Report on the distribution of agrochemical residues in royal jelly and beeswax

Deliverable D2.5

December 2021

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PoshBee

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

Prepared under contract from the European Commission

Grant agreement No. 773921
EU Horizon 2020 Research and Innovation action

Project acronym:	**PoshBee**
Project full title:	**Pan-european assessment, monitoring, and mitigation of stressors on the health of bees**
Start of the project:	June 2018
Duration:	60 months
Project coordinator:	Professor Mark Brown Royal Holloway, University of London www.poshbee.eu
Deliverable title:	Report on the distribution of agrochemical residues in royal jelly and beeswax
Deliverable n°:	D2.5
Nature of the deliverable:	Report
Dissemination level:	Public
WP responsible:	WP2
Lead beneficiary:	CREA
Citation:	Serra, G., Colombo R., Cardaio I., Costa, C. (2021). *Report on the distribution of agrochemical residues in royal jelly and beeswax*. Deliverable D2.5 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.
Due date of deliverable:	Month n° 42
Actual submission date:	Month n° 43

Deliverable status:

Version	Status	Date	Author(s)
1.0	Final	15 December 2021	Serra, G., Colombo R., Cardaio I., Costa, C. CREA

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Summary

In recent years, honey bee populations have been subject to decline and beekeepers have suffered unprecedented losses. This decline has been linked to several factors, including the use of pesticides which can have toxic effects on bees. 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. Lipophilic substances present in the bee hive or colony can reside in the wax. Both adult and larval stages of honey bees and bumble bees are continuously in contact with the wax, thus, its contamination could be a source of chronic sublethal exposure. Royal jelly can also become contaminated with pesticide residues resulting from treatments applied in the agricultural environment or inside beehives, and as it is used in the colony for feeding the queen and the young larvae, its contamination could have potential effects on the whole colony.

In the POSHBEE project freshly produced wax produced by honey bees and bumble bees, and royal jelly produced by honey bees, were collected from hives and colonies placed in apple orchards and oilseed rape fields characterised by different levels of agricultural intensity, to understand whether the surrounding landscape could contaminate these important hive products. Three countries representing the three European climatic zones for pesticide modelling and registration were chosen for the study of residues in beeswax and royal jelly: Sweden for the Northern zone, Germany for the Central zone, and Italy for the Southern zone. 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 24 analysed samples of beeswax, 17 (71%) of them were found to be positive for at least one a.i.. 15 active ingredients were found. The highest number of positive samples was found in the German oilseed rape sites, followed by Swedish apple orchards. In beeswax produced in the Italian oilseed rape sites, no positive samples were detected. The a.i. with the highest number of positive samples was Tau-Fluvalinate, an a.i. also used in bee hives for control of the parasitic mite *Varroa destructor*. The insecticide Bifenthrin was also detected in several samples in the Swedish sites. In Germany, the synergizer Piperonyl Butxide, used in association with natural pirethrines, was detected.

Of the 24 analysed samples of bumble bee wax, 19 (79%) of them were found to be positive for at least one a.i.. 24 active ingredients were found. Samples from the apple sites in Italy were the ones with the highest number of positive samples and the highest number of different a.i. detected, followed by apple sites in Germany. Residue levels in bumble bee wax highlighted high contamination in the German apple sites by the Captan metabolite, 1,2,3,6-Tetrahydrophthalimide. This a.i. was detected also in Italian apple sites, but with lower residue levels.

Of the 24 analysed samples of royal jelly, 13 (54%) were found to be positive for at least one a.i.. The highest number of positive samples were found in the German oilseed rape samples, and represented by samples positive for the insecticide Bifenthrin. In Italy, several samples were positive for coumaphos, an insecticide that is used against the *Varroa* mite.

1. Introduction

Beeswax is a natural product secreted by specific glands of worker honey bees and bumble bees (Snodgrass, 1956) and consists primarily of complex mixtures of paraffinic hydrocarbons, free fatty acids, esters of fatty acids and fatty alcohol, and di-esters (Tulloch, 1980).

