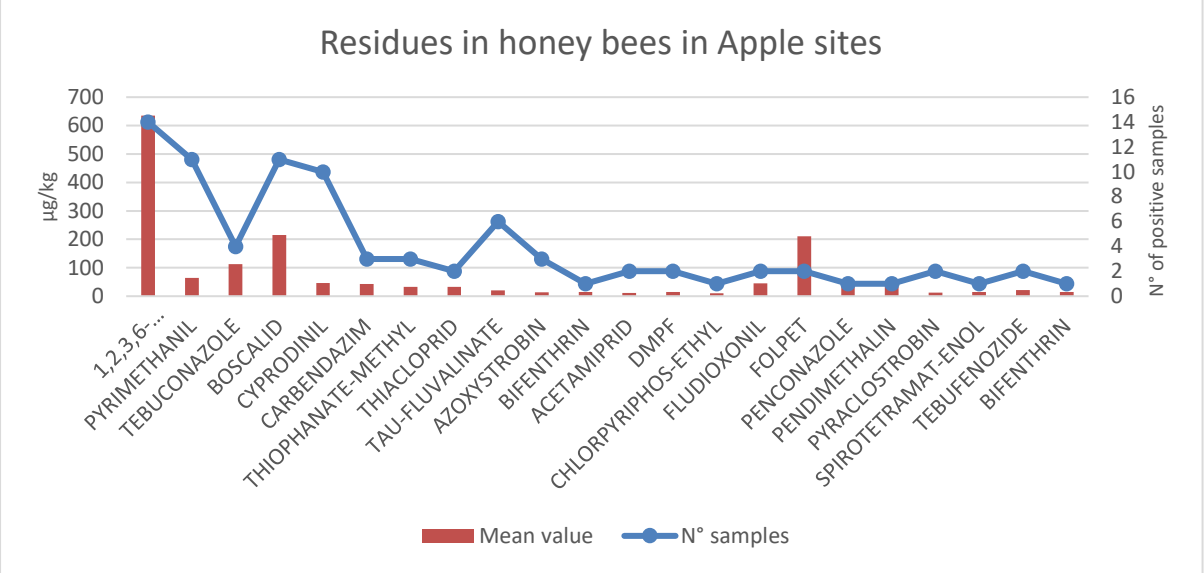

Figure 4: Mean levels of residues of the active ingredients detected in honey bee samples in the apple sites in the site network (red bars) and number of positive samples for each a.i.

Details for the different country sites are reported in Table 2, which highlights that Germany and Italy had the highest numbers of sites with positive samples. The highest numbers of detected a.i. were in Germany and Great Britain sites, where the most frequently detected a.i. was found to be 1,2,3,6-Tetrahydrophthalimide, the metabolite of the fungicide Captan. In Italy and Sweden the most frequent a.i. was the fungicide Pyrimethanil, in Switzerland the fungicide Cyprodinil, in Ireland Boscalid (again a fungicide), while in Spain the most frequent a.i. was the insecticide Tau-Fluvalinate.

Table 2: Overview of presence of residues in apple sites across the network. F = fungicide; I = insecticide.

Country	N° of sites with residues	Average N° of a.i. per site	N° total detected a.i.	Most frequent a.i.
CHE	5	2.8	7	Cyprodinil (F)
ESP	6	1.5	4	Tau-Fluvalinate (I)
EST	0	0	0	none
GBR	6	3	7	1,2,3,6-Tetrahydrophthalimide (F), Boscalid (F)
GER	7	2	7	1,2,3,6-Tetrahydrophthalimide (F)
IRE	6	1.2	4	Boscalid (F)
ITA	7	2.1	6	Pyrimethanil (F)
SWE	4	2	6	Pyrimethanil (F), Acetamiprid (I)

In Figure 5 the residue levels in honey bees from the apple network sites are shown. In all countries apart from Great Britain, residue levels were on average below 200 µg/kg. In Estonia, no positive

samples were detected in any apple site. In Sweden, only half of the sites had positive samples, and residue levels were low. The lowest average residue level was found in Spain.