Due to the lipid nature of beeswax, lipophilic substances present in the bee hive or colony can accumulate in the wax. The main source of contamination of honey bee wax is acaricides. In honey bee hives, beeswax can accumulate residues of acaricides used to control the parasitic mite *Varroa destructor* (Bogdanov et al, 1998). In wild bumble bees, the main source of exposure is likely to be environmental contaminants that are brought back to the nest. Both adult and larval stages of honey bees and bumble bees are continuously in contact with the wax, thus, its contamination could be a source of chronic sublethal exposure.

Royal jelly is a highly sugary and proteinaceous substance, produced by the hypopharyngeal and mandibular glands of worker honey bees. It can become contaminated with pesticides and/or antibiotic residues resulting from treatments applied either inside beehives or in the agricultural environment (Karazafiris et al., 2008). Since royal jelly is used in honey bee colonies for feeding the queen and the young larvae, its contamination could have potential effects on the whole colony.

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.

Honey bees and bumble bees forage nectar, pollen, water, and resin from a wide area surrounding their hive (nb., water and resin collection are predominantly honey bee activities). 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 or nest, where they can accumulate and cause chronic and sublethal effects (Johnson, 2015; Sanchez-Bayo & Goka, 2016).

In the POSHBEE project beeswax produced by honey bees and bumble bees, and royal jelly produced by honey bees were collected from hives and colonies placed in apple orchards and oilseed rape fields characterised by different levels of agricultural intensity, to understand whether the surrounding landscape could contaminate these important bee products.

2. Methods

2.1. Field collection of beeswax samples

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 of beeswax and royal jelly were collected from sites in three countries representing the three European climatic zones for pesticide modelling and registration: Sweden for the Northern zone, Germany for the Central zone, and Italy for the Southern zone. Samples were collected from the hives and colonies 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, which were performed at a CREA laboratory specialised in analyses of honey bee products, accredited according to UNI CEI EN ISO/IEC 17025 norm. Samples were posted on dry ice.

2.1.1. Honey bee wax

Samples of beeswax were collected at the end of the flowering period from the site network apiaries (4 OSR and 4 APP sites). The collection of newly produced wax was ensured by the placement of an empty frame (i.e., no comb or foundation) next to the brood cluster, during the second week of blooming. With a strong nectar flow, a normal-size colony easily produces wax to draw a comb in that position within a few days. A minimum of 2 g of new wax was collected from each of the 3 colonies present in each site. The samples were pooled per site.

2.1.2. Bumble bee wax

To ensure collection of clean wax, cotton wool was not placed in the colonies in the 4 sites chosen for wax collection. The bumble bee wax produced during the flowering period was collected at the end of the flowering period and after the bumble bee colonies were removed from the field and frozen for dissection and analysis. If the colony had produced a wax cover, this was used as the wax sample. Otherwise, the newest wax covering the egg clusters and young larvae was collected (the wax from older larvae was not collected as it is difficult to separate it from the cocoons).

2.1. Field collection of royal jelly samples

During regular colony inspections, presence, location and developmental stage of newly started queen-cells was noted, and when the cell was close to closure it was removed from the hive and stored until reaching the laboratory. Here royal jelly was removed with a spatula from all the queen-cells collected at a particular site, thus yielding a pooled sample of royal jelly for each site.

2.2. Chemical analyses

The method for the analysis of pesticides in royal jelly and beeswax has been developed and validated.

The total number of molecules that were screened for, inclusive of isomers and metabolites, was 374. The extraction method is a simplified QuEChERS, which consists of an extraction and a purification stage: in the extraction stage, MgSO4 salts together with a solution of water and acetonitrile were added. The sample was 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 or LC-MS/MS analysis.

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

3. Results

3.1. Beeswax

3.1.1. Positive samples and number of pesticides in honey bee wax

All of the 24 received samples of honey bee wax were analysed, and 17 (71%) of them were found to be positive for at least one a.i.. 15 active ingredients were found: 1,2,3,6-Tetrahydrophthalimide, Azoxystrobin, Bifenthrin, Boscalid, Carbendazim, Chlorthalonil, Coumaphos, Cyprodinil, Fludioxonil, Piperonyl Butxide, Pyrimethanil, Tau-Fluvalinate, Tebuconazole, Thiacloprid, and Tetramethrin.

3.1.2. Detected active ingredients in honey bee wax

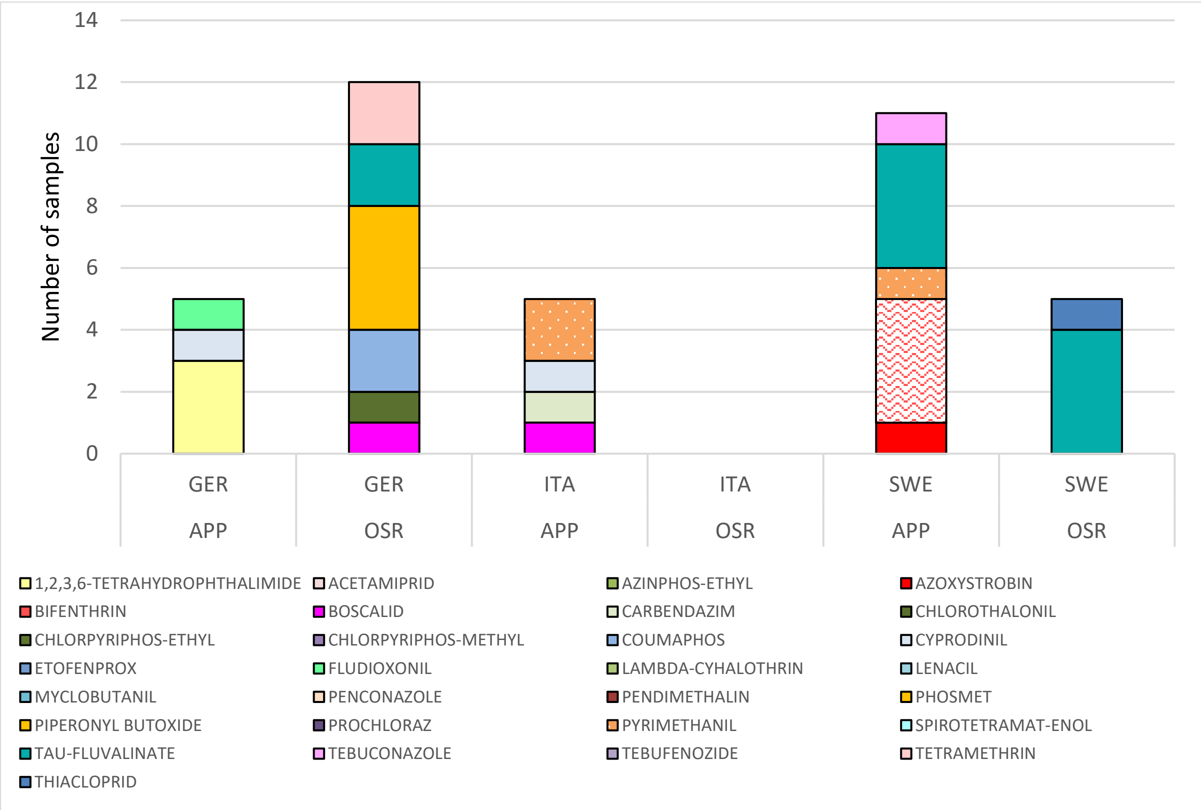

Figure 1: Number of positive samples per a.i., per crop type and per country. APP = apple sites; OSR = oilseed rape sites; GER = Germany; ITA = Italy; SWE = Sweden.

The highest number of positive samples was found in the German oilseed rape sites, followed by Swedish apple orchards (Figure 1). In beeswax produced in the Italian oilseed rape sites, no positive samples were detected. The a.i. with the highest number of positive samples was Tau-Fluvalinate, an a.i. also used in honey bee hives for control of the parasitic mite *Varroa destructor*. The insecticide Bifenthrin was also detected in several samples in the Swedish sites. In Germany, the synergizer Piperonyl Butxide, used in association with natural pirethrines, was detected in 4 samples.