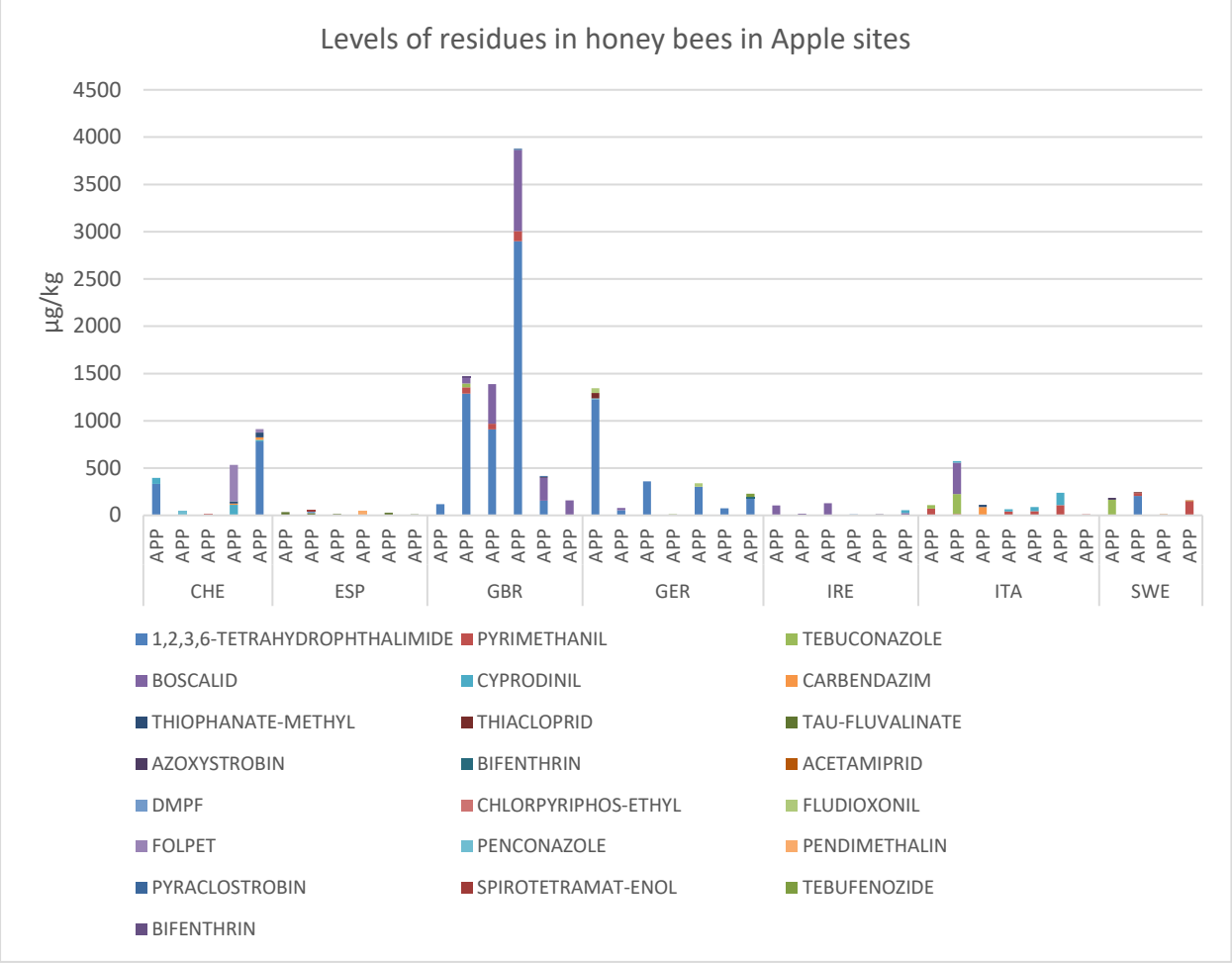

Figure 5: Levels of residues of the active ingredients detected in honey bee samples in the apple sites in the site network countries. In Estonian apple sites, no positive samples were detected. APP = apple sites; CHE = Switzerland; ESP = Spain; EST = Estonia; GBR = Great Britain; GER = Germany; IRE = Ireland; ITA = Italy; SWE = Sweden.

3.1.3. Residues in honey bees in oilseed rape sites

In honey bees collected in the oilseed rape sites the a.i. that was present in the highest number of samples was Tau-Fluvalinate, while the one with the highest level of residues was Boscalid, a synthetic carboxamide fungicide which is used as a seed treatment and for control of fungal flowering diseases (Sclerotinia, Alternaria and Botrytis) in rapeseed (Figure 6).