3.1.3. Levels of residues in honey bee wax

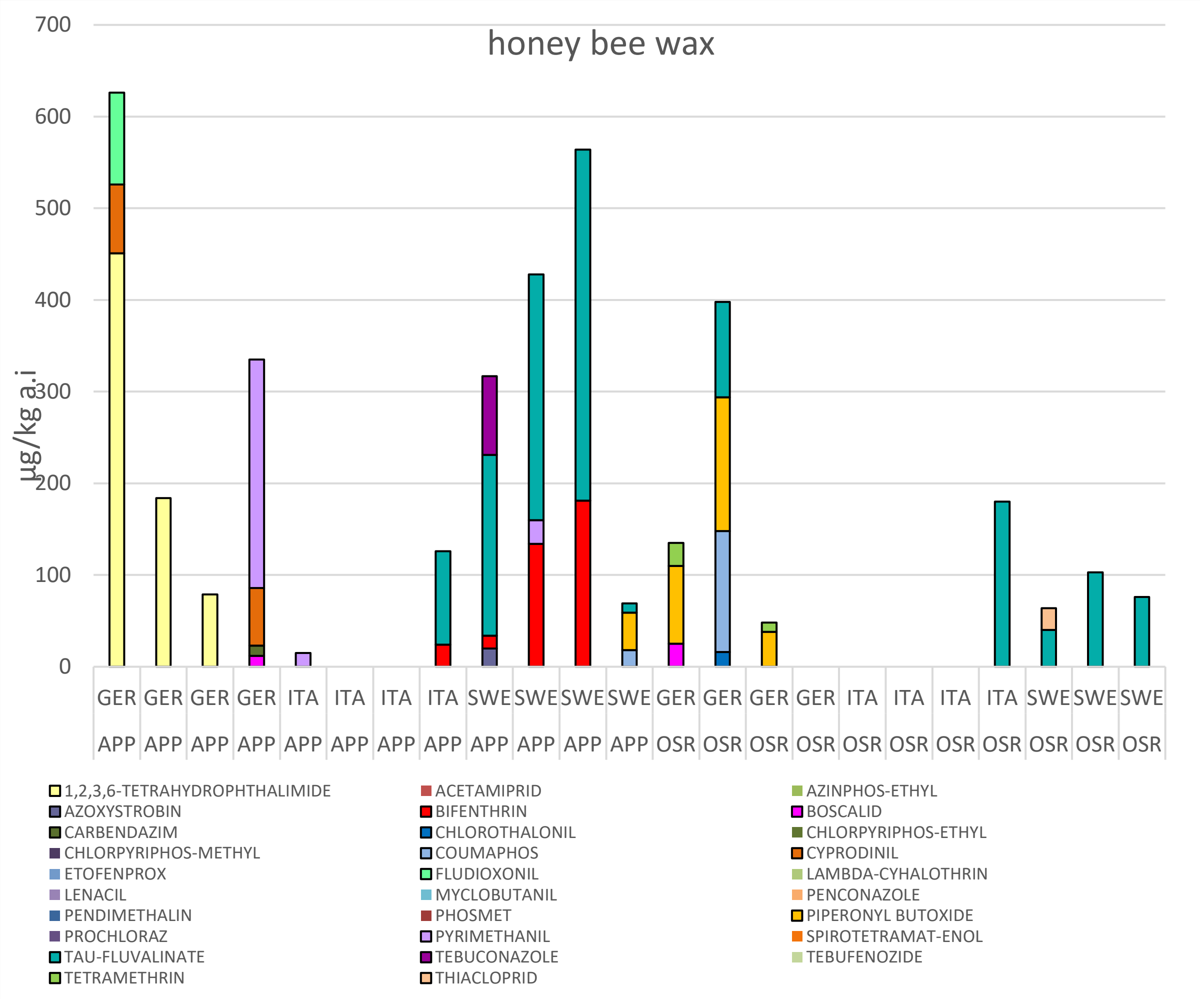

Figure 2: Average residue levels of active ingredients in all monitoring sites. APP = apple sites; OSR = oilseed rape sites; GER = Germany; ITA = Italy; SWE = Sweden.

The a.i. with the highest residue levels were 1,2,3,6-Tetrahydrophthalimide, the metabolite of the fungicide Captan, in one German apple site; the insecticide and acaricide Tau-Fluvalinate found mainly in the Swedish sites, in both apple and oilseed rape sites; and the fungicide Pyrimethanil, again in a German apple orchard. In Swedish apple sites, Bifenthrin was observed with residue levels over 100 µg/kg.