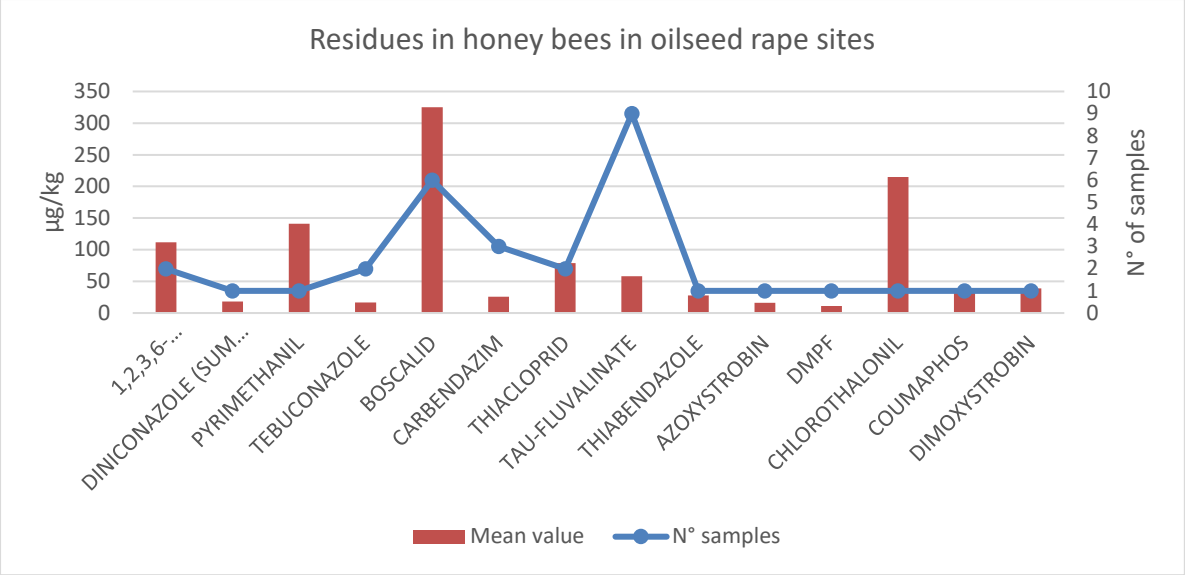

Figure 6: Mean levels of residues of the active ingredients detected in honey bee samples in the oilseed rape sites in the site network (red bars) and N° of positive samples for each a.i.

Details for the different country sites are reported in Table 3, which highlights that Spain and Sweden had the highest numbers of sites with positive samples. The highest number of detected a.i. was 5, while in Ireland no positive samples were detected.

Table 3: Overview of presence of residues in honey bees in oilseed rape sites across the network. F = fungicide; I = insecticide.

Country	N° of sites with residues	Average N° of a.i. per site	N° total detected a.i.	A.i. with highest residue level
CHE	1	2	2	1,2,3,6-Tetrahydrophthalimide (F)
ESP	7	1.4	4	Coumaphos (I)
EST	3	1.3	4	Boscalid (F)
GBR	3	1.3	4	Tau-Fluvalinate (I)
GER	3	1	2	Carbendazim (F)
IRE	0	0	0	none
ITA	2	1	2	1,2,3,6-Tetrahydrophthalimide (F)
SWE	5	1.4	5	Boscalid (F)

In Figure 7 the residue levels in honey bees from the oilseed rape network sites are shown. The a.i. with the highest residue level was Boscalid, from the Swedish sites, followed by Tau-Fluvalinate, found with highest levels (around 400 µg/kg) in the Great Britain sites. In Ireland, no positive samples were found in the oil rape seed sites.

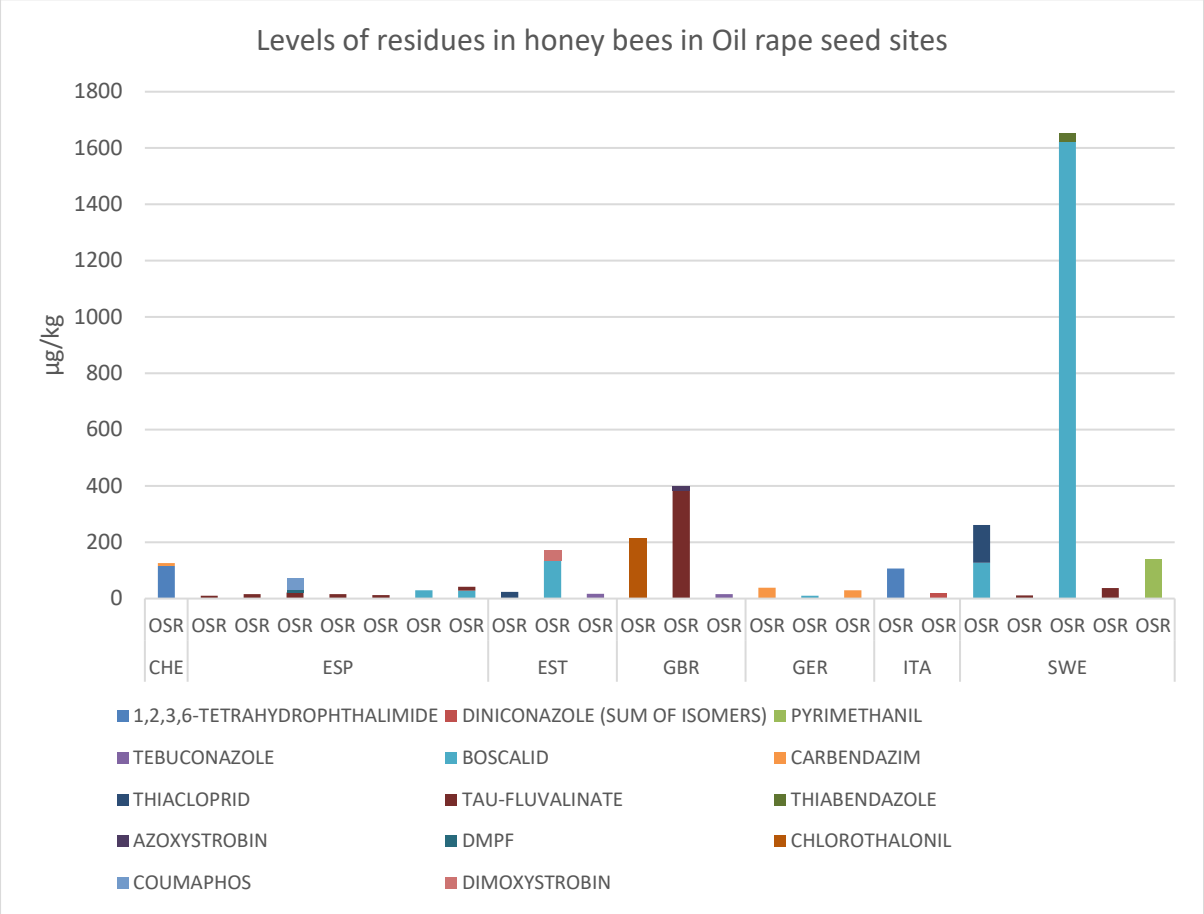

Figure 7: Levels of residues of the active ingredients detected in honey bee samples in the oilseed rape sites in the site network countries. In Irish oilseed rape sites, no positive samples were detected. OSR = oilseed rape sites; CHE = Switzerland; ESP = Spain; EST = Estonia; GBR = Great Britain; GER = Germany; IRE = Ireland; ITA = Italy; SWE = Sweden.

3.1. Bumble bees

3.1.1. Residue type, level and rate in bumble bees

All of the 128 received samples of bumble bees were analysed. 47% of these were positive for at least one a.i., and a total number of 24 a.i. were detected across all samples. Of these, the fungicides Chlorothalonil and Fenhexamid, and the insecticide Phosmet were not detected in honey bees. Figure 8 shows the distribution of the a.i. across the site network countries and in the two different crops. A higher number of positive samples was found in apple orchards in all countries apart from Estonia, similar to what was observed in honey bees. Bumble bees confirmed findings in honey bees that apple orchards in Great Britain had the highest number of positive samples per a.i., but differently from honey bees, bumble bees highlighted a high number of positive samples per a.i. in Ireland. Bumble bees confirmed the relatively high number of positive samples in Germany, Italy and Switzerland. In Spain, no positive samples were detected in any of the oilseed rape sites.