3.1.4. Positive samples and number of pesticides in bumble bee wax

All of the 24 received samples of bumble bee wax were analysed, and 19 (79%) of them were found to be positive for at least one a.i.. 24 active ingredients were found: 1,2,3,6-Tetrahydrophthalimide, Acetamaprid, Azinphos-Ethyl, Boscalid, Carbendazim, Chlorthalonil, Chlorpyriphos-Ethyl, Chlorpyriphos-Methyl, Cyprodinil, Etofenprox, Fludioxonil, Lambda-Cyhalothrin, Lenacil, Myclobutanil, Penconazole, Pendimethalin, Phosmet, Prochloraz, Pyrimethanil, Spirotetramat-enol, Tau-Fluvalinate, Tebuconazole, Tebufenozide, and Thiacloprid.

3.1.5. Detected active ingredients in bumble bee wax

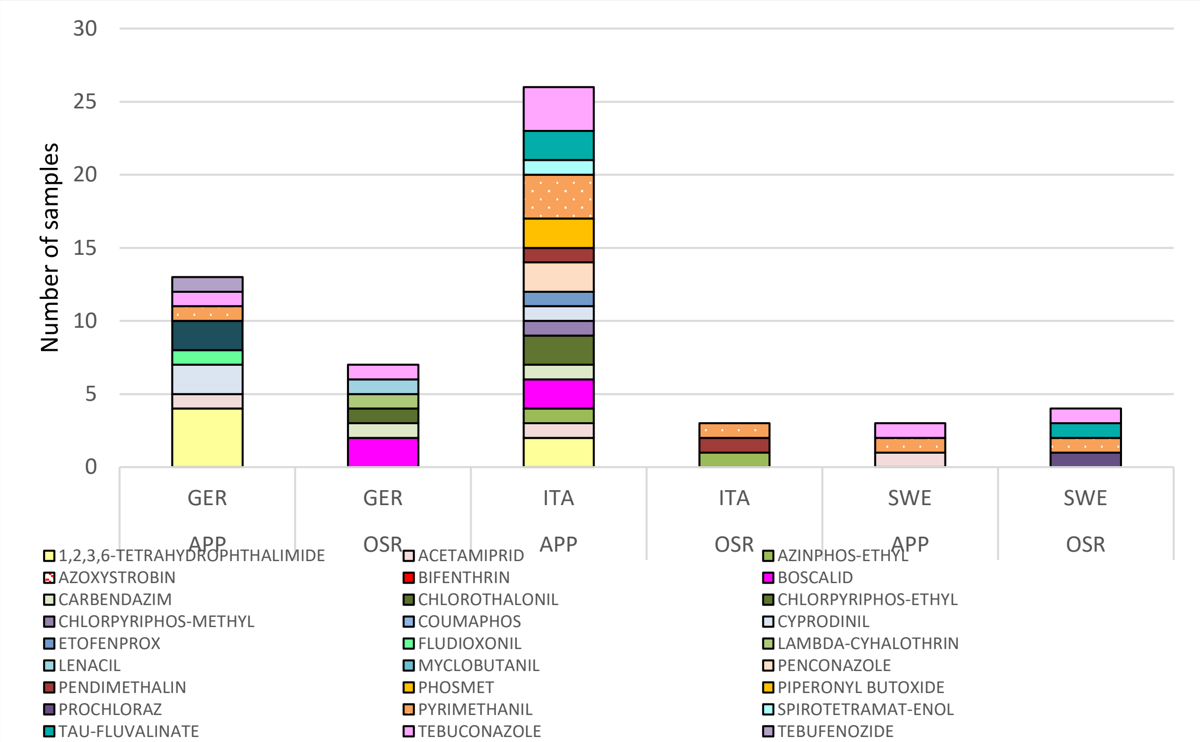

Figure 3: Average residue levels of active ingredients in bumblebees' wax in all monitoring sites. APP = apple sites; OSR = oilseed rape sites; GER = Germany; ITA = Italy; SWE = Sweden.

Samples from the apple sites in Italy were the ones with the highest number of positive samples and the highest number of different a.i. detected, followed by apple sites in Germany.