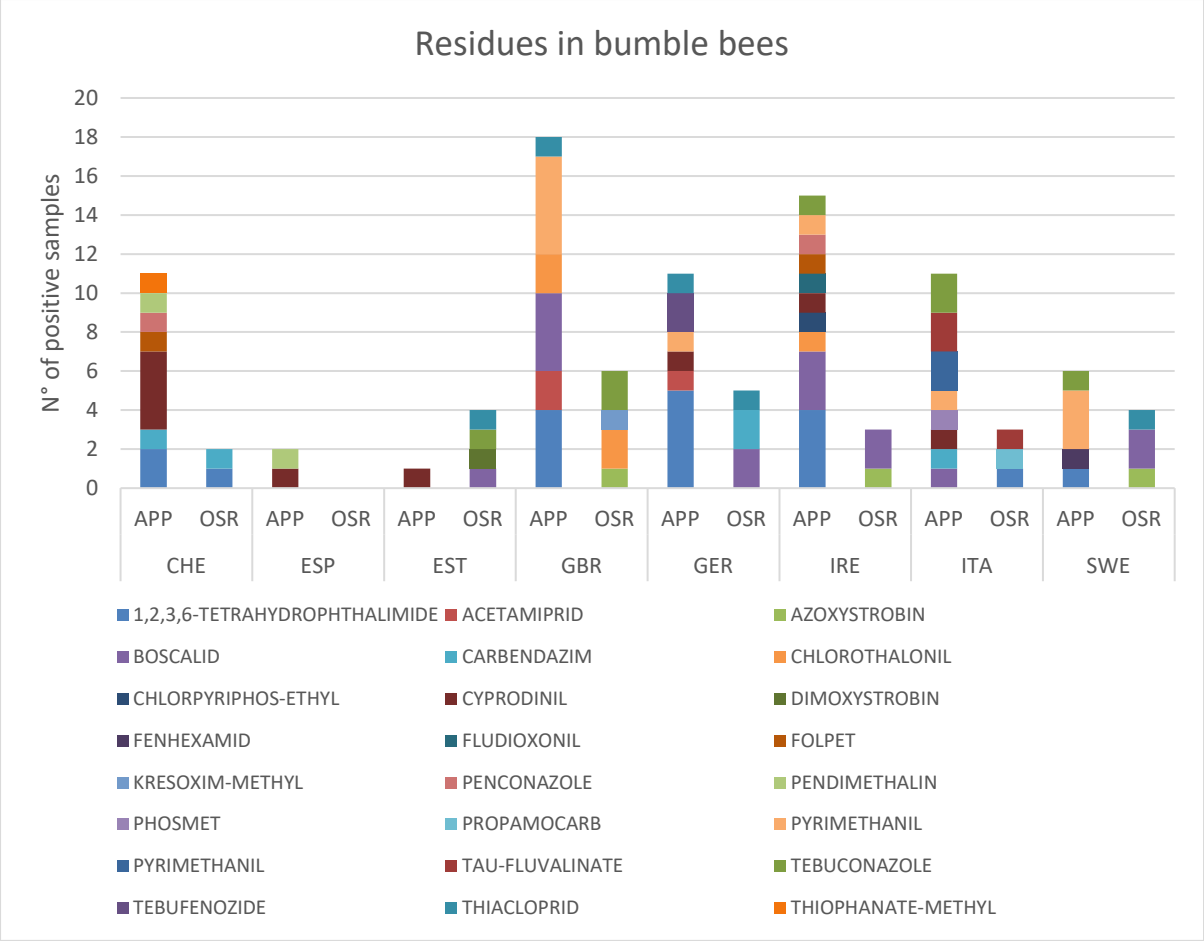

Figure 8: Active ingredients (a.i.) detected in bumble bee samples across the site network and number of positive samples per a.i. in different countries and crop type. APP = apple sites; OSR = oilseed rape sites; CHE = Switzerland; ESP = Spain; EST = Estonia; GBR = Great Britain; GER = Germany; IRE = Ireland; ITA = Italy; SWE = Sweden.

3.1.2. Residues in bumble bees in apple sites

As was observed for honey bees, in bumble bees collected in apple sites the a.i. that was present in the highest number of samples and which also had the highest level of residues was 1,2,3,6-Tetrahydrophthalimide, a metabolite of the fungicide Captan, one of the most widely used fungicides in fruit orchards. In bumble bees, other a.i. found with numbers of positive samples were the fungicides Pyrimethanil and Cyprodinil; in these cases however the residue levels were low (< 100 µg/kg).

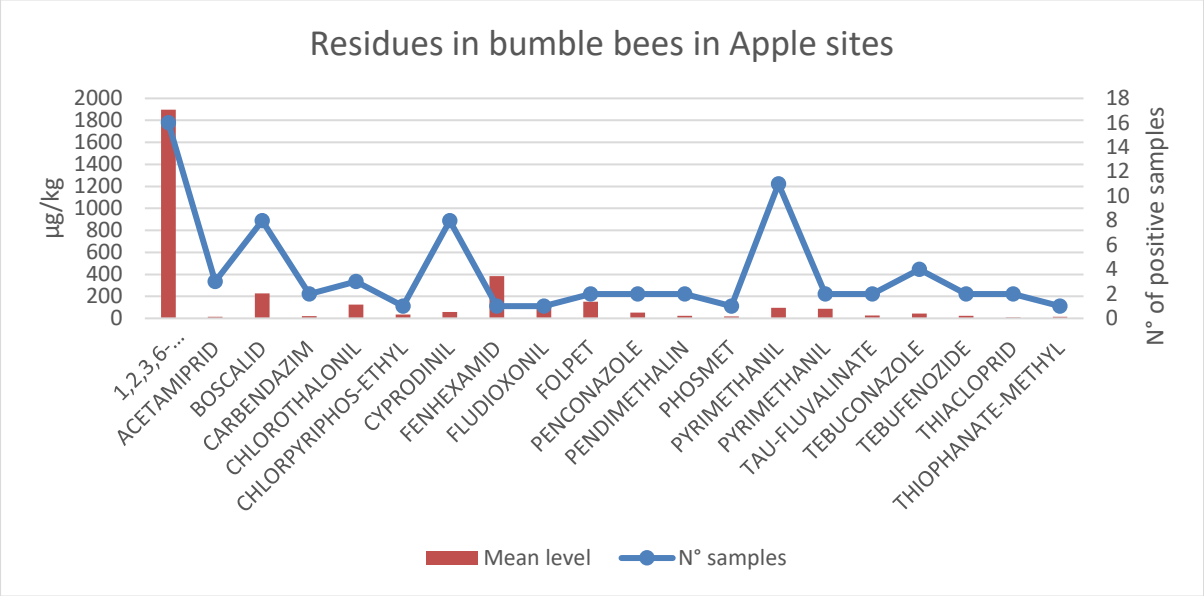

Figure 9: Mean levels of residues of the active ingredients detected in bumble bee samples in the apple sites in the site network (red bars) and number of positive samples for each a.i.