3.1.6. Levels of residues in bumble bee wax

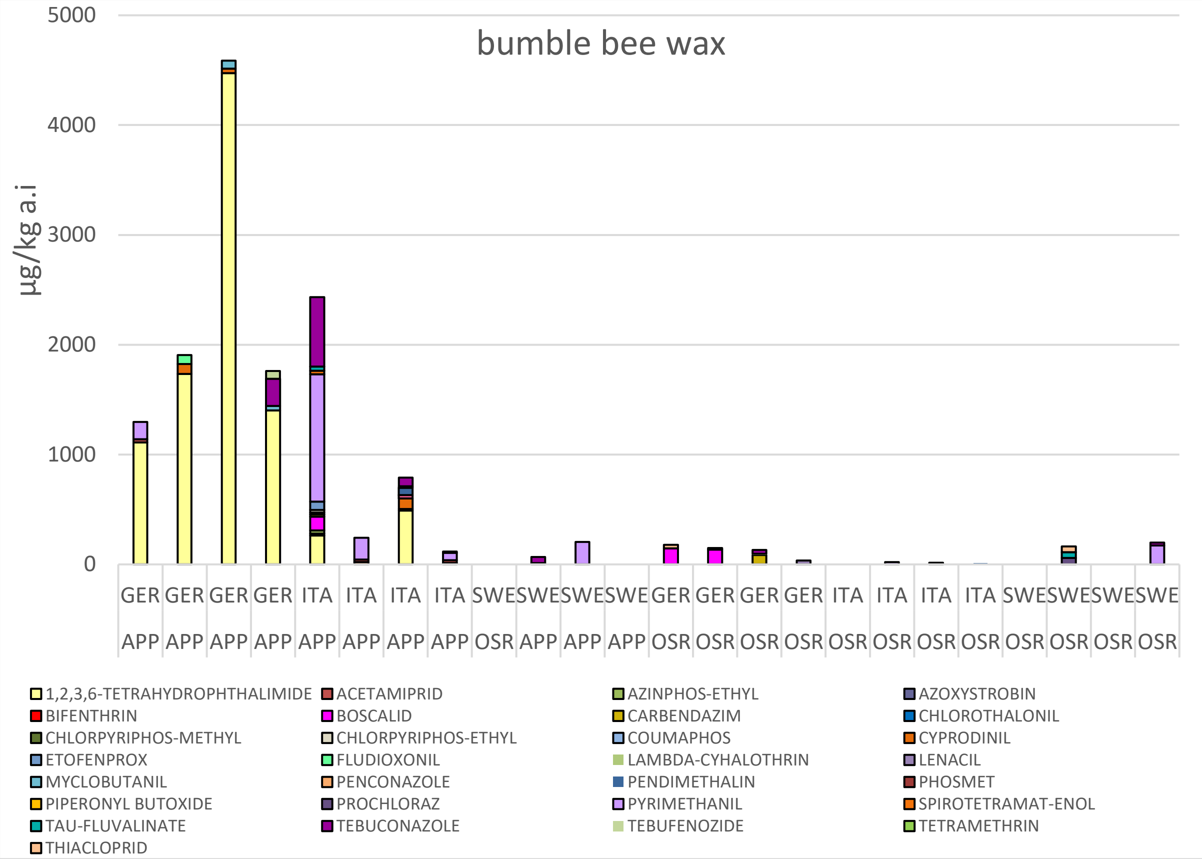

Figure 4: Average residue levels of active ingredients in in all monitoring sites. APP = apple sites; OSR = oilseed rape sites; GER = Germany; ITA = Italy; SWE = Sweden.

Residue levels in bumble bee wax highlighted high contamination in the German apple sites by the Captan metabolite, 1,2,3,6-Tetrahydrophthalimide. This a.i. was also detected in Italian apple sites, but with lower residue levels (Figure 4). Conversely, two other fungicides, Pyrimethanil and Tebuconazole, were found with high levels in one Italian apple site. The bumble bee wax in oilseed rape sites had residue levels below 200 µg/kg.