Details for the different country sites are reported in Table 4, which highlights that Ireland, followed by Germany, Great Britain and Italy had the highest numbers of sites with positive samples. Also, the highest numbers of detected a.i. were in Ireland (n=10), in contrast to what was observed in honey bees samples (n=4). The number of a.i. detected in positive samples in Germany, Great Britain and Italy sites was similar to the pattern seen in honey bees. Again, as in honey bees, in Germany and Ireland the most frequently detected a.i. was found to be 1,2,3,6-Tetrahydrophthalimide, the metabolite of the fungicide Captan. In Italy, Great Britain and Sweden the fungicide Pyrimethanil was the most frequently detected a.i., although in Italy tebuconazole and the insecticide Tau-Fluvalinate were detected at the same frequency. In Switzerland and Estonia the fungicide Cyprodinil was the most frequently detected a.i.

Table 4: Overview of presence of residues in bumble bee samples in apple sites across the network. F = fungicide; I = insecticide.

Country	N° of sites with residues	Average N° of a.i. per site	N° total detected a.i.	Most present a.i.
CHE	4	2.8	7	Cyprodinil (F)
ESP	1	1	1	Pendimethalin (F)
EST	1	1	1	Cyprodinil (F)
GBR	6	3	6	Pyrimethanil (F)
GER	6	1.8	6	1,2,3,6-Tetrahydrophthalimide (F)
IRE	7	2.1	10	1,2,3,6-Tetrahydrophthalimide (F)
ITA	6	1.8	6	Pyrimethanil (F), Tau-Fluvalinate (I), Tebuconazole (F)
SWE	5	1.2	4	Pyrimethanil (F)

In Figure 10 the residue levels in bumble bees from the apple network sites are shown. Compared to honey bees, residue levels are on average 3-fold higher in bumble bees. The a.i. with the highest residue levels in bumble bees is 1,2,3,6-Tetrahydrophthalimide (F), the metabolite of the fungicide Captan. In one Irish site, 14000 µg/kg were detected, while the highest value found in honey bees was ~2900 µg/kg. In Estonia, Italy and Spain, residue levels were on average below 100 µg/kg.