3.2. Royal jelly

3.2.1. Positive samples and number of pesticides

All of the 24 received samples of royal jelly were analysed, and 13 (54%) of them were found to be positive for at least one a.i.. The active detected a.i. were: Acetamiprid, Bifenthrin, Boscalid, Coumaphos, Endrin Aldehyde, Tau-Fluvalinate, Thiacloprid, and 1,2,3,6-Tetrahydrophthalimide.

3.2.2. Detected active ingredients

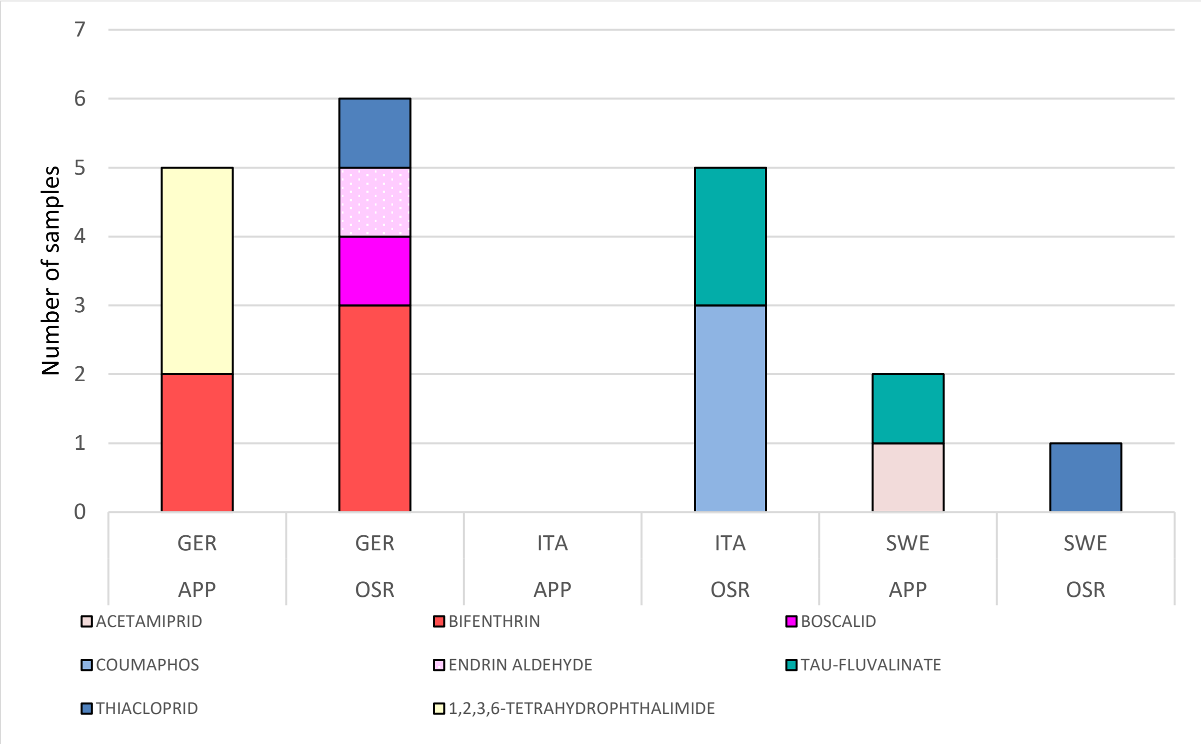

Figure 5: Number of positive samples of royal jelly per a.i., per crop type and per country. APP = apple sites; OSR = oilseed rape sites; GER = Germany; ITA = Italy; SWE = Sweden.

The highest number of positive samples were found in the German oilseed rape samples, and these included samples positive for the insecticide Bifenthrin. In Italy, several samples were positive with coumaphos, an insecticide that used to be used against *Varroa* mites.

3.2.3. Levels of residues

In royal jelly from the German apple sites, as found in honey bee wax and bumble bee wax, high residue levels of the Captan metabolite were detected. Detectable levels of Tau-Fluvalinate were also observed in Sweden (apple site) and in Italy (oil rape seed sites), possibly of in-hive origin.