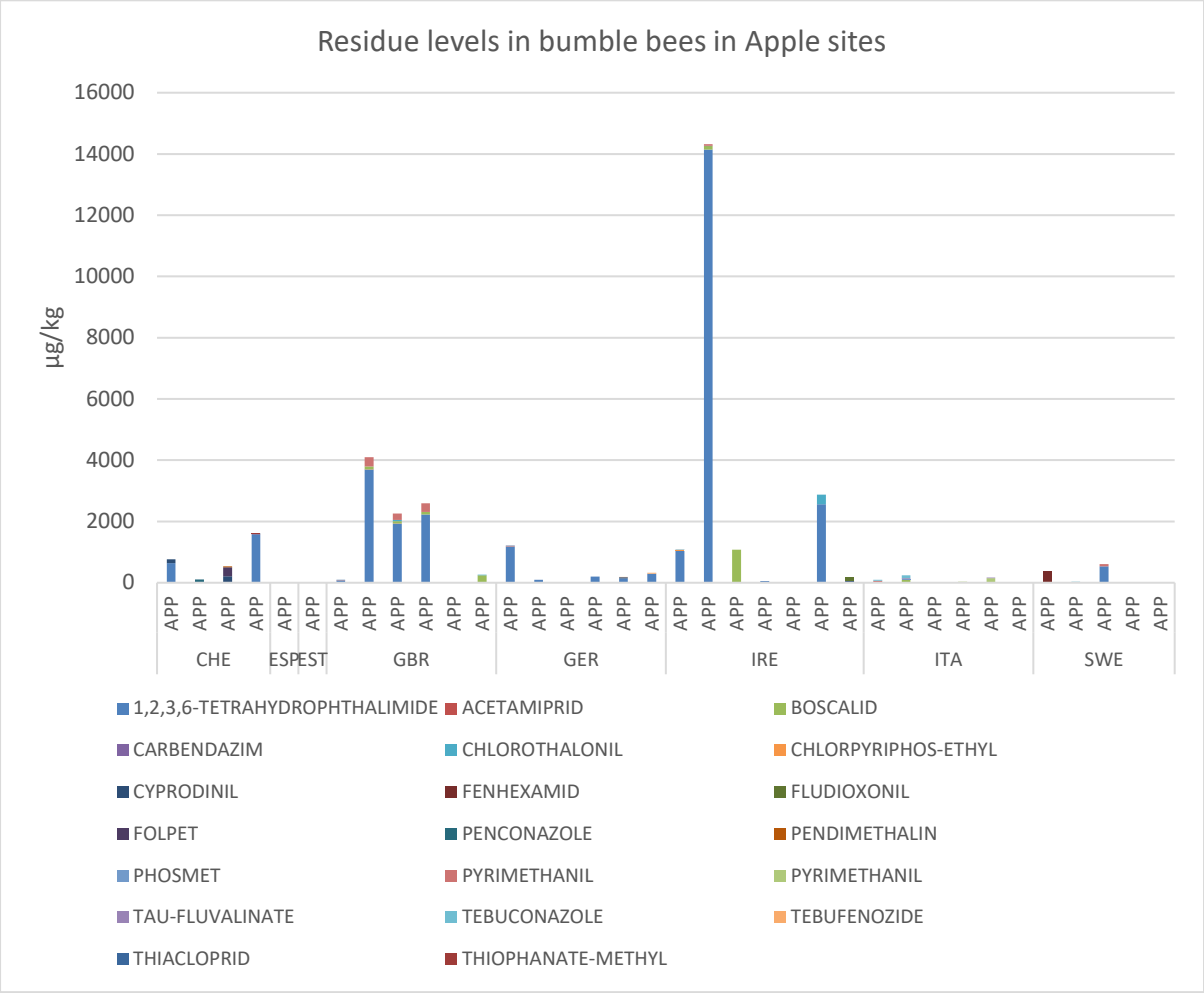

Figure 10: Levels of residues of the active ingredients detected in bumble bee samples in the apple sites in the site network countries. APP = apple sites; CHE = Switzerland; ESP = Spain; EST = Estonia; GBR = Great Britain; GER = Germany; IRE = Ireland; ITA = Italy; SWE = Sweden.

3.1.3. Residues in bumble bees in oilseed rape sites

In bumble bees collected in the oilseed rape sites the a.i. that was present in the highest number of samples was Boscalid, which in honey bees had the highest level of residues, while the one with the highest level of residues in bumble bees was the Captan metabolite 1,2,3,6-Tetrahydrophthalimide (Figure 11).

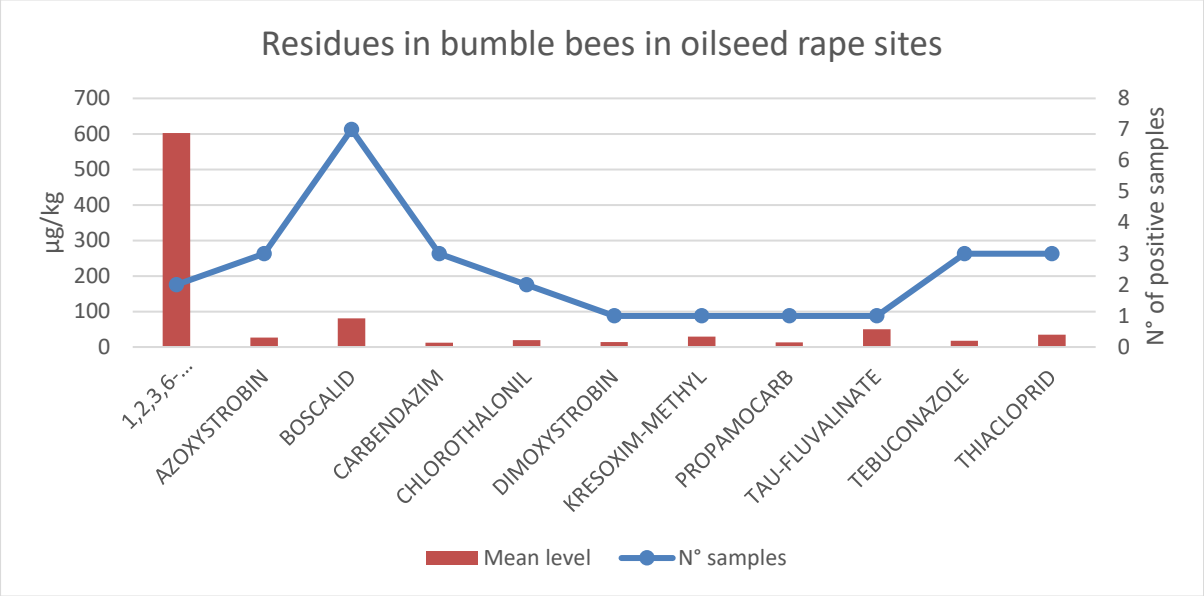

Figure 11: Mean levels of residues of the active ingredients detected in bumble bee samples from the oilseed rape sites in the site network (red bars) and number of positive samples for each a.i.

Details for the different country sites are reported in Table 5, which highlights that Great Britain and Sweden had the highest numbers of sites with positive samples. The highest number of detected a.i. was 4, in Estonia and Great Britain, while in Spain no positive samples were detected.

Table 5: Overview of presence of residues in bumble bee samples in Oil rape seed sites across the network. F = fungicide; I = insecticide.

Country	N° of sites with residues	Average N° of a.i. per site	N° total detected a.i.	A.i. with highest residue level
CHE	2	1	2	1,2,3,6-Tetrahydrophthalimide (F)
ESP	0			
EST	2	2	4	Boscalid (F)
GBR	5	1.2	4	Tau-Fluvalinate (I)
GER	4	1.2	3	Boscalid (F)
IRE	3	1	2	none
ITA	2	1.5	3	1,2,3,6-Tetrahydrophthalimide (F)
SWE	5	1.5	2	Boscalid (F)

In Figure 12 the residue levels in bumble bees from the oilseed rape network sites are shown. The a.i. with the highest residue level was 1,2,3,6-Tetrahydrophthalimide in one Italian site, much higher than levels found in honey bees, followed by Boscalid in one Swedish site.

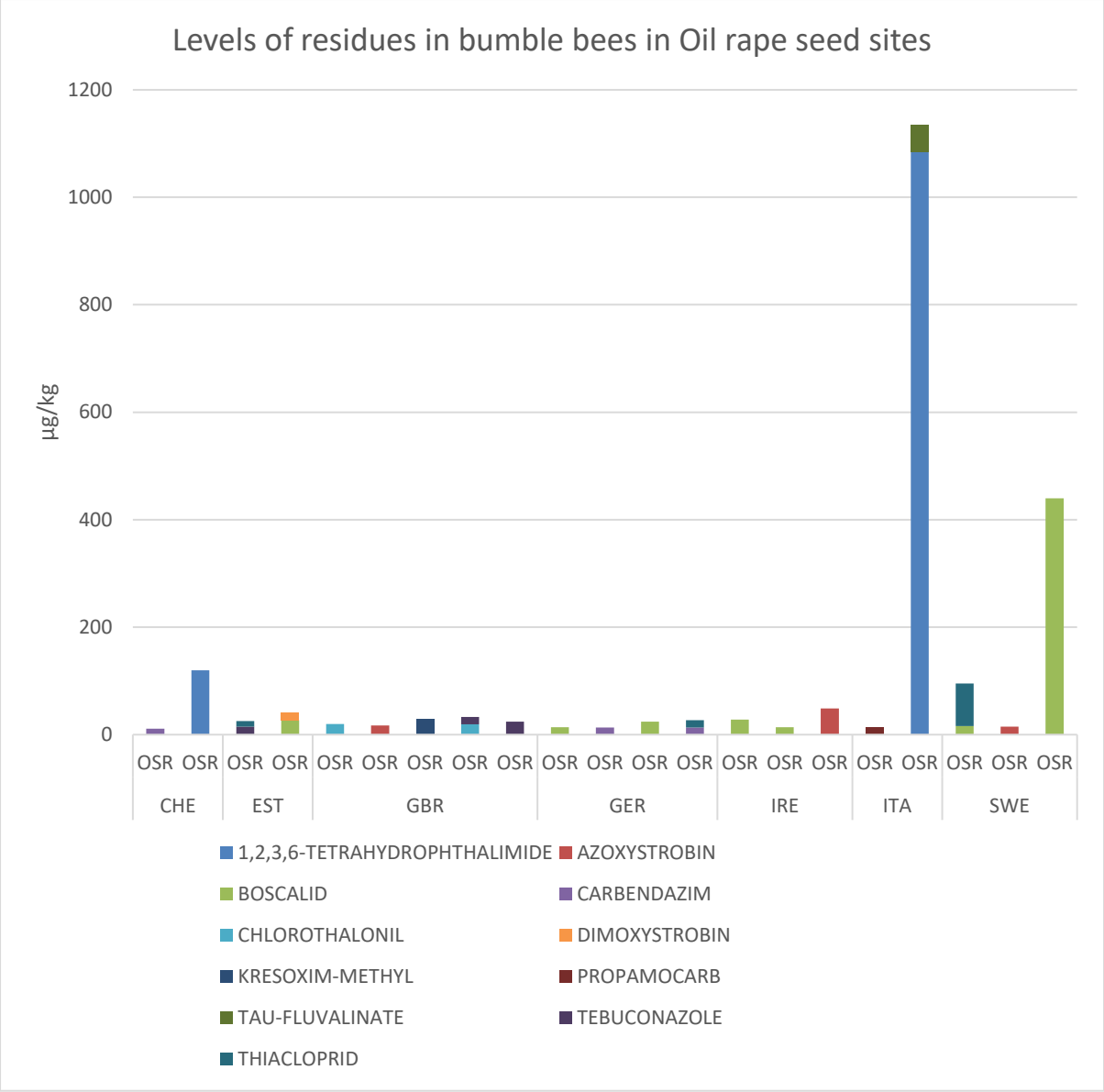

Figure 8: Levels of residues of the active ingredients detected in bumble bees in the oilseed rape sites in the site network countries. In Spanish oilseed rape sites, no positive samples were detected. OSR = oilseed rape sites; CHE = Switzerland; ESP = Spain; EST = Estonia; GBR = Great Britain; GER = Germany; IRE = Ireland; ITA = Italy; SWE = Sweden.

4. Conclusions

Approximately half of the analysed samples of both honey bees and bumble bees were positive for at least one a.i. In general, both species were found to contain more a.i. and with higher levels in the apple sites. Residue types and levels differed between the species, with bumble bees presenting some a.i. not detected in honey bees and vice versa. In apple sites, bumble bees had an average higher level of residues, while in oilseed rape sites the difference was less marked, possibly reflecting more varied environmental conditions around the apple orchards, resulting in different foraging choices.

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