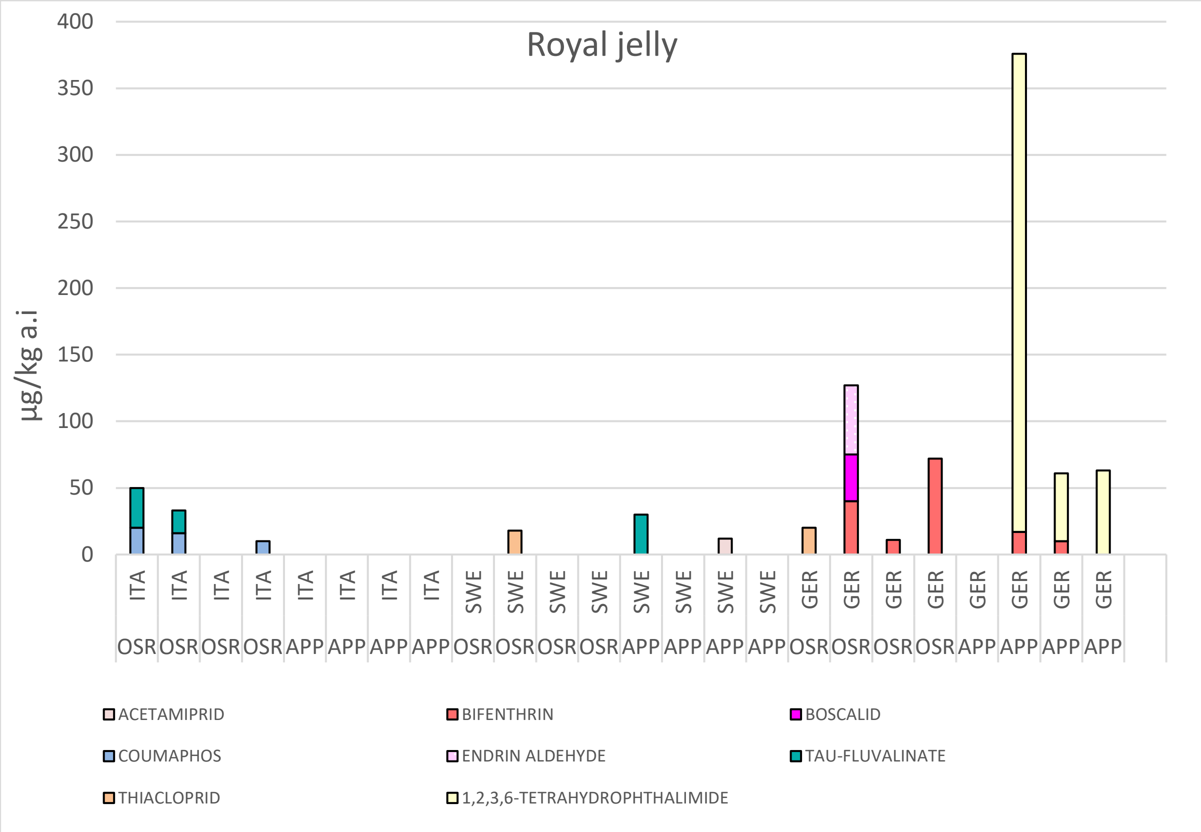

Figure 6: Residue levels in royal jelly across the selected site network, per a.i., per crop type and per country. APP = apple sites; OSR = oilseed rape sites; GER = Germany; ITA = Italy; SWE = Sweden.

4. Conclusions

Beeswax produced by honey bees and bumble bees was contaminated by a.i. used for plant protection, as can be seen by the high rates of positive samples – more than half for all three matrixes. The intensive use of the fungicide Captan was highlighted by the presence of its metabolite 1,2,3,6-Tetrahydrophthalimide in all three matrices.

In contrast to adult honey bees, as described in the PoshBee Deliverable D2.2 Report on exposure of bees to agrochemicals (Serra et al., 2021), beeswax and royal jelly highlighted the frequent presence in the hives of products traditionally used to control the Varroa mite, such as Tau-Fluvalinate and Coumaphos, which can accumulate in wax and even pass onto virgin wax from the old combs present in the hive (Lodesani et al., 2008; Boi et al., 2016). The presence of Bifenthrin in several samples of beeswax and of royal jelly, which was found in a single sample of adult honey bees (in a German site) suggests that the detected residues are due to use for hive treatment against Varroa.

5. References

